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Comparison of larval shell morphology of two coastal turbinid snails, *Turbo (Batillus) cornutus* and *Turbo (Marmarostoma) stenogyrus* (Vetigastropoda: Turbinidae)

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Abstract Larval shell morphology was compared to determine criteria for the identification of larvae and juveniles of two coastal turbinid snails, *Turbo (Batillus) cornutus* and *Turbo (Marmarostoma) stenogyrus*: long axial length, short axial length, aperture diameter and surface sculpture. For all three morphological parameters of the larval shell, *T. cornutus* was smaller than *T. stenogyrus*, for example the mean long axial length being 267 μm and 303 μm, respectively. Several differences were detected between the two species in the surface sculpture of the larval shells based on scanning electron microscope observations. Distinction could be achieved between the two species even for snails in which the postlarval shell had partially covered the larval shell. The current results allow the differentiation for all life stages of the postlarval stages between *T. cornutus* and *T. stenogyrus* and therefore facilitate ecological studies of the postlarval stages.

Key words: larval shell morphology, Turbinidae, SEM, *Turbo (Batillus) cornutus*, *Turbo (Marmarostoma) stenogyrus*

Turbo cornutus is distributed around Japan, and *Turbo stenogyrus* is distributed from central to western Japan and in the tropical Pacific (Okada, 1982). The two species inhabit rocky shores sympatrically, and their distributions overlap from central to western Japan.

Adults of *T. cornutus* grow up to 10 centimeters in shell height and often develop spines on postlarval shells, while those of *T. stenogyrus* grow up to only 35 millimeters and have no spines (Okada, 1982). Therefore adults of two species are easily distinguished on shell morphology. However distinction of two species in the early life stages is difficult, as the postlarval shell morphologies of juveniles are similar to each other (Yamazaki and Ishiwata, 1987). The trochophore larva of the two species secretes a larval shell and then develops into a veliger larva. *T. cornutus* hatches as the trochophore larva and has a planktonic stage for a few days after hatching. *T. stenogyrus* remains in the egg-capsule from the trochophore to the veliger larval stage and hatches out as a benthic juvenile. The veliger larva of the two species forms a postlarval shell from the aperture of the larval shell after settlement. Therefore, the two species just after settlement have no development of postlarval shell (Ai, 1965; Hayashi, 1983a; Kono and Yamakawa, 1999).

Before settlement, larvae of the two species are not collected together because of the difference of developmental pattern mentioned above. At just after settlement, it is possible to distinguish the two species by differences of the lar-

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val shell morphology, i.e., aperture shape, surface sculpture and shell size (Yamazaki, 1992). Further, for juveniles from 2.5-10.0 millimeter shell height, it is possible to separate the two species by differences in the postlarval shell morphology, i.e., the shape of the lower part of a columella, the shape of 3 spiral ribs, the occurrence of 2 weak ribs between the cords, and spines on the operculum (Yamazaki and Ishiwata, 1987). However there is no information about criteria for the distinction of the two species in the size range from just after settlement to 2.5 millimeter shell height. To estimate the mortality rate in the early life stage after settlement, it is important to distinguish the two species for this stage.

The objective of this study is to determine the criteria to distinguish *T. cornutus* and *T. stenogyrus* in the size from just after settlement. If it is possible to distinguish the two species in this stage, distinction through all the early life stages after settlement will be achieved. This will contribute to the promotion of ecological field studies of the early life history of both these species.

Materials and Methods

Specimens of *T. cornutus* were larvae and juveniles reared for stock enhancement purposes in 1990 and 1992 at the Kanagawa Prefectural Fisheries Research Institute (Misaki City) and were sampled at 1, 14 and 40 days after settlement. Those of *T. stenogyrus* were juveniles reared for experiments on its early developmental biology (Kono and Yamakawa, 1999) and were sampled at 5, 50, 60 and 70 days after settlement. Specimens were fixed with neutralized 10% formalin-seawater and then preserved in 70% ethyl alcohol until measurements and observations.

Length of long axis, short axis, and aperture diameter were measured for the comparison of the larval shell morphology. Measurements were made according to Hayashi (1983b); the length of each part was measured on the adapical side of the larval shell (Fig. 1).

All measurements were made using a compound microscope equipped with a calibrated ocular micrometer. As the postlarval shell develops discoidal for the first whorl and has been shown to progressively cover the periphery of the larval shell in *T. cornutus* (Ai, 1965; Hayashi, 1983a), the measured values of the observable part of the larval shell are estimated as smaller than the real size with the development of the postlarval shell. Taking this into account, measurements were made on specimens which were classified into the following three stages based on the degree of the postlarval

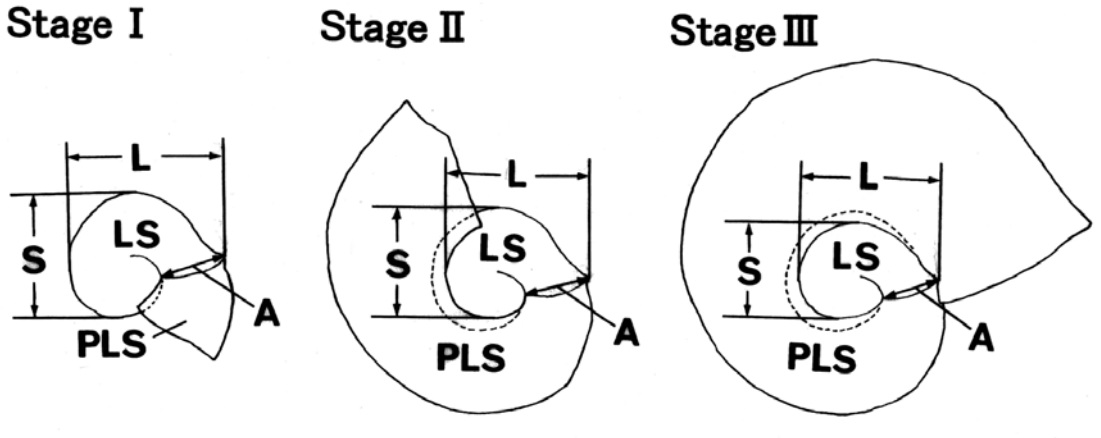

Fig. 1. Measurement baselines (L: long axial length; S: short axial length; A: aperture diameter) of the larval shell (adapical side) at the three developmental stages. LS: larval shell; PLS: postlarval shell.
Stage I : specimen with less than 1/4 of a whorl of the postlarval shell;
Stage II : from 1/2 to 3/4 whorl; Stage III : more than one whorl.

Table 1. Details of specimens and comparision of the size (mean±SD) of larval between *Turbo cornutus* (Tc) and *Turbo stenogyrus* (Ts). Data in parentheses represent the size range.

Stage	I		*t*	II		*t*	III		*t*
Species	Tc	Ts		Tc	Ts		Tc	Ts	
Days after hatching(Days)	1	5		14	50, 60 and 70		40	50, 60 and 70	
Sample number	61	41		61	5		11	6	
Shellwidth(μm)	348±44 (269～ 450)	365±43 (317～ 482)		629±36 (450～ 606)	698±104 (563～ 838)		1111±143 (865～ 1297)	1025±117 (813～ 1138)	
Long axial length(μm)	270±6 (253～ 283)	328±7 (317～ 346)	45.090**	259±8 (241～ 278)	307±11 (297～ 325)	12.352**	267±10 (255～ 284)	303±7 (291～ 313)	7.879**
Short axial length(μm)	201±6 (188～ 216)	249±6 (237～ 269)	39.127**	193±8 (173～ 206)	231±5 (223～ 238)	10.255**	189±15 (165～ 206)	203±9 (197～ 219)	2.140*
Aperture diameter(μm)	136±12 (114～ 175)	158±8 (140～ 173)	10.055**	134±10 (113～ 159)	150±7 (144～ 163)	3.430**	133±7 (124～ 148)	148±6 (141～ 156)	4.525**

*: $p<0.05$; **: $p<0.001$

shell development (Fig. 1, Table 1), namely, stage I : specimens with less than 1/4 of a whorl of the postlarval shell, stage II : from 1/2 to 3/4 whorl, stage III : more than one whorl.

Specimens for surface sculpture observations were dehydrated in 99.5 % ethyl alcohol. After air-drying on a petri dish, they were coated with gold using a Hitachi E-1030 ion spattering apparatus. All observations were made using a Hitachi S-4000 scanning electron microscope under an accelerating voltage of 10kV.

Results

Measurements of larval shells

Among the measured parts of the larval shells, the lengths of *T. cornutus* were shorter than those of *T. stenogyrus* for all stages (Table 1). All measured values of the larval shells significantly differed (t-test, short axis in stage III : $p<0.05$, all other measurements: $p<0.001$). For the long axis of all stages, and the short axis of stages I and II , the range of the lengths did not overlap between the two species.

The exposed part of the larval shells remained observable on the postlarval shells of *T. stenogyrus* in stage II (70 days after settlement) and *T. cornutus* in stage III (40 days) (Fig. 2-E, F). At these stages it was possible to measure the long axial length, short axial length and aperture diameter, were 327, 233 and 155 μm in *T. stenogyrus* versus 280, 208 and 138 μm in *T. cornutus*, respectively.

Observations of the larval shells using SEM

There were some similarities between the two species in the pattern of surface sculpture and the aperture shape on the larval shells; (1) the pattern is formed by many irregular ornaments scattered on the larval shell (Fig. 2-A, B) and repeats in all specimens although there are slight variations in the size and the form of the ornaments among individuals, (2) the size and height of the ornaments reduce toward the central part of the larval shell (Fig. 2-C, D), and (3) the density of the ornaments is roughly the same between the adapical and abapical sides, and (4) the apertures of the larval shells have a rapid outward expansion.

On the other hand, there were some differences between the two species in the pattern of the surface sculpture and the aperture shape. The characters of each species and the differences between the species are described as follows. In *T. stenogyrus*, the ornaments on larval shell are relatively dense, frequently connect with each other and form a reticular pattern. No spiral ridges and radiated patterns are observed. The expansion of the aperture is more marked than that of *T. cornutus*. In *T. cornutus*, ornaments on the larval shell is relatively sparse, a few discontinuous spiral cords are formed parallel on the periphery of the larval shell. Between the ridges, about 10 discontinuous

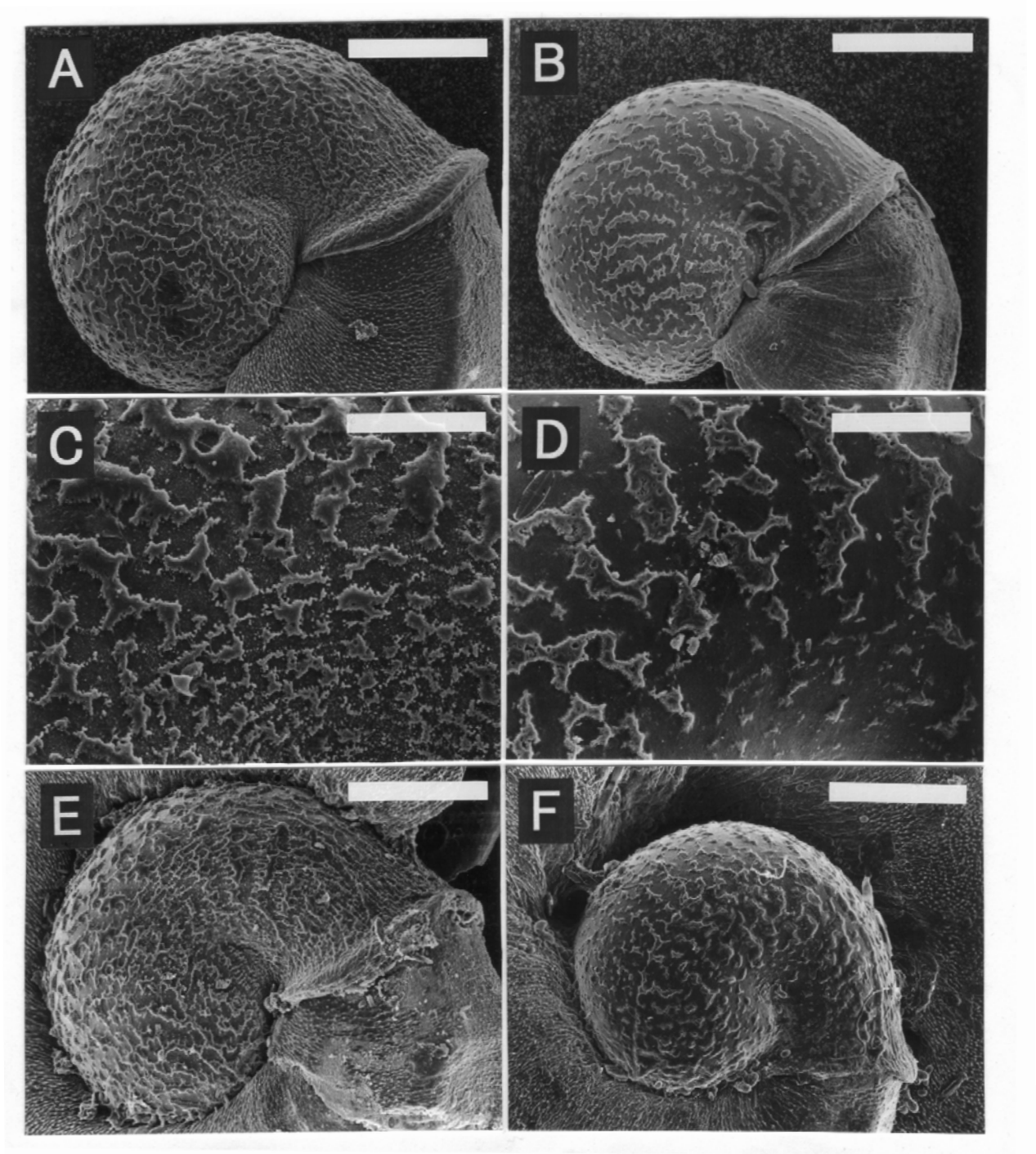

Fig. 2. Scanning electron micrographs of the larval shell of the two turbinid snails, *Turbo (Marmarostoma) stenogyrus* and *Turbo (Batillus) cornutus*. Left side (A, C, E): *T. stenogyrus*; right side (B, D, F): *T. cornutus*. A-B: Newly settled juveniles, 5 days (left) and 14 days (right) after settlement. Scale=100 μm. C-D: Central section of the larval shell, 5 days (left) and 14 days (right) after settlement. Scale=30μm. E-F: Larval shell of juveniles, 70 days (left) and 40 days (right) after settlement. Scale=100μm.

patterns often radiate from the center of the larval shell toward the periphery. The expansion of the aperture is relatively weak. No differences were found in the surface sculpture on the larval shells between the 1990 and 1992 specimens.

On the larval shell of *T. stenogyrus* in stage Ⅱ and *T. cornutus* in stage Ⅲ, it was possible to readily detect using SEM the characters of each species as stated above (Fig. 2-E, F).

Discussion

The larval shell of the haliotid, *Haliotis discus hannai*, begins to mineralize 21 hours after hatching, and the planispiral morphology is completed by 40-45 hours. No growth increments in the larval shell were observed on the surface thereafter (Iwata, 1980). This indicates that the outer shape of the larval shell such as the size and the surface sculpture does not change after the completion. The formation of the larval shell requires 27 hours to complete after hatching in *T. cornutus* and 32 hours in *T. stenogyrus* (Ai, 1965; Kono and Yamakawa, 1999). If characteristic features of each species occur in the morphology of the larval shell, they are effective criteria for the identification between these two species.

In all three stages used in this study, the range of the long axial length of the larval shell of *T. cornutus* and *T. stenogyrus* did not overlap and therefore it was possible to distinguish the two species after settlement based on measurements of the long axial length. Yamazaki (1992) showed that the long axial length of *T. cornutus* was smaller than that of *T. stenogyrus* just after settlement, equivalent to stage I in this study, and their ranges did not overlap. This result agrees with the findings of this study. It was, therefore, found that the long axial length is an important criterion to distinguish juveniles of the two species with a postlarval shell, as well as for juveniles just after settlement.

In both species, distinct patterns of surface sculpture and the aperture shape of larval shells were recognizable from the SEM observations. The surface sculpture of *T. stenogyrus* was denser than that of *T. cornutus* and formed a reticular pattern. The expansion of the aperture of *T. stenogyrus* was more marked than that of *T. cornutus*. Hayashi (1983a) described a sudden outward expansion of the aperture just before settlement, some discontinuous cords running in the direction of growth and irregular forms of projections among the cords based on SEM observations of larval shells of *T. cornutus*. Present study supports these previous observations. Yamazaki (1992) showed that it is possible to distinguish the two species just after settlement based on differences of the surface sculpture and aperture shape under an optical microscope; ornaments of *T. cornutus* were more discontinuous than that of *T. stenogyrus* and the expansion of the aperture of *T. cornutus* was smaller than that of *T. stenogyrus*. However there are only illustrations of part of the surface sculpture in Yamazaki (1992). Moreover the ornaments of *T. stenogyrus*, were described as "well-developed and continuous", were not illustrated with a reticular pattern. The illustration of *T. stenogyrus* in Yamazaki (1992) is similar to that of *T. cornutus* in this study. This difference may be caused by differences in the resolution and the depth of field between observation techniques. Therefore it is necessary to note that an available standard of the surface sculpture is different by a kind of a microscope used. The increase of shell height leads to a decrease in the light transmittance and makes observations of the surface sculpture under an optical microscope difficult. Observations under SEM as carried out in this study allow greater clarity of the distinction of the two species with a postlarval shell because of the greater depth of field.

Hayashi (1983a) pointed out that the surface sculpture variation at the species level might be caused by differences of environment factors such as water temperature, salinity etc. during development. For *T. cornutus*, no differences between the 1990 and 1992 specimens were found in the surface sculpture. Accordingly the surface sculpture variation might not be caused by differences of environment factors.

In both species, the larval shell was exposed on the postlarval shell for several months after settlement. Moreover, it was possible to distinguish the two species by measurements of the long axial length of the larval shell and observations of the surface sculpture and development of prominent axial ridge at larval-postlarval shell boundary under SEM in the postlarval stage. Hayashi (1983a) reported that it was possible to recognize the surface sculpture on the

larval shell of haliotids, *Haliotis* (*Nordotis*) *discus discus*, for 40 days after settlement using SEM. In some prosobranch species, the larval shell remains on the postlarval shell for at least several months after the onset of the formation of the postlarval shell (e.g., Iwata, 1980; Lima and Lutz, 1990; Ramón, 1990). This indicates that observations of the larval shell using SEM can be used as an effective method for the distinction between species for several months after the onset of the formation of the postlarval shell.

In conclusion, it was possible to distinguish between juveniles of *Turbo cornutus* and *Turbo stenogyrus* in the size range from just after settlement (<0.4mm) to a few millimeters in shell height even if the periphery of the larval shell is partially covered by the postlarval shell. Firstly measurements of the long axial length of the larval shell and/or observations of the surface sculpture and development of prominent axial ridge at larval-postlarval shell boundary under an optical microscope are recommended to differentiate between the two species because there are easy and reliable methods. Observations of the surface sculpture and development of ridge under SEM are effective if it is not possible to measure the long axial length because of the loss of a part of the larval and postlarval shell or to observe the surface sculpture and development of ridge under an optical microscope because of the decrease of transmittancy with the growth of the postlarval shell.

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