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Development and Growth of the Feather Star *Oxycomanthus japonicus* to Sexual Maturity

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Crinoids, including feather stars, are the most basal group among extant echinoderm classes and share a basic body plan. In spite of their importance for evolutionary developmental study, information on the development of crinoids has been limited, because there are not many species whose spawning season is known, and artificial spawning is impossible. Therefore, it is not easy to obtain fertilized eggs of crinoids. We have observed the spawning and development of the feather star *Oxycomanthus japonicus* for 7 years. We have established a cultivation system that has enabled us to culture large numbers of *O. japonicus* from eggs through to sexually mature adults. In the present study, we show that (1) individuals take 2 years to reach sexual maturity; (2) the skeleton of the theca of a stalked juvenile consists of five orals, five basals, five radials, five infrabasals, and an anal plate; and (3) the onset of spawning has shifted by about two weeks since 60 years ago. Our cultivation system can provide enough embryos, larvae, juveniles, and adults for further experiments, extending the possibilities for crinoid research.

Key words: crinoid, feather star, development, long-term culture, sexual maturation, spawning season

INTRODUCTION

Crinoids are considered to be the most basal group among extant echinoderm classes and are important for understanding the evolution of the body plans of echinoderms and deuterostomes (Paul and Smith, 1984; Smith et al, 2004). They possess a well-developed aboral nervous system, which has degenerated in other echinoderm classes. Also, crinoids have strong regenerative capability, which is dependent on the nervous system (Przibram, 1901, quoted by Hyman, 1955; Amemiya and Oji, 1992). Thus, crinoids are potentially important model organisms for evolutionary developmental biology and regenerative biology. Among crinoids, feather stars (stalkless crinoids) live in relatively shallow waters, and up to 530 species have been described (Clark, 1931, 1941, 1947, 1950; Clark and Clark, 1967; Messing and White, 2001). Many observations of the gonads, eggs, spawning, and early development of feather stars have been reported (Carpenter, 1866; Mortensen, 1920; Kubota, 1969, 1970; Holland and Kubota, 1975; Holland et al., 1975; Holland 1978, 1991; Mladenov and Chia, 1983; Lahaye and Jangoux, 1987; Kohtsuka, 2001; Kohtsuka and Nakano, 2005). However, information on development after settlement is limited.

The feather star *Oxycomanthus japonicus* inhabits relatively shallow rocky seashores around Japan and has been especially well studied in Sagami Bay. Spawning of *O. japonicus* was first described by K. Dan and J. C. Dan (1941), who showed that spawning occurred in early autumn. Dan and Kubota (1960), who observed *O. japonicus* in the field continuously over a period of 19 years (1937–1955), found that spawning occurred on a neap-tide day early in October, between 3 PM and 5 PM. Development has been described up to the juvenile stage (J. C. Dan and K. Dan, 1941; Kubota, 1969, 1970; Grimmer et al., 1984), but subsequent development through to mature adults remained completely unknown.

We have succeeded in rearing large numbers of *O. japonicus* to sexual maturity. Here we present the first complete description of the development of a crinoid, focusing on skeletal developmental in stalked-stage juveniles; establish improved methods for cultivation; and report that the date of spawning has shifted later by one tide cycle for the past seven years.

MATERIALS AND METHODS

Collecting adults

Several weeks before spawning, sexually mature individuals of *Oxycomanthus japonicus* were collected by SCUBA from a submarine rocky terrace about 5 m deep, near the Misaki Marine Bio-

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logical Station, Sagami Bay, central Japan. Approximately 30 animals were kept in cages in the sea at a depth of 3 m until observation started.

Spawning

A few days before the neap tide, sexually mature feather stars were transferred from the cage in the bay to containers in the laboratory. Males and females were kept separately in different containers in flowing seawater. Spawning occurred spontaneously between 4 PM and 7 PM. When spawning occurred, the temperature of the water in the containers was about 20°C .

Culture and rearing

After spawning (Fig. 1A, B), eggs and sperm were collected with a pipette. Eggs were inseminated by mixing with a sperm suspension. Eggs dissected from gonads prior to spawning were immature, suggesting that egg maturation may not be completed prior to spawning. Fertilized eggs were cultured in plastic containers 30 cm in diameter, with approximately 7000 eggs in 10 liters of seawater in each container (Fig. 1C). The containers were covered with plastic film to prevent water evaporation. The seawater in the culture containers was replaced with freshly filtered seawater every other day. The room temperature was 18–22°C.

Most larvae had settled onto the wall or bottom of the container and metamorphosed into stalked juveniles by 7 days after fertilization. The culture containers in which juveniles settled were placed in net bags of 1-cm mesh, and the openings of the bags were tied. (Fig. 2A, B). To avoid the accumulation of sediment, the containers were hung upside down from a float anchored in the bay (Fig. 2C). The juveniles seemed to obtain enough plankton, even though the containers were upside down. Sessile organisms such as ascidians, barnacles, mussels, and sea mosses that settled on the containers and nets were removed every other month. The feather stars remained in the containers until they grew into sexually mature adults. We modified the method of Grimmer et al. (1984) for long-term culture.

Observation of skeletal development

Living larvae and juveniles, and juveniles fixed in 70% ethanol, were observed under a Nikon BIOPHOT polarizing microscope. For observaton by scanning electron microscope (SEM), the softparts of fixed juveniles were removed by treatment with NaClO (1–5%). After washing with distilled water, the juveniles were air-dried. Specimens were coated with platinum palladium in a Sanyo Denshi SC-701AT sputter coatter and then observed with a Hitachi S-2400S SEM.

RESULTS

Spawning dates

Spawning dates from 2000–2006 are shown in Table 1. The animals spawned around neap tide, between 4 PM and 7 PM. There was individual variation (±5 days) in the timing of spawning. Generally, the males spawned before the females.

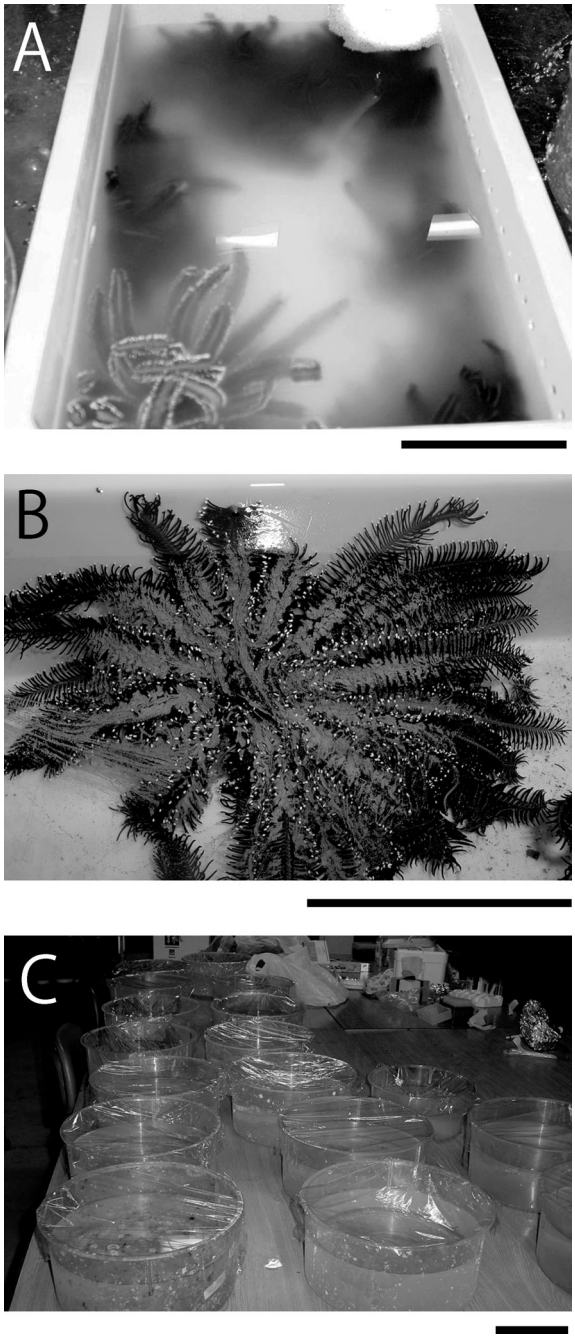

Fig. 1. **(A)** Spawning of males of *O. japonicus*. **(B)** Spawning of females of *O. japonicus*. **(C)** Culture of embryos and planktonic larvae in the laboratory. Scale bars, 10 cm.

Skeletal development in the planktonic and stalked stages

Development of plates in the theca

About 50 hours after fertilization, skeletal plates are formed in the doliolaria. The plates can be classified into columnals, basals, and orals according to their position in the larva (Fig. 3A). The plates are all small and almost identical in shape. There are five basals and five orals. Two days after fertilization, the larvae settle onto the walls of the plastic containers and enter the cystidean stage (Fig. 3B). The basals and orals develop into an asterisk shape, with some branches. An additional five skeletal plates are formed between the basals and columnals. These newly formed plates were identified as infrabasals, judging from their relative position in the larva. The columnals thicken and grow longitudinally in the developing stalk. By three days after settlement, infrabasals, basals, and orals grow and develop into a structure called the reticular stereom (Smith, 1980). The infrabasals are located in positions alternating with the basals (Fig. 3C). Subsequently, the infrabasals move inside

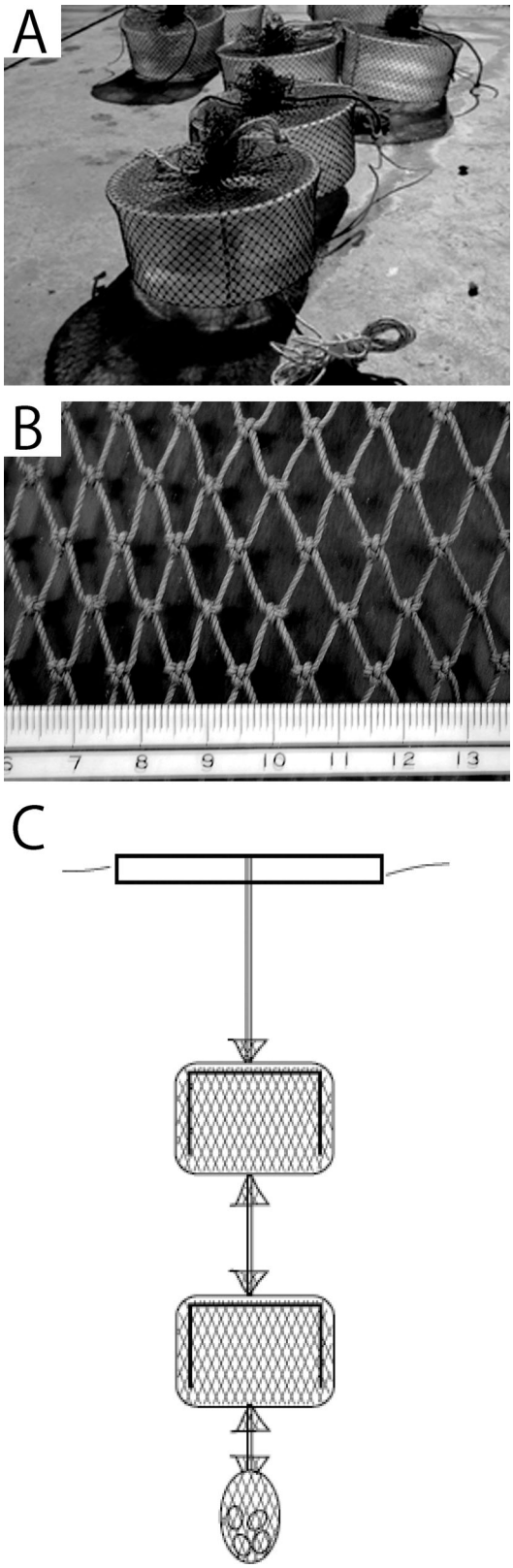

Fig. 2. **(A)** Containers with 30 cm in diameter, enveloped in nets. Several thousand stalked juveniles inhabit each container. **(B)** The enveloping net with a 1-cm mesh size. **(C)** Illustration of the cultivation in seawater. The containers are set upside down and hung from a float in the sea.

Table 1. Spawning dates from 2000 to 2006. The "Difference" column indicates individual variation in the timing of spawning, measured in days before (–) or after (+) the day of neap tide.

Year	Spawning date and time	Sex	Difference
2000	Oct. 21, 1610	Male	+1
	Oct. 23, 1610	Male	+3
	Oct. 23, 1625	Female	+3
	Oct. 23, 1630	Female	+3
	Oct. 23, 1652	Female	+3
	Oct. 25, evening	Male	+5
	Oct. 25, evening	Female	+5
2001	Oct. 23, 1700	Male	–1
	Oct. 23, 1900	Female	–1
2002	Oct. 24, 1707	Male	–5
	Oct. 24, 1710	Female	–5
	Oct. 31, before 1930	Male	+2
	Oct. 31, 1930	Female	+2
	Oct. 31, 2200	Male	+2
	Oct. 31, 2200	Female	+2
	Nov. 1, night	Male	+3
	Nov. 1, night	Female	+3
2003	Oct. 16, 1600	Male	–2
	Oct. 17, 1600	Male	–1
	Oct. 17, 1730	Female	–1
	Oct. 19, before 1800	Male	+1
	Oct. 19, 1800	Female	+1
2004	Oct. 21, before 1650	Male	0
	Oct. 21, 1650	Female	0
	Oct. 23, before 1400	Male	+2
	Oct. 23, 1400	Female	+2
2005	Oct. 11, 1555	Male	0
	Oct. 11, 1640	Female	0
	Oct. 13, before 0930	Male	+2
	Oct. 13, before 0930	Female	+2
	Oct. 24, 1520	Male	–1
	Oct. 24, 1645	Female	–1
	Oct. 25, 1725	Male	0
	Oct. 27, 1900	Male	+2
2006	Oct. 13, 1630	Female	–1
	Oct. 28, 1545	Male	–2
	Oct. 28, 1800	Female	–2
	Oct. 30, 1540	Male	0
	Oct. 31, 1540	Male	0
	Oct. 31, 1800	Female	+1
	Oct. 31, 1820	Female	+1

the basal circlet (Fig. 3D), eventually becoming completely covered by the basals. SEM examination of the skeletal plates in the theca of the pentacrinoid stage (stalked juvenile, 27 days after fertilization) showed the five infrabasals alternating with the five basals (Fig. 4A). Approximately 2 weeks after settlement, two additional types of plates form: five radials and one anal plate. The radials are located alternately between the orals and basals (Fig. 4B). The anal plate is located between two of the radials (Fig. 4C, D).

Ultrastructure of columnals and the articulation

The columnals in larvae just after settlement form a cylindrical structure called the galleried stereom (Smith,

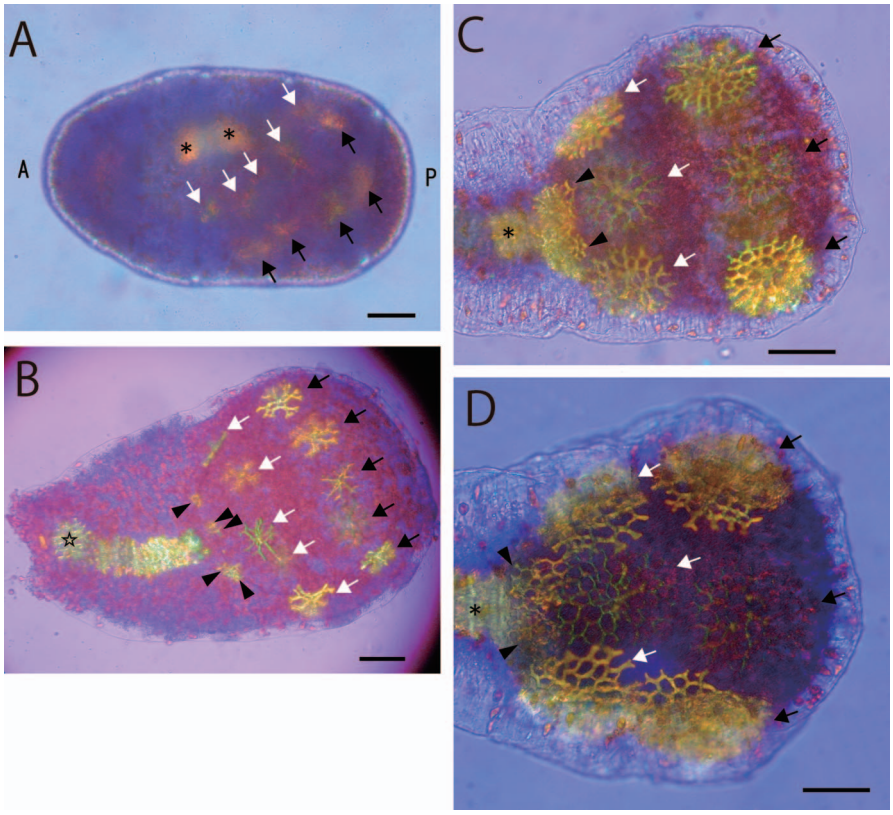

Fig. 3. **(A)** Doliolaria, fifty hours after fertilization. **(B)** Cystidean, two days after fertilization. It settled to the wall of the container and was removed for observation. **(C)** Crown of a cystidean, six days after fertilization. **(D)** Crown of a cystidean, seven days after fertilization. A, anterior; P, posterior; black arrows, orals; white arrows, basals; asterisks, columnals; black arrowheads, infrabasals; star, attachment disc. Scale bars, 50 μm.

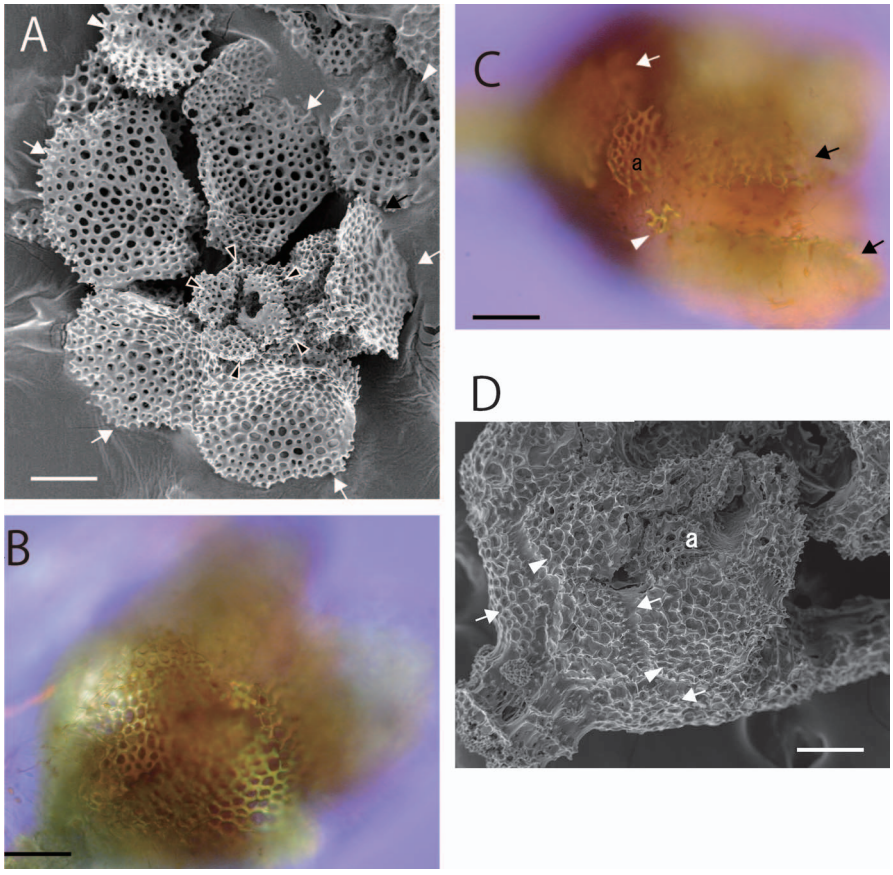

Fig. 4. **(A)** Skeletal plates in the theca of a pentacrinoid, 27 days after fertilization. Inside view of the skeletal structures of the opened theca observed by SEM. **(B)** Skeletal structures of the theca of 18-day-old pentacrinoid observed by polarizing microscopy. **(C)** Lateral view of the same specimen in (B). **(D)** Crown of a 39-day-old pentacrinoid, observed by SEM. Black arrows, orals; white arrows, basals; black arrowheads, infrabasals; white arrowheads, radials; a, anal plate. Scale bars, 100 μm (A, B, C), 150 μm (D).

1980). Columnals identical in diameter are arranged in tandem in a single series and interlock by tooth-like protrusions on their ends (Fig. 5A, B), a type of articulation called simplexy (Moore et al., 1978). Simplexy allows very limited movements. In the pentacrinoid stage, each columnal is elongated (Fig. 5C), and eventually both tips thicken (Fig. 5D). Each tip of the columnals bears a fulcral ridge that connects to an adjacent columnal, a mode of architecture called synarthry (Fig. 5E–H). In a single columnal, the directions of the upper and lower fulcral ridges differ by about 90°; synarthry thus allows flexible movements of the joined columnals. In the later pentacrinoid stage, long columnals of irregular shape appear in the proximal part of the stalk. These appear to form by fusion of short columnals, but this process is unclear, and further observations are necessary to confirm the fusion of columnals (Fig. 5I).

Development in the free-living stage

About 2 months after fertilization, juveniles discard the stalk and begin a free-living mode of life (Fig. 6). Average arm length is approximately 0.9 cm. The juveniles settle on substrates (shells of barnacles and tubes of clam worms attached to the container) by grasping them with their cirri. The arms grow at the average rate of 1 cm a month, but this rate decreases after the arms reach approximately 15 cm in length.

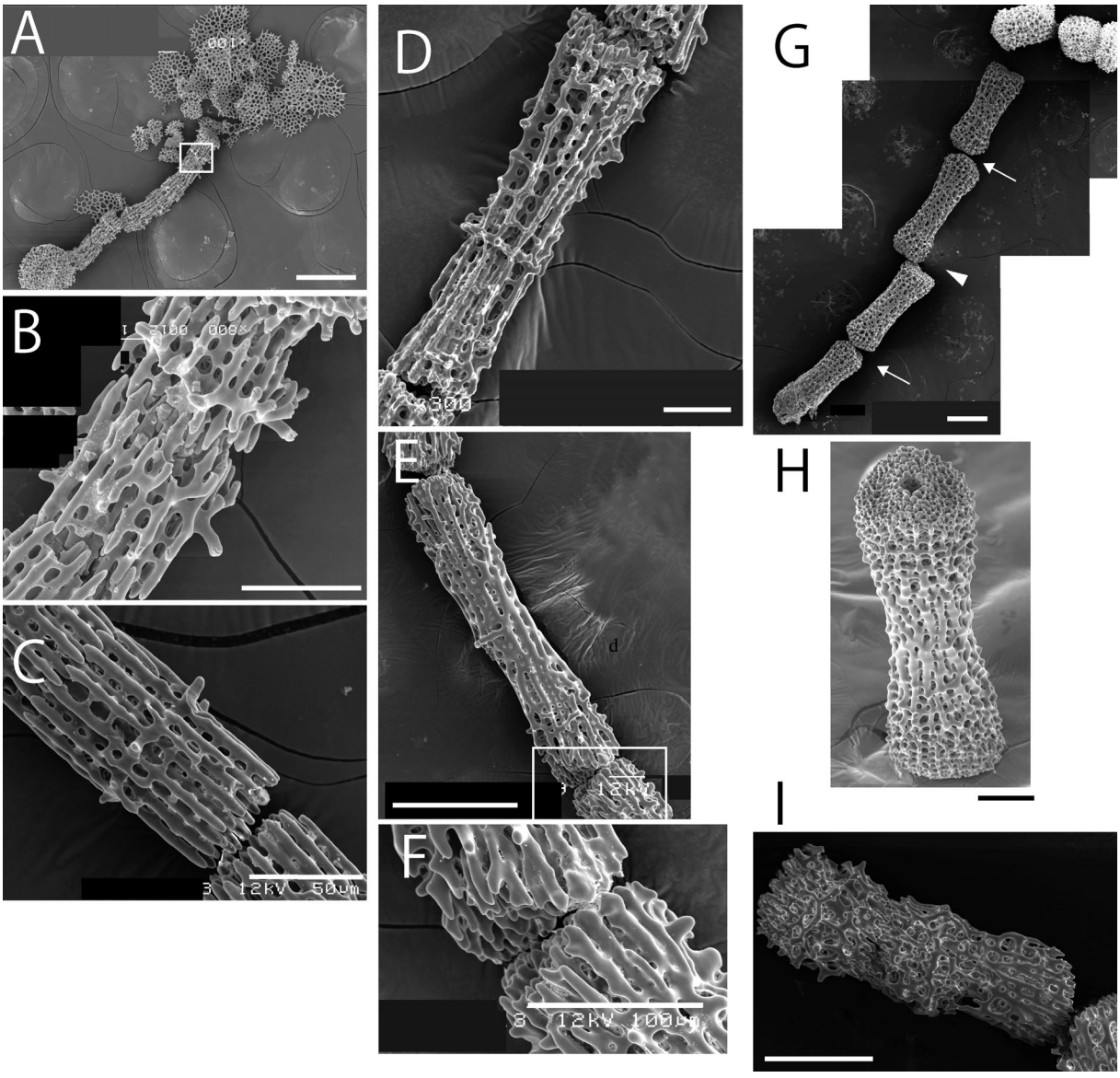

Fig. 5. Skeleton of the stalks of juveniles observed by SEM. **(A)** Skeleton of an 8 day-old cystidean. **(B)** High magnification of white box in (A), showing that columnals interlock with each other. **(C)** Columnal of an 11-day-old cystidean. **(D)** Columnal of a 27-day-old pentacrinoid with thickened ends. **(E)** Columnal of a 36-day-old pentacrinoid. **(F)** High magnification of white box in (E) showing synarthrial articulation. **(G)** Columnals of a 39-day-old pentacrinoid showing synarthries alternating by 90°. **(H)** Articulation facet of a columnal of a 39-day-old pentacrinoid. **(I)** Columnal of a 27-day-old pentacrinoid, which seems to be formed by fusion of short columnals. Scale bars, 200 μm (A, E, G), 50 μm (B, C, D), 100 μm (F, H, I).

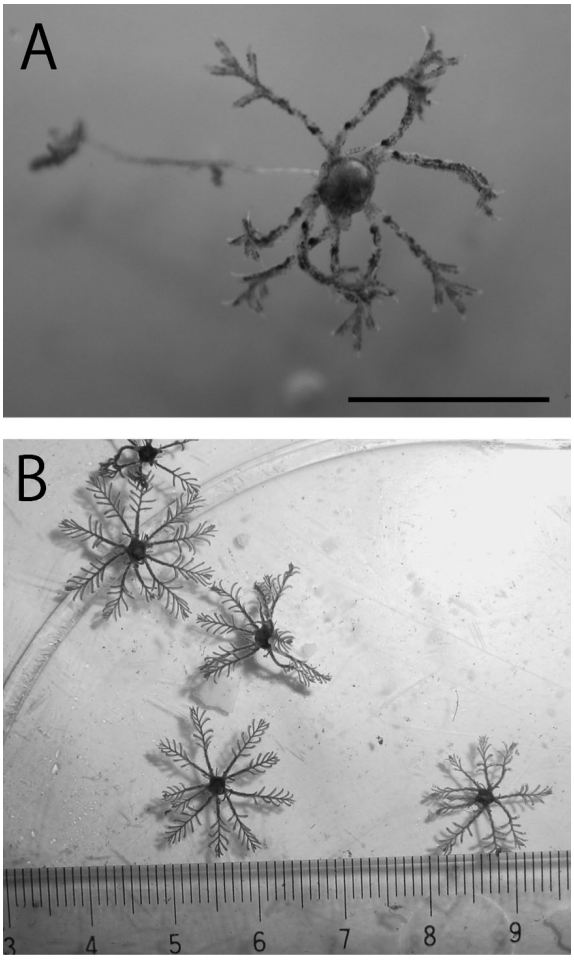

Fig. 6. Two-month-old juveniles. **(A)** Juvenile that has not detached its stalk. Scale bar, 500 mm. **(B)** Juveniles that have detached their stalks. The length of an arm is about 8 mm.

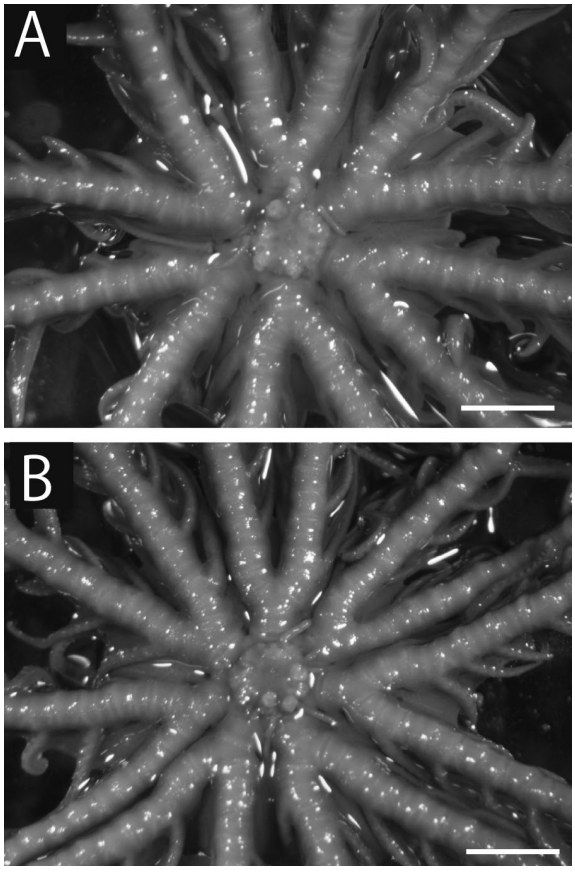

Fig. 7. Aboral view of 8-month-old juveniles bearing an extra ray(s); specimen was fixed in 70% ethanol. The cirri were removed to observe the rays. **(A)** Juvenile bearing 6 rays. **(B)** Juvenile bearing 7 rays. Scale bars, 2 mm.

Juveniles originally have ten arms. Several months after fertilization, new arms form by branching at a plate called the axillary, in the following manner. The animal autotomizes the original arms at a ligamentary articulation called a syzygy, which is considered to be an articulation specialized for autotomy (Wilkie, 2001). In the first phase of regeneration, a structure shaped like a small heart forms at the point of amputation. Eventually, two new arms regenerate at this point, increasing the number of arms (for the manner of increase in arm number, see Shibata and Oji, 2003). The average number of arms reaches 13 in individuals one year old.

By 17 months after fertilization, almost all the original arms have branched. Newly formed arms branch in the same manner as previous arms, by autotomy followed by regeneration of a pair of new arms. The number of arms ranges from 17–25 in individuals two years old.

Most of the animals had five rays; however, in the cultivation in 2000, approximately one-fourth of the individuals formed one or two extra rays (Fig. 7). Morphology of the arms was almost identical between normal individuals and those with extra rays. Each of the primary arms extended from the theca and branched, as in the normal animals. The number of ambulacral grooves also showed the same variation, corresponding with the number of rays. In the individuals with a supernumerary ray, each ambulacral groove started from the mouth, as in the normal individuals. Branching of the groove was observed in some individuals. Individuals with a supernumerary ray also grew normally and reached sexual maturity in two years, as did the normal individuals. When the density of individuals in the container was kept lower, supernumerary rays were not observed. In 2000, the density was 14,000–20,000 eggs per container. From 2001, the density was about 7,000 per container. It is possible that high-density culture induced the supernumerary rays.

Sexual maturation

Most animals cultured in the cultivation system grew to sexual maturity two years after fertilization. Swelling of arm pinnules, in which the germ cells develop, was evident early in October. Spawning occurred in the evening of neap-tide days in October, during the same period in which adults spawn in the natural environment.

We cultured descendants of the abnormal individuals with extra rays for 10 days after fertilization; however, because of the small size of these offspring, we could not confirm how many rays they had.

DISCUSSION

We succeeded in cultivating *Oxycomanthus japonicus* from eggs to sexually mature adults. This is the first report of the complete life cycle of a crinoid. The key point in cultivation is the feeding system after the juvenile stage. Kohtsuka and Nakano (2005) previously cultivated juveniles of another species of feather star, *Decametra tigrna*, in a container hung in the seawater of a bay; however, growth stopped approximately 300 days after settlement, and all the animals died. The cultivation methods we employed are almost the same as those of Kohtsuka and Nakano (2005). To maintain good circulation of seawater, we often removed seaweeds and sessile animals such as ascidians and acorn barnacles from containers and nets. Good circulation of seawater may be necessary to allow sufficient feeding by the growing juveniles.

In 2000, some animals formed six or seven rays. These animals developed and grew normally into sexually mature adults. This suggests that the number of rays is not strictly regulated, but is flexible to some extent depending on the conditions of development, such as the density of individuals.

Recent delay in the spawning date

Continuous monitoring of spawning through the years at the Misaki Marine Biological Station (MMBS) revealed that the spawning date of *O. japonicus* has been delayed for approximately two weeks in recent years, compared with half a century ago. Previous reports from 1937–1955 indicated that spawning occurred between 3 and 19 Oct. (Dan and Kubota, 1960), whereas from 2000–2006 we observed spawning between 11 Oct. and 1 Nov. The animals tend to spawn after the seawater temperature decreases below 22°C (Kubota, 1988). The seawater temperature before spawning would influence the rate of sexual maturation and the timing of spawning. Although we do not have data on water temperature from 1937–1955, average seawater temperature in September and October (Table 2) was significantly higher from 2000–2006 than from 1964–1973, the oldest period for which records are available at MMBS. It is possible that an increase in the surface seawater temperature caused this delay in spawning to the next neap-tide cycle. Although we do not know the mechanism, the animals must be able to recognize the neap tide and adjust the timing of spawning.

The spawning of the cultivated animals also occured exactly in the period of a neap tide. It is unlikely that the animals felt a change in water pressure during the tidal cycle, since thy were hung from a float, and the water pressure on them should have been constant, and independent of the tide level. To clarify the mechanism of induction of spawning, other factors, such as rate of tidal currents introducing substances released from wild animals, or slight changes in gravity, should be examined.

Skeletal development

Theca

Previous studies showed that the theca in the stalked

Table 2. Comparison of monthly seawater temperatures (1 m deep) between the period from 1964–1973 and that from 2000–2006. Average seawater temperatures in March, May, September, and October for 2000–2006 were significantly lower than those for 1964–1973 ($p<0.01$; T-test).

	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	Mean	SD
Jan		14.4	14.0	13.8	13.8	14.0	13.3	14.7	15.1	11.9	13.9	0.93
Feb		12.8	14.0	12.4	12.8	11.5	12.3	12.5	12.6	12.5	12.6	0.67
Mar		13.1	13.5	12.5	12.3	12.7	11.9	11.7	12.9	12.7	12.6	0.56
Apr		13.8	15.4	15.0	14.5	15.3	13.6	14.1	15.2	17.2	14.9	1.08
May		17.9	18.1	19.1	17.6	18.7	18.2	16.8	18.5	18.3	18.1	0.67
Jun		21.7	20.9	22.3	20.7	20.8	20.2	20.1	20.9	20.4	20.9	0.70
Jul		23.0	23.3	25.0	22.7	22.9	22.4	22.9	24.0	24.1	23.4	0.86
Aug		26.2	25.3	26.6	26.3	24.9	25.1	25.9	25.6	26.2	25.8	0.59
Sept	24.0	22.9	24.8	24.5	22.8	23.7	24.3	23.3	24.0	23.6	23.8	0.68
Oct	20.7	19.5	21.2	20.2	19.5	19.7	21.7	20.1	20.9	21.4	20.5	0.82
Nov	18.3	18.2	18.6	18.3	17.1	17.4	18.7	16.7	18.3	18.0	18.0	0.67
Dec	16.4	14.2	16.7	14.9	14.9	15.2	14.6	14.6	15.1	14.2	15.1	0.87

	2000	2001	2002	2003	2004	2005	2006	Mean	SD	T-test	
Jan	13.9	14.3	11.8	12.8	13.7	13.9	12.9	13.3	0.89	Jan	0.227
Feb	12.4	12.3	11.7	11.9	12.4	13.0	11.9	12.2	0.43	Feb	0.216
Mar	13.4	13.0	13.7	12.9	13.7	13.3	13.6	13.4	0.32	Mar	0.007
Apr	14.7	15.5	17.2	15.3	16.6	15.9	14.9	15.7	0.92	Apr	0.136
May	19.7	18.9	19.6	19.6	19.3	18.4	18.7	19.2	0.49	May	0.004
Jun	21.6	21.7	21.2	22.4	22.2	21.4	21.1	21.7	0.48	Jun	0.026
Jul	25.0	25.2	23.9	22.5	25.1	23.2	22.9	24.0	1.13	Jul	0.237
Aug	26.3	26.2	26.6	25.2	26.1	26.5	25.3	26.0	0.57	Aug	0.401
Sept	25.3	24.9	25.0	24.0	25.2	24.6	24.7	24.8	0.42	Sept	0.003
Oct	22.1	21.5	21.6	20.8	22.1	21.5	22.0	21.7	0.45	Oct	0.003
Nov	17.8	17.7	16.0	18.9	19.8	19.3	18.6	18.3	1.27	Nov	0.492
Dec	16.2	15.0	15.0	15.5	16.4	14.5	14.6	15.3	0.76	Dec	0.589

stage consists of five orals, five radials, five basals, and one anal plate (Clark, 1915; Mortensen, 1920; Mladenov and Chia, 1983; Lahaye and Jangoux, 1987). In addition to these plates, a different type of plate called infrabasals was reported in some species (Mortensen, 1920). However, the number of the infrabasals in the theca remained unclear. In *Antedon bifida*, the existence of infrabasals is controversial (Mortensen, 1920; Lahaye and Jangoux, 1987). In this study, we observed the development of skeletal plates in detail and confirmed the existence of five infrabasals in the theca of *O. japonicus*, in addition to the plates mentioned above. Kubota (1969, 1970, 1988) previously observed skeletal development in the stalked stages of *O. japonicus*; however, these studies did not identify the infrabasals. The basals cover the infrabasals as the larva grows, possibly concealing the latter from external view. Although we cannot exclude the possibility that infrabasals vary in number in different species, the inconsistency in number could be due to the difficulty of observation of these plates.

Flexibility of stalk movement

The alternate direction of the ridge in the synarthries of the pentacrinoid stalk allows flexible movements of the joined columnals. The angle of the directions of the upper and lower fulcral ridges in *O. japonicus* (90°) is different from that in *A. bifida* (60°) (Lahaye and Jangoux, 1987). Further observations in different species are required to understand the variation in this angle in the evolution of crinoids, in the context of the phylogeny of stalkless crinoids reported by Rouse et al. (2006).

Arm number after sexual maturation

The number of arms of sexually mature adults two years old ranged from 17–25, which was less than in fully grown adults in the wild (approximately 40), although the length of the arms of the cultured individuals was almost the same as in individuals in the wild. We speculate that branching of the arms continues after sexual maturity until the arm number reaches up to 40. At present, we do not know how long it takes for an animal to become a fully grown adult.

Oxycomanthus japonicus as a new experimental model echinoderm

The long-term cultivation system for crinoids that we have developed allows us to provide an ample number of embryos, larvae, juveniles, and adults at any stages to zoologists with various interests in crinoids. Up to now, sea urchins have been considered as representative of echinoderms, although they have lost many features of the original echinoderm body plan, such as stalks. Our work makes it possible to use crinoids as experimental animals representative of more-basal echinoderms. We are trying to develop various experimental techniques and a laboratory cultivation system that will enable us to obtain sexually mature adults in all seasons of the year. These efforts are expected to promote further studies of crinoids as an experimental model organism.

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