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SHORT NOTE

LARVAL DEVELOPMENT OF THE SEA CUCUMBER, *ACTINOPYGA ECHINITES* (ECHINODERMATA: HOLOTHUROIDEA)[1]

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Chang-Po Chen and Ching-Sung Chian (1990) Larval development of the sea cucumber, *Actinopyga echinites* (Echinodermata: Holothuroidea). *Bull. Inst. Zool., Academia Sinica* **29**(2): 127-133. Gonad-matured adults of *A. echinites* were induced to spawn by ultra-violet light (UV)-irradiated warm seawater. The embryos hatched at 14 h after semination when reared at 25-28°C under continuous lighting of fluorescent light (100 lux). The larvae were fed with the alga *Isochrysis* aff. *galbana* at 10^4 to 10^5 cells/ml. The larvae grew to the auricularia with hydrocoel at 10 days, to the doliolaria at 15 days and to the pentactula with one podium at 16 days. The pentactula settled on the substratum and became juveniles.

Key words: Inducing spawning, UV-irradiated seawater.

The sea cucumber *Actinopyga echinites* (Jaeger) is widely distributed on shallow-water rocky plateforms of the Indo-West Pacific (Clark and Row, 1971). In New Caledonia, the gonads of *A. echinites* grow fast from September to November and then spawn annually in the warm period of January-February (Conand, 1982). During spawning, *A. echinites* elevates the anterior end to vertical position, slowly waves it and then sheds gametes from the gonopore (Shelly, 1982 in McEuen 1988; personal observation). Larvae of *A. serratidens* have been reared from naturally spawned gametes to metamorphosis, but information is fragmentary (Mortensen, 1937). Up to date, only a few planktotrophic sea cucumber larvae have been well studied (McEuen, 1987). They are commercially important species, i. e. *Stichopus japonicus* (Houkou Production Team *et al.*, 1976; Ishida, 1979) and *Parastichopus californicus* (McEuen, 1987; Cameron and Fankboner, 1989).

Reliable methods of inducing spawning have not been developed for most holothurians (McEuen, 1987). Recently, we are able to induce *Actinopyga echinites* spawn by UV-irradiated seawater. The purpose of present report is to describe larval development of *A. echinites* from fertilization to juvenile.

MATERIALS AND METHODS

Adult *Actinopyga echinites* were collected from Ao-Ta, northern Taiwan (121°55′E, 25°03′N) during the breeding seasons of June and August, 1988 and

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1989. The animals were kept in a 80 l tank which was supplied with running seawater (35‰ S, 25-27°C) under 100 lux fluorescent light for 2 to 5 days before inducing spawning.

To induce spawning, sea cucumbers were taken from the holding tank to 2 l beakers and bathed in seawater which had been warmed up to 30°C and irradiated by ultraviolet (UV) light for 1 h before the induction. The irradiation of UV was conducted with a 8 watt UV light (Aquanetics Inc, Model Q8IL) at 13.2 l/min flow rate in 10 l of sea water. Spawned mature eggs were seminated artificially and then washed with filtered seawater to remove excess sperm within 1-2 min. The procedures of culturing larvae are similar to the method reported in Chen and Run (1988). The larvae were raised in 1 l beakers containing filtered seawater (35‰ S) at 25-28°C and 100 lux fluorescent light. The alga *Isochrysis* aff. *galbana* were added to culture beakers to make algal concentration between 10^4 to 10^5 cells/ml in every morning, starting from the second day till the end of culturing experiment. Seawater was gently aerated and changed every 1 to 3 days.

The embryos, larvae and post-metamorphosed juveniles of *Actinopyga echinites* were taken from the culture beaker at appropriate intervals, observed and photographed with a Nikon compound microscope under a transmitted light, polarized or dark field.

RESULTS

Gametes shedding and early development of larvae

Actinopyga echinites increased their body movement after the shock of UV-irradiated, warm seawater. It took about 30 min for males to spawn and 1 h for females. Males released intermittent puffs of sperm through 1 to 3 gonopores and could continue releasing for 2 h. Females spawned out all eggs in 1 or 2 powerful explosive bursts through one gonopore.

Table 1
Chronology of the larval development of *Actinopyga echinites* feeding with the alga *Isochrysis galbana* at the concentration of 10^{4-5} cells/ml at 25-28°C

Developmental stage	Time
2-cell	1.5 h
4-cell	2 h
8-cell	3 h
16-cell	3.5 h
Blastula	7 h
Gastrula	20 h
Auricularia (gut divides into 3 parts)	3 d
Auricularia (hydrocoel)	10 d
Auricularia (somatocoel)	11 d
Auricularia (five lobulations of hydrocoel)	13 d
Doliolaria	15 d
Pentactula (one podium)	16 d
Juvenile (two podia)	18 d

Fig. 1. Larval development and metamorphosis of *Actinopyga echinites*. 2 min., fertilized egg surrounded by the fertilization membrane (FM), N: nucleus breakdown; PB: polar body. Bar indicates 100 μm for all figures.

Fig. 2. 1.5 h, 2-cell stage embryo, dark field.

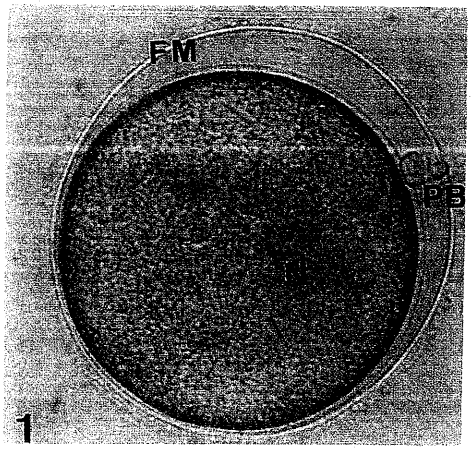
Fig. 3. 3 h, 8-cell stage embryos.

Fig. 4. 3 day, early auricularia, focused at ventral surface, AL: anal lobe; POL: preoral lob.

Fig. 5. same individual as above but focused at middle, E: esophagus; HP: hydropore; O: ossicle; S: stomach.

Fig. 6. 13 day, late auricularia, many sinuous extention and spherules (S) surrounded the body; dorsal view.

Fig. 7. Enlargement of the previous one, H: hydrocoel; L: left somatocoel; R: right somatocoel.



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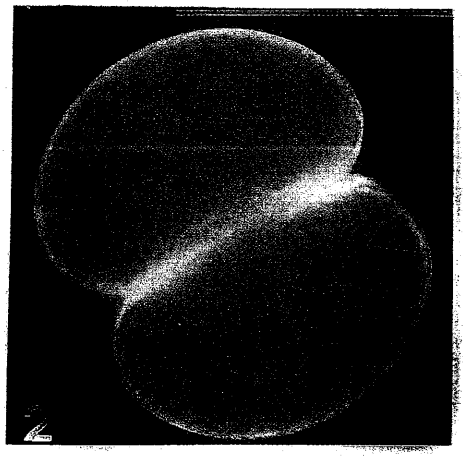
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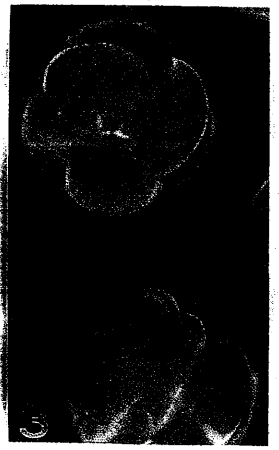
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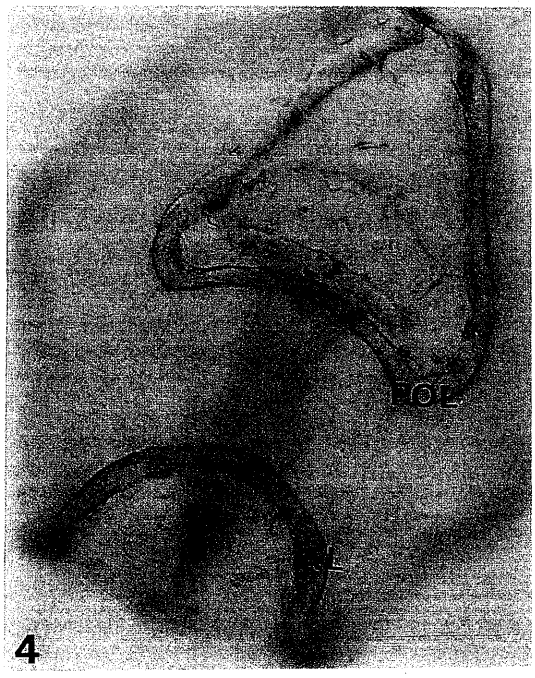

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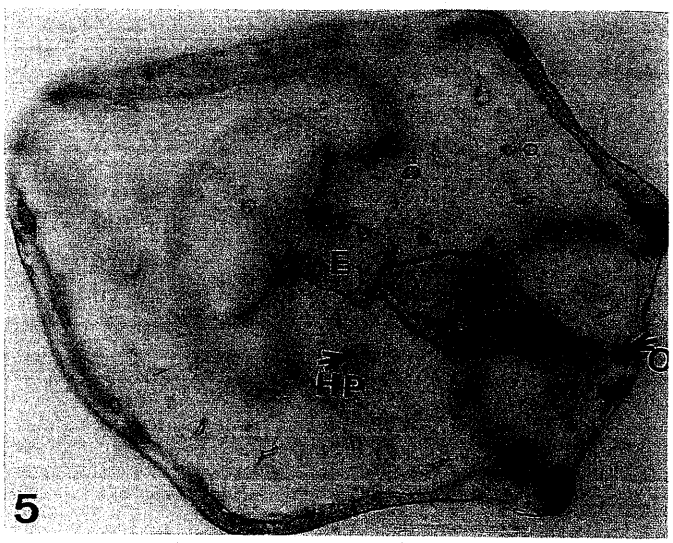

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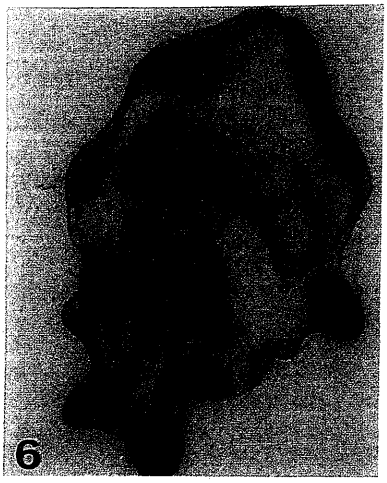
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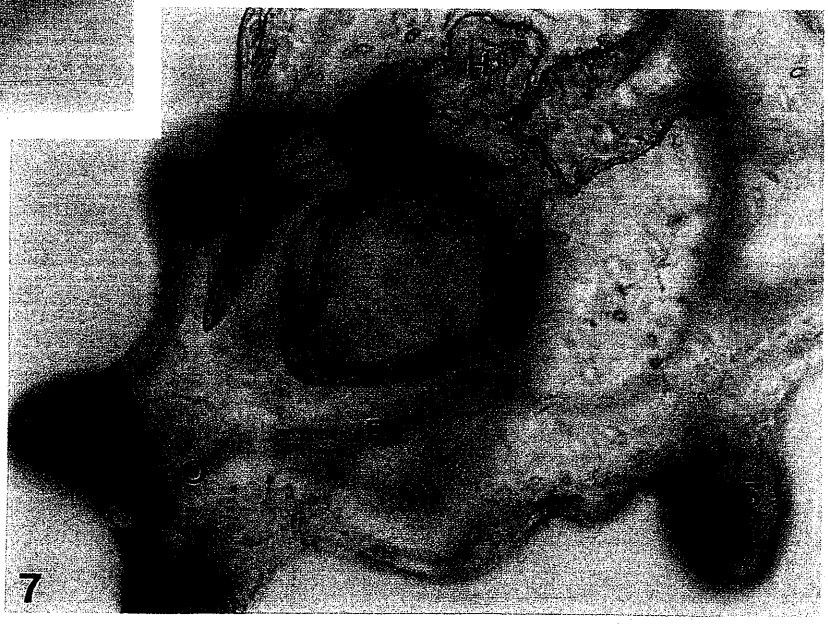
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The chronology of larval development of *Actinopyga echinites* at 25-28°C is presented in Table 1. The living unfertilized egg, was opaque, red, homolecithal, sunken to the bottom and about 140 μm in diameter. A fertilization membrane was formed a few minutes after insemination. Polar bodies were then extruded through oocyte surface (Fig. 1). The first cleavage was completed 1.5 h after fertilization (Fig. 2) and the third equilateral cleavage plan was visible at 3 h (Fig. 3). About 12 h after fertilization the embryo began to spin within the fertilization membrane. Embryos hatched from fertilization membrane 14 h after insemination. As gastrulation proceeded, the larva became more dorsal-ventrally flattened, and mesenchymal cells began migrating from the archenteron tip into blastocoel. The hydroporic canal opened on the dorsal surface as the hydropore at 48 h. At 3 d the larva developed to typical auricularia with the appearance of a single looped band of oilia. A preoral lobe covered the anterior mid-ventral stomach and anal lobe covered the posterior mid-ventral stomach (Fig. 4). The gut was divided into three parts: oesophagus, stomach and intestine (Fig. 5), and rhythmic contraction and relaxation of stomach commenced. Calcareous ossicles, characterized by irregular star shape, were also observed in the center of the rear of the larvae (Fig. 5).

Late development and metamorphosis of the larvae

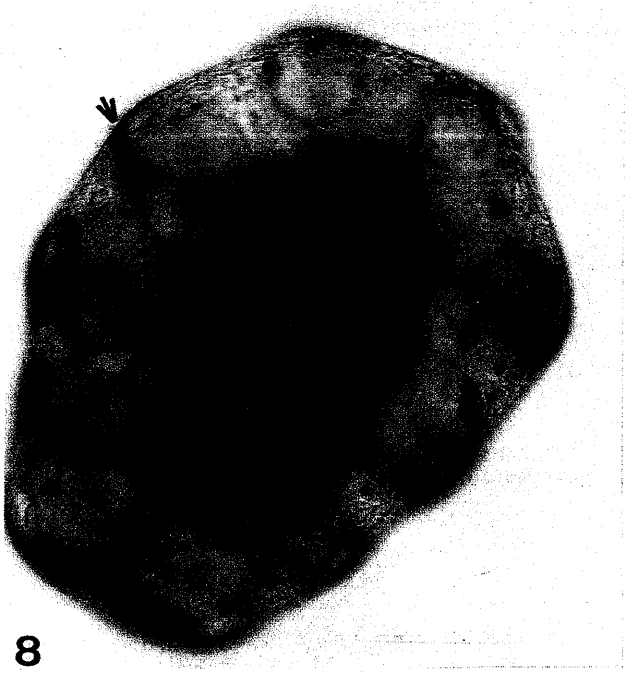
The late auricularia of *Actinopyga echinites* was about 800 μm long and 250 μm wide in well-developed one, but with considerable variation in size among individuals. Further growth along the margins of the body produced more sinuous extentions over which the continuous ciliary band was looped. The hydrocoel appeared at 10 d and the somatocoel first observed at 11 d. The hydrocoel formed 5 lobulations at 13 d and the spherules associated with each of the lateral lobes were conspicuous (Figs. 6, 7). At about 15 d, metamorphosis resulted in a brown compact barrel-shaped doliolaria that body length diminished and 5 transverse rings of cilia appeared (Fig. 8). The pentactula larva was observed at 16 d that primary tentacles pushed out through the oral indentation and a single ventroposterior podium lengthened (Fig. 9). Finally, the tips of the tentacles attached to the substratum and commenced the benthic life. Two podia juvenile was first presented at 18 d (Figs. 10, 11). At this time, five tentacles existed outside the body and adult ossicles of needle, star and irregular network shapes embeded in the tentacles and body wall (Fig. 12).

DISCUSSION

The developmental pattern of embryo and larvae of *Actinopyga echinites* is similar to that of another aspidochirote holothurians which have a planktotrophic larval phase (Montensen, 1937; Hyman, 1955; Ishida, 1979; Maruyama, 1980; McEuen, 1987).

UV-irradiated seawater induces spawning of *Actinopyga echinites* (present study) and the abalone, *Haliotis discus hannai* (Kikuchi and Uki, 1984). However, the mechanism of inducing spawning is still unknown. The oocytes of holothurians remain in the first prophase of meiosis until spawning (Holland, 1981).

Fig. 8. 15 day, doliolaria with many conspious orange spherules (S), arow: cilliary tuft.
Fig. 9. 16 day, pentactula with tentacles (T) and first podium (P).
Figs. 10, 11, 12. 18 day, juvenile with five tentacles (T) and two podia (P) observed with low magnification, brightfield or polarized light, AO: adult ossicle.



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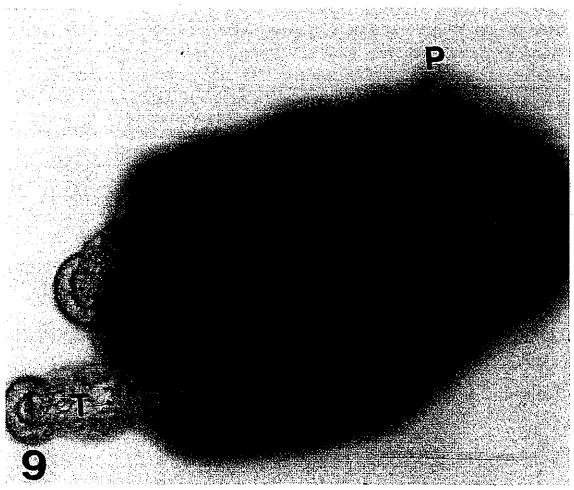

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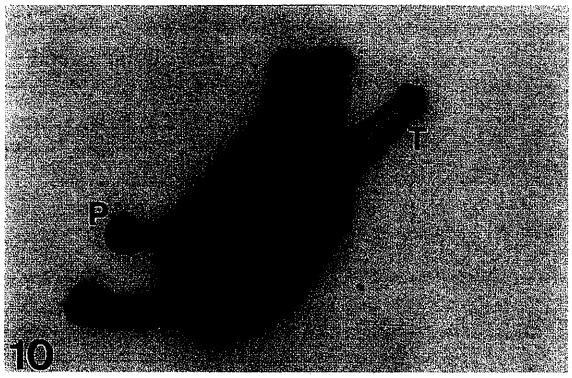

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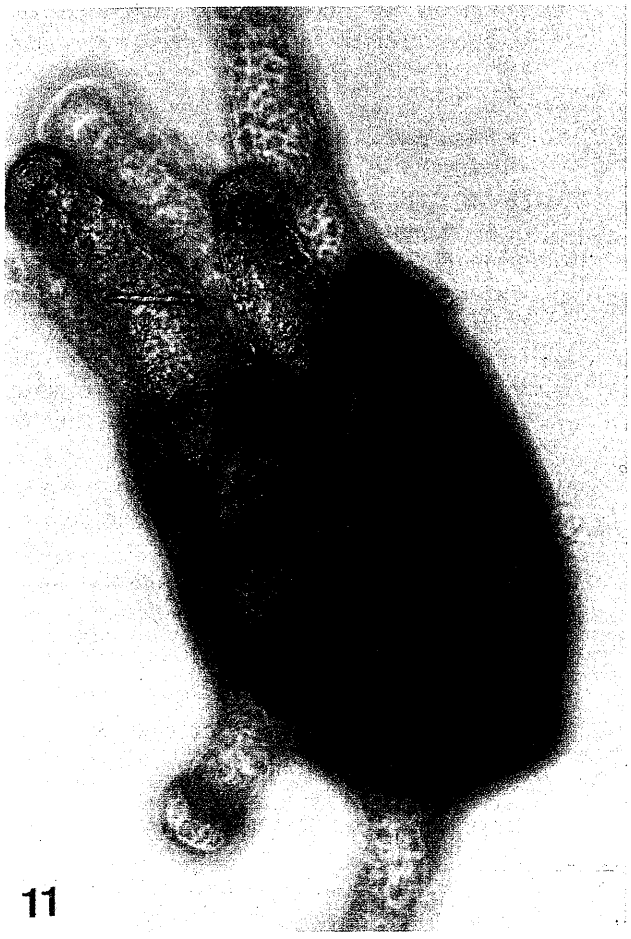

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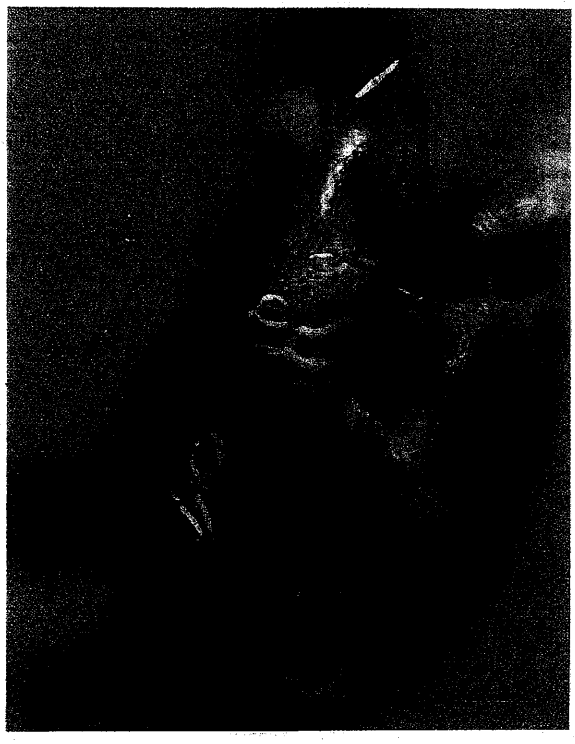

The maturation of oocytes is enhanced by the gonad-stimulating substance (GSS) of radial nerve (Maruyama, 1985) and the maturation inducing substance (MIS) of oocytes (Smiley, 1988). The relationship between UV-irradiated warm seawater and these maturation inducers of GSS and MIS needs more studies.

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棘輻肛參 (*Actinopyga echinites*) 之幼生發育

陳 章 波　　江 建 嵩

生殖腺成熟的棘輻肛參經紫外線照射及加溫之海水處理後誘引產卵或排精。胚胎飼育於日光燈 (100 lux) 照明，25-28°C 之水槽中。受精後 14 小時孵化。幼生餌以浮游藻 *Isochrysis* aff. *galbana* (10^{4-5} cells/ml)。於第 10 天，幼生發育至有水腔 (Hydrocoel) 的耳幼生 (auricularia)，第 15 天變態成樽形幼生 (doliolaria)，於 16 天長出有一管足的觸手幼生 (pentactula)，而後營底棲之稚海參。