

## THE DIVERSITY OF SPONGE ASSOCIATED FAUNA (SAF) IN FOUR SELECTED MARINE SPONGES (CLASS: DEMOSPONGIAE) AT CORAL REEFS OF PULAU BATU RUSA, MALAYSIA

AL-JERIA ABDUL<sup>1</sup>, MELISSA BEATA MARTIN<sup>2,3</sup>, JASNIZAT SAIDIN<sup>2,4</sup> AND WEE HIN BOO<sup>3,5\*</sup>

<sup>1</sup>SEADLING Sdn. Bhd., KKIP Industrial Zone 4, 88450 Kota Kinabalu, Sabah, Malaysia. <sup>2</sup>Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia. <sup>3</sup>Institute of Oceanography and Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia. <sup>4</sup>Institute of Climate Adaptation and Marine Biotechnology, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia. <sup>5</sup>Institute of Climate Change, Earth Observation Centre Building, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia.

\*Corresponding author: weehinboo@ukm.edu.my

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**Abstract:** Marine sponges are known to host a range of marine organisms, especially reef-associated sponges. However, limited studies have been conducted to evaluate the diversity of Sponge Associated Fauna (SAF) in the South China Sea. This study aims to document the SAF diversity of four common reef-associated sponges (*Theonella swinhoei*, *Theonella cupola*, *Neopetrosia* cf. *chaliniformis*, and *Stylissa carteri*) from the Pulau Batu Rusa coral reef, Terengganu. Sponges were collected and washed with 70% alcohol to extract the SAF they hosted. Echinodermata *Ophiactis Seigny* (brittle star) and *Synaptula* sp. (sea cucumber) are the most common SAF in all host sponges. A clustering analysis showed differences between SAF composition hosted and the phylogenetic distance of the host sponges, particularly with the position of *S. carteri* to the two *Theonella* species. In contrast, the assemblages of SAF are consistent with the prevailing environmental conditions, substrate characteristics of the host habitat, and the host's morphological structure. As a result, it can be inferred that the composition and abundance of SAF are not dictated by the phylogenetic lineage of the host species, but rather by the interplay of environmental factors and host morphology.

Keywords: Porifera, coral reef, Terengganu, fauna, symbiosis.

### Introduction

Habitat plays a crucial role in shaping the variety and spatial arrangement of benthic marine ecosystems. This principle extends particularly to biogenic habitats formed by organisms, where the assortment of host organisms can significantly impact the richness and structure of associated faunal communities (Morrison *et al.*, 2008; Badano & Marquet, 2009). Typically, large multicellular organisms harbour communities of small associated fauna, which inhabit either the internal regions (endobiont) or exterior surfaces (epibiont) of the host organism. These assemblages collectively form a distinct ecological entity known as a holobiont (Margulis *et al.*, 1991). These smaller associated faunal populations encompass a range of ecological roles, encompassing parasitic, mutualistic, or commensalistic relationships with their host

(symbiont). Consequently, the composition of these associated faunal communities assumes significance, as it can serve as an indicator of host well-being, environmental cues, and host species differentiation (Betancourt-Lozano *et al.*, 1998; Selvin *et al.*, 2009; Glasl *et al.*, 2018; Pita *et al.*, 2018; Yang *et al.*, 2019). This aspect becomes even more crucial in the case of sessile marine organisms specialised for particular environments. Sessile organisms, permanently attached to substrates, develop specialised traits to excel in defined conditions, encompassing factors such as temperature, currents, and nutrient availability. These adaptations give insight into the dynamics of organism-environment, underscoring the importance of associated fauna in maintaining ecosystem health and balance. Exploring the composition of associated fauna

in these sessile organisms offer insights into intricate environmental relationships.

Marine sponges are an important constituent of the marine benthic community (Bell & Barnes, 2000; Bell, 2008; Maldonado *et al.*, 2015; Anjum *et al.*, 2016; Pomponi *et al.*, 2019). They are sessile organisms and exist in diverse morphological architectures and sizes that include encrusting, massive, erect, branching, and digitate (Boury-Esnault & Rutzler, 1997; Hooper & Van Soest, 2002; Regueiras *et al.*, 2019; Łukowiak, 2020; Dahihande & Thakur, 2021; Anteneh *et al.*, 2022). The internal aquifer of sponges varies from a simple to an extraordinarily complex arrangement that comprises a vast network of canals of varying diameter (Boury-Esnault & Rutzler, 1997; Hooper & Van Soest, 2002; Duris *et al.*, 2011; Chin *et al.*, 2020). Thus, they are ideal hosts for other small “inquiline” fauna (organisms that live in the body of another animal) to occupy as they provide nursery grounds, habitat defences and food via their feeding habits (Beazley *et al.*, 2013; Kazanidis *et al.*, 2016; Hawkes *et al.*, 2019; Folkers & Rombouts, 2020).

Greater aquifer complexity offers optimal microhabitats and shelter and enhances the abundance of surface hiding spaces for sponge inquilines, herein referred to as sponge-associated fauna (SAF) (Rützler, 1976; Koukouras *et al.*, 1996; Duris *et al.*, 2011; Chin *et al.*, 2020). In addition to shelter, sponges also provide food and spawning ground for invertebrates (Bell, 2008; Duris *et al.*, 2011; Çinar *et al.*, 2019).

Some sponges were recorded to produce toxic secondary metabolites as a defence mechanism to deter some of the SAF (Skilleter *et al.*, 2005). However, many organisms are able to suppress or surpass the defence mechanism. Thousands of individual polychaetes, amphipods, hydrozoans, ostracods, isopods, shrimps, and ophiuroids have been found inside individual sponges (Koukouras *et al.*, 1996; Amsler *et al.*, 2009; Duris *et al.*, 2011; Maggioni *et al.*, 2017; Ismet *et al.*, 2020). The nature of the reported species included everything

from commensals to parasites, however, the relationship between SAF and sponge hosts is unclear. The abundance and richness of the SAF are influenced by various factors. These include the species of sponge, its size and the diameter of the host sponge’s aquiferous canals (Chin *et al.*, 2020). Environmental factors also play a role, such as the habitat type, water depth (Çinar *et al.*, 2002; 2019; Chin *et al.*, 2020), and seasonal changes of the location the host was collected (Koukouras *et al.*, 1996; Çinar *et al.*, 2019).

The symbiotic association between sponge hosts and the SAF presents an intriguing ecological dynamic, necessitating comprehensive investigation (Ismet *et al.*, 2020). However, there is a scarcity of documented instances of SAF that reside within sponges, particularly within the context of Malaysia. This scarcity emphasises the significance of this research, given Malaysia’s strategic position at the Coral Triangle, a marine biodiversity hotspot (Hoeksema, 2007; Veron *et al.*, 2009; 2011). The high marine biodiversity is attributed to the well-established coral reef ecosystem within the region, which provided a safe haven for associated biodiversity (Moberg & Folke, 1999; Sorokin, 2013). The fact that limited studies were conducted for reef-associated sponges and their SAF provided the opportunity to examine the diversity they hosted (Woo *et al.*, 2010). Furthermore, Pulau Batu Rusa, a small, rocky outcrop solely occupied by a lighthouse, offers a pristine environment with the minimal human influence on for studying SAF communities potentially distinct from those in more heavily impacted regions. This research can bridge the knowledge gap regarding reef-associated sponge diversity and their associated fauna, particularly in Malaysia.

Therefore, this study aims to investigate and compare the SAF communities in four sponge species found at coral reefs, two of which are from the family Theonellidae (*Theonella swinhoei* Gray, 1868 and *Theonella cupola* Burton, 1928), and representative one species from each family: Petrosiidae (*Neopetrosia* cf. *chaliniformis* Thiele, 1899) and Scopalinidae (*Stylissa carteri*

Dendy, 1889). We hypothesise that (1) “Similar Morphology, Similar Communities”: *Theonella* species share comparable morphological characteristics, leading us to expect similar SAF communities within these hosts; (2) “Distinct Morphology, Distinct Communities”: The significant morphological divergence of *N. cf. chaliniformis* against other sponges suggests it will house distinct SAF communities; (3) “Genetic Similarity, Varied Diversity”: Despite morphological differences between *S. cateri* and *Theonella*, their relative genetic closeness suggests they may harbour similar SAF communities, potentially with variations in species diversity (Hooper & Van Soest, 2002).

## Methods and Methodology

### *Samples Collection*

The sampling location was based at Pulau Batu Rusa, which is a small rocky island, with the surrounding water composed of branching corals. Four whole individual sponge species were collected via Scuba divers at a depth of 18 m from one sampling location (5.56 N, 102.99 E) at Pulau Batu Rusa, Terengganu, Malaysia

(Figure 1). The selected samples were *Theonella swinhoei* (Voucher Number = SP01016026; GenBank Accession = MK903075), *Theonella cupola* (SPO1016004, MK473904), *Neopetrosia cf. chaliniformis* (SPO1016013, not available), and *Stylissa cateri* (SP01016006; MK43890) (Figure 2). The molecular identification of samples was reported by Bakar *et al.* (2024). Individual samples (approximately 1 kg wet weight for each species representative) were packed in Ziplock bags underwater to trap any escaping SAF immediately. These sponge species were chosen for the fact that the 1 kg sample extracted from each species accurately represents an individual organism from a specific locality, rather than comprising distinct individuals from scattered locations.

The selection of the four sponge species from the studied location is based on their ability to offer substantial biomass within a limited geographical range. This approach mitigates the inclination to sample more sporadic sponge species with lower abundance, consequently enhancing the efficiency of species identification and minimising labour-intensive search efforts. In addition, this strategy ensures

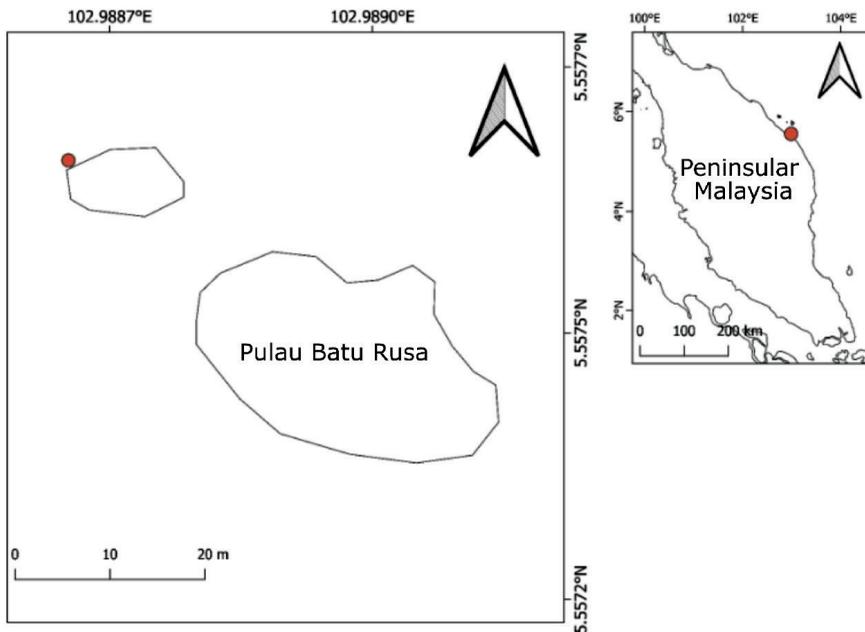


Figure 1: Map of the sampling site in relation to Pulau Batu Rusa (left) and Peninsular Malaysia (right)

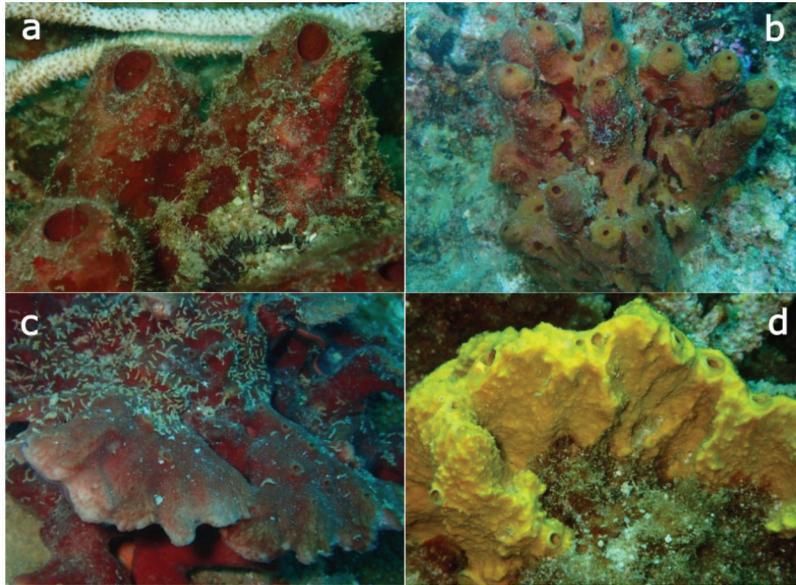


Figure 2: Sampled sponges of this study, representatives from the Class Demospongiae. (a) *Theonella swinhoei*, (b) *Theonella cupola*, (c) *Neopetrosia cf. chaliniformis*, and (d) *Stylissa carteri*

the maintenance of consistent environmental parameters, including temperature, salinity, and light intensity. The influence of light intensity and timing on endosymbiont activity such as feeding and predation has been empirically established. Standardising the volume of organisms present at any given time further contributed to the homogeneity of experimental conditions.

After retrieval, the specimens were promptly immersed in a vessel containing seawater upon emergence, with the addition of between 5% and 10% alcohol to facilitate the disengagement of the SAF from the host. The SAFs were collected and preserved in 70% alcohol. The intact sponge samples were kept in an ice chest and transported to a laboratory at the Institute of Oceanography and Environment (INOS) at Universiti Malaysia Terengganu (UMT). To extract the rest of the fauna from the samples, the sponges were cut into 1 cm thick slices with a knife and fauna was extracted using a fine pair of forceps. The fauna collected were identified to the lowest taxa possible. The morphological identification of both hosts and SAF was carried out based on established sources for various taxonomic groups: Sponges (Rützler, 2002;

Wilson *et al.*, 2003; Dahms, 2007; Hooper, 2021); echinoderms (Hendler *et al.*, 1995; Lane & Vandenspiegel, 2003; Fujita, 2016; Tan *et al.*, 2018); crustacean arthropods (Davie *et al.*, 2009; Crab Database, 2016); molluscs (Russell, 1993; Edwards, 2002; Leena & Arshad, 2011; Apte, 2017) and other documented invertebrates (Palomares & Pauly, 2021). Anatomised sponge host specimens were collected (after SAF extraction) and kept in the South China Sea Repository and Reference Centre (RRC) at UMT. At the RRC Voucher numbers (Appendix 1) of the type specimens were designated based on the hosts they were extracted from for easy reference.

#### ***Host Species Phylogenetic Distance***

Samples were identified by cytochrome oxidase 1 (CO1) mtDNA sequencing according to Folmer *et al.* (1994), except *Neopetrosia cf. chaliniformis*, which was identified using conventional taxonomy, as barcoding did not produce any viable identification (Bakar *et al.*, 2024).

To identify the species distance between the host, a genetic distance method was used.

Host identities were determined by Bakar *et al.* (2024), using morphological and phylogenetic methods. However, only *Theonella swinhoei*, *Theonella cupola*, and *Stylissa carteri* were able to be identified phylogenetically, since sequences were easily obtained from our samples. *Neopetrosia* cf. *chaliniformis* on the other hand was distinguished based on morphological attributes (Hooper & Van Soest, 2002; Cheng *et al.*, 2008; Pereira & Raghunathan, 2023). Thus, phylogenetic sequences of *T. cupola* (MK473904) and *N.* cf. *chaliniformis* (MK833935) were acquired through the GenBank repository. The sequences were imported into MEGA11 (Tamura *et al.*, 2021) and aligned using the MUSCLE algorithm. Pairwise genetic distances of the sponges were calculated using the Tamura 3-parameter uniform rates model.

### Data Analysis

The diversity of the SAF in each sponge host was calculated based on indices: Shannon-Wiener Diversity Index (SWDI), Simpson Diversity Index (SDI), Fisher's alpha ( $F\alpha$ ), and Pielou's Evenness (PE) (Fisher *et al.*, 1943; Hurlbert, 1971). The SAF community compositions in the four sponges were analysed using techniques described in Oksanen (2015) using RStudio software (R version 4.0.3) loaded with "vegan", "Mass" and "dplyr" packages (R Core Team, 2020; R Studio Team, 2020). The data set was arranged in Bray-Curti's dissimilarity matrix, to obtain the dissimilarity distance of the pairwise composition of SAF between each sponge (Oksanen, 2015). Cluster analysis was performed using the *hclust* function and plotted to identify composition distances through a dendrogram.

The weighted similarity percentage of each SAF was calculated between sponges using similarity percentages (SIMPER) analysis. A Regularised Discriminant Analysis (RDA) function was used to generate an RDA plot to identify the symbiont that influenced the SIMPER value the most.

## Results

### *Sponge Host Morphological and Genetic Profile*

Both samples from species *Theonella swinhoei* and *Theonella cupola* are from the family Theonellidae (Subclass Heteroscleromorpha, Order Tetractinellida, Suborder Astrophorina) were closely similar in morphological characteristics. Body structure of both individuals were hard in consistency and were collected with between five and seven chimneys growth attached to a rocky substrate. *Theonella swinhoei* is identified by its massive, short chimneys (cylindrical) with a broad base; measures 3.5 cm high and about 4 cm in diameter (maximum recorded size: 40 cm across); walls approximately 0.6 cm thick; a single large osculum, about 1-0.7 cm wide, located in the middle of the short cylindrical; outer side of the sponge is supplied with irregular thick ridges. The species have smooth, velvety surfaces; and maroon-brown to dark brown in colouration (Fassbender *et al.*, 2021) [Figure 2 (a)].

*Theonella cupola* is characterised by robust, irregular cylindrical structures that manifest as chimneys. These chimneys reach a height of approximately 8 cm and have a transverse diameter ranging between 0.9 cm and 3.0 cm. The structures exhibit multiple projections, each having a broad base. The surface of these chimneys appears smooth, with a depression situated off-centre on the upper surface. This depression accommodates a solitary oscule. Notably, at the chimney's apex, there exists a solitary, sizable osculum, the diameter around 2 cm. This osculum is encompassed by a delicate membranous margin that resembles a velum. Adjacent to the aperture of the osculum lies a spacious chamber that receives the openings of numerous exhalant canals. The coloration tends to be a greyish beige hue, sometimes leaning towards maroon-brown. Internally, the sponge comprises exclusively slender canals, measuring 0.5 mm or smaller in diameter (Burton, 1928) [Figure 2 (b)].

The collected *N. cf. chaliniformis* (Subclass Heteroscleromorpha, Order Haplosclerida, Family Petrosiidae) forms a thin layer encrusting small areas between 10 cm and 20 cm and is layered with ornamented brittle digitate appendages of up to 30 cm in height with a width of between 1.5 and 2 and a thickness of between 0.5 cm and 1.2 cm. Tiny oscula (of between 0.5 cm and 1.0 cm) at the top of folds and scattered elsewhere. The sponge was obtained from coral rubble, a thin layer encrusted with irregular low “walls”, folds, knobs, and lobes. The texture is smooth and velvety (not rubbery or glossy) since the surface has a layer of tiny needle-shaped spicules (oxeas). Their colouration is often ochre to golden and orangey brown. In deeper water, it may have taller lobes or turrets to 30 cm (Cheng *et al.*, 2008; Pereira & Raghunathan, 2023)[Figure 2 (c)].

*Stylissa carteri* (Subclass Heteroscleromorpha, Order Scopalinida, Family Scopalinidae) is identified from the flabellate (fan-shaped or branching) form with a ribbed surface, 45 cm in height, 30 cm in width and 2 to 8 cm in thickness: Surface conulose, finely hispid and fleshy, with few oscules on sides of the fan, 0.2 to 0.5 cm in diameter. The surface appears rough and crinkly, corrugated longitudinally with shallow ridges. It was collected from a sandy region on the fringe of an *Acropora* coral reef area (Fassbender *et al.*, 2021) [Figure 2 (d)].

Genetic distance analyses of host sponges showed that *T. swinhoei* and *T. cupola* were identical, based on the COI mtDNA marker (Table 1). On the contrary, *N. cf. chaliniformis* had the largest genetic differences with *Theonella* spp., whereas *S. carteri* fell in the middle of both clusters. Thus, the genetic

distance (from close to furthest) was based on *T. swinhoei* and *T. cupola* being closely similar, followed by *S. carteri*, and the furthest was *N. cf. chaliniformis*.

### SAF Community Structure in Sponge Host

A total of 919 individual specimens from 55 taxa were collected from all four Demospongiae sponges, with each sponge species hosting 19 to 28 SAF taxa. *T. swinhoei* had the most symbionts of 319 individuals and 28 species found, followed by *T. cupola* with 272 individuals from 27 species, *S. carteri* with 202 individuals from 19 species while *N. cf. chaliniformis* had the least symbionts with 125 individuals from 19 species (Table 2). Representatives from the phylum Echinodermata constitute more than half of the SAF from host sponges. Brittle star *Ophiactis savignyi* (Echinodermata: Ophiuroidea: Ophiactidae) was the dominant SAF in *T. swinhoei* (n = 183, 57.4%), *T. cf. cylindrica* (n = 201, 73.9%) and *N. cf. chaliniformis* (n = 61, 48.8%). *Synaptula* sp. (Echinodermata: Holothuroidea: Synaptidae) was the dominant SAF hosted by *S. carteri* (n = 62, 30.7%).

*Stylissa carteri* (Table 3) has the highest values for SWDI (2.085), SDI (0.826), and PE (0.708), with the lowest Fa (5.140) among sponge hosts. In contrast, the *T. cupola* has the lowest values of SWDI (1.277), SDI (0.446) and PE (0.388), with the highest Fa (7.449). *T. swinhoei* followed closely to *T. cupola* in terms of all diversity indices (Table 3). *Ophiactis savignyi* is the most common symbiont found in *T. swinhoei*, *T. cupola*, *N. cf. chaliniformis*; while *Synaptula* sp. was the most common symbiont found in *S. carteri*.

Table 1: Genetic differences between the host sponges based on COI mtDNA

Sponge Host	<i>Stylissa carteri</i> (KY263266)	<i>Neopetrosia cf. chaliniformis</i> (MK833935)	<i>Theonella swinhoei</i> (HM592745)
<i>Neopetrosia cf. chaliniformis</i> (MK833935)	0.291		
<i>Theonella swinhoei</i> (HM592745)	0.274	0.326	
<i>Theonella cupola</i> (KJ494353)	0.274	0.326	0

Cluster analysis (Figure 3) showed that *T. swinhoei* and *T. cupola* share the highest similarity composition among sponges at 75% (dissimilarity: 0.25). This was followed by *N. cf. chaliniformis* which was 45% (dissimilarity: 0.55) similar to the Theonellidae sponge cluster. Lastly, *S. carteri* has the least common composition of symbiont among the four, with only 30% (dissimilarity: 0.70) similar to the rest. Both *T. cupola* (201 individuals) and *T. swinhoei* (183 individuals) has a high number of brittle stars *Ophactis savingyi* when compared with the

other sponges, while *S. carteri* composed mostly of sea cucumber *Synaptula* sp. (62 individuals), glass shrimp *Pasiphaea* sp. (39 individuals) and brittle star *Ophiothrix* sp. (30 individuals) (Figure 4). The most abundant symbiont in *N. cf. chaliniformis* is the porcelain crab *Pachycheles* sp., with 32 individuals.

Based on SIMPER analysis, *Ophactis savingnyi* is the main driver of dissimilarity almost among all sponges, contributing 11.76% to 60.60% of dissimilarity. *Synaptula* sp. is the

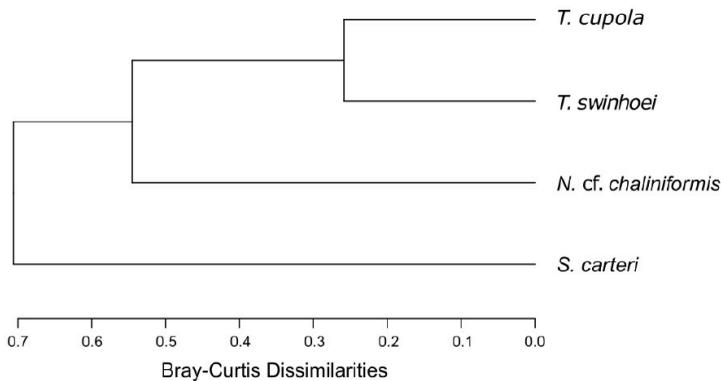


Figure 3: Cluster analysis dendrogram based on symbiont composition variation of each sponge host. Scale height indicates the dissimilarity among symbiont composition in hosts

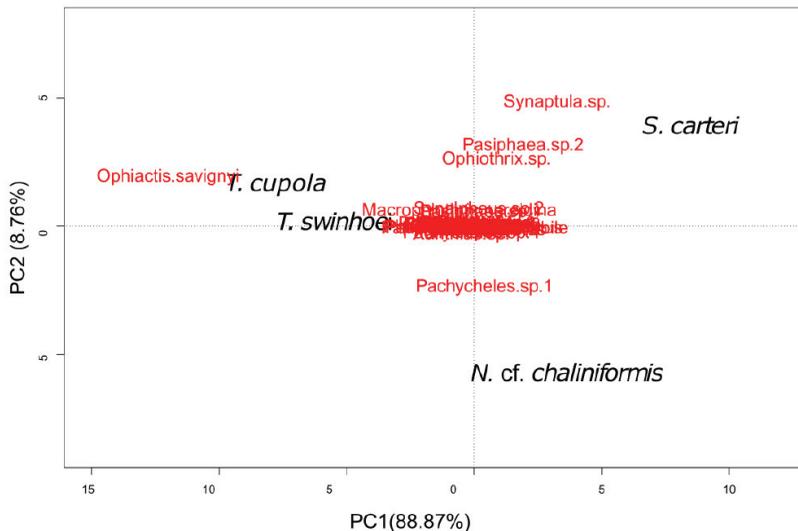


Figure 4: Regularised Discriminant Analysis (PC1 & 2 = 97.63%) showing the contribution of symbiont species components driving the composition found in each host. The plot axes represent the eigenvalues and the distance of each species from the distance from the point of origin of each species is the eigenvector length

Table 2: Count of sponge-associated fauna (SAF) grouped in Phylum and species for each host: *Theonella swinhoei*, *Theonella cupola*, *Neopetrosia cf. chaliniformis*, and *Stylissa carteri*

Phylum	Species	<i>Theonella swinhoei</i>	<i>Theonella cupola</i>	<i>Neopetrosia cf. chaliniformis</i>	<i>Stylissa carteri</i>
Echinodermata	<i>Macrophiothrix nereidina</i>	3	20	4	5
	<i>Ophiactis savignyi</i>	183	201	61	23
	<i>Ophiothrix</i> sp.	12	7	0	30
	<i>Synaptula</i> sp.	0	0	0	62
Arthropoda	<i>Pyromaia</i> sp.	0	1	0	0
	<i>Polyonyx</i> sp.	0	1	0	0
	<i>Pachycheles</i> sp. 1	20	7	32	12
	<i>Pachycheles</i> sp. 2	7	1	0	0
	<i>Pachycheles</i> sp. 3	2	0	0	0
	<i>Pachycheles</i> sp. 4	4	1	3	0
	<i>Petrolisthes galathinus</i>	1	0	1	0
	<i>Xanthias</i> sp.	17	4	6	2
	Xanthidae (family)	0	1	0	0
	Unidentified	1	0	0	0
	Sphaeromatidae (family)	0	0	3	1
	<i>Synalpheus</i> sp. 1	3	1	0	0
	<i>Synalpheus</i> sp. 2	8	1	1	9
	<i>Alpheus</i> sp.	19	1	2	4
	<i>Betaeus lilianae</i>	0	1	0	0
	<i>Philarius venustus</i>	1	0	1	0
	<i>Pandalus</i> sp.	1	1	0	0
	<i>Pasiphaea</i> sp. 1	0	3	1	7
	<i>Pasiphaea</i> sp. 2	0	3	0	39
	<i>Balanus</i> sp.	0	0	1	0
Chordata	Ascidiacea (class)	1	0	0	1
Mollusca	<i>Cypraea</i> sp.	1	0	0	0
	<i>Melongena</i> sp.	0	1	0	0
	<i>Anadara</i> sp.	0	1	0	0
	Arcidae (family)	1	0	0	0
	<i>Tellimya</i> sp.	1	0	0	0
	<i>Crenella</i> sp.	0	0	0	1
	Ostreidae 1 (family)	1	0	0	0
	Ostreidae 2 (family)	0	0	1	0
	<i>Palliolium incomparabile</i>	0	0	1	0
	<i>Pteria</i> sp.	0	0	0	1
Unidentified 1	1	0	0	0	

	Unidentified 2	0	0	1	0
	Unidentified 3	0	1	0	0
	<i>Neopilina</i> sp.	5	3	0	0
Sipuncula	<i>Phascolosoma</i> sp.	20	4	3	1
Annelida	<i>Capitella capitata</i>	1	0	0	0
	<i>Lysidice</i> sp.	0	1	0	0
	<i>Marphysa</i> sp.	0	1	0	0
	Nereididae (family)	1	0	0	0
	Orbiibidae (family)	0	1	0	0
	<i>Eteone</i> sp.	1	0	0	0
	Phyllodocidae (family)	0	0	0	1
	<i>Phyllodocida</i> sp.	0	0	1	0
	<i>Synelmis</i> sp.	1	0	0	0
	<i>Harmothoe</i> sp.	2	3	0	0
	Sigalionidae (family)	0	1	0	0
	Spionidae (family)	0	0	0	1
	Enchytraeidae (family)	0	0	0	1
	Naididae (family)	0	0	1	0
Unidentified	Unidentified	0	0	1	1
	<b>TOTAL</b>	<b>319</b>	<b>272</b>	<b>125</b>	<b>202</b>

Table 3: Details of the number of individuals and species, and diversity indices (Shannon Wiener Diversity Index, Simpson Diversity Index, Fisher's alpha, and Peilou's Evenness) for the four host species

	<i>T. swinhoei</i>	<i>T. cupola</i>	<i>N. cf. chaliniformis</i>	<i>S. carteri</i>
Total individual specimen	<b>319</b>	272	<u>125</u>	202
Number of species	<b>28</b>	27	<u>19</u>	<u>19</u>
Shannon Wiener Diversity Index	1.814	<u>1.277</u>	1.714	<b>2.085</b>
Simpson Diversity Index	0.653	<u>0.446</u>	0.69	<b>0.825</b>
Fisher alpha	7.392	<b>7.449</b>	6.236	<u>5.14</u>
Pielou Evenness	0.545	<u>0.388</u>	0.582	<b>0.708</b>

Bold values represent the highest values, while underline values represent the lowest among the hosts.

main driver (26.84%) of dissimilarity between *N. cf. chaliniformis* and *S. carteri*. *Synaptula* sp. also contributed to the dissimilarity between *S. carteri* with *T. cupola* (16.85%) and *T. swinhoei* (15.96%). Other major contributors to the dissimilarity of the composition of symbionts among sponges are *Pasiphae* sp., *Pachycheles* sp., and *Alpheus* sp.

## Discussion

This is the first study of SAF hosted by sponges in the coral reefs of Malaysia, as most previous SAF studies are concentrated in the Mediterranean Sea and Australia (Evcen & Çinar, 2001; Longo *et al.*, 2005; Fremont *et al.*, 2012; 2016; Przeslawski, 2015; Łukowiak, 2016; Topaloğlu *et al.*, 2016; Santín *et al.*, 2021).

The results showed that marine sponges host a wide variety of SAF, particularly echinoderms (brittle star and sea cucumber), molluscs, crabs, barnacles, crustaceans, and polychaetes (Bierbaum, 1981; Kelly-Borges & Richmond, 1997; Çinar *et al.*, 2002; Sivadas *et al.*, 2014). In total, 919 individuals of SAF were counted, *T. swinhoei* recorded the most SAF (34% of total) and *N. cf. chaliniformis* (13%) recorded the lowest number of individuals. Approximately 230 individuals of SAF were hosted by each sponge, which was less than what was recorded in the Aegean Sea (average 265 individuals) of a similar study (Çinar *et al.*, 2002).

### **Structure of the Sponge-associated Fauna Community**

In total, we found 55 taxa of SAF housed in the four host sponges. Individually, each host recorded 19 to 28 taxa, which was in line with adjacent studies in the Indian Ocean (Sivadas *et al.*, 2014). Due to the dominance of brittle star (*Ophiactis savignyi*) for SAF in *T. swinhoei*, *T. cupola*, *N. cf. chaliniformis*, the SAF diversity was also impacted. The dominance of a single species such as the brittle star *Ophiactis savignyi* can significantly impact other SAF species within the same ecological niche. When a particular species becomes overwhelmingly abundant, it can lead to various ecological effects that influence the dynamics of the entire SAF community such as resource competition, habitat modification, predation pressure, and altered community composition. This can be seen in the *T. cupola*, with almost 74% of the SAF found represented by the brittle star, which was nearly 20 times more than the next common SAF hosted (*Macrophiothrix nereidina*,  $n = 20$ ). This resulted in the diversity indices (Shannon-Wiener and Simpsons) and evenness (Pielou) being the lowest among all. Hence, low diversity for the SAF hosted by *T. cupola*, although there was a higher number of SAF individuals recorded. On the contrary, *S. carteri* was the inverse of *T. cupola*, with a relatively evenly distributed SAF species composition, even though with a lower species number. This

is because the most dominant SAF in *Synaptula* sp. was only 59% more abundant than the next most abundant SAF (*Pasiphaea* sp.,  $n = 39$ ).

*Ophiactis savignyi* and *Synaptula* sp. are the most common echinoderms for this study, which represents more than half of the total SAF counted (66.56%). Furthermore, *T. cylindrica* again showed that it has the lowest diversity/ evenness with 83.82% recorded of the SAF found to be Echinodermata. As most brittle stars and Synaptid sea cucumbers are detritus feeders, the base of the sponges become their site for finding foods. Hence, it is probably the main reason for the dominance of these groups of organisms (Westinga & Hoetjes, 1981). The higher dominance of these organisms is due to the conducive environment of which host sponges provide for breeding and nursery ground, hiding from predation and food sources (Westinga & Hoetjes, 1981; Mladenov & Emson, 1988). However, there are also documented instances of a parasitic association between SAF and its host (Henkel & Pawlik, 2014), in which the observed brittle star, *Ophiothrix lineata*, preys on *Callyspongia vaginalis* larvae. These observations have been made in both controlled laboratory settings and under natural conditions.

The fragile star *Ophiactis savignyi* emerges as the predominant SAF in investigations beyond the confines of the South China Sea, as evidenced by previous studies (Çinar *et al.*, 2002; 2019; Sivadas *et al.*, 2014; Sadhukhan *et al.*, 2020). This species exhibits a widespread presence in tropical and subtropical shallow waters, a fact well-documented (McGovern, 2002; Sadhukhan *et al.*, 2020). Sivadas *et al.* (2014) intriguingly observed that *Ophiactis savignyi* maintains a year-round association with the host sponge *Ircinia fusca*, utilising the sponge surfaces as a breeding habitat. Conversely, an earlier investigation (Çinar *et al.*, 2002) portrayed this species as an assertive species, responsible for dominating SAF abundance within host sponges. Interestingly, subsequent work (Çinar, 2019) identified *Ophiactis savignyi* as an alien species in the Mediterranean Sea, a revelation that has implications for the diversity and uniformity

of SAF. This study unearths a correlational aspect in our observations. However, within the context of this study and its current stage, we remain unable to definitively ascertain the true nature of this brittle star species, particularly its potential predation on sponge larvae or other co-inhabitants within the SAF community.

*Synaptula* sp. is the dominant SAF in the sponge host *Stylissa carteri*. The echinoderm has been well documented as a common SAF of sponges (Woo *et al.*, 2010; Cleary *et al.*, 2019), occasionally covering the host sponges. It is hypothesised that dissolved organic matter (DOM) found in host sponges could be utilised by many holothuroids of the genus *Synaptula*. These feedings may include: (1) Grazing on accumulated adhesive detritus material on the surface of host sponges and/or (2) DOM leaked from the host sponges. The former is more plausible, as holothuroidea SAF are usually found outside of the sponge where detritus accumulates; and absent from large spongocoel (inner pockets of sponges) where detritus is rather lacking (Hammond & Wilkinson, 1985; Mucharin *et al.*, 2019).

The phylum Crustacea (Arthropoda) emerged as the second most prevalent group of SAF, comprising 25.71% of all SAF individuals tallied, representing a minimum of 20 distinct species. In other studies, Çınar *et al.* (2002) reported eight decapod species present in *Sarcotragus muscarum*, while Koukouras *et al.* (1985) reported 27 decapod species in seven different sponges. Crustaceans favour the internal canal of sponges (Wulff, 2006) due to the role of sponges as a direct or indirect source of nutrition (Becerro *et al.*, 1997). Such an association is likely to have established a commensal relationship, as the associated organisms do not harm the host species, as reported in Duffy (1992) that studied the relationship between *Synalpheus* sp. and its host. In our study, members of Alpheidae (represented by *Synalpheus* and *Alpheus*) have a high number of male and female individuals and species numbers present, thus, in agreement with Reed *et al.* (1982) and Duffy (1992) on

possible commensalism between its host and the inquiline.

This study reports an unusual low number of polychaetes found, in contrast to other studies (Ribeiro *et al.*, 2003; Gerovasileiou *et al.*, 2016) that showed that polychaetes dominate the sampled sponges. The dominance of other organisms (i.e., Echinodermata and Crustacea) resulted in minimal available space and acted as competitors to polychaetes. It is also suggested that since most brittle stars consume small organisms such as polychaetes and crustaceans (Çınar *et al.*, 2002), they could limit the competitiveness of other SAF. The dominance of *Ophiactis savignyi* may explain the low number of polychaetes. Furthermore, Beepat (2014) claimed that sponges containing *Balanus* sp. (Crustacea) will have a low number of polychaetes. In this present study, despite the presence of a *Balanus* sp. sample in *N. cf. chaliniformis*, all the sponges in this research had a low number of polychaetes associated with them. Thus, no conclusive remarks can be made with his hypothetical approach. The barnacles located at the external pores of the sponge canal, however, may block the internal access of polychaetes to the hosts, but this claim needs to be validated.

#### ***Host-SAF Ecological Niche-alien Species Indicator (possible)***

The parameters (depth 19 m and temperature 24°C) of the obtained samples were identical in each sample; they were collected from the same sampling area at the same time. However, there were a few factors that might affect the composition of the SAF such as sponge species, morphological characteristics, and habitat/growing niche/substrate. The two samples from the Theonellidae family showed similar symbiont composition based on the cluster analysis dendrogram (Figure 3). This might be due to the similar growth characteristic and growing niche/substrate, where both samples were collected from rocky substrate. *Neopetrosia cf. chaliniformis*, which shared higher relative similarities to *Theonella* spp. was

attached to dead corals within coral reef areas during collection, while *S. carteri* was collected at the fringe of the coral reefs, closer to the sandy bottom. A study by Çinar *et al.* (2002) indicates that the diversity of SAF composition from a sponge species *Sarcotragus muscarum*, was greatly influenced by the nature of the substratum around the sponge. Therefore, the environment/substrate/habitat in which the host sponges were found could be an indication of the diversity of SAF (Çinar *et al.*, 2002; 2019; Beepat *et al.*, 2014). Conducted by Abdo (2007), a study delved into the composition and diversity of SAF in two distinct species of *Haliclona*, identified as *Haliclona* sp. 1 (green in colour) and *Haliclona* sp. 2 (brown in colour). The findings indicated that the green *Haliclona* harboured a greater overall biomass of endofauna compared with its brown counterpart. Additionally, the study revealed a correlation between the abundance and biomass of the associated endo fauna and the variances in the morphologies, sizes, and internal structures of sponge species. Significant distinctions were observed in the endofaunal assemblages of both sponge species, although it is worth noting that only the endofaunal assemblage within the green *Haliclona* exhibited significant differences across various locations.

This study indicated that the morphology of the host sponges may influence the diversity and number of SAFs hosted. Chin *et al.* (2020) concluded that sponges are large in size, with more internal space supporting a higher abundance of SAF. These two studies (Çinar *et al.*, 2002; Chin *et al.*, 2020) agree with the findings from our Theonellidae family, which was collected from the same sampling location, habitat type, and with almost similar morphological characteristics. This also agrees with the findings of *N. cf. chaliniformis* exhibiting the lowest number of SAF. Despite the wet weight of all the collected samples being similar, *N. cf. chaliniformis* is an encrusted species with thin appendages, thus, offering less internal space for SAF to inhabit. This explains the number of SAF found at each host, whereby Theonellidae sponges recorded high SAF, while

*N. cf. Chaliniformis* recorded low individual abundance.

In general, studies have shown that larger internal space hosts more variety of SAFs (Kaikuras, 1995; Çinar, 2019; Chin *et al.*, 2020), while a few (Beepat, 2014) underplay this notion by stating as loosely related. The discrepancy between SAF abundance and composition with gross morphological complexity occurs and was discussed elsewhere (Chin, 2020). This indicated that the gross morphological complexity alone may be inadequate as predictors/indicators of SAF abundance and composition due to the complexity of the phylum Porifera itself, with more than 9,000 described species with diverse gross morphologies and complex metabolic pathways that could either promote or inhibit colonisation by specific SAF. Furthermore, the metabolites produced by some sponges were derived from their symbiotic microorganisms, which were influenced by geography (Ivanisevic *et al.*, 2011; Bayona *et al.*, 2020).

Contrary, Porifera is well known for its morphological plasticity, which is not related to phylogenetic distance among comparisons. Where some closely related species (or in some cases the same species) by phylogenetic analysis might be totally different in gross morphology (Hill & Hill, 2001; Erwin & Thacker, 2007). Therefore, the phylogenetic relationship cannot be used to predict the abundance and composition of SAF in sponges.

## Conclusions

Examination of the SAF found in four reef-associated host sponges in Malaysia showed that diversity is driven by substrate/habitat, followed by host morphology. The phylogeny of hosts does not play an important role in driving the composition of the SAF. This agrees with previous studies in other parts of the world (Kaikuras, 1995; Çinar *et al.*, 2002; 2019; Chin *et al.*, 2020), with substrate/habitat and morphology preceding the species of the hosts.

Echinoderms (especially *Ophiactis savignyi* and *Synaptula* sp.) dominated the SAF of all

host sponges, followed by crustaceans. These findings were well-founded compared to other studies from other regions of the world. However, it should be noted that SAF such as *O. savignyi* is known to be aggressive and opportunistic organisms, which will dominate the composition of SAF by predation of other SAF. In contrast, *Synaptula* sp. and Crustacea representatives were mostly ecologically mutualistic with the hosts. Ironically, there was a relatively low number of polychaetes (Annelida: Polychaeta) in this study, contradicting most previous findings. This might be the result of overdominance of predatory organisms of polychaetes, such as *O. savignyi* and other crustaceans.

This provided an important initial insight into the ecology of SAF in different reef-associated sponges in the South China Sea. However, it should be noted that the research was carried out only in a very small number of hosts ( $n = 4$ ) found in one locality, without replication. However, the analyses were detailed enough to show that there was a relationship of environments/habitats/substrates to the diversity of the SAF, and loosely coupled with morphology of the host. The results of the study hope to incite more studies on the environmental driver related to SAF diversity in reef-associated sponges. Mostly on:

- (1) The diversity of SAF of different host sponges found in the same reef substratum/habitat will determine the host-driven diversity.
- (2) The diversity of SAF of a single species of ubiquitous host sponge can be found in different fringing reef habitats. This will be a good experimental design to determine the indicator and niche species in different environments.

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### Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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