DESCRIPTION OF THE NAUPLIAR STAGES OF MEGABALANUS TINTINNABULUM (CIRRIPEDIA: BALANIDAE)

V. Thiyagarajan, V. P. Venugopalan, T. Subramoniam, and K. V. K. Nair

ABSTRACT

Larval development of Megabalanus tintinnabulum was studied in the laboratory. At 26 ± 1°C the development from nauplius I–VI required about 4 days, when the diatom Chaetoceros wighami was used as food. Detailed morphological drawings and setation formulae of appendages of all 6 naupliar stages are presented for the first time. Notable features of the nauplii include the presence of a spinulated lateral margin, a trilobed labrum with teeth, long dorsal and posterior shield spines, a single tooth on the inner prong of the antennal gnathobase, comb-shaped cuspidate setae on the antennae, and spines and simple denticulate setae on the mandibular endopodite in naupliar stage II. The present description of the nauplii is not in full agreement with the previous description of this species from this coast by Daniel (1958). Differences in stage sizes, naupliar morphology, and setation of appendages are used to distinguish this species from other megabalanines and the nauplii of megabalanines from those of balanines in the Balanus amphitrite group.

Megabalanus tintinnabulum (L.) is a large acorn barnacle commonly encountered in the shallow waters of both east and west coasts of India. It has been reported as a major component of fouling communities from both coasts (Anil, 1986; Venugopalan et al., 1990; Rajagopal, 1991). Sasikumar (1991) reported various aspects of the biology of this barnacle, especially those related to reproduction and settlement. However, studies on the larval stages of this important barnacle species are sparse. Daniel (1958) briefly described the larval development in this species. Nevertheless, his report is incomplete with respect to detailed description of diagnostic features, such as carapace spine, labrum, abdominal process, and setation of appendages. In this paper, we give a detailed account of the larval development of M. tintinnabulum, emphasizing the changes in larval size, shape, general morphology and setation of appendages, along with data on duration of each stage.

MATERIALS AND METHODS

Larval Rearing.—Adult M. tintinnabulum were collected from the approach jetty piers of the Madras Atomic Power Station, Kalpakkam (12°23'N, 80°11'E), east coast of India. Release of the first (sometimes second) stage larvae from mature broods occurred after 24 h of aerial exposure, followed by immersion in clean sea water (Thiyagarajan et al., 1996). Larvae were cultured following the method of Rittschof et al. (1984) with certain modifications, such as (1) instead of Skeletonema costatum (Grev.), another common diatom, Chaetoceros wighami Brightwell (13–17 × 10^5 cells/ml), was used as food, and (2) larvae were reared at 26 ± 1°C in Millipore (0.22 μm)-filtered sea water (1 larva/ml and salinity 30 ppt) under constant illumination. Every 6 h, 3-ml aliquots were removed from each replicate culture (6) and the larvae were observed to determine their stage of development and percentage of survival. Naupliar stages were determined using the key provided by Karande (1974a).

Description of Larvae.—The nauplii were observed under a Nikon Optiphot microscope for detailed analysis of larval morphology and limb setation. Drawings were made using a camera lucida and size was determined with the help of a calibrated ocular micrometer. Total length (TL) of larvae was determined by measuring the distance between the frontal margin of the carapace and the tip of the caudal spine (CS) or the abdominal furcal tip (whichever was longer). The carapace width (CW) was measured at the widest point behind the naupliar eye. Ten larvae of each stage were examined for determining size, shape, morphology, and setal formulae. The setal types present on the limbs are expressed by alphabetical formulae (Newman, 1965). The terminology used to describe the naupliar morphology follows that given by Kado and Hirano (1994).

RESULTS

The intermolt period and percentage survival of the naupliar stages are given in Table 1. Stage II nauplii reached stage VI within 4 days. The mean values of total length (TL) and carapace width (CW) of various larval stages are given in Table 2. Typical setal types and shape used in the present study are illustrated in Fig. 1. The outline of the larval carapace (dorsal view), abdominal processes (AP) (lateral view), and details of the labrum

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Table 1. *Megabalanus tintinnabulum*. Time taken for the appearance of naupliar stages in cultures along with percentage survival at each stage (culture temperature 26 ± 1°C).

<table>
<thead>
<tr>
<th>Nauplius stage</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>89</td>
<td>82</td>
</tr>
<tr>
<td>Time of appearance*</td>
<td>0</td>
<td>0</td>
<td>1.6</td>
<td>2</td>
<td>3.6</td>
<td>4</td>
</tr>
</tbody>
</table>

* Mean time (days) after which the naupliar stage appeared in culture (calculated from the initial time of release).

are shown in Figs. 2–4, respectively. The setal formulae of the antennule, antenna, mandible, and maxilla are given in Table 3 and illustrated in Figs. 5, 6, 7.

Each naupliar stage shows characters which are helpful in larval identification. Significant features of the various larval stages are given below.

**Nauplius I**
Figs. 2A, 3A, 4A, 5A, 6A, 7A

The body is elongated and pear-shaped with a mean total length of 264 ± 6 μm. The anterior margin of the carapace is markedly convex. Frontal horns (FH) are folded back toward the long axis of the body. The trilobed labrum is visible without teeth. Frontal filaments (FF) cannot be seen and the setae of the limbs are simple. The abdominal process (AP) and caudal spine (CS) are not fully differentiated.

The presence of posteriorly folded FH and simple setae on the appendages are characteristic of this stage. The larvae swim actively using three pairs of limbs and molt to stage II within 30 min.

**Nauplius II**
Figs. 2B, 3B, 4B, 5B, 6B, 7B

At this stage, the nauplius has a mean length of 404 ± 21 μm and mean width of 203 ± 4 μm. The carapace has extended in all directions, becoming bell-shaped with a less convex anterior margin. The FH are 93 ± 15 μm in length and are extended either anteriorly or are perpendicular to the long axis of the body. A pair of FF 80 ± 6 μm long can now be observed. The entire lateral margin of the carapace is spinulous with numerous small spines and with a pair of prominent spines. The labrum bears many short setules on each lobe. The distal margin of the median labral lobe bears teeth from this stage onward.

Table 2. Total length (TL) and carapace width (CW) in microns of naupliar stages of *Megabalanus tintinnabulum* (MT)—present study; *Balanus amphitrite* (BA)—Egan and Anderson 1986; *Megabalanus rosa* (MR) and *Megabalanus volcano* (MV)—Kado and Hirano, 1994; and *Megabalanus tintinnabulum* (MT-D)—Daniel, 1958.

<table>
<thead>
<tr>
<th>Stages</th>
<th>MT</th>
<th>BA</th>
<th>MR</th>
<th>MV</th>
<th>MT-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>TL</td>
<td>264</td>
<td>220</td>
<td>245</td>
<td>229</td>
</tr>
<tr>
<td></td>
<td>CW</td>
<td>160</td>
<td>140</td>
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<td>126</td>
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<td>II</td>
<td>TL</td>
<td>404</td>
<td>350</td>
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<td>214</td>
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<td>200</td>
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</tr>
<tr>
<td></td>
<td>CW</td>
<td>364</td>
<td>200</td>
<td>334</td>
<td>312</td>
</tr>
<tr>
<td>V</td>
<td>TL</td>
<td>784</td>
<td>470</td>
<td>780</td>
<td>670</td>
</tr>
<tr>
<td></td>
<td>CW</td>
<td>480</td>
<td>270</td>
<td>437</td>
<td>416</td>
</tr>
<tr>
<td>VI</td>
<td>TL</td>
<td>880</td>
<td>540</td>
<td>963</td>
<td>941</td>
</tr>
<tr>
<td></td>
<td>CW</td>
<td>560</td>
<td>310</td>
<td>540</td>
<td>543</td>
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</tbody>
</table>

The AP has a pair of large serrated series 1 spines and a long distal forked portion (furca). The furcal stem has circlets of spines at the distal end. The CS is longer than the AP. The CS and furcal branches are covered with small spines from this stage onward.

The maxillae appear as long setae on the AP. At this stage, no preaxial setae are pres-

Fig. 1. Setal types used in the setation formulae of naupliar stages of *Megabalanus tintinnabulum*; S = simple, S° = simple-denticulate, P = plumose, D = plumodenticulate, MC = mandible-cuspidate, AC = antenna-cuspidate, AG = antennal gnathobase, MG = mandibular gnathobase setae, B₁ = series 1 bristle, B₂ = series 2 bristle, ss = setiform spine.
Table 3. Setal formulae (Newman, 1965) of six naupliar stages of *Megabalanus tintinnabulum* (MT)—present study; *Megabalanus rosa* (MR) and *Megabalanus volcano* (MV)—Kado and Hirano, 1994; *Balanus amphitrite* (BA)—Egan and Anderson, 1986. s = setiform spine, S = simple seta, P = plumose, C = cuspidate, G = gnathobase, S^p = simple denticulate, and D = denticulate.

<table>
<thead>
<tr>
<th>Stages</th>
<th>Antenna</th>
<th>Mandible</th>
<th>Maxilla</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exopodite</td>
<td>Endopodite</td>
<td>Exopodite</td>
</tr>
</tbody>
</table>

Consequent on the antennules (Fig. 5B). The antennal gnathobase is well developed with three apical spines (inner, median, and outer prongs) and series 1 bristle (Fig. 6B). Simple denticulate setae (S^p) on the endopodite of the mandible are present. This stage molts to stage III in 38 h.

**Nauplius III**

Figs. 2C, 3C, 4C, 5C, 6C, 7C

Stage III nauplii are 516 ± 12 μm long and 280 ± 2 μm wide. The carapace is more bulbous than in the earlier stages. The prominent spines on the lateral margin of the carapace have disappeared, but small spines are retained throughout the naupliar stages. The AP bears only a pair of series 1 (distal) spines. A pair of dorsal shield spines (DSS) appears in this stage and is retained till stage VI. The maxillae appear as long simple setae in the thoracic region of AP. The first preaxial setae appears on the antennule. Other setation features of this stage are given in Table 3. Nauplii of this stage molt to stage IV within 10 h.

**Nauplius IV**

Figs. 2D, 3D, 4D, 5D, 6D, 7D

The larvae are 610 ± 10 μm in total length and the shield is 364 ± 4 μm in width. The FH and FF are 96 ± 12 μm and 100 ± 8 μm long, respectively. The median lobe of the labrum bears serrated setules, but the lateral lobes still have only simple short setules. In the posterior region of the larva, the AP has separated from the carapace, so that it now has an entire posterior margin, bearing a pair of posterior shield spines (PSS). This carapace form is retained until stage VI. The AP bears series 1 (distal) and series 2 (proximal) spines. The series 1 spines are larger than the series 2 spines. A median nonserrated spine appears between the pair of serrated series 2 spines. CS and AP are almost equal in length. The maxillae are seen as long simple setae. The antennule now bears two preaxial setae. From this stage onward the antennal endopodite bears 15 setae.

The presence of an entire shield, long PSS, two preaxial setae on the antennule, and series 1 and 2 spines on the AP are character-
Fig. 2. *Megabalanus tintinnabulum*: A–F, shield outline (dorsal view) of naupliar stages I–VI. Scale bar = 100 μm.

Characteristic features of this stage. These larvae molt to stage V after 38 h.

**Nauplius V**
Figs. 2E, 3E, 4E, 5E, 6E, 7E

Stage V larvae are 784 ± 35 μm long and 480 ± 57 μm wide. The shield margin is bulbous with a slightly convex anterior margin. The well-developed long DSS are now seen along with many small spines on the shield. The PSS are now 122 ± 4 μm long. The thoracic area is swollen and segmented. A pair of series 3 spines appears anterior to the furcal stem, in addition to the series 1 and 2 spines. The antennule now has three preaxial setae and five postaxial setae.

The presence of 12 setae on the antennule and three series of spines (1, 2, and 3) on the AP (Fig. 3) are characteristic of this stage. This stage molts to stage VI within 10 h.

**Nauplius VI**
Figs. 2F, 3F, 4F, 5F, 6F, 7F

The larva measures 880 ± 2 μm in length and 560 ± 3 μm in width. The FF and FH measure about 136 ± 4 μm and 124 ± 8 μm, respectively. A median spine makes its appearance between the pair of DSS. The PSS is 163 μm long. Six pairs of series 2 spines (the primordial thoracopode) are present along the ventral side of the thoraco-abdominal process in two parallel lines. The anten-
nule is provided with three preaxial and six postaxial setae. Paired lateral compound eyes are seen on either side of the unpaired median naupliar eye. However, the eyes became fully pigmented only toward the end of the period.

Presence of series 2 spines (6 pairs) and 13 antennular setae (S:P:P:PSPP:SP:P:PS:S) (see legend for Fig. 1) are the distinguishing characters of this final stage.

**DISCUSSION**

Four of the common and most abundant barnacles encountered on the east coast of India are *Megabalanus tintinnabulum*, *Balanus reticulatus* Utinomi, *B. cirratus* Darwin, and *B. amphitrite* Darwin (see Sasikumar, 1991; Fernando, 1978). Larval development of all these species has already been reported (Pillai, 1958; Daniel, 1958; Karande, 1973, 1974a; Thiyagarajan et al., 1996, in press). The larvae of these tropical balanids show many similarities. Hence, in the absence of detailed descriptions of key features, such as carapace shape and spines, AP spines, and limb setation, nauplii of these species are likely to be wrongly identified in plankton samples. On the other hand, the chthamalid *Tetraclitella* and pedunculate barnacle larvae available along this coast can be easily distinguished by their well-established characteristics (Karande, 1974b; Karande and Thomas, 1976; Molares et al., 1994). The ability to identify balanid larvae in plankton samples would be very helpful to cirriped ecologists trying to understand larval distribution in

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**Fig. 3.** *Megabalanus tintinnabulum*: A–F, ventral view of labrum of stages I–VI. Scale bar = 100 μm.
coastal waters. The larval description of *M. tintinnabulum* by Daniel (1958) is insufficient to fully differentiate the species from other closely related balanids.

Larval Development

Larval development times of all tropical balanids studied in the laboratory are similar (Karande, 1974a). Variations in duration of larval development in the laboratory are possibly due to differences in culture conditions (Kado and Kim, 1996). Daniel (1958) reported the appearance of cyprids of *M. tintinnabulum* in laboratory culture within 48 h (in the present study it took four days), but he did not describe the culture conditions used. It is possible that sea water used for Daniel's culture might have been contaminated by larval barnacles.

Larval Morphology

Except body size, larval morphological features such as body shape, spines on the abdomen, and setation of appendages are not likely to vary between larvae reared in the laboratory and those collected from natural plankton (Ovsyannikova and Korn, 1984; Miller and Roughgarden, 1994). Therefore,
the size of laboratory-reared larvae is of little value in the identification of species. However, other features, such as shape of the carapace, spines on the AP, and setation of appendages are considered crucial in larval identification (Lang, 1979; Egan and Anderson, 1986; Miller and Roughgarden, 1994).

The margin of the carapace of *M. tintinnabulum* (present study), *M. volcano* (Pilsbry), (see Kado and Hirano, 1994), *B. reticulatus* Utinomi (see Thiyagarajan et al., in press b), *B. cirratus* and *B. amaryllus euamaryllus* Broch (see Karande, 1974a, b) is spinulated, whereas in *B. amphitrite* (see Karande, 1973;
Fig. 6. Antennae of naupliar stages I–VI (A–F) of Megabalanus tintinnabulum. Scale bar = 100 μm.

Egan and Anderson, 1986, B. albicostatus Pilsbry (see Lee and Kim, 1991), and M. rosa (Pilsbry) (see Kado and Hirano, 1994), it is smooth. The presence of DSS in naupliar stages III–VI distinguishes larvae of M. tintinnabulum from other balanid larvae in plankton samples in Indian waters. However, it has been reported for other megabalanines, such as M. rosa and M. volcano (see Choi et al., 1992; Kado and Hirano, 1994) from Japan. The DSS, therefore, are helpful only in differentiating naupliar stages III–VI of this species from other balanid larvae available locally. The carapace becomes entire and develops a pair of long PSS in stage IV as in other barnacles, except in chthamalids (Karande and Thomas, 1976). These large spines are visible under a dissection microscope (104 μm in stage IV, 122 μm in stage V, and 163 μm in stage VI). No other cirriped larva described from Indian waters has a PSS longer than 110 μm (Karande, 1974b). Thus, it is also possible to separate this species from others by virtue of the presence of this long PSS. The presence of teeth on the trilobed labrum of this species is a feature similar to that of many other barnacle species. However, in most balanid species the teeth are lost after stage II (Egan and Anderson, 1986), while in megabalanids they are retained to stage VI.

The relative length of CS and AP may vary with the orientation in which the larvae are
observed. Therefore, it is not of much value in the determination of a stage or identification of species. In many balanids, the sequence of appearance and nature of spines on the AP are of diagnostic value. In *B. cirratus* both the proximal and distal AP spines appear in stage II (Karande, 1974a). The presence of a median spine between the series 2 spines of stage IV of *M. tintinnabulum* is characteristic of many balanids except *B. amphitrite* (see Karande, 1973).

The salient morphological features of nauplii of *M. tintinnabulum* are, therefore, their large size (Table 2), spinulated lateral margin, trilobed labrum with teeth, long posterior shield spines, and presence of dorsal shield spines. The present observations differ from those of Daniel (1958) with regard to larval size, shape, and morphology, such as FF in stage I, development of paired compound eyes in stage V, appearance of six pairs of series 2 spines in stage V, and the setation of antennules and mandibles (see below).

**Setation of Appendages**

The antennular setation of *M. tintinnabulum*, in general, conforms to that of other cirripeds thus far described. That is, there are no preaxial setae in stages I and II; the setae appear in the following order: one in stage III, two in stage IV, and three in stages V and VI. Stage VI larva has six setae on the postaxial side. Thus, it is possible to stage the nauplii by looking at antennular setation with the help of a dissection microscope. Among the three preaxial setae, which develop on the antennule during stages V and VI, two setae are always plumose in all megabalanids, whereas in other balanids, namely, *B. reticulatus, B. cirratus*, and *B. amphitrite* (Table 3),
only one seta is plumose and two are simple. In *B. reticulatus*, the presence of preaxial “hairs” on the antennule has been considered a distinguishing character (Thiyagarajan et al., in press b). In *M. tintinnabulum* such preaxial “hairs” are absent.

In naupliar stages IV–VI of *M. rosa* the antenna has three denticulate setae (Table 3) on the fifth setal quadrate (Kado and Hirano, 1994). In other balanids, including the present species, not more than one such seta is observed. The size and shape of the antennal gnathobase in successive naupliar stages of this species are similar to those of *B. amphitrite*, *M. rosa*, and *M. volcano*. However, in *M. rosa* and *M. volcano*, two teeth have been reported on the inner prong, whereas in the present species only one such tooth was observed. Moreover, in *M. tintinnabulum*, Daniel (1958) observed only 21, 22, and 22 setae on the antenna of stages IV, V, and VI, respectively, whereas we observed 23, 25, and 26, respectively, confirming the observations of others who have worked on megabalanids (Miller and Roughgarden, 1994; Kado and Hirano, 1994).

In general, no differences are observed in the setation of mandibles among the species mentioned above, except that in *M. rosa* and *M. volcano* 2–4 denticulate setae appear during naupliar stages II–VI (Table 3). In *M. tintinnabulum* and *B. amphitrite*, not more than three setae appear during the same period. Daniel (1958) also observed fewer setae on the mandibles (19, instead of 20, 21, and 22 in stages IV, V, and VI, respectively).

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**LITERATURE CITED**


Daniel, A. 1958. The development and metamorphosis of three species of sessile barnacles.—Journal of Madras University (B) 28: 23–47.


——. 1974b. Larval development of the barnacle *Tetraclitella karandei* reared in the laboratory.—Biological Bulletin 146: 249–257.


Ovsyannikova, I. I., and 0. M. Korn. 1984. Naupliar development of the barnacle *Balanus crenatus* in Peter
the Great Bay (Sea of Japan).—Biologya Morya 5: 34–40.


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