Zoological Studies **49**(5): 601-607 (2010)

Zoological Studies

Reproduction of the Bath Sponge *Spongia ceylonensis* (Dictyoceratida: Spongiidae) from Penghu, Taiwan

I-Fu Chung[1], Yen-Ming Huang[1], Tsung-Han Lee[2], and Li-Lian Liu[1,*]

[1]*Institute of Marine Biology and Asia-Pacific Ocean Research Center-Kuroshio Research Group, National Sun Yat-sen University, Kaohsiung 804, Taiwan*

[2]*Department of Life Sciences, National Chung-Hsing University, Taichung, 402, Taiwan*

(Accepted March 12, 2010)

I-Fu Chung, Yen-Ming Huang, Tsung-Han Lee, and Li-Lian Liu (2010) Reproduction of the bath sponge *Spongia ceylonensis* (Dictyoceratida: Spongiidae) from Penghu, Taiwan. *Zoological Studies* **49**(5): 601-607. The reproductive cycle of the bath sponge *Spongia ceylonensis* Dendy, 1905 was studied over a period of 1.5 yr in the intertidal zone at Penghu, Taiwan. Results indicate that *S. ceylonensis* is gonochoric and viviparous. Early-stage oocytes were first observed in Mar. in the mesohyl. Embryos and larvae were found to be gathering in the vicinity of the aquiferous canals from Apr. to Sept. Their sizes ranged from 346.0 ± 57.0 to 395.6 ± 51.2 μm, and the mean number of embryos/larvae per mm^3 of tissue varied from 2.7 to 10.7. The larvae belong to the parenchymella type and have a black pigmented ring with long flagella surrounding their anterior. The reproductive period of *S. ceylonensis* generally coincided with an increase in water temperature, and it remained active in the temperature range of 19-28°C. http://zoolstud.sinica.edu.tw/Journals/49.5/601.pdf

Key words: *Spongia ceylonensis*, Bath sponge, Reproduction, Taiwan.

Bath sponges, belonging to the order Dictyoceratida, are comprised of *Hippospongia* and *Spongia* species which have spongin skeleton as their supporting structure. They are largely distributed in shallow water, on littoral rocky or sandy bottoms, e.g., *S. officinalis*, *S. agaricina*, *S. zimocca*, and *H. communis* (Josupeit 1991). These sponges have been harvested from the Mediterranean Sea, Caribbean, and Micronesian areas since ancient times (Pronzato 1999).

Spongia ceylonensis Dendy, 1905 is an Asian bath sponge species, distributed in Sri Lanka, Indonesia, the Philippines, Thailand, Singapore, and Hong Kong from the intertidal to a depth of 54 m (Dendy 1905, Van Soest 1982 2009, Kesava 2008). In Taiwan, this bath sponge is abundant in some intertidal areas covered with sand, silt, and coral rubble of the Penghu Archipelago in the Taiwan Strait.

It is massive and spherical, with a springy, compressible, and elastic texture (Dendy 1905, Van Soest 1982 2009, Kesava 2008). Its size can reach to > 30 cm in diameter. The black surface is covered with low, even conules, and the interior is orange or yellow (Fig. 1A). Its primary fibers are reduced in number, and the highly developed secondary fibers make up the bulk of the skeleton. The primary fibers contain a central axis of foreign spicules and/or sand grains which are mostly found near the sponge surface (Fig. 1B). In contrast, secondary fibers contain no foreign materials.

Although this bath sponge is widely distributed and has the potential for commercial use, we know virtually nothing about its biology. We conducted a study of its reproductive biology, which is expected to improve our ability to design efficient strategies for future, sustainable exploitation of this species.

*To whom correspondence and reprint requests should be addressed. Tel: 886-7-5252000 ext. 5108. Fax: 886-7-5255100. E-mail:lilian@mail.nsysu.edu.tw

MATERIALS AND METHODS

The reproduction of *S. ceylonensis* was studied at an intertidal area of Wa-ton, Penghu (119°34'E, 23°39'N) (Fig. 2). Five to 22 sponge individuals with a diameter of 20-25 cm were collected monthly from June 2000 to Nov. 2001. Sponges were sampled at least 30 m apart from each other. Water temperature and salinity were also recorded at the same time.

Ten 1 cm^3 sponge tissues including the upper, middle, and lower mesohyl were taken from each individual for a light-microscopic investigation. The fresh-cut sponge tissues were embedded in optimal cutting temperature compound (OCT 4583, Tissue-Tek®, Sakura, IL, USA) for cryosectioning. Serial sections were cut at 5-8 μm thick using a Cryostat Microtome (HM 505E, Microm, Walldorf, Germany) at -25°C. The sections were then stained with hematoxylin and eosin (Katz and Watson 1985) and examined under a light microscope (Olympus BH-2, Tokyo, Japan). Occasionally, by slicing live sponges, free-swimming larvae were obtained, and these were placed in Petri dishes for further observation.

Then, five 125 mm^3 sponge tissues from each individual were taken to determine the number and size of the embryos and larvae under a dissecting microscope. After that, the remaining sponge samples were used for studies of secondary metabolites and symbiotic invertebrates. For present evaluation, all phases of embryonic development and larvae were counted as a whole. Data of larval density were analyzed and compared among different months, using a one-way analysis of variance (ANOVA) and Tukey's multiple-comparisons test.

RESULTS

During the study period of June 2000 to Nov. 2001, sea surface temperatures ranged from a low of 16.0°C to a high of 27.6°C (Fig. 3), and the salinity was 32-36 psu. Reproduction of *S. ceylonensis* was quantified based on 222 examined individuals. The percent males, females, and brooding females were 5.0%, 3.6%, and 20.3% of the total. In the monthly samples, reproductive individuals ranged 0%-70% (Fig. 3). The proportions of males, females, and brooding females varied in the ranges of 0%-30%, 0%-20%, and 0%-67%, respectively. The peak period of reproduction was from June through Aug., and no reproductive specimens were observed from Oct. to Feb. In general, the reproductive period of *S. ceylonensis* coincided with increased water temperatures, and it remained active in the temperature range of 19-28°C.

Female and male gametes never occurred in the same individuals in our sponge samples, clearly showing this species to be gonochoristic. Spermatic cysts in clumps of 3 or 4 were first observed in the mesohyl in Apr. (Fig. 4A). Single oocytes were first seen in Mar. Early oocytes had a size about 25 μm, and late oocytes (at ca. 70 μm) with large yolk spheres filling the cytoplasm were occasionally found (Figs. 4B, C).

Embryos had a polygonal shape and always gathered together in the vicinity of the aquiferous canals (Figs. 1A, 4D, E, 5). The developed embryos and larvae found in sponge tissues

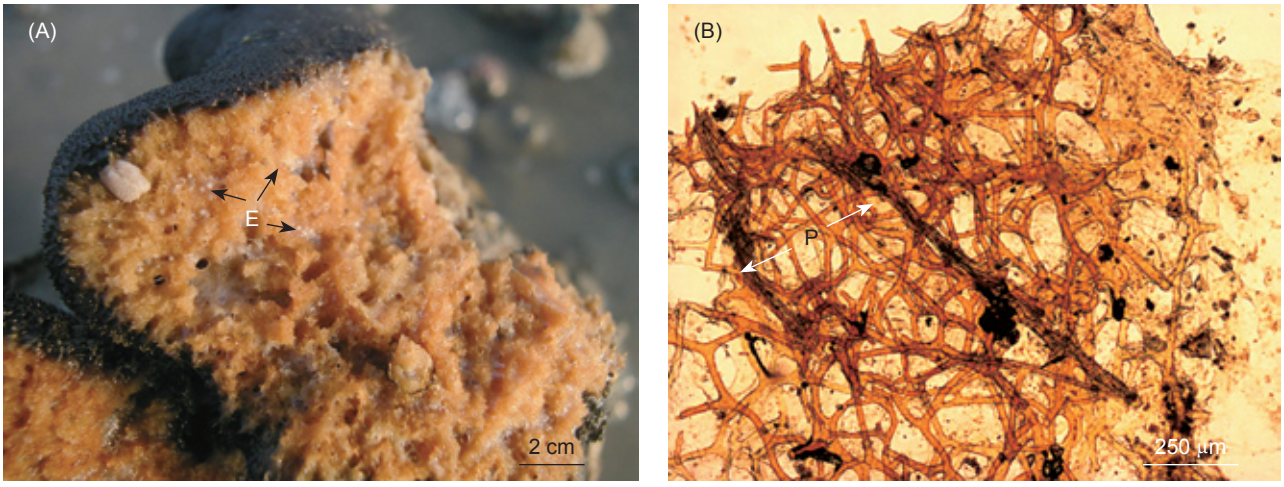

Fig. 1. *Spongia ceylonensis*. (A) Gross morphology. (B) Fiber skeleton. E, embryos; P, primary fiber with spicules.

indicated that *S. ceylonensis* was viviparous. Within a single specimen, embryo/larval diameters ranged 225-700 μm. Their development was asynchronous in that within a clump, early white embryos coexisted with mature parenchymellae which were gray or black (Fig. 5).

Monthly variations in the mean diameters of embryos/larvae were within the ranges of 346.0 ± 57.0-395.6 ± 51.2 μm (Fig. 6A). The mean number of embryos/larvae per mm^3 of tissue varied from

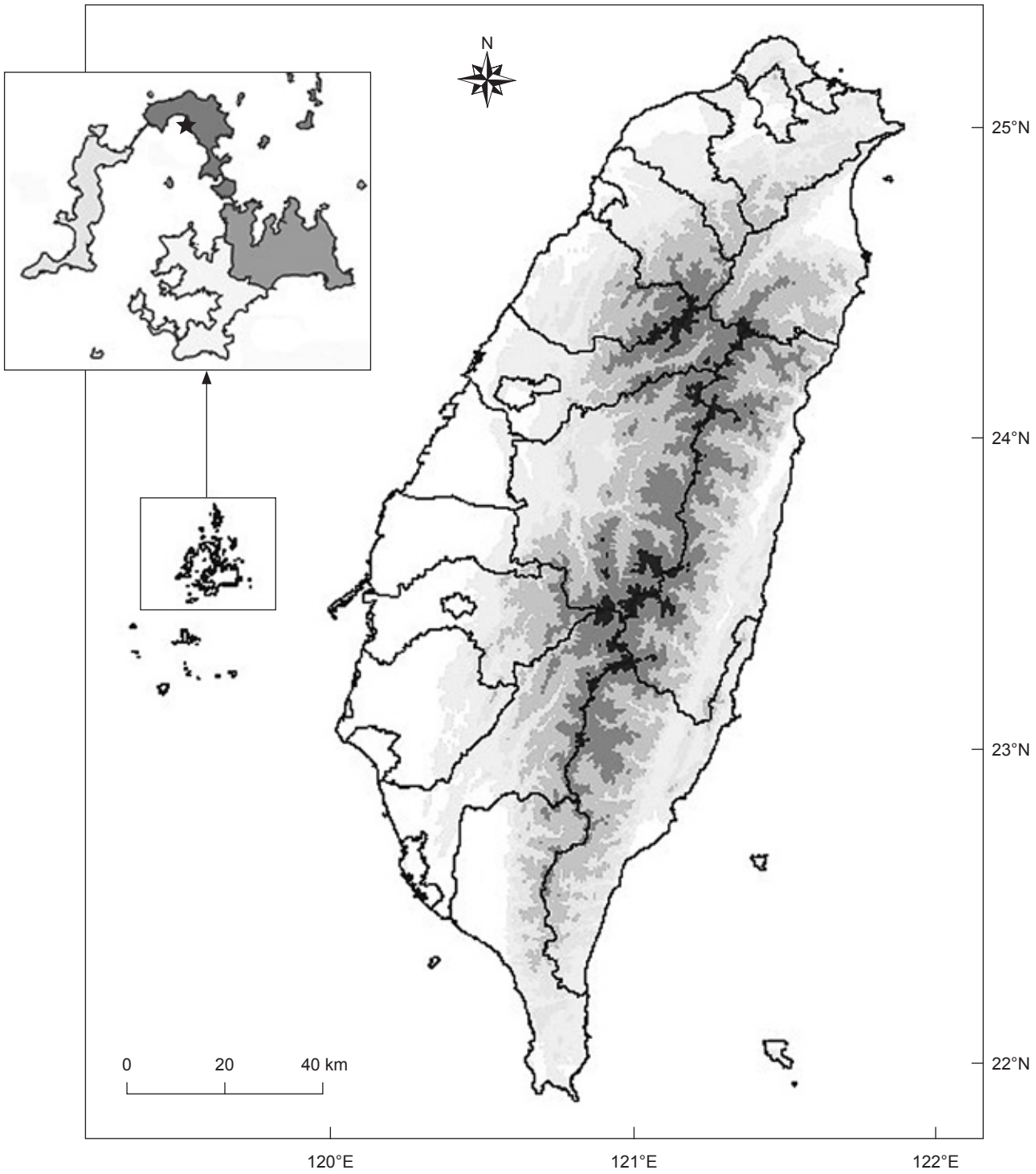

Fig. 2. Location of the sampling site at Wa-ton, Penghu Archipelago, Taiwan. ★: Sampling site.

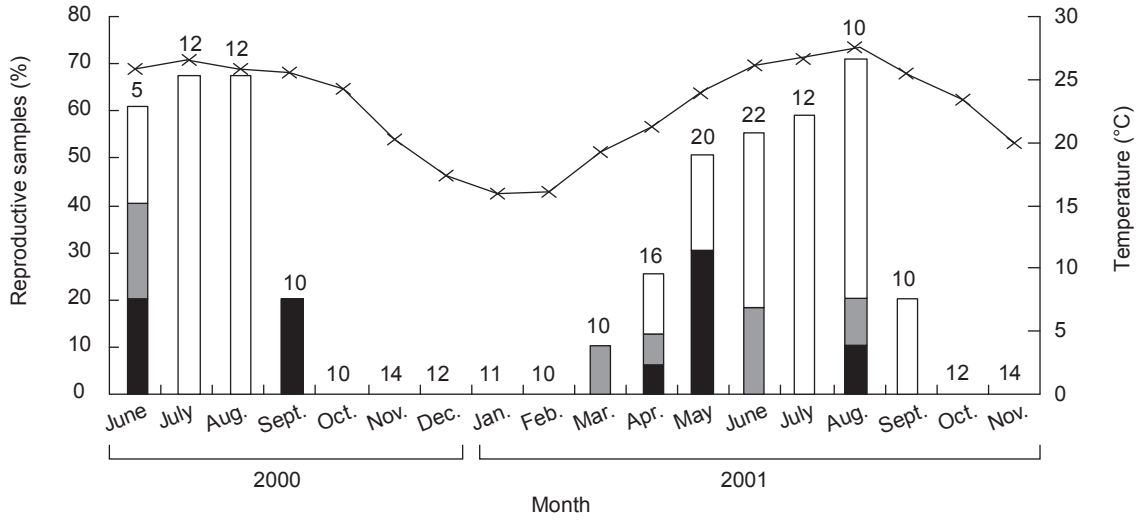

Fig. 3. Total numbers of reproductive *Spongia ceylonensis* in relation to water temperatures. Numbers of examined specimens are listed at the top of the histograms. ■, sperm; ■, eggs; □, embryos/larvae; x, temperature.

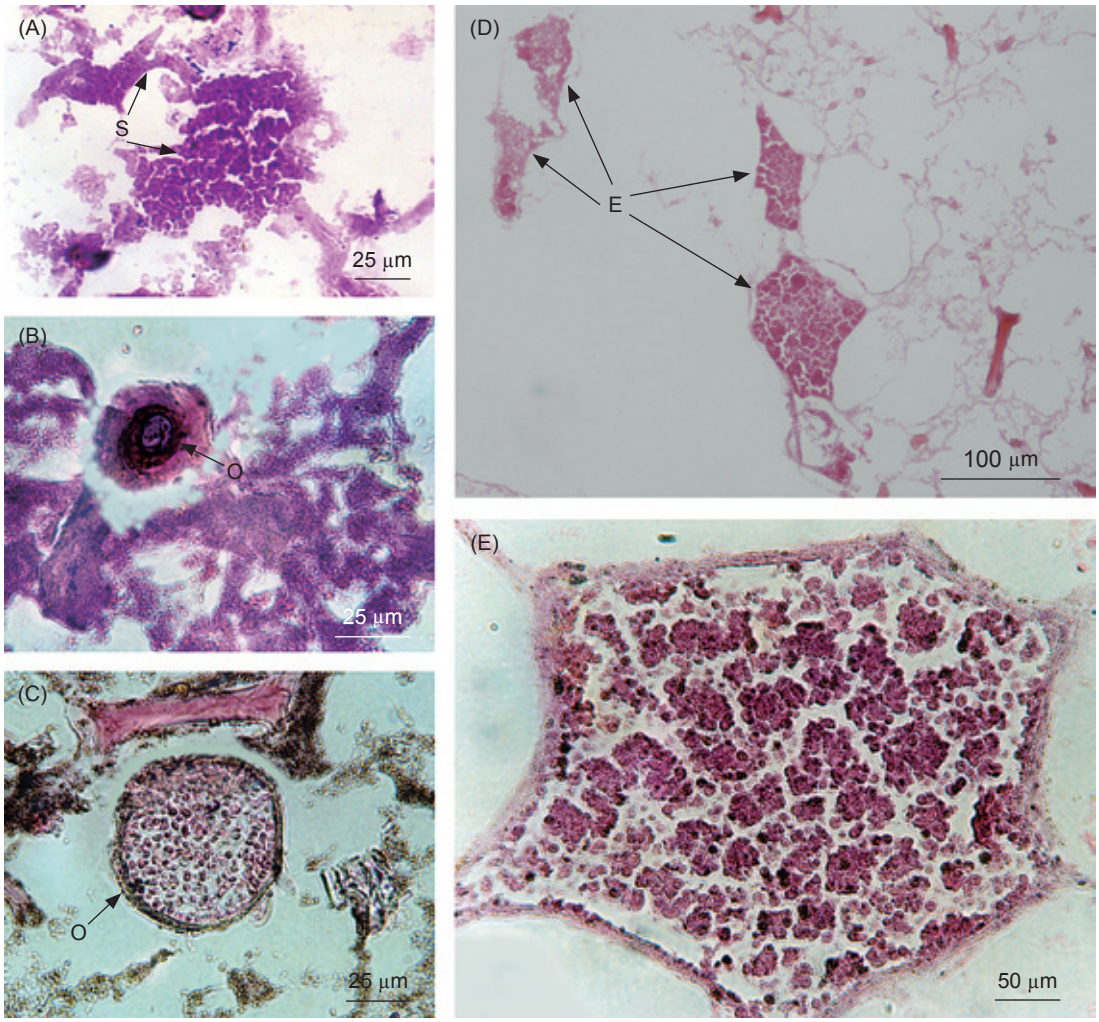

Fig. 4. *Spongia ceylonensis*. (A) Spermatic cysts. (B) An early oocyte. (C) A late oocyte. (D) Embryos. (E) An enlarged embryo. E, embryo; O, oocyte; S, spermatic cyst.

2.7 to 10.7, and the peak density occurred in May (Fig. 6B). The results of the ANOVA and Tukey's tests on the density of embryos/larvae showed significant differences among monthly samples which were in the order of May > Apr., June, and July > Aug. ($p < 0.05$). Released larvae had a black pigmented ring with long flagella surrounding their anterior (Fig. 7). They were tufted parenchymella larvae at 441.5 ± 47.7 μm long and 311.7 ± 38.1 μm wide ($n = 33$).

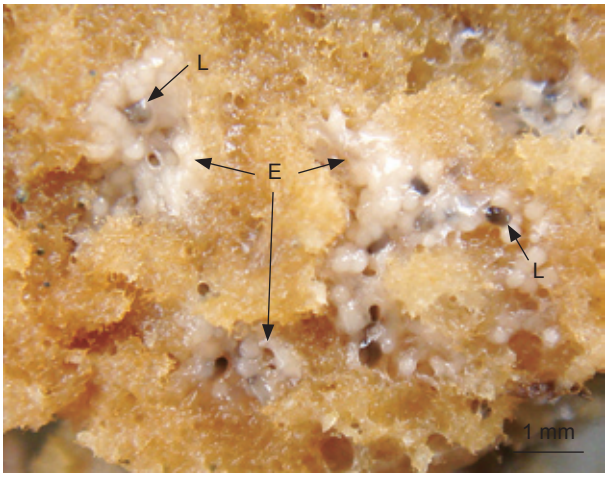

Fig. 5. View of embryos and larvae of *Spongia ceylonensis*. E, embryo; L, larva.

DISCUSSION

Spongia ceylonensis appears to be gonochoric and viviparous. Its reproductive cycle extends from Mar. to Sept., with a peak period occurring in June to Aug. Our results confirm a prevalent gonochoric condition as in other *Spongia* species, such as *S. barbara*, *S. cheiris*, and *S. graminea* (Kaye and Reiswig 1991). Additionally, hermaphroditic samples like in *S. officinalis* were not seen in the present study (Baldacconi et al. 2007).

The general patterns of gametogenesis and embryonic development in *S. ceylonensis* were similar to those reported for other viviparous

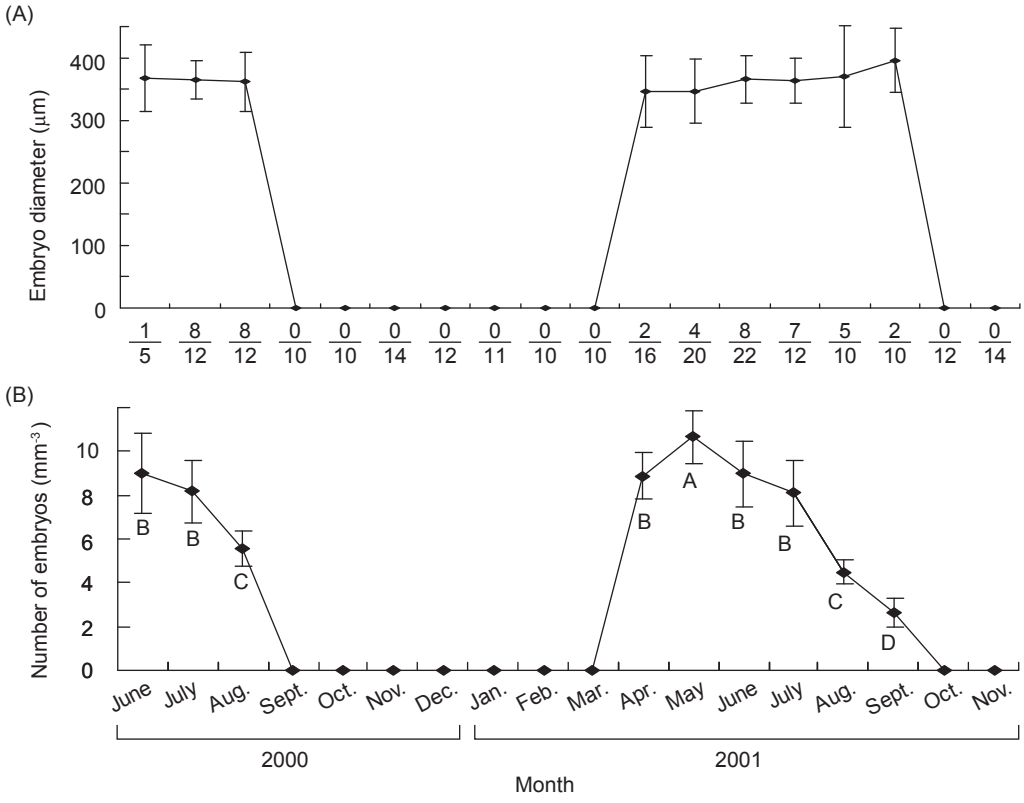

Fig. 6. Size and density of *Spongia ceylonensis* embryos and larvae. (A) Mean embryo size. (B) Mean embryo density. Vertical lines indicate the standard deviation of the mean. Numbers of examined specimens and specimens with reproductive elements are listed at the bottom of figure A. Means significantly differing from each other as analyzed by ANOVA and Tukey's tests are indicated by different letters ($p < 0.05$).

demosponges (Saller and Weissenfels 1985, Saller 1988, Kaye and Reiswig 1991, Leys and Ereskovsky 2006, Maldonado 2006, Baldacconi et al. 2007, Maldonado and Riesgo 2008b 2009). Oogenesis begins with the differentiation of primary oocytes. In Caribbean *Spongia* species (Kaye 1991, Kaye and Reiswig 1991), oocytes were about 15 μm then grew to about 200 μm. In our study, only a few oocytes (< 70 μm) were occasionally observed in the mesohyl, but large numbers of embryos and larvae were commonly seen clumping in the canal areas. There was an obvious lack of intermediate stages between late oocytes and early embryos. It was proposed that oogenesis in some sponges involves oocyte growth and also a migration process of mature spermatic cysts and oocytes from the mesohyl to the exhalant aquiferous canals for spawning (Maldonado and Riesgo 2008b). Recently, this developmental pattern was reported in the oviparous sponge, *Petrosia ficiformis* (Maldonado and Riesgo 2009). Its early oocytes sparsely occur in the mesohyl, and late oocytes aggregate below the endopinacoderm of the exhalant canals prior to spawning. If a similar process was found in *S. ceylonensis*, a lack of intermediate oogenic stages could be explained. Because our sections were prepared from small portions of individuals, it is possible that oocyte-aggregating areas were missed.

In addition, our study was based on random samples within a population, and the entire monitoring period was 1.5 yr. A low percentage of samples undergoing gametogenesis and a low male-to-female ratio were observed. This is similar to some other marine demosponge studies, e.g., *Mycale contarenii*, *Tethya citrine* and *Tethya aurantium* (Corriero et al. 1996 1998). Although the sex ratio of sponges usually departs from 1:1 with frequent cases of female dominance (Maldonado and Riesgo 2008b), other factors may also have influenced our female-biased sex ratio.

For instance, males being less often detected than females may be due to a shorter period of spermatogenesis than of oogenesis. The duration of spermatogenesis of *Axinella damicornis*, *Corticium candelabrum*, *Raspaciona aculeata*, and *Chondrosia reniformis* is a few weeks at the individual level (Riesgo and Maldonado 2008). In contrast, the oogenic period is much longer, i.e., 7-8 mo for *A. damicornis* and *C. candelabrum*, 3-5 mo for *R. aculeata*, and 3 mo for *C. reniformis*. Recently, a 2.5 wks spermatogenesis contrasting with 7 mo of oogenesis in *P. ficiformis* was reported (Maldonado and Riesgo 2009). Another case is that *S. officinalis* developed spermatic cysts only from Sept. to Dec., while oocytes occurred year round (Baldacconi et al. 2010). In the present study, we only sampled once a month, and we did not apply a tagging technique. Therefore, there was no way to determine the time period of gametogenesis at the individual level.

In addition, sponges continually moving out of our sampling site might have seriously affected the reproductive status of the population. The potential problems resulting from progressively removing individuals include modification of the sex ratio, fertilization rate, and embryo production (Baldacconi et al. 2007). In contrast, with a non-destructive tagging method, successive sampling of the same individual over time can truly estimate its gametogenesis, and the number of spermatic cysts, oocytes, or embryos. Therefore, a better understanding of the reproductive nature of the species can be obtained. In fact, this method was applied in many studies, such as with *S. offcinalis*, *Halichondria panacea*, *Chondrilla australiensis*, *Chondrilla nucula*, and *P. ficiformis* (Witte et al. 1994, Usher et al. 2004, Sidri et al. 2005, Baldacconi et al. 2007, Maldonado and Riesgo 2009).

The mean number of embryos/larvae per mm^3 in mother sponges of *S. ceylonensis* was 2.7-10.7. This is comparable to the intertidal sponge *Haliclona permollis* (5/mm^3) (Elvin 1976) but lower than that of *C. candelabrum* (21.3 ± 12.0/mm^3) (Maldonado and Riesgo 2008a). In contrast,

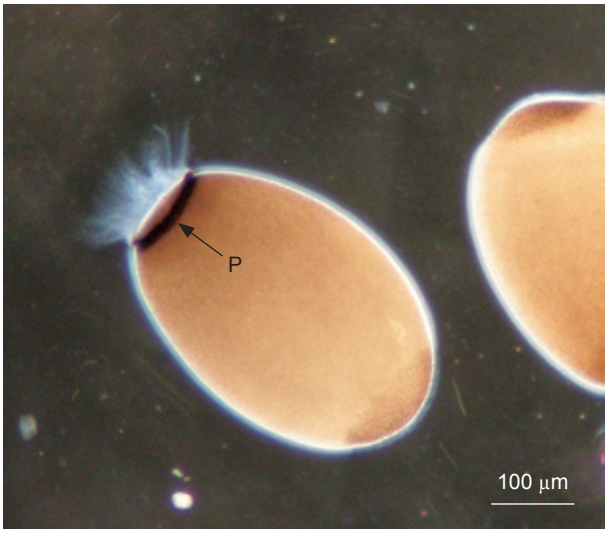

Fig. 7. A newly released parenchymella larva of *Spongia ceylonensis*. P, pigmented ring.

embryo densities of *M. contarenii* (0.07-0.20/mm^3), *S. officinalis* (2.4-0.6/mm^3), *Chondropsis* sp. (0.1/mm^3), *Stylopus* sp. (0.2/mm^3), and *Anchinoe* sp. (2/mm^3) were less than that of *S. ceylonensis* (Alying 1980, Corriero et al. 1998, Baldacconi et al. 2007 2010). Compared to these viviparous species, *S. ceylonensis* occupies a middle position in the range reported for reproductive output. The fact that this species has a relatively moderate to high reproductive output is worth further evaluating its commercial potential to meet the increased interest in exploiting natural bath sponge resources.

Acknowledgments: We are grateful to the anonymous reviewers for their constructive comments which substantially improved the manuscript. We thank Ms. M.T. Tang, Y.C. Lu, Y. Chou, and Mr. C.H. Chang for sample collection. We also thank Dr. N.J. de Voogd, National Museum of Natural History, Leiden, the Netherlands and Mr. L.S. Cheng, Tropical Marine Science Institute, National University of Singapore for their help in providing valuable information on species identification. This study was supported by the National Science Council, Taiwan (NSC96-2621-Z-110-001), and the Asia-Pacific Ocean Research Center–Kuroshio Research Group, National Sun Yat-sen Univ. funded by the Ministry of Education, Taiwan.

REFERENCES

Alying AL. 1980. Patterns of sexuality, asexual reproduction and recruitment in some subtidal marine Demospongiae. Biol. Bull. **158:** 271-282.

Baldacconi R, F Cardone, C Longo, M Mercurio, CN Marzano, E Gaino, G Corriero. 2010. Transplantation of *Spongia officinalis* L. (Porifera, Demospongiae): a technical approach for restocking this endangered species. Mar. Ecol. doi:10.1111/j.1439-0485.2009.00299.x.

Baldacconi R, C Nonnis-Marzano, E Gaino, G Corriero. 2007. Sexual reproduction, larval development and release in *Spongia officinalis* L. (Porifera, Demospongiae) from the Apulian coast. Mar. Biol. **152:** 969-979.

Corriero G, LS Liaci, CN Marzano, E Gaino. 1998. Reproductive strategies of *Mycale contarenii*. Mar. Biol. **131:** 319-327.

Corriero G, M Sara, P Vaccaro. 1996. Sexual and asexual reproduction in two species of *Tethya* (Porifera: Demospongiae) from a Mediterranean coastal lagoon. Mar. Biol. **126:** 175-181.

Dendy A. 1905. Report on the sponges collected by Professor Herdman, at Ceylon, in 1902. *In* WA Herdman, ed. Report to the government of Ceylon on the pearl oyster fisheries of the Gulf of Manaar. Vol. **3 (Supplement 18):** 57-246. London: Royal Society.

Elvin DW. 1976. Seasonal growth and reproduction of an intertidal sponge, *Haliclona permollis* (Bowerbank). Biol. Bull. **151:** 108-125.

Josupeit H. 1991. Sponge world production and trade. Infofish Int. **91:** 21-26.

Katz MJ, LF Watson. 1985. Intensifier for Bodian staining of tissue sections and cell cultures. Stain Tech. **60:** 81-87.

Kaye HR. 1991. Sexual reproduction in four Caribbean commercial sponges. II. Oogenesis and transfer of bacterial symbionts. Invertebr. reprod. Dev. **19:** 13-24.

Kaye HR, HM Reiswig. 1991. Sexual reproduction in four Caribbean commercial sponges. I. Reproductive cycles and spermatogenesis. Invertebr. Reprod. Dev. **19:** 1-11.

Kesava S. 2008. A spongy kind of love. Singapore: Straits Times, p. D10.

Leys SP, AV Ereskovsky. 2006. Embryogenesis and larval differentiation in sponges. Can. J. Zool. **84:** 262-287.

Maldonado M. 2006. The ecology of the sponge larva. Can. J. Zool. **84:** 175-194.

Maldonado M, A Riesgo. 2008a. Reproductive output in a Mediterranean population of the homosclerophorid *Corticium candelabrum* (Porifera, Demospongiae), with notes on the ultrastructure and behavior of the larva. Mar. Ecol. **29:** 298-316.

Maldonado M, A Riesgo. 2008b. Reproduction in Porifera: a synoptic overview. Treballs SCB. **59:** 29-49.

Maldonado M, A Riesgo. 2009. Gametogenesis, embryogenesis, and larval features of the oviparous sponge *Petrosia ficiformis* (Haplosclerida, Demospongiae). Mar. Biol. **156:** 2181-2197.

Pronzato R. 1999. Sponge-fishing, disease and farming in the Mediterranean Sea. Aquat. Conserv. Mar. Freshw. Ecosyst. **9:** 485-493.

Riesgo A, M Maldonado. 2008. Differences in reproductive timing among sponges sharing habitat and thermal regime. Invertebr. Biol. **127:** 357–367.

Saller U. 1988. Oogenesis and larval development of *Ephydatia fluviatilis* (Porifera, Spongillidae). Zoomorphology **108:** 23-28.

Saller U, N Weissenfels. 1985. The development of *Spongilla lacustris* from the oocyte to the free larva (Porifera, Spongillidae). Zoomorphology **105:** 367-374.

Sidri M, M Milanese, F Bümmer. 2005. First observations on egg release in the oviparous sponge *Chondrilla nucula* (Demospongiae, Chondrosida, Chondrillidae) in the Mediterranean Sea. Invertebr. Biol. **124:** 91-97.

Usher KM, D Sutton, S Toze, J Kuo, J Fromont. 2004. Sexual reproduction in *Chondrilla australiensis* (Porifera: Demospongiae). Mar. Freshwater Res. **55:** 123-134.

Van Soest RWM. 1982. A small collection of sponges (Porifera) from Hong Kong. *In* BS Morton, CK Tseng, eds. The Marine flora and fauna of Hong Kong and southern China. Proceedings of the 1st International Marine Biology Workshop, pp. 85-95.

Van Soest RWM. 2009. *Spongia* (*Spongia*) *ceylonensis* Dendy, 1905. *In* RWM Van Soest, N Boury-Esnault, JNA Hooper, K Rützler, NJ de Voogd, B Alvarez, E Hajdu, AB Pisera, J Vacelet, R Manconi, C Schoenberg, D Janussen, KR Tabachnick, M Klautau, eds. World Porifera database. Available at http://www.marinespecies.org/porifera/ (Accessed 17 May 2009)

Witte U, D Barthel, OS Tendal. 1994. The reproductive cycle of the sponge *Halichondria panicea* Pallas (1766) and its relationship to temperature and salinity. J. Exp. Mar. Biol. Ecol. **183:** 41-52.