

Larval Development of *Balanus trigonus* Darwin (Cirripedia: Thoracica: Balanidae) reared in the laboratory

Chu LEE and Chang-Hyun KIM

Department of Biology, College of Natural Sciences, Pusan National University,
Pusan 609-735, Korea

Sessile barnacle, *Balanus trigonus* Darwin, was collected from the intertidal rocks and the external shells of bivalve *Mytilus coruscus*. The nauplius and cyprid larvae were cultured in a constant temperature cabinet at a temperature of 20°C and a light regime of 14 h light and 10 h darkness. Larval development includes the six nauplius stages and a cyprid stage prior to settlement and metamorphosis to the young adult. Morphological characteristics including antennules, antennae and mandibles are described and illustrated. All nauplius larvae have trilobed labra typical in balanoides. The developmental time taken from newly hatched nauplius I to cyprid was 9~13 days and averaged 11 days.

Introduction

Balanus trigonus Darwin is a cosmopolitan barnacle species which inhabits the intertidal zone of warm waters (Ross et al., 1964; Utinomi, 1970; Newman and Ross, 1976; Kim, 1985). The planktonic larvae of genus *Balanus* are so similar each other in their morphology that their identification is practically difficult without detailed descriptions of appendages at each stage. Therefore many investigators have centered on the descriptions of the cultured larvae rather than ones from plankton samples in the genus *Balanus* (Bassindale, 1936; Hudinaga and Kasahara, 1942; Pyefinch, 1949; Jones and Crisp, 1954; Costlow and Bookhout, 1957; Barnes and Barnes, 1959; Crisp, 1962; Sandison, 1967; Molenock and Gomez, 1972; Barker, 1976; Lang, 1979; Branscomb and Vedder, 1982; Brown and Roughgarden, 1985; Egan and Anderson, 1986; Lee and Kim, 1991).

B. trigonus is prominent member of the intertidal fauna of the coast of Korea and it is attached to the rocks, bivalve shells, hermit crab shells, and sponge. There are several descriptions on the larvae of *Balanus trigonus* by Hirano (1953), Sandison

(1954), and Lang (1979), but their descriptions are not detailed enough to distinguish the larvae of *B. trigonus* from those of other species. Therefore, it is the purpose of the present study to describe detailed nauplius and cyprid stages and to compare the morphological characteristics with those of the other known larvae in the genus *Balanus*.

Materials and Methods

Sessile barnacle, *Balanus trigonus* Darwin, was collected from intertidal rocks and shells of bivalve *Mytilus coruscus* occurring in the lower tidal marks adjacent to Kadōcto Island near Pusan. The barnacles were reared in the several aquaria, 30×40 cm by 40 cm in depth. The shells of some barnacles were opened for the examination of the presence of embryos within the mantle cavity by the method of Walley (1965).

Shortly after hatching as nauplius I, the larvae, being positively phototactic, were concentrated in the light of a lamp for removal of the first nauplii with a Pasteur pipette. The larvae were reared in 6-well tissue culture plates containing 10 nauplii

per well. The culture was carried out in a cabinet under a photoperiod of 14 h light and 10 h darkness and at a temperature of 25 °C. The basic culture method was derived from those of Bookhout and Costlow (1959), and Brown and Roughgarden (1985). Larvae were transferred to the filtered sea water of 33 ppt every day and fed with the diatom, *Nitzschia closterium* at a density of 1.0×10^4 to 2.0×10^4 cells/ml. Some larvae as well as exuviae were preserved in 70% alcohol for the examination of the morphological characteristics at each stage.

Dissections of all larvae were performed under a binocular dissecting microscope with fine needles in a mixture of glycerine and alcohol. Drawings were made with the aid of a camera lucida. Measurements were made with an ocular micrometer: total body length from the anterior margin of the shield to the tip of the dorsal thoracic spine; shield length from the anterior margin of the shield to the hind shield margin; shield width at the greatest distance of the body.

Abbreviations: ap, abdominal process; as-1, abdominal spine-1; as-2, abdominal spine-2; ce, compound eye; dts, dorsal thoracic spine; f, furca; ff, frontal filament; la, anterior region of labrum; lb,

posterior region of labrum; m, maxillule; ne, nauplius eye; o, oil cell; pss, posterior shield spine; ts, thoracic spine.

Results

Larval development includes the six nauplius stages and one cyprid stage prior to settlement and metamorphosis to the young adult. Nauplius larvae have a cephalic shield with a pair of frontolateral horns, an abdominal process with a dorsal thoracic spine, and a pair of posterior shield spines at the stages IV, V and VI. The thorax has frontal filaments except for the stage I, a trilobed labrum and three pairs of appendages such as the antennules, antennae, and mandibles. Ten larvae were measured to give means with standard deviation at each stage and measurements are given in Table 1. The dorsal and lateral regions of all larvae are shown in the Figs. 1 and 2. In addition to the drawings of the antennules, antennae, and mandibles, numerical setation (Bassindale, 1936) and alphabetical setation (Newman, 1965) are shown in Tables 2 and 3.

Table 1. Dimensions of the larvae of the six nauplius stages of *Balanus trigonus* Darwin

Stage	Total length(μm)	Shield width(μm)	Shield Length(μm)
I	240 ± 13	144 ± 11	
II	404 ± 21	179 ± 19	
III	480 ± 18	240 ± 13	
IV	548 ± 21	313 ± 11	324 ± 9
V	711 ± 21	400 ± 13	451 ± 11
VI	880 ± 23	526 ± 15	596 ± 17

Table 2. Numerical setation(Bassindale, 1936) of the six nauplius stages of *Balanus trigonus* Darwin

Stage	Antennule	Antenna	Exopodite-Endopodite	Mandible	Exopodite-Endopodite
I	04211		014-03222G		013-03222G
II	04211		025-03223G		014-03232G
III	14211		025-03224G		014-03333G
IV	114211		036-05324G		014-04343G
V	11142111		038-05324G		015-04443G
VI	11142121		048-05324G		015-04443G

Table 3. Alphabetical setation (Newman, 1965) of the six nauplius stages of *Balanus trigonus* Darwin.
Setal types: S, simple; P, plumose; D, plumodenticulate; C, cuspidate; G, gnathobase; S(p), simple sometimes plumose; P(s), plumose sometimes simple; sp, spine

Naupliar stage	Antennule	Antenna		Mandible	
		Exopodite	Endopodite	Exopodite	Endopodite
I	SSSS:SS:S:S	S:4S	3S:2S:2S:2S:G	S:3S	3S:2S:2S:2S:G
II	SSPS:SP:P:S	2P:RPS	2PS:SP:PD:SPC:G	P:3PS	3S:SP:PCS(p):spPC:G
III	S:PSPP:SP:P:S	2P:5P	3P:SP:PD:SPCP:G	P:3PP	3S:SPS:PCP:spPPC:G
IV	S:P:PSPP:SP:P:S	3P:5PS	3P2S:SPS:PD:SPCP:G	P:3PP(s)	4S:SPP:SPCP:spPCP:G
V	S:S:P:PSPP:SP:S:P:S	3P:6PS	3PSP:SPP:PD:SPCP:G	P:4PS	4S:SPSP:SPCP:spPCP:G
VI	S:S:P:PSPP:SP:P:PS:S	4P:8P	3PSP:SPP:PD:SPCP:G	P:5P	4S:SPSP:SPCP:spPCP:G

Nauplius I (Figs. 1A, 1H, 2A, 3A, 4A, 5A)

The abdominal process and dorsal thoracic spine are short and blunt. The frontal filaments are not visible. The pear-shaped carapace is projected posteriorly, with short frontolateral horns.

Nauplius II (Figs. 1B, 2B, 2G, 2L, 3B, 4B, 5B)

The frontolateral horns are slightly curved and slender, and are held at the right angle to the longitudinal axis of the body. The dorsal thoracic spines, generously barbed, are much longer than the abdominal process. There are 3 to 5 small spines on the stem of the abdominal process.

Nauplius III (Figs. 1C, 1J, 2C, 2H, 2M, 3C, 4C, 5C)

The cephalic shield has increased in size. A pair of abdominal spines are extended on the abdominal process. There are a row of small spines on the stem of the abdominal process. Maxillules consist of 1 thick and 4 to 5 thin setae.

Nauplius IV (Figs. 1D, 1K, 2D, 2I, 2N, 3D, 4D, 5D)

The cephalic shield is smooth and more round, and a pair of posterior shield spines have appeared. The frontolateral horns are stouter than those of the stage III, but not increased in length according to the increase in body size. The abdominal process bears a pair of abdominal spines. The thoracic spines are present as a stout and three small spines crossing the thoracic region. There are a row of small spines on the thoracic region. Maxillules consist of 4 long and 2 short setae.

Nauplius V (Figs. 1E, 1L, 2E, 2J, 2O, 3E, 4E, 5E)

The cephalic shield remains the same as in the stage IV except for its enlargement. The abdominal process bears 2 pairs of abdominal spines and the first pair is relatively longer than the second. There are 6 irregular small thoracic spines on the thoracic region. Maxillules consisting of 4 long setae, stretch at parallel to the longitudinal axis of the body.

Nauplius VI (Figs. 1F, 1M, 2F, 2K, 2P, 3F, 4F, 5F)

There are 6 pairs of thoracic spines. It is easily distinguished with other stages by paired compound eyes and the primordia of the cyprid thoracic appendages under the thoracic region.

Cyprid (Fig. 1G)

The bivalved carapace is completely sculptured. Compound and a median eyes are present and the head of cyprid is packed with numerous oil cells.

Discussion

Identification of the larvae belonging to the balanomorph is difficult if not provided with detailed description at each stage because the larvae are very similar each other. Therefore, description of barnacle larvae has been conducted over the last three decades for the classification of larvae which is very important for the studies of larval ecology and

planktology (Jones and Crisp, 1954; Costlow and Bookhout, 1958; Molenock and Gomez, 1972; Branscomb and Vedder, 1982; Brown and Roughgarden, 1985; Achituv, 1986; Dineen, 1987; Miller et al., 1989).

Bassindale (1936) suggested a numerical setation on the setal number of appendages and location of setae in the larvae of three English barnacles, but it was not suitable to explain the setal type and relative location of setae. Lee and Kim (1991) proposed it convenient to make plot of the numerical setation of the antennae against that of the mandibles for the identification of numerous species larvae because numerical setation of the antennules at each stage is almost the same in all barnacle species. We found it to be effective to use an alphabetical setation by the method of Newman (1965) in addition to the plot of numerical setation as useful method for the species identification.

Sandison (1954) described that there are a pair of small spines on the lateral cephalic shield of the larvae of the stages II and III of *Balanus trigonus*. According to the present study, we could not find out a pair of small spines on the lateral cephalic shield. Lang (1979), who described briefly the nauplius stages of *B. trigonus*, reported the presence of plumodenticulate setae on the second, third and fourth group of the mandibular endopodite of the stages V and VI, while we found only plumose setae at the same positions of the mandibular endopodite.

It is possible to separate nauplii of *B. trigonus* from nauplii of *B. perforatus* (Norris and Crisp, 1953), *B. amphitrite* (Egan and Anderson, 1986), *B. eburneus* (Costlow and Bookhout, 1957), and *B. improvisus* (Jones and Crisp, 1954) on the basis of several characters such as total length of larvae, shape of labrum, number of thoracic spines, and setation of antennae and mandibles (Table 4). The labrum has been used as a character to distinguish balanid nauplii having a trilobed labrum with nauplii of other genus. It is available in separating nauplii of *B. trigonus* and *B. amphitrite* from those of other three species by the presence of serrated setules in the middle labral lobe. At the stage V, nauplii of *B. trigonus*, having one median and two

pairs of thoracic spines, can be also separated from nauplii of *B. perforatus*, *B. amphitrite* and *B. improvisus*, having one median and a pair of thoracic spines on the thoracic region.

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Table 4. Comparison of the nauplius characteristics in the five *Balanus* species

Stage	Total length(mm)	Labrum	The number of Thoracic spine	Setation	
				Antenna	Mandible
<i>B. perforatus</i> (Norris and Crisp, 1953)					
I	0.28			014-03222G	013-03222G
II	0.43			025-03223G	013-03232G
III	0.50			025-03224G	014-03333G
IV	0.56		1 pair, 1 median	036-04324G	014-04333G
V	0.72		1 pair, 1 median	038-04324G	015-04443G
VI	0.86		6 pairs	048-04324G	015-04443G
<i>B. amphitrite</i> (Egan and Anderson, 1986)					
I	0.22			023-03222G	013-03222G
II	0.35			025-03223G	013-03232G
III	0.37	The middle		025-03224G	014-03333G
IV	0.40	labral lobe	1 pair, 1 median	036-05324G	014-04343G
V	0.47	with serrated	1 pair, 1 median	047-05324G	015-04443G
VI	0.54	setules	6 pairs	048-05324G	015-04443G
<i>B. eburneus</i> (Costlow and Bookhout, 1957)					
I	0.29~0.23			014-03222G	013-03222G
II	0.32~0.34			024-03222G	014-03232G
III	0.35~0.38			025-03222G	014-03232G
IV	0.40~0.42			035-05324G	014-04243G
V	0.44~0.48			038-05324G	015-04443G
VI	0.54~0.60		6 pairs	048-05324G	015-04443G
<i>B. improvisus</i> (Jones and Crisp, 1954)					
I	0.19			014-03222G	013-03222G
II	0.32			025-03223G	013-03232G
III	0.36			025-03224G	014-03333G
IV	0.40		1 pair, 1 median	027-05324G	014-04343G
V	0.50		1 pair, 1 median	038-05324G	015-04443G
VI	0.62		6 pairs	048-05324G	015-04443G
<i>B. trigonus</i> (The present study)					
I	0.24			014-03222G	013-03222G
II	0.40	The middle		025-03223G	014-03232G
III	0.48	labral lobe		025-03224G	014-03333G
IV	0.54	with serrated	1 pair, 1 median	036-05324G	014-04343G
V	0.71	setules	2 pair, 1 median	038-05324G	015-04443G
VI	0.88		6 pairs	048-05324G	015-04443G

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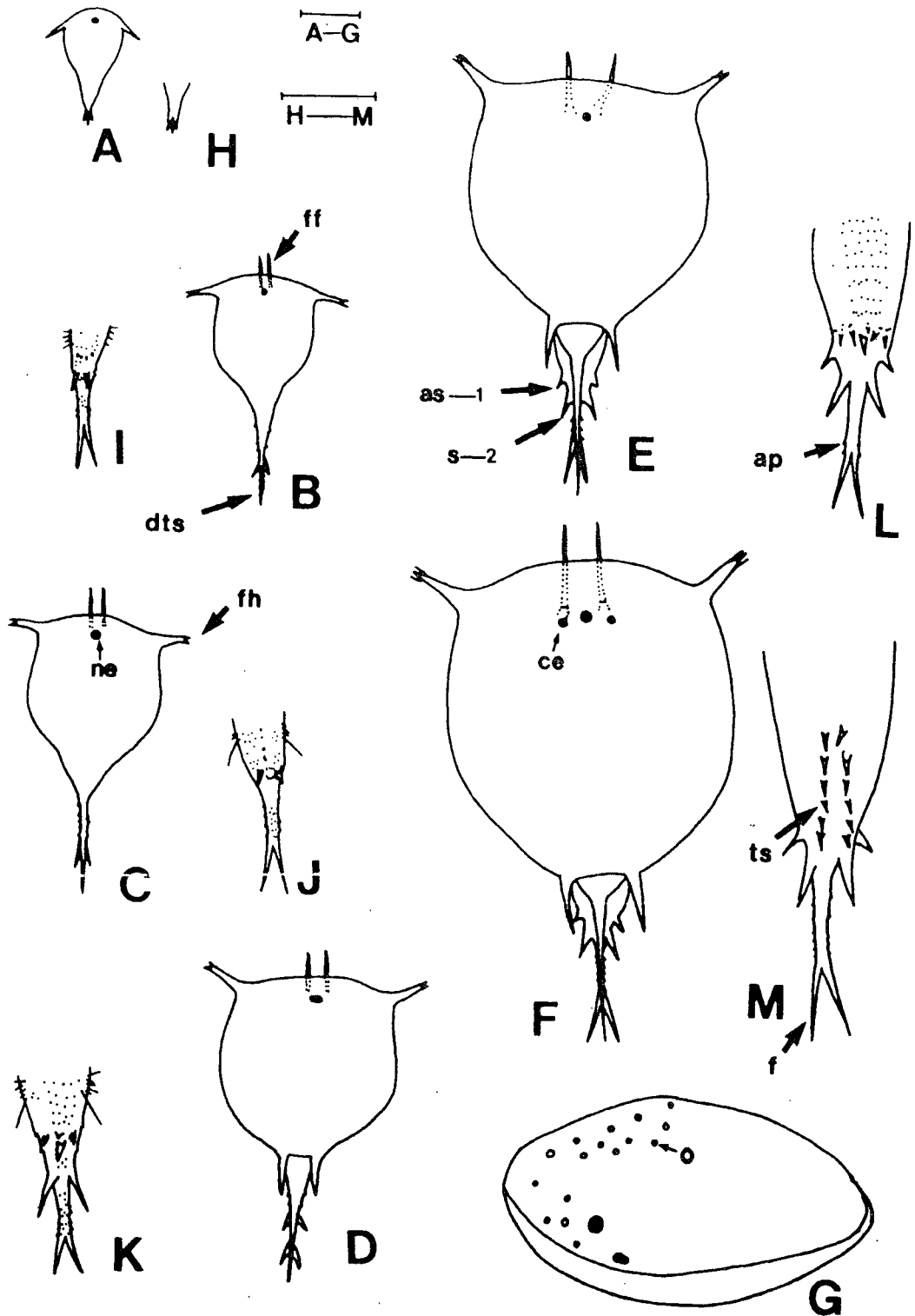


Fig. 1. Outline drawing of the six nauplius and cyprid stages of *Balanus trigonus* Darwin. Nauplius and cyprid stages are indicated with alphabetical numerals: A. H, Stage I; B. I, Stage II; C. J, Stage III; D. K, Stage IV; E. L, Stage V; F. M, Stage VI; G, Cyprid. Scale bar=100 μm.

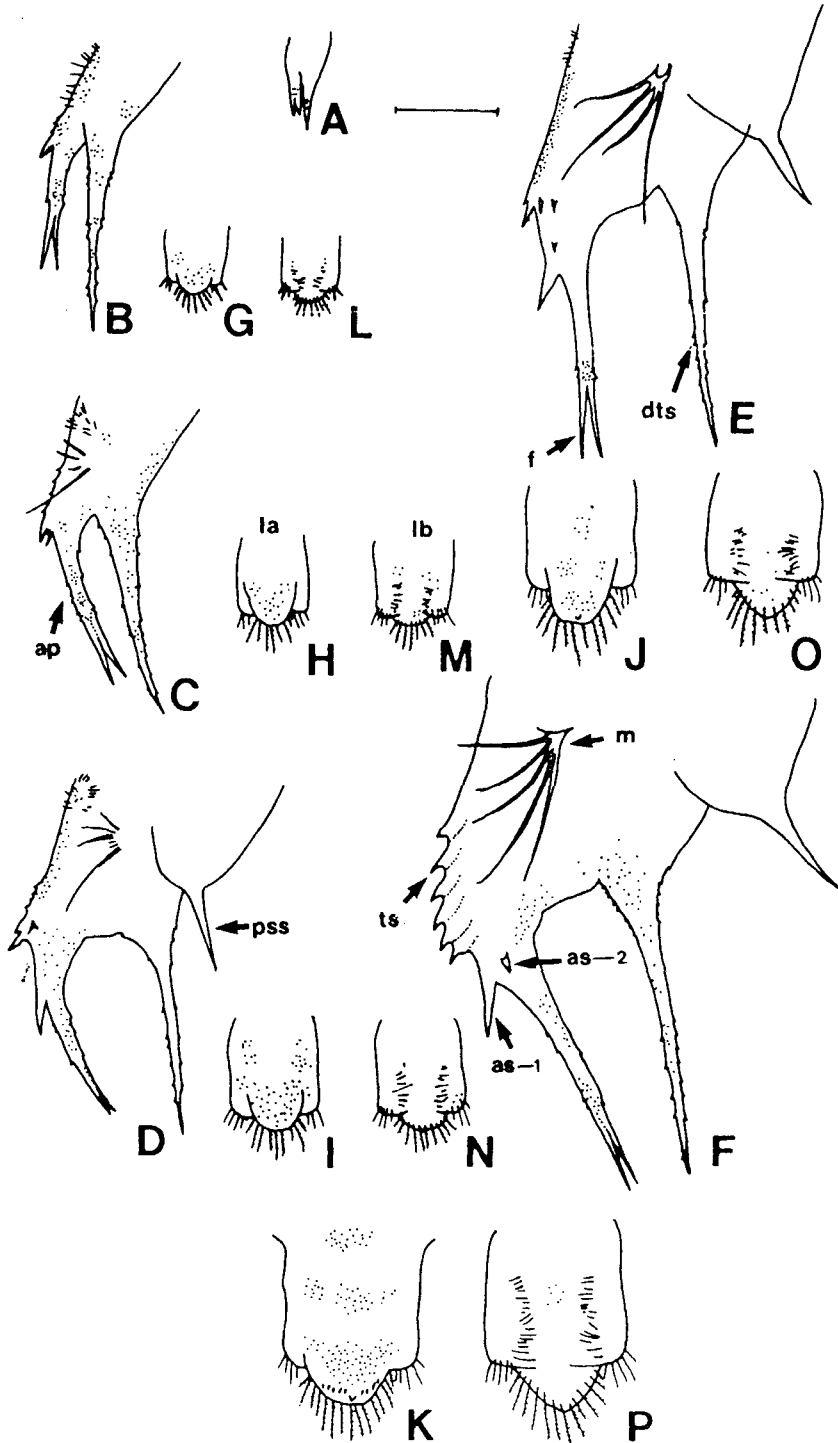


Fig. 2. Outline drawing of lateral view of the six nauplii and labra of *Balanus trigonus* Darwin. Alphabetical numerals indicate nauplius stage and labrum: A, Stage I; B. G. L, Stage II; C. H. M, Stage III; D. I. N, Stage IV; E. J. O, Stage V; F. K. P, Stage VI. Scale bar=100 μ m.

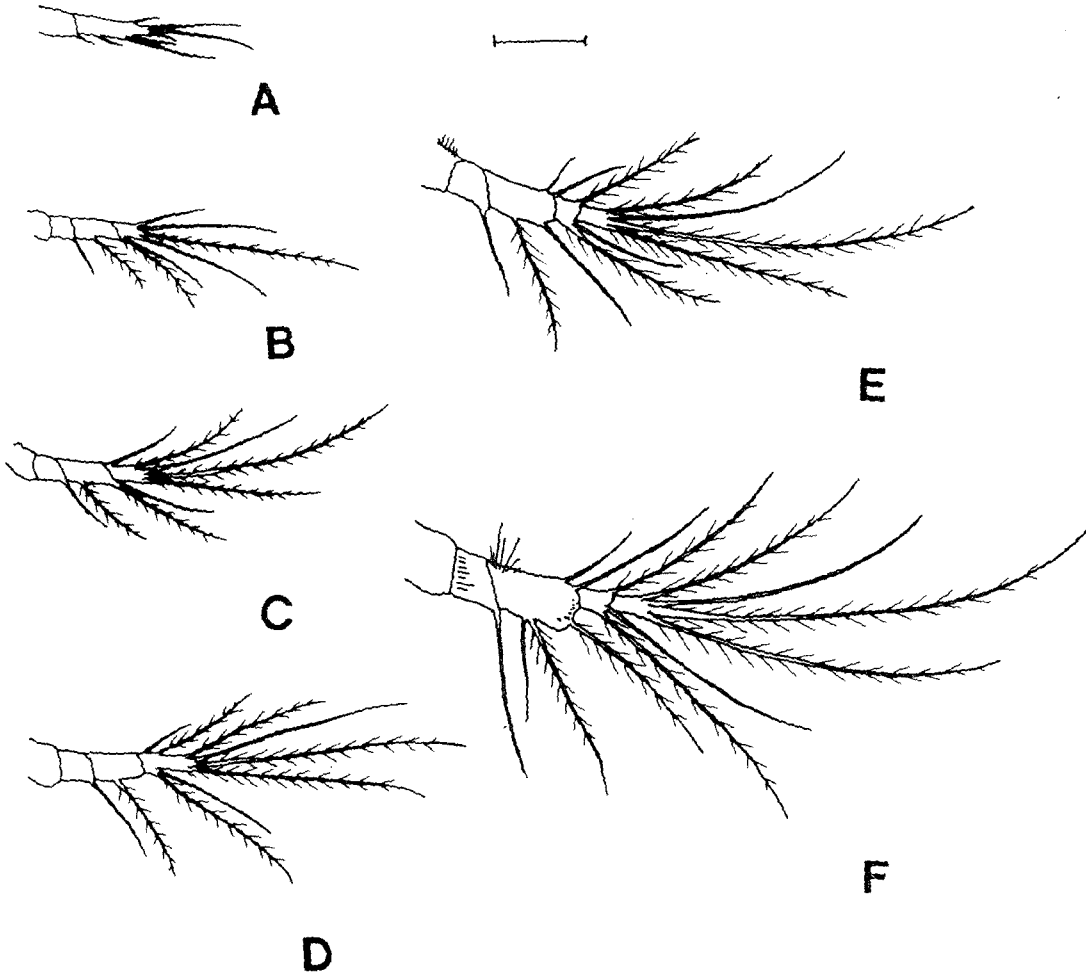


Fig. 3. Antennules of the six nauplius stages of *Balanus trigonus* Darwin. Nauplius stages are indicated with alphabetical numerals: A, Stage I; B, Stage II; C, Stage III; D, Stage IV; E, Stage V; F, Stage VI. Scale bar = 100 μm .

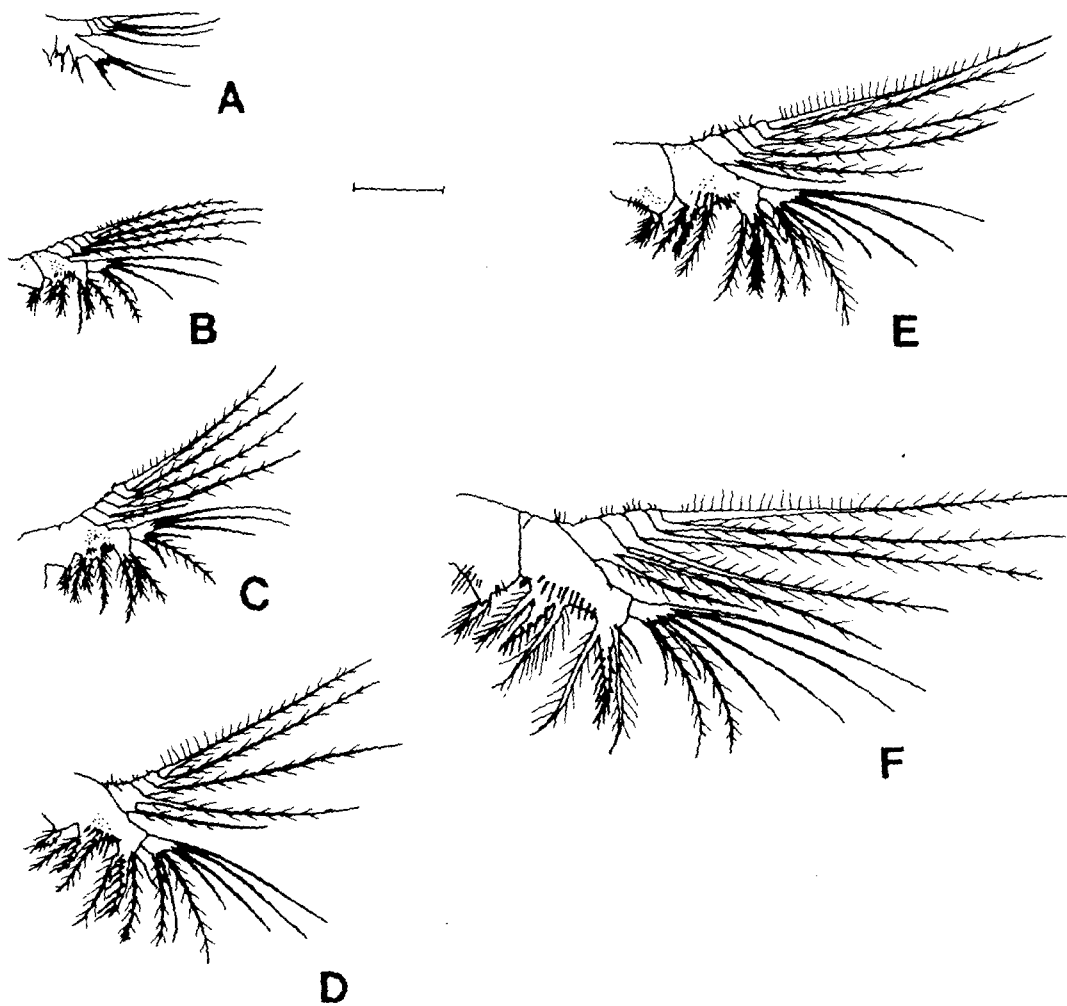


Fig. 4. Antennae of the six nauplius stages of *Balanus trigonus* Darwin. Nauplius stages are indicated with alphabetical numerals: A, Stage I; B, Stage II; C, Stage III; D, Stage IV; E, Stage V; F, Stage VI. Scale bar = 100 μ m.

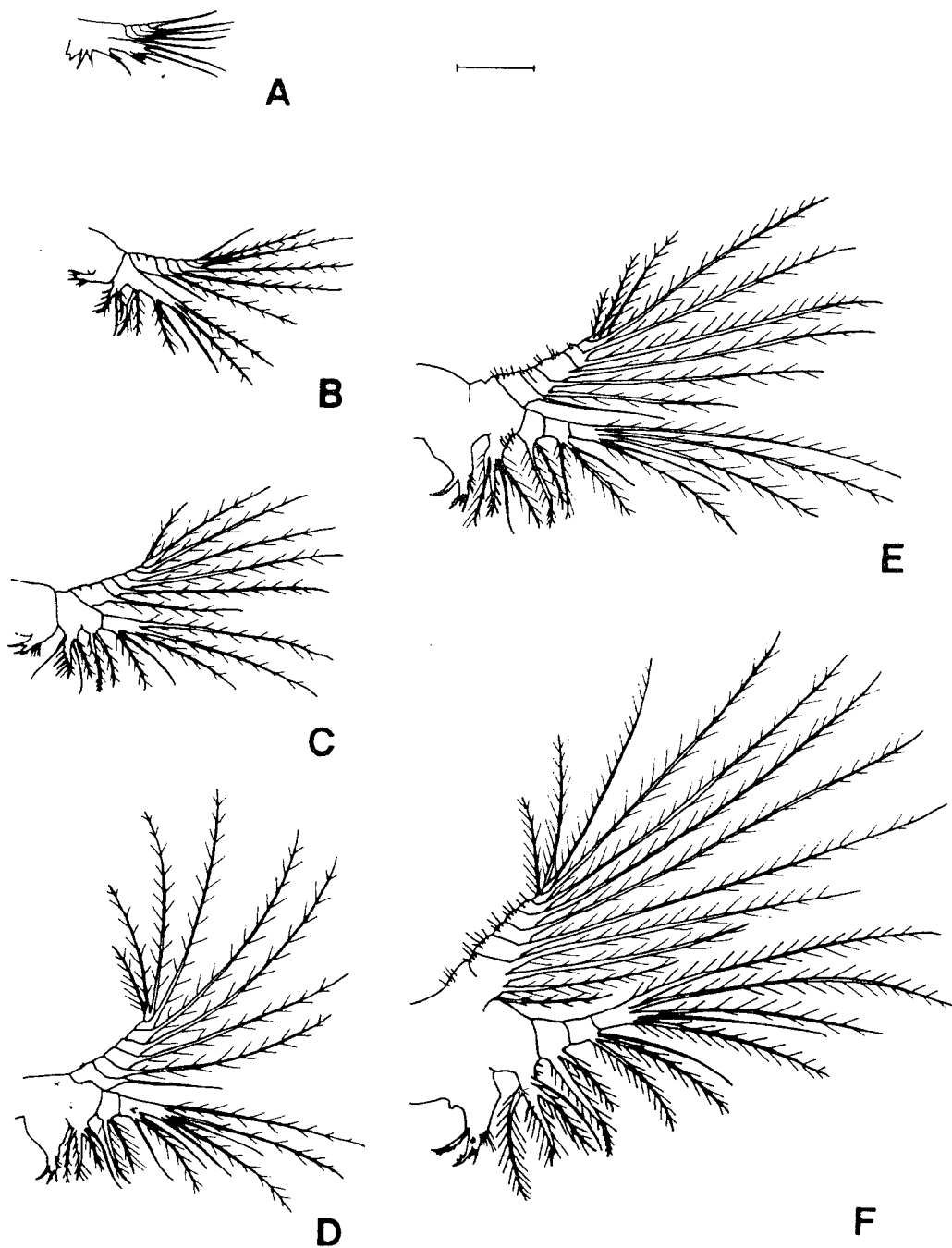


Fig. 5. Mandibles of the six nauplius stages of *Balanus trigonus* Darwin. Nauplius stages are indicated with alphabetical numerals: A, Stage I; B, Stage II; C, Stage III; D, Stage IV; E, Stage V; F, Stage VI. Scale bar = 100 μ m.