Fatty Acid Profiles of Separated Host-Symbiont Fractions From Five Symbiotic Corals: Applications of Chemotaxonomic and Trophic Biomarkers

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Abstract

Fatty acids (FAs) are the main components of lipids in corals. We examined FA profiles from five symbiotic coral species belonging to five different genera (*Acropora*, *Pavona*, *Turbinaria*, *Favites* and *Platygyra*) and four different families (Acroporidae, Agariciidae, Dendrophyllidae, Faviidae). We separated symbionts from coral host tissue to investigate the interaction of FA between symbionts and host tissue. After separation, we used FA profiles, in particular specific FAs (*e.g.* 16:0, 18:0, 18:3n-3, 20:5n-3, 22:6n-3) and their ratios (EPA:DHA, PUFA:SFA) as biomarkers to examine chemotaxonomic indication and trophic level (autotrophy *vs.* heterotrophy) of each coral species. Gas chromatography-mass spectrometry (GC-MS) was performed to identify and quantify FA. For quantification, the dry weight of total lipids was used to normalize FA concentration (μg mg^{-1}). We found that 1) FA profiles and their abundance in coral host tissue and symbionts are distinct (with the exception of autotrophic species) 2) our five different coral species are defined by species-specific FA profiles, 3) certain FAs are useful biomarkers to determine relative trophic strategies (*i.e.* autotrophy and/or heterotrophy, 4) the application of FA ratios to define trophic level requires caution in research application and data interpretation. Considering the limitations of FA ratios determined herein, we suggest it to be more appropriate if these FA ratios were only applied to examine response to environmental change within species. Going forward, our study provides important FA baseline data that builds the foundation to future investigations on impacts of environmental changes on nutrition and metabolism in symbiotic corals.

Introduction

Lipids are essential macromolecules in marine organisms and source of energy (Bergé and Barnathan 2005; Lee et al. 2006; Parrish 2013). In comparison to other macromolecules, protein and carbohydrates (ca. 17 kJ g^{-1} and 18 kJ g^{-1}, respectively), lipids have the highest energy content (ca. 39 kJ g^{-1}). Additionally, lipids are important components to maintain cell membranes, and they are related to antioxidants (tocopherol, pigments), hormone regulation (*e.g.* ecdysone), anti-inflammatory regulation and immune system response, as well as to achieve buoyancy (neutral lipids) (Ackman 1999; Tocher 2003; Lee et al. 2006; Parrish 2013). Thus, lipids play a crucial role in the physiology of marine organisms (*e.g.* respiration, cell renewal and reproduction) and therefore reflect the biochemical and ecological conditions of the marine environment (Arai et al. 1993; Ward 1995; Tarrant 2005; Wang et al. 2013). In particular, fatty acids (FAs) are the main components of lipids in most marine organism, and strongly correlated with environmental conditions (Budge et al. 2006). They are transferred throughout the food web without experiencing changes in different trophic levels, which encourages their use as effective biomarkers in ecological studies (Parrish et al. 2000; Alfaro et al. 2006). Indeed, many studies have applied FAs as biomarkers in respect to various marine organisms such as bacteria, diatoms, dinoflagellates, plankton, macroalgae, invertebrates, fishes, and marine mammals (Rajendran et al. 1993; Parrish et al. 2000; Falk-Petersen et al. 2002; Howell et al. 2003; Bergé and Barnathan 2005; Lee et al. 2006; Kelly et al. 2008; Shin et al. 2008; Ju et al. 2011; Sardenne et al. 2017).

Lipids are the main biochemical compounds in corals as well, where they are used for energy storage that take up 10–50 % of the dried coral tissue (Joseph 1979; Harland et al. 1992; Harland et al. 1993; Al-Lihaibi et al. 1998; Yamashiro et al. 1999; Oku et al. 2003b; Yamashiro et al. 2005; Seemann et al. 2013). FA profiles of different coral species have been used as chemotaxonomic indicator, since FA are often species-specific (Latyshev et al. 1991; Imbs et al. 2007; Imbs et al. 2010b; Imbs et al. 2016; Lopes et al. 2016). For example, octocorals can be identified by their synthesis of 24:5n-3 and 24:6n-6, as these FA are absent in hexacorals (Svetashev and Vysotskii 1998; Imbs et al. 2010a). Symbiotic corals can be identified by high level of 18:3n-6 (GLA) and 18:4n-3 (SDA), as these FAs are present in symbiotic algae, while they are very low in non-symbiotic corals (Papina et al. 2003; Imbs et al. 2007; Imbs et al. 2010b; Imbs 2013; Lopes et al. 2016). Imbs et al. (2007) classified five different families (Acroporidae, Faviidae, Fungidae, Pocilloporidae and Poritidae) of the scleractinian corals based on their specific polyunsaturated fatty acids (PUFAs), as they found that several FAs can only be detected in certain taxonomic groups of corals.

Most scleractinian corals have symbiotic relationships with dinoflagellates, namely the family Symbiodiniaceae (hereafter 'symbionts'), which are photosynthetic microalgae conducting carbon dioxide fixation (Wooldridge 2014). Their photosynthesis contributes significantly to the supply of biomolecules used to feed themselves and the nutrients are also translocated to the coral host for growth, reproduction, and metabolism (Muscatine et al. 1981). The main photosynthates transferred from symbionts to the coral host tissue include lipids, glycerol, glucose and amino acids (Kellogg and Patton 1983; Gates et al. 1995; Papina et al. 2003; Whitehead and Douglas 2003; Reynaud et al. 2009), where lipids predominate (Patton and Burris 1983). Of the lipids, FA in particular, is one of the most important nutrient transferred across the plant-animal interface in the aquatic food web (Dalsgaard et al. 2003; Allan et al. 2010). It has been reported that photosynthetic organisms (*e.g.* plants, macro- microalgae, dinoflagellates etc.) produce omega-3 PUFAs (*e.g.* 18:3n-3, 18:4n-3, 20:5n-3, 22:6n-3) and omega-6 PUFAs (*e.g.* 18:2n-6, 18:3n-6), while animals rarely do so (Papina et al. 2003; Bachok et al. 2006; Revel et al. 2016). Thus, the PUFAs synthesized by symbionts can act as biomarkers to understand the coral-symbiont relationship among coral species.

On the other hand, certain essential FAs cannot be synthesized in higher trophic positions, although they are incorporated into heterotrophic species in the marine ecosystems (Arai et al. 2015). Moreover, FAs mirror nutritional input, making it possible to use them as biomarkers to trace diet and quantify feeding relationships (Dalsgaard et al. 2003; Bay et al. 2013; Mies et al. 2018). For example, 20:5n-3 is abundant in diatoms and 22:6n-3 is abundant in dinoflagellates. Both are highly conserved in the marine food web, which allows food source tracing (Viso and Marty 1993; Scott et al. 2002; Dalsgaard et al. 2003). The ratio of 20:5n-3 to 22:6n-3 (EPA:DHA) has been used to define trophic level (autotrophy or heterotrophy) and feeding type (herbivore, omnivore, carnivore) in zooplankton (Graeve et al. 1994; Dalsgaard et al. 2003). Additionally, carnivorous zooplankton tend to have higher PUFAs than herbivorous species, hence the PUFA:SFA ratio offers an index of carnivory (Cripps and Atkinson 2000; Dalsgaard et al. 2003; Stevens et al. 2004). A few coral studies successfully applied these FA indices to define trophic level in coral reef

ecosystems (Tolosa et al. 2011; Seemann et al. 2013; Salvo et al. 2017; Radice et al. 2019; Rocker et al. 2019).

Although FA profiles have shown to present excellent opportunities for chemotaxonomic and trophic biomarkers in symbiotic corals, applying FA profiles to corals and symbionts separately, as opposed to a two-organism unity, remains understudied. Specifically, symbiotic corals are able to obtain essential FAs either via autotrophy which are being translocated from symbionts, or via heterotrophy through external food sources, *e.g.* phytoplankton, zooplankton, particulate organic matter (Sebens et al. 1996; Ferrier-Pagès and Gattuso 1998; Anthony and Fabricius 2000; Yahel et al. 2004; Rocker et al. 2019). Furthermore, corals have a high trophic plasticity, switching between autotrophic and heterotrophic feeding strategies to cope with environmental changes (Goreau et al. 1971; Porter 1976; Muscatine et al. 1989; Anthony and Fabricius 2000; Ferrier-Pagès et al. 2003; Fabricius 2005). Therefore, their symbiotic relationship and trophic plasticity provide challenges to the application of FA biomarkers.

In this study, we characterized FA profiles from five symbiotic coral species belonging to five different genera (*Acropora*, *Pavona*, *Turbinaria*, *Favites* and *Platygyra*) and four different families (Acroporidae, Agariciidae, Dendrophyllidae, Faviidae). To do so, we firstly separated symbionts from coral host tissue, and then investigated the interaction of FA between symbionts and host tissue. After their separation, we used these FA profiles, in particular specific FAs and their ratios (EPA:DHA, PUFA:SFA) as biomarkers to examine chemotaxonomic indication and trophic level (autotrophy *vs.* heterotrophy) of each coral species. We applied only coral host tissue samples to exclude disturbance from symbionts. We tested the following hypotheses: 1) FA profiles of coral host tissue are distinctly different from those of their associated symbionts as FA profiles are organism-specific; 2) Symbiotic corals can be classified by FA profiles, as FA profiles are species-specific.; 3) Symbiotic corals have a specific trophic level which can be defined by certain FA biomarkers.

Materials And Methods

Coral sampling

In May 2016, five different symbiotic coral species (*Acropora samoensis* of the family Acroporidae, *Pavona decussata* of the family Agariciidae, *Turbinaria peltata* of the family Dendrophyllidae, *Favites abdita* and *Platygyra carnosa* of the family Faviidae) were collected from four mesocosm coral tanks at the Swire Institute of Marine Science (SWIMS) located at the marine reserve area of Cape d'Aguilar in Hong Kong SAR. Samples were collected in May 2016. Hong Kong has alternating wet/dry seasons; the wet season is from March to October, characterized by higher temperatures, and the dry season is from November to March. We collected our samples during the transition time between dry and wet season, with an average water temperature of 26.0 ℃. Branching and foliaceous corals (*A. samoensis, P. decussata*) were collected by scissors, and plate corals (*T. peltata*) or boulder corals (*F. abdita, P. carnosa*) were collected by hammer and chisel. Each nubbin was taken from different colonies (n = 4) in each tank. The collected samples were immediately rinsed with deionized water and kept frozen at -70 ℃ until

further analyses. Open circulation flow-through tanks at SWIMS were used and seawater was supplied directly from the bay after a primary step of physical filtering (sand filter). The seawater included natural food sources such as plankton, particulate organic matter, hence additional feeding was not necessary. Seawater was kept running consistently through the tanks (≈ 5 l min^{-1}).

Coral-Symbiont separation

For lipid analysis, the coral fragments were rinsed with buffer (5 mM EDTA), 20 µl ml^{-1} butylated hydroxytoluene in distilled water to prevent FA oxidation and development of artefacts. Coral tissue was extracted from the skeleton using an airbrush with the same buffer solution and collected in 50 ml tubes through a funnel. Slurry tissue, which included coral host tissue and symbionts was homogenized on ice with a tissue grinder for 30 s. The homogenate was centrifuged for 5 min at 4 °C at initially 300 rcf, increasing up to 800 rcf depending on tissue thickness, amount of mucus, and species (Table S1). The centrifugation separates symbionts (pellets) and coral tissue (supernatant). The supernatant is then transferred to another 50 ml tube and centrifuged in deionized water three times for 5 min at 4 °C with different velocity of centrifugation depending on the species, to eliminate any remaining symbionts. A drop of supernatant was observed under a microscope to verify no symbiotic cells remained in the coral host tissue sample. The pellet was resuspended in 20–30 ml of DI water and centrifuged at 10 rcf for 30 s to remove any carbonate residue. The supernatant was immediately transferred to another tube and then centrifuged three times for 5 min at 4 °C at varying velocity of centrifugation depending on species (Table S1). After the 3rd centrifugation, the pellet was recollected. Both coral host tissue and symbiont samples were kept in the freezer at -80 °C. Prior to chemical analyses, the samples were freeze-dried and kept as powder.

Extraction lipid and fatty acids

Total lipid (TL) was extracted from dried samples of the coral host tissue and symbionts separately using Folch solution (2:1; methanol: chloroform). TL samples were spiked with internal standard (19:0; nonadecanoic acid, 1 µg l^{-1} in dichloromethane). Alkaline hydrolysis was performed at 70 °C in an oven for 60 min using aqueous 1 M potassium hydroxide (KOH) to release the esterified FA. The total FAs were then collected by liquid-liquid extraction using hexane:diethyl ether (9:1 v/v), and the solvent was evaporated immediately under nitrogen gas and derivatized with boron trifluoride (BF_3) at 70 °C for 30 min to form fatty acid methyl esters (FAMEs). FAMEs were extracted three times with hexane:diethyl ether (9:1 v/v) and then pooled. The extracted FAMEs were dried under nitrogen gas and re-suspended in dichloromethane for gas chromatography mass spectrometry (GC-MS) analysis set at electron ionization (EI) mode. A set of external standards (37 Component FAME mix, Supleco, USA) was used to identify and quantify each FAME. GC-MS (Agilent 5977A mass selective detector interfaced with an Agilent 7890B gas chromatograph) was performed using an SP-2560 capillary column (100 m x 0.25 mm, 0.2 µm film thickness, Sigma-Aldrich, USA) to detect the FA. Helium was used as the carrier gas with a flow rate of 1 ml min^{-1}. A volume of 1 µl of the derivatized sample was injected into the GC with a split ratio of 1:100. The oven temperature was programmed to ramp from 100°C to 140°C at 10°C min^{-1} and from 100°C to

240°C at 4°C min^{-1}. Each FAME was identified by using the target and qualifier ion listed in Table S3. FAMEs were quantified by relating the peak area of the individual FAME with the peak of the internal standard. For quantification, the dry weight of TL was used to normalize FAME concentration (μg mg^{-1}).

Statistics

ANOVA test was performed using the software SPSS (ver. 19.0, IBM, NY, USA). Statistical differences among the five species were determined for TL and FA ratios by one-way analysis of variance (ANOVA) and Tukey's post hoc test for pairwise comparison. Values of at least $p < 0.05$ were considered significant in the analysis. Further, we performed multivariate analyses on square root transformed value of the 19 FA profiles using the software PRIMER version 5. Hierarchical cluster analysis was conducted to assess the degree of similarity between symbionts and tissue of their coral hosts. FA profiles were compared among the five coral species by non-metric multidimensional scaling (nMDS) and analysis of similarity (ANOSIM) on the Bray-Curtis similarity index. The contribution of each fatty acid to these clusters was determined by similarity percent (SIMPER) analysis. A principal component analysis (PCA) was performed among coral species to highlight certain FA profiles.

Results

Total lipid and fatty acids of 5 different coral species

Overall, TL in symbionts showed to be much higher than in host tissue of the five coral species (average 30.7 ± 4.2 % in symbionts, 13.5 ± 3.0 % in coral host tissue) (Fig. 1). TL of symbionts in *P. carnosa* showed the highest value (36.1 ± 5.5 %) compared to four other species, but it was not significantly different among the species ($p > 0.05$). There was no significant difference in TL of coral host tissue between *A. samoensis* (8.9 ± 1.4 %) and *P. decussata* (12.4 ± 3.9 %). TL in host tissue of *A. samoensis* was significantly lower compared to *T. peltata* ($p < 0.05$), *F. abdita* ($p < 0.005$) and *P. carnosa* ($p < 0.005$). There were no significant differences between *P. decussata*, *T. peltata*, *F. abdita* and *P. carnosa*.

Table 1
Fatty acid profiles in symbionts and their host tissue from five different coral species.

FA	*Acropora samoensis*		*Pavona decussata*		*Turbinaria peltata*		*Favites abdita*		*Platygyra carnosa*	
	S	H	S	H	S	H	S	H	S	H
14:0	7.6 ± 1.3	3.6 ± 1.3	7.4 ± 2.6	2.9 ± 0.4	1.5 ± 0.6	0.6 ± 0.1	4.0 ± 0.5	1.0 ± 0.2	2.4 ± 1.0	1.2 ± 0.8
16:0	50.1 ± 9.7	42.0 ± 14.5	63.4 ± 24.0	46.4 ± 2.9	35.4 ± 3.5	20.7 ± 3.1	49.9 ± 2.2	30.9 ± 0.4	23.8 ± 6.1	35.3 ± 4.7
18:0	12.9 ± 5.1	19.4 ± 4.4	15.1 ± 2.4	16.9 ± 1.4	16.1 ± 1.4	23.2 ± 4.3	19.0 ± 1.8	19.7 ± 2.5	8.8 ± 2.3	17.8 ± 2.8
20:0	0.3 ± 0.1	0.8 ± 0.2	0.6 ± 0.1	0.6 ± 0.1	0.2 ± 0.02	0.1 ± 0.02	0.4 ± 0.05	0.3 ± 0.05	0.1 ± 0.02	0.5 ± 0.1
22:0	0.4 ± 0.1	0.2 ± 0.01	0.2 ± 0.04	0.2 ± 0.03	ND	ND	0.1 ± 0.0	ND	0.1 ± 0.0	ND
Σ SFA	71.2 ± 15.6	66.1 ± 20.1	86.7 ± 28.2	67.0 ± 2.1	53.1 ± 4.9	44.6 ± 5.2	73.5 ± 4.2	51.9 ± 2.3	35.2 ± 8.8	54.7 ± 5.0
16:1	5.2 ± 3.0	3.1 ± 0.5	6.5 ± 2.2	3.4 ± 0.3	1.9 ± 0.2	0.3 ± 0.02	2.0 ± 0.2	1.1 ± 0.2	1.8 ± 0.5	2.0 ± 0.8
18:1n-9	5.3 ± 1.2	3.6 ± 0.3	5.5 ± 1.9	3.9 ± 0.4	5.0 ± 0.5	2.0 ± 0.3	6.7 ± 0.3	4.0 ± 0.7	1.4 ± 0.4	2.1 ± 0.4
20:1n-9	1.1 ± 1.3	3.9 ± 0.1	ND	0.2 ± 0.04	1.3 ± 0.1	ND	0.6 ± 0.3	0.5 ± 0.1	ND	0.3 ± 0.04
22:1n-9	0.3 ± 0.1	0.3 ± 0.03	0.2 ± 0.1	0.2 ± 0.05	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.03	0.3 ± 0.1	0.1 ± 0.02	0.1 ± 0.04
Σ MUFA	11.9 ± 4.0	11.0 ± 0.6	12.1 ± 4.1	7.7 ± 0.4	8.5 ± 0.7	2.5 ± 0.3	9.5 ± 0.4	6.0 ± 0.9	3.3 ± 0.9	4.5 ± 1.2
18:3n-3 (ALA)	5.3 ± 4.5	16.2 ± 3.7	0.3 ± 0.1	ND	5.8 ± 0.9	ND	2.8 ± 0.6	2.3 ± 0.4	ND	0.9 ± 0.4
18:4n-3 (SDA)	23.4 ± 7.3	1.7 ± 0.7	6.6 ± 2.7	0.6 ± 0.1	7.7 ± 1.3	0.8 ± 0.1	8.4 ± 1.3	0.3 ± 0.1	5.6 ± 3.5	0.7 ± 0.1
20:5n-3 (EPA)	30.9 ± 22.0	7.7 ± 4.2	6.7 ± 2.3	0.7 ± 0.1	17.7 ± 3.5	1.3 ± 0.3	11.1 ± 2.0	0.9 ± 0.3	4.2 ± 0.5	0.6 ± 0.2

Value given as mean ± SD, $n = 4$. FA concentration: μg mg^{-1}.

FA	*Acropora samoensis*		*Pavona decussata*		*Turbinaria peltata*		*Favites abdita*		*Platygyra carnosa*	
	S	H	S	H	S	H	S	H	S	H
22:5n-3 (DPA)	0.9 ± 0.4	1.3 ± 0.5	1.2 ± 0.8	1.2 ± 0.2	2.0 ± 0.2	0.4 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	5.7 ± 2.3	6.3 ± 1.4
22:6n-3 (DHA)	44.1 ± 16.7	9.0 ± 1.3	11.6 ± 2.8	2.4 ± 0.8	22.7 ± 3.7	1.5 ± 0.2	27.2 ± 3.8	2.2 ± 1.1	11.9 ± 2.5	2.1 ± 0.4
Σ n-3 PUFA	104.6 ± 42.5	35.8 ± 9.6	26.4 ± 7.4	4.9 ± 0.8	56.0 ± 9.4	4.0 ± 0.6	49.9 ± 6.9	5.9 ± 0.8	27.4 ± 1.7	10.6 ± 1.0
18:2n-6 (LA)	2.1 ± 0.7	1.1 ± 0.1	3.8 ± 1.7	2.1 ± 0.2	1.4 ± 0.3	0.5 ± 0.1	4.8 ± 0.3	1.8 ± 0.2	1.7 ± 0.4	1.9 ± 0.4
18:3n-6 (GLA)	30.5 ± 10.7	7.4 ± 1.4	14.4 ± 5.2	4.6 ± 0.8	9.7 ± 1.7	0.8 ± 0.1	44.6 ± 6.2	4.8 ± 1.6	19.0 ± 3.9	4.1 ± 1.1
20:3n-6 (DGLA)	0.8 ± 0.4	1.4 ± 0.2	0.7 ± 0.4	0.8 ± 0.1	1.3 ± 0.3	0.5 ± 0.1	1.5 ± 0.1	1.1 ± 0.1	0.2 ± 0.1	0.6 ± 0.1
20:4n-6 (ARA)	2.9 ± 0.6	9.7 ± 1.3	2.8 ± 1.3	2.7 ± 0.3	17.4 ± 3.0	10.7 ± 2.7	9.3 ± 1.1	11.8 ± 4.1	3.7 ± 1.5	18.9 ± 4.1
22:4n-6 (AdA)	1.1 ± 0.2	2.7 ± 0.5	1.2 ± 0.1	2.3 ± 0.5	4.3 ± 0.3	1.9 ± 0.7	2.1 ± 0.3	2.1 ± 0.7	0.7 ± 0.3	2.7 ± 0.3
Σ n-6 PUFA	37.4 ± 11.9	22.3 ± 2.7	23.0 ± 7.6	12.5 ± 1.0	34.0 ± 3.8	14.3 ± 3.4	62.3 ± 7.6	21.6 ± 6.5	25.2 ± 5.9	28.2 ± 5.7
Σ PUFA	142.0 ± 38.0	58.1 ± 11.0	49.4 ± 13.7	17.4 ± 1.4	90.0 ± 12.9	18.3 ± 3.2	112.2 ± 14.2	27.5 ± 7.0	52.7 ± 7.1	38.8 ± 5.7
EPA:DHA	-	0.8 ± 0.4^{a,b}	-	0.3 ± 0.1^{a,c}	-	0.9 ± 0.1^{b}	-	0.5 ± 0.4^{c,d}	-	0.3 ± 0.04^{a,b,d}
PUFA:SFA	-	0.9 ± 0.2^{a}	-	0.3 ± 0.03^{b}	-	0.4 ± 0.1^{c}	-	0.5 ± 0.1^{d}	-	0.7 ± 0.1^{e}

Value given as mean ± SD, $n = 4$. FA concentration: μg mg^{-1}.

Superscripts sharing different letters indicate significant differences (one-way ANOVA, Tukey's test, $p < 0.05$) among five coral species.

S: symbionts, H: coral host tissue, ND: not detectable.

Out of the 37 FAMEs, we evaluated 26 types of long chain FAs (> 16C) where a total of 19 were detected (Table 1). The most predominant FA in both symbionts and coral host tissue for all species was 16:0. The concentration of 16:0 in symbionts ranged from 23.8 ± 6.1 μg mg^{-1} (*P. carnosa*) to 63.4 ± 24.0 μg mg^{-1}

(*P. decussata*). In the coral host tissue, it ranged from 20.7 ± 3.1 μg mg^{-1} (*T. peltata*) to 46.4 ± 2.9 μg mg^{-1} (*P. decussata*) (Table 1). The highest concentration of 16:0 was found in both symbionts and host tissue of *P. decussata*. The second most dominant SFA in both symbionts and coral host tissue for all species was 18:0 (ranging from 8.8 ± 2.3 μg mg^{-1} to 19.0 ± 1.8 μg mg^{-1} in symbionts, and from 16.9 ± 1.4 μg mg^{-1} to 23.2 ± 4.3 μg mg^{-1} in coral host tissue). The 16:0 in symbionts showed to be higher than the host tissue except for those of *P. carnosa*, whereas 18:0 constantly showed to be higher in the host tissue than in symbionts. The major MUFA for all species was 18:1n-9, followed by 16:1. The concentration of 18:1n-9 ranged from 1.4 ± 0.4 μg mg^{-1} (*P. carnosa*) to 6.7 ± 0.3 μg mg^{-1} (*F. abdita*) in symbionts, and from 2.0 ± 0.3 μg mg^{-1} (*T. peltata*) to 4.0 ± 0.7 μg mg^{-1} (*F. abdita*) in the coral host tissue. The highest concentration of 18:1n-9 was found in both symbionts and the host tissue of *F. abdita*.

A. samoensis was characterized by the highest concentration of total PUFA in both symbionts and the host tissue, 142.0 ± 38.0 μg mg^{-1} and 58.1 ± 11.0 μg mg^{-1} respectively (Table 1). However, *P. decussata* was characterized by the lowest value in both symbionts and host tissue, 49.4 ± 13.7 μg mg^{-1} and 17.4 ± 1.4 μg mg^{-1} respectively. In general, the major PUFAs were 18:4n-3, 20:5n-3, 22:6n-3, 18:3n-6 and 20:4n-6. PUFAs namely 18:4n-3, 20:5n-3, 22:6n-3 and 18:3n-6 in symbionts for all species showed higher concentrations than the coral host tissue. The highest concentrations of PUFAs (18:4n-3, 20:5n-3, 22:6n-3, 18:3n-6) except for 20:4n-6 in the host tissue were observed in *A. samoensis* (1.7 ± 0.7 μg mg^{-1}, 7.7 ± 4.2 μg mg^{-1}, 9.0 ± 1.3 μg mg^{-1} and 7.4 ± 1.4 μg mg^{-1} respectively). However, the lowest value of 18:4n-3 was found in *F. abdita* (0.3 ± 0.1 μg mg^{-1}), for 20:5n-3 in *P. carnosa* (0.6 ± 0.2 μg mg^{-1}), and for 22:6n-3 and 18:3n-6 in *T. peltata* (1.5 ± 0.2 μg mg^{-1}, 0.8 ± 0.1 μg mg^{-1}, respectively). The concentration of 18:4n-3, 20:5n-3 and 22:6n-3 in symbionts of *A. samoensis* showed a much higher value (23.4 ± 7.3 μg mg^{-1}, 30.9 ± 22.0 μg mg^{-1} and 44.1 ± 16.7 μg mg^{-1} respectively) compared to the symbionts of other species (average 7.1 ± 2.2 μg mg^{-1}, 9.9 ± 2.1 μg mg^{-1} and 18.4 ± 3.2 μg mg^{-1}, respectively). In addition, 18:3n-3 in symbionts of *A. samoensis* showed to be the highest (16.2 ± 3.7 μg mg^{-1}) although values for *P. decussata* and *T. peltata* were not detectable. The concentration of 20:4n-6 in the host tissue showed higher levels than 20:5n-3 and 22:6n-3 among all species. In particular, 20:4n-6 in the host tissue of *T. peltata*, *F. abdita* and *P. ca*mosa had the highest concentration among all PUFAs (10.7 ± 2.7 μg mg^{-1}, 11.8 ± 4.1 μg mg^{-1} and 18.9 ± 4.1 μg mg^{-1} respectively), where *P. carnosa* had the highest level among all species.

We applied FA ratios namely 20:5n-3 to 22:6n-3 (EPA:DHA) and PUFA:SFA as biomarkers for coral feeding or determining trophic level in this study. The ratio is an indicator for trophic level (higher value indicates relatively autotrophic, lower value indicates relatively heterotrophic feeding strategies) (Rocker et al. 2019). The ratio of EPA:DHA was significantly higher in *T. peltata* and *A. samoensis* (0.9 ± 0.1, 0.8 ± 0.4, respectively) compared to the other three species (Table 1). PUFA:SFA has been suggested as indicator for carnivory (higher value indicates relatively carnivorous, lower value indicates relatively herbivorous). PUFA:SFA showed the highest value in *A. samoensis* (0.9 ± 0.2), followed by *P. carnosa* (0.7 ± 0.1), *F. abdita* (0.5 ± 0.1), *T. peltata* (0.4 ± 0.1) while *P. decussata* had the lowest value (0.3 ± 0.03).

Multivariate analysis of coral host and symbiont fatty acid profiles

Cluster analysis was conducted on 19 different FAs (as noted in Table 1) to evaluate whether FA profiles from symbionts and their coral host tissue are statistically separated. As shown, two groups were formed at the 75 % similarity level (Fig. 2). Group I is composed of all coral host tissues except for host tissue from *A. samoensis*, whereas Group II is composed of the symbionts from the five species and including the host tissue of *A. samoensis*.

The nMDS plot based on the 19 FA profiles clearly revealed a distinct grouping among the five different species. ANOSIM analysis also confirmed a clear separation among the groups ($R = 0.999$; $p < 0.05$) (Fig. 3). SIMPER analysis showed that each group had high similarity (> 90 %) within the group (Table S4). In all species groups, two main FAs namely 16:0 and 18:0, explained 25.1–39.8 % of the group similarity. In the *T. peltata*, *F. abdita* and *P. carnosa* group, 20:4n-6 explained 13.4 %, 10.8 % and 13.5 % respectively of the group similarity. While in the *A. samoensis* group, 18:3n-3 explained 9.5 % of the group similarity (Table S5). Dissimilarity between *A. samoensis* and *T. peltata* showed to be the highest (30.3 %), which was explained by the contribution of the following FA, in decreasing order of importance: 18:3n-3, 20:1n-9, 16:0, 18:3n-6, 22:6n-3 (Table S5). Moreover, the dissimilarity between *F. abdita* and *P. carnosa* (same family: Faviidae) was the lowest (12.6 %), which is explained by the contribution of the following FAs, in decreasing order of importance: 22:5n-3, 20:4n-6, 18:3n-3, 18:1n-9.

In PCA, PC1, PC2, and PC3 accounted for 48.5 %, 17.7 % and 13.2 % respectively (only PC1 and PC2 are shown in Fig. 4). PC1 separated *A. samoensis* and *P. decussata* (positive scores) from the other species (negative scores), explaining 48.5 % of the variability between the FA profiles of all species. The main FAs driving this distinction for *A. samoensis* are 18:3n-3, 20:5n-3, 22:6n-3, 20:3n-6 and 20:1n-9 (Fig. 4). *A. samoensis* had the highest PC1 scores, whereas *T. peltata* had the lowest. In contrast, PC2 separated *P. decussata* (negative scores) from the other species (positive scores), explaining 17.7 % of the variation in FA profiles. In particular, *P. decussata* was characterized by 18:2n-6, *F. abdita* and *P. carnosa* were characterized by 20:4n-6.

Discussion

Our results showed TL in holobionts (unity of symbionts and coral host tissue) ranged from 19 % to 26 % in average. Indeed, a similar trend was found in Okinawan corals (14–37 %), Caribbean corals (12–32 %) and Red Sea corals (12–32 %) (Harland et al. 1993; Yamashiro et al. 1999). TL in symbionts separated from coral holobionts were much higher (27–36 %) than in coral host tissue (9–16 %). Culture experiments on five different marine dinoflagellate taxa have shown that they store 6–16 % lipid, in particular *Symbiodinium microdiriaticum*, which is one of the symbiotic dinoflagellates associated with corals, contained 15 % TL (Mansour et al. 1999). Therefore, it is likely that TL contents in symbionts and in their coral host separately show distinct different values. However, there are no such studies comparing TL between symbionts and their coral host tissue. TL in corals are their main source of energy storage,

and symbionts can translocate lipids to their coral host to contribute to the coral's health (Harland et al. 1993; Papina et al. 2003; Treignier et al. 2009; Imbs et al. 2014). No significant species-specific differences in TL of symbionts from the five target species was found which indicates that symbionts are limited to contain lipids as energy storage regardless of coral host species. Furthermore, it is known that the same Symbiodiniaceae genus (C1) is hosted in all of our target coral species (Wong et al. 2016). Thus, we hypothesized that lipid production and capability of storage might not be different in symbionts from either coral species. However, TL in the host tissue of *F. abdita* and *P. carnosa* were significantly higher than in host tissue of other species, which can be explained by 1) these two species might be able to feed more than other species. In fact, fed corals showed to have higher lipid contents than unfed corals (Al-Moghrabi et al. 2008; Treignier et al. 2008); 2) these two species have a higher lipid translocation rate from symbiont to the host tissue compared to the other species. On the other hand, TL in *A. samoensis* has shown to be significantly lower than TL in *T. peltata*, *F. abdita*, *P. carnosa*, which reversely may indicate *A. samoensis* relies on relatively less feeding.

Hierarchical cluster analysis confirmed that FA profiles and their abundance in coral host tissue and symbionts are significantly different, with the exception of *A. samoensis* host tissue, which categorized in the group with symbionts. Indeed, FA profiles in *A. samoensis* showed subtle differences between the host tissue and symbionts. In addition, PUFAs in symbionts of *A. samoensis* showed much higher concentration compared to symbionts of other species, since PUFAs are the main FAs that are being transferred from symbionts to the coral host tissue (Ward 1995; Papina et al. 2003; Al-Moghrabi et al. 2008). As a result, we suggest that *A. samoensis* relies relatively on FA from symbionts rather than on external feeding. Indeed, the *Acropora* species has been found to be relatively autotrophic in studies assessing FA profiles and stable isotopic values (Seemann 2013; Seemann et al. 2013; Conti-Jerpe et al. 2020). Moreover, among the studies on photosynthesis in corals found that the genus *Acropora* exhibited a higher maximum quantum yield (F_v/F_m) than other genera, which is indicative of higher photosynthesis efficiency (McIlroy et al. 2019). PUFAs in corals are directly coupled with photosynthesis of *in hospite* symbionts of corals (Oku et al. 2003a). Thus, high concentration of PUFAs in *A. samoensis* host tissue provides further evidence that this species is autotrophic.

Although our target species are associated with the same genus of symbionts (Wong et al. 2016), higher concentration of major PUFAs in symbionts of *A. samoensis* when compared to the other species could potentially be explained by different physiology that depends on the species of coral host due to specific coral morphology (*i.e.* growth rate, tissue thickness, polyp size) and the absence of disturbances of light penetration (*i.e.* particulate matter or sedimentation) in our coral tanks, which is beneficial to autotrophic species. Theoretically, symbionts in *A. samoensis* perform the best biochemistry via photosynthesis, where surplus FA can be transferred to the host tissue. In fact, the concentration of each FA in symbionts and the host tissue alike was very similar to each other in this species. Therefore, we confirm that *A. samoensis* is the most autotrophic species among the five target species. However, the PUFA:SFA ratio, which is an indicator of carnivory, was the highest in *A. samoensis*, which contrasts our above interpretation. Although the highest PUFA was observed in *A. samoensis*, SFA did not vary within the five

species. This indicates that PUFA is the determinant for the high value of PUFA:SFA ratio in *A. samoensis* which in turn, is responsible for the contradicting indications on trophic level. Therefore, applying FA ratios to determine trophic level in corals has its limitations due to the mixture of possible food sources.

The diversity of FA profiles exhibited by our five target coral species is in accordance with previous coral FA studies (Imbs et al. 2007; Imbs et al. 2010b; Imbs et al. 2014). These studies confirmed that hard corals can be distinguished on family or subclass level on the basis of PUFA profiles. In this study, we took this even further and managed to clearly separate all our five corals on genus level by multivariate analyses using19 FA profiles including not only PUFAs but also SFAs and MUFAs. However, we should be careful when interpreting data since FA profiles in corals can be modulated by external nutritional input (Parrish et al. 2000; Dalsgaard et al. 2003; Alfaro et al. 2006), as well as by environmental factors, such as season, site, depth, and temperature (Imbs 2013; Seemann et al. 2013; Rocker et al. 2019). Furthermore, our coral samples were collected in flow mesocosm tanks, where sea water comes from the marine protected area of Hong Kong after being primarily filtered through a sand filtration system to remove suspended matter and sedimentation. This implies, coral colonies are exposed to more stable environmental conditions when compared to wild corals in the bay. Regarding season, we sampled in May that falls within the transition period between dry and wet season when it is climatically less extreme. In addition, the average water temperature was 25.7 ℃, which is close to the all-year-average water temperature (26.0 ℃ in 2016). In contrast, Imbs et al. (2007) collected their coral samples in the shallow waters of Vietnam in the South China Sea. Despite the same sampling region, season, depth etc. as our set-up, FAs in corals from natural habitats can be influenced by a variety of food sources (Imbs et al. 2010b). Therefore, we believe that the consistent and stable environmental condition in our coral tanks was the key to distinguish distinct groups among the five coral species.

The five distinct species groups are associated to 16:0 and 18:0 which are known to be transferred from glucose, symbionts and dietary sources (Imbs et al. 2011; Revel et al. 2016). Numerous studies on FA profiles of hard corals have also documented that these FAs are the most abundant FAs since their first report by Meyers (1977) (Latyshev et al. 1991; Harland et al. 1993; Yamashiro et al. 1999; Imbs et al. 2007; Imbs et al. 2010b; Imbs 2013). The 16:0 in host cnidarian tissue can be *de novo* synthesized from symbiont photosynthesis-driven glucose (Oku et al. 2003a; Revel et al. 2016), while a high 16:0 in symbionts is originated from their photosynthesis products. In *P. decussata*, 16:0 in both symbionts and their host tissue showed the highest concentration, suggesting 16:0 in this species relies on symbionts. However, 16:0 in host tissue can be originated from both symbionts and dietary sources, such as zooplankton which is known to have abundant 16:0 (Lee et al. 2006). An elevated concentration of 16:0 in the *P. carnosa* host tissue than in its symbionts, as oppose to the other four species, suggests that *P. carnosa* obtains additional 16:0 from external food sources. Upon obtaining 16:0, the biosynthesis pathway 16:0 → 18:0 → 18:1n-9 can be consistently metabolized in both symbionts and their coral host tissue (Treignier et al. 2009; Revel et al. 2016). However, the coral host cannot synthesize further from 18:1n-9 to 18:2n-6, which is the precursor of n-6 PUFA pathway, due to the lack of Δ^{12}-desaturase enzyme

(Dunn et al. 2012; Matthews et al. 2018). Therefore, we assume that the coral host stores energy in the form of 16:0 or 18:0 in lipids, but it is species-specific and depends on the coral's nutrition.

Although, *F. abdita* and *P. carnosa* belong to the same family (Faviidae), their FA profiles differed. The dissimilarity between FA profiles of *F. abdita* and *P. carnosa* was the lowest (12.6 %), which indicated that these two species have comparable FA profiles relative to any other combination of species. Both species were also characterized by the highest concentration of 20:4n-6, and further exhibited the highest concentration among all the PUFAs. Unfortunately, there are very few studies on 20:4n-6 in corals. Rocker et al. (2019) documented that symbiont density has a negative correlation with 20:4n-6 concentration, which implies that this FA in the coral host tissue might not be derived from symbionts, hence 20:4n-6 suggest to be a potential indicator of coral feeding. Accordingly, a few studies surmised that 20:4n-6 in corals might originate from external food sources *e.g.* phytoplankton and/or herbivorous zooplankton (Seemann et al. 2013; Imbs et al. 2016). Indeed, high 20:4n-6 content in phytoplankton has been documented (Jónasdóttir 2019) Subsequently, higher 20:4n-6 in *P. carnosa*, *F. abdita* and *T. peltata* than in other species show that these species are more heterotrophic. In addition, *P. decussata* is characterized by a negative relationship with 20:4n-6, which confirms the relative autotrophy of this species.

On the other hand, the most dissimilar species coupling with *P. carnosa* and/or *F. abdita* was *A. samoensis*. This species was determined by the predominance of 16:0 and 18:0, followed by 18:3n-3. Especially, 18:3n-3 in *A. samoensis* was the most abundant of all the PUFA, and it should be noted 18:3n-3 plays a key role in further synthesizing essential n-3 PUFA. However, this FA cannot be biochemically synthesized by coral hosts due to their lack of Δ^{15}-desaturase enzyme, indicating that 18:3n-3 originate from symbionts via photosynthesis and subsequently transferred to the coral host. Moreover, the high concentration of 18:3n-3 in the host tissue of *A. samoensis* indicates that this species is heavily dependent on autotrophy through its symbionts. The highest dissimilarity (30.3 %) of FA profiles among the five coral species was in between *A. samoensis* and *T. peltata*, and 18:3n-3 contributed to this separation the most. In contrast to the high concentration of 18:3n-3 in *A. samoensis*, no 18:3n-3 could effectively be detected in *T. peltata*. In previous studies, on a different species of the same genus, *T. reniformis*, no 18:3n-3 was observed in representatives under starvation conditions, but the concentration was high in corals fed with zooplankton (*Artemia salina* nauplii) (Tolosa et al. 2011). Although we did not quantify the extent of food availability in our coral tanks, a lack of zooplankton is possible. However, *T. peltata* showed a high concentration of 20:4n-6, which is an indicator of heterotrophic feeding (mainly phytoplankton) as previously discussed. This incompatible result is due to the abundance of different food sources (here, zooplankton *vs.* phytoplankton), which causes a variation of 18:3n-3 and 20:4n-6 in this species. On the other hand, symbionts of *T. peltata* showed the highest concentration of 18:3n-3 among all five species indicating a low translocation rate from symbionts to host tissue in this species might be responsible for our finding and unique characteristic of *T. peltata*. Exceptional values of 18:3n-3 due to species-specific characteristics have been reported by Papina et al. (2003). They observed low 18:3n-3 in *Montipora digitata* and postulated species-specific differences are responsible.

Besides a high concentration of 18:3n-3, *A. samoensis* is also characterized by high concentrations of 20:5n-3 and 22:6n-3. These FAs are known to be essential PUFAs in marine organisms, including corals, as they act as functional FAs for growth, reproduction, and health (Wacker and von Elert 2001; Pernet et al. 2002; Figueiredo et al. 2012). It is known 22:6n-3 is highly conserved through the food web (Scott et al. 2002; Dalsgaard et al. 2003), whereas 20:5n-3 is the dominant FA in symbionts and transferred to the coral host tissue (Revel et al. 2016). Thus, the ratio of 20:5n-3 to 22:6n-3 (EPA:DHA) has been applied to define trophic level (low value means heterotrophy, high value means autotrophy) (Rocker et al. 2019). According to the EPA:DHA ratio, *A. samoensis* and *T. peltata* are defined as relatively autotrophic, whereas *P. decussata*, *F. abdita* and *P. carnosa.* are relatively heterotrophic species. This ratio confirms our previous conclusions drawn from FA profiles that *A. samoensis* is autotrophic, *F. abdita and P. carnosa* are heterotrophic. However, this ratio contrasts with *P. ducussata* and *T. peltata* when compared to our findings, as well as to investigations from previous studies (Treignier et al. 2008). We may explain this controversy with the fact that corals are polytrophic, thus they can switch their trophic strategies depending on specific environmental conditions such as food sources and amount or symbiont density influenced by water quality (Dalsgaard et al. 2003; Seemann et al. 2013; Rocker et al. 2019). Thus, the most plausible reason is that not only 20:5n-3, but also 22:6n-3 in coral host tissue can be either transferred from symbionts (Revel et al. 2016) or taken up externally through planktonic food sources (20:5n-3 enriched in diatom, 22:6n-3 enriched in dinoflagellate) (Dalsgaard et al. 2003; Figueiredo et al. 2012; Revel et al. 2016).

In summary, TL and FA profiles proved a powerful tool as chemotaxonomic indicators on genus level of symbiotic corals, especially when host tissue and symbionts are investigated separately. This confirms our first two hypotheses, 1) FA profiles being distinctly different between coral host tissue and associated symbionts, and 2) FA profiles being species-specific, enabling a classification of symbiotic corals. In addition, our study provides new insights into the interaction of coral-symbionts endosymbiosis and species-specific trophic strategies. We hypothesized that the trophic level of symbiotic corals can be deduced from specific biomarkers. However, we realized that interpretation based on the accepted and widely used FA ratio indicators: EPA:DHA or PUFA:SFA to define specific trophic levels needs to be handled with more caution in research application and data interpretation. Since FA in coral host tissue can be driven by different sources such as symbionts and external food sources, interpretations may not be as straight forward as currently applied. Hence, our third hypothesis is true only when rephrased as certain FA biomarkers giving indications on the relative trophic level of symbiotic corals. Given these limitations of FA ratios, we suggest that it to be more appropriate if these FA ratios were only applied to examine response to environmental change within species. Going forward, our study provides important FA baseline data that builds the foundation to future investigation on impacts of environmental changes on nutrition and metabolism in symbiotic corals.

Abbreviations

TL	Total lipids
FA	Fatty acid
SFA	Saturated fatty acid
MUFA	Monounsaturated fatty acid
PUFA	Polyunsaturated fatty acid
PA	Palmitic acid (16:0)
SA	Stearic acid (18:0)
OA	Oleic acid (18:1n-9)
LA	Linolenic acid (18:2n-6)
ALA	α-linolenic acid (18:3n-3)
SDA	Stearidonic acid (18:4n-3)
EPA	Eicosapentaenoic acid (20:5n-3)
DPA	Docosapentaenoic acid (22:5n-3)
DHA	Docosahexaenoic acid (22:6n-3)
LA	Linoleic acid (18:2n-6)
GLA	γ-Linolenic acid (18:3n-6)
DGLA	Dihomo-γ-linolenic acid (20:3n-6)
ARA	Arachidonic acid (20:4n-6)
AdA	Adrenic acid (22:4n-6)

Declarations

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figures

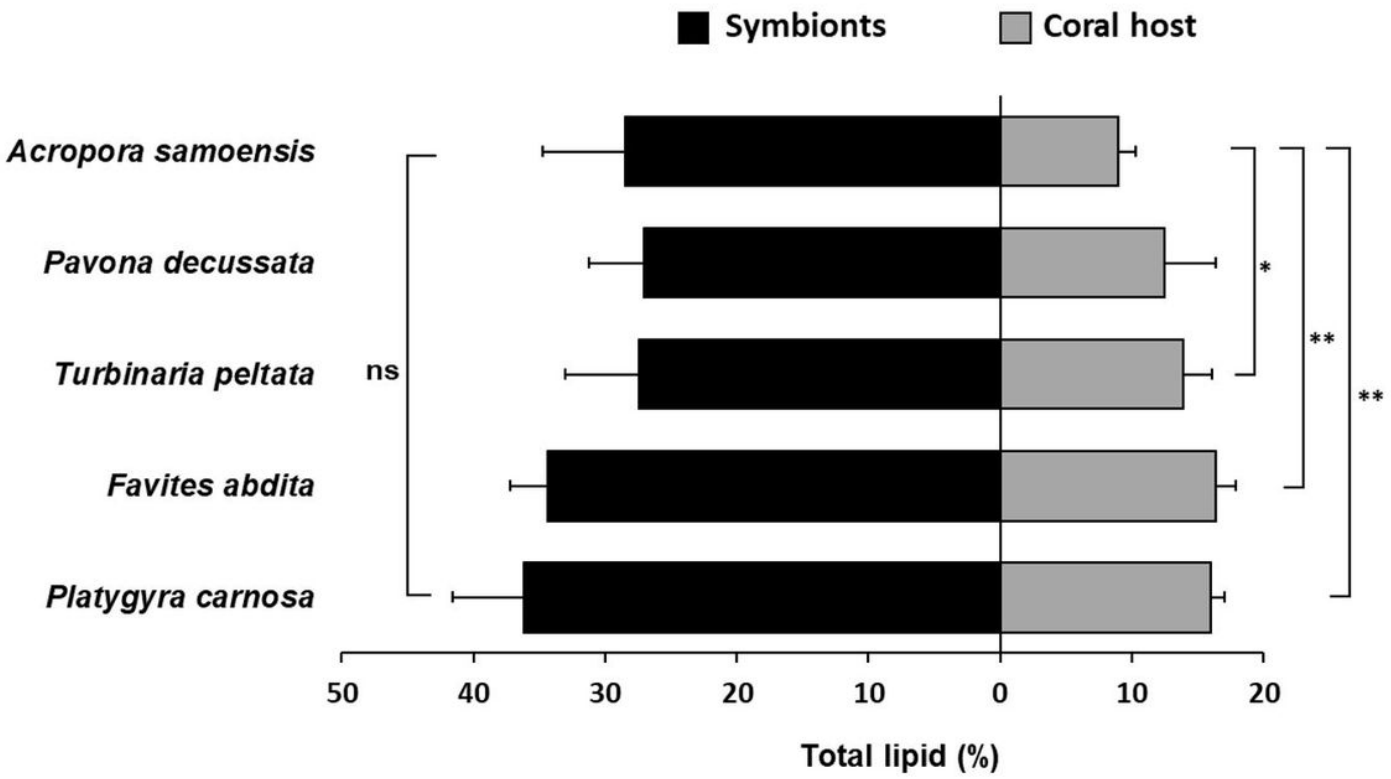

Figure 1

Total lipids in symbionts and their host tissue from five different coral species. Statistically significant differences ($p < 0.05$) in each symbiont and coral host tissue are indicated by asterisk (*): * indicates $p < 0.05$, ** indicates $p < 0.005$, ns indicates no significance (one-way ANOVA, Tukey's test) between species.

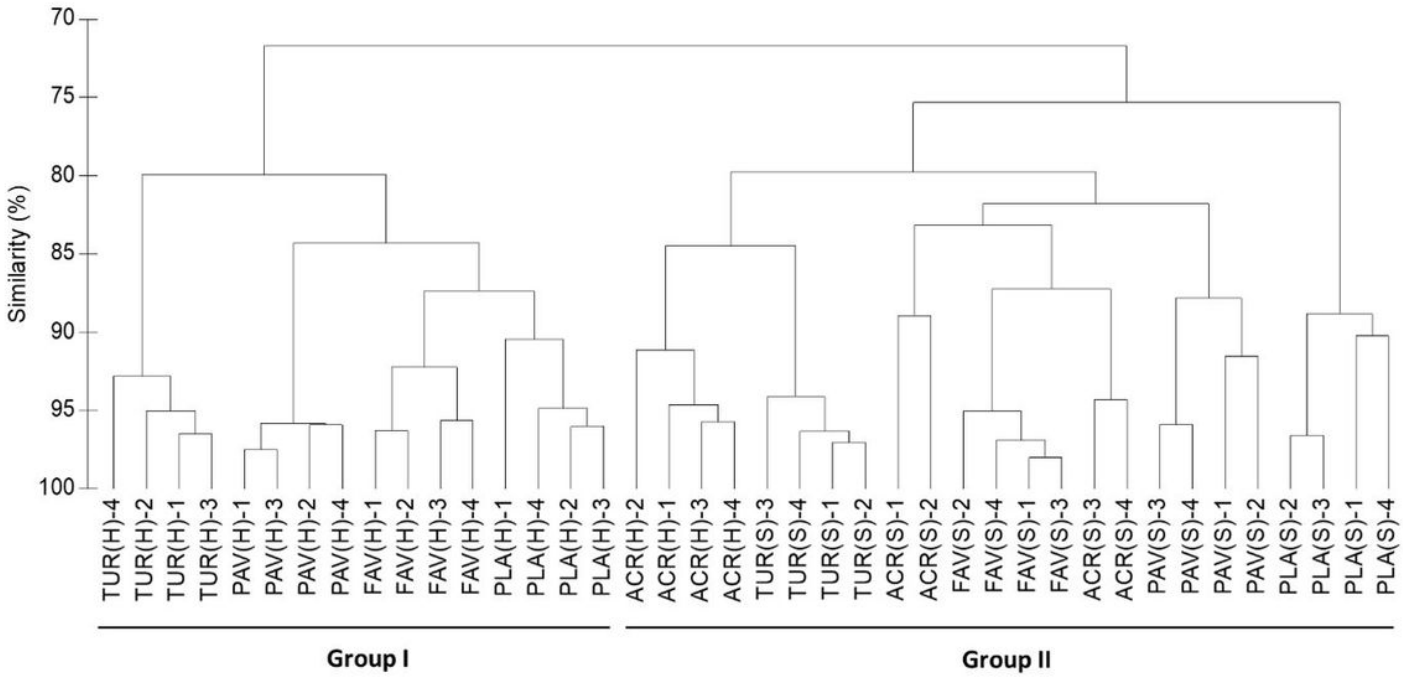

Figure 2

Hierarchical cluster analysis based on a Bray-Curtis similarity matrix of fatty acid profiles (µg mg-1) from five different symbiotic coral species (n = 4): ACR - Acropora samoensis, PAV - Pavona decussata, TUR - Turbinaria peltata, FAV - Favites abdita, PLA - Platygyra carnosa. (S) indicates symbionts, (H) indicates coral host tissue. Group I and Group II derived at 75% similarity level.

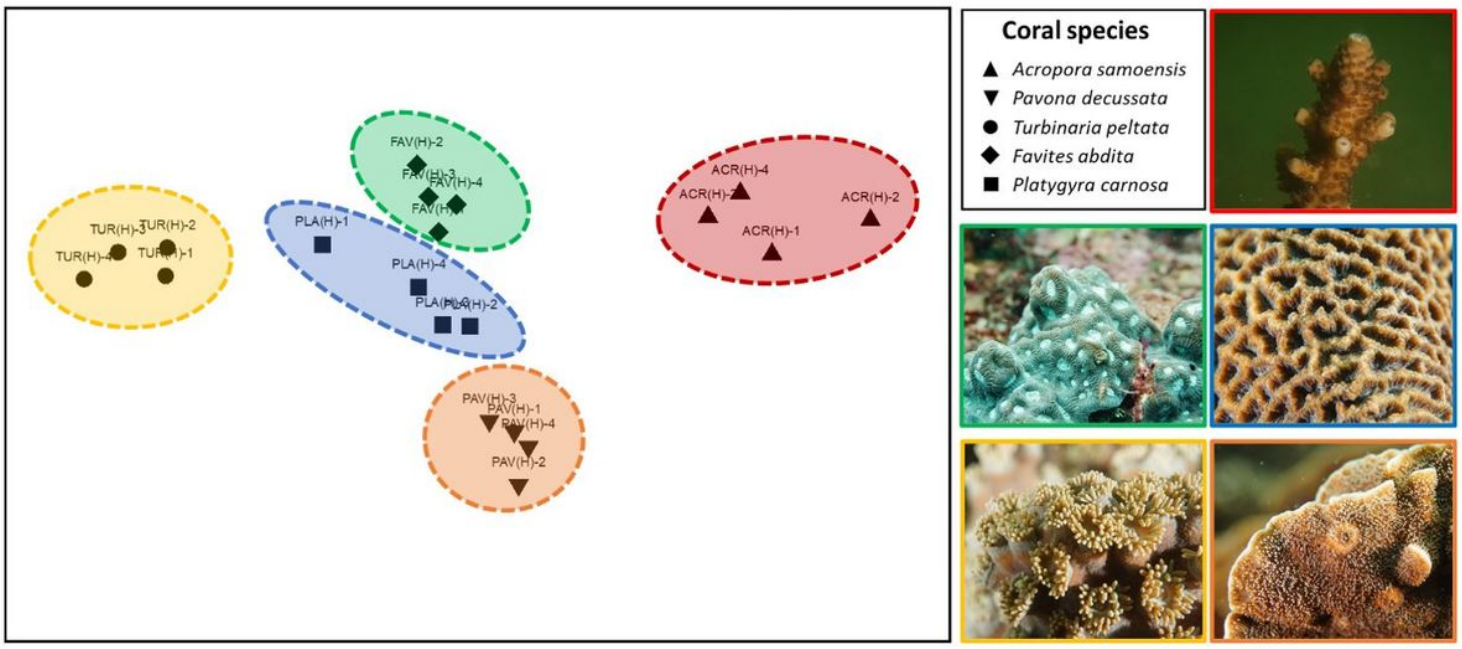

Figure 3

Non-metric multidimensional scaling (nMDS) plot of fatty acid profiles of host tissue from five different symbiotic coral species: ACR - Acropora samoensis (▲), PAV - Pavona decussata (▼), TUR - Turbinaria peltata (●), FAV - Favites abdita (♦), PLA - Platygyra carnosa (■). (H) indicates coral host tissue. Each symbol indicates one coral species. Dotted lines (---) indicate homogenous groups as determined by ANOSIM (R = 0.998, P < 0.05).

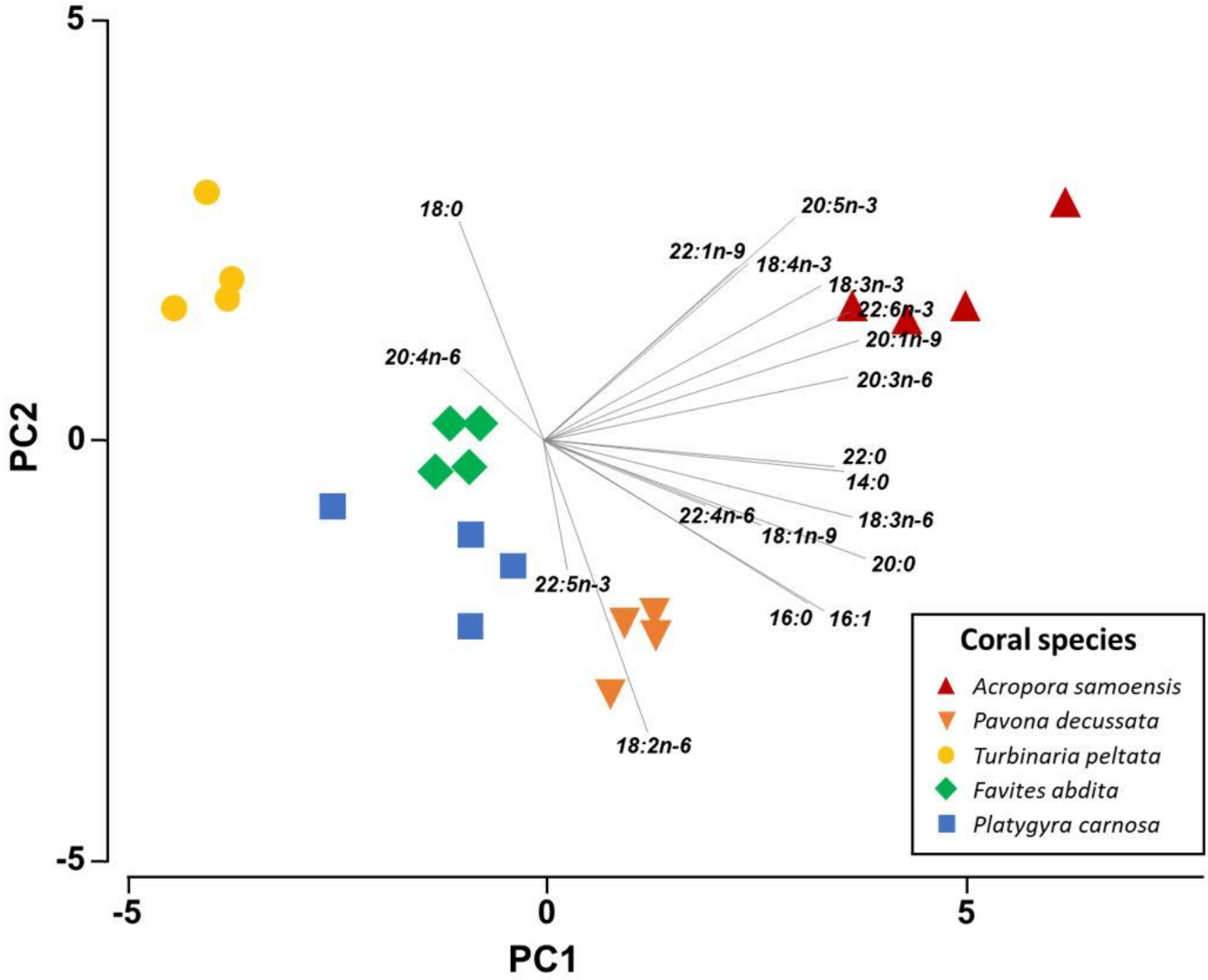

Figure 4

Principal component analysis (PCA) of fatty acid profiles from five different coral species.

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