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Larval Development of *Chirona cristatus* (Cirripedia, Thoracica) Reared in the Laboratory

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ABSTRACT

Larval development of *Chirona cristatus* Ren and Riu, 1978, found in the low part of rocks in the intertidal zone or the shell of scallops, was described in detail and compared with those of other known barnacles. Durations from nauplius through cyprid to pinhead stage are three weeks at 20±0.5°C. Trilobed labrum bearing three groups of slender hairs and frontolateral horns folded under the anterior cephalic shield margin are diagnostic features through all nauplius stages. The posterior border of the cephalic shield bears a pair of cephalic shield spines in nauplius stages IV, V and VI. There is no specific hispid seta at the fourth group of the antennal endpodite. The dorsal thoracic spine, abdominal process and posterior shield spine have numerous small spines. 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 antennule in this species are found to be practically helpful for intraspecific identification of barnacle nauplius stages without dissection.

Key words: *Chirona cristatus*, sessile barnacle, larval development

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INTRODUCTION

Chirona criststus Ren and Riu, 1978, a predominant species attached to the intertidal rocks lying in concealment from sunlight, is known from Korea to China (Kim, 1998). Since the larval development of barnacles belonging to this order is well investigated, more information is required due to the simplicity and similarity of the morphological features available for larval classification of barnacle larvae. Studies of *C. cristatus* have not resolved the morphological features of the six nauplius stages and the cyprid. Comparisons of morphological characteristics between the larvae of *C. cristatus* and other barnacles would provide helpful information for the study of marine ecology and planktology. The purpose of the present study is to describe and illustrate the detailed morphological characteristics of the nauplius and cyprid larvae of *C. cristatus*, and to compare them with those of the other known barnacle inhabiting Korean coastal waters.

MATERIALS AND METHODS

Chirona cristatus Ren and Riu, 1978 was collected from the rocks of the intertidal zone near Busan, Korea. The barnacles were placed in an aquarium containing the filtered seawater. They were fed daily on *Brachionus* sp. and newly hatched *Artemia* nauplii. The hatched nauplii concentrated near the light source were removed with a Pasteur pipette and transferred into several 6-well tissue culture plates to examine the developmental process. The basic culture method was derived from that of Brown and Roughgarden (1985). Preserved larvae and exuviae were dissected under a stereomicroscope using fine tungsten needles in a mixture of glycerin and alcohol. Drawings were made with the aid of a camera lucida. At least ten specimens for 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 the dorsal thoracic spines. Shield width was measured at its widest point and shield length from the frontal side of the cephalic shield to the end of the posterior shield spines.

RESULTS

The larvae cultured in the laboratory pass through six nauplius stages and one cyprid before becoming a young barnacle. The outline shape of nauplius larvae consists of a cephalic shield, frontolateral horns, labrum, appendages, abdominal process from nauplius stages I-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 for each stage are given in Figs. 1-6. In addition to the numerical setation formula in Table 2, alphabetical setation formula (Newman, 1965) is given in Table 3. The detailed morphological characteristics for each stage are as follows:

Nauplius I

The frontal margin of these larvae is a round arch when compared with those of later stages.

Table 1. Total length and shield width of larval stage of *Chirona cristatus* Ren and Riu, 1978 (n = 10).

Larval stage	Total length (μm)			Shield width (μm)		
	Mean	SD	Range	Mean	SD	Range
I	172	13	166-185	106	4	100-111
II	270	11	265-280	132	14	126-146
III	313	14	307-326	157	28	145-181
IV	406	13	401-412	222	13	204-239
V	460	11	455-468	232	11	224-245
VI	557	10	548-567	297	12	288-312
Cyprid	590	11	581-613			

Table 2. Comparison of morphological features between the larvae of *Chirona cristatus* Ren and Riu, 1978, in the present study and those of *Chirona hameri* Ascanius, 1767, described by Crisp (1962).

Morphological feature	*Chirona cristatus*	*Chirona hameri*
Shield length in stages II-III	265-326 μm	798-895 μm
Patterns of thoracic spine in stage IV	4 rows of 3 small thoracic spines	A parallel row of 3 spines
Total length in stages IV-VI	401-567 μm	1120-1563 μm
Setation of mandible stages V	015-04333G	015-04443G
Length of cephalic shield in stages IV-VI	264-380 μm	608-1120 μm

Table 3. The numerical setal formulae of appendages in nauplii of *Chirona cristatus*.

Stage	Antennule	Antenna	Mandible
I	04211	014-03222G	013-03222G
II	04211	025-03223G	014-03232G
III	14211	025-03224G	014-03333G
IV	114211	026-04324G	014-04333G
V	11142111	028-04324G	015-04333G
VI	11142121	028-04324G	015-04343G

Abdominal process and dorsal thoracic spine are undeveloped and rudimentary. The appendages of antennule, antenna and mandible have simple setae without fine setules. Frontolateral horns are significantly bent and project backward along the side of the body. Frontal filaments are not found. A nauplius eye is present through all nauplius stages. The trilobed labrum has small teeth on the frontal side.

Nauplius II

The frontolateral horns are swollen and directed perpendicular to the body axis. The cephalic shield bears numerous small spines on lateral margin. The frontal filaments are present and remain without change in all the subsequent stages. The labrum is trilobed, with slender hairs through all

Table 4. Morphological characteristics of barnacle larvae inhabiting the coastal region of Korea. Abbreviations are as follows: S = stage, SP = species, TL = total length, SW = shield width, LB = shape of labrum, CSS = shape of cephalic shield spine, ST = specific setal type observed in 4th segment of antennal endopodite, DTS = dorsal thoracic spine, PSS = posterior shield spine, PSSD = posterior shield spine plus dorsal shield spine. Species abbreviations are: CM = *Capitulum mitella*, CC = *Chirona cristatus*, MR = *Megabalanus rosa*, BR = *Balanus reticulatus*, BA = *Balanus amphitrite*, BAL = *Balanus albicostatus*

S	SP	TL (μm)	SW (μm)	LB	CSS	ST	Setal type of antennule	Setal type of antenna	Setal type of mandible
II	CM	500	235	Unilobed	DTS	Hispid	SP2S:SP:S:S	2P:4PS-2PS:PS:PD:SHP:G	P:3PS-3S:SP:PC P :PC:G
II	MR	470	230	Trilobed	DTS	Cuspidate	2SPS:SP:P:S	SP:4PS-2PS:SP:PD:SPC:G	P:3PS-3S:SP:PCS:PC:G
II	CC	270	132	Trilobed	DTS	Cuspidate	2SPS:SP:P:S	SP:4PS-P2S:SP:2P:PCS:G	P:3PS-3S:SP:PCS:PC:G
II	BR	342	168	Trilobed	DTS	Cuspidate	2SPS:SP:P:S	SP:4PS-2PS:SP:PD:SC:G	P:3PS-3S:SP:PDP:PC:G
II	BA	350	150	Trilobed	DTS	Cuspidate	2SPS:PS:P:S	SP:4PS-2PS:SP:PD:SPC:G	P:3PS-3S:SP:SPC:PC:G
II	BAL	363	164	Trilobed	DTS	Cuspidate	2SPS:2S:P:S	SP:4PS-2PS:SP:PD:PC:G	P:3PS-3S:2S:PDP:PC:G
III	CM	520	252	Unilobed	DTS	Hispid	S:PS2P:SP:P:S	2P:5P-2PSP:PS:PD:SHSP:G	P:4P-5S:S2PS: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:SPS:PCP:2PC:G
III	CC	313	157	Trilobed	DTS	Cuspidate	S:PS2P:SP:P:S	SP:5P-3P:SP:2P:PSCS:G	P:3PS-3S:SPS:PCP:PCP:G
III	BR	375	192	Trilobed	DTS	Cuspidate	S:PS2P:SP:P:S	2P:5P-3P:SP:PD:SCP:G	P:3PS-3S:SPS:PDP:PC:G
III	BA	370	200	Trilobed	DTS	Cuspidate	S:PS2P:PS:P:S	2P:5P-3P:SP:PD:2SPC:G	P:3PS-3S:SPS:DPC:DPC:G
III	BAL	468	228	Trilobed	DTS	Cuspidate	S:PS2P:2S:P:S	2P:5P-3P:SP:PD:PSC:G	P:3PS-3S:SPS:PDP:PC:G
IV	CM	570	312	Unilobed	PSS	Hispid	S:P:PS2P:SP:P:S	2P:7P-2PSPS:SPS:PDS:SHPS:G	P:4P-5S:S2PS:PCP:PCP:G
IV	MR	610	350	Trilobed	PSSD	Cuspidate	S:P:PS2P:SP:P:S	3P:6P-3P2S:SPS:PD:SPCP:G	P:3PS-4S:S2P:PSCP:PCP:G
IV	CC	406	222	Trilobed	PSS	Cuspidate	S:P:PS2P:SP:P:S	2P:6P-3PS:SPS:2P:PSCS:G	P:4P-4S:S2P:PCP:PCP:G
IV	BR	482	263	Trilobed	PSS	Cuspidate	S:S:PS2P:SP:P:S	2P:6PS-2P2SP:SPS:PD:PC2P: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:S2P:DSPC:DPC:G
IV	BAL	470	247	Trilobed	PSS	Cuspidate	S:P:PS2P:SP:P:S	3P:5PS-3PS:SPS:PD:PSC:G	P:4P-4S:S2P:PDP:2PC:G
V	CM	760	460	Unilobed	PSS	Hispid	S:P:P:PS2P:SP:S:P:S	3P:8P-5P:2PS:PDP:SPHP:G	P:5P-5S:S2PS:PCP:PCP:G
V	MR	760	460	Trilobed	PSSD	Cuspidate	S:P:P:PS2P:SP:S:P:S	3P:7PS-3PSP:S2P:PD:SDCP:G	P:4PS-4S:SPSP:SPCP:2PC:G
V	CC	460	232	Trilobed	PSS	Cuspidate	S:P:P:PS2P:SP:S:P:S	2P:7PS-3PS:SPS:2P:PSCS:G	P:4PS-4S:S2P:PCP:PCP:G
V	BR	591	324	Trilobed	PSS	Cuspidate	S:S:P:PS2P:2P:P:S:S	3P:7P-2PS2P:SPS:PD:PC2S:G	P:4PS-4S:S2PS:SPDP:PCP:G
V	BA	470	270	Trilobed	PSS	Cuspidate	S:S:P:PS2P:PS:P:P:S	4P:6PS-4PS:S2P:PD:S2PC:G	P:4PS-4S:2S2D:SDPC:PDC:G
V	BAL	561	277	Trilobed	PSS	Cuspidate	S:S:P:PS2P:SP:P:S:S	4P:6P-5P:S2P:PD:PCSP:G	P:4PS-4S:S2P:SPDP:2PC:G
VI	CM	741	432	Unilobed	PSS	Hispid	S:P:P:PS2P:SP:P:PS:P	3P:8P-5P:2PS:PDP:SPHP:G	P:5P-5S:S2PS:PCP:PCP:G
VI	MR	980	560	Trilobed	PSSD	Cuspidate	S:P:P:PS2P:SP:P:PS:S	4P:8P-3PSP:S2P:PD:SPCP:G	P:5P-4S:SPSP:PSCP:PCP:G
VI	CC	557	297	Trilobed	PSS	Cuspidate	S:P:P:PS2P:SP:P:PS:P	2P:8P-4P:SPS:2P:PSCS:G	P:4PS-4S:S2P:SPCP:PCP:G
VI	BR	642	331	Trilobed	PSS	Cuspidate	S:S:P:PS2P:2P:P:PS:P	4P:8P-2PS2P:SPS:PD:PC2S:G	P:5P-4S:S2PS:SPDP:PCP:G
VI	BA	540	310	Trilobed	PSS	Cuspidate	S:S:P:PS2P:PS:P:PS:S	4P:8P-4PS:S2P:PD:S2PC:G	P:5P-4S:2S2D:S2PC:PDC:G
VI	BAL	630	305	Trilobed	PSS	Cuspidate	S:P:P:PS2P:SP:P:PS:P	4P:8P-5P:S2P:PD:PC2P:G	P:5P-4S:S2P:SPDP:2PC:G

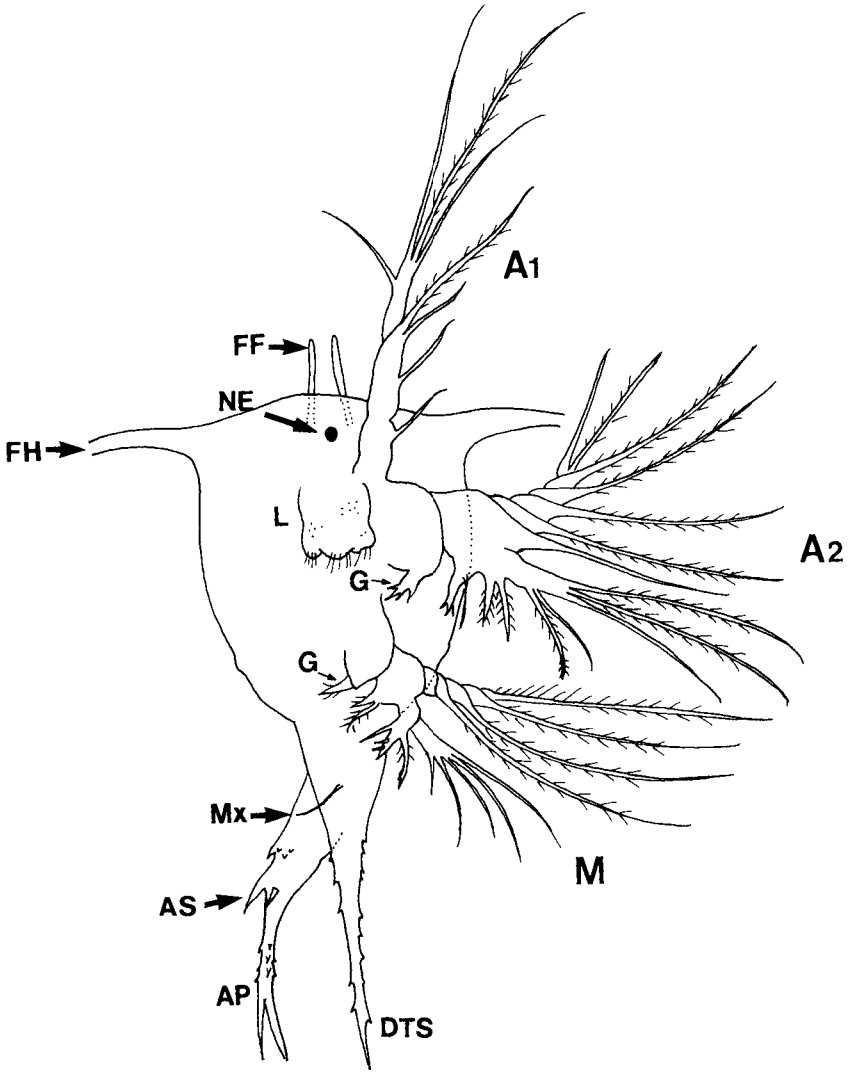

Fig. 1. Schematic drawing of the second nauplius with right appendages. A1, antennule; A2, antenna; AP, abdominal process; AS, abdominal spine; DTS, dorsal thoracic spine; FF, frontal filament; FH, frontolateral horn; G, gnathobase; Lb, labrum; M, mandible; Mx, maxillule. Scale = 100 μm.

subsequent stages. A pair of abdominal spines appears on the apex of the abdominal process. Some setae of the appendages bear minute setules. There are 10~12 irregular small teeth on a pair of abdominal spines.

Nauplius III

The size of the larvae is increased in total length and width when compared with that of stage II. The outline shape of cephalic shield is more rounded than those of stage II. A preaxial seta is present on the antennules as a diagnostic feature of these nauplii. The abdominal process bears a pair of abdominal spines. Irregular small thoracic teeth of stage II are replaced with 5 transverse

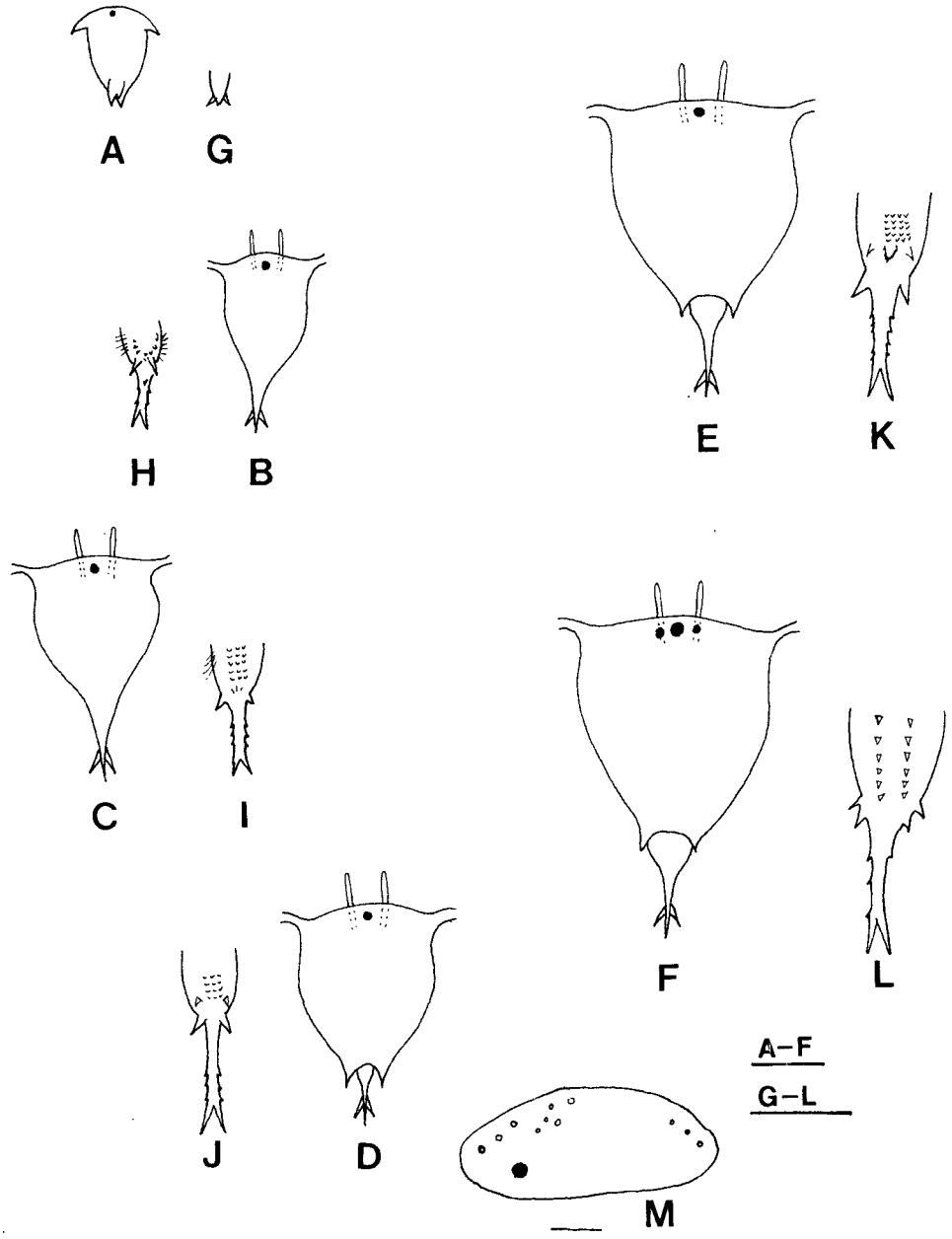

Fig. 2. Outline drawing of six nauplius stages and cyprid of *Chirona cristatus* Ren and Riu, 1978. Nauplius stages are indicated with alphabetical numerals: A, G, stage I; B, H, stage II; C, I, stage III; D, J, stage IV; E, K, stage V; F, L, stage VI; M, cyprid stage. Scales = 100 μm.

rows with 3-4 small teeth in the median region on a pair of abdominal spines.

Nauplius IV

There is a pair of posterior shield spines at this stage and the spine marks the posterior edge.

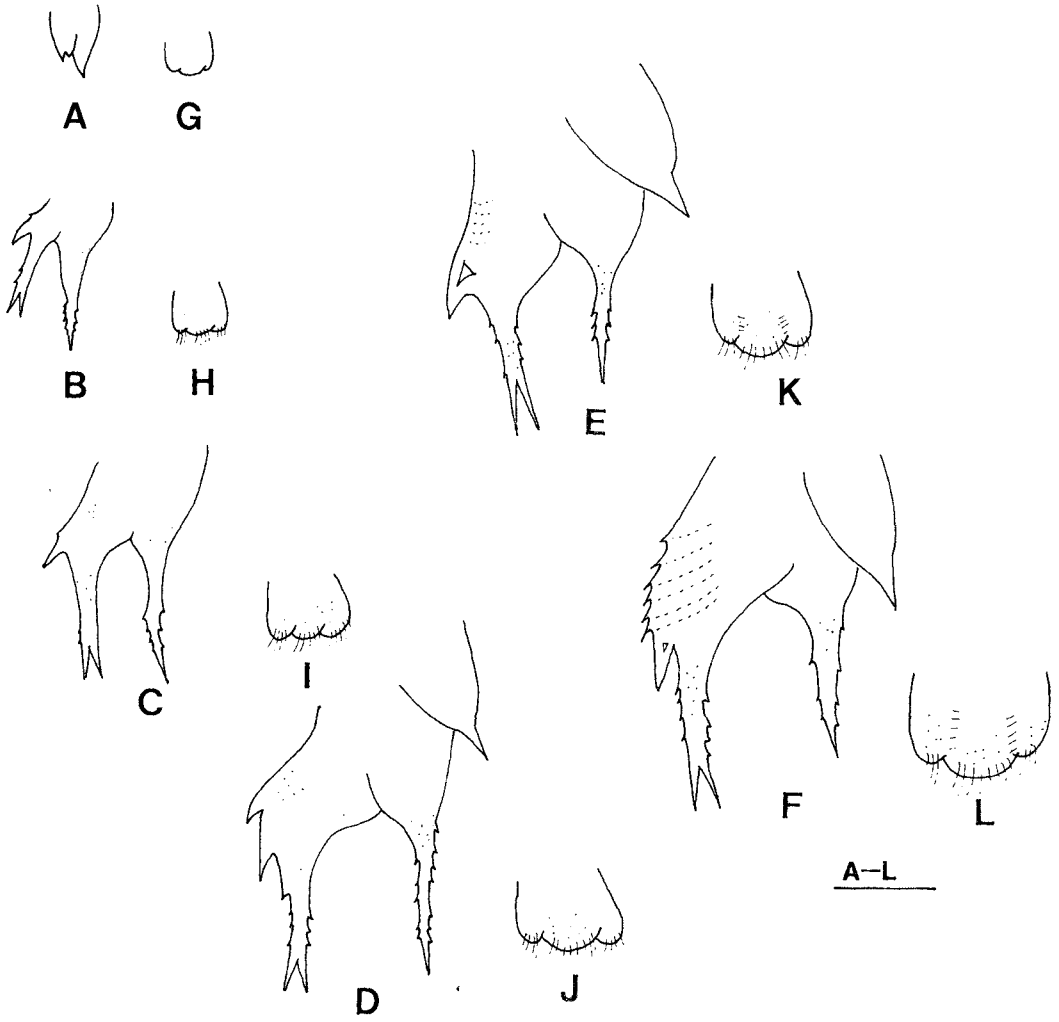

Fig. 3. Outline drawing of lateral view in posterior part and labra of six nauplius stages of *Chirona cristatus* Ren and Riu, 1978. Nauplius stages are indicated with alphabetical numerals: A, G, stage I; B, H, stage II; C, I, stage III; D, J, stage IV; E, K, stage V; F, L, stage VI. Scale = 100 μm.

Two preaxial setae are present on the antennules as a diagnostic feature of these nauplii. There are five rows of 5-6 small thoracic teeth and a pair of small abdominal spines (series 2 spines) on the abdominal spines. Several rows of minute spinules are present on the surface of the abdominal process. The length of dorsal thoracic spine is two-thirds longer than that of abdominal process.

Nauplius V

The cephalic shield bears long posterior shield spines as in nauplius IV except for its enlargement. Three preaxial setae and 5 postaxial setae are present on the antennules as a diagnostic feature of these nauplii. The dorsal thoracic spine becomes shorter than that of stage IV. There are three rows of 3-4 small thoracic teeth between the small abdominal spines (series 2

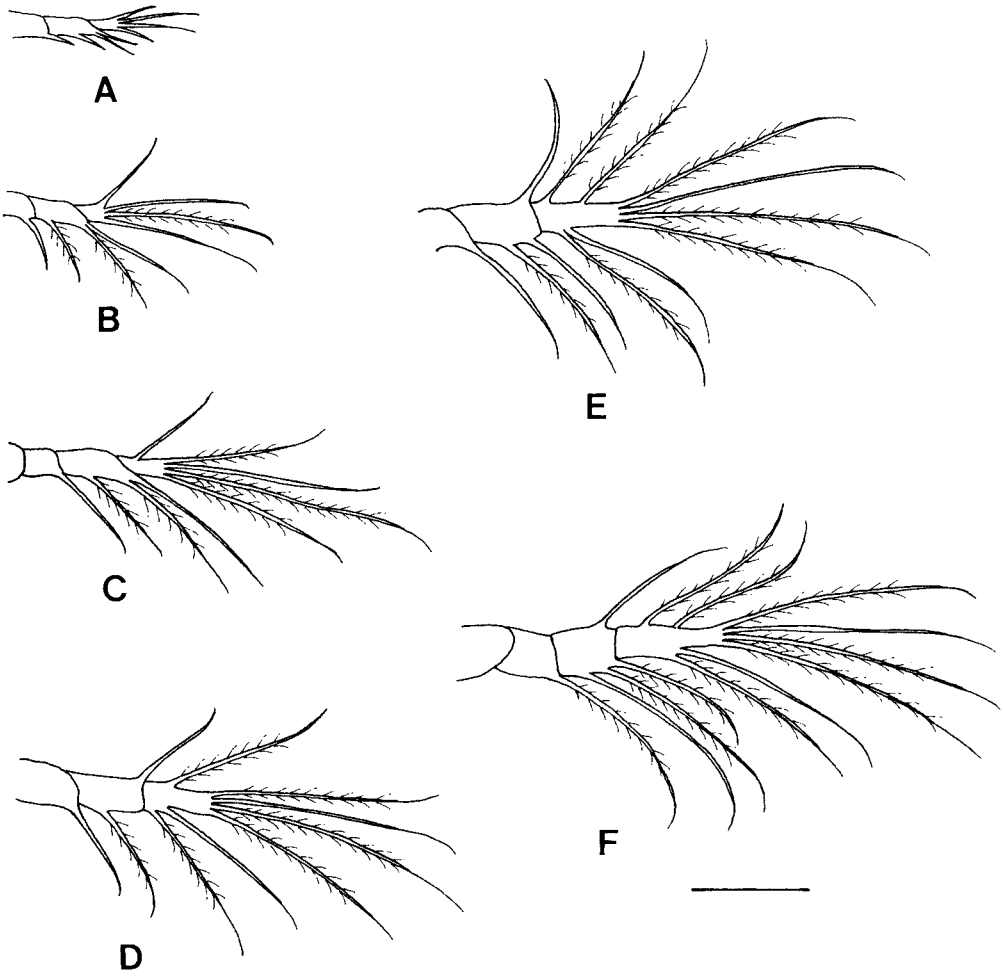

Fig. 4. Antennules of six nauplius stages of *Chirona cristatus* Ren and Riu, 1978. Nauplius stages are indicated with alphabetical numerals: A, stage I; B, stage II; C, stage III; D, stage IV; E, stage V; F, stage VI. Scale = 100 μm.

spines). Several rows of fine spinules are present on the thoracic spines. 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 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 these nauplii. Paired compound eyes appear in the later period of this stage.

Cyprid

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

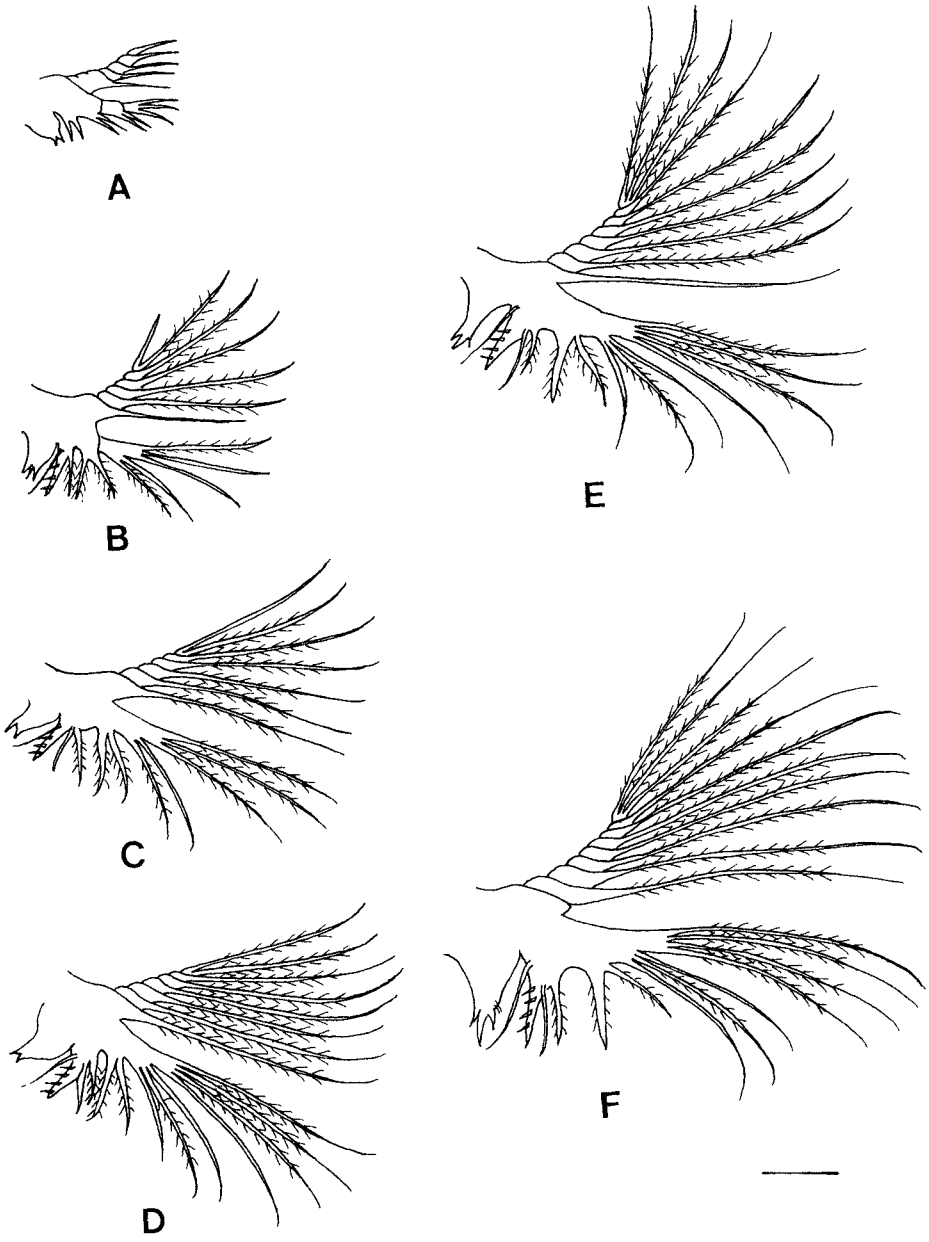

Fig. 5. Antennae of six nauplius stages of *Chirona cristatus* Ren and Riu, 1978. Nauplius stages are indicated with alphabetical numerals: A, stage I; B, stage II; C, stage III; D, stage IV; E, stage V; F, stage VI. Scale = 100 μm.

DISCUSSION

The biology of many adult cirripedes is relatively well documented, but less is known about their

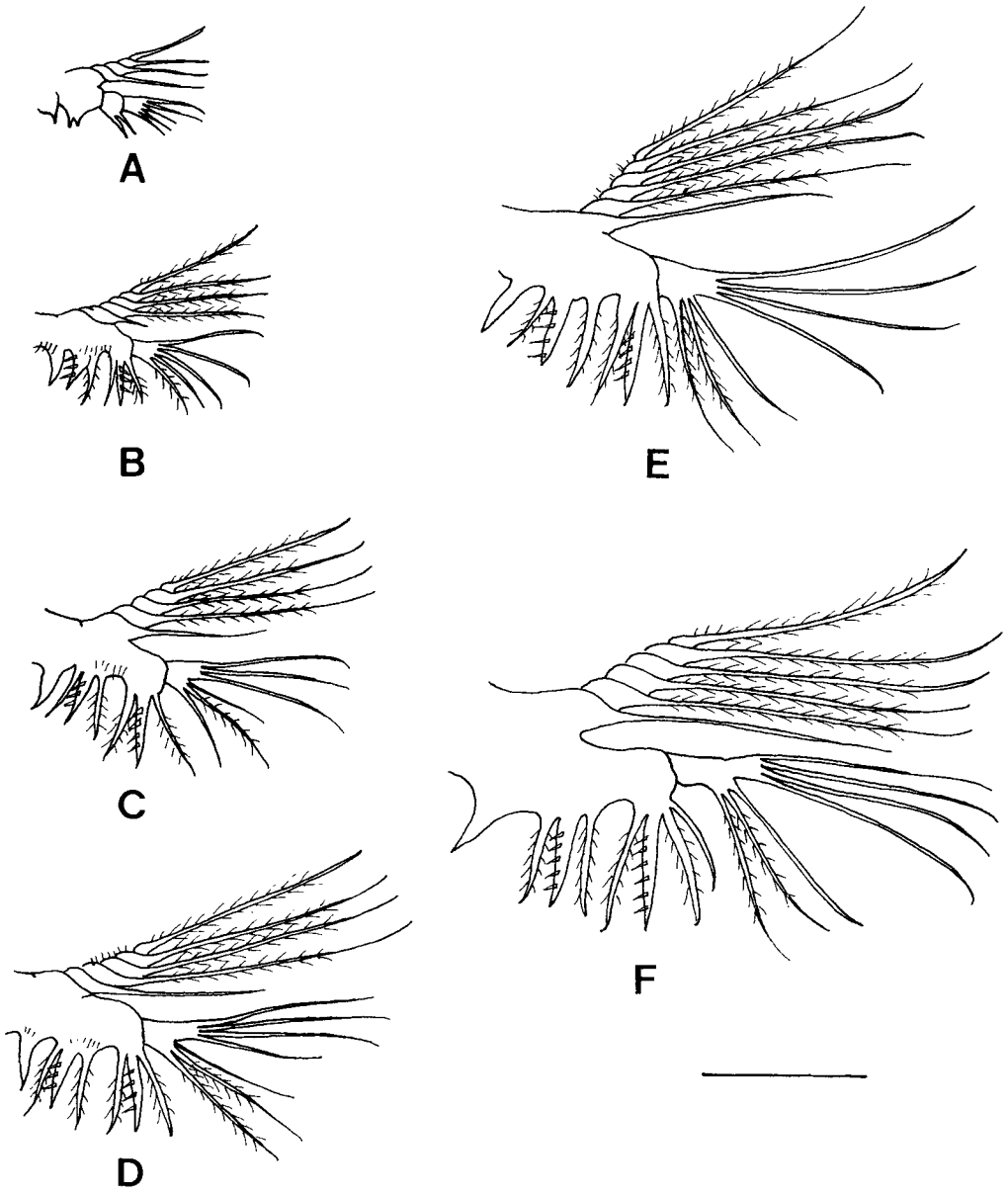

Fig. 6. Mandibles of six nauplius stages of *Chirona cristatus* Ren and Riu, 1978. Nauplius stages are indicated with alphabetical numerals: A, stage I; B, stage II; C, stage III; D, stage IV; E, stage V; F, stage VI. Scale = 100 μm.

larval biology. The identification of barnacle larvae is important for the studies of planktonic ecology. The morphological resemblance of barnacle larvae at each stage between different species made it difficult to separate the six nauplius stages from those of other barnacle species. For the larval descriptions, most investigators now use cultured material rather than those obtained from

plankton samples (Costlow and Bookhout, 1958; Barker, 1976; Barnes and Achituv, 1981; Egan and Anderson, 1987; Lee *et al.*, 1999; Lee and Shim, 2000; Lee *et al.*, 2000). Because of the close morphological similarities among barnacle larvae, their identification has not been easy until specific key characters are discovered. Of the nauplius characters, the antennular setation is widely used for identifying its various stages (Lee *et al.*, 2000): stageI, antennule without preaxial setae and fine setules; stage II, antennule without preaxial setae but some other setae with fine setules; stage III, antennule with a preaxial seta; stage IV, antennule with two preaxial setae; stage V, antennule with three preaxial setae and five postaxial setae; stage VI, antennule with three preaxial setae and six postaxial setae. However, it is not useful for interspecific identification of barnacle nauplii. To overcome this problem, it was suggested that the numerical, graphical and alphabetical setations involving the number, relative location and types of setae on appendages such as antennule, antenna and mandible are essential for the interspecific identification of the barnacle larvae (Bassindale, 1936; Newman, 1965; Sandison, 1967). Lee and Kim (1991) found that it is convenient in morphological identification of the larvae to plot the numerical setation (antennae against mandibles) of all species, with some modification because the numerical setation of the antennules is almost the same in all of the species of the Balanomorph. Miller and Roughgarden (1994) have suggested that according to morphological characteristics, the larvae can be separated into two classes: Class II with (1) unilobed labrum and (2) hispid or feathery setae, and Class II with (1) trilobed labrum and (2) no hispid or feathery setae. Table 4 shows the total length and shield width, shape of labrum, presence of posterior shield spine, alphabetical setal formulae of appendages, and setal type of 4th segment in antennal endopodite from stages II-VI depending on the specific species. *Capitulum mitella* nauplii belong to class I since they have a unilobed labrum and hispid seta in the setal type of 4th segment in antennal endopodite whereas the larvae of *B. reticulatus, B. albicostatus* and *B. amphitrite* belong to class II because the nauplii have a trilobed labrum and no hispid seta through all subsequent stages.

In addition to the above morphological characteristics, shape of the thoracic spines over the abdominal process are helpful in not only recognizing the nauplius species but distinguishing the nauplius stages since they vary in each species and stage of barnacle nauplii. For instance, there are only open circle of seven small thoracic spines over abdominal process in *C. cristatus* (the present study), open circle of ten small thoracic spines in *B. amphitrite* (Egan and Anderson, 1986) and open circle of eight small thoracic spines in *B. albicostatus* (Lee and Kim, 1991) in the nauplii of stageII while the nauplii of *B. reticulatus* (Lee *et al.*, 1999) possess a pair of small thoracic spines on the abdominal process. In the nauplii of stage III, there are five rows of three small thoracic spines over abdominal process in *C. cristatus*, one row of four small thoracic spines lying at right angles to the abdominal process in *B. amphitrite* and five rows of 3-5 irregular small thoracic spines in *B. albicostatus* while the nauplii of *B. reticulatus* possess one row of three small thoracic spines on the abdominal process. In the nauplii of stage IV, there are four rows of three small thoracic spines, parallel row of four small thoracic spines, and five rows of 4-5 small thoracic spines in *C. cristatus*, *B. amphitrite* and *B. albicostatus*, respectively whereas the nauplii of *B. reticulatus* have one row of four small thoracic spines on the abdominal process. In the nauplii of stage V, there are five rows of 4-5 irregular small thoracic spines and a median spine between series two spines on abdominal process in *C. cristatus*, a parallel row of three thoracic spines

between series 2 spines in *B. amphitrite* and three small thoracic spines in *B. albicostatus* while the nauplii of *B. reticulatus* possess three small thoracic spines and a median spine on them. The nauplii of stages VI have consistently six pairs of thoracic spines arranged in a transverse row on abdominal process. Therefore, it is likely that the number and arranged shape of thoracic spines over abdominal process are useful not only in identifying the species but in distinguishing the nauplius stages.

No descriptions have yet been made of morphological characteristics of six nauplius stages and a cyprid on the barnacle *C. cristatus*. However, there has been a reference on larval stages of *C. hameri* (as *B. hameri*) partly described by Crisp (1962). It is shown that some morphological differences exist between *C. cristatus* larvae and *C. hameri* larvae at each stage. Antennal endopodites of *C. cristatus* and *C. hameri* consistently possess thirteen setal numbers (-04324G setation) from stage IV to stage VI while antennal exopodite shows a progressive increase in setal number with each stage: beginning with nine setal numbers (027- setation) of stage IV and ending with eleven setal numbers (038- setation) of stages V-VI in *C. hameri*, while beginning with eight setal numbers (026- setation) of stage IV and ending with ten setal numbers (028- setation) of stages V-VI in *C. cristatus*. Mandibular endpodites of the former possess twelve setal numbers (-03333G setation) at stage III, fourteen setal numbers (-04343G) from stage IV and fifteen setal numbers in stages V-VI whereas those of the latter show a progressive increase in setal number with each stage, beginning with twelve setal numbers (-03333G setation) of stage III and thirteen setal numbers (-04333G) of stages IV-V and ending with fourteen setal numbers (-04343G setation) at stage VI. The former nauplii possess four rows of three small thoracic spines over abdominal spine at stage IV while the latter nauplii show a parallel row of three spines over the abdominal spine (Table 2).

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하구별따개비 (만각아강, 완흉상목)의 유생 발생

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요 약

유생의 형태발생이 미발표된 별따개비속의 하구별따개비는 조간대의 암석 기저부에 부착하거나 가리비의 껍질에 부착하여 생활하는 따개비이다. 유생을 실험실에서 최초로 사육하여 유생발생단계를 형태적으로 도시 및 기재하였다. 노플리우스에서 어린 성체 직전까지 유생사육 기간은 20±0.5°C에서 3주일이 소요되었다. 각 유생단계별로 유생의 크기 및 강모식을 설명하고 하구별따개비 유생의 유생기별 전장, 갑폭, 촉각 내지에 위치한 4절의 강모형태, 상순, 4-6기의 노플리우스 두순극의 형태 및 제 1 촉각, 제 2 촉각, 대악의 강모식이 본 따개비유생의 동정에 유용함을 설명하였다. 위에서 언급한 형태학적 특징 뿐만 아니라 복부돌기에 있는 미세가습가시의 형태와 미세가시수도 따개비유생의 종내 및 종간 동정에 매우 유용함을 구명하였다.