# Larval Development of *Chthamalus challengeri* Hoek (Cirripedia: Thoracica: Chthamalidae) with Keys to Barnacle Larvae of Korean Coastal Waters

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Key Words:
Chthamalus challengeri
Larval development
Barnacle key
Cyprid

Chthamalus challengeri Hoek was collected from intertidal rocks to rear the larvae from hatching through nauplius to cyprid in the laboratory. Larval development consists of six nauplius stages and a cyprid. Unilobed labrum with a prominent protuberance and the frontolateral horns folded under the anterior cephalic shield margin are diagnostic features through all nauplius stages. The posterior border of the cephalic shield bears no posterior shield spines in nauplius stages IV-VI. There is a specific hispid seta in the fourth group of the antennal endopodite. Morphological features such as the cephalic shield, labrum, abdominal process, antennules, antennae and mandibles in all nauplius and cyprid stages are illustrated and described. The numerical setations of the antennule are found to aid in the intraspecific identification of barnacle nauplius stages without dissection. The keys to each stage of the barnacle larvae in Korean coastal waters are provided based on the reared nauplii of seven species: Pollicipes mitella, Octomeris sulcata, Chthamalus challengeri, Balanus albicostatus, B. trigonus, B. amphitrite, and B. improvisus inhabiting Korean coastal waters.

Chthamalus challengeri Hoek, a predominant species attached to rocks in the intertidal zone, is present in Korea, Japan and the Philippines (Newman and Ross 1976; Kim, 1985, 1998). The larvae of Chthamalus stellatus from England and Southern India (Bassindale, 1936; Daniel, 1958), C. aestuarii from South Africa (Sandison, 1967), C. malayensis from India (Karande and Thomas, 1976), C. dalli from Russia (Korn and Ovsyannikova, 1979), C. fragilis from North America (Lang, 1979), C. dentatus from South Africa (Achituv, 1986), and C. antennatus from Australia (Egan and Anderson, 1989) have been described, but there is no larval description for the C. challengeri species. The purpose of this study is to describe the detailed morphology of the nauplius and cyprid stages of C. challengeri reared in the laboratory and to provide keys to the barnacle larvae of seven species found in Korean coastal waters.

## Materials and Methods

Chthamalus challengeri Hoek was collected from rocks in the intertidal zone near Pusan, Korea. The barnacles were placed in an aquarium containing the filtered sea water. They were fed daily on newly hatched *Artemia* nauplii.

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The hatched barnacle nauplii concentrated near the light source were removed with a Pasteur pipette. They were transferred into several 6-well tissue culture plates containing 10 nauplii per well and a 800 ml beaker containing filtered sea water to examine the developmental process. Nitzschia closterium, a diatom, was supplied as food. Streptomycin sulphate and penicillin were used to inhibit bacterial contamination. The basic culture method was derived from that of Lee and Kim (1991). Some of the newly hatched larvae were immediately preserved in 70% alcohol because of rapid molting to stage II. The culturing was carried out in a cabinet with conditions of 14L:10D photoperiod, temperature of 25°C and salinity of 33%. Live larvae, as well as exuviae, were preserved in 70% alcohol. Preserved larvae and exuviae were dissected under a stereomicroscope using fine tungsten needles in a mixture of glycerin and alcohol. Dissected specimens were stained with gentian violet to observe and describe the morphological characteristics of cephalic shield and the type of appendage setae. Drawings were made with the aid of a camera lucida. At least ten specimens at each stage were examined.

Measurements were made with an ocular micrometer. Total length was measured from the frontal margin of the cephalic shield to the end of dorsal caudal spines. Shield width was measured at its widest point and shield length from the frontal side of

Table 1. Dimension (  $\overline{\mathbf{x}} \times SD$ ) of the larvae of Chthamalus challengeri (n=10)

Stage	Total length (µm)	Shield width (µm)	Shield length (µm)
1	223±11	165±15	
II	373±18	175±13	-
111	473±21	238±19	-
١٧	540±16	$323 \pm 15$	325±17
V	587±17	$357 \pm 13$	394±15
VI	$638 \pm 23$	$398 \pm 15$	421±19
Cyprid	587±21	$268 \pm 13$	-

the cephalic shield to the end of posterior shield spines.

#### Results

The larvae of *C. challengeri* cultured in the laboratory pass through six nauplius stages and one cyprid before settling to the pin-head stage. Nauplius larvae consist of a cephalic shield, frontolateral horns, nauplius eye, labrum, appendages, abdominal process, dorsal thoracic spine from nauplius stagesI-VI and posterior shield spines at stages IV, V and VI. The mean size of the larval stage is given in Table 1. Descriptive drawings at each stage are given in Figs. 1-6. In addition to the drawing of appendages, the numerical (Bassindale, 1936) and alphabetical setation formula (Newman, 1965) are given in Tables 2 and 3. The detailed morphological characteristics at each stage are as follows:

## Nauplius I

The frontal margin of this larvae is more arched than those of later stages. The cephalic shield is more or less ovoid and very slender. Frontolateral horns are significantly bent and project backwards along the side of the body. Frontal filaments are not found. The caudal portion is short and blunt. The abdominal process and dorsal caudal spine are short and more or less equal in size. A median eye is present through all nauplius stages. Setae of each appendage bear no setules. The unilobed labrum has small teeth on the frontal side.

# Nauplius II

The frontolateral horns are swollen, bent toward the upside direction and directed perpendicular to the

Table 2. Numerical setal formulae of six nauplius stages of *Chthamalus challengeri* 

Stage	Appendages		
Stage	Antennule	Antenna	Mandible
1	04211	024-03222G	013-032220
н	04211	025-03234G	014-032320
111	14211	025-03234G	014-033330
IV	114211	037-05334G	014-043330
V	11142111	038-05444G	015-04433G
VI	11142121	038-05454G	015-055430

body axis. The cephalic shield has small fine spinules in the lateral region and bears small fine teeth on the lower part near the dorsal thoracic spine. The abdominal process and dorsal caudal spine are elongated. The frontal filaments are present and remain without change in all subsequent stages. Labrum is unilobed, with slender hairs through all subsequent stages. A pair of abdominal spines appear on the apex of the abdominal process. Some setae of appendages bear minute setules. There is a hispid seta in the fourth group of the antennal endopodite. A circlet of five small thoracic spines is present on a pair of abdominal spines.

## Nauplius III

The size of larvae increases in total length and width when compared to that of stage II. The frontal margin is flatter than that of stage II. A pair of fine teeth are not seen in the lateral region of the cephalic shield. A preaxial seta is present on antennules as a diagnostic feature of this nauplii. The abdominal process bears a pair of abdominal spines. There is a hispid seta in the fourth group of the antennal endopodite through all subsequent stages. A circlet of 5 small thoracic spines of stage II is replaced into a small thoracic spine in the median region of a pair of abdominal spines.

#### Nauplius IV

There are no posterior shield spines at this stage. A pair of short carapace spines mark the posterior edge. Two preaxial setae are present on antennules as a diagnostic feature of this nauplii. A pair of thoracic spines and a median spine are observed on a pair of abdominal spines over the abdominal process. Several rows of minute spinules are present on the surface of the abdominal process.

#### Nauplius V

Cephalic shield bears no posterior shield spines as in nauplius IV except for its enlargement. Three preaxial setae and five postaxial setae are present on antennules as a diagnostic feature of this nauplii. The dorsal thoracic spine becomes shorter than that of stage IV. The abdominal process bears 2 pairs of abdominal spines and the first pair is relatively longer than the second.

# Nauplius VI

Six pairs of thoracic spines are present under the thorax and the primordia of the cyprid thoracic appendages are observed underneath the exoskeleton of the thoracic spines. Three preaxial setae and six postaxial setae are present on antennules as a diagnostic feature of this nauplii. Paired compound eyes appear in the later period of this stage.

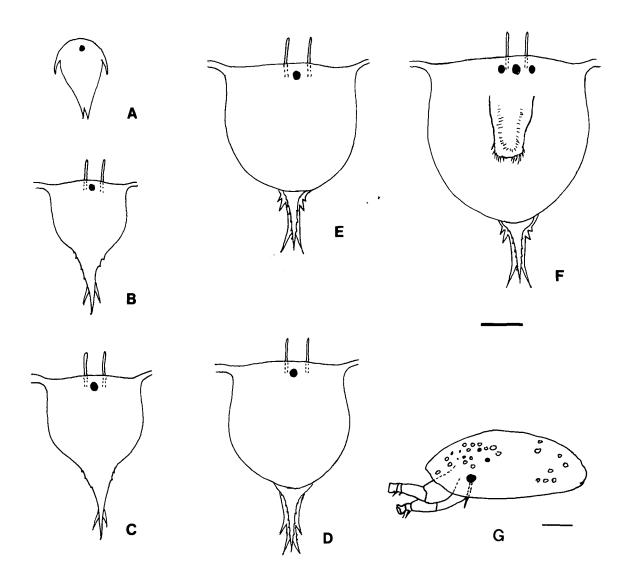


Fig. 1. Outline drawing of six nauplius stages and cyprid of *Chthamalus challengeri*. A, Nauplius I. B, Nauplius II. C, Nauplius III. D, Nauplius IV. E, Nauplius V. F, Nauplius VI. G, Cyprid. Scale bars=100 μm.

# Cyprid

Six pairs of thoracic appendages are present. The head is packed with oil cells. Segments of antennules are reduced when compared with those of nauplli.

# Discussion

It may be possible to classify many larval features in the barnacle species with adult taxonomy in Korea because larval morphologies of several barnacle species are described and illustrated. Chthamaloid larvae including those of *C. challengeri* have unique features which clearly separate them from the larvae of Balanoidea, and more closely resemble the lepado-

morph larvae since morphological characteristics such as the hispid seta and a unilobed labrum are shared. However, they lack the plumodenticulate setae found in the balanomorph larvae. Chthamaloid larvae possess a unilobed labrum with prominent protuberance, but lack a pair of posterior shield spines in the nauplius stages IV-VI. They have a specific hispid seta in the fourth group of the antennal endopodite and the frontolateral horns folded under the anterior cephalic shield margin. In contrast, Balanoidea larvae have a trilobed labrum with serrated setae, a pair of posterior shield spines in the nauplius stages IV-VI, but lack a hispid seta in the fourth group of the antennal endopodite and frontolateral horns that are perpen-

Table 3. Alphabetical setal formulae of larvae of Chthamalus challengeri

Stage	Appendages					
	Antennule		Antenna		Mandible	
		Exopod	Endopod	Exopod	Endopod	
I II III	4S:SS:S:S 2SPS:SP:P:S S:2SPS:SP:P:S S:P:2P2S:SP:P:S	2S:4S SP:4PS 2P:5P 3P:7P	3S:2S:2S:2S:G 2PS:2S:PDS:SCPH:G 3S:PS:PDS:SCPH:G 4PS:SPS:PDS:SCPH:G	S:3S P:3PS P:3PS P:4P	3S:2S:2S:2S:G 3S:SP:PDP:PC:G 3S:SPS:PDP:PCP:G 4S:S2P:PDP:PCP:G 2S:2P:S3P:PDP:PCP:G	
V VI	S:P:P:2PSP:SP:S:P:S S:P:P:2PSP:SP:P:2S:P	3P:7PS 3P:8P	4PS:2SPS:PDSP:SCPH:G 5P:2SPS:SPDSP:SCPH:G	P:5P P:5P	2SPSP:S3PS:PDP:PCP:	

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

dicular to the long axis of the cephalic shield. Antennal endopodite of stage VI nauplii of all chthamaloid shows at least 16 setae. For example, there are 18 setae in Chthamalus stellatus from Bassindale (1936) and Daniel (1958), 19 setae in C. aestuarii from Sandison (1967), 19 setae in C. dalli from Korn and Ovsyannikova (1979), 18 setae in C. fragilis from Lang (1979), 17 setae in C. dentatus from Achituv (1986), and 16 setae in C. antennatus from Egan and Anderson (1989). However, the setal number of the antennal endopodite does not exceed 14 in the Balanoidea larvae, that is, 14 setae are found in B. improvisus from Lee et al. (1998), 14 setae in B. albicostatus from Lee and Kim (1991), 14 setae in B. trigonus from Lee and Kim (1990), and 14 setae in B. amphitrite from Egan and Anderson (1986). Therefore, since the larvae of C. dalli from Russia (Korn and Ovsyannikova, 1979) within the Chthamalus genus are not frequently observed in the waters of the intertidal zone of Korea, it is easy to separate the larvae of C. challengeri from those of other barnacle species.

The numerical setation formulae of the antennule are helpful in the intraspecific identification of barnacle nauplius stages without dissection: stage I without preaxial setae and fine setule in all setae; stage II without preaxial setae but having some setae with fine setules; stage III with a preaxial seta and 4 postaxial setae; stage IV with 2 preaxial setae; stage V with 3 preaxial setae and 5 postaxial setae; stage VI with 3 preaxial setae and 6 postaxial setae.

Key to nauplius stages of 7 barnacle species inhabiting Korean coastal waters

- 1a. Antennule without preaxial seta-----2
- 1b. Antennule with preaxial seta-----3
- 2a. Frontolateral horns folded back; abdominal process and dorsal thoracic spine rudimentary; appendages without fine setules in setae; antennule with setation formula of 04211 ----- Nauplius I
- 2b. Antennule without a preaxial seta; abdominal process and dorsal caudal spine extended; appendages with fine setules in some setae; tip of

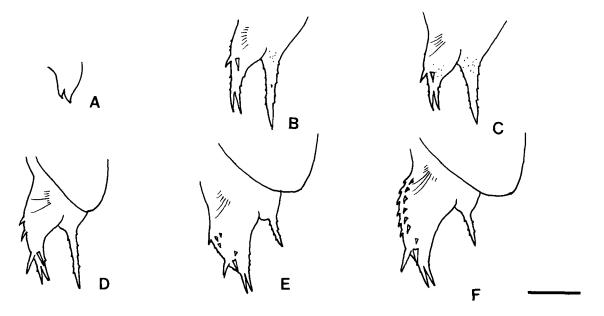


Fig. 2. Outline drawing of lateral view of six nauplius stages of Chthamalus challengeri. A, Nauplius I. B, Nauplius II. C, Nauplius III. D, Nauplius IV. E, Nauplius V. F, Stage VI. Scale bar=100 μm.

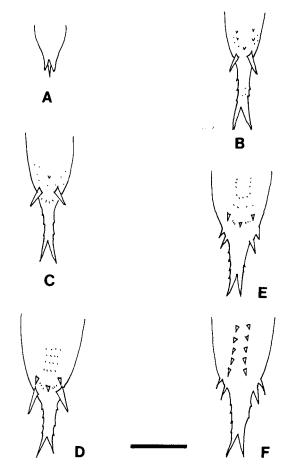


Fig. 3. Outline drawing of abdominal process of six nauplius stages of <code>Chthamalus challengeri</code>. A, Nauplius I. B, Nauplius II. C, Nauplius III. D, Nauplius IV. E, Nauplius V. F, Stage VI. Scale <code>bar=100 \mum</code>.

frontolateral horns without fine tuft; antennule with setation formula of 04211; a pair of abdominal spines present ------ Nauplius II 3a. Posterior shield margin absent; antennule with one preaxial seta; tip of frontolateral horns with fine tuft; abdominal process extended; antennular setation formula of 14211; a pair of abdominal spines present----- Nauplius III 3b. Posterior shield margin present-----4 4a. Compound eye absent-----5 4b. Compound eye present; 6 pairs of thoracic spines; antennule with 3 preaxial setae; presense of posterior shield margin with or without antennular setation formula of 11142121----- Nauplius VI 5a. Antennule with 2 preaxial setae; posterior shield margin with or without posterior shield spine; 2 pairs of abdominal spines present; antennular setation formula of 114211----- Nauplius IV 5b. Antennule with 3 preaxial setae; posterior shield margin with or without posterior shield spine; 2 pairs of abdominal spines present; antennular setation formula of 11142111----- Nauplius V

Based on morphological characteristics of the barnacle larvae reared in the laboratory, it is possible to produce keys to the nauplius stages of *Pollicipes mitella* (see Lee, 1992), *Octomeris sulcata* (see Kim, 1989), *Chthamalus challengeri* (the present study), *Balanus albicostatus* (see Lee and Kim, 1991), *B. trigonus* (see Lee and Kim, 1990), *B. amphitrite* (see Egan and Anderson, 1989), and *B. improvisus* (see Lee et al., 1998) inhabiting Korean coastal waters. As nauplii of stage I are rarely found in plankton samples because of rapid metamorphosis into nauplius stage II within 4 h and show little variations, the keys are defined for nauplius stages II-VI.

Key for nauplius II of 7 barnacle species inhabiting Korean coastal waters

1a.	Labrum unilobed2
	Labrum trilobed4
2a.	Cephalic shield round; dorsal caudal spine shorter
	than cephalic shield3
2b.	- 1
	spine longer than cephalic shield; a pair of ce-
	phalic shield spine present; antennal setation
	formula of SP:4PS-2PS:2S:PDS:SCPH:G; mandi-
	bular setation formula of P:3PS-3S:SP:PDP:PC:
	GPollicipes mitella
За.	Lateral margin of labrum with several teeth; a
	hispid seta at fourth group of antennal endopodite
	present; antennal setation formula of SP:5P-2PS:
	SP:PD:SPC:G; mandibular setation formula of P:
٥.	3PS-3S:SP:2PC:PC:GOctomeris sulcata
3b.	
	hispid seta at fourth group of antennal endopodite
	present; dorsal caudal spine and abdominal process with numerous small spinules; antennal
	setation formula of SP:4PS-2PS:2S:PDS:SCPH:G;
	mandibular setation formula of P:3PS-3S:SP:PDP:
	PC:G
12	PC:GChthamalus challengeri Frontolateral horns directed perpendicularly or re- ward5
<del>4</del> a.	ward5
4h	Frontolateral horns directed distinctly forward6
5a	An open circle of 6 small thoracic spines on abdo-
ou.	minal spine present; antennal setation formula of
	SP:4PS-2PS:SP:PD:SC:G; mandibular setation
	formula of P:3PS-3S:SP:SPC:PC:G; larvae less
	than 350 µm in total lengthBalanus amphitrite
5b.	An open circle of 8-9 small thoracic spines on
	abdominal spine present; antennal setation for-
	mula of SP:4PS-2PS:SP:PD:PC:G; mandibular seta-
	tion formula of P:3PS-3S:2S:PDP:PC:G; larvae
	less than 380 μm in total length
	Balanus albicostatus
6a.	An open circle of 6 small thoracic spines on
	abdominal spine present; median lobe of labrum
	bearing small teeth; antennal setation formula of
	SP:4SP-2PS:PS:2P:SCP:G; mandibular setation for-

mula of P:3PS-3S:PS:PCS:PC:G; larvae less than

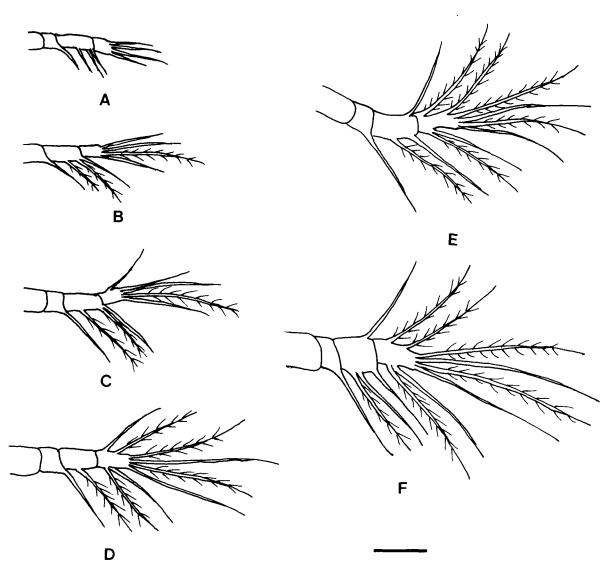


Fig. 4. Antennules of six nauplius stages of Chthamalus challengeri. A, Nauplius I. B, Nauplius II. C, Nauplius III. D, Nauplius IV. E, Nauplius V. F Stage VI. Scale bar=100 μm.

Key for nauplius III of 7 barnacle species inhabiting Korean coastal waters

- 1a. Labrum unilobed-----2
- 1b. Labrum trilobed-------4

  2a. Cephalic shield round; dorsal thoracic spine shorter than cephalic shield--------3
- 2b. Cephalic shield inverted triangle; dorsal thoracic spine longer than cephalic shield; a pair of ce-

- 3a. Lateral margin of labrum with several teeth; armoured shape under abdominal spine absent; a hispid seta at fourth group of antennal endopodite present; antennal setation formula of 2P:5P-3S:PS:PDS:SCPH:G; mandibular setation formula of P:3PS-3S:SPS:PDP:PCP:G-------

------Octomeris sulcata

3b. Lateral margin of labrum with sparse teeth; a small thoracic spine on abdominal spine present; a hispid seta at fourth group of antennal endopodite present; dorsal caudal spine and abdominal process with numerous small spicules; antennal setation formula of 2P:5P-3S:PS:PDS:SCPH:G; mandi-

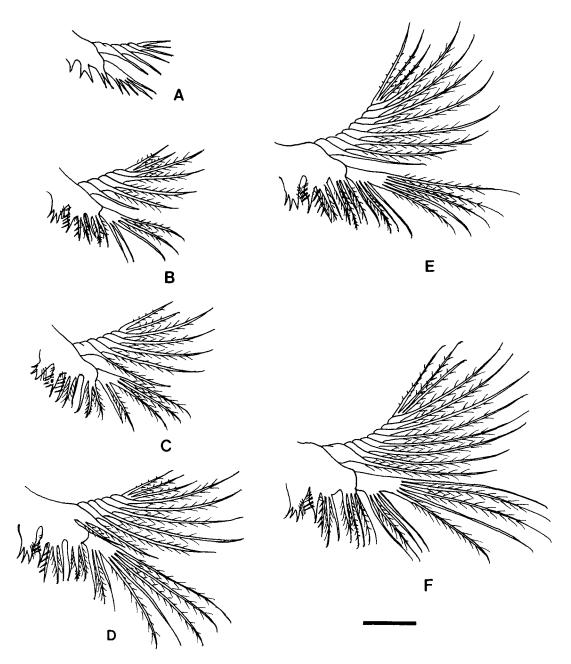


Fig. 5. Antennae of six nauplius stages of Chthamalus challengeri. A, Nauplius I. B, Nauplius II. C, Nauplius III. D, Nauplius IV. E, Nauplius V. F, Stage VI. Scale bar=100 µm.

- bular setation formula of P:3PS-3S:SPS:PDP:PCP: G------Chthamalus challengeri
- 4a. Frontolateral horns directed perpendicularly or reward-----5
- 4b. Frontolateral horns directed distinctly forward------6

- 6a. A transverse row of 3 small thoracic spines on abdominal spine present; median lobe of labrum bearing small teeth; antennal setation formula of 2P:5P-4P:PS:2P:SCPS:G; mandibular setation formula of P:3PS-3S:SPS:PCP:PCP:G; larvae less

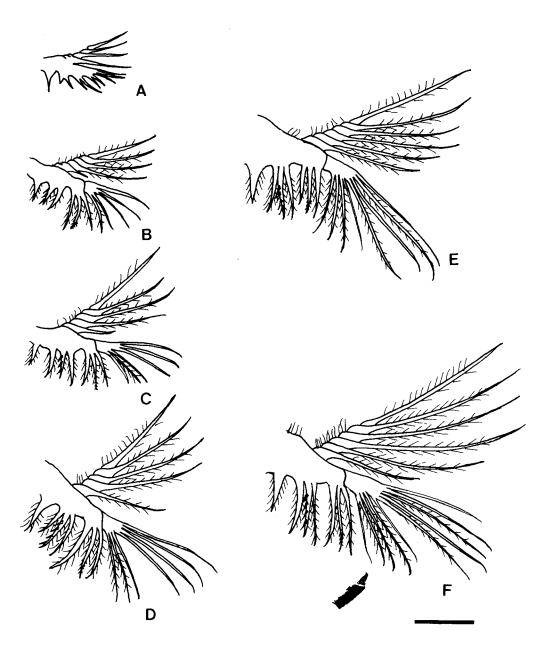


Fig. 6. Mandibles of six nauplius stages of *Chthamalus challengeri*. A, Nauplius II. B, Nauplius II. C, Nauplius III. D, Nauplius IV. E, Nauplius V. F, Stage VI. Scale bar=100  $\mu$ m.

than 390 μm intotal length-----Balanus improvisus 6b. A vertical row of 2 small thoracic spines on abdominal spine present; antennal setation formula of 2P:5P-3P:SP:PD:SPCP:G; mandibular setation formula of P:4P-3S:SPS:PCP:2PC:G; larvae less than 500 μm in total length---Balanus trigonus

Key for nauplius IV of 7 barnacle species inhabiting Korean coastal waters

1a. Labrum unilobed-----2

- 1b. Labrum trilobed-----4
- 2a. Cephalic shield round; dorsal thoracic spine shorter than cephalic shield------3

- 3a. Lateral margin of labrum with several teeth; a pair of short posterior shield spines present; a hispid seta at fourth group of antennal endopodite present; antennal setation formula of 2P:7P-2PSPS: 3S:PSP:SPSC:G; mandibular setation formula of P:4P-5S:P2S:PCP:PCD:G-------Octomeris sulcata
- 3b. Lateral margin of labrum with sparse teeth; rounded posterior shield region without a pair of posterior shield spines; a hispid seta at fourth group of antennal endopodite present; dorsal thoracic spine and abdominal process with numerous, small spicules; antennal setation formula of 3P: 7P-4PS:SPS:PDS:SCPH:G; mandibular setation formula of P:4P-4S:S2P:PDP:PCP:G-------
- -----Chthamalus challengeri
   4a. Frontolateral horns directed perpendicularly or reward------5
- 4b. Frontolateral horns directed distinctly forward------

Key for nauplius V of 7 barnacle species inhabiting Korean coastal waters

- 2a. Cephalic shield round; dorsal thoracic spine shorter than cephalic shield------3
- 2b. Cephalic shield inverted triangle; dorsal thoracic spine longer than cephalic shield; a pair of long posterior shield spines being equivalent to two thirds of cephalic shield present; antennal setation formula of 3P:8P-5P:2PS:PDP:SPHP:G; mandibu-

- lar setation formula of P:5P-5S:S2PS:PCP:PCP:G-------Pollicipes mitella
- 3a. Lateral margin of labrum with several teeth; a pair of short, posterior shield spines present; a hispid seta at fourth group of antennal endopodite present; antennal setation formula of 3P:8P-2PS2P: 3S:PS2PD:S2PC:G; mandibular setation formula of P:5P-5S:SD2P:SPCP:2PC:G-----Octomeris sulcata
- 4a. Frontolateral horns directed perpendicularly or reward------5
- 4b. Frontolateral horns directed distinctly forward------6

- 6b. Six irregular small thoracic spines on abdominal spine present; antennal setation formula of 3P: 6PS-3PSP:S2P:PD:SPCP:G; mandibular setation formula of P:4PS-4S:SPSP:SPCP:PCP:G; larvae more than 680 μm in total length-------Balanus trigonus

Key for nauplius VI of 7 barnacle species inhabiting Korean coastal waters

- 1a. Labrum unilobed-----2
- 1b. Labrum trilobed-----4
- 2a. Cephalic shield round; dorsal thoracic spine shorter than cephalic shield------3
- 2b. Cephalic shield inverted triangle; dorsal thoracic spine longer than length of cephalic shield; a pair of long posterior shield spines being equivalent to two thirds of cephalic shield present; antennal

- 3a. Lateral margin of labrum with several teeth; a pair of short posterior shield spines present; hispid seta at fourth group of antennal endopodite present; antennal setation formula of 4P:7PS-5P:3S: S2PD:2PC:G; mandibular setation formula of P: 5P-5S:SD2P:SDCP:2PC:G-------Octomeris sulcata
- 3b. Lateral margin of labrum with sparse teeth; rounded posterior shield region without a pair of posterior shield spines; hispid seta at fourth group of antennal endopodite present; dorsal thoracic spine and abdominal process with numerous small spicules; antennal setation formula of 3P:8P-5P: 2SPS:SPDSP:SCPH:G; mandibular setation formula of P:5P-2SPSP:S3PS:PDP:PCP:G-------
- 4a. Frontolateral horns directed perpendicularly or reward------5
- 4b. Frontolateral horns directed distinctly forward-----6
- 5a. Median lobe of labrum bearing serrated setules; antennal setation formula of 4P:8P-4PS:S2P:PD: S2PC:G; mandibular setation formula of P:5P-4S: 2S2D:S2PC:PDC:G; larvae less than 540 μm in total length-------Balanus amphitrite
- 6a. Median lobe of labrum bearing small, irregular teeth; antennal setation formula of 4P:8P-4PS: 2PS:2P:SCPS:G; mandibular setation formula of P:5P-4S:2P2S:SPCP:SPCP:G; larvae less than 640 μm in total length-------Balanus improvisus

#### Acknowledgements

We are grateful to Dr. I.H. Kim of Kangnung National University for classification of adult barnacles. We thank two anonymous referees whose insightful comments helped to improve this paper.

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[Received November 26, 1998; accepted December 18, 1998]