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ホトトギスガイとコウロエンカワヒバリガイの D型幼生から初期稚貝の形態

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Larval and Post-larval Shell Morphology of Two Mytilid Species *Musculista senhousia* (Benson) and *Limnoperna fortunei kikuchii* Habe

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Abstract: In order to aid identification of larval and post-larval stages of two mytilid species *Musculista senhousia* and *Limnoperna fortunei kikuchii*, which are both predominant among the intertidal macrobenthos in Lake Hamana along the Pacific coast of middle Honshu, the eggs and planktonic larvae of the two species were cultured in the laboratory until the larvae grew up to post-larval stages. Based these specimens of larval and post-larval stages, morphological features of bivalve shells, especially hinge apparatus, were examined using SEM and optical microscopy, with the result that all stages of the two species are clearly distinguished from each other in several characteristics.

Introduction

It is suggested that population dynamics of marine benthic invertebrates including fouling animals have been considerably influenced by a supply of planktonic and settled larvae, and the mortality of settled larvae and early juveniles (Connell, 1985; Roughgarden *et al.*, 1988). In order to clarify larval recruitment processes by which population dynamics of marine benthic invertebrates are determined, first of all, we need to identify, in a specific level, larvae and early juveniles of the invertebrates which are identifiable in adult forms. Unfortunately, there is very little information on morphological features of larvae and early juveniles of most marine benthic invertebrates, resulting that larval recruitment processes remain open to be clarified.

The fouling bivalves of the family Mytilidae, especially *Mytilus edulis* and *M. galloprovincialis*, have extensively been studied owing to their importance as sea food and fouling animals (Lutz and Kennish, 1992). Identification of larval mytilids is usually based on hinge morphology (Chanley and Andrew, 1971; Le Pennec, 1980). Recently, SEM (scanning

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electron microscopy) has made it possible to clarify detailed morphological features of hinge. Based on the features of hinge from D-shaped larval to post-larval stages of bivalves, identification of several species of larval mytilids has been made available by Lutz and Hidu (1979), Siddall (1980) and Fuller and Lutz (1989).

In Japan, 94 species of Mytilidae have been reported (Higo and Goto, 1993). Of these, however, larval morphology of only 10 species have been described (Yoshida, 1964; Tanaka, 1979 a,b,c; Xu, 1983; Sakai and Sekiguchi, 1992).

Except for D-shaped larvae, the morphological features of larval to post-larval stages of the two mytilid species *Musculista senhousia* and *Limnoperna fortunei kikuchii* have already been described. The former was done by Yoshida (1937), Tanaka (1979 a,b,c) and Xu (1983) using optical microscopy, and by Xu (1983), and Sakai and Sekiguchi (1992) using SEM, while the later was done by Xu (1983) using optical microscopy and SEM. However, descriptions for *L. fortunei kikuchii* by Xu are not sufficient for identifying the larvae in field samples. So, when we examine planktonic larvae of mytilids collected in inlet waters, especially in Lake Hamana where these two species are predominant among intertidal macrobenthos, we feel that it is difficult to distinguish planktonic larvae of these two species from each other. In this paper, we describe morphological features of larval to post-larval stages of these two species using optical microscopy and SEM, based on their cultivation in the laboratory.

Materials and Methods

On the intertidal flats of Inohana and Shonai inlets in Lake Hamana located along the Pacific coast of middle Japan, 25 – 100 specimens of *M. senhousia* and *L. fortunei kikuchii* were collected once a week from August 1991 to August 1993.

In order to induce artificial spawning in laboratory, each specimen was put separately in a multi-dish which was filled with whatman GF/C filtered seawater (20‰) and kept at room-temperature. When the water temperature exceeded 25°C around the sampling site, specimens were first put in 5°C for 2 hours and then transferred into a room-temperature condition in order to give a stimulus to promote artificial spawning. The fertilized eggs were kept at 25°C in a 800 ml vessel filled with whatman GF/C filtered seawater. The water was changed once a day, and dense droplets of a diatom *Chaetoceros calcitorans* (2.0×10^6 cell/ml per dish) were supplied every day. In the course of keeping alive the eggs and larvae of the two species of bivalves, several larvae were sampled from the dish every day, fixed with 70% alcohol, and then deposited in a small glass vial and placed within a refrigerator at 5°C until they were used for morphological examination. Features of hinge in bivalve shells were examined using SEM (JSM-T, Nippon Denshi Ltd.) and/or optical microscopy according to the method by Sakai and Sekiguchi (1990, 1992).

We succeeded to induce artificial spawning of *M. senhousia* and *L. fortunei kikuchii*, and to obtain fertilized eggs: four times for *M. senhousia*, on October 1 and 18, 1991, and on September 3 and 30, 1992, while three times for *L. fortunei kikuchii*, on September 1 and 11, and on December 8, 1992. As the results, a total of 58 specimens in larval and post-larval stages of *M. senhousia* were reared (37 specimens in D-shaped larvae, 14 in umbo larval stage, and 7 in post-larval stage), while 84 specimens of *L. fortunei kikuchii*

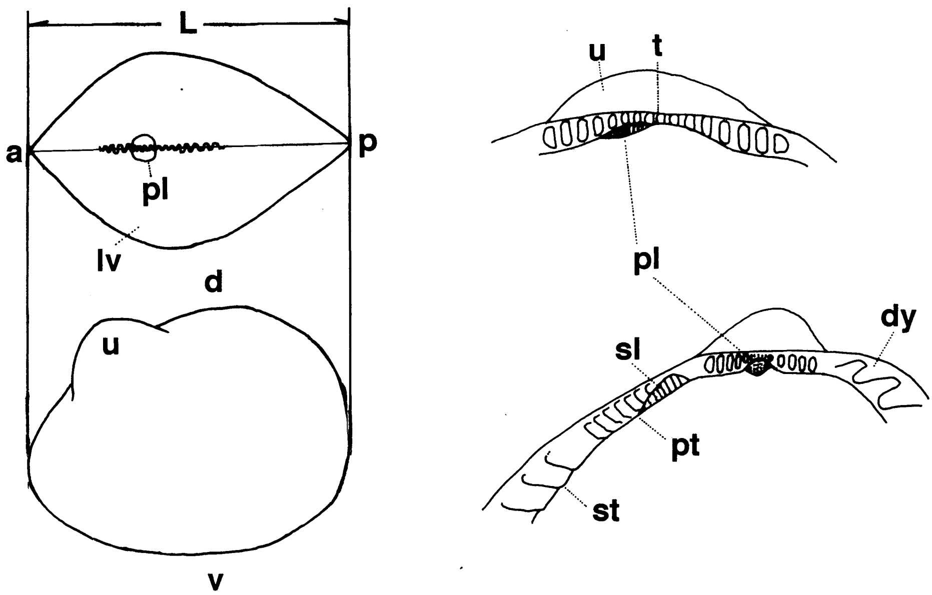


Fig. 1. Terminology for larval to post-larval shell and hinge in Mytilidae. イガイ科幼生の各部名称.
L: Shell length 殻長, **a**: anterior 前, **d**: dorsal 背方, **dy**: dysodont teeth 前側歯, **lv**: left valve 左殻, **p**: posterior 後, **pl**: primary ligament 第1靱帯, **pt**: primary lateral teeth 第1側歯, **sl**: secondary ligament 第2靱帯, **st**: secondary lateral teeth 第2側歯, **t**: teeth 交歯, **u**: umbo 殻頂, **v**: ventral 腹方.

(36 specimens in D-shaped larvae, 41 in umbo larval stage, and 7 in post-larval stage).

In the present paper, the terminology is defined as follows: planktonic larvae include both D-shaped and umbo stage larvae, D-shaped larvae is larvae before forming an umbo, umbo stage larva is larva after forming an umbo until the larvae settled onto the bottom, and settled bivalves are larvae in the post-larval stage after settled on the bottom.

Furthermore, terminology for hinge and teeth in larval and post-larval shells follows Fuller and Lutz (1989) (Fig. 1).

Results: Morphological features of the two mytilid species

Musculista senhousia (Benson)

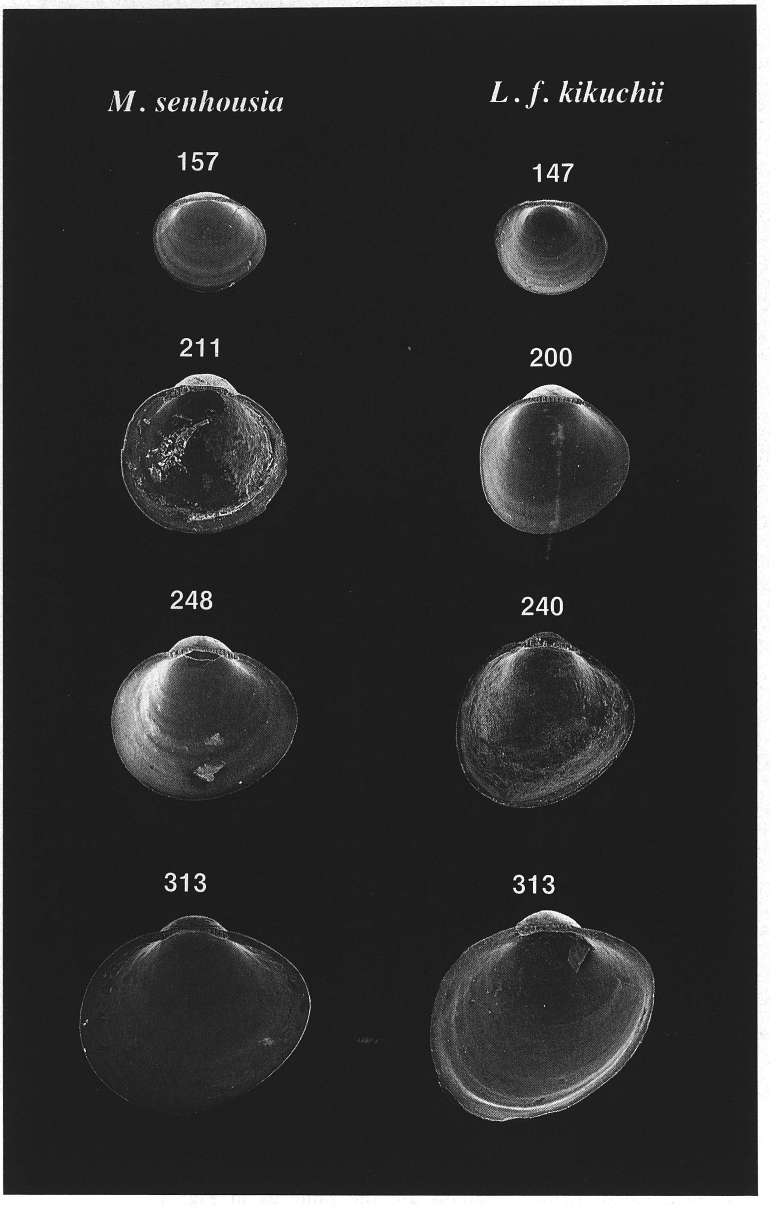
The number of teeth is 14-15 in D-shaped larvae with shell length of 70-120 μm, and each tooth has almost the same height (Figs. 2 and 4).

Larval shells with ca. 120 μm long have an umbo at the middle portion of the shell (Fig. 2). Umbo stage larvae with shell length of 120-300 μm are oval in shape with a round posterior and pointed anterior margins. The number of teeth in umbo stage larvae is 18-20 throughout the stage, and an uninterrupted series of teeth becomes smaller with growth in the middle portion than those at both ends (Figs. 2 and 4). Shells with length of ca. 200 μm develope a primary ligament pit below the teeth between post and median teeth, and this pit progressively encroaches onto the teeth with growth (Figs. 4 and 5). When shells of 260 μm and longer are observed from the dorsal view with an optical microscopy, the median teeth appear to be weak and indistinct, and a rounded primary ligament is visible between the post and median teeth (Fig. 6).

In the post-larval stage with length of 550 μm, shells increase their growth along the postero-ventral margin which is angular toward the posterior margin, developing an obsolete beginning of a secondary ligament pit behind the posterior teeth (Figs. 3 and 5). Shells of the post-larval stage have three types of lateral teeth, i.e., primary lateral teeth being visible on shells with length of 500 μm and the number increases with growth, secondary lateral teeth being visible on shells with length of 1500 μm and more, and dysodont teeth visible on shells with length of 2000 μm (Figs. 3 and 5).

Remarks: Of the *Musculista* species, larval morphologies have been known for *M. senhousia* and *M. perfragilis* (Tanaka, 1979b). General morphology of larval to post-larval stages of *M. senhousia* were already described (Yoshida, 1937; Tanaka, 1979a,b,c; Xu, 1983; Sakai and Sekiguchi, 1992). But the secondary ligament was not referred to in these past studies. Furthermore, the number of teeth in umbo stage larvae is 18–20 in the present study, while it was reported to be 22–23 in Sakai and Sekiguchi (1992).

Fig. 2. SEM pictures of the left valve shell of larvae of *M. senhousia* and *L. fortunei kikuchii*. ホトトギスガイとコウロエンカワヒバリガイのD型および殻頂期幼生の左殻の電子顕微鏡像. Numerical values: shell length (μm).



M. senhousia
L. f. kikuchii
157
147
211
200
248
240
313
313

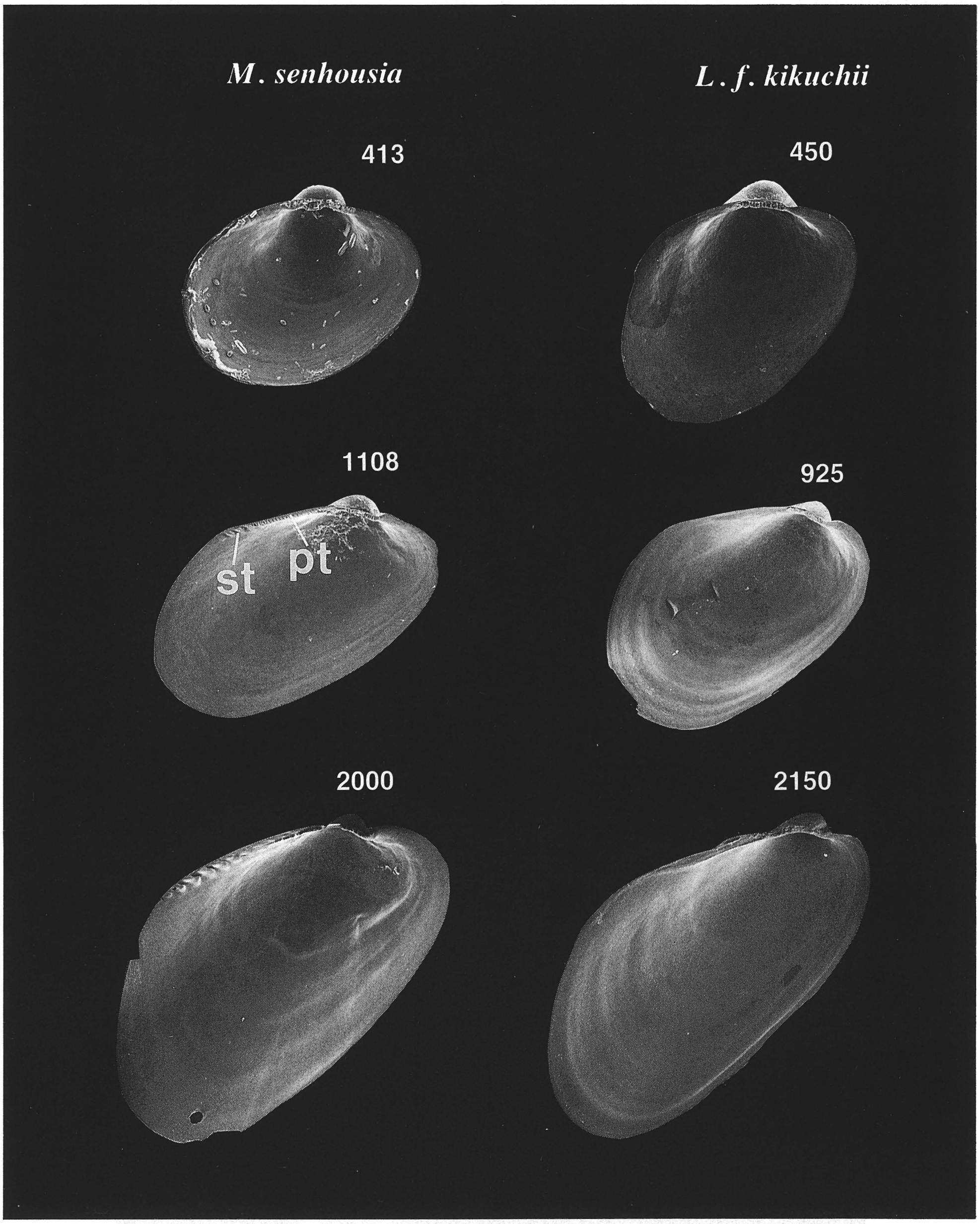


Fig. 3. SEM pictures of the left valve shell of post-larval stages of *M. senhousia* and *L. fortunei kikuchii*. ホトトギスガイとコウロエンカワヒバリガイの初期稚貝の左殻の電子顕微鏡像. Numerical values: shell length (μm). Symbols are the same as in Fig. 1.

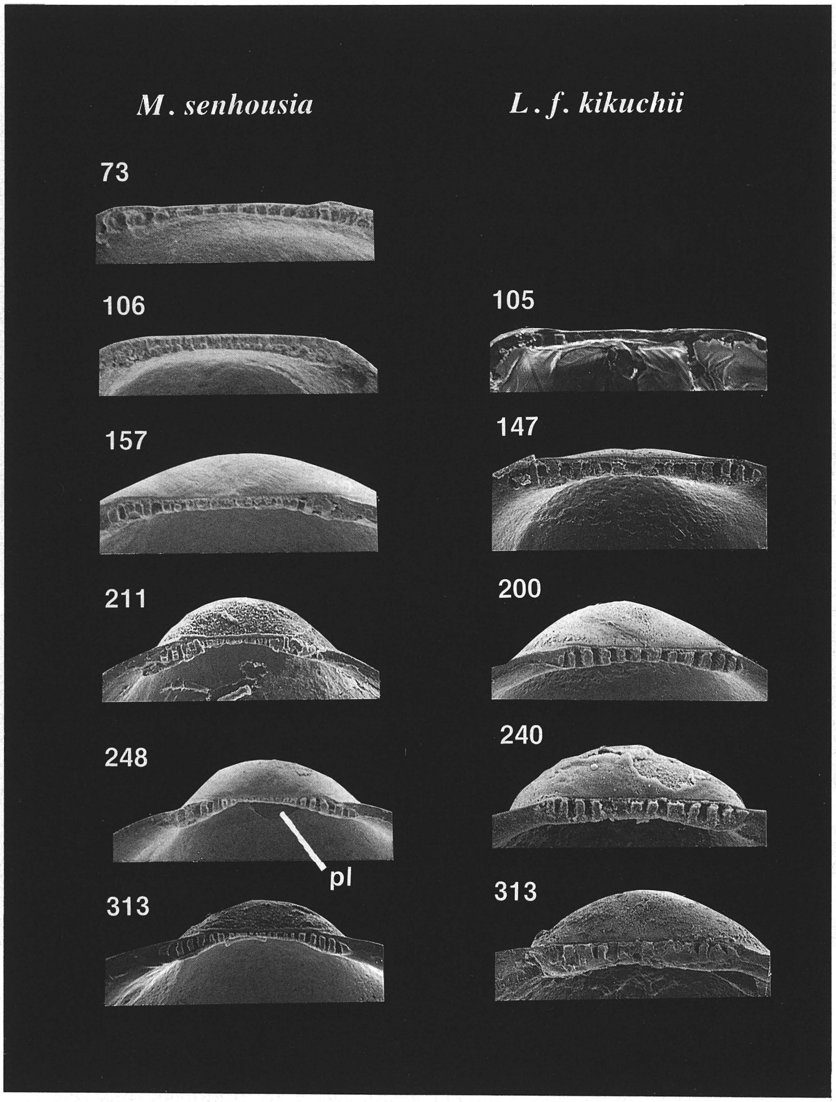


Fig. 4. SEM pictures of hinge of the left valve shell of larvae of *M. senhousia* and *L. fortunei kikuchii*. ホトトギスガイとコウロエンカワヒバリガイのD型および殻頂期幼生の左殻交装の電子顕微鏡像. Numerical values: shell length (μm). Symbols are the same as in Fig. 1.

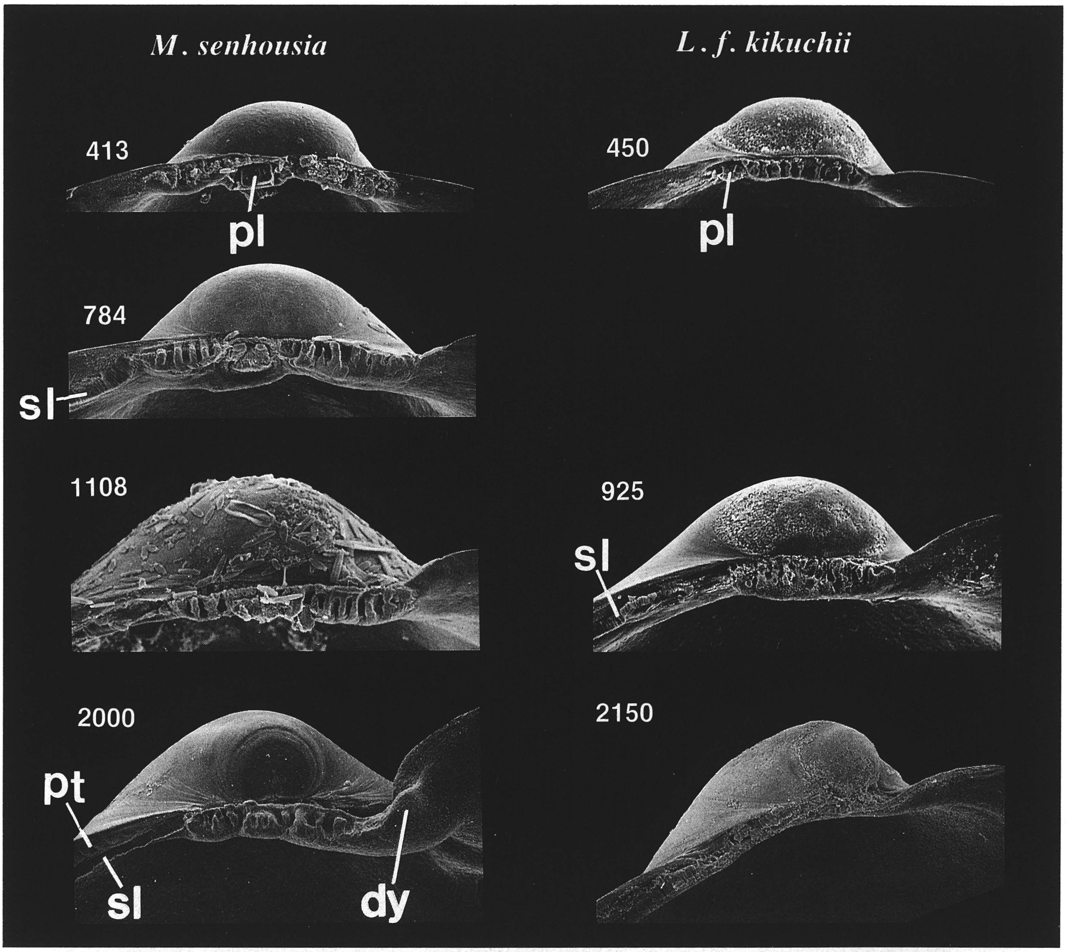


Fig. 5. SEM pictures of hinge of the left valve shell of post-larval stages of *M. senhousia* and *L. fortunei kikuchii*. ホトトギスガイとコウロエンカワヒバリガイの初期稚貝の左殻交装の電子顕微鏡像. Numerical values: shell length (μm). Symbols are the same as in Fig. 1.

Limnoperna fortunei kikuchii Habe

The number of teeth is 9–11 in D-shaped larvae with shell length of 80–134 μm, and each teeth has almost the same height (Fig. 4).

Larval shells with ca. 140 μm long have an umbo which inclined anteriorly (Fig. 2). Umbo stage larvae with shell length of 140–355 μm are triangular in shape with a pointed anterior margin (Fig. 2). The number of teeth in umbo stage larvae is 9–13 throughout the stage, and these teeth have almost the same height throughout the stage, though the teeth of shells of 350 μm and longer become (Figs. 4 and 5). Shells of ca. 250 μm long have a primary ligament pit behind the posterior teeth. When these shells are observed from a dorsal view with an optical microscopy, a rectangular primary ligament is visible,

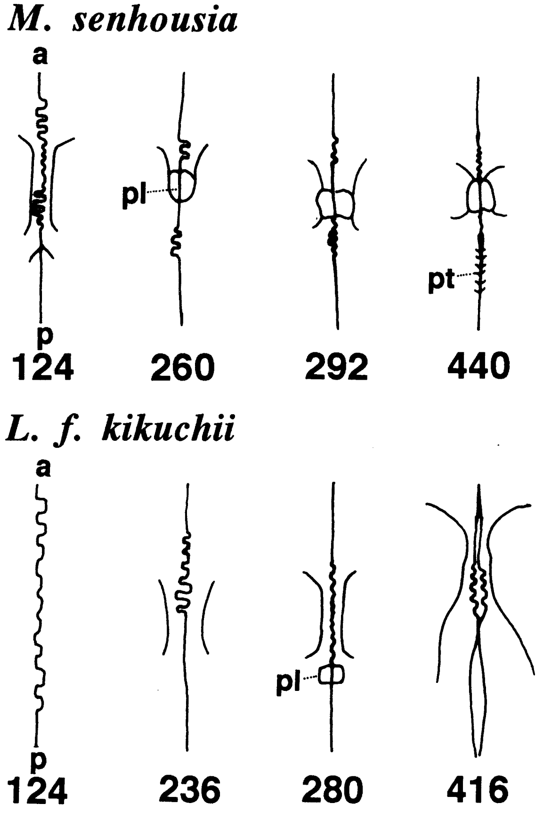


Fig. 6. Optical micrographs of hinge of larval to post-larval stages of *M. senhousia* and *L. fortunei kikuchii*. ホトトギスガイとコウロエンカワヒバリガイD型幼生から初期稚貝の交装の変化. Numerical values: shell length (μm). Symbols are the same as in Fig. 1.

though indistinctly, behind the posterior teeth (Fig. 6). Shells of ca. 350 μm and longer develop an obsolete beginning of a secondary ligament pit behind the posterior teeth, and the primary and secondary ligaments seem to be overlapped (Fig. 5).

In the post-larval stage, with 450 μm and longer shells increase their growth along the postero-ventral margin. Lateral teeth are not found even in shells reached 2000 μm and longer, while the teeth that were observed in the umbo stage larvae disappear (Figs. 3 and 5).

Remarks: Of the *Limnoperna* species, larval morphology has been known only on *L. fortunei kikuchii* (Xu, 1983). Morphological features of D-shaped larvae, hinges of umbo stage to post-larval stage, and the secondary ligament are here described for the first time by the present study.

Table 1. Summary of morphological features of larval and post-larval stages in *M. senhousia* and *L. fortunei kikuchii.*
ホトトギスガイとコウロエンカワヒバリガイのD型幼生から着底稚貝の形態比較.

D-shaped larvae

Species	Shell shape	Teeth	Lateral teeth	Primary ligament	Secondary ligament
M. senhousia	D-shaped	14–15, same height	lacked	lacked	lacked
L. f. kikuchii	D-shaped	9–11, same height	lacked	lacked	lacked

Umbo stage larvae

Species	Shell shape	Teeth	Lateral teeth	Primary ligament	Secondary ligament
M. senhousia	egg-shaped	18–20, lower height in median teeth	lacked	occurred between posterior and median teeth	lacked
L. f. kikuchii	triangular	9–13, same height	lacked	occurred behind posterior teeth	lacked

Post-larval stage

Species	Shell shape	Teeth	Lateral teeth	Primary ligament	Secondary ligament
M. senhousia	elongated triangular	9–13, encroached median teeth	occurred, 3 types	occurred between posterior and median teeth	occurred behind posterior teeth
L. f. kikuchii	elongated triangular	encroached and then disappeared	lacked	occurred behind posterior teeth	occurred behind posterior teeth

Discussion

Based on detailed examination on morphological features of D-shaped and umbo stage larvae, settled bivalves and early juveniles in the two mytilid species, *M. senhousia* and *L. fortunei kikuchii*, these two species are clearly distinguishable from each other using several morphological characteristics (Table 1).

In D-shaped larvae, shell length of *L. fortunei kikuchii* tends to be larger than that of *M. senhousia*, while the number of teeth of the latter species was more greater than that of the former. D-shaped larvae of the two mytilid species are thus distinguished from each other.

In umbo stage larvae, shells of *M. senhousia* are more round than those of *L. fortunei kikuchii*, and the latter species has well-developed anterior, central and posterior teeth while the former has only fine central teeth. In *M. senhousia*, the primary ligament is formed between the post and median teeth, while it is formed behind the posterior teeth in *L. fortunei kikuchii*. Umbo stage larvae of these two species are clearly distinguishable from each other.

In settled and juvenile bivalve stages, shells of *M. senhousia* have three types of lateral teeth, while *L. fortunei kikuchii* lacks those teeth.

Furthermore, in umbo stage larvae to juveniles, the umbo in shells of *L. fortunei kikuchii* more inclines anterior by as compared with *M. senhousia*.

In Japan, except for *M. senhousia* and *L. fortunei kikuchii* in the present study, all larval stages of mytilid species have not been described. Lutz and Hidu (1979), Siddall (1980) and Fuller and Lutz (1989) described 9 species of mytilids from D-shaped larval to post-larval stages using SEM. Larval and post-larval stages of these 9 species are clearly distinguished from those of *M. senhousia* and *L. fortunei kikuchii* using shell and hinge characteristics.

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要　約

ホトトギスガイ *Musculista senhousia* とコウロエンカワヒバリガイ *Limnoperna fortunei kikuchii* は，静岡県西部に位置する浜名湖の奥部の潮間帯に優占するイガイ類である。筆者らはこれらの幼生を室内飼育し，得られた試料をもとに2種のD型幼生から初期稚貝までの外部形態および交装を比較した。試料は SEM と光学顕微鏡を用いて観察した。その結果，D型幼生，殻頂期幼生および初期稚貝のすべての成長段階で2種の間には，形態に相違が認められた。D型幼生ではコウロエンカワヒバリガイの方がホトトギスガイよりも殻長が大きい傾向があったが，計測値は重複しているので，D型幼生の種を殻長のみから同定することは困難である。しかし，D型幼生の交歯は，ホトトギスガイが14 - 15個であるのに対し，コウロエンカワヒバリガイでは9 - 11個と差異がみられた。殻頂期幼生では，ホトトギスガイの中央の交歯は小さくなり，第1靭帯が交歯中央やや後方に形成される。殻の輪郭は卵型で，殻頂は中央に位置する。これに対し，コウロエンカワヒバリガイでは，殻頂期幼生の交歯は

同大であり，第１靱帯は交歯後端に形成される。殻の輪郭はほぼ三角形で，殻頂は前方に偏る。初期稚貝では，ホトトギスガイは3種類の側歯を持つのに対し，コウロエンカワヒバリガイは側歯類を欠く。殻頂の位置は，コウロエンカワヒバリガイの方がホトトギスガイよりも前方に偏る。

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