Morphology, abundance and the invasiveness of coral-killing sponge *Chalinula nematifera* (Porifera: Demosponigiae) from Con Dao National Park, Vietnam

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Abstract

This is the first study on morphology, abundance and invasion of coral-killing sponge *Chalinula nematifera* (De Laubenfels, 1954) from offshore waters in Con Dao islands, Vietnam. The results of the study show that the morphology is similar to that in the Western Pacific, but differs in skeletal structure compared to the description in the Eastern Pacific. The density of *C. nematifera* (average \pm standard deviation) is 2.02 \pm 5.03 colonies per 100 m² (present at 12/15 survey stations) with the number of colonization belonging to diameter group 5–20 cm. They invade 30 species belonging to 23 genera of Scleractinia corals (44.7% on foliose/laminar and encrusting coral, 23.86% on massive coral and 21.97% on branching coral). The ability to invade corals of *C. nematifera* does not limit the host. So, this is considered a potential hazard to the East Vietnam Sea coral reefs in the future.

Keywords: Coral-killing sponge, Con Dao, coral reefs, Vietnam.

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INTRODUCTION

Sponges are not only a strong competitor for living space on coral reefs [1–4] but also resist and destroy coral [5–8]. Three species *Terpios hoshinota* Rützler & Muzik, 1993; *Chalinula nematifera* (de Laubenfels, 1954); *Chalinula milnei* (de Laubenfels, 1954) are considered a potential threat to coral reefs in the Asia Pacific region [9]. The mechanism of attack of coral-killing sponge overgrows coral cover and smoothens the surface of hard corals. They use the ability to grow at a high rate and combine to release allelopathic compounds to destroy coral tissue [1].

Chalinula nematifera was first described with scientific name Nana nematifera by de Laubenfels [10], which was collected in Ebon Atoll and Ailinglaplap Atoll belonging to the Marshall islands. Later, it was found in the Western and Eastern Pacific regions including Central and Southern Great Barrier Reef [11], Komodo National Park and the eastern coast of Sulawesi island - Indonesia [9, 12], Okinawa island - Japan [13], Nha Trang bay - Vietnam [14, 15], Isla Isabel National Park and Cabo Pulmo National Park (Mexican Pacific Ocean) [16, 17]. These species have the ability to grow on living corals, dead coral that is covered by sediments or algae and coralline algae, with the very thin surface with bright purple color and white filaments produced by symbiotic fungi [9]. The behaviour of C. nematifera is unclear in terms of living coral hosts and distribution depth limits [9]. Their growth rate is very fast about 50 mm/month on the reef [12]. The difference in light intensity between the outer space and space within the coral group has a branching shape that significantly influences the ability of C. nematifera to colonize Pocillopora corals [17].

The Con Dao National Park is one of 16 marine protect areas of Vietnam, that has coral reef, mangroves, seagrass beds and dead coral tidal flats with 300 species of hard corals, 4 species of soft coral, 202 species of reef fish, 88 species of mangrove plants, 148 species of mollusks, 110 species of crustaceans, 44 species of echinoderms, 39 species of polychaetes [18, 19]. Only one species of sponge *Ciocalypta foetida* (Dendy) was

recorded in Con Dao by Dawydoff (1952) [20]. *C. nematifera* has been recorded in Con Dao since 2016 by the author. In this study, we focus on description of morphology, distribution, abundance and invasion of *C. nematifera* in coral reef ecosystems from the Con Dao National Park.

MATERIALS AND METHODS

The abundance and specimens of *C. nematifera* were surveyed in situ using SCUBA in Feb. & Oct. 2019 at 15 sites around Con Dao islands (Poulo Condore) (figure 1), in Southeastern Vietnam, which is located at $8^{\circ}34'-8^{\circ}49'$ North latitude and $106^{\circ}31'-106^{\circ}45'$ East longitude.

The data-recording method is applied in the way of estimating the density of invertebrates on coral reefs by English et al., (1997) [21]. Four 20 m survey sections are completed along a 100 m transect set up on two zones of the reef flat (2–5 m) and reef slope (6–10 m) of coral reefs. Surveyor completes a Z-shaped search patterns on a 5×20 m wide belt to record the number and size (maximum dimensions) of *C. nematifera* and the number of corals invaded by the sponge within the belt transects, listed by genus with document of Veron et al., (2000) [22].

A smaller sample was collected and stored in 80% ethanol, and analyzed in the laboratory in terms of the morphology of skeleton slice and spicule size. Methods of classifying and describing specimens were based on the anatomical characteristics of the skeleton structure and the brooch according to the instructions of Hooper (2003); Hooper and Van Soest (2002) [23, 24]. The skeleton samples and spicules were then dehydrated before mounted on glass microscope slide in Canada balsam for light microscope (ACCU-Scope EXC 350). bony dimension parameters were measured with ImageJ image analysis software (V.1.5.2 K). 30 spicules of each sample were measured including minimum - mean maximum of length and width size. The classification documents follow Laubenfels (1954); Cruz-Barraza et al., (2008); Rossi et al., (2015) [10, 12, 16]. Statistical analyses were performed in the Microsoft Excel 2016.



Analyses were based on differences in density; sponge abundance among study sites was tested

v; using one factor ANOVA.

Figure 1. Map of the study sites at Con Dao islands, in Southeast Vietnam. Cycle size scale indicates the average number of colonies *C. nematifera* per 100 m². The study sites are described in table 1 (Open source base map: Open Street Map; Mercator projection, Datum WGS84)

No.	Study sites	Depth	Latitude	Longitude	Time of survey
1	Dam Tre bay 1	3–12 m	8.746150	106.660630	Oct. 2019
2	Dam Tre bay 2	2–12 m	8.746040	106.659679	Oct. 2019
3	Tau Be cape	4–13 m	8.703890	106.651260	Oct. 2019
4	Cua Ga	5–7 m	8.671330	106.609140	Oct. 2019
5	Da Trang	3–10 m	8.655820	106.607840	Oct. 2019
6	Tai islet	2–15 m	8.639910	106.632260	Oct. 2019
7	Bong Lan islet	4–13 m	8.652100	106.674640	Feb. 2019
8	Cau islet	2–10 m	8.684000	106.738660	Feb. 2019
9	Ben Dam	4–5 m	8.666330	106.564200	Feb. 2019
10	Vung islet	2–10 m	8.631253	106.556924	Oct. 2019
11	Dam Quoc	3–6 m	8.640316	106.552461	Oct. 2019
12	Tre Lon islet	2–18 m	8.707702	106.549878	Oct. 2019
13	Ong Cau beach	2–12 m	8.703631	106.577527	Oct. 2019
14	Ong Dung beach	2–10 m	8.712680	106.587510	Feb. 2019
15	Ong Cuong beach	2–10 m	8.747150	106.630370	Feb. 2019

Table 1. Study sites in Feb. and Oct. 2019 at Con Dao Islands.

RESULTS

Systematics description

Class: Demospongiae Order: Haploslerida Topsent, 1928 Family: Chalinidae Gray, 1867 Genus: *Chalinula* Schmidt, 1868 *Chalinula nematifera* (de Laubenfels, 1954)

(figure 2) *Synonym:* Haliclona nematifera (De Laubenfels, 1954), Nara nematifera de Laubenfels, 1954.

Description: The body forms a thin layer covering a hard surface (about 1–4 mm thick), often covering the surface of living corals. The surface is smooth when viewed with the naked eye, but spongy and fragile, easily torn into small patches with white filaments. The body has a bright purple colour when it is alive and fades when soaked in ethanol. On the surface of the body, white filaments form interwovenly and lead in the direction of the drainage (Oscules). Oscule drainage holes have a diameter of about 2 mm, scattered on the surface with a distance between the drainage holes about 1.5–2 mm. (figures 2A–2B).

Skeleton: No specific surface layer (Ectosomal skeletal) is formed. This surface layer is formed by a network of isodictyal reticulation from choanosomal region (figure 2C). The grid cells have the form of a polygon grid with three different sizes, sometimes in the form of squares and diamond blocks (figure 2D). Inside the bone axis there are 1–2 oxea spicules arranged along the length of the bone axis (figure 2F). The connect between the individual spicule forms star-shaped point with 5–6 clustered spines.

Spicules: Only microscleres present are oxeas (a single axis with two pointed heads) with small size. The shape of the oxea spicules may be straight to slightly curved middle part (figs. 2E–2F). Size of spicules with length: $85.1-116.5 \mu m$ (average $104.3 \pm 6.4 \mu m$, n = 30) and width: $2.3-5.1 \mu m$ (average $3.6 \pm 0.7 \mu m$, n = 30).

Ecology: They overgrow on dead coral with surfaces covered with coralline algae and sediments. Then they invade the hard coral's living space by releasing toxins that destroy the tissue of corals, and grow to cover the surface.

They are usually distributed at a depth of 3-15 m in Con Dao reefs.

Distribution on the world: Ebon Atoll and Ailinglaplap Atoll (Marshall islands), Great Barrier Reef (Australia), Komodo National Park, eastern coast of Sulawesi island (Indonesia), Okinawa island (Japan), Isla Isabel National Park and Cabo Pulmo National Park (Mexican Pacific Ocean), Nha Trang bay and Con Dao islands (Vietnam) (figure 3, sea also Hooper, 2008; de Laubenfels, 1954; Rossi et al., 2015; Eva et al., 2018; Chervyakova, 2007; Cruz-Barraza et al., 2008; Ávila et al., 2009; OBIS USA, 2017).

Remarks: The specimens were compared with the original description of the spicule size and the skeletal morphology of the species Nara nematifera de Laubenfels, 1954. The adjacent wharf was compared with the image, the size of the thorns as described by Cruz-Barraza et al., (2008) and Rossi, G. et al., (2015). The results of comparison with the documents show that the outer morphology of C. nematifera distributed in Con Dao is similar in colour and morphology of drainage holes, white silk on the body surface compared to descriptions in the Western Pacific region by Laubenfels (1954); Hooper (2008); Eva et al., (2018); Rossi et al., (2015); Chervyakova (2007) [9–12, 14]. However, there are differences with the descriptions of Cruz-Barraza et al., (2008) and Avila et al., (2009) [16, 17] as follows: Darker colour, the thinner body and the smaller diameter of the drainage hole. The skeletal structure of the specimens in Con Dao is isodictyal reticulation and is more chaotic than the skeleton structure in the description of Cruz-Barraza et al., (2008) [16]. Compared to the original description, both of these descriptions have a similarity in the structure of grid cells formed by one to two simple brooch bones, one after the other, but have difference in the shape of the grid cells (rectangular). In the description of Cruz-Barraza et al., (2008), the irregular cubes from square to diamond form were used to measure the length and width of bone brooches between similar documents. They are similar in length, but the width value of the bone is larger than in the original description (table 2).

Figure 2. Chalinula nematifera (de Laubenfels, 1954) overgrowing on coral at Con Dao. (A–B) sponge overgrowing on *Oxypora* spp. and *Acropora* spp. (white arrow shows oscula of sponges);
(C) Skeleton look under stereo microscope; (D and F) Skeleton look under light microscope; (E) Oxea spicules. Scale bars: (A–B) 2 cm; (C–D) 200 µm; (E) 50 µm; (F) 100 µm

Table 2. Comparison of size of spicules between references

No.	Spicules	References		
1	Oxea: $90-95 \times 1-2 \ \mu m$	Laubenfels (1954)		
2	Oxea: 87.5–112.5 (98.7) × 2.5–5.0 (4.4) μm.	Cruz-Barraza et al., (2008).		
3	Oxea: $80-105 \times 2-2.5 \ \mu m$.	Rossi et al., (2018)		
4	Oxea: 85.1–116.5 (104.3 ± 6.4) × 2.3–5.1 (3.6 ± 0.7) μ m	This study		

Abundance of *Chalinula namatifera* on the Con Dao reefs

In Con Dao islands, *C. nematifera* is found in 12/15 survey sites (2–15 m depth) with an average 2.02 \pm 5.03 colonies/100 m². The density (mean \pm standard deviation) was highest in Cua Ga (11.25 \pm 21.84 colonies/100 m²) and lowest in Dam Tre bay (1.0 \pm 1.41 colonies/100 m²) (table 3 and figure 3). The abundance was significantly different between study sites (p = 0.021). *C. nematifera* has a high density in the reef slope, this trend is found in all survey sites. Survey sites in the east and south of Con Dao islands have a higher density than sites in the north and west of the island (figure 3).

Table 3. Average number of colonies *Chalinula nematifera* per 100 m² following depth and colony size on coral reef (Number in () is SD)

No.	Site name	Average	Transect		Colony size (cm)				
			Reef flat	Reef slope	≤ 5	> 5-10	> 10–20	> 20–30	> 30
1	Dam Tre bay 1	0	0	0	0	0	0	0	0
2	Dam Tre bay 2	1.00 (1.41)	0	2.00 (1.41)	0.13 (0.35)	0.25 (0.46)	0.63 (1.06)	0	0
3	Tau Be cape	0.38 (0.74)	0.50 (1.00)	0.25 (0.50)	0	0.25 (0.71)	0.13 (0.35)	0	0
4	Cua Ga	11.25 (21.84)	11.25 (21.84)	0	4.25 (7.85)	5.00 (10)	1.75 (3.50)	0	0.25 (0.50)
5	Da Trang	2.63 (4.21)	0	5.25 (4.79)	1 (2.14)	0.75 (1.16)	0.63 (0.92)	0.13 (0.35)	0.13 (0.35)
6	Tai islet	1.50 (2.07)	2 (2.31)	1 (2.00)	0.25 (0.46)	0.63 (1.06)	0.63 (1.19)	0	0
7	Bong Lan islet	1.25 (2.05)	2.25 (2.63)	0.25 (0.50)	0.38 (1.06)	0.13 (0.35)	0.63 (0.74)	0	0.13 (0.35)
8	Cau islet	0.63 (1.41)	0.25 (0.50)	1.00 (2.00)	0.38 (0.74)	0	0.25 (0.71)	0	0
9	Ben Dam bay	0	0	0	0	0	0	0	0
10	Vung Islet	6.88 (4.82)	4.50 (5.45)	9.25 (3.1)	1.38 (1.19)	2.75 (2.82)	2.13 (2.95)	0.38 (0.52)	0.25 (0.46)
11	Dam Quoc	2.13 (2.36)	0.50 (1.00)	3.75 (2.22)	0.13 (0.35)	0.5 (0.76)	1 (1.41)	0.25 (0.46)	0.25 (0.46)
12	Tre Lon Islet	1.63 (2.07)	0	3.25 (1.71)	0.5 (0.76)	0.5 (0.53)	0.63 (1.06)	0	0
13	Ong Cau beach	0	0	0	0	0	0	0	0
14	Ong Dung beach	0.13 (0.35)	0	0.25 (0.50)	0	0	0	0.13 (0.35)	0
15	Ong Cuong beach	5.50 (5.58)	1.25 (1.89)	9.75 (4.57)	0.63 (0.74)	1.88 (1.81)	2.25 (2.96)	0.63 (1.19)	0.13 (0.35)
C	Grand total	2.02 (5.03)	1.50 (5.92)	2.57 (3.82)	0.47 (1.69)	0.7 (2.19)	0.67 (1.54)	0.10 (0.40)	0.07 (0.25)

The percentage of density by size group at 12/15 surveyed sites around the island shows that the density of group > 5–10 cm and > 10–20 cm is present at most stations (10/12 stations) and dominates in numbers (table 3). Smallsized group < 5 cm and largesized group > 30 cm are mainly recorded at stations in the area of Con Son beach and southeast islands). Survey stations in the southeast of Con Son island are more diverse in size group than stations in the north and west of the island.

Figure 3. Distribution of *Chalinula nematifera* (de Laubenfels, 1954) in the world (yellow circle is the area of this study)

The effects of *Chalinula nematifera* on Scleractinia coral

90.53% of total colonies of C. nematifera were found to overgrow on living corals, the remaining 9.47% on rock and dead corals. Foliose/laminar and encrusting corals have been recorded to be most eroded (44.7%), followed by the massive coral (23.86%), and the branching coral (21.97%). The genera affected by coral-killing sponge include (11.74%), Pavona (15.53%),Acropora Mycedium (7.95%)*Stylophora* (7.58%),Pectinia (6.82%) and Favia (6.82%), other genera less than 1%.

30 species of coral belonging to 23 genera were recorded to be eroded by C. nematifera on the Con Dao reef, including Pavona cactus, P. decussata, P. varians, Acopora divaricata, A. millepora, A. robusta, A. millepora, Mycedium elephantotus, Stylophora pistillata, Pectinia alciconis, P. latuca, Favia spp., F. speciose, Pocillopora danyconis, P. verucosa, Cyphatrea chalciclium, Eyphyllia divisa, **Favites** pentagonus, Galaxea fatcicularis, Goniastrea aspera, Hydrophora exesa, Lobophyllia spp., L. hemprichii, L. robusta, Merulina ampliata, Montipora spp., M. aequituberculata, Oxypora spp., Pachyceric speciose, Platygyra sinnensis and Porites spp. Compared to previous publications, this study recorded more corroded corals than Rossi et al., (2015) [12]. In addition, the results also confirmed that hard corals with laminar and encrusting morphology are more susceptible to invasion than branching coral as compared to the study of Ávila et al., (2009) [9] and Eva et al., (2018) [17].

CONCLUSION

The morphology of Chalinula nematifera collected at Con Dao is similar to other descriptions in the Western Pacific region but differs in the skeleton structure from samples in the Eastern Pacific region. This species is able to colonize most corals and settle on both living and dead corals. High densities on reef slope with conglomerate size mainly belong to the group > 5-20 cm. The abundance of Chalinula nematifera is not high, but the ability to invade corals does not limit the host. This is considered a potential hazard to coral reefs in case of outbreak. The monitoring program should be added to this species to the coral reef quality monitoring index in the Western Pacific region.

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