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# Development of the Brittle Star, *Ophioplocus japonicus* H.L. Clark. I

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**ABSTRACT**—The entire process of development from eggs to juveniles in the brittle star, *Ophioplocus japonicus*, is described with special attention to external morphology. The breeding of this brittle star in Toyama Bay, Sea of Japan, occurs in August. The eggs are spherical, orange, and 300 $\mu$m in average diameter. Cleavage is total and radial. Gastrulation takes place by invagination. Larvae possessing tube-foot buds and ophiuroid rudiments on the posterior part of their bodies, develop into the vitellaria stage. Vitellariae of the present species have transverse ciliary bands, but no larval skeletons. Six days after fertilization at 25°C, metamorphosis is completed and the resulting juvenile is pentagonal in outline and about 400 $\mu$m in diameter. The present study shows that ciliary bands are formed in an earlier larval stage than was reported previously.

## INTRODUCTION

A considerable number of feeding (planktotrophic) larvae, the ophiopluteus, has been reported in Ophiuroidea [5]. Development of ophiopluteus larvae is generally called an indirect or planktotrophic type [5, 9]. However, ophiuroids with yolky eggs have diverse developmental courses as will be shown below. The developmental pattern was classified into 7 types based on the breeding and brooding styles, the nature of the larvae, and other features [20]. On the other hand, Hendler [5] designated 3 types of development (planktotrophic, direct and abbreviated) based on the egg size, life span, larval size and some other factors. From his view, development of the vitellaria belongs to the abbreviated type. However, the abbreviated type has some heterogeneous larval forms, whereas the planktotrophic and direct types are distinctive. Thus, agreement has not yet been achieved on what are the developmental types and on their nomenclature in Ophiuroidea. This is partly because of the comparative paucity of knowledge available on the development passing through other than typical ophiopluteus larval stages.

At present, 8 species are known to possess vitellaria larvae. They are *Ophiocoma pumila, Ophioderma brevispina, O. cinereum, O. longicauda, Ophiolepis cincta, O. elegans, Ophionereis annulata* and *O. squamulosa* [1, 3, 5, 7, 8, 12, 14, 15, 19]. To date, however, detailed studies on development through vitellaria have been done only in 2 species; *O. brevispina* [1] and *O. squamulosa* [14]. In addition, descriptions on the gastrulation, including formation of the archenteron, are very limited. Indeed, no regular and convenient method, such as KCl and 1-methyladenine which induces spawning in echinoids and asteroids, respectively, has been reported to induce spawning in ophiuroids. However, as the result of several chances of spawning the authors have gotten embryos of this species. The present paper describes the larval development of the brittle star, *Ophioplocus japonicus* through metamorphosis.

## MATERIALS AND METHODS

### Specimen collection

*Ophioplocus japonicus* H. L. Clark have their habitat under stones on a sandy bottom. Specimens were collected from the seashores of Uozu,

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Toyama Prefecture, of Tsukumo Bay, Ishikawa Prefecture, and of Sado Island, Niigata Prefecture, all of them facing the Sea of Japan, for nearly every summer between 1980 and 1990. This brittle star is gonochoric. In both sexes, the gonad was arranged in pairs in each interradius. In the breeding season, the body cavity is almost completely occupied with gonads. Ovaries in this season are orange or brown in color and the testes are milky white.

*Breeding season*

In August 15, 1980, natural spawning occurred in a glass container with small amounts of seawater in which about 20 individuals were kept. They had been en route to a laboratory at Toyama University after they had been collected at Uozu. Natural spawning also occurred in a tank with running seawater at Noto Marine Laboratory, Ishikawa Prefecture, on August 13, 1985, and at Sado Marine Laboratory, Niigata Prefecture, on August 9, 1990, a few days after collection. Specimens, which were held in a glass container containing as much seawater as needed to immerse the dorsal surface of their disk, spawned spontaneously at Noto Marine Laboratory, on August 25, 1983, August 21, 1984, August 6 and 9, 1986, and July 31, 1991. These ova were fertilized using a dilute suspension of spermatozoa from the dissected testes of homologous male specimens.

*Culture of embryos and larvae*

Temperature in the laboratory culture was approximately 25°C, which is close to the water temperature at the natural habitat in August. The embryos and larvae were taken from the culture dish at appropriate intervals and observed under dissecting and light microscopes. They were measured live with an ocular micrometer. The skeletal system, from fresh squash preparations, was examined under standard and Nomarski differential interference microscopes, and from samples preserved by the following method. Embryos and larvae were fixed in 70% alcohol and then macerated in a 10% aqueous solution of potassium hydroxide.

For histological observation, the embryos and larvae were fixed with Bouin's solution. The fixed material was embedded in paraffin, serially sectioned at a thickness of 6 $\mu$m, and stained with Delafield's hematoxylin and eosin. Embryos and larvae were fixed for scanning electron microscopy in 2% $OsO_4$ in 50 mM Na-cacodylate buffer (pH 7.4); the osmolarity of the fixative was adjusted by the addition of sucrose (final conc. 0.6 M). The fixed materials were dehydrated in an ethanol series and dried with a critical point dryer (Hitachi, HCP-2). They were observed with a scanning microscope (Hitachi, S-510) after being coated with gold-palladium (Hitachi, E101 Ion Sputter).

## RESULTS

*Annual reproductive cycle*

In order to study the annual reproductive cycle, specimens were collected at a fixed locality of the coast of Himi along Toyama Bay, Toyama Prefecture. Sampling of the animals was made once a month during the period from March, 1979 through October, 1981. At each collection, 5 individuals of both sexes and those which were more than 10 mm in disk diameter were selected. Soon after collection, the disk was cut off from the arms and the fresh weight was determined. Gonads were then dissected out and weighed. The gonad index for the gross estimation of the annual reproductive cycle was calculated by the following formula:

$$\text{Gonad Index} = (\text{Fresh Weight of Gonads} / \text{Fresh Weight of Total Disk}) \times 100$$

The annual reproductive cycle was determined in the population of *O. japonicus* at Himi as shown in Figure 1. Although there were some differences throughout the years, the gonad indices of males and females were highest in June, 1981 and July, 1980, while they were lower in August of these 2 years. After September, the value for both sexes increased gradually. According to the transition of the gonad index, and the natural and inductive spawnings, the spawning of this species is presumed to occur in August.

*Embryos of cleavage stage*

In the present study, the entire process of de-

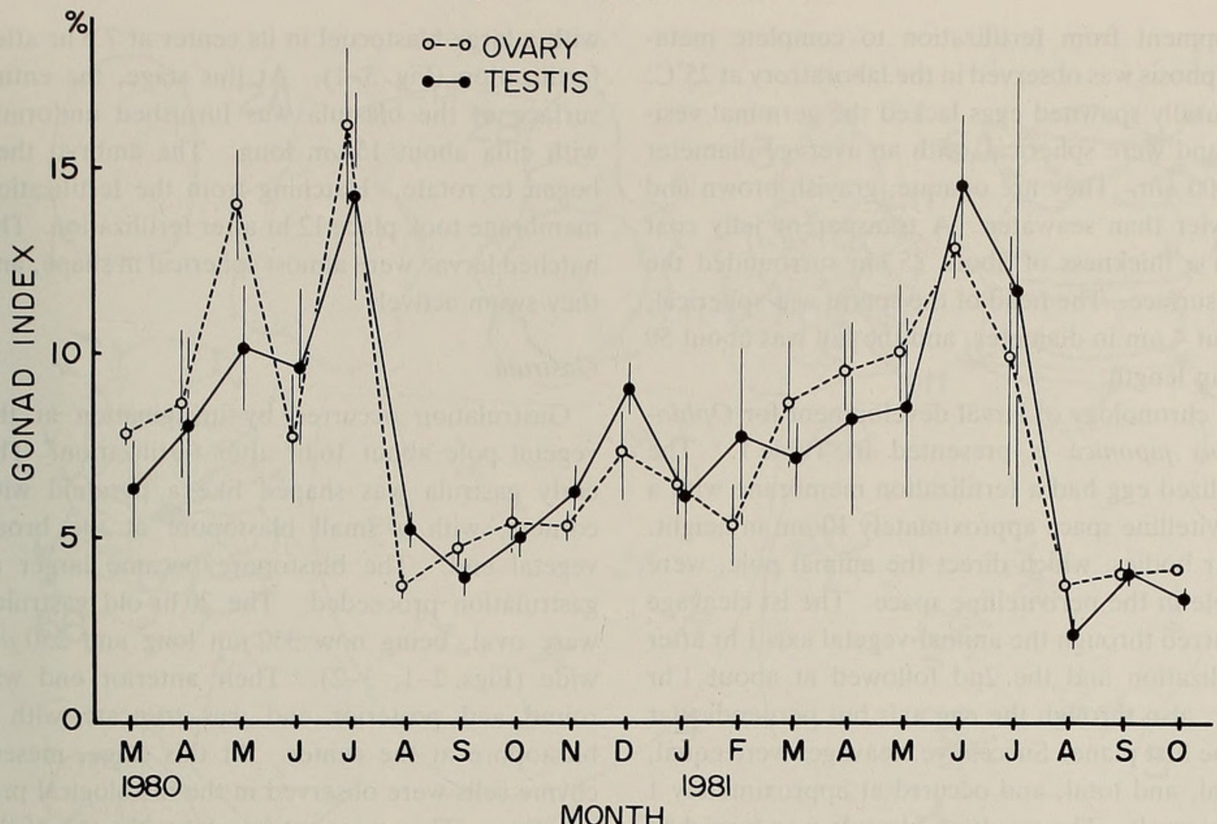


FIG. 1. Monthly variations of the gonad index in *Ophioplocus japonicus*, from March, 1980 through October, 1980. Each point shows an average of 5 individuals and standard error (vertical bar).

TABLE 1. Chronology of development of *Ophioplocus japonicus* (25°C)

| Time (after fertilization) | Stage |
|---|---|
| 1 hr | 1st cleavage |
| 2 hrs | 2nd cleavage |
| 3 hrs | 8-cell stage |
| 4 hrs | 16-cell stage |
| 5 hrs | 32-cell stage |
| 6 hrs | 64-cell stage |
| 7.5 hrs | Blastula with blastocoel |
| 12 hrs | Hatching |
| 16 hrs | Gastrulation |
| 20 hrs | Gastrula, 350 $\mu$m long and 250 $\mu$m wide |
| 30 hrs | Larva with 5 visible hydrolobes |
| 36 hrs | Vitellaria bearing tube-foot buds in hydrolobe, ciliary bands appear |
| 2 days | Terminal tentacles and oral tube-feet present |
| 2.5 days | Vitellaria being rectangular in outline on the posterior potion |
| 3 days | Rudiments of adult skeleton appear as spicules |
| 3.5 days | Vitellaria with 4 distinct transverse ciliary bands on dorsal side, 530 $\mu$m long and 350 $\mu$m wide |
| 4 days | Preoral lobe begins to be resorbed |
| 5 days | Oral plates are apparent |
| 6 days | Metamorphosis completed, 500 $\mu$m diameter |
| 14 days | Juvenile, 600 $\mu$m diameter |

velopment from fertilization to complete metamorphosis was observed in the laboratrory at 25°C. Naturally spawned eggs lacked the germinal vesicle and were spherical, with an average diameter of 300 $\mu$m. They are opaque, grayish brown and heavier than seawater. A transparent jelly coat with a thickness of about 25 $\mu$m surrounded the egg surface. The head of the sperm was spherical, about 4 $\mu$m in diameter, and the tail was about 50 $\mu$m in length.

A chronology of larval development for *Ophioplocus japonica* is presented in Table 1. The fertilized egg had a fertilization membrane with a perivitelline space approximately 10 $\mu$m in height. Polar bodies, which direct the animal pole, were visible in the perivitelline space. The lst cleavage occurred through the animal-vegetal axis 1 hr after fertilization and the 2nd followed at about 1 hr later, also through the egg axis but perpendicular to the first plane. Successive cleavages were equal, radial, and total, and occurrd at approximately 1 hr intervals. The resulting blastula was furnished with a large blastocoel in its center at 7.5 hr after fertilization (Fig. 3–1). At this stage, the entire surface of the blastula was furnished uniformly with cilia about 15 $\mu$m long. The embryo then began to rotate. Hatching from the fertilization membrane took place 12 hr after fertilization. The hatched larvae were almost spherical in shape, and they swam actively.

*Gastrula*

Gastrulation occurred by invagination at the vegetal pole about 16 hr after fertilization. The early gastrula was shaped like a pyramid with corners, with a small blastopore at the broad vegetal end. The blastopore became larger as gastrulation proceeded. The 20 hr-old gastrulae were oval, being now 350 $\mu$m long and 250 $\mu$m wide (Figs. 2–1, 3–2). Their anterior end was round and posterior end was truncate with a blastopore at the center. At this stage, mesenchyme cells were observed in the histological preparations. They were set free from the top of the

Fig. 2. Development of *Ophioplocus japonicus*. Each drawing was made from a living specimen. 1. Twenty hr, gastrula. 2. Thirty hr, early vitellaria larva bearing 5 hydrolobes. 2A; dorsal side, 2B; ventral side. 3. Two and a half days, vitellaria larva with tube-feet and oral tube-feet in a ventral view. 4. Three days, spicules of the rudimental skeletal plate of the adult. 5. More developed spicules than those shown in Fig. 2–4. 6. Three and a half days, vitellaria larva, arrows show transverse ciliary bands. 6A; dorsal side, 6B; ventral side, 6C; spicules of the rudimental skeletal plate of the adult on the dorsal surface of the larva. Dotted lines represent 5 hydrolobes with 2 pairs of tube-feet each. 7. Five days, metamorphosing larva with the absorbed preoral lobe. 7A; dorsal side, 7B; ventral side. 8. Six days, metamorphosed juvenile with a pen-tamerous shape. 8A; aboral side, 8B; oral side. 9. Eight days, juvenile bearing 5 pairs of lateral arm plates in the radius. 9A; aboral side, 9B; oral side. 10. Three months, juvenile with a long arm, 10A; aboral side, 10B; oral side. Symbols: dcp; dorsocentral plate, hl; hydrolobe, lap; lateral arm plate, m; mouth, mpp; madreporic plate, op; oral plate, otf; oral tube-foot, pl; preoral lobe, rp; radial plate, tf; tube-foot, tfp; tube-foot pore, to; tooth, tp; terminal plate, tt; terminal tentacle, vp; ventral plate. Magnification: ca. 65× in all drawings except for 4, 5 and 6C (ca. 200×).

Fig. 3. Development of *Ophioplocus japonicus*. 1 and 2 are micrographs of sections (6 $\mu$m) of specimens and others are scanning electron micrographs. 1. Seven and a half hr, blastula with blastocoel (blc). 2. Thirty hr, gastrula showing outer ectodermal layer and inner endodermal layer, same stage as shown in Fig. 2–1. 3. Twenty-four hr, late gastrula. Note a rise (arrows) corresponding to the area of rudiments of a ciliary band. 4, 5 and 6. Thirty-six hr, early vitellaria larva with primary ciliary bands. 4A; dorsal side. Flaglet and arrow show ciliary bands on the preoral lobe and in the posterior portion, respectively. 4B; magnified view of the anterior left lateral part, 5; ventrolateral (left) side showing hydrolobes (hl). Note the ciliary band on the preoral lobe (flaglet), in the middle of the posterior portion (arrow), and below the hydrolobe (arrowhead). 6; ventral side. Note rudiments (○) of tube-foot on hydrolobe. Flaglet shows ciliary band on the preoral lobe. 7. Two days. 7A; ventral side of a vitellaria larva bearing apparent tube-foot (tf), oral tube-foot (otf) and terminal tentacle (tt) on the hydrolobe, and spiral ciliary band (flaglets) on the preoral lobe, 7B; Magnified view of spiral ciliary band (flaglets) on the preoral lobe. 8 and 9. Two and a half days. 8; dorsal side of vitellaria larva showing 3 parts (arrows) of the upper ciliary band on the posterior portion and the ciliary bands (arrowheads) at the posterior end, 9; magnified view of a posterior part of a specimen. Note ciliary bands (arrowheads) in the future interradius. Each of the hydrolobes in the future radius has a terminal tentacle (✢), 1 pair of tube-feet (○) and a pair of oral tube-feet (⦿). Scale; 100 $\mu$m in 1–4A, 5–7A and 8, and 20 $\mu$m in 4B, 7B and 9.

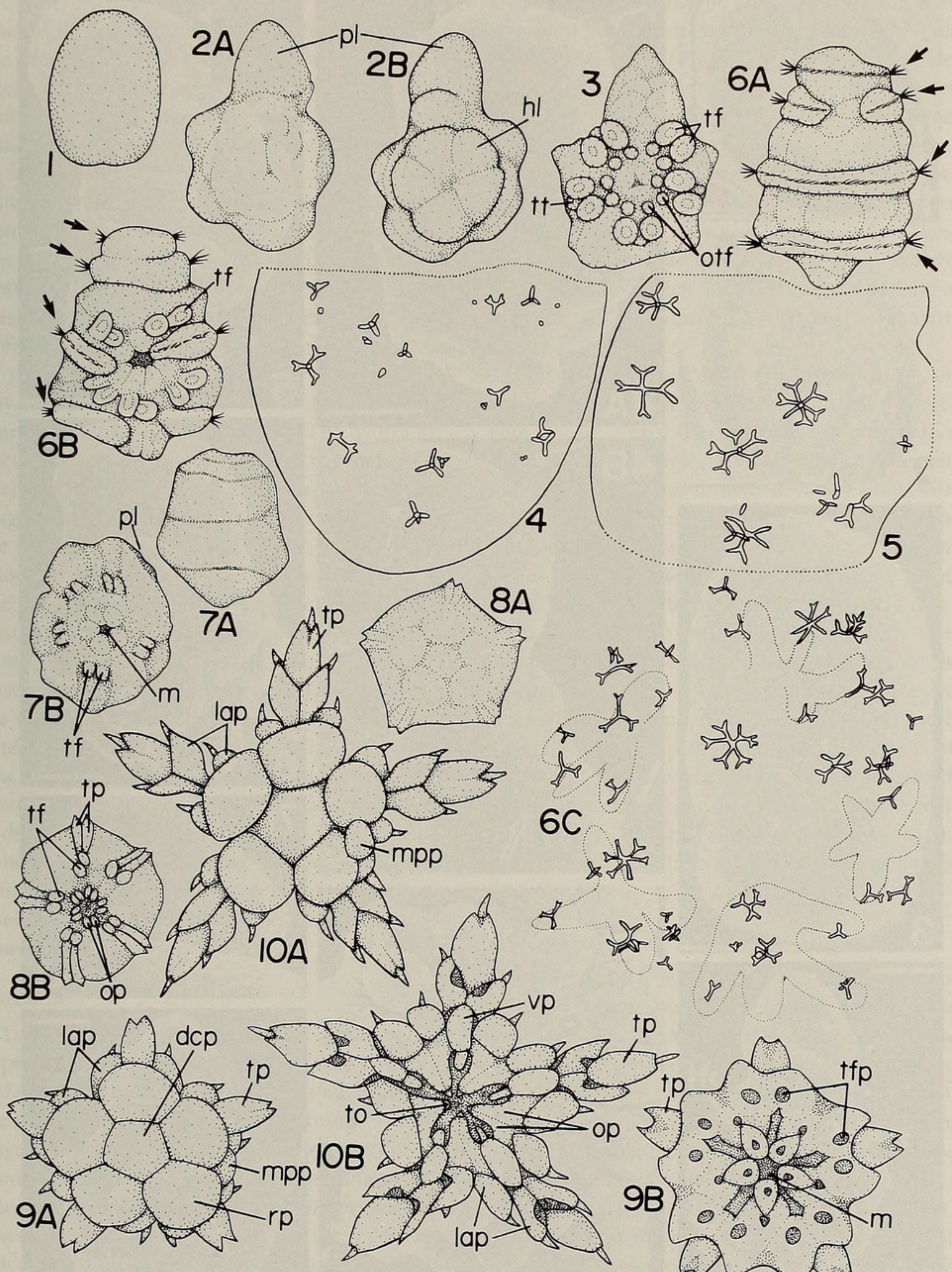
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FIG. 2.

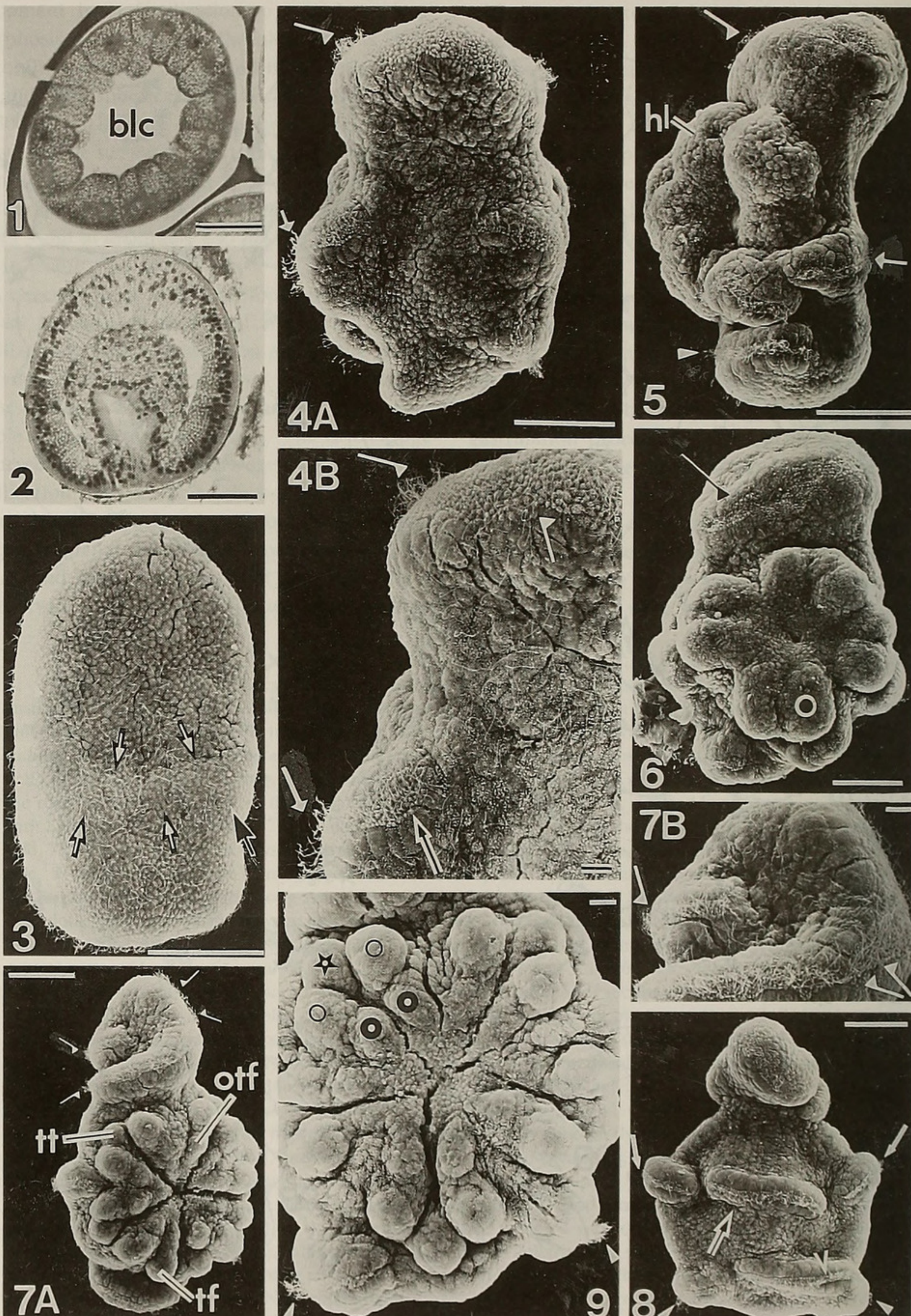



FIG. 3.

archenteron into the blastocoel. Around 25 hrs after fertilization, the area of the middle portion of the body, where the ciliary band will be formed, became apparent as a ridge (Fig. 3–3). The larvae had a distinct constriction which separated the anterior portion from the posterior portion by 30 hr after fertilization (Fig. 2–2A, 2–2B). The anterior portion was seen as tapering and corresponded to the preoral lobe. It disappeared during the metamorphosis into the adult form. The posterior portion, which forms the juvenile body, was more or less pentaradiate in this stage. Projections of the rudiments of the hydrolobes appeared in the future oral side of the larva. In the 1.5 day-old larva, each of the 5 hydrolobes bore one pair of tube-foot buds (Fig. 3–6). The hydrolobes encircled a concave portion, which becomes the oral area of the adult after metamorphosis. At that stage, a ciliary band surrounded the middle part of the preoral lobe (Fig. 3–4A, 3–4B, 3–5, 3–6). In addition to the transverse ciliary band on the preoral lobe, a band encircling the middle of the posterior portion became discontinuous at the ventral and dorsal sides of the body, and could be observed from both lateral sides (Fig. 3–5). On the ventral side, the lateral parts of the ciliary band extended across the hydrolobe (Fig. 3–5), which was situated in the posterior end of the body. There was a transverse ciliary band below the hydrolobes on the ventral side (Fig. 3–5), although it had not yet been formed on the dorsal side. Thus, the larva at this stage should be called a vitellaria, because a transverse ciliary band was now apparent.

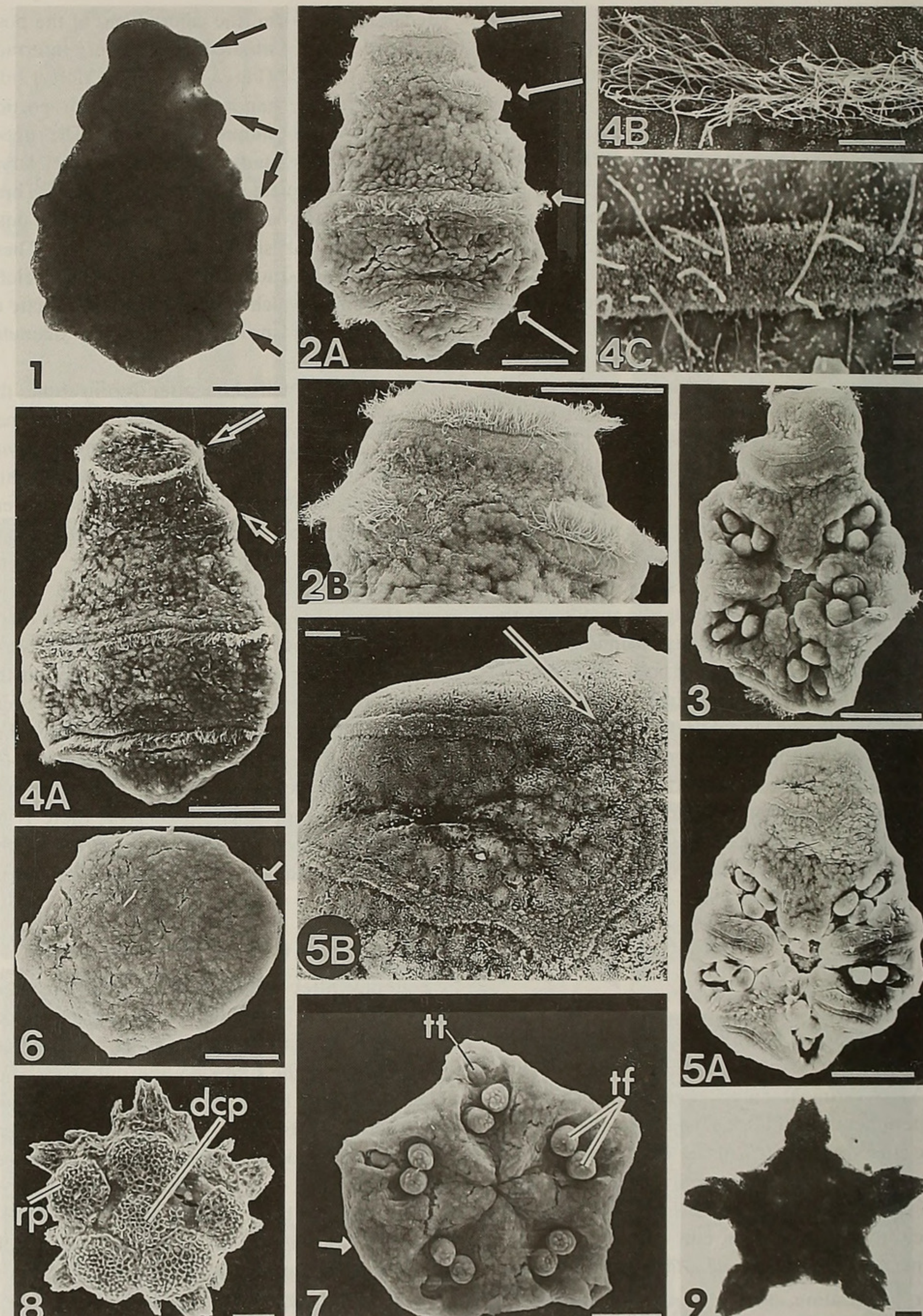
*Vitellaria*

Two days after fertilization, an oral tube-foot appeared in the aboral side of each tube foot (Fig. 3–7A). A terminal tentacle was distinguishable on the peripheral portion of each hydrolobe. At this stage, the ventral parts of the ciliary band on the preoral lobe assumed a spiral form (Fig. 3–7B). The ciliary band below the hydrolobe was displaced to the posterior end of the body and extended to the dorsal side. Two and a half days after fertilization, the posterior portion of the vitellaria larva became clearly rectangular in outline (Fig. 2–3). Because of the change of the vitellaria larval form, the ciliary band of the posterior end became situated in the future interradii of the ventral side (Fig. 3–9). On the dorsal side, each of 2 ciliary bands in the posterior portion were almost continuous (Fig. 3–8). The upper ciliary band consisted of 3 parts: 2 short larval parts and a long median one between them. Three day-old vitellaria larvae had spicules in the posterior portion, as shown in Figure 2–4. These spicules are the rudiments of the adult skeletal plates, some of which later become tetraradiate or hexaradiate, measuring 20–40 $\mu$m in diameter (Fig. 2–5).

Three and a half days after fertilization, the vitellaria larvae became dorso-ventrally flattened (Fig. 2–6A, 2–6B). The vitellaria was now 530 $\mu$m long and 350 $\mu$m wide. By this stage, the juvenile mouth opened. Four transverse ciliary bands were distinct on the dorsal side (Fig. 4–1, 4–2A) and were completely continuous except for the 2nd anterior band, which consisted of small lateral parts (Fig. 4–2B). SEM observation showed that 2 (the 1st and 2nd) anterior bands, which were situated in the preoral lobe, made contact on the ventral side (Fig. 4–3). At this stage, the tube-feet were functional and the vitellariae could either swim freely or creep on the substrata. Figure 2–6C shows spicules viewed from the future aboral side of the juvenile specimen. However, there is no trace of the larval skeleton, in contrast to the findings in the vitellaria of *Ophionereis annulata* by Hendler [8].

*Metamorphosis*

Four days after fertilization, the anterior portion of the vitellaria, the preoral lobe, began to degenerate (Fig. 4–4A). By this stage, the cilia had disappeared on the oral side and only a trace of ridges of ciliary bands remained (Fig. 4–5A, 4–5B). On the dorsal side, the cilia on the incomplete ciliary bands decreased in number and became short (Fig. 4–4C). On the other hand, 3 transverse ciliary bands were retained (Fig. 4–4B). Absorption of the preoral lobe proceeded rapidly after the commencement of the metamorphosis. The metamorphic stage shown in Figure 4–6 is at approximately half a day after the commencement of metamorphosis. Five days after fertilization,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only a trace of the preoral lobe remained (Figs. 2–7A, 2–7B, 4–7). By this stage, the skeletal system of the adult started to grow and the larval body became more rigid except for the tube-feet. A pair of the oral plate was now apparent in each radius. Six days after fertilization, the anterior portion of the larva disappeared and thus metamorphosis was completed (Fig. 2–8A, 2–8B). Metamorphosed juveniles, 500 μm in diameter, bore 5 rudimental arms, each with a terminal plate at its tip. They were benthonic and moved on the substratum by means of their tube-feet. On the aboral side, rudiments of the skeletal plate were apparent. The aboral side of the disk became convex 1 day later. One dorsocentral plate and 5 radial plates around the former became firm at this stage.

*Juvenile*

Eight days after fertilization (2 days after the completion of metamorphosis), 1 pair of lateral arm plates each of which bore 1 spine, were formed in both sides of the arm (Fig. 2–9A). The madreporic plate was now recognizable on 1 inter-radius. Skeletal plates of the oral side of the disk were indistinct except for the oral and terminal ones (Fig. 2–9B). One pair of tube-foot pores were seen to exist in each radius. Ten days after fertilization, the 1st ventral arm plates appeared. Figure 4–8 shows the aboral view of a juvenile at 2 weeks after fertilization. The skeletal plates of the aboral side had many pore-like sieves. The juvenile seen here is 600 μm in diameter. One month after fertilization, juveniles grew to 700 μm in diameter. Although a number of juveniles were kept alive in the laboratory for several months, no further differentiation could be observed. Three month-old juveniles measured 500 μm in disk diameter and 250 μm in arm length (Figs. 2–10A, 2–10B, 4–9). They bore 2 arm segments from the development of the 2nd lateral arm plates. Each arm was furnished with 3 pairs of tube-feet.

## DISCUSSION

Detailed studies on the development through metamorphosis are very limited in ophiuroids, although larvae of ophiuroid have been recognized in about 80 species [5].

It was reported that gastrulation takes place by invagination at the vegetal pole [6, 10, 17, 21]. Little is known about the formation of the archenteron in the species with the vitellaria larva. It was reported that the archenteron is formed by splitting of the central core in *Ophioderma brevispina* [1]. On the other hand, judging from the figure by Mortensen ([14], Plate XXXI, Fig. 5) the archenteron seems to be formed by invagination in a 26 hr-old larva of *Ophionereis squamulosa*. In the present species, gastrulation is apparently achieved by an invagination. Thus, formation of the archenteron seems to be diverse in different species in ophiuroids with vitellaria larvae. However, further studies are needed to promote a deeper understanding of the archenteron formation.

*Ophioplocus japonicus* is the 9th ophiuroid species that has been shown to have vitellaria larvae. The larva of *O. brevispina* was called worm-like larva by Müller [16]. Brooks and Grave [1] described in some detail the larva of *O. brevispina*. Later the term, vitellaria, was proposed by Fell [2] for a distinctive larval type of echinoderm which

FIG. 4. Development of *Ophioplocus japonicus*. 2–8 views are scanning electron micrographs. 1 and 9 are light (regular) micrographs. 1, 2 and 3. Three and a half days, same stage as that shown in Figs. 2–6 and 4–2A. 1; vitellaria larva from the dorsal side with 4 transverse ciliary bands (arrows), 2A; dorsal side, 2B; magnified view of the anterior part, 3; ventral side. 4 and 5. Four days. 4A; dorsal side, vitellaria larva showing the disappearance of cilia on the ciliary band (short arrow). The long arrow indicates a ciliary band on the preoral lobe. 4B; magnified view of the ciliary band (the long arrow shown in Fig. 4–4A) on the preoral lobe, 4C; magnified view of the ciliary band (the short arrow shown in Fig. 4–4A) showing the disappearance of cilia, 5A; ventral side of the vitellaria, 5B; magnified view of the anterior part. Arrow indicates the junction of two ciliary bands on the preoral lobe. 6. Four and a half days, metamorphosing vitellaria with reduced preoral lobe (arrow), view from dorsal side. 7. Five days, metamorphosing vitellaria showing absorbed preoral lobe (arrow), view from ventral side, same stage as that shown in Fig. 2–7. 8. Two weeks, juvenile with a dorsal central plate and 5 radial plates on the dorsal side. 9. Three months, juvenile with arm segments, view from aboral side, same stage as shown in Fig. 2–10. Scale: 100 μm in 1–4A, 5A, and 7–9, 10 μm in 4B and 5B, and 1 μm in 4C.

corresponds to the larva of *O. brevispina*. Occurrence of ciliary rings is one of the characteristics of the vitellaria: the existence of 4 ciliary bands has been reported in the vitellariae of *Ophioderma longicauda* [3] and *O. brevispina* [1]. In fact, Hendler [8] noted that ophiuroid vitellariae generally have 4 transverse ciliary bands. However, the number of the ciliary bands varies in different species. There are 3 ciliary bands in *Ophionereis annulata* because of the lack of the 4th transverse band [8]. Mortensen [15] described that vitellariae of *Ophiolepis cincta* are furnished with a ciliated tuft in addition to 4 transverse ciliary bands. Judging from his figure (Fig. 26, p. 48), the actual number of ciliary bands seems to be 3; the 3rd and the 4th bands are continuous and should be counted as 1. The number of ciliary bands was not described in *Ophioderma cinereum* [7], *Ophiolepis elegans* [19] or *O. squamulosa* [14]. However, it can be determined in the latter 2 species from available figures (Fig. 1c, p. 9; Figs. 2 and 3, Pl. XXXI). Each of these vitellariae is furnished with 3 ciliary bands and 1 ciliated tuft. In the vitellaria of the present species, no ciliated tuft was observed at the anterior portion of the body by both LM and SEM observations. Ciliation of *O. japonica* is, therefore, different from that of *O. cincta, O. squamulosa* and *O. elegans*. Thus, ciliation of the ophiuroid vitellaria differs in different species. The vitellaria larva of *O. japonica* seems to have 4 transverse ciliary bands when observed from the dorsal view. However, the actual number of ciliary bands is 3, since 2 anterior ciliary bands on the preoral lobe are continuous on the ventral side. Therefore, detailed observation of ciliation of the vitellaria should be one of the points to be investigated in the future.

As noted before, the classification of the developmental pattern in ophiuroids seems to be different judging from the literature. Hendler [5] classified the development of ophiuroids into 3 types; planktonic, direct, and abbreviated. According to his view, development that includes vitellariae belongs to the abbreviated development. The abbreviated development category includes *Gorgonocephalus caryi* [18], which develops through only a pear-shaped larva without ciliary bands. Thus, the abbreviated type comprises heterogeneous larval forms. Hendler's proposal was supported by Mladenov [11]. Strathmann and Rumrill [20] later called the abbreviated development a pelagic or demersal development with lecithotrophic larvae.

According to the original proposals by Fell [2], the vitellaria larva is a larval form which bears transverse ciliary bands. Therefore, in terms of abbreviated development, it is reasonable to distinguish development with vitellariae from that with other types of larvae.

The relation between developmental type and egg size in ophiuroids was shown by Hendler [5] and then revised by Mladenov [11]. According to the latter, the ranges of the egg's diameter in the direct and planktotrophic developments are 100–1000 $\mu$m and 70–200 $\mu$m, respectively. Thus, there is a considerable spread of egg size in these 2 developmental types. On the other hand, the diameter of an egg of the species undergoing the abbreviated development type is greater than 130 $\mu$m and less than 350 $\mu$m.

It is likely that ophiuroid eggs, being from 200 to 350 $\mu$m in diameter, develop through the vitellaria larva. As a matter of fact, egg of *O. japonicus* is approximately 300 $\mu$m in diameter. The egg of *Ophiocoma pumila* is very small (73 $\mu$m) among the ophiuroids with vitellaria larvae. However, this species is exceptional since *O. pumila* passes through both the vitellaria and the planktotrophic pluteus stages.

In the present species, larva completes metamorphosis 6 days after fertilization at about 25°C. Stancyk [19] reported that in *O. annulata*, metamorphosis is completed 3 days after fertilization at 24°C. The majority of species which have vitellaria larvae were reported to complete metamorphosis within 4 or 5 days, although the temperatures were not indicated [4, 13, 15]. On the other hand, in *Amphipholis kochii* which has planktotrophic development with eight-armed ophiopluteus, metamorphosis is incipient 12 days after fertilization at 23°C [21]. This term seems to be the shortest among planktotrophic developers according to the table by Mladenov ([11], p. 60). Thus, it may be concluded that developments through the vitellaria larva proceed faster than those through the ophiopluteus larva.

In *O. japonicus,* no larval skeleton is present in the vitellariae. No trace of any larval skeleton was observed in either the vitellaria larvae of *O. squamulosa* or *O. brevispina* [1, 14]. On the other hand, occurrence of a larval skeleton was reported in *O. annulata* [8]. Furthermore, an irregular calcareous structure was found in the vitellaria of *O. cincta* [15]. It is, therefore, interesting that there are 2 types of vitellariae, one with a pluteus-like skeleton and the other with no skeleton at all.

*O. japonicus* is one of the most common ophiuroids in Japan, with its habitat being under the stones of shallow water. In the present study, the larva of this species is demonstrated as being vitellaria, not ophiopluteus, which is thought to be a typical planktotrophic larva of ophiuroids [5, 9, 21]. Twenty-four species of ophiuroids have been known to pass through ophiopluteus larvae. They belong to 5 families of 2 suborders, Gnathophiurina and Chilophiurina. On the other hand, vitellariae have been found in 9 species including the present species, and all belong to 4 families of 1 suborder, Chilophiurina. These families are Ophiodermatidae, Ophiocomidae, Ophiuridae and Ophionereidae. Thus, the occurrence of vitellaria is very limited and this may be phylogenetically significant.

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## REFERENCES

1 Brooks WK Grave C (1899) *Ophiura brevispina.* Mem natn Acad Sci 8: 79–100

2 Fell HB (1945) A revision of the current theory of echinoderm embryology. Trans Roy Soc New Zealand 75: 73–101

3 Fenaux L (1969) Le développmen larvaire chez *Ophioderma longicauda* (Retzius). Cah Biol Mar 15: 59–62

4 Grave C (1916) *Ophiura brevispina* II. An embryological con tribution and a study of the effect of yolk substance upon development and developmental processes. J Morph 27: 413–453

5 Hendler G (1975) Adaptational significance of the patterns of ophiuroid development. Amer Zool 15: 691–715

6 Hendler G (1977) Development of *Amphioplus abditus* (Verrill) (Echinodermata: Ophiuroidea) I. Larval biology. Biol Bull 152: 51–63

7 Hendler G (1979) Reproductive periodicity of ophiuroids (Echinodermata: Ophiuroidea) on the Atlantic and Pacific coasts of Panamá In "Reproductive Ecology of Marine Invertebrates" Ed by SE Stancyk, Univ of South Carolina Press, USA, pp 145–156

8 Hendler G (1982) An echinoderm vitellaria with a bilateral larval skeleton: evidence for the evolution of ophiuroid vitellariae from ophioplutei. Biol Bull 163: 431–437

9 Hyman L (1955) The Invertebrates VolIV Echinodermata. McGraw-Hill, New York, pp 763

10 MacBride EW (1914) Invertebrate In "Text-Book of Embryology" Ed by W Heape, Macmillan, London, Vol IV, pp 692

11 Mladenov PV (1979) Unusual lecithotrophic development of the Caribbean brittle star *Ophiothrix oerstedi.* Mar Biol 55:55–62

12 Mladenov PV (1985) Development and metamorphosis of the brittle star *Ophiocoma pumila*: evolutionary and ecological implication. Biol Bull 168: 285–295

13 Mortensen Th (1898) Die Echinodermenlarven der Plankton-Expedition nebst neiner systematischen Revision der bisher bekannten Echinodermenlarven. Ergebnis Plankton-Exped Humboldt-Stift 2J: 1–118

14 Mortensen Th (1921) Studies of the Development and Larval Forms of Echinoderms. GEC Gad, Copenhagen, pp 261

15 Mortensen Th (1938) Contributions to the study of the development and larval forms of Echinoderms IV. Kgl Dan Vidensk Selsk Skr, Naturvid Math Afd, Ser 9, 7(3): 1–45

16 Müller J (1850) Ueber die Larven und die Metamorphose der Echinodermen. Dritte Abhandlung Abhandl Konigl Preuss Akad Wiss Berlin, 1849: 35–72

17 Oguro C, Shōsaku T, Komatsu M (1982) Development of the brittle-star, *Amphiolis japonica* Matsumoto In "Echinoderms" Ed by JM Lawrence, AA Balkema, Rotterdam, pp 491–496

18 Patent DH (1970) The early embryology of the basket star *Gorgonocephalus caryi* (Echinodermata, Ophiuroidea). Mar Biol 6: 262–267

19 Stancyk SE (1973) Development of *Ophiolepis elegans* (Echinodermata: Ophiuroidea) and its implications in the estuarine environment. Mar Biol 21: 7–12

20 Strathmann MF, Rumrill SS (1987) Phylum Echi-

nodermata, Class Ophiuroidea In "Reproduction and Development of Marine Invertebrates of the North Pacific Coast" Ed by MF Strathmann, The Univ Was Press, USA, pp 556–573

21 Yamashita M (1985) Embryonic development of the brittle-star *Amphipholis kochii* in laboratory culture. Biol Bull 169: 131–142