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DIVERSITY, DISTRIBUTION AND BIOLOGICAL ACTIVITY OF SOFT CORALS (OCTOCORALLIA, ALCYONACEA) IN SINGAPORE

Beverly Pi Lee Goh, Grace Enhui Tan, and Lik Tong Tan**

Natural Sciences and Science Education, National Institute of Education, Nanyang Technological University, 1 Nanyang Walk, Singapore 637616

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ABSTRACT

The Southern Islands of Singapore are known to contain coral reefs which are high in biodiversity. However, the diversity of soft corals had received little attention to date. This study was conducted to determine the soft coral diversity in Singapore reefs as well as to conduct preliminary bioactivity tests on the organic extracts from these soft corals. A 100-meter line transect was used to survey soft corals at a 3m depth at ten different sample sites. Sclerites from samples were used to identify the soft corals to the generic level. This study uncovered the following genera of soft corals: Carijoa spp., Cladiella spp., Sinularia spp., Lobophytum spp., Sarcophyton spp., Stereonephthya spp., and Nephthea spp. In addition, an unidentified genus of soft coral was observed at Kusu Island. Cladiella spp. yielded the highest number of colonies, and Sarcophyton spp. had the highest coverage in terms of total colony diameter. The brine shrimp (Artemia salina) toxicity assay was carried out to screen for toxicity of the soft coral extracts at concentrations of 10, 100, and 1000 ppm. Results showed high levels of toxicity in extracts of Sarcophyton spp. and Cladiella spp., indicating that these soft corals are potentially good sources of bioactive compounds for drug discovery.

Key words: Octocorallia diversity, Singapore, toxicity of octocorals, brine shrimp lethality assay

Correspondance : Phone: +65 6790 3820; Fax: +65 6896 9432; E-mails: beverly.goh@nie.edu.sg, liktong.tan@nie.edu.sg

INTRODUCTION

Soft coral communities (Octocorallia, Alyconacea), excluding the gorgonians (Suborder Holaxonia, Calcaxonia and Scleraxonia) have been documented in coral reef studies from Southern Taiwan (Benayahu *et al*., 2004), Indonesia (Manuputty, 1992), Thailand (Satapoomin and Sudara, 1991) and the Red Sea, Israel (Benayahu *et al.*, 2002). In all, a total of 21 genera have been reported from Indo-Pacific reefs (Satapoomin and Sudara, 1991; Manuputty, 1992). There have been no comprehensive studies of soft corals undertaken in the reefs around the Southern Islands of Singapore and very little is known about their diversity and distribution. Although much information has been gathered on coral reefs in Singapore, these studies have not given much attention to soft corals, but rather have focused on other reef organisms, particularly hard corals (Chou and Teo, 1983; Chong, 1985; Chong, 1986;

Chou, 1988; Goh *et al.*, 1990; Chua, 1990; Goh and Chou, 1991; Low and Chou, 1991; Low and Chou, 1992; Leng and Lim, 1992; Goh and Chou, 1993; Chou *et al*., 1994a). A total of 197 hard corals species from 55 genera have been recorded here, accounting for almost 25% of the global total (Chou *et al*., 1994a; Chou and Goh, 1998; Tan *et al.*, 2007). In addition, studies on gorgonians in Singapore have also been documented (Goh and Chou, 1998; Goh *et al.*, 1999; Koh *et al.*, 2000). Information on soft coral communities reported in previous studies however, did not give any account of their diversity (Goh and Chou, 1991; Goh and Chou, 1994).

The coral reefs located around the Southern Islands of Singapore are known to be rich in biodiversity (Loo *et al*., 1991; Chou and Tun, 2004). However, these coral reefs have seen a steady decline over the past few years (Chua and Chou, 1992; Chou *et al*., 1994b; Chou, 2002). It has been estimated that 60% of the reefs have been lost through foreshore reclamation since the late 1960's (Chou and Goh, 1998). Extensive reclamation has had negatives impacts on the reefs and it is almost impossible to reverse the declining status (Chou *et al.*, 1994b). It has been estimated that the coral reef cover today has been reduced to only about 30 km^2 (Chou and Tun, 2004). This has led to growing concerns about coral reef conservation in Singapore. With increasing focus on conservation of reefs in Singapore, an assessment of the biodiversity of soft corals obtained from this survey will provide a good reference for further comparative studies.

Much has been documented about the significant role of reefs, particularly their potential use in biomedical science. The dense concentration of marine species makes them potential source for drug discovery (Newman and Cragg, 2004). Soft corals in particular have been a prolific source of bioactive compounds belonging mainly to the terpenoid structural class. One example is a cembranoid compound, sarcophytol A, isolated from the soft coral *Sarcophyton glaucum* having potent antitumor as well as cancer chemopreventive activity (Yokomatsu *et al*., 1994). Extracts with bioactive properties have also been screened from gorgonians in Singapore (Goh *et al.*, 1995). However, no research has so far focused on other Alyconacea in Singapore, in particular the soft corals. This study aims to document the diversity and distribution of soft corals in Singapore reefs as well as to carry out preliminary screening for bioactive compounds based on the brine shrimp toxicity assay from these soft corals.

MATERIALS AND METHODS

Sample sites.

The study was conducted at selected sites at the Southern Islands of Singapore (**Fig. 1**). A total of 10 sites were surveyed from January 2008 to April 2008. These were fringing reefs surrounding the islands of Raffles Lighthouse, (1^o10'N, 103^o45'E), Pulau (P.) Semakau (1^o 12'N, 103^o45.5'E), Sister's island (1^o 13'N, 103^o50'E), Kusu Island (1^o13'N, 103^o51'E), two patch reefs west of P. Hantu (1^o 13'N, 103^o45'E) and a jetty located in the south of P. Hantu.

Sampling method.

The depth-specific 100m line transect as described in English *et al*. (1994) was used to sample reef communities at a depth of 3meters from the reef crest for all sites except the jetty at P. Hantu. At P. Hantu jetty, pillars supporting the jetty were surveyed by observation at a depth of 3 meters. Since this was not a coral reef on which a transect could be laid, only total colony counts of soft corals were recorded from the four pillars. All surveys were

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carried out using SCUBA. Soft corals encountered along the transects were measured (colony diameter, m) and sections of the colonies were collected in separate bags.

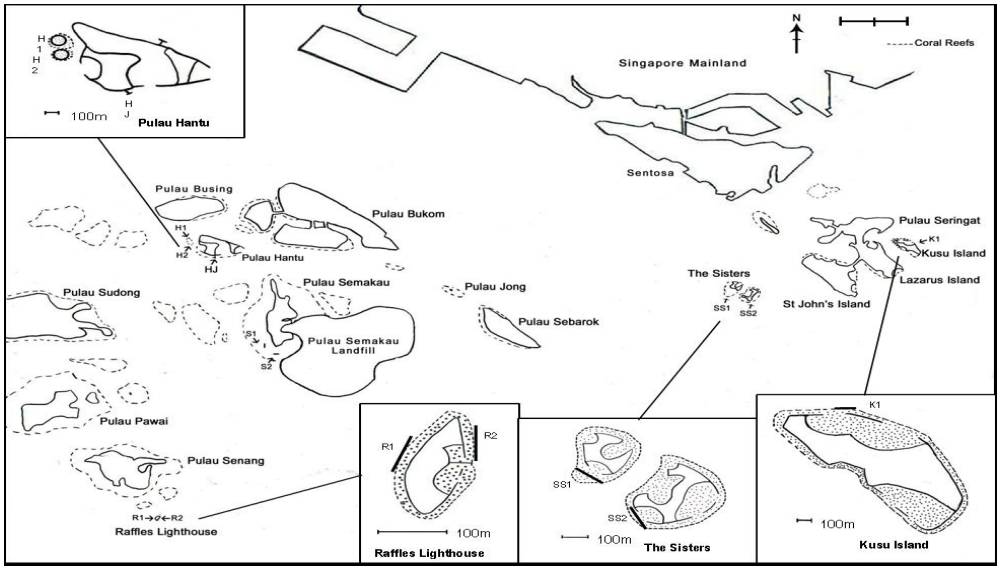

Fig 1. Map of the Southern Islands of Singapore showing the location of sample sites. Inset: Enlarged maps of individual sample sites indicating transects. (H1: P. Hantu 1, H2: P. Hantu 2, K1: Kusu Island 1, R1: Raffles Light House 1, R2: Raffles Light House 2, S1: P. Semakau 1, S2: P. Semakau 2, SS1: The Sister's Island 1, SS2: The Sister's island 2. HJ: P. Hantu Jetty)

Samples of soft corals were frozen immediately. In the laboratory, about 0.5cm^2 of soft coral tissues were removed and dissolved in 10% sodium hypochlorite to obtain sclerites for identification. Identification was carried out to the genus level with the use of keys from the Australian Institute of Marine Science (Fabricius and Alderslade, 2001). There are no published resources for identifying soft corals to the species level. Soft corals obtained in this study were therefore identified only to the genus level.

Brine shrimp (Artemia salina) toxicity assay.

The brine shrimp toxicity assay based on Meyer *et al*. (1982) was used to test for biological activity from samples of the soft corals. Extracts were obtained by soaking samples in MeOH for 24h and followed by filtration using the buchner funnel. Removal of organic solvent was achieved using the roto-evaporator. Three different concentrations of extracts were used to test for toxicity by re-suspending dried extracts in artificial sea water in 20 ml glass vials to give resulting concentrations of 100 ppm, 100 ppm, and 10 ppm. A total of five replicates were tested for each concentration, together with controls containing no extracts. Brine shrimps (*Artemia salina*) larvae were hatched from cysts 48h prior to the commencement of the assay. For each test concentration and controls, 20 brine shrimp larvae were introduced into each vial using long stemmed pipettes. The percentage mortality of the brine shrimp was observed after 24h.

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RESULTS AND DISCUSSION

Results

A total of seven genera of soft corals were identified at the ten sites surveyed, representing the families Clavulariidea, Alcyoniidae and Neptheidae (**Table 1**). In addition, four colonies of an unidentified soft coral genus were found at Kusu Island. The largest coverage of soft corals, in terms of colony diameter, was found at Kusu Island (total of 4.76m) while the lowest coverage was found at Sister's Island 1 (total of 0.31m). In terms of abundance (number of colonies), P. Semakau yielded the highest number of soft corals with *Cladiella* spp. having the highest occurrence at all sample sites (**Table 1**). Furthermore, *Sarcophyton* spp. was observed to have the highest cover at all sites.

Table 1. Total soft coral cover (colony diameter in m) for each genus along a 100m transect at sample sites in the Southern Islands of Singapore. The abundance (number of colonies) of each genus is stated in parentheses. (H1: P. Hantu 1, H2: P. Hantu 2, K: Kusu Island 1, R1: Raffles Light House 1, R2: Raffles Light House 2, S1: P. Semakau 1, S2: P. Semakau 2, SS1: The Sister's Island 1, SS2: The Sister's island 2. *HJ: P. Hantu Jetty (only number of colonies indicated), Car: *Carijoa* spp.; Cla: *Cladiella* spp.; Sin: *Sinularia* spp.; Lob: *Lobophytum* spp.; Nep: *Nephthea* spp.; Sar: *Sarcophyton* spp.; Ste: *Stereonephthya* spp.; UG: Unknown).

	Clavulariidae	Alcyoniidae				Neptheidae			
Sample sites	Car	Cla	Lob	Sar	Sin	Nep	Ste	UG	Total
H1		0.32 (7)					0.10 (1)		0.42 (8)
H2		0.11 (2)				0.23 (1)	0.12 (3)		0.46 (6)
K		1.63 (3)		0.72 (3)		0.92 (5)	0.12 (1)	1.37 (4)	4.76 (16)
R1		0.80 (6)	0.53 (3)	0.17 (1)	0.20 (2)				1.70 (12)
R2		0.11 (1)	1.48 (7)	0.64 (2)			0.11 (1)		2.34 (11)
S1				0.11 (1)		0.40(4)	0.21 (3)		0.72 (8)
S2			0.56 (1)	2.23(15)	0.17 (2)	0.57 (3)	0.28 (4)		3.80 (25)
SS1					0.10 (1)		0.21 (3)		0.31(4)
SS2		1.06 (6)		0.28 (1)	0.23 (5)		0.46 (2)		2.03(14)
*HJ	(5)								
Total	(5)	4.03(25)	2.57(11)	4.15(23)	0.7 (10)	2.12(13)	1.61(18)	1.37 (4)	

Extracts of six soft coral species (*Sarcophyton* spp., *Sinularia* spp., *Cladellia* spp., *Nephthea* spp., *Carijoa* spp, and the unidentified soft coral from P. Hantu) showed significant biological activity with mortality greater than 80% at 1000 ppm (**Fig. 2**). Both extracts of *Sacrophyton* spp. and *Cladellia* spp. indicated high toxicity (> 80% mortality) when tested at a lower concentration of 100 ppm. No significant toxicity responses were observed at the lowest concentration of 10 ppm for all extracts. All controls indicated no mortality of brine shrimp larvae.

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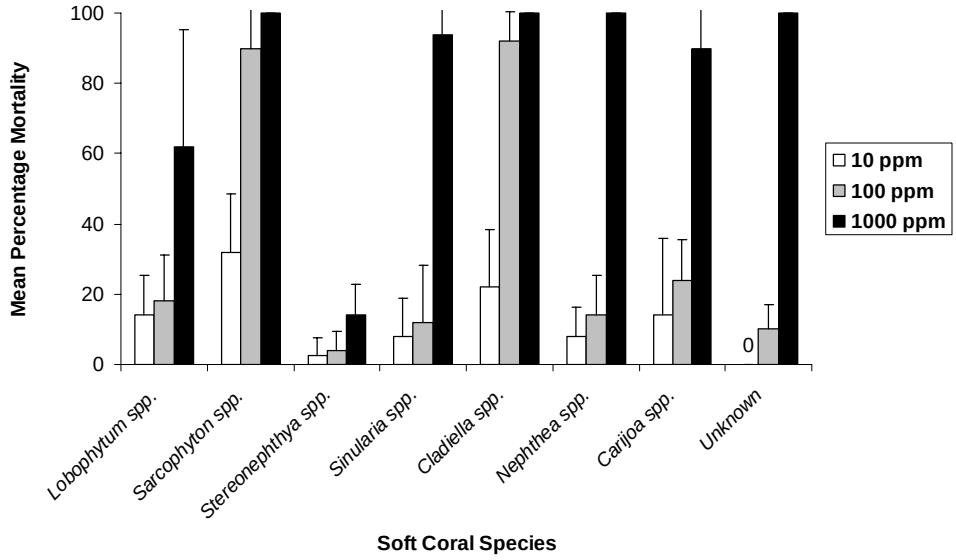

Fig 2. Graph showing mean percentage mortality of brine shrimp larvae (pooling of five replicates) at three test concentrations of soft coral extracts.

Discussion

This study documents for the first time seven named genera of soft corals in the reefs of Singapore, and Alcyoniidae as the most abundant family observed. Past records of soft corals from Singapore obtained from preserved specimens at the Raffles Museum of Biodiversity Research (NUS) and reports (Wee and Ng, 1994) have listed the genera: *Sinularia* spp., *Cladiella* spp., *Lobophytum* spp., *Sarcophyton* spp. and *Dendronephthya* spp. Only *Dendropnephthya* spp. was not sampled in this study.

The soft coral diversity observed in this study was low compared to records of hard corals from the Southern Islands of Singapore over the past few years. Past records have reported over 150 species of hard corals from 55 genera (Chou *et al*., 1994a; Chou and Goh, 1998). The results here also indicate fewer soft corals in terms of abundance and diversity compared to other countries in the region. A survey conducted in Indonesia, Seribu Island, reported 17 genera from six different families (Manuputty, 1992). Records from the gulf of Thailand showed ten different genera from five families (Satapoomin and Sudara, 1992), and reefs in Taiwan were observed to have 22 genera from seven families (Benayahu *et al*., 2004). Our study surveyed a relatively small area compared to the other studies, and a more comprehensive survey covering a wider area of reefs in Singapore may reveal more species of soft corals.

In this study, *Cladiellia* spp. was the most abundant with 25 collected throughout the survey, and hence it may be the dominant soft coral genus in Singapore. The survey concentrated on areas of the coral reef close to the reef flat, which is a region which experiences high wave action. *Cladiella* spp. is a thick encrusting octocoral, and has been reported to be able to tolerate some wave action (Fabricius and Alderslade, 2001). This may explain its high abundance in this study. *Cladiella* spp. was also found to be one of the dominant genera in the gulf of Thailand (Satapoomin and Sudara, 1991).

Members of the Alcyoniidae, such as *Sarcophyton* spp., *Sinularia* spp. and *Lobophytum* spp. have been reported to be

fast growing and able to grow well in moderately turbid, well lit coastal areas (Fabricius and Alderslade, 2001). These soft corals were frequently sampled at all the survey locations in this study, and it may be an indication of the higher tolerance of these particular genera for sedimented waters, like those occurring in Singapore .

The low soft coral abundance observed in the study could be attributed to the high sedimentation rate in Singapore waters. Average sedimentation rates of 5-20mg cm^{-2} day^{-1} have been reported with maximum rates of up to 44.64 cm^{-2} day^{-1} recorded (Low and Chou, 1994). Rogers (1990) postulated that "normal" sedimentation rates in coral reefs are in the order of approximately 10mg cm^{-2} day^{-1}. Heavy sediment loading form land reclamation, dredging and dumping have contributed to a high sedimentation rate in Singapore waters, causing anthropogenic stress to reefs (Chou *et al.*, 1994a). In addition, sedimentation also results in a reduction in light penetration and thus also affects coral reef organisms that photosynthesize (Goh and Chou, 1991). Chua and Chou (1992) have further reported that the deeper zones of the coral reef have become denuded as a result of a reduction in light penetration. Thus may also explain why the soft corals sampled in this study were located in the shallow reef crest.

Chou (2002) documented a steady decrease in live coral cover at most reef sites since 1986. Even Raffles Lighthouse, the island furthest from Singapore mainland was also reported to suffer a decrease in live coral cover, from 76.35% in 1987 to 48% in 1997. *Sinularia* spp., the only soft coral monitored in a 1998 study showed complete mortality during a mass bleaching event in 1998. The overall reduction in total live coral cover through bleaching was most evident at the reef crest and this was attributed to an increase in sea surface temperature causing thermal stress to reefs organisms (Chou, 2002). Hence the two factors: sedimentation and temperature increase could have lead to the decline of the reef community in terms of abundance and species richness (Chou *et al.*, 1994a), and may also explain the low abundance of soft corals observed in this study.

Other factors also play a role in reef diversity. In particular, substrate type determines how reef organisms may be established. Satapoomin and Sudara (1991) stated that a stable substrate was important for attachment by soft corals. Loo *et al*. (1991) documented the sea floor of the southern islands of Singapore to be covered with unconsolidated sand and mud. Hence the low diversity and abundance of soft corals at the patch reefs of P. Hantu in this study may be due to the fact that the bottom substrate is mainly composed of dead coral covered with sediment (personal observation).

A study of coral establishment on submerged concrete pillars at P. Hantu conducted by Chong (1985) focussed on hard corals, and no soft corals were documented. In the present study, *Carijoa* spp. was the only genus found at the pillars of the P. Hantu jetty, and it was not sampled at the other nine study sites. *Carijoa* spp. is a common fouling organism on jetties and wrecks (Fabricius and Alderslade, 2001), and this may explain its occurrence at the P. Hantu jetty.

Although the soft coral fauna of Singapore's reefs represent a small proportion of the total reef community, it is believed to play a significant role in the reef ecosystem through many interspecific interactions (Goh and Chou, 1991).

Results of the brine shrimp toxicity assay indicated the presence of bioactive compounds in the soft corals collected in this study. Extracts from *Stereonephythya* spp. yielded the lowest mortality rates at every test concentration, and hence had the lowest toxicity effect. *Sarcophyton* spp. and *Cladiella* spp. were shown to be active even at 100 ppm, and thus their extracts may be considered to be more toxic than those from the other genera. Therefore, this preliminary

test of bioactivity in soft corals from Singapore showed promising results, particularly for the genera *Sarcophyton* spp. and *Cladiella* spp., and may be investigated further as source of bioactive compounds for drug discovery.

The use of the brine shrimp toxicity assay in this study has proven to be a quick and cost effective method to establish preliminary toxicity activity in these soft corals. A search of the literature has shown that a number of pharmaceutically important marine-derived compounds, such as curacin A (Gerwick *et al.*, 1994), have been identified through the use of this assay. Furthermore, studies have shown good correlations between brine shrimp toxicity data and cell-based cytotoxicity data (Carballo *et al*., 2002). In spite of the small sample size of just eight soft coral species used in this study, high incidence of toxicity data was indicated in six of them. This further demonstrates that in general marine organisms from local waters are significant source of bioactive compounds for drug discovery and highlights the importance of conserving local marine flora and fauna.

CONCLUSION

In conclusion, this preliminary study revealed seven known genera and one unknown soft coral genus from the Southern Islands of Singapore. The brine shrimp toxicity assays indicated the presence of bioactive compounds from the soft coral extracts, particularly from *Sarcophyton* spp. and *Cladiell*a spp. This suggests a good potential for drug discovery in these two genera and further analysis of the active compounds from the soft coral extracts should be conducted to uncover the specific compound(s) responsible for the toxicity. It is evident that further research should be conducted to identify the soft corals in Singapore to the species level, as well as identify and classify the unknown soft coral genus into an appropriate taxonomic group for future reference. In addition, more sites in the Southern Islands of Singapore could be surveyed for a more comprehensive study of soft coral diversity and ecology.

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