

First Description of Phyllosoma Larva in the Genus *Projasus* (Crustacea: Decapoda: Palinuridae)

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A giant phyllosoma larva, assigned to the final stage, was collected from off Peru. This specimen was determined to be *Projasus bahamondei* George, 1976 by DNA barcoding, and morphological description was given for the first time in the genus *Projasus* George and Grindley, 1964. The present phyllosoma was characteristic of having very wide cephalic shield (about 2.3 times of thorax width), median rostrum, eminent setose exopods on the second and third maxillipeds, and five stout spines on basal endite of maxillule. *Projasus* phyllosoma has morphological characteristics of both ‘Silentes’ and ‘Stridentes’ groups in the family Palinuridae.

Key Words: larval morphology, plankton, *Projasus bahamondei*, giant phyllosoma.

Introduction

The palinurid genus *Projasus* George and Grindley, 1964 was established for deep-sea spiny lobsters, which includes only two species (George and Grindley 1964). They are deep-water dwellers found in south American, south African, and southern Pacific regions (Holthuis 1991). In the previous life history studies on *Projasus*, only the postlarval puerulus stage has been described from the Chilean and Australian waters (Berry 1974; Webber and Booth 1988; Báez and Ruiz 2000; Arana 2014). No information about the larval phyllosoma stage of *Projasus* has been presented to date.

We obtained a giant phyllosoma specimen from off Peru. The phyllosoma was similar to that of *Jasus* Paker, 1883 or *Sagmariasus* Holthuis, 1991 in general appearance, but it was different in having an exopod on the maxilliped 3, eminently wide cephalic shield and very large body size. DNA barcoding revealed this giant phyllosoma to be *Projasus bahamondei* George, 1976. Here, we describe *P. bahamondei* phyllosoma with certain parentage for the first time in the genus *Projasus*.

Materials and Methods

A large phyllosoma (Fig. 1), was obtained during the R/V Kaiyou-Maru cruise in a project on the evaluation of status of international fishery resources by Fisheries Agency of Japan. This phyllosoma was caught using a “larva capture” net (LC-100²-R3, Nichimo Co. Ltd.; 100m² opening, 36m long, 6mm mesh, cod-end bucket) on 31 January 2012 at 17°59’S, 84°00’W. The LC net was towed in nighttime (21:57–22:25 hours) at layer shallower than 20m. Water

temperature was 23.1°C at the surface and 20.9°C at a depth of 20m. This larva was fixed in 80% ethanol on board and transferred to the laboratory. A small tissue piece dissected from a broken pereopod was washed with tap water well and homogenized, from which crude DNA was obtained using a DNA extraction kit (Qiagen Inc.). PCR primers to amplify a 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 U of EX Taq HS polymerase (TaKaRa). The reaction mixture was preheated at 94°C for 5 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 7 min. The PCR product was treated with ExoSap-IT (Amersham Biosciences) to remove primers and used as template DNA for cycle sequencing reactions using the 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 sequence determined was subjected to BLAST homology searches (Altschul et al. 1990) in GenBank to find the identical or similar sequences. Sequence alignment, calculation of the Kimura’s two parameter distance (K2P) among sequences, and construction of a phylogenetic tree were performed by MEGA 6 (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) were measured according to Higa and Shokita (2004) and Palero et al. (2008). In addition, Pleon width (PW) was measured at the widest position. The voucher specimen was deposited at the

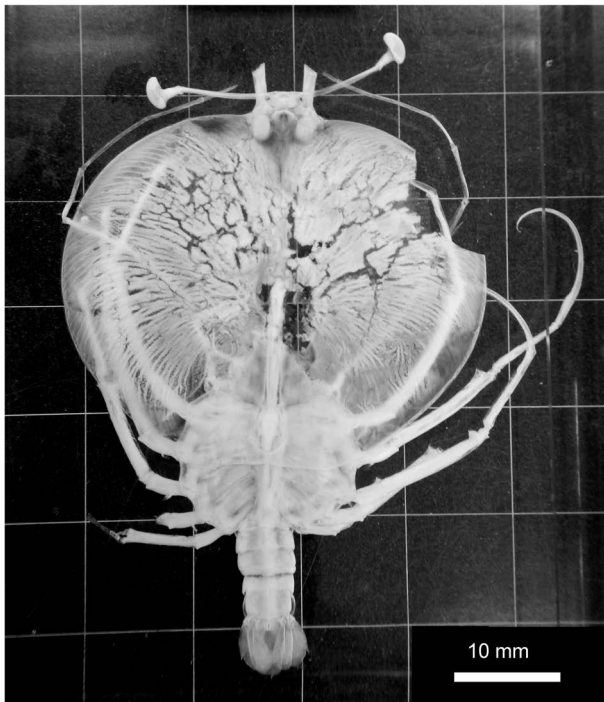


Fig. 1. Photograph of a giant phyllosoma of *Projasus bahamondi* George, 1976, (ICHUM-6253) in dorsal view collected from off Peru.

Hokkaido University Museum under the accession number of ICHUM-6253.

Results

DNA analysis. Nucleotide sequence of the present specimen determined was 553 bp and available in database under the GenBank accession number of LC619701. BLAST top hit species for this sequence was 16S rDNA of *Projasus bahamondi* (AF502948 and FJ174897 in GenBank), and we arbitrarily selected 16S rDNA sequences of 'Silentes' spiny lobster species (see George and Main 1967) of the genera *Jasus*, *Sagmariasus* and *Projasus* as well as the Japanese spiny lobster [*Panulirus japonicus* (von Sieboldt, 1824)] as an outgroup from database for constructing phylogenetic tree. The phylogenetic tree (Fig. 2) unambiguously determined this phyllosoma to be *Projasus bahamondi*, which was also supported by the small K2P distances between the larval and the adult reference sequences (0.232–0.368%).

Description of final phyllosoma stage of *Projasus bahamondi*. Dimensions: TL=54.3 mm; CL=34.9 mm; CW=39.2 mm; TW=16.8 mm; PW=4.6 mm.

Cephalothorax (Figs 1, 3A, B): cephalon circular, wider than long (CL/CW ratio=0.89), much wider than thorax (CW/TW ratio=2.33). Median rounded rostrum shortly

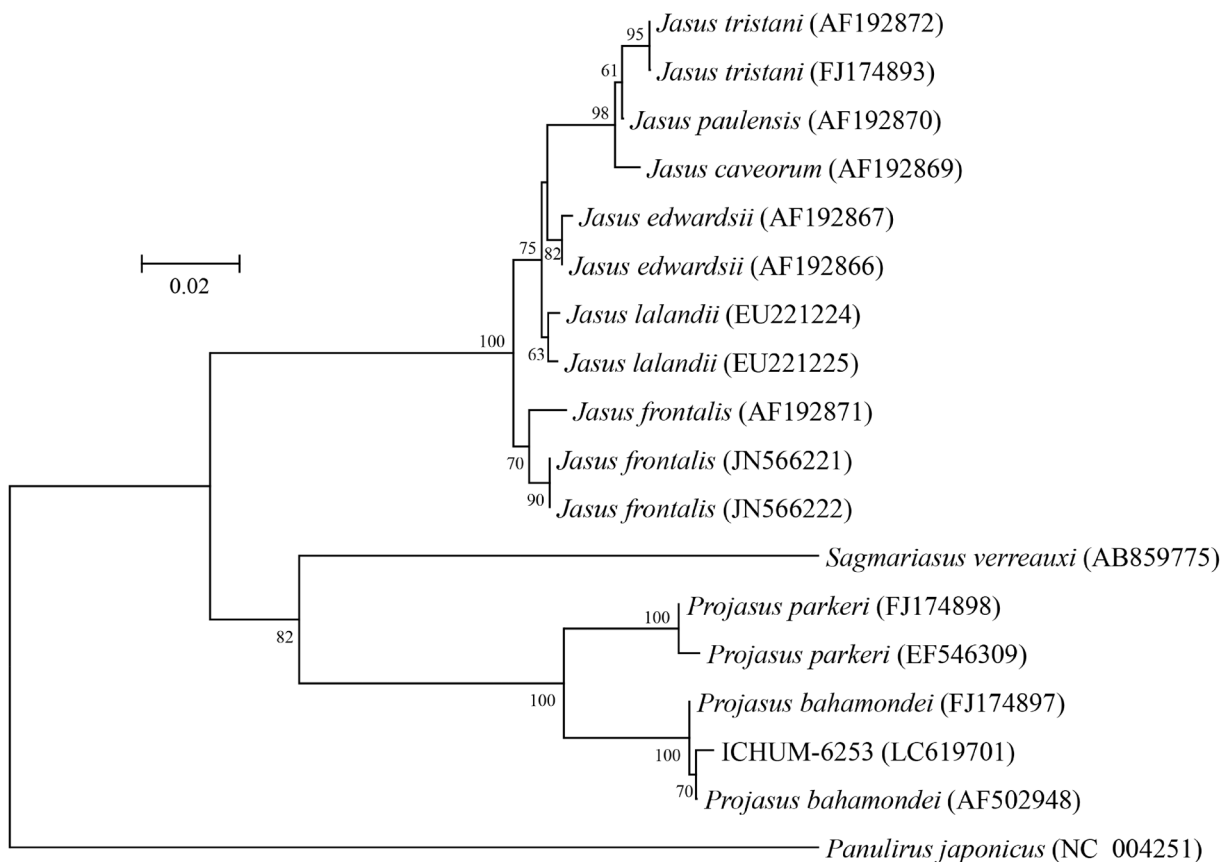


Fig. 2. Neighbor-joining phylogenetic tree drawn based on Kimura-2-parameter distances of partial 16S sequences among 9 'Silentes' spiny lobster species and the Japanese spiny lobster (*Panulirus japonicus*) as an outgroup. Accession numbers are shown in the parenthesis. Bootstrap values of >50% (out of 1000 replicates) are shown at the nodes. The same tree topology was obtained by maximum likelihood and maximum parsimony methods.

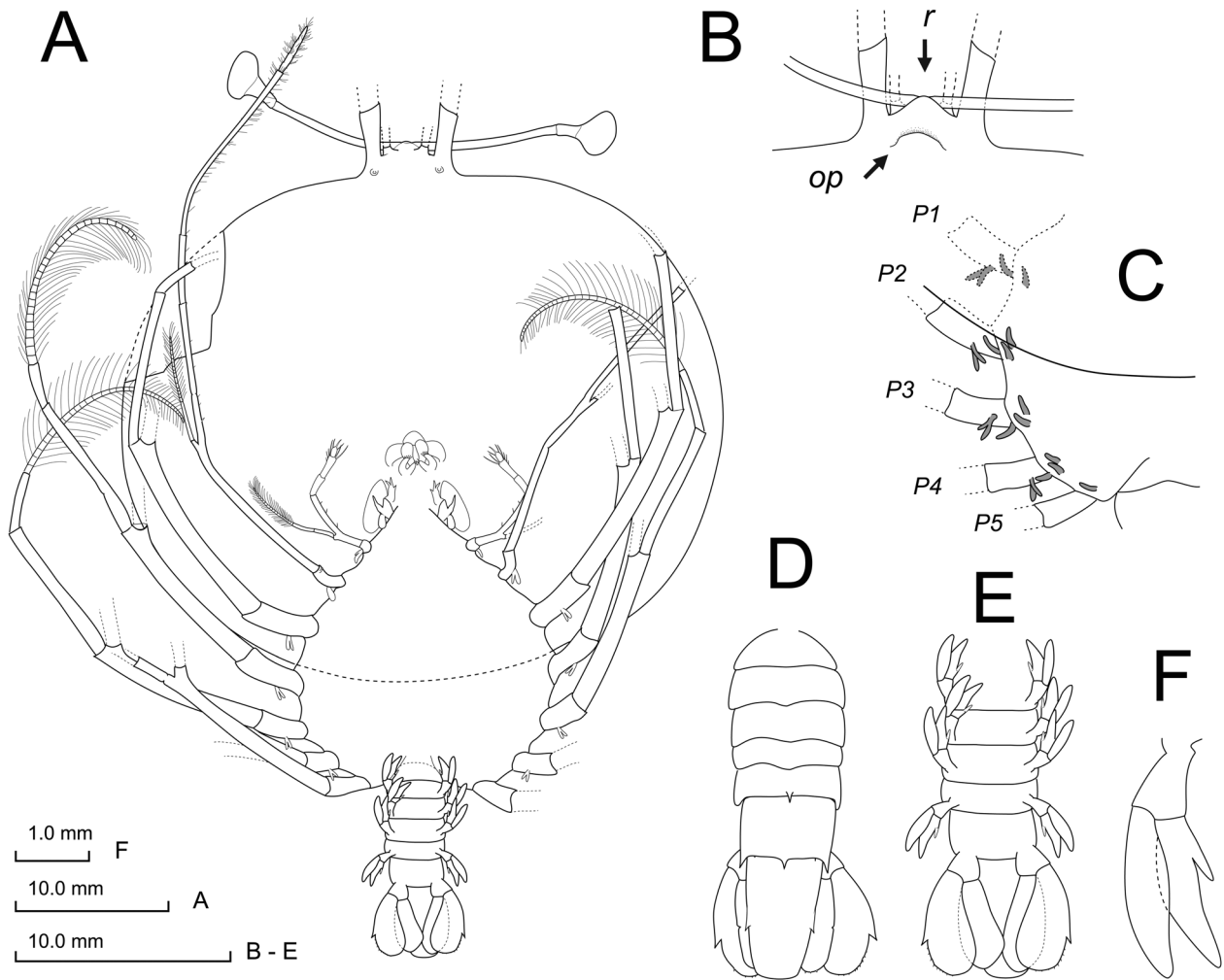


Fig. 3. *Projasus bahamondei* George, 1976, final stage (gilled) phyllosoma. A, Whole body in ventral view; B, frontal region of cephalic shield in dorsal view [upper and lower arrows indicating an overhang plate (op) and a rostrum (r), respectively]; C, gill buds on pereopods (P1–5: pereopods 1–5); D, pleon in dorsal view; E, telson in ventral view; F, right pleopod 4.

protruded (Fig. 3B: r). Small overhang plate behind the base of eyestalk (Fig. 3B: op). Eyes stalked, 9.7 mm in stalk length.

Antennule: missing in the present specimen.

Antenna: missing in the present specimen.

Mandibles (Fig. 4A): slightly flattened dorso-ventrally, asymmetrical in dentition. Incisor process, medial gnathal edge with a series of teeth. Molar process crowned by many denticles and minute papilla. Labrum, paragnath (Fig. 4A') well-developed, cover distal inner half portion of mandibles.

Maxillule (Fig. 4B): rudimentary endopod projection with two, long and minute, simple setae, near anterior base. Basal endite with 5 stout spines (Fig. 4B: ss) and 10 subterminal setae. Coxal endite with 11 moderate lengths setae.

Maxilla (Fig. 4C): endopod with 3 distal setae and a minute subterminal seta. Basal and coxal endite with 6 and 3 setae, respectively. Scaphognathite with 126 marginal plumose setae.

Maxilliped 1 (Fig. 4D): exopod incipiently 2-segmented process. Epipod rudimentary lobe.

Maxilliped 2 (Fig. 4E): biramous, with gill rudiments. Endopod 3-segmented, with no setae on proximal, 11 distal

setae on second, and a long stout spine and 13 setae on distal segment. Exopod with 17 annulations, each annulation with a pair of natatory plumose setae.

Maxilliped 3 (Fig. 4F): biramous, with gill rudiments. Endopod 3-segmented, numerous setae on distal segment. Exopod with 17 annulations, each annulation with a pair of natatory plumose setae.

Pereopods 1–5 (Fig. 3A, E): biramous, with gill rudiments except for pereopod 5 (Fig. 4G), but distal part of endopod, merus to dactylus, missing. No subexopodal spine was confirmed in pereopods 1–3 and 5, while pereopod 4 missing (Fig. 3A). Gill rudiments on thorax pleura (Fig. 3C).

Pleon (Fig. 3D–F): much narrower than thorax ($PW/TW=0.27$), fully segmented with four pairs of biramous pleopods (Fig. 3E, F) on somites 2–5. Pleonites 5 and 6 with strong dorsal median spine (Fig. 3E). Uropods biramous, posterior margins of endopod and exopod not outreaching posterior end of telson (Fig. 3D, E). Telson rectangular, slightly narrowing posteriorly, with pair of lateral spines (Fig. 3D).

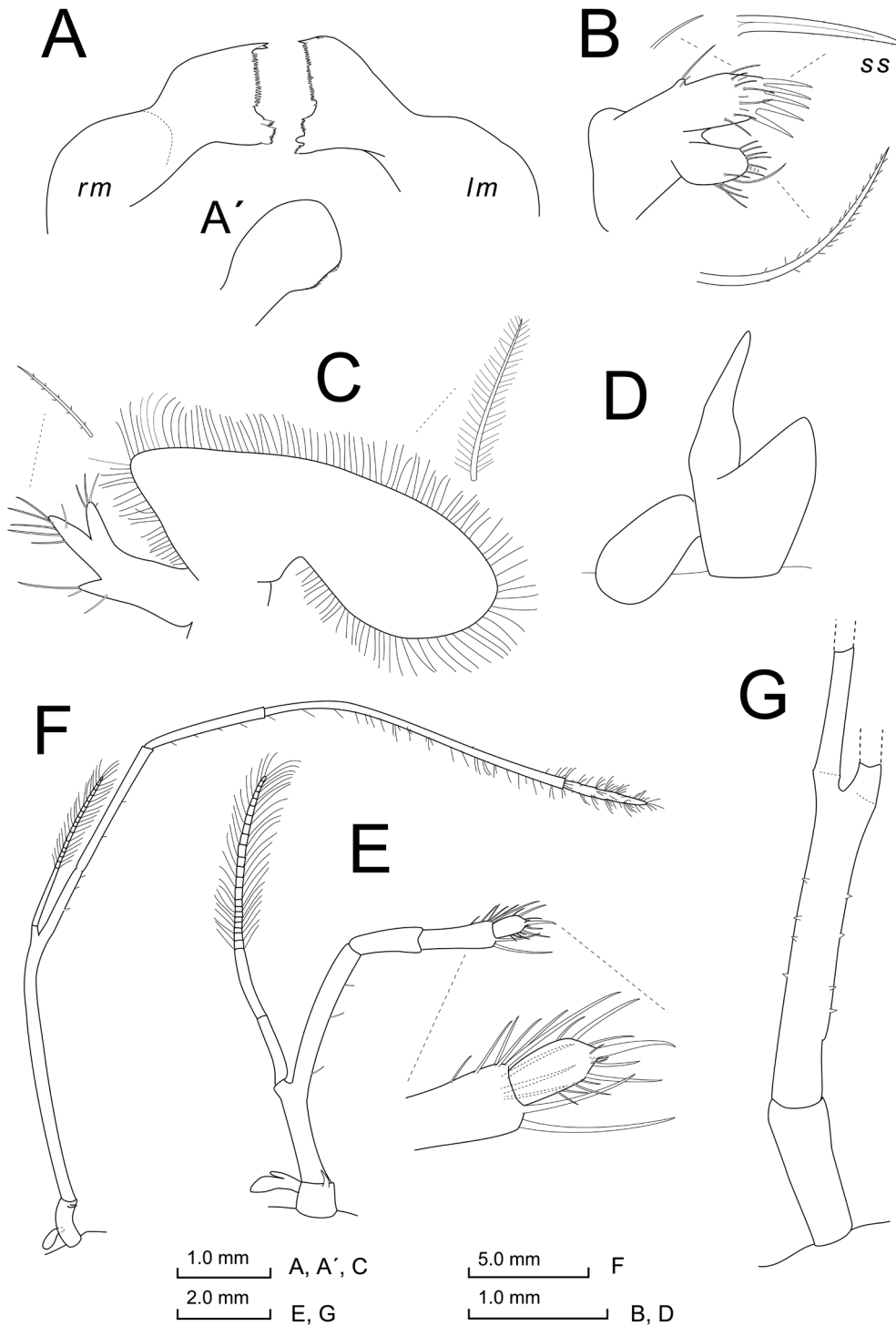


Fig. 4. *Projasus bahamondei* George, 1976, final stage (gilled) phyllosoma. A, Mandibles in ventral view, (lm: left mandible, rm: right mandible); A', right paragnath; B, maxillule (ss: stout spine); C, maxilla; D, maxilliped 1; E, maxilliped 2; F, maxilliped 3; G, left pereopod 5 in dorsal view, enlarged.

Discussion

Although the antennae and pereopodal endopods were damaged during tow-net collection, the present specimen still carried some valuable morphological characters for larval identification. Table 1 compares main morphological characteristics of the final or latest stage phyllosomas pre-

viously reported in the family Palinuridae (Bouvier 1914; Gurney 1936; Sims 1966; Michel 1970; Johnson and Robertson 1970; Berry 1974; McWilliam and Phillips 1987; Sekiguchi et al. 1996; Goldstein et al. 2008; Palero et al. 2010; Konishi et al. 2021). The cephalic shield of the present specimen is circular, and CW/TW ratio (2.33) is the largest in the family Palinuridae reported to date. Possession of a median rounded rostrum is also unique, and this character can be

Table 1. Main larval characteristics in the final or latest stages of phyllosoma in 10 palinurid genera.*

Genus	Cephalic shield				CW/TW ratio**	Maxillae	Mxp 2	Mxp 3	Pereiopod	Pleon	References
	outline	rostrum	overlap on thorax	overlap on Mxp 3 coxa		stout spines on BE	exopod	exopod	chelate	DMS on pleonite	
'Silentes' group											
<i>Projasus</i>	circular	+	No	Yes	2.33	5	+	+	?	1	This study
<i>Sagmariasus</i>	circular	–	No	Yes	1.93	4	r	–	No	–	1
<i>Jasus</i>	circular	–	No	Yes	2.25	3	r	–	No	–	1
<i>Palinurellus</i>	rectangular	+	Yes	Yes	2.25	3	+	+	No	–	2, 3

'Stridentes' group											
<i>Palinurus</i>	oval	–	No	Yes	1.71	3	+	+	No	–	4, 5, 6
<i>Panulirus</i>	oval	–	No	Yes	0.83	3	+	+	No	–	7
<i>Justitia</i>	oval	–	No	No	1.06	2	+	+	Yes (P1,P3)	–	8, 9
<i>Nupalirus</i>	oval	–	No	Yes	0.91	2	+	+	Yes (P1,P3,P4)	–	8, 9
<i>Palinustus</i>	subrectangular	–	No	Yes	1.76	2	+	+	Yes (P1,P3,P4)	2	10
<i>Puerulus</i>	circular	–	Yes	Yes	1.98	2	+	+	No	1	11

*No late stage phyllosoma with evident identification has been described in the genera *Palibythus* and *Linuparus* to date. **Latest stage. BE: basal endite; CW: cephalic shield width; DMS: dorsal median spine; Mxp: maxilliped; P: pereiopod; r: rudimentary bud; TW: thoax width; "–" means absent; "+" means present. References: ¹McWilliam and Phillips (1987); ²Sims (1966); ³Michel (1970); ⁴Gurney (1936); ⁵Berry (1974); ⁶Bouvier (1914); ⁷Goldstein et al. (2008); ⁸Johnson and Robertson (1970); ⁹Konishi et al. (2021); ¹⁰Palero et al. (2010); ¹¹Sekiguchi et al. (1996).

found only in phyllosomas of the genus *Palinurellus* von Martens, 1878 which was previously classified as a separate family 'Synaxidae'. Dorsal median spines on the pleonites are observed in the sub-final and final stage phyllosomas of limited groups of Achelata such as *Palinustus* Milne-Edwards, 1880, *Scyllarides* Gill, 1898, and *Ibacus* Leach, 1815 (Ritz and Thomas 1973; Phillips et al. 1981; Palero et al. 2010, 2016). The family Palinuridae is morphologically subdivided into two groups 'Silentes' and 'Stridentes' (George and Main 1967) in adult phase. These two groups have been supported by comprehensive molecular phylogenetic analyses (Palero et al. 2009; Tsang et al. 2009), in which *Jasus/Sagmariasus/Projasus* form a well-supported clade as 'Silentes'. Since the present *Projasus* phyllosoma has eminent setose exopods in maxillipeds 2 and 3, which is common in 'Stridentes' but different from other members of 'Silentes' (Gurney 1936; Báez 1973; Lesser 1978; Miller 1985; Kittaka et al. 1997), the setose exopods cannot be a diagnostic characteristic for separating phyllosoma larvae of 'Stridentes' and 'Silentes' species. Discrepancy between adults and larvae based on morphology, such as in this case, should be resolved by further accumulation of morphological and molecular data in future.

Báez (1973) described a late stage phyllosoma specimen (TL=19.2 mm) of unknown parentage as 'phyllosoma X' from the Chilean coast. Since this phyllosoma possessed exopods on the maxillipeds 2 and 3, Báez (1973) considered this phyllosoma not to be of *Jasus* but suggested that the phyllosoma could be a larva of *Projasus* mainly based on distribution of adult palinurid species, and his postulation was followed by Sekiguchi (1989). Báez (1973: 127) also commented about that the final stage phyllosoma '*Palinurus* sp.' (TL=50 mm) collected in the south Atlantic described by Gurney (1936) was possibly belonging to *Projasus*. On the other hand, Webber and Booth (1988) suggested that the 'phyllosoma X' was too small to be a larva of *P. bahamondei*. Báez and Ruiz (2000: 23) insisted their belief that 'phyllosoma X' belonged to *Projasus*, since *P. bahamondei*

and *Jasus frontalis* (Milne-Edwards, 1837) were only 'Silentes' palinurid lobsters occurring in the southeastern Pacific. However, exopods on the maxillipeds 2 and 3 of 'phyllosoma X' are incipient with very few setae. Such incipient or bud-like exopods without setae on the maxillipeds have been observed in the late stage phyllosomas of *Jasus* (Báez 1973; Lesser 1978; Miller 1985; McWilliam and Phillips 1987; Nishida et al. 1990; Mujica et al., 2010), whereas exopods on the maxillipeds 2 and 3 of the present specimen and Gurney's '*Palinurus* sp.' are eminent and equipped with bunch of setae (Fig. 2A; Gurney 1936: fig. 15). Furthermore, a new species of the genus *Jasus* has been discovered in the southeastern Pacific (Webber and Booth 1995). Pereiopods of *Jasus* phyllosoma are usually equipped with subexopodal spine (Báez 1973; Lesser 1978; Miller 1985; McWilliam and Phillips 1987), a feature shared with 'phyllosoma X' (see Báez 1973). In contrast, *P. bahamondei* phyllosoma had no subexopodal spine on the pereiopods (Fig. 3A), a feature shared with *Sagmariasus* phyllosoma (McWilliam and Phillips 1987; Kittaka et al. 1997; Pollack and Webber 1998). Cephalic shield of Gurney's '*Palinurus* sp.' is wider than thorax (CW/TW=1.5, measured from his figure) but not comparable with that of the present specimen (CW/TW=2.33). Thus, neither Báez's 'phyllosoma X' nor Gurney's '*Palinurus* sp.' are of *P. bahamondei*.

The largest phyllosoma collection in the family Palinuridae was the final stage of *Palinustus* sp. recorded by Palero et al. (2010), measuring 53.7 mm in total length. The present specimen of *Projasus* phyllosoma measuring 54.3 mm in total length exceeds the previous records and now become the largest specimen among the palinurid phyllosomas. Báez and Ruiz (2000) noted that the pueruli of *Projasus* are larger than those described forms of other palinurid species, and this fact implicates the correlation between the large sized phyllosoma and puerulus stages in *P. bahamondei*. Since the pueruli of *Projasus parkeri* are reported to be larger (TL=54–61.4 mm, CL=21.1–26.8 mm) (Berry 1974; Webber and Booth 1988) than those of *P. bahamondei* (TL=

48.9–50.3 mm, CL=19.9–20.3 mm) (Báez and Ruiz 2000), *P. bahamondei* might surrender its title as the world's largest palinurid phyllosoma to *P. parkeri* when it is found in future.

Acknowledgments

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