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Mid- to late stage phyllosoma larvae of *Panulirus brunneiflagellum* Sekiguchi & George, 2005 collected south of the Ogasawara Islands, Japan

Kooichi Konishi*, Takashi Yanagimoto, Seinen Chow

National Research Institute of Fisheries Science, 2-12-4 Fukuura, Yokohama, Kanagawa 236-8648, Japan

*Corresponding author; e-mail: kzoea@fra.affrc.go.jp

Abstract

Mid- to late stage phyllosoma larvae, assigned to 7th and 8th stages, collected south of the Ogasawara Islands, Japan, were determined to be *Panulirus brunneiflagellum* Sekiguchi & George, 2005 by DNA barcoding, and morphological description was given for the first time. Phylogenetic analysis based on the mtDNA COI sequences indicated that the most closely related species to *P. brunneiflagellum* was *P. marginatus*, and hence *P. brunneiflagellum* was indicated to belong to '*Panulirus longipes* group'. The larval morphology also indicated *P. brunneiflagellum* to be a member of the same group, in which the ratio of carapace shield width to thoracic width was larger than that of *P. japonicus* but similar to those of the other species of '*Panulirus longipes* group' previously reported.

Key words: Crustacea; Palinuridae; larva; DNA barcoding; plankton

Introduction

Spiny lobsters of the genus *Panulirus* have been target species for economically important fishery. *Panulirus longipes* (A. Milne-Edwards, 1868) is a species complex, which has been splitted into five nominal species (*P. brunneiflagellum*, *P. cygnus*, *P. longipes bispinosus*, *P. longipes longipes* and *P. femoristriga*) (Sekiguchi 2008). Sekiguchi (1991) noted two distinct types in *Panulirus longipes* in the Ogasawara Islands, Japan and tentatively named one of them to be 'Aka-ebi' in Japanese. This 'Aka-ebi' was later described as a new species *P. brunneiflagellum* by Sekiguchi and George (2005). Very long planktonic period is characteristics of phyllosoma larvae of spiny lobsters, and *P. brunneiflagellum* may not be the exception. However, *P. brunneiflagellum* is endemic of the Ogasawara Islands, unlike most species of '*Panulirus longipes* group' having very wide distribution throughout the Indo-western Pacific (Holthuis 1991). Although genetically distinct status of *P. brunneiflagellum* was presented (Chow et al. 2006a; Shirai et al. 2006), no information of larval morphology has been known yet. We examined the plankton specimens of phyllosomata collected around the Ogasawara Islands in the research cruise of R/V Shunyo-Maru of the Fisheries Research Agency in February 2007. The general morphology of the specimens suggested that these provisionally belong to the genus *Panulirus* by the diagnostic keys (Sekiguchi 1986; Konishi 2015). Consequently, two specimens were found to be *P. brunneiflagellum* *via* DNA analysis. In this study, we introduce the results of morphological description on these two phyllosoma larvae of this species for the first time.

Materials and Methods

A total of 14 phyllosoma larvae of the genus *Panulirus* were collected in February 2007 around the Ogasawara Islands. Crude DNA was extracted from a pereiopod, and approximately 1300 bp fragment of mitochondrial COI region was amplified as described in Chow et al. (2006a). Amplified fragments digested by *Alu* I and *Taq* I restriction enzymes were electrophoresed on agarose gel to tentatively identify lobster species (Chow et al. 2006a). Five larvae were determined to be *P. brunneiflagellum*, of which two specimens (designated as St.6NS3 and St.6NM4) (Table 1) were selected for further morphological and nucleotide sequence analyses because of the relatively well-conserved condition. We used forward primers (COI65F1~4) (Chow et al. 2006a) for nucleotide sequence analysis, as the upstream region of COI had enough information for species discrimination. COI sequences of closely related species were derived from database (Table 2), and nucleotide sequence alignment, model selection, calculation of K2P distance, and construction of phylogenetic tree were performed using MEGA6 (Tamura et al. 2013). Nucleotide sequences determined are available in the DDBJ-EMBL-GenBank database (accession numbers LC472814 and LC475101). Observations and drawings were made with an aid of drawing tube attached to an Olympus BX51 microscope and a SZX10 stereomicroscope. Stage definition of phyllosoma is followed after Matsuda and Yamakawa (2000). Measuring method is followed after Matsuda (2006). The voucher specimens are deposited at the Hokkaido University Museum under the accession No. ICHUM-5961-5964.

Table 1. Sampling information for two phyllosoma specimens collected at 24 February 2007.

	specimen	St6NS3	St6NM4
Net set at 30 m			
	Local time (hh:mm)	18:21	19:38
	Lat. (N)	25.983	25.905
	Long. (E)	142.999	143.002
Net lift starting			
	Local time (hh:mm)	18:51	20:08
	Lat. (N)	25.937	25.869
	Long. (E)	143.001	143.005

Table 2. *Panulirus* lobster COI sequences derived from database.

species	Accession No.
Panulirus argus	AF339452, FJ174969
Panulirus brunneiflagellum	AB193077*, AB193078, AB193079
Panulirus cygnus	AF339453, KT696496
Panulirus femoristriga	MG062676
Panulirus japonicus	AB071201, JN591364
Panulirus longipes bispinosus	MK371327, MK371329
Panulirus longipes longipes	AF339464, JQ229879
Panulirus marginatus	KF828012, KF828013
Panulirus ornatus	HM446347
Panulirus pascuensis	AF339466

*larval form.

Results

Phylogenetic analysis

Nucleotide sequences determined were 607 bp for St6NS3 and St6NM4. K2P distance between these two sequences was 0.5 ± 0.3 % S.E. In maximum likelihood analysis (ML), T92+G+I was selected as the best fitted model. ML phylogenetic tree is shown in Fig. 1, indicating intimate affinity of St6NS3 and St6NM4 to *P. brunneiflagellum*. K2P distance between St6NS3 and St6NM4 and *P. brunneiflagellum* ranged from 0.2 to 2.0 % with a mean of 1.1 ± 0.3 % S.E. All these results indicate St6NS3 and St6NM4 to be *P. brunneiflagellum.* The closest species to *P. brunneiflagellum* was *P. marginatus*, in which K2P distance between these two species ranged from 4.2 to 5.1 % with a mean of 4.6 ± 0.9 % S.E., comparable with or even smaller than that (5.7 ± 1.0 % S.E.) between *P. l. bispinosus* and *P. l. longipes*.

Morphological description of the phyllosoma specimens

The larval stages of the present specimens are identified to be the 7th and 8th stage based on morphological definition by Matsuda and Yamakawa (2000), mainly by the condition of maxilliped 2, pereiopod 5 and pleopods. Detailed morphology is given as below.

Stage 7 (St6NS3)

Dimensions: Total body length (TL) = 15.9 mm, cephalic shield length (CL) = 12.0 mm,

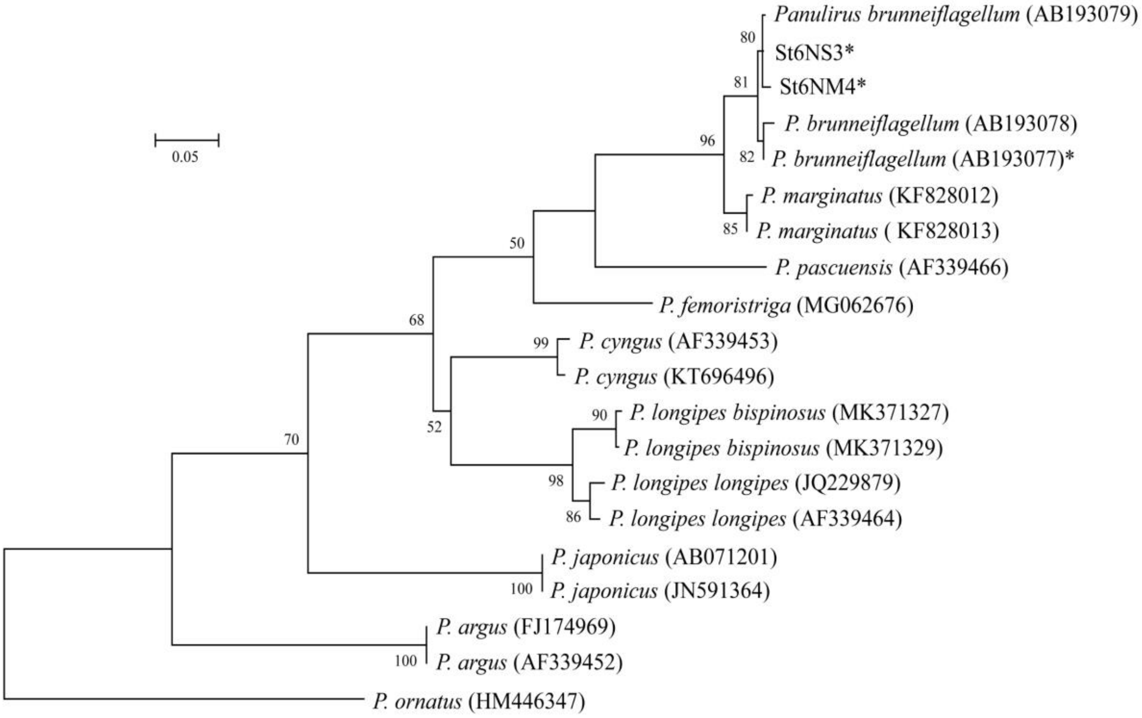

Fig. 1. Phylogenetic tree of mtDNA COI sequences in '*Panulirus longipes* group' created using the maximum likelihood method (ML). COI sequence of *Panulirus ornatus* was used as a root. Accession numbers are shown in the parenthesis. Bootstrap values of ≥ 50% (out of 1000 replicates) are shown on the nodes. *Larval form.

cephalic shield width (CW) = 7.4 mm, thorax width (TW) = 8.8 mm.

Cephalothorax: Cephalic shied (CS) ovate in outline (Fig. 2A), longer than wide, CW/TW ratio = 0.84. Posterior margin of thorax slightly concave between the base of the pair of 4th pereiopods.

Antennule (Fig. 2B): Biramous, peduncle 3-segmented. Outer flagellum (exopod) with aesthetascs on about half of its inner margin. Inner flagellum about 1/4 length of outer flagellum, without setae.

Antenna (Fig. 2C): Uniramous, 5-segmented, shorter but as nearly long as antennule.

Mandibles: Slightly flattened dorso-ventrally. 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, covering distal inner half portion of mandible.

Maxillule (Fig. 2D): Two short setae (arrow), showing presumptive endopod site, near anterior base. Basial endite with 2 stout spines and a subterminal seta while coxal endite with 3 stout spines.

Maxilla (Fig. E): 2-segmented bud. Basal segment with 2 thin anterior thin setae. Distal segment (scaphognathite) with an apical seta.

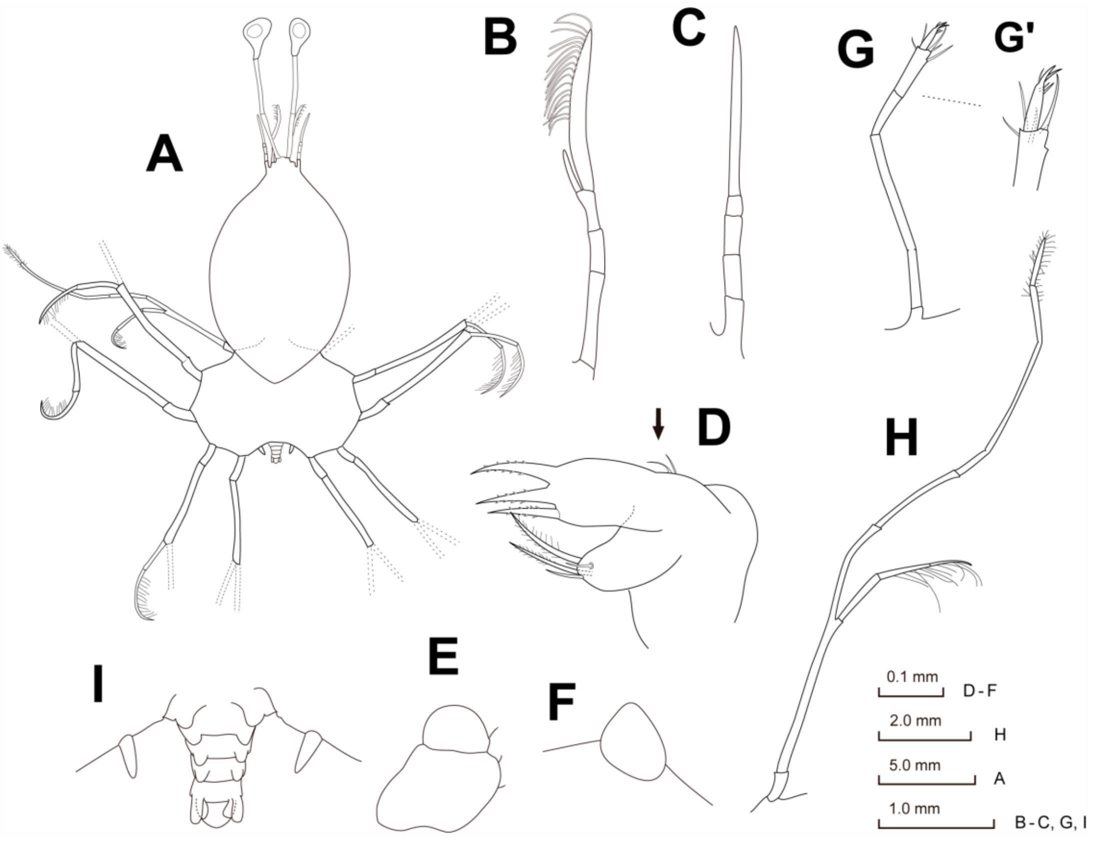

Fig. 2. *Panulirus brunneiflagellum*, stage 7 phyllosoma (St6NS3). A: whole animal, dotted lines showing presumptive missing part of pereopods, B: antennule, C: antenna, D: maxillule, E: maxilla, F: maxilliped 1, G: maxilliped 2, G’: tip of the same, enlarged, H: maxilliped 3, I: abdomen. A and H in ventral view; B-G in dorsal view.

Maxilliped 1 (Fi. 2F): Present as small bud near maxilla.

Maxilliped 2 (ig. 2G): Uniramous with 5 segments. No exopod rudiment.

Maxilliped 3 (Fi. 2H): Endopod 4-segmented, many setae on distal segment. Exopod two-segmented, distal segment slightly curved with natatory plumose setae.

Pereiopod 1-4: Distal part of endopod, merus to dactylus, missing. No subexopodal spine was observed on all pereiopods.

Pereiopod 5 (Fig. 2I): Uniramous, unsegmented projection.

Abdomen (Fig. 2J): Somite segmented, with uniramous rudiments of pleopods and biramous uropod buds.

Stage 8 (St6NM4)

Dimensions: TL = 22.2 mm, CL = 15.9 mm, CW = 10.4 mm, TW = 12.1 mm.

Cephalothorax: Outline of CS ovate, longer than wide (Fig. 3A), CW/TW ratio = 0.86. Others as in the stage 7.

Antennule (Fig. 3B): Aesthetascs on outer flagellum increased, inner flagellum slightly elongate with 3 short setae, others as in stage 7.

Antenna (Fig. 3C): 5-segmented, now longer than antennule.

Manibles (Fig. 3D): 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

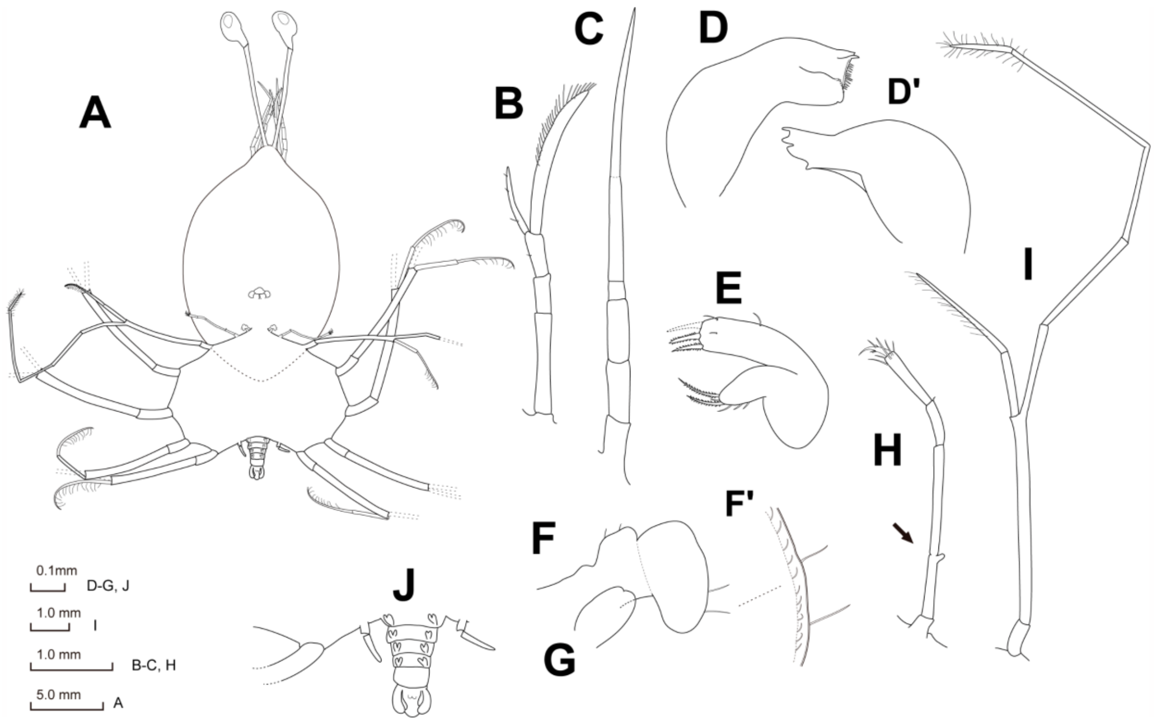

Fig. 3. *Panulirus brunneiflagellum*, stage 8 phyllosoma (St6NM4). A: whole animal, B: antennule, C: antenna, D: left mandible in inner view, D’: same in outer view, E: maxillule, F: maxilla, F’: same, enlarged distal segment, G: maxilliped 1, H: maxilliped 2, I: maxilliped 3, J: abdomen. A and I in dorsal view; B-H and J in ventral view.

paragnath well-developed, covers distal inner half portion of mandible.

Maxillule (Fig. 3E): A short seta, showing presumptive endopod site, near anterior base. Basial endite with 2 stout spines and 3 subterminal setae while coxal endite with 3 stout spines and 2 setae.

Maxilla (Fig. 3F): Basal segment with 2 thin anterior setae. Distal segment with 2 marginal thin setae; many marginal setae of next stage are visible under the cuticle (Fig. 3F').

Maxilliped 1 (Fig. 3G): Elongated, distal part slightly cleft.

Maxilliped 2 (Fig. 3H): Exopod rudiment visible as a bud in left side (arrow).

Maxilliped 3 (Fig. 3I): Endopod 4-segmented, many setae on distal segment. Exopod two-segmented, distal segment with natatory plumose setae.

Pereiopod 1-4: Distal part of endopod, merus to dactylus, missing. A small papilla on ventral side of thorax, near the base of appendage. Others as in the stage 7.

Pereiopod 5 (Fig. 3J): 2-segmented, uniramous projection.

Abdomen (Fig. 3J): Somite segmented, rudiments of pleopods and uropod as biramous buds. Scaphognathite with 7 or 8 marginal plumose setae and an apical process.

Table 3. Main dimensions of 7th and 8th stage phyllosoma in four *Panulirus* species.

Species	Stage	N	TL	CW	TW	CW/TW
Panulirus japonicus[1]	VII	4	12.7±0.6	5.3±0.3	7.2±0.4	0.74±0.01
	VIII	26	20.3±1.2	8.5±0.4	11.2±1.8	0.76±0.02
P. brunneiflagellum[2]	VII	1	15.9	7.4	8.8	0.84
	VIII	1	22.2	10.4	12.1	0.86
P. longipes bispinosus[1]	VII	14	16.6±1.5	7.6±0.6	9.3±0.7	0.82±0.01
	VIII	32	21.5±1.2	9.7±0.5	11.5±0.6	0.84±0.02
P. longipes longipes[1]	VII	1	16.9	8.0	9.2	0.87
	VIII	3	20.2±0.7	9.0±0.6	10.8±0.7	0.83±0.01
P. femoristriga[1]	VII	1	14.0	6.7	7.7	0.87
P. marginatus[3 †]	VII	1	18.2	8.7	9.6	0.91
	VIII	1	23.6	10.9	12.2	0.90
P. penicillatus[1]	VII	8	17.0±1.3	9.4±0.7	9.5±0.6	0.99±0.02
	VIII	16	22.6±1.5	12.1±0.8	12.2±0.8	0.99±0.03
P. homarus[4 †]	VII	1	9.4	4.7	4.6	1.02
	VIII	1	16.2	8.2	7.4	1.10
P. versicolor[1]	VIII	1	16.7	8.8	7.9	1.11
P. ornatus[1]	VIII	1	17.3	9.4	8.4	1.12

[1]Chow et al. (2006b), [2]this study, [3]Johnson (1968), [4]Berry (1974), [†]measured from the figures.

Remarks

Morphological description of the phyllosoma of *P. brunneiflagellum* is incomplete yet, because not a few parts of thoracic appendages were missing in the present plankton samples, probably by accidental damages during net collection. Nevertheless, in the present phyllosoma specimens, general feature apparently resembles that of *P. japonicus*, and of '*Panulirus longipes* group' (*P. longipes bispinosus*, *P. longipes longipes*, *P.*

femoristriga, and *P. marginatus*). Main dimensions of 7th and 8th stages of *P. brunneiflagellum* specimens are apparently larger than that of *P. japonicus*, closely allied with those in '*Panulirus longipes* group', but smaller than those of *P. penicillatus*, *P. homarus*, *P. versicolor* and *P. ornatus* (Table 3, Fig. 4). Thus, mid- to late stage phyllosomata of the genus *Panulirus* may be divided into three major groups by CW/TW ratio, i.e., larger than 1.00, 1.00-0.80, and less than 0.80. *P. brunneiflagellum* is included in the second group. At present we are not able to discuss in detail about this condition, since in this study the dimensions are based only one specimen in each stage. More additional data will resolve this statistical difficulty. The phyllosomata of *P. brunneiflagellum*, evidently belong to the phyllosoma species-group 1 defined by McWilliam (1995) as follows: CW is obviously narrower than TW, no ventral coxal spines or subexopodal spines on pereiopods. Thus,

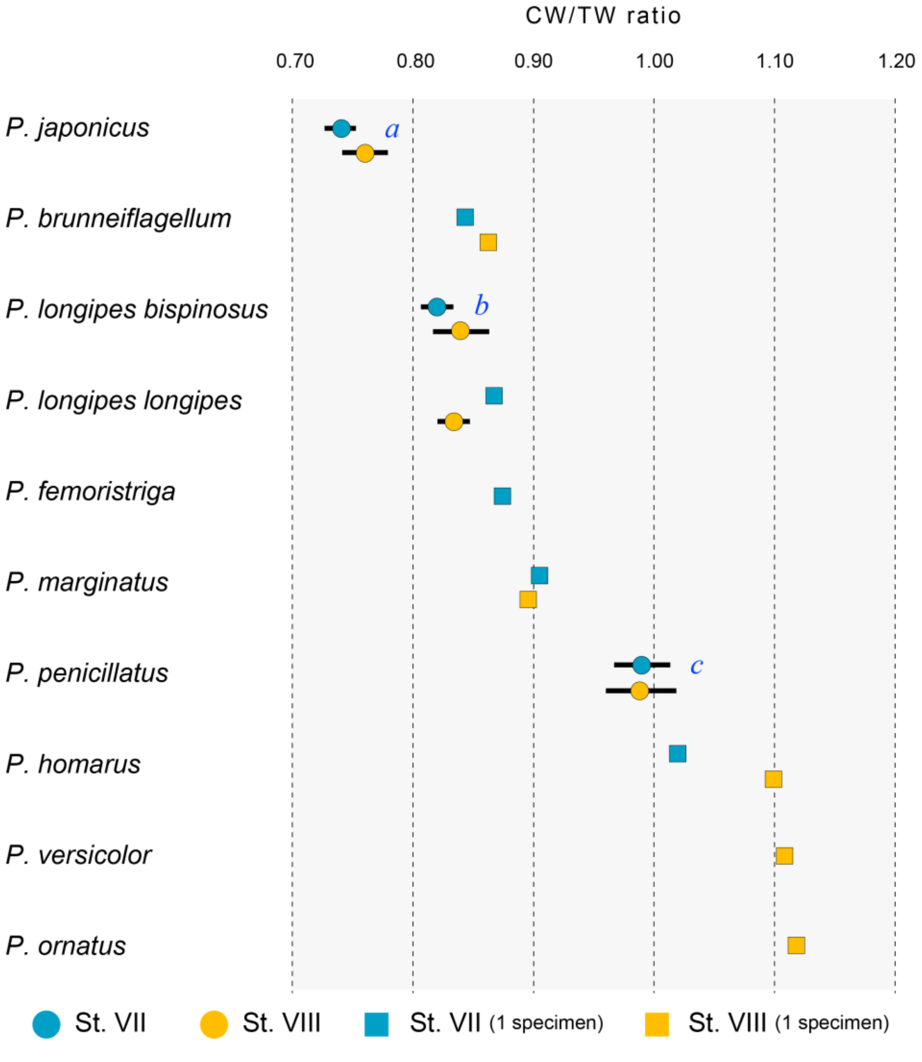

Fig.4. CW/TW ratio of 7th (blue) and 8th (orange) stage phyllosoma in ten *Panulirus* species. Horizontal bars on plotted circles indicate standard deviation. Squares mean the data based on single specimen. CW/TW ratio data of 7th and 8th stage phyllosoma were pooled to statistically compare three species (*P. japonicus*, *P. longipes bispinosus* and *P. penicillatus*), in which averages carrying different alphabet were significantly different one another ($p < 0.001$). See Table 3 for detail.

the phyllosomata of *Panulirus* species of Japanese coasts, including *P. brunneiflagellum*, are classified into 3 larval groups as shown in Table 4. This result also is in accordance with the group based on adult forms by George and Holthuis (1965) and George and Main (1967). The setal number on the proximal maxillar segment is 2 in the present specimens, while 3 for the most of *Panurilus* species. Matsuda (2006) noted that this setal number is useful for identification in early phyllosoma stages based on laboratory rearing. However, variation of the number has also been found even in the same species, i.e., *P. echinatus* and *P. guttatus* (Abrunhosa et al. 2004; Baisre and Alfonso 1994). It seems that this setal number is not constant at least after mid-later phyllosoma stages. In the previous larval works, this larval character sometimes has not been described. More detailed description of larval morphology should be required in future.

Acknowledgments

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Table 4. Comparison of setal number on the proximal segment of maxilla in *Panulirus* species.

Phyllosoma group§	Species	Setal number	References
Group 1	*P. argus*	3	Lewis (1951)
Group 1	*P. brunneiflagellum**	2	this study
Group 1	*P. echinatus*	2 or 3†	Abrunhosa et al. (2004), Konishi et al. (2006)
Group 1	*P. japonicus**	3	Matsuda (2006)
Group 1	*P. longipes bispinosus**	3	Matsuda and Yamakawa (2000), Matsuda (2006)
Group 2	*P. guttatus*	2 or 3¶	Baisre and Alfonso (1994)
Group 2	*P. interruptus*	3	Johnson (1956)
Group 2	*P. penicillatus**	2	Minagawa (1990), Matsuda (2006)
Group 3	*P. inflatus*	3	Johnson and Knight (1966)
Group 4	*P. homarus**	3	Prasad et al. (1975)
Group 4	*P. laevicauda*	3	Abrunhosa et al. (2004)
Group 4	*P. ornatus**	3	Prasad et al. (1975)
Group 4	*P. polyphagus*	3	Uchida and Dotsu (1973)
Group 4	*P. versicolor**	3	Deshmukh (1968)

§phyllosoma group determined by McWilliam (1995), *species found in the coasts of Japan, †2 setae in Konishi et al. (2006), ¶3 setae in the stage X.

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小笠原海域から採集されたアカイセエビの中・後期フィロソーマ幼生について

小西光一[*]・柳本 卓・張 成年

水産研究・教育機構中央水産研究所，神奈川県横浜市金沢区福浦 2-12-4

小笠原諸島の南海域で採集された 7 および 8 期フィロソーマ幼生の COI 遺伝子配列を分析したところ、アカイセエビ（*Panulirus brunneiflagellum*）と判定され、その形態観察を行った。系統解析で示された本種の最近縁種はハワイイセエビ（*P. marginatus*）であり、カノコイセエビ群（‘*Panulirus longipes*’group）に属することが示された。また頭甲幅/胸部幅の比においても、アカイセエビはイセエビ（*P. japonicus*）より値が大きく、よりカノコイセエビ群に近いことが示された。

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