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Larval development of the barnacle *Ibla cumingi* (Cirripedia: Pendunculata: Iblidae) reared in the laboratory

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Larvae of *Ibla cumingi* from Daya Bay, China were cultured in the laboratory. Larval development includes six naupliar stages and a non-feeding cypris stage following the ground patterns of cirripeds. Larvae reached the cypris stage in nine days at ~25°C after hatching. Morphological features including the cephalic shield, frontal horns, labrum, abdominal process, antennules, antennae and mandibles in all nauplii were described and illustrated using light microscopy. A full morphological description of cyprid larvae was provided using scanning electron microscopy. Attempts were made to compare the difference between the larval development model of *I. cumingi* and that of *Ibla* species.

INTRODUCTION

Ibla cumingi Darwin, 1851 is an Indo-West Pacific species; its distribution extends southwards of southern China to Taiwan, the Philippines, Fiji and northern Australia and northwards to southern Japan and westwards to the Red Sea, Persian Gulf and Madagascar (Klepal, 1985; Liu & Ren, 1985). *Ibla cumingi* are common in the eulittoral of Daya Bay, China and largely restricted to intertidal crevices, such as rock cracks and fissures between oyster and barnacle shells.

In the genus *Ibla*, other than *I. cumingi*, four more species of *Ibla* are recognized: *I. idiotica*, *I. atlantica*, *I. quadrivalvis* and *I. segmentata* (Klepal, 1985). Among the five known species, the larvae of three species have been described, *I. quadrivalvis* (Anderson, 1965), *I. idiotica* (Batham, 1946) and *I. cumingi* (Karande, 1974). Although the larval development of *I. cumingi* has been briefly described from the Indian Ocean (Karande, 1974), this report is not complete as setation formulae of this species are still unknown (Korn, 1995). The present paper provides a morphological description of nauplii and cyprids of *I. cumingi* reared in the laboratory.

MATERIALS AND METHODS

Adult *Ibla cumingi* were collected from March–April 2003 at mid-shore in Daya Bay, China. The barnacles were maintained in an aquarium containing filtered seawater, and fed daily on newly hatched *Artemia* nauplii. The hatched Nauplii I of the barnacle were concentrated with a beam of light, and transferred with a wide pipette to 1-l autoclaved glass vessels. Culture techniques were derived from that of Yan & Chan (2001) and Yan (2003). The larvae were fed with a mixed diet of the flagellate *Isochrysis galbana* ($\sim 1 \times 10^5$ cells/ml) and *Skeletonema costatum* ($\sim 5 \times 10^5$ cells/ml).

Preserved exuviae and larvae were stained and dissected with fine needles and observed under a light microscope. Drawings were made using a *camera lucida* attachment and measurements made with a calibrated ocular micrometer. The total length of the nauplii was measured from the frontal margin of the cephalic shield to the tip of the caudal spine or abdominal process, whichever was longer. The shield width of the nauplii was measured at its widest point and shield length from the anterior margin of the shield to the hind shield margin, excluding the posterior

Table 1. *Setal formulae (after Newman, 1965) of* Ibla cumingi *through six naupliar stages.*

Naupliar stage	Antennule	Antenna		Mandible	
		Exopod	Endopod	Exopod	Endopod
VI	S: P:P: PSSS: SP: SPS: S	4P: 8P	P4S: 5S: FSPPS: SFSH: G	P:5P	6S: PPSS: PCPP: PCP: G
V	P:P: PSSS: SP: SP: S	3P: 8P	P4S: 4S: FSPPS: SFSH: G	P:5P	5S: PPSS: PCPS: PCP: G
IV	S:P: PSSS: SP: P: S	3P: 6P	P4S: 3S: FPP: SFSH: G	P:4P	5S: PPS: PCPS: PCP: G
III	P: PSSS: SP: P: S	2P: 5P	PSS: 3S: FPS: SFSH: G	P:3PS	3S: SPS: PCP: PCP: G
II	PSSS: S: P: S	SP: 4PS	PSS: SS: FP: SFH: G	P:3P	3S: SP: PCP: PC: G
I	SSSS: S: S: S	S: 4S	SSS: SS: SS: SSH: G	S:3S	3S: SS: SS: SS: G

S, simple seta; P, plumose seta; C, cuspidate seta; G, gnathobase seta; H, hispid seta; F, feathery seta. Colon indicates different segment.

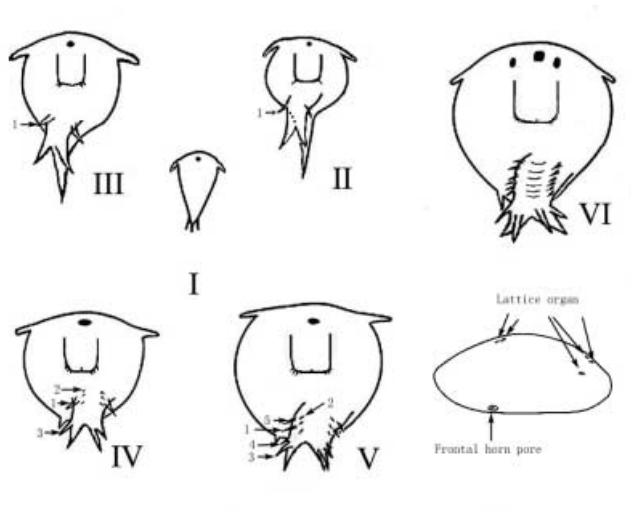

Figure 1. Body forms (ventral view) of naupliar Stages I to VI of *Ibla cumingi*. Scale bar: 100 μm.

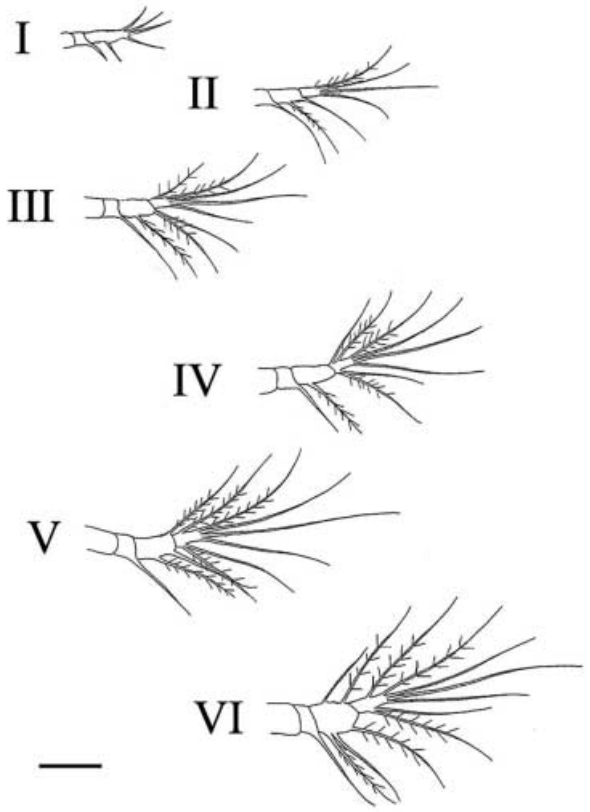

Figure 2. Antennules of naupliar Stages I to VI of *Ibla cumingi*. Scale bar: 100 μm.

shield spines in naupliar Stages IV, V and VI. The width (depth) of the cypris larvae was measured as the maximum distance between the dorsal and ventral margins of the carapace at the deepest point, and cypris length from the anterior to the posterior carapace margins. The morphology of the antennules, antennae and mandibles was described using the setation formulae of Newman (1965) and setal terminology based on Lang (1979).

The external morphology of the cyprid larvae was investigated using scanning electron microscopy (SEM,

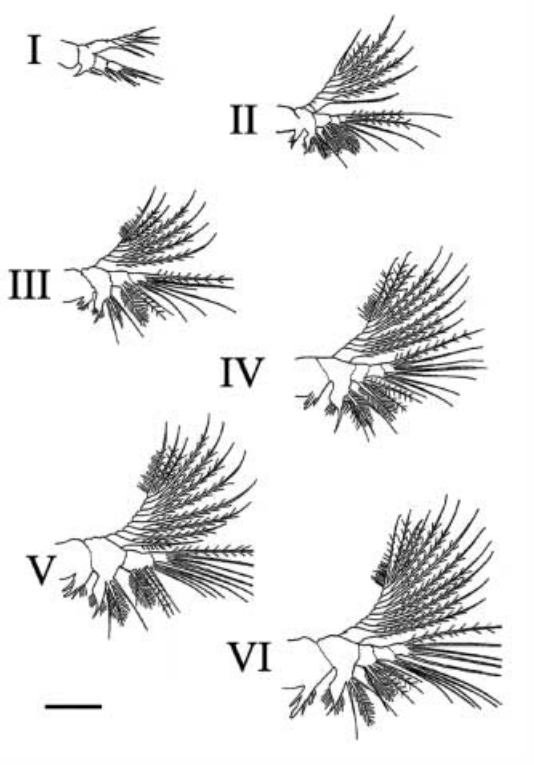

Figure 3. Antennae of naupliar Stages I to VI of *Ibla cumingi*. Scale bar: 100 μm.

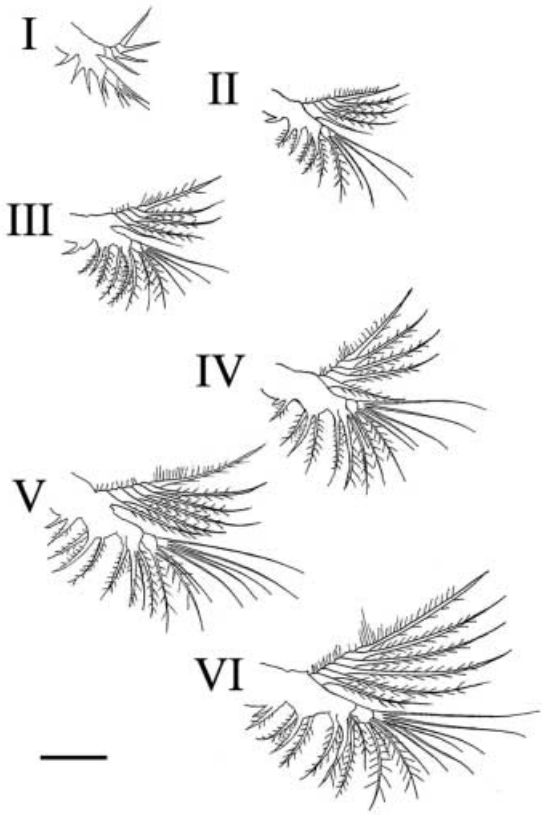

Figure 4. Mandibles of naupliar Stages I to VI of *Ibla cumingi*. Scale bar: 100 μm.

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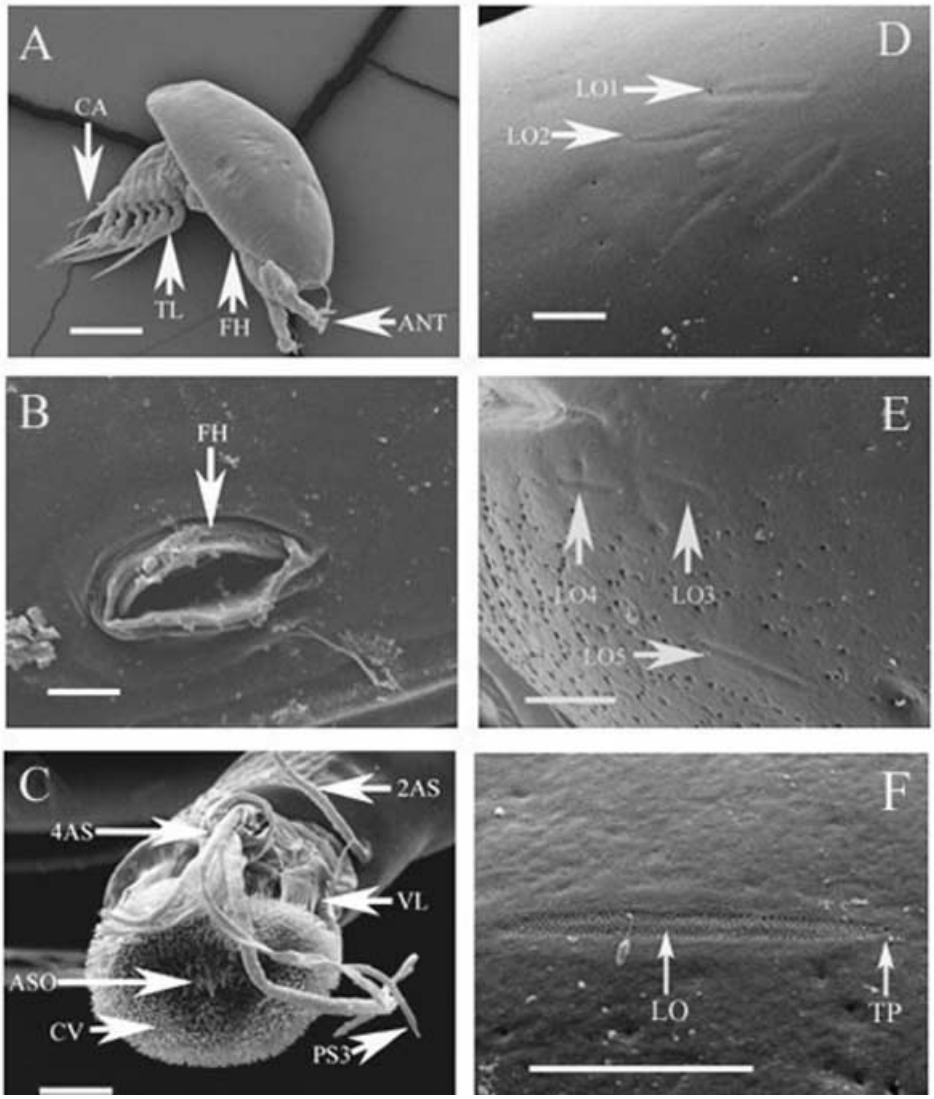

Figure 5. Morphology of cyprid of *Ibla cumingi* under SEM. (A) Lateral view of a cyprid showing the antennuals (ANT), thoracic limbs (TL) and caudal appendages (CA); (B) a left front horn pore (FH) located on the ventral surface of the anterior part of the carapace close to ventral margin of valve; (C) antennular attachment disc surface, composing of the central sense (SO), setae (SE) and velum (VL); (D) dorsal view displaying two pairs of lattice organs (LO1 and LO2); (E) dorsal view of lattice organ pairs 3–5 (LO3, LO4 and LO5) near the apex of the posterior carapace slit (CS) and (F) view of the lattice organ (LO) and the terminal pore (TP). Scale bars: A, 100 μm; B–F, 10 μm.

Leica Stereoscan 440). Larvae were fixed in 2.5% glutaraldehyde (made up in seawater) for 1 h, rinsed in distilled water for 1 min, then dried progressively in graded ethanols (30%, 50%, 75%, 95% and 100%), critical-point dried and coated in gold-palladium before observation using the SEM.

RESULTS

Larval development of *Ibla cumingi* consists of six naupliar stages and a non-feeding cyprid stage following the ground pattern of developments in cirripeds. Larval development from Stage I to cyprids took ~9 d at 25 ±1 °C.

Nauplii of *Ibla cumingi* have a convex, globular cephalic shield with a posterior spine at Stages IV–VI. A pair of frontal filaments is evident at Stages I–VI. The frontolateral horns are directed ventrally and the unilobed labrum bears numerous teeth. In addition to common simple (S) and plumose (P) setae, the mandibles bear cuspidate (C) setae and the antennae possess one hispid (H) and several feathery (F) setae (Table 1).

Nauplius I

This larval stage has a mean length of 291 ±6 μm. The pear-shaped cephalic shield has a pair of anterior frontolateral horns folded at angles to the long axis of the body and a dorsal shield spine. The dorsal thoracic spine and trunk are blunt and similar in length (Figure 1). All setae are simple (Figures 2–4).

Nauplius II

The dorsal thoracic spine has become extended in length (372 μm) and convex in shape. The frontal filaments are present and remain in all the subsequent stages.

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The labrum is unilobed, with a pair of large teeth on the distolateral portion. The trunk bears a pair of abdominal spines (series-1).

Nauplius III

The dorsal thoracic spine is barbed and consistently longer than the trunk (Figure 1). The frontolateral horns have become thickened and shorter in length. Three small teeth on each posteriolateral margin of the labrum and a preaxial seta present on the antennules are diagnostic features of Stage III nauplii.

Nauplius IV

The dorsal thoracic spine disappears at this stage and all the subsequent stages. The cephalic shield bears short posterior shield spines (Figure 1). The labrum has 5–6 small teeth with slender hairs at each corner of the distal margin. A pair of large spines (series-2) has emerged close to the base of the furca. Three pairs of small spines (series-3) appear on the ventral side of the trunk (Figure 1).

Nauplius V

The cephalic shield has increased in size, but the general shape remains similar through to Nauplii VI (Figure 1). The number of teeth on the labrum of this stage is the same as for Stage IV, and also subsequently at Stage VI (Figure 1) except that the number of spines on the surface of the labrum varies. One pair of spines (series-4) appears close to the series-3 spines and one pair of small spines (series-5) close to the series-2 spines on the ventral side of the trunk (Figure 1). The presence of two pairs of spines close to the base of the furca is a diagnostic feature of this nauplius stage.

Nauplius VI

The body shape of this stage is similar to Stage V except in size. In the later development of this stage, a pair of compound eyes becomes clearly visible on either side of the median nauplius eye. The mean larval length at this stage is $514 \pm 13\,\mu$m. The Nauplius VI stage is easily distinguished from other stages by the six pairs of thoracic spines occurring on the trunk (Figure 1).

Cyprid

The bivalve carapace of the cyprid is $528 \pm 9\,\mu$m in mean total length and $260 \pm 5\,\mu$m in mean height. Under the SEM, the carapace has a relatively smooth outer surface (Figure 5A). A pair of frontal horn pores is located on the ventral surface of the anterior part of the carapace close to the ventral margin of valve (Figure 5A,B). The antennule ends in a cup-shaped third segment consisting of an almost circular attachment disc, a raised rim (velum or skirt) and sense organs (Figure 5C). Five pairs, two anterior (Figure 5D) and three posterior (Figure 5E), of lattice organs (Figure 5F) are also observed. The distribution of the five pairs of lattice organs (LO) was also noted (Figure 1).

DISCUSSION

When comparing larvae of *Ibla cumingi* with *I. quadrivalvis* and *I. idiotica*, they exhibit distinctive characters, which can be observed in the larval development model. *Ibla cumingi* developed successfully from Nauplius I to cyprid when the larvae were provided with algae culture, showing that these larvae need to feed on algae to meet the energy demands for the larval development and metamorphosis, although we found that Nauplii I can develop to Nauplius V stage from starved cultures (personal unpublished data). *Ibla quadrivalvis* and *I. idiotica*, however, have an entirely different type of development. The free-swimming but non-feeding nauplius of *Ibla quadrivalvis* does not grow in size, but moults regularly, and its limb setation is simple. The duration of larval development is abbreviated and the development of cypris features begins precociously; the cyprids themselves appear in seven days (Anderson, 1965). In *I. idiotica* the direct development to the cypris stage is completed in the female mantle cavity. The body of the larva is full of yolk, the cephalic shield is hemispherical, the frontolateral horns are reduced, and the nauplius is almost motionless. Antennae and mandibles are absent, whereas the cypris thoracic limbs are precociously developed (Batham, 1946).

In *Ibla cumingi*, the teeth of the labrum are very useful in distinguishing Stages II and III–VI larvae. The unilobed labrum has one pair of teeth at the corners of the free distal end at Stage II, and small teeth with slender hairs at Stages III–VI. The labrum is also a diagnostic character to separate *I. cumingi* from another stalked barnacle *Capitulum mitella*, which occurs with *I. cumingi* in Daya Bay, China. The labrum of *Capitulum mitella* possesses two pairs of teeth on each posteriolateral margin (Lee et al., 2000).

Recognition of the free swimming cyprid stages of cirripeds is not only essential for studies of larval supply and plankton ecology, but also significant in thecotracan phylogeny (Høeg et al., 2004). The results of the present study show that the relatively smooth cyprid carapace of *I. cumingi* is similar to that of *Tetraclita squamosa* and *T. japonica* (Chan, 2003), but different from that of *Capitulum mitella* and *Chthamalus malayensis* with a honeycombed surface (Jensen et al., 1994; Yan & Chan, 2001). Although the 2+3 pairing pattern of lattice organs was confirmed on the outer surface of cyprid in *I. cumingi* which also has a similar arrangement of lattice organs to *Capitulum mitella*, *Chthamalus malayensis*, *T. squamosa* and *T. japonica* (Jensen et al., 1994; Yan & Chan, 2001; Chan, 2003), the conspicuous terminal pore of lattice organs in *I. cumingi* is different from that of *Capitulum mitella*, *Chthamalus malayensis*, whilst similar to that of *T. squamosa* and *T. japonica* (Chan, 2003).

Ibla cumingi larvae possess a rounded, convex cephalic shield, short ventrally directed frontolateral horns, a very short abdominal process and an unilobed labrum, which are similar to those of chthamalids. Larvae of *I. cumingi*, however, have unique features such that the dorsal thoracic spine disappears and the larvae have a pair of short posterior cephalic shield spines at Stages IV–VI.

The observation of larval morphology in *Ibla cumingi* suggests that the surface of the cephalic shield, frontolateral horn, the shape and teeth of the labrum, setation of appendages and outer surface of cyprids carapace are important features to separate the nauplii and cyprids of

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Ibla cumingi from other barnacle species. The results of the observation also provide data about this species for future comparative studies of barnacle larval morphology and for phylogenetic implications of larval differences in Cirripedia.

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REFERENCES

Anderson, D.T., 1965. Embryonic and larval development and segment formation in *Ibla quadrivalvis* Cuv. (Cirripedia). *Australian Journal of Zoology*, **13**, 1–15.

Batham, E.J., 1946. Description of female, male and larval forms of a tiny stalked barnacle, *Ibla idiotica* n.sp. *Transactions of the Royal Society of New Zealand*, **75**, 347–356.

Chan, B.K.K., 2003. Studies on *Tetraclita squamosa* and *Tetraclita japonica* (Cirripedia: Thoracica) II: larval morphology and development. *Journal of Crustacean Biology*, **23**, 522–547.

Høeg, J.T., Lagersson, N.C. & Glenner, H., 2004. The complete cypris larva and its significance in thecostracan phylogeny. In *Evolutionary developmental biology of crustacean* (ed. G. Scholtz), pp. 197–215. Rotterdam: A.A. Balkema. [*Crustacean Issues*, **15**.]

Jensen, P.G., Moyse, J., Høeg, J. & Al-Yahya, H., 1994. Comparative SEM studies of lattice organs: putative sensory structures on the carapace of larvae from Ascothoracida and Cirripedia (Crustacea Maxillopoda Thecostraca). *Acta Zoologica*, **75**, 125–142.

Karande, A.A., 1974. Developemnt of the pedenculate barnacle *Ibla cumingi* Darwin. *Indian Journal of Marine Science*, **3**, 173–177.

Klepal, W., 1985. *Ibla cumingi* (Crustacea, Cirripedia)—a gonochoristic species (anatomy, dwarfing and systematic implications). *P.S.Z.N.I.: Marine Ecology*, **6**, 47–119.

Korn, O.M., 1995. Naupliar evidence for cirripede taxonomy and phylogeny. In *New frontiers in barnacle evolution* (ed. F.R. Schram and J.T. Høeg), pp. 87–122. Rotterdam: A.A. Balkema. [*Crustacean Issues*, **10**.]

Lang, W.H., 1979. Larval development of shallow water barnacles of the Carolinas (Cirripedia: Thoracica) with keys to naupliar stages. *National Oceanic and Atmospheric Administration Technical Report, NMFS*, no. 421, 1–39.

Lee, C., Shim, J.M. & Kim, C.H., 2000. Larval development of *Capitulum mitella* (Cirripedia: Pedunculata) reared in the laboratory. *Journal of the Marine Biological Association of the United Kingdom*, **80**, 457–464.

Liu, R. & Ren, X., 1985. Studies on Chinese Cirripedia (Crustacea) V. Suborder Lepadomorpha. *Studia Marina Sinica*, **25**, 179–281.

Newman, W.A., 1965. Prospectus on larval cirriped setation formulae. *Crustaceana*, **9**, 51–56.

Yan, Y., 2003. Larval development of the barnacle *Chinochthamalus scutelliformis* (Cirripedia: Chthamalidae) reared in the laboratory. *Journal of Crustacean Biology*, **23**, 513–521.

Yan, Y. & Chan, B.K.K., 2001. Larval development of *Chthamalus malayensis* (Cirripedia: Thoracica) reared in the laboratory. *Journal of the Marine Biological Association of the United Kingdom*, **81**, 623–632.

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