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Colobocentrotus mertensii: Emergence of the Peculiar Form of Spines

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Source: Zoological Science, 21(3): 265-274
Published By: Zoological Society of Japan
URL: https://doi.org/10.2108/zsj.21.265
Larval and Juvenile Development of the Echinometrid Sea Urchin *Colobocentrotus mertensii*: Emergence of the Peculiar Form of Spines

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ABSTRACT—The development of *Colobocentrotus mertensii* from embryos to larvae and early juveniles was observed to give the first detailed description of larval and juvenile formation and skeletal structures in echinometrid sea urchins. The first larval spicules appeared at the mesenchyme blastula stage, whereas, in many echinoids, spicules were formed after gastrulation. From late eight-armed larva to juvenile, body color of *C. mertensii* was deep red, which has never been described for any echinoid before.

The adult form of *C. mertensii* is characteristic in that the spines at the aboral side are short, truncated and pavement-like. The first sign of peculiar adult features could be seen in the juvenile spines and adult spines, which are broader than those of closely related *Anthocidaris crassispina*. The primary podia emerged on the left side of larval body were more stout and thicker in *C. mertensii* than in *A. crassispina*. The present study shows that developmental process of larval structure of *C. mertensii* is in general similar to the *A. crassispina* and the differences is first seen in juvenile structure including the distribution of pigment spots and morphology of adult spine.

Key words: echinometrid, larva, juvenile, spicule, spine

INTRODUCTION

Living echinoids are subdivided into two groups, regular and irregular (Hyman, 1955). *Colobocentrotus mertensii*, an endemic Japanese sea urchin species, is closely related to *Anthocidaris crassispina*, occurs in the warm current from the Miyake Island in Izu, Ogasawara Islands and the Kii Peninsula to Ryūkyū Islands (Shigei, 1974), and is classified as a regular urchin. The test of regular urchins is characterized by its globose shape and long needle-like spines (Hyman, 1955). However, *C. mertensii* is different from other regular sea urchins in that it has a hat-shaped test. More-
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over, the spines of the aboral side is very short, truncate, and form pavement-like polygonal plates covering the whole aboral side. The marginal spines are broad, flat and chisel-shaped (Fig. 1A). On the oral side, the tube-feet are well developed, and covered by short and flat spines. Thus *C. mertensii* has an unusual morphology and peculiar spines in adulthood, but it is classified in the family of Echinometridae (Shigei, 1986). In the echinometrid sea urchins, spines of adults are usually needle-like, such as seen in *A. crassispina*, while those of *C. mertensii* are flat in shape.

The aim of the present study is to observe the development of *C. mertensii* to confirm that its morphology in the early stages is similar to those of other members of the family Echinometridae. Here we report that the difference in morphology of *C. mertensii* and *A. crassispina* is first seen in larval and juvenile stages.

As for the development of sea urchins of genus *Colobocentrotus*, Mortensen (1921) reported the development of early stages up to four-armed pluteus of *C. atratus* in Hawaii. There has been, however, no available information about *C. mertensii*. In the case of *A. crassispina*, Onoda (1931) described its development from early stages up to juvenile.

The observations made in the present study demonstrated that the peculiar form of adult spine in *C. mertensii* emerges first in juvenile stages and that the comparison of its developmental morphology with those of other Echinometridae members clearly places *C. mertensii* among Echinometridae.

**MATERIAL AND METHOD**

Adult specimens of *C. mertensii* were collected from Chichijima Island, the Ogasawara (Bonin) Islands. *A. crassispina* and *Pseudocentrotus depressus* from Bousou Peninsula. Urchins were induced to spawn by intracoelomic injection of 0.5 to 1.0 ml of 0.5 M KCl. The natural sea water was used for culture after filtration and sterilization at 80°C for 20 min. Eggs were washed three times in sterilized sea water and fertilized with a diluted sperm solution. Larvae were cultured with constant stirring of sea water by a paddle at a speed of 30 r.p.m in a 3L glass beaker. They were kept at 27°C for *C. mertensii* and 23°C for *A. crassispina*. After larvae reached early four-armed stage, they were fed diatom *Chaetoceros gracilis* cultivated in the laboratory with medium KW-21, every day or every other day. In this paper, Mortensen’s (1921) terminology was used for larval arms and spicules. Observations of metamorphosis were made on late larvae with well-developed primary podia and juvenile spines. Metamorphosing larvae were put into glass bowls with approximately 150 ml of filtered sea water. Each bowl contained a small rock (4–5 cm³) which had been collected from adult habitats and held in the laboratory sea water table prior to use.

**Preparation of the skeletal specimens**

To make the preparations for drawing or photography, larvae were fixed on the depression slide with a drop of 5% formaline solution in the sea water. Preparation of the larval skeleton and the corona of juvenile was done according to Goldern (1926). The materials fixed in 70% ethanol or living larvae in sea water were washed in the depression slides with distilled water to remove alcohol or salts. Next, the muscular parts of the materials were macerated with 1M KOH and the specimens were rinsed thoroughly in distilled water to remove KOH. They were then washed three times with distilled water and, in order to make the specimens transparent, put in a 50% glycerine in water. Finally, a drop of pure glycerine.
was put on the skeletal specimens and slides were covered with a cover glass. Two polarizing plates were used to distinguish overlapped skeletons separately by their respective birefringence. Drawing were made with Nikon's drawing apparatus.

RESULTS

The average developmental time course of *C. mertensii* reared at 27°C is summarized in Table 1. In general, the variation in developmental stages among cultures increased with time, although larvae appeared healthy throughout the culture period. For example, after 37 days, when the first larvae metamorphosed completely into juveniles, others had only partially developed juvenile structures while still others showed no external sign of juvenile rudiment development.

**Early larval development**

The mature egg of *C. mertensii* was 69 μm in diameter and slightly yellowish in color and quite opaque. Early cleavage and development followed the pattern similar to those reported for *A. crassispina* (Onoda, 1931). It is to be noted that the appearance of first spicules of *C. mertensii* and *A. crassispina* was earlier than that of non-Echinometridae sea urchin eggs.

![Table 1. Summary of developmental stages of *Colobocentrotus mertensii*.](Downloaded From: https://bioone.org/journals/Zoological-Science on 25 Jul 2019 Terms of Use: https://bioone.org/terms-of-use)

**Table 1. Summary of developmental stages of *Colobocentrotus mertensii*.**

<table>
<thead>
<tr>
<th>Time after fertilization</th>
<th>Developmental stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.75 hr</td>
<td>Eight cell embryo.</td>
</tr>
<tr>
<td>3.5 hr</td>
<td>Sixteen cell embryo.</td>
</tr>
<tr>
<td>12 hr</td>
<td>Mesenchyme blastula. Triradiate spicules evident.</td>
</tr>
<tr>
<td>13.5 hr</td>
<td>Gastrula. Archenteron began to invaginate into blastocoel.</td>
</tr>
<tr>
<td>17 hr</td>
<td>Late gastrula. The archenteron touched the prospective oral part of the ectoderm.</td>
</tr>
<tr>
<td>28 hr</td>
<td>Early two-armed larva. A pair of coelomic vesicle developed on the top of archenteron.</td>
</tr>
<tr>
<td>32 hr</td>
<td>Two-armed larva. Fenestrated postoral arms were formed and red pigment spots were scattered through the larval arms.</td>
</tr>
<tr>
<td>3 d</td>
<td>Early four-armed larva. After anterolateral arms well developed, larva was fed on <em>C. gracilis</em>.</td>
</tr>
<tr>
<td>7 d</td>
<td>Late four-armed larva. Postoral arms well developed, 630 μm long (measured from posterior curve of the larval body to arm tip).</td>
</tr>
<tr>
<td>16 d</td>
<td>Posterodorsal arms were about one-third shorter than postoral arms. Buds of preoral arms present.</td>
</tr>
<tr>
<td>23 d</td>
<td>Echinus rudiment began to form. Preoral arm developed from dorsal arch. Posterior transverse spicule present.</td>
</tr>
<tr>
<td>32 d</td>
<td>Three ciliated bands developed. Postoral arms approx. 800 μm long. The more advanced larva had five primary podia and three pedicellariae and juvenile spines.</td>
</tr>
<tr>
<td>35 d</td>
<td>The most advanced larva showed reduction in arm length.</td>
</tr>
<tr>
<td>37 d</td>
<td>First larva metamorphosed and the juvenile became deep red. Juvenile test diameter approx. 770 μm including the spine.</td>
</tr>
</tbody>
</table>

Larvae were reared at 27°C.

![Fig. 2. Birefringence of spicules of mesenchyme blastula stage embryo of *Colobocentrotus mertensii* (A) and mid gastrula stage embryo of *Pseudocentrotus depressus* (B) photographed with polarization microscope. Note that first pair of triradiate spicules appears much earlier in *C. mertensii* than in *P. depressus*. Scale bars in A and B, 50 μm.](Downloaded From: https://bioone.org/journals/Zoological-Science on 25 Jul 2019 Terms of Use: https://bioone.org/terms-of-use)
urchin species. In *C. mertensii* triradiate spicules (trs) were formed in mesenchyme blastula stage before gastrulation (Fig. 2A) while in many sea urchin species, spicules were formed at mid gastrula stage (Fig. 2B, *Pseudocentrotus depressus*).

At 42 hr, in addition to two fenestrated postoral arms (poa), two anterolateral arms (ala) having simple rod appeared, forming the four-armed echinopluteus. The preoral ciliated band (pr.ci.b) developed in front of the mouth. Red pigment spots (pig) which were initially present throughout the larval arms became increasingly concentrated at the tips after this stage. The four-armed stage persisted through the 7th day after fertilization (Fig. 3A).

Seven to 9 days after fertilization, bases of postoral arms bulged and a triradiate spicule, a rudiment of postero-dorsal rod, was formed in each bulge. Soon later, the 3rd triradiate spicule, a rudiment of dorsal arch, was formed. The fenestrated postero-dorsal arms (pda) characteristic of the six-armed stage appeared 12 days after fertilization and fully developed by the 16th day (Fig. 3B).

**Late larval development**

Between the 18th and 20th day, the posterior area of the larval body gradually became flat and dorsal arch (da) elongated anteriorly and formed two preoral arms (proa) indicating the initiation of the eight-armed stage. With further development, a pair of anterior ciliated band (a.ci.b) (vibratile type) grew on the dorsal and ventral surface (Fig. 4A). During the late six-armed stage, the left and right coelomic vesicles were divided into anterior and posterior parts. Left anterior coelomic vesicle formed a hydrocoel (hy), a rudiment of adult water-vascular system (MacBride, 1903, 1914; Fukushi, 1960), and an axocoel (ax). Twenty three days after fertilization, the ectodermal epithelium between the left posterodorsal arm (l.pda) and the left postoral arm (l.poa), directly above the hydrocoel (hy), began to invaginate, which then became the vestibule (v) (Fig. 4A). Combination of the hydrocoel (hy) and the vestibule (v) made up the echinus rudiment (Fig. 5A). The floor of the vestibule bulged out forming five primary podia (p.p) and echinus rudiment continued to increase in size and came to dominate the left side of the larva (Fig. 5B).

The late eight-armed larva of *C. mertensii* looked ruddy and could be recognized by the naked eye. Three ciliated bands developed on the body: the preoral ciliated band (pr.ci.b) and anterior ciliated band (a.ci.b), were formed on the ventral and dorsal surfaces of the larval body, and the posterior ciliated band (p.ci.b) on the right and left sides of the posterior end of the body encircling the posterior area of the larva (Fig. 5B). On the right side of the larva were three pedicellariae (ped). The first pedicellaria was formed in the middle of the posterior part of the larva. The others were formed at the base of the right posterodorsal arm (pda) and postoral arm (poa) (see below).

In the later larval developmental stage, there were no particular differences between morphology of *C. mertensii* and *A. crassispina* except for the distribution of the pigment spots. The larval body of *C. mertensii* was vividly colored.

**Fig. 3.** Early larval stages of *Colobocentrotus mertensii*. A. Seven-day-old four-armed larva with well developed postoral arm and anterolateral arm. Brownish red pigment spots are observed at the tip of the larval arms. Dorsal view. B. Sixteen-day-old late six-armed larva with two pairs of posterodorsal arms and well-developed preoral ciliated band. Ventral view. Scale bars, 100 µm.
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with red pigment spots, especially at the tip of the arms (Fig. 4A). In *A. crassispina* (Fig. 4B), pigment spots were dispersed along the arms like other echinometrid larvae (Onoda, 1936).

**Metamorphosis**

When metamorphosis began, the anterolateral, posterodorsal and preoral arms were absorbed together with the skeletons and epidermis, first from left side of the larva (Fig. 6A). On the other hand, the spines covered with epithelium distended the vestibular wall and brisk primary podia (p.p) and spines (sp) protruded through the opening of the vestibular pore. Then, the primary podia and spines completely emerged to the exterior of the body. Thereafter,
the newly metamorphosed juvenile turned left side down so that the echinus rudiment became the lower surface and the opposite part, including the right side of postoral (r.poa) and posterodorsal arms (r.pda), three pedicellariae (ped), and four juvenile spines (g.jp), became the upper surface of larval body (Fig. 6B). Metamorphosis took about one hour for
complete emergence of the primary podia and spines. Thus, the process of metamorphosis of *C. mertensii* was almost the same as that of *A. crassispina* (Onoda, 1931). One of the morphological features of *C. mertensii* and *A. crassispina* was found in the mode of the absorption of the arms. In these species, the epidermis and skeletons were absorbed simultaneously, whereas in some species, it has been claimed that absorption occurs only in the epidermis resulting in the naked skeletons (Emlet, 1988; Gosselin, 1998).

**Juvenile structures**

The form of the juvenile was penta-radial as the adult. Three pedicellariae (ped) and the juvenile spines (g.jp) were formed on the aboral surface of the juvenile, while the ciliated bands were absorbed (Fig. 6C). Juvenile morphology of *C. mertensii* began to deviate markedly from that of juveniles of other echinometrids. The juvenile of *C. mertensii* was dotted with a lot of pigment spots and became deep-red in color (Fig. 1B) while that of *A. crassispina* was light brown. The aboral region was circular and five ambulacral areas (am.a) and interambulacral areas (i.am.a) were arranged alternately. In each ambulacral area, there were one central primary podium (p.p) and two broad juvenile spines (j.sp) in aboral position (Fig. 6C). Each podium was extensible and had a terminal disc of about 77 µm in diameter which was more stout and thicker than that of *A. crassispina* which had a diameter of about 62 µm with more slender disc (Fig. 6D). In the interambulacral areas were a group of four adult spines (a.sp) that began to demonstrate their adult features. The adult spines (Fig. 6C) were broader than those of *A. crassispina* (Fig. 6D). Besides, in *C. mertensii*, one adult spine (a.sp) (shown by the arrows in Figs. 6C and 8B) was smaller than other three spines, while in *A. crassispina*, four adult spines (a.sp) (Fig. 6D) were the same size.

**Features of larval and juvenile skeletal structures**

Since the formation and structure of skeletal system of larva and juvenile are important for the classification of sea urchins, we made close examination on the skeletal system of *C. mertensii* in comparison with that of *A. crassispina*.

The early larval skeleton is formed by the elongation of triradiate spicules (Okazaki, 1975). In the posterior part of the larval body develops a compound basket structure. At the later developmental stage, a new unpaired posterior transverse spicule was formed where the basket structure had disintegrated. During the mid eight-armed larva stage, posterior transverse rod (ptr) (Fig. 7A) became well developed and on each side there were two branches: one was directing upward obliquely and the other was long and directing downward. In *C. mertensii* (Fig. 7A) the lower branches were smooth, whereas in *A. crassispina* (Fig. 7B) they were furnished with a series of 5-6 thorns along their lower edge. The fenestrated postoral rod (por) and postero-dorsal rods (pdr) of *C. mertensii* had only small numbers of smooth thorns (Fig. 7A) but in *A. crassispina* they had many thorns (Fig. 7B).

The base of each postoral rod (por) expanded into a dense network (stereom) and formed a highly fenestrated plate (f.p) (Fig. 8A). A slightly smaller fenestrated plate

![Fig. 7. Skeletal specimen of mid eight-armed larvae. (A). Twenty-five-day-old larva of *Colobocentrotus mertensii* (A) and twenty-seven-day-old larva of *Anthocidaris crassispina* (B). A shows that two pairs of fenestrated skeletal rods and bifurcated unpaired posterior transverse rod. Ventral view. B shows that posterior transverse rod (ptr) with two pairs of small posterior processes and lower branches were furnished with a series of 5-6 thorns along their lower edge. Three triradiate spicules, presumptive jaws of the pedicellaria, are visible in the posterior region. Ventral view. Note that skeletal rods possess smooth thorns (arrow) in *C. mertensii*, but many sharp thorns (arrow) in *A. crassispina*. Scale bars, 100 µm.](https://bioone.org/journals/Zoological-Science/271.pdf)
Fig. 8. Skeletal specimen of late eight-armed larva and juvenile of Colobocentrotus mertensii. A. Thirty-five-day-old larva, showing five genital plates (G-1~G-5) and two terminal plates (T-4, T-5). G-1 develops from new triradiate spicules and other plates from the larval skeletal elements. Terminal plates are formed at the base of left posterodorsal and postoral rod. Dorsal view. B. A juvenile two days after metamorphosis. The aboral surface of juvenile is occupied with five genital plates (G-1~G-5) and an anal plate (ap). Four broad adult spines in interambulacral area form a lozenge. Arrows indicate one of the four adult spines that is smaller in size than others. Juvenile spines are formed in ambulacral area. Dorsal View. C. A set of the adult spines in an interambulacral area. One adult spine located in the dorsal side is smaller in size than other three spines. Scale bars, 100 µm.
developed at the base of each posterodorsal rod (pdr). Together, the fenestrated plates originating from the postoral rods (por) and the posterodorsal rods (pdr) formed a truncated pyramidal structure. At the posterior end of the larva, first pedicellaria (1st.ped) (Figs. 5B and 8A) and a pair of juvenile spines (g.jp) (Fig. 8A) were formed in association with the posterior transverse rod. The second pedicellaria (2nd.ped) and juvenile spine (g.jp) (Fig. 8A) were formed at the base of the right posterodorsal rod (r.pdr), and the third pedicellaria (3rd.ped) (Figs. 5B and 8A) and juveniles spine (g.jp) (Fig. 8A) were formed at the base of the right postoral rod (r.por). Later on, these skeletal networks were integrated into the test of the juvenile where they formed the G-3 and G-5 genital plates (Fig. 8B). In the same way, the future G-2 genital plate developed at the basal part of the dorsal arch (da). Finally, the G-4 plate developed at the base of posterior transverse rod and the G-1 plate in the center of the right lateral field (Fig. 8A). The terminal plates (T-4, T-5) were formed at the base of the skeletal rods sustaining, respectively, the left postoral (l.pdr) and postoral arms (l.por).

The other major parts of juvenile appendages, such as terminal plates (T-1, T-2, T-3), coronal plates, spines, primary podia and the five teeth of Aristotle's lantern, developed in the echinus rudiment.

During the metamorphosis, the coronal plates, ambulacral plate and interambulacral plate came to contact and formed the outline of a rigid test. In a 2-day juvenile urchin, the aboral area included five genital plates (G-1~G-5) and two types of juvenile and adult spines. These plates (G-1~G-5) were attached to one another and touched the corona valves. (T-4, T-5) were formed at the base of the skeletal rods sustaining, respectively, the left postoral (l.pdr) and postoral arms (l.por).

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The family Echinometridae includes six species according to the classification by Shigei (1986) and commonly possesses the following morphological characters. (1) The valves of the globiferous pedicellariae have one unpaired lateral tooth. (2) The larval skeleton shows a compound basket structure. (3) The optical crystal axis of calcite of coronal plate is perpendicular to the plate of test (Shigei, 1974). However, the shape of spines demonstrates variety among them. A. crassispina, Echinometra mathaei, Echinostrephus acicularis and Echinostrephus molaris have needle-like spines, whereas other two species, C. mertensii and Heterocentrotus mammillatus, have peculiar spines. In H. mammillatus, some spines on the aboral side are long, thick and very strongly developed like a pencil, while others are short and flat-topped forming mosaic over the surface (Hyman, 1955; Matsuoka, 1989). In C. mertensii, spines on the aboral side are short and flat-topped and look like pavement stones. Some spines around the edge of the test are broad, flat and chisel-shaped (Fig. 1A). These differences in adult forms of Echinometrid species raise the question whether they are truly in the same family.

The developmental process of larval structure of C. mertensii is very similar to that of A. crassispina. Additionally, we compared the morphological features in three species of echinometrid larvae and found that the larval structure of C. mertensii resembles those of other echinometrid sea urchins. Especially, it is to be noted that triradiate spicules, the first sign of larval skeleton, were formed at the mesenchyme blastula stage in C. mertensii (Fig. 2A) and other Echinometrid urchins including A. crassispina. In many other species, i.e., Echinostrephus moralis, Strongylocentrotus pulcherrimus, Toxopneustes pileolus and Pseudocentro-
tus depressus (Onoda, 1936, Fig. 2B), the triradiate spicules appeared after gastrulation. Thus, from the developmental viewpoints, larvae of Echinometridae family members share the common fundamental characteristics.

In newly metamorphosed juvenile of C. mertensii (Fig. 6B) and A. crassispina, larval arms were absorbed together with the skeletons and epidermis. On the contrary, in Eucidaris thouarsi (Emlet, 1988) and Paracentrotus lividus (Gosse

In conclusion, this study demonstrates that the development of larval structure of C. mertensii may be due to the species difference.

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(Received September 3, 2003 / Accepted November 7, 2003)

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(Received September 3, 2003 / Accepted November 7, 2003)