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**Photosymbiotic Ascidians from Nakanoshima Island and Takarajima Island  
(the Tokara Islands, Ryukyu Archipelago, Japan) with Remarks on the Status of  
*Diplosoma midori* (Tokioka, 1954)\***

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**Abstract** We studied the fauna of photosymbiotic ascidians in the shallow reef shore of the Tokara Islands. We collected *Lissoclinum bistratum*, *L. punctatum*, *Trididemnum clinides*, *T. cyclops*, *T. sp.* (cf. *T. paraclinides*), *Diplosoma ooru*, *D. simile*, *D. simileguwa*, *D. virens*, *D. sp. A*, and *D. sp. B* (cf. *D. multipapillatum*) from four sites off Nakanoshima Island and one site off Takarajima Island. *Diplosoma midori* (Tokioka, 1954) is a photosymbiotic ascidian that was originally described from Nakanoshima Island and Takarajima Island. Although there is argument as to the taxonomical status of this species, the conditions of the syntype specimens were inadequate for examining detailed features for identification. Based on the topotype specimens from Takarajima Island, we concluded that *D. midori* should be regarded as an invalid species, and is a junior synonym of *D. simile* or *D. virens*.

**Key words:** ascidian-algal symbiosis, species list, northern distribution limit, topotype, Didemnidae

### Introduction

In tropical or subtropical waters, some ascidian species of the family Didemnidae are known to have obligate symbiotic relationships with the prokaryotic algae *Prochloron*, *Synechocystis*, and/or unknown cyanophytes (see Parry and Kott, 1988; Lewin and Cheng, 1989). About 30 species in the four didemnid genera have been described as host species to date (see Kott, 2001). Photosymbiosis was supposed to be established at least once in each genus (Kott, 1982), and molecular phylogeny supported this hypothesis (Yokobori et al., 2006). Since the first record from the Tokara Islands (Tokioka, 1954), many photosymbiotic didemnids have been recorded in Japan, mainly from the Ryukyu Archipelago. Recently, two photosymbiotic species were newly described from the Ryukyu Archipelago (Oka et al., 2005). In general, more photosymbiotic species are distributed at lower latitudes: 15 species of photosymbiotic didemnid have been recorded from the Yaeyama Islands (Hirose et al., 2004; Oka and Hirose, 2005) and 10 species from Amamiyohshima Island (Oka et al., 2007), whereas only one species, *Diplosoma midori* (Tokioka, 1954), has been recorded from the main islands of Japan (see Nishikawa, 1995). For many of the photosymbiotic species, the northern limits of their distributions in Japan are thought to occur in the Ryukyu Archipelago. Because the photosymbiont *Prochloron* shows no photosynthetic activity at 20°C or lower *in vitro* (Dionisio-Sese et al., 2001), water temperature is probably the primary factor restricting the distribution of photosymbiotic didemnids. The increase in water temperature caused by global warming may result in a northward shift in the range of each species in the future (cf. Walther et al., 2002). Therefore, photosymbiotic didemnids are potentially useful for monitoring the effects of global warming on marine organisms, and it is important to record the present distribution of each species in the Ryukyu

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Archipelago and the southern part of the main islands of Japan.

Tokioka (1954) found two photosymbiotic didemnids in the intertidal zones of Nakanoshima Island and Takarajima Island (the Tokara Islands), i.e., *Didemnum (Didemnum) pulvinum* and *Leptoclinium midori* (= *Diplosoma midori*). Whereas the former species is generally regarded as a junior synonym of *Lissoclinium bistratum* (Sluiter, 1905) (cf. Kott, 1980), the systematic position of *D. midori* is open to question. The original description of *D. midori* did not mention larvae or retractor muscles (Tokioka, 1954), and so *D. midori* does not have definite features with which to differentiate it from some photosymbiotic congeners, e.g., *D. simile* and *D. virens*. Recently, Kott (2001) assigned *D. midori* as a junior synonym of *D. simile*. In our previous study (Oka et al., 2005), we could not conclude the status of *D. midori* based on its syntype specimens because the zooids were poorly preserved. Thus, we decided that the next best attempt would be a survey of topotype specimens of *D. midori*. In 2004, we investigated the photosymbiotic ascidian fauna of the Tokara Islands; here, we report the occurrence of several photosymbiotic species, including *D. simile* and *D. virens*.

### Material and Methods

Ascidian colonies were collected during snorkeling surveys at four sites off Nakanoshima Island and one site off Takarajima Island in 2004 (Fig. 1). Collection sites and dates were as follows: for Nakanoshima Island, Yoriki (29 July), Nanatsuyama (30 July), Nakanoshima Port (31 July), and Kebushi (1 August); for Takarajima Island, Oh-gomori (3 August). The colonies were fixed in 10% formalin-seawater after anesthesia with menthol and 0.37 M MgCl<sub>2</sub>. The specimens were dissected under a binocular stereomicroscope. Drawings were prepared upon observation with a light microscope equipped with Nomarski differential interference contrast optics and a camera lucida. The taxonomic identification of specimens was mainly performed following Nishikawa (1995) and Kott (2001). Some of the specimens were deposited in the Nagoya University Museum (NUM).

In *Diplosoma* spp., the number of stigmata in respective rows in the branchial sac is an important characteristic for species identification. We describe the numbers as four digits in double quotation

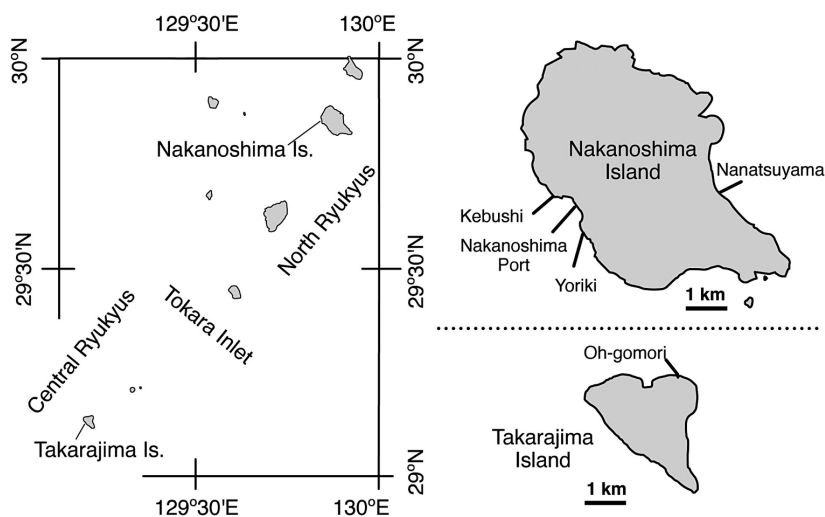


Fig. 1. Map of the Tokara Islands and enlargements of Nakanoshima Island (upper right) and Takarajima Island (lower right).

marks, which digits indicate the number of stigmata from the first (top) row to the fourth (bottom) row.

### Results and Discussion

Photosymbiotic ascidians distributed in the Tokara Islands

The photosymbiotic didemnids recorded in the survey are listed below (see also Table 1). Although we collected 11 species, three of them could not be identified to the species level.

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

Collection Sites: Yoriki, Nanatsuyama, Nakanoshima Port, Kebushi (Nakanoshima Island); Oh-gomori (Takarajima Island).

Specimens: NUM-Az 0561, Yoriki.

The colonies were commonly found at all of the five sites investigated. Tokioka (1954) recorded this species as *Didemnum* (*Didemnum*) *pulvinum*, which was assigned as a junior synonym of *Lissoclinum bistratum* (see Kott, 1980).

#### *Lissoclinum punctatum* Kott, 1977

Collection Sites: Yoriki, Nanatsuyama (Nakanoshima Island).

Specimens: NUM-Az 0562, Yoriki.

The colonies are small cushions (<5 mm in diameter) or narrow sheets (<20 mm long). Spicules form a capsule-like aggregation enveloping each zooid.

#### *Trididemnum clinides* Kott, 1977

Collection Sites: Yoriki, Nanatsuyama (Nakanoshima Island).

Specimens: NUM-Az 0563, Nanatsuyama.

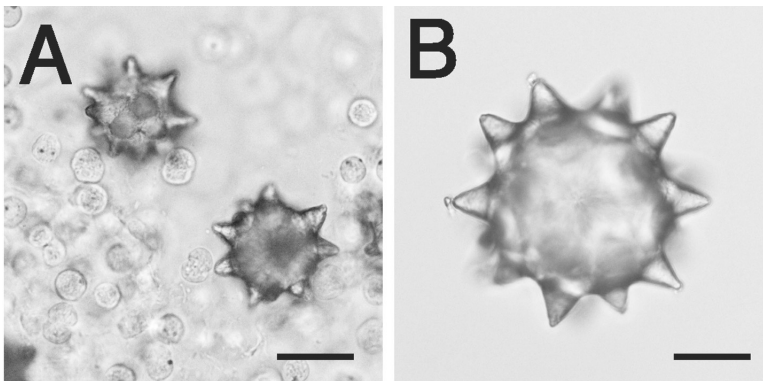
The colonies are oval cushion-like forms up to 4 mm in the long axis. The colonies contain stellate spicules of 30  $\mu\text{m}$  diameter on the average ( $n = 100$ ,  $SD = 4.8$ ) (Fig. 2A).

#### *Trididemnum cyclops* Michaelsen, 1921

Collection Sites: Nanatsuyama, Kebushi (Nakanoshima Island); Oh-gomori (Takarajima Island).

Specimens: NUM-Az 0564, Nanatsuyama.

The small colonies are round or oval and up to 3 mm in the long axis.



**Fig. 2.** Spicules of *Trididemnum clinides* (A: NUM-Az 0563) and *Trididemnum* sp. (cf. *T. paraclinides*) (B: NUM-Az 0565). Scale bars, 20  $\mu\text{m}$ .

***Trididemnum* sp. (cf. *T. paraclinides* Kott, 1982)**

Collection Sites: Kebushi-zaki (Nakanoshima Island).

Specimens: NUM-Az 0565, Kebushi-zaki.

The colonies are irregularly shaped sheets, with some over 10 mm long. The zooids are about 0.8 mm long and are similar to those of *T. clinides*, but the diameter of the spicules is 56  $\mu$ m on the average (n = 100, SD = 9.2) (Fig. 2B). These features (colony and spicules) appear to correspond with those of *T. paraclinides*, rather than *T. clinides* (Kott, 1982, 2001).

***Diplosoma ooru* Hirose et Suetsugu, 2005**

Collection Sites: Yoriki, Nakanoshima Port, Kebushi (Nakanoshima Island).

Specimens: NUM-Az 0566, Nakanoshima Port.

The colonies are thin sheets less than 1 mm in thickness. The zooids are about 0.5 mm long. The numbers of stigmata are "5654" (i.e., five stigmata in the first and third rows, six in the second row, and four in the fourth row; see Materials and Methods). The retractor muscle emerges from the underside of the thorax.

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

Collection Sites: Yoriki, Kebushi (Nakanoshima Island); Oh-gomori (Takarajima Island).

Specimens: NUM-Az 0567, Oh-gomori.

The colonies are irregularly shaped sheets 1-2 mm thick. They were found commonly and were particularly abundant at Kebushi. The zooids are about 1 mm long. The numbers of stigmata are "6665," and the retractor muscle emerges from the underside of the thorax (Fig. 3A). The stomach often tapers to the pylorus, whereas the shape is variable among zooids, even within the same colony (Fig. 3B). The well-developed embryo has three adhesive organs and two pairs of ampullae.

***Diplosoma simileguwa* Oka et Hirose, 2005**

Collection Sites: Nakanoshima Port (Nakanoshima Island); Oh-gomori (Takarajima Island).

Specimens: NUM-Az 0568, Nakanoshima Port.

The colonies are thin sheets approximately 1 mm thick. The zooids are about 0.5 mm long. The numbers of stigmata are "4543," and the retractor muscle emerges from the underside of the thorax.

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

Collection Sites: Yoriki, Nanatsuyama, Nakanoshima Port, Kebushi (Nakanoshima Island); Oh-gomori (Takarajima Island).

Specimens: NUM-Az 0569, Oh-gomori; NUM-Az 0570, Oh-gomori.

The colonies are irregularly shaped sheets usually 2 mm thick or more. They were common at all five sites investigated. At sites where *D. simile* was also found, the habitats of the two species overlapped. Thus, it is difficult to discriminate *D. virens* colonies that are about 2 mm thick from those of *D. simile* without dissection. The zooids are about 1 mm long. The numbers of stigmata are "6665," and the retractor muscle emerges from halfway along the esophagus (Fig. 3C). The pyloric part of the stomach is prominent in some stomachs, but not in others (Fig. 3D). The well-developed embryo (NUM-Az 0570) has three adhesive organs and four pairs of ampullae. Although *Diplosoma matie* Monniot et Monniot, 1987 and *D. pavonia* Monniot et Monniot, 1987 also have the stigmatal pattern of "6665" and the retractor muscle emerging from halfway along the esophagus, both of the latter species have larvae with more than four adhesive organs (Monniot and Monniot, 1987).

***Diplosoma* sp. A**

Collection Sites: Nakanoshima Port (Nakanoshima Island).

Specimens: NUM-Az 0572, Nakanoshima Port.

The colonies are very thin sheets approximately 0.5 mm thick. The zooids are about 0.8 mm

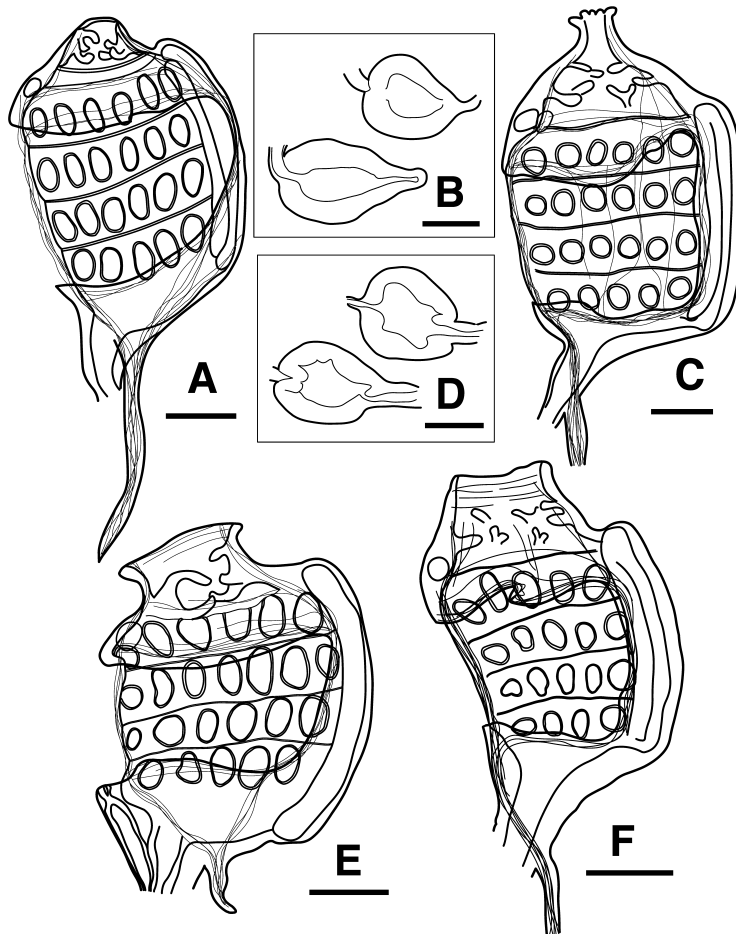
long. The numbers of stigmata are "6765," and the retractor muscle emerges from the underside of the thorax (Fig. 3E). Our specimens did not contain any well-developed embryos. Although photosymbiotic *Diplosoma* spp. with the "6765" stigmatal pattern have never been described to date, colonies with the same pattern were also collected in the Yaeyama Islands (South Ryukyus, Japan; Oka, unpublished data). Further investigation is required to clarify the taxonomic identification using more specimens containing embryos.

*Diplosoma* sp. B (cf. *D. multipapillatum* Kott, 1980)

Collection Sites: Nanatsuyama, Nakanoshima Port (Nakanoshima Island).

Specimens: NUM-Az 0571, Nanatsuyama.

The colonies are small cushions 2-3 mm in diameter and approximately 1 mm thick. The zooids are about 0.8 mm long. The numbers of stigmata are "5554," and the retractor muscle emerges from halfway along the esophagus (Fig. 3F). The "5554" stigmatal pattern is reminiscent of the "5555"



**Fig. 3.** Drawings of the thorax (A, C, E, F) and stomach (B, D) of some photosymbiotic *Diplosoma* species. *Diplosoma simile* (A, B: NUM-Az 0567), *D. virens* (C, D: NUM-Az 0569), *Diplosoma* sp. A (E: NUM-Az 0572), and *Diplosoma* sp. B (F: specimen from Nakanoshima Port). Cardia is the left side in B and D. Scale bars, 0.1 mm.

**Table.1.** List of the photosymbiotic didemnids from Nakanoshima Is. and Takarajima Is.

Species	Nakanoshima Is.				Takarajima Is.
	Yoriki	Nanatsuyama	Nakanoshima Port	Kebushi	Oh-gomori
<i>Lissoclinum bistratum</i>	+	+	+	+	+
<i>Lissoclinum punctatum</i>	+	–	+	–	–
<i>Trididemnum clinides</i>	+	+	–	–	–
<i>Trididemnum cyclops</i>	–	+	–	+	+
<i>Trididemnum</i> sp. (cf. <i>T. paraclinides</i> )	–	–	–	+	–
<i>Diplosoma ooru</i>	+	–	+	+	–
<i>Diplosoma simile</i>	+	–	–	+	+
<i>Diplosoma simileguwa</i>	–	–	+	–	+
<i>Diplosoma virens</i>	+	+	+	+	+
<i>Diplosoma</i> sp. A	–	–	+	–	–
<i>Diplosoma</i> sp. B (cf. <i>D. multipapillatum</i> )	–	+	+	+	–

pattern in *D. multipapillatum*, which also has retractor muscles emerging from halfway along the esophagus. Whereas *D. multipapillatum* is characterized by an unusually large number of adhesive organs, our specimens unfortunately lacked larvae and well-developed embryos. Colonies with the same pattern, i.e., "5554," were collected in the Yaeyama Islands (South Ryukyus, Japan; Oka, unpublished data), but neither embryos nor larvae have ever been found in these specimens.

The Tokara Islands range from 29° N to 30° N and are biogeographically divided into two areas by the Tokara inlet, with Nakanoshima Island and Takarajima Island belonging to the North Tokara and the South Tokara, respectively. The number of species found was fewer off Takarajima Island than off Nakanoshima Island, probably because of the fact that we could survey only one site off Takarajima Island, whereas we surveyed four sites off Nakanoshima Island. Only two photosymbiotic didemnids were reported from the Tokara Islands by Tokioka (1954), who mainly examined the intertidal zone. We found nine additional photosymbiotic species, probably because we surveyed the subtidal zone by snorkeling. Alternatively, it may be possible that several species have colonized from further south within the 50 years since Tokioka's 1953 survey because of increased water temperature due to global warming. Because the number of species listed here is comparable to that recorded from Amamiohshima Island (ca. 28° N; Oka et al., 2007), our list almost encompasses all the photosymbiotic didemnid species that commonly occur on the shallow reefs in the Tokara Islands. *Didemnum molle* (Herdman, 1886) and *Trididemnum nubilum* (Kott, 1980) were recorded from Amamiohshima Island, but not from the Tokara Islands. The northern distribution limit for these two species possibly lies between Amamiohshima Island and Takarajima Island.

#### Taxonomic status of *Diplosoma midori*

*Diplosoma midori* (Tokioka, 1954) was first recorded from Nakanoshima Island and Takarajima Island. According to the original description (Tokioka, 1954), i.e., a few colonies from Takarajima (Type 132) and many colonies (TK. No. 76) from Nakanoshima, the colonies are 2 mm in thickness, the thorax and abdomen of zooids are about 500  $\mu$ m and 640  $\mu$ m in length, respectively, the stigmatal pattern is "6665," the retractor muscle is short, and the shape of the stomach is tapering to pylorus, which discriminates this species from *D. virens*. However, there were no descriptions of the locations of retractor muscle emergence, the embryos, and the distinctions from *D. simile*. Moreover,

because the shape of the stomach varies somewhat, even among the zooids of the same colony (Fig. 3B and D), the stomach tapering to pylorus is not a decisive feature in differentiating these photosymbiotic *Diplosoma* species. Kott (1980) identified *Diplosoma* colonies collected in Tonga as *D. midori*. Later, Kott (2001) regarded *D. midori* as a junior synonym of *D. simile* because there is no significant difference between the two species. Kott (2001) did not mention the possibility that the *D. midori* is a junior synonym of *D. virens*.

Aside from *D. midori*, four photosymbiotic species thus far have been described as *Diplosoma*, with five or six stigmata in respective rows. They are *D. matie*, *D. pavonia*, *D. simile*, and *D. virens*. The location of emergence of the retractor muscle is a critical key in distinguishing these species from one another: the muscle emerges from the underside of the thorax in *D. simile*, but from halfway along the esophagus in the others. The significance of this characteristic is supported by the molecular phylogeny inferred from 18S rDNA sequences (Yokobori et al., 2006). However, we could not make any conclusions based on the syntype specimen of *D. midori* because the zooids were poorly preserved (Oka et al., 2005). We collected many *Diplosoma* species with the "6665" pattern from Takarajima Island, as well as from Nakanoshima Island, and these should include topotype specimens of *D. midori*. Because all specimens with the "6665" pattern were identified as either *Diplosoma simile* or *D. virens*, we concluded that *D. midori* should be regarded to be an invalid species and a junior synonym of *D. simile* or *D. virens*. It is difficult for us to specify whether the senior synonym of *D. midori* should be *D. simile* or *D. virens*. Both *D. simile* and *D. virens* were often found in the same habitat in both Nakanoshima Island and Takarajima Island; thus, the two species were possibly regarded as the single species *D. midori* by Tokioka (1954). Alternatively, only one of the two species was distributed in the Tokara Islands 50 years ago, and the other species settled there after the 1953 survey by Tokioka, following the increase in water temperature. Although *Diplosoma midori* has been recorded from Kushimoto (Wakayama Prefecture, Japan) (cf. Nishikawa, 1995), it may be *D. simile*, *D. virens*, or other photosymbiotic *Diplosoma* species. Further geographic surveys at higher latitudes would reveal which species has the northern distribution limit.

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