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Molecular Identification of Mid to Final Stage Slipper Lobster Phyllosoma Larvae of the Genus *Chelarctus* (Crustacea: Decapoda: Scyllaridae) Collected in the Pacific with Descriptions of Their Larval Morphology

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Morphological descriptions of phyllosoma larvae are essential for correct species identification and investigating the spatiotemporal distribution and recruitment process of spiny and slipper lobsters. Species identification of the phyllosoma larvae in the Scyllarinae subfamily is particularly difficult because of the morphological similarities among species and the scarcity of morphological information describing correct species identity. We extracted mid- to final-stage (V to VIII) phyllosoma larvae (*n* = 12) belonging to the subfamily Scyllarinae from several plankton samples collected in the Pacific and then performed molecular species identification using mitochondrial DNA *COI* and 16S rDNA sequence analyses. Three larvae collected around the Ryukyu Archipelago were identified as *Chelarctus aureus* (stage VI to VIII), and four collected around the Ryukyu Archipelago and Ogasawara Islands were identified as *C. virgosus* (V to VIII). One larva (V) collected in the central South Pacific was determined to be a subspecies of *C. crosnieri*. DNA barcodes could not be made for the remaining four larvae (V to VIII) collected around the Ryukyu Archipelago (designated by ?*Chelarctus* sp-1). Based on the morphological characteristics of the *C. virgosus* phyllosoma described in this study and the adult distributions reported to date, *C. cultrifer* phyllosomas previously reported in Japanese and Taiwanese waters are likely to be *C. virgosus*. This paper also presents a set of diagnostic morphological characteristics that can be used to discriminate among these four species of *Chelarctus* and from other genera in the subfamily Scyllarinae.

Key words: Phyllosoma larvae, Slipper lobster, Molecular analysis, Morphology, Identification, *Chelarctus*.

BACKGROUND

Species identification is difficult for spiny and slipper lobster phyllosoma larvae in the Palinuridae and Scyllaridae families because closely related species are morphologically similar and morphological descriptions based on correct species identifications are scarce. There have been numerous morphological descriptions of wild-caught phyllosoma larvae, most of which are not based on confident species identities. Scyllaridae, which comprises 19 extant genera and at least 88 extant species, is more diverse than Palinuridae, which

contains 12 extant genera and 58 or more extant species (Yang and Chan 2010; Chan et al. 2013; Chan 2019). Scyllaridae comprises four subfamilies: Arctidinae, Ibacinae, Scyllarinae, and Theninae (Holthuis 1991). While the subfamily Scyllarinae used to contain only one genus, *Scyllarus*, the Holthuis (2002) revised the genus and divided it into 14 genera, in which more than 52 species have been described (Holthuis 2002; Chan 2010; Yang et al. 2008 2011 2012 2014 2017; Yang and Chan 2012). Scyllarinae is now the most diverse subfamily within the Scyllaridae with an increasing number of descriptions for new species (Yang et al. 2008 2011 2012 2014 2017; Yang and Chan 2012). However, species identification of the phyllosomas within this subfamily may be extremely difficult using morphological keys alone, as most larvae have been given a tentative alphabetic or numeric name such as *Scyllarus* sp., A, B, I, II, 1, 2 or ? (Prasad and Tampi 1957 1960; Johnson 1971a b; Berry 1974; Phillips et al. 1981; Barnett et al. 1986; McWilliam et al. 1995; Webber and Booth 2001). Nevertheless, complete larval development in the laboratory has been achieved in only six of the species in this subfamily (Robertson 1968 1979; Ito and Lucas 1990; Higa et al. 2005; Kumar et al. 2009; Fernández et al. 2010), leaving researchers to rely on other methods of identification. DNA barcoding has been shown to be useful for linking larvae or juveniles to adults of marine animals (Chen et al. 2013; Wong et al. 2014 2020; Li et al. 2019; Wei et al. 2021) and has been applied to wild-caught phyllosomas of only five species of the subfamily Scyllarinae (Palero et al. 2008 2011 2014; Genis-Armero et al. 2017; Wakabayashi et al. 2017 2020).

In this study, we report the results of our genetic and morphological analyses of mid-to final-stage phyllosoma larvae belonging to the Scyllarinae collected in the Northwest Pacific and central South Pacific Ocean.

MATERIALS AND METHODS

Wild-caught phyllosoma specimens

Phyllosomas were collected by three research vessels: [1] the RV *Shunyo-Maru* (Japan Fisheries Research and Education Agency), which cruised around the Ryukyu Archipelago (22°00'N−29°15'N, 123°30'E−130°00'E) from May 31 to June 26, 2009 (SHU-09); [2] the RV *Koyo-Maru* (the Tokyo Ogasawara Marine Products Center), which completed two cruises in the coastal waters of the Ogasawara Islands (26°32'N−27°09'N, 142°01'E−142°38'E) in 2015 and 2018 (KY15 and 18); and [3] the RV *Hakuho-*

Maru (the University of Tokyo), which completed a cruise in the South Pacific (5°00'S−25°00'S, 160°00'E−140°00'W) from August 8 to September 28, 2016 (KH 16-4). The survey area and collection location of each phyllosomas used in this study are shown in figure 1. Phyllosomas were sorted on board and fixed in 80–90% ethanol before being transferred to the laboratory. In the laboratory, we first extracted the phyllosomas with round-, oval-, or lemon-shaped cephalic shields and a tapered abdomen that continues from the thorax (see Phillips et al. 1981; Sekiguchi 1986) from each cruise sample. This procedure allowed us to select the phyllosomas from the subfamily Scyllarinae. Subsequently, mid- to final-stage larvae (V–VIII) were selected based on the criteria described by Inoue and Sekiguchi (2006); stage V: 5th pereiopod unsegmented or 2-segmented, pleopods bud, uropods unbiloed or slightly bilobed; stage VI: 5th pereiopod 2 or 3-segmented, pleopods unbilobed, uropods bilobed; stage VII: 5th pereiopod 3-segmented, pleopods bilobed; stage VIII: 1st to 4th pereiopods gilled, 5th pereiopod 4-segmented. We further selected 12 larvae that were as morphologically intact as possible (Table 1). These phyllosoma specimens were deposited into the National Museum of Nature and Science, Tokyo, Japan (NSMT-Cr29022–29033). Body length (BL), cephalic shield length (CL), cephalic shield width (CW), thorax width (TW) and abdominal length (AL) were measured as described by Higa and Shokita (2004) and Palero et al. (2008). Detailed morphological investigations were performed using an Olympus LV100 optical microscope. Mandible and detailed setation were not included in the drawing because they are not useful for species identification. Arrangement of subexopodal spine was not described, since intra-specific and intra-individual variations have been reported in palinurid phyllosoma larvae (Chow et al. 2006a). In the same species, only the earliest developmental stage is completely described in detail, and in subsequent stages only the changes were described.

Molecular genetic analysis

The selected specimens were rinsed thoroughly with tap water, and one pereiopod was then selected and subjected to dissection. Pereiopods were further rinsed well with tap water, homogenized, and subjected to crude DNA extraction using a blood and tissue kit (Qiagen Inc.). Since we frequently failed to obtain good sequence electropherograms using universal primers for mitochondrial cytochrome oxidase subunit I gene (*COI*) (Folmer et al. 1994) and for 16S rRNA gene (16S) (Palumbi et al. 1991), we used mixed primers: COI65F1-F4/COI1342R1-R4 (Chow et al. 2006b) for

amplifying *COI* sequence and a newly designed primer pair (16SUF: 5'-TGACTGTGCGAAGGTAGCAT-3'/16SR2: 5'-ACGCCGGTCTGAACTCAAAT-3') for 16S. PCR was performed in 10 µl of total reaction mixture containing 5–10 ng template DNA, 1 µl of 10 × buffer, 1 µl each dNTPs, 2×10^{-3} mol m^{-3} each primer, and 0.5 U of EX Taq HS DNA polymerase (Takara, Shiga, Japan) on an ABI 9700 Thermal cycler (Applied
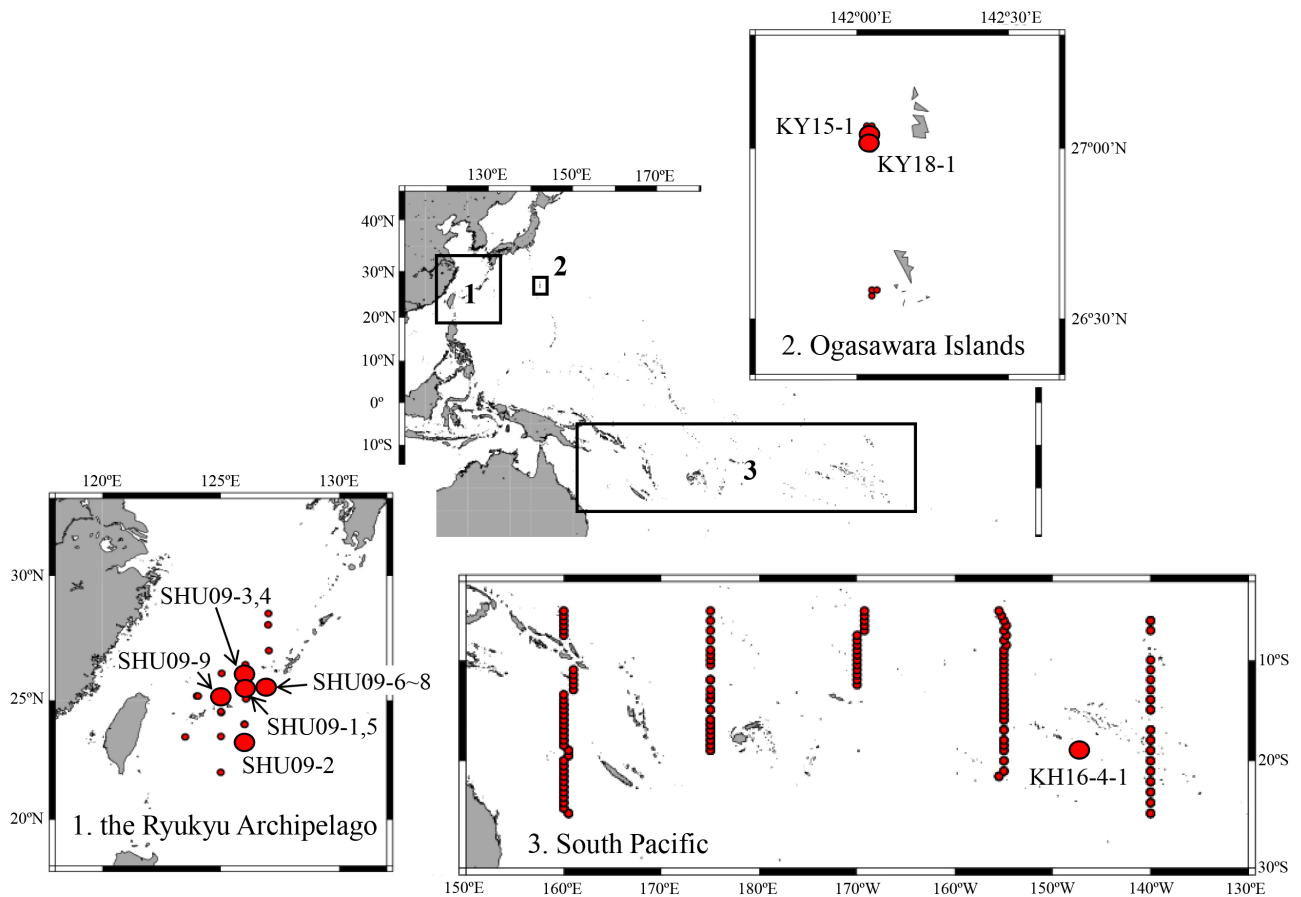

Fig. 1. Survey area and collection locations for the phyllosoma larvae sampled by the three research vessels: RV *Shunyo-maru* (SHU), *Koyo-maru* (KY), and RV *Hakuho-maru* (KH). Closed red circles indicate the sampling stations where Scyllarinae phyllosomas were collected. The specimens analyzed in this study were collected from the large circle stations. See table 1 for specimen ID.

Table 1. Summary of the sampling information, stage and DNA sequence for each of the phyllosoma specimens used in this study

Specimen ID	Cruise	Location*	Coordinate	St. No.	Date	Stage	Sequences determined	Specimen voucher
KY15-1	KY15	NWP	27°07'N, 142°05'E	-	30/01/2015	VI	*COI*, 16S	NSMT-Cr29022
KY18-1	KY18	NWP	27°02'N, 142°05'E	-	31/01/2018	V	*COI*, 16S	NSMT-Cr29023
SHU09-1	SHU09	NWP	25°30'N, 126°05'E	7	08/06/2009	VII	*COI*, 16S	NSMT-Cr29024
SHU09-2	SHU09	NWP	23°19'N, 126°09'E	22	09/06/2009	VIII	*COI*, 16S	NSMT-Cr29025
SHU09-3	SHU09	NWP	26°09'N, 126°01'E	23	10/06/2009	VI	16S	NSMT-Cr29026
SHU09-4	SHU09	NWP	26°09'N, 126°01'E	23	10/06/2009	VII	16S	NSMT-Cr29027
SHU09-5	SHU09	NWP	25°30'N, 126°05'E	7	08/06/2009	VIII	*COI*	NSMT-Cr29028
SHU09-6	SHU09	NWP	25°33'N, 127°02'E	2a	23/06/2009	V	16S	NSMT-Cr29029
SHU09-7	SHU09	NWP	25°33'N, 127°02'E	2a	23/06/2009	VI	*COI*, 16S	NSMT-Cr29030
SHU09-8	SHU09	NWP	25°33'N, 127°02'E	2a	23/06/2009	VII	*COI*, 16S	NSMT-Cr29031
SHU09-9	SHU09	NWP	25°16'N, 124°58'E	20a	19/06/2009	VIII	*COI*, 16S	NSMT-Cr29032
KH16-4-1	KH16-4	CSP	19°00'S, 147°30'W	226	11/09/2016	V	*COI*, 16S	NSMT-Cr29033

*NWP: Northwest Pacific, CSP: central South Pacific.

Biosystems, Foster City, CA, USA). Reaction mixtures were preheated at 94°C for 2 min, followed by 40 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 7 min. The PCR product was purified using a PCR purification kit (Qiagen, Hilden, Germany) and was used as the DNA template for cycle sequencing reactions using a Big Dye Terminator Cycle Sequencing Kit (Ver. 3.1, Applied Biosystems) with COI65F1-F4 and 16SR2 primers. Sequencing was conducted using an ABI Prism 3730XL instrument (Applied Biosystems). Nucleotide sequences of one per species or subspecies for each of the 13 genera in the subfamily Scyllarinae, were derived from the database, and those of *Scyllarides squammosus* (JN701654 and JN701689) were adopted as an outgroup. These sequences were aligned with the sequences obtained in this study using ATGC (Genetix, Osaka, Japan) followed by an eye examination. A neighbor-joining (NJ) tree based on the uncorrected Kimura's two-parameter distances (K2P) between sequences was constructed using MEGA ver. 6 (Tamura et al. 2013).

RESULTS

DNA barcoding for phyllosoma larvae

We produced partial *COI* (564 to 573 bp) and 16S (333 to 404 bp) sequences from nine and 11 phyllosomas, respectively (Table 1). All sequences were deposited into the International Nucleotide Sequence Database Collection (INSDC) under accession numbers LC632672 to LC632682 for 16S and LC632947 to LC632955 for *COI*. Phylogenetic trees generated using these sequences are shown in figures 2 and 3, and *Bathyarctus* was the only genus that was commonly monophyletic in both the *COI* and 16S trees.

These phylogenetic trees unambiguously identified the four larvae (KY15-1, KY18-1, SHU09-1, and SHU09-2) collected around the Ryukyu Archipelago and Ogasawara Islands as *Chelarctus virgosus* and the three larvae (SHU09-3 to -5) collected around the Ryukyu Archipelago as *C. aureus*. The K2P distances between the larval and adult reference sequences were 1.0 to 1.9% in *COI* and 0.3% in 16S (Tables 2 and 3), which were well below the typical values between congeneric species (Hebert et al. 2003; Kochzius et al. 2010).

Sample KH16-4-1 collected in the South Pacific was closely related to *C. crosnieri* (JX 486086) in the *COI* tree (Fig. 2). The K2P distance (6.9% in *COI*) (Table 2) between KH16-4-1 and *C. crosnieri* was smaller than those between valid species of slipper lobsters (Yang et

al. 2008) and comparable to that between the *C. cultrifer* subspecies (*C. cultrifer cultrifer* and *C. cultrifer meridionalis*) (5.6 to 8.3%) (Yang and Chan 2012), so we determined that KH16-4-1 is likely a subspecies of *C. crosnieri* and designated it as *C. crosnieri* sub sp.1.

The remaining four larvae (SHU09-6 to -9) collected around the Ryukyu Archipelago could not be assigned to any species, even using a BLAST search. The K2P distances among these larvae were 1.4 to 1.6% in *COI* and 0.0 to 0.6% in 16S (Tables 2 and 3), indicating that these four specimens were conspecific. These four larvae were nested with other *Chelarctus* species (Figs. 2 and 3), so we tentatively determined that they belonged to the genus *Chelarctus* and designated these samples as ?*Chelarctus* sp-1. The *COI* and 16S trees (Figs. 2 and 3) suggested that the closest kin for ?*Chelarctus* sp-1 may be *C. crosnieri* and *C. crosnieri* sub sp.1 (KH16-4-1), in which the K2P distances between ?*Chelarctus* sp-1 and the latter two species ranged from 14.9 to 15.9% in *COI* and from 7.8 to 8.2% in 16S (Tables 2 and 3).

Morphological descriptions of phyllosomas

Chelarctus virgosus (Yang and Chan, 2012)

Stage V (KY18-1, Fig. 4)

TL = 8.45 mm; CL = 5.73 mm; CW = 6.71 mm; TW = 3.50 mm; AL = 1.24 mm; CW/CL = 1.17; CW/TW = 1.92. This description was completed for the specimen collected at 27°02'N, 142°05'E (offshore of the Ogasawara Islands) on 31 July 2018.

Cephalic shield (Fig. 4A): sub-pentagonal; slightly wider than long, nearly twice as wide as thorax. Antennule (Fig. 4B): biramous, 3-segmented; distal segment with two flagella (primary and accessory); primary flagellum unsegmented with 9–10 rows of sensory setae, slightly shorter than accessory flagellum. Antenna (Fig. 4B): bifurcated, unsegmented, half H-shaped (⊦), exceeding antennule; short lateral process projecting horizontally. Maxillule 1 (Fig. 4E): anterior lobe with denticulate three strong setae; posterior lobe with denticulate two strong setae. Maxillule 2 (Fig. 4C): rudimentary flattened. Maxilliped 1 (Fig. 4C): rudimentary bud. Maxilliped 2 (Fig. 4C, D): 5-segmented, coxal spine absent; three short setae on dactylus; at least five long setae on proximal margin of propodus (Fig. 4D). Maxilliped 3 (Fig. 4A): 5-segmented, tiny ventral coxal spine; distal parts of propodus and dactylus setosed densely; exopod absent. Pereiopods 1–4 (Fig. 4A): biramous, tiny coxal spine and 5-segmented endopod; ischio-merus fused to basis, one stronger and one smaller distal spines on outer and

inner sides of ischio-merus; a tiny distal spine on carpus; a dense of setae covering ischio-merus. Pereiopod 5 (Fig. 4F, G): elongated bud, coxal spine absent, much shorter than abdomen. Abdomen (Fig. 4F, G): four pairs of undeveloped pleopod buds; slightly bilobed uropod, rounded posterior margin locating far anterior of central margin of telson; prominent posterolateral spines of telson far beyond posterior central margin of telson, more than three times the length of uropod.

Stage VI (KY15-1, Fig. 5)

TL = 12.66 mm; CL = 7.96 mm; CW = 9.73 mm; TW = 4.58 mm; AL = 2.41 mm; CW/CL = 1.22; CW/TW = 2.12. This description was completed for the specimen collected at 27°07'N, 142°05'E (offshore of Ogasawara Islands), on 30 January 2015.

Cephalic shield (Fig. 5A): more than twice as wide as thorax. Antennule (Fig. 5B): primary flagellum
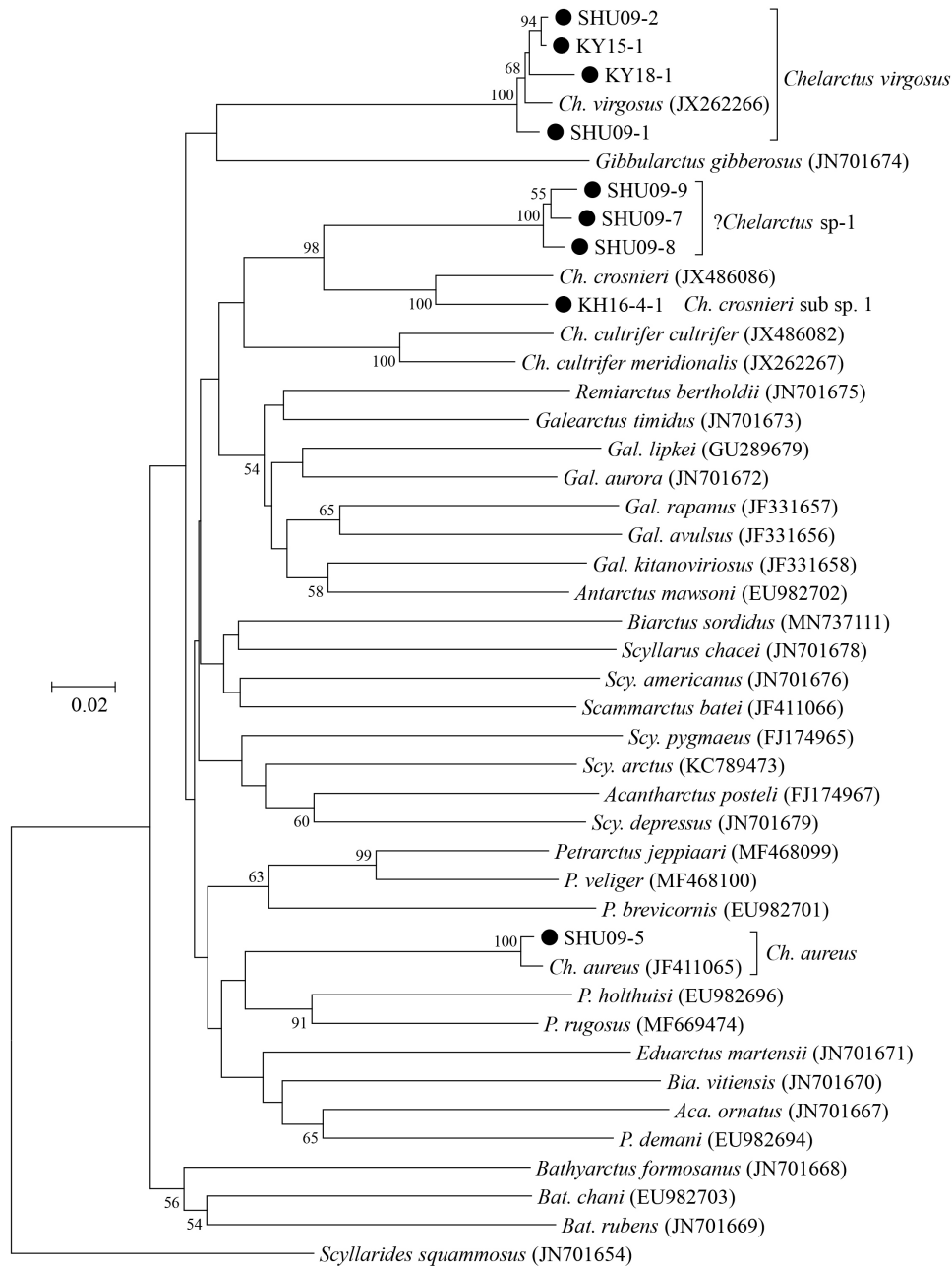

Fig. 2. Neighbor-joining phylogenetic tree drawn using the Kimura-2-parameter distances for each of the partial *COI* sequences between the subfamily Scyllarinae species. *Scyllarides brasiliensis* was used as the outgroup. Closed squares indicate the phyllosoma specimens analyzed in this study. Bootstrap values of > 50% (from 1000 replicates) are shown at each node.

similar in length to accessory flagellum. Antenna (Fig. 5B): similar in length to antennule. Maxilliped 1 (Fig. 5C): cone-shaped buds. Maxilliped 3 (Fig. 5A): propodus more densely setose than stage V (at least 20 setae). Pereiopods 1−4 (Fig. 5A): coxal spines more developed than those during stage V. Pereiopod 5 (Fig. 5F, G): 2-segmented, shorter than half of abdomen. Abdomen (Fig. 5F, G): four pairs of unbilobed

pleopods; bilobed uropods; posterolateral spines of telson twice as long as uropod.

Stage VII (SHU09-1, Fig. 6)

TL = 18.61 mm; CL = 11.89 mm; CW = 14.11 mm; TW = 7.67 mm, AL = 4.66 mm; CW/CL = 1.18; CW/TW = 1.84. This description was completed for the
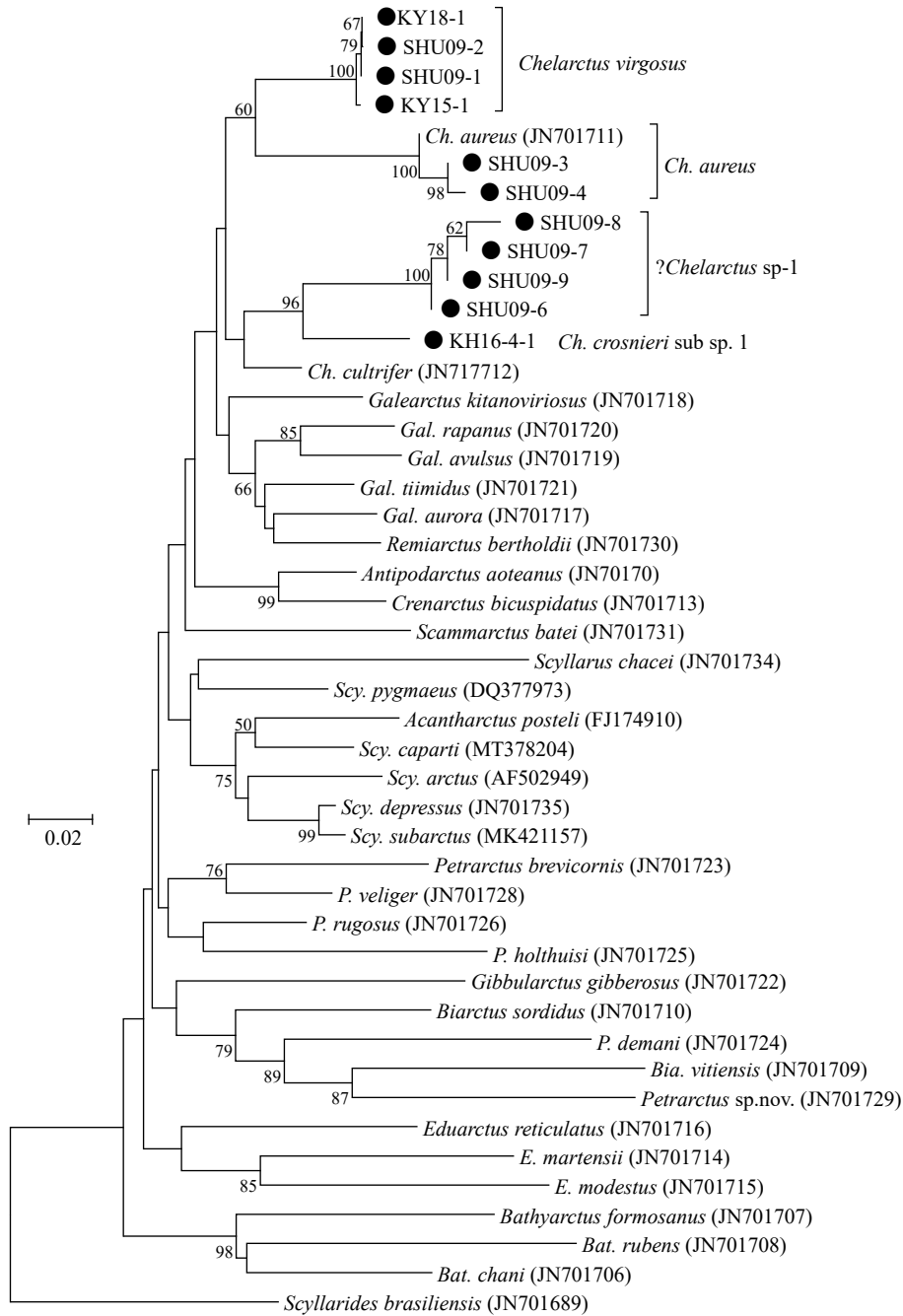

Fig. 3. Neighbor-joining phylogenetic tree drawn using the Kimura-2-parameter distances for each of the partial 16S rDNA sequences for species belonging to the subfamily Scyllarinae. *Scyllarides brasiliensis* was used as the outgroup. Closed squares indicate the phyllosoma specimens analyzed in this study. Bootstrap values of > 50% (from 1000 replicates) are shown at each node.

specimen collected at 25°30'N, 126°05'E (around the Ryukyu Archipelago) on 8 June 2009.

Antennule (Fig. 6B): accessory flagellum longer than primary flagellum; larger number of setae on primary flagella than during stage VI. Antenna (Fig. 6B): lateral process stouter than that of stage VI. Maxillule 2 (Fig. 6C): rudimentary bilobed. Maxilliped 1 (Fig. 6C): slightly biramous. Maxilliped 2 (Fig. 6C, D): four setae on dactylus; two long terminal setae on propodus with four short simple setae. Maxilliped 3 (Fig. 6A): exopod bud, at least 19 setae on propodus.

Pereiopods 1−4 (Fig. 6A): more spine-like setae on basis surface than during stage VI; at least 26 setae scattered over the surface of the P1 propodus; P4 longer than other pereiopods. Pereiopod 5 (Fig. 6F, G): 3-segmented, tiny coxal spine, about half of abdomen. Abdomen (Fig. 6F, G): pleopod biramous; posterolateral spines slightly longer than uropod.

Stage VIII (SHU09-2, Fig. 7)

TL = 20.66 mm; CL = 12.30 mm; CW = 15.97 mm;

Table 2. Uncorrecred K2P distance (lower diagonal) and standard error (upper diagonal) for the partial *COI* sequences from the nine phyllosoma larvae analyzed in this study and the five reference *Chelarctus* sequences from the database

	Location*	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1: *C. aureus*	Taiwan		0.021	0.024	0.023	0.026	0.027	0.026	0.026	0.027	0.004	0.022	0.022	0.022	0.023
2: *C. crosnieri*	Vanuatu	0.187		0.022	0.021	0.024	0.025	0.024	0.023	0.024	0.022	0.020	0.020	0.021	0.012
3: *C. cultrifer cultirfer*	Indonesia	0.217	0.190		0.014	0.024	0.024	0.024	0.023	0.024	0.024	0.022	0.022	0.023	0.022
4: *C. cultrifer meridionalis*	Philippines	0.201	0.178	0.086		0.022	0.023	0.022	0.021	0.022	0.023	0.023	0.023	0.023	0.022
5: *C. virgosus*	Taiwan	0.242	0.213	0.208	0.184		0.006	0.005	0.005	0.005	0.026	0.024	0.024	0.024	0.023
6: KY15-1	NWP	0.245	0.225	0.208	0.192	0.019		0.005	0.006	0.006	0.026	0.024	0.024	0.025	0.023
7: KY18-1	NWP	0.242	0.214	0.211	0.184	0.012	0.014		0.004	0.002	0.026	0.023	0.023	0.023	0.022
8: SHU09-1	NWP	0.236	0.211	0.200	0.178	0.013	0.019	0.010		0.005	0.025	0.023	0.023	0.023	0.022
9: SHU09-2	NWP	0.245	0.217	0.213	0.181	0.014	0.016	0.002	0.012		0.026	0.023	0.023	0.024	0.023
10: SHU09-5	NWP	0.010	0.195	0.212	0.196	0.233	0.236	0.233	0.227	0.236		0.022	0.022	0.022	0.022
11: SHU09-7	NWP	0.190	0.150	0.190	0.196	0.213	0.216	0.197	0.199	0.199	0.193		0.006	0.005	0.020
12: SHU09-8	NWP	0.188	0.155	0.188	0.199	0.207	0.208	0.194	0.196	0.197	0.190	0.016		0.005	0.019
13: SHU09-9	NWP	0.190	0.158	0.198	0.199	0.216	0.219	0.199	0.202	0.202	0.193	0.014	0.014		0.020
14: KH16-4-1	CSP	0.208	0.069	0.194	0.196	0.210	0.210	0.202	0.199	0.205	0.205	0.154	0.149	0.159	

Accession numbers: JF411065 (1), JX486086 (2), JX486082 (3), JX486085 (4), JX262266 (5), LC632949 (6), LC632948 (7), LC632950−LC632955 (8-13), LC632947 (14). *NWP: Northwest Pacific, CSP: central South Pacific.

Table 3. Uncorrecred K2P distance (lower diagonal) and standard error (upper diagonal) for the partial 16S rDNA sequences from the 11 phyllosoma larvae analyzed in this study and the two *Chelarctus* reference sequences from the database

	Location*	1	2	3	4	5	6	7	8	9	10	11	12	13
1: *Chelarctus aureus*	Taiwan		0.022	0.019	0.020	0.019	0.019	0.003	0.003	0.027	0.027	0.027	0.027	0.023
2: *Chelarctus cultrifer*	Philippines	0.117		0.016	0.017	0.016	0.016	0.023	0.023	0.017	0.018	0.017	0.017	0.016
3: KY15-1	NWP	0.102	0.071		0.003	0.000	0.000	0.020	0.020	0.021	0.021	0.021	0.021	0.018
4: KY18-1	NWP	0.098	0.068	0.003		0.003	0.003	0.020	0.020	0.021	0.021	0.022	0.022	0.018
5: SHU09-1	NWP	0.098	0.068	0.003	0.000		0.000	0.020	0.020	0.021	0.021	0.021	0.021	0.018
6: SHU09-2	NWP	0.098	0.068	0.003	0.000	0.000		0.020	0.020	0.021	0.021	0.021	0.021	0.018
7: SHU09-3	NWP	0.003	0.121	0.106	0.102	0.102	0.102		0.000	0.027	0.026	0.027	0.027	0.023
8: SHU09-4	NWP	0.003	0.121	0.106	0.102	0.102	0.102	0.000		0.027	0.026	0.027	0.027	0.023
9: SHU09-6	NWP	0.169	0.085	0.115	0.111	0.111	0.111	0.164	0.164		0.004	0.003	0.003	0.018
10: SHU09-7	NWP	0.165	0.088	0.111	0.108	0.108	0.108	0.160	0.160	0.006		0.003	0.003	0.017
11: SHU09-8	NWP	0.169	0.085	0.115	0.111	0.111	0.111	0.165	0.165	0.003	0.003		0.000	0.018
12: SHU09-9	NWP	0.169	0.085	0.115	0.111	0.111	0.111	0.165	0.165	0.003	0.003	0.000		0.018
13: KH16-4-1	CSP	0.132	0.074	0.089	0.085	0.085	0.085	0.136	0.136	0.085	0.078	0.082	0.082	

Accession numbers: JN701711 (1), JN701712 (2), LC632673 (3), LC632672 (4), LC632674−LC632682 (5−13). *NWP: Northwest Pacific, CSP: central South Pacific.

TW = 7.76 mm; AL = 5.92 mm; CW/CL = 1.25; CW/TW = 2.06. This description was completed for the specimen collected at 23°19'N, 126°09'E (around the Ryukyu Archipelago) on 9 June 2009.

Antenna (Fig. 7B): exceeding antennule. Maxillule 1 (Fig. 7E): six short terminal setae, two long denticulate setae and two normal terminal setae on the posterior lobe and two sub-terminal setae on the outer side of the posterior lobe. Maxillule 2 (Fig. 7C): apparently biramous, more developed posterior lobe than during stage VII. Maxilliped 1 (Fig. 7C): unsegmented and bilobed, outer lobe flattened and rounded. Maxilliped 2 (Fig. 7C, D): an exopod bud. Maxilliped 3: coxal spine absent; propodus more densely setose than stage VII. Pereiopods 1−4 (Fig. 7A): gill buds on base of coxa; at least 33 setae scattered over the surface of the propodus in P1. Pereiopod 5 (Fig. 7F, G): 4-segmented, much longer than half of abdomen. Abdomen (Fig. 7F, G): biramous pleopods larger than stage VII; round uropod margin near reaching but never exceeding posterior central margin of telson; posterolateral spines of telson similar in length of uropod.

Chelarctus aureus (Holthuis, 1963)

Stage VI (SHU09-3, Fig. 8)

TL = 9.40 mm; CL = 5.81 mm; CW = 8.88 mm; TW = 4.50 mm; AL = 1.26 mm; CW/CL = 1.30; CW/TW = 2.1. This description was completed for the specimen collected at 26°09'N, 126°01'E (around the Ryukyu Archipelago) on 10 June 2009.

Cephalic shield (Fig. 8A): wide sub-rectangular; wider than long, more than twice as wide as thorax, slightly concaved posterior edge. Antennule (Fig. 8B): biramous, 3-segmented, exceeding antenna; distal
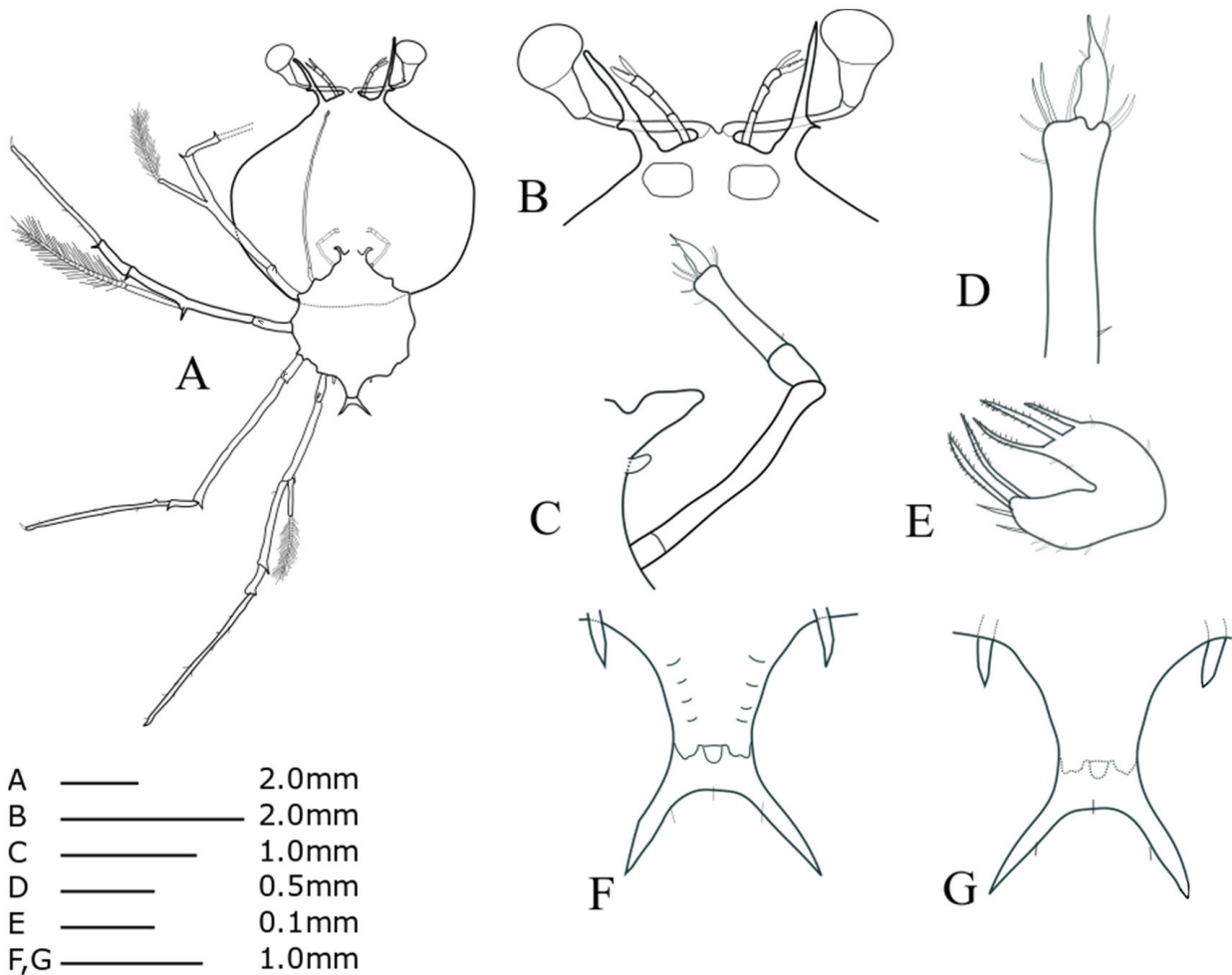

A	———	2.0mm
B	—————	2.0mm
C	———	1.0mm
D	——	0.5mm
E	——	0.1mm
F,G	————	1.0mm

Fig. 4. Phyllosoma larva of *Chelarctus virgosus*, stage V. A, ventral view; B, ventral view of eye, antennule and antenna; C, 2nd maxillule, 1st and 2nd maxilliped; D, distal end of 2nd maxilliped; E ventral view of 1st maxillule; F, ventral view of 5th pereiopod and abdomen; G, dorsal view of 5th pereiopod and abdomen.

segment with two flagella (primary and accessory); primary flagellum unsegmented with at least six rows of sensory setae, slightly longer than accessory flagellum. Antenna (Fig. 8B): bifurcated, unsegmented, half H-shaped (⊢); short lateral process projecting horizontally. Maxillule 1 (Fig. 8E): anterior lobe with denticulate three strong setae; posterior lobe with denticulate three strong setae. Maxillule 2 (Fig. 8C): rudimentary bilobed. Maxilliped 1 (Fig. 8C): elongated bud. Maxilliped 2 (Fig. 8C, D): 5-segmented, tiny coxal spine; four setae on dactylus; three long setae on distal margin of propodus. Maxilliped 3 (Fig. 8A): damaged propodus; tiny spine on ventral margin of coxa; exopod absent. Pereiopods 1–4 (Fig. 8A): biramous, distinct coxal spine, 5-segmented endopod; ischio-merus fused to basis; two short distal spines on basis; two sharp distal spines on ischio-merus; two distal spines on the carpus; a dense of setae covering ischio-merus. Pereiopod 5 (Fig. 8F, G): 2-segmented, tiny coxal spine, extending to half of abdomen. Abdomen (Fig. 8F, G): four pairs of unbilobed pleopods; bilobed uropods, rounded posterior margin locating far anterior of central margin of telson; posterolateral spines of telson similar in length with uropod exceeding posterior central margin of telson.

Stage VII (SHU09-4, Fig. 9)

TL = 17.78 mm; CL = 13.09 mm; CW = 18.19 mm; TW = 7.17 mm; AL= 4.57 mm; CW/CL = 1.34; CW/TW = 2.54. This description was completed for the specimen collected at 26°10'N, 126°01'E (around the Ryukyu Archipelago) on 10 June 2009.

Cephalic shield (Fig. 9A): more than 2.5-times wider than thorax, concaved posterior edge. Antennule (Fig. 9B): primary flagellum with at least 14 rows of sensory setae, similar in length to accessory flagellum. Maxillule 2 (Fig. 9C): slightly bilobed. Maxilliped 1 (Fig. 9C): rudimentary bilobed. Maxilliped 2 (Fig. 9C, D): bearing six short setae on dactylus; exopod bud. Maxilliped 3 (Fig. 9A): 5-segmented, tiny coxal spine; dense of setae covering surface of fourth and fifth segments. Pereiopod 5 (Fig. 9F, G): 3-segmented, distinct coxal spine, exceeding half of abdomen. Abdomen (Fig. 9F, G): four pairs of bilobed pleopods; posterolateral spines of telson slightly shorter than
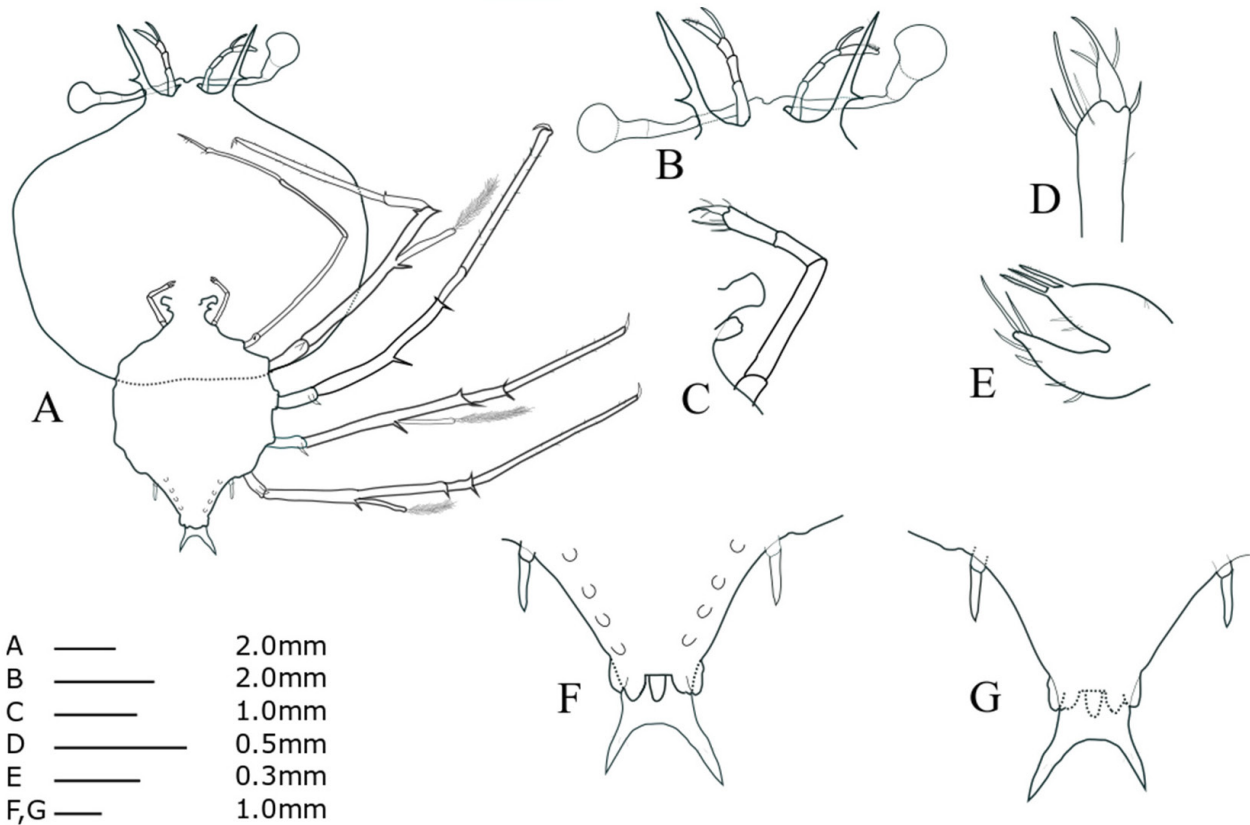

A	——	2.0mm
B	——	2.0mm
C	——	1.0mm
D	——	0.5mm
E	——	0.3mm
F,G	——	1.0mm

Fig. 5. Phyllosoma larva of *Chelarctus virgosus*, stage VI. A, ventral view; B, ventral view of eye, antennule and antenna; C, 2nd maxillule, 1st and 2nd maxilliped; D, distal end of 2nd maxilliped; E, ventral view of 1st maxillule; F, ventral view of 5th pereiopod and abdomen; G, dorsal view of 5th pereiopod and abdomen.

uropod.

Stage VIII (SHU09-5, Fig. 10)

TL = 23.38 mm; CL = 16.12 mm; CW = 21.99 mm; TW = 9.85 mm; AL = 8.43 mm; CW/CL = 1.36; CW/TW = 2.23. This description was completed for the specimen collected at 25°30'N, 126°05'E (around the Ryukyu Archipelago) on 8 June 2009.

Antennule (Fig. 10B): primary flagellum with 15 rows of sensory setae, shorter than accessory flagellum. Antenna (Fig. 10B): lateral process slightly projecting antero-diagonally. Maxillule 2 (Fig. 10C): bilobed, more flattened and anterior and posterior lobes extend more than stage VII. Maxilliped 1 (Fig. 10C): bilobed; posterior lobe extending backwards and larger than anterior lobe. Maxilliped 2 (Fig. 10C): distinct coxal spine. Pereiopods 1−4 (Fig. 10A): gill buds present on the base of the coxa, short strong coxal spine, at least 20 pairs of short spine-like setae scattered over the surface of propodus. Pereiopod 5 (Fig. 10F, G): 4-segmented

extending more than during stage VII. Abdomen (Fig. 10F, G): round uropod margin near reaching but never exceeding posterior central margin of telson; posterolateral spines of telson shorter than uropod.

Chelarctus crosnieri sub sp. 1

Stage V (KH16-4-1, Fig. 11)

TL = 6.60 mm; CL = 4.70 mm; CW = 5.62 mm; TW = 3.05 mm; AL = 0.61 mm; CW/CL = 1.19; CW/TW = 1.84. This description was completed for the specimen collected from 19°00'S, 147°30'W (offshore of French Polynesia, the south Pacific) on 11 September 2016.

Cephalic shield (Fig. 11A): sub-pentagon, slightly wider than long, nearly twice as wide as thorax. Antennule (Fig. 11B): biramous, 3-segmented, exceeding antenna; distal segment with two flagella (primary and accessory); primary flagellum unsegmented with at least eight rows of sensory setae,
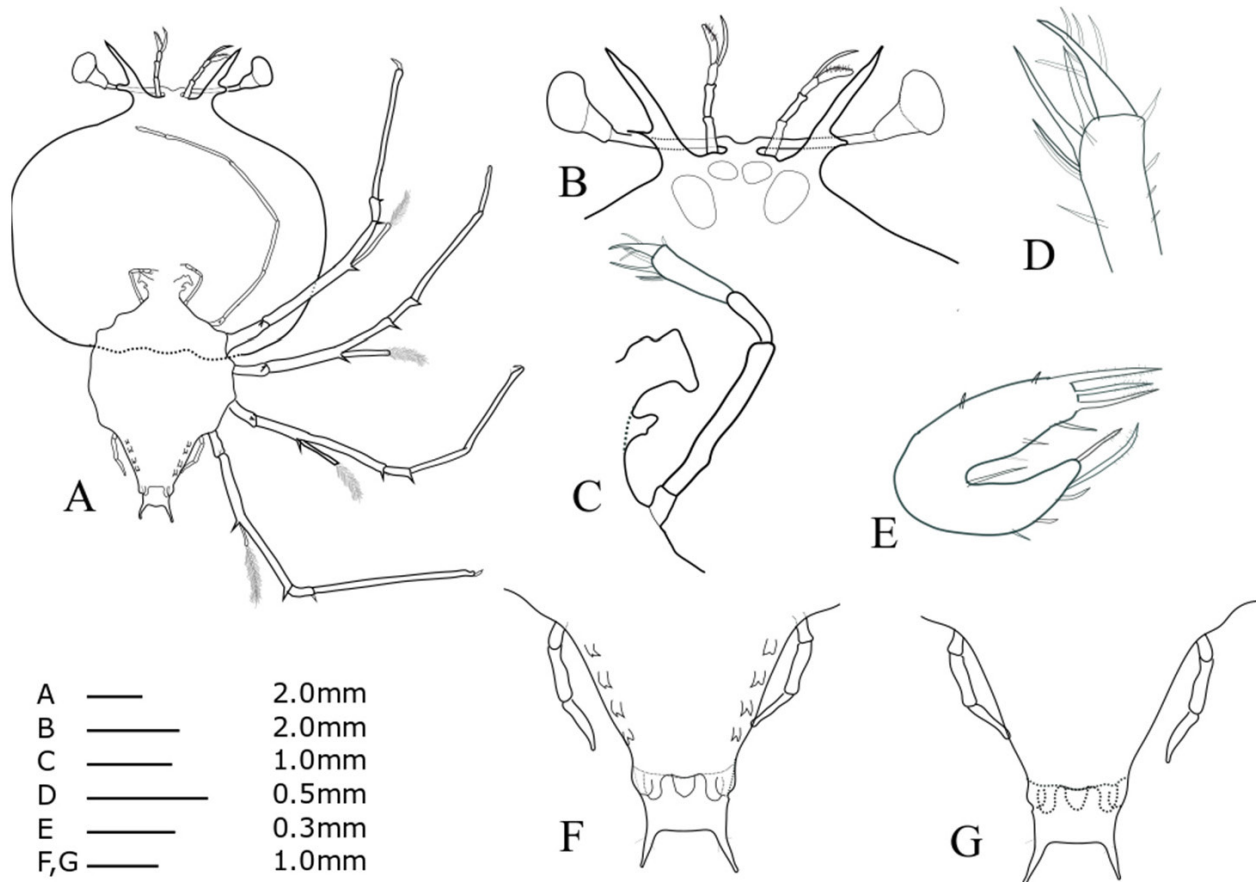

A	——	2.0mm
B	———	2.0mm
C	——	1.0mm
D	———	0.5mm
E	——	0.3mm
F,G	———	1.0mm

Fig. 6. Phyllosoma larva of *Chelarctus virgosus*, stage VII. A, ventral view; B, ventral view of eye, antennule and antenna; C, 2nd maxillule, 1st and 2nd maxilliped; D, distal end of 2nd maxilliped; E, ventral view of 1st maxillule; F, ventral view of 5th pereiopod and abdomen; G, dorsal view of 5th pereiopod and abdomen.

much longer than accessory flagellum. Antenna (Fig. 11B): bifurcated, unsegmented, half H-shaped (⊢), short lateral process projecting horizontally. Maxillule 1 (Fig. 11E): anterior lobe with denticulate three strong setae; posterior lobe with denticulate two strong setae Maxillule 2 (Fig. 11C): rudimentary flattened. Maxilliped 1 (Fig. 11C): rudimentary bud. Maxilliped 2 (Fig. 11C, D): 5-segmented, coxal spine absent, four long terminal setae on propodus, at least four fine setae on dactylus. Maxilliped 3 (Fig. 11A): 5-segmented, ventral coxal spine, distal part of the propodus and dactylus densely setosed; exopod absent. Pereiopods 1–4 (Fig. 11A): biramous, distinct coxal spine and 5-segmented endopod, ischio-merus fused to basis, single long distal spine on basis, two distal spines on ischio-merus and carpus. Pereiopod 5 (Fig. 11F, G): two-segmented, coxal spine absent, extending to half of abdomen. Abdomen (Fig. 11F, G): four pairs of pleopod bud; slightly bilobed uropod, rounded posterior margin locating far anterior of central margin of telson; not well developed posterolateral spines of telson exceeding posterior central margin of telson.

?*Chelarctus* sp-1

Stage V (SHU09-6, Fig. 12)

TL = 9.64 mm; CL = 6.09 mm; CW = 7.69 mm; TW = 4.22 mm, AL = 1.24 mm, CW/CL = 1.26, CW/TW = 1.82. This description was completed for the specimen collected at 25°33'N, 127°02'E, Ryukyu Archipelago, on 23 June 2009.

Cephalic shield (Fig. 12A): sub-pentagon; wider than long, nearly twice as wide as thorax. Antennule (Fig. 12B): similar in length to antenna; biramous, 3-segmented, distal segment with two flagella (primary and accessory); primary flagellum unsegmented with at least eight rows of sensory setae, slightly longer than accessory flagellum. Antenna (Fig. 12B): bifurcated, unsegmented, half H-shaped (⊢), short lateral process projecting horizontally. Maxillule 1 (Fig. 12E): anterior lobe with denticulate three strong setae; posterior lobe with denticulate two strong setae. Maxillule 2 (Fig. 12C): unbilobed. Maxilliped 1 (Fig. 12C): rudimentary bud. Maxilliped 2 (Fig. 12C, D): 5-segmented, coxal
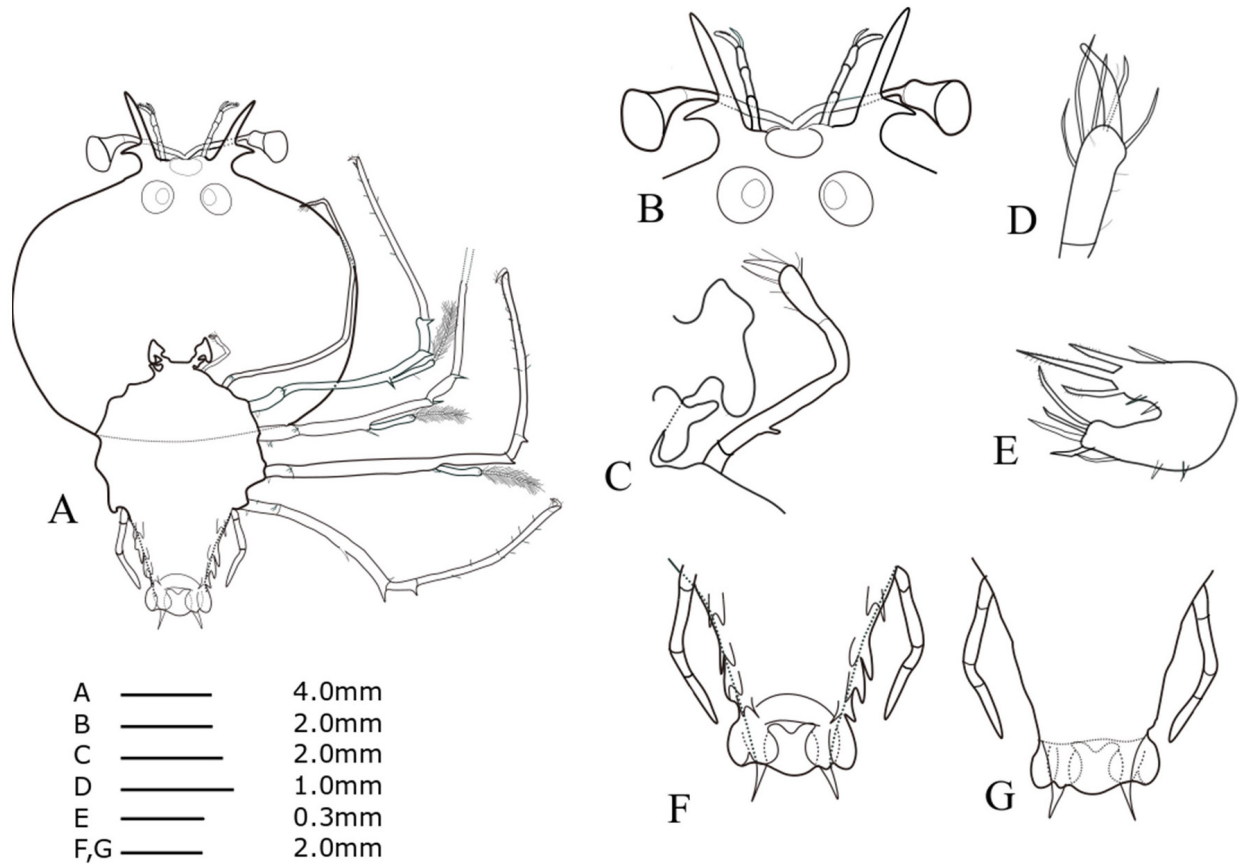

A	———	4.0mm
B	———	2.0mm
C	———	2.0mm
D	———	1.0mm
E	———	0.3mm
F,G	———	2.0mm

Fig. 7. Phyllosoma larva of *Chelarctus virgosus*, stage VIII. A, ventral view; B, ventral view of eye, antennule and antenna; C, 2nd maxillule, 1st and 2nd maxilliped; D, distal end of 2nd maxilliped; E ventral view of 1st maxillule; F, ventral view of 5th pereiopod and abdomen; G, dorsal view of 5th pereiopod and abdomen.

spine absent, three short setae on dactylus; six long setae on posterior margin of propodus (Fig. 12D). Maxilliped 3 (Fig. 12A): 5-segmented, distinct coxal spine; distal parts of propodus and dactylus setosed densely; exopod absent. Pereiopods 1–4 (Fig. 12A): biramous, distinct coxal spine and 5-segmented endopod; ischio-merus fused to basis; two distal spines on ischio-merus; two distal spines on the carpus. Pereiopod 5 (Fig. 12F, G): 2-segmented, coxal spine absent, extending to half of abdomen. Abdomen (Fig. 12F, G): four pairs of undeveloped pleopod buds; slightly bilobed uropod, rounded posterior margin locating well anterior of central margin of telson; posterolateral spine of telson well exceeding posterior central margin of telson, similar in length to uropod.

Stage VI (SHU09-7, Fig. 13)

TL = 13.71 mm; CL = 10.02 mm; CW= 13.04 mm; TW = 5.66 mm; AL = 2.88 mm; CW/CL = 1.30; CW/TW = 2.30. This description was completed for the specimen collected at 25°33'N, 127°02'E, Ryukyu Archipelago, on 23 June 2009.

Cephalic shield (Fig. 13A): wide sub-rectangular; larger CW/CL and CW/TW than in stage V. Maxillule 2 (Fig. 13C): posterior lobe slightly projecting backward.

Maxilliped 1 (Fig. 13C): bud. Maxilliped 2 (Fig. 13C, D): tiny coxal spine; four setae on dactylus. Pereiopod 5 (Fig. 13F, G): 3-segmented, distinct coxal spine, exceeding half of abdomen. Abdomen (Fig. 13F, G): bilobed uropods.

Stage VII (SHU09-8, Fig. 14)

TL = 17.41 mm; CL = 10.95 mm; CW = 16.20 mm; TW = 6.46 mm; AL = 4.69 mm; CW/CL = 1.47; CW/TW = 2.51. This description was completed for the specimen collected at 25°33'N, 127°02'E, Ryukyu Archipelago, on 23 June 2009.

Cephalic shield (Fig. 14A): larger CW/CL and CW/TW than in stage VI. Antennule (Fig. 14B): primary flagellum similar in length to accessory flagellum. Maxilliped 1 (Fig. 14C): rudimentary biramous. Maxilliped 2 (Fig. 14C): distinct coxal spine. Maxilliped 3 (Fig. 14A): damaged. Abdomen (Fig. 14F, G): four pairs of bilobed pleopods.

Stage VIII (SHU09-9, Fig. 15)

TL = 23.65 mm; CL = 13.86 mm; CW = 18.84 mm; TW = 8.4 mm; AL = 7.36 mm; CW/CL = 1.35; CW/TW = 2.24. This description was completed for the specimen
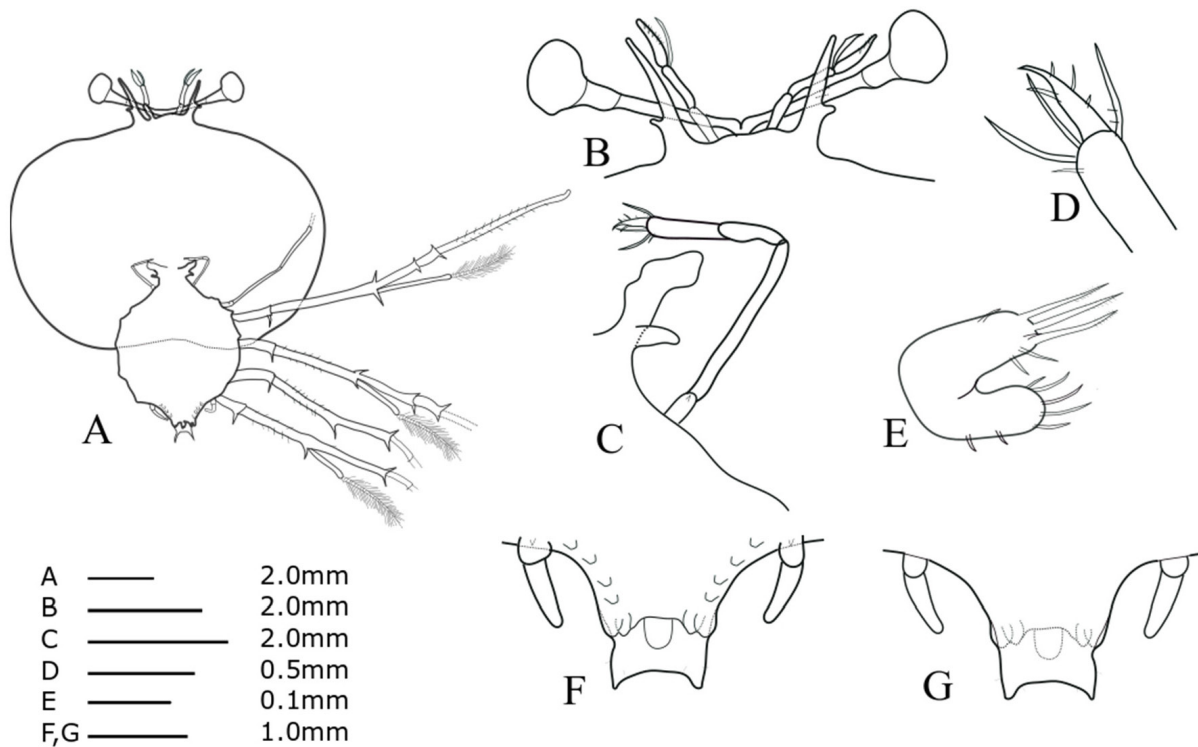

A	——	2.0mm
B	———	2.0mm
C	————	2.0mm
D	———	0.5mm
E	——	0.1mm
F,G	————	1.0mm

Fig. 8. Phyllosoma larva of *Chelarctus aureus*, stage VI. A, ventral view; B, ventral view of eye, antennule and antenna; C, 2nd maxillule, 1st and 2nd maxilliped; D, distal end of 2nd maxilliped; E, ventral view of 1st maxillule; F, ventral view of 5th pereiopod and abdomen; G, dorsal view of 5th pereiopod and abdomen.

collected at 25°16'N, 124°58'E, on 19 June 2009.

Cephalic shield (Fig. 15A): smaller CW/CL and CW/TW than in stage VII. Antenna (Fig. 15B): lateral process projecting slightly antero-diagonally. Maxillule 1 (Fig. 15E): posterior lobe with denticulate two strong setae. Maxillule 2 (Fig. 15C): flattened and outer lobe extending backward. Maxilliped 1 (Fig. 15C): biramous; anterior lobe shorter than posterior lobe. Pereiopods 1−4 (Fig. 15A): gill buds. Pereiopod 5 (Fig. 15F, G): 4-segmented. Abdomen (Fig. 15F, G): round uropod margin near reaching but never exceeding posterior central margin of telson; posterolateral spines of telson shorter than uropod.

DISCUSSION

We noticed that the four species investigated in the present study share morphological characteristics among their phyllosomas: sub-pentagon or sub-rectangular shaped cephalic shield, wide cephalic shield (CW/CL = 1.17 to 1.47), half H-shaped antenna, round posterior margin of uropod not exceeding posterior central margin of telson, and eminent posterolateral spines exceeding posterior central margin of telson. This combination of these morphological characteristics is not observed in many species of the other genera of the subfamily Scyllarinae described to date (Robertson 1968 1979; Johnson 1971a b; Ito and Lucas 1990; Higa et al. 2005; Kumar et al. 2009; Palero et al. 2008 2011; Fernández et al. 2010; Genis-Armero et al. 2017; Wakabayashi et al. 2017 2020), except for *Scyllarus pygmaeus* from the Mediterranean (Palero et al. 2008), *Scyllarus depressus* from the Atlantic (Robertson 1971, figs. 7–10), and *Scyllarus delfini* (*Acantharctus delfini*) from the Southeast Pacific (Johnson 1971c, figs. 1–3; Báez 1973, figs. 5 and 6).

The morphological characteristics of the phyllosomas investigated in this study are summarized in table 4. The phyllosomas were divided into two types of cephalic shield shapes: the phyllosomas of *C. virgosus* (Figs. 4 to 7) and *C. crosnieri* sub sp. 1 (only stage V) (Fig. 11), which contain a sub-pentagon-shaped cephalic shield, and those of *C. aureus* (Figs. 8–10) and ?*Chelarctus* sp-1 (except for stage V) (Figs. 13–15), which contain a sub-rectangular-shaped cephalic shield. CW/CL ranged from 1.17 to 1.25 in *C. virgosus* and *C. crosnieri* sub sp. 1, and 1.26 to 1.47 in *C. aureus* and
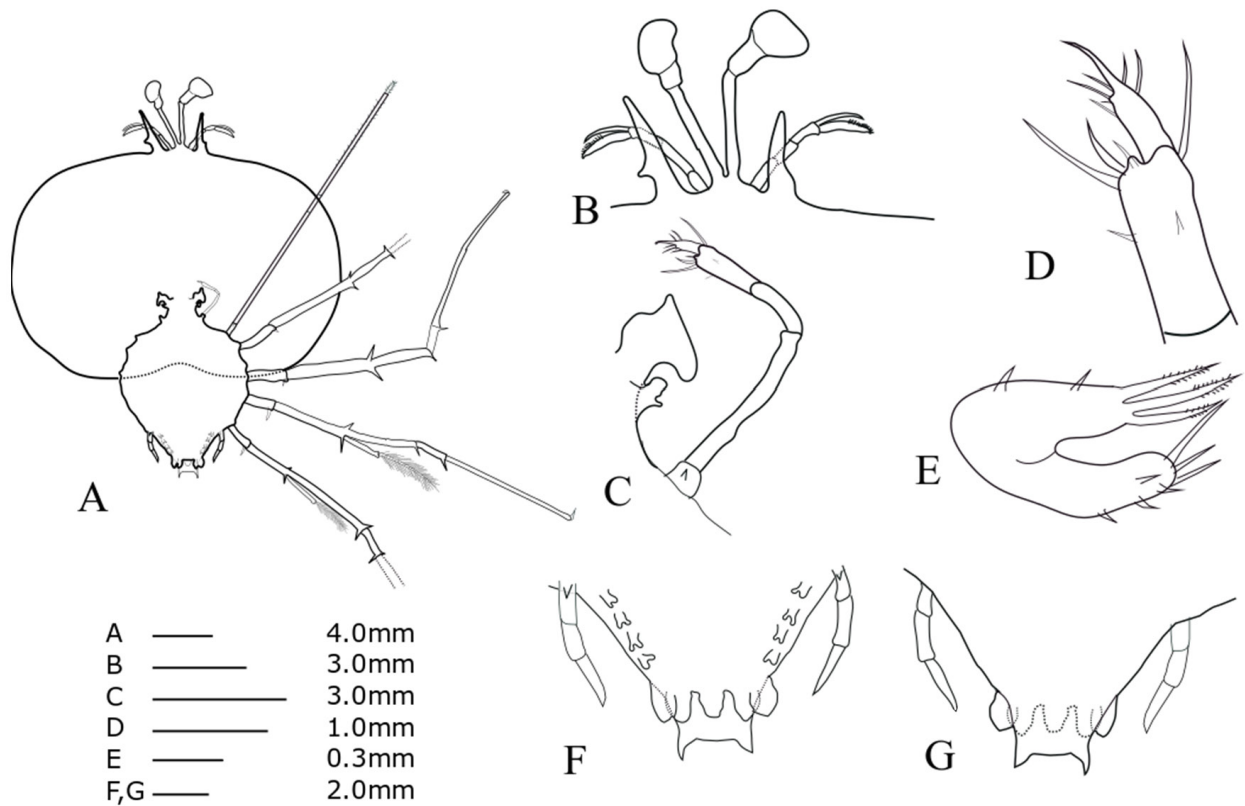

Fig. 9. Phyllosoma larva of *Chelarctus aureus*, stage VII. A, ventral view; B, ventral view of eye, antennule and antenna; C, 2nd maxillule, 1st and 2nd maxilliped; D, distal end of 2nd maxilliped; E ventral view of 1st maxillule; F, ventral view of 5th pereiopod and abdomen; G, dorsal view of 5th pereiopod and abdomen.

?*Chelarctus* sp-1. In the sub-pentagon group, stage V phyllosoma of *C. crosnieri* sub sp. 1 had a more rounded cephalic shield than that of *C. virgosus*. Phyllosomas of *C. virgosus* and *C. crosnieri* sub sp. 1 can be distinguished using the relative length of antennule and antenna. The antenna of *C. virgosus* phyllosoma was exceeding its antennules during stages V and VIII (Figs. 4 and 7) and almost the same as its antennule during stages VI and VII (Figs. 5, 6). Antennules were exceeding the antenna during stages VI–VIII *C. aureus* phyllosomas (Figs. 8 to 10) and V phyllosoma of *C. crosnieri* sub sp. 1 (Fig. 11). Antennule and antenna of ?*Chelarctus* sp-1 phyllosomas were approximately the same length at all stages (Figs. 12 to 15). Posterolateral spines of the telson in *C. virgosus* were specifically longer than those of the other species examined.

It was not challenging to interpret the phylogeny of the slipper lobsters from the subfamily Scyllarinae in this study; however, the results obtained here are not concordant with the taxonomic revisions suggested by Holthuis (2002). Insufficient genetic information and taxon sampling, saturation of the nucleotide substitution, and/or long-branch attraction may cause such discrepancies; alternatively, the taxonomic revisions suggested by Holthuis (2002) may not be an accurate representation of the natural evolution of these animals. The most comprehensive molecular phylogenetic analysis of the slipper lobsters to date (Yang et al. 2012; Palero et al. 2009) and a more recent phylogenetic analysis of Achelata lobsters (Davis et al. 2015) suggest that the Scyllarinae subfamily may be largely subdivided into *Bathyarctus* and others, which agrees with our phylogenetic results.

Chelarctus cultrifer is known to occur widely in the Indo-western Pacific (Holthuis 2002). Yang and Chan (2012) split this species into two subspecies—*C. cultrifer cultrifer* from the Indian Ocean and Indonesia and *C. cultrifer meridionalis* from Indonesia and the Philippines—with relatively large genetic distances (5.6 to 8.3% nucleotide sequence divergence in *COI*). Yang and Chan (2012) also found many phenotypic differences and large genetic divergence (15.5 to 16.5% in *COI*) between the adult forms of "*C. cultrifer*" from Taiwan-Japan and *C. cultrifer* from the Philippines and described the former as a new species the *C. virgosus*. *Chelarctus virgosus* is widely distributed across the Indian Ocean and extends to northeastern Asia, whereas *C. cultrifer* ssp. is restricted to more southern localities
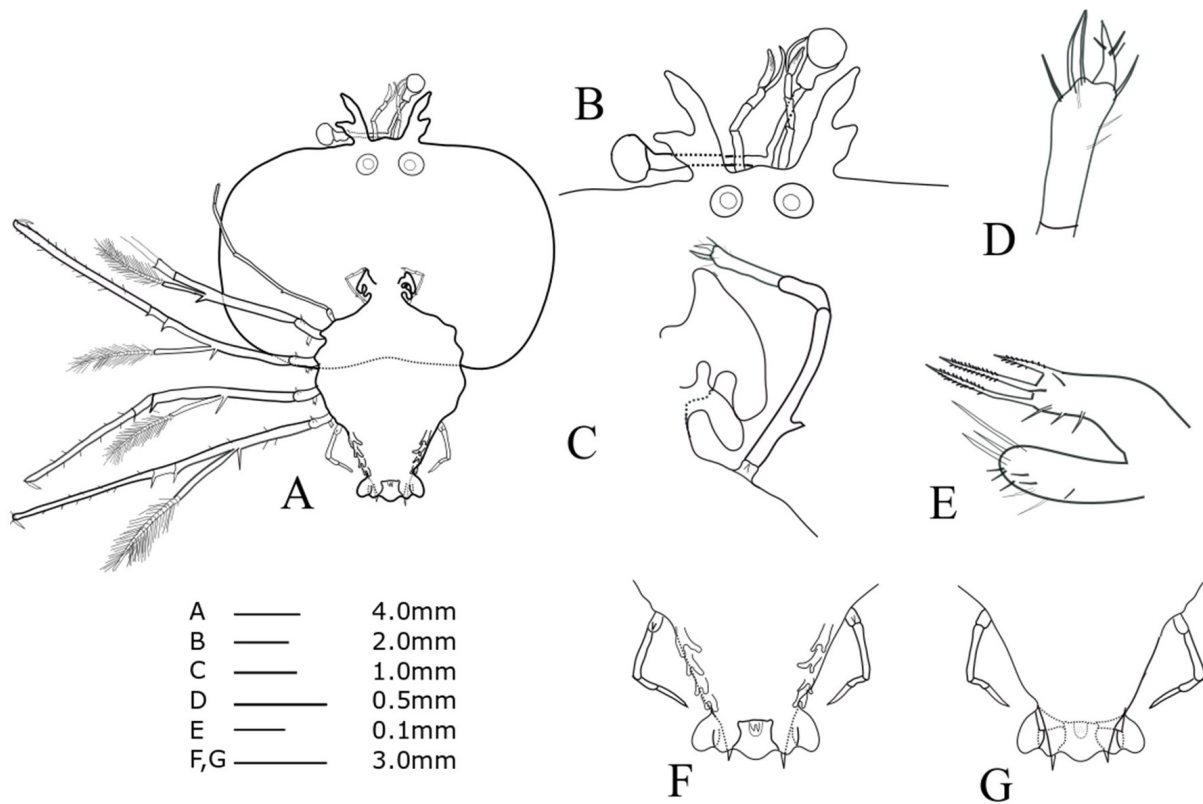

A ⎯⎯	4.0mm
B ⎯	2.0mm
C ⎯	1.0mm
D ⎯⎯	0.5mm
E ⎯	0.1mm
F,G ⎯⎯⎯	3.0mm

Fig. 10. Phyllosoma larva of *Chelarctus aureus*, stage VIII. A, ventral view; B, ventral view of eye, antennule and antenna; C, 2nd maxillule, 1st and 2nd maxilliped; D, distal end of 2nd maxilliped; E ventral view of 1st maxillule; F, ventral view of 5th pereiopod and abdomen; G, dorsal view of 5th pereiopod and abdomen.

of the Philippines and the Indian Ocean (Yang and Chan 2012), corresponding to the collection area of the *C. virgosus* phyllosomas identified in this study. *Chelarctus virgosus* phyllosomas have a sub-pentagon-shaped cephalic shield, antenna exceeding the antennules, and noticeably long posterolateral spines on the telson. Mid- to final-stage phyllosomas of *C. cultrifer* (*Scyllarus cultrifer*) from Japanese waters have been reported by Higa and Shokita (2004) and Inoue and Sekiguch (2006), in which their morphological descriptions and body size (stage f-4, f-2, f-1 and final stage in the former and stage IV to VIII in the later) agreed well with those obtained in the present study. Thus, the *C. cultrifer* phyllosomas previously described in Japanese waters may actually be *C. virgosus*. The *Scyllarus bicuspidatus* phyllosoma from Mariana waters (Sekiguchi 1990, fig. 2) and the Indian Ocean (Phillips et al. 1981, fig. 4) also shows great morphological agreement with *C. virgosus*, with this similarity was already noted by Inoue et al. (2000). True *S. bicuspidatus* (*Crearctus bicuspidatus*) phyllosomas reported by Inoue and Sekiguchi (2006) have round cephalic shield, V-shaped antennae, short

posterolateral spine of the telson, and much smaller bodies than *C. virgosus*. *Scyllarus* sp. A from the South China Sea (Johnson 1971a, figs. 47−53); *Scyllarus* sp.? from Hawaii (Johnson 1971b, fig. 34); *Scyllarus* sp. from Hawaii (Johnson 1977, fig. 1); *Scyllarus* sp. 1, 9, and 13 from off the coast of Japan (Johnson 1979, figs. 5−14); and *Scyllarus* sp. Z from New Zealand (Webber and Booth 2001, figs. 6−10) may all be *C. virgosus*, as all of these larvae share half H-shaped antenna, sub-pentagon shaped cephalic shield, and conspicuously long posterolateral spines of telson. However, this inference is still premature because *C. cultrifer* phyllosomas have not yet been fully catalogued and described in those areas. There have been several reports describing *C. cultrifer* (*Scyllarus cultrifer*) phyllosomas identified in the plankton samples collected in the Indian Ocean and off the coast of South Africa (Berry 1974, fig. 49; Prasad et al. 1975, fig. 9; Tampi and George 1975, figs. 29−33). However, all of these larvae had a significantly narrower cephalic shield than the *Chelarctus* species examined in this study.
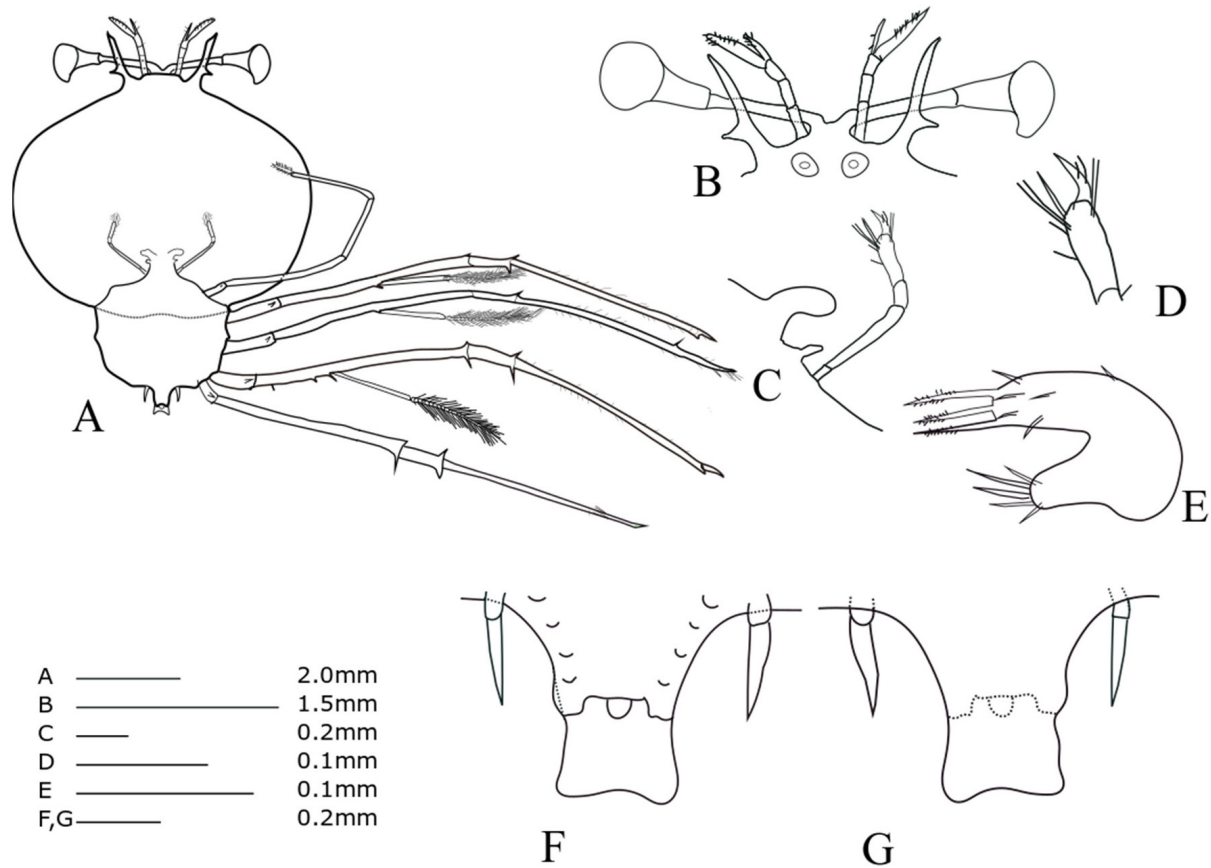
Adults of *C. aureus* have been described in

A	———	2.0mm
B	————————	1.5mm
C	—	0.2mm
D	——	0.1mm
E	—————	0.1mm
F,G	———	0.2mm

Fig. 11. Phyllosoma larva of *Chelarctus crosnieri* sub sp., stage V. A, ventral view; B, ventral view of eye, antennule and antenna; C, 2nd maxillule, 1st and 2nd maxilliped; D, distal end of 2nd maxilliped; E, ventral view of 1st maxillule; F, ventral view of 5th pereiopod and abdomen; G, dorsal view of 5th pereiopod and abdomen.

the Philippines, Indonesia, Fiji Islands (Holthuis 2002). More recently, Yang et al. (2014) reported that the distribution of this species extends to Taiwan, corresponding to our collection location of *C. aureus* phyllosomas (SHU09-3 to SHU09-5) around the Ryukyu Archipelago. A *Scyllarus* sp. d phyllosoma (designated as stage VI) collected off the north coast of Taiwan (Inoue et al. 2001, fig. 5) showed a great affinity for *C. aureus* in the present study as it had a wide sub-rectangular-shaped cephalic shield, antennule exceeding antenna, and posterolateral spines of telsons similar in length to the uropod. *Chelarctus aureus* is not known from Japanese waters (Sekiguchi and Inoue 2002), so the phyllosoma larvae may not be settled in the mainland of Japan.

One phyllosoma specimen (KH16-4-1) collected in the central South Pacific was closely allied with *C. crosnieri* in the *COI* tree and designated as *C. crosnieri* sub sp. 1. Holthuis (2002, figs. 30, 31, 68) described the new species *C. crosnieri* based on the holotype collected in Tonga, and Yang and Chan (2012) collected *C. crosnieri* in Vanuatu. Although the collection location of KH16-4-1 is geographically distant from Tonga (c.a.

2500 km away) and Vanuatu (c.a. 4700 km away), the genetic divergence (6.9% in *COI*) between KH16-4-1 and *C. crosnieri* (JX486086 by Yang and Chan 2012) is too large to consider them as two extremes of the same species. Therefore, it is likely that there is a distinct population of undescribed species close to *C. crosnieri* or a subspecies in the tropical to subtropical areas of the central South Pacific. KH16-4-1 (stage V) was the smallest of the phyllosomas examined in this study and had a sub-pentagonal cephalic shield and antennule well exceeding the antenna, which did not match with any phyllosoma larvae reported to date.

Since ?*Chelarctus* sp-1 was nested with several other *Chelarctus* species in the phylogenetic tree (Figs. 2, 3), we determined that this species belongs to the genus *Chelarctus*. A total of five species and subspecies of this genus have been described in the Indo-western Pacific (Holthuis 2002; Yang and Chan 2012; Yang et al. 2012 2014), and *COI* and 16S sequence data for all these species have been reported (Yang and Chan 2012; Yang et al. 2012 2014). Distinct differences in the *COI* and 16S sequences between the ?*Chelarctus* sp-1 and those other *Chelarctus* species, as well as
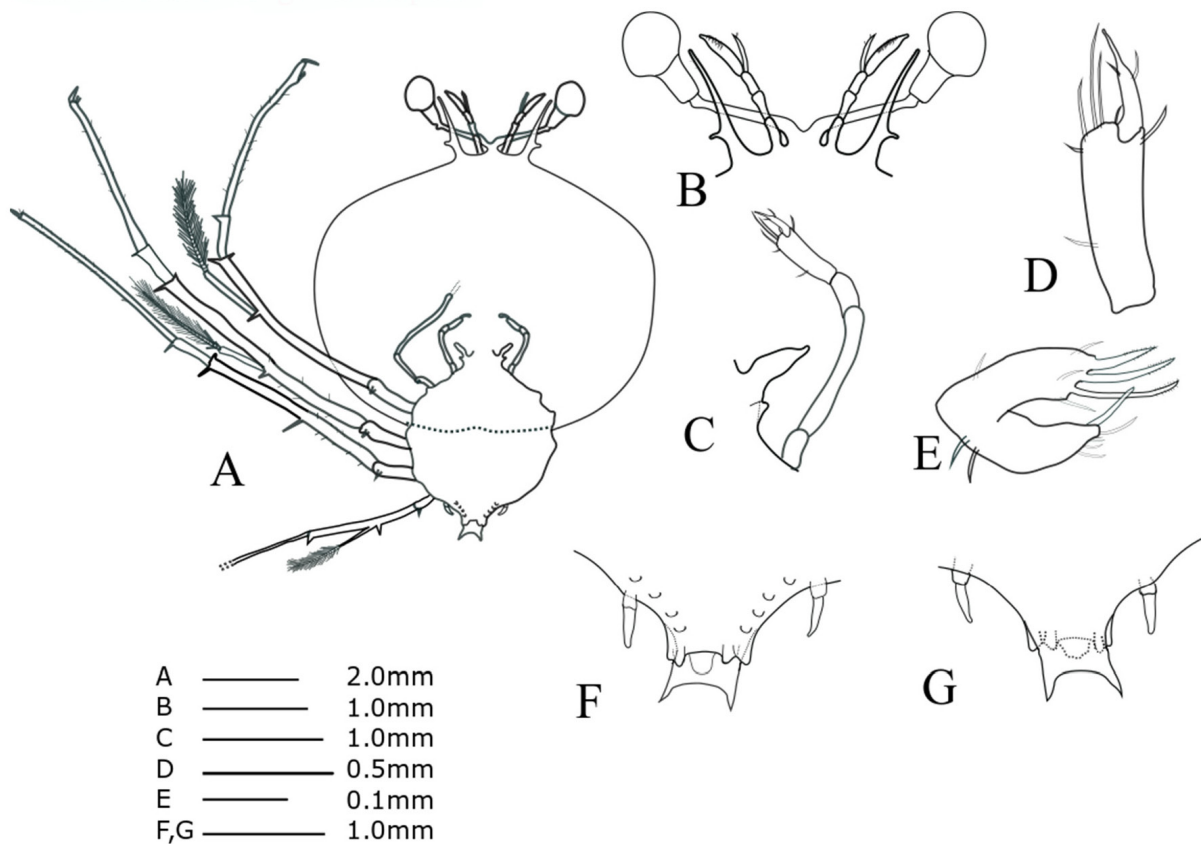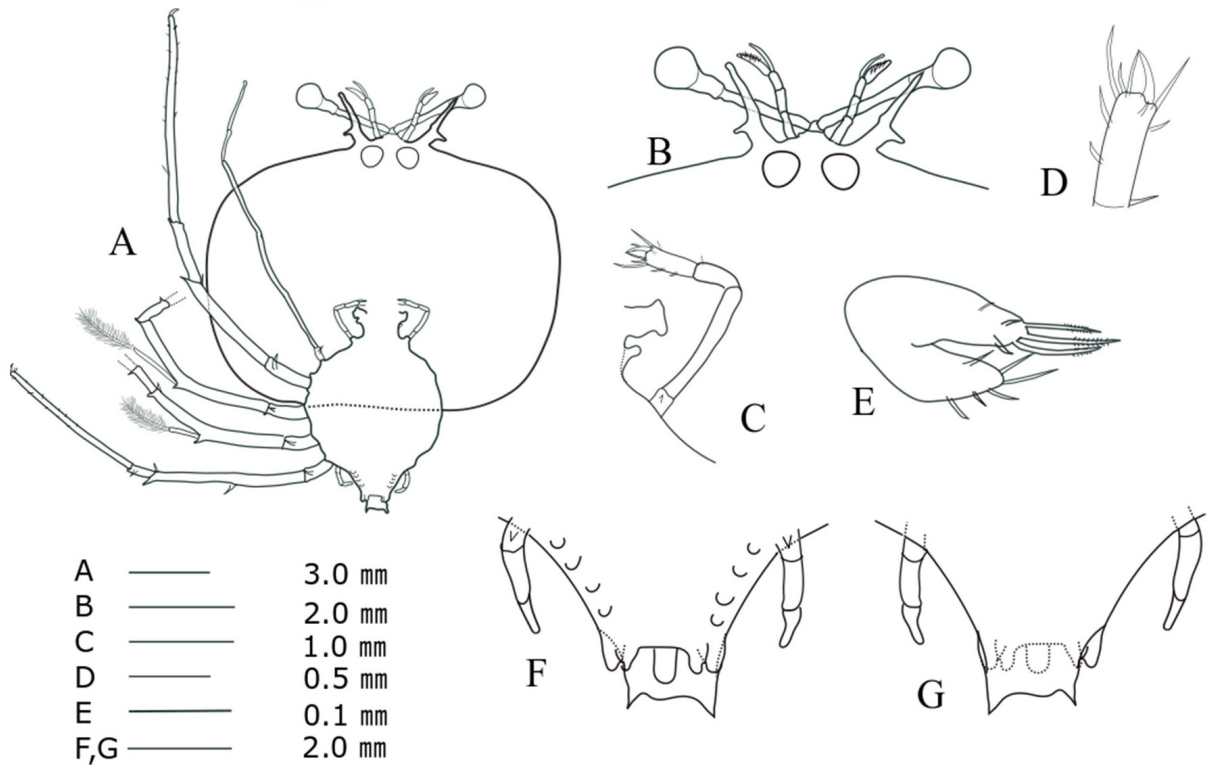

Fig. 12. Phyllosoma larva of ?*Chelarctus* sp-1, stage V. A, ventral view; B, ventral view of eye, antennule and antenna; C, 2nd maxillule, 1st and 2nd maxilliped; D, distal end of 2nd maxilliped; E, ventral view of 1st maxillule; F, ventral view of 5th pereiopod and abdomen; G, dorsal view of 5th pereiopod and abdomen.
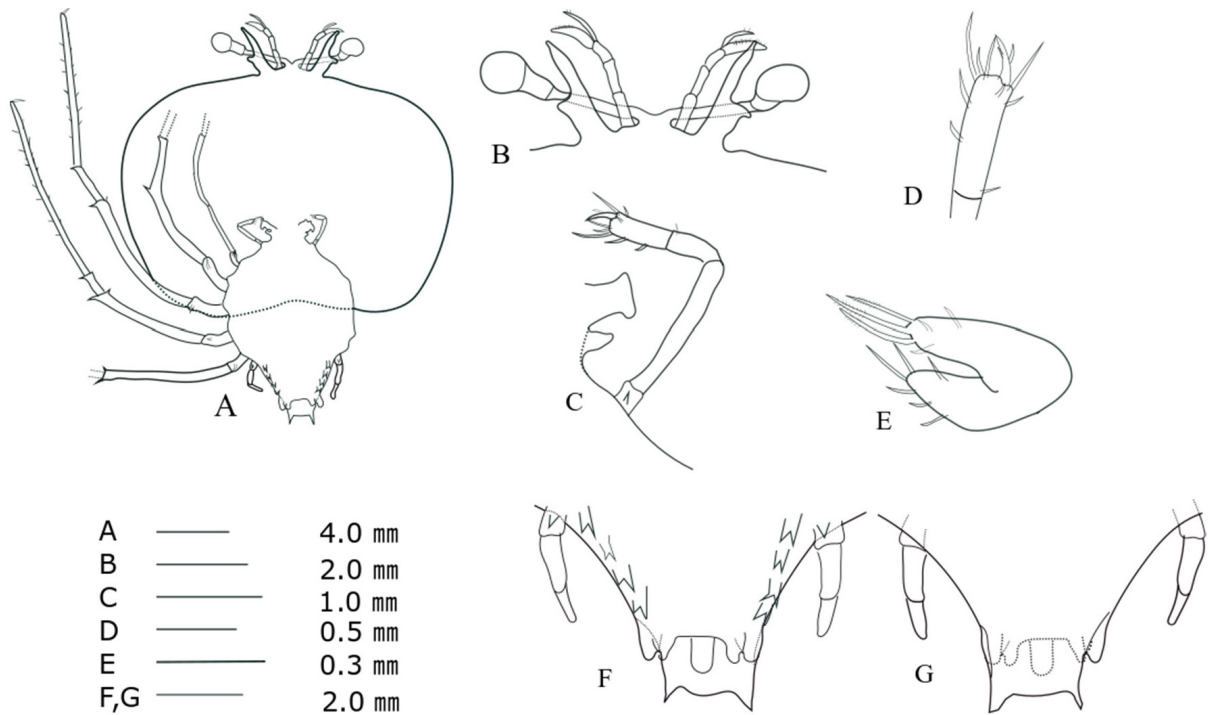
Fig. 13. Phyllosoma larva of ?*Chelarctus* sp-1, stage VI. A, ventral view; B, ventral view of eye, antennule and antenna; C, 2nd maxillule, 1st and 2nd maxilliped; D, distal end of 2nd maxilliped; E, ventral view of 1st maxillule; F, ventral view of 5th pereiopod and abdomen; G, dorsal view of 5th pereiopod and abdomen.

Fig. 14. Phyllosoma larva of ?*Chelarctus* sp-1, stage VII. A, ventral view; B, ventral view of eye, antennule and antenna; C, 2nd maxillule, 1st and 2nd maxilliped; D, distal end of 2nd maxilliped; E ventral view of 1st maxillule; F, ventral view of 5th pereiopod and abdomen; G, dorsal view of 5th pereiopod and abdomen.

the morphological uniqueness of ?*Chelarctus* sp-1 phyllosomas indicate that ?*Chelarctus* sp-1 may be a unique, previously undescribed species. Nevertheless, the genus to which this species belongs is still uncertain, and we need to obtain the adult specimen to resolve this issue.

Previous studies have suggested that the shape of the antenna, relative arrangement of the tips of the antennule and antenna, relative arrangement of the posterior margins of the uropods and telson, and length
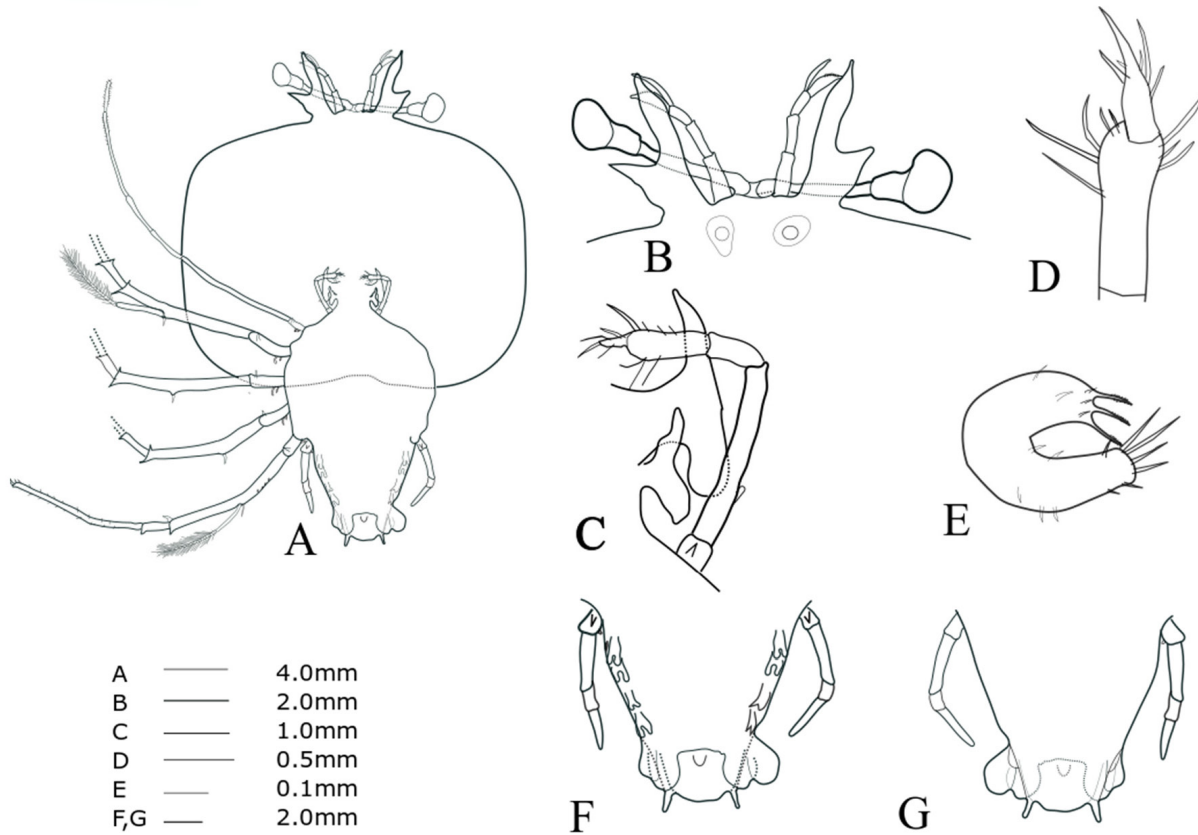

Fig. 15. Phyllosoma larva of ?*Chelarctus* sp-1, stage VIII. A, ventral view; B, ventral view of eye, antennule and antenna; C, 2nd maxillule, 1st and 2nd maxilliped; D, distal end of 2nd maxilliped; E ventral view of 1st maxillule; F, ventral view of 5th pereiopod and abdomen; G, dorsal view of 5th pereiopod and abdomen.

Table 4. Morphological characteristics of mid (V) to final (VIII) stage phyllosoma larvae of four *Chelarctus* species

Stage	Specimen ID	Species	Body length (mm)	Cephalic shield shape	CW/CL	CW/TW	Antennule and antenna*	Coxal spines on 2nd maxilliped	Coxal spines on 5th pereiopod	Relative length of posterolateral spines of telson to uropod
V	KY18-1	*C. virgosus*	8.45	sub-pentagon	1.17	1.92	TX	absent	absent	longer
	SHU09-6	?*Chelarctus* sp-1	9.64	sub-pentagon	1.26	1.82	S	absent	absent	almost the same
	KH16-4-1	*C. crosnieri* sub sp. 1	6.60	sub-pentagon	1.19	1.84	NX	absent	absent	almost the same
VI	KY15-1	*C. virgosus*	12.66	sub-pentagon	1.22	2.12	S	absent	absent	longer
	SHU09-3	*C. aureus*	9.40	sub-rectangular	1.30	2.10	NX	tiny	tiny	almost the same
	SHU09-7	?*Chelarctus* sp-1	13.71	sub-rectangular	1.30	2.30	S	tiny	distinct	almost the same
VII	SHU09-1	*C. virgosus*	18.61	sub-pentagon	1.18	1.84	S	absent	tiny	longer
	SHU09-4	*C. aureus*	17.78	sub-rectangular	1.34	2.54	NX	tiny	distinct	shorter
	SHU09-8	?*Chelarctus* sp-1	17.41	sub-rectangular.	1.47	2.51	S	distinct	distinct	shorter
VIII	SHU09-2	*C. virgosus*	20.7	sub-pentagon	1.25	2.06	TX	absent	tiny	almost the same
	SHU09-5	*C. aureus*	23.38	sub-rectangular	1.36	2.23	NX	distinct	distinct	shorter
	SHU09-9	?*Chelarctus* sp-1	23.65	sub-rectangular	1.35	2.24	S	distinct	distinct	shorter

*antenna exceeding antennule (TX), antennule exceeding antenna (NX), almost the same (S).

of the posterolateral spines on the telson are not very important for species identification at the phyllosoma stage. However, the morphological investigations based on DNA barcoding performed in this study suggest that these characteristics may be useful for species identification. Thus, morphological investigation based on DNA barcoding could allow for the identification of morphological characteristics that are useful and not useful for identifying target species of larval samples. Simple but reliable morphological keys for species identification could facilitate the elucidation of the distribution, transportation, and recruitment processes for various phyllosoma larvae at the species level.

CONCLUSIONS

DNA barcoding analysis using mitochondrial cytochrome oxidase I (*COI*) and 16S rDNA sequences was performed to identify species of mid to final stage (V to VIII) phyllosoma larvae of the subfamily Scyllarinae collected in the Northwest and central South Pacific. Four larvae collected around the Ryukyu Archipelago and the Ogasawara Islands were identified as *Chelarctus virgosus*. Three larvae collected around the Ryukyu Archipelago were identified as *Chelarctus aureus*. DNA barcodes could not be generated for the four larvae collected around the Ryukyu Archipelago, and thus designated as ?*Chelarctus* sp-1. One larva collected in the central South Pacific was designated as *Chelarctus crosnieri* sub sp. 1. Half H-shaped antenna, wide sub-pentagon or sub-rectangular shaped cephalic shield, round posterior margin of the uropod not exceeding the central margin of the telson, and long posterolateral spines of the telson were shared among phyllosomas of these four species, and we suggest that a combination of these characteristics can be diagnostic for discriminating these *Chelarctus* larvae from the other genera of the subfamily Scyllarinae. Species of these four phyllosoma could be distinguished using the shape of the cephalic shield, relative length of the antenna and antennule, and length of the posterolateral spines of the telson.

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