Distribution Patterns of the Renieramycin-Producing Sponge, *Xestospongia* sp., and Its Association with Other Reef Organisms in the Gulf of Thailand

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Distribution patterns of the renieramycin-producing sponge, *Xestospongia* sp., and its association with other reef organisms in the Gulf of Thailand. *Zoological Studies* **46**(6): xxx-xxx. The renieramycin-producing sponge, *Xestospongia* sp., is a coral reef inhabitant occurring in the Gulf of Thailand. The distribution patterns of *Xestospongia* sp. and its association with other organisms were investigated, with results showing that the most frequently coexisting organisms were the massive coral, *Porites lutea*, and the colonial zooanthid, *Palythoa caesia*, but it also inhabited algal patches and dead coral rubble. The largest individuals of *Xestospongia* sp. were found growing on *Pa. caesia* while the smallest individuals were found on algal patches. The results also showed that concentrations of renieramycin M, the main alkaloid with highly potent cytotoxicity, extracted from this sponge differed significantly among sites ($p < 0.05$).
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Key words: *Xestospongia* sp., renieramycin concentration, coexisting organisms, Gulf of Thailand.

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The genus *Xestospongia* is distributed worldwide, from the Indo-Pacific to the
Caribbean, and is particularly common and diverse in northwestern Australia, the
Great Barrier Reef, Papua New Guinea, the Solomon Is., the Palau Archipelago, the
West-Central Pacific, the Gulf of Thailand, and the Indo-Malay Peninsula (de
Laubenfels 1954, Bergquist 1965, Bergquist and Tizard 1967, Bergquist et al. 1971,
Fromont 1991, Amir 1992, Kerr and Borges 1994, Pulitzer-Finali 1996, Kritsanapuntu
et al. 2001, Desqueyroux-Faúndez and Valentine. 2002). *Xestospongia* is known to
settle and grow on a variety of substrates, such as sand, rock beds, dead coral rubble,
and coral heads (Zea 1993, Hooper 1994, Moyer et al. 2003, Bell and Smith 2004,
Armstrong et al. 2006). Although *Xestospongia* is found in a range of localities and is
associated with a number of different organisms, its morphological heterogeneity has
not yet been correlated with microhabitat or geographical factors, although habitat
heterogeneity at the micro-scale and macro-scales appears to impact variations in the
chemical concentrations produced. For example, factors influencing chemical
concentrations include the spatial scale, local adaptations, inter- and intra-individual
variations, habitat differences, and physical stresses (Chanas and Pawlik 1997,
Swearingen and Pawlik 1998, Schupp et al. 1999, Wulff 2005). The chemicals
produced by sponges are known to be effective as a feeding deterrent, as being
allelopathic to competitors, and as possessing anti-fouling and antimicrobial activities
(Frincker and Faulkner 1982, Thacker et al. 1998, Engle and Pawlik 2000). Some of
the chemicals produced by sponges are not restricted to a single species. For example,
the blue sponge, *Xestospongia* sp., from the Gulf of Thailand, studied herein,
produces a similar class of compounds, renieramycins, as does *Reniera* sp. and
Haliclona (Reniera) cribricutis from the Indian Ocean (Frincker and Faulkner 1982,
Parameswaran 1998, Suwanborirux et al. 2003, Amnuaypol et al. 2004, Saito et al.
2004a b). The possibility of either common biosynthetic pathways or associated
microorganisms which might be the true producers has been suggested.
Renieramycins are a class of bistetrahydroisoquinoline alkaloids which show very
potent cytotoxicities against several cancer cell lines. With its promising anticancer

properties, our group has attempted to isolate a number of renieramycins in high yields from the Thai blue sponge, *Xestospongia* sp. (Suwanborirux et al. 2003, Amnuoypol et al. 2004).

The objectives of the present study were to investigate the distribution pattern of *Xestospongia* sp. and its association with other organisms, and seek correlations with variations in the amounts of renieramycins produced by individuals among sites in the Gulf of Thailand.

MATERIALS AND METHODS

Animal materials

The blue sponge, *Xestospongia* sp., in our study has unique characteristics which differ from other renieramycin-producing sponges such as *Reniera* sp. (accepted as *Haliclona (Reniera)* sp., Van Soest et al. 2005), *Haliclona cribricutis* (accepted as *Haliclona (Reniera) cribricutis*, Van Soest et al. 2005), *Cribrochalina* sp., *Neopetrosia* spp. (Frincke and Faulkner 1982, Parameswaran et al. 1998, Pettit et al. 2000, Oku et al. 2003, Nakao et al. 2004, Van Soest et al. 2005), and other species in the genus *Xestospongia* that previously have been documented. It is likely that the species is undescribed although a more-rigorous taxonomic investigation of this large genus would be necessary prior to describing and naming it as a new species. This sponge differs from renierid, cribrochalinid, and neopetrosid sponges by its choanosomal skeleton with pauci- to multispicular tracts, and ectosomal skeleton with a tangential disordered network and no specialized structure. Renierid sponges have a unispicular, isotropic reticulation of the choanosomal skeleton and a tangential, unispicular, isotropic reticulation of the ectosomal skeleton (de Weerdt 2002), while cribrochalinid sponges have an ectosomal network consisting of a palisade of spicule brushes covered by a fine membrane (crust) (Desqueyroux-Faúndez and Valentine 2002a). Although this sponge is very similar to neopetrosid sponges, its oxea megascleres are longer than 200 μm while those of neopetrosid sponges are shorter than 200 μm. In addition, neopetrosid sponges have a secondary subectosomal tangential network not present in our species (Desqueyroux-Faúndez and Valentine 2002b). The blue *Xestospogia* sp. can be distinguished from other previously

described species of *Xestospongia* such as the Indo-Pacific species *X. exigua* (accepted as *Neopetrosia exigua*, Van Soest et al. 2005), *X. testudinaria,* and *X. bergquistia*, and the Caribbean species *X. carbonaria* and *X. muta* as follows. *Xestospogia exigua* is sticky to the touch when alive and preserved, and its ectosome adheres to the fingers. Both *X. testudinaria* and *X. bergquistia* are volcano-shaped while the Caribbean *X. muta* is barrel-shaped (Fromont 1991). The major differentiating morphological characteristic from *X. carbonaria* is the hispid blue surface of *Xestospongia* sp. compared to a smooth surface, black live coloration, and volcano-shaped elevation of the oscules (STRI 2006).

Samples of blue *Xestospongia* sp., collected from the coral reefs in the Gulf of Thailand, were found to coexist with other reef organisms (e.g., corals, algae, and other sponges) as well as settling on rock beds and dead coral rubble. This species is thickly encrusting and mostly lobate in growth form; the texture is hard, brittle, and friable; the surface is prominently bulbous, almost digitate-like; the color is light blue externally, yellowish-gray internally when alive and yellowish-brown in ethanol; and the oscules are numerous and mostly found on the apices of the surface lobes. The ectosomal skeleton forms a tangential disordered network with no specialized spiculation. The choanosomal skeleton exhibits isotropic reticulation of paucispicular to multispicular tracts of oxeas forming tight oval meshes. There are no visible fibers and only a small amount of collagen in the mesohyl. The oxeas are straight or slightly curved at the center, sharply pointed and hastate, and 218, 241, and 257 µm long and 10, 17, and 20 µm wide (Fig. 1).
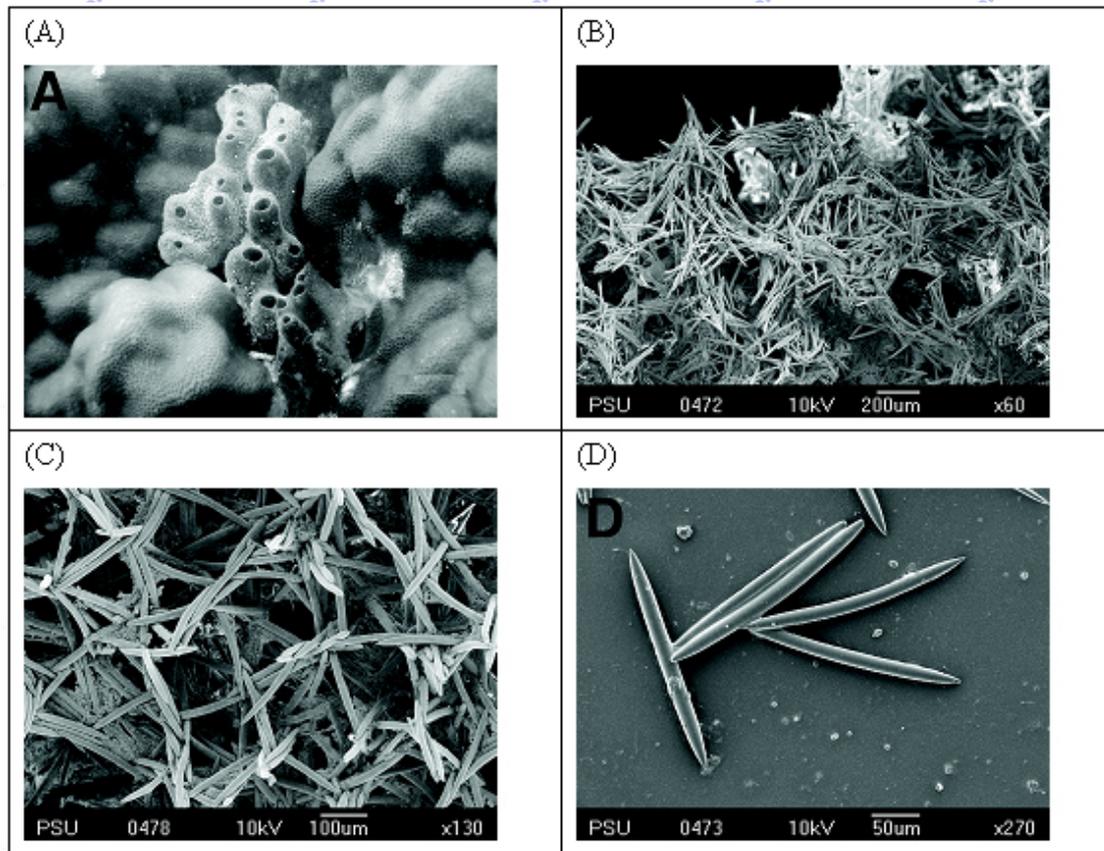
Fig. 1. (A) Blue sponge *Xestospongia* sp. coexisting with the hard coral, *Porites lutea*. (B) Choanosomal skeleton, showing a highly dense network of multispicular tracts. (C) Longitudinal section through the surface, showing an ectosomal tangential disordered network of spicule brushes. (D) Oxeas.

Study sites

Five reef sites were examined in the Gulf of Thailand, Samui (9°28'05"N, 99°55'52"E), Hin Kob (10°40'33"N, 99°19'54"E), Khlong Wan (11°45'53"N, 99°47'59"E), Chang (12°08'43"N, 102°16'06"E), and Sumpayu (13°11'18"N, 100°47'53"E) (Fig. 2).
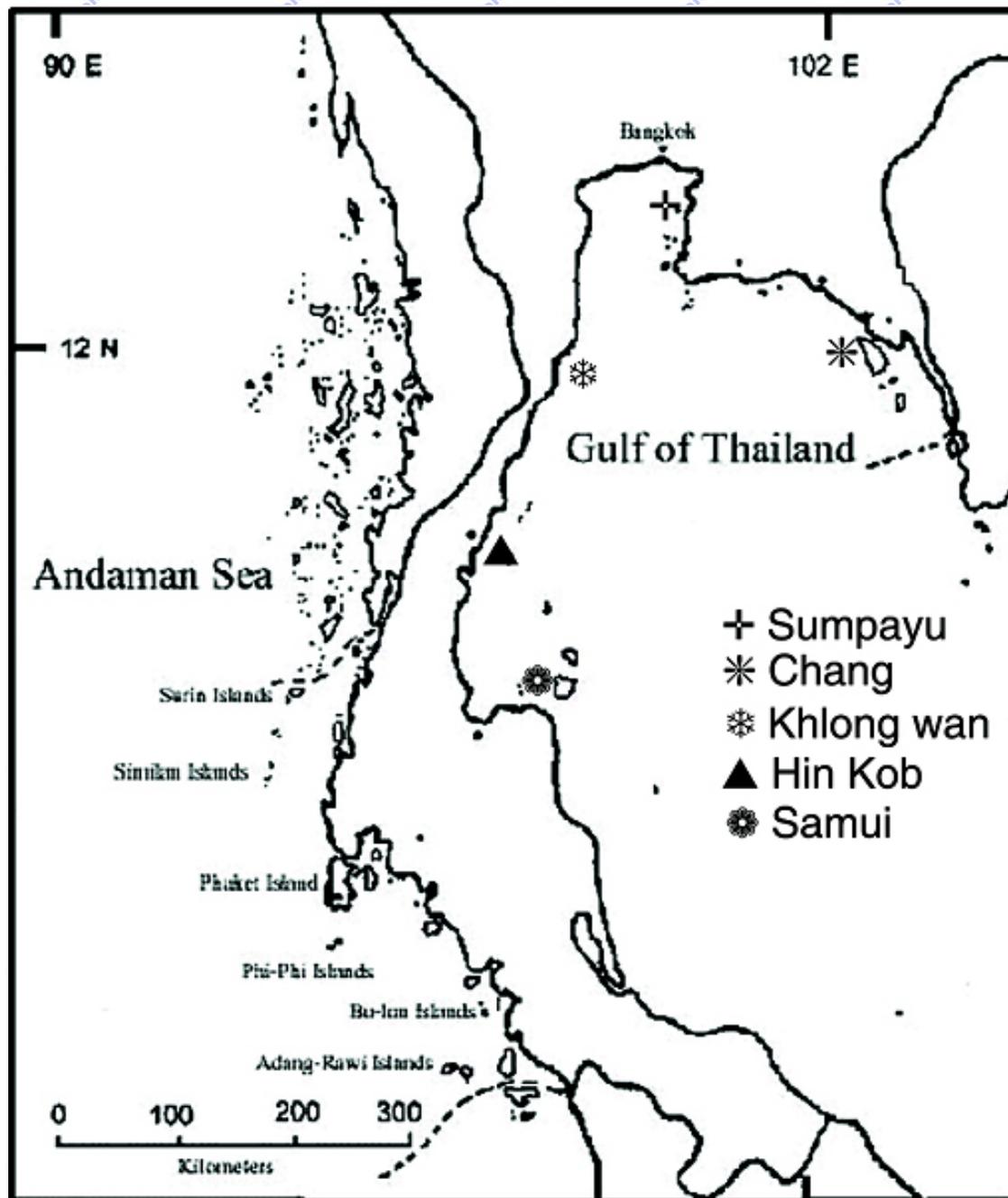
Fig. 2. Study sites along the Gulf of Thailand.

Sample collection

Surveys were conducted along a 50-m line transect with a 2-m-wide quadrat.
Organisms coexisting with *Xestospongia* sp. were recorded and identified to the
lowest taxonomic level, and the maximum length and width of individual sponges
were measured to estimate sponge coverage. We collected 2-3 g (wet weight) of each

sponge individual by hand, and samples were kept in net bags while scuba diving or snorkeling, with a total of 15 replicates taken at each site. To reduce the amount of saltwater, each specimen was cut into 2-cm^2-sized pieces and dried with tissue paper for a minute, twice. The semi-dried specimens were placed in plastic bags in an icebox during transportation to the lab. The specimens were then stored at -20°C until extraction.

Crude extract preparation

Frozen specimens were left in the container for a few minutes until reaching ambient temperature. Each specimen was cut into fine pieces and accurately weighed to the nearest 1500 mg. The sample was macerated with 10 mM potassium cyanide in phosphate buffer solution (6.00 mL, pH 7.0) for 5 h. Then the suspension was extracted with methanol (24.00 mL) for 1 h. After centrifugation at 5000 rpm for 5 min, the supernatant (3.00 mL) was partitioned with ethyl acetate (9.00 mL) and a brine solution (6.00 mL). The ethyl acetate layer (3.00 mL) was evaporated until dry. The dried residue was dissolved in methanol (1.00 mL) containing 300 ng acenapthene as an internal standard. The sample solutions were filtered through 0.45-nm nylon syringe tip filters before the high-performance liquid chromatographic (HPLC) analysis.

Standard calibration solution

A stock standard solution of renieramycin M, at a concentration of 1 mg/mL was prepared in methanol. Calibration solutions containing 2.4, 4.8, 9.0, 180.0, 375.0, 750.0, and 1500.0 ng/mL were prepared by appropriate dilutions of the stock solution with methanol containing 800 ng acetonapthene as an internal standard.

HPLC conditions

A Waters 2690 Controller (Waters, USA) was used with a Shimadzu SPD-10 VP class Absorbance Detector (Shimadzu, Japan) operated at 270 nm. Separation was achieved on a LiChrospher®100RP-18 reversed-phase column (5 μm, spherical, 4.0 x

125 mm (Merck, Germany) with methanol-water (7:3) as the mobile phase at a flow rate of 0.70 mL/min.

RESULTS

We found that *Xestospongia* sp. coexisted with several organisms including algae, bivalves, cnidarians, and other sponges (Fig. 3). At Samui, the major coexisting organism was algae (with a 71.43% frequency), while at Hin Kob and Chang *Xestospongia* sp. was mainly found coexisting with *Porites lutea* (at 46.78% and 54.84% frequencies, respectively) (Fig. 3). *Xestospongia* sp. was most abundant at Sumpayu (86 individuals/100 m^2) and least abundant at Samui (7 individuals/100 m^2) (Fig. 4).

Legend:
- ■ Algae
- ▨ Demospongia
- □ *Porites lutea*
- ≡ *Lobophyllia hemprichii*
- ◣ *Palythoa caesia*
- ▨ *Palythoa tuberculosa*
- ▦ *Heteractis sp.*
- ▥ Hydrozoa
- ▨ *Barbataria belbingia*
- ▣ Dead coral rubble
- ▨ Rock
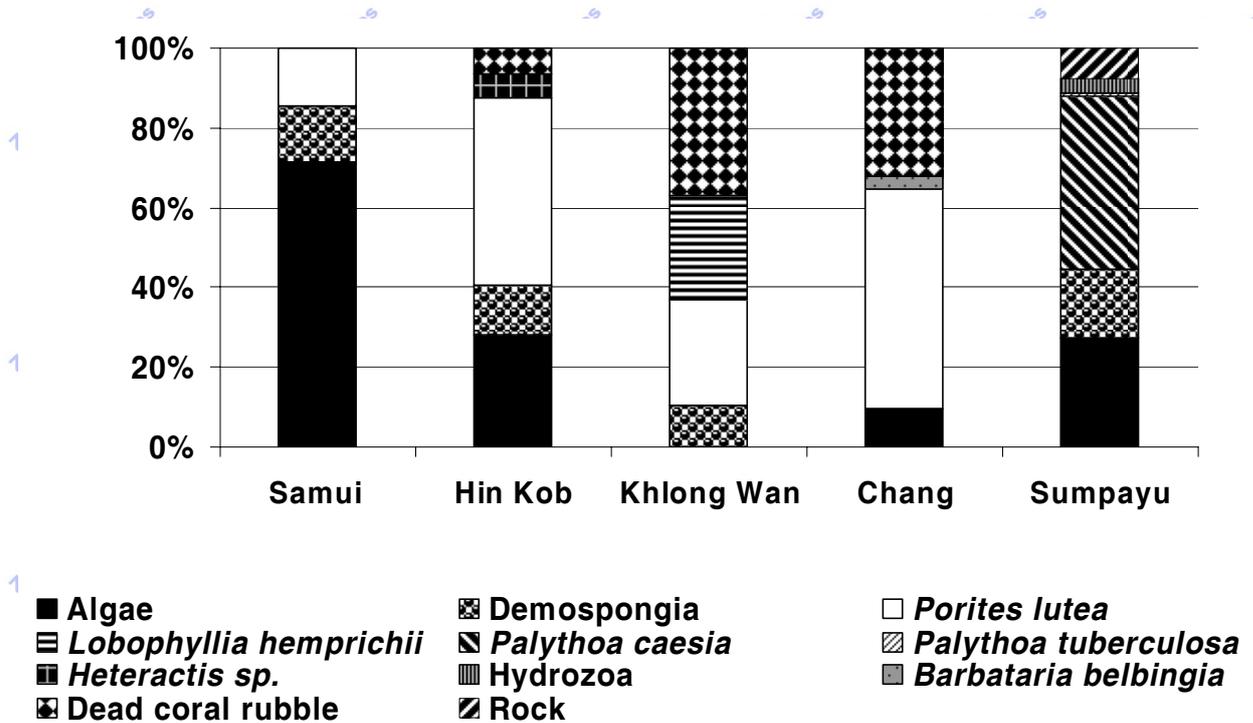
Fig. 3. Accumulated percent frequency of organisms and substrata that *Xestospongia* sponge coexisted with or inhabited.

Xestospongia sp. at the Sumpayu site had the highest maximum area of cover compared to other sites. The largest individuals were found at Sumpayu (500 cm^2) and Chang (451 cm^2) where they coexisted with *Palythoa caesia* and *Po. lutea* respectively (Figs. 4, 5). Hin Kob had the lowest maximum cover of *Xestospongia* sp.

(96 cm^2) (Fig. 4), and overall, the highest average area of cover was at Chang (74 cm^2) (Fig. 4). *Xestospongia* sp. coexisting with *Pa. caesia* had the largest range of average area of coverage compared to other coexisting organisms (Fig. 5), whereas the smallest range of average cover was found for *Xestospongia* sp. coexisting with the sea anemone, *Heteractis* sp. (Fig. 5).
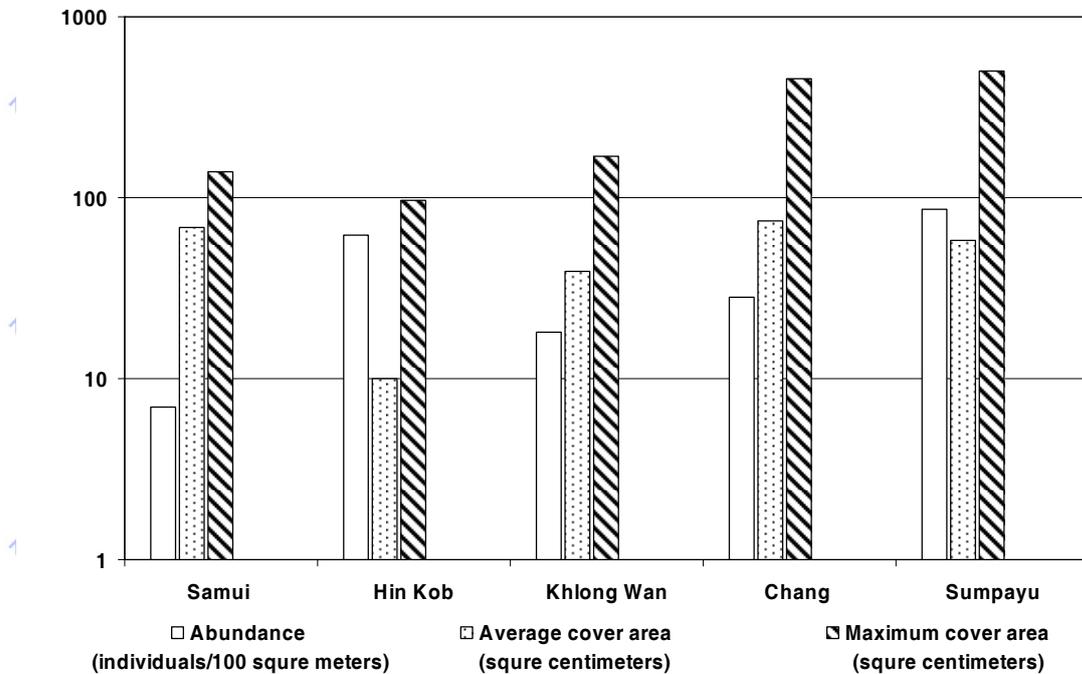
Fig. 4. Abundance, average area of coverage, and maximum area of coverage of *Xestospongia* sp. at different sites.
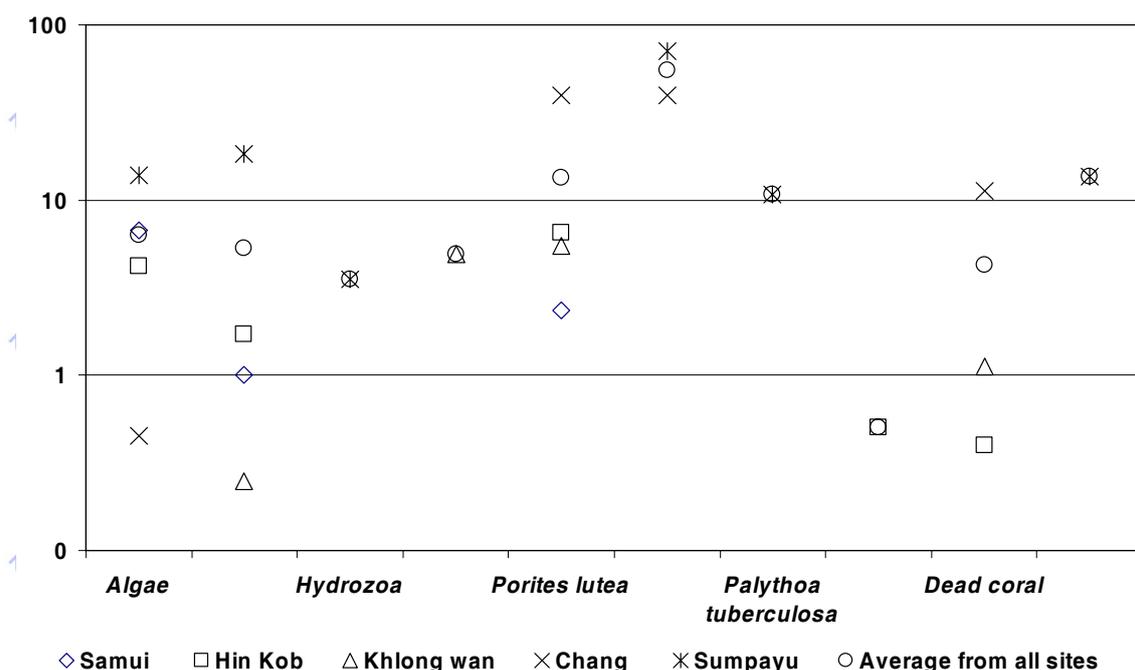
Fig. 5. Average cover area of *Xestospongia* sp. coexisting with different organisms and habitats among the different sites.

There were significant differences in the average percentage of renieramycin M concentrations of *Xestospongia* sp. at different sites (ANOVA, $p < 0.05$) (Fig. 6). The highest renieramycin M concentrations were found at Hin Kob and Chang (Fig. 6).
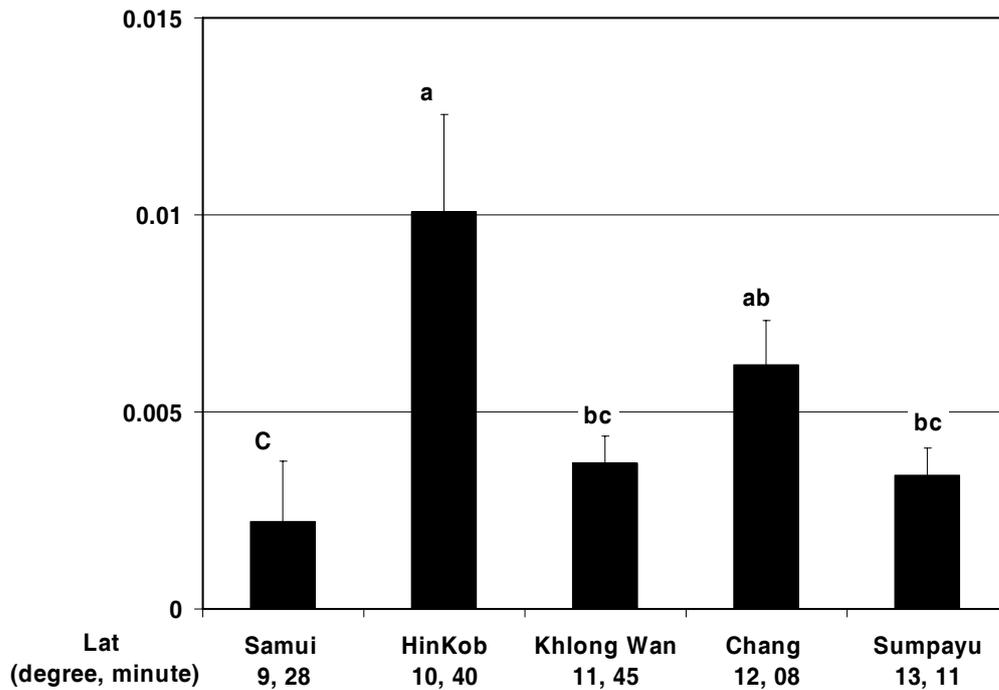
Fig. 6. Percent renieramycin M concentrations (mean ± SE) in semi-dried weight of *Xestospongia* sp. The means of groups with the same letter above the columns do not significantly differ (ANOVA and Tukey's test).

However, there was no relationship between renieramycin M concentrations and the latitude of the collection site. There were distinct patterns between the frequency of occurrence of *Xestospongia* sp. associated with other organisms, renieramycin M concentration, and percent area of cover at different sites. The major coexisting organism with the highest frequency of occurrence had the highest average renieramycin M concentration and the highest average area of cover at every site except at Khlong Wan (Tables 1, 2, Figs. 3, 5). At Hin Kob and Chang, *Xestospongia* sp. mainly coexisted with *Po. lutea* and had the largest cover (96 and 451 cm^2, respectively), and also the highest renieramycin M concentrations (0.009% and

0.005% w/w, respectively) (Table 2). At Samui and Sumpayu there were similar patterns as at Hin Kob and Chang, although the coexisting organisms differed. At Samui, *Xestospongia* sp. coexisting with algae had the highest frequency, highest cover area, and highest renieramycin M concentration, while at Sumpayu, *Xestospongia* sp. coexisting with *Pa. caesia* had the highest frequency, highest cover area, and highest renieramycin M concentration (Table 2). Statistical analysis using a 2-factor analysis between sites and coexisting organisms showed that there were significant differences between sites and coexisting organisms, and both had effects on the average cover area and average renieramycin M concentrations (Table 3).

Table 1. Average percent of renieramycin M concentration of *Xestospongia* among sites with different coexisting organisms and habitats (-, no coexisting organisms occurred at that site; ND, non-detectable concentration of renieramycin M)

	Site				
Coexisting organism	Samui	Hin Kob	Khlong Wan	Chang	Sumpayu
Algae	0.005	0.004	-	ND	ND
Demospongiae	ND	0.001	0.001	-	ND
Hydrozoan	-	-	-	-	ND
Lobophyllia hemprichii	-	-	0.002	-	-
Porites lutea	ND	0.009	0.001	0.005	-
Palythoa caesia	-	-	-	-	0.003
Palythoa tuberculosa	-	-	-	-	0.001
Heteractis sp.	-	ND	-	-	-
Barbataria belbingia	-	-	-	ND	-
Dead coral rubble	-	ND	0.002	0.003	-
Rocks	-	-	-	-	ND

Table 2. Maximum renieramycin M concentration, maximum percent frequency, and maximum percent area of coverage of coexisting organisms found with *Xestospongia* sp. at different sites

Maximum	Site				
	Samui	Hin Kob	Khlong Wan	Chang	Sumpayu
Renieramycin M concentration	Algae	*Porites lutea*	Algae	*Porites lutea*	*Palythoa caesia*
Average frequency	Algae	*Porites lutea*	*Lobophyllia hemprichii* Dead coral	*Porites lutea*	*Palythoa. caesia*
Average cover area	Algae	Algae	*Lobophyllia hemprichii*	*Porites lutea*	*Palythoa caesia*

Table 3. p values of the 2-factor analysis of coexisting organisms and sites on the average area of coverage and average renieramycin M concentration

	Average cover area	Average renieramycin M
Site	0.99	< 0.001
Coexisting organisms	< 0.001	< 0.001
Site x coexisting organisms	< 0.001	< 0.001

DISCUSSION

The abundance of sponges at any particular locality can be influenced by a great number of factors, including the presence of corals and the aggressiveness of each sponge species (Aerts and Van Soest 1997, Ward-Paige et al. 2005). Other biotic and abiotic factors that influence sponge distributions and abundances include coexisting organisms that may either facilitate or be detrimental to the settlement and survival of sponge individuals (Zea, 1993). In this study, *Xestospongia* sp. was found mainly coexisting with *Po. lutea* and *Palythoa* spp. compared to other coral species such as *Lobophyllia hemprichii* and other organisms such as other sponges (*Neopetrosia* sp.), sea anemones (*Heteractis* sp.), and bivalves (*Barbataria belbingia*). Species of *Xestospongia* have been reported in other studies to prefer hard substrates in coral reefs (Asa et al. 2000, Barnes and Bell 2002). In this study, we determined that *Xestospongia* sp. preferred to grow on massive corals (mostly *Po. lutea*), rock beds, algal patches, and dead coral rubble. Coexisting with the coral *Po. lutea*, *Xestospongia* sp. was mostly observed growing over dead areas of the coral, presumably being responsible for killing the living tissues of the coral through smothering or chemical offense, with the sponge having a competitive spatial advantage through its likely faster growth rates than the coral (Aerts and Van Soest 1997, Aerts 1998).

Physical factors such as waves, turbidity, desiccation, and nutrient availability in the water column have been documented as important factors in limiting or promoting the distribution and size of sponges (e.g., de Voogd et al. 1999 and literature cited therein). At Sumpayu, on an isolated rock standing in the ocean and subjected to wave action both from the northeast and southwest monsoons, we recorded the highest abundance of *Xestospongia* sp. compared to other sites. At Hin Kob, directly exposed to the northeast monsoon, 73% of sponge individuals were < 10 cm^2 in area of coverage, while at Khlong Wan, a semi-enclosed area and less susceptible to wave action, only 6% of individuals of < 10 cm^2 in cover were observed. Sponge size can also be influenced by the amount of nutrients in the water column (Ward-Paige et al. 2005). At Sumpayu, where the largest sponge individual (500 cm^2 in cover area) was found, a high ammonium ion concentration (400 μg/L NH$_4^+$) was detected in the water column (this study, and Pollution Control Department 2005). These nutrient

loads are derived from the Bang Pa Kong river mouth, industrial lands, and Leam Chabang deep seaport. At Hin Kob, the largest area of sponge coverage was 96 cm^2 whereas only 200 μg/L NH$_4^+$ was measured in the water column (this study, and Pollution Control Department 2005). Fresh water discharge is a major input at this site.

Sponge/coral interactions were unique among the coexisting organisms and may be classified into 4 categories: overgrowth, peripheral contact, tissue contact, and non-contact (Aerts and Van Soest 1997). In this study, we found that interactions between *Xestospongia* sp. and the corals *Po. lutea* and *L. hemprichii* were of the overgrowth type. In the case of *Xestospongia* sp. coexisting with *L. hemprichii*, inter-corallite spaces represent microhabitats for *Xestospongia* sp., and we observed that these corallites were severely affected by overgrowth of the sponge. The overgrown areas were pale, bleached, necrotic, and eroded. Unlike *L. hemprichii*, *Po. lutea* does not have inter-corallite spaces, although ultimately sponge overgrowth had the same effects on the living coral. A combination of interactions among overgrowth, peripheral contact, and tissue contact was observed in the case where *Xestospongia* sp. coexisted with *Palythoa* spp. and another sponge, *Neopetrosia* sp. *Xestospongia* sp. coexisting with *Pa. caesia* was observed originating from the inter-colonial space of the latter. However, neither necrotic scars nor wounds were observed on surface areas of *Pa. caesia* in contact with *Xestospongia* sp. A similar situation was observed with *Xestospongia* sp. coexisting with *Neopetrosia* sp. Moreover, in the intertidal zone of places such as Samui and Hin Kob, we observed no partnership between *Xestospongia* sp. and *Neopetrosia* sp. The volcano-shaped *Xestospongia* spp. were not found in the same habitat as *Xestospongia* sp. either. In addition, *Xestospongia* sp. was frequently observed growing along the margin of *Pa. caesia,* overgrowing and pushing *Palythoa* away, and competing for settlement space. Conversely, no contact interactions were observed between *Xestospongia* sp. coexisting with algae, hydrozoans, or the anemone *Heteractis* sp.

Published records of variability in chemical defense among sessile marine organisms reveal geographical variations and differences in defense reactions in different habitats (Green 1977, Chanas and Pawlik 1997, Puglisi et al. 2000). Our results found no correlation between the latitude of collection and chemical concentrations, but we did record differences in renieramycin concentrations between the different collection sites (Fig. 5), and variations in chemical concentrations appeared to be dependent on

partnerships with coexisting species. From this latter result, we suggest that there was a direct correlation between the maximum renieramycin M concentration and the maximum average frequency of organisms coexisting with *Xestospongia* sp. At Samui, HinKob, Chang, and Sumpunyu, the partnerships between *Xestospongia* sp. and the algae, *Po. lutea* and *Pa. caesia,* respectively, showed this pattern (Table 2). However, in 2 partnerships (*Xestospongia* sp. with a hydrozoan and *Xestospongia* sp. with *Heteractis* sp.), non-contact interactions were not detectable for any concentration of renieramycin M, while this was not so with tissue contact between *Xestospongia* sp. and *Po. lutea* (Table 2). Thus, *Xestospongia* sp. may produce renieramycin M for spatial competition but only via the tissue-contact mode. Although bioassay testing of renieramycins as potential pharmaceuticals has been carried out for decades (Frincker and Faulkner 1982, Suwanborirux et al. 2003, Amnuoypol et al. 2004, Saito et al. 2004a b), more ecological studies are needed in order to better understand the mechanisms that govern its biosynthesis and the environmental effects on both biosynthetic pathways and its effectiveness in nature as a chemical defensive strategy.

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