Juvenile development of *Pagurus criniticornis* (Dana, 1852) (Anomura, Paguridae) in the laboratory

Ana L. Brossi-Garcia and Nilton J. Hebling

(ALBG, NJH) Departamento de Zoologia, Instituto de Biociências, Unesp, 13506-900, Rio Claro, São Paulo, Brasil. C.P. 199.
(ALBG) E-mail: abrossi@rc.unesp.br

Abstract

The objective of the present study was to recognize the juvenile phase of *Pagurus criniticornis* hermit crabs. The development of this species was followed in the laboratory over a period of 2 years of rearing, when animals reached a mean shield length 2.2 mm and 34 ecdysis. The first five juvenile instars were analyzed morphologically and some information on the duration and survival were done. The relationship between shield length and propodus length of the right chelipod of 25 postlarval instars was studied to evaluate the growth pattern and the instars, which characterize it.

Key words: Juvenile, Development, Decapod, *Pagurus, P. criniticornis.*

Introduction

Hermit crabs of the families Paguridae and Diogenidae have been relatively well studied from the viewpoint of larval development. Some aspects of metamorphosis are usually presented simultaneously with morphological descriptions of larval forms obtained in laboratory culture.

With respect to juvenile development, which starts with ecdysis of the megalopa, few studies are available and they have been limited to descriptions of *Dardanus pectinatus* by Forest (1954), *Clibanarius misanthropus* by Dechancé (1958), *Paguristes sericeus* by Provenzano and Rice (1966), *Clibanarius sclopetarius* and *Clibanarius vittatus* by Brossi-Garcia (1987, 1988), of the Diogenidae family, and of *Pagurus longicarpus* and *P. annulipes* studied by Thompson (1903), *P. samuelis* by Coffin (1960), *P. bernhardus* by Carvacho (1988), *P. kennerlyi* and *P. ochotensis* by McLaughlin *et al.* (1989, 1992) of the Paguridae family, respectively.

In the present study, the first five juvenile instars of *Pagurus criniticornis* were analyzed morphologically. The zoea and megalopa of this crustacean have been previously described by Hebling and Brossi-Garcia (1981). The relationship between shield length and propodus length of the right chelipod of 25 postlarval instars was studied in parallel to evaluate the growth pattern and the instars, which characterize it.

Material and Methods

Pagurus criniticornis (Dana, 1852) is a shallow water (0-50 m) species which has been reported to occur in the Gulf of Mexico, Caribbean, Brazil (from Pernambuco to Rio Grande do Sul), Uruguay and Argentina (Hebling and Rieger, 1986; Melo, 1999).

Ovigerous females were collected in the Araçá beach, São Sebastião (23°40'7"S - 45°23'9"W) from tide pools at ebb tide.

The procedure for larviculture up to the first juvenile instar was the same as described by Hebling and Brossi-Garcia (1981). Starting from the megalopa instar, all animals were maintained individually on Petri dishes containing 20 to 30 ml seawater of 30‰ salinity, in a climatic room at 25 ± 1°C under natural photoperiod conditions. Three to four small empty shells of *Littorina* sp. were offered to each megalopa in order to observe at what instar the animals start to occupy them as shelter. After the passage to the first juvenile instar, the shells were left there and replaced with larger ones as the animals underwent new ecdysis. Feeding consisted of *Artemia nauplii* offered daily after the water was changed and ecdysis were recorded. Dead animals and the exuviae of each instar were fixed and stored in 1:1 ethyl alcohol and glycerine. Animal length is expressed as shield length on the dorsal surface from the tip of the rostrum to midpoint of the posterior margin of shield. The length of the propodus of the right chelipod was measured between the median posterior margin of the propodus and the tip of the fixed finger.

The relationship between shield length (x) and the length of the propodus of the right chelipod (y) was plotted graphically according to the expression log y = log b + a log x, which is considered to be the simplest equation for the expression of data concerning relative growth (Hartnoll, 1978, 1982). The equations of the lines, which best fit the relationships detected, were calculated by the least squares method. The linear correlation coefficient of Pearson was also calculated.

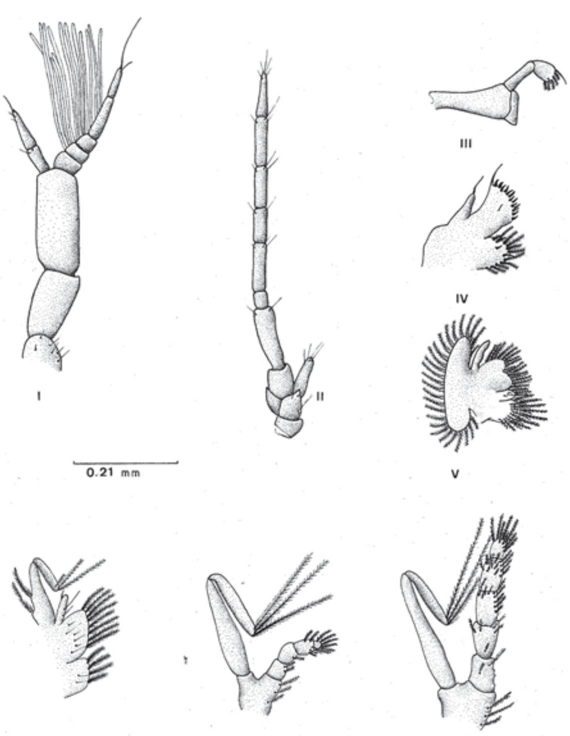

Figure 1. *Pagurus criniticornis*. First juvenile instar. I. Antennula. II. Antenna. III. Mandible. IV. Maxillula. V. Maxilla. VI. 1st Maxilliped. VII. 2nd Maxilliped. VIII. 3rd Maxilliped.

Results

1. Considerations on postlarval growth

The development of *P. criniticornis* was followed in the laboratory from larval hatching to the 34 th postlarval instar over a period of 2 years of rearing.

As is the case for the remaining species in the genus, *P. criniticornis* presents 4 zoeal instars and one megalopa instar. Mean duration was 4 days for each zoeal instar and 6.6 days for the megalopa instar (Hebling and Brossi-Garcia, *op. cit.*).

Juvenile or postlarval development starts from the ecdysis undergone by the megalopa, but its end is not as clearly determined as that of the larval instar. However, by analyzing relative growth, it is possible

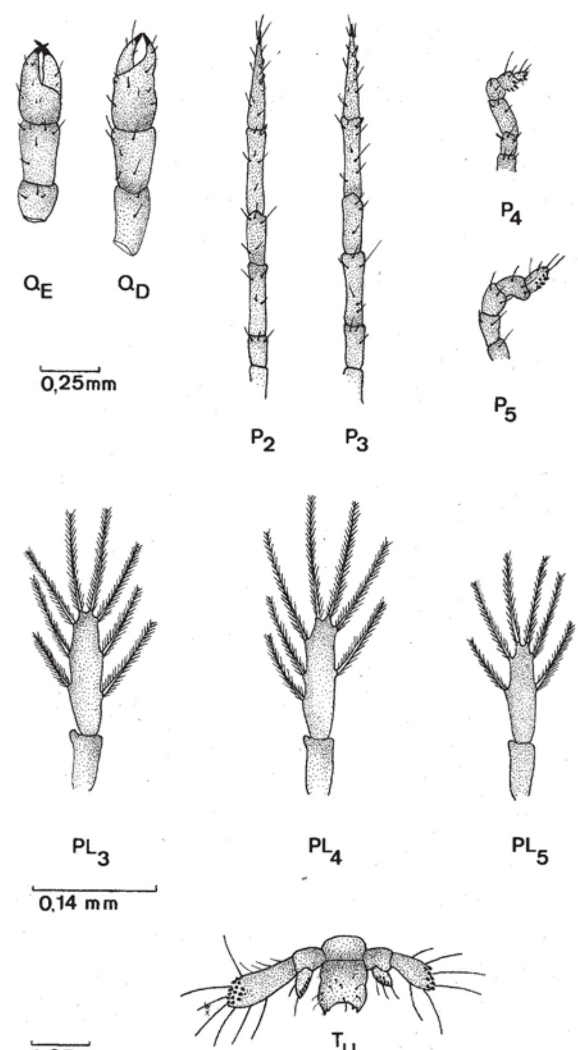

Figure 2. *Pagurus criniticornis*. First juvenile instar. Pereiopods (Q_E, Q_D, P_2, P_3, P_4, P_5); Pleopods (PL_3, PL_4, PL_5). Telson and uropods (T_U).

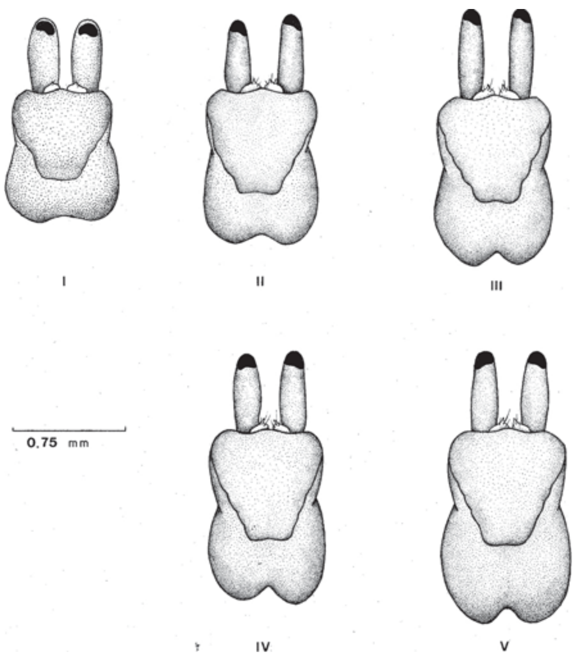

Figure 3. *Pagurus criniticornis.* Dorsal view of shield, 1st to 5th juvenile instars.

to characterize the juvenile, prepuberal and puberal instars. The first two are immature phases and the transition from one to the other is marked by a prepuberal molt which is not perceptible in isolated specimens but which is characterized by a small inflection in the growth curve. In contrast, the passage from the prepuberal to the puberal instar is marked by a sharp discontinuity in the growth curve caused by a critical molt (puberty molt) which precedes gonadal maturity. The passage between these phases always involves several intermolt cycles. The relationship between carapace length (x) and length of the propodus of the right chelipod (y) was investigated in 25 instars involving approximately 250 individuals, with an average of 10 individuals per instar.

Data concerning XXVI to XXXIV juvenile were not considered because they were represented by a single individual.

The pattern of point dispersal indicated that the proportion between x and y is modified at the same rate for the first 6 juvenile instars. The slope of the line (a = 1) characterizes growth of the isometric type.

The typical gonopores of each sex become clearly visible from the 7th juvenile instar, a time which coincides with a small inflection in the growth curve characterizing the transition between the juvenile and the prepuberal phase.

Males and females, identified by gonopore position starting from instar VII juvenile, showed significant differences in growth rate. Females presented growth of the isometric type (a = 1), and males presented growth of the allometric type. The correlation coefficients obtained for males (r = 0.9385, r^2 = 88%) and females (r = 0.9093, r^2 = 83%) were highly significant, adequately expressing the relationship investigated.

2. Biometry and morphology of postlarvae (Tables I and II)

The more marked morphological modifications were detected between the first 5 juvenile instars, which were analyzed in detail and whose structures were described and drawn. Table I presents growth between the successive juvenile instars and Table II the data on instar duration and animal survival from juvenile I-V.

2.1. First juvenile instar

Antennula (Fig. 1-I). Three-segmented peduncle. Exopod 5-segmented; 2nd, 3rd and 4th segments with countless aesthetascs (12-14). Endopod bisegmented with few minute setae.

Antenna (Fig. 1-II). Peduncle 5-segmented. Antennal flagellum with 6-8 articles with small simple setae.

Mandible (Fig. 1-III). Two cutting blades. Palp bisegmented; distal segment with plumose simple setae.

Maxillule (Fig. 1-IV). Endopod not segmented with 1 apical setae. Basal endite with approximately 13 spiniform projections arranged distally and subdistally, plus 2 or 3 simple setae. Coxal endite with 12 marginal plumose setae and 1 or 2 simple submarginal setae.

Maxilla (Fig. 1-V). Scaphognathite 0.22 mm long with 26 to 31 marginal plumose setae. Endopod not segmented, without setae. Bilobate basal and coxal endites. Distal lobe of the basal endite with approximately 8 setae and proximal lobe with 9. Distal lobe of the coxal endite with 3 marginal and 3 submarginal plumose setae. Proximal lobe of the coxal endite with 7 marginal and

Table I. *P. criniticornis*. Growth between the successive juvenile instars.

Juvenile Stages (J)	Individuals number	Shield length (mm)	Standard deviation	Growth factor
JI	08	0,55	0,02	
JII	07	0,59	0,05	1,07
JIII	09	065	0,06	1,10
JIV	10	0,6	0,07	1,06
JV	09	0,7	0,69	1,07
JVI	08	0,86	0,10	1,16
JVII	10	1,03	0,14	1,19
JVIII	13	1,24	0,11	1,20
JIX	13	1,33	0,13	1,07
JX	11	1,42	0,11	1,06
JXI	16	1,44	0,12	1,01
JXII	12	1,58	0,18	1,09
JXIII	12	1,68	0,25	1,06
JXIV	11	1,73	0,17	1,02
JXV	13	1,80	0,20	1,04
JXVI	11	1,90	0,08	1,05
JXVII	07	1,90	0,21	1,00
JXVIII	05	1,92	0,14	1,01
JXIX	09	2,00	0,19	1,04
JXX	09	2,00	0,17	1,00
JXXI	07	2,00	0,19	1,00
JXXII	07	2,10	0,17	1,05
JXXIII	07	2,10	0,22	1,00
JXXIV	09	2,11	0,50	1,00
JXXV	03	2,10	0,20	1,00

Table II. *P. criniticornis*. Duration and survival of I-V juvenile instars. X, mean duration (days) of each instar; (X), cumulative mean duration from eclosion; S.D., standard deviation; D-D', minimum and maximum duration; n, number of young alive; S%, percentage of survival from eclosion.

	JI	JII	JIII	JIV	JV
X (X)	7,4 (29)	6,5 (35,5)	7,0 (42,5)	7,5 (50)	6,1 (56,1)
S.D.	4,5	3,9	3,4	2,6	1,5
D-D'	4-21	4-23	4-16	4-13	3-9
N	35	33	32	30	20
S%	23,3	22	21,3	20	13,3

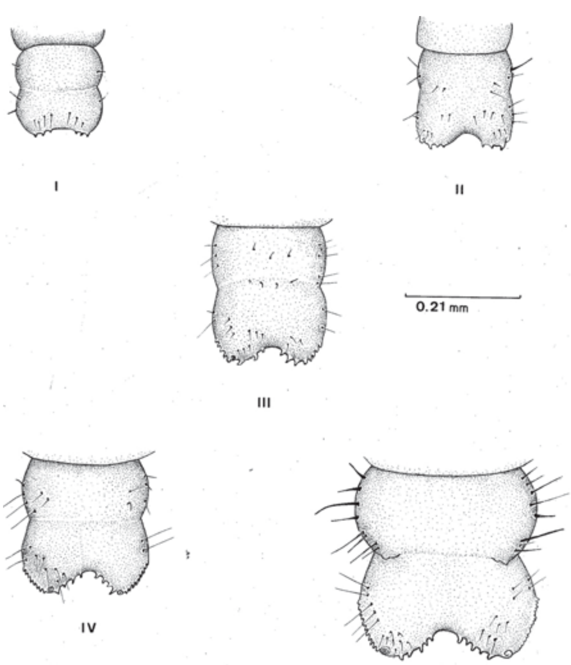

Figure 4. *Pagurus criniticornis*. Telson, 1st to 5th juvenile instars.

approximately 9 submarginal plumose setae. Minute non-plumose setae can be observed on the endite surface.

First Maxilliped (Fig. 1-VI). Exopod bisegmented with 3 to 4 plumose setae on the distal segment and 2 on the proximal segment. Endopod not segmented. Basal endite with 8 to 13 marginal plumose setae and coxal endite with 4 marginal plumose setae. Non-plumose setae are observed on the surface of the two endites.

Second Maxilliped (Fig. 1-VII). Exopod bisegmented. Distal segment forming an acute angle with the proximal segment and having 3 long plumose setae on the apex. Endopod 4-segmented with a larger number of plumose setae on the propodus (5) and dactyl (6). Basipod with 3 plumose setae.

Third Maxilliped (Fig. 1-VIII). Exopod unchanged in relation to the second maxilliped.

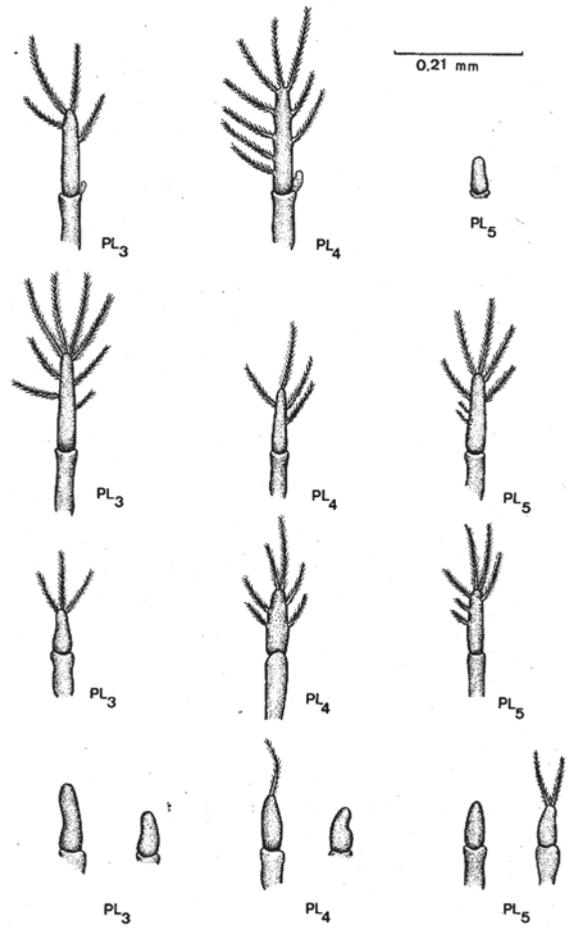

Figure 5. *Pagurus criniticornis.* Pleopods variability, 2nd juvenile instar (males).

Endopod 5-segmented. Ischiopod with a "crista dentate" having 3 to 6 teeth. The remaining segments of the endopod have a large number of plumose setae, specially on the carpo (9), propodus (11) and dactyl (8). Basipod with 3 plumose setae.

Pereiopods (Fig. 2-A). Subequal chelipods, the left (Q_E) being slightly smaller than the right one (Q_D). The second (P_2) and third (P_3) pereiopods are identical, with 2 small spines on the dactyl and 1 on propodus. P_4 and P_5 with countless tubercles on their respective dactyls.

Pleopods (Fig. 2-B). Three uneven pleopods, with only the exopod considerably developed and having 4 to 8 plumose setae for Pl_3 and Pl_4 and 4 to 6 for Pl_5.

Uropods (Fig. 2-C). Left uropod (0.5 mm) larger than the right one (0.3 mm). Both with countless tubercles and approximately 6 to 7 marginal plumose setae. Endopods also having tubercles and setae, but in smaller numbers.

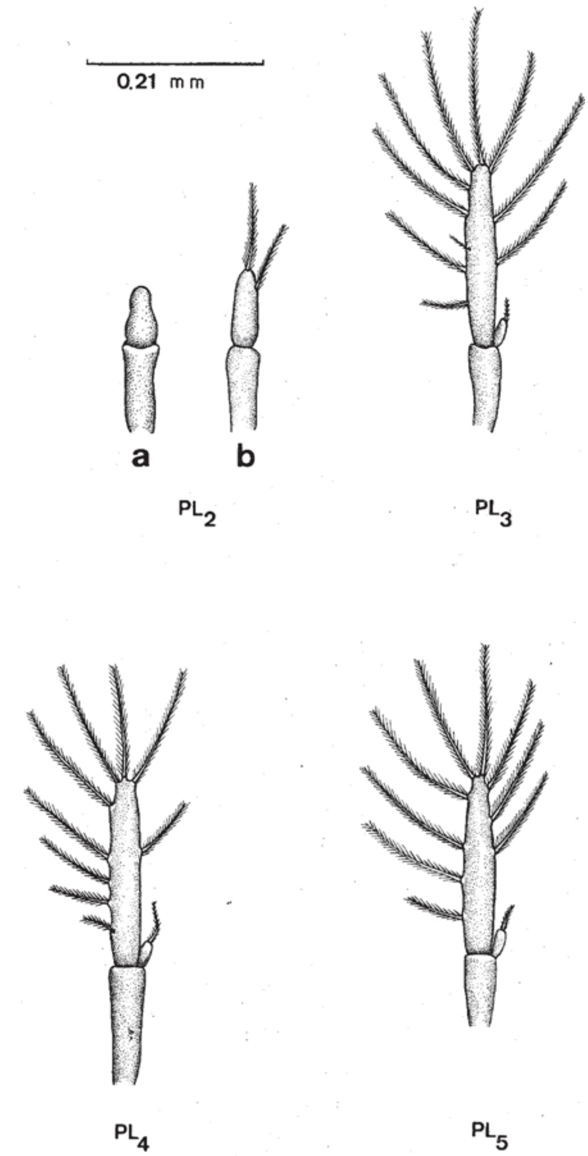

Figure 6. *Pagurus criniticornis.* Pleopods variability, 2nd juvenile instar (females).

2.2. Second to fifth juvenile instars (Tables III, IV, and V)

Tables III and IV show the evolution of the major morphological traits of easy visualization that permit the identification of the first 5 juvenile instars.

Pleopods (Table V and Figs. 2-B and 5 to 11). The ontogeny of the pleopods of *P. criniticornis,* analyzed for the first instars of juvenile development, permits sex identification before the onset of the gonopores.

As shown in Table V, during the first juvenile instar all specimens analyzed presented three odd pleo-

Table III. *P. criniticornis*. Measurements (mm) of the shield and of the eye stalks, from the 1st to the 5th juvenile instar. (n = 10)

	JI	JII	JIII	JIV	JV
shield	0,56	0,59	0,65	0,70	0,75
length	(0,54-0,58)	(0,53-0,69)	(0,52-0,72)	(0,64-0,83)	(0,64-0,83)
shield	0,58	0,61	0,66	0,71	0,76
width	(0,52-0,60)	(0,55-0,70)	(0,55-0,76)	(0,65-0,82)	(0,64-0,84)
eye stalks	0,38	0,43	0,48	0,54	0,54
length	(0,32-0,44)	(0,40-0,52)	(0,44-0,56)	(0,52-0,56)	(0,48-0,60)
eye stalks	0,16	0,17	0,17	0,17	0,19
width	(0,15-0,20)	(0,15-0,20)	(0,16-0,18)	(0,16-0,19)	(0,16-0,20)

Table IV. *P. criniticornis*. Morphological traits that permit the identification of the 1st to 5th juvenile instar. (n = 10)

	JI	JII	JIII	JIV	JV
number of segments of the antennular exopod	5	5	7	7	8
	(5-7)		(7-8)	(7-10)	
number of segments of the antennular endopod	2	2	2	2-3	2-3
number of articles of the antennal flagellum	6	8	11	13	15
	(6-8)	(6-11)	(10-13)	(11-15)	(12-18)
number of marginal setae of the scaphognatithe of the maxilla	27	34	45	47	61
	(27-31)	(27-47)	(40-48)	(38-58)	(46-71)
number of teeth of the "crista dentata" of the 3rd maxilliped	6	7	8	9	9
	(5-6)	(6-8)	(7-10)	(8-10)	(8-10)

Table V. *P. criniticornis*. Pleopods (Pl_2-Pl_5) from the 1st to the 5th juvenile instar.

		JI	JII	JIII	JIV	JV
Pl_2	male	absent	absent	absent	absent	absent
	female	absent	rudimentary or little developed	rudimentary	rudimentary	rudimentary
Pl3	male	well developed	rudimentary, little or well developed	rudimentary or well-developed	well-developed	well-developed
	female	well-developed	well-developed	well-developed	well-developed	well-developed
Pl4	male	well-developed	rudimentary, little or well developed	rudimentary, little or well developed	well-developed	well-developed
	female	well-developed	well-developed	well-developed	well-developed	well-developed
Pl5	male	well-developed	rudimentary, little or well developed	rudimentary, little or well developed	little or well developed	well-developed
	female	well-developed	well-developed	well-developed	well-developed	well-developed

pods (Pl_3 to Pl_5) on the left side of the abdomen. These were characterized by a well-developed exopod with long marginal plumose setae and a forming endopod. Starting at the second juvenile instar (JII), Pl_2 continues to be absent in males and becomes visible in females, a situation that lasts until the adult stage.

The development of these appendages is marked by wide morphological variation, specially during the second and third juvenile instars, with a tendency to uniformity starting at juvenile IV. Thus, the Pl_3, Pl_4, and Pl_5 of juvenile II and III males may either appear as rudimentary organs or as little or well-developed organs. In the more rudimentary forms, there is only one clearly visible exopod of varying length (0.06 to 0.12 mm), but usually without setae. In the slightly more developed forms, exopod and protopod are equally visible, with no setae or with 1 or 2 apical plumose setae on the exopod. In contrast, in well-developed forms, endopods can usually be visualized in addition to an increase in number of setae and in size (Figs. 5, 7, 9 and 11-A).

Females present four odd pleopods (Pl_2, Pl_3, Pl_4 and Pl_5) on the left side of the abdomen, except during the first juvenile instar. Only Pl_2 can appear in the rudimentary or little developed form, whereas Pl_3, Pl_4 and Pl_5 are well developed (Figs. 6, 8,10 and 11-B).

Nauplius

Table VI. *P. criniticornis*. Comparison of morphology of the 1st juvenile stage of *P. criniticornis* with other *Pagurus* species.

JUVENILE I	*P. criniticornis*	*P. kennerlyi*	*P. ochotensis*	*P. bernhardus*
Antennule: number of exopod segments	05	05 (06)	05	06
Antennule: number of endopod segments	02	03	03	03
Antenna: number of flagellum articles	6-8	16	27-31	18-24
Mandibular palp	2-segmented	3-segmented	2-segmented	3-segmented
Scaphognatithe: number of marginal plumose setae	26-31	32-38	45-52	44
Maxilliped 1: number of exopod plumose setae	2 + 3 (4)	5 + 6 (7)	6 + 5 (6)	?
Maxilliped 2: number of exopod plumose setae	0 + 3	1 + 7	1 + 6	?
Maxilliped 3: number of exopod plumose setae	0 + 3	2 + 7	1 + 6	?

Discussion

Since the first studies on the metamorphosis of pagurids, there has always been a special interest regarding the moment and the sequence of the morphological alterations that occur in the symmetrical pleopods of the larvae, transforming them into asymmetric appendages of the adults.

With respect to this particular item, *P. criniticornis* loses the symmetry of its pleopods precociously, following the ecdysis of the megalopa to the first juvenile stage. In addition to losing all the pleopods on the right side, the second pleopod on the left side (Pl_2), also fails to appear in the first juvenile stage, with only the third, fourth and fifth (Pl_3, Pl_4 e Pl_5) developing fully in most individuals. Beginning in the second juvenile stage, although there is great variation in development and morphology, the pleopods are numerically constant, according to the sex of the animal, with the left Pl_2 appearing in the female but not in the male.

In *P. annulipes* and *P. longicarpus,* studied by Thompson (1903), the first juvenile stage presents a rudiment of the left Pl_2, and 19% of individuals still preserve one or more rudimentary pleopods

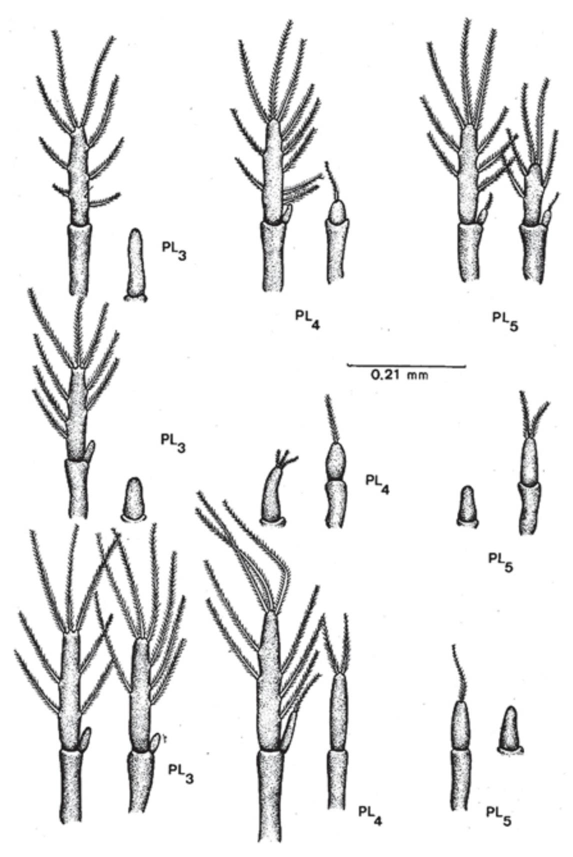

Figure 7. *Pagurus criniticornis*. Pleopods variability, 3rd juvenile instar (males).

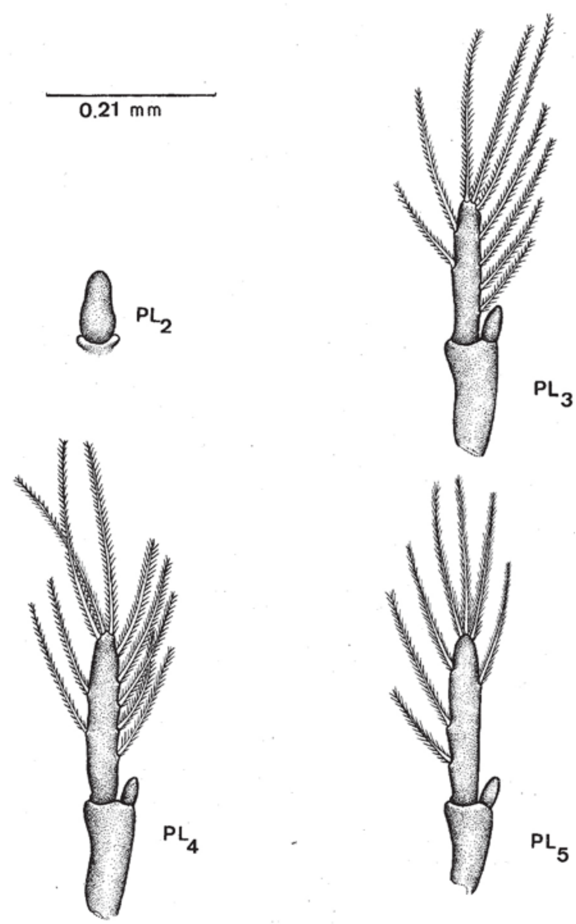

Figure 8. *Pagurus criniticornis*. Pleopods variability, 3rd juvenile instar (females).

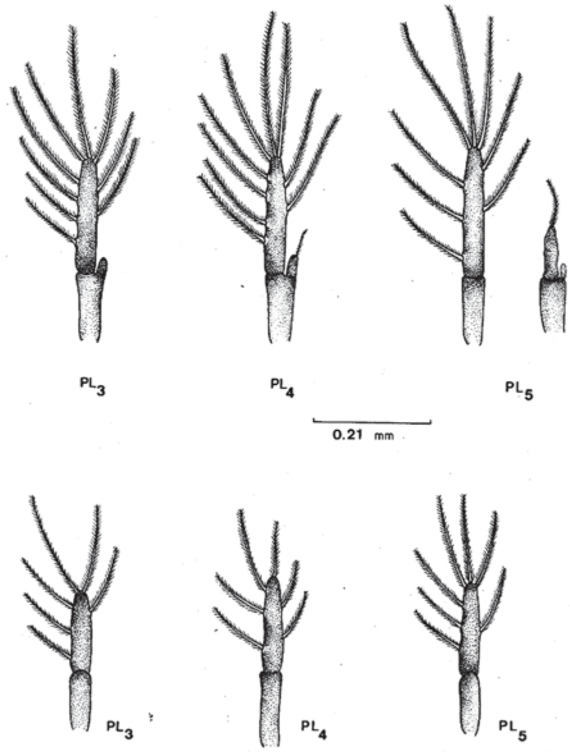

Figure 9. *Pagurus criniticornis.* Pleopods variability, 4th juvenile instar (males).

on the right side. The sex of these two species, as in *P. criniticornis,* can be identified in the second juvenile stage by the absence of the left Pl_2 in the males. In *P. samuelis,* according to Coffin (1960), only three odd pleopods appear in the first juvenile stage on the left side, similar to *P. criniticornis.* However, in this same species, studied in Japan by Kurata (1968), the first juvenile stage is illustrated with four pairs of rudimentary pleopods (Pl_2 a Pl_5). In *P. bernhardus,* according to Carvacho (1988), the rudimentary left Pl_2 is present in the first juvenile stage of the females, but there are apparently no pleopods on the right side. MacLaughlin *et al.* (1989) described and illustrated the first juvenile stage of the *P. kennerlyi* with the Pl_2 to Pl_5 pairs more markedly asymmetrical, and those on the right smaller, without an endopod. In the second stage, the pleopods on the right side, as well as the left Pl_2, are completely absent, or represented by minuscule bulbs. In *P. ochotensis,* according to McLaughlin *et al.* (1992), the first two juvenile stages have pleopod pairs, although those on the right side, and the left Pl_2, are quite reduced, uniramous.

Through analysis of the morphology of the other appendages of the first juvenile stages in the species of *Pagurus,* whose development is known, *P. criniticornis* was found to be more simplifies with respect to the number of segments and bristles that compose these structures. The more significant difference are presented in the Table VI.

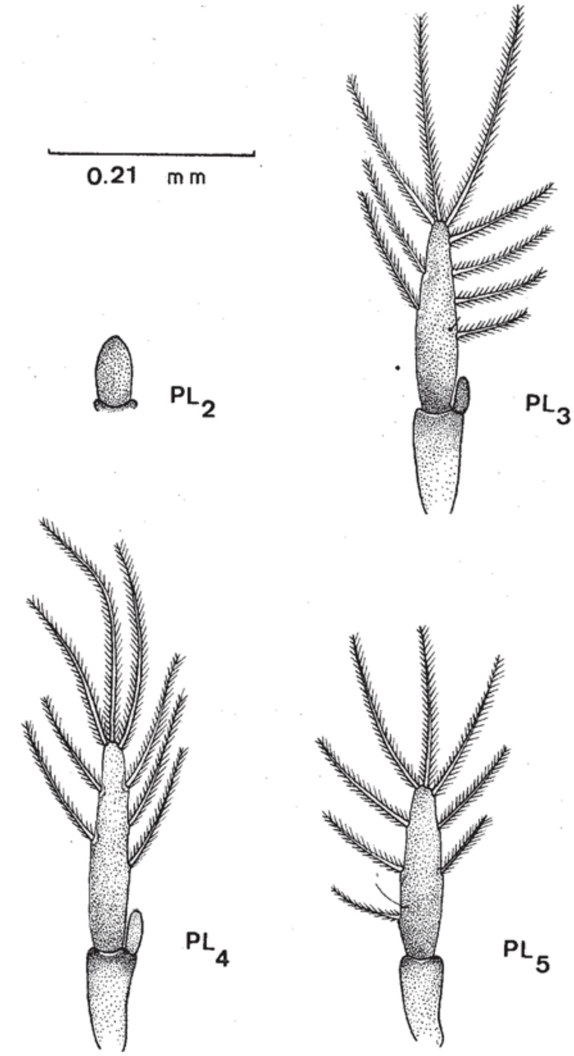

Figure 10. *Pagurus criniticornis.* Pleopods variability, 4th juvenile instar (females).

Considering the increments in size between the successive ecdysis, it can be inferred that *P. criniticornis* should reach sexual maturity around the XII stage, when the animals acquires a shield equivalent in length to that found in the smaller ovigerous females collected in nature, which presumably should occur six months after eclosion.

On the other hand, the laboratory cultures demonstrate that, although the animals survive for close to two years, their carapaces do not exceed 2.2 mm in length, which in nature, can reach 3 mm in the females and 4,5 mm in the larger males.

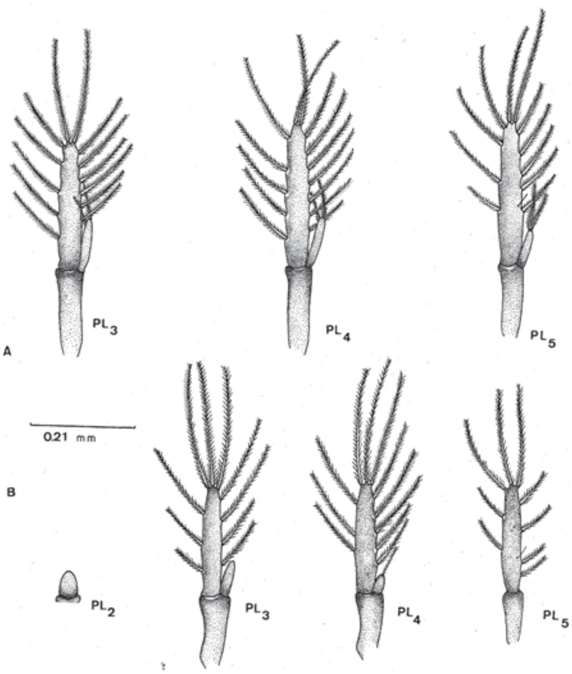

Figure 11. *Pagurus criniticornis.* Pleopods variability, 5th juvenile instar (A, males; B, females).

References

Brossi-Garcia, A.L. 1987. Juvenile development of *Clibanarius sclopetarius* (Herbst, 1796) (Crustacea, Paguridea, Diogenidae) in the laboratory. Journal of Crustacean Biology, 7: 338-357.

Brossi-Garcia, A.L. 1988. Juvenile development of *Clibanarius vittatus* (Bosc, 1802) (Crustacea, Decapoda, Anomura) in the laboratory. Crustaceana, 54: 294-313.

Carvacho, A. 1988. Developpement juvénile de *Pagurus bernhardus* L. (Crustacea, Decapoda). Cahiers de Biolgie Marine – France, 29: 109-133.

Coffin, H.G. 1960. The ovulation, embryology and developmental stages of the hermit crab *Pagurus samuelis* (Stimpson). Walla Walla College Publications, 25: 1-30.

Dechancé, M. 1958. Caractérization de la glaucothoe et des premiers stades Pagure chez *Clibanarius misanthropus* (Risso) (Crust. (Décapode Anomoure). Comptes Rendus des Séances de L´Académie des Sciences Paris, 246: 839-842.

Forest, M.J. 1954. Sur les premiers stades post-larvaires du Pagure *Dardanus pectinatus* (Ortman). Comptes Rendus des Séances de L´Académie des Sciences Paris, 239: 1697-1699.

Hartnoll, R.G. 1978. The determination of relative growth in Crustacea. Crustaceana, 34: 281-293.

Hartnoll, R.G. 1982. Growth. In: Bliss, D.E. (ed). The biology of Crustacea. Embryology, morphology and genectics. Academic Press, New York, vol.2.

Hebling, N.J. and Brossi-Garcia, A.L. 1981. Desenvolvimento pós-embrionário de *Pagurus criniticornis* (Dana, 1852) (Crustacea, Paguridae) em laboratório. Revista Brasileira de Biologia, 41: 765-779.

Hebling, N.J. and Rieger, P.J. 1986. Os ermitões (Crustacea, Decapoda: Paguridae e Diogenidae) do litoral do Rio Grande do Sul, Brasil. Atlântica Rio Grande, 8: 63-77.

Kurata, H. 1968. Larvae of Decapoda Anomura of Arasaki, Sagami Bay. I. *Pagurus samuelis* (Stimpson) Paguridae. Bulletin of the Tokay Regional Fisheries Research Laboratory. Japan, 55: 256-269.

McLaughlin, P.A., Gore, R.H. and Buce, W.R. 1989. Studies on the Provenzanoi and other Pagurid groups. III. The larval and early juvenile stages of *Pagurus kennerlyi* (Stimpson) (Decapoda: Anomura: Paguridae) reared in the laboratory. Journal of Crustacean Biology, 9: 626-644.

McLaughlin, P.A., Crain, J.A. and Gore, R.H. 1992. Studies of the Provenzanoi and other pagurid groups. VI. Larval and early juvenile stages of *Pagurus ochotensis* Brandt (Decapoda, Anomura, Paguridae) from a northeastern Pacific population, reared under laboratory conditions. Journal of Natural History, 26: 507-531.

Melo, G.A.S. de 1999. Manual de identificação dos Crustacea Decapoda do Litoral Brasileiro: Anomura, Thalassinidea, Palinuridea e Astacidea. São Paulo: Ed. Plêiade, 1999. 551p.

Provenzano, A.J. and Rice, A.L. 1966. Juvenile morphology and the development of taxonomic characters in *Paguristes sericeus* A. Milne-Edwards (Decapoda, Diogenidae). Crustaceana, 10: 53-69.

Thompson, M.T. 1903. The metamorphoses of the hermit crab. Proceedings of the Boston society of Natural History, 31: 147-209.

Received: December 2005
Accepted: September 2006