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THE COMPLETE LARVAL DEVELOPMENT OF THE PRONGHORN SPINY LOBSTER *PANULIRUS PENICILLATUS* (DECAPODA: PALINURIDAE) IN CULTURE

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A B S T R A C T

The ability to culture larval lobsters is of paramount importance to the commercial development of effective aquaculture methods. Recently, we developed two separate laboratory culturing strategies that yielded the complete larval development from egg to puerulus (post-larva) for the commercially important and transpacific Pronghorn spiny lobster, *Panulirus penicillatus* (Olivier, 1791). Individual phyllosomal culture of 10 newly hatched animals was carried out in a static seawater system. Two of the 10 phyllosomata held at 24.5-26.0°C metamorphosed after 22 molts to the puerulus stage at 256 and 294 days respectively (final body lengths = 30.80 mm and 32.00 mm). Mass culture of 500 newly hatched phyllosomata was also carried out in two specialized acrylic flow-through seawater tanks. Of the 500 larval animals, 215 were randomly sampled and morphologically staged (10 distinct stages were observed and documented as well as two sub-stages). Seven phyllosomata that were mass cultured metamorphosed to the puerulus stage under a constant temperature regime of 24°C (mean days = 302.4 and mean final body length = 32.133 mm). This species is now one of eight palinurid lobsters and only the fourth *Panulirus* spp. to be cultured completely from hatch to settlement stage. The biological understanding of larval development for this species promotes the feasibility for aquaculture and potentially facilitates future modeling of larval dispersal and duration in the field.

INTRODUCTION

The planktotrophic larval phase (phyllosoma) of spiny lobsters (palinurid) possesses a highly complex and often long-lived pelagic period. As such, phyllosomal form (transparent, 'leaf-like', and dorso-ventrally compressed) reflects their function as hydrodynamic specialists, capable of directed and long-distance movements (e.g., phototaxis, vertical migrations), active feeding, and extended dispersal throughout oceanic waters (Phillips and Sastry, 1980; Phillips and McWilliam, 1986; Vogel, 1994; McWilliam, 1995; Cobb, 1997; Bradford et al., 2005). Our ability to fully discern phyllosomal development is precluded however by difficulties in both accurate identification of field samples and technological shortcomings in culturing (Kittaka and Booth, 2000).

Directed attempts at laboratory culture of *Panulirus* lobsters from hatched larvae have been made to fully interpret their complete development (Saisho, 1962; Ong, 1967; Dexter, 1972). However, success has been limited by the challenges in sustaining such a long-lived larval phase (often, exceeding 300 days) and adequately controlling biological factors such as disease, diet, and optimal abiotic environmental conditions (reviewed in Kittaka, 2000). Of the 21 extant species of *Panulirus* lobsters (George, 2005), only three to date (*P. japonicus*, *P. longipes* and *P. homarus*) have been cultured from hatch to puerulus (post-larva) (Yamakawa et al., 1989; Kittaka and Kimura, 1989; Matsuda and Yamakawa, 2000; Murakami, personal communication). Thus, the development of new culturing techniques (see Matsuda and Takenouchi, 2005) becomes a valuable tool for disseminating and resolving the early life history and field identification for these ecologically and economically important marine species.

The Pronghorn spiny lobster *Panulirus penicillatus* (Olivier, 1791) is one such species. It is geographically the most widely distributed palinurid lobster, found throughout tropical and subtropical areas of the Indo-Pacific region from East Africa and the Red Sea across to Pacific Mexico and Central America (Holthuis, 1991), commonly inhabiting high energy (and turbid) surf-zones of coral reefs and large rocky outcroppings (Holthuis, 1991; Coutures and Chauvet, 2002). Considered a major commercial shallow-water species, *P. penicillatus* is fished extensively, throughout its range, dominated by an artisinal fishery and a more limited trap fishery (Munro, 2000). Wide-ranging stock assessments and biological reference points such as abundance, growth, and fishing mortality (MacDonald, 1982; Munro, 1988; Coutures and Chauvet, 2002), size at sexual maturity, and reproduction (MacDonald, 1982; Juinio, 1987) have been examined, however catch statistics are difficult to interpret since they are often lumped together with data from other lobster species fished concomitantly (Prescott, 1988; Munro, 2000).

Apart from any juvenile or adult life history characteristics, other studies have sought to document phyllosomata of *P. penicillatus* using field-caught plankton samples (Prasad and Tampi, 1959; Johnson, 1968, 1971a, b; Prasad et al., 1975; Tampi and George, 1975). Through the efforts

of Minagawa (1990), *P. penicillatus* phylosomata were cultured and described up unto middle-stages (maximum 10.98 mm body length, BL) from hatch. Despite these noble efforts, we still lack a major body of data that links all developmental and morphological early-life phases for this species. Moreover, no study either by itself or in tandem with others gives a complete snap-shot over the entire suite of phyllosomal development for this species.

Hence, we set out to culture newly hatched phyllosomata of *P. penicillatus* to the puerulus stage under two separate laboratory culturing regimes. Here, we document and quantify all phyllosomal stages for this species, including growth, larval duration, and changes in morphological features. Our findings represent only the fourth *Panulirus* spp. to be cultured to completion. Consequently, this contribution further helps to elucidate field identification and distributions and provides viable methods for future commercial culturing.

Materials and Methods

Terminology

For purposes of clarity, the term 'instar' refers to the intermolt period between two successive ecdyses, while 'stage' denotes one or more specific morphological characteristics unique to that phyllosoma (plural phyllosomata). Thus, a stage can include one or more instars (Mikami and Greenwood, 1997), depending on its duration, and these are documented herein. The nektonic or puerulus (plural pueruli) stage is defined here as the transitional phase between the planktonic and benthic life stages and is specific to species of the family Palinuridae (Phillips and McWilliam, 1986; Jeffs et al., 2005).

Specimens and Larval Source

A single ovigerous *P. penicillatus* female (79.7 mm carapace length) was collected by handnet from the southwestern coast of Japan off Amami-ohshima Island (28°22′N, 129°30′E) on 5 September 2000, packed in a foam polystyrol box with sawdust, and transferred to the Fisheries Research Division in Hamajima, Mie Prefecture, Japan. Prior to hatching (September 19), this lobster was maintained in a flow-through holding tank supplied with sand-filtered seawater (24.2°-26.0°C and 34-35 psu) that was pumped 400 m from the shoreline of the Kumano-nada Sea. The lobster was fed once daily with the mussel, *Mytilus galloprovincialis*. Newly hatched and strong swimming phyllosomata (assayed via surface lighting, see behavior section in results) were sampled and designated for culturing.

Larval Culture

Phyllosomata were cultured under two treatment regimes: 1) individual cultures using small glass bowls (120 and 400 mL) in a static-seawater system for individual growth variation, and 2) group cultures using 40 L acrylic tanks (see Sekine et al., 2000 for tank details) in a flow-through system designed to obtain samples for morphological measurements and behavioral observations. For both trials seawater (33-35 psu) was sand-filtered and processed down through a 0.2 μm membrane filter. A total of 10 and 500 newly hatched phyllosomata were used for individual and group cultures, respectively. Culturing methods in this study followed similarly to those used for *P. longipes* phyllosomata (see Matsuda and Yamakawa, 2000 for details). Lighting conditions were controlled using full-spectrum fluorescent bulbs equipped with electric timers with photoperiods for both treatments regulated at 12L : 12D. Light intensity during the light phase measured ~30 μmol/m^2/s for individual cultures and 5 μmol/m^2/s for group cultures.

Individual Culture.—For individual cultures, larvae were placed into 120 mL glass bowls with 100 mL seawater until the 100th day of culture, after which animals were transferred into 400 mL glass bowls with 350 mL seawater. Seawater was changed daily in each bowl after checking for exuviae and any mortalities. Culturing vessels were placed in a temperature-regulated water bath (Model RZ-150Y, Rei Sea Ltd., Tokyo, Japan) and maintained at 26.0°C until 110 days after hatching (DAH) (mean body length (BL) = 12.9 mm, n = 7), and then kept at 24.0°C according to culturing methods by Matsuda and Yamakawa (1997) for *P. japonicus* phyllosomata. Intermolt period and molt increment in BL were monitored regularly for each phyllosoma. Pueruli that metamorphosed from phyllosomata were cultured in 400 mL glass bowls using methods similar to phyllosomal culture. Pueruli were not fed since this life phase uses energy stored as internal lipids prior to metamorphosis and do not contain functional mouthpart apparatii conducive to exogenous feeding (Lemmens, 1994; Jeffs et al., 2005).

Individually cultured phyllosomata from first to fourth instar were fed solely *Artemia* spp. nauplii (~ 0.6 mm BL) at a density of two individuals per mL once per day and upon reaching the fifth instar were fed a combination of *Artemia* cultured with the diatom, *Phaeodactylum tricornutum*, and finely minced mussel gonad. *Artemia* size was gradually increased to 4-5 mm BL as phyllosomata developed; accordingly, *Artemia* density was decreased to 0.3 individuals per mL. Mussel gonad, fed at rations of 10-12 pieces per glass bowl, was also increased from ~1-4 mm^3 as larvae grew. Dead *Artemia* and uneaten mussel gonad were removed daily and replaced with fresh material daily.

Group Cultures.—Newly hatched larvae (n = 500) were placed into two 40 L specialized acrylic culture tanks in a flow-through seawater system (20-60 L/h). Due to operational constraints, seawater temperature was fixed at 24°C (salinity 33-35 psu) throughout the entire culture duration using an Aquatron-portable APS-206A (Koito Industries Ltd., Japan). Additionally, incoming seawater was processed through a complex series of filtration treatments, which included sand, 1.0 μm wound mesh, and 0.2 μm membrane filters. Phyllosomata were fed similarly to individual cultures described earlier. *Artemia* densities for group cultures were decreased from 1.0 to 0.03 individuals per mL relative to increases in *Artemia* BL. Approximately 50-60 pieces of minced mussel gonad were prepared and fed once daily for each group tank. At 37 and 50 DAH, 135 and 73 phyllosomata were thinned out respectively to reduce larval density to more optimal conditions. Survival rate (S) for group cultures was calculated as follows:

$$S\ (\%) = 100 \times \prod_{i=0}^{n-1} S_i$$

where S_i is the survival rate during the period from ith sampling to $(i+1)$th for all morphological observations. The thinnings at 37 and 50 DAH were treated the same as samplings for calculating the survival rate.

Morphological Measurements

At regular intervals 6-10 phyllosomata of each instar (1-6) were sampled from group cultures. Since the number of instars for each phyllosoma could not be recognized beyond 6th instar, 10 larvae were randomly sampled each week between 50-115 DAH thereafter, five larvae were sampled every three weeks between 121-259 DAH. Beyond 260 DAH, six larvae were sampled semi-regularly until the completion of culture. Consequently, a cumulative total of 215 phyllosomata were sampled from

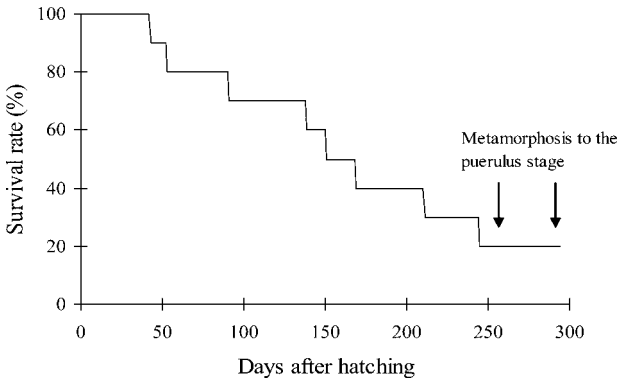


Fig. 1. Survival of phyllosomata of *Panulirus penicillatrus* for individual cultures in small glass bowls (120 or 400-mL).

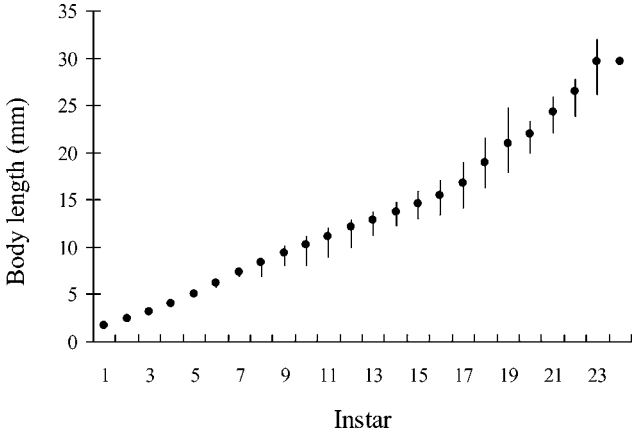


Fig. 2. Body length with development of *Panulirus penicillatus* phyllosomata for individual cultures. Dots and vertical bars indicate the mean and the range of body length.

group cultures, fixed in 5% buffered formalin and then archive-preserved in 70% ethanol. Measurements and drawings of all phyllosomata were made with a Nikon profile projector (model V-12A, Nikon Ltd., Japan) and later digitized using Adobe Illustrator CS2 (Adobe Systems Inc., San Jose, CA, USA).

For individually cultured animals, BL was measured 1-7 days after each ecdysis, with special care being taken to avoid damage to the new instar. Body dimensions of specimens preserved were measured 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), at the widest section of the cephalic shield; thorax width (TW), at the widest section of the thorax; and pleonal length (AL), from a level line with the base of the pleon to the posterior end of the pleon.

Whole body and appendages of phyllosomata were quantified according to Matsuda and Yamakawa (2000). The number of pairs of exopodal natatory setae on the 2nd-3rd maxillipeds and 1st-4th pereiopods were also counted (coupled setae on a segment of exopod were counted as one pair, and a non-coupled seta was counted as 1/2). Mandibles were not described in this study since their small structure precluded accurate and detailed drawings.

For pueruli, body length (BLp) was measured between the anterior margin of the supraorbital plate (which develops into supraorbital spines) to the posterior margin of the telson. Cephalothorax length (CLp) was measured between the anterior margin of the supraorbital plate to the posterior margin of cephalothorax. Molt shells of individuals that reached the juvenile stage in the group cultures, were used for morphological observations after being fixed in 5% buffered formalin and then archive-preserved in 70% ethanol (dead specimens swelled up and were not suitable for observations). Whole body and appendages of pueruli were observed and measured according to previous studies (Briones-Fourzán and McWilliam, 1997; Inoue et al., 2002). Drawings were made similarly as described above.

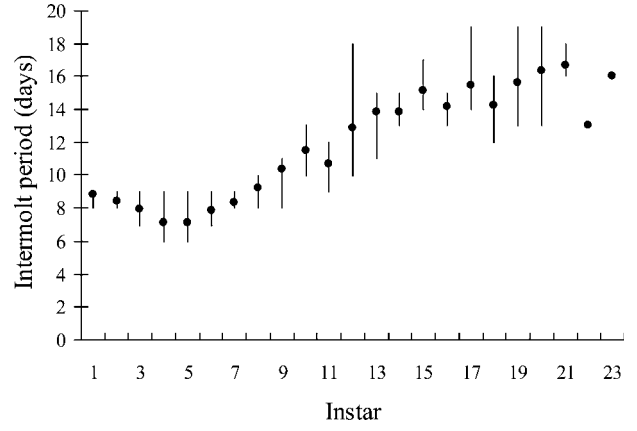


Fig. 3. Intermolt period with development of *Panulirus penicillatus* phyllosomata for individual cultures. Dots and vertical bars indicate the mean and the range of intermolt period.

Table 1. Key to phyllosoma stages of *Panulirus penicillatus*, modified from key for *Panulirus longipes* by Matsuda and Yamakawa (2000).

1. Eyestalk unsegmented	Stage I
Eyestalk segmented	2
2. Expod of 3rd pereiopod not setose	Stage II
Expod of 3rd pereiopod setose	3
3. Fourth pereiopod unsegmented	Stage III
Fourth pereiopod with two or more segments	4
4. Exopod of fourth pereiopod not setose	Stage IV
Exopod of fourth pereiopod setose	5
5. Antennule with three segments	Stage V
Antennule with four segments	6
6. Uropod bud uncleft	Stage VI
Telson not differentiated	VI-1
Telson differentiated	VI-2
Uropod bud cleft or bifid	7
7. Pleopod bud uncleft	Stage VII
Pleopod bud cleft or bifid	8
8. Exopod of 2nd maxilliped not setose	Stage VIII
Exopod of 2nd maxilliped setose	9
9. Gill bud absent or present as rudiment or papilla	Stage IX
Coxae of 1st to 4th pereiopods with bilobed gill buds	Stage X

RESULTS

Individual Cultures

Of 10 individually cultured phyllosomata, two (P-1 and P-2) metamorphosed successfully to the puerulus stage at 256 and 294 days (mean = 275.0 days) (Fig. 1). Body lengths in the final phyllosomal instar of P-1 and P-2 were 30.80 and 32.00 mm, respectively, with a total of 22 instars. The remaining eight mortalities occurring between 43-245 DAH were subject to bacterial infection, confirmed by observing cloudiness in the antennal gland, midgut and intestine, and from complications due to molting, two of the most common forms of phyllosomal mortality (Matsuda and Takenouchi, 2005).

Mean BLs for 1st instar (newly hatched) phyllosomata in individual cultures were 1.78 mm (SD = 0.01, n = 10) increasing linearly with development until the 17th instar

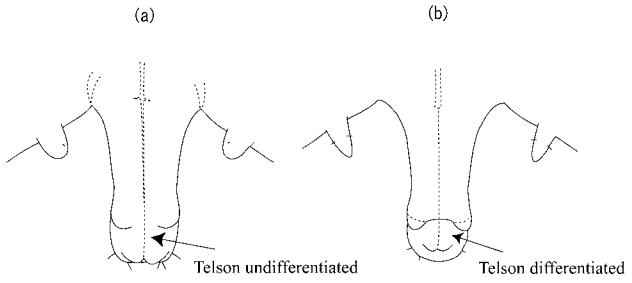


Fig. 4. Developmental states of telson in stage VI-1 (a) and stage VI-2 (b) of *Panulirus penicillatus* phyllosomata.

Table 2. Developmental summary characteristics for phyllosomata of *Panulirus penicillatus*. (biram) biramous; (diff) differentiated; (exop) exopod; (fs) fringing setae; (lts) long terminal setae; (ped) peduncle; (rect) rectangular; (rud) rudimentary; (seg) segmented; (segs) segments; (st) strong terminal spine; (tps) terminal plumose setae; (unseg) unsegmented. =, same as in the previous stage. *1: Integral numbers indicate the number of paired setae; 0.5 denotes the existence of non-paired seta.

				First maxilla						Ventral coxal spine			
Stage	Eyestalk	Antennule	Antenna	Coxal endite	Basal endite	Second maxilliped	First maxilliped	Fourth Pereiopod	Fifth pereiopod	Third maxilliped	First pereiopod	Second pereiopod	Third pereiopod
I	Unseg	Unseg	2 segs (slightly)	2 lts	2 st	4 tps	Bud	Absent	Absent	Present (very short)	Present	Present	Present
II	Seg	=	2 segs	=	=	=	=	Rud bud	=	=	=	=	=
III	=	=	=	=	=	=	Small bud	Bud	Absent or bud	=	=	=	=
IV	=	2-3 segs	=	=	=	=	=	2 segs	Bud	=	=	=	=
V	=	3 segs	=	=	=	1-4 tps	Bud	5 segs	=	=	=	=	=
VI-1	=	4 segs	2-3 segs	=	2-3 st	0-4 tps or 5 fs	=	=	=	=	Present or absent	Present or absent	Present or absent
VI-2	=	=	3-5 segs	=	=	=	Conical bud	=	=	Present or absent	=	=	=
VII	=	=	5 segs	=	3 st	4-30 fs	=	=	Elongated bud or 2 segs	=	Absent	Absent	Absent
VIII	=	=	=	=	=	26-57 fs	Conical bud or trilobed bud	=	2-4 segs	Absent	=	=	=
IX	=	=	=	=	=	48-62 fs	Rect bud or trilobed bud	=	=	=	=	=	=
X	=	=	=	2-3 lts	=	61-77 fs	Trilobed bud	=	5 segs	=	=	=	=

Table 2. Extended.

No. of pairs of natatory setae on exopod of*1										
Second maxilliped	Third maxilliped	First pereiopod	Second pereiopod	Third pereiopod	Fourth pereiopod	Pleopod	Uropod	Telson	Exterior of abdomen	Gill buds
No exop	3	5	5	Exop bud	Absent	Absent	Absent	Absent	Unseg	Absent
=	3	6	6	Elongated exop bud	No exop	=	=	=	=	=
=	3.5-5.5	7-10	7-10	3-7	Exop bud	=	=	=	=	=
=	6-7	11.5-13	11-13	8-9	Elongated exop bud	=	=	=	=	=
=	7.5-8.5	12.5-15	13-14.5	9.5-11.5	3.5-5.5	=	=	=	=	=
=	8-17	14-23	13.5-23	10.5-20.5	5-19	=	Absent or rud bud	=	=	=
=	13-20	18.5-25	18.5-25.5	16.5-23	13-22	Absent or rud bud	Bud	Diff	=	=
No exop or small exop bud	19-26	27-31.5	24-31	22.5-29	23-29	Rud bud or bud	Cleft bud or biram	=	=	=
Exop bud	25.5-30	31-36	29.5-34.5	26-35	27-31	Bifid or biram	Biram	=	Unseg or seg	=
1-3	28.5-29.5	33-35	33-35	30-32	29.5-31.5	+ Rud appendix interna	+ Lateral serration	=	Seg	Absent or unilobed bud
1.5-3.5	29-33	33.5-40	33.5-38.5	31-37	31-35.5	=	=	=	=	Bilobed bud

(Fig. 2) compared with 10.28 mm for the 10th instar (SD = 0.98, n = 8) and 14.58 mm for the 15th instar (SD = 1.28, n = 6). An inflection point was reached around the 17th instar in the relationship between BL and molt increment, only increasing slightly thereafter. The mean BL for the 20th instar was 22.05 mm (SD = 1.80, n = 3). Overall mean instar duration averaged 8.8 days (SD = 0.4, n = 10) for the 1st instar, gradually decreasing to 7.1 days (SD = 0.9, n = 10) for the 4th instar (Fig. 3) after which increasing relative to larval growth over durations of the 10th (11.5 days, SD = 1.1, n = 8), 15th (15.2 days, SD = 1.2, n = 6), and 20th instars (16.3 days, SD = 3.1, n = 3). It appears that a decrease in culture temperature from 26.0 to 24.0°C at 110 DAH did not affect overall instar duration.

Group Cultures

Group cultures progressed favorably without significant mortalities until very late (332 DAH). Survival rates for group cultures were 96.9% at 35 DAH, 88.8% at 80 DAH, 68.5% at 155 DAH, 59.9% at 245 DAH and 41.8% at 314 DAH. A total of seven phyllosomata from group cultures metamorphosed successfully to the puerulus stage between 244 and 330 DAH (mean = 302.4 days). Group culture duration showed a slightly longer trend than that of individual culture (mean = 275.0 days) but was not significant (Mann-Whitney U-test, $U = 3$, $P = 0.241$). Body lengths for the final instar among the seven phyllosomata (range = 29.40-34.60 mm, mean = 32.133 mm) showed no significant difference compared with the final BL between group and individual cultured phyllosomata (Mann-Whitney U-test, $U = 5$, $P = 0.721$).

In accordance with staging criteria for *Panulirus longipes* phyllosomata by Matsuda and Yamakawa (2000) (Table 1), 215 *P. penicillatus* phyllosomata were divided into 10 stages. Stage VI showed a considerably wider range in BL than did any other thus we divided this stage further into two substages based on developmental features of the uropod (Fig. 4). A cumulative summary in developmental sequence traits is given in Table 2, and various body dimensions for each phyllosoma stage are presented in Table 3.

Phyllosomal Behaviors

At the onset of hatching, almost all phyllosomata showed a strong positively phototactic response demonstrated by fast (> 7 BLs/s) and directed swimming towards light, as was apparent by dense aggregations of animals at the surface where dim light was present. This behavior was seen even more intensely when we used a small halogen light (4W) to attract phyllosomata to a specific area of the surface for collection. In addition, group cultured animals (individual cultures were difficult to assess due to shallow and small containers) continued to display positive phototaxis for ~20 DAH; attraction to light was often observed by patchy clusters of phyllosomata at or near the surface in response to discontinuities in light refraction. Animals then began to show negative phototaxis and gathered near the bottom of the tanks in the daytime to escape light that was illuminated from the ceiling of culture room. This behavior continued until the end of the phyllosomal phase. Just prior to metamorphosis to the puerulus stage, final stage phyllosomata became extremely docile and metamorphosed to the puerulus stage on the bottom.

Pueruli

The two pueruli (P-1 and P-2) that metamorphosed in the individual cultures measured 20.30 and 20.80 mm BL. Puerulus P-1 died one day following metamorphosis, while P-2 molted to the first juvenile instar 19 days later. Of the seven pueruli obtained from the group culture, two reached the juvenile stage 16 and 18 days after metamorphosis. Pueruli were characterized similarly to previous studies (Phillips and Sastry, 1980; McWilliam, 1995; George, 2005) as having dorso-ventrally flattened and cylindrically transparent bodies, pigmented eyes, and active forward swimming using their setose pleopods. However we also noted seven distinct white horizontal bands on each antenna just before metamorphosis; the bands remained on the antennae of pueruli even after their metamorphosis, gradually decreasing to 3-4 bands one day prior to metamorphosis to the first juvenile instar.

Phyllosoma Descriptions

Phyllosoma Stage I (n = 7).—Cephalic shield (Fig. 5a). Pear-shaped in outline, distinctly wider than thorax, mean values of CW/CL and CW/TW 0.843 (range = 0.817-0.864)

Table 3. Morphometric size comparisons for phyllosomata of *Panulirus penicillatus* reared in the laboratory. (BL) body length; (CL) cephalic shield length; (CW) cephalic shield width; (TW) thorax width; (AL) abdomen length.

Stage	I	II	III	IV	V	VI-2	VII-2	VII	VIII	IX	X
(N)	(7)	(9)	(18)	(10)	(12)	(70)	(48)	(20)	(10)	(6)	(5)
Body Dimension:											
BL (mm)											
mean	1.776	2.435	3.721	5.147	6.125	8.887	11.405	15.878	21.495	24.600	30.200
min.	1.75	2.34	3.00	4.86	5.75	6.05	9.20	13.40	18.70	21.50	27.00
max.	1.82	2.51	4.28	5.31	6.53	11.30	13.80	18.10	24.80	26.70	33.60
CL (mm)											
mean	1.014	1.537	2.631	3.766	4.585	6.808	8.814	12.223	15.620	17.075	19.500
min.	0.99	1.46	2.06	3.56	4.3	4.45	7.05	10.40	14.20	15.55	18.30
max.	1.03	1.62	3.73	3.92	4.90	8.60	10.70	13.75	17.40	18.00	21.20
CW (mm)											
mean	0.855	1.135	1.698	2.334	2.742	4.081	5.382	7.818	10.440	11.633	13.180
min.	0.83	1.06	1.44	2.16	2.58	2.70	4.15	6.10	9.40	10.60	12.50
max.	0.88	1.18	1.94	2.46	2.90	5.40	6.80	8.90	11.65	12.60	14.30
TW (mm)											
mean	0.627	0.903	1.601	2.408	3.008	4.619	6.026	8.520	11.120	12.350	13.780
min.	0.61	0.87	1.26	2.30	2.85	3.00	4.65	7.10	9.70	11.40	12.90
max.	0.64	0.93	1.94	2.54	3.15	6.05	7.35	9.60	12.20	13.20	14.80
AL (mm)											
mean	0.255	0.271	0.341	0.377	0.413	0.545	0.663	1.038	2.465	3.833	6.560
min.	0.24	0.25	0.30	0.36	0.38	0.40	0.55	0.80	1.40	2.60	5.00
max.	0.27	0.29	0.38	0.41	0.45	0.70	0.80	1.40	4.70	5.00	7.80

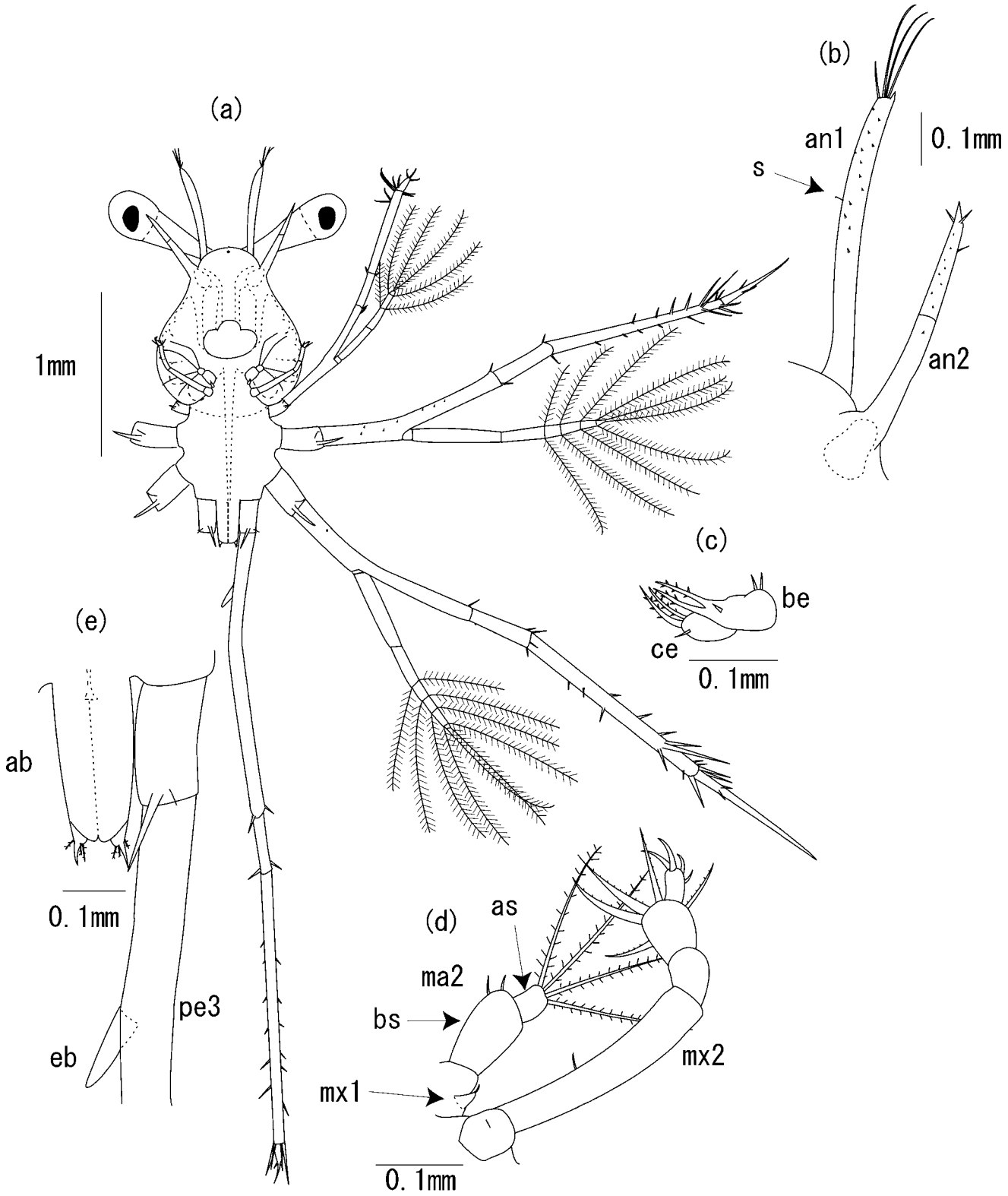


Fig. 5. Phyllosoma Stage I of *Panulirus penicillatus*. a) ventral view; b) left antennule (an1) and antenna (an2), s shows a seta located on the position of future segmentation; c) coxal and basal endites (ce, be) of left 1st maxilla, ventral; d) ventral view of left 2nd maxilla (ma2), 1st maxilliped (mx1) and 2nd maxilliped (mx2); e) 3rd pereiopod (pe3) with exopod bud (eb) and abdomen (ab), ventral.

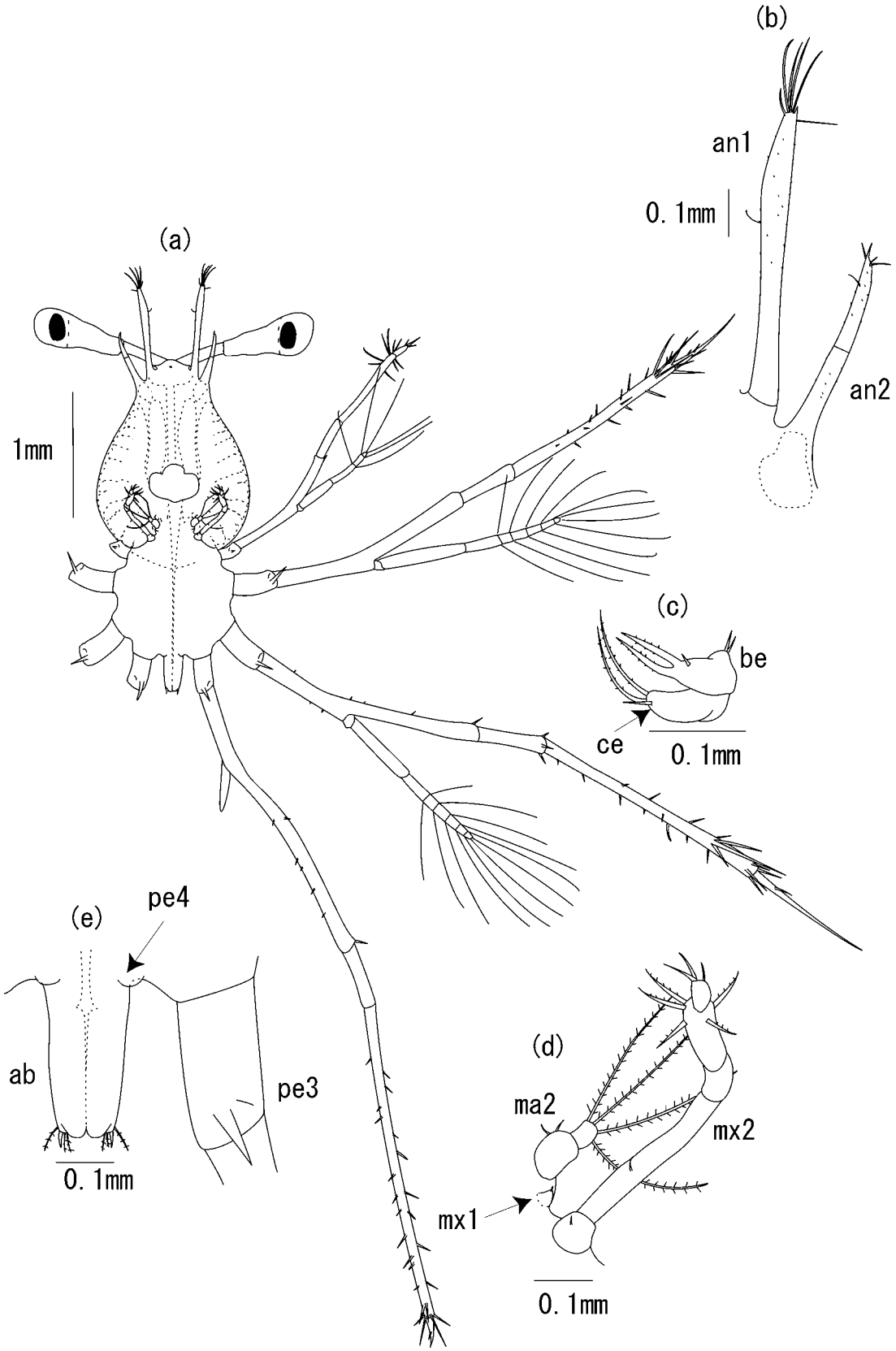
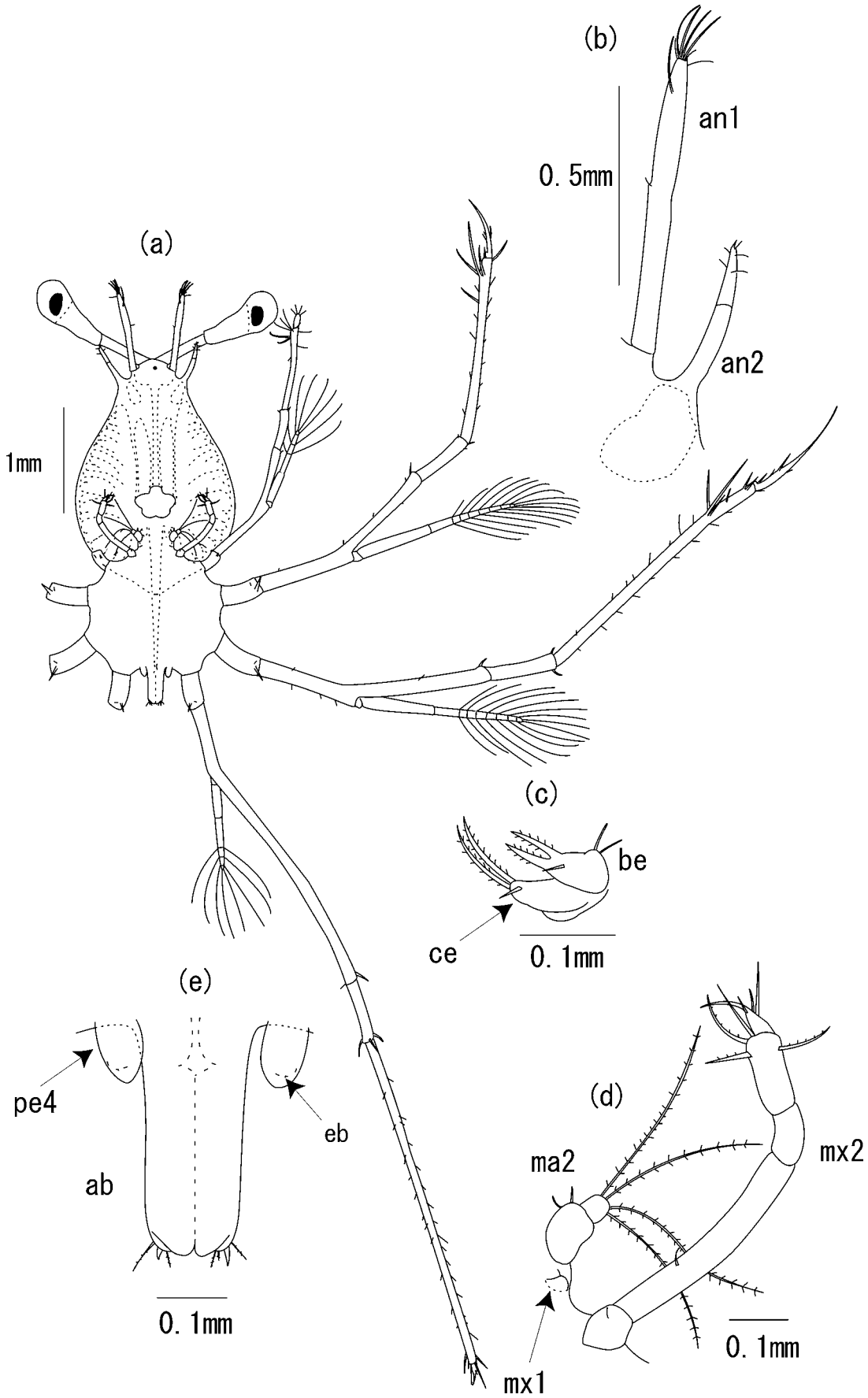


Fig. 6. Phyllosoma Stage II of *Panulirus penicillatus*. a) ventral view; b) left antennule (an1) and antenna (an2); c) coxal and basal endites (ce, be) of left 1st maxilla, ventral; d) ventral view of left 2nd maxilla (ma2), 1st maxilliped (mx1) and 2nd maxilliped (mx2); e) coxa of 3rd pereiopod (pe3), rudimentary 4th pereiopod (pe4) and abdomen (ab), ventral. Setules on plumose setae of exopod of pereiopod are not shown.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an2
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ce
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(d)
ma2
mx2
mx1
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(e)
pe4
eb
ab
0. 1mm

and 1.364 (range = 1.310-1.403), respectively; eyestalk unsegmented.

Antennule (Fig. 5b). Uniramous and unsegmented, with scattered small spines; 3 long sensory setae and 1 short seta present at distal end; 1 short seta present at position of future segmentation (middle of antennule).

Antenna (Fig. 5b). Uniramous, slightly shorter than antennule; a slight segmentation at middle.

First maxilla (Fig. 5c). Coxal endite with 2 long serrated terminal setae and 1 short subterminal seta; basal endite with 2 strong serrated terminal spines and 1 short subterminal seta, along with 2 short setae on vestigial palp.

Second maxilla (Fig. 5d). 2-segmented; larger basal segment with 2 short setae on anterior margin; smaller distal segment bearing 4 long plumose setae.

Maxillipeds (Fig. 5a, d). 1st maxilliped present as small bud with 1 seta at top, located near base of 2nd maxilla; 2nd maxilliped 5-segmented, without exopod; 3rd maxilliped well-developed, having exopod bearing 3 pairs of natatory plumose setae, with 1 short ventral coxal spine.

Pereiopods (Fig. 5a, e). 1st and 2nd pereiopods 5-segmented, exopod with 5 pairs of natatory plumose setae; exopod of 3rd pereiopod present as small bud; each 1st-3rd pereiopod with 1 ventral coxal spine and 1 accessory seta.

Abdomen (Fig. 5e). Parallel-sided, longer than coxa of 3rd pereiopod, bearing 1 postero-lateral spine with 2-3 short basal setae at each side; pleopod, uropod and telson not differentiated.

Phyllosoma Stage II (n = 9).—Cephalic shield (Fig. 6a). Becoming elongated, mean values of CW/CL and CW/TW 0.739 (range = 0.723-0.758) and 1.257 (range = 1.206-1.287), respectively; eyestalk segmented.

Antennule (Fig. 6b). With 4 long sensory setae and 1 short seta at distal end.

Antenna (Fig. 6b). No marked change.

First maxilla (Fig. 6c). No marked change.

Second maxilla (Fig. 6d). No marked change.

Maxillipeds (Fig. 6a, d). No marked change.

Pereiopods (Fig. 6a, e). Exopods of 1st and 2nd pereiopods with 6 pairs of natatory plumose setae; exopod of 3rd pereiopod as long as abdomen, still without plumose setae; 4th pereiopod present as minute swelling.

Abdomen (Fig. 6e). No marked change.

Phyllosoma Stage III (n = 18).—Cephalic shield (Fig. 7a). Becoming more elongated, medial part in posterior margin slightly projecting posteriorly, mean value of CW/CL 0.663 (range = 0.621-0.709); nearly as wide as thorax, mean value of CW/TW 1.071 (range = 0.990-1.159).

Antennule (Fig. 7b). With 1 subterminal sensory seta; minute protuberance at middle.

Antenna (Fig. 7b). No marked change.

First maxilla (Fig. 7c). Coxal endite with 1-2 short subterminal setae.

Second maxilla (Fig. 7d). No marked change.

Maxillipeds (Fig. 7a, d). Exopod of 3rd maxilliped with 3.5-5.5 pairs of natatory plumose setae.

Pereiopods (Fig. 7a, e). Exopod of 3rd pereiopod with 3-7 pairs of natatory plumose setae; 4th pereiopod elongated, shorter than or as long as abdomen, still without segmentation, bearing minute exopod bud dorsally; 5th pereiopod present as minute swelling in larger individuals.

Abdomen (Fig. 7e). No marked change.

Phyllosoma Stage IV (n = 10).—Cephalic shield (Fig. 8a). Mean value of CW/CL 0.620 (range = 0.607-0.632); narrower than thorax in most individuals, mean value of CW/TW 0.967 (range = 0.939-1.035).

Antennule (Fig. 8b). 2-segmented; proximal segment with a partial segmentation in many individuals, antero-lateral margin developing to small lump; distal segment with 2 rows of subterminal sensory setae.

Antenna (Fig. 8b). No marked change.

First maxilla (Fig. 8c). Coxal endite with 2 short subterminal setae; basal endite with 1-2 short subterminal setae and 2 short setae on vestigial palp.

Second maxilla (Fig. 8d). No marked change.

Maxillipeds (Fig. 8a, d). 1st maxilliped becoming relatively smaller than in Stage III, apical seta disappearing in many individuals.

Pereiopods (Fig. 8a, e). 4th pereiopod 2-segmented, exopod bud elongated and without plumose setae; 5 pereiopod present as small bud at base of abdomen.

Abdomen (Fig. 8e). No marked change.

Phyllosoma Stage V (n = 12).—Cephalic shield (Fig. 9a). This stage, together with stage VI-1, having the smallest value of CW/CL, 0.598 (range = 0.590-0.605) throughout phyllosoma stages, mean value of CW/TW 0.911 (range = 0.877-0.949).

Antennule (Fig. 9b). 3-segmented; proximal segment with a slight partial segmentation in many individuals; middle segment possessing short process on antero-lateral margin.

Antenna (Fig. 9b). No marked change.

First maxilla (Fig. 9c). Coxal endite with 2-3 short subterminal setae.

Second maxilla (Fig. 9d). Segmentation between basal segment and distal one becoming indistinct; distal segment with 1-4 apical natatory setae.

Maxillipeds (Fig. 9a, d). No marked change.

Pereiopods (Fig. 9a, e). 4th pereiopod 5-segmented, exopod with 3.5-5.5 pairs of plumose setae.

Abdomen (Fig. 9e). No marked change.

Phyllosoma Stage VI-1 (n = 70).—Cephalic shield (Fig. 10a). Lemon-shaped in outline, the widest part located near to middle of length, mean values of CW/CL and CW/TW 0.599 (range = 0.568-0.647) and 0.884 (range = 0.832-0.942), respectively.

Antennule (Fig. 10b). 4-segmented in all individuals; 3rd segment with finger-like process on antero-lateral margin;

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Fig. 7. Phyllosoma Stage III of *Panulirus penicillatus*. a) ventral view; b) left antennule (an1) and antenna (an2); c) coxal and basal endites (ce, be) of left 1st maxilla, ventral; d) ventral view of left 2nd maxilla (ma2), 1st maxilliped (mx1) and 2nd maxilliped (mx2); e) 4th pereiopod (pe4) with exopod bud (eb) and abdomen (ab), ventral. Setules on plumose setae of exopod of pereiopod are not shown.

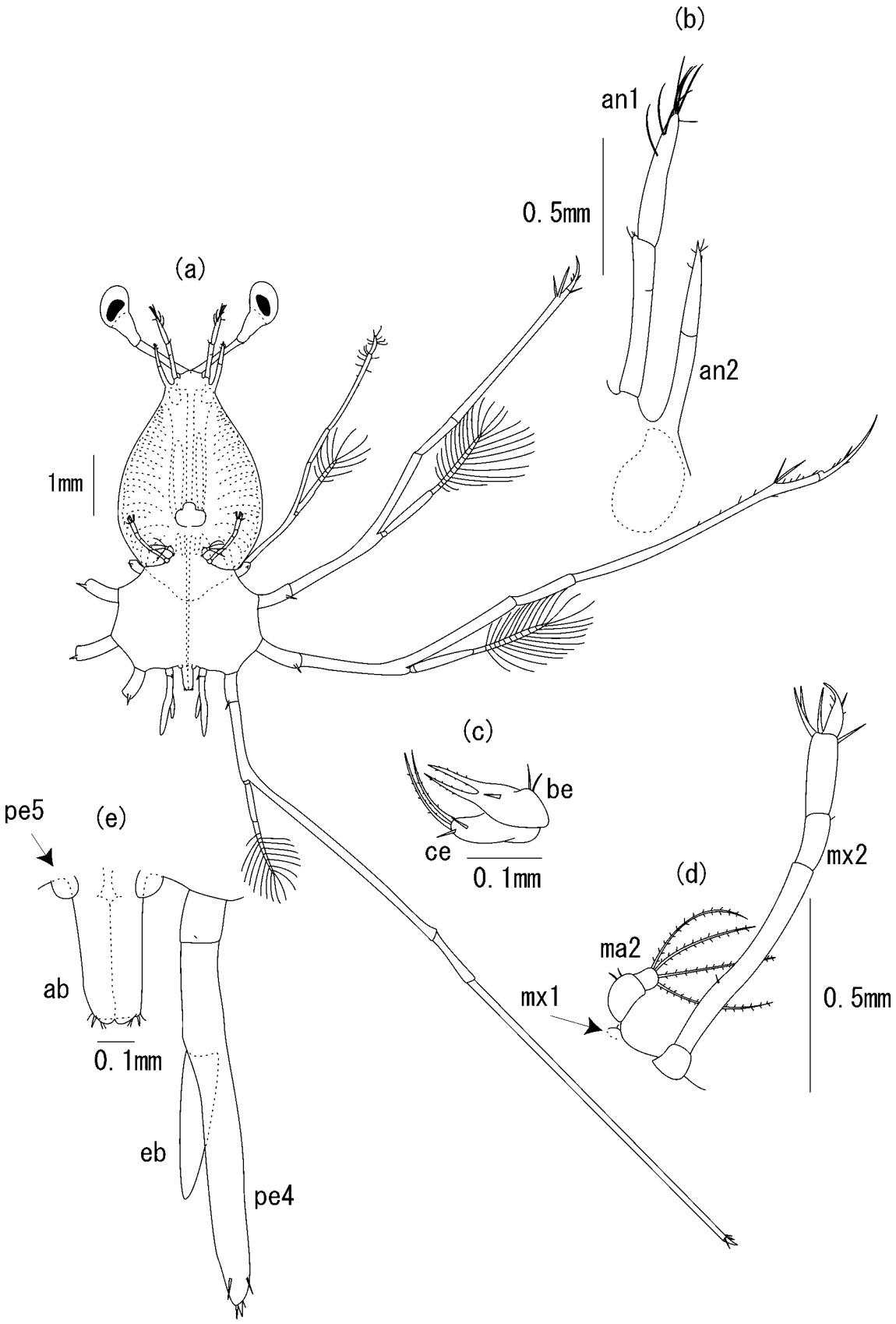


Fig. 8. Phyllosoma Stage IV of *Panulirus penicillatus*. a) ventral view; b) left antennule (an1) and antenna (an2); c) coxal and basal endites (ce, be) of left 1st maxilla, ventral; d) ventral view of left 2nd maxilla (ma2), 1st maxilliped (mx1) and 2nd maxilliped (mx2); e) 4th pereiopod (pe4) with exopod bud (eb), 5th pereiopod (pe5) and abdomen (ab), ventral. Setules on plumose setae of exopod of pereiopod are not shown.

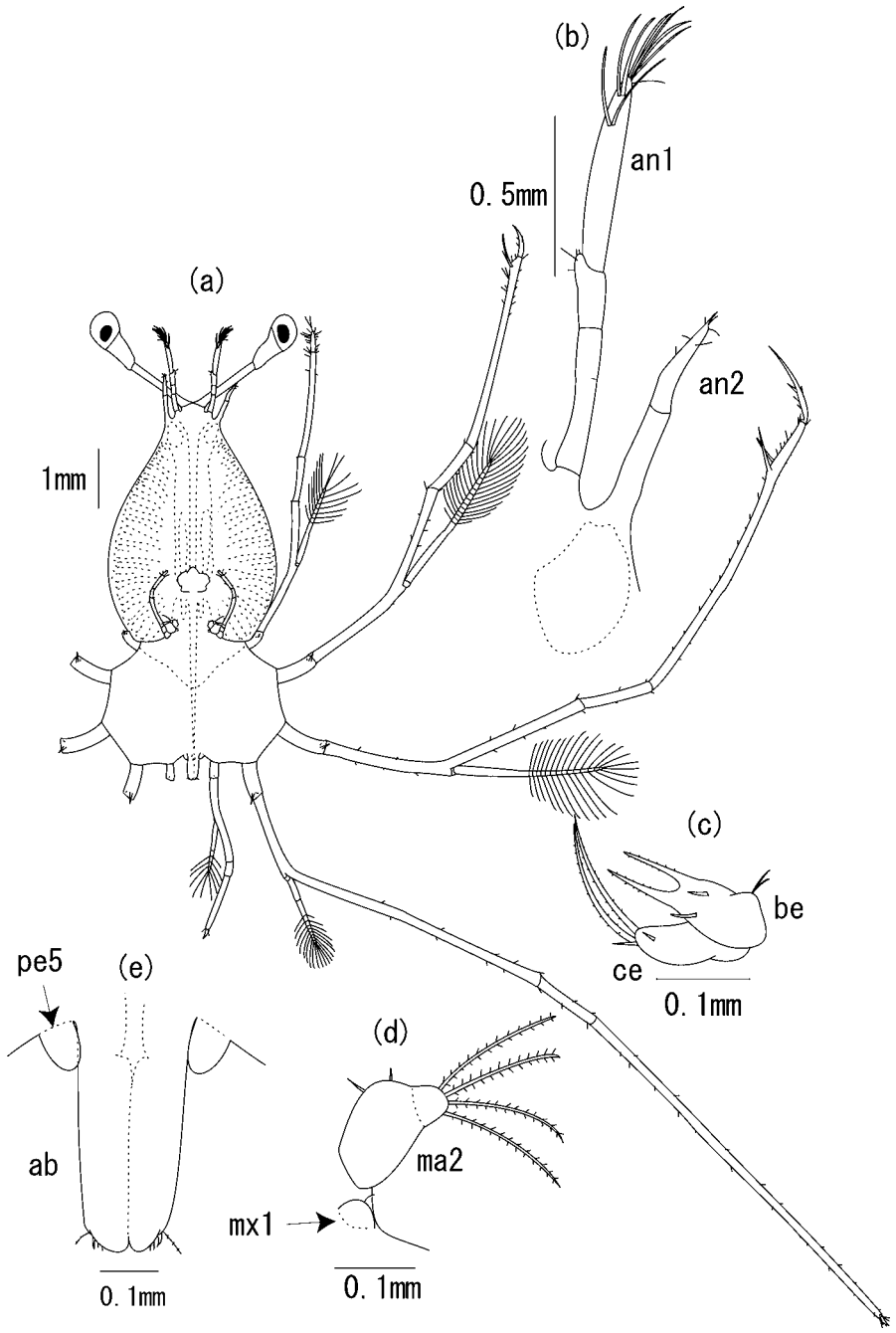


Fig. 9. Phyllosoma Stage V of *Panulirus penicillatus*. a) ventral view; b) left antennule (an1) and antenna (an2); c) coxal and basal endites (ce, be) of left 1st maxilla, ventral; d) ventral view of left 2nd maxilla (ma2) and rudimentary 1st maxilliped (mx1); e) ventral view of 5th pereiopod (pe5) and abdomen (ab). Setules on plumose setae of exopod of pereiopods are not shown.

4th (distal) segment with 2-8 rows of subterminal sensory setae.

Antenna (Fig. 10b). 2-4-segmented.

First maxilla (Fig. 10c). Coxal endite with 2-6 short subterminal setae; basal endite with 2-3 strong serrated terminal spines.

Second maxilla (Fig. 10d). Somewhat broader, with 0-4 apical plumose setae.

Maxillipeds (Fig. 10a, d). 1st maxilliped developing to conical bud; otherwise unchanged.

Pereiopods (Fig. 10a, e). 5th pereiopod located at distance from base of abdomen; ventral coxal spines on 1st-3rd pereiopods disappearing in some individuals; minute sternal spines present near the coxae of 1st-3rd pereiopods in advanced individuals.

Abdomen (Fig. 10e). Uropod differentiated as faint swelling in many individuals; pleopod and telson absent.

Phyllosoma Stage VI-2 (n = 48).—Cephalic shield (Fig. 11a). Mean value of CW/CL and CW/TW 0.610

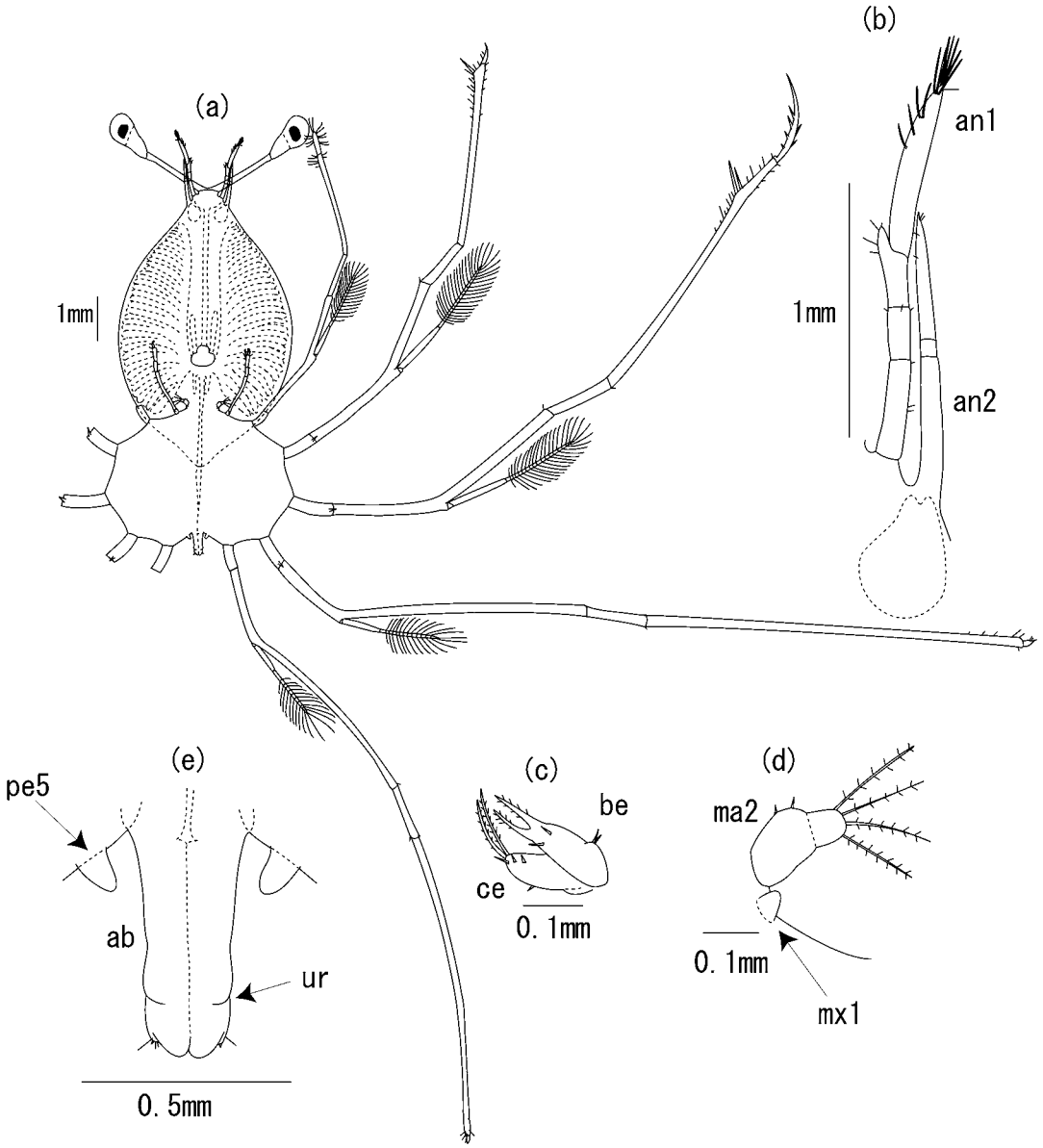


Fig. 10. Phyllosoma Stage VI-1 of *Panulirus penicillatus*. a) ventral view; b) left antennule (an1) and antenna (an2); c) coxal and basal endites (ce, be) of left 1st maxilla, ventral; d) ventral view of left 2nd maxilla (ma2) and 1st maxilliped (mx1); e) 5th pereiopod (pe5) and abdomen (ab) (ur = uropod), ventral. Setules on plumose setae of exopod of pereiopod are not shown.

(range = 0.575-0.656) and 0.893 (range = 0.853-0.979), respectively.

Antennule (Fig. 11b). 4 segmented; finger-like process on antero-lateral margin of 3rd segment elongated; 4th segment with 4-12 rows of subterminal sensory setae.

Antenna (Fig. 11b). 3-5-segmented, shorter than or nearly as long as antennule.

First maxilla (Fig. 11c). Basal endite with 2-3 strong serrated terminal spines and 2-3 short subterminal setae, along with 2 short setae on vestigial palp.

Second maxilla (Fig. 11d). Becoming more swollen than in previous stage; proximal portion with 0-3 short setae on anterior margin; apical plumose setae lacking in most individuals.

Maxillipeds (Fig. 11a, d). Sternal spine present near the coxa of 3rd maxilliped in advanced individuals.

Pereiopods (Fig. 11a, e). Ventral coxal spine on 1st pereiopod remaining in half of the specimens observed, but 2nd-3rd pereiopods lacking the spine in most individuals; sternal spines present near the coxae of 1st-3rd pereiopods in all specimens plus 4th pereiopod in advanced individuals.

Abdomen (Fig. 11e). Broadened at base and minimum width at middle of length; pleopod present as 1-2 pairs of slight swelling in well-advanced individuals; uropod small bud; telson differentiated.

Phyllosoma Stage VII (n = 20).—Cephalic shield (Fig. 12a). Maximum width located slightly closer to anterior part rather than to middle of length, mean values of CW/CL and CW/TW 0.634 (range = 0.587-0.672) and 0.917 (range = 0.859-0.967), respectively.

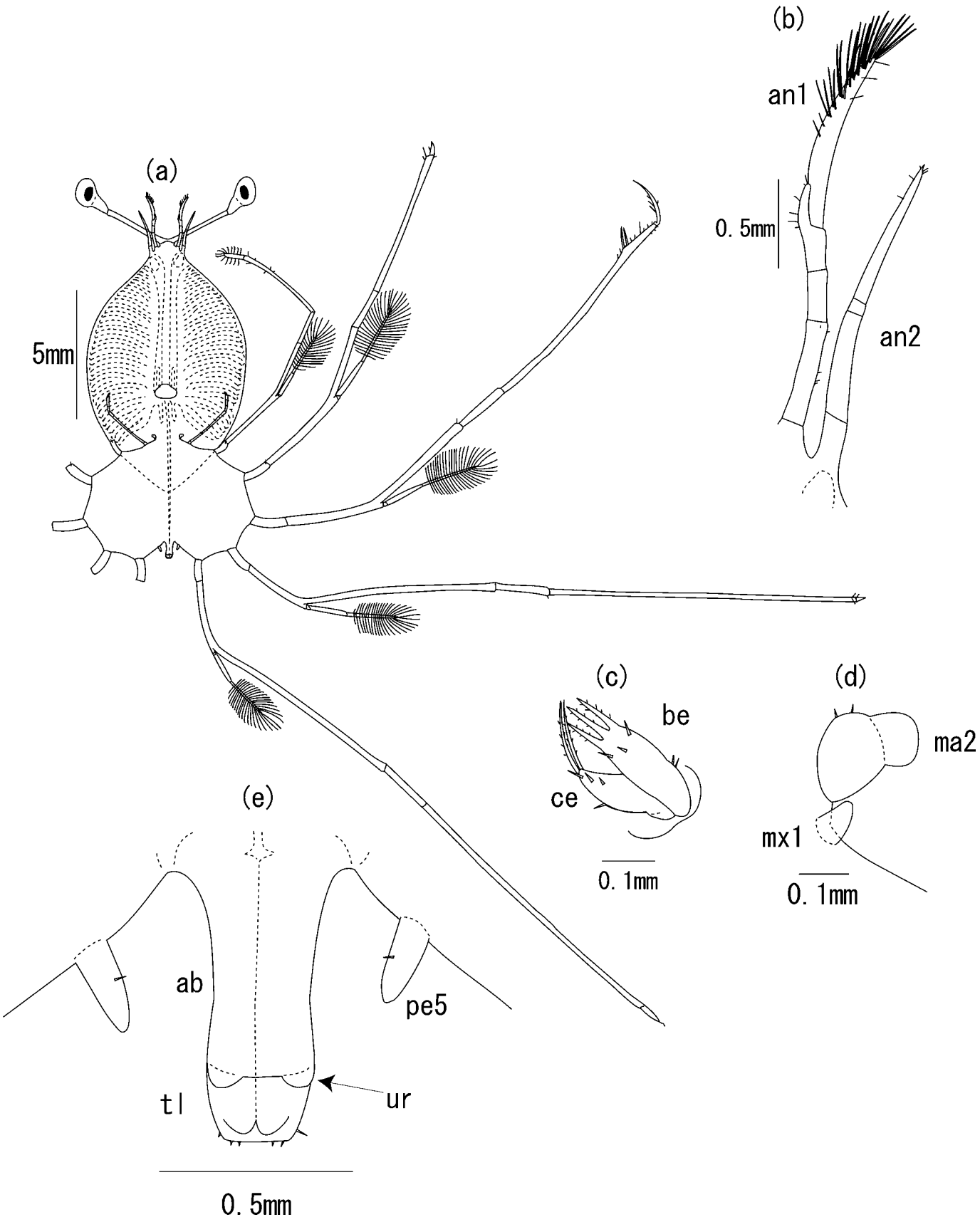
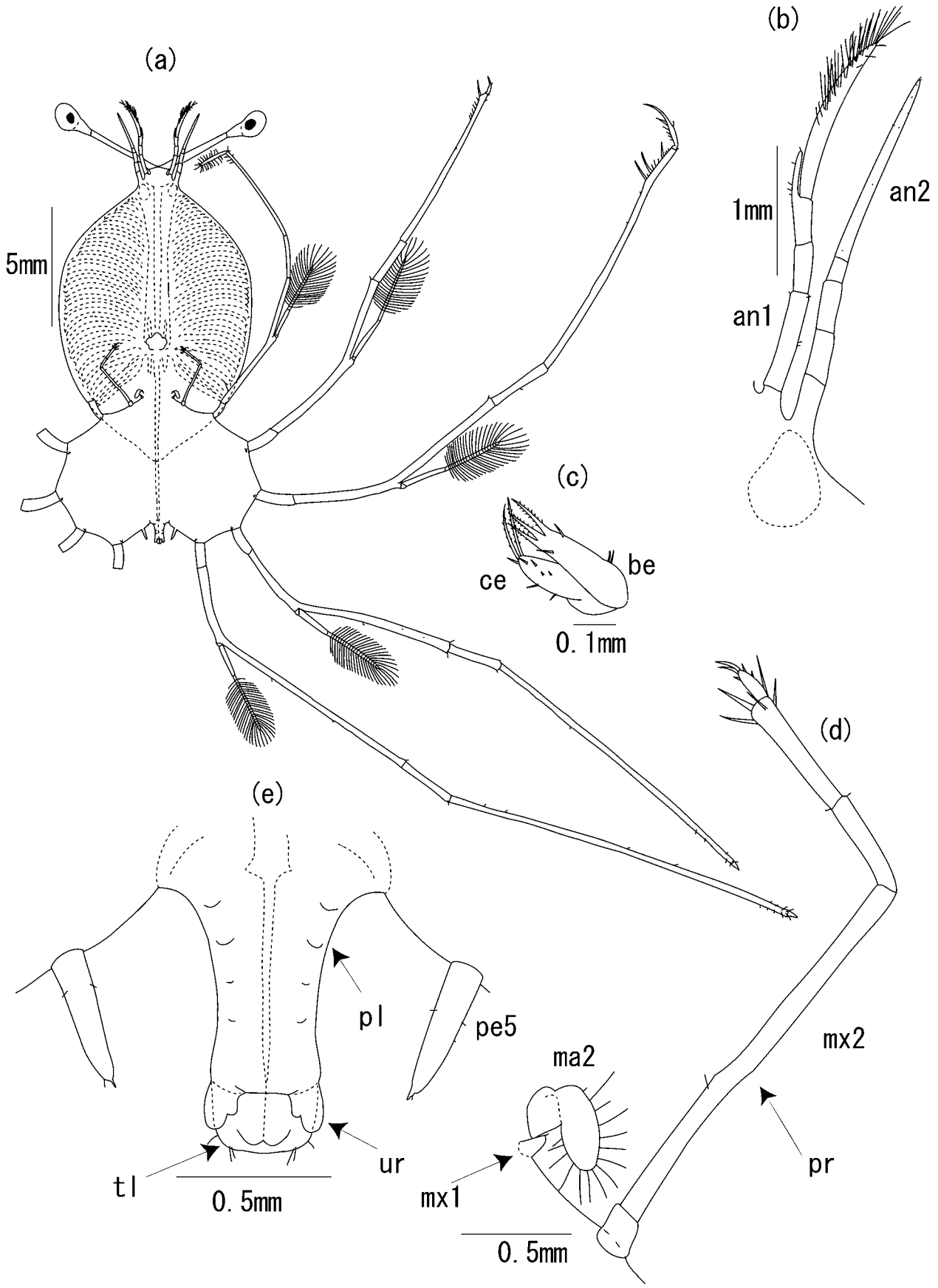


Fig. 11. Phyllosoma Stage VI-2 of *Panulirus penicillatus*. a) ventral view; b) left antennule (an1) and antenna (an2); c) coxal and basal endites (ce, be) of left 1st maxilla, ventral; d) ventral view of left 2nd maxilla (ma2) and 1st maxilliped (mx1); e) 5th pereiopod (pe5) and abdomen (ab) (ur = uropod, tl = telson), ventral. Setules on plumose setae of exopod of pereiopod are not shown.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Antennule (Fig. 12b). 4th segment with 7-18 rows of subterminal sensory setae.

Antenna (Fig. 12b). 5-segmented, longer than or as long as antennule.

First maxilla (Fig. 12c). Coxal endite with 5-8 short subterminal setae; basal endite with 3 strong serrated terminal spines.

Second maxilla (Fig. 12d). Distal portion distinctly expanding posteriorly, fringed with 4-30 short setae.

Maxillipeds (Fig. 12a, d). Exopod of 2nd maxilliped present as minute protuberance in some individuals; sternal spine present near the coxa of 3rd maxilliped.

Pereiopods (Fig. 12a, e). 5th pereiopod elongated, 1/3-1/2 length of abdomen; ventral coxal spines on 1st-3rd pereiopods absent; sternal spines present near the coxae of 1st-4th pereiopods.

Abdomen (Fig. 12e). Pleopod 4 pairs of small buds; uropod cleft bud or biramous.

Phyllosoma Stage VIII (n = 9).—Cephalic shield (Fig. 13a). Mean values of CW/CL and CW/TW 0.667 (range = 0.645-0.693) and 0.940 (range = 0.891-0.969), respectively.

Antennule (Fig. 13b). 4th segment lacking terminal sensory setae in some individuals, with 17-24 rows of subterminal sensory setae.

Antenna (Fig. 13b). Elongated, about 1.5-2 times as long as antennule.

First maxilla (Fig. 13c). Coxal endite with 7-10 short subterminal setae; basal endite with 3 short subterminal setae and 2 short setae on vestigial palp.

Second maxilla (Fig. 13d). Distal portion expanding widely and posteriorly more than in stage VII, bordered by 26-57 short setae; anterior margin of proximal portion slightly projecting anteriorly, with 1-4 short setae.

Maxillipeds (Fig. 13a, d). 1st maxilliped conical or rectangular bud, with short lobe on lateral margin in well-advanced individuals; exopod of 2nd maxilliped still without plumose setae.

Pereiopods (Fig. 13a, e). 5th pereiopod 2-3 segmented, about 1/2-3/4 length of abdomen.

Abdomen (Fig. 13e). Weakly segmented; pleopod 4 pairs of bifid bud or biramous; uropod distinctly biramous, reaching posterior margin of telson.

Phyllosoma Stage IX (n = 6).—Cephalic shield (Fig. 14a). Mean values of CW/CL and CW/TW 0.681 (range = 0.670-0.700) and 0.942 (range = 0.920-0.976), respectively.

Antennule (Fig. 14b). Terminal sensory setae of 4th segment absent in all individuals; with 21-26 rows of subterminal sensory setae.

Antenna (Fig. 14b). About 2-2.5 times as long as antennule.

First maxilla (Fig. 14c). No remarked change.

Second maxilla (Fig. 14d). Distal portion fringed with 48-62 short setae; anterior margin of proximal portion with 2 knobs, bearing 1-4 short setae.

Maxillipeds (Fig. 14a, d). 1st maxilliped rectangular bud or bilobed-trilobed bud; exopod of 2nd maxilliped with 1-3 pairs of plumose setae.

Pereiopods (Fig. 14a, e). Sternal spines present near the coxae of 1st-5th pereiopods.

Abdomen (Fig. 14e). Segmentation becoming apparent; both rami of pleopod elongated, inner ramus with rudiment of appendix interna in well-advanced individuals; uropod with serrations on lateral margin of both rami in most individuals.

Gill buds. Rudimentary or short bud on 2nd and 3rd maxillipeds and 1st-5th pereiopods present in some individuals.

Phyllosoma Stage X (n = 6).—Cephalic shield (Fig. 15a, b). Maximum width located anterior part of the shield; mean values of CW/CL and CW/TW 0.676 (range = 0.656-0.687) and 0.953 (range = 0.907-0.977), respectively.

Antennule (Fig. 15c). 4th segment with 27-33 rows of subterminal sensory setae.

Antenna (Fig. 15c). About 2.5-3 times as long as antennule.

First maxilla (Fig. 15d). Coxal endite with 2-3 long serrated terminal setae and 8-11 short subterminal setae; basal endite with 3-4 strong serrated terminal spines.

Second maxilla (Fig. 15e). Distal portion fringed with 61-77 short setae; anterior margin of proximal portion with 2 conspicuous knobs, bearing 2-4 short setae.

Maxillipeds (Fig. 15a, e). 1st maxilliped trilobed bud, posterior lobe expanding greatly; exopod of 2nd maxilliped with 1.5-3.5 pairs of plumose setae.

Pereiopods (Fig. 15a, f, g). 5th pereiopod 5-segmented.

Abdomen (Fig. 15f, g). Pleopod segmented, inner ramus with appendix interna; serrations on lateral margin of uropod developed.

Gill buds (Fig. 15h-k). Full complement of gill buds present on 2nd-3rd maxillipeds and 1st-5th pereiopods, gill buds on coxae of 2nd-3rd maxillipeds and 1st-4th pereiopods bilobed.

Puerulus Description

Puerulus Stage (n = 2).—Whole body and cephalothorax (Fig. 16a). 23.08 and 20.30 mm BLp, 9.05 and 8.60 mm CLp, respectively; 4 pairs of major spines (suborbital spine, anterolateral spine, spine posterior to anterolateral spine and branchial spine) and several minute spines on cephalothorax, supraorbital spine is not counted in this study because it is in a undeveloped state forming a plate; cervical groove and median carina invisible.

Antennule (Fig. 16b). Peduncle with 3 segments; proximal segment having several short setae on anterior

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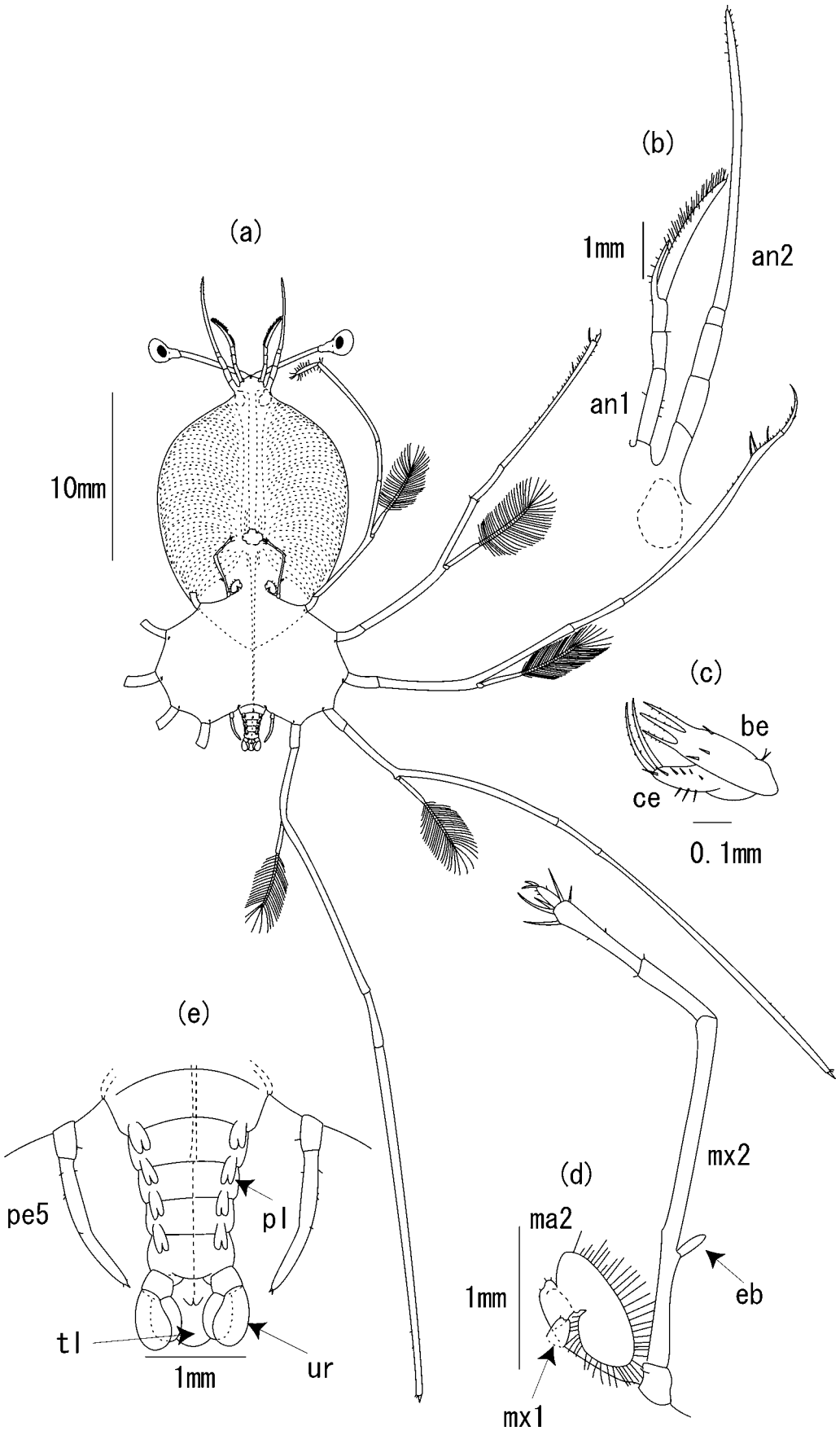
Fig. 12. Phyllosoma Stage VII of *Panulirus penicillatus*. a) ventral view; b) left antennule (an1) and antenna (an2); c) coxal and basal endites (ce, be) of left 1st maxilla, ventral; d) ventral view of left 2nd maxilla (ma2), 1st maxilliped (mx1), and 2nd maxilliped (mx2) with slight protuberance (pr); e) 5th pereiopod (pe5) and abdomen (pl = pleopod, ur = uropod, tl = telson), ventral. Setules on plumose setae of exopod are not shown.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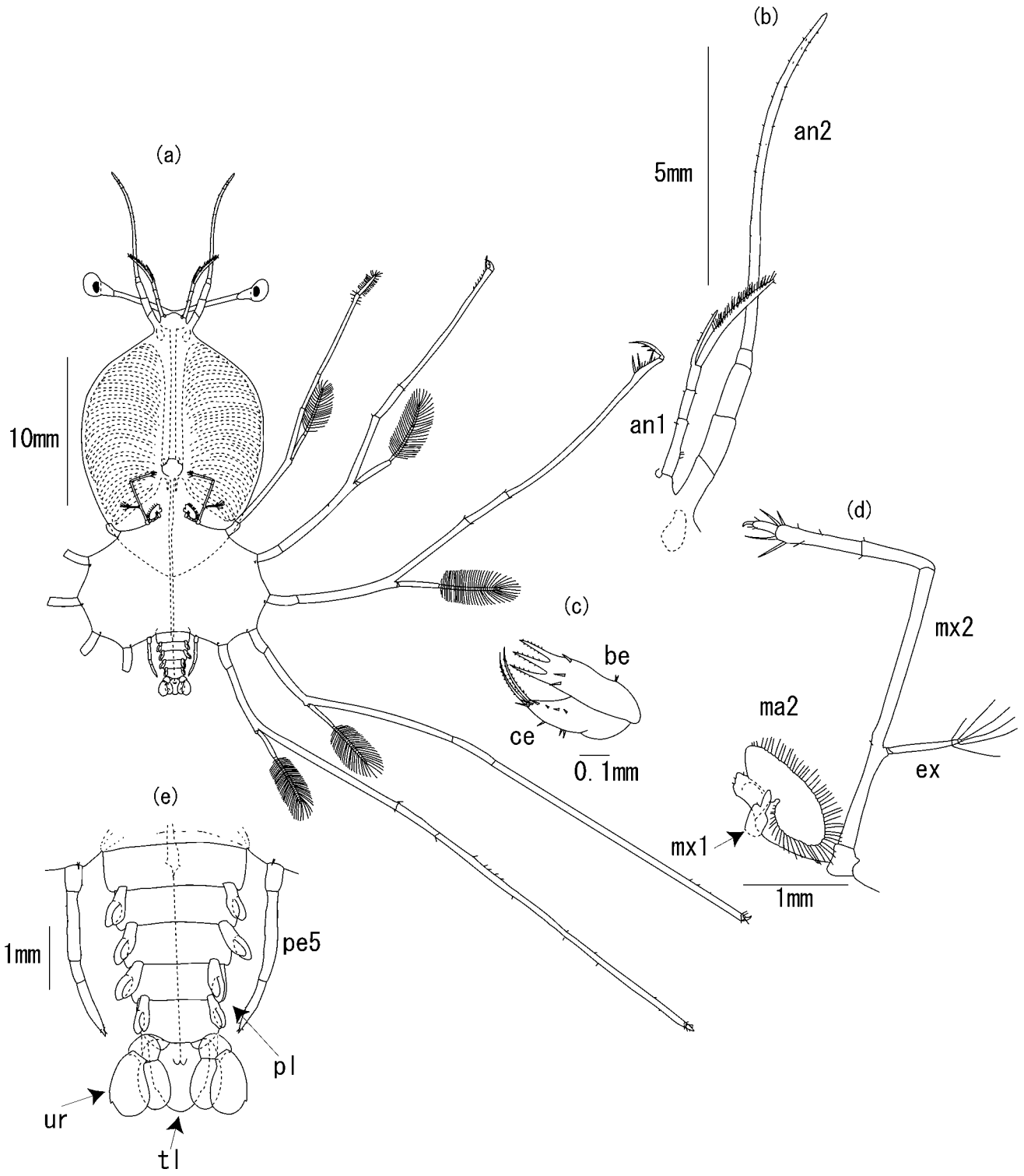

Fig. 14. Phyllosoma Stage IX of *Panulirus penicillatus*. a) ventral view; b) left antennule (an1) and antenna (an2); c) coxal and basal endites (ce, be) of left 1st maxilla, ventral; d) ventral view of left 2nd maxilla (ma2), 1st maxilliped (mx1) and 2nd maxilliped (mx2) with exopod (ex); e) ventral view of 5th pereiopod (pe5) and abdomen (pl = pleopod, ur = uropod, tl = telson). Setules on plumose setae of exopod of pereiopod are not shown.

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Fig. 13. Phyllosoma Stage VIII of *Panulirus penicillatus*. a) ventral view; b) left antennule (an1) and antenna (an2); c) coxal and basal endites (ce, be) of left 1st maxilla, ventral; d) ventral view of left 2nd maxilla (ma2), 1st maxilliped (mx1) and 2nd maxilliped (mx2) with exopod bud (eb); e) 5th pereiopod (pe5) and abdomen (pl = pleopod, ur = uropod, tl = telson), ventral. Setules on plumose setae of exopod of pereiopod are not shown.

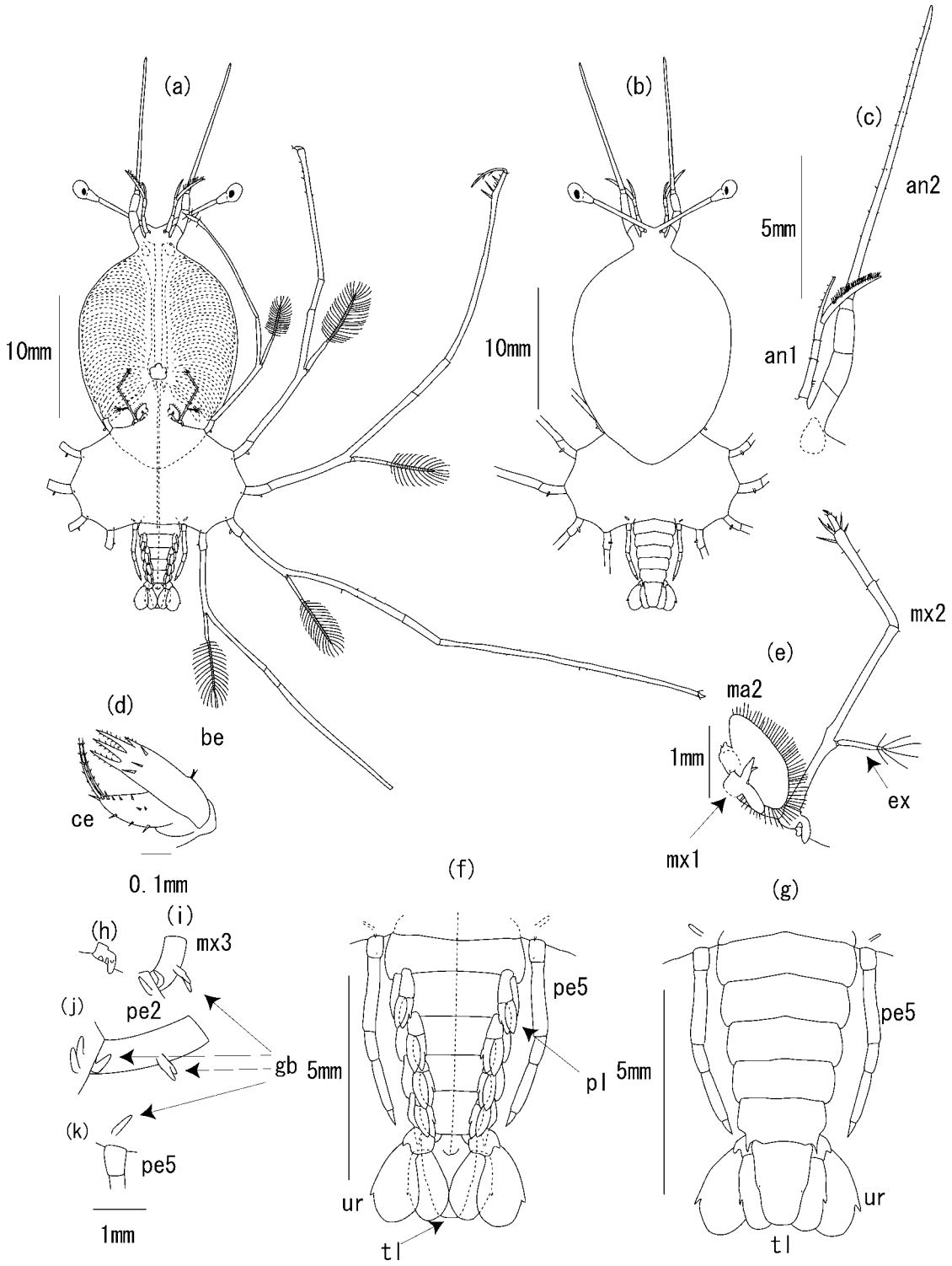

Fig. 15. Phyllosoma Stage X of *Panulirus penicillatus*. a) ventral view; b) dorsal view; c) left antennule (an1) and antenna (an2), ventral; d) coxal and basal endites (ce, be) of left 1st maxilla, ventral; e) ventral view of left 2nd maxilla (ma2), 1st maxilliped (mx1) and 2nd maxilliped (mx2) with exopod (ex); f) 5th pereiopod (pe5) and abdomen (pl = pleopod, ur = uropod, tl = telson), ventral; g) 5th pereiopod (pe5) and abdomen abdomen (ur = uropod, tl = telson), dorsal; h-k) gill buds (gb) on dorsal surface of left 2nd and 3rd maxillipeds (mx3), 2nd and 5th pereiopods (pe2 and pe5), respectively. Setules on plumose setae of exopod of pereiopod are not shown.

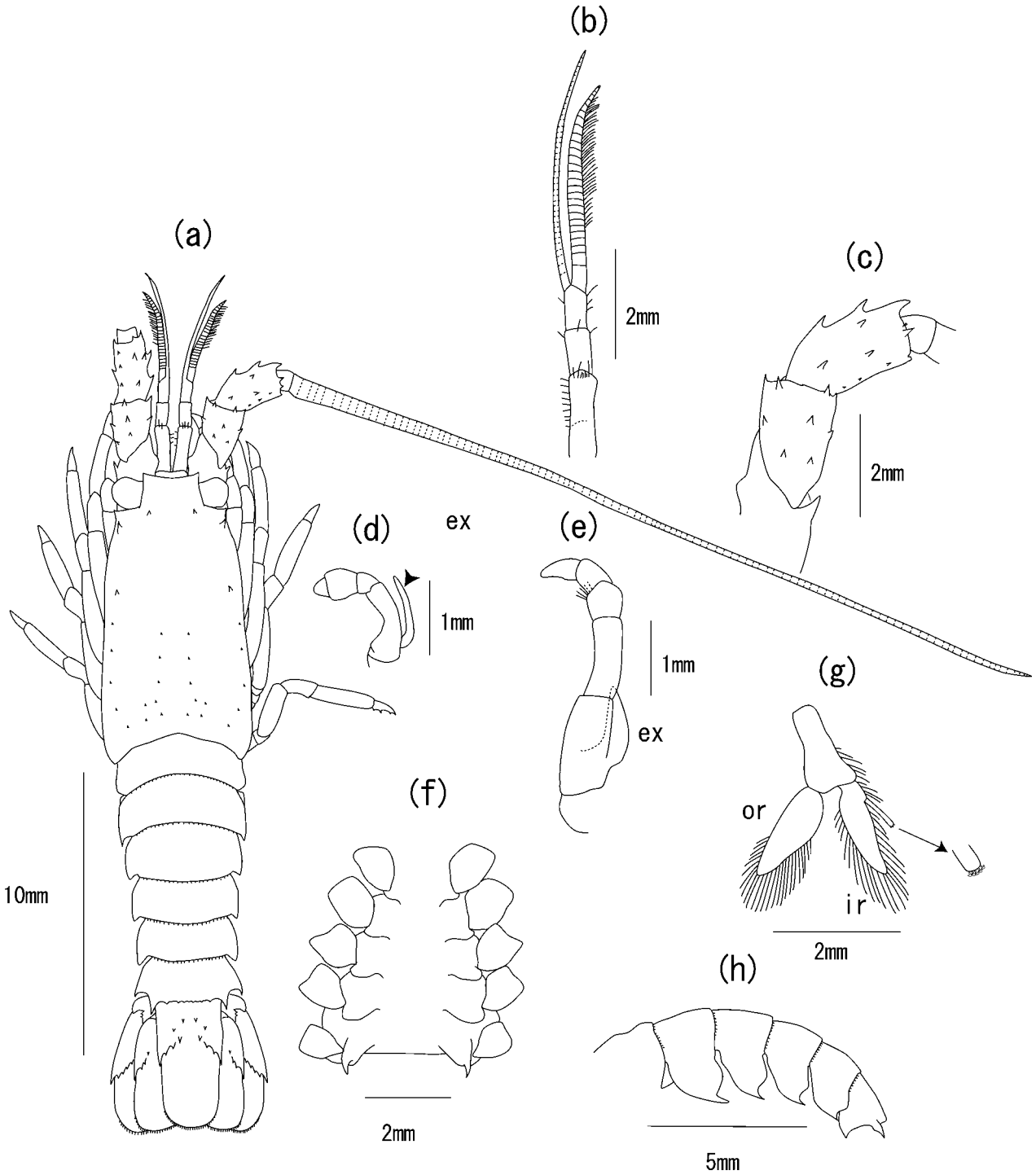

Fig. 16. Puerulus Stage of *Panulirus penicillatus*. a) dorsal view; b) right antennule, dorsal; c) right antenna, dorsal; d) ventral view of left 2nd maxilliped with exopod (ex); e) left 3rd maxilliped with exopod (ex), ventral; f) ventral thoracic sternum; g) right 2nd pleopod (or = outer ramus, ir = inner ramus) and tip of appendix interna, ventral; h) lateral view of abdomen.

margin and inner lateral margin, longer than other segments; outer flagellum with dense rows of setae on outer margin, shorter than inner flagellum.

Antenna (Fig. 16a, c). About 1.0 and 1.5 times as long as BLp (1.0 is not a normal rate because the antenna appears to be damaged), having peduncle with 4 segments and long tapered flagellum; 1st segment with anterolateral spine; 2nd segment with 8 spines on dorsal surface; 3rd segment with 8 strong and 2-3 minute spines on dorsal surface.

Maxillipeds (Fig. 16d, e). 2nd maxilliped with long exopod reaching anterior margin of proximal segment, lacking any setae; 3rd segment of 3 maxilliped with several setae on anterior margin, exopod beyond proximal segment.

Thoracic sternum (Fig. 16f). Rounded protuberances near bases of 1st to 4th pereiopods on thoracic sternum, strong posterolateral sternal spine near base of 5th pereiopod.

Pleon (Fig. 16g, h). 2nd to 6th pleural plates produced into strong spines; posterior margin of abdominal tergites of 2nd to 5th somites fringed with short setae, posterior margin of 6th tergite with minute serrations; pleopods present on 2nd to 5th somites, inner and outer rami with plumose setae, inner ramus having appendix interna with anchor-shaped hooks (cincinnuli).

Discussion

In this study, two individually cultured (static system) and seven group cultured (flow-through system) *Panulirus penicillatus* phyllosomata metamorphosed successfully to the puerulus stage. *P. penicillatus* is only the eighth palinurid lobster and only the fourth species of *Panulirus* to be cultured completely to settlement out of 21 extant species. Moreover, this investigation documents a variety of biological metrics, e.g., instar durations, growth variation, and stage identification, that help to characterize these phyllosomata.

Phyllosomal Growth

Two larval *P. penicillatus* that reached the puerulus stage in individual cultures were reared for 256 and 294 days in the phyllosomal phase (mean = 275.0 days), while seven phyllosomata in group cultures were reared for longer periods ranging from 244 to 330 days (mean = 302.4 days). Sekine et al. (2000) also observed a similar trend in *Panulirus japonicus* phyllosomata cultured using similar methods. We surmise two factors may have contributed to these differences in the growth between individual and group cultured animals: phyllosomal density and food abundance. Phyllosomata of *Panulirus* spp. are characterized by a very long and slender third maxilliped and pereiopods. Hence, phyllosomal group interactions incurred appendage damage or loss; in particular their pereiopods were often lost shortly after molting while bodies were still soft. Most likely, losses in pereiopods prevented larvae from capturing an adequate amount of food and as such, we lowered the total density of *Artemia* throughout group culturing to keep the culturing tanks as clean as possible. As a result, we documented slower growth among group cultured phyllosomata.

Johnson (1971b) examined the relationship between phyllosomal size and distributional spread in the tropical east Pacific for *P. penicillatus* and noted that total phyllosomal duration in the ocean probably exceeded 7-8 months which is fairly consistent with what we observed in this present study (8-11 months). The reported total larval duration period for two species of *Panulirus* (*P. japonicus* and *P. longipes*) that were successfully cultured through the phyllosomal phase ranged from 231-417 days (mean = 319.4 days, n = 325) at 24-27°C for *P. japonicus* (Sekine et al., 2000) and 281-294 days (mean = 287.5 days, n = 2) at 24.5-26 °C for *P. longipes* (Matsuda and Yamakawa, 2000). In this study *P. penicillatus* appears to have a similar total phyllosomal duration as those of *P. japonicus* and *P. longipes*.

Phyllosomal Stages of *P. penicillatus*

Prasad and Tampi (1959) described phyllosomal stages I-XI (1.50-20.75 mm BL) of *P. penicillatus* for the first time based on plankton samples from the Indian Ocean. However, Johnson (1971a) noted that phyllosomata from Prasad and Tampi were commonly misidentified as *Panulirus versicolor*. Minagawa (1990) cultured *P. penicillatus* phyllosomata from hatch to stage VII (1.61-10.98 mm BL) in the laboratory and described each stage. He pointed out that Prasad and Tampi's identification was accurate until stage III based on the number of short setae on the anterior margin of proximal segment of the second maxilla. The number of short setae on the anterior margin of second maxilla is considered to be a key attribute in distinguishing *P. penicillatus* from other *Panulirus* spp., e.g., comparing evolutionary sequence groups I-IV (see George and Main, 1967; also McWilliam, 1995), which can commonly possess three setae until ~10 mm BL (Minagawa, 1990). In this study, most *P. penicillatus* phyllosomata up until stage V (mean = 6.13 mm BL) possessed two short setae on the anterior margin of the proximal segment of their second maxilla (one seta in some individuals). This trait is partially coincident with Minagawa's description, however the number of short setae in phyllosomata beyond stage V varied widely among individuals from zero to five, indicating that this metric is not useful in distinguishing phyllosomata of *P. penicillatus* from those of other *Panulirus* lobsters beyond stage V.

Johnson (1968) described and illustrated *P. penicillatus* phyllosomal stages VI-XI collected from Hawaii and later reported a phyllosoma of stage VIII (21 mm BL) caught in the South China Sea (Johnson, 1971a), while Prasad et al. (1975) described stages VII-XII collected in tows in the Indian Ocean. Cultured phyllosomata from this study appear to have many similarities to their field-caught counterparts with the exception of BLs and the cephalic shield (CS) shape of the final (gilled) stage. The final phyllosomal stage from our study is smaller than field-caught specimens (24.8-33.6 mm BL versus 37.6-39.9 mm and 38.0-43.0 mm BL) (Johnson, 1968 and Prasad et al., 1975, respectively). Moreover, cultured phyllosomata from this study showed narrower CSs than field-caught specimens. Cephalic shield ratios of width to length were 0.68 for cultured phyllosoma versus 0.75 for ones described by Johnson (1968) and Prasad et al. (1975). In some palinurid and scyllarid lobsters, differences in the development between wild-caught and laboratory reared specimens have been observed (Ong, 1967; Robertson, 1969). A plausible explanation for the discrepancy may be attributed to differences, for example, in prey choice (wild zooplankton assemblages versus more limited lab diets) or abundance, e.g., frequency and duration of feedings. We suggest that these comparisons need to be investigated in much more detail.

Another explanation for differences between field and lab-cultured animals is that the phyllosomata of Johnson (1968) and Prasad et al. (1975) may in fact belong to another panulurid species, and as mentioned earlier, wild-caught

specimens do not always provide definitive identification. To clarify why such discrepancies were observed, it is necessary to compare morphologically cultured phyllosomata with plankton specimens that are identified with accurate methods such as DNA analyses (Siraishi, 2000). Cultured *P. penicillatus* phyllosomata described by Minagawa (1990) are similar to those cultured here. However stages I-VII of Minagawa correspond with stages I , II, III, IV-V, V, VI, and VI since the staging criteria of Minagawa differs from that in our study.

Puerulus Stage of *P. penicillatus*

Michel (1971) briefly described the puerulus stage of *P. penicillatus* from three specimens caught by net in the south tropical and equatorial Pacific area. This description appears to have similarities with the puerulus stage documented here with respect to antennal flagellar apex, antennal length relative to BL and exopod conditions of the 2nd and 3rd maxillipeds, all criteria related to species-specific pueruli groupings within *Panulirus* spp. proposed by McWilliam (1995). The presence of strong posterolateral sternal spines in our study (also a criterion for grouping) was not mentioned by Michel (1971). Tanaka et al. (1984) described a puerulus dubbed 'type B' collected in shallow waters off the central Pacific coast of Japan, (Chiba Prefecture) which was confirmed as *P. penicillatus* after several molts in the juvenile stage (Tanaka, 1987). In both studies, however, wild-caught pueruli were larger than those of our lab-cultured specimens. Body lengths of pueruli collected in the wild ranged from 26.0 to 27.9 mm (Tanaka et al., 1984) and from 28.5 to 29.3 mm (Michel, 1971), while cultured pueruli in this study ranged in BL from 20.30 to 23.08 mm.

Pueruli cultured in this study were completely transparent except for their eyes and seven distinct white bands on each antenna just after metamorphosis. Bands gradually disappeared except for 3-4 faint bands that remained upon molting to the first juvenile stage. By comparison, lab-cultured pueruli of *P. japonicus* and *P. longipes* showed no discernable antennal bands (Yamakawa et al., 1989; Kittaka and Kimura, 1989; Matsuda and Yamakawa, 2000) although in *P. japonicus* pueruli, a few bands gradually appeared 2-4 days after metamorphosis increasing to 7 or more (Matsuda et al., 2001). Wild-caught pueruli of the rock lobster *Jasus edwardsii* also lack pigmentation except for the eye, the tip of antenna and sometimes the legs after which a series of 4-6 brown bands on each antenna are observed (Booth, 1979). At present, the characteristic colored banding observed and documented here appears to be a unique identifier to *P. penicillatus*.

Implications

The culturing techniques and subsequent biological characteristics obtained and described for the early-life development of the Pronghorn spiny lobster (*P. penicillatus*) completes the early-life identification of a commercially important and ecologically significant marine resource and advances future development for commercially viable lobster production. Additionally, another powerful application exists. Because of the vast distribution of this species, i.e., the only transpacific spiny lobster species) (Williams, 1988) our set of lab-generated biological data provides an initial step (along with behavior and physical oceanography) for future modeling of the scale, rate, and potential connectivity of this species across regional or local domains. Information of this kind has already been used to model other related marine species (Katz et al., 1994; Cobb et al., 1997; Sale and Kritzner, 2003; Cowen et al., 2006) providing a template for quantifying relevant patterns of recruitment and settlement.

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