

## Larval Development of a Sessile Barnacle *Balanus improvisus* Darwin (Cirripedia: Thoracica)

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The larvae of *Balanus improvisus* were reared in the laboratory from hatching to cyprid. Larval development consists of six nauplius stages and a cyprid. Morphological features of all the nauplius stages are illustrated and described.

Key words: *Balanus improvisus*, larvae, development, cyprid

### Introduction

*Balanus improvisus* Darwin is a predominant species attached to pilings used for *Porphyra* farming and rocks of intertidal zone (Kim, 1985). The larvae of *B. improvisus* have been described by Jones and Crisp (1954) for the first time, but they only relied on the method of planktonic sampling. Lang (1979) have described the setation of the limbs and outer shape of *B. improvisus* larvae but have not reported complete description of the larvae at each stage. The purpose of the present study is to describe the nauplius and cyprid stages of *B. improvisus* reared in the laboratory and to compare the morphological characteristics with those of the other known larvae in the family Balanidae.

### Materials and Methods

*B. improvisus* was collected from the *Porphyra* farming of Myongji near Pusan, Korea. The barnacles were placed in an aquarium (30 cm×40 cm×40 cm) containing the filtered sea water. They were daily fed on newly hatched *Artemia* nauplii.

The hatched nauplii concentrated near the light source were removed with a Pasteur pipette to transfer into several 6-well tissue culture plates containing 10 nauplii per well. One thousand nauplii were transferred into a 800 ml beaker containing filtered sea water to know developmental process. *Nitzschia*

*closterium* was supplied for feed. Streptomycin sulphate and penicillin were used to inhibit bacterial contamination. The basic culture method was derived from that of Bookhout & Costlow (1959), and Brown and Roughgarden (1985).

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 under 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 with fine tungsten needles in a mixture of glycerin and alcohol under a stereomicroscope. Dissected specimens were stained with a gentian violet to observe and describe the morphological characteristics of carapace and the type of appendage setae. Drawings were made by a camera lucida, based on at least ten specimens at each stage.

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

The numerical setation formulae (Bassindale, 1936) and the alphabetical setation formulae (Newman, 1965) were used here in order to distinguish the nauplius larvae.

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## Results

The larvae of *B. improvisus* cultured in the laboratory passed through six nauplius stages and one cyprid before settling to the pin-head stage. Nauplius larvae consisted of a cephalic shield, frontolateral horns, nauplius eye, labrum, appendages, abdominal process, dorsal thoracic spine and posterior shield spines at stages IV, V and VI. Mean size of larval stage was given in Table 1. Descriptive drawings at each stage were given in Figs. 1~5. The nauplii were rather small and broad as compared with other species of *Balanus*.

The frontal margin of the larvae of stage I is more arched than that of later stages. The labrum is a trilobed, with a slightly long median lobe. In addition to the drawing of appendages, numerical setation and alphabetical setation were given in Tables 2 and 3. The detailed morphological characteristics on the larvae were as follows:

### Nauplius Stage I

(Figs. 1A, 1G, 2A, 2G, 3A, 4A, 5A)

The cephalic shield is rounded. Frontolateral horns are short and project backwards along the side of the body. Frontal filaments are not found. The abdominal process and dorsal thoracic spine are short and more or less equal in size. A median eye is present through all nauplius stages.

### Nauplius Stage II

(Figs. 1B, 1H, 2B, 2H, 3B, 4B, 5B)

The cephalic shield has been extended in length and width. The abdominal process and dorsal thoracic spine have been elongated. The frontal filaments are present and remain the same as in all the subsequent stages. Labrum is trilobed, with slender hairs. A pair of abdominal spines and three pairs of small spines above abdominal spines on

Table 1. Dimension of the larvae of *Balanus improvisus*. Ten larvae were measured to give means with standard deviation at each stage

Stage	Total length ( $\mu\text{m}$ )	Shield width ( $\mu\text{m}$ )	Shield length ( $\mu\text{m}$ )
Nauplius I	228 $\pm$ 13	118 $\pm$ 17	—
II	302 $\pm$ 13	154 $\pm$ 19	—
III	375 $\pm$ 11	187 $\pm$ 15	—
IV	423 $\pm$ 23	270 $\pm$ 21	283 $\pm$ 16
V	492 $\pm$ 19	282 $\pm$ 13	342 $\pm$ 19
VI	624 $\pm$ 15	337 $\pm$ 18	461 $\pm$ 13
Cyprid	523 $\pm$ 21	223 $\pm$ 19	—

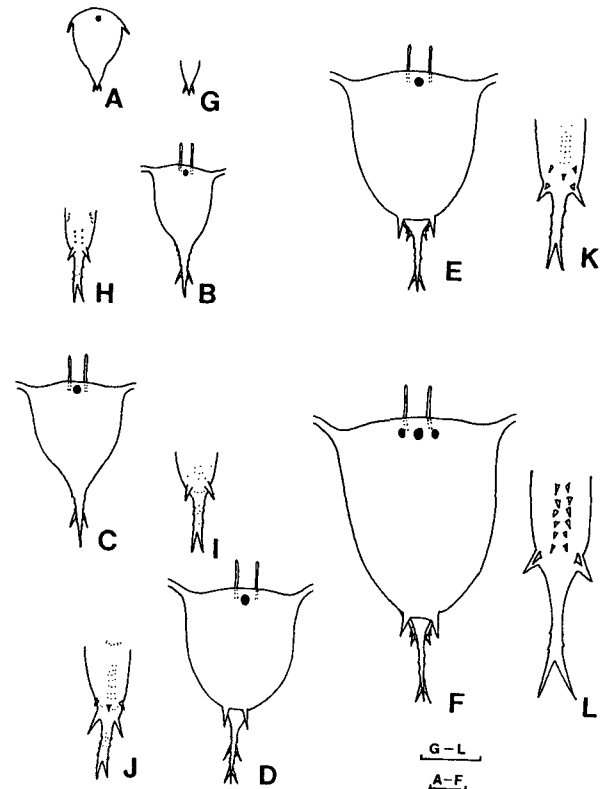


Fig. 1. Outline drawings and abdominal processes (ventral view) of the six nauplius stages of *Balanus improvisus*. A, G, Stage I; B, H, Stage II; C, I, Stage III; D, J, Stage IV; E, K, Stage V; F, L, Stage VI. Scale bar=100  $\mu\text{m}$

Table 2. Numerical setal formulae of six nauplius stages of *Balanus improvisus*

Stage	Appendages		
	Antennule	Antenna	Mandible
Nauplius I	04211	013-03222G	013-03222G
II	04211	025-03223G	014-03232G
III	14211	025-03224G	014-03333G
IV	114211	027-05324G	014-04343G
V	11142111	038-05324G	015-04443G
VI	11142121	048-05324G	015-04443G

thoraco-abdominal process appear on the apex of an abdominal process.

### Nauplius Stage III

(Figs. 1C, 1I, 2C, 2I, 3C, 4C, 5C)

The size of larvae has increased in total length and width when compared with that of stage II. The frontal margin is more flatter than that of stage II. A pair of abdominal spines appear on the apex of an abdominal process. Three pairs of small spines above abdominal spines on thoraco-abdominal process are not observed in this stage.

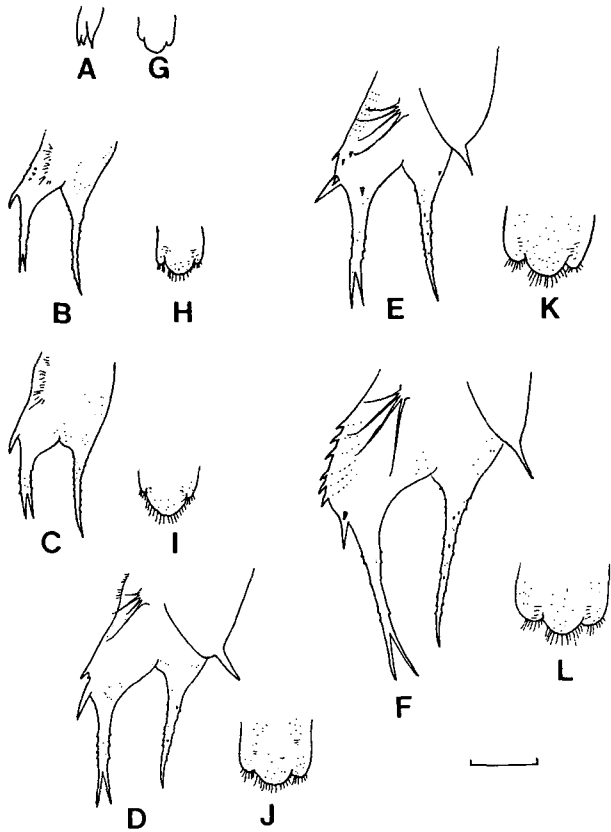


Fig. 2. Lateral view and labra of the six nauplius stages of *Balanus improvisus*. A, G, Stage I; B, H, Stage II; C, I, Stage III; D, J, Stage IV; E, K, Stage V; F, L, Stage VI. Scale bar=100  $\mu$ m

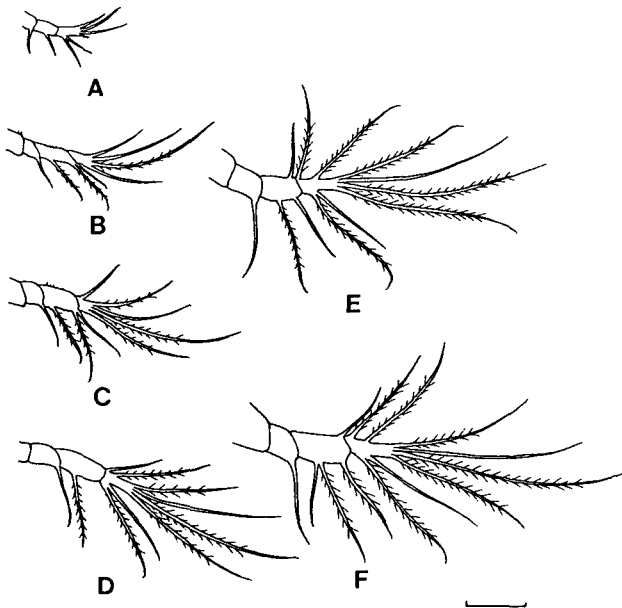


Fig. 3. Antennules of the six nauplius stages of *Balanus improvisus*. A, Stage I; B, Stage II; C, Stage III; D, Stage IV; E, Stage V; F, Stage VI. Scale bar=100  $\mu$ m

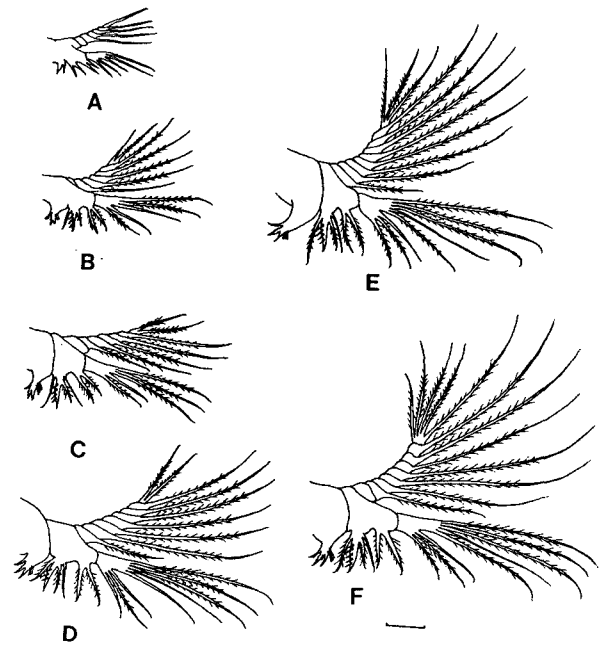


Fig. 4. Antennae of the six nauplius stages of *Balanus improvisus*. A, Stage I; B, Stage II; C, Stage III; D, Stage IV; E, Stage V; F, Stage VI. Scale bar=100  $\mu$ m

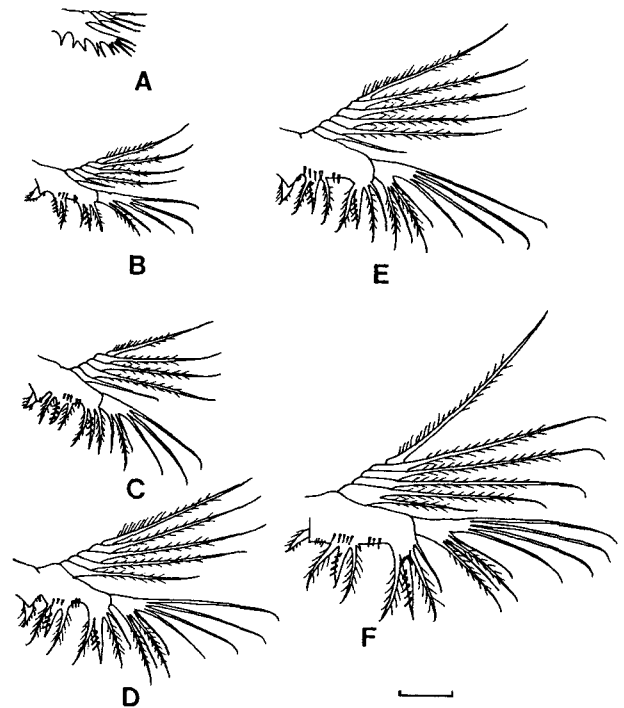


Fig. 5. Mandibles of the six nauplius stages of *Balanus improvisus*. A, Stage I; B, Stage II; C, Stage III; D, Stage IV; E, Stage V; F, Stage VI. Scale bar=100  $\mu$ m

Table 3. Alphabetical setal formulae of the larvae of *Balanus improvisus*. Setal types: S, simple; P, plumose; C, cuspidate; G, gnathobase

Stage	Appendages				
	Antennule	Antenna		Mandible	
		Exopod	Endopod	Exopod	Endopod
I	4S:SS:S:S	S:4S	3S:2S:2S:2S:G	S:3S	3S:2S:2S:2S:G
II	2SPS:SP:P:S	SP:4SP	2PS:PS:2P:SCP:G	P:3PS	3S:PS:PCS:PC:G
III	S:PSPP:SP:P:S	2P:5P	4P:PS:2P:SCPS:G	P:3PS	3S:SPS:PCP:PCP:G
IV	S:PSPP:SP:P:S	2P:7P	3P2S:SPS:2P:SCPS:G	P:4P	4S:2PS:SPCP:PCP:G
V	S:P:P:PSPP:SP:S:P:S	3P:7PS	4PS:2PS:2P:SCPS:G	P:4PS	4S:2P2S:SPCP:SPCP:G
VI	S:P:P:PSPP:SP:P:PS:P	4P:8P	4PS:2PS:2P:SCPS:G	P:5P	4S:2P2S:SPCP:SPCP:G

## Nauplius Stage IV

(Figs. 1D, 1J, 2D, 2J, 3D, 4D, 5D)

A pair of short carapace spines marks the posterior edge. A pair of small spines (series 2 spines) and a minute median spines present on a pair of abdominal spines (series 1 spines) above the thoraco-abdominal process. Several rows of minute bristles present on the ventral surface of large abdominal spines.

## Nauplius Stage V

(Figs. 1E, 1K, 2E, 2K, 3E, 4E, 5E)

Cephalic shield remains the same as in nauplius IV except for its enlargement. The dorsal thoracic spine becomes shorter than that of stage IV, being the same length as the thoraco-abdominal process. Thoraco-abdominal process now bears three smaller spines above two pairs of abdominal spines.

## Nauplius Stage VI

(Figs. 1F, 1L, 2F, 2L, 3F, 4F, 5F)

Six pairs of series 2 spines and a pair of series 1 spines present under the thorax and primordia of the cyprid thoracic appendages are observed under the exoskeleton of the thoracic spines. Paired compound eyes appear in the later period of this stage.

## Cyprid

(Fig. 6C)

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

## Discussion

The morphological identification of the barnacle larvae has recently been important for the studies of larval ecology and planktology, especially as the base for the solution of fouling problem and its prevention. Many investigators have focused on the descriptions of the cultured larvae rather than ones from plankton samples (Sandison, 1954; Crisp, 1962; Barker, 1976; Branscomb & Vedder, 1982; Egan and Anderson, 1985).

As distinguishing and identifying numerous nauplii have been essential to the classification of the barnacle larvae, students have concentrated their efforts on finding of several characters for a key. It was suggested that the numerical, graphical and alphabetical setations involving number, relative location and types of setae on appendages including the antennules, antennae and mandibles have been useful (Bassindale, 1936; Newman, 1965; Sandison, 1967; Lang, 1979). The setations of antennule have been practically helpful for intraspecific identification of barnacle nauplii without dissection: Stage I, without preaxial setae and fine setule; Stage II, without preaxial setae but some setae with fine setules; Stage III, with a preaxial seta; Stage IV, with two preaxial setae; Stage V, with three preaxial setae and five postaxial setae; Stage VI, with three preaxial setae and six postaxial setae.

However, the setation of antennule was not practically useful for interspecific identification of barnacle nauplii. Bassindale (1936) suggested it being valuable in the specific recognition of barnacle nauplii to make a numerical setation showing the setal number and relative location of setae on antennules, antennae and mandibles. Lee

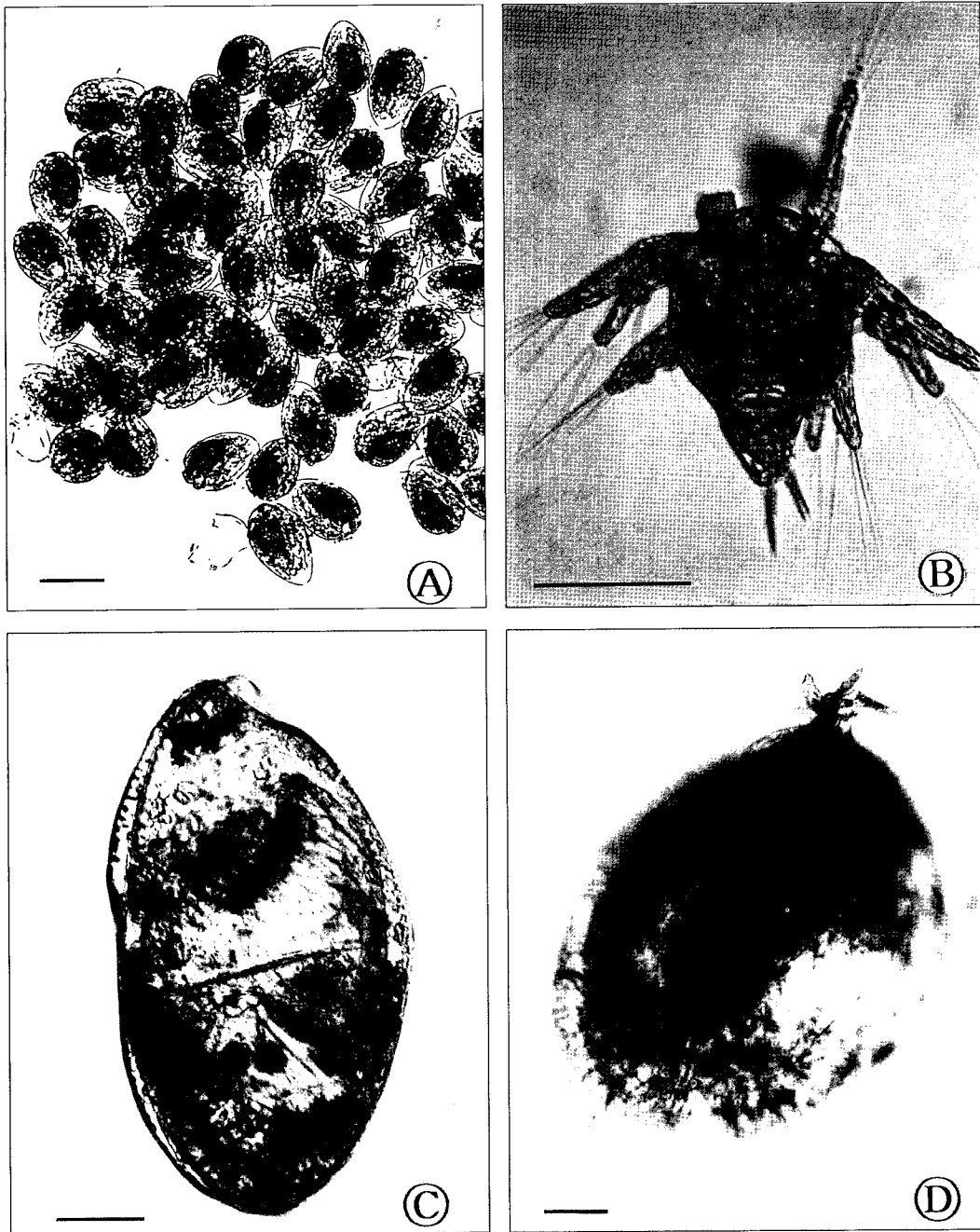


Fig. 6. Photomicroscopic view of eggs, nauplius I, cyprid larva and juvenile sessile barnacle of *Balanus improvisus*. A, eggs with a nauplius eye, rudimentary appendages, abdominal process and dorsal thoracic spine immediately before hatching from egg membrane; B, nauplius I immediately after hatching from egg membrane; C, a cyprid metamorphosed from nauplius VI after the sixth molting; D, juvenile sessile barnacle with undifferentiated opercular valves and numerous slender hairs on basal region. Scale bar=100  $\mu$ m

and Kim (1991) found it convenient to make a plot of the numerical setation (antennae against mandibles) of all the species for interspecific identification, with some modification since numerical setation of the antennules is almost the same in all of the species of the Balanomorph. However, it was

not sufficient to identify the larvae at each species by only the numerical setation of appendages within genus *Balanus*.

Lang (1979) described briefly shield outlines and abdominal process of nauplius stages II-VI, antenna and mandible of stage IV, and labrum of stage II

and IV in *B. improvisus*. He explained that the numerical setal formula of *B. improvisus* was nearly identical to that of *B. eburneus* except of five terminal setae in the antennal endopod of stage IV as opposed to four setae in the latter while Jones and Crisp (1954) reported only four terminal setae at the same position, in agreement with present observation. Present observation varies in the numerical setation of mandibular exopod of nauplius stage II from the description of Jones and Crisp (1954). They described three terminal setae in the mandibular exopod while we observed a simple seta in addition to these setae. Jones and Crisp (1954), and Lang (1979) reported a pair of lateral spines and a small median spine on abdominal process in nauplius stages IV and VI whereas we found two pairs of lateral spines and a small median spine for both stages.

In addition to these characters, the type of labrum, the shape of cephalic shield, the size of larvae and the ratio of abdominal process/dorsal thoracic spine were emphasized as identification keys (Branscomb and Vedder, 1982). *B. improvisus* larvae are separated from *B. amphitrite* larvae (Costlow and Bookhout, 1958; Egan and Anderson, 1986) in presence of small distinct teeth on the median labral lobe and lack of small lateral shield spine. *B. improvisus* larvae reported by Jones and Crisp (1954) are relatively small in total length compared with our measurement. Smaller size of the larvae can be explained with two reasons: a real phenomenon using planktonic materials and regional variation depending on environmental difference. It is possible to separate the nauplii of genus *Balanus* and genus *Chthamalus* depending on type of labrum, setae and posterior shield spine. *Chthamalus* larvae have unilobed labrum with a pair of lateral teeth and numerous small denticles, no posterior shield spine, and hispid and feathered setae, while *Balanus* larvae possess trilobed labrum with a median lobe extending slightly beyond the lateral lobes, a pair of posterior shield spines, and no hispid and feathered setae.

It can be suggested that small spines and spinules on the thoraco-abdominal process are helpful not only in identifying the nauplius species but also in distinguishing the nauplius stages since they show differences in each species and stage of nauplii. In the nauplii of stage II of *B. improvisus* there are only three pairs of small spines and a pair of abdominal spines on thoraco-abdominal process

and, while nauplii of *B. amphitrite* possess a pair of a pair of abdominal spines and an open circle of small spines extending forwards anterior to the abdominal spines. In stage III the former nauplii show no small spines on the abdominal process except for a pair of abdominal spines, but the latter nauplii own two rows of fine setules lying at right angles above abdominal spines. The former nauplii of stages IV have a pair of small spines (series 2 spines) and a minute median spines present on a pair of abdominal spines (series 1 spines) above the thoraco-abdominal process whereas the latter nauplii show a pair of large, smooth spines (series 1 spines) and parallel rows of fine setules above the series 2 spines on the thoraco-abdominal process. In stage V the former possess three smaller spines above two pairs of simple spines (series 1 and series 2 spines) while the latter possess rows of spinules or hairs above series 2 spines, with two rows of small spines on the thoracic region, parallel to the long axis of the body.

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