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Molecular Diet Analysis of Phyllosoma Larvae of the Japanese Spiny Lobster *Panulirus japonicus* (Decapoda: Crustacea)

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Abstract

To clarify the natural diet of phyllosoma larvae of the Japanese spiny lobster *Panulirus japonicus*, the sources of 18S rDNA clones obtained from the hepatopancreas were investigated. Of a total of 1537 clones examined, 160 had different restriction profiles from the host larvae, in which 21 restriction types were observed. Nucleotide sequences of 16 of 21 restriction types were successfully determined and their assignments were investigated by homology search and phylogenetic analysis. From seven late-stage larvae collected in spring to early summer, eukaryote DNA molecules of Teleostei, Oomycetes, Mycetozoa, and Fungi were identified. Exogenous DNA from four younger phyllosoma larvae collected in late autumn could not be recovered. A previous study identified DNAs of cnidarians and urochordates in late-stage phyllosoma larvae of a closely related species collected in winter. This indicates that the phyllosoma larvae are opportunistic carnivores, whose diets correlate with the relative abundance of prey organisms in the ambient water.

Keywords: 18S rDNA — natural prey — opportunistic carnivore — *Panulirus japonicus* — phyllosoma larvae

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Introduction

Spiny and slipper lobsters (Palinuridae and Scyllaridae, respectively) are important crustacean species in commercial fisheries all over the world. Much attention has been paid to biological investigations of the bottom-living phase of the young and adult stages, while biological studies of the planktonic larval stage called phyllosoma are scarce. This is mainly due to small sample sizes of wild-caught larvae, as the phyllosoma larvae disperse extensively in the open ocean and have a long planktonic period extending from several months to more than a year (Chittleborough and Thomas 1969; Inoue 1981; Matsuda and Yamakawa 2000). Specifically, information on natural diets for larvae may greatly contribute to the development of better artificial feeds, and thereby enhance survival during seed production. Although feeding of *Artemia nauplii* supplemented with gonads of common mussel (*Mytilus edulis*) has achieved successful metamorphosis in several spiny lobster species (Yamakawa et al. 1989; Kittaka 1997; Kittaka et al. 1997; Matsuda and Yamakawa 2000), the mortality is still very high.

Laboratory experiments offering a variety of plankton species have indicated that the soft-bodied zooplanktons may be major prey items for mid to late-stage phyllosoma larvae of spiny and slipper lobsters (Mitchell 1971; Kittaka 1994). This agrees with our previous molecular diet analysis, which observed 18S rDNA molecules of cnidarians and urochordates in the larval hepatopancreas of two spiny (*Panulirus longipes bispinosus* and *P. echinatus*) lobsters and one slipper (*Scyllarus* sp.) lobster (Suzuki et al. 2006). However, the phyllosoma larvae may exploit a much wider variety of plankton prey,

as experiments using enzyme profiles and lipid signatures in both cultured and wild phyllosoma have indicated that spiny lobster larvae are capable of digesting a wide range of zooplankton prey items (Jeffs et al. 2004; Johnston et al. 2004). Because Suzuki et al. (2006) analyzed a small number of specimens collected from a limited area, their results may not reflect the full extent of phyllosoma diets.

We have continued sample collection of lobster phyllosoma larvae, and we here detail the results of the 18S rDNA analysis applied to *Panulirus japonicus* phyllosoma larvae collected from different seasons and localities.

Materials and Methods

Phyllosoma Specimens and DNA Extraction Of a total of 11 *Panulirus japonicus* phyllosoma larvae used in this study, seven late-stage larvae were collected in spring to early summer (May 2003 and June 1995) and four mid-stage larvae in late autumn (November 2004) (Table 1). All these larvae were caught around the Yaeyama Islands of the Ryukyu Archipelago. The developmental stages of these phyllosoma larvae were determined according to previous descriptions (Johnson 1971; Inoue 1981; Matsuda and Yamakawa 2000). Species identification of all larvae was performed using restriction fragment length polymorphism (RFLP) or nucleotide sequence analyses on the mitochondrial *COI* or 16S rRNA genes (Ptacek et al. 2001; Chow et al. 2006a,b).

To avoid possible contaminants from the body surface before DNA extraction, the phyllosoma specimens were thoroughly washed with sterile distilled water. This procedure was probed to effectively eliminate contaminants from the body surface (Suzuki et al. 2006), and washing water was recovered for DNA extraction as a negative control. Hepatopancreas tissues with the contents were pipetted out from a small incision made in the cephalic region of the specimens. Each set of the contents and the water was subjected to crude DNA extraction using a standard proteinase K digestion/phenol-chloroform extraction.

Polymerase Chain Reaction (PCR) Amplifications of 18S rDNA, Screening, and Sequencing The experimental procedure of the 18S rDNA analysis has been fully described in the previous study (Suzuki et al. 2006). Two sets of the universal primers were used for nested PCR to amplify the central domain of the 18S rDNA gene. The first PCR was made with 18SU467F (5′-ATCCAAGGAAGGCAGCAGGC-3′) and 18SL1310R (5′-CTCCACCAACTAAGAACG GC-3′), and a second PCR with 18SU615F (5′-C AAGTCTGGTGCCAGCAGCC-3′) and 18SL1170R (5′-GTGCCCTTCCGTCAATTCCT-3′). The labeled numbers of each primer marginally correspond to the nucleotide positions of human 18S rDNA (McCallum and Maden 1985). The second PCR products were subjected to TA cloning. Colony-direct PCR using the second primer pair was performed to select clones containing 18S rDNA fragments, and restriction profiles of these clones were assayed by digestion with endonuclease mixture (*Alu*I and *Mse*I). The digested products were electrophoresed on a 2.5% to 3.0% BioGel (BIO101, Carlsbad, CA) to select clones possessing restriction profiles distinct from those of the host larvae.

One or a few clones from each restriction type were arbitrarily selected and purified via ExoSAP-IT (Amersham, San Francisco, CA) for nucleotide sequencing. The sequences were determined with an automated DNA sequencer (ABI 310, Applied Biosystems, Foster City, CA), using the BigDye Terminator Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems) with the 18SU615F primer. Sequences determined in this study are deposited in DDBJ/EMBL/GenBank (accession numbers AB248104 to AB248116 and AB267883 to AB267885).

Table 1. Collection information of the Japanese spiny lobster phyllosoma larvae used in this study

Specimen ID	*Date captured*	*Locality*	*BL (mm)*	*Stage*
SHN160	June 1995	29°45′ N, 129°00′ E	22.9	IX
SHN176	June 1995	25°30′ N, 129°00′ E	18.2	VIII
Tr5-26	May 2003	24°00′ N, 124°30′ E	22.7	IX
Tr6-2	May 2003	24°00′ N, 124°30′ E	26.3	IX
Tr6-6	May 2003	24°00′ N, 124°30′ E	17.4	VIII
Tr6-11	May 2003	24°00′ N, 124°30′ E	22.5	IX
Tr6-23	May 2003	24°00′ N, 124°30′ E	23.8	IX
SP32	Nov. 2004	22°33′ N, 124°45′ E	13.2	VII
SP45	Nov. 2004	22°24′ N, 124°46′ E	11.8	VII
SP93	Nov. 2004	21°57′ N, 125°58′ E	12.6	VII
SP94	Nov. 2004	21°57′ N, 125°58′ E	13.2	VII

Homology Search and Sequence Analysis The sequences obtained were subjected to homology searches using the FASTA algorithm through the DNA Data Bank of Japan (DDBJ) to determine source of eukaryotes. CHECK_CHIMERA search (Maidak et al. 2000) was also performed to all sequences to detect potential chimeric artifacts. Suspected sequences were subdivided into upper and lower half fragments, which were subjected to sequence similarity search (BLAST) to determine the source organisms. Among the high-ranking candidates nominated by FASTA, those showing identities higher than 90% were selected and incorporated into sequence alignments followed by phylogenetic analysis. Alignments of the DNA sequences were performed with the ClustalW algorithm (Thompson et al. 1994) implemented in MEGA 3.1 (Kumar et al. 2004). The aligned sequences were manually edited and imported into MEGA 3.1 to construct a neighbor-joining phylogenetic tree (NJ) to determine the phylogenetic positions of the cloned DNAs. Kimura's two-parameter (K2P) substitution model with either the pairwise or complete deletion options was attempted on MEGA to consider equivocal sequence alignment especially observed between higher taxa. Maximum likelihood (ML) analysis was also adopted using the PHYLIP package ver. 3.6 (Felsenstein 2004) and PHYML software (Guindon and Gascuel 2003) based on the optimal substitution model (GTR+I+Γ) selected by Modeltest 3.06 (Posada and Crandall 1998). Reliability of each node of the NJ and ML trees was assessed by bootstrap probabilities with 1000 replications.

Results

Detection of Foreign DNA Clones Strong amplifications of the target region of 18S rDNA were obtained from hepatopancreas contents of all the specimens, whereas little amplification was found from the washing water as shown in the previous report.

Endonuclease digestion was performed on amplicons from 72 to 179 clones per larva (1537 clones in total), and a total of 21 restriction types among 160 clones were observed to have different restriction profiles from those of the host larvae. Nucleotide sequence analysis failed in five of 21 restriction types, and sequences of the remaining 16 types were used for subsequent analyses.

Assignment of Foreign DNAs Recovered from Hepatopancreas FASTA homology search for the remaining 16 restriction types nominated sequences of Crustacea (palinurid and scyllarid lobsters), Gastrotricha (Oomycetes), Teleostei, Mycetozoa, Fungi, and unknown (homology score with nominated sequences was less than 90%). CHECK_CHIMERA search indicated the three unknown sequences (AB248105, AB248115, and AB248116) likely to be chimeric. BLAST search indicated that the source organisms for these sequences were "lobster/fungus," "fungus/teleost," and "chaetonotida/teleost," respectively, which were excluded from further analysis. Alignment using sequences of the remaining 13 restriction types and those nominated by FASTA can be obtained at http://fsf.fra.affrc.go.jp/chow/phyll_18S.txt. The central domain and 5′ and 3′ regions of the sequences were well conserved even among higher taxa (phylum). The other regions were observed to be much less conserved, making sequence alignment between higher taxa be equivocal. However, essentially the same tree topologies were obtained between pairwise and complete deletion options for NJ tree as well as between NJ and ML trees.

Five clusters corresponding to the five eukaryote taxa (Mycetozoa, Oomyceta, Fungi, Crustacea, and Teleostei) were represented in the resultant NJ tree with the pairwise deletion option (Figure 1), supported by high bootstrap probabilities (98% to 100% and 99% to 100% in NJ and ML, respectively). Two sequences (AB248106 and AB248112) inferred to be from Protista were assigned to Mycetozoa and Oomyceta, respectively. One (AB248109) of the four sequences in the Fungi cluster was almost identical with sequences of *Cryptococcus* fungi (98.6% to 100% nucleotide identity in FASTA), whereas the other three (AB248104, AB248107, and AB248113) were similar to that of *Sclerotium* fungus. The three sequences (AB267883 to AB267885) obtained from three larvae were closely related to lobster's 18S rDNA, and we determined these to be host variants. Three (AB248110, AB248111, and AB248114) of the four sequences assigned to Teleostei were almost identical to one another and appeared to be closely related to perciforms. The other one (AB248108) was distantly related to other teleost fishes (96.2% to 96.4% nucleotide identity), and the nearest taxon could not be identified. The assignment of foreign DNAs recovered from hepatopancreas is summarized in Table 2.

Eukaryote components obtained from hepatopancreas of each larva, from which the host variant clones and chimeric artifacts were excluded, are also shown in Table 2. A wide variety of eukaryotes was observed in the late larvae (stages VIII and IX) collected in the spring to early summer, while no exogenous DNA sequence was obtained in the mid-stage larvae (VII) collected in the autumn.

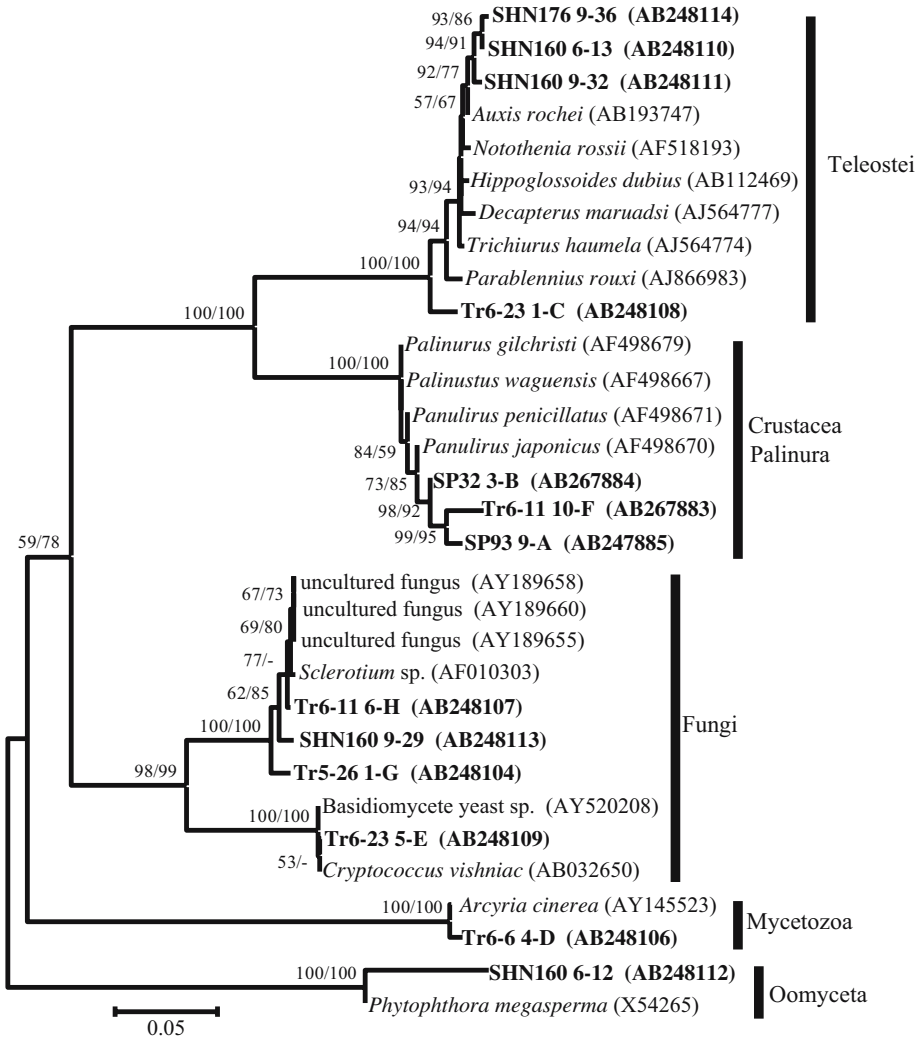

Figure 1. Neighbor-joining tree for affiliating 18S rDNA sequences obtained from the phyllosoma hepatopancreas. Sequences obtained in the present study are shown in boldface. Eukaryote 18S rDNA sequences nominated by the FASTA search are shown in lightface. All sequences are given with the accession numbers in parentheses. The numbers on the branches indicate bootstrap probabilities (NJ/ML) with 1000 replications.

Discussion

The majority of the DNA clones observed in this analysis (66.7% to 100% per specimen) were microbial eukaryotes comprising of Fungi, Mycetozoa, and Oomycetes (Table 2). Although the possibility to utilize microscopic eukaryotes as a nutrient source has been discussed in phyllosoma larvae (e.g., Rodriguez Souza et al. 1999), it is unclear if these microbial organisms are symbiotic or parasitic and contributing to the host or not.

Laboratory experiments offering a variety of plankton species indicated that soft-bodied zooplanktons may be major prey items for mid- to late-stage phyllosoma larvae of spiny and slipper lobsters (Mitchell 1971; Kittaka 1994). These implications were strongly supported by our previous molecular analysis detecting 18S rDNA molecules of cnidarians and urochordates from two late-stage larvae of spiny lobsters (*Panulirus longipes bispinosus* and *P. echinatus*) and one of slipper lobster (*Scyllarus* sp.) (Suzuki et al. 2006). Although the present study examined more than twice the number of samples (n=11) compared with the previous analysis (n=5) (Suzuki et al. 2006), no DNA of these gelatinous animals was obtained. Alternatively, 18S rDNA molecules of teleost fishes were found from three of the seven late-stage phyllosoma larvae of Japanese spiny lobster collected in spring to early summer (Table 2). Stage and size of *P. longipes bispinosus* and *P. echinatus* larvae used in the previous study (Suzuki et al. 2006) were comparable with those of *P. japonicus* larvae analyzed in this study. No apparent difference in the functional morphology of

Table 2. Results of eukaryotes DNA detection using wild-caught phyllosoma larvae

Specimen ID	No. of clones examined	No. of clones[a]		Identified eukaryote[b] (no. of clones: % for possible prey DNAs)			
		Host	Non-host				
SHN160	150	139	11	Teleost[2c]	(3: 33.3)	AB248110	AB248111
				Oomycetes[1]	(1: 11.1)	AB248112	
				Fungus[1]	(5: 55.6)	AB248113	
				nd[1]	(2)		
SHN176	152	134	18	Teleost[1]	(3: 100)	AB248114	
				Fungus[1]	(10: 76.9)	AB248115	
				Chimera[1]	(15)	AB248116	
Tr5-26	129	118	11	Fungus[1]	(10: 100)	AB248104	
				nd[1]	(1)		
Tr6-2	81	69	12	Chimera[1]	(12)	AB248105	
Tr6-6	163	120	43	Mycetozoa[1]	(42: 100)	AB248106	
				Host variant[1]	(1)	AB267883	
Tr6-11	150	122	28	Fungus[1]	(27: 100)	AB248107	
				Host variant	(1)	AB267883	
Tr6-23	72	58	14	Teleost[1]	(3: 21.4)	AB248108	
				Fungus[1]	(11: 78.6)	AB248109	
SP32	164	160	4	Host variant[2]	(4)	AB267884	
SP45	179	171	8	nd[3]	(4)		
				Host variant[2]	(4)		
SP93	147	140	7	Host variant[2]	(7)	AB267885	
SP94	150	146	4	Host variant[1]	(4)		

[a]Selected via RFLP analysis.
[b]Determined via nucleotide sequence analysis.
[c]Number of restriction types.
nd, not determined.

feeding apparatuses has been observed between these congeneric species (Matsuda and Yamakawa 2000; Konishi et al. 2006), suggesting that the late-stage larvae of these species may share similar prey organisms. In the laboratory, complete metamorphosis was attained in both *P. japonicus* and *P. longipes* under the similar feeding condition (Kittaka and Kimura 1989; Yamakawa et al. 1989; Matsuda and Yamakawa 2000). Therefore, any differences in the feeding habit between these closely related species in natural environment may be negligible. Seasonal or spatial differences in prey organisms available for lobster larvae may be responsible for the contrasting results between the present and previous molecular studies. The lobster larva of *P. longipes bispinosus* used by Suzuki et al. (2006) was caught at southern water of Japan (27°30′ to 30°27′ N: 133°20′ to 135° E) in winter (January), while the late-stage larvae of *P. japonicus* used in this study were collected during spring to early summer off southern part of the Ryukyu Archipelago. Huang and Chiu (1998) investigated seasonal density of ichthyoplankton in the waters off northeastern Taiwan adjacent to the Ryukyu Archipelago, where they found that the density of fish larvae was the lowest in winter, increased in spring, reached a maximum in summer, and declined in autumn. In the laboratory, we also observed that stage II phyllosoma larvae of *P. japonicus* fed on newly hatched larvae of anchovy (*Engraulis japonicus*) (unpublished data). Thus, the lobster phyllosoma larvae may be opportunistic carnivores in the wild rather than oligophagous feeder, and their diets may be correlated with the relative abundance of prey organisms in the ambient water. This implication is supported by comparative analysis on lipid class and fatty acid and sterol composition between phyllosoma larvae of the spiny lobster *Jasus edwardsii* and its potential prey items (Jeffs et al. 2004).

Detection of no polychaete DNA in this study may be reasonable, as pelagic polychaetes are much less abundant compared with arrow worms and fish larvae. On the other hand, it is less clear why no chaetognath DNA has been detected, though the arrow worms appear to be one of the most abundant macro-zooplankton all the year round. Several chaetognath 18S rDNA sequences obtained from database were observed to share identical sequences with all primers used in this study. Further, in our onboard feeding attempts, phyllosoma larvae of scyllarid and synaxid lobsters were observed to readily consume a wide variety of animals particularly pelagic polychaetes and arrow worms followed by larval fish (unpublished observation).

The analysis method developed and adopted in our previous and present studies appears not to be quantitative because the universal primers used must selectively amplify the vast amount of the host DNA. This may also consequently mask the amplification of the much lower amounts of exogenous DNAs, accompanied with arbitrary amplification independent of the quantity of DNA.

We have detected no possible prey DNA from all mid-stage larvae of *Panulirus japonicus* (stage VII, 11.8 to 13.2 mm BL); four in the present study collected in late autumn and one in the previous study collected in winter. It is unlikely that the feeding habit of younger larvae is considerably different from that of late-stage larvae, as we have observed stage II larvae of *P. japonicus* could capture and consume fish larvae in the laboratory conditions. As detection of consumed prey depends on the amount of tissue that remains and the DNA degradation status in the digestive tract (Wallace 2004; Deagle et al. 2005), smaller quantity of prey if any consumed by the younger phyllosoma larvae may be partially responsible for the lack of detection of exogenous DNA molecules.

In the present study, possible prey items of wild late-stage phyllosoma larvae of *Panulirus japonicus* could be presented. Seasonal differences in prey organisms were also suggested, but the whole picture of the feeding habit throughout the larval stages in relation to spatio-temporal dynamics of prey composition is not understood. Further technical development to reduce the effects of amplification of the host and microorganism DNAs and to identify species may be necessary.

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References

Chittleborough RG, Thomas LR (1969) Larval ecology of the western Australian marine crayfish, with notes upon other panulirid larvae from the eastern Indian Ocean. Aust J Mar Freshw Res 20, 199–223

Chow S, Suzuki N, Imai N, Yoshimura T (2006a) Molecular species identification of spiny lobster phyllosoma larvae of the genus *Panulirus* from the northwestern Pacific. Mar Biotechnol 8, 260–267

Chow S, Yamada H, Suzuki N (2006b) Identification of mid to final stage lobster phyllosoma larvae of the genus *Panulirus* White, 1847 collected in the Ryukyu Archipelago. Crustaceana 79, 745–764

Deagle BE, Tollit DJ, Jarman SN, Hindell MA, Trites AW, Gales NJ (2005) Molecular scatology as a tool to study diet: analysis of prey DNA in scats from captive Steller sea lions. Mol Ecol 14, 1831–1842

Felsenstein J (2004) PHYLIP (Phylogeny Inference Package), Version 3.6. Distributed by the Author. Department of Genome Sciences, University of Washington, Seattle, WA

Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol 52, 696–704

Huang JB, Chiu TS (1998) Seasonal and hydrographic variations of ichthyoplankton density and composition in the Kuroshio edge exchange area off northeastern Taiwan. Zool Stud 37, 63–73

Inoue M (1981) Studies on the cultured phyllosoma larvae of the Japanese spiny lobster, *Panulirus japonicus* (V. Siebold). Sp Rep Kanagawa Pref Fish Exp St 1, 1–91 (In Japanese)

Jeffs AG, Nichols PD, Mooney BD, Phillips KL, Phleger CF (2004) Identifying potential prey of the pelagic larvae of the spiny lobster *Jasus edwardsii* using lipid signatures. Comp Biochem Physiol B 137, 487–507

Johnson MW (1971) On palinurid and scyllarid lobster larvae and their distribution in the South China Sea (Decapoda, Palinuridea). Crustaceana 21, 247–282

Johnston D, Ritar A, Thomas C, Jeffs A (2004) Digestive enzyme profiles of spiny lobster *Jasus edwardsii* phyllosoma larvae. Mar Ecol Prog Ser 275, 219–230

Kittaka J (1994) Culture of phyllosomas of spiny lobster and its application to studies of larval recruitment and aquaculture. Crustaceana 66, 258–270

Kittaka J (1997) Culture of larval spiny lobsters: a review of work done in northern Japan. Mar Freshw Res 48, 923–930

Kittaka J, Kimura K (1989) Culture of the Japanese spiny lobster *Panulirus japonicus* from egg to juvenile stage. Nippon Suisan Gakkaishi 55, 963–970

Kittaka J, Ono K, Booth JD (1997) Complete development of the green rock lobster, *Jasus verreauxi* from egg to juvenile. Bull Mar Sci 61, 57–71

Konishi K, Suzuki N, Chow S (2006) A late stage phyllosoma larva of the spiny lobster *Panulirus echinatus* Smith, 1869 (Crustacea: Palinuridae) identified by DNA analysis. J Plankton Res 28, 841–845

Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinf 5, 150–163

Maidak BL, Cole JR, Lilburn TG, Parker CT, Saxman PRJr, Stredwick JM, Garrity GM, Li B, Olsen GJ, Pramanik S, Schmidt TM, Tiedje JM (2000) The RDP (Ribosomal Database Project) continues. Nucleic Acids Res 28, 173–174

Matsuda H, Yamakawa T (2000) The complete development and morphological changes of larval *Panulirus longipes* (Decapoda, Palinuridae) under laboratory conditions. Fish Sci 66, 278–293

McCallum FS, Maden BE (1985) Human 18 S ribosomal RNA sequence inferred from DNA sequence. Variations in 18 S sequences and secondary modification patterns between vertebrates. Biochem J 232, 725–733

Mitchell JR (1971) Food preferences, feeding mechanisms and related behavior in phyllosoma larvae of the California spiny lobster, *Panulirus interruptus* (Randall). MS Thesis, San Diego State College, San Diego, CA

Posada D, Crandall KA (1998) Model test: testing the model of DNA substitution. Bioinformatics 14, 817–818

Ptacek MB, Sarver SK, Childress MJ, Herrnkind WF (2001) Molecular phylogeny of the spiny lobster genus *Panulirus* (Decapoda: Palinuridae). Mar Freshw Res 52, 1037–1047

Rodriguez Souza JC, Strüssmann CA, Takashima F, Sekine S, Shima Y (1999) Absorption of dissolved and dispersed nutrients from sea-water by *Panulirus japonicus* phyllosoma larvae. Aquac Nutr 5, 41–51

Suzuki N, Murakami K, Takeyama H, Chow S (2006) Molecular attempt to identify prey organisms of lobster phyllosoma larvae. Fish Sci 72, 342–349

Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res 22, 4673–4680

Wallace SK (2004) Molecular gut analysis of carabids (Coleoptera: Carabidae) using aphid primers. MS Thesis, Montana State University, Bozeman, MT

Yamakawa T, Nishimura M, Matsuda H, Tsujigado A, Kamiya N (1989) Complete larval rearing of the Japanese spiny lobster *Panulirus japonicus*. Nippon Suisan Gakkaishi 55, 745