

Latitudinal Difference in the Species Richness of Photosymbiotic Ascidians Along the East Coast of Taiwan

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Some didemnid ascidians harbor cyanobacterial symbionts, and this is the only obligate photosymbiosis system known in chordates. These photosymbiotic ascidians are found only in tropical and subtropical waters, probably because the photosymbionts are vulnerable to low temperatures. We surveyed the photosymbiotic ascidian fauna along the east coast of Taiwan. The present and previous reports recorded 13 species in Taiwan, and the species richness and composition is different in five areas along the east coast. Along the middle-east, southeast, and south coasts, five or more species have been recorded, whereas only one species has been found along the northeast coast, and no species have been recorded on the north coast. This gap in the species richness is probably related to the Kuroshio Current, which travels from south to north along the east coast of Taiwan but changes to an easterly direction off the northeast coast. Increases in water temperature due to global warming could cause northward expansion of the distribution ranges of these ascidians in the future. Hence, the photosymbiotic ascidian fauna could be an environmental indicator in subtropical, shallow water, and the present study provides a basic dataset that illustrates the current status of photosymbiotic ascidians in Taiwan.

Key words: Biogeography, Coral reef, Didemnid ascidians, Cyanobacterial symbiosis, Latitude, Species diversity.

BACKGROUND

Obligate symbiosis between photosynthetic organisms and chordates is known to occur in some colonial ascidians inhabiting coral reefs, and the most common photosymbiont is the green, unicellular coccoid *Prochloron*, a unique cyanobacterium containing chlorophyll *b* and lacking phycobilin (reviewed in Lewin and Cheng 1989; Hirose et al. 2009a; Hirose 2015). Whereas about 30 host ascidians have been described from four genera of Didemnidae (e.g., Kott 2001), they occur exclusively on tropical-subtropical coral reefs. In the Ryukyu Archipelago, which stretches from 24°N to 31°N, the species richness of photosymbiotic ascidians

is greater at lower latitudes: 13 species were recorded from Haterumajima Is. (ca. 24°N), while only four were recorded from Tanegashima Is. (ca. 30°30'N) (Oka and Hirose 2005; Hirose et al. 2009b). Water temperature is supposed to be an important factor limiting the distribution range of the host ascidian because of the vulnerability of *Prochloron* to low temperature (Dionisio-Sese et al. 2001). Since an increase in water temperature due to global warming may extend the distribution range of the host ascidians towards higher latitudes in the future (e.g., Walther et al. 2002), the species composition and richness of these ascidians can be a potential indicator for coastal environments. In contrast to the susceptibility to low temperature,

photosynthetic activity of *Prochloron* showed low light adaptation but did not show photoinhibition, even at strong irradiation equivalent to full sunlight (Kühl et al. 2012; Nielsen et al. 2015).

Several studies have reported that outbreaks of photosymbiotic ascidians can threaten coral reef communities (Littler and Littler 1995; Vargas-Ángel et al. 2009; Sommer et al. 2010; Rodriguez-Martinez et al. 2012; Li et al. 2016; Tebbett et al. 2019). Therefore, we need to pay attention to these ascidians because invasive species can alter local ecosystems. For instance, *Diplosoma simile* (Sluiter, 1909), a photosymbiotic species, is common in Pacific coral reefs and reported from the Red Sea to the Caribbean Sea (Hirose et al. 2012; Hirose and Hirose 2013; Koplovitz et al. 2015); its occurrence in Caribbean waters is thought to be the result of an artificial introduction. Accordingly, it is important to record the current status of the distribution of each species. We have carried out biogeographic surveys of the photosymbiotic ascidian species in the Japan–Taiwan area (Hirose et al. 2004 2007 2014 2015; Oka and Hirose 2005 2008; Oka et al. 2007; Hirose and Nozawa 2010; Hirose and Su 2011; Hirose 2013 2014).

In Taiwan, 11 species of the photosymbiotic ascidians have been recorded from Kenting, Lyudao (Green Island) and Penghu, but not were found on the north coast (Hirose and Nozawa 2010; Hirose and Su 2011; Hirose et al. 2014 2015). Apart from an exceptional occurrence in Penghu, the distribution pattern of the photosymbiotic species apparently overlaps with the reef community of scleractinian corals in Taiwan (Chen 1999). The difference in biota between the northern and southern coasts of Taiwan was also documented for intertidal fish (Wen et al. 2018). In the present study, we surveyed the photosymbiotic ascidian fauna along the east coast of Taiwan, expecting a latitudinal difference in species composition and richness.

MATERIALS AND METHODS

Samples were collected by snorkeling in the shallow subtidal zone at the lowest tide around the spring tides. Ascidian colonies were photographed *in situ* before being collected. Collection sites and dates were as follows: Maobitou (5 Sept. 2017: 21°56'N, 120°44'E), Gongzibi (7 Sept. 2017: 22°09'N, 120°53'E), Jialeshuei (8 Sept. 2017: 21°58'N, 120°50'E), Shengao (7 Sept. 2018: 25°8'N, 121°48'E), He-mei (8 Sept. 2018: 25°5'N, 121°55'E), Shimen (13 and 16 Sept. 2019: 23°31'N, 121°30'E), Wushibi (14 Sept. 2019: 23°13'N, 121°25'E), and Xiaoyeliu (15 Sept. 2019: 22°48'N, 121°12'E) (Fig. 1). Sampling permissions are

#10630870200 for Kenting, #1073329756 for Dong-Bei-Jiao (northeast coast of Taiwan), #1080052641 for Hualien, and #1080000399 for Taitung.

Specimens were anesthetized with menthol and 0.37 M MgCl₂ for approximately 2 h, and then fixed with 10% formalin-seawater. The fixed colonies were dissected under a binocular stereomicroscope. Zooids and spicules were observed under a microscope equipped with differential interference contrast optics. In some microphotographs, several images were combined to increase the depth of field using the post-processing image software Helicon Focus Pro 6.4.3 (Helicon Soft, Ltd., Kharkov, Ukraine). Species identification was confirmed following Kott (2001), Hirose and Su (2011), and Hirose et al. (2014). For *Diplosoma* species, we counted the number of stigmata in each row in the branchial basket and located the liberating position of the retractor muscle. The specimens were donated to the National Museum of Natural Science (NMNS), Taichung, Taiwan under code NSMS-8141.

RESULTS

The present survey recorded the following 11 photosymbiotic species across four didemnid genera. Among them, *Diplosoma watanabei* and *Lissoclinium punctatum* were recorded in Taiwan for the first time, including from sites at Penghu and Lyudao.

Didemnum molle (Herdman, 1886)

Specimens: NMNS-8141-015 (Shimen), -016 (Wushibi).

Dome- or irregularly-shaped colonies of about 5 mm or larger were attached to hydrozoan colonies (Fig. 2A). Each colony had one or a few cloacal apertures (white arrows in Fig. 2A). Colonies were entirely white with brown patches. Globular spicules in the tunic reflected light, resulting in the white color of the colony (Fig. 2B). Several morphotypes are genetically defined in *D. molle* (Hirose et al. 2009c 2010a). Based on the colony size and coloration, all colonies in the present survey were “small type”, which was also recorded from Tiao-shi and Wanlitung in Kenting, while “large type” was recorded from Lyudao (Hirose and Nozawa 2010).

The expansion of *D. molle* was reported on the Great Barrier Reef (GBR); the colony density increased significantly following a mass coral bleaching (Tebbett et al. 2019). The *D. molle* colonies do not smother the live corals, but they may reduce the number of settlement sites suitable for coral recruits. Based on the photographs in Tebbett et al. (2019), the morphotype of

the expanding colonies appears to be the “brown type” that was recorded in the Ryukyus but not in Taiwan.

Diplosoma gumavirens Hirose and Oka, 2009

Specimens: NMNS-8141-001 (Maobitou), -017 (Shimen), -018 (Wushibi), -019 (Xiaoyeliu).

D. gumavirens colonies occurred on coral limestone in Maobitou, and many were also found on hydrozoan colonies in Shimen, Wushibi, and Xiaoyeliu (Fig. 2C). The colonies were small cushions of about 1 mm thick and entirely green due to *Prochloron* cells in the common cloacal cavity. There are no tunic spicules in *Diplosoma* species. In the thorax, there were 4 rows

of stigmata, and the pattern of stigmata was always 5-5-5-4 that means 5 stigmata in the first (top) row, 5 in the second row, 5 in the third row, and 4 in the fourth (bottom) row (Fig. 2D). The retractor muscle emerged from halfway down the esophagus (Fig. 2D).

Diplosoma ooru Suetsugu and Hirose, 2005

Specimens: NMNS-8141-002 (Gongzibi), -003 (Jialeshuei), -020 (Shimen).

Colonies were thin, irregularly shaped sheets of ≤ 1 mm thick (Fig. 2E). The pattern of stigmata was 5-6-5-4, and the retractor muscle emerged from the bottom of the thorax (Fig. 2F).

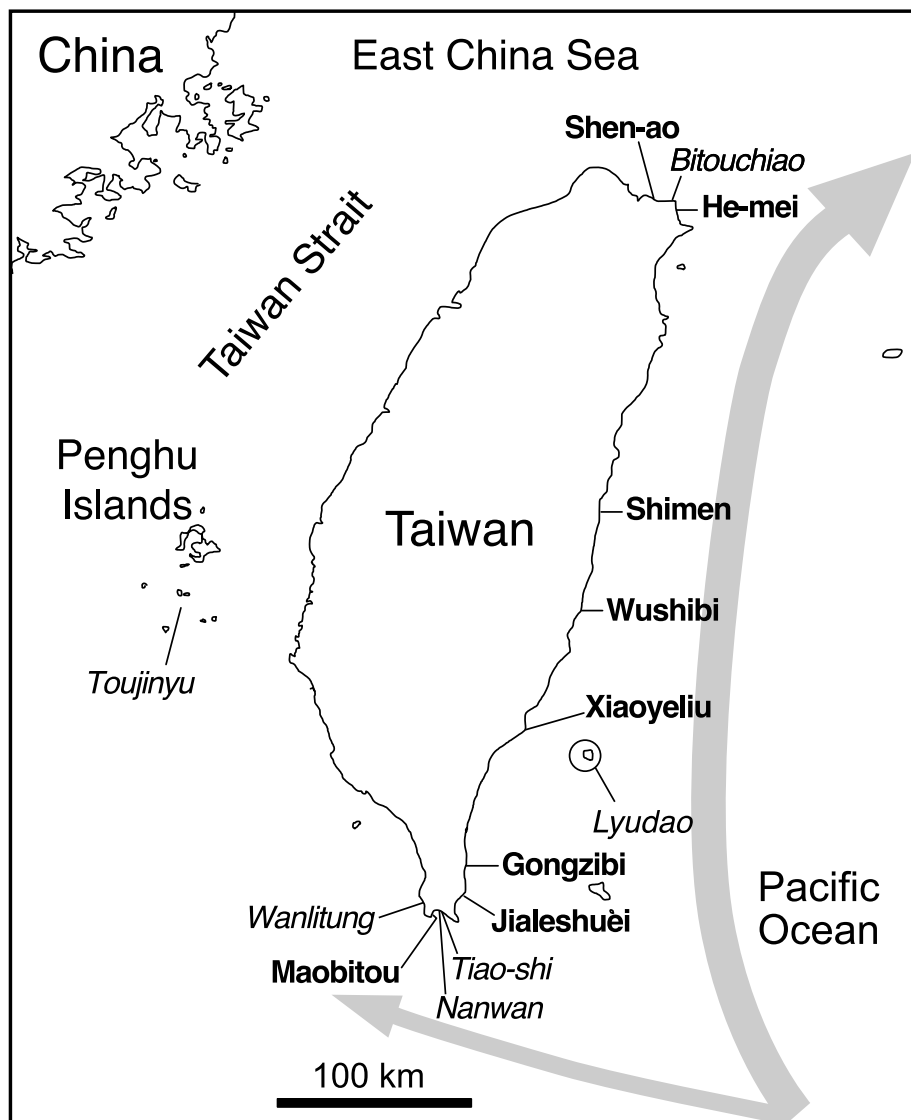


Fig. 1. Map of Taiwan. Collection sites are indicated in bold letters for the present study and in italics for the previous reports (Hirose and Nozawa 2010; Hirose and Su 2011; Hirose et al. 2014 2015). Gray arrows indicate the Kuroshio Current modified from Ujiie et al. (2003 2016).

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

Specimens: NMNS-8141-004 (Maobitou), -021 (Shimen), -022 (Wushibi).

Colonies were irregularly shaped sheets of 1-2 mm thick and firmly attached to the dead coral branch and coral limestone (Fig. 2G). The pattern of stigmata was 6-6-6-5, and the retractor muscle emerged from the bottom of the thorax (Fig. 2H).

An outbreak of *D. simile* colonies has threatened coral reef communities in Fiji and American Sāmoa (Littler and Littler 1995; Vargas-Ángel et al. 2009). It is possible that a *Diplosoma* sp. killing *Acropora* corals on Hainan Is. (South China Sea) may be *D. simile* (Li et al. 2016), although the zooid morphology should be examined to confirm the species identity.

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

Specimens: NMNS-8141-004 (Maobitou), -005 (Gongzibi), -023 (Wushibi).

Colonies were irregularly shaped sheets about 2-5 mm thick (Fig. 2I). The tunic was tougher than in other photosymbiotic *Diplosoma* species. The pattern of stigmata was 6-6-6-5 (Fig. 2J), and the retractor muscle emerged from halfway down the esophagus.

***Diplosoma watanabei* Hirose and Oka, 2009**

Specimens: NMNS-8141-014 (He-mei).

Colonies were thin, irregularly shaped sheets of ≤ 1 mm thick (Fig. 2K). The pattern of stigmata was 6-7-6-5 (Fig. 2L), and the retractor muscle emerged from the bottom of the thorax. This is the first time *D. watanabei* has been recorded from Taiwan.

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

Specimens: NMNS-8141-007 (Maobitou), -008 (Gongzibi), -009 (Jialeshuei).

Colonies were flat, irregularly shaped sheets and usually green due to the *Prochloron* distributed in the common cloacal cavities (Fig. 3A). The peripheries and the substratum side of the colony sheets were white due to globular spicules densely distributed in the tunic (Fig. 3B).

The present species shares many morphological features with *Lissoclinum timorense* (Sluiter 1909), and the absence of the stellate spicules distinguishes *L. bistratum* from *L. timorense* (Kott, 2001). Monniot and Monniot (2001), however, considered the variability in

the spicule composition to be intraspecific and proposed that *L. timorense* be assigned as a junior synonym of *L. bistratum*. Because there were no stellate spicules, the present specimens were identified as *L. bistratum sensu stricto*. There were no records of *L. timorense* from Taiwan, although this species is widely distributed in the tropical west Pacific from the Ryukyu Archipelago to GBR. The coral reefs in the Ryukyus are usually barrier reefs, and *L. bistratum* and *L. timorense* are often found at the same site; *L. bistratum* inhabits the reef flat and reef edge, while *L. timorense* inhabits the back reef. The reefs in Taiwan are mainly fringing reefs and lack back reefs, and the difference in reef structure is probably why *L. timorense* has never been recorded from Taiwan.

***Lissoclinum midui* Hirose and Hirose, 2011**

Specimens: NMNS-8141-010 (Gongzibi), -011 (Jialeshuei).

Small colonies comprising 10 or fewer zooids often attached to or partly fused with neighbor colonies (Fig. 3C). Colonies were entirely green due to *Prochloron* cells distributed in the tunic. There was an unknown organ beneath the thorax of each zooid (Fig. 3D). This character is unique to *L. midui*. Tunic spicules were globular (Fig. 3E). The type locality of the species was the out reef off Shinri-hama (Kumejima Is., Ryukyu Archipelago, Japan) (Hirose and Hirose 2011), and it has not been recorded from any other site in the Ryukyus. In Taiwan, this species was first recorded from Lyudao (Hirose et al. 2014), and the present report is the second distribution record. It is uncertain why the distribution of *L. midui* is sporadic, but it is possible that microhabitats in Kumejima Is., Lyudao, and the southeast coast may have shared features.

***Lissoclinum punctatum* Kott, 1977**

Specimens: NMNS-8141-012 (Gongzibi), -013 (Jialeshuei).

Colonies were irregularly shaped, fragile sheets (Fig. 3F). They were entirely green due to *Prochloron* cells in the common cloacal cavity and tunic. Most the *Prochloron* cells in the tunic were located in the tunic cells, *i.e.*, tunic phycocytes (Hirose et al. 1996) (Fig. 3G). Intracellular distribution of the cyanobacterial cells is unique to *L. punctatum*, and photosymbionts are not associated with any tunic cells in the other host ascidians harboring cyanobacteria in the tunic (reviewed in Hirose 2015). Tunic spicules were globular and densely aggregated around each zooid (Fig. 3H). This is a new record of *L. punctatum* from Taiwan.

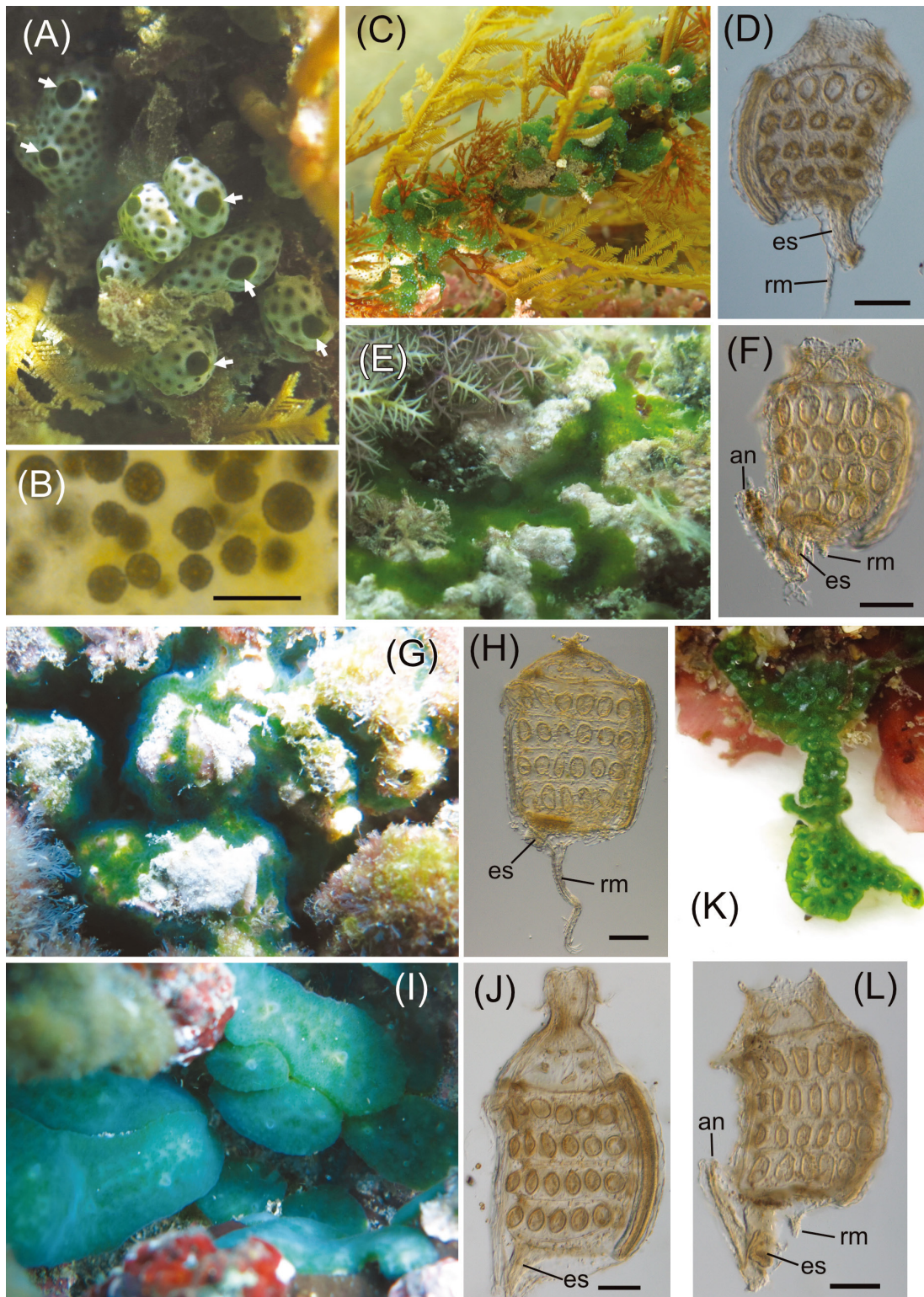


Fig. 2. *Didemnum* and *Diplosoma* species. (A, B), *Didemnum molle* at Shimen (A, colonies; B, tunic spicules). (C, D), *Diplosoma gumavirens* at Xiaoyeliu (C, colonies; D, thorax with 5-5-5-4 pattern of stigmata). (E, F), *Diplosoma ooru* at Gonzibi (E, colonies; D, thorax with 5-6-5-4 pattern of stigmata). (G, H), *Diplosoma simile* at Maobitou (G, colonies; H, thorax with 6-6-6-5 pattern of stigmata). (I, J), *Diplosoma virens* at Gonzibi (I, colonies; J, thorax with 6-6-6-5 pattern of stigmata). (K, L), *Diplosoma watanabei* at He-mei (K, colonies; L, thorax with 6-7-6-5 pattern of stigmata). White arrows in A indicate some cloacal aperture. an, anus; es, esophagus; rm, retractor muscle. Scale bars: B = 20 μ m; D, F, H, J, L = 0.1 mm.

***Trididemnum clinides* Kott, 1977**

Specimens: NMNS-8141-024 (Shimen).

Irregularly shaped colonies were attached to hydrozoan colonies. Live colonies were yellow or light brown (Fig. 3I). The fixed specimens gradually turned greenish, probably because the yellowish colors were dissolved or discolored in the fixative. Tunic spicules consisted of rod-like rays (Fig. 3J). Three types of cyanobacterial species were found in the tunic, *i.e.*, greenish-unicellular, reddish-unicellular, and reddish-

filamentous cyanobacteria (Fig. 3K and L).

***Trididemnum cyclops* Michaelsen, 1921**

Specimens: NMNS-8141-025 (Shimen).

The colonies were oval or elongated cushions and aggregated on a side wall of a tide pool (Fig. 3M). They were green due to *Prochloron* in the common cloacal cavities, while the colony peripheries and substratum side were white due to tunic spicules with burred surfaces (Fig. 3N). In an enlarged image of the colonies,

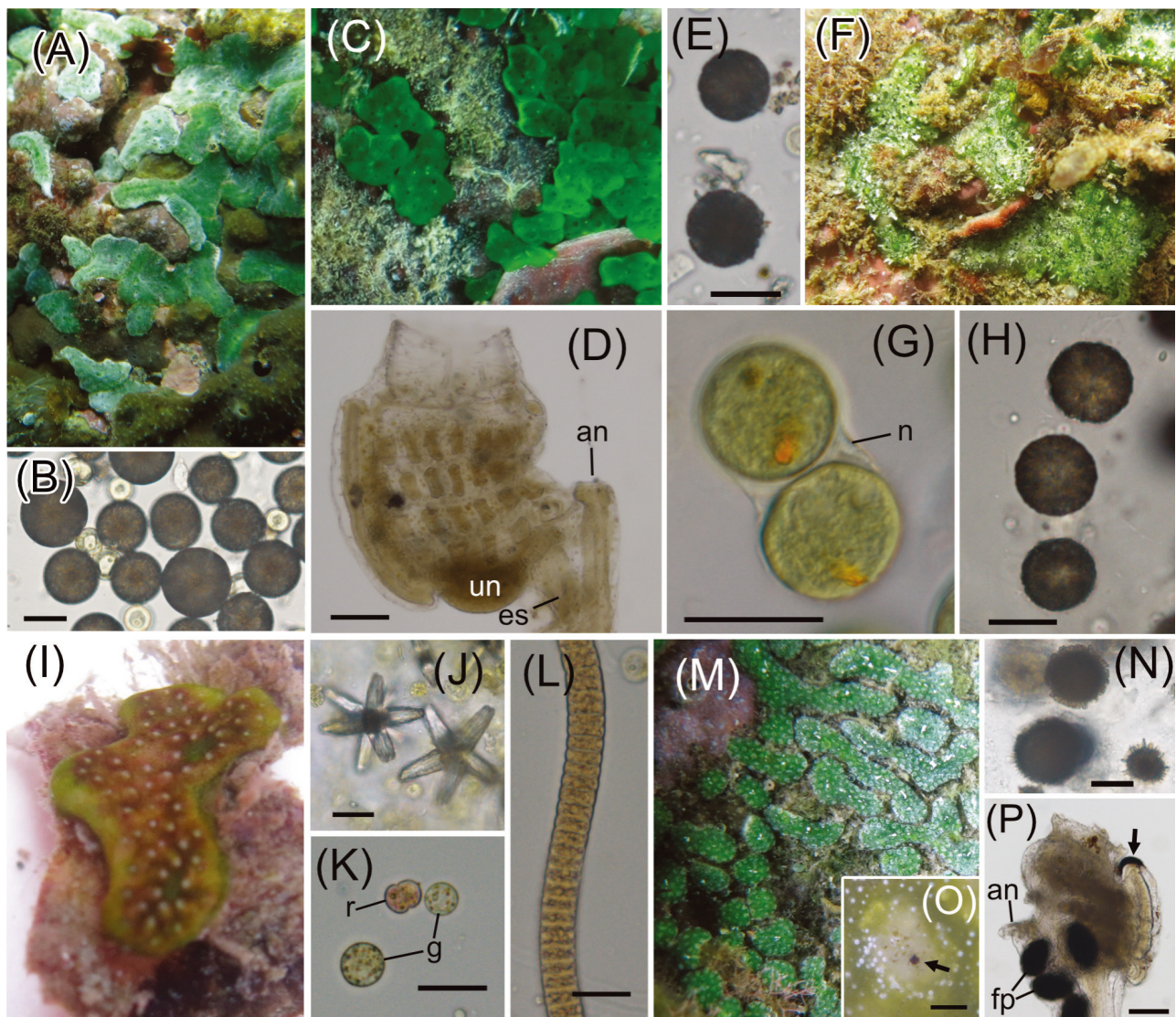


Fig. 3. *Lissoclinum* and *Trididemnum* species. (A, B), *Lissoclinum bistratum* at Gonzibi (A, colony; B, tunic spicules). (C–E), *Lissoclinum midui* at Gonzibi (C, colony; D, thorax; E, tunic spicules). (F–H), *Lissoclinum punctatum* at Gonzibi (F, colony; G tunic phycocyte; H, tunic spicules). (I–L), *Trididemnum clinides* at Shimen (I, colony; J, tunic spicules; K, unicellular cyanobacteria in the tunic; L, filamentous, multicellular cyanobacteria in the tunic). (M–P), *Trididemnum cyclops* at Shimen (M, colony; N, tunic spicules; O, enlargement of colony surface showing a zooid; P, thorax). Arrows in O and P indicate pigmentation at the top of endostyle (pigment cap). an, anus; fp, fecal pellet; g, greenish cyanobacteria; n, nucleus of tunic phycocyte; r, reddish cyanobacterium; un, unknown organ. Scale bars: B, E, G, H, J–L, N, and O = 20 μm; D and P = 0.1 mm; O = 0.2 mm.

there was a black spot in each zooid (Fig. 3O). This is a black (or dark brown) pigmentation at the apical end of the endostyle referred to as pigment cap (Fig. 3P), which is unique to the *T. cyclops* and *Trididemnum paracyclops* (Kott, 1980). Although *T. paracyclops* is distinguished from *T. cyclops* by several characters such as larger colony size, colony shape, larger zooids, and the number of coils in the vas deferens (Kott 1980 2001), Monniot and Monniot (1987) made no distinction between the two species. Although the size of the tunic spicules differed significantly between *T. cyclops* and *T. paracyclops*, the molecular phylogeny inferred from the partial *COI* sequences did not recognize two species (Hirose et al. 2010b). Based on the colony size and shape, the present specimens were identified as *T. cyclops*.

DISCUSSION

Thirteen photosymbiotic didemnid species have been recorded from Taiwan in the present and previous surveys (Hirose and Nozawa 2010; Hirose and Su 2011; Hirose et al. 2014 2015). Table 1 summarizes the occurrence of the ascidian species at each site with approximate latitude. Among the species in the table, *Diplosoma aggregatum* Hirose and Hirose, 2009 was reported from Lyudao (Hirose and Nozawa 2010) but not recorded in the present survey on the Taiwan east coast. Whereas this species is very similar in zooid morphology to *D. virens*, the partial sequence of mitochondrial cytochrome *c* oxidase subunit I clearly discriminates these species (Hirose and Hirose

2009) as does the difference in colony form (Fig. 4). Moving from the north towards the south, the number of species were 0 in the north area (2 sites), 1 in the northeast area (1 site), 7 in the middle-east area (3 sites), 5 in the southeast area (2 sites), and 8 in the south area (4 sites). There seems to be a barrier between the northeast and middle-east areas and/or between the north and northeast areas. The barrier is probably related to the course of the Kuroshio Current, which goes north along the east coast of Taiwan but changes the direction towards the east off the northeast area (Fig. 1). The Kuroshio is a warm current and causes marked differences in water temperature between the south-east coast and the north-west coast, particularly in winter (Japan Meteorological Agency 2020). The distribution pattern of photosymbiotic didemnid species in Taiwan appears to be consistent with that of reef-building species of scleractinian corals (Chen 1999). Yang and Dai (1982) also reported that the coral communities are typical marginal communities characterized by low coral coverage and limited reef-building activity in Yenliao Bay (25°3'N, 121°56'E) on the northeastern coast of Taiwan. On the other hand, the coral recruitment rates at Yenliao Bay were comparable to those of tropical reefs, suggesting that “recruitment might not be a limiting factor for the maintenance and development of local coral communities” and “light intensity is possibly the primary factor controlling settlement and survival of coral recruits” (Ho and Dai 2014).

It is possible that post-settlement mortality rather than recruitment causes the absence or rare occurrence of photosymbiotic ascidians in the north and northeast coasts of Taiwan. We suggest that water temperature

Table 1. Photosymbiotic ascidian fauna in Taiwan

Area (No. of species)	Penghu (1)	North (0)		Northeast (1)	Middle east (7)			Lyudao (10)	Southeast (5)		South (8)			
Location		Shin-ao	Bitouchiao	He-mei	Shimen	Wushibi	Xiaoyeliu		Gongzibi	Jialeshuei	Wanlitung	Tiao-shi	Nanwan	Maobitou
Approx. latitude	23°17' N	25°08'	25°07'	25°05'	23°31'	23°14'	22°48'	22°38–41'	22°09'	21°58'	21°59'	21°57'	21°57'	21°55'
<i>Didemnum molle</i>					✓	✓		✓						✓
<i>Diplosoma aggregatum</i>								✓						
<i>Diplosoma gumavirens</i>	✓				✓	✓	✓	✓			✓			✓
<i>Diplosoma ooru</i>					✓	✓		✓	✓	✓	✓		✓	✓
<i>Diplosoma simile</i>					✓	✓		✓			✓	✓		✓
<i>Diplosoma simileguwa</i>													✓	
<i>Diplosoma virens</i>						✓		✓	✓		✓	✓		✓
<i>Diplosoma watanabei</i>				✓										
<i>Lissoclinum bistratum</i>								✓	✓	✓	✓	✓		✓
<i>Lissoclinum punctatum</i>									✓	✓				
<i>Lissoclinum midui</i>								✓	✓	✓				
<i>Trididemnum clinides</i>					✓			✓			✓			
<i>Trididemnum cyclops</i>					✓			✓						
References*	4	1						1, 3			1	1	2	

* 1, Hirose and Nozawa 2010; 2, Hirose and Su 2011; 3, Hirose et al. 2014; 4, Hirose et al. 2015.

would be more significant than light intensity in limiting the distribution range of each photosymbiotic ascidian species, considering the species richness along the Ryukyu Archipelago ranging from 24°N to 31°N, *e.g.*, 13 species from Haterumajima Is. (ca. 24°N) and four from Tanegashima Is. (ca. 30°30'N) (Oka and Hirose 2005; Hirose et al. 2009b). The latitudinal gradient of ascidian diversity was also documented for the species on eelgrass from New Jersey to Newfoundland along the Gulf Stream (Carman et al. 2016 2019). Because growth rates are often temperature-dependent for sessile benthic organisms, climate change is expected to affect interspecific competition for space and latitudinal range of species distribution (Lord and Whitlatch 2015). Accordingly, global warming may promote northward expansion of the distribution ranges of the photosymbiotic ascidians in Taiwan.

There is also a difference in species composition between the survey areas (Table 1); *L. midui* was found only in the southeast and Lyudao, and *L. punctatum* was common in the southeast but was not found in other areas. Among the photosymbiotic ascidians so far reported, *D. simile* is the most widely distributed species and has been recorded from some oceanic islands such as Hawaii and Ogasawara (Bonin) Islands (Abbot et al. 1997; Hirose et al. 2007; Hirose and Hirose 2013), suggesting that it has a high capacity for dispersal. Therefore, the distribution range of this species is possibly underestimated in Taiwan, or it could potentially expand in the near future. In contrast, *D. watanabei* was found exclusively in the northeast area and was the only photosymbiotic ascidian species

found there. The type series of *D. watanabei* were also collected at Takezaki (30°22'16"N, 130°55'37"E) and Shimama (30°29'10"N, 130°52'56"E) on Tanegashima Island (Satsunan Islands, Japan), located near the north end of the Ryukyu Archipelago (Hirose et al. 2009b). Such a distribution may indicate that, among the photosymbiotic ascidians, this species has greater resistance to low temperatures, and cold-resistance is a possible reason why *D. watanabei* was the only species recorded from the northeast coast area in the present study. In the Ryukyus, *D. watanabei* were also recorded from the middle and south Ryukyu area, and therefore, it is possible that this species is also distributed in southern Taiwan. The occurrence or disappearance of these species could indicate environmental changes in the future.

All the species listed in Table 1 have been recorded from the Ryukyu Archipelago, Japan. These 13 species would not completely cover the photosymbiotic ascidian fauna in Taiwan because of the limitation of sampling sites and sampling efforts, but we believe that they represent the major species in Taiwan. The species richness in Taiwan is poorer than that of the Ryukyus, in particular, south Ryukyus. For instance, 13 species were recorded from two sites on Haterumajima Is. (Oka and Hirose 2005), whereas the largest number of species in Taiwan was 10 in Lyudao, where we surveyed five sites several times (Hirose and Nozawa 2010; Hirose et al. 2014). As mentioned in the description of *L. bistratum*, the species that inhabit back reefs on the barrier reef of the Ryukyus are probably absent or rarely occur in the fringing reefs of Taiwan.

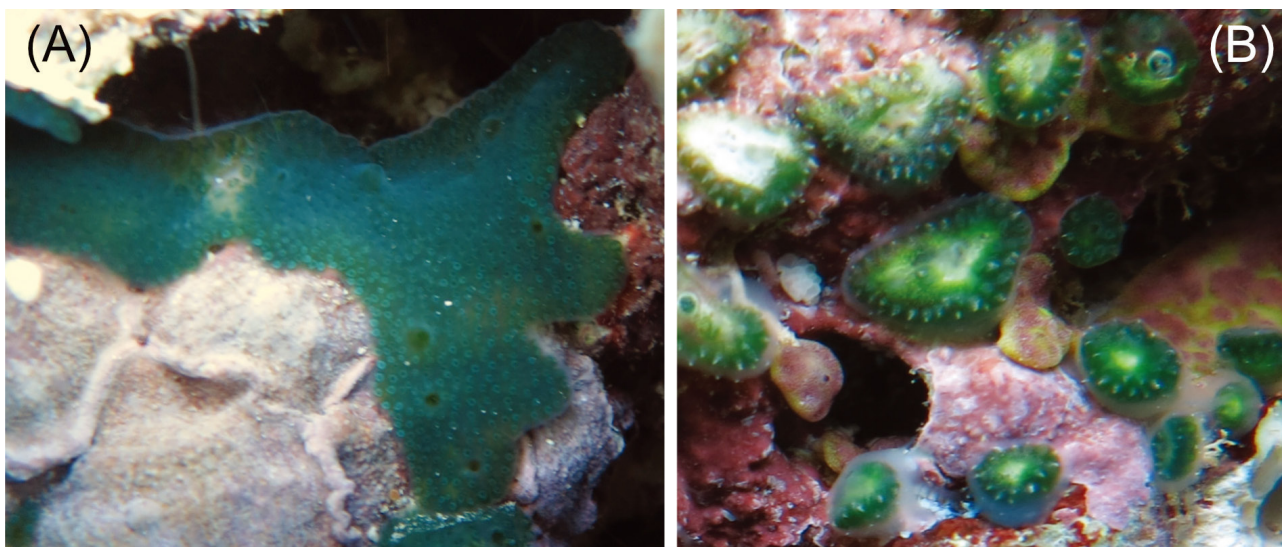


Fig. 4. Irregularly shaped sheet of *Diplosoma virens* colony at Maobitou (A) and small oval colonies of *D. aggregatum* at Dabaisha in Lyudao (B).

CONCLUSIONS

Photosymbiotic ascidians were absent or rarely occurred on the north-northeast coast of Taiwan, probably because of the vulnerability of the cyanobacterial symbionts to low temperature. The photosymbiotic ascidian fauna is believed to be an environmental indicator in subtropical, shallow waters. An increase in water temperature will extend the distribution range of each species towards higher latitudes, and an outbreak of some of the species will be a potential threat to the benthic community, as reported for *Diplosoma* spp. (Littler and Littler 1995; Vargas-Ángel et al. 2009; Li et al. 2016) and *D. molle* (Tebbett et al. 2019). The present study provides a baseline for the photosymbiotic ascidian fauna in Taiwan to monitor future changes in distribution ranges and species diversity. Since species identification of these species is difficult, an identification key is provided below.

Key for the photosymbiotic ascidians recorded in Taiwan

1. Cyanobacterial cells are exclusively located in the tunic 2
 - Green cyanobacterial cells are exclusively located in the cloacal cavity 3
 - Green cyanobacterial cells are located in both tunic and cloacal cavity, those in the tunic are contained in the tunic cells *Lissoclinum punctatum*
2. All cyanobacterial cells are green, unicellular coccoids. Thorax has 4 rows of stigmata *Lissoclinum midui**
 - Yellowish colony with 3 types of cyanobacteria including multicellular filaments. Thorax has 3 rows of stigmata *Trididemnum clinides***
3. Dome-shaped colony with a few large cloacal apertures *Didemnum molle*
 - Colony is an irregularly shaped sheet or cushion 4
4. Tunic spicules are present 5
 - Tunic spicules are absent, *i.e.*, *Diplosoma* spp. 6
5. Each zooid has a pigment cap at the apical end of endostyle. Thorax has 3 rows of stigmata *Trididemnum cyclops*
 - Zooid has no pigment cap. Thorax has 4 rows of stigmata. All tunic spicules are globular *Lissoclinum bistratum****
6. Retractor muscle emerges from underside of thorax 7
 - Retractor muscle emerges from halfway along esophagus 8
7. Pattern of stigmata is 4-5-4-3 *Diplosoma simileguwa*
 - Pattern of stigmata is 5-6-5-4 *Diplosoma ooru*
 - Pattern of stigmata is 6-6-6-5 *Diplosoma simile*
 - Pattern of stigmata is 6-7-6-5 or 6-7-6-4 *Diplosoma watanabei*
8. Pattern of stigmata is 5-5-5-4 *Diplosoma gumavirens*
 - Pattern of stigmata is 6-6-6-5 9
9. Colony is an irregularly shaped sheet *Diplosoma virens*
 - Oval colonies (≤ 10 mm) form an aggregate *Diplosoma aggregatum*

*Consider *Trididemnum miniatum* (unrecorded in Taiwan), when thorax has 3 rows of stigmata.

**Consider *Trididemnum nubilum* (unrecorded in

Taiwan), when colonies are dark brown, and all cyanobacterial cells are reddish coccoids.

*** Consider *Lissoclinum timorensis* (unrecorded in Taiwan), when the conical cones emerged at the colony periphery, and some tunic spicules are stellate.

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