

## Larval development of *Balanus reticulatus* Utinomi, 1967 (Cirripedia, Thoracica) and a comparison with other barnacle larvae

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**Abstract.** The morphological features of the cephalic shield, labrum, abdominal process, antennules, antennae and mandibles of *Balanus reticulatus* are described and illustrated. The size and setation formulae of the larvae are given at each stage. The trilobed labrum and lateral margin of the cephalic shield with numerous small spines are diagnostic features for all the subsequent nauplius stages. Numerous small denticulate processes on the surface of the cyprid carapace are major morphological characteristics not found in other balanomorph species. We have constructed keys from stage II to stage VI for the predominant barnacle nauplii of Korean coastal waters, based on morphological traits such as total length, shield width, labrum shape, the presence or absence of posterior shield spines and dorsal shield spines in stages IV, V and VI, the specific setal type in the fourth segment of the antennal endopodite, and setation formulae of *Pollicipes mitella*, *Chthamalus challenger*, *Megabalanus rosa*, *B. reticulatus*, *Balanus amphitrite* and *Balanus albicostatus*.

### Introduction

Within the genus *Balanus*, larval development is relatively well known for 13 species, as described and illustrated from Branscomb and Vedder (1982) for *B. crenatus*, Barnes and Costlow (1961) for *B. balanus*, Costlow and Bookout (1957) for *B. eburneus*, Brown and Roughgarden (1985) for *B. glandula*, Egan and Anderson (1986) for *B. amphitrite*, Lee and Kim (1991) for *B. albicostatus*, Jones and Crisp (1954) for *B. improvisus*, Karande (1979) for *B. kondakovi*, Barnes and Barnes (1959) for *B. nubilis*, Sandison (1967) for *B. pallidus*, Barker (1976) for *B. trigonus*, Egan and Anderson (1986) for *B. variegatus*, and Lang (1979) for *B. venustus*. Although the larval development of barnacles belonging to this genus is well investigated, more information is required due to the simplicity and similarity of the morphological features available for larval classification. Studies of *B. reticulatus* Utinomi, 1967 have not resolved the morphological features of the six nauplius stages and the cyprid. Comparisons of morphological characteristics between the larvae of *B. reticulatus* and other barnacles would provide helpful information for the study of marine ecology and planktology. The purpose of the present study is to describe the detailed morphological characteristics of the nauplius and cyprid larvae of *B. reticulatus*, and to compare them with those of the other known barnacles inhabiting Korean coastal waters.

### Method

#### *Culture of adult barnacles*

The sessile barnacle *B. reticulatus* Utinomi, 1967 was collected from intertidal rocks at Kyokpo, located on the southwestern coast of Korea (Kim, 1985). Adult

barnacles attached to rocks were thoroughly rinsed with filtered sea water in the laboratory and then reared in several aquaria. They were fed daily on newly hatched *Artemia* nauplii, reared in the laboratory, and copepods collected from a plankton net.

### *Larval rearing*

Shells of some barnacles were broken open to investigate the degree of egg maturity. Egg masses containing ova with a nauplius eye were transferred to a beaker filled with filtered sea water plus antibiotics. Hatching of these eggs occurred in a couple of days. Hatched nauplii, which concentrated near the light source, were removed with a Pasteur pipette, transferred into 6-well tissue culture plates (10 nauplii per well), and separated into a beaker containing 1000 ml of filtered sea water for mass culture. *Nitzschia closterium* was supplied as food for nauplii. The basic culture method was modified slightly from that of Brown and Roughgarden (1985). The culture was carried out in a constant-temperature cabinet under conditions of a 14 h light:10 h dark photoperiod at a temperature of 25°C.

### *Larval descriptions*

Owing to their rapid molting to stage II, some of the newly hatched larvae were immediately preserved in 70% alcohol after hatching. Forty larvae at each stage were removed with the aid of a stereomicroscope. Preserved exuviae and larvae were dissected with fine tungsten needles in a mixture of glycerin and alcohol. Appendages at each stage were stained with 1% gentian violet for 2 min. Drawings were made with a camera lucida and measurements with an ocular micrometer. The total length was measured from the anterior margin of the shield to the tip of the dorsal thoracic spine. Shield length and width were measured from the anterior margin of the cephalic shield to the hind shield margin, and at the greatest distance across the body, respectively.

## **Results**

Nauplius larvae of *B.reticulatus* have a uniform cephalic shield with numerous small spines in the lateral margin (Figure 1). A pair of frontolateral horns, an abdominal process and dorsal thoracic spines are present. A pair of posterior shield spines are shown at stages IV, V and VI. The thorax has a pair of frontal filaments, a trilobed labrum and three pairs of appendages, including the antennule, antenna and mandible. A nauplius eye is present at all nauplius stages. The larval measurements are given in Table I. Tables II and III show the numerical (Bassindale, 1936) and alphabetical setal formulae (Newman, 1965) of appendages, respectively. The detailed morphological characteristics of the larvae are as follows (Figures 1–7).

**Table I.** Dimensions of the larvae of *B. reticulatus*. Ten larvae were measured to give means with the standard deviation at each stage

Stage	Total length (µm)	Shield width (µm)	Shield length (µm)
I	235 ± 15	134 ± 15	
II	342 ± 19	168 ± 19	
III	375 ± 21	1920 ± 13	
IV	482 ± 23	263 ± 18	325 ± 17
V	591 ± 17	324 ± 21	414 ± 13
VI	642 ± 24	331 ± 25	438 ± 19
C	613 ± 21	245 ± 17	

**Table II.** The numerical setal formulae of antennule, antenna and mandible in the nauplii of *B. reticulatus*

Stage	Antennule	Antenna	Mandible
I	04211	014-03222G	013-03222G
II	04211	025-03222G	014-03232G
III	14211	025-03223G	014-03332G
IV	114211	036-05324G	014-04343G
V	11142111	037-05324G	015-04443G
VI	11142121	048-05324G	015-04443G

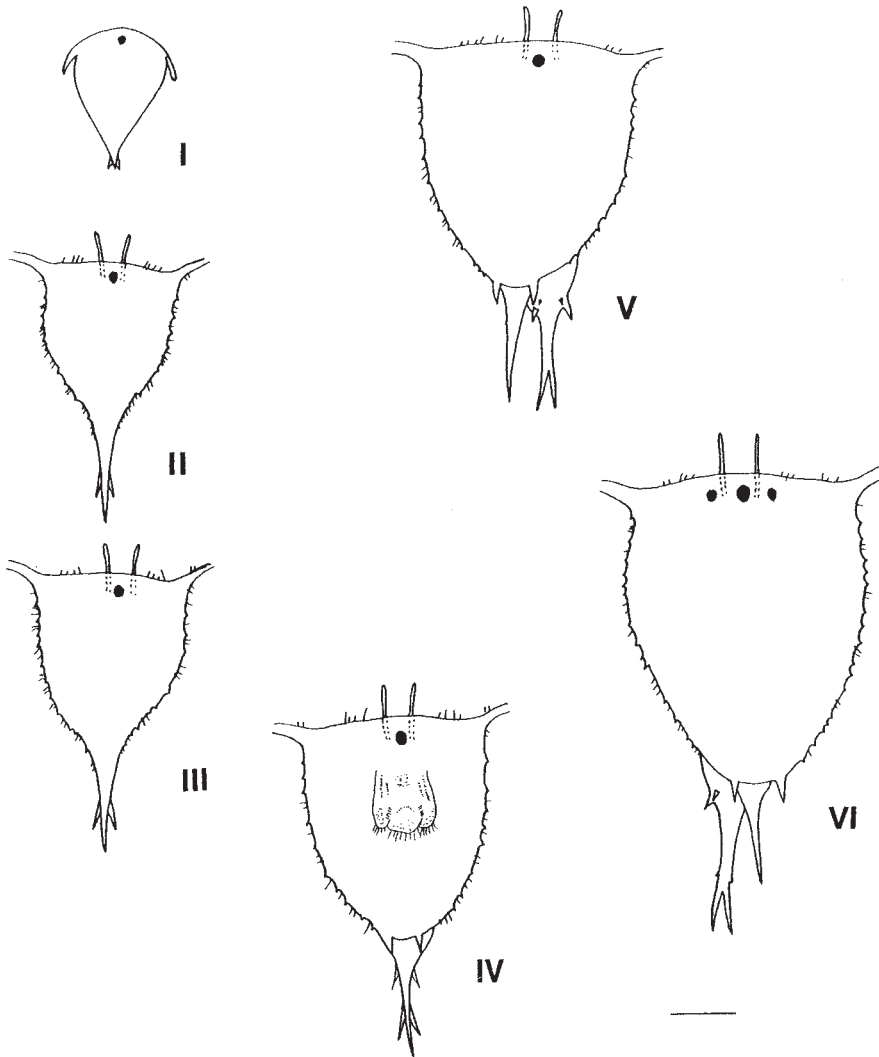
**Table III.** The alphabetical setal formulae of antennule, antenna and mandible in the nauplii of *B. reticulatus*

Stage	Antennule	Antenna		Mandible	
		Exopodite	Endopodite	Exopodite	Endopodite
I	4S:2S:S:S	S:4S	3S:2S:2S:2S:G	S:3S	3S:2S:2S:2S:G
II	2SPS:SP:P:S	SP:4PS	2PS:SP:PD:SC:G	P:3PS	3S:SP:PDP:PC:G
III	S:PS2P:SP:P:S	2P:5P	3P:SP:PD:SCP:G	P:3PS	3S:SPS:PDP:PC:G
IV	S:S:PS2P:SP:P:S	3P:5PS	2P2SP:SPS:PD:PC2S:G	P:4P	3S:SP2S:SPDP:PCP:G
V	S:S:P:PS2P:2P:P:S:S	3P:7P	2PS2P:SPS:PD:PC2S:G	P:4PS	4S:S2PS:SPDP:PCP:G
VI	S:S:P:PS2P:2P:P:PS:P	4P:8P	2PS2P:SPS:PD:PC2S:G	P:5P	4S:S2PS:SPDP:PCP:G

Setal types: S, simple; P, plumose; D, plumodenticulate; C, cuspidate; G, gnathobase.

### Stage I

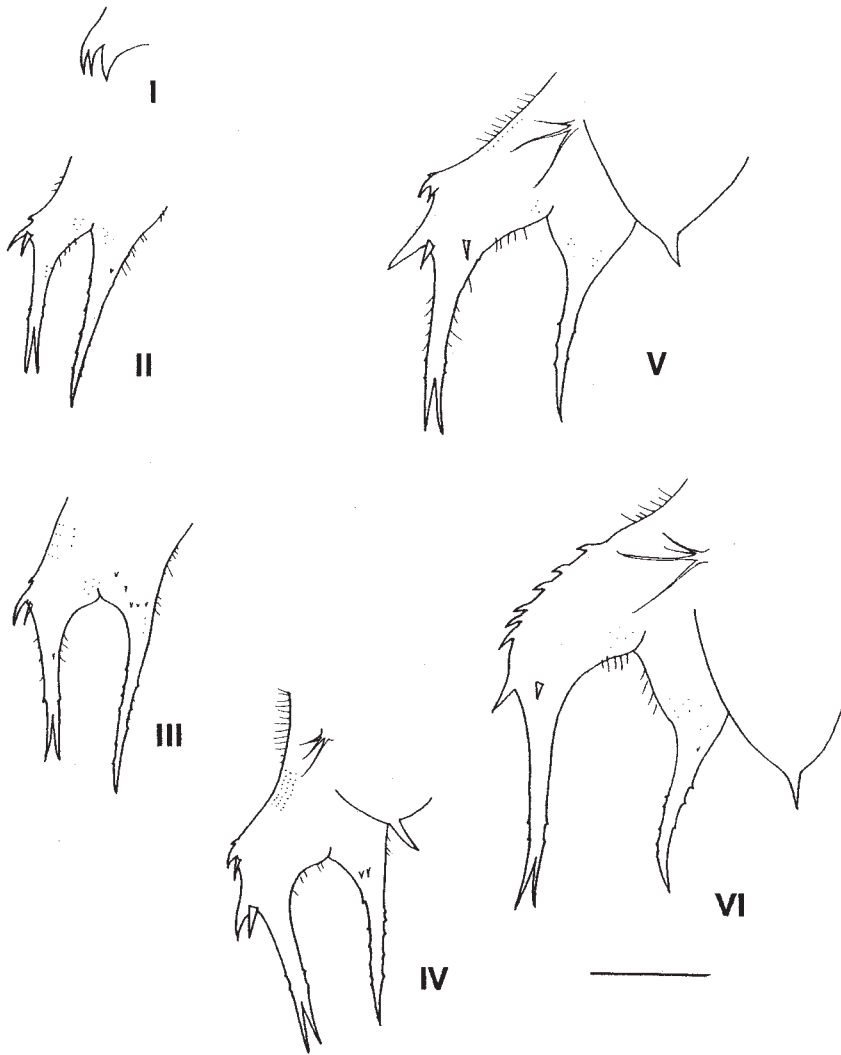
The pear-shaped cephalic shield has a pair of frontolateral horns and a dorsal thoracic spine. The anterior margin of the cephalic shield is strongly convex under the dorsal region. Frontal filaments are not seen on the frontal side. The nauplius eye is present in a frontomedian position and remains constant throughout all the subsequent stages. The abdominal process and dorsal thoracic spine are rudimentary and almost similar in length. Appendages such as antennule, antenna and mandible have simple setae without fine setules.



**Fig. 1.** Outline drawing of the six nauplius stages of *B.reticulatus*. Scale bar = 100  $\mu$ m.

### Stage II

The lateral margin of the cephalic shield is covered with numerous small spines. The frontolateral horns are now extended and parallel with the frontal side of the cephalic shield. Frontal filaments are present on the anterior shield margin of the nauplius. The abdominal process and dorsal thoracic spine are prominently developed, the latter being longer than the former. The abdominal process terminates in a bifurcated ramus and has a pair of abdominal spines. Two thoracic spines and a pair of lateral small spines are present over the abdominal process. There are

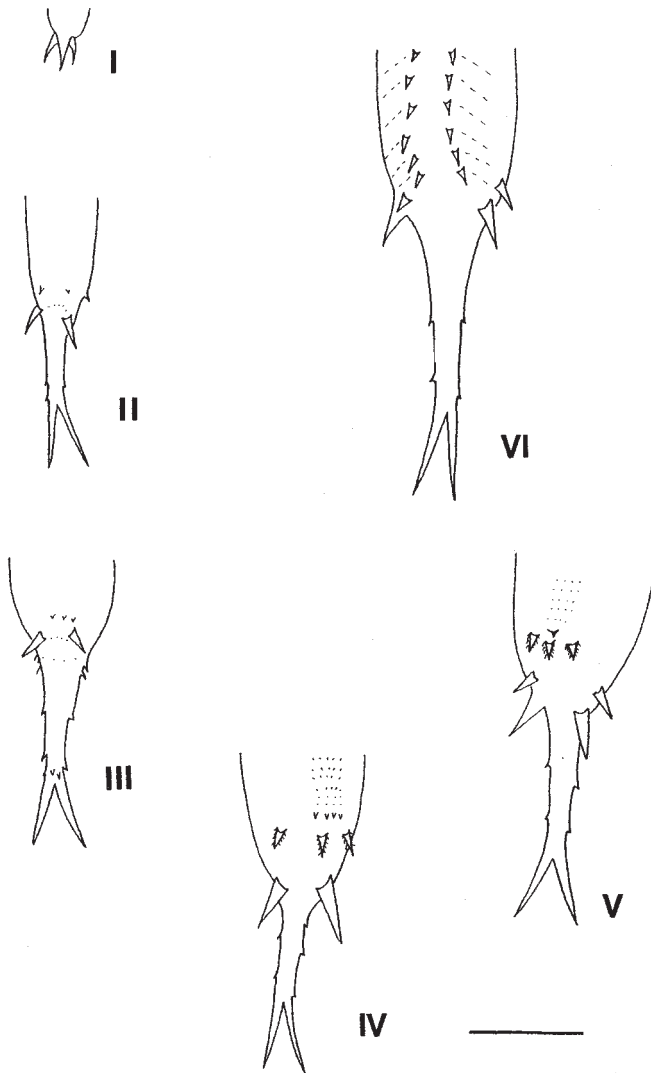


**Fig. 2.** Outline drawing of the lateral view of the six nauplius stages of *B. reticulatus*. Scale bar = 100  $\mu\text{m}$ .

numerous fine setules on some setae of the three appendages. No pre-axial seta is observed on the antennule.

### Stage III

The lateral margin of the cephalic shield has numerous small spines and is more rounded when compared with that of the previous stage nauplii. Frontolateral horns become somewhat thickened and appear diminished in length. The dorsal thoracic spine is barbed and constantly longer than the abdominal process. Two

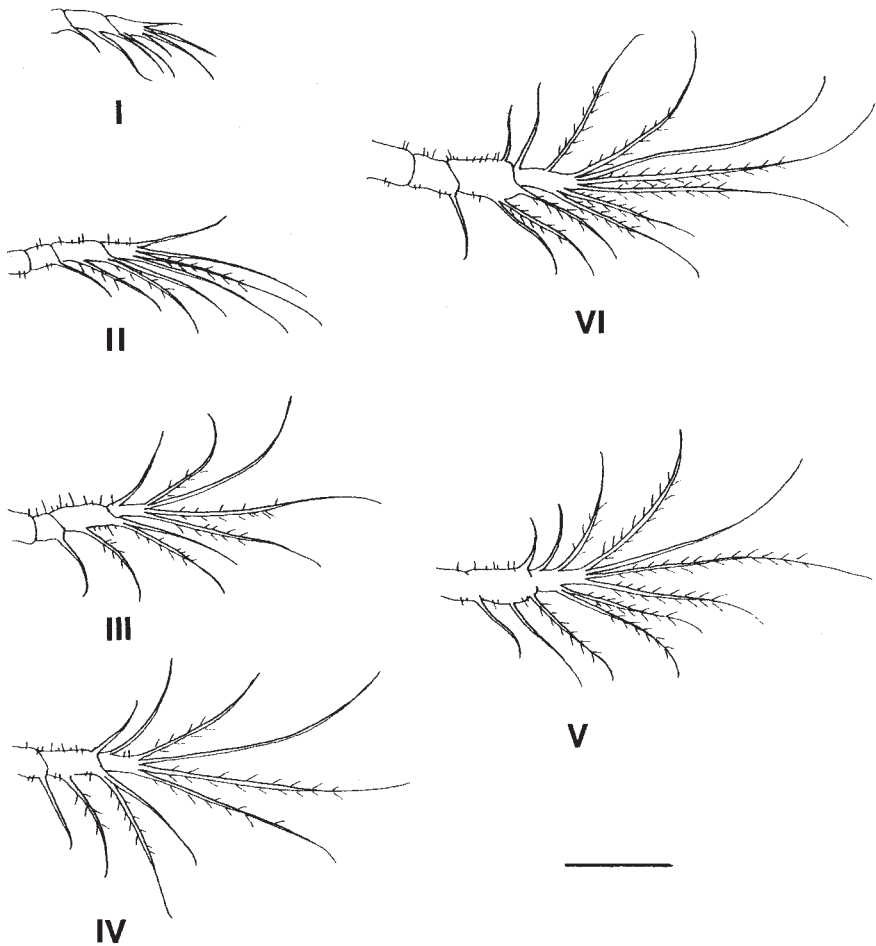


**Fig. 3.** Outline drawing of the abdominal process of the six nauplius stages of *B.reticulatus*. Scale bar = 100  $\mu$ m.

thoracic spines and lateral spines on the abdominal spines of nauplius stage II replace the three thoracic spines at this stage. A pre-axial seta, present on the antennules, is a diagnostic feature of these nauplii.

#### Stage IV

The lateral margin of the cephalic shield is covered with numerous small spines. The cephalic shield has a distinct posterior border bearing a pair of posterior



**Fig. 4.** Antennules of the six nauplius stages of *B. reticulatus*. Scale bar = 100  $\mu$ m.

shield spines. The dorsal thoracic spine is more or less shorter than the abdominal spine. There are three thoracic spines on the abdominal process and four small spines on the thoracic spines that are arranged in a transverse row. Two pre-axial setae, present on the antennules, are a diagnostic feature of these nauplii.

#### *Stage V*

The lateral margin of the cephalic shield is covered with numerous small spines. The cephalic shield has an increased size and length, but the general shape remains the same as in the fourth nauplius stage. The dorsal thoracic spine remains barbed, but is now shorter than the abdominal process. A pair of abdominal spines (series 2 spines) has appeared just anterior to the series 1 spines. The series 1 spines are relatively longer than the series 2 spines. Armored shape is observed on the

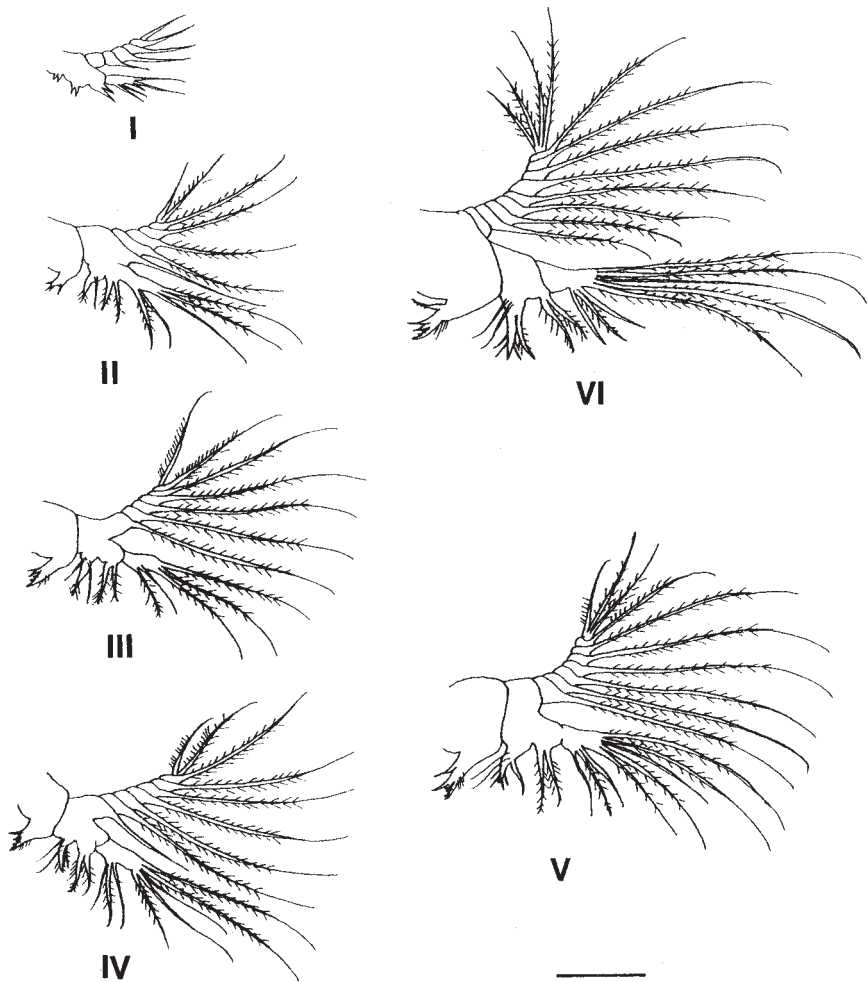


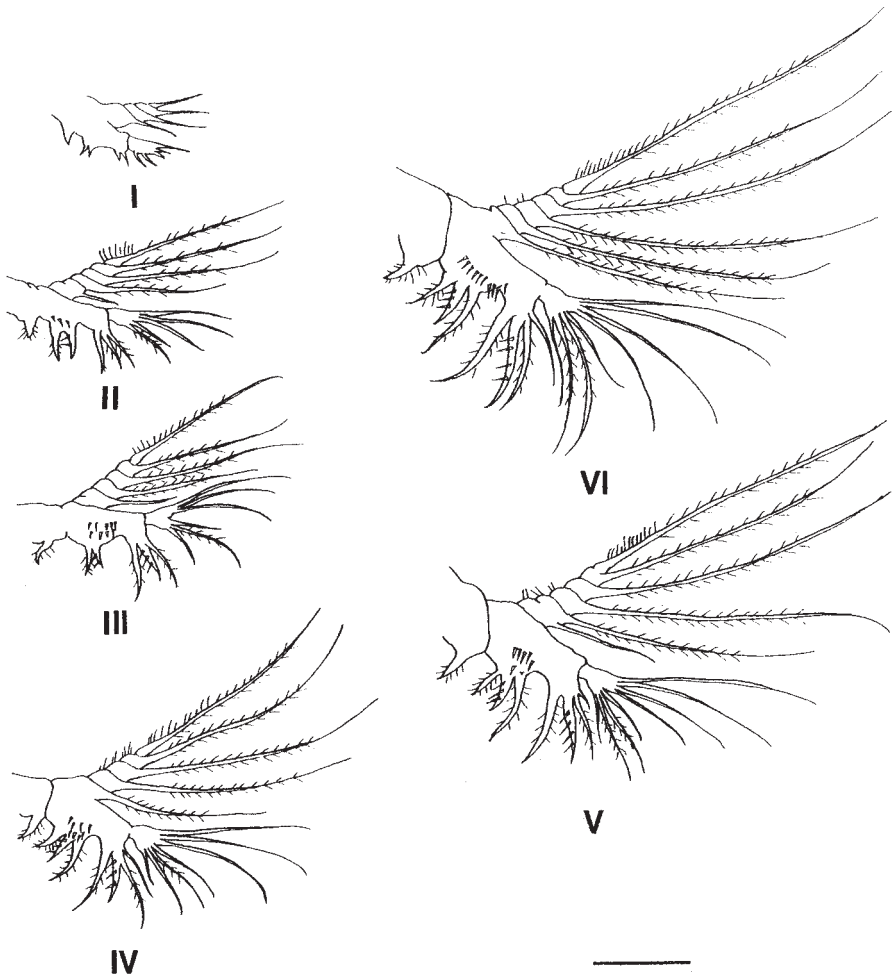
Fig. 5. Antennae of the six nauplius stages of *B. reticulatus*. Scale bar = 100  $\mu$ m.

thoracic spines. There are three thoracic spines, arranged in a transverse row, and a small spine on the thoracic spines. Three pre-axial setae and five post-axial setae, present on the antennules, are diagnostic features of these nauplii.

### Stage VI

The lateral margin of the cephalic shield is covered with numerous small spines. This stage larva is easily distinguished from the larvae of the other stages by six pairs of thoracic spines occurring on the series 1 and 2 abdominal spines. The primordia of the cyprid thoracic appendages are now seen beneath them. A pair of compound eyes is present lateral to the nauplius eye. Three pre-axial setae and



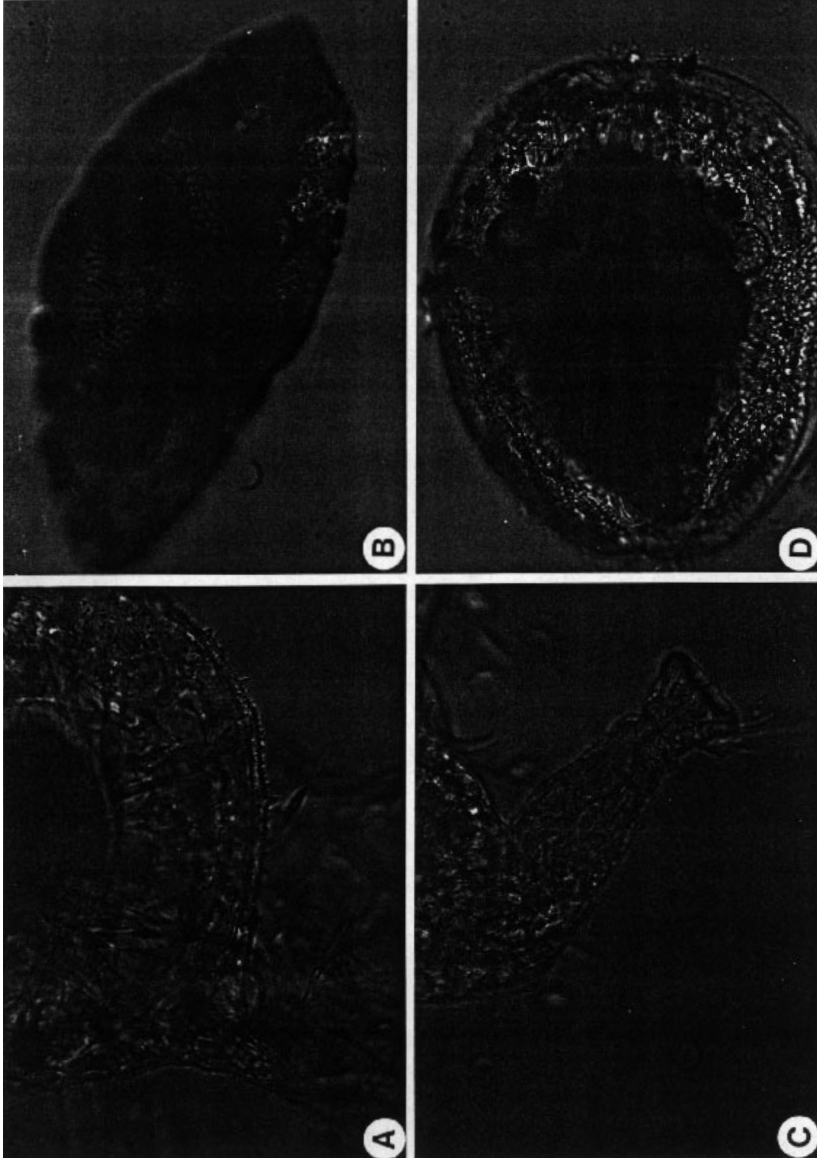


**Fig. 6.** Mandibles of the six nauplius stages of *B. reticulatus*. Scale bar = 100  $\mu$ m.

six post-axial setae, which occur on the antennules, are diagnostic features of these nauplii.

#### *Cyprid stage*

The surface of the carapace is covered with numerous small denticulate processes, typical of this species. The head is packed with numerous oil droplets. The antennule is reduced and terminates with the fourth segment. There are six pairs of thoracic appendages and a caudal furca on the thoracic region. Median and compound eyes appear on the anterior region of this larva.



**Fig. 7.** Nauplius, cyprid and young adult of *B. reticulatus*. (A) Lateral margin of the cephalic shield with numerous small spines in nauplius of stage II. (B) A cyprid with numerous small denticulate processes on the carapace surface and with a median eye. (C) Antennule of the cyprid terminated in the fourth segment. (D) Juvenile young adult with compound eyes in front of the anterior region.

## Discussion

In coastal waters, where barnacle larvae of several species are hatched synchronously, the morphological similarities of cyprid larvae of different species make identification difficult. Lang (1979) noted that a study of their pigmentation, size variations and fine carapace detail may eventually result in methods which could distinguish all cyprids, since cyprid larvae are very similar in most barnacle species, and size and even length to width ratios of the carapace vary with rearing conditions. Miller and Roughgarden (1994) suggested that the identification of cyprids must take into account their relative sizes and body forms since species-specific setulation has not been established in barnacle cyprids. The results of the present study indicate that it is possible to distinguish the cyprid of *B. reticulatus* from other balanoid cyprids by the presence of numerous small denticulate processes on the surface of the cyprid carapace.

A comparison of the nauplii of *B. reticulatus* with those of *B. albicostatus* revealed that the former differed markedly from the latter in the shape of the lateral margin of the cephalic shield and numerous fine setules on the antennule. The nauplii, however, were similar in total length, shield width and numerical setation of the appendages. Numerous small spines and slender hairs on the lateral margin of the cephalic shield, and the presence of significant fine setules on the antennule, enable easy separation of *B. reticulatus* nauplii from those of other balanoid genera. It is possible to distinguish the larvae of *B. reticulatus* from those of *B. amphitrite* (Egan and Anderson, 1986) by the arrangement, shape and number of thoracic spines above the abdominal spines on the abdominal process. The former nauplii possess an open circle of small thoracic spines extending forwards, anterior to the abdominal spines in stage II, two rows of fine setules lying at right angles to the long axis of the body in stage III, parallel rows of fine setules on three smaller thoracic spines in stage IV, and two rows of small thoracic spines on two distinct thoracic spines in stage V. The nauplii of *B. amphitrite* have three smaller thoracic spines on the abdominal spines in stages II and III, three thoracic spines, four smaller thoracic spines and a parallel row of fine setules in stage IV, and a median spine on three serrated thoracic spines in stage V.

*Chthamalus* nauplii have a cephalic shield nearly as broad as it is long, with a highly convex dorsal surface. They lack a pair of posterior shield spines in stages IV, V and VI, have the frontolateral horns folded under the anterior shield margin, and retain a unilobed labrum (Sandison, 1954, 1967; Korn and Ovsyannikova, 1979; Achituv, 1986). The appendages of nauplii of *Chthamalus* differ in both setal type and number from those of balanoid nauplii. Egan and Anderson (1989) noted that chthamaloid nauplii possess two setal types, feathered and hispid, which are never found on the limbs of coronuloid and balanoid nauplii. *Chthamalus challengerii* nauplii possess a hispid seta on the fourth segment of the antennal endopodite and an increased setal number compared with the setal type and number of balanoid nauplii. Chthamaloid larvae have at least 16 setae on the antennal endopodite of stage VI, but no more than 14 setae occur in the balanoid larvae.

The larvae of *Chthamalus dalli* and *Chthamalus fusus* are similar to those of *Pollicipes polymerus* in general shape, size and the setation of appendages, but

differences between these species exist in the setal types occurring at different development stages and, in a few instances, the setal number (Miller and Roughgarden, 1994). The larvae of *C.challengeri* are morphologically similar to those of the pedunculate barnacle *Pollicipes mitella*, both in setal type of the antennal endopodite and the shape of the labrum. They both have similar unilobed labra and hispid seta on the fourth segment of the antennal endopodite, which are never found on the appendages of balanid nauplii. However, morphological differences separate the larvae of the two species. The nauplii of the former have a rounder cephalic shield than those of the latter and lack posterior shield spines, and the nauplii of the latter are easily separated by the presence of an inverted triangular cephalic shield and a pair of posterior shield spines in stages IV, V and VI. Lang (1979) concluded that chthamaloid larvae differ in many aspects from coronuloid and balanoid larvae, and perhaps most closely resemble lepadomorph larvae. Egan and Anderson (1989) suggested that chthamaloid nauplii share morphological similarities with the planktotrophic nauplii of lepadomorphs and verrucosomorphs, which bear a unilobed labrum, feathered setae and similar shape and orientation of the gnathobase. They also deduced that fossil evidence and comparative functional morphological evidence support the evolution of chthamaloid larvae from a scalpellid–lepadomorph ancestry. Newman (1987) explained that the transition from a pedunculate to a sessile way of life was evolutionarily more complicated, and that this has a significant bearing on our understanding of the paleoecology as well as the evolution of sessile barnacles.

Dorsal shield spines are a prominent characteristic of the nauplii of *Megabalanus rosa* (Choi *et al.*, 1992), but their phylogenetic function remains unknown. They are also found in the larvae of the lepadomorphs *Lepas pectinata* (Lang, 1979), *Lepas fascicularis* (Bainbridge and Roskell, 1966) and *Conchoderma auritum* (Dalley, 1984), and in the balanomorphs *Balanus venustus* (Lang, 1979), *Conopea galeata* (Molenock and Gomez, 1972) and *Hexaminius popeiana* (Egan and Anderson, 1985). A pair of dorsal shield spines occur in nauplius stages IV–VI of *M.rosa* and stages III–VI of *Conchoderma auritum*, while the larvae of *Conopea galeata* and *H.popeiana* have a median dorsal shield spine in stage II, and a pair of dorsal shield spines in nauplius stages III–VI. Egan and Anderson (1989) suggest that the disjunct distribution of dorsal shield spines in lepadomorphs and balanoids requires further functional investigation before its evolutionary significance can be assessed.

We constructed keys from stage II to stage VI for the predominant barnacle nauplii of Korean coastal waters, based on the morphological characteristics such as total length, shield width, shape of the labrum, the presence or absence of posterior shield spines and dorsal shield spines in stages IV, V and VI, specific setal type in the fourth segment of the antennal endopodite, and setation formulae of the appendages described and illustrated from Lee (1992) for *Pollicipes mitella*, Lee (1999) for *C.challengeri*, Choi *et al.* (1992) for *M.rosa*, the present study for *B.reticulatus*, Egan and Anderson (1986) for *B.amphitrite* and Lee and Kim (1991) for *B.albicostatus* (Table IV). The first letters of the genus and species names abbreviate the species name. The following keys to barnacle larvae are helpful for the interspecific identification of unknown larvae collected in the field.

**Table IV.** Morphological characteristics of larvae depending on larval stage

S	SP	TL (µm)	SW (µm)	LB	SS	ST	Setal type of antennule	Setal type of antenna	Setal type of mandible
II	PM	500	235	Unilobed	DTS	Hispid	SP2S:SP:S	2P-4PS-2PS:PS:PD:SPC:G	P-3PS-3S:SP:PCP:PC:G
II	MR	470	230	Trilobed	DTS	Cuspidate	2SPS:SP:P:S	SP-4PS-2PS:SP:PD:SPC:G	P-3PS-3S:SP:PCP:PC:G
II	CC	373	175	Unilobed	DTS	Hispid	2SPS:SP:P:S	SP-4PS-2PS:PS:PD:SCPH:G	P-3PS-3S:SP:PCP:PC:G
II	BR	342	168	Trilobed	DTS	Cuspidate	2SPS:SP:P:S	SP-4PS-2PS:SP:PD:SC:G	P-3PS-3S:SP:PCP:PC:G
II	BA	350	150	Trilobed	DTS	Cuspidate	2SPS:SP:P:S	SP-4PS-2PS:SP:PD:SPC:G	P-3PS-3S:SP:PCP:PC:G
II	BAL	363	164	Trilobed	DTS	Cuspidate	2SPS:2S:P:S	SP-4PS-2PS:SP:PD:PC:G	P-3PS-3S:2S:PCP:PC:G
III	PM	520	252	Unilobed	DTS	Hispid	S:PS2P:SP:P:S	2P-5P-2PSP:PS:PD:SPC:G	P-4P-5S:2PS:PCP:2PC:G
III	MR	530	290	Trilobed	DTS	Cuspidate	S:PS2P:SP:P:S	2P-5P-3P:SP:PD:SPCS:G	P-3PS-3S:SP:PCP:2PC:G
III	CC	473	238	Unilobed	DTS	Hispid	S:2SPS:SP:P:S	2P-5P-3S:PS:PD:SCPH:G	P-3PS-3S:SP:PCP:PC:G
III	BR	375	192	Trilobed	DTS	Cuspidate	S:PS2P:SP:P:S	2P-5P-3P:SP:PD:SCPH:G	P-3PS-3S:SP:PCP:PC:G
III	BA	370	200	Trilobed	DTS	Cuspidate	S:PS2P:SP:P:S	2P-5P-3P:SP:PD:2SPC:G	P-3PS-3S:SP:PCP:PC:G
III	BAL	468	228	Trilobed	DTS	Cuspidate	S:PS2P:2S:P:S	2P-5P-3P:SP:PD:PC:G	P-3PS-3S:SP:PCP:PC:G
IV	PM	570	312	Unilobed	PSS	Hispid	S:P:PS2P:SP:P:S	2P-7P-2PSPS:SPS:PD:SPC:G	P-4P-5S:2PS:PCP:PC:G
IV	MR	610	350	Trilobed	DTS	Cuspidate	S:P:PS2P:SP:P:S	3P-6P-3P2S:SPS:PD:SPC:G	P-3PS-4S:2P:PCP:PC:G
IV	CC	540	323	Unilobed	NPSS	Hispid	S:P:2P2S:SP:P:S	2P-7P-4PS:SPS:PD:SCPH:G	P-4P-4S:2P:PCP:PC:G
IV	BR	482	263	Trilobed	PSS	Cuspidate	S:S:PS2P:SP:P:S	3P-5PS-2P2SP:S:PS:PD:PC2S:G	P-4P-3S:SP2S:SPDP:PCP:G
IV	BA	400	200	Trilobed	PSS	Cuspidate	S:S:PS2P:PS:P:S	3P-5PS-3P2S:SPS:PD:S2PC:G	P-3PS-4S:2P:DSPC:DPC:G
IV	BAL	470	247	Trilobed	PSS	Cuspidate	S:P:PS2P:SP:P:S	3P-5PS-3PS:SPS:PD:PC:G	P-4P-4S:2P:PCP:PC:G
V	PM	760	460	Unilobed	PSS	Hispid	S:P:P:PS2P:SP:P:S	3P-8P-5P:2PS:SPDP:SPC:G	P-5P-5S:2PS:PCP:PC:G
V	MR	760	460	Trilobed	PSS	Cuspidate	S:P:P:PS2P:SP:P:S	3P-7PS-3PS:SP:SP:SDCP:G	P-4PS-4S:SPSP:SPCP:2PC:G
V	CC	587	357	Unilobed	NPSS	Hispid	S:P:P:2PSP:SP:P:S	3P-7PS-4PS:2PSP:SDSP:SCPH:G	P-5P-SP:SP:SP:PCP:PC:G
V	BR	591	324	Trilobed	PSS	Cuspidate	S:S:P:PS2P:2P:P:S	3P-7P-4PS:SPS:PD:PC2S:G	P-4PS-4S:2PS:SPDP:PCP:G
V	BA	470	270	Trilobed	PSS	Cuspidate	S:S:P:PS2P:PS:P:S	4P-6PS-4PS:SP:PD:2PC:G	P-4PS-4S:2SD:SDFC:PC:G
V	BAL	161	277	Trilobed	PSS	Cuspidate	S:S:P:PS2P:SP:P:S	4P-6P-5P:2P:PD:PC3SP:G	P-4PS-4S:2P:SPDP:2PC:G
VI	PM	142	432	Unilobed	PSS	Hispid	S:P:P:PS2P:SP:P:P:S	3P-8P-5P:2PS:PCP:PC:G	P-5P-5S:2PS:PCP:PC:G
VI	MR	986	596	Trilobed	NPSS	Cuspidate	S:P:P:PS2P:SP:P:P:S	4P-8P-3P:SP:SP:PCP:PC:G	P-5P-5S:SPSP:SPCP:PCP:G
VI	CC	638	398	Unilobed	NPSS	Hispid	S:P:P:2PSP:SP:P:P:S	3P-8P-5P:2PS:SPDP:SCPH:G	P-5P-2PSP:SP3PS:PCP:PC:G
VI	BR	642	331	Trilobed	PSS	Cuspidate	S:S:P:PS2P:2P:P:PS:P	4P-8P-2P2SP:SPS:PD:PC2S:G	P-5P-4S:2PS:SPDP:PCP:G
VI	BA	540	310	Trilobed	PSS	Cuspidate	S:S:P:PS2P:PS:P:S	4P-8P-4PS:SP:PD:2PC:G	P-5P-4S:2SD:SDFC:PC:G
VI	BAL	630	305	Trilobed	PSS	Cuspidate	S:P:P:PS2P:SP:P:P:S	4P-8P-5P:SP:PD:PC2P:G	P-5P-4S:2P:SPDP:2PC:G

S, stage; SP, species; TL, total length; LB, shape of labrum; SS, shape of shield spine; ST, specific setal type observed in the fourth segment of antennal endopodite; DTS, dorsal thoracic spine; PSS, posterior shield spine; NPSS, no posterior shield spine; PM, *Politicipes mitella*; CC, *Chthamalus chalcogaster*; MR, *Megalobalanus rosa*; BR, *Balanus reticulatus*; BA, *Balanus amphitrite*; BAL, *Balanus albicostatus*.

**Key to nauplius stage II of barnacle species in the coastal waters**

- 1a. Labrum unilobed .....2
- 1b. Labrum trilobed.....3
- 2a. Cephalic shield rounded; anterior cephalic shield margin more curved under ventrally; dorsal thoracic spine shorter than the length of cephalic shield; hispid seta on fourth group of antennal endopodite present; antennal setation formula of 025-03233G; mandibular setation formula of 014-03232G .....*Chthamalus challengerii*
- 2b. Cephalic shield inverted triangular; dorsal thoracic spine longer than the length of cephalic shield; hispid seta on fourth group of antennal endopodite present; a pair of cephalic shield spines present; antennal setation formula of 025-03223G; mandibular setation formula of 014-03232G.....*Pollicipes mitella*
- 3a. Lateral margin of cephalic shield with small spines and slender hairs; a pair of thoracic spines on abdominal spine present; antennular segments with slender hairs; antennal setation formula of 025-03222G; mandibular setation formula of 014-03232G.....*Balanus reticulatus*
- 3b. Lateral margin of cephalic shield without small spines and slender hairs; antennular segments without numerous slender hairs .....4
- 4a. An open circle of small thoracic spines on abdominal spines present .....5
- 4b. Three to five ubiquitous small thoracic spines on abdominal spines present; antennal setation formula of 025-03223G; mandibular setation formula of 014-03232G.....*Megabalanus rosa*
- 5a. An open circle of 8–9 small thoracic spines on abdominal spines present; larvae >350 µm in total length; antennal setation formula of 025-03222G; mandibular setation formula of 014-03232G .....*Balanus albicostatus*
- 5b. An open circle of six small thoracic spines on abdominal spines present; larvae <350 µm in total length; antennal setation formula of 025-03223G; mandibular setation formula of 014-03232G.....*Balanus amphitrite*

**Key to nauplius stage III of barnacle species in the coastal waters**

- 1a. Labrum unilobed .....2
- 1b. Labrum trilobed.....3
- 2a. Cephalic shield rounded; anterior cephalic shield margin more curved under ventrally; dorsal thoracic spine shorter than the length of cephalic shield; hispid seta on fourth group of antennal endopodite present; antennal setation formula of 025-03233G; mandibular setation formula of 014-03333G .....*Chthamalus challengerii*
- 2b. Cephalic shield inverted triangular; dorsal thoracic spine longer than the length of cephalic shield; hispid seta on fourth group of antennal endopodite present; a pair of cephalic shield spines present; antennal setation formula of 025-03224G; mandibular setation formula of 014-05433G .....*Pollicipes mitella*
- 3a. Lateral margin of cephalic shield with numerous small spines; three horizontal thoracic spines on abdominal spine present; antennular segments with slender hairs; antennal setation formula of 025-03223G; mandibular setation formula of 014-03332G.....*Balanus reticulatus*



- 3b. Lateral margin of cephalic shield without numerous small spines; antennular segments without slender hairs.....4
- 4a. A small thoracic spine on abdominal spines absent .....5
- 4b. A horizontal row of three small thoracic spines on abdominal spines present; antennal setation formula of 025-03224G; mandibular setation formula of 014-03333G.....*Megabalanus rosa*
- 5a. Five horizontal rows of fine setules on abdominal spines present; larvae >400 µm in total length; antennal setation formula of 025-03223G; mandibular setation formula of 014-03332G.....*Balanus albicostatus*
- 5b. Two horizontal rows of fine setules on abdominal spines present; larvae <380 µm in total length; antennal setation formula of 025-03224G; mandibular setation formula of 014-03333G .....*Balanus amphitrite*

### Key to nauplius stage IV of barnacle species in the coastal waters

- 1a. Labrum unilobed; posterior shield margin present.....2
- 1b. Labrum trilobed; posterior shield margin present .....3
- 2a. Cephalic shield rounded; anterior margin of cephalic shield more curved under ventrally; rounded posterior shield margin without posterior shield spines; dorsal thoracic spine shorter than the length of cephalic shield; hispid seta on fourth group of antennal endopodite present; antennal setation formula of 036-05324G; mandibular setation formula of 014-04333G .....*Chthamalus challengerii*
- 2b. Cephalic shield inverted triangular; dorsal thoracic spine longer than the length of cephalic shield; hispid seta on fourth group of antennal endopodite present; a pair of long posterior shield spines being equivalent to two-thirds of cephalic shield present; antennal setation formula of 027-05334G; mandibular setation formula of 014-05433G .....*Pollicipes mitella*
- 3a. Lateral margin of cephalic shield with numerous small spines; four transverse small thoracic spines and three horizontal thoracic spines on abdominal spine present; antennular segments with slender hairs; a pair of posterior shield spines present; antennal setation formula of 036-05324G; mandibular setation formula of 014-04343G.....*Balanus reticulatus*
- 3b. Lateral margin of cephalic shield without numerous small spines; antennular segments without slender hairs; a pair of posterior shield spines present .....4
- 4a. A pair of dorsal shield spines on cephalic shield absent .....5
- 4b. A pair of dorsal shield spines on cephalic shield present; a stout and three horizontal spines on abdominal spines present; antennal setation formula of 027-05324G; mandibular setation formula of 014-04343G .....*Megabalanus rosa*
- 5a. A pair of thoracic spines and a median small spine on abdominal spines present; larvae >460 µm in total length; antennal setation formula of 036-04323G; mandibular setation formula of 014-04333G.....*Balanus albicostatus*
- 5b. A horizontal row of three smaller thoracic spines on abdominal spines present; larvae <460 µm in total length; antennal setation formula of 036-05324G; mandibular setation formula of 014-04343G .....*Balanus amphitrite*

**Key to nauplius stage V of barnacle species in the coastal waters**

- 1a. Labrum unilobed; posterior shield margin present; two pairs of abdominal spines on abdominal process present.....2
- 1b. Labrum trilobed; posterior shield margin present; two pairs of abdominal spines on abdominal process present.....3
- 2a. Cephalic shield rounded; anterior cephalic shield margin more curved under ventrally; rounded posterior shield margin without posterior shield spines; dorsal thoracic spine shorter than the length of cephalic shield; hispid seta on fourth group of antennal endopodite present; antennal setation formula of 038-05444G; mandibular setation formula of 015-05433G .....*Chthamalus challengerii*
- 2b. Cephalic shield inverted triangular; dorsal thoracic spine longer than the length of cephalic shield; hispid seta on fourth group of antennal endopodite present; a pair of long posterior shield spines being equivalent to two-thirds of the cephalic shield present; antennal setation formula of 038-05334G; mandibular setation formula of 015-05433G .....*Pollicipes mitella*
- 3a. Lateral margin of cephalic shield with numerous small spines; a small spine and three horizontal thoracic spines on abdominal spine present; antennular segments with slender hairs; a pair of posterior shield spines present; antennal setation formula of 037-05324G; mandibular setation formula of 015-04443G .....*Balanus reticulatus*
- 3b. Lateral margin of cephalic shield without numerous small spines; antennular segments without slender hairs; a pair of posterior shield spines present .....4
- 4a. A pair of dorsal shield spines on cephalic shield absent .....5
- 4b. A pair of dorsal shield spines on cephalic shield present; a stout and three horizontal spines on abdominal spines present; antennal setation formula of 038-05324G; mandibular setation formula of 015-04443G .....*Megabalanus rosa*
- 5a. A horizontal row of three thoracic spines on abdominal spines present; larvae >540 µm in total length; antennal setation formula of 046-05324G; mandibular setation formula of 015-04343G.....*Balanus albicostatus*
- 5b. Parallel rows of fine setules and a pair of small thoracic spines on abdominal spines present; larvae <540 µm in total length; antennal setation formula of 037-05324G; mandibular setation formula of 015-04443G .....*Balanus amphitrite*

**Key to nauplius stage VI of barnacle species in the coastal waters**

- 1a. Labrum unilobed; posterior shield margin present; two pairs of abdominal spines on abdominal process present; six pairs of thoracic spines present ....2
- 1b. Labrum trilobed; posterior shield margin present; two pairs of abdominal spines on abdominal process present; six pairs of thoracic spines present ....3
- 2a. Cephalic shield rounded; anterior cephalic shield margin more curved under ventrally; rounded posterior shield margin without posterior shield spines; dorsal thoracic spine shorter than the length of cephalic shield; hispid seta on fourth group of antennal endopodite present; antennal



- setation formula of 038-05554G; mandibular setation formula of 015-0544G .....*Chthamalus challenger*
- 2b. Cephalic shield inverted triangular; dorsal thoracic spine longer than the length of cephalic shield; hispid seta on fourth group of antennal endopodite present; a pair of long posterior shield spines being equivalent to two-thirds of cephalic shield present; antennal setation formula of 038-05334G; mandibular setation formula of 015-05433G .....*Pollicipes mitella*
- 3a. Lateral margin of cephalic shield with numerous small spines; antennular segments with slender hairs; a pair of posterior shield spines present; antennal setation formula of 048-05324G; mandibular setation formula of 015-04443G.....*Balanus reticulatus*
- 3b. Lateral margin of cephalic shield without numerous small spines; antennular segments without slender hairs; a pair of posterior shield spines present .....4
- 4a. A pair of dorsal shield spines on cephalic shield absent .....5
- 4b. A pair of dorsal shield spines on cephalic shield present; a stout and three horizontal spines on abdominal spines present; antennal setation formula of 039-05324G; mandibular setation formula of 015-04443G.....*Megabalanus rosa*
- 5a. Larvae >600 µm in total length; antennal setation formula of 048-05324G; mandibular setation formula of 015-04343G .....*Balanus albicostatus*
- 5b. Larvae <600 µm in total length; antennal setation formula of 048-05324G; mandibular setation formula of 015-04443G.....*Balanus amphitrite*

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