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Chemical diversity of scleractinian corals revealed by untargeted metabolomics and molecular networking

Jiying Pei[1], Yuxia Zhou[1], Shiguo Chen[1], Kefu Yu[1, 2*], Zhenjun Qin[1], Ruijie Zhang[1], Yitong Wang[1]

[1] Coral Reef Research Center of China, Guangxi Laboratory on the Study of Coral Reefs in the South China Sea, School of Marine Sciences, Guangxi University, Nanning 530000, China

[2] Southern Marine Science and Engineering Guangdong Laboratory, Zhuhai 519080, China

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Abstract

The chemical diversity of scleractinian corals is closely related to their physiological, ecological, and evolutionary status, and can be influenced by both genetic background and environmental variables. To investigate intraspecific variation in the metabolites of these corals, the metabolomes of four species (*Pocillopora meandrina*, *Seriatopora hystrix*, *Acropora formosa*, and *Fungia fungites*) from the South China Sea were analyzed using untargeted mass spectrometry-based metabolomics. The results showed that a variety of metabolites, including amino acids, peptides, lipids, and other small molecules, were differentially distributed among the four species, leading to their significant separation in principal component analysis and hierarchical clustering plots. The higher content of storage lipids in branching corals (*P. meandrina*, *S. hystrix*, and *A. formosa*) compared to the solitary coral (*F. fungites*) may be due to the high densities of zooxanthellae in their tissues. The high content of aromatic amino acids in *P. meandrina* may help the coral protect against ultraviolet damage and promote growth in shallow seawater, while nitrogen-rich compounds may enable *S. hystrix* to survive in various challenging environments. The metabolites enriched in *F. fungites*, including amino acids, dipeptides, phospholipids, and other small molecules, may be related to the composition of the coral's mucus and its life-history, such as its ability to move freely and live solitarily. Studying the chemical diversity of scleractinian corals not only provides insight into their environmental adaptation, but also holds potential for the chemotaxonomy of corals and the discovery of novel bioactive natural products.

Key words: scleractinian coral, chemical diversity, metabolomics, molecular networking, environmental adaptation

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1 Introduction

Coral reefs, which are made up of scleractinian corals, are among the most biodiverse ecosystems in the marine environment. However, they have faced significant degradation in recent decades due to global climate change and human disturbance (Hughes et al., 2017; Rocha et al., 2018). This has caused a shift in the dominant coral species on reefs, from more complex branching, foliaceous, and massive colonies to simpler massive ones (Yu et al., 2019), resulting in a simplification of assemblage structures and a homogenization of reef functions. This shift suggests that different coral species has varying levels of susceptibility to environmental stress. In general, branching corals with competitive life-histories are more sensitive to environmental stress. For example, the high temperatures in Hawaii in 2019 led to severe bleaching in many *Pocillopora meandrina* colonies (Jones et al., 2021), while *Acropora formosa*, which used to be found in Weizhou Island, northern South China Sea, has disappeared since the 21st century (Yu et al., 2019). However, not all branching corals are equally sensitive to environmental stress. Some, like branching *Seriatopora hystrix*, are able to colonize a variety of disturbed environments and thrive (Sinniger et al., 2013). Solitary corals, which live independently, may be at risk of invasion by colonial neighbors during competition for space or light on reefs. They may coexist with colonial corals through chemical communication or behavioral interaction (Quévrain et al., 2014; Chadwick, 1988). The chemical makeup of scleractinian corals, which is influenced by both genetics (Sogin et al., 2014; Farag et al., 2016; Hayes et al., 2021) and the environment (He et al., 2014; Farag et al., 2016), may provide insight into their adaptive mechanisms to the surroundings.

Metabolomic profiling can reveal a coral's resistance, resilience, and function, and is a powerful tool for characterizing and comparing coral metabolism. Recently, innovative untargeted metabolomics approaches have been used to explore the chemical diversity of various biosystems (Subbaraj et al., 2019; Tang and Hatzakis, 2020). For example, metabolomic analysis of differ-

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*Corresponding author, E-mail: kefuyu@scsio.ac.cn

ent coral species in the deep sea showed distinct metabolite fingerprints and richness. The unique core ions identified in Callogorgia Delta were found to be diterpenes, which may have biologically important functions such as deterring predators, preventing fouling, and exhibiting allelopathy (Vohsen et al., 2019). Mass spectrometry (MS) is a widely used technique for metabonomic analysis due to its high sensitivity, resolution, and wide metabolite coverage (Dettmer et al., 2007). Combined with separation techniques like liquid chromatography (LC), MS allows for the simultaneous analysis of a range of metabolites with high throughput (Metz et al., 2007). Metabolic profiling based on chromatographic fingerprints can provide a deeper understanding of chemical composition. Metabolomics strategies have recently emerged as a way to gain a better understanding of the physiological (Pei et al., 2022b) and ecological adaptation mechanisms of corals to environmental conditions, as well as the differentiation of coral species (Hartmann et al., 2017; Farag et al., 2016).

In this study, we used LC-MS and molecular networking to explore the metabolomic diversity of four coral species (*P. meandrina*, *S. hystrix*, *A. formosa*, and *Fungia fungites*) with different biological characteristics. Multivariate data analysis was used to identify differential metabolites among the four species, and global natural product social molecular networking (GNPS) (Zhao et al., 2022; Wang et al., 2016) was used to identify the metabolites. The potential physiological functions of these species-enriched metabolites were then discussed. As far as we know, the first time high-throughput untargeted metabolomics has been used to compare the chemical diversity of multiple coral species with different biological traits, which helps to shed light on the diversity of corals' physiological responses to environmental stressors.

2 Materials and methods

2.1 *Sample collection and extraction*

On March 15, 2019, *P. meandrina* (eight colonies), *S. hystrix* (four colonies), *A. formosa* (six colonies), and *F. fungites* (eleven colonies) were collected from Panshi Atoll (16°02′–16°05′, 111°45′–111°50′) in the Xisha Islands of the South China Sea (SCS). The water quality at Panshi Atoll, including salinity, nutrients, and turbidity, has been previously reported in the literature (Qin et al., 2021). Coral fragments (5 cm × 5 cm) were carefully collected from colonies or solitary individuals using a hammer and chisel, taking care to minimize damage to the remaining coral. After collection, the coral samples were immediately placed in a bucket with seawater and transferred to a –20℃ refrigerator.

Coral tissue was extracted using a previously described method (Fig. S1) (Pei et al., 2022b). Briefly, coral tissue was removed from the skeleton using a water toothpick and chilled ultrapure milli-Q water. Then, 10 mg of lyophilized coral tissue containing an internal standard (caffeine-D9, 3 μg) was extracted with 0.5 mL of ice-cold methanol/water (*v*/*v*, 7:3). The coral tissue was then extracted a further two times, and the three supernatants (total 1.5 mL) were combined, filtered through a 0.22 μm nylon syringe filter, and stored at –80°C until analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS).

2.2 *MS data collection and pre-processing*

The extracts were analyzed on a Thermo™ Q-Exactive™ mass spectrometer coupled to a Dionex UltiMate 3000 UHPLC system. Chromatographic separation was performed at 30℃ using an ACQUITY CSH C_{18} column (2.1 mm× 100 mm; 1.7 μm; Waters, MA, USA) with the parameters listed in Table S1. The mobile phase consisted of a mixture of methanol and water with 0.1% formic acid, which was gradually changed from 5% methanol to 95% methanol over 25 min. The flow rate of the mobile phase and the injection volume of the coral extracted in LC were 0.2 mL/min and 2 μL, respectively. Mass spectrometry data acquisition was performed in positive electrospray ionization (ESI) mode with data-dependent acquisition (alternating collection of full mass spectra from *m/z* 100 to *m/z* 1 000 and MS/MS spectra of the top 10 most intense compounds). Raw files (.raw) were converted to .mzXML format before chromatographic feature extraction. The mass spectrometry data were organized into two files in .csv (a two-dimensional data matrix including retention time, *m/z*, and peak intensity) and .mgf format (MS/MS spectra) for feature-based molecular networking (FBMN) analysis using the open-source MZmine software (v2.51). The data underwent several stages of preprocessing in Mzmine: spectral filtering (MS mass detection and MS/MS mass detection), peak detection (ADAP chromatogram builder for chromatogram building and local minimum search algorithm for chromatogram deconvolution), alignment (join aligner algorithm), and normalization (normalized by the signal intensity of the internal standard, caffeine-D9). Detailed parameters can be found in the literature (Pei et al., 2022b).

2.3 *Statistical analysis*

Principal component analysis (PCA) and partial least square discriminant analysis (OPLS-DA) models were used to distinguish the four coral species using SIMCA-P (v14.1, Umetrics, Umea, Sweden). All data were unit variance and Pareto scaled before PCA and OPLS-DA analysis, respectively. The fitting parameter (R^2) and the predictive ability parameter (Q^2) were used to evaluate the quality of the models. In addition, sevenfold cross-validation and 200 random permutations of the class membership variable were performed to assess the predictiveness of the OPLS model. Potentially distinguishing metabolites among the four groups were identified based on the variable importance in the projection (VIP) values obtained from the OPLS-DA model. Generally, variables with VIP > 1 were considered relevant for group discrimination, but to strictly screen variables that significantly contribute to the separation of the four groups, the criterion of VIP > 2 was adopted. One-way analysis of variance (ANOVA) with Tukey's post hoc test was applied to compare the signal intensities of the variables with VIP > 2 among the four coral species using SPSS statistical analysis (SPSS, Version 23.0). A p-value < 0.05 was considered statistically significant. Histogram and box-plot were plotted using Origin 2021, whereas Venn diagram was plotted using Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/index.html).

2.4 *Molecular network analysis*

FBMN was conducted on the GNPS website (https://gnps.ucsd.edu/ProteoSAFe/static/gnps-splash.jsp?redirect=auth). MS/MS fragment ions were filtered to retain only the top 6 fragment ions within a ±50 Da window across the spectrum, and fragment ions within ±17 Da of the precursor *m/z* were removed. Both the precursor ion and MS/MS fragment ion tolerances were set to 0.02 Da. An edge in the network was created when the cosine score was greater than 0.7 and the number of matched peaks exceeded 2. The maximum size of a molecular family was set to 100 by removing the lowest scoring edges until the molecular family size was below this threshold. The library spectra were filtered in the same manner as the input data. The FBMN analys-

is results were then imported into Cytoscape 3.8.0 software for visualization. The FBMN job conducted in this study is available at: https://gnps.ucsd.edu/ProteoSAFe/status.jsp?task=ae3692556f2649e1904cd23f0b04928c.

2.5 *Quality control*

Quality control (QC) was conducted throughout the study. QC samples were prepared by pooling 2 mg of each coral tissue dry powder and extracted using the same method as the samples. To monitor instrument stability, QC samples were repeatedly injected five times at the beginning of LC-MS/MS analysis and alternately injected every seven samples. After extracting the metabolite features with MZmine, we only retained metabolites with a coefficient of variation (CV) < 30% in the QC samples. The maximum allowable error for the identification of metabolites using a Thermo Q-Exactive mass spectrometer was five parts per million.

3 Results

3.1 *Ion distribution among four coral species*

To compare the chemical diversity of four coral species, eight *P. meandrina*, four *S. hystrix*, six *A. formosa*, and eleven *F. fungites* fragments were extracted for LC-MS/MS-based untargeted metabolomics analysis. While untargeted metabolomics aims to gather as much information as possible about an organism's metabolites (Kusano et al., 2015), the metabolite coverage is restricted by the extraction protocol and detection instrument. LC-ESI-MS/MS, a common analytical technique for metabolomics analysis (De Vos et al., 2007), is used to characterize polar and weak polar compounds in organisms. Therefore, a polar solvent such as methanol/water is often used as the extraction solvent (Pei et al., 2022a, 2022b). This protocol, which was also used in this study, has been widely applied in untargeted metabolomics analysis of coral samples (Hartmann et al., 2017; Williams et al., 2021; Roach et al., 2021). In this research, a total of 6 784 feature peaks were extracted from the metabolomics dataset of the four coral species using MZmine. The numbers of metabolites for *P. meandrina*, *S. hystrix*, *A. formosa*, and *F. fungites* were 5 425, 4 721, 4 902, and 5 531, respectively. To examine the distribution of ions among the coral samples, the frequency of occurrence of each ion in the parallel coral samples was determined (Fig. 1). For *P. meandrina*, for example, 7.7% (420/5 425) of ions were only detected in one colony, while 38.4% (2 085/5 425) of ions were present in all eight colonies. Among the metabolites found in a particular coral species, the ones that were shared by all samples made up the largest proportion. The remaining metabolites, which were shared by 1 to *n*–1 (where *n* is the total number of samples for that coral species) samples, made up a roughly equal proportion of the rest.

Of the 6 784 metabolites, 3 241 are common to the four coral species, comprising 47.8% of the total number of metabolites and indicating that these are present in all coral species. There were

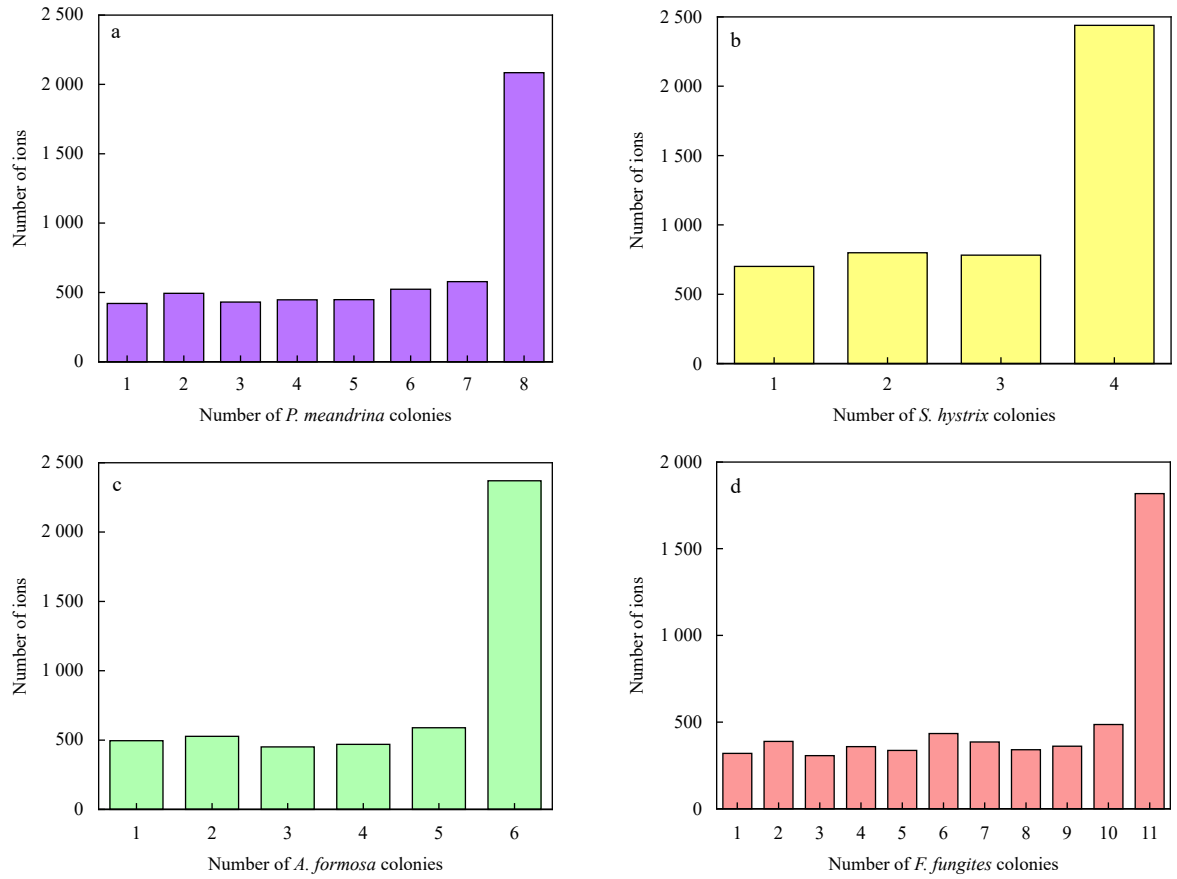

Fig. 1. Histogram showing the frequencies of the detection of each ion in eight colonies of *Pocillopora meandrina* (a), four colonies of *Seriatopora hystrix* (b), six colonies of *Acropora formosa* (c), and eleven colonies of *Fungia fungites* (d). The *X*-axis represents the total number of coral colonies detected with the same ion, and the *Y*-axis represents the number of ions commonly detected in 1 to *n* (*n* being the total sample number for a coral species) coral colonies. The total number of detected ions in *P. meandrina*, *S. hystrix*, *A. formosa*, and *F. fungites* were 5 425, 4 721, 4 902, and 5 531, respectively.

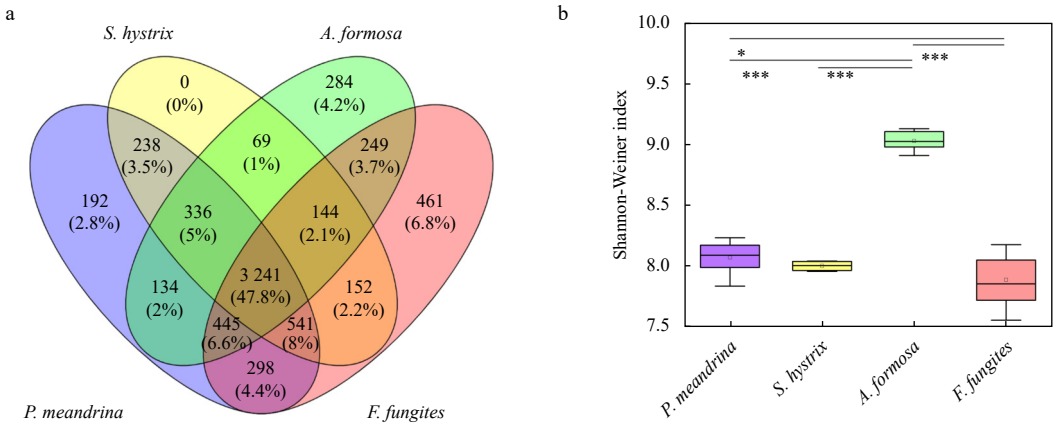

Fig. 2. Venn diagram showing the metabolites detected in *Pocillopora meandrina*, *Seriatopora hystrix*, *Acropora formosa*, and *Fungia fungites* (a). Shannon-Weiner diversity index of metabolites in the four coral species. (b) Note: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

192, 284, and 461 metabolites unique to *P. meandrina*, *A. formosa*, and *F. fungites*, respectively, while none were unique to *S. hystrix* (Fig. 2a). To compare the chemical diversity between different coral species, the Shannon-Weiner diversity index was calculated. The results showed that the Shannon-Weiner indexes for *P. meandrina*, *S. hystrix*, *A. formosa*, and *F. fungites* were 8.07, 8.00, 9.03, and 7.89, respectively. There were significant differences between *P. meandrina* and *A. formosa*, *P. meandrina* and *F. fungites*, *S. hystrix* and *A. formosa*, and *A. formosa* and *F. fungites* (Fig. 2b).

3.2 *Multivariate statistical analysis based on the metabolic profiling*

It is believed that the metabolic profiles of different coral species are positively correlated with their phylogenetic relationships. To test this hypothesis, multivariate statistical analysis was performed on a dataset obtained using LC-MS/MS. Both the unsupervised PCA model and supervised OPLS-DA model showed that the four coral species were clearly separated in the score plots (Fig. 3). Additionally, the distance between these species in the score plots was positively correlated with their phylogenetic relationships. The coral species belonging to different families (Pocillopora: *P. meandrina* and *S. hystrix*; Acropora: *A. formosa*; Fungia: *F. fungites*) were significantly separated (Fig. 3a and b). The distance between *P. meandrina* and *S. hystrix* was relatively small compared to the distances between *P. meandrina* and *A. formosa* or *P. meandrina* and *F. fungites*. However, *P. meandrina* and *S. hystrix* were still well separated in the PCA and OPLS-DA plots (Fig. S2). The replicates of each coral species clustered together, indicating good biological repeatability and close phylogenetic relationships. To evaluate the ability of the OPLS-DA model to distinguish between coral species, seven rounds of cross-validation and 200 random permutation tests were conducted. The results showed that the model had good predictability without overfitting (Fig. S3).

3.3 *Screening and identification of differential metabolites among the four coral species*

To identify differential metabolites among the four coral species, the VIP values of each metabolite were calculated in the OPLS-DA model. Two hundred and sixty-five metabolites were identified as differential with a VIP value greater than 2, with 11 of them being removed due to a p-value less than 0.05. These differential metabolites were then searched against LC-MS/MS databases and the GNPS libraries, resulting in the identification of 50 metabolites. The remaining unknown differential metabolites were annotated using molecular networking and manual interpretation, with known metabolites serving as "seeds" (Figs S4 and S5). This process led to the annotation of an additional 13 differential metabolites, including dipeptides, phospholipids,

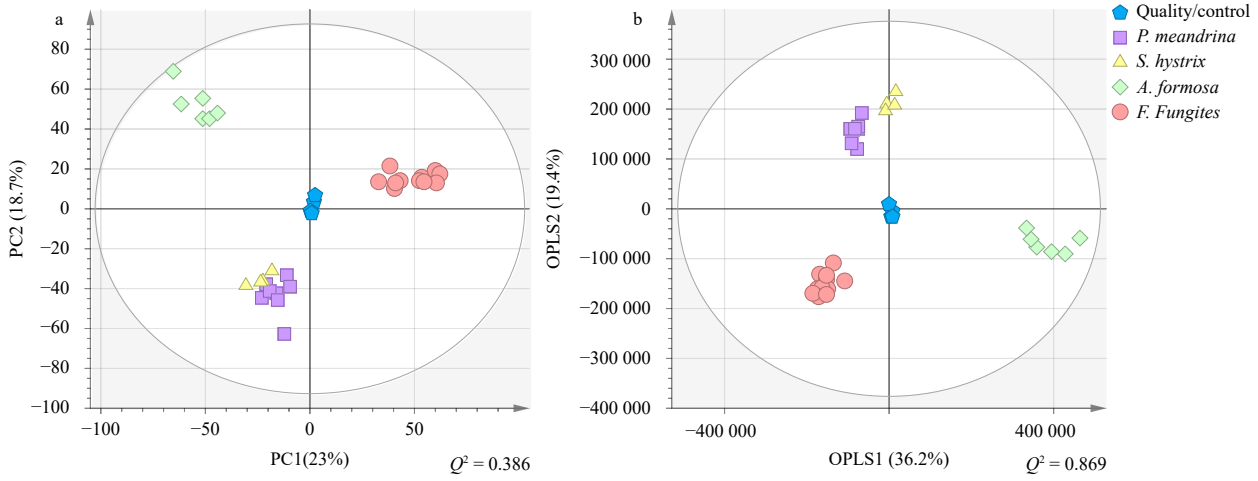

Fig. 3. PCA (a) and OPLS-DA (b) plots for differentiating the four coral species (*Pocillopora meandrina*, *Seriatopora hystrix*, *Acropora formosa* and *Fungia fungites*).

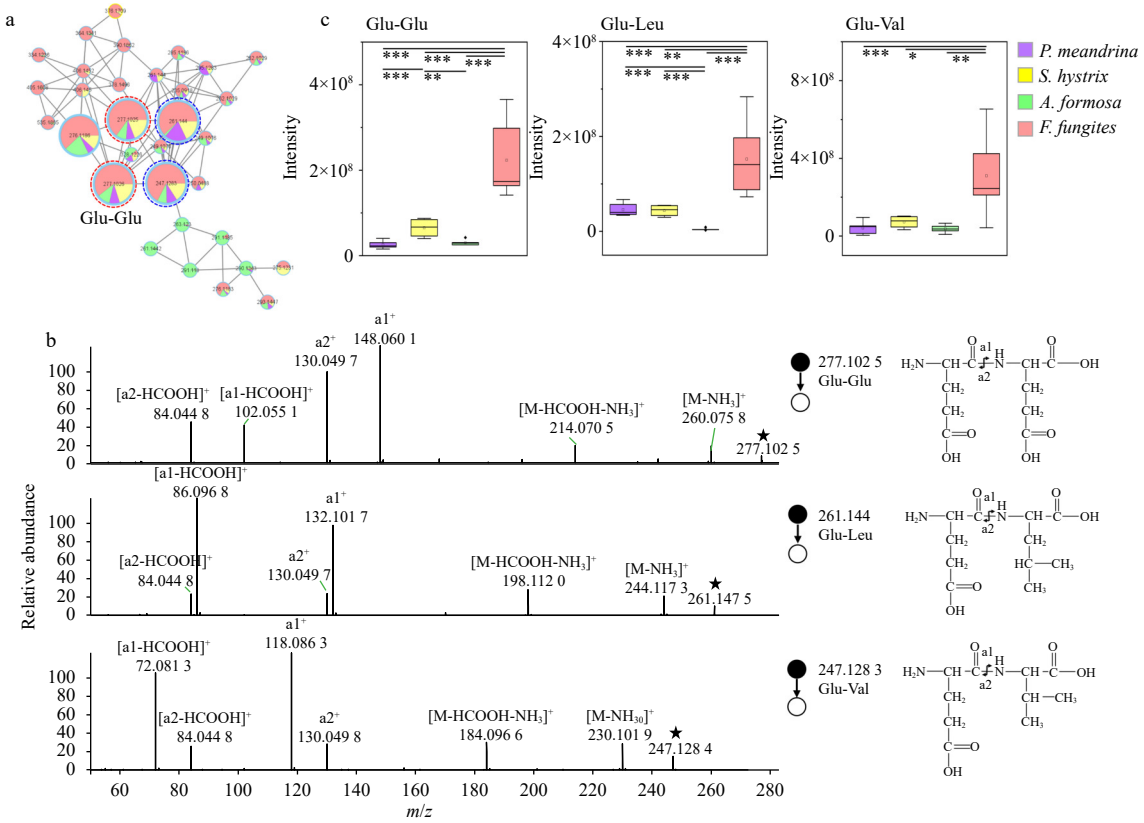

Fig. 4. Molecular networking of the peptide family (a). Mass spectra of peptides (b) and the corresponding box diagrams of the signal intensities among *Pocillopora meandrina, Seriatopora hystrix, Acropora formosa* and *Fungia fungites* (c) . Note: *, p <0.05; **, p <0.01; ***, p <0.001.

and other small molecules. In general, compounds with similar structural backbones will produce similar MS/MS fragmentation patterns in collision-induced dissociation (CID) spectra and cluster together in molecular networking. For example, the dipeptide Glu-Glu (*m/z* 277.102 5) was linked to two unknown metabolites with *m/z* 261.144 and 247.128 3 in the molecular networking (Fig. 4). Examining their MS/MS spectra showed that the three parent ions shared common fragment ions, including *m/z* 130.049 7 and 84.044 8, which were assigned as the glutamate residue and the decarboxylation group, respectively. This characteristic fragmentation pattern indicated that the three metabolites contained a common glutamate residue in their structural backbone. Additionally, the fragment ions of *m/z* 261.144 and 247.128 3 showed a constant mass shift of 15.955 Da (O) and 29.974 1 Da (O_2-H_2) from the fragment ions of *m/z* 277.102 5, respectively, suggesting that the metabolites with *m/z* 261.144 and 247.128 3 are protonated Glu-Leu and Glu-Val, respectively.

Hierarchical clustering was performed on the intensities of the extracted features in each sample to analyze the distribution of 63 differential metabolites in four coral species. It was found that various metabolites were differently distributed in different coral species (Fig. 5, Table S2). Fatty acids (FA 20 : 4, FA 22 : 4, FA 20 : 5, FA 22 : 5, FA 22 : 6, 17(S)-HETE, FA 20 : 6) and FAME (FAME 18 : 1, FAME 20 : 5) were more abundant in branching colonial corals (*P. meandrina*, *S. hystrix*, *A. formosa*) than in the solitary coral (*F. fungites*). Phospholipids (lyso-PAF C-16, lyso PC(16 : 0), lyso PC(18 : 0)), amino acids (proline, glutamate, asparagine, glutamine, pyroglutamate, serine, lysine, histidine, aspartate, methionine, arginine), dipeptides (Glu-Val, Glu-Leu, Glu-Glu), octopine, and alanopine were more enriched in *F. fungites*. Aromatic amino acids (phenylalanine, 2-hydroxyphenylalanine, tyrosine, and tryptophan) were more enriched in *P. meandrina* and *A. formosa* (especially tryptophan). Nitrogen-rich compounds including hypoxanthine, 2′-deoxy-guanosine, cytidine, adenosine, creatine, inosine, guanosine, 2′-deoxyinosine, arginine, g-guanidinobutyrate, and thymine were more enriched in *S. hystrix*. In addition, hierarchical clustering analysis (HCA) confirmed the results of the PCA and OPLS-DA models. Specifically, the distance between species was positively correlated with genetic relationships. The coral samples from the same species tended to cluster together preferentially; then the coral species belonging to the same family clustered together preferentially compared to coral species belonging to different families.

4 Discussion

Coral species vary in their susceptibility to environmental stress. The chemical diversity that results from genetic diversity is an important factor in an organism's ability to adapt and survive. Studying the metabolomic diversity among coral species can provide important insights into the interactions between coral and their environment. The four coral species in this study, *P. meandrina* and *A. formosa*, are characterized by fast growth rates and high efficiency at competing for resources in productive environments and are considered to have a "competitive" life-history strategy. *S. hystrix*, on the other hand, is characterized by fast growth and opportunism in competing for resources in unfavorable environments and is considered to have a "weedy" life-history strategy *F. fungites*, with its slow growth rate and high tolerance to chronic stress or variable environments, is considered to have a "stress-tolerant" life-history strategy (Darling et al., 2012).

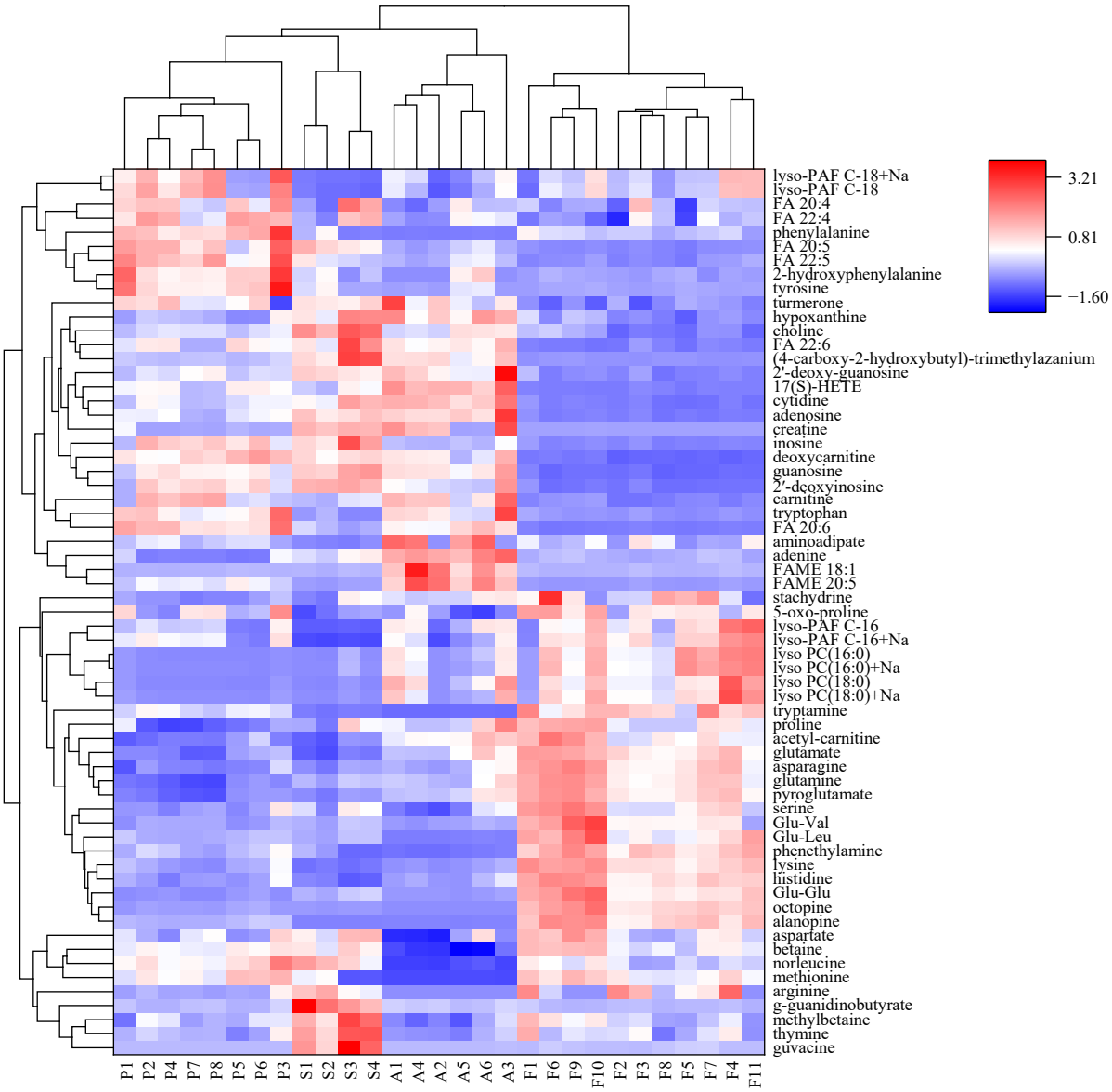

Fig. 5. HCA of the 63 differential metabolites in *Pocillopora meandrina, Seriatopora hystrix, Acropora formosa* and *Fungia fungites*. The letters of P, S, A, F in *X*-axis represent *Pocillopora meandrina*, *Seriatopora hystrix*, *Acropora formosa* and *Fungia fungites* respectively.

Different coral species adapt to their environment in a variety of ways.

Metabolites in organisms can be divided into primary and secondary metabolites, with primary metabolites making up the majority. Primary metabolites, such as amino acids, nucleotides, polysaccharides, and lipids, are essential for the growth and reproduction of organisms and can be found in all coral individuals, regardless of species or individual differences. However, their levels may vary among individuals, which makes metabolic profiling a useful tool for identifying coral species (Hayes et al., 2021). Differential metabolites found in different coral species may be important for their physiological, ecological, and evolutionary status.

Fungia fungites is a solitary coral species that is able to move freely. To protect itself from environmental stressors like sedimentation, desiccation, UV radiation, and temperature changes, it secretes a large amount of mucus (Han et al., 2020; Drollet et al., 1993; Brown and Bythell, 2005). Since mucus is rich in protein and carbohydrate polymers but lacks lipids (Stabili et al., 2014; Hadaidi et al., 2019), the extract from *F. fungites* has high levels of amino acids and peptides but low levels of fatty acids and FAME. Peptides have a variety of biological functions, such as anti-inflammatory/antioxidant, antibacterial, antiviral, defense, and others (Schmidt et al., 2019). PAFs are also immune factors that play a role in resistance to interspecies competition (d'Auriac et al., 2018). The high content of peptides and lyso-PAFs in *F. fungites* may help it survive on reefs dominated by various colonial coral species and planktons, similar to the function of nematocysts (Karabulut et al., 2022). Octopine and alanopine (Murphy and Richmond, 2016) are two metabolites involved in the anaerobic metabolism of organisms. As *F. fungites* moves freely on the seafloor, it may encounter various obstacles and threatening organisms, and anaerobic metabolism may be an alternative pathway for *F. fungites* to adjust its energy metabolism in case of emergency

Aromatic amino acids contain a benzene ring in their mo-

lecular structure and have the ability to absorb ultraviolet light due to their π-conjugated structure, which helps protect organisms from UV damage. *Pocillopora meandrina* and *A. formosa*, which have a "competitive" life-history strategy and are generally found in shallow, high-light environments, may use aromatic amino acids to absorb excess energy and protect themselves from UV damage, similar to the function of mycosporine-like amino acids (Shinzato et al., 2011; Rosic and Dove, 2011). Aromatic amino acids also serve as building blocks for proteins and as precursors to many primary and specialized metabolites (Shinzato et al., 2011; Rosic and Dove, 2011). Derivatives of aromatic amino acids have diverse functions in organisms, ranging from the formation of photosynthetic electron carriers to the synthesis of natural products with biological activities (Maeda and Dudareva, 2012). For example, phenylalanine is a precursor to lignin, anthocyanins, flavonoids, isoflavonoids, and tannins (Hou et al., 2022; Maeda and Dudareva, 2012); tyrosine is a precursor for quinones, betalains, isoquinoline alkaloids, and lignin (Lynch and Dudareva, 2020); and tryptophan participates in the synthesis of auxin, alkaloids, indole-glucosinolates, and phytoalexins (Lynch and Dudareva, 2020). Plants direct about 30% of photosynthetically fixed carbon towards aromatic amino acid formation to support growth, development, reproduction, defense, and environmental responses (Dudareva, 2015). For example, the downregulation of aromatic amino acid biosynthesis in the coral endosymbiont Cladocopium goreaui can suppress its reproduction (Tang et al., 2022). In summary, the high content of aromatic amino acids in *P. meandrina* and *A. formosa* may contribute to their fast growth rate in ideal environments by improving resource utilization efficiency (Darling et al., 2012).

The high levels of nitrogen-rich compounds in *S. hystrix* may be related to its habitat. *Seriatopora hystrix* has a "weedy" life-history strategy (Pei et al., 2022a), which allows it to survive by colonizing a variety of disturbed environments, such as heavily fished reefs or shallow back reef lagoons. These stressful or variable environments may result in specific physiological and ecological adaptations. Previous research has shown that scleractinian corals can turn to planktonic diazotrophs to help with bleaching recovery by incorporating more nitrogen (Meunier et al., 2019). Marcelino et al. also found that *S. hystrix* has a relatively high abundance of diazotrophs (Marcelino et al., 2017). Therefore, it is likely that *S. hystrix* uses diazotrophs to synthesize more nitrogen-rich compounds in order to cope with unfavorable environments.

The higher content of storage lipids (FA, FAME) in branching corals (*P. meandrina*, *S. hystrix*, and *A. formosa*) compared to the solitary coral (*F. fungites*) may be related to the density of zooxanthellae. While the density of zooxanthellae based on unit area is similar for Pocilloporidae, Acroporidae, and Fungiidae (Qin et al., 2019), the tissue thickness of branching coral is generally significantly lower than that of massive coral (Qin et al., 2020), resulting in a higher density of zooxanthellae based on tissue weight in *P. meandrina*, *S. hystrix*, and *A. formosa* compared to *F. fungites*. It is well known that coral hosts and symbiotic zooxanthellae have a mutually beneficial relationship. The coral host provides inorganic nutrients to the zooxanthellae for photosynthesis, while the zooxanthellae offer photosynthates to the coral host to support growth, respiration, reproduction, and biocalcification (Kopp et al., 2015). A reduction in the density of zooxanthellae will lead to a decrease in the rate at which photosynthates are translocated to the coral host. Therefore, we infer that the high density of zooxanthellae in branching corals is responsible for the high storage lipid content of coral holobiont, which promotes their fast growth (Oku et al., 2002). As previously reported, bleaching results in the loss of symbionts from the coral host, along with a reduction in both storage and structural lipids (Imbs and Yakovleva, 2012; Yamashiro et al., 2005).

5 Conclusions

In summary, untargeted mass spectrometry-based metabolomics was used to analyze the chemical diversity of *P. meandrina*, *S. hystrix*, *A. formosa*, and *F. fungites*. A variety of metabolites, including amino acids, peptides, lipids, and other small molecules, were differentially distributed among the four coral species. The unique metabolites that were particularly enriched in a specific coral species were believed to play a vital role in its physiological and ecological status. For example, aromatic amino acids enriched in *P. meandrina* and *A. formosa* were thought to help alleviate UV damage and promote coral growth. Nitrogen-rich compounds were thought to help *S. hystrix* withstand environmental stress. The special metabolites in *F. fungites* were believed to be related to the composition of mucus and the species' life-history traits. Investigating the chemical diversity of scleractinian corals provides a biological indication of environmental adaptation and can serve as a potential tool for the chemotaxonomy of scleractinian corals in future research. Combining untargeted metabolomics with molecular networking also helps to discover novel coral-based natural products with potential physiological and pharmacological activity.

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Supplementary information:

Fig. S1. Workflow diagram for exploring the chemical diversity of *Pocillopora meandrina, Seriatopora hystrix, Acropora formosa, and Fungia fungites*.

Fig. S2. PCA (a) and OPLS-DA (b) plots for differentiating *Pocillopora meandrina* and *Seriatopora hystrix*.

Fig. S3. Permutation test (n = 200 times) of the OPLS-DA models for distinguishing the four coral species (a), *Pocillopora meandrina* and *Seriatopora hystrix* (b).

Fig. S4. Overview of the molecular networking of all the metabolites in the four coral species.

Fig. S5. Molecular networking of phospholipid family (a); mass spectra of phospholipids (b) and the corresponding box diagrams of the signal intensities among the four coral species (c).

Table S1. Liquid chromatography conditions for separations.

Table S2. Parameters about the 63 differential metabolites among the four coral species.

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