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Zoeal Stages of Two Majoidea Crabs, *Doclea muricata* (Herbst, 1788) and *Acanthonyx limbatus* (A. Milne Edwards, 1862), Reared under Laboratory Conditions

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Abstract: The early developmental stages of the Pisidae crab, *Doclea muricata* (Herbst, 1788), and the Epialtidae crab, *Acanthonyx limbatus* (A. Milne Edwards, 1862), from Pakistan are described for the first time. Zoeae I and II of *Doclea muricata* and *Acanthonyx limbatus* were obtained from field-collected ovigerous females that were kept under laboratory conditions. These larval stages are described, illustrated, and compared with those of earlier studies of known species of the genera.

The larval stages of these 2 species can be differentiated by the presence of spines on the posterolateral angles of abdominal somites 3-5 and a pair of lateral spines on the telson furca in *A. limbatus*, whereas these spines are absent in *D. muricata*. As the present study was based only on early larval stages, complete information about the development of these species will be needed in the future.

Key Words: Majoidea, Pisidae, Epialtidae, Zoeal stages, *Doclea muricata*, *Acanthonyx limbatus*, Pakistan

Introduction

According to Provenzano and Brownell (1977), there are approximately 900 species of majid crabs, all of which are marine and widely distributed throughout the world. Forty-eight majid crab species were reported from Pakistani waters of the northern Arabian Sea by Kazmi (1997). The genus *Doclea* contains 3 species, *D. gracilipes*, *D. aduncus*, and *D. muricata*, and the genus *Acanthonyx* is also represented by 3 species, *A. limbatus*, *A. elongates*, and *A. scutellatus*.

Knowledge of the larval morphology of Pakistani majoid crabs is restricted to 7 species [*Elamena sindensis* and *Schizophrys aspera* (Tirmizi and Kazmi, 1987), *Micippa platipes* and *Achaeus lacertosus* (Siddiqui, 1996, 1999, respectively), *Schizophrys pakistaniensis* (Siddiqui and Kazmi, 2000), and *Menaethiops nodulosus* and *M. bicornis* (Ghory and Siddiqui, 2002, 2005, respectively)].

Morphological studies of larvae have played an important role in the taxonomy of majoid crabs, which was previously discussed by Campodonico and Guzman (1972), Rice (1980), Webber and Wear (1981), Negreiros-Fransozo and Fransozo (1991), and Guerao and Abello (1996).

The present study was based on the morphological study of zoea I and II of the families Pisidae and Epialtidae; in general, both families have only 2 zoeal stages (Rice, 1980) followed by a megalopal stage that completes their developmental cycle. Herein *Doclea muricata* and *Acanthonyx limbatus* are described for the first time from Pakistan. We present illustrations and descriptions, and also a comparison with previously described larvae of Indian, Brazilian, and Spanish species of the respective genera given in Tables 1 and 2.

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Table 1. Comparison between zoea I and zoea II of *Doclea muricata* (Herbst, 1788) from Pakistan, and *D. muricata* (Herbst, 1788), *D. hybrida* (Fabricius, 1798), *D. ovis* (Herbst, 1788), and *D. gracilipes* Stimpson, 1857, all from India.

Zoea I					
Characters	<i>D. muricata</i> Present study Pakistan	<i>D. muricata</i> Kirishna & Kannupandi (1987) India	<i>D. hybrida</i> Sankolli & Shenoy (1975) India	<i>D. ovis</i> Mohan & Kannupandi (1985) India	<i>D. gracilipes</i> Kirishna & Kannupandi (1988) India
Carapace					
rostral spine:	present	present	present	present	absent
Antennule:					
aesthetascs	2	4	4	4	4
setae	2	1	2	2	absent
Maxillule:					
endopod	2-segmented	non-segmented	non-segmented	non-segmented	2-segmented
Maxilla					
setae:					
coxal endite	6 + 3	3	5 + 4	4 + 5	3 + 3
basial endite	5 + 4	2 + 3	5 + 4	5 + 4	3 + 3
endopod	5	4	5	5	5
scaphognathite	11 + 1	16	11	12	13
Maxilliped I					
setae:					
basis	10	8	8	9	9
endopod	13	12	14	13	15
Maxilliped II					
setae:					
endopod	6	4	5	5	4
Zoea II					
Characters	<i>D. muricata</i> Present study Pakistan	<i>D. muricata</i> Kirishna & Kannupandi (1987) India	<i>D. hybrida</i> Sankolli & Shenoy (1975) India	<i>D. ovis</i> Mohan & Kannupandi (1985) India	<i>D. gracilipes</i> Kirishna & Kannupandi (1988) India
Carapace					
rostral spine:	present	present	present	present	absent
Antennule:					
aesthetascs	5	5	8	5	6
setae	absent	1	absent	2	1
Maxillule					
setae:					
coxal endite	7	6	8	7	7
basial endite	7	8	9	9	9
exopod seta	absent	1	absent	1	absent
Maxilla					
setae:					
coxal endite	5 + 4	4 + 4	5 + 5	5 + 4	5 + 3
basial endite	5 + 4	4 + 4	5 + 5	6 + 5	5 + 3
scaphognathite	17-24	30	25-26	26	27

Table 2. Comparison between zoea I and zoea II of *Acanthonyx limbatus* (A. Milne Edwards, 1862) from Pakistan, and *A. petiveri* H. Milne Edwards, 1834 from Brazil and *A. lunulatus* (Risso, 1816) from Spain.

Zoea I			
Characters	<i>A. limbatus</i> Present study Pakistan	<i>A. petiveri</i> Hiyodo, Fransozo & Fransozo (1994) Brazil	<i>A. lunulatus</i> Guerao & Abello (1996) Spain
Carapace:			
dorsal spine	well developed	very small	small
Antennule:			
aesthetascs	2	3	3
setae	2	1	1
Maxillule			
setae:			
coxal endite	6	7	7
basial endite	8	7	7
endopod	with 7 setae	with 4 setae	with 4 setae
Maxilla			
setae:			
coxal endite	3 + 3	3 + 5	5 + 3
basial endite	5 + 5	4 + 5	5 + 4
scaphognathite	13 + 1	11	11 + 1
Zoea II			
Characters	<i>A. limbatus</i> Present study Pakistan	<i>A. petiveri</i> Hiyodo, Fransozo & Fransozo (1994) Brazil	<i>A. lunulatus</i> Guerao & Abello (1996) Spain
Antennule			
setae:	1	2	2
Maxillule			
setae:			
basial endite	9	10	10
endopod	7	4	4
Maxilla			
setae:			
coxal endite	4 + 4	4 + 5	5 + 3
basial endite	4 + 6	3 + 5	5 + 5
scaphognathite	28	22	22

Materials and Methods

An ovigerous *D. muricata* female was collected from Pitti Creek using a Tukri net on 27 September 1994. In addition, an ovigerous *A. limbatus* female was collected by hand from Manora Island on 4 December 1994. These specimens were reared in laboratory aquaria with filtered seawater (salinity: 32‰-35‰) at room temperature (15-30 °C).

After 5 days of incubation in the laboratory, *D. muricata* larvae hatched on 2 October 1994. The first zoeae of *A. limbatus* were obtained on 26 December 1994. For each species, 10 first stage larvae were placed into each of 5 glass beakers filled with 500 ml of filtered seawater (salinity: 32‰-35‰) at 15-30 °C. Each beaker was examined daily for exuviae and dead larvae. All the *D. muricata* first zoeae molted into

second zoeae on 7 October 1994. *A. limbatus* zoeae molted to the second stage on 30 December 1994. Upon molting, surviving larvae were transferred to clean beakers filled with freshly collected filtered seawater and provided with newly hatched *Artemia* nauplii.

Exuviae and dead larvae were preserved in 5% formalin. Temporary slides of each stage were also made by using 5% formalin plus glycerin in a ratio of 1:3. The specimens were dissected with a tungsten needle under an Ogawa Seiki dissecting microscope at high magnification (WF 10 × 4.5). An Olympus BH2 microscope (magnification 1.25 × 4, 10, and 20) with Nomarski interference contrast and a *camera lucida* attachment were used for illustrations. Measurements of each stage were taken with a micrometer. Total length (TL) was determined by adding the carapace length (CL), measured from the tip of the rostral spine to the posterior midpoint of the carapace, to the abdominal length, the center of the second abdominal somite to the mid-posterior margin of the telson.

The spent females and remaining larvae were deposited in the Marine Reference Collection and Resource Centre, University of Karachi, catalogue no. BRAC. 713, 714.

Results

The detailed morphology of the first and second zoeal stages of each species, *D. muricata* and *A. limbatus*, are given below. Table 1 summarizes the successive development of the morphological characters of the 2 species.

Doclea muricata (Herbst, 1788)

Zoea I (Figure 1a-k).

Size: CL = 0.51-0.62 mm; TL = 1.99-2.01 mm (5 specimens examined). Duration: 6 days.

Carapace (Figure 1a, a', a''): Dorsal spine long and curved backwards; rostral spine small; lateral spines absent; one pair of posterodorsal setae present; posterolateral margin with 6 + 6 plumose setae (Figure 1a). Eyes sessile.

Antennule (Figure 1b): Uniramous, terminally with 2 aesthetascs and 2 setae, with a protuberance feature of inner ramous.

Antenna (Figure 1c): Biramous, protopod developed; distal 1/6 of the length as a spinous process; exopod with 3 terminal cuspidate setae; endopod rudimentary.

Mandible (Figure 1d): With well-developed incisor and molar processes; palp absent.

Maxillule (Figure 1e): Coxal endite with 7 plumodenticulate setae; basal endite with 4 cuspidate and 3 plumodenticulate setae; endopod 2-segmented with 1, 2 + 4 plumodenticulate setae from proximal to distal segments, respectively.

Maxilla (Figure 1f): Coxal and basal endites bilobed with 6 + 3 and 5 + 4 plumodenticulate setae from proximal to distal segments, respectively; endopod non-segmented with 5 plumodenticulate setae; scaphognathite with 11 marginal plumose setae and with a posterior plumose process.

Maxilliped I (Figure 1g): Coxa with 1 seta; basis with 10 plumodenticulate setae (arranged 2 + 2 + 3 + 3); endopod 5-segmented with 3, 2, 1, 2, and 1 + 4 plumodenticulate setae from proximal to distal segments, respectively; exopod partially 2-segmented, proximal segment with 4 terminal natatory plumose setae.

Maxilliped II (Figure 1h): Coxa with 1 seta; basis with 3 plumodenticulate setae; endopod 3-segmented with 0, 1, 2 + 3 plumodenticulate setae from proximal to distal segments, respectively; exopod partially 2-segmented, proximal segment with 4 terminal natatory plumose setae.

Maxilliped III (Figure 1i): Rudimentary, biramous.

Periopods I-V (Figure 1j): Rudimentary.

Abdomen (Figure 1k): Somite 1 with 2 dorso-median setae; somites 2-5 with 2 posterodorsal setae; somite 2 with a pair of forward-directed, curved dorsolateral spine-like knobs; posterolateral angles of somites 2-5 rounded.

Telson (Figure 1k): Forked, inner posterior margin with 3 pairs of spinulate setae; furca without lateral spines.

Zoea II (Figures 2a- k).

Size: CL = 0.53-0.65 mm; TL = 2.02-3.0 mm (3 specimens examined). Duration of survival: 3 days.

Carapace (Figure 2a,a'): Unchanged setation, except a slight increase in size and posterolateral margins with 10 + 10 setae. Eyes stalked.

Antennule (Figure 2b): Biramous, outer ramous with 5 aesthetascs, inner ramous rudimentary.

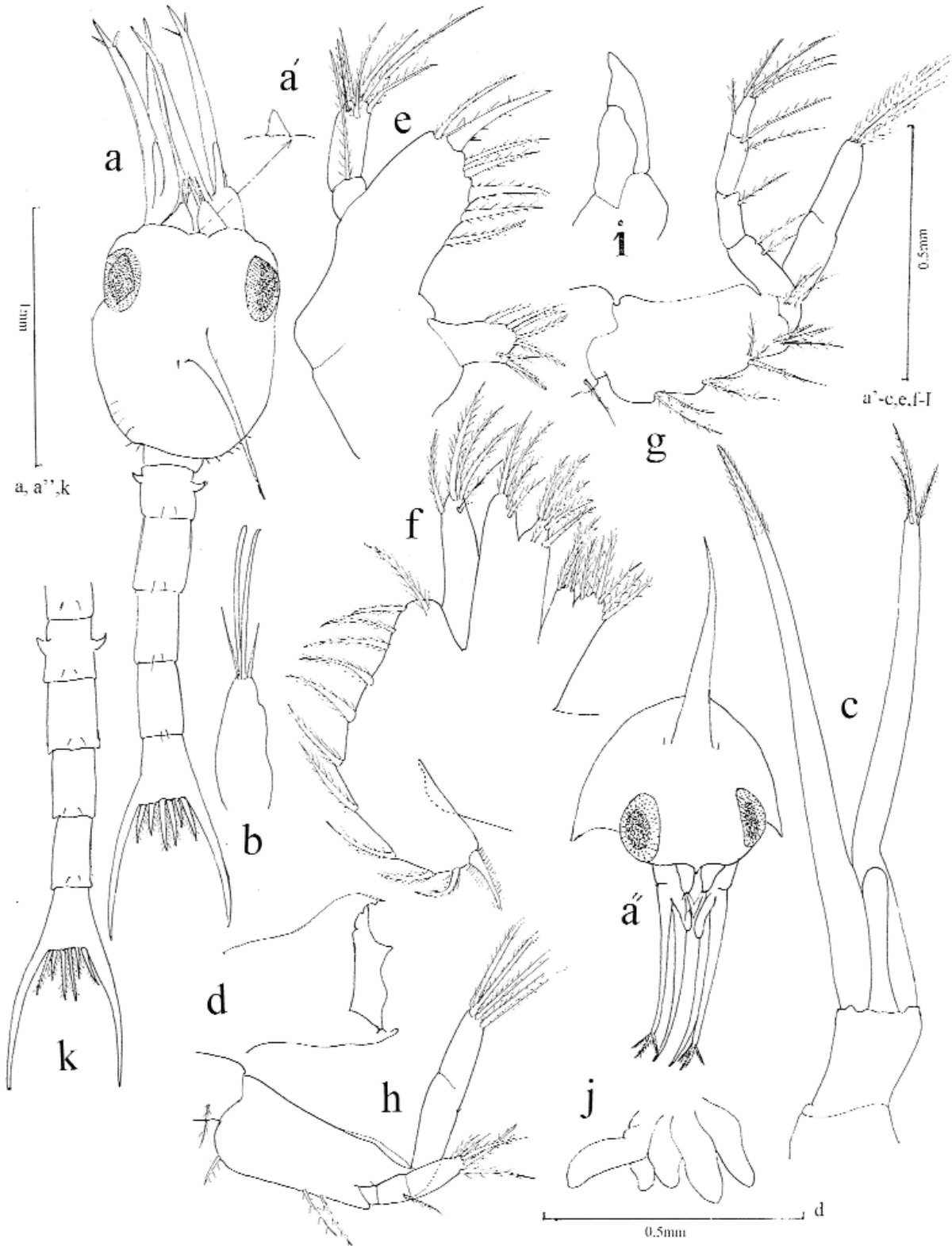


Figure 1. *Doclea muricata* (Herbst, 1788). Zoea I: (a) dorsal view; (a') rostrum, enlarged; (a'') dorso-frontal view; (b) antennule; (c) antenna; (d) mandible; (e) maxillule; (f) maxilla; (g-i) maxillipeds I-III; (j) periopods I-V; (k) abdomen with telson, dorsal view.

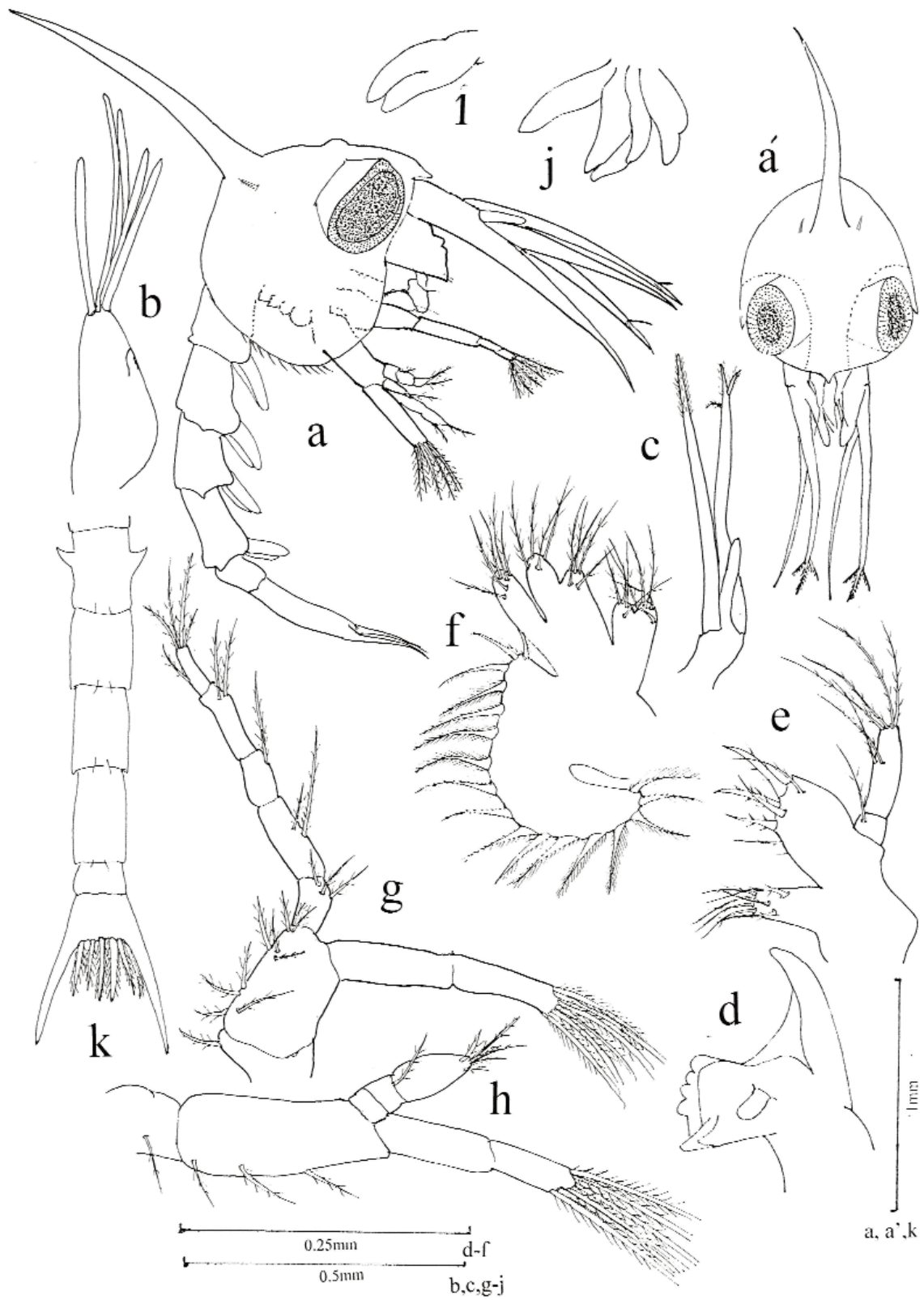


Figure 2. *Doclea muricata* (Herbst, 1788). Zoea II: (a) lateral view; (a') dorso-frontal view; (b) antennule; (c) antenna; (d) mandible; (e) maxillule; (f) maxilla; (g-i) maxillipeds I-III; (j) pereopods I-V; (k) abdomen with telson, dorsal view.

Antenna (Figure 2c): Protopod, exopod, and endopod unchanged.

Mandible (Figure 2d): Palp present.

Maxillule (Figure 2e): Unchanged.

Maxilla (Figure 2f): Coxal and basal endites bilobed, each with 5 + 4 plumodenticulate setae from proximal to distal segments, respectively; endopod unchanged; scaphognathite with 17-24 marginal plumose setae.

Maxilliped I (Figure 2g): Coxa, basis, and endopod unchanged; exopod partially 2-segmented, proximal segment with 6 terminal natatory plumose setae.

Maxilliped II (Figure 2h): Coxa, basis, and endopod unchanged; exopod partially 2-segmented, proximal segment with 6 terminal natatory plumose setae.

Maxilliped III (Figure 2i): Unchanged.

Periopods I-V (Figure 2j): Buds slightly developed.

Abdomen (Figure 2k): Abdomen with 6 somites and a pair of pleopodal buds on somites 2-5.

Telson (Figure 2k): Unchanged.

***Acanthonyx limbatus* A. Milne Edwards, 1862**

Zoea I (Figure 3a- k).

Size: CL = 0.74-0.92 mm, TL = 1.97-2.74 mm (5 specimens examined). Duration: 5 days.

Carapace (Figure 3a,a'): Dorsal spine long and curved backwards; rostral spine small; lateral spine absent; 1 pair of posterodorsal setae present; posterolateral margins with 11 + 11 plumose setae. Eyes sessile.

Antennule (Figure 3b): Uniramous, terminally with 2 aesthetascs and 2 setae.

Antenna (Figure 3c): Biramous, protopod developed, distal 1/6 of the length as a spinous process; exopod with 3 terminal cuspidate setae; endopod rudimentary.

Mandible (Figure 3d): With well-developed incisor and molar processes; palp absent.

Maxillule (Figure 3e): Coxal endite with 6 plumodenticulate setae; basal endite with 3 cuspidate and 5 plumodenticulate setae; endopod 2-segmented with 1, 2 + 4 plumodenticulate setae from proximal to distal segments, respectively.

Maxilla (Figure 3f): Coxal and basal endite bilobed with 3 + 3 and 5 + 5 plumodenticulate setae from proximal to distal lobes, respectively; endopod with 5

plumodenticulate setae; scaphognathite with 13 marginal plumose setae and with a posterior plumose process.

Maxilliped I (Figure 3g): Coxa without setae; basis with 10 plumodenticulate setae (arranged 2 + 2 + 3 + 3); endopod 5-segmented with 3, 2, 1, 2, and 4 + 1 plumodenticulate setae from proximal to distal segments, respectively; exopod 2-segmented, proximal segment with 4 terminal natatory plumose setae.

Maxilliped II (Figure 3h): Coxa without setae; basis with 3 plumodenticulate setae; endopod 3-segmented with 0, 1, 2 + 2 plumodenticulate setae; exopod 2-segmented, proximal segment with 4 terminal natatory plumose setae.

Maxilliped III (Figure 3i): Rudimentary, biramous.

Periopods I-V (Figure 3j): Rudimentary; periopod I chelate.

Abdomen (Figure 3k): 5 somites, each with a pair of dorso-median setules on their posterior margin; somite 2 with a pair of forwardly directed, curved dorsolateral spine-like knobs; posterolateral angle of somite 2 rounded, somites 3-5 pointed and angles of somite 3 relatively enlarged.

Telson (Figure 3k): Forked, with a pair of lateral spines; inner posterior margin with 3 pairs of spinulate setae.

Zoea II (Figures 4a-l).

Size: CL = 1.37-1.75 mm; TL = 2.89-3.18 mm (5 specimens examined). Duration of survival: 2 days.

Carapace (Figure 4a): Unchanged, except for an increase in size; posterolateral margins with 16 + 16 plumose setae. Eyes stalked.

Antennule (Figure 4b): Biramous, outer ramous with 6 aesthetascs and 1 seta, inner ramous rudimentary.

Antenna (Figure 4c): Unchanged, except that endopod increased in size.

Mandible (Figure 4d): Palp present.

Maxillule (Figure 4e): Coxal endite with 7 plumodenticulate setae; basal endite with 5 cuspidate and 4 plumodenticulate setae; endopod 2-segmented with 1, 2 + 4 plumodenticulate setae from proximal to distal segments, respectively.

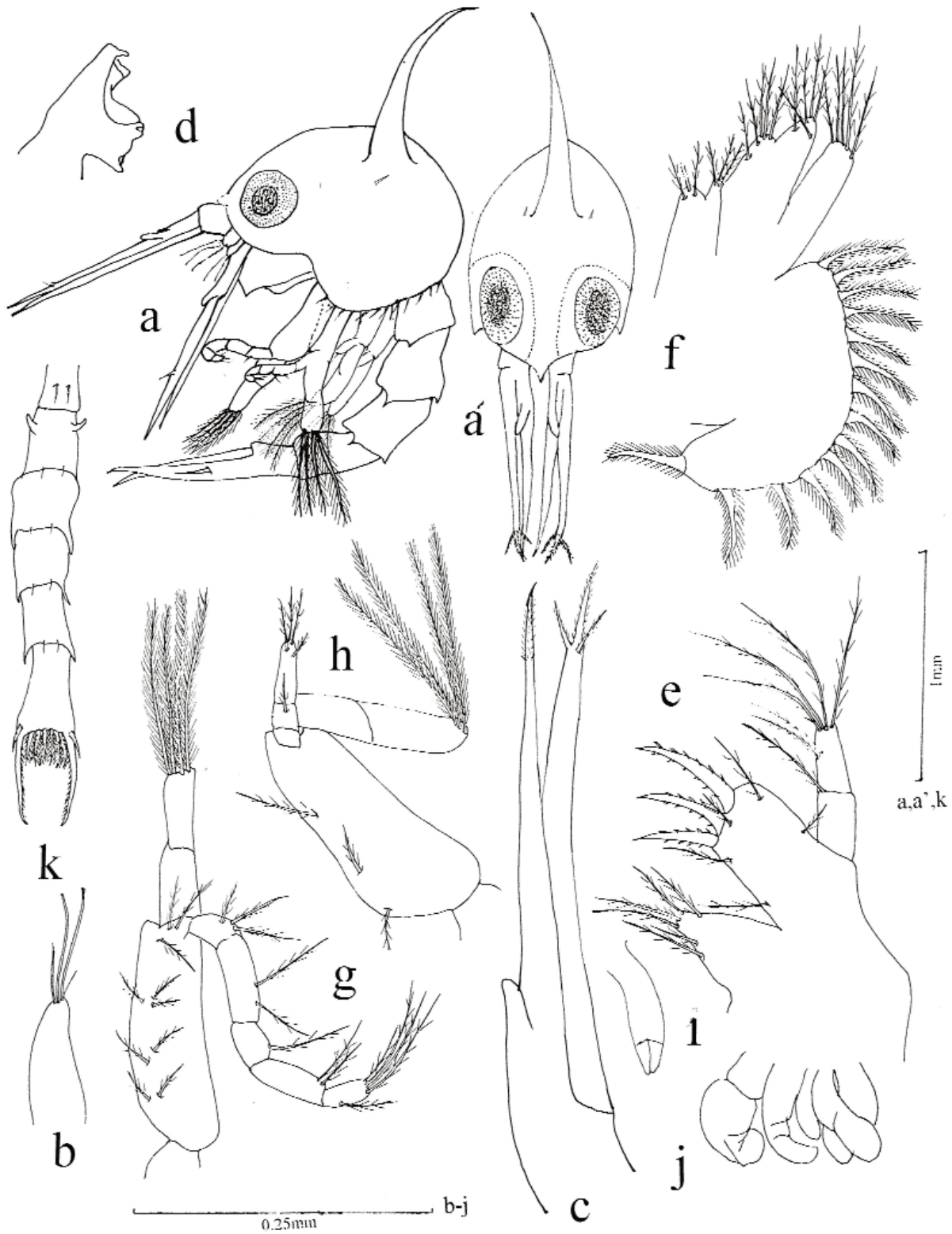


Figure 3. *Acanthonyx limbatus* (A. Milne Edwards, 1862). Zoea I: (a) lateral view; (a') dorso-frontal view; (b) antennule; (c) antenna; (d) mandible; (e) maxillule; (f) maxilla; (g-i) maxillipeds I- III; (j) pereopods I-V; (k) abdomen with telson, dorsal view.

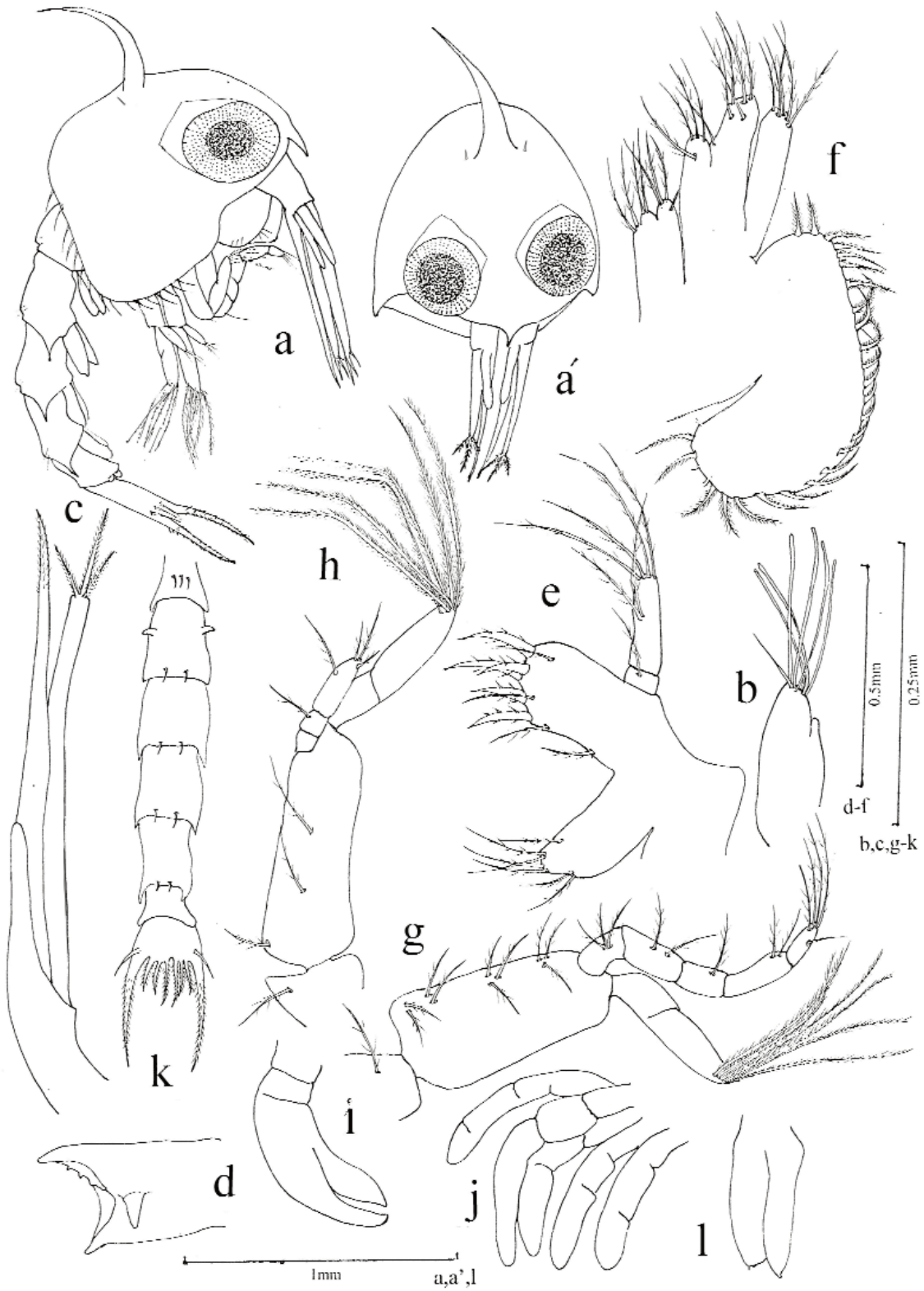


Figure 4. *Acanthonyx limbatus* (A. Milne Edwards, 1862). Zoea II: (a) lateral view; (a') dorso-frontal view; (b) antennule; (c) antenna; (d) mandible; (e) maxillule; (f) maxilla; (g-i) maxillipeds I-III; (j) pereopods I-V; (k) abdomen with telson, dorsal view; (l) pleopod I.

Maxilla (Figure 4f): Coxal and basal endite bilobed with 4 + 4 and 4 + 6 plumodenticulate setae from proximal to distal lobes, respectively; endopod unchanged; scaphognathite with 28 marginal plumose setae.

Maxilliped I (Figure 4g): Coxa with 1 seta; basis and endopod unchanged; exopod 2-segmented, proximal segment with 6 terminal natatory plumose setae.

Maxilliped II (Figure 4h): Coxa with 1 seta; basis with 3 plumodenticulate setae; endopod 3-segmented with 0, 1, 2 + 2 plumodenticulate setae; exopod 2-segmented, proximal segment with 6 terminal natatory plumose setae.

Maxilliped III (Figure 4i): Rudimentary, biramous.

Periopods I-V (Figure 4j): Rudimentary, buds increased in size; periopod I chelate.

Abdomen (Figure. 4k): Somite 1 with 3 and Somite 2-5 each with 2 dorso-median setules. Somites 2-5 each with a pair of pleopodal buds (Figure 4l).

Telson (Figure 4k): Unchanged.

Concluding Remarks

Zoeae of the 2 Pakistani species described herein, *Doclea muricata* and *Acanthonyx limbatus*, each differ from their congeners and even conspecifics in several important ways, as highlighted in Tables 1 and 2, and as described below. Zoea I and II of Pakistani *D. muricata* (present study) differed from those of Indian conspecifics described by Kirishna and Kannupandi (1987). Most notably, 2 antennular aesthetascs are present in zoea I of Pakistani *D. muricata*, whereas 4 are found in Indian conspecifics and in 3 other congeners (citations). In addition, maxillule of Pakistani *D. muricata* had 2-segmented endopodites, similar to *D. gracilipes*, while these are non-segmented in Indian *D. muricata*, *D. hybrida*, and *D. ovis*.

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Morphological differences were also evident between the *D. muricata* described here and elsewhere in *D. hybrida* (Sankolli and Shenoy, 1975), *D. ovis* (Mohan and Kannupandi, 1985), and *D. gracilipes* (Kirishna and Kannupandi, 1988). The small rostral spine present in zoea I and II of *D. muricata* (Pakistani and Indian), *D. hybrida*, and *D. ovis*, is absent in *D. gracilipes*. In addition, 5 antennular aesthetascs are present in zoeal II stage *D. muricata*, (Pakistani and Indian) and *D. ovis*, whereas 8 and 6 are present in *D. hybrida* and *D. gracilipes*, respectively

Acanthonyx limbatus larvae (zoea I and II) chiefly differ from Brazilian *A. petiveri* (Hiyodo, et al., 1994) and *A. lunulatus* in the size of the dorsal spine. The dorsal spine of *A. limbatus* is well developed, but it is very small in *A. petiveri* and *A. lunulatus*. Similarly, *A. limbatus* has 2 antennular aesthetascs, whereas *A. petiveri* and *A. lunulatus* have 3.

The number of maxillule, maxilla, and maxilliped setae in 5 species of the genus *Doclea* and 3 species of the genus *Acanthonyx* are very different, the details of setal differences are noted in Tables 1 and 2.

The present study shows that Pakistani and Indian *D. muricata* have some significant differences, which sheds doubt on their designation as a single species found in both areas; however, further studies are needed to confirm their species status.

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