

## The Smallest Oocytes among Broadcast-Spawning Actiniarians and a Unique Lunar Reproductive Cycle in a Unisexual Population of the Sea Anemone, *Aiptasia pulchella* (Anthozoa: Actiniaria)

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**Chienhsun Chen, Keryea Soong, and Chaolun Allen Chen (2008)** The smallest oocytes among broadcast-spawning actiniarians and a unique lunar reproductive cycle in a unisexual population of the sea anemone, *Aiptasia pulchella* (Anthozoa: Actiniaria). *Zoological Studies* 47(1): 37-45. *Aiptasia pulchella*, an aquarium species and invasive pest, is used as a common model actinarian for laboratory-based experiments to study environmental regulation of asexual reproduction, mutualism of cnidarian-dinoflagellate intracellular symbiosis, and cnidarian bleaching mechanisms. In this study, gametogenesis and the reproductive cycle of *A. pulchella* were studied in a population from the outlet of Tungkang Marine Station, southern Taiwan using histological sections. Samples were taken on a monthly basis in 1993 and 1994, and at 6 d intervals in Aug. to Oct. 1997. Additional sampling was conducted in 1998 to examine the relationship between sexuality and body size. Among the 211 individual sea anemones examined, only females ( $n = 173$ ) and non-reproductive ( $n = 38$ ) anemones were observed; no male individuals were recorded throughout the course of these sampling periods. Gonads of *A. pulchella* females were located in one of the paired primary mesenteries and two of the paired secondary mesenteries. The body size of *A. pulchella* was significantly larger in individuals with developed gonads in both primary and secondary mesenteries compared with those with developed gonads only in the primary mesenteries and with sterile individuals. The mature oocytes of *A. pulchella*, which ranged 60-100  $\mu\text{m}$  in diameter, are the smallest recorded among broadcasting actiniarians. Gametogenesis in this unisexual population of *A. pulchella* followed the lunar cycle, and spawning occurred between the 24th and 29th lunar days year round, which is also first reported for actiniarians. <http://zoolstud.sinica.edu.tw/Journals/47.1/37.pdf>

**Key words:** Gametogenesis, Unisexual population, *Aiptasia pulchella*, Lunar cycle, Smallest oocyte.

Sea anemones exhibit various patterns of sexual reproduction and even different breeding patterns among species of the same genus (reviewed in Stephenson 1928, Shick 1991, Fautin 1992). Revealing the sexual reproduction of these primitive invertebrates will not only provide useful information for understanding the evolution of reproductive adaptations, but also help understand a basic model system in modern biology (reviewed in Darling et al. 2005).

Sexual reproduction has been studied using histological methods in about 30 species or sub-

species of sea anemones since 1976 (Table 1). Most of the examined species are gonochoristic and exhibit an annual reproductive cycle in nature (Tables 1, 2). In contrast, a biannual reproductive cycle was noted for *Actinia equina* (Chia 1976), *Batholomea annulata* (Jennison 1981), *Gonactinia prolifera* (Chia et al. 1989), and *Sagartia troglodytes* (Shaw 1989), while a prolonged reproductive cycle was observed in *Actinoporus elongates* (Clayton and Collins 1992). It has been suggested that sexual reproductive cycles of sea anemones are influenced by various annual

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changes in environmental factors, such as water temperature (Jennison 1979, Wedi and Dunn 1983, Shaw 1989, Lin et al. 1992a) or food availability (Lin et al. 1992a). The influences of some of these environmental factors have been verified in laboratory experiments. For example, *Anthopleura dixoniana* sea anemones maintained with high feeding frequencies had significantly larger oocytes than those maintained with low feeding frequencies or in starved conditions (Lin et al. 1992a). Furthermore, other environmental factors such as a higher light intensity and longer photoperiod also enhanced the growth of oocytes (Lin et al. 1992a, Chen 2000). Under laboratory

conditions, a weekly sexual reproduction cycle was induced in the sea anemone, *Nematostella vectensis*, by the presence of light, high temperatures, and increased amounts of food, although an annual sexual reproduction cycle is reportedly exhibited in natural populations (Hand and Uhlinger 1992, Fritzenwanker and Technau 2002). Recently, because of its high frequency of sexual reproduction in optimal laboratory conditions, *Nematostella* has become a new model system in developmental biology (Magie et al. 2005), genomics (Sullivan et al. 2006), and evolution (reviewed in Darling et al. 2005).

*Aiptasia* spp. have a worldwide distribution on

**Table 1.** Review of the sexual reproduction of sea anemones (Order Actiniaria): 1, broadcast spawning; 2, external brooding; 3, internal brooding; F, female only; G, gonochoristic; H, hermaphroditic; n.a., not available

Suborder	Family	Species	Sexuality	Sexual reproduction	Mature egg diameter (μm)	Sources
Protantheae	Gonaactiniidae	<i>Gonactinia prolifera</i>	G	3	60-80	Chia et al. 1989
Nynantheae	Edwardsiidae	<i>Nematostella vectensis</i>	G	1	170-240	Hand and Uhlinger 1992
	Aiptasiidae	<i>Aiptasia pulchella</i>	F	1	60-80	present study
		<i>Bartholomea annulata</i>	H	1	150-200	Jennison 1981
	Holoclavidae	<i>Peachia quinquecapitata</i>	G	1	120	Spaulding 1974
	Hormathiidae	<i>Amphianthus inornata</i>	G	1	100-150	Bronsdon et al. 1993
		<i>Paracalliactis stephensoni</i>	H	1	150-180	van-Praet 1990
	Metridiidae	<i>Metridium senile</i>	G	1	150	Bucklin 1982
	Sagartiidae	<i>Cereus pedunculatus</i>	H	3	130-150	Shaw 1989
		<i>Kadosactis commensalis</i>	H	1	n.a.	Bronsdon et al. 1993
		<i>Sagartia elegans</i>	G	1	130-140	Shaw 1989
		<i>Sagartia troglodytes</i>	G	1	100-160	Hummel and Bogaards 1991, Shaw 1989
	Actiniidae	<i>Actinia equina</i>	G	3	180-200	Chia and Rostron 1970, Gashout and Ormond 1979
		<i>Actinia equine mesembryanthemum</i>	G	3	n.a.	Carter and Thorp 1979
		<i>Actinia tenebrosa</i>	G	3	n.a.	Ottaway 1979
		<i>Anemonia alicemartinar</i>	F	n.a.	n.a.	Haussermann and Forsterra 2001
		<i>Anthopleura asiatica</i>	G	1	370-380	Fujii 1991
		<i>Anthopleura dixoniana</i>	G	1	200-250	Lin et al. 1992a
		<i>Anthopleura elegantissima</i>	G	1	140-250	Ford 1964, Jennison 1979, Sebens 1981
		<i>Anthopleura handi</i>	H	3	300	Dunn 1982
		<i>Anthopleura xanthogrammica</i>	G	1	150-200	Sebens 1981
		<i>Condylactis gigantea</i>	H	1	150-500	Jennison 1981
		<i>Condylactis</i> sp.	F	1	400-500	Tsai 2003
		<i>Entacmaea quadricolor</i>	G	1	n.a.	Scott and Harrison 2005
		<i>Epiactis prolifera</i>	H	2	400	Dunn 1975
		<i>Haliplanella luciae</i>	G	1	100-150	Dunn 1982, Fukui 1995
	<i>Tealia (=Urticina) crassicornis</i>	G	1	500-700	Chia and Spaulding 1972	
	<i>Urticina lofotensis</i>	G	1	700-800	Wedi and Dunn 1983	
	Capneidae	<i>Actinoporus elongatus</i>	G	1	400-450	Clayton and Collins 1992
	Phymanthiidae	<i>Phymanthus crucifer</i>	G	3	450-500	Jennison 1981
		<i>Phymanthus loligo</i>	G	3	550-620	Lin et al. 1999
		<i>Phymanthus strandesi</i>	G	1	550-560	Lin et al. 2001
	Actinostolidae	<i>Actinostola spetsbergenensis</i>	G	3	400-750	Riemann-Zurmeck 1976
	Stichodactylidae	<i>Heteractis crispa</i>	G	1	n.a.	Scott and Harrison 2005

intertidal rocky shores in temperate and tropical seas (Carlgren 1949). Forty-two species of *Aiptasia* have been described, and 16 of these were considered to be valid species in a recent review (Fautin 2006). Most of the valid species are distributed in the Atlantic Ocean and Mediterranean Sea; only *A. californica* (Carlgren 1952) and *A. pulchella* (Carlgren 1943) have been reported in the Pacific Ocean (Fautin 2006). *Aiptasia pulchella* was first described in a subtidal area of Japan by Carlgren in 1943, and subsequently reported in the Hawaiian Is. (Cutress 1977), Marshall Is. (Cutress and Arneson 1987), and Taiwan (Lin et al. 1992b, Chen 2000). Individuals of *A. pulchella* produce asexual offspring by pedal laceration, by which fragments are torn, constricted, or pinched-off from the junction of the column and basal disc (Lin et al. 1992b). Unlike binary fission, pedal laceration produces multiple daughter individuals that are far smaller than the parent (reviewed in Stephenson 1928, Shick 1991). Recently, some of them have been reported as non-indigenous species that were introduced through vectors of aquaculture or the

aquarium trade (Mito and Uesugi 2004).

*Aiptasia pulchella* has also been used as a common model organism for laboratory-based experiments to study environmental regulation of asexual reproduction (Hunter 1984), cnidarian bleaching mechanisms (Sawyer and Muscatine 2001), and mutualism of cnidarian-dinoflagellate intracellular symbiosis (Wang and Douglas 1997 1998 1999, Kuo et al. 2004, Rodriguez-Lanetty et al. 2006). However, details of its sexual reproduction are not well known. In this study, gametogenesis, mesentery development, and periodicity of sexual reproduction of *A. pulchella* were investigated using histological analyses, and characteristics of its sexual reproduction pattern are discussed.

## MATERIALS AND METHODS

### Sampling location

*Aiptasia pulchella* samples were collected from a seawater outlet of a marine station at Tungkan (22.25°E, 120.26°N), a facility of the

**Table 2.** Reproductive cycles of sea anemones: 1/Y, gametes mature once a year; 2/Y, twice a year; 1/M, once a month; 1/W, once a week; con., no peak of maturity and a protracted period of gamete release

Reproduction	Species	Maturation frequency					Sources
		1/Y	2/Y	1/M	1/W	Other	
Broadcast spawning	<i>Aiptasia pulchella</i>			✓			this study
	<i>Amphianthus inornata</i>	✓					Bronsdon et al. 1993
	<i>Haliplanella luciae</i>	✓					Dunn 1982, Fukui 1995
	<i>Sagartia troglodytes</i>		✓				Hummel and Bogaards 1991, Shaw 1989
	<i>Sagartia elegans</i>	✓					Shaw 1989
	<i>Anthopleura elegantissima</i>	✓					Ford 1964, Jennison 1979, Sebens 1981
	<i>Metridium senile</i>	✓					Bucklin 1982
	<i>Paracalliactis stephensoni</i>	✓					van-Praet 1990
	<i>Anthopleura xanthogrammica</i>	✓					Sebens 1981
	<i>Bartholomea annulata</i>		✓				Jennison 1981
	<i>Nematostella vectensis</i>				✓		Hand and Uhlinger 1992
	<i>Anthopleura dixoniana</i>	✓					Lin et al. 1992
	<i>Condylactis gigantea</i>	✓					Jennison 1981
	<i>Anthopleura asiatica</i>	✓					Fujii 1991
	<i>Condylactis</i> sp.	✓					Tsai 2003
	<i>Actinoporus elongatus</i>					con.	Clayton and Collins 1992
	<i>Phymanthus strandesi</i>	✓					Lin et al. 2001
	<i>Urticina lofotensis</i>	✓					Wedi and Dunn 1983
	Brooding	<i>Gonactinia prolifera</i>		✓			
<i>Cereus pedunculatus</i>		✓					Shaw 1989
<i>Actinia equina</i>			✓				Chia and Rostron 1970, Gashout and Ormond 1979
<i>Epiactis prolifera</i>		✓					Dunn 1975
<i>Phymanthus crucifer</i>		✓					Jennison 1981
<i>Phymanthus loligo</i>		✓					Lin et al. 1999

Taiwan Fishery Research Institute in southern Taiwan. The aquaculture effluent passes through a seawater outlet, and its benthos are patchily covered with invertebrates, such as isopods, sponges, sea anemones, and sea urchins. The sea anemone, *A. pulchella*, was the dominant invertebrate of the sampling location (Lin et al. 1992b, Chen 2000).

### Histology of gametogenesis and the sexual reproductive cycle

Sampling was conducted on a monthly basis in Mar., Apr., and May 1993, and restarted from Nov. 1993 to Mar. 1994 followed by 2 samplings in May and July 1994. Because no significant pattern of gametogenesis was revealed by this sampling strategy (see "Results and Discussion"), additional sea anemones were also collected at 6 d intervals from Aug. to Oct. 1997. About 50 specimens were sampled in each collection.

After each collection period, all specimens were narcotized and relaxed in 7% (w/v) MgCl<sub>2</sub>, then fixed in Zenker's seawater solution for at least 24 h (Clark 1980). After fixation, 2-10 individuals were randomly chosen and dehydrated in alcohol, cleared in xylene, and embedded in paraffin. Histological sections were made at a 5 µm thickness and stained with basic fuchsin and indigo carmine, then observed under a microscope (Lin et al. 1992a). The reproductive mesenteries of each individual were recorded. From each specimen, the longest and shortest axes were measured with a calibrated ocular micrometer under a compound microscope from at least 50 randomly chosen oocytes of each specimen. These oocytes were sectioned through the nuclei and measured at x400 magnification.

### Sexuality and mesentery development

Because no male individuals were discovered in our previous study, detailed surveys of the sexuality and the possibility of sexuality related to body size were initiated on 15 Apr. 1998. The size of each *A. pulchella* was indicated by the pedal disc diameter because a significant correlation between its dry weight and pedal disc diameter was detected in our preliminary study (Chen 2000). In order to collect intact *A. pulchella*, 13 PVC plastic plates, 15 x 15 cm and 0.5 cm thick, were placed 5 cm under the surface of the seawater at the outlet channel and collected before an estimated spawning event. All sea anemones on the plates were

narcotized and fixed as described above. Seventy-five sea anemones of the fixed samples were randomly chosen and dissected under a microscope. The longest axis of the pedal disc and the axis perpendicular to that were measured with a ruler, and the numbers, development, and orientation of the mesenteries were recorded. These sea anemones were sectioned to determine their sexuality by the method described above. These sea anemones were classified according to sexuality and different developmental stages of the reproductive mesenteries, and the classification was manually verified statistically by Dunn's multiple comparison (Gibbons 1976).

## RESULTS AND DISCUSSION

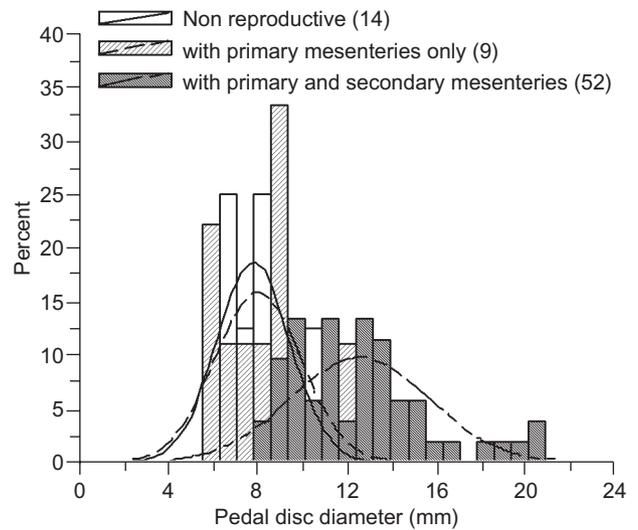
### A female-dominant population

In total, 136 *A. pulchella* individuals were sectioned and observed from sample collections in 1993, 1994, and 1997. Histological observations revealed that only female ( $n = 112$ ) and non-reproductive ( $n = 24$ ) *A. pulchella* occurred at the outlet of the Tungkuang Marine Station. In addition, there were also only female ( $n = 61$ ) and non-reproductive ( $n = 14$ ) individuals in samples from 1998. No male individual was observed during this study. Gonads of female *A. pulchella* develop between the mesenteric filaments and the retractor muscles which are located in one of the paired primary mesenteries and two of the paired secondary mesenteries (Fig. 1A, B).

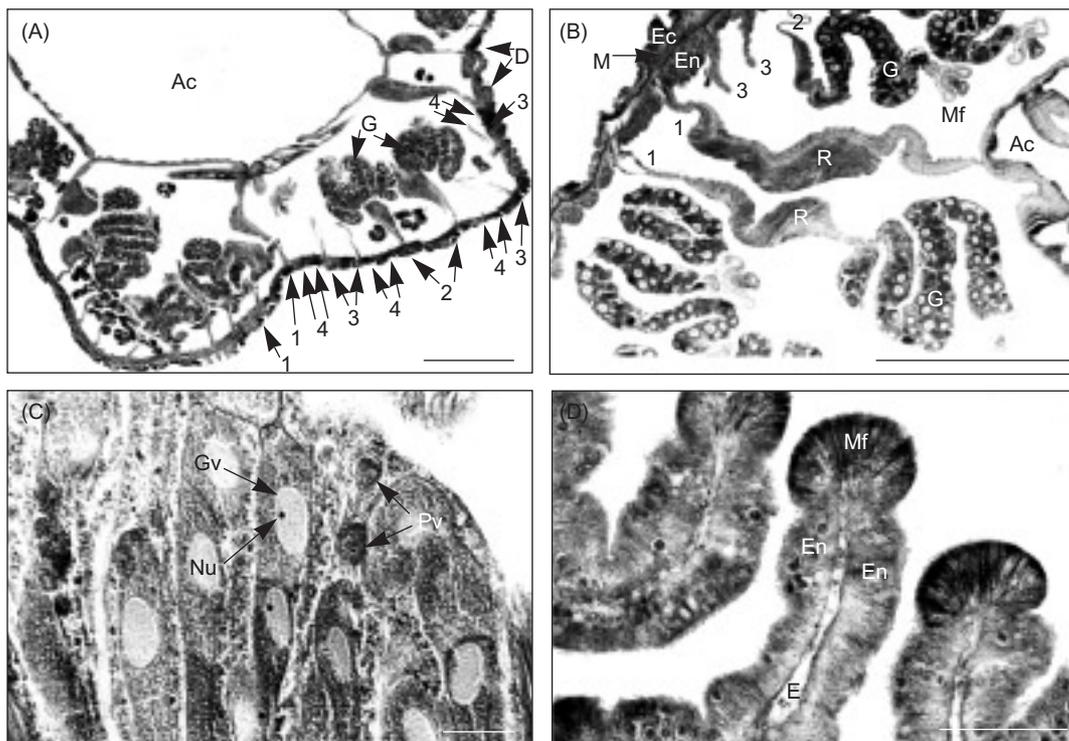
Gonad development was influenced by the body size of sea anemones. Observations from the collection in 1998 revealed that the body size of the sea anemones with developed gonads in both the primary and secondary mesenteries ranged from 8.0 to 20.8 mm (mean  $\pm$  SD = 12.59  $\pm$  3.12;  $n = 52$ ) in mean pedal disc diameter. These sea anemones were significantly larger than non-reproductive individuals or fertile individuals with developed gonads only in the primary mesenteries ( $p < 0.01$ , Dunn's multiple comparison, Fig. 2). Mean pedal disc diameter of sterile individuals did not significantly differ from those of individuals with developed gonads only in the primary mesenteries (size range 5.5-10.9 and 5.6-11.8 mm, respectively; Fig. 2).

*Aiptasia pulchella* at the outlet of the Tungkuang Marine Station appears to be a gonochoristic sea anemone, although only female and non-reproductive individuals were observed. The

prevalence of female *A. pulchella* may have been caused by a founder effect resulting from initial colonization by a female and subsequent extensive pedal laceration in the study area (Chen 2000). A similar phenomenon of unisexual sea anemone populations has also been reported in *Anthopleura asiatica* (Fujii 1987), *Ant. elegantissima* (Sebens 1982), *Anemonia alicemartinae* (Haussermann and Forsterra 2001), *Haliplanella luciae* (Shick and Lamb 1977, Fukui 1995), and *Condylactis* sp. (Tsai 2003). It has been suggested that the absence of one sex or the skewness of sex ratios in sea anemone populations results from frequent asexual propagation in those populations (Shick and Lamb 1977, Sebens 1982, Tsai 2003). Other research using allozyme electrophoresis and RAPD analysis also suggested that field populations of the sea anemones, *H. luciae* and *Nematostella vectensis*, belonged to a single clone or a general-purpose genotype that possibly resulted from asexual reproduction of a single or a few founder individuals (Shick and Lamb 1977, Pearson et al. 2002).



**Fig. 2.** Size-frequency distribution of the pedal disc diameter of *Aiptasia pulchella* at different reproductive and development stages. Numbers in parentheses are the sample sizes of sea anemones.



**Fig. 1.** Oogenesis stages of *Aiptasia pulchella* (a) transverse section of mesentery development, (b) transverse section through gametogenic mesenteries from a female, (c) section through a female gametogenic mesentery containing gametes in different stages of development, (d) a female gonad following spawning. 1, primary mesenteries; 2, secondary mesenteries; 3, tertiary mesenteries; 4, quarternary mesenteries; Ac, actinopharynx; D, directive mesenteries; E, empty space within mesoglea after spawning; Ec, ectoderm; En, endoderm; G, gonad; Gv, germinal vesicle; M, mesoglea; Mf, mesenterial filament; Nu, nucleolus; Pv, pre-vitelligenous oocyte; R, retractor muscle of mesentery; scale bar: 1 mm (1a, 1b), 50  $\mu$ m (1c, 1d).

An alternative hypothesis to explain the unisexual population of *A. pulchella* is that the species might be hermaphroditic, and the population maintains relatively few male spermaries, or spermatogenesis develops during a relatively short period. Another hypothesis is that rare reproductive specimens are males, but the samples were taken outside of their spermatogenic cycles. Hermaphroditic reproduction has been reported for some sea anemones, e.g., *Ant. handi*, *Condylactis gigantean*, *Kadosactis commensalis*, and *Paracalliactis stephensoni* (Table 1). An extreme case of a mixed reproduction pattern was reported for *P. stephensoni* for which only 1 hermaphroditic individual was discovered among hundreds of gonochoristic sea anemones (van-Praet 1990). Furthermore, male spermaries might be indistinguishable on histological slides, if they develop rapidly and during the late period of sexual reproduction. If males are present, the local *A. pulchella* population might contribute to recruitment by producing asexual propagules and also participate in production of sexual offspring. Future application of hypervariable molecular markers, such as microsatellites, can be utilized to reveal the contributions of different reproductive modes and to resolve the clonal structure of mixed *A. pulchella* populations (Avisé 2004).

#### **Gametogenesis and the smallest oocyte among broadcast-spawning sea anemones**

The earliest recognizable pre-vitellogenic oogonia were 5  $\mu\text{m}$  in diameter, and they were distributed at the border of the mesenteric mesoglea or surrounding the larger oocytes within mesoglea (Fig. 1C). The germinal vesicle occupied most of the pre-vitellogenic oogonia. During vitellogenesis, the germinal vesicles of pre-vitellogenic oocytes increased in size and moved to the margin of oocytes, and the yolk granules of pre-vitellogenic oocytes increased in both number and volume. The sizes of most post-vitellogenic oocytes were between 60 and 80  $\mu\text{m}$  in diameter, with the largest oocytes having a maximum diameter of 100  $\mu\text{m}$  (Fig. 1C). Oogenesis of *A. pulchella* is similar to those of other sea anemones (Shick 1991, Fautin 1992). No trophonema-like structure, a supposed nutrient transportation zone, was recorded during vitellogenesis, and no brooded larvae were observed during any stage of oogenesis. The size of mature oocytes is close to that of the brooding sea anemone *Gonactinia prolifera*, but is the smallest among broadcast-spawning sea

anemones known so far (Table 1). According to Chia's (1976) scheme, sexual reproduction in *A. pulchella* fits the most primitive type of oviparous-pelagic-planktotrophic development. This type of reproductive pattern is characterized by small eggs that are spawned and fertilized externally.

#### **Lunar sexual reproductive cycle**

The oocyte size distributions in 1993 and 1994 revealed that there was no obvious annual reproductive cycle for *A. pulchella*, when arranged by calendar date (Fig. 3A). The pre-vitellogenic oocytes were recorded in almost every collection, except for the Apr. 1993 and Feb. and May 1994 collections. In some cases, they co-occurred with large post-vitellogenic oocytes, for example in May and Dec. 1993 and Jan. 1994, suggesting that samples were taken prior to approaching spawning events. However, a lunar reproductive cycle for *A. pulchella* was evident when the oocyte distribution was arranged by lunar date (Fig. 3B). Post-vitellogenic oocytes increased in size, and then most of them disappeared after the 25th lunar day. At this time, pre-vitellogenic oocytes appeared, signaling the beginning of the next period of gametogenesis.

A lunar cycle of sexual reproduction was confirmed by intensive sampling between Aug. and Oct. 1997 (Fig. 4). Female *A. pulchella* produced small oocytes around the 23rd and 24th lunar days, and small oocytes grew to maturity at about 100  $\mu\text{m}$  in diameter around the 17th-24th lunar days. The disappearance of large post-vitellogenic oocytes suggested that gamete release occurred between the 24th and 29th lunar days, and empty spaces within mesenteries were observed in histological sections after the gametes had been released (Fig. 1D). At the same time, newly produced small oogonia were present in the mesenteries, and were presumably prepared to develop to maturity during the next lunar cycle of gametogenesis.

Sexual reproduction attuned to the regular lunar cycle by *A. pulchella* is the first report in sea anemones (Table 2). In addition to our histological studies, further research recorded a monthly spawning event which verifies the proposed pattern of sexual reproduction for *A. pulchella* (Chang 2003). The gametogenic cycle of *A. pulchella* differs from those of other sea anemones, most of which produce sexual offspring only once per year (Table 2). Frequent sexual reproductive cycles have been reported for broadcast-spawning sea

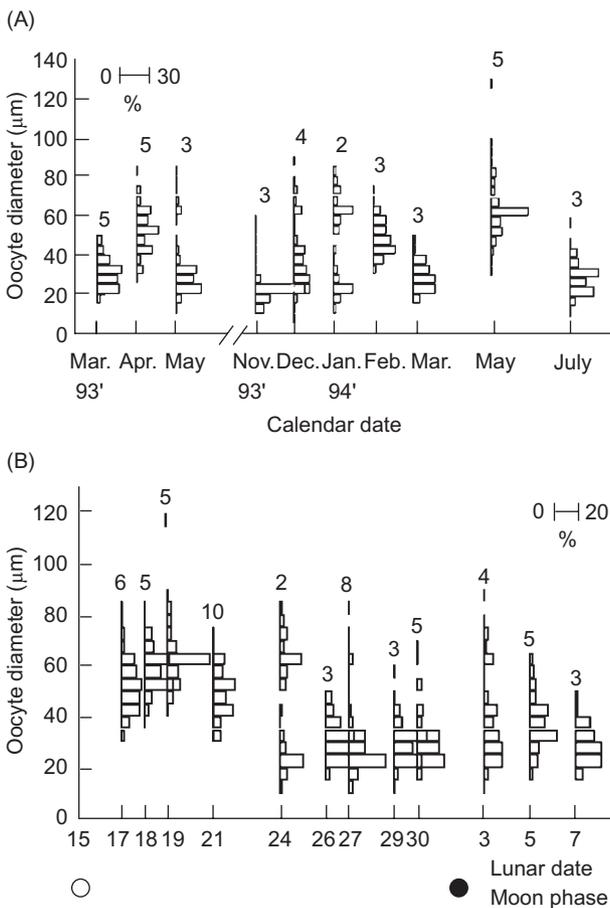
anemones such as *Actinoporus elongates* (Clayton and Collins 1992), *Bartholomea annulata* (Jennison 1981), *Nematostella vectensis* (Hand and Uhlinger 1992), and *Sagartia troglodytes* (Hummel and Bogaards 1991), and for brooding sea anemones such as *Actinia equina* (Chia and Rostron 1970) and *Gonactinia prolifera* (Chia et al. 1989); however, most sea anemones exhibit a biannual cycle (Table 2). The most rapid and frequent cycle of gametogenesis occurs in *N. vectensis*, which displays a weekly cycle under optimal laboratory conditions, but is thought to have an annual reproductive cycle in nature (Hand and Uhlinger 1992, Fritzenwanker and Technau 2002).

The lunar reproductive cycles of *A. pulchella* may be a result of the abundant food available in the aquaculture effluent of the study area. It was

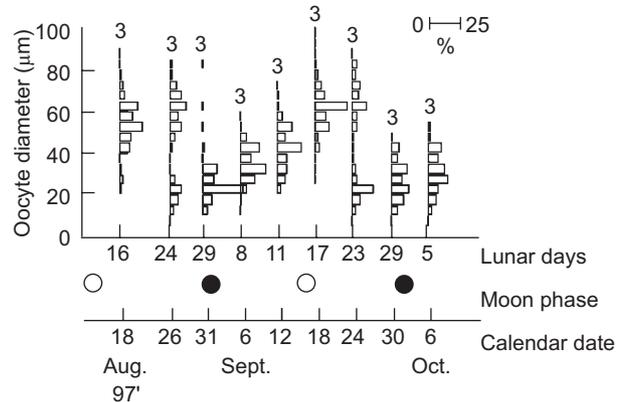
suggested that oogenesis of *A. pulchella* is significantly accelerated when supplied with increasing food and light intensity in the laboratory (Chen 2000). A similar phenomenon of plasticity in sexual reproductive cycles has been observed and verified in other sea anemones (Hand and Uhlinger 1992, Lin et al. 1992a). It was suggested that both energy storage and triggers of gamete release play important roles in regulating sexual reproduction. Alternatively, the lunar reproductive cycle of the examined *A. pulchella* population may also be an artifact of the artificial seawater outlet where they were collected. It is possible that natural populations may reproduce just once or a few times a year, as has been recorded in *Nematostella vectensis* (Hand and Uhlinger 1992).

**A putative model organism for research on marine invasive organism**

To date, it remains unclear whether the study population represents a sea anemone indigenous to Taiwan. However, its appearance is generally affiliated with local aquaculture farms or a commercial aquarium, which implies the possibility of introduction through aquaculture or the aquarium trade (Chen 2000). As globalization has increased, biological invaders are now widely recognized as one of our most pressing conservation threats and as a major cause of loss of global biodiversity (Parker et al. 2003). *Aiptasia pulchella* possesses several facets of a successful alien species, such as preempting space and excluding competitors using venom-containing acontia and rapidly establishing local populations by both frequent sexual and asexual reproduction. Hence, in addition to serving as a model organism to study environmen-



**Fig. 3.** Size-frequency distribution of oocyte diameter of *Aiptasia pulchella* collected from Mar. 1993 to July 1994 (A) arranged by calendar date and (B) lunar date. Numbers above the bars are the sample sizes of sea anemones. ●, New moon; ○, full moon. Note: There are 29 or 30 d in a lunar cycle; the 1st lunar day is the new moon, and day 15 is the full moon.



**Fig. 4.** Frequency distribution of oocyte diameters of *Aiptasia pulchella* from Aug. to Oct. 1997. Numbers above the bars are the sample sizes of sea anemones.

tal regulation of asexual reproduction and cnidarian-dinoflagellate symbiosis, *A. pulchella* could be considered a model organism for research on marine invasive species in the future.

## CONCLUSIONS

In conclusion, examination of *Aiptasia pulchella* by histological sections revealed that only female and sterile individuals were observed for the population at the outlet of the Tungkang Marine Station. This unisexual population probably resulted from extensive propagation by pedal laceration. Mature oocytes of *A. pulchella* are about 60-100  $\mu\text{m}$  in diameter which is the smallest case recorded among broadcast-spawning sea anemones. Gametogenesis follows the lunar cycles throughout the year, with suggests spawning events between the 24th and 29th lunar days in this unisexual population of *A. pulchella*.

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