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Larval development of *Chthamalus malayensis* (Cirripedia: Thoracica) reared in the laboratory

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Larvae of *Chthamalus malayensis* (Cirripedia: Thoracica) from Hong Kong were cultured in the laboratory. Larval development includes six naupliar stages and a non-feeding cypris stage. Larvae reached the cypris stage in 20 d at $\sim 21^\circ\text{C}$ compared to 14 d at $\sim 28^\circ\text{C}$. Morphological features including the cephalic shield, frontal horns, labrum, abdominal process, antennules, antennae and mandibles in all nauplius and cypris stages were described and illustrated using a combination of light microscopy and scanning electron microscopy. Attempts were made to compare morphological differences between the nauplii and cyprid of *C. malayensis* with those of other *Chthamalus* species including *C. stellatus*, *C. montagui*, *C. dentatus*, *C. fragilis*, *C. dalli*, *C. antennatus*, *C. fissus* and *C. challengerii*. The present description of the nauplii of *C. malayensis* is not in agreement with the previous description of this species.

INTRODUCTION

Chthamalus malayensis Pilsbry (1916) is an intertidal barnacle common in the Indo-Pacific region (Pope, 1965). In Hong Kong, *C. malayensis* inhabits the high intertidal on exposed, oceanic shores (Morton & Morton, 1983). The larvae of only nine *Chthamalus* species have been described, namely *C. stellatus* (Bassindale, 1936; Daniel, 1958; Burrows et al., 1999); *C. montagui* (Burrows et al., 1999); *C. dentatus* (Achituv, 1986); *C. malayensis* (Karande & Thomas, 1976); *C. fragilis* (Lang, 1979); *C. dalli* (Korn & Ovsyannikova, 1979; Miller et al., 1989); *C. antennatus* (Egan & Anderson, 1989); *C. fissus* (Miller et al., 1989) and *C. challengerii* (Lee, 1999). Although the larval development of *C. malayensis* has been reported from the Indian Ocean (Karande & Thomas, 1976), this description is not detailed, as light microscope examination alone of the larval development of barnacles has been found to be inadequate. To maximize taxonomic resolution, descriptions of barnacle larvae often involve the use of scanning electron microscopy (SEM) to investigate larval structures in more detail (e.g. frontal horns, surface sculpture, and antennular segment and carapace of the cyprid) and to discover more diagnostic characters of species (see Rainbow & Walker, 1976; Walker & Lee, 1976; Clare & Nott, 1994; Elfimov, 1995; Glenner & Høeg, 1995; Moysse et al., 1995; Walossek et al., 1996; Karande, 1999).

This paper presents observations made with both light microscopy and SEM of morphological characteristics of the nauplii and cypris larvae of *Chthamalus malayensis* reared in the laboratory. Morphological differences between the nauplii and cyprids of this species from Hong Kong and Mumbai, India (Karande & Thomas, 1976) are compared. A detailed comparison of the

described larvae of other members of the genus *Chthamalus* is also included.

MATERIALS AND METHODS

Larval rearing

Adult *Chthamalus malayensis* were collected in May–November 2000 from high intertidal rocks at Cape d'Aguilar, on the south-east coast of Hong Kong island. Egg masses containing embryos with eyes were removed from mantle cavities and transferred to filtered seawater containing $50\ \mu\text{g ml}^{-1}$ streptomycin sulphate (to inhibit Gram-negative bacteria) and $10\ \mu\text{g ml}^{-1}$ penicillin (to inhibit Gram-positive bacteria) (Landau & d'Agostino, 1977).

Hatched larvae were cultured in 1-l autoclaved glass vessels maintained in culture cabinets under 14 h light: 10 h dark photoperiod and temperatures of 21 ± 2 or $28 \pm 2^\circ\text{C}$. These two temperatures covered the range of Hong Kong seawater temperatures during the reproductive season (Morton & Morton, 1983). Autoclaved seawater (30 psu) containing antibiotics was changed every three days, and larvae were fed with the flagellate *Isochrysis galbana* at 1×10^5 – 5×10^5 cells ml^{-1} concentration.

During the larval development, 30 larvae at each stage (stage I to cyprids) were collected and preserved in 30% ethanol in order to monitor their morphological characters (Miller, 1994). Preserved exuviae and larvae were dissected with fine needles and observed under a light microscope. Drawings were made using a *camera lucida* attachment and measurements made with a calibrated ocular micrometer. The total length of the nauplii was measured from the frontal margin of the cephalic shield

to the tip of the dorsal thoracic spine or abdominal process, whichever was longer. The shield width of the nauplii was measured at its widest point and shield length from the anterior margin of the shield to the posterior border. The width (depth) of the cypris larvae was measured as the maximum distance between the dorsal and ventral margins of the carapace at the deepest point, and cypris length from the anterior to the posterior carapace margins. The morphology of the antennules, antennae and mandibles was described using the setation formulae of Newman (1965) and setal terminology based on Lang (1979) and Branscomb & Vedder (1982).

Scanning electron microscopy (SEM)

The structure and surface sculpturing of the carapace, labrum, frontal horns and thoraco-abdominal processes were investigated using the scanning electron microscope (SEM, Leica Stereoscan 440). Larvae were fixed in 2.5% glutaraldehyde (in seawater) for 1 h, rinsed in distilled water for 1 min, then dried progressively in graded ethanols (30, 50, 75, 95 and 100%), critical-point dried and coated in gold-palladium before observation using SEM.

RESULTS

Larval culture and development

Larval development of *Chthamalus malayensis* consists of six naupliar stages and a non-feeding cypris stage. Larval development from stage I to cyprid took 20 d at $21 \pm 2^\circ\text{C}$ and 14 d at $28 \pm 2^\circ\text{C}$ (Table 1).

Larval morphology of *Chthamalus malayensis*

Nauplii of *C. malayensis* have a convex, globular cephalic shield with a posterior spine at stages II and III; the posterior spine is absent from stages IV to VI. The frontolateral horns are directed ventrally and the unilobed labrum bears numerous teeth. The caudal processes are considerably shorter than the cephalic shield itself. In addition to common simple (S) and plumose (P) setae, the mandibles bear cuspidate (C) setae and the antennae possess one hispid (H) and several feathered (F) setae. Setal formulae (after Newman, 1965) are given in Table 2.

Naupliar length increased from $239 \mu\text{m}$ at stage I to $526 \mu\text{m}$ at stage VI (Table 3), while cyprid length ranged

Table 1. Time taken (days) for the appearance of the larval stages of *Chthamalus malayensis* at the two culture temperatures.

Culture temperature ($^\circ\text{C}$)	Larval stage						
	I	II	III	IV	V	VI	Cyprid
21 ± 2	1	1	4	6	9	14	20
28 ± 2	1	1	2	4	7	10	14

I–VI, naupliar stages.

Table 2. Setal formulae for the nauplii of *Chthamalus malayensis*.

Naupliar stage	Antennule	Antenna exopod	Endopod	Mandible exopod	Endopod
I	S.SSS:SS.S:S	S:4S	3S:2S:2S:2S:G	S:3S	3S:2S:2S:2S:G
II	2SPS:SP.P:S	SP:4P:S	2PS.2S:2F:SFH:G	P:3P:S	3S.SP:PCP:2P:G
III	S.PSPS:SP.P:S	2P:5P	P3S:2S:2F:SFPH:G	P:3P:S	3S.SPS:PCP:PCP:G
IV	S.P.PSPS.SP:P:S	2P:7P	P4S:3S:FSF:SFPH:G	P:4P	4S:SPP:PCP:PCP:G
V	S:P.P.PSPS.SP:S.P:S	3P:8P	2PS2P:4S:FPF:SFPH:G	P:4P:S	5S:SPSP:PCP:PCP:G
VI	S:P.P.PSPS.SP:P.PS:S	4P:8P	2PS2P:5S:SFFF:SFPH:G	P:5P	5S:SPSP:SPCP:PCP:G

Setal types: S, simple; P, plumose; C, cuspidate; G, gnathobase; H, hispid; F, feathered.

Table 3. Dimensions of cultured larval stages of *Chthamalus malayensis*.

Stage	N	Total length (μm)		Shield width (μm)		Shield length (μm)	
		Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range
I	30	239 ± 12	225–260	126 ± 7	120–140		
II	30	332 ± 10	320–340	217 ± 9	210–225		
III	30	379 ± 15	350–390	267 ± 10	255–280		
IV	30	428 ± 10	410–445	310 ± 10	290–320	312 ± 9	310–330
V	30	475 ± 17	440–490	350 ± 11	330–370	342 ± 24	310–385
VI	30	526 ± 19	490–510	424 ± 16	400–450	429 ± 13	420–450
Cyprid	20	425 ± 13	410–440	221 ± 9	210–230		

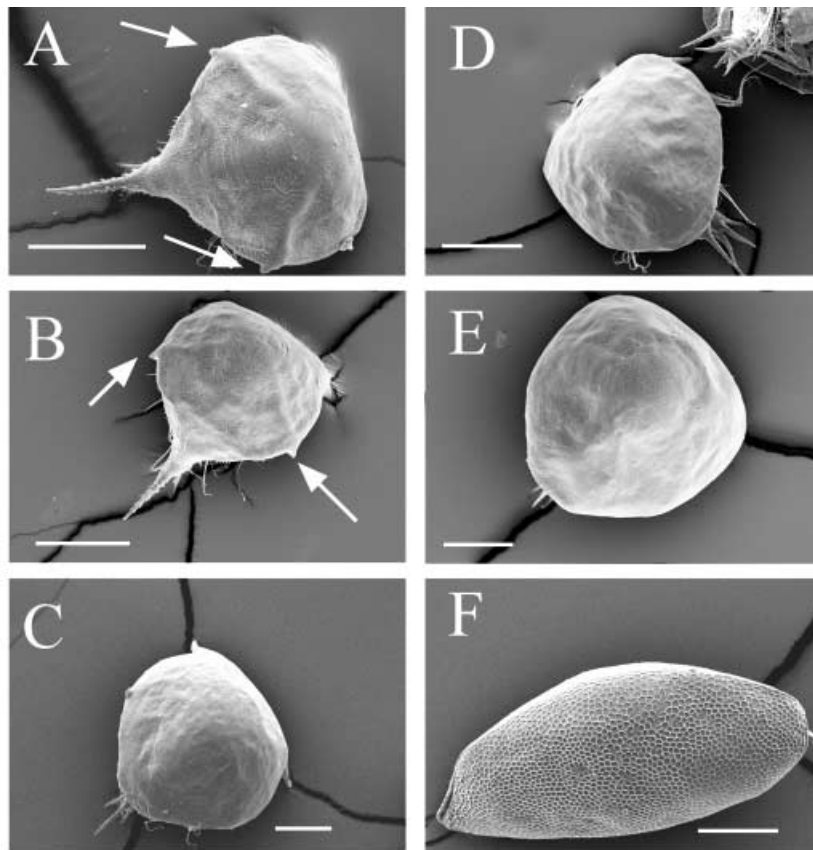


Figure 1. Body forms (dorsal shield outline) of larvae of *Chthamalus malayensis*: (A–F) representing naupliar stages II–VI and cyprid. Note the presence of lateral gland spine (indicated by arrows). Scale bar: 100 μm .

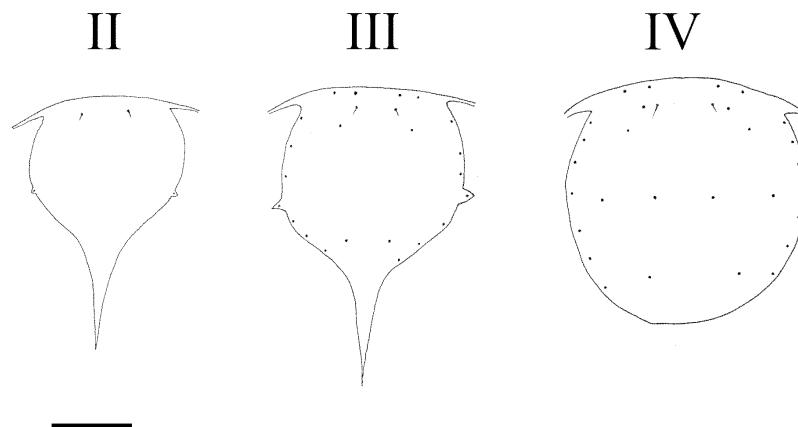


Figure 2. The position of the pores and setae on the dorsal cephalic shield of naupliar stage II, III and IV recorded under SEM investigation. Scale bar: 100 μm .

from 410–440 μm (Table 3). The detailed morphological characteristics of the larvae are as follows:

Nauplius I

This larval stage has a mean length of $239 \pm 12 \mu\text{m}$. The pear-shaped cephalic shield has a pair of anterior frontolateral horns folded back parallel with the long axis of the body and a dorsal thoracic spine. No frontal filaments were observed. The labrum is smooth and has no teeth. The dorsal thoracic spine and abdominal processes are blunt and similar in length. The setae are

simple, but many small spines decorate all appendages when observed under SEM.

Nauplius II

The cephalic shield (Figure 1A) has become extended in length (332 μm) and convex in shape. A pair of lateral gland spines was observed (Figure 1A). The anterior shield margin is smooth, but the posterolateral shield margin is spinulated with 5–9 fine spines. No pores were observed on the dorsal surface of the cephalic shield, but a pair of setae was recorded on the frontal

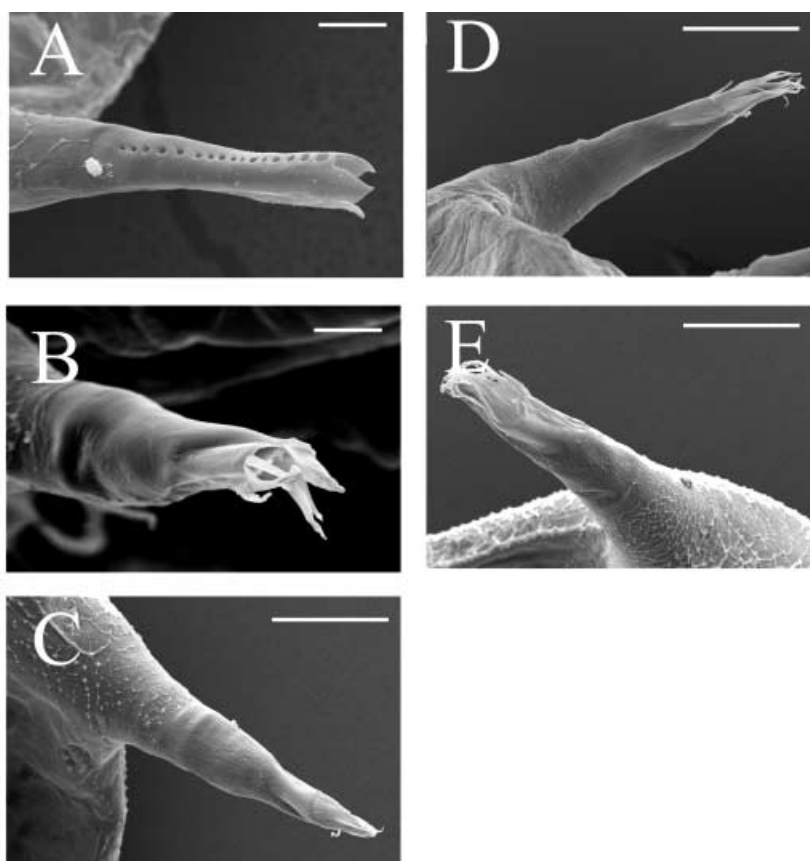


Figure 3. Frontal horns of larvae of *Chthamalus malayensis* under SEM: (A–E) of naupliar stages II–VI. Note the pores are only present at stage II. Scale bar: 10 μ m.

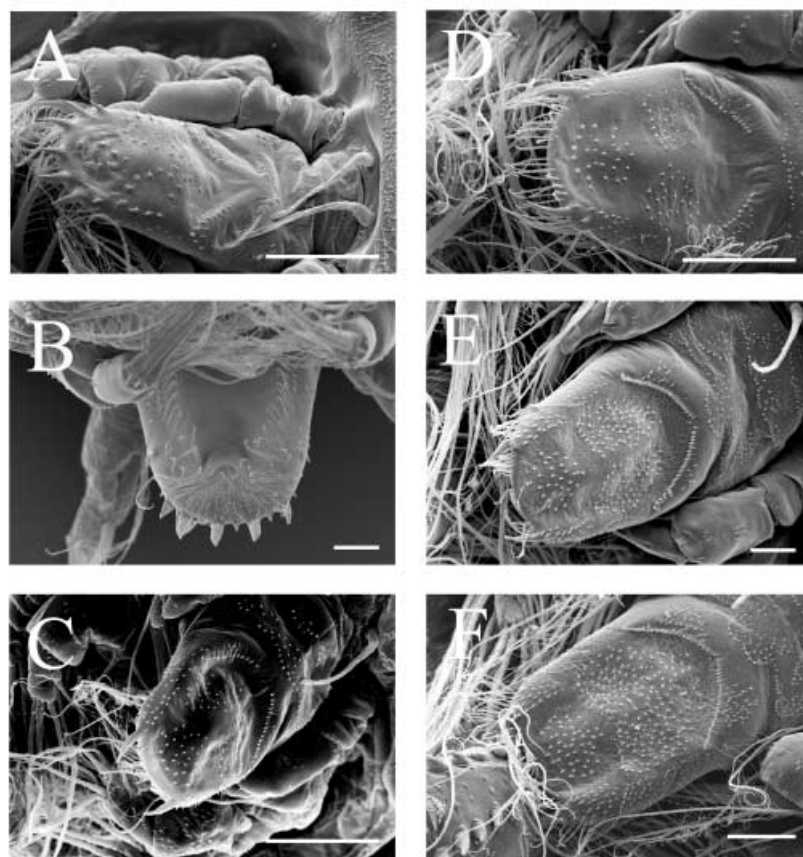


Figure 4. Labrum of naupliar stages II to VI of *Chthamalus malayensis*: (A,C–F) ventral views; and (B) dorsal view of naupliar stages II. Scale bar: 10 μ m.

Table 4. Comparison of morphological features between the larvae of *Chthamalus malayensis* in the present study and those described by Karande & Thomas (1976) in India.

Morphological features	<i>Chthamalus malayensis</i> in present study	<i>Chthamalus malayensis</i> described by Karande & Thomas (1976)
Cephalic shield in stages II and III	No fine spinulation along the posterior lateral margin and with 5–9 spines.	A fine spinulation along its posterior lateral margin with 7–8 large spines.
Thoraco-abdominal process at stage II	A row of 5 fine large spinules and 10 small spinules in the ramus.	A row of 7–8 fine spinules along the ventrolateral side of each ramus.
Thoraco-abdominal process at stage VI	A row of fine spinules on the lower margin of the dorsal thoracic spine and 6–7 unequal spinules on the upper margin of the dorsal thoracic spine. Spines absent from the dorsal surface.	A row of 4–5 unequal spinules on a caudally directed distal abdominal spine. Spines present on the dorsal surface.
Thoraco-abdominal process at stage VI	Six pairs of thoracic spines on the abdominal process. Three pairs of spines at the end of the thoraco-abdominal process.	Four spines in all, forming a crown pattern at the free end of the thoraco-abdominal process.
Labrum	5–9 teeth at stage II, one pair of teeth at stage III and two pairs of teeth at stages IV, V and VI.	7–8 teeth at stage II and only two teeth at stages III–VI.
Setal type in antenna	Simple and plumose seta in the first group of endopodites in all stages. Feathered seta in the fourth group of endopodites in stages II–VI.	Additional stub-like seta in the first group endopodites in stages III, IV and VI. Simple or plumose seta in the fourth group of endopodites.
Total length in stages IV–VI	428–526 μm .	415–450 μm .

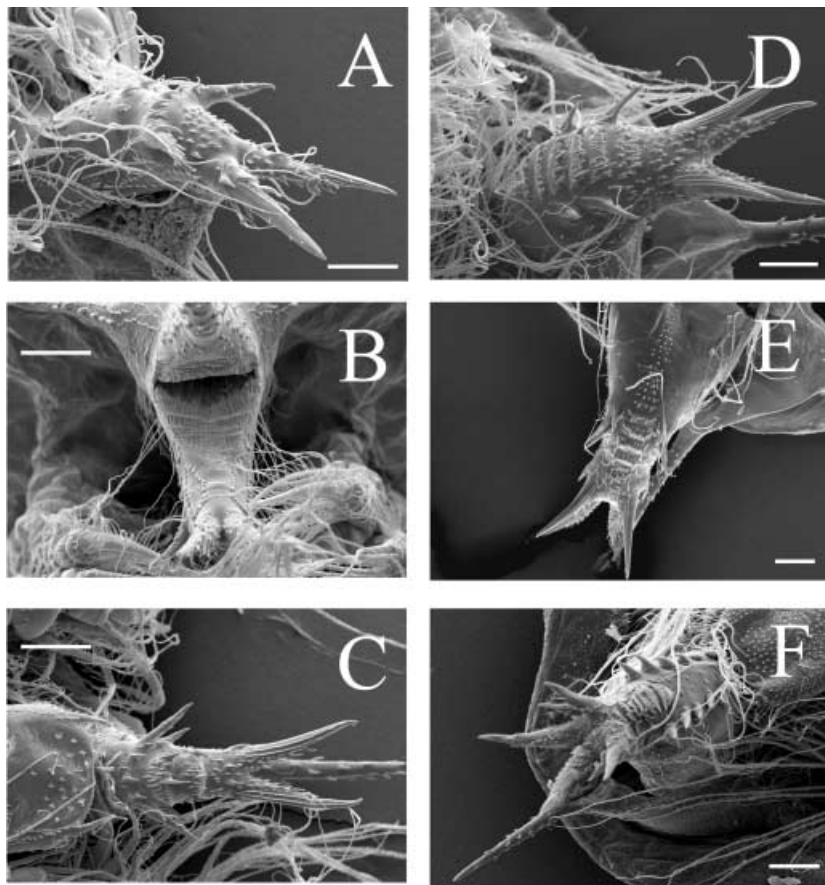


Figure 5. Thoraco-abdominal process of naupliar stages II to VI of *Chthamalus malayensis*: (A,C–F) ventral views; (B) dorsal view, at stage II. Scale bar: 20 μm .

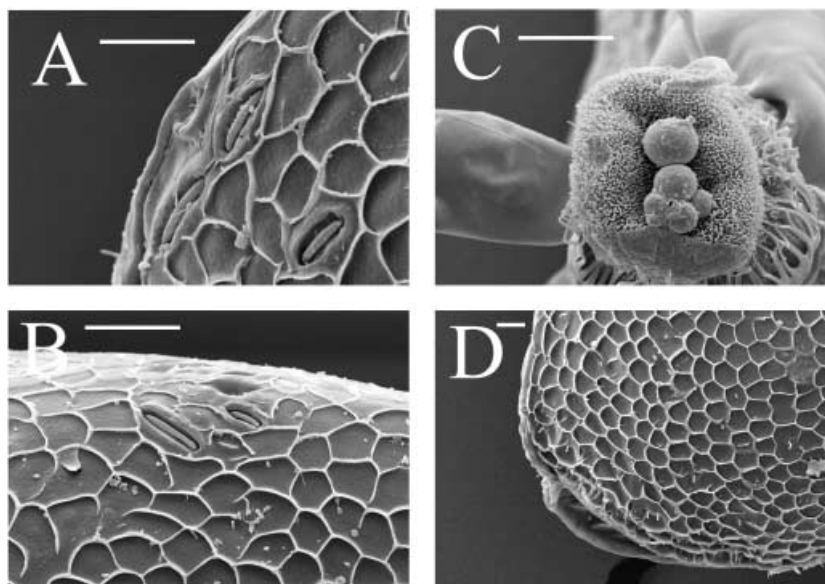


Figure 6. Surface structures of a cyprid of *Chthamalus malayensis*: (A) three posterior lattice organs; (B) two anterior lattice organs; (C) shape of third segment; and (D) setae. Scale bar: 10 μm .

part of the shield (Figure 2). The frontolateral horns are bent slightly in an anterior direction when compared to stage I and have 16–18 pores (Figure 3A); a short dorsal stylet is present which is not fused with the rim of the open distal end of the horn. The labrum bears 5–9 spines plus slender setae (Figure 4A,B). A

pair of frontal filaments was observed on the anterior side which continue to be present in all the subsequent stages. The dorsal thoracic spine has small teeth and is longer than the abdominal process, which has a pair of large serrated spines (series 1) and a bifurcated ramus (Figure 5A,B).

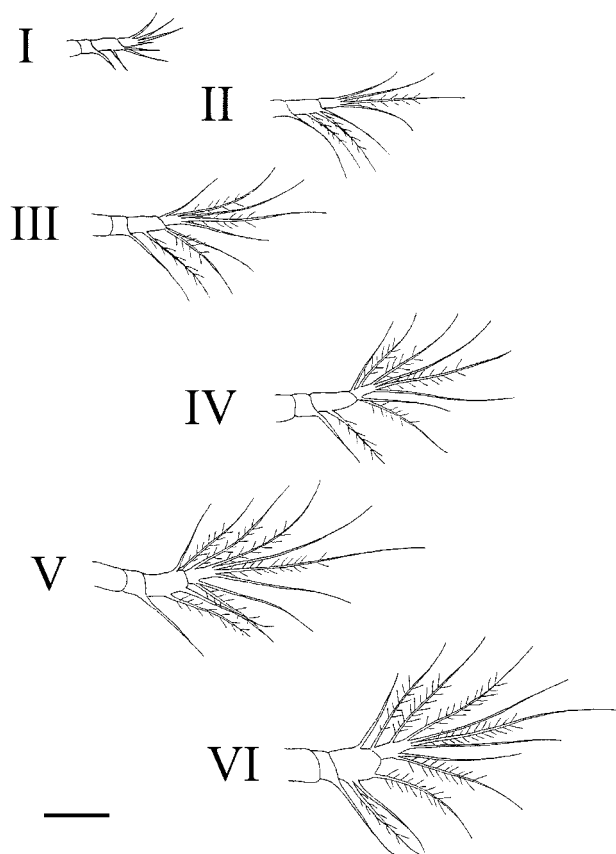


Figure 7. Antennules of naupliar stages I–VI of *Chthamalus malayensis*. Scale bar: 100 μm .

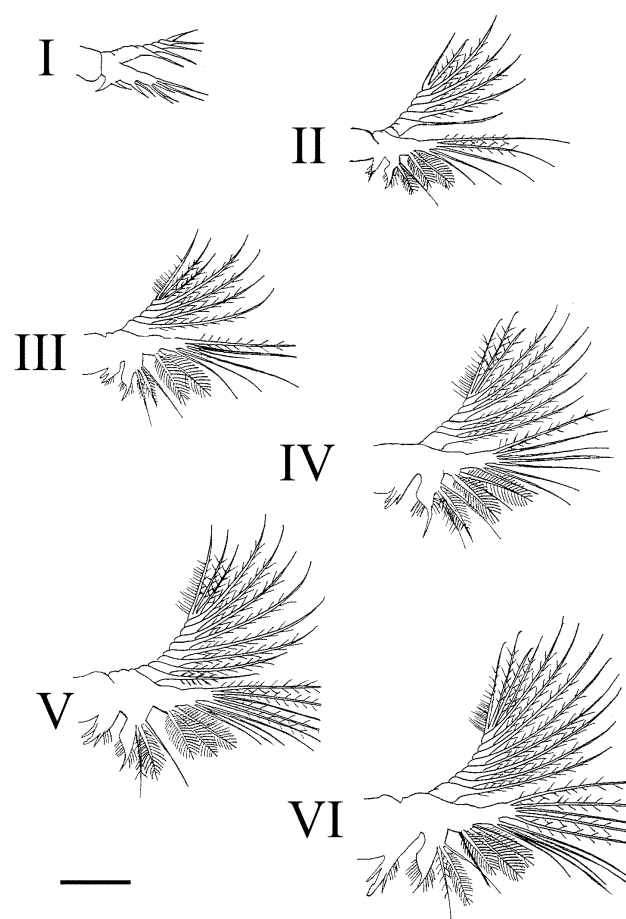


Figure 8. Antennae of naupliar stages I–VI of *Chthamalus malayensis*. Scale bar: 100 μm .

Nauplius III

The larva (Figure 1B) has increased in total length (379 μm) and width (267 μm) when compared to stage II. Similar to stage II, one pair of lateral spines and eight pairs of pores appear on the dorsal cephalic shield surface: two pairs of pores on the anterior, three anterior lateral, three posterior lateral, and a further two pairs of pores on the central area of the dorsal surface: one anterior and another posterior (a total 22 pores, Figure 2). The frontolateral horns have become thickened and shorter in length (Figure 3B). The ventral perforations have been lost and the structural arrangement at the distal end is altered. Two teeth on each posteriolateral margin of the labrum (Figure 4C) and a preaxial seta present on the antennules are diagnostic features of stage III nauplii. The dorsal thoracic spine is now barbed and consistently longer than the abdominal process. A row of four spines with spinules appears behind the series 1 spine (Figure 5C).

Nauplius IV

This stage (Figures 1C) has the caudal spine separated from the cephalic shield. In addition to the 22 pores arranged on the dorsal cephalic shield (see in stage III), another three pairs of pores on the central area of the dorsal surface are present, giving a total of 28 pores (Figure 2). The frontolateral horns of this stage are different from those of stage III (Figure 3C), but remain morphologically similar in subsequent stages

(Figure 3D,E). There is a fine dorsal spine, a ventral spine and a row of tapering filaments surrounding the distal end of the horns. An additional pair of lateral teeth occur on the labrum (Figure 4D) which together with the presence of two pre-axial setae on the antennules are diagnostic features of this nauplius stage. The second pair of large spines (series 2) has emerged close to the base of the furca and the dorsal thoracic spine is still longer than the abdominal spine (Figure 5D).

Nauplius V

The cephalic shield has increased in size (Table 3), but the general shape remains similar through to nauplii VI (Figure 1D). The position and number of pores on the dorsal surface of the cephalic shield remain the same. The number of teeth on the labrum of this stage (Figure 4E) is the same as for stage IV, and subsequently stage VI (Figure 4F), except that the number of spines on the surface of the labrum varies. The dorsal thoracic spine remains barbed, but is now shorter than the abdominal process where a third pair of spines (series 3) has appeared (Figure 5E). The presence of three pre-axial and five postaxial setae on the antennules is a diagnostic feature of this nauplius stage.

Nauplius VI

The body shape of this stage (Figure 1E) is similar to stage V except in size. In the latter development of this stage, a pair of compound eyes becomes clearly visible on

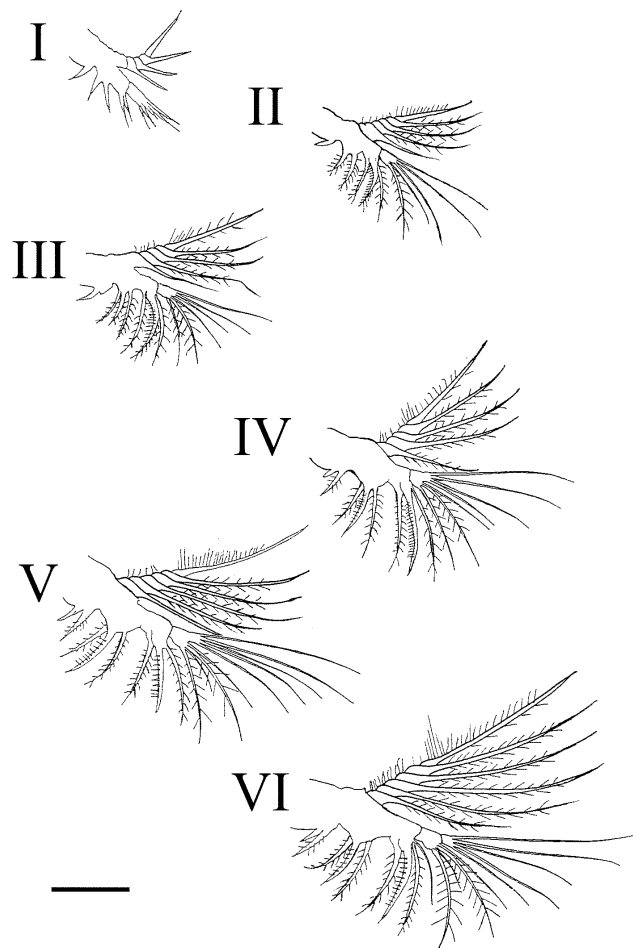


Figure 9. Mandibles of naupliar stages I–VI of *Chthamalus malayensis*. Scale bar: 100 μm .

either side of the median nauplius eye. The nauplius VI stage is easily distinguished from other stages by the six pairs of thoracic spines occurring on the abdominal process and the primordia of the cypris thoracic appendages beneath the exoskeleton of the thoracic spines (Figure 5F). Three distal teeth are present on the inner prong of the antennal gnathobase of the third group of endopodites of this stage, similar to those of stages III–V.

Cyprid

The bivalve carapace of the cyprid (Figure 1F) is 425 μm in mean total length and 221 μm in mean height (Table 3). The anterior region of the body contains numerous oil droplets. There are six pairs of thoracic limbs and a posterior caudal furca. Median and compound eyes are present in the anterior region of this larva. Under the SEM, the carapace surface has a honeycombed pattern with a large number of small projecting setae, which are uniformly distributed over the entire carapace (Figure 6D). Five pairs, two anterior (Figure 6B) and three posterior (Figure 6A), of lattice organs (LO) are also obvious. The antennule ends in a cup-shaped third segment consisting of an almost circular attachment disc, a raised rim (velum or skirt) and sense organs (Figure 6C).

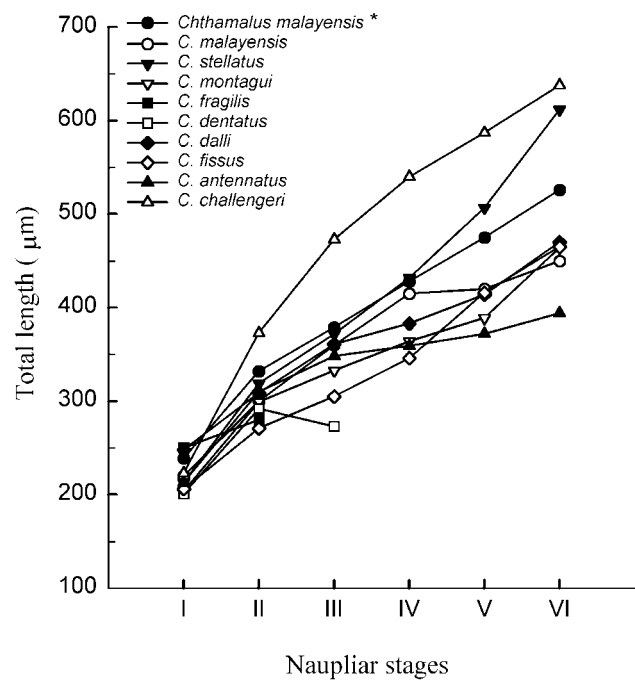


Figure 10. Total length (μm) of naupliar stages of *Chthamalus malayensis** (present study), *C. malayensis* (Karande & Thomas, 1976), *C. stellatus* and *C. montagui* (Burrows et al., 1999), *C. fragilis* (Lang, 1979), *C. dentatus* (Achituv, 1986), *C. dalli* and *C. fissus* (Miller et al., 1989), *C. antennatus* (Egan & Anderson, 1989) and *C. challengerii* (Lee, 1999).

DISCUSSION

Comparison of the larval description of *Chthamalus malayensis* in the present study with that of Karande & Thomas (1976)

Larval development

Observations showed that *Chthamalus malayensis* requires at least 20 days to reach the cypris stage under laboratory culture at 21°C. This period was longer than for the same species from Mumbai, India, which only required 7–12 days (Karande & Thomas 1976). The difference may be due to the culture temperatures employed. Although Karande & Thomas (1976) did not give their culturing temperature, when other batches of larvae in the present study were cultured at 28°C, cyprids appeared within 14 days.

Larval morphology

The present larval observations of *C. malayensis* differ from those of Karande & Thomas (1976) with regard to larval size and morphology, such as spines along the posterior lateral margin of the cephalic shield at stages II and III, thoraco-abdominal process at stage II and stage IV, teeth of the labrum and setation of the antennae (Table 4), possibly reflecting ecological and biogeographical variation.

Comparison with other *Chthamalus* species

Larval size and shape

Comparison of size ranges among *Chthamalus* species show that the nauplii of *C. malayensis* occur in the middle of the range (Figure 10). The nauplii of

C. challengerii are the largest at every larval stage and the larvae of *C. fragilis* and *C. antennatus* are smallest. In some cases, the size of larvae at each stage is probably the simplest distinguishing character i.e. between *C. stellatus* and *C. montagui* (Burrows et al., 1999), and also separates *C. fragilis* and *C. dalli* at the same stages (Miller & Blower, 1989). The size of larvae reared in the laboratory, however, is often of little value in the identification of species, as variation in culture conditions such as temperature (Miller & Blower, 1989; Burrows et al., 1999), larval density (Lewis, 1975) and food types (Stone, 1988) can affect larval size.

Shield length was approximately equal to shield width (ratio=1.01–1.03) in *C. malayensis*, which is similar to other *Chthamalus* species (ratio of length to width=1.0–1.15; Lang, 1979; Achituv, 1986; Miller et al., 1989; Egan & Anderson, 1989; Burrows et al., 1999; Lee, 1999). Shield shape, therefore, is not a diagnostic character to separate species in the genus *Chthamalus*.

Sculpturing of the cephalic shield

There is no recorded information about the dorsal surface sculpturing of the cephalic shield in *Chthamalus* nauplius larvae and very little for the Cirripedia in general. Scanning electron microscopy images of *Semibalanus balanoides* nauplii showed 32 dorsal pores and two pairs of minute, mid-dorsal setae on the cephalic shield surface in the sixth nauplius stage (Walker & Lee, 1976). In the present study, the nauplii of *C. malayensis* have 22 dorsal pores at stage III and 28 at stage IV and also one pair of frontal setae from stages II–VI. These observations might provide useful for future comparative studies of the sculpturing of the cephalic shield of cirripede nauplii. It is likely that the dorsal ornamentation of the cephalic shield and the number and positions of the pores and setae may vary from species to species.

Labrum

The teeth of the labrum in *Chthamalus* species are a diagnostic character in the separation of the larval stages of a given species and also the larvae of different species. In *C. malayensis*, the teeth of the labrum are very useful in distinguishing stages II, III and IV larvae. The unilobed labrum has 5–9 teeth at stage II, one pair of teeth at the corners of the free distal end at stage III, and two pairs of teeth from stages IV–VI. A similar pattern has also been observed in *C. stellatus*, *C. montagui*, *C. fissus* and *C. dalli*. The teeth of the labrum of some *C. malayensis* stages, however, are different from those of *C. antennatus*, *C. dentatus*, *C. fragilis* and *C. challengerii*. The setose labrum in stages IV, V and VI of *C. antennatus* have two lateral teeth on the distal margin (Egan & Anderson, 1989), whereas in *C. dentatus*, the labrum at stage IV has a pair of lateral teeth (Achituv, 1986). The labrum in *C. fragilis* has small denticles at stage II and two pairs of lateral teeth separated by numerous small denticles, which are lost at stage VI, and a median protuberance that becomes increasingly larger in later stages (Lang, 1979). The labrum has slender setae with sparse teeth from stage II to stage VI in *C. challengerii* (Lee, 1999).

Abdominal processes

At all six stages, the pattern and emergence of spines on the abdominal process observed in *C. malayensis* was the same as that in *C. montagui*, *C. stellatus*, *C. dalli* and *C. fissus*, *C. antennatus* and *C. fragilis*, except for stage III. There are no differences in the abdominal process between larvae in stages II and III for some *Chthamalus* species, such as *C. montagui*, *C. stellatus*, *C. dalli*, *C. fissus*, *C. antennatus*, *C. fragilis*, *C. challengerii* and *C. dentatus* (Lang, 1979; Achituv, 1986; Egan & Anderson, 1989; Miller et al., 1989; Burrows et al., 1999; Lee 1999), although differences are present in *Chthamalus malayensis* between nauplius II and III. In nauplius II of *C. malayensis*, only a group of fine spinules on the posterior trunk were observed, but in nauplius III, two rows of fine spinules with some bigger spinules (3–4) were present.

Setation of appendages

Little attention has been paid to the morphological variability of the antennal coxal gnathobase in nauplii of *Chthamalus* species. The gnathobases of two *Chthamaloid* species (*Octomeris sulcata* and *C. challengerii*) and one Lepadomorph (*Capitulum mitella*) barnacle were reported to be very similar to each other with two distal teeth on the inner prong of the antennal gnathobase (Kado & Hirano, 1994). Scanning electron microscopy images of the antennal gnathobases of *Chthamalus malayensis* revealed three teeth on the inner prong of the antennal gnathobase, which indicates there are differences between *C. malayensis* and *C. challengerii*, suggesting the antennal gnathobase may be a useful feature to separate *Chthamalus* species.

The review of larval morphology in the nine *Chthamalus* species from a wide geographic range suggests that the differences in size of larvae, the shape and teeth of the labrum, the number and position of abdominal process spines and setation of appendages are important features to separate the nauplii of *Chthamalus* species.

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