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Reproduction and Development of a Miniature Sand Dollar, *Sinaechinocyamus mai* (Echinodermata: Echinoidea) in Taiwan[1]

Bih-Yuh Chen and Chang-Po Chen[2]

Institute of Zoology, Academia Sinica,
Nankang, Taipei, Taiwan 115,
Republic of China

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Bih-Yuh Chen and Chang-Po Chen (1993) Reproduction and development of a miniature sand dollar, *Sinaechinocyamus mai* (Echinodermata: Echinoidea) in Taiwan. *Bull. Inst. Zool., Academia Sinica* **32**(2): 100-110. The reproduction biology of the miniature sand dollar *Sinaechinocyamus mai* (Wang) was examined from June, 1990 through October, 1991 at Tunghsiao in western Taiwan. Monthly measurements of gonad indexes plus histological examinations of both gonads and capacity for spawning induction reveal that *S. mai* has an annual reproductive cycle which includes spawning in October and November. During gametogenesis, the degree of development of nutritive phagocytes varies inversely with the number of differentiating gametes. The mature eggs of *S. mai* are white in color and 120 μm in diameter. Red pigment granules were found in the gelatinous coating of the eggs. Early cleavages were found to be equal, radial, and holoblastic. *Sinaechinocyamus mai* produces planktotrophic larvae which metamorphose completely nine days after fertilization when reared in seawater (33‰ S) at 25~28°C.

Key words: Sand dollar, Reproductive cycle, Embryogenesis, Larval Development.

Sinaechinocyamus is a genus of extremely small clypeasteroids with only two extant species; *S. planus* Liao occurs in Yellow Sea off the China coast (Liao 1979), and *S. mai* (Wang) occurs only off the coast of western Taiwan (Wang 1984). *Sinaechinocyamus mai* commonly occurs in the intertidal and nearshore subtidal waters of western Taiwan (Wang 1984); it never exceeds 11 mm in length.

Since adult *Sinaechinocyamus mai* closely resemble juveniles of the genus *Scaphechinus* in morphology, Mooi (1990) has suggested that *Sinaechinocyamus* is a progenetic miniature form derived from *Scaphechinus. Scaphechinus* occurs in waters surrounding Japan (Nisiyama 1968), and has been found in Pliocene fossil form in Taiwan (Wang 1984).

However, no data are available on the reproductive biology of *Sinaechinocyamus*. Therefore, we have researched *Sinaechinocyamus mai* reproductive biology from Taiwan specimens, including reproductive periodicity, embryogenesis, and larval develop-

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2. To whom reprint requests should be sent.

ment. The developmental events of *S. mai* are compared with those previously observed in some clypeastorids, and the reproductive strategy of *S. mai* is discussed.

MATERIALS AND METHODS

Reproduction

Sinaechinocyamus mai specimens were collected monthly from June, 1990 through October, 1991 from the low tide zone of Tunghsiao (120°39'45"E; 24°29'21"N), near Miaoli in western Taiwan. Individuals were collected from sand with a 0.5 mm mesh screen sieve. Specimens were fixed and preserved in 10% neutral formalin. Specimen lengths were measured as the longest diameter through the anus. The collected sand dollars were blotted dry with absorbent paper and weighed. Testes were dissolved with dilute HCl, after which gonads were removed, blotted, and weighed with a Mettler AE 240 balance to the nearest 0.01 mg. Gonad index was calculated as the ratio of gonad weight to body length x 10^4.

After weighing, gonads were fixed in Bouin's solution for histological analysis. Paraffin sections (5 μm thick) were stained with hematoxylin and eosin. The diameters of fifty oocytes sectioned through the nucleolus were measured for each female.

Development

Adults were collected during the spawning season (October, 1990). To induce spawning in the laboratory, 0.1 ml 0.5 M KCl was injected into the coelom through the peristomial membrane. Mature eggs were then fertilized artificially and washed with 0.45 μm filtered seawater. Larvae were reared in 250 ml glass bowls containing seawater (33‰ S) at 25~28°C under continuous light (200 lux); seawater was changed daily. Following the methods described by Chen and Run (1989) developing larvae were fed daily with 10^4 cells/ml of cultured *Isochrysis galbana*. *S. mai* embryos, larvae, and post-metamorphosed juveniles were observed and photographed with a Nikon compound microscope camera. Following the methods of Chen and Chen (1992) the time taken to attain any single developmental stage was defined as the time at which more than half the embryos reached that stage.

RESULTS

Reproduction

Gonad index cycle

The *Sinaechinocyamus mai* adults collected for this study had a mean body weight of 6.4 $\pm$ 2.7 mg ($\pm$ 1 SE, n = 184) and mean body length of 7.24 $\pm$ 1.06 mm. The gonad index of *S. mai* increased sharply from July to September, 1989, reached a peak in September, and then decreased rapidly (Fig. 1a). The gonad index also peaked in September of the following year. It should be noted that in western Taiwan, the east-north winter monsoon creates harsh conditions for intertidal animals; therefore during this season, the population size of *S. mai* in intertidal pools decreases. Thus, no individuals were collected from January to March, 1991. The gonad of one individual collected in April, 1991 was too small to be weighed.

Gametogenic cycle

The gonads we inspected were filled with many nutritive phagocytes (eosinphile globules), as well as small oocytes (between 15 μm and 30 μm in diameter) occurring along the periphery epithelia (June and July, 1990) (Figs. 1b, 2a). As gametogenesis progressed, the small oocytes increased in size

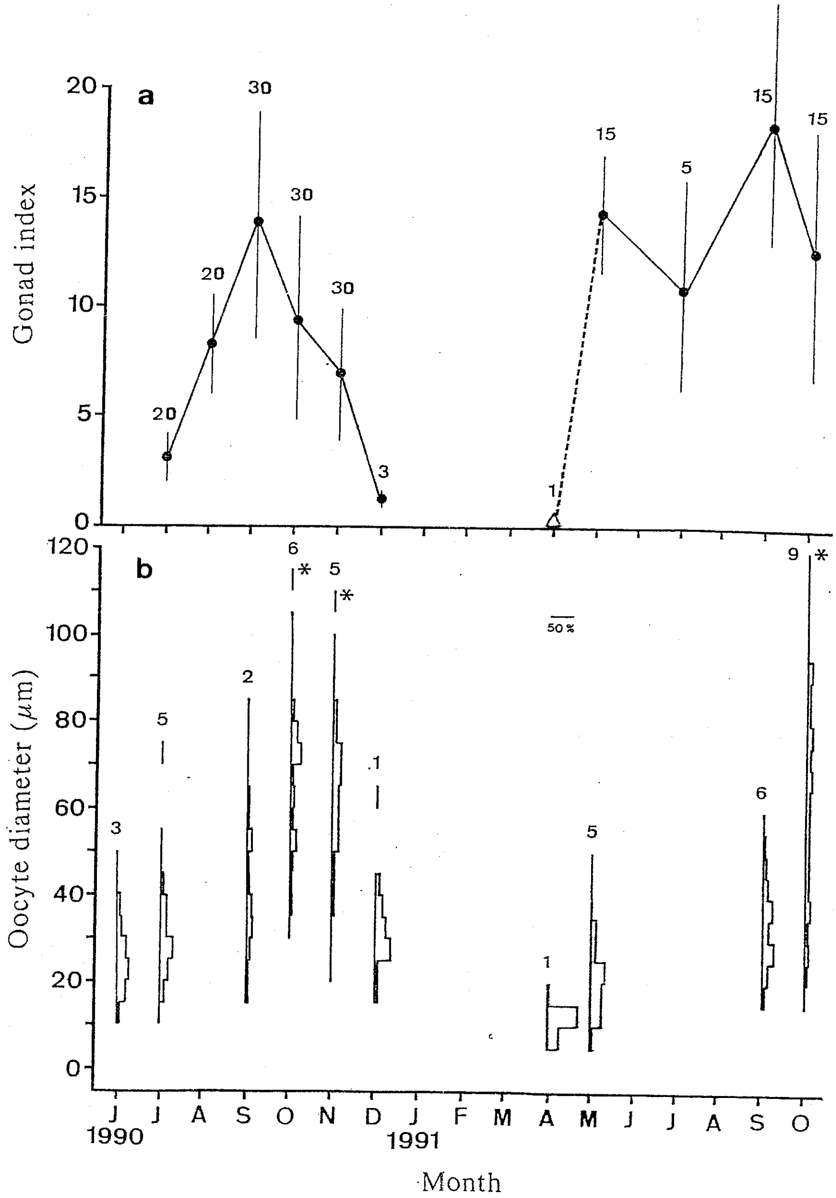

Fig. 1. Reproductive cycle of *Sinaechinocyamus mai* showing monthly changes in mean gonad indices (a) (mean and one sd are given) and size-frequency distribution of oocytes (b). Numbers indicate the number of individuals examined. Asterisks indicate spawning induction success.

and moved away from the germinal epithelium. Oocyte size increased greatly in September (Fig. 2b), and large oocytes (between 50 μm and 80 μm) were observed in *S. mai* gonads from October to November, 1990 (Fig. 1b). As oocytes matured along the ovarian wall, the size of nutritive phagocytes decreased. At this time it was possible to induce individuals to spawn. In December, 1990 we observed residual oocytes being resorbed; only very few nutritive phagocytes were still present within the gonads at this time (Fig. 2c). However, only residual oocytes were found in the gonads in April, 1991 (Fig. 1b). Small oocytes again occurred along the periphery epithelia of gonads in May, 1991 (the beginning of the next reproductive cycle).

The male and female gametogenic cycles observed were similar. In the first stage, nutritive phagocytes were present in the testes (Fig. 2d); the number of spermatogonia increased as the size of nutritive phagocytes decreased. The spermatogenic cells in the testes merged, thereby completely lining the base of the germinal epithelium (Fig. 2e). In the spawning stage, sperm aggregated and filled the testis lumen (Fig. 2f).

Embryonic and larval development

Eggs were spherical, 120.7 ± 3.9 μm (n = 30) in diameter, and coated with a gelatinous cover containing red pigment granules (Fig. 3a). Table 1 presents the chronology of larval development in *Sinaechinocyamus mai* at 25~28°C. Cleaving was holoblastic and radial, with the first cleavage occurring at 1 hour (Fig. 3b) and the second occurring 1.5 hours after fertilization. The 8, 16, and 32-cell stages were recorded 1.8, 2.8, and 3 hours after fertilization, respectively. Eggs underwent a series of rapid cleavages to form an early blastula 3.5 hours after fertilization (Fig. 3c). Initial gastrula rotated within fertilization membranes 6 hours after fertilization.

S. mai larvae are planktotrophic and in need of suitable food supplies in order to develop. The first pair of arms (the postoral arms of the pluteus) appeared 30 hours after fertilization (Fig. 3d). At this stage, a complete gut was formed, allowing the larvae to actively feed. On the second day the second pair of arms (the anterolateral arms of the pluteus) appeared (Figs. 3e, 3f), and the third pair of arms (the postdorsal arms of the pluteus) appeared during the third day (Fig. 4a). Also during the third day, the hydrocoel was distinguishable on the left side of the pluteus (Fig. 4a). Four days after fertilization, each larva became a fully-developed pluteus with the appearance of a pair of preoral arms (Fig. 4b). Eight days later, urchin rudiments became visible through the larval body wall on the left side, and tube feet were observable (Fig. 4c). After nine days, the pluteus completed its metamorphosis (Figs. 4d, 4e).

DISCUSSION

Reproduction

The gonad index, histological characteristics, and spawning induction capacity of *Sinaechinocyamus mai* in the Tunghsiao area indicate that there is one major annual spawning period which occurs in the fall (October-November). The gonad index is the quantitative method most widely-used for estimating reproductive activity (Giese and Pearse 1974). Although the gonad index of *Sinaechinocyamus mai* peaked in September, *S. mai* oocytes only reached full size in October. As Giese and Pearse (1974) suggested, a gonad index is more successful in estimating reproductive changes in a population when combined with a histological analysis of the gonads plus observations of spawning.

Gametogenic cycle

Gametogenesis characteristics in *Sinaechinocyamus mai* are similar to those of most echinoids (Fuji 1960, Pearse and Giese 1966, Pearse 1970, Lane and Lawrence 1979). In *S. mai*, the presence of small oocytes does not indicate the beginning of oocyte growth, which is abrupt and easily observed. *S. mai* eggs matured within two to three months following the initiation of

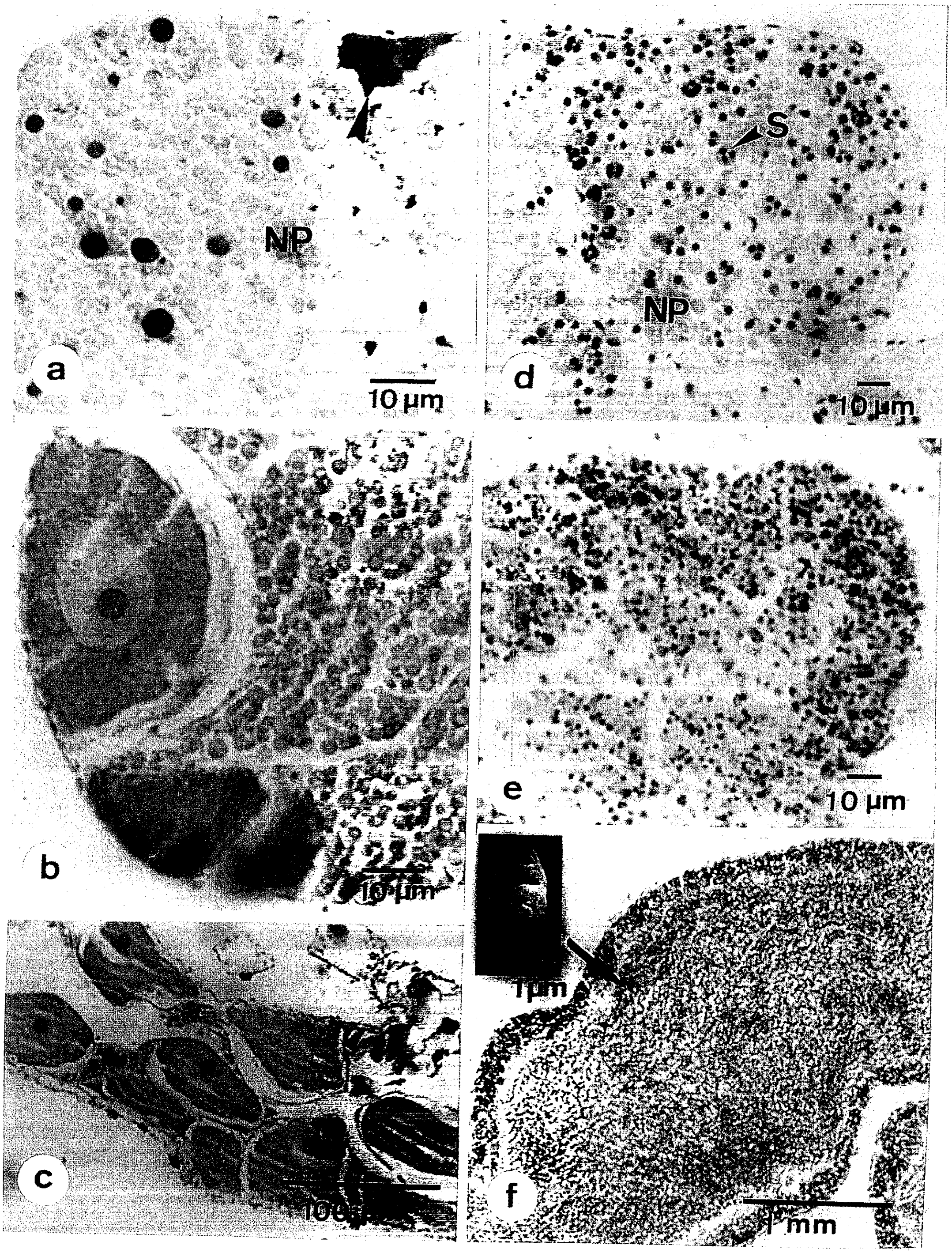

Fig. 2. Histological sections of *Sinaechinocyamus mai* gonads stained with hematoxylin and eosin. (a): a newly-developing oocyte (arrow) and nutritive phagocytes (NP) are fully within the gonad tubule (July, 1990); (b): small oocytes occurring near the peripheral epithelium of the ovary tubule (September, 1990); (c): mature oocytes (October, 1990); (d): developing spermatocytes (S) in testes filled with nutritive phagocytes (July, 1990); (e): developing spermatocytes (September, 1990); (f): mature sperm in the lumen of a specimen (October, 1990). The morphology of the sperm can be seen in the upper left corner of the SEM photo.

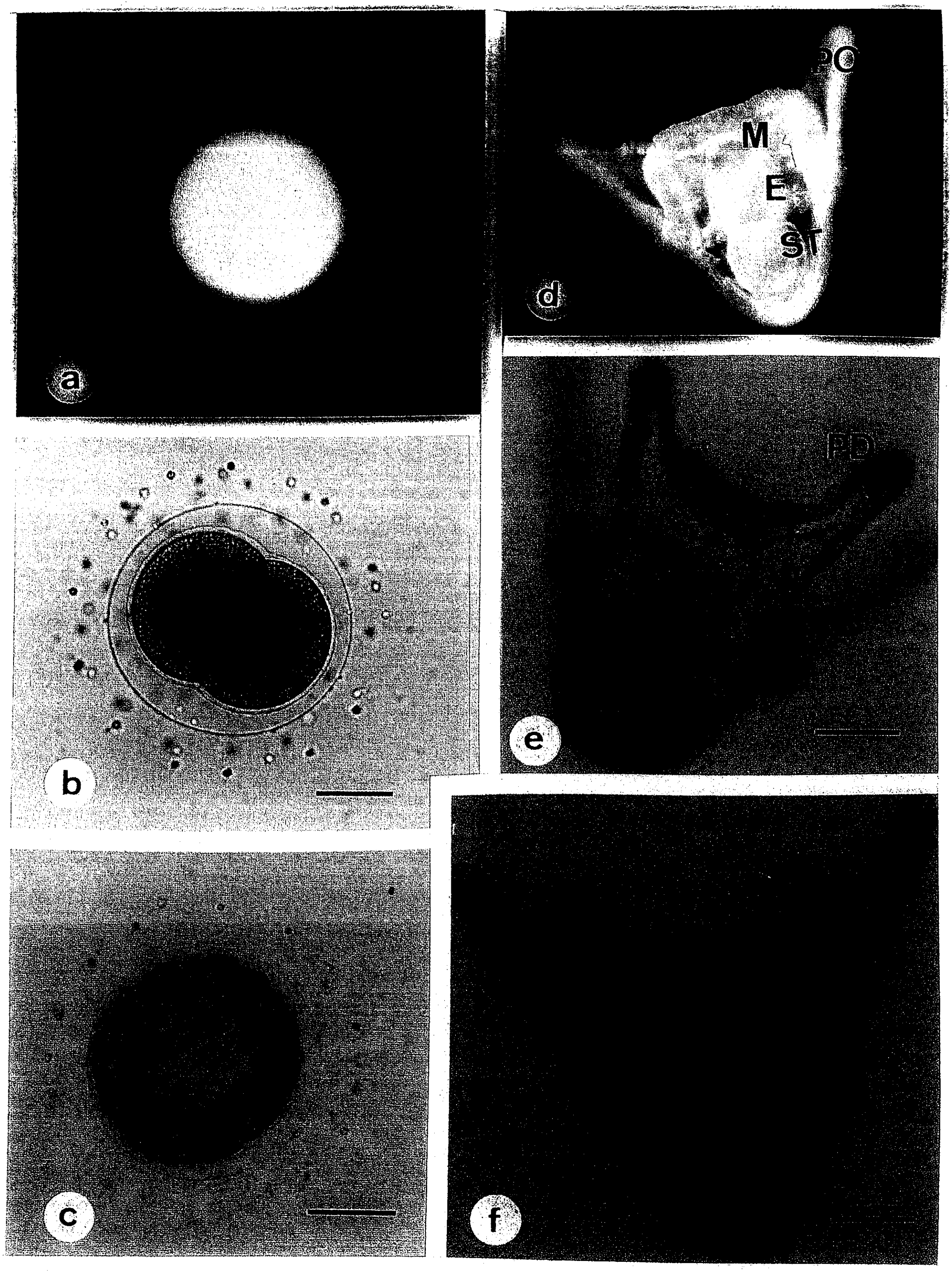

Fig. 3. Development of *Sinaechinocyamus mai*. (a) fertilized egg showing F: fertilization membrane and P: pigment granule; (b) 1st cleavage; (c) blastula embryo (6h); (d) 2-armed pluteus with a completed gut (30h), PO: postoral arm, E: esophagus, M: mouth, ST: stomach; (e) front view of 4-armed pluteus (2 days). PD: postdorsal arm; (f) hind view of 4-armed pluteus (2 days). Scale bars = 100 μm. a, d = dark field; b, c, e, f = transmitted light.

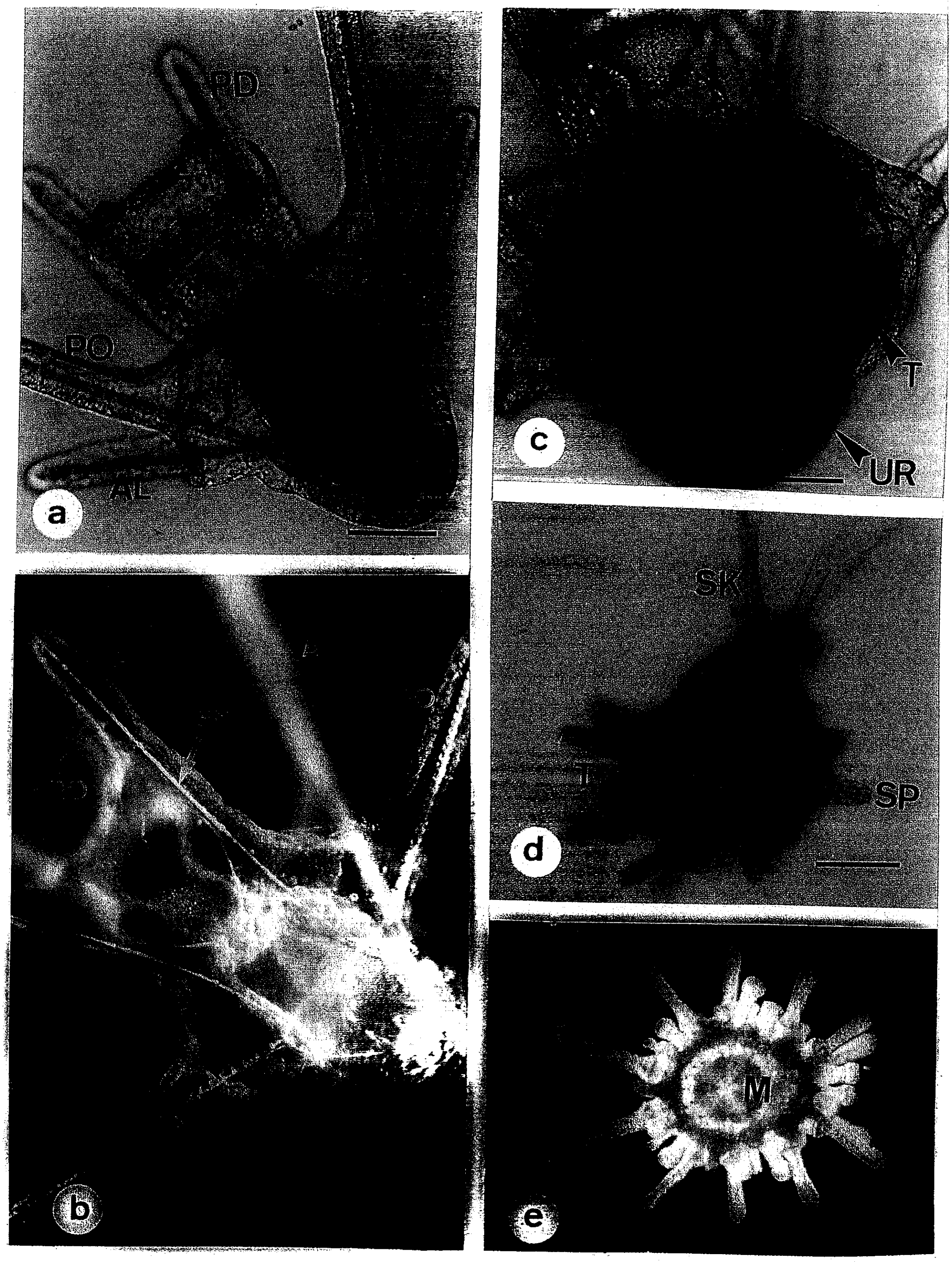

Fig. 4. Continued development of *Sinaechinocyamus mai.* (a) 6-armed pluteus (3 days), AL: anterolateral arm, H: hydrocoel, PD: postdorsal arm, PO: postoral arm; (b) 8-armed pluteus (4 days), PRO: preoral arm, SK: skeleton; (c) competent larva and well-developed urchin rudiment (UR) is formed by the seventh day, T: tube feet; (d) a metamorphosing larva (8 days), SK: skeleton, SP: spine; (e) a newly-metamorphosed juvenile (9 days), M: mouth. Scale bars = 100 μm. a, c, d = transmitted light; b, e = dark field.

Table 1. Chronology of *Sinaechinocyamus mai* development at 25~28°C in the laboratory.

Developmental stage	Time	
2 cells	1	hour
4 cells	1.5	hours
8 cells	1.8	hours
16 cells	2.8	hours
32 cells	3	hours
Blastula	3.5	hours
Gastrula	6	hours
Pluteus	30	hours
4 arms	2	days
6 arms	3	days
8 arms	4	days
Urchin rudiment (tube feet)	7	days
Metamorphosis	9	days

oocyte growth, and were then maintained for three months before spawning. Although male gametogenesis is difficult to measure in *S. mai*, the maturation of sperm from primary spermatocytes is more rapid—approximately one month.

Nutritive phagocytes, which store nutrients before gametogenesis, are usually large in size. Many investigators have suggested that nutritive phagocytes store nutrients for use during gametogenesis both in PAS-positive, eosinphile globules and as cytoplasmic glycogen (Fuji 1960, Cathlynne 1969, Lane and Lawrence 1979; also see Walker 1982). Nutritive input to these specially-adapted storage cells occurs both prior to and during gametogenesis (Takashima 1976). Studies by Moss and Lawrence (1977) on *Mellita quinquiesperforata* indicate peaks in the biochemical constituents (lipid, protein) of coelomic fluid and gonads preceeding gametogenesis. Such results can be interpreted as evidence of nutrient supply and storage by nutritive phagocytes. However, there are no ultrastructural studies available on the cytology of nutritive phagocytes.

Larval development

Most marine invertebrates that produce planktonic larvae also produce numerous small eggs which develop into planktotrophic larvae; several marine invertebrates, however, produce a few large eggs that develop into lecithotrophic larvae (Emlet *et al.* 1987). The egg sizes, developmental modes and geographical distribution of eleven species of clypeasteroids are given in Table 2. The eggs of these clypeasteroids are small compared with other species of echinoids (Emlet *et al.* 1987). Most clypeasteroids have small eggs (100~150 μm in diameter) and produce planktotrophic larvae; these are two exceptions: *Clypeaster rosaceus*, which has larger eggs (280 μm), facultative planktotrophic larvae, and observable metamorphosis on the 7th day following fertilization (Emlet 1986), and *Peronella japonica*, which has larger eggs (300 μm), lecithotrophic larvae, and an observable metamorphosis at 2.5 day after fertilization (Okazaki 1975). The time needed for the development of clypeasteroid larvae increases with latitude. The size of eggs, developmental mode, developmental time, and size at metamorphosis of the miniature sand dollar *Sinaechinocyamus mai* are typical when compared to those of the large sand dollar *Arachnoides placenta* (Felician 1933, Huang and Chen 1989) and *Mellita quinquiesperforata* (Caldwell 1972).

Reproductive strategy

S. mai has a distinct, synchronized seasonal spawning period (in October-November) and larvae that are planktotrophic. Environmental factors considered important for the synchronization of reproductive periodicities in marine animals include sea temperature (Crump 1971, Chen

Table 2. Developmental mode, larval development, and latitudinal distribution of Clypeasteroida. Species order follows the latitudinal increase in home range.

Species	Egg diam (μm)	Devel. mode*	Lat (°)	Temp (°C)	Devel. time[@]	Metam. size[#] (μm)	Reference
Clypeaster rosaceus	280	P-L	10N	27	7d	340	Emlet 1986
Clypeaster subdepressus	152	P	10N	27	16d	340	Emlet 1986
Arachnoides placenta	110	P	15N		10d		Felician 1933
	~120	P	24N	26	7d	260	Huang & Chen 1989
Sinaechinocyamus mai	120	P	24N	25-28	9d	~300	Present study
Laganum depressum	100	P	27N	28	2wk	310	Emlet *et al.* 1987
Mellita quinquiesperforata	150	P	29N	27	7-9d	350	Caldwell 1972
Peronella japonica	300	L	34N	28	2.5d	312	Okazaki & Dan 1954 Okazaki 1975
Scaphechinus tenuis	100	P	34N				Emlet *et al.* 1987
Dendraster excentricus	120	P	37N	15	3wk	360	Emlet *et al.* 1987
Echinarachnius parma	145	P	41N	10	4-5wk	375	Emlet *et al.* 1987
Echinocyamus pusillus	100	P	58N		45d	260	Emlet *et al.* 1987

*Developmental mode: P, planktotrophy; L, lecithotrophy; P-L, facultative.
[@]Developmental time: d, days; wk, week.
[#]Size at metamorphosis: test diameter.

and Chang 1981), photoperiod (Pearse and Ernisse 1982), salinity (Giese and Pearse 1974), tide (Pearse 1972), lunar cycle (Pearse 1990), food (Boolootian 1966) and the occurrence of phytoplankton blooms (Starr et al. 1991). In our present study, photoperiod did not correspond with gametogenesis, and salinity varied within 2‰ during the study period; in addition, planktonic algae occurs abundantly every September in western Taiwan (Huang 1986). Therefore, these factors cannot be connected with reproduction. However, the sea temperature at Tunghsiao does show seasonal variation: the average high temperature (31°C) occurs between May and August, and the average low temperature (18°C) occurs between December and March (Chen and Chen 1991). However, the gonad index, size of oocytes, and timing of spawning all corresponded with sea temperature variation, thus suggesting that sea temperature may be the major factor affecting the synchronization of spawning in *S. mai* in the Tunghsiao area.

Sinaechinocyamus mai larvae have a short pelagic development period (9 days) which may decrease the chances of mortality due to temperature shock, predators, or food shortages. In winter, the temperature sometimes drops abruptly to 14°C (Chu 1971); individuals have not yet been found in nearshore subtidal zones during winter. Small *S. mai* (ca. 3.5 mm in diameter of body length) with gonopores recruited abundantly in April and May (Chen and Chen 1991). In such harsh conditions, it is not known how larvae and newly-metamorphosed juveniles survive. Whether or not *S. mai* migrates or burrows in order to avoid unfavorable environmental conditions requires further study.

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小型化海錢，馬氏海錢 *Sinaechinocyamus mai* (Wang) 的生殖與發生

陳碧玉　陳章波

馬氏海錢 *Sinaechinocyamus mai* (Wang) 自 1990 年六月至 1991 年十月，採自臺灣西部潮間帶低潮線之沙地。從每月測量生殖腺指數、檢查組織切片和人工誘引產卵，可確定馬氏海錢的生殖季在十～十一月。在配子發育期間，營養吞噬細胞的量與配子分化的程度及數量呈相逆關係。馬氏海錢的卵直徑大小約 120μm。在卵膠囊上出現許多紅色的色素顆粒。早期的卵割爲完全、對等和輻射分割。馬氏海錢的幼生屬浮游物營養型，在室溫 25～28°C，海水鹽度 33‰的環境下飼育，幼生在受精後第九天完成變態。