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A Coral-Killing Sponge, *Terpios hoshinota*, Releases Larvae Harboring Cyanobacterial Symbionts: An Implication of Dispersal

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Jih-Terng Wang, Euichi Hirose, Chia-Min Hsu, Yi-Yun Chen, Pei-Jie Meng, and Chaolun Allen Chen (2012) A coral-killing sponge, *Terpios hoshinota*, releases larvae harboring cyanobacterial symbionts: an implication of dispersal. *Zoological Studies* **51**(3): 314-320. *Terpios hoshinota*, an encrusting sponge, overgrows hard corals on a relatively large scale, raising concerns for coral survival in the Indo-West Pacific region. However, mechanisms of dispersal of this sponge remain unknown. This study examined the ultrastructure of parenchymella larvae collected from *T. hoshinota* to infer potential mechanisms of dispersal and outbreaks of this threatening sponge. The ovum-shaped parenchymella larva has negative buoyancy and a limited swimming capability even though cilia cover its entire surface. Furthermore, larvae settled within 1 d in an aquarium, indicating a larval stage of short duration. These characteristics suggest that dispersion distances of *Terpios* larvae are short. An ultrastructural examination also indicated that larvae are filled with cyanobacteria, and lack spicules and a mesophyl structure as found in other sponge larvae. Most cyanobacteria in the larvae appeared intact and dividing as are found in adult *Terpios*, but some found within sponge cells had disintegrated. Cyanobacteria being engulfed in *Terpios* cells implies a trophic function of the microbes during larval development. http://zoolstud.sinica.edu.tw/Journals/51.3/314.pdf

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Benthic coral-reef communities exhibit constant competition for space and light (Benayahu and Loya 1981, González-Rivero et al. 2011), which can compound survival and has fundamental consequences for the physical and biological structure of a coral-reef community. Among these sessile organisms, sponges are one of the major competitors of corals in tropical reef systems through overgrowth, substrate accretion, and erosion. In most cases, sponges outcompete corals for space when the 2 taxa confront each other, potentially causing degradation of large zones of coral reef communities (González-Rivero et al. 2011).

Terpios hoshinota, a cyanobacteriosponge which occurs in shallow-water Pacific coral reefs, is a typical example of a sponge that can outcompete corals and cause damage to coral-reef communities (Plucer-Rosario 1987, Liao et al. 2007, Chen et al. 2009). Bryan (1973) reported the 1st outbreak of a coral-killing sponge in Guam, which was described later as the new species *T. hoshinota* Rützler

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and Muzik 1993. Since then, coral community loss (30%-80%) from *Terpios* outbreaks, the so-called 'black disease' of corals, has sporadically been reported throughout the western Pacific. Infected coral reefs include those at Truk Lagoon of American Samoa, Cebu I. of the Philippines, Thailand (Plucer-Rosario 1987), the Ryukyu Is. of Japan (Rützler and Muzik 1993, Reimer et al. 2011), Green I. (*Lyudao*) of Taiwan (Liao et al. 2007, Chen et al. 2009), and Lizard I. of the Great Barrier Reef (Fujii et al. 2011). Nevertheless, the life history and factors associated with outbreaks of *T. hoshinota* remain uncertain.

Field observations indicated that *T. hoshinota* mainly forms a thin black sheet that encrusts live corals (Liao et al. 2007, Chen et al. 2009), and its color may be derived from dense populations of cyanobacteria (Hirose and Murakami 2011). In some cyanobacteriosponges, the endosymbiotic cyanobacterium was shown to provide its host sponge with nitrogenous and carbonic nutrients via nitrogen fixation and photosynthesis (Wilkinson and Fay 1979, Wilkinson 1983, Cheshire et al. 1997), and was engulfed and digested by its host as a source of nutriments (Rützler 1988, Maldonado and Young 1998). However, nutritional interactions between *T. hoshinota* and cyanobacteria are unknown. Endosymbiotic cyanobacteria can also produce bioactive compounds for chemical defense and competition with other marine organisms (reviewed in Uemura et al. 2009). The *Terpios* sponge can efficiently expand its territory, although the mechanism of this species' dispersion is poorly understood.

Terpios-like larvae were first described by Yamaguchi (1986) with a sponge sample from Tokunoshima Is. (Ryukyus, Japan), but no photographic data were presented. The original description of *T. hoshinota* reported no reproductive cells in histological sections (Rützler and Muzik 1993). However, fine-structural studies of *T. hoshinota* demonstrated that the sponge has spermaries and possibly oocytes in summer but not in winter in Okinawa, Japan (Hirose and Murakami 2011), suggesting that sexual reproduction in *T. hoshinota* on subtropical coral reefs is seasonal.

A collection of *T. hoshinota* at Green I., Taiwan in Nov. 2010 produced a specimen bearing larvae. The larvae were barely able to swim even with cilia, and harbored numerous symbiotic cyanobacteria. Herein, the behavior and fine structures of the parenchymella larvae released from *T. hoshinota* are reported, and potential dispersal and outbreak mechanisms are proposed.

MATERIAL AND METHODS

Specimen collection and maintenance

Individuals of *T. hoshinota* (around 5 cm in diameter or 10 cm long) encrusting a coral skeleton were collected in Nov. 2010 by scuba diving from 3-5 m in depth at Dabaisha, southwestern Green I., Taiwan (22°39'N, 121°29'E), and then delivered to the laboratory in a sealed bag with 1/2 seawater and air within 5 h. The sampling date was 20 Nov. 2010, during a full moon. The samples were placed in a tank (90 × 45 × 45 cm) containing 40 L of recirculating seawater (around 1.2 L/min) and illuminated at around 150 μE/m^2/s photosynthetically active radiation under a 12-h/12-h light/dark regime. In order to observe the reproduction of the *Terpios* sponge, the number of larvae was counted every day, and their moving behaviors and body structure were examined directly or under a microscope. Some larval specimens were fixed in 4% glutaraldehyde containing 0.25 M sucrose and 0.1 M sodium cacodylate (pH 7.2-7.4) and stored at 4°C for microscopic examination.

Light microscopy of whole-mounted specimens

Some glutaraldehyde-fixed specimens were observed with a light microscope equipped with differential interference contrast optics. Several microscopic images were combined to increase the depth of field using the post-processing image software, Helicon Focus Pro 4.2.6 (Helicon Soft, Kharkov, Ukraine). Cyanobacteria in the larvae were also examined for chlorophyll autofluorescence under an epifluorescence microscope (Nikon, Eclipse 50i, Tokyo, Japan) at excitation of 450-490 nm.

Histology and electron microscopy

Glutaraldehyde-fixed specimens were rinsed with 0.45 M sucrose containing 0.1 M sodium cacodylate, postfixed with 1% osmium tetroxide containing 0.1 M sodium cacodylate for 1.5 h, and then dehydrated with a graded ethanol series.

Some specimens were immersed in *t*-butanol and freeze-dried for scanning electron microscopic (SEM) observation of whole-mounted specimens. They were sputter-coated with gold-palladium and

examined using an SEM (JEOL, JSM-6060LV, Tokyo, Japan) at 15 kV.

The remaining specimens were cleared with *n*-butyl glycidyl ether and embedded in epoxy resin for histological and transmission electron microscopic (TEM) observations. Sections 0.5-1.0 μm thick were stained with toluidine blue for light microscopic observations. Thin sections stained with uranyl acetate and lead citrate were then examined with a TEM (JEOL, JEM-1011).

RESULTS

One (at around 50 cm^2) of 44 specimens of *T. hoshinota* from Green I., Taiwan was found to bear larvae, and it continued to release larvae for 3 d following collection. Numbers of released larva reached a peak (of 343 individuals (ind.)) on the 2nd d following collection, compared to only 160 and 60 individuals on the 1st and 3rd d, respectively. No further larval release was observed after the 3rd d. The larval density of the specimen was estimated to be about 11.3 ind./cm^2. *Terpios* larvae were released from the osculum and immediately dropped to the bottom of the aquarium tank due to negative buoyancy and a limited swimming capability. Even though larvae might be expected to move toward light so that their endosymbiotic cyanobacteria could conduct photosynthesis, they did not exhibit a significant response to directional light. Released larvae were observed to settle on glass surfaces or coral skeletons within 1 d when the movement of the seawater was nearly quiescent.

Larvae had an ovum shape and a bare posterior pole, were 650 ± 98 μm (*n* = 15) long and 350 ± 40 μm (*n* = 15) wide (Fig. 1A), and had no blastocoels or spicules. The brown color of the larvae was attributed to numerous internal symbiotic cyanobacteria (Fig. 1A). When observed under an epifluorescence microscope (with excitation at 450-490 nm), all larvae displayed red autofluorescence from the chlorophyll contained

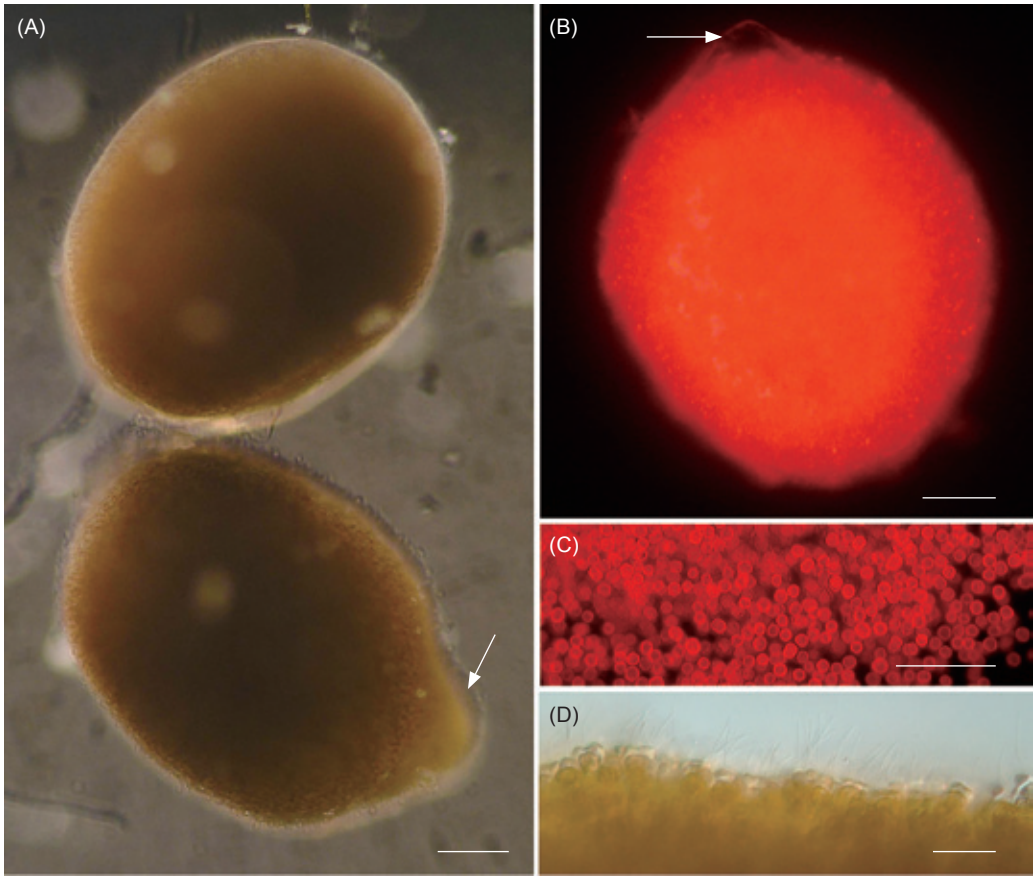

Fig. 1. Larvae of *Terpios hoshinota*. (A) Whole larva under light microscopy; (B) autofluorescence of cyanobacterial symbionts in a whole larva with green-light excitation; (C) enlargement of B; (D) ciliated ectoderm (differential interference contrast). Arrows in A and B indicate the posterior poles. Scale bars: A, B = 10 μm; C = 50 μm; D = 20 μm.

in the endosymbiotic cyanobacteria, indicating that the functional cyanobacteria were evenly distributed in the larvae (Fig. 1B, C). Even though the endosymbiotic cyanobacteria were densely packed within most larval cells, the microbes seemed to be absent from the posterior pole (Fig. 1B). The larval surface was entirely covered by cilia (Fig. 1D), which could possibly allow the larvae to slowly move by rotation or swimming.

In the SEM observations, many parts of the ectoderm had been exfoliated from the larvae during specimen preservation and microscopic sample preparation (Fig. 2A). Overall, ectodermal cells supported many cilia (Fig. 2B). In lateral view, the ectoderm was a monolayer of ciliated columnar cells (Fig. 2C) that were loosely attached to one another by lateral membranes. Although there were often thin gaps among neighboring cells, it is possible that the gaps were artifacts of sample preparation, e.g., freeze-drying. Cyanobacterial cells were sometimes found in the basal part of the ectodermal layer (arrow in Fig. 2C). Figure 2D also shows 1 cilium emerging from each ectodermal cell.

The inner part of a larva was occupied by spherical cyanobacterial cells and amoeboid sponge cells; these cells were loosely packed and did not directly contact each other (Fig. 3A, B). No bacteria other than the cyanobacterial symbionts were found in the larvae (Fig. 3B). A prominent mesohyl, a major matrix structure in adult sponges, was not found, although there were loose, fibrous materials within spaces among the cells (white arrows in Fig. 3B-D). Cyanobacterial cells had thylakoid membranes in the cell periphery, several vacuoles in other parts of the cell, and some granular inclusions in the cytoplasm (Fig. 3B, C). Dividing cyanobacteria were often found, indicating active proliferation within the larvae (Fig. 3C). Occasional sponge cells contained cyanobacterial cells, but subcellular structures of the microbes had disintegrated (Fig. 3D).

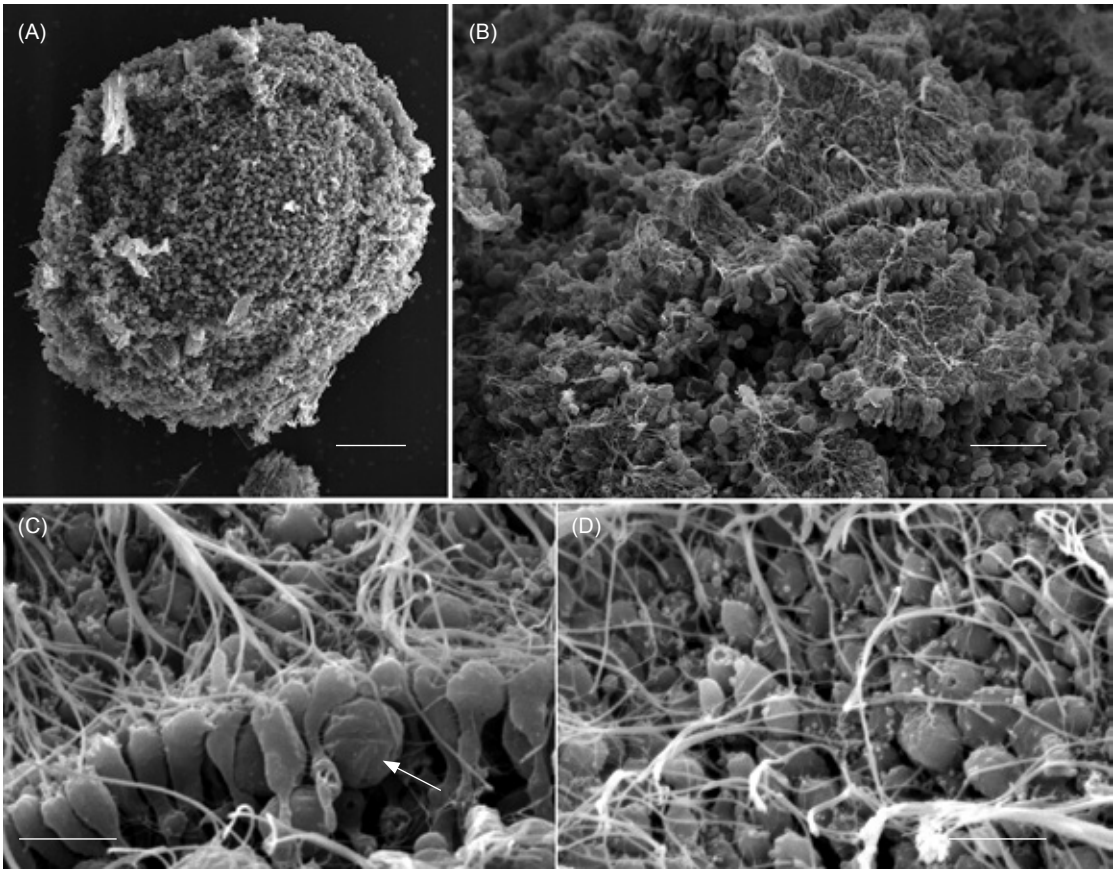

Fig. 2. SEM images of larvae of *Terpios hoshinota*. (A) Whole larva. Many parts of the ectoderm are exfoliated, and inner cells are exposed. (B) Fragments of the ectoderm remaining on a larva. (C) Lateral view of the ectoderm consisting of columnar cells. The arrow indicates a cyanobacterial cell. (D) Apical surface of the ectoderm, showing a cilium emerging from each cell. Scale bars: A= 50 μm; B = 20 μm; C, D = 5 μm.

DISCUSSION

In this study, we present the 1st detailed description of larvae released by the coral-killing sponge, *T. hoshinota*. Consistent with many other parenchymella larvae, *Terpios* larvae are solid and entirely ciliated, but we could not confirm whether the posterior pole was covered by cilia or not because the larval ectoderm was fragile and easily exfoliated. The larvae can be categorized as a parenchymella (Maldonado and Bergquist 2002, Mariani et al. 2005). Once the larvae left the parent *Terpios*, they settled within 1 d in the aquarium and rarely moved before settling down when the water flow was turned off. This short duration of the larval stage is consistent with field observations made by Dr. Nozawa's group in 2011 (pers. comm.). These observations suggest that larvae might have short dispersal distances, which may also partly explain the sporadic distribution of *T. hoshinota*. The short larval duration found for *T. hoshinota* might also be supported by their larval structure. It was found that adherence among ectodermal cells was not robust, implying that larvae tend to fall apart if they stay in the larval stage too long.

Most of the cyanobacteria found in *Terpios* larvae were the same as those cyanobacterial symbionts in the adult sponge (Rützler and Muzik 1993, Hirose and Murakami 2011). However, it

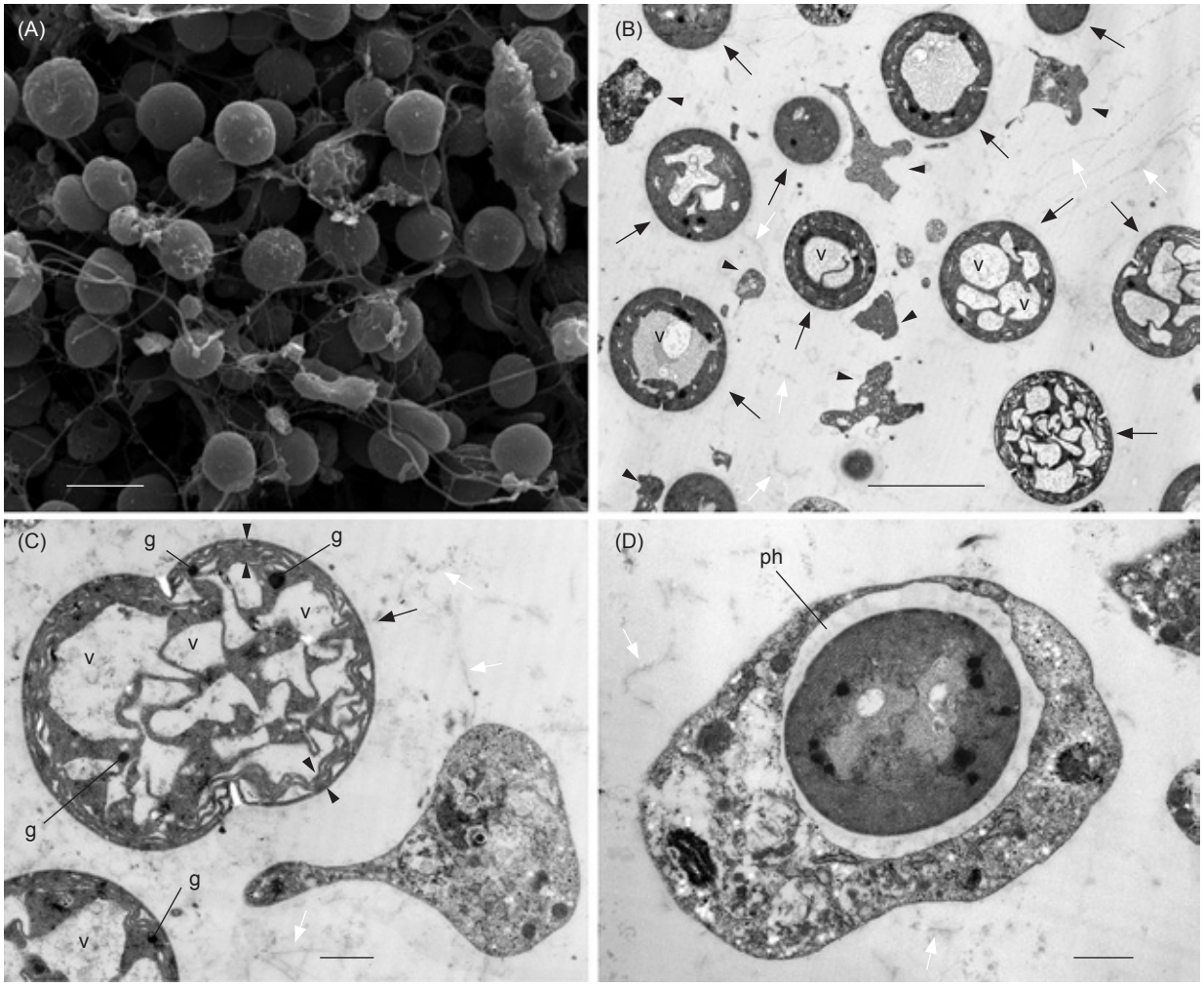

Fig. 3. Inner structures of larvae observed with SEM (A) and TEM (B-D). (A) Spherical cyanobacteria and amoeboid sponge cells forming a loose mass in larvae. (B) Cyanobacterial (black arrows) and sponge cells (arrowheads) not directly contacting one another. (C) Cyanobacterial cells in larvae with stacks of thylakoid membranes (facing arrowheads) in the peripheral cytoplasm and appearing with an intact ultrastructure. Some cells are dividing (black arrow). (D) Sponge cell engulfing a cyanobacterial cell, the ultrastructure of which has disintegrated. White arrows indicate fibrous materials. g, granular inclusions; ph, phagosome; v, vacuoles. Scale bars: A, B = 5 μm; C, D = 1 μm.

was also found that some cyanobacteria were located inside sponge cells. This might not be a case of intracellular symbiosis because those cyanobacteria appeared to have been digested by the sponge cells, as indicated by the disintegrated thylakoid membranes, within phagosomes. Digesting endosymbiotic cyanobacteria as a nutrient source was also described in other sponges (Berthold et al. 1982, Borowitzka and Hinde 1999). Therefore, cyanobacteria being engulfed implies a trophic function of the microbes in the development of larvae.

Since freshly released larvae harbor numerous cyanobacteria, these photosymbionts should be acquired during oogenesis and/or embryogenesis in the parent individual. While gametes and embryos are surrounded in the mesohyl where there are many cyanobacteria, the mechanism of symbiont transfer across generations is unresolved. In another viviparous cyanobacteriosponge, *Diacarnus erythraenus*, the larvae always harbor cyanobacteria identical to those of the parent individuals as determined by comparing ultrastructures and 16S ribosomal DNA sequences (Oren et al. 2005). The endosymbiotic cyanobacteria in *T. hosinota* and *D. erythraenus* share several common features. They all have intact ultrastructures and are extracellularly distributed in both sponge individuals and larvae. Dividing cells were often found, and some were engulfed and probably had been digested by the sponge cells. However, ultrastructures of cyanobacterial symbionts greatly differ between the 2 host origins, indicating they are not the same species. It would be revealing to know if the modes of symbiont transmission were similar between the 2 sponge species, since they probably independently developed photosymbiosis with each symbiont species.

This is the 1st report of larval release in the cyanobacteriosponge, *T. hoshinota*, and should contribute our knowledge of the dispersal of this coral-killing sponge. A recent discovery of the dispersal mechanism of *T. hoshinota* suggests that the sponge occasionally forms tissue threads to extend its territory (Soong et al. 2009) and produces spherical fragments of tissues (Wang, pers. observ.). Therefore, the black sponge *T. hoshinota* has several methods of dispersal to achieve sporadic outbreaks in coral reefs.

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