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Morphological descriptions for late stage phyllosomas of furrow lobsters (Crustacea, Decapoda, Achelata, Palinuridae) collected off Okinawa Islands, Japan

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Abstract

Two late stage phyllosomas collected from off Okinawa Islands were first determined to be *Justitia* or *Nupalirus* because of the chelate pereipods. DNA analysis indicated these to be *Justitia longimana* and *Nupalirus japonicus*. Morphological larval description with note on palinurid phyllosomas is given.

Key words: *Justitia*; *Nupalirus*; phyllosoma; morphology; taxonomy

Introduction

Seven palinurid genera, *Justitia*, *Linuparus*, *Nupalirus*, *Palinustus*, *Panulirus*, *Puerulus*, and *Palinurellus* have been recorded in Japanese and the adjacent waters, (Holthuis 1991; Chan and Yu 1993; Miyake 1998; De Grave et al. 2009). Among them, furrow spiny lobsters of the genera *Justitia* and *Nupalirus* have been known as rare species (Holthuis 1991), and unique appearance among palinurids as “chelate Achelata”, at least in the male of *Justitia*. The larval development of *Justitia* and *Nupalirus* has been described based exclusively on plankton samples to date (Johnson 1969; Robertson 1969; Baisre 1969; Johnson and Robertson 1970; Aoyama et al. 1984; Inoue et al. 2004). Thus, no morphological descriptions of the phyllosomas of furrow spiny lobsters has been done by more convincing method which insure accurate parentage yet, i.e., laboratory-rearing or DNA-barcording.

During our research cruises in southern Japan waters, two late stage phyllosomas, different from typical palinurid species in having chelate or subchelate pereiopods, were obtained from the planktons collected off Okinawa Islands. Primary investigation of the morphology based on key characters from previous larval works (Johnson and Robertson 1970; Sekiguchi 1986a, b) indicated that these phyllosomas belonged to the genera *Justitia* or *Nupalirus*, but the species could not be identified. In this study, we analysed mtDNA 16S rDNA sequences of these phyllosomas, then identified them to be *Justitia longimana* and *Nupalirus japonicus*. The aims of this study are to give morphological descriptions of the phyllosomas and to compare their morphological characters with those known for the other genera of palinurid lobsters distributing Japanese waters.

Materials and Methods

Two late stage phyllosoma larvae were collected using MOHT (Matsuda, Oozeki & Hu Midwater Trawl) net towed stepwise at 50 m and 35 m depth for 10 min each in off Okinawa Islands, of which one (designated by SP30) was caught on 10 November 2004 at 22°33’N 124°44’E and the other (designated by KY1404) on 19 November 2014 at 24°00'N 124°00'E. The larval specimens were fixed with 80 % ethanol, photographed and measured on board, and transferred to the laboratory for subsequent molecular and morphological analyses. Total DNA was extracted from a piece of pereiopod using a DNA extraction kit (Qiagen inc.). PCR primers to amplify partial mtDNA 16S rDNA region were 16Sar-L and 16Sbr-H by Palumbi et al. (2002). PCR was performed in 10 µl of total reaction mixture containing 5–10 ng template DNA, 1 µl of 10 × buffer, 1 µl of dNTP (2.5 mM each), 0.5 µl of each primer (10 µM), and 0.25 units of EX Taq HS DNA polymerase (Takara, Shiga, Japan) on an ABI 9700 Thermal cycler (Applied Biosystems, Foster City, CA, USA). Reaction mixtures were preheated at 94°C for 2 min, followed by 35 amplification cycles (94°C for 30 s, 55°C for 30 s, and 72°C for 50 s), with a final extension at 72°C for 7min. The PCR products were treated with ExoSap-IT (Amersham Biosciences) to remove primers and used as template DNA for cycle sequencing reactions using Big Dye Terminator Cycle Sequencing Kit (Ver.3.1, Applied Biosystems) with PCR primers. Sequencing was conducted on an ABI Prism 3730XL (Applied Biosystems). Nucleotide sequences determined were subjected to BLAST homology searches (Altshul et al. 1990) in GenBank to find identical or similar sequences. Calculation of Kimura's two parameter distance (K2P) between sequences was performed using MEGA6 (Tamura et al. 2013).

After the molecular analysis, the appendages were dissected using fine insect pins. Observations and drawings were made with an aid of drawing tube attached to an Olympus BX51 microscope and a SZX10 stereomicroscope. Total body length (TL), width (CW) and length (CL) of cephalic shield (CS), and thorax width (TW) (see Fig. 1) were measured according to Higa and Shokita (2004) and Palero et al. (2008).

The voucher specimens are deposited at the Hokkaido University Museum under the accession No. ICHUM-6251-6252.

Results

Molecular identification

Nucleotide sequences determined are 508 bp for SP30 and 505 bp for KY1404, which are available in the International Nucleotide Sequence Database Collection (INSDC) under accession numbers of LC619699 and LC619700. BLAST top hit sequence for SP30 was *Justitia longimana* (AF502953) with K2P distance of 0.2 % and that for KY1404 was *Nupalirus japonicus* (KF828188) with K2P distance of 0.4%. These values are sufficiently small for species identity (see Vences et al. 2005; Lianming et al. 2014; Kannan et al. 2020). K2P distance between SP30 and KY1404 was 27.3 %.

Morphological description of the phyllosomas

Justitia longimana

Stage VII (SP30, Figs. 1, 2)

Dimensions: TL = 17.2 mm, CL = 12.4 mm, CW = 10.9 mm, TW = 10.3 mm.

Cephalothorax (Fig. 1A): CS nearly circular in outline, slightly longer than wide 1.14 in CL/CW ratio, and 1.06 in CW/TW ratio. Posterolateral margin of the CS not covering the base of maxilliped 3. Posterior margin of thorax concave between the coxae of pereiopod 3. Eyes stalked, stalk longer than antennule and antenna.

Antennule (Fig. 2A): Biramous, peduncle 3-segmented, each segment without setae. Inner flagellum shorter than outer flagellum.

Antenna (Fig. 2B): Uniramous, 2-segmented, slightly shorter than antennule.

Mandibles (Fig. 2C): Slightly flattened dorso-ventrally, asymmetrical in dentition. Incisor process and medial gnathal edge with a series of teeth. Molar process crowned by many denticules and minute papillae. Labrum and paragnath well-developed, covers distal inner half portion of mandible.

Maxillule (Fig. 2D): Basal endite with 2 stout cuspidate spines and 4 subterminal setae while coxal endite with 2 stout setae and 3 short setae. Two setae on the presumptive endopod area (Fig. 2D, arrow).

Maxilla (Fig. 2E): Basis with 2 thin anterior setae. Scaphognathite with 37 marginal thin plumose setae.

Maxilliped 1 (Fig. 2F): Small bud, 3 setae on basal part. Exopod short process without setae.

Maxilliped 2 (Fig. 2G, 2H): Endopod 3-segmented, with a stout long seta and 2 short setae on its distal part. Exopod unsegmented with 4 distal plumose setae.

Maxilliped 3 (Fig. 1A): Endopod 3-segmented, many setae on distal segment. Exopod with 19 annulations, each annulation with a pair of natatory plumose setae.

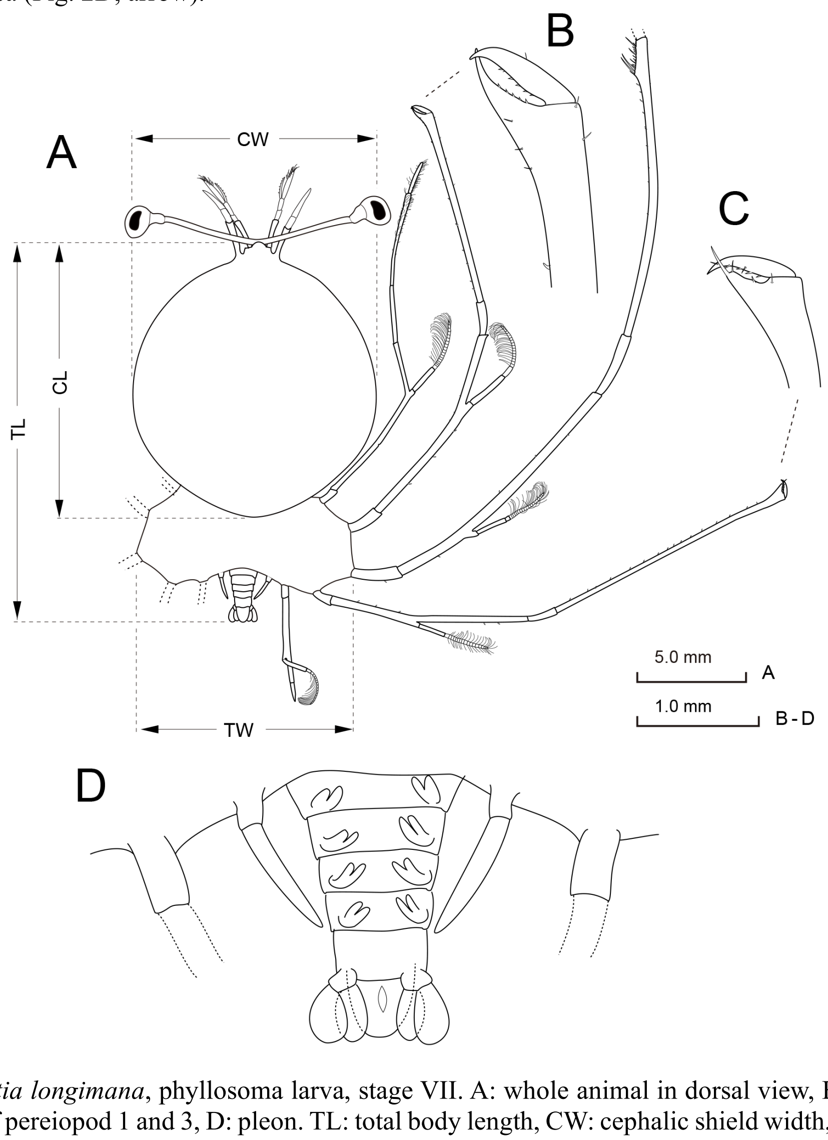

Fig. 1. *Justitia longimana*, phyllosoma larva, stage VII. A: whole animal in dorsal view, B-C: enlarged distal part of pereiopod 1 and 3, D: pleon. TL: total body length, CW: cephalic shield width, CL: cephalic shield length, TW: thorax width. Scale bars: A = 5.0 mm; B-D = 1.0 mm.

Pereiopod 1 (Fig. 1A, 1B): Endopod 3-segmented, chelate in distal part. Exopod with 17 annulations, each annulation with a pair of natatory plumose setae. No conspicuous coxal and subexopodal spines on all pereiopods.

Pereiopod 2 (Fig. 1A): Longest among pereiopods. Dactylus of endopod long sickle-shaped with setae and spines on its inner margin, resembling raptorial claw. Exopod with 18 annulations, otherwise as in pereiopod 1.

Pereiopod 3 (Fig. 1A): Distal part of endopod subchelate with the propodus extending distally and terminating in 2 stout setae between which the dactylus close (Fig. 1C). Exopod as in pereiopod 1.

Pereiopod 4 (Fig. 1A): Endopod 2-segmented. Exopod with 16 annulations, otherwise as in pereiopod 1.

Pereiopod 5 (Fig. 1D): Two-segmented, uniramous projection, reaching posterior margin of pleonal somite 4.

Pleon (Fig. 1D): Somites segmented, rudiments of pleopods and uropod as biramous buds.

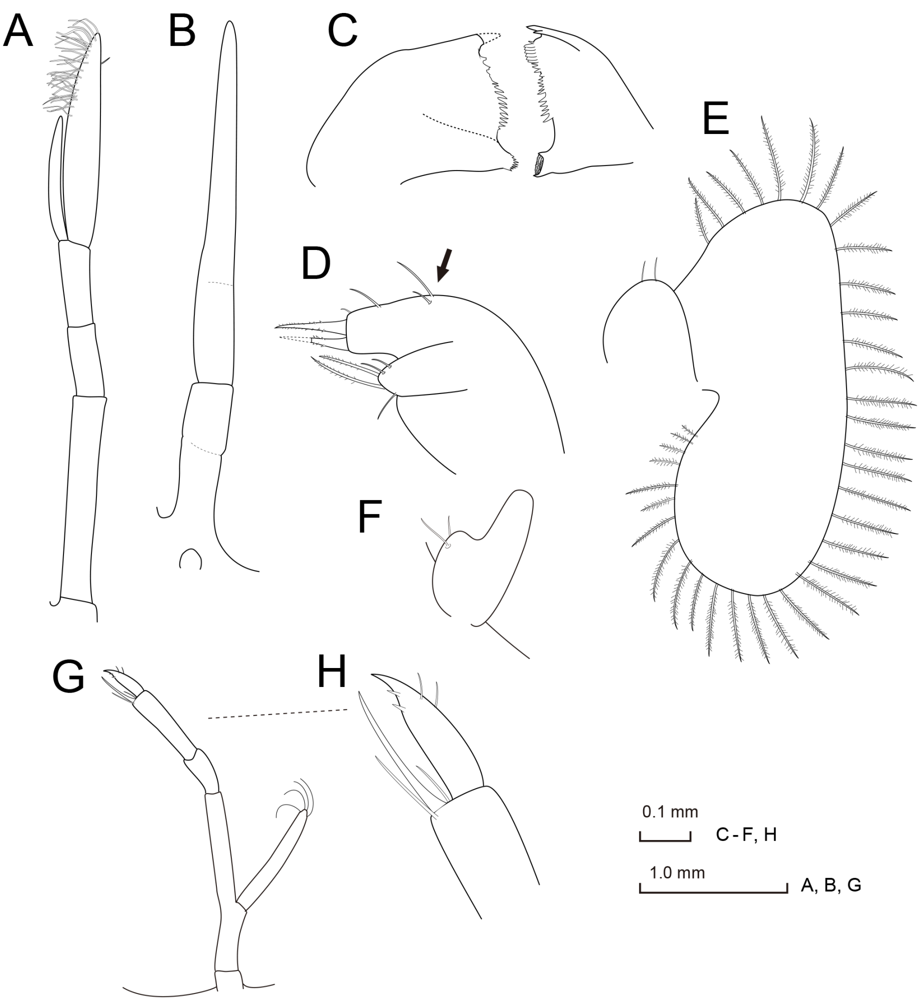

Fig. 2. *Justitia longimana*, phyllosoma larva, stage VII. A: antennule, B: antenna, C: mandibles in dorsal view, D: maxillule, arrow indicating presumptive endopod, E: maxilla, F: maxilliped 1, G: maxilliped 2, H: enlarged distal part of endopod of maxilliped 2. Scale bars: A, B, G = 1.0 mm; C-F, H = 0.1 mm.

Nupalirus japonicus

Stage VIII (KY1404, Figs. 3-5)

Dimensions: TL= 17.4 mm, CL = 12.8 mm, CW = 9.5 mm, TW = 10.4 mm.

Cephalothorax (Fig. 3A): CS oval in outline, longer than wide, 1.35 in CL/CW ratio and 0.98 in CW/TW ratio. Posterolateral margin of the CS covering the base of maxilliped 3. Posterior margin of thorax concave from the coxae of the pereiopod 3. Eyes stalked, stalk longer than antennule and antenna.

Antennule (Fig. 4A): Biramous, peduncle 3-segmented, each segment without seate. Inner flagellum shorter than outer flagellum.

Antenna (Fig. 4B): Three-segmented, longer than antennule, but distal part missing.

Mandibles (Fig. 4C): Slightly flattened dorso-ventrally, asymmetrical in dentition. Incisor process and medial gnathal edge with a series of teeth which densely in left mandible. Molar process crowned by many denticules and minute papillae.

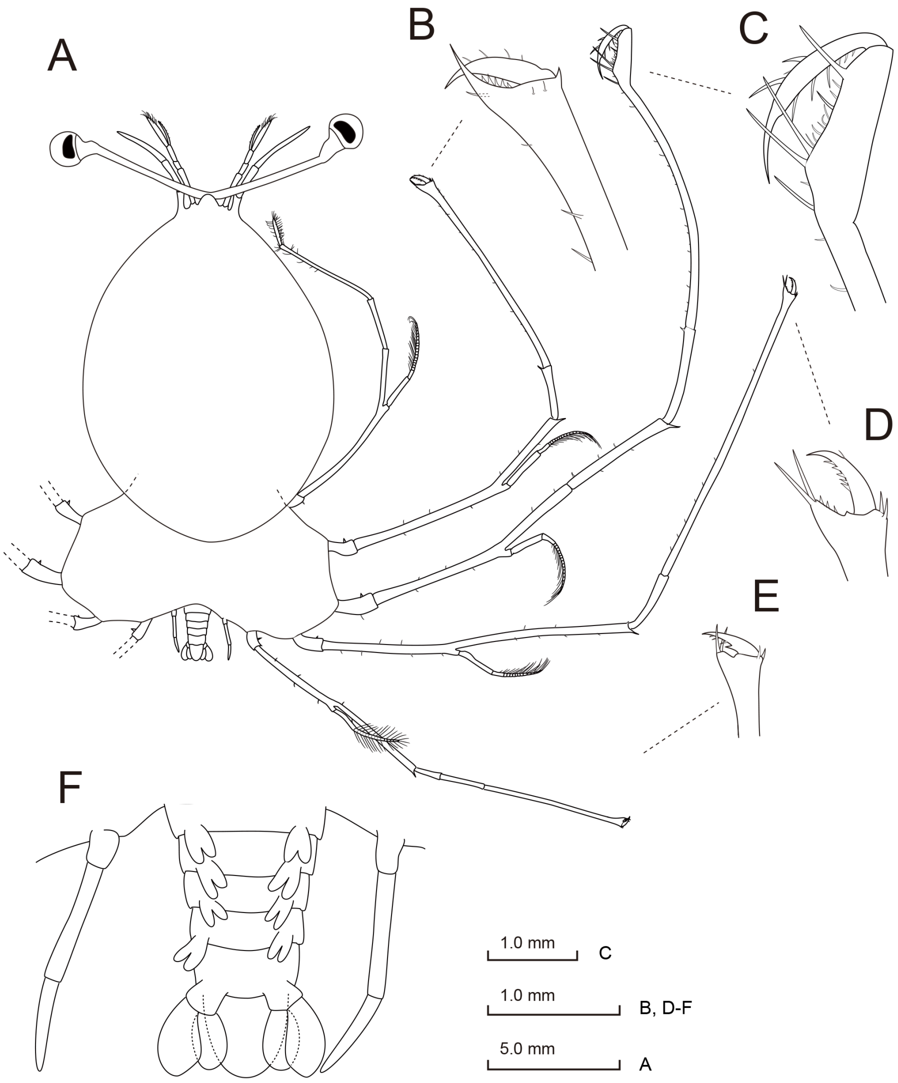

Fig. 3. *Nupalirus japonicus*, phyllosoma larva, stage VIII. A: whole animal in dorsal view, B-E: enlarged distal part of pereiopod 1-4, F: abdomen in ventral view. Scale bars: A = 5.0 mm; B-F = 1.0 mm.

Maxillule (Fig. 4D): Basal endite with 2 stout cuspidate spines and 2 subterminal setae while coxal endite with 1 stout spine and 8 setae. Endopod small bud with 2 distal short setae.

Maxilla (Fig. 4E): Basis with 4 thin anterior setae. Scaphognathite with 45 marginal thin plumose setae.

Maxilliped 1 (Fig. 4F): Basal part with 2 short setae. Exopod tip extending beyond basal segment of maxilla.

Maxilliped 2 (Fig. 4G): Endopod 3-segmented, one seta on proximal, 6 setae and a stout spine on second, and 4 setae and a spine on distal segment. Exopod with 5 annulations, each annulation with a pair of natatory plumose setae.

Maxilliped 3 (Fig. 3A): Endopod 4-segmented, many setae on distal segment. Exopod with 16 annulations, each annulation with a pair of natatory plumose setae.

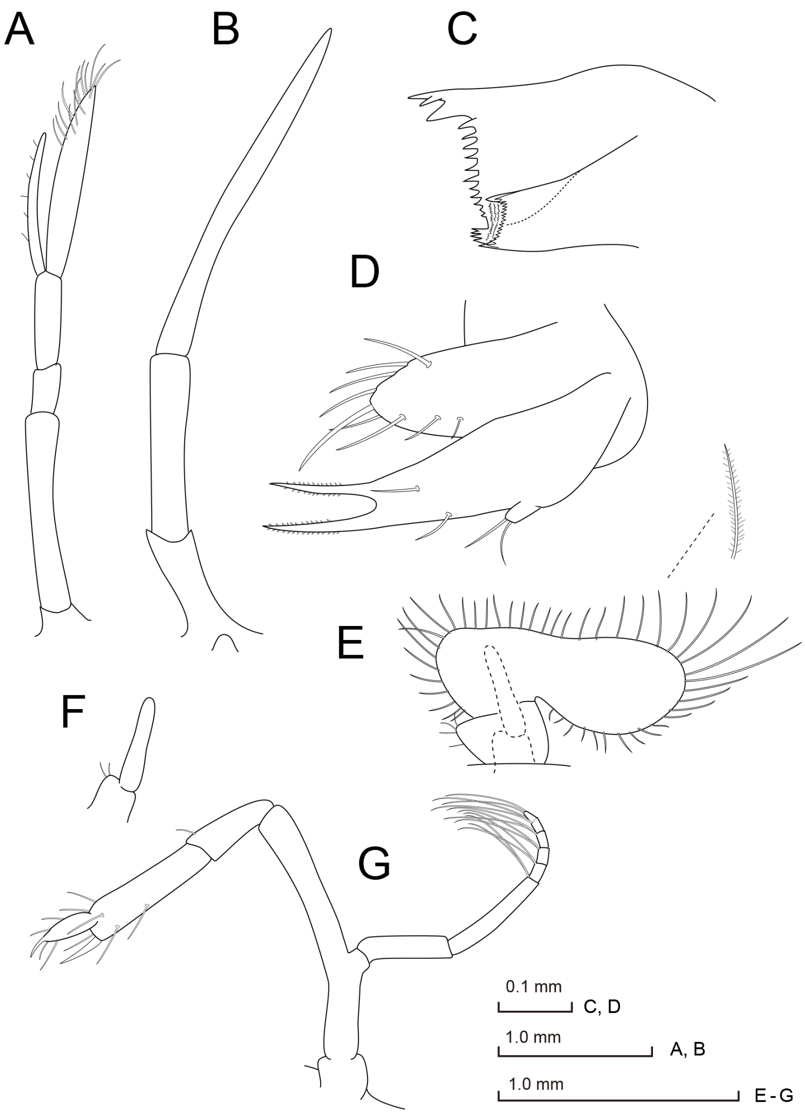

Fig. 4. *Nupalirus japonicus*, phyllosoma larva, stage VIII. A: antennule, B: antenna, C: mandible (right) in dorsal view, D: maxillule, E: maxilla, dotted line shows the position of maxilliped 1, F: maxilliped 1, G: maxilliped 2. Scale bars: A, B, E-G = 1.0 mm; C, D = 0.1 mm.

Pereiopod 1 (Fig. 3B): Distal part of endopod, chelate. Exopod with 16-17 annulations, each annulation with a pair of natatory plumose setae. No subexopodal spine was observed on all pereiopods.

Pereiopod 2 (Fig. 3C): Longest among pereiopods. Dactylus of endopod sickle-shape as in the previous species. Exopod with 19 annulations, otherwise as in the pereiopod 1.

Pereiopod 3 (Fig. 3D): Subchelate. Exopod as in the pereiopod 1.

Pereiopod 4 (Fig. 3E): Subchelate. Exopod with 15-16 annulations, otherwise as in the pereiopod 1.

Pereiopod 5 (Fig. 3F): Three-segmented, uniramous projection, reaching posterior margin of telson.

Pleon (Fig. 3F): Somites segmented, rudiments of pleopods biramous, and uropod developed.

Color in living specimen (Fig. 5): Transparent, but patches of orange red chromatophores on appendages and mouth parts (cf. Konishi et al. 2015).

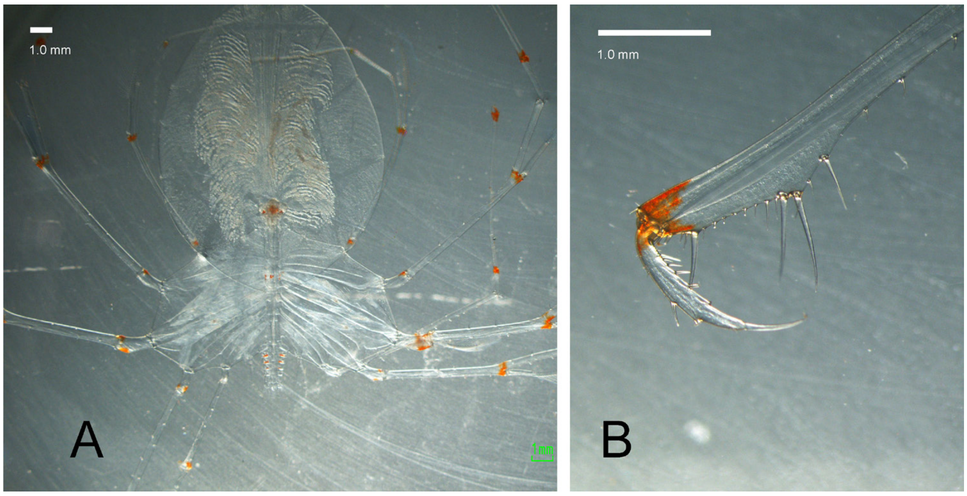

Fig. 5. *Nupalirus japonicus*, the living phyllosoma larva, stage VIII. A: dorsal view, B: tip of pereiopod 2.

Table 1. Main staging characters of *Justitia* phyllosoma larvae based on planktons by Robertson (1969).

Stage	TL (mm)	Antennule		Antenna	MXP1	MXP2	P3	P4	P5	Pleopod	Uropod
		IF	PSG	exopod							
I				(unknown)							
II	2.5, 2.6	-	-	A1>A2	-	-	r	-	-	-	
III	3.9	-	-	A1>A2	-	-	+	r	-	-	-
IV	4.7, 5.3	r	-	A1>A2	-	-	+	rb	r	-	-
V	7-10	+	+	A1>A2	-	-	+	+	+	-	r
VI	12-15	+	+	A1>A2	-	r	+	+	+	r	r
VII	18-19	+	+	A1>A2 [1)]	+	+	+	+	+	r	rb
VIII	23, 24	+	+	A1≥A2 [2)]	+ [3)]	+	+	+	+	rb	+
IX	26-29	+	+	A1<A2	+	+	+	+	+	rb	+
X	37	+	+	A1<A2	+	+	+	+	+	rb	+

A1: antennule, A1P: antennular peduncle, A2: antenna, IF: inner flagellum, MXP: maxilliped, P: pereiopod, PSG: peduncular segment, r: rudiment, rb: bifurcate rudiment, TL: total length. 1) antenna length nearly same as antennular penduncle, 2) eye stalk lengh longer than antenna, 3) exopod bud exceed maxillar basal segmet.

Table 2. Comparison of main larval characteristics in phyllosomas of *Nupalirus* and *Justitia*.

Species	*Justitia longimana*							*Nupalirus japonicus*			*Justitia* sp.
Reference	this study	1)	1)	2)	3)	4)	1)	this study	2) †	3) †	5)
locality	off Okinawa Is., Japan	Western Atlantic	Western Atlantic	Mindanao, Philippine	off Okinawa Is., Japan	Oahu, Pacific	Western Atlantic	off Okinawa Is., Japan	Batjan Is., Moluccas	off Okinawa Is., Japan	off Irōzaki, Japan
estimated stage	VII	VII	VIII	VIII	VIII**	IX (?)	X	VIII	final	IX (final)**	final (?)
TL (mm)	17.2	18.0	23.0	15.9	27.2	21.5	37.0	17.4	29.8	32.7	28.4
CL (mm)	12.4	14.1*	18.2*	12.0*	19.0	15.1	23.0*	12.8	19.5*	21.0	18.0
CW (mm)	10.9	10.0*	13.6*	9.2*	15.0	13.4	19.4*	9.5	15.6*	16.1	12.3
TW (mm)	10.3	11.1*	14.1*	9.9*	16.0	13.2*	19.8*	10.4	15.7*	16.7	11.5
CL/CW	1.14	1.42	1.29	1.30	1.27	1.12	1.24*	1.35	1.25	1.30	1.46
CW/TW	1.06	0.90	0.96	0.93	0.94	1.02	0.98*	0.91	0.99	0.96	1.07
A1L/A2L	1.10	1.39*	1.17*	1.51*	0.61*	1.20*	0.47*	0.85	0.41*	0.42*	0.32
maxillule BE spine	2	2	ND	ND	2	2	ND	2	ND	2	2
CS overlap MXP3 base	no	no	no	no	no	no	no	yes	yes	yes	yes
pereiopod 1	chelate	chelate	chelate	chelate	chelate	chelate	chelate	chelate	chelate	chelate	chelate
pereiopod 2	sickle-shape	sickle-shape	sickle-shape	sickle-shape	sickle-shape	sickle-shape	sickle-shape	sickle-shape	sickle-shape	sickle-shape	sickle-shape
pereiopod 3	subchelate	subchelate	subchelate	subchelate	subchelate	subchelate	subchelate	subchelate	subchelate	subchelate	subchelate
pereiopod 4	NFD	NFD	NFD	NFD	NFD	non-chelate	non-chelate	subchelate	subchelate	subchelate	non-chelate
pereiopod 5 (segment)	2	2	ND	2	5	2	more than 3	3	5	5	5
gill buds	-	-	-	-	-	-	+	-	+	+	+

1) Robertson (1969), 2) Johnson and Robertson (1970), 3) Inoue et al. (2004), 4) Johnson (1969), 5) Aoyama et al. (1984). †: as '*Justitia japonica*', *: measured from the figure, **: based on stage definition by Braine et al. (1979), -: absent, +: present. A1L: antennule length, A2L: antenna length, BE: basal endite, CL: cephalic shield length, CS: cephalic shield, CW: cephalic shield width, MXP: maxilliped, ND: no data, TL: total length, TW: thorax width.

Discussion

Based on the characters of appendages according to Robertson (1969) (Table 1), the stages of the phyllosoma specimens of *J. longimana* and *N. japonicus* are identified to be VII and VIII, respectively. Although body dimensions of the late phyllosomas are variable in the previous larval studies (see Table 2), other main characters assure that our specimen of *J. longimana* is identified to stage VII: e.g., tip of the exopodal bud in maxilliped 1 do not exceed the basal segment of maxilla, and the maxilliped 2 has setose exopod. The dimensional difference may be derived from individual variation due to internal physiological or external environmental conditions. It should be mind that the "stage" of phyllosomas has been determined arbitrarily from a series of instars which are possibly variable in number. No complete larval development has been known in *Justitia* and *Nupalirus* to date. Complete series of larval descriptions by laboratory-rearing should be required for construction of more exact developmental stage tables.

Inoue et al. (2004) also described both *J. longimana* and *N. japonicus* (as *Justitia japonica*) collected from almost the same area in the present study. In Japanese waters, another collection record of these phyllosomas is off Izu, Shizuoka (Aoyama et al. 1984). The final stage phyllosoma specimens described by Aoyama et al. (1984) as *Justitia japonica* or *Justitia* sp. are closely similar to that of *N. japonicus* in general morphology, as shown in Table 2, although the tip of the pereiopod 4 of their phyllosomas is not chelate.

Table 3 compares selected larval characters of the late stage phyllosomas of palinurid spiny lobster genera in Japanese waters, except for *Linuparus* of which larval description has been not yet presented. Phyllosoma of furrow lobsters, *Justitia* and *Nupalirus* are distinguished from those of other palinurids as follows. In early stage phyllosoma (stage II-IV), length of antenna is less than half of antennule (Robertson 1969), while more than half of antennule in the other palinurid genera. In the late stage phyllosoma (stage V-X), posterior margin of thorax is concaved between pereiopod 3 coxae (Johnson and Robertson 1970), while between pereiopod 4 coxae or not concaved in the other palinurid genera. Although the concaved posterior margin in the X stage is shallower than in the previous stages, this marginal concavity is a diagnostic larval character in the palinurid phyllosomas. The chelate pereiopod has been regarded as the

Table 3. Diagnostic larval characteristics in late stage phyllosoma of six Japanese palinurid genera†.

Genus	*Justitia*	*Nupalirus*	*Palinustus*	*Panulirus*	*Puerulus*	*Palinurellus*
CS outline	oval	oval	subrectangular	oval	circular	rectangular
CS rostrum	-	-	-	-	-	+
CS overlaps thorax completely	no	no	no	no	yes	no
CS overlaps MXP3 coxae	no	yes	yes	yes	yes	yes
posterior margin of thorax	concaved between P3 coxae	concaved between P3 coxae	concaved between P4 coxae or not	concaved between P4 coxae	not concaved	not concaved
maxillue basal endite	2 spines	2 spines	2 spines	3 spines	2 spines	3 spines
pereiopod subexopodal spine	-	-	+	-	+	-
chelate/subchelate pereiopod	P1, P3	P1, P3, P4	P1, P3, P4	-	-	-

†: No late stage has been described in *Linuparus* among Japanese palinurid genera. CS: cephalic shield, MXP: maxilliped, P: pereiopod, -: absent, +: present.

major diagnostic character for *Justitia* and *Nupalirus* phyllosomas, but it was shown that the chelate pereiopod are also found in *Palinustus* phyllosomas (Palero et al. 2010). It is of interest that the molecular analyses in the palinurid species showed that *Justitia* and *Nupalirus* are much closer to *Linuparus* than to *Palinustus* (Palero et al. 2009; Tsang et al. 2009). Furthermore, stage IV phyllosoma of *Linuparus* sp. proposed by Johnson (1971) had subchelate pereiopod 1, suggesting that *Linuparus* may be belonging to the chelate larval group.

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沖縄海域から採集されたリョウマエビ類の後期フィロソーマ幼生について

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沖縄県石垣島の南方海域で鋏脚を持つ後期フィロソーマ幼生 2 個体が採集された。これらの 16S rDNA 配列を解析し、また Robertson（1969）の発育段階表に基づき形態を調べたところ、ウデナガリョウマエビ（*Justitia longimana*）の 7 期及びリョウマエビ（*Nupalirus japonicus*）の 8 期幼生と判定された。また後期幼生の報告例がないハコエビ属（*Linuparus*）を除いた、国内産のイセエビ科 6 属における既知の後期フィロソーマの主な形態形質と比較したところ、リョウマエビ類 2 属は、Johnson and Robertson（1970）が指摘した胸部の後縁が第 3 胸脚基部の間で凹む点で、第 4 胸脚基部の間かまたは凹まない他のイセエビ科の属と異なることを再確認した。

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