The early phyllosoma stages of spiny lobster *Panulirus echinatus* Smith, 1869 (Decapoda: Palinuridae) reared in the laboratory

Abrunhosa, FA.[a]*, Santiago, AP.*[b]* and Abrunhosa, JP.*[a]

[a]Núcleo de Estudos Costeiros, Campus de Bragança, Universidade Federal do Pará – UFPA, Alameda Leandro Ribeiro, Bairro Aldeia, CEP 68600-000, Bragança, PA, Brazil

[b]Laboratório de Recursos Aquáticos, Universidade Federal do Ceará – UFC, Av. Mister Hull, s/n, Pici, CEP 60455-760, Fortaleza, CE, Brazil

*e-mail: faraujo@ufpa.br, aps@hotmail.com

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(With 6 figures)

Abstract

The early stages of the *Panulirus echinatus* were hatched and reared in the laboratory. Ovigerous females were captured in their habitat and carefully transported to the laboratory. Larvae were transferred in a recirculation water tank at a density of 10 larvae.L^{-1}. The larvae were fed on *Artemia* and gonads of mussel *Brachydonts* sp. Microalgae *Dunaliella viridis* was added at a concentration of 150 x 10^4 cell.mL^{-1}. Larvae and exuviae of each zoeal stage were preserved in an alcohol 70% + glycerin (1:1) solution. The phyllosomas moulted eight times; the intermoulting period of each instar averaged about 7 to 10 days. The main morphological changes of each appendage were described in detail, illustrated and compared with previous reports.

Keywords: spiny lobster, phyllosoma, larval development, morphology.

Os primeiros estágios de filosoma da lagosta *Panulirus echinatus* (Decapoda: Palinuridae) cultivados em laboratório

Resumo

Os estágios iniciais de lagosta tropical *Panulirus echinatus* eclodiram e foram cultivados em laboratório. Fêmeas ovígeras foram capturadas em seu habitat e cuidadosamente transportadas para o laboratório. As larvas foram cultivadas em tanques de recirculação de água e alimentadas com náuplios de *Artemia* e gônadas mexilhão *Brachydonts* sp. Microalga *Dunaliella viridis* foi adicionada em cada tanque de cultivo na concentração de 150 x 10^4 cel.mL^{-1}. Os filosomas mudaram oito vezes e o período de intermuda de cada instar foi de cerca de 7 a 10 dias. As principais mudanças morfológicas de cada apêndice foram descritas, ilustradas e comparadas com trabalhos anteriores.

Palavras-chave: lagosta, filosoma, desenvolvimento larval, morfologia.

1. Introduction

The Brazilian spiny lobsters of the *Panulirus* genus have high economic value. The species *P. argus* (Latreille, 1804) and *P. laevicauda* (Latreille, 1817) are captured extensively, representing one of the most important fishery resources in the world (Phillips et al., 1980). For a long time another species, *P. echinatus*, was occasionally captured, however, an increase in their production has been observed (Santiago, 2001). Despite the economic significance that this latter species has represented in the fisheries, few studies concerning their biological aspects have been reported.

Information on the biology of phyllosomas is considered important and necessary to elucidate aspects related to larval biology and culture. The larval development of spiny lobster has been reported for specimens collected in the sea (Lewis, 1951; Baisre, 1964; Phillips and Sastry, 1980) and for those reared in the laboratory progressed through the metamorphic puerulus stage (Inoue, 1981; Kittaka and Kimura, 1989; Yamakawa et al., 1989; Matsuda and Yamakawa, 2000). The long larval period (about one year) is considered an important obstacle for phyllosoma culture research. The lack of detailed morphological description of some or all phyllosoma stages has also hindered studies of larval distribution and ecology.

Successful studies on culture from egg to the pueruli stage of *Panulirus* were achieved for *P. japonicus* (Von Siebold, 1824) (Kittaka and Kimura, 1989; Yamakawa et al., 1989) and *P. longipes* (A. Milne-Edwards, 1868) (Matsuda and Yamakawa, 2000). Some attempts at rearing phyllosoma larvae were reported for *P. homarus*

(Linnaeus, 1758) (Radhakrishnan and Vijayakumaran, 1995) and for *P. ornatus* (Fabricius, 1776) (Duggan and McKinnon, 2003) in which, 10 and 9 early instars were reared and described, respectively. More recently, Abrunhosa et al. (2004) described and compared the morphological characteristics of three early instars of *P. laevicauda* and *P. echinatus*, contributing to the identification of phyllosomas that occur on the Brazilian coast. These three early larval instars of *P. echinatus* are again reviewed in the present study.

The present study describes in detail and illustrates the 5 early stages and instars of each stage of the species *P. echinatus* in the laboratory. Morphological comparisons with larvae of its congeneric species, previously described, are briefly discussed.

2. Material and Methods

Ovigerous females of *P. echinatus* were obtained at Iparana Beach (Caucaia/CE) in October 2001 by autonomous diving in the coastal shallow waters. They were transported to the laboratory in plastic baskets (10 L) containing local seawater.

In the laboratory the females were acclimated and then placed in a circular tank (cap. 1000 L) with biological filter and constant aeration.

After hatching, the phyllosomas were carefully transferred to three transparent water recirculation tanks at a density of 60 larvae.L^{-1}. These tanks are similar to those used for culture of many species of spiny lobsters of the family Palinuridae (Kittaka, 1994). The tanks, which were confectioned with acrylic (70 cm Ø and 30 cm deeper), allow free circulation of flowing water and total visualization of the floating elements is possible. Salinity was maintained at 35‰. The larvae were fed *Artemia* nauplii for the 3 first instars and then, with gonads of *Brachyodonts* sp. Microalgae *Dunaliella viridis* Teodoresco 1906 were added daily in the culture system.

Samples of larvae were removed after each instar and fixed in 10% formalin solution for 24 hours and preserved in a glycerol+alcohol 70% (1:1) solution. The larvae were dissected under an optical binocular microscope. The illustrations of each larval instar, including appendages, were made using a camera lucida.

For the species *P. homarus*, information on the average length, number of setae in the apical segment of the 2nd maxilla and number of the exopod setae of each appendage were based on the illustrations reported by Radhakrishnan and Vijayakumaran (1995).

The terminologies STAGES and INSTAR were used in order to make comparisons with other previously described species. Both terms were extensively used by the authors in descriptions of larval development of the spiny lobster.

3. Results

3.1. On the larval culture

The larval hatching was closely related to the moon phases, in which the majority of the larval hatching was carried out on the first quarter and full moon, indicating that lunar cycle had direct influence in the life cycle in the laboratory.

The phyllosomas readily feed on *Artemia* nauplii in stages I to III. Then, the phyllosomas seemed to have difficulty in catching *Artemia* so other foods were tested. Some foods were initially well accepted by the phyllosomas, but later were rejected. This behaviour may be related to nutritional aspects, because many

Table 1. Intermoulting period, accumulative days and maim characteristics of each stage for phyllosomas of *Panulirus echinatus* reared in the laboratory.

Stage	Intermoult (Mean days)	Accumulative days	Main characteristics
I	9	9	Ocular peduncle unsegmented. Exopod of the 1st and 2nd pereiopods with 5 pairs of setae.
II	9	18	Eyes segmented; exopod of the 1st and 2nd pereiopods with 6 pairs of setae.
IIIa	7	25	Fourth pereiopod present; exopod of the 1st and 2nd pereiopods with 7 pairs of setae.
IIIb	5	30	1st and 2nd pereiopods with 8 pairs of setae.
IVa	5	35	Fifth pereiopod present; exopod of the 1st and 2nd pereiopods with 9 pairs of setae.
IVb	5	40	exopod of the 1st and 2nd pereiopods with 10 pairs of setae.
IVc	7	47	exopod of the 1st and 2nd pereiopods with 11 pairs of setae.
IVd	8	55	exopod of the 1st and 2nd pereiopods with 12 pairs of setae.
V	>8*	>63	Antennule 3-segmented; exopod of the 1st and 1st and 2nd pereiopods with 13 pairs of setae.

*No phyllosoma succeeded in moulting to the next instar.

phyllosomas failed to moult to the subsequent stage. This fact was more evident for larvae in the VIII instar.

The larvae moulted eight times, once at stages I to II, two times at stage III and four times at stage IV (Table 1).

There was not a specific day or night period in which the larvae moulted to the next instar. The moult of the phyllosomas from stage I to II and II to III ranged from 7 to 11 and 7 days, averaging in 9 and 7 days, respectively. For successive instars, it was not possible to determine accurately the intermoult period for later instars, because the phyllosomas were not reared individually. The intermoult average for each instar is shown in Table 1.

The largest mortality of the phyllosomas was on the 4th day of rearing, when about 50% of the larvae died in both systems. After this period, low mortality rates were observed.

3.2. On gross morphology

The morphological features were fully described only for instar I. For the subsequent instars, only main morphological changes were reported. The morphological changes and the main characteristics of each stage are fully described in the Table 2.

STAGE I with one instar (Figure 1a)

Cephalic shield: Mean length 1.04 mm (range: 1.00-1.07), semicircular, pear like in shape.

Eyes: Ocular peduncle unsegmented.

Antennule (Figure 2a): Uniramous, unsegmented, longer than antenna, ending in a short spine, 3 long aesthetascs and 2 simple setae.

Antenna (Figure 2b): Uniramous, 2-incompletely segmented, ending in a short curved spine and 2-3 simple setae, 2 simple setae in the distal region near to the apex.

First Maxilla (Figure 2c): Coxal endite with 2 long serrulate setae and 1 short simple seta; basal endite with 2 strong serrulate setae distally and 1-2 sub-distally; endopod short, unsegmented with 2 setae.

Second Maxilla (Figure 2d): Basal segment enlarged with 2-3 short setae, distal segment short with 4 long plumose setae.

First Maxilliped: Small, uniramous and unsegmented protuberance.

Second Maxilliped (Figure 2e): Endopod 5-segmented, distal segment ending in 1 strong distal and 2 sub-distal spines, sub-distal segment rounded distally with long serrulate setae; exopod absent.

Third Maxilliped: Endopod well developed, 5-segmented, segment distal with long setae rounding the distal margin and 2 long setae on the sub-distal margin; exopod with 3 pairs of long and plumose setae.

First and second Pereiopods: Endopod 5-segmented, completely developed, distal segment ending in a strong spine, dorsal and ventral sub-exopodal spines absent, coxal spine present but not elongated (disposition of setae as illustrated); exopod with 5 pairs of long and plumose setae.

Third Pereiopod: Endopod very long, 5-segmented, ending in a strong curved spine, coxal spine present; exopod undeveloped showing a small unarmed protuberance.

Abdomen: Short, unsegmented with 3 setae and 1 spine at each ramous on the posterior portion.

STAGE II with one instar (Figure 1b)

Cephalic shield: Mean length 1.25 mm (range: 1.18-1.30 mm).

Eyes: Ocular peduncle segmented.

Table 2. Cephalic length, number of setae of the 2nd maxilla and exopod of maxillipeds and pereiopods of *Panulirus echinatus* reared in the laboratory.

Stage	Instar	Average of Cephalic shield Length (mm)	2nd maxilla (Distal seg.)	Pairs of setae in the exopods					
				3rd maxill.	1st Pereiop.	2nd Pereiop.	3rd Pereiop.	4th Pereiop.	5th Pereiop.
I	I	1.04	4	3	5	5	0	absent	absent
II	II	1.25	4	3	6	6	0	absent	absent
III	III	1.55	4	4	7	7	3	bud	absent
	IV	1.72	4	4	8	8	4	bud	absent
IV	V	2.09	4	5	9	9	5	bud birram.	bud
	VI	2.17	4	5	10	10	6	bud birram.	bud
	VII	2.25	4	6	11	11	7	bud birram.	bud
	VIII	2.50	4	7	12	12	8	0	bud
V*	IX	2.58	4	8	13	13	9	0	bud

*The culture finished at the first instar of the stage V (nine instars were reared.)

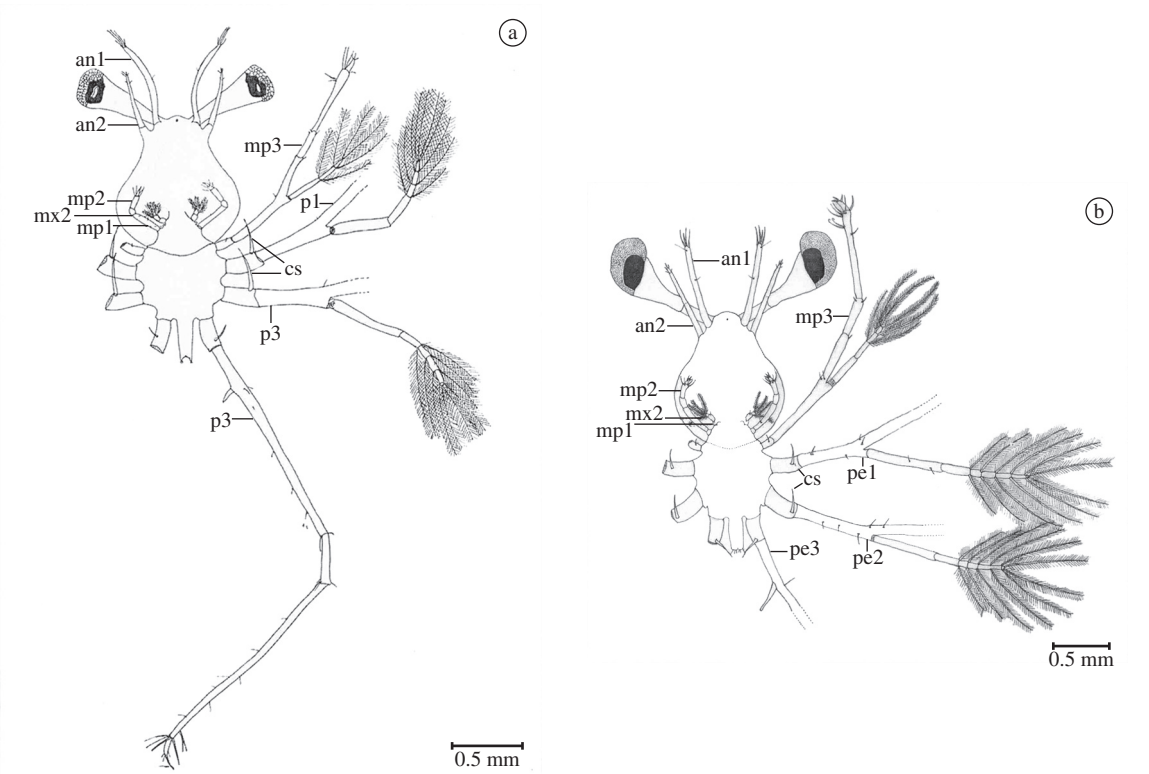

Figure 1. *Panulirus echinatus* (in ventral view): a) Stage I; b) Stage II. Abbreviations: an1 = antennule; an2 = antenna; cs = coxal spine; mp1 = 1st maxilliped; mp2 = 2nd maxilliped; mp3 = 3rd maxilliped; mx2 = maxilla; pe1 = 1st pereiopod; pe2 = 2nd pereiopod; pe3 = 3rd pereiopod.

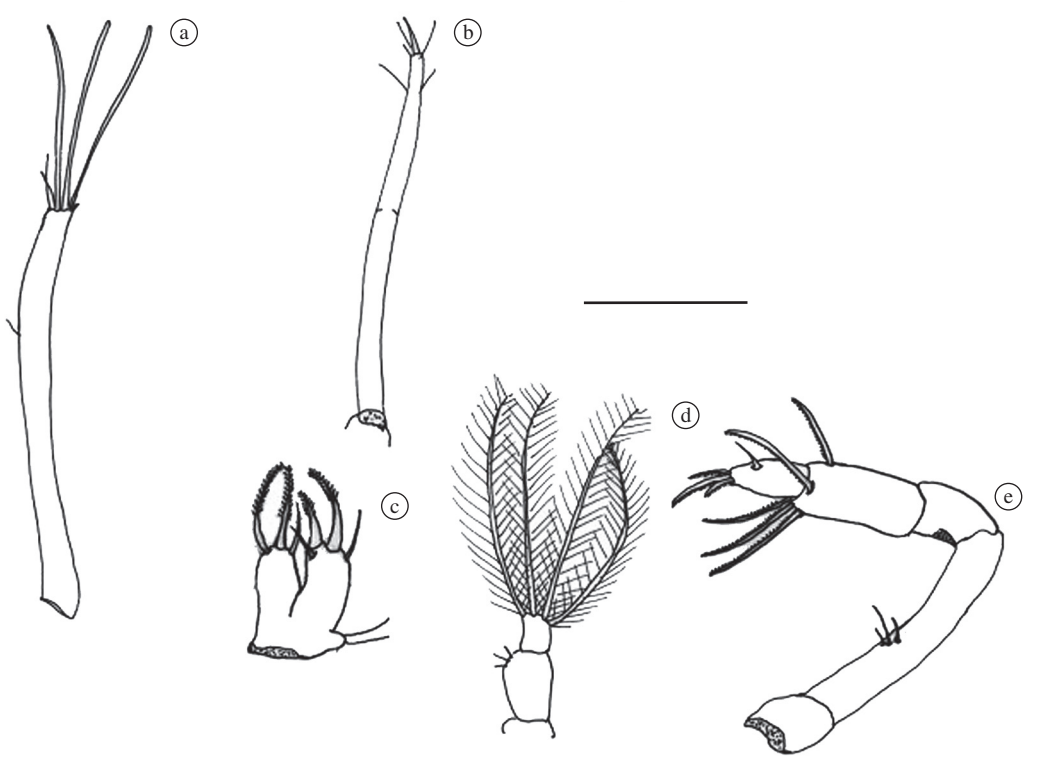

Figure 2. *Panulirus echinatus*: a) antennule, b) antenna, c) maxillule, d) maxilla, and e) 2nd maxilliped. Scale bar = 0.5 mm.

First and second Pereiopods: Exopod with 6 pairs of long and plumose setae.

Third Pereiopod: Exopod a little more developed compared to the first instar, lacking setae.

STAGE III with two instars

INSTAR IIIa (Figure 3a)

Cephalic shield: Mean length 1.55 mm (range: 1.38-1.74 mm).

Third Maxilliped: Exopod with 4 pairs of long and plumose setae.

First and second Pereiopods: Exopod with 7 pairs of long and plumose setae.

Third Pereiopod: Exopod well developed compared to previous instars with 3 pairs of long and plumose setae.

Fourth Pereiopod: Unsegmented and biramous bud.

INSTAR IIIb (Figure 3b)

Cephalic shield: Mean length 1.72 mm (range: 1.70-1.75 mm).

First and second Pereiopods: Exopod with 8 pairs of long and plumose setae.

Third Pereiopod: Exopod with 4 pairs of long and plumose setae.

STAGE IV with 4 instars

INSTAR IVa (Figure 4a)

Cephalic shield: Mean length 2.09 mm, narrower than previous instars, about 1/3 longer than large (range: 1.90-2.28 mm).

Third Maxilliped: Exopod with 5 pairs of long and plumose setae.

First and second Pereiopods: Exopod with 9 pairs of long and plumose setae.

Third Pereiopod: Exopod with 5 pairs of long and plumose setae.

Fourth Pereiopod: More developed in relation to previous instar (as illustrated), lacking setae.

Fifth Pereiopod: Present but unsegmented, bud and lacking setae.

INSTAR IVb (Figure 4b)

Cephalic shield: Mean length 2.17 mm (range: 2.05-2.39 mm).

First and second Pereiopods: Exopod with 10 pairs of long and plumose setae.

Third Pereiopod: Exopod with 6 pairs of long and plumose setae.

INSTAR IVc (Figure 5a)

Cephalic shield: Mean length 2.25 mm (range: 2.15-2.35 mm).

Third Maxilliped: Exopod with 6 pairs of long and plumose setae.

First and second Pereiopods: Exopod with 11 pairs of long and plumose setae.

Third Pereiopod: Exopod with 7 pairs of long and plumose setae.

INSTAR IVd (Figure 5b)

Cephalic shield: Mean length 2.50 mm (range: 2.48-2.52 mm).

Antennule: Segmented, proximal segment longer than the distal one.

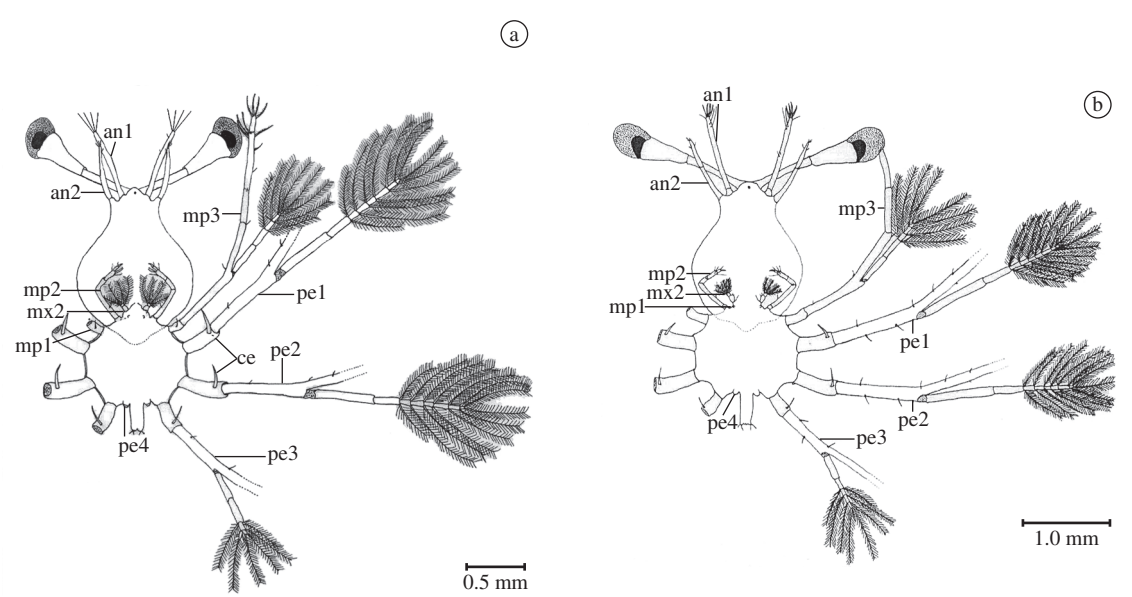

Figure 3. *Panulirus echinatus* (in ventral view): a) Stage IIIa, b) Stage IIIb. Abbreviations: an1 = antennule; an2 = antenna; cs = coxal spine; mp1 = 1st maxilliped; mp2 = 2nd maxilliped; mp3 = 3rd maxilliped; mx2 = maxilla; pe1 = 1st pereiopod; pe2 = 2nd pereiopod; pe3 = 3rd pereiopod; pe4 = 4th pereiopod.

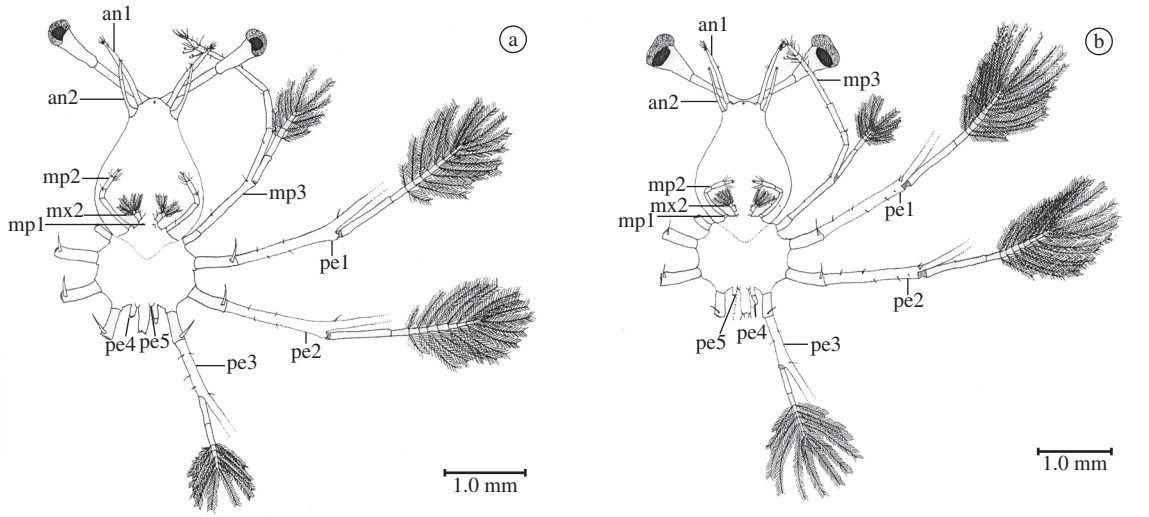

Figure 4. *Panulirus echinatus* (in ventral view): a) Stage IVa, b) StageIVb. Abbreviations: an1 = antennule; an2 = antenna; mp1 = 1st maxilliped; mp2 = 2nd maxilliped; mp3 = 3rd maxilliped; mx2 = maxilla; pe1 = 1st pereiopod; pe2 = 2nd pereiopod; pe3 = 3rd pereiopod; pe4 = 4th pereiopod; pe5 = 5th pereiopod.

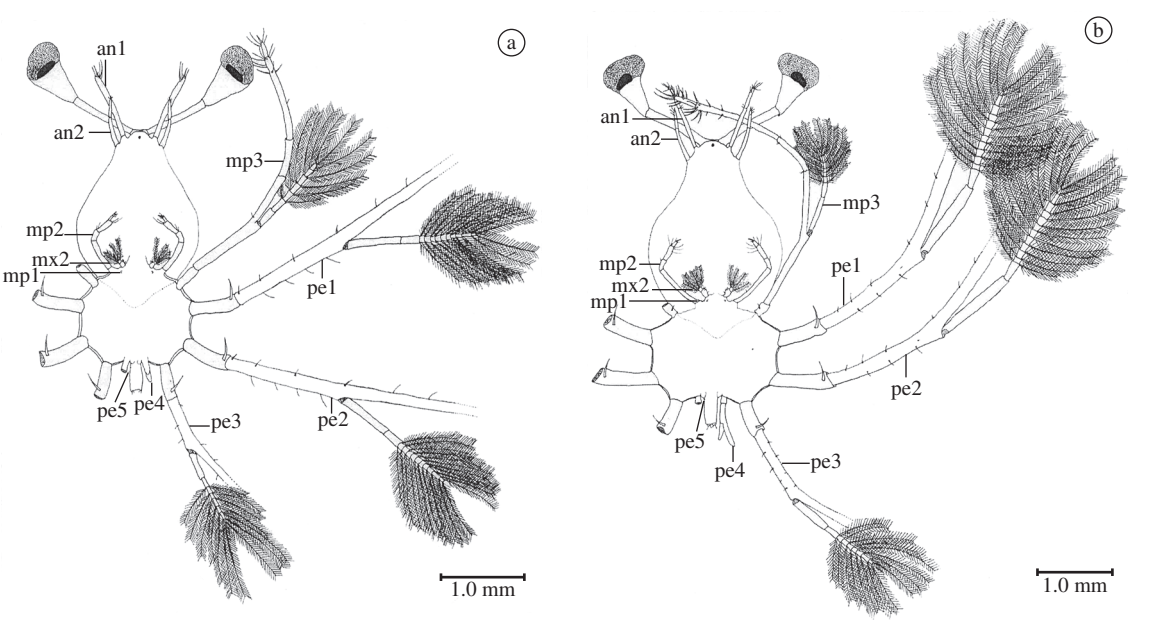

Figure 5. *Panulirus echinatus* (in ventral view): a) Stage IVc, b) Stage IVd. Abbreviations: an1 = antennule; an2 = antenna; mp1 = 1st maxilliped; mp2 = 2nd maxilliped; mp3 = 3rd maxilliped; mx2 = maxilla; pe1 = 1st pereiopod; pe2 = 2nd pereiopod; pe3 = 3rd pereiopod; pe4 = 4th pereiopod; pe5 = 5th pereiopod.

Third Maxilliped: Exopod with 7 pairs of long and plumose setae.

First and second Pereiopods: Exopod with 12 pairs of long and plumose setae.

Third Pereiopod: Exopod with 8 pairs of long and plumose setae.

Fourth Pereiopod: More developed in relation to previous instar (as illustrated), surpassing abdomen. Exopod more developed, lacking setae.

STAGE V (Figure 6)

Cephalic shield: Mean length 2.58 mm (range: 2.50-2.58 mm).

Antennule: 3-segmented, about 2 times the antennal size.

Third Maxilliped: Exopod with 8 pairs of long and plumose setae.

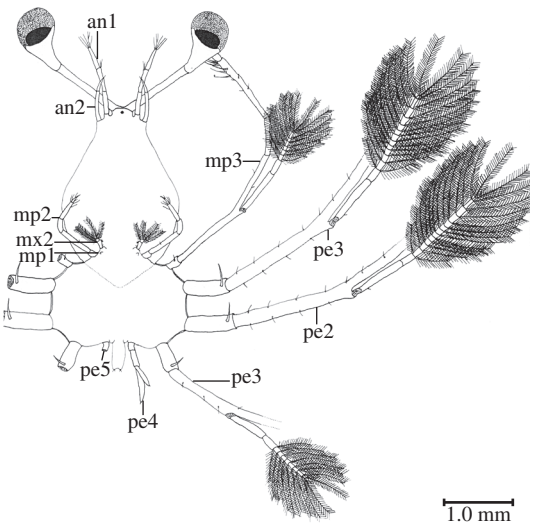

Figure 6. *Panulirus echinatus* (in ventral view): Stage V. Abbreviations: an1 = antennule; an2 = antenna; mp1 = 1st maxilliped; mp2 = 2nd maxilliped; mp3 = 3rd maxilliped; mx2 = maxilla; pe1 = 1st pereiopod; pe2 = 2nd pereiopod; pe3 = 3rd pereiopod; pe4 = 4th pereiopod; pe5 = 5th pereiopod.

First and second Pereiopods: Exopod with 13 pairs of long and plumose setae.

Third Pereiopod: Exopod with 9 pairs of long and plumose setae.

Fourth Pereiopod: Surpassing about 2 times the abdomen.

Fifth Pereiopod: Still bud and lacking setae.

4. Discussion

In the laboratory, phyllosoma larvae moulted eight times comprising five larval stages considering the developmental criteria of description adopted by the authors in previous studies, Inoue (1978, 1981) for *P. japonicus*, Radhakrishnan and Vijayakumaran (1995) for *P. homarus*, Matsuda and Yamakawa (2000) for *P. longipes* and Coutures, (2000) for palinurid species.

The term instar, which corresponds to each moulting of the phyllosoma, was used for palinurid and scyllarid species by Kittaka (1994), Booth and Phillips (1994) and Kittaka and Abrunhosa (1997). In general, the number of setae increased in the exopods of the phyllosoma appendages. In the case of *P. echinatus*, an additional pair of setae arises on each successive larval moulting (Table 2). Such morphological change appears to be a characteristic of the palinurid species, in which each instar could be considered a larval stage. In this case, comparisons with other species will become easier to observe.

Morphologically, the phyllosomas of *P. echinatus* are very similar to other palinurid species. At stage I the mean cephalic length is relatively large compared with other reported *Panulirus* species such as *P. laevicauda* (by Abrunhosa, 2004), *P. ornatus* (by Duggan and McKinnon, 2003) *P. japonicus* (Inoue, 1981), *P. longipes* (by Matsuka and Yamakawa), *P. homarus* (by Radhakrishnan and Vijayakumaran, 1995) and *P. inflatus* (by Inoue, 1981). The initial number of setae 3, 5, 5 in the exopods of the third maxilliped and pereopods and their gradual increase are also similar to the reported species.

On the other hand, the distinct difference among other species is the presence of coxal spines on *P. echinatus*, which is included in group B, according to Gurney, 1936 and Baisre and Ruiz de Quevedo, 1982. According to Abrunhosa et al. (2004), phyllosomas of the Brazilian species, *P. echinatus* and *P. argus*, bear only the coxal spines while *P. laevicauda* exhibited both the coxal and sub-exopodal spines placing these species in different groups.

The Intermoult period appears to be distinct for palinurid species and may vary in accordance to the rearing conditions in which the larvae are submitted, such as, adaptation, type of food administered, density, water quality, and others (Kittaka and Ikegani, 1988; Kittaka et al., 1988; Kittaka and Ikegami, 1988; Kittaka and Kimura, 1989; Radhakrishnan and Vijayakumaran, 1995; Kittaka et al., 1998; Matsuda and Yamakawa, 2000; Sekine et al., 2000).

Kittaka and Kimura (1989) rearing phyllosomas of *P. japonicus*, obtained an intermoult period of 6 to 7 days for instar I. However, a gradual increase in that period was observed for the subsequent instars. Matsuda and Yamakawa (2000) observed an intermoult period for the first two instars of *P. longipes* similar to *P. echinatus* of 7 to 10 days. But, for *P. homarus*, cultured individually, this period increased to 8 to 10 days. When *P. homarus* were submitted to mass culture, this period increased to 12 to 14 days (Radhakrishnan and Vijayakumaran, 1995). These facts may be related to the elevated density.

Feeding is considered a critical factor in the success of the phyllosoma culture. Delay or decrease of the amount of food requested by the larva may prolong the intermoulting period or cause death during the ecdyse (Abrunhosa and Kittaka, 1997). High mortality was observed in the instar IV of *P. echinatus*. This fact may be related to the nutritional requirement needed for moulting as many phyllosomas failed to moult to the subsequent stage. An interesting study on larval feeding of *P. elephas* revealed their ability to capture large-sized fish larvae (Kittaka and Abrunhosa, 1997). Presumably, fish larvae may compose the natural diet for phyllosomas in the sea.

Many kinds of food were tested in phyllosoma tanks from instar III. Larvae of *P. japonicus* were cultured being fed with adult *Artemia* and pieces of mussel gonads (Sekine et al., 2000). Kittaka and Kimura (1989) also obtained success in the culture of phyllosomas of this species feeding pieces of gonads of *Mytilus edulis*. Dexter (1972) reported that phyllosoma larvae of *P. interruptus*

eat large food items: fish larvae, chaetognaths and hydromedusae. In the current study, the phyllosomas were fed with gonads of mollusk *Brachyodonts* sp. from instar III and this kind of food has demonstrated to be suitable until instar VIII but it was not effective for instar IX because failure moult was observed on many phyllosomas in the tanks. These facts suggest that further research is required about the feeding and nutrition of *P. echinatus*.

Water quality is another decisive factor in the success of the phyllosoma culture. Phyllosomas with good activity and free of contaminations by protozoa and bacteria depend on rigid control of the water. Any negligence in the water quality can completely affect the larviculture (Radhakrishnan and Vijayakumaran, 1995). Intensive monitoring of the water quality was accomplished during the culture of *P. echinatus*. The results of phyllosoma culture may be considered satisfactory compared with those reported previously (Kittaka, 1988; Kittaka et al., 1988; Kittaka and Ikegami, 1988).

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