A late-stage phyllosoma larva of the spiny lobster *Panulirus echinatus* Smith, 1869 (Crustacea: Palinuridae) identified by DNA analysis

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A late-stage phyllosoma larva of the genus *Panulirus* was caught in the central Atlantic in October 2000. Nucleotide sequence analysis of mitochondrial 16S rDNA identified this larva as *Panulirus echinatus*, which has been undescribed to date. Morphological examination indicated that this eighth stage larva belonged to phyllosoma species group 2, distinguishable from the other Atlantic species of the genus but closely related to an Indo-Pacific species, *P. penicillatus*, which is in accordance with the results of a molecular phylogenetic analysis.

INTRODUCTION

Five spiny lobster species of the genus *Panulirus* White, 1847 are distributed in the Atlantic Ocean (Holthuis, 1991): *Panulirus argus*, *P. echinatus*, *P. guttatus*, *P. laevicauda* and *P. regius*. Morphological descriptions of the pelagic larval phase (phyllosoma) have been published for all Atlantic species except for *P. echinatus* (Lebour, 1950; Lewis, 1951; Crosnier, 1971; Baisre and Ruiz de Quevedo, 1982; Baisre and Alfonso, 1994). Identification of planktonic phyllosoma specimens is very difficult because of the morphological similarity between species and variation within species. Usually, several lobster species share similar distribution ranges (Holthuis, 1991), and their long-lived teleplanic larvae (Chittleborough and Thomas, 1969) may be transported to distant waters. Recent advances in molecular analysis and database enhancement, however, have significantly improved species identification of lobster phyllosoma larvae. Silberman and Walsh (Silberman and Walsh, 1992) successfully discriminated between phyllosoma larvae of two northwestern Atlantic *Panulirus* species (*P. argus* and *P. guttatus*) using restriction fragment length polymorphism (RFLP) analysis based on polymerase chain reaction (PCR) amplification of 28S rDNA. The phylogenetic analyses of Ptacek et al. (Ptacek et al., 2001) analysed partial nucleotide sequences of two mitochondrial DNA segments (cytochrome oxidase subunit I and 16S ribosomal DNA) of almost all lobster species in the genus *Panulirus*. Yamauchi et al. (Yamauchi et al., 2002) reported the nucleotide sequence of the entire mitochondrial DNA of the Japanese spiny lobster *P. japonicus*. More recently, Chow et al. (Chow et al., 2006a,b) applied nucleotide sequence analysis for *Panulirus* phyllosoma samples collected in the Japanese waters and successfully showed the samples to comprise eight species.

In October 2000, one late-stage phyllosoma was collected in the central Atlantic Ocean by the research cruise of RV Shoyo-Maru, Fisheries Agency of Japan. Preliminary morphological observation indicated that the larva did not belong to a larval form of any Atlantic species published to date.

In this study, we report the result of our molecular species identification and give a morphological description of this phyllosoma larva.
METHODS

A late-stage phyllosoma larva (designated Atl-2) was caught at night (22:00–23:00 hours) using a IKMT (Isaacs–Kidd Midwater Trawl) net towed stepwise at 100 and 50 m depth for 10 min each in the equatorial central south Atlantic, about 1000 km east Recife, Brazil (8°45′ S and 24°57′ W). The larva was fixed in 90% ethanol, photographed onboard (Fig. 1) and transferred to the laboratory for subsequent molecular and morphological analyses. Crude DNA was extracted from a piece of pereiopod using a DNA extraction kit (GenomicPrep Cells and Tissue DNA Isolation Kit; Amersham Bioscience). Mitochondrial 16S ribosomal DNA (16S rDNA) was amplified by PCR using universal primers (16SL and 16SH; see http://inbio.byu.edu/Faculty/kac/crandall_lab/Primers.html). Procedures for PCR amplification and nucleotide sequence analysis are according to Chow et al. (Chow et al., 2006a). Nucleotide sequence alignment and construction of neighbour-joining phylogenetic tree (NJ) are based on Kimura’s two-parameter model (K2P), and the maximum parsimony tree (MP) is constructed using MEGA version 3.1 (Kumar et al., 2004). 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 BX microscope.

RESULTS AND DISCUSSION

DNA analysis

The length of partial 16S rDNA sequence of Atl-2 determined was 472 bp; the sequence was deposited to DNA Data Bank of Japan (DDBJ) under accession number AB248090. The 16S rDNA data of Atl-2 were incorporated with those reported by Ptacek et al. (Ptacek et al., 2001). As the tree topologies obtained by NJ and MP methods were almost in complete agreement, only the NJ tree is shown in Fig. 2. The phylogenetic tree unambiguously indicated Atl-2 sequence to have greatest affinity with P. echinatus, being well separated from all other species and formed a sister group with P. penicillatus. The distance (K2P) (0.007 ± 0.004) between Atl-2 and P. echinatus sequences was considerably smaller than those (0.028–0.238) between good species of the genus Panulirus and comparable with or much smaller than those between subspecies of P. homarus homarus and P. homarus megasculpta (0.007) and those between P. longipes femoristriga and P. longipes longipes (0.078) (Ptacek et al., 2001). Therefore, we conclude that the phyllosoma specimen (Atl-2) is P. echinatus.

Description of phyllosoma stage

Dimensions of the body (Figs 1 and 3) measured just after fixation: total body length = 21.3 mm, cephalic shield length (CSL) = 16.0 mm, cephalic shield width (CSW) = 11.4 mm and thorax width (TW) = 11.6 mm. In addition, based on Johnson’s method (Johnson, 1968), the distance from the midpoint between the
coxal segments of the second maxillipeds (a) to the frontal margin of mouthparts (b), expressed as a proportion b/a, is 1.1. Cephalic shield (=cephalon) oval in outline (Fig. 3a), CSW/TW ratio, 0.98.

Antennule (Fig. 4a): Biramous, peduncle three-segmented.

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

Mandibles (Fig. 4c): Slightly flattened dorsoventrally, 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 (labium) well developed covers distal inner half of mandible. Upper inner margin of paragnath with a tuft of thin setae and spinules on sub-distal edge (Fig. 4d').

Maxillule (Fig. 4d): A short seta (arrow), presumptive endopod site, near anterior base. Basal endite with three stout spines and three subterminal setae while coxal endite with two stout spines and five setae.

Maxilla (Fig. 4e): Basis with two thin anterior setae. Scaphognathite with 13 marginal thin setae.

Maxilliped 1 (Fig. 4f): Small uniramous bud.

Maxilliped 2 (Fig. 4g): Exopod rudiment visible as a bud on left side, while very small on right side.

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

Pereiopods 1–4 (Figs 3a and 4i): In each case, distal part of endopod, merus to dactylus, missing. A small papilla on ventral side of thorax, near the base of appendage (Fig. 4i). No subexopodal spine was observed on any pereiopod.

Pereiopod 5 (Fig. 3a): Two-segmented, uniramous projection.

Abdomen (Fig. 3b): Segmented, rudiments of four pairs of pleopods and uropod present as biramous buds.

**Morphological remarks**

The present phyllosoma specimen is identified to be the eighth stage based on the condition of the appendages according to the 10-stage categories of Matsuda and Yamakawa (Matsuda and Yamakawa, 2000). Morphological characteristics of *P. echinatus* and some...
related congeners are summarized in Table I. The phyllosoma of *P. echinatus* may be distinguished from those of the other species in the Atlantic Ocean by a combination of the CSW/TW ratio, the b/a value and the ornamentation of pereiopods.

Adult species groups put forward by George and Main (George and Main, 1967) and phyllosoma species groups put forward by McWilliam (McWilliam, 1995) show only moderate correlation. George and Main (George and Main, 1967) proposed that *P. echinatus* belonged to the group II of *Panulirus* species in adult morphology together with *P. guttatus* and *P. penicillatus*. McWilliam (McWilliam, 1995) hypothesized *Panulirus B* described by Gurney (Gurney, 1936) to be the larvae of *P. echinatus* and placed *P. echinatus* larvae in the phyllosoma species group 1, which includes *P. argus*, *P. cygnus*, *P. japonicus*, *P. longipes* sp. and *P. marginatus*. The present study, however, indicates that the assignment of *P. echinatus* larvae should be to phyllosoma species group 2 and that *Panulirus B* described by Gurney (Gurney, 1936) appears not to be of *P. echinatus* larvae. The particularly narrow cephalic shield (CSW/TW ratio = 0.653, calculated from the Fig. 22) of Gurney’s late-stage phyllosoma (stage IX) of *Panulirus B* cannot be explained by shrinkage or other physical disturbance. As Gurney (Gurney, 1936) suggested, his *Panulirus B* may be larva of *P. argus*.

This study shows that molecular analyses coupled with morphological investigations may significantly contribute to refine species identification in larval form of the genus *Panulirus*. On the basis of the nucleotide sequence analysis, for instance, Chow et al. (Chow et al., 2006b) compared late-stage phyllosoma larvae of *P. ornatus* and *P. versicolor* and revealed that the arrangement of subexopodal spines may not to be a diagnostic key character for separating these species.

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