

Photosymbiotic Ascidians from Kenting and Lyudao in Taiwan

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Euichi Hirose and Yoko Nozawa (2010) Photosymbiotic ascidians from Kenting and Lyudao in Taiwan. *Zoological Studies* 49(5): 681-687. The photosymbiotic ascidian fauna was surveyed in Keelung, Kenting, and Lyudao (Green I.), Taiwan. Nine species in total were recorded from Kenting and Lyudao, but no photosymbiotic species were found in Keelung, probably due to the cold surface water in winter (ca. 16°C) in that region. Eight of the 9 species harbored *Prochloron* as their symbiotic cyanophytes, and *Trididemnum clinides* harbored some other cyanophyte species. All of these species have also been recorded from the Ryukyu Archipelago, Japan, whereas some species common to the Ryukyus were not abundant in Taiwan. Differences in reef structures between Taiwan and the Ryukyus may have caused some differences and the diversity of the microhabitats therein, resulting in the observed differences in species distributions. <http://zoolstud.sinica.edu.tw/Journals/49.5/681.pdf>

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In tropical and subtropical waters, some colonial ascidians belonging to the Family Didemnidae have obligate symbiotic relationships with prokaryotic algae *Prochloron*, *Synechocystis*, and/or unknown cyanophytes (see Parry and Kott 1988, Lewin and Cheng 1989, Hirose et al. 2009c). Whereas about 30 species in the 4 didemnid genera were described as host species by Kott (2001), recent taxonomic studies disclosed several new species from the Ryukyu Archipelago of Japan (Oka et al. 2005, Hirose and Oka 2008, Hirose and Hirose 2009, Hirose et al. 2009a). The symbiosis between didemnid ascidians and cyanophytes is the only obligate photosymbiosis known in the phylum Chordata, and the major symbiont, *Prochloron*, is a unique cyanophyte that has chlorophyll *a* and *b* but lacks phycobilins. Therefore, this is a unique symbiotic system from the viewpoints of phycology, physiology, evolution, and ecology, and researchers of biochemical and pharmaceutical science have also paid great

attention to photosymbiotic ascidians as resources of bioactive compounds (see Hirose et al. 2009b).

A larger number of photosymbiotic ascidian species are generally distributed at lower latitudes, and thus the distribution of these species is a potential indicator of the warming of seawater. *Prochloron* cells isolated from host ascidians in Palau showed no photosynthetic activity at $\leq 20^{\circ}\text{C}$ in vitro (Dionisio-Sese et al. 2001), indicating the susceptibility of the photosymbionts to low temperatures. Thus, water temperature may be crucial in limiting the distribution of photosymbiotic ascidians. On the other hand, an increase in water temperature caused by global warming may result in shifts in the range of species in the future (e.g., Walther et al. 2002).

Recently, we carried out a biogeographic survey of photosymbiotic didemnids in the Ryukyus to record the current distribution range for each species. To date, at least 19 photosymbiotic species are known to be distributed in Japan,

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mainly in the Ryukyu Archipelago (Oka and Hirose 2008, Hirose and Hirose 2009, and references therein). The species numbers gradually decrease northward in the Ryukyu Archipelago which ranges from nearly 24°N to 31°N. Nineteen species have been recorded from the Yaeyama Is. (the southernmost island group: at ca. 24°N), whereas only 4 species were recorded from Yakushima and Tanegashima (the northernmost islands: at ca. 30°-31°N).

Taiwan is positioned close to the southernmost island group of the Ryukyu Archipelago (i.e., the Yaeyama Is.), and the survey of the photosymbiotic ascidian fauna here is, therefore, indispensable for a better understanding of their biogeography in the subtropical West Pacific. A few species of photosymbiotic ascidians were reported from Taiwan (see Chen 2001 2002), but the records appear to include misidentifications. In 2009, we surveyed the photosymbiotic ascidian fauna in Keelung, Kenting, and Lyudao (Green I.) with the generous collaboration of members of the Biodiversity Research Center, Academia Sinica, Taipei, Taiwan. Herein, we record 9 species of photosymbiotic didemnid species as a contribution to the marine benthic fauna of Taiwan.

MATERIALS AND METHODS

Samples were collected by snorkeling in the shallow subtidal zone and by scuba diving at depths of 5-15 m. Ascidian colonies were photographed in situ before being collected. Some specimens were kindly provided by Dr. Masami Obuchi (Rising Star Program, Univ. of the Ryukyus, Okinawa, Japan). Collection sites and dates were as follows: Kenting (Wanlitung and Tiao-shi, 4 Sept. 2009: sampling permission #980904/1400), Keelung (Bitouchiao, 26 Sept. 2009), and Lyudao (Shihlan, 1 Oct. 2009; Dabaisha, 2 Oct. 2009; Gueiwan, 3-4 Nov. 2009).

Specimens were anesthetized with menthol and 0.37 M MgCl₂ for approximately 2 h, and then fixed with 10% formalin-seawater. Some *Didemnum molle* colonies were also fixed in ethanol for future DNA sequencing. The fixed colonies were dissected under a binocular stereomicroscope. Taxa were mainly identified following Nishikawa (1995), Kott (2001), and Hirose et al. (2009a). Some of the specimens were deposited in National Museum of Natural Science (NMNS), Taichung, Taiwan.

RESULTS

Nine species of didemnid ascidians harboring prokaryotic algae were recorded from Kenting and Lyudao. Their occurrences at each site are listed in table 1. Among them, 8 species harbored *Prochloron* as their symbiotic cyanophytes, while *Trididemnum clinides* harbored some other cyanophyte species. No photosymbiotic species were found in Keelung in the present survey.

Didemnum molle (Herdman, 1886)

Specimens: NMNS-6235-007 (Tiao-shi, subtidal), NMNS-6235-021 (Gueiwan, 16 m).

The dome-shaped colonies from Kenting were ≤ 5 mm in diameter (Fig. 1). They were irregularly smaller than colonies (< 5 cm) commonly found in the Ryukyus, Great Barrier Reef, and many other sites. It is uncertain whether these small colonies were not fully grown, but we did not find larger colonies in the present survey. The colonies from Lyudao were irregular in shape and sometimes over 5 cm on the long axis (Fig. 2) and had gray patches on the white colony. Several morphotypes are known within this species, and a molecular phylogeny based on partial cytochrome oxidase subunit I (COI) gene sequence discriminated 4 morphotypes (i.e., white, gray, brown, and large) from the Ryukyus, indicating that each morphotype is a distinct species (Hirose et al. 2009). The colony color of Kenting's specimens was white or brown, but these small colonies may be a new morphotype. The colonies from Lyudao apparently corresponded to the large morphotype. Future molecular phylogenetic analyses are necessary to clarify these observations.

Table 1. Distribution records of the photosymbiotic didemnid ascidians in Taiwan

Location	Kenting	Lyudao	Keelung
Approx. latitude	21°40'N	22°40'N	25°N
<i>Didemnum molle</i>	+	+	
<i>Trididemnum clinides</i>	+	+	
<i>Trididemnum cyclops</i>			+
<i>Lissoclinium bistratum</i>	+	+	
<i>Diplosoma aggregatum</i>			+
<i>Diplosoma gumavirens</i>	+	+	
<i>Diplosoma ooru</i>	+	+	
<i>Diplosoma simile</i>	+	+	
<i>Diplosoma virens</i>	+	+	

***Trididemnum clinides* Kott, 1977**

Specimens: NMNS-6235-001 (Wanlitung, 5 m), NMNS-6235-015 (Shihlan, subtidal).

Colonies from Wanlitung, Kenting were oval cushions of 2-4 mm on the long axis, and those from Shihlan, Lyudao were up to 8 mm on the long axis (Fig. 3). The colonies were white to brown due to various concentrations of stellate spicules and cyanophyte symbionts in the tunic (Fig. 4). The colonies turned pale green in 10% formalin seawater, probably due to phycobilins of the symbiotic cyanophytes becoming discolored or extracted in the fixation medium. Three types of cyanophytes were contained in the tunic, and they appeared to be the same as those observed in specimens from Okinawajima I. (Hirose et al. 2009c). Diameters of the tunic spicules differed between specimens from Wanlitung (Kenting) and Shihlan (Lyudao): about 30 μm or less in Wanlitung's specimens and sometimes > 45 μm in Shihlan's specimens.

***Trididemnum cyclops* Michaelsen, 1921**

Specimens: NMNS-6235-020 (Dabaisha, subtidal), NMNS-6235-024 and NMNS-6235-025 (Gueiwan, subtidal).

Colonies were oval or irregularly shaped cushions of 2-5 mm on the long axis (Fig. 5). Each zooid had a black dot, due to a pigment mass at the top of the endostyle. While specimens were collected in the subtidal zone, colonies were also found at depths of 5-15 m. A tailed embryo was found in a colony at Gueiwan, Lyudao (Fig. 5 inset). Berry-like spicules were distributed in the tunic (Fig. 6).

Blister-like spheres were found on one of the colonies from Gueiwan, Lyudao (Fig. 7: NMNS-6235-024), which proved to be egg pouches of an unknown animal: there was a round opening at the top of the spheres and embryos covered the inner wall of the lower hemispheres. The embryos were at the 4-cell stage with many yolk granules (Fig. 8). In *T. cyclops* colonies from Okinawajima I., we have found an egg pouch of an unknown gastropod (Kuze and Hirose, 2009). Therefore, the egg pouches of the present specimens were likely laid by a gastropod species.

***Lissoclinum bistratum* (Sluiter, 1905)**

Specimens: NMNS-6235-002 (Wanlitung, subtidal), NMNS-6235-008 (Tiao-shi, subtidal), NMNS-6235-026 (Gueiwan, subtidal).

Colonies were flat sheets of irregular shapes (Fig. 9). While colonies were usually green due to the symbiotic alga *Prochloron*, the substratum side of the colonies was white due to calcareous tunic spicules. The spicules were always round and $\leq 40 \mu\text{m}$ in diameter (Fig. 10). This species was recorded in Taiwan by Chen (2001 2002) as *Lissodinum* (cf. *bistratum*).

This species is very similar in morphology to *Lissoclinum timorense*, and the presence or absence of the stellate spicules discriminates these 2 species (Kott 2001). However, Monniot and Monniot (2001) proposed that *L. timorense* is a junior synonym of *L. bistratum*, as they consider the variations of spicules to be intraspecific. Moreover, these 2 species were indistinguishable in a molecular phylogeny inferred from the partial sequences of the COI gene (Hirose et al. 2010).

***Diplosoma aggregatum* Hirose and Hirose, 2009**

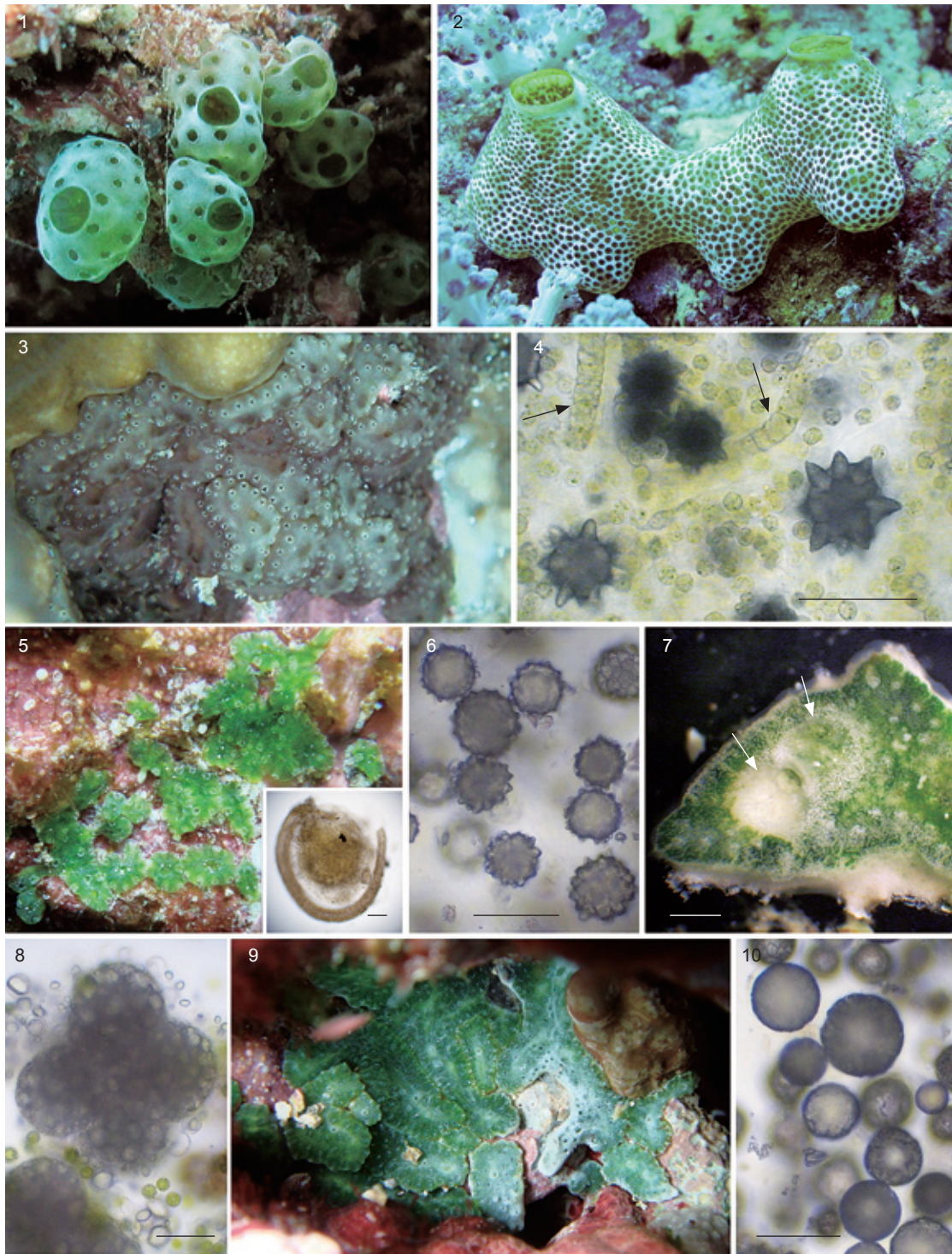
Specimens: NMNS-6235-016 (Dabaisha, subtidal), NMNS-6235-023 (Gueiwan, subtidal).

Colonies were small oval cushions about 2-5 mm on the long axis (Fig. 11). The colonies often formed mosaic-like aggregates on the substrata but never formed a larger sheet of colonies such as seen in *Diplosoma virens*. In *Diplosoma* species, there are 4 rows of stigmata on both the right and left halves of the branchial sac. In this species, there were 6 stigmata in the 1st (top) row, 6 in the 2nd row, 6 in the 3rd row, and 5 in the 4th (bottom) row, i.e., a 6-6-6-5 stigma pattern. The retractor muscle emerged from halfway down the esophageal neck (N type).

***Diplosoma gumavirens* Hirose and Oka, 2009**

Specimens: NMNS-6235-003 (Wanlitung, subtidal), NMNS-6235-013 (Shihlan, subtidal), NMNS-6235-017 (Dabaisha, subtidal).

Colonies were small cushions of about 1 mm thick. The branchial aperture of each zooid was often bordered with a light-blue ring (Fig. 12). The stigmata in each half of the branchial sac numbered 5, 5, 5, and 4 from the 1st to the 4th rows, i.e., 5-5-5-4. The retractor muscle emerged



Figs. 1-10. Photosymbiotic ascidians with tunic spicules. **1.** Small colonies of *Didemnum molle*, Wanlitung, Kenting, Taiwan (5 m). Colonies are approximately 5 mm in diameter. **2.** Large colony of *Didemnum molle*, Gueiwan, Lyudao, Taiwan (15 m). The colony is approximately 5 cm on the long axis. **3.** *Trididemnum clinides*, Shihlan, Lyudao, Taiwan (subtidal). **4.** Tunic spicules, and filamentous (arrows) and unicellular cyanophytes embedded in the tunic of *Trididemnum clinides*. Scale bar = 50 μ m. **5.** *Trididemnum cyclops*, Gueiwan, Lyudao, Taiwan (subtidal) and a tailed embryo brooded in the colony (inset). Scale bar = 50 μ m. **6.** Tunic spicules of *Trididemnum cyclops*. Scale bar = 50 μ m. **7.** *Trididemnum cyclops* colony (NMNS-6235-024) with blister-like egg pouches (arrows) from an unknown animal. Scale bar = 1 mm. **8.** Embryos in an egg pouch laid in the tunic of *Trididemnum cyclops*. Scale bar = 1 mm. **9.** *Lissoclinum bistratum*, Gueiwan, Lyudao, Taiwan (subtidal). **10.** Tunic spicules of *Lissoclinum bistratum*. Scale bar = 50 μ m.

from halfway down the esophageal neck (N type).

***Diplosoma ooru* Hirose and Suetsugu, 2005**

Specimens: NMNS-6235-004 (Wanlitung, subtidal), NMNS-6235-012 (Shihlan, subtidal)

Colonies were thin, irregularly shaped sheets of ≤ 1 mm thick (Fig. 13). This species was characterized by a 5-6-5-4 stigma pattern and by the retractor muscle which emerged from the bottom of the thorax (T type).

***Diplosoma simile* (Sluiter, 1909)**

Specimens: NMNS-6235-005 (Wanlitung, subtidal), NMNS-6235-009 (Tiao-shi, subtidal), NMNS-6235-014 (Shihlan, subtidal), NMNS-6235-018 (Dabaisha, subtidal).

Colonies were irregularly shaped sheets 1-2 mm thick (Fig. 14). This species was characterized by a 6-6-6-5 stigma pattern and T-type retractor muscle.

***Diplosoma virens* (Hartmeyer, 1909)**

Specimens: NMNS-6235-006 (Wanlitung, subtidal), NMNS-6235-010 (Tiao-shi, subtidal), NMNS-6235-019 (Dabaisha, subtidal), NMNS-6235-022 (Gueiwan, subtidal).

Colonies were irregularly shaped sheets about 2-5 mm thick (Fig. 15). This species was characterized by a 6-6-6-5 stigma pattern and an N-type retractor muscle as in *Dip. aggregatum*, but the colonies were much larger (compare with Fig. 11). COI sequences could discriminate these species (Hirose and Hirose 2009), although we conducted no molecular analyses in the present study. A female notodelphyid copepod was found from a colony collected at Tiao-shi, Kenting (NMNS-6235-011). Since colonies of this species in the Ryukyus are often parasitized by *Loboixys ryukyuensis* (see Ooishi 2006), the present notodelphyid is likely to be a conspecific or closely related species.

DISCUSSION

This is the 1st faunal study of the photosymbiotic ascidians of Taiwan. The 9 species listed here are likely far from the entire coverage of the photosymbiotic ascidian fauna in Taiwan, because the present survey was done over a very

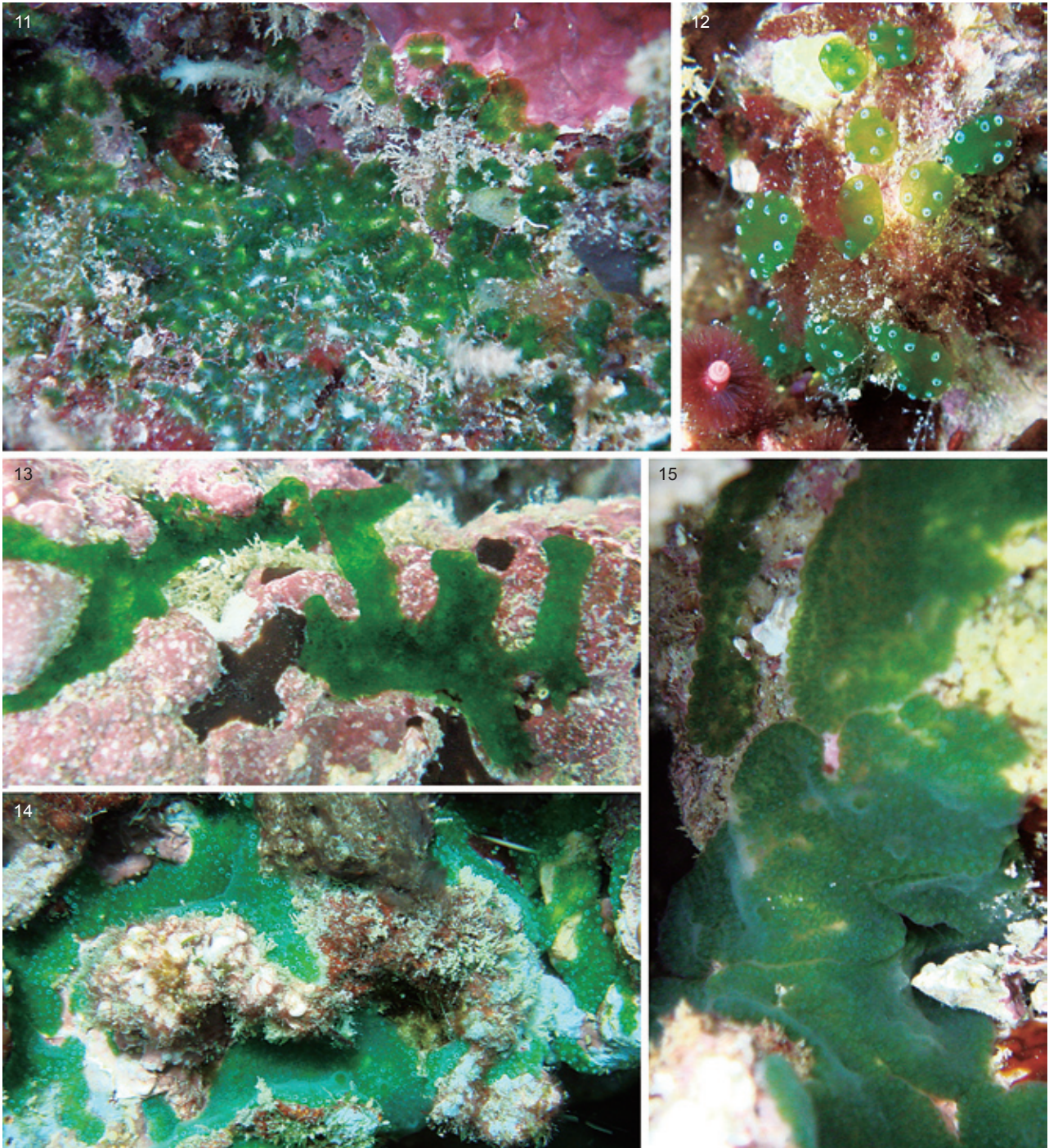
short term and at limited sites. It is also possible that some undescribed species still remain to be disclosed. Interestingly, no photosymbiotic species were found in Keelung in the present survey, although the latitude is comparable to that of the Miyakojima Is. (South Ryukyus) from which 12 photosymbiotic species were recorded. Although these results do not conclusively demonstrate the absence of the photosymbiotic ascidians from Keelung, photosymbiotic species must be rare there. Interestingly, distribution patterns of photosymbiotic ascidians in Taiwan and the Ryukyus agree with the coral communities there: the number of coral species off northeastern coast of Taiwan is much smaller than those in the Central and South Ryukyus and southern Taiwan (Chen and Shashank 2009). This is likely due to the fact that the waters of the northern coast of Taiwan are colder than most of the Ryukyus. The minimum winter temperature of the sea surface is about 16°C on the northern and northwestern coasts of Taiwan due to the cold China coastal water, and the scleractinian distribution there is considered a “non-reefal community” (Chen 1999, Chen and Shashank 2009, see also Veron 1995). In contrast, the minimum winter temperature is about 19°C at Okinawajima I. Since *Prochloron* is susceptible to low temperatures (Dionisio-Sese et al. 2001), cold water in winter probably causes the absence or rare occurrence of photosymbiotic ascidians on the coast of Keelung. If any photosymbiotic ascidians become prominent there in the near future, it could be interpreted as a sign of local ocean warming.

All of the photosymbiotic ascidians described here were also recorded from the Ryukyu Archipelago, Japan. More species, including undescribed ones, are potentially distributed in Taiwan, as 19 and 12 species were respectively recorded from the Yaeyama Is. and Miyako Is. in the South Ryukyus (E Hirose unpublished data). It is noteworthy that some species common in the Ryukyus were not abundant in Kenting or Lyudao.

In the Ryukyus, *Did. molle* and *L. bistratum* are common on the subtidal coral reefs, but they were not abundant in Taiwan. Regarding *Did. molle*, 4 morphotypes were found in the Ryukyus, and each type is genetically distinct based on partial COI gene sequences (Hirose et al. 2009). In Taiwan, 2 morphotypes of *Did. molle* were found, but they were rare in subtidal coral reefs. Some differences in the photosymbiotic ascidian distributions could have been caused by differences in the coral reef structures between Taiwan and the Ryukyus. The coral reefs in

Kenting and Lyudao are categorized as “fringing reefs” that are directly attached to the shore, whereas the reefs in the Ryukyus are often “barrier reefs” with a reef lagoon (moat) and back reef. Differences in reef structures cause differences

and diversity of the microhabitats therein. Extensive surveys and continuous monitoring in more locations are necessary to complete an overview of the photosymbiotic ascidian fauna of Taiwan.



Figs. 11-15. Photosymbiotic ascidians without tunic spicules, i.e., *Diplosoma* spp. **11.** *Diplosoma aggregatum* colonies that had formed a mosaic-like aggregate, Dabaisha, Lyudao (subtidal). **12.** *Diplosoma gumavirens*, Dabaisha, Lyudao (subtidal). **13.** *Diplosoma ooru*, Wanlitung, Kenting (subtidal). **14.** *Diplosoma simile*, Tiao-shi, Kenting (subtidal). **15.** *Diplosoma virens*, Dabaisha, Lyudao (subtidal).

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