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RESEARCH ON THE LIPID CHEMISTRY OF TWO HYDROCORAL SPECIES, *Millepora dichotoma* AND *Millepora platyphylla*, IN VIETNAM

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I. INTRODUCTION TO THE DISSERTATION

1. The urgency of the dissertation

Coral reefs are among the most biologically diverse ecosystems in the oceans. In tropical seas, they contribute to the formation and protection of thousands of islands, safeguard coastlines, and help maintain the ecological balance of the marine environment. Coral species form the foundation of coral reef communities, with hydrocorals playing a crucial role as the second most important reef builders after hard corals. They significantly contribute to reducing reef degradation and play an essential role in coral reef restoration. Many scientific laboratories have focused their efforts on studying coral ecosystems, biodiversity, and biochemistry.

Lipids are a vital chemical component, accounting for up to 40% of the dry biomass of corals. In modern research, the composition and content of lipid classes, as well as fatty acids, reflect biochemical diversity, nutritional sources, and symbiotic relationships. These aspects serve as key factors in studying biosynthetic pathways and the transfer of primary lipid forms through the food chain in organisms in general, and corals in particular. Lipids belong to specific classes, but they vary in terms of the acyl group composition of fatty acids and their positional distribution within lipid molecules, which are referred to as the "molecular species" of that lipid class. Each molecular species is a distinct chemical compound, and each lipid class consists of a mixture of dozens of molecular species. Research on the chemistry and biological activity of lipids is highly complex, and isolating individual lipid compounds is challenging. Their application as pharmaceutical products is also limited due to these structural characteristics. With the aid of modern research equipment, significant advances have been made in analyzing the molecular species composition of certain non-polar lipid classes, such as triglycerides derived from vegetable oils. However, information on the molecular composition of lipids from animals, particularly polar lipids, remains much more limited.

In Vietnam, previous studies on coral lipids have primarily focused on ecological diversity, assessments of coral reef status, and preliminary biochemical analyses, which have yielded data on total lipid content, fatty acid composition, and lipid class composition. Some studies have explored the composition of non-polar lipid classes in greater detail. However, to date, very few studies have investigated the molecular composition of polar phospholipids—a class that holds valuable information on the lipid biosynthesis process in hydrocorals, both in Vietnam and globally.

Recognizing the importance of in-depth lipid research for applications in ecology and biochemistry, particularly in establishing nutritional, symbiotic, and taxonomic relationships in marine species studies, we have chosen the dissertation topic: "**Research on the lipid chemistry of two hydrocoral species *Millepora dichotoma* and *Millepora platyphylla* in Vietnam "**

2. Research objectives of the dissertation

- Comprehensively study the lipid chemistry of two hydrocoral species, *Millepora dichotoma* and *Millepora platyphyll* collected from Nha Trang sea, Vietnam. This includes investigating total lipid content, composition, and distribution of lipid classes, fatty acids, and polar phospholipid layers, as well as determining the molecular species of polar phospholipid in these Vietnamese hydrocorals .

- Monitor the changes in the composition and content of lipid classes, fatty acids, and polar phospholipid layers in the total lipid of the two studied hydrocoral samples over 12 months.

- Conduct an initial survey on the symbiotic relationship between the hydrcoral *Millepora* and the symbiotic microorganism *zooxanthellae*.

3. Research content of the dissertation

- Determination of total lipid content, composition, and lipid classes distribution in two hydrocoral species, *Millepora dichotoma* and *Millepora platyphylla*, over 12 months

- Determination of the composition and content of fatty acids in two hydrocoral species, *M.dichotoma* and *M.platyphylla,* over 12 months.

- Determination of the composition and content of polar phospholipid layers in two hydrocoral samples over 12 months.

- Identification of molecular species in the polar phospholipid of the studied samples.

- Survey of chlorophyll A content in two *Millepora* species over 12 months.

4. New contributions of the dissertation

- This is the first comprehensive study on the lipidomic of two hydrocoral species, *M. dichotoma* and *M.platyphylla,* collected from Nha Trang Sea, Vietnam.

- This is the first study investigating the 12-month variations in lipid composition of the two Vietnamese hydrocoral species, *M.dichotoma* and

M.platyphylla. The study includes fluctuations in total lipid content, composition and content of lipid classes in total lipid, composition and content of fatty acids in total lipid, composition and content of polar lipids.

- For the first time, molecular species in the polar phospholipid classes of the two hydrocoral species *M.dichotoma* and *M.platyphylla* have been studied. A total of 77 phospholipid molecular species of phospholipids were identified in *M.dichotoma* and 78 phospholipid molecular species in *M.platyphylla* .

- This is the first study on the symbiotic relationship between *zooxanthellae* and two *Millepora* species collected from Nha Trang, Vietnam.

5. Structure of the dissertation

The dissertation consists of 138 pages (excluding the Appendix), including 29 tables, 60 figures. The main contents is structured as follows: Introduction (3 pages); Chapter 1: Overview (34 pages); Chapter 2: Research objects and methods (22 pages); Chapter 3: Results and discussion (62 pages); Conclusions and recommendations (2 pages); List of the publications related to the dissertation (1 page); References (14 pages).

II. CONTENT OF THE DISSERTATION

INTRODUCTION

The introduction mentions the scientific significance, objects and problems to be researched in the thesis as well as the new points that the thesis brings.

CHAPTER 1. OVERVIEW

The overview consists of 5 parts: part 1 Overview of Corals and Hydrocoral; part 2 Lipids in corals (general introduction to lipids and lipid classification in general); part 3 studies on lipids in corals and hydrocorals; part 4 introduction to symbiotic microorganisms *Zooxanthellae*; part 5 introduction to the two studied species (*M.dichotoma* and *M.platyphylla*)

CHAPTER 2. RESEARCH OBJECTS AND METHODS

This chapter clearly defines the research subjects of the dissertation and provides a detailed description of the experimental procedures conducted on the research samples based on the previously outlined methods. These include:

lipid extraction experiments to determine total lipid content; quantification of the components within total lipids; analysis of phospholipid classes; and fatty acid composition. Experiments were also conducted to identify molecular species within the phospholipid classes, presenting the mass spectrometric data obtained for each molecular species in the polar phospholipid subclasses. Additionally, the chapter addresses the experimental determination of chlorophyll A content in the samples.

2.1. Research subjects

The hydrocoral samples *Millepora dichotoma* and *Millepora platyphylla* were collected for 12 consecutive months, once a month in the waters of Nha Trang, Khanh Hoa. The samples were named by Associate Professor, Dr. Hoang Xuan Ben and colleagues at the Institute of Oceanography - Nha Trang.

2.2. Research method

2.2.1. Sample collection and preservation methods

Samples were collected by SCUBA diving, transported in seawater using a covered device. After being brought to the laboratory, the samples were de-stained and stored at -18 ^{0}C.

2.2.2. Total lipid extraction method

Total lipid extraction by modified Folch JF method.

2.2.3. Method for determining fatty acid composition and content

Fatty acid methyl ester mixtures were analyzed by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS), using the NIST standard spectrum library for comparison.

2.2.4. Method for determining the composition and content of lipid layers

The composition and content of lipid classes were determined by TLC method combined with the Sorbfil TLC Videodensitometer DV image analysis program (Krasnodar, Russia) .

2.2.5. Method for determining the composition and content of phospholipid layers

Qualitative analysis of the substance classes: Using 2-dimensional TLC, combining Ninhydrin and Molybdate reagents.

Quantitative analysis of substance layers: Using 1-dimensional TLC, H_2SO_4/MeOH 5% reagent, combined with the image analysis program Sorbfil TLC Videodensitometer DV (Krasnodar, Russia) .

2.2.6. Method for determining molecular species of polar lipids

Using high-performance liquid chromatography coupled to high-resolution mass spectrometry (HPLC-HRMS), the content and structure of molecular species were analyzed by LCMS-IT-TOF system (Shimadzu, Japan).

2.2.7. Method for determining chlorophyll A content

Chlorophyll A content was analyzed following the method of Parsons et al. (1984).

CHAPTER 3. RESULTS AND DISCUSSION

Two hydrocoral samples, *M.dichotoma* and *M.platyphylla*, were studied according to the general study diagram (Figure 3.1) and obtained the following results:

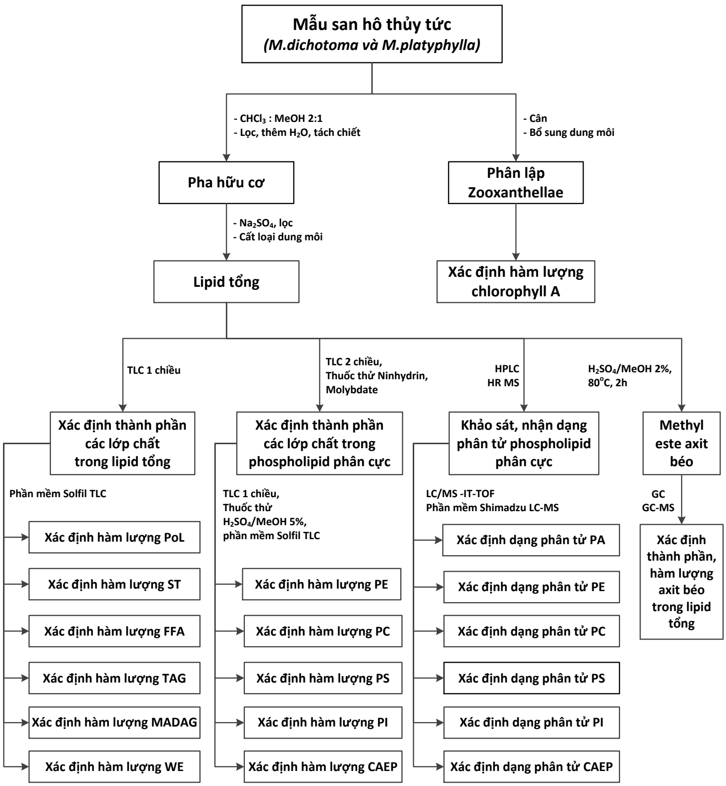

Figure 3.1. General study diagram

3.1. Study on lipid composition of hydrocoral samples *Millepora dichotoma* and *Millepora platyphylla*

3.1.1. Total lipid content of two Millepora samples

Two samples of hydrocorals *M. dichotoma* and *M. platyphylla* were monitored for changes in total lipid (TL) content over 12 months of the year. The results of determining the total lipid content of the studied samples are presented in the table. 3.1

Table 3.1. Total lipid content over 12 months of two hydrocoral species M.dichotoma and M.platyphylla

TT	Time	Total lipid content * (%)	
		M. dichotoma	*M. platyphylla*
1	January	0.49 ± 0.06	0.48 ± 0.05
2	February	0.50 ± 0.12	0.45 ± 0.08
3	March	0.46 ± 0.16	0.47 ± 0.13
4	April	0.64 ± 0.10	0.50 ± 0.08
5	May	0.42 ± 0.02	0.51 ± 0.05
6	June	0.45 ± 0.15	0.63 ± 0.12
7	July	0.53 ± 0.16	0.40 ± 0.15
8	August	0.55 ± 0.12	0.54 ± 0.06
9	September	0.48 ± 0.07	0.41 ± 0.15
10	October	0.45 ± 0.15	0.31 ± 0.08
11	November	0.35 ± 0.06	0.30 ± 0.04
12	December	0.32 ± 0.08	0.26 ± 0.09
Medium		**0.48**	**0.44**

*: % of dry weight

The results of Table 3.1 show that the average total lipid content of both studied samples is quite similar, 0.48% dr.w with *M.dichotoma* and 0.44% dr.w with *M.platyphylla.* The total lipid content of the two species tends to increase in the first months of the year and summer; and decreases in the winter months.

There was a significant difference between the total lipid content of *Millepora* corals and that of hard corals (10.87 - 17.03 % dr.w) and soft corals (14.1 - 37.0% dr.w). The results of total lipid content in the study can be considered as scientific evidence for the species classification of these coral samples.

3.1.2. Composition and content of lipid classes in total lipid

Table 3.2. Composition and content of lipid classes in total lipid of M.dichotoma coral in 12 months

Sample	Lipid content (%)							Sw temp (^0C)
	PoL	ST	FFA	TAG	MADAG	WE	Other	
Md1	18.3±4.4	8.6 ±2.2	2.19±0.14	17.6±3.2	12.6±2.1	11.7±2.1	1.76±0.11	25.3
Md2	17.7±3.6	8.7±2.4	2.75±0.28	19.6±1.6	17.8±2.8	16.4±2.6	1.36±0.16	25.3
Md3	12.6±2.3	5.3±0.6	1.97±0.10	17.8±1.5	18.7±1.6	21.8±0.9	-	28.0
Md4	16.1±2.9	8.9±1.5	1.31±0.16	28.1±2.7	24.5±2.6	22.9±3.3	-	28.3
Md5	24.8±3.8	10.5±1.6	1.34±0.16	29.7±3.3	27.5±2.3	23.8±2.8	1.38±0.10	28.1
Md6	25.6±2.7	12.2±1.2	1.75±0.34	40.8±4.9	33.0±2.5	33.5±2.6	1.72±0.22	28.2
Md7	24.1±2.5	11.6±1.2	1.51±0.25	34.4±2.8	31.9±2.8	34.8±3.1	0.29±0.05	28.4
Md8	26.1±2.8	9.0±1.3	1.18±0.11	25.4±2.1	27.9±2.3	25.0±2.4	-	28.5
Md9	30.8±10.1	10.0±2.9	2.14±0.22	27.1±3.3	27.3±4.7	21.1±5.4	1.11±0.09	28.3
Md10	23.1±4.3	10.6±1.6	2.60±0.46	34.5±7.6	19.2±5.9	16.0±4.5	0.59±0.10	26.5
Md11	18.8±3.0	9.1±1.3	2.02±0.26	22.3±4.9	16.3±4.9	14.2±2.8	1.72±0.14	25.7
Md12	23.4±2.0	12.1±1.5	3.63±0.31	26.4±6.2	11.3±4.3	10.0±2.7	1.71±0.10	25.4
Medium	**21.78**	**9.72**	**2.03**	**26.96**	**22.33**	**20.93**	**0.97**	

Table 3.3. Composition and content of lipid classes in total lipid of M.platyphylla coral sample in 12 months

Sample	Lipid content (%)							Sw temp (^0C)
	PoL	ST	FFA	TAG	MADAG	WE	Other	
Mp1	17.6 ±0.2	9.2 ±0.06	1.6 ±0.02	30.6 ±0.6	19.3 ±0.4	19.9 ±0.2	1.76 ±0.02	25.3
Mp2	17.1 ±0.2	8.0 ±0.03	1.4 ±0.02	27.6 ±0.3	20.4 ±0.4	24.2 ±0.3	1.36 ±0.01	25.3
Mp3	17.3 ±0.4	7.7 ±0.01	1.4 ±0.02	27.9 ±0.1	20.9 ±0.4	24.7 ±0.4	-	28.0
Mp4	16.4 ±0.1	7.8 ±0.09	1.3 ±0.03	31.3 ±0.7	21.1 ±0.3	22.1 ±0.4	-	28.3
Mp5	11.1 ±0.1	7.5 ±0.07	1.2 ±0.00	32.1 ±0.3	22.5 ±0.3	24.2 ±0.1	1.38 ±0.01	28.1
Mp6	12.7 ±0.1	7.0 ±0.09	1.2 ±0.01	30.4 ±0.6	22.2 ±0.5	24.8 ±0.2	1.72 ±0.01	28.2
Mp7	12.1 ±0.2	7.2 ±0.02	1.0 ±0.02	31.7 ±0.5	22.8 ±0.3	25.0 ±0.4	0.29 ±0.02	28.4
Mp8	14.1 ±0.1	6.8 ±0.09	1.1 ±0.03	34.3 ±0.2	22.1 ±0.3	21.6 ±0.2	-	28.5
Mp9	14.2 ±0.1	7.2 ±0.01	1.2 ±0.02	32.6 ±0.7	20.2 ±0.4	22.8 ±0.4	1.11 ±0.01	28.3

Mp10	14.0 ±0.1	7.3 ±0.06	1.6 ±0.02	35.3 ±0.3	22.6 ±0.2	18.7 ±0.2	0.59 ±0.00	26.5
Mp11	18.8 ±0.9	9.2 ±0.06	1.3 ±0.01	29.1 ±0.4	20.9 ±0.2	18.9 ±0.3	1.72 ±0.01	25.7
Mp12	18.9 ±0.3	9.9 ±0.07	1.5 ±0.02	29.9 ±0.9	18.4 ±0.2	19.7 ±0.3	1.71 ±0.00	25.4
Medium	**15.35**	**7.89**	**1.32**	**31.12**	**21.12**	**22.21**	**0.99**	

From the results of Table 3.2 and Table 3.3, it can be seen that in the total lipid of the two species of hydrocoral, there are 5 main classes: the structural lipid classes are PoL and ST, the main reserve lipid classes are WE, TAG and MADAG and the secondary component is FFA, these fatty acids have small content and change insignificantly during the year, on average 2.03% with *M.dichotoma* and 1.32% with *M.platyphylla* .

The research results showed that the two reserve lipid classes (WE, TAG, MADAG) and the structure (PoL and ST) had quite clear seasonal fluctuations. For *M.dichotoma* , there was a similarity between the two reserve lipid classes and the structure, which was that the content of these two classes increased in the summer months and decreased in the winter months; For *M.platyphylla* , the content of FFA, ST, PoL decreased in the summer months, increased in the winter months and vice versa for the MADAG, WE, TAG classes.

3.1.3. Composition and content of the polar phospholipid layers of two Millepora samples

Qualitative and quantitative analysis of the phospholipid classes in the total lipids of the research samples were performed according to the method described in section 2.2.5. The results of the analysis of the composition and content of the phospholipid classes in M. dichotoma and M. platyphylla are presented in Tables 3.4 and 3.5.

Table 3.4. Composition and content of phospholipid classes according to 12 months of the year of M.dichotoma species

Sample symbol	**Content of phospholipid classes (%)**					**Sea water temperature (^{o}C)**
	PI	PS	CAEP	PC	PE	
Md1	4.48±0.44	5.30±0.98	19.67±1.27	39.72±1.12	30.84±1.50	23.7
Md2	5.14±0.36	5.28±0.82	22.81±1.34	36.35±1.03	30.42±0.58	25.1
Md3	4.80±0.39	5.47±0.97	20.63±0.77	34.95±0.55	34.15±0.97	25.7
Md4	4.81±0.19	5.35±0.21	21.04±0.71	37.01±0.40	31.80±0.72	27.8
Md5	3.64±0.39	4.74±0.30	18.38±0.74	40.85±1.70	32.39±1.61	29.1
Md6	3.38±0.20	4.90±0.55	17.28±0.62	38.84±0.63	35.59±0.97	29.8
Md7	3.46±0.31	4.57±0.86	20.94±1.31	36.55±1.13	34.48±1.07	28.7
Md8	4.23±0.32	3.01±0.42	20.38±0.64	35.32±0.53	37.06±1.26	28.6

Md9	4.49±0.26	4.09±0.44	17.34±0.61	37.05±0.36	37.04±1.23	27.6
Md10	5.26±0.51	5.55±0.66	20.49±0.82	33.45±0.82	35.25±0.54	27.2
Md11	4.32±0.25	5.90±0.95	18.70±0.74	35.10±0.88	35.98±1.02	26.7
Md12	4.11±0.26	5.84±0.43	20.09±0.52	34.30±0.75	35.66±0.66	24.4
Medium	**4.34**	**5.01**	**19.81**	**36.62**	**34.22**	

Table 3.5. Composition and content of phospholipid classes according to 12 months of the year of M.platyphylla species

Sample symbol	**Content of phospholipid classes (%)**					**Sea water temperature (°C)**
	PI	PS	CAEP	PC	PE	
Mp1	6.28 ±0.02	7.99 ±0.01	17.64 ±0.25	35.10 ±0.32	32.99 ±0.29	25.3
Mp2	5.34 ±0.02	5.25 ±0.03	17.36 ±0.21	37.43 ±0.03	34.62 ±0.32	25.3
Mp3	4.98 ±0.03	5.33 ±0.02	20.39 ±0.16	37.15 ±0.51	32.16 ±0.36	28.0
Mp4	4.04 ±0.01	5.74 ±0.05	19.25 ±0.23	40.04 ±0.63	30.93 ±0.20	28.3
Mp5	4.14 ±0.04	5.85 ±0.08	18.16 ±0.22	40.14 ±0.16	31.71 ±0.23	28.1
Mp6	4.50 ±0.02	6.08 ±0.06	18.08 ±0.12	39.57 ±0.47	31.77 ±0.30	28.2
Mp7	5.70 ±0.02	6.01 ±0.02	16.89 ±0.28	39.38 ±0.39	32.02 ±0.22	28.4
Mp8	5.25 ±0.03	5.92 ±0.04	18.03 ±0.10	37.44 ±0.53	33.36 ±0.39	28.5
Mp9	5.55 ±0.01	5.29 ±0.02	16.36 ±0.36	38.91 ±0.23	33.89 ±0.12	28.3
Mp10	5.73 ±0.03	5.51 ±0.02	16.48 ±0.13	39.24 ±0.15	33.04 ±0.27	26.5
Mp11	6.04 ±0.06	8.85 ±0.06	19.49 ±0.15	34.43 ±0.36	31.19 ±0.28	25.7
Mp12	6.75 ±0.04	7.39 ±0.03	19.69 ±0.12	34.70 ±0.12	31.47 ±0.18	25.4
Medium	**5.36**	**6.27**	**18.15**	**37.79**	**32.43**	

In the polar phospholipids of two species of hydrocorals, there are phospholipid subclasses typical of Cnidarian animals, which are PE, PC, PS, PI, CAEP subclasses.

In the 5 phospholipid classes, the highest content is PC, PE, followed by CAEP, the lowest content is PS, PI.

Research on the variation in composition and content of phospholipid groups of *M.dichotoma* and *M.platyphylla* samples over 12 months of the year showed that , with *M.dichotoma* samples , the content of the main groups PE, PC changed significantly according to the season,

increasing in summer and decreasing in winter, the remaining groups CAEP, PS, PI had insignificant content variation over 12 months.
For the hydrocoral *M.platyphylla*, the variation in The content of polar phospholipid groups was negligible. This means that changes in environmental conditions during the year did not significantly affect the phospholipid classes in the *M. platyphylla* sample .

Therefore, from the results of this study, it can be assessed that the cell membrane structure of the hydrocoral *M. platyphylla* is more stable than the cell membrane structure of the *M. dichotoma species* , under environmental changes in natural living conditions during the study period.

3.1.4. Composition and content of fatty acids in total lipids of two Millepora species

The composition and content of fatty acids in two *Millepora* species were determined over 12 months using the method described in Section 2.2.3. The analysis results are presented in Table 3.6 and Table 3.7.

Results of analysis of composition and fatty acid content of *M. dichotoma* and *M.platyphylla species* according to 12 months of the year, the fatty acid composition of hydrocoral samples contained fatty acids with carbon chain lengths from C12 to C22.

M.dichotoma species :

- SFA acid group: Average content ranges from 30.08 - 48.06%. Palmitic acid C16:0 (16.09-26.09%) has the highest content, followed by C18:0 acid (5.60-16.96%), C20:0 acid has a lower content (2.14-3.67%), C12:0 and C22:0 acids only exist in trace form. In general, SFA content increases in summer and decreases in winter.
- MUFA acid group: 5 fatty acids have been identified including 16:1n-7 , 18:1n-9 ; 18:1n-7 , 20:1n-9 ; 22:1 with average content fluctuating in about 2.04 - 12.93%. 16:1n-7 acid accounts for the highest proportion of MUFAs (from 0.12-8.93%), this acid fluctuates seasonally, decreasing in the summer months and increasing sharply in the winter months. The remaining MUFAs do not fluctuate seasonally.
- PUFA acid group: Accounts for the highest proportion with content fluctuating between 44.59 - 56.03%, generally without large dispersion during the year.

M.platyphylla species :

- The content of unsaturated fatty acids ranges from 53.54 - 63.15%, dominant over saturated fatty acids (ranging from 27.99 - 45.92%).
- The PUFA acid composition has very low content of 20:4n-6 and 20:5n-3 fatty acids, very high content of 22:6n-3 acid and contains significant content of rare fatty acid 22:5n-6.
- There was no significant difference between months in SFA and MUFA acid content, this gap narrowed in summer months and increased in winter months.

Table 3.6. Composition and content (%) of fatty acids in total lipids over 12 months of M.dichotoma species

TT	Fatty acids	Md1	Md2	Md3	Md4	Md5	Md6	Md7	Md8	Md9	Md10	Md11	Md12
1	12:0	0.15 ± 0.01	0.14 ± 0.02	0.12 ± 0.01	0.12 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	0.14 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.16 ± 0.02	0.21 ± 0.04
2	14:0	3.07 ± 0.69	2.04 ± 0.31	2.14 ± 0.23	2.48 ± 0.18	1.46 ± 0.17	0.98 ± 0.28	1.91 ± 0.14	1.69 ± 0.13	1.54 ± 0.15	6.61 ± 0.91	7.35 ± 0.97	5.03 ± 0.90
3	15:0	0.21 ± 0.02	0.16 ± 0.02	0.15 ± 0.01	0.16 ± 0.01	0.10 ± 0.02	0.11 ± 0.01	0.16 ± 0.02	0.26 ± 0.05	0.16 ± 0.01	0.33 ± 0.03	0.32 ± 0.03	0.28 ± 0.02
4	16:0	22.02±1.03	21.35±1.06	21.84±1.03	22.91±1.39	25.90±1.20	24.63±0.97	24.72±1.17	21.97±1.11	26.09±1.41	21.81±0.81	18.63±1.15	16.09±1.10
5	16:1n-7	3.36 ± 0.95	0.33 ± 0.03	0.61 ± 0.10	1.28 ± 0.28	0.24 ± 0.02	0.12 ± 0.01	0.21 ± 0.03	0.26 ± 0.04	0.29 ± 0.04	8.93 ± 0.97	8.47 ± 0.96	6.02 ± 0.86
6	17:1	0.78 ± 0.19	0.66 ± 0.20	0.77 ± 0.23	0.76 ± 0.16	0.88 ± 0.29	1.09 ± 0.23	0.93 ± 0.17	1.05 ± 0.24	1.08 ± 0.23	2.66 ± 0.35	2.80 ± 0.34	3.05 ± 0.33
7	18:0	11.39±1.16	13.85±1.33	11.30±0.73	11.49±1.66	16.89±1.98	14.39±1.42	14.33±1.46	11.77±1.21	14.85±1.27	7.91±0.81	6.54±0.65	5.60±0.80
8	18:1n-9	2.13 ± 0.17	3.41 ± 0.75	3.72 ± 0.78	2.93 ± 0.63	1.30 ± 0.18	2.48 ± 0.28	1.49 ± 0.07	2.06 ± 0.19	1.56 ± 0.09	2.69 ± 0.20	2.14 ± 0.25	2.92 ± 0.20
9	18:1n-7	0.66 ± 0.11	0.29 ± 0.09	0.30 ± 0.03	0.30 ± 0.02	0.17 ± 0.02	0.07 ± 0.01	0.06 ± 0.01	0.15 ± 0.01	0.23 ± 0.02	1.47 ± 0.18	1.30 ± 0.17	1.24 ± 0.12
10	18:2n-6	1.55 ± 0.24	1.41 ± 0.31	2.12 ± 0.37	1.30 ± 0.12	0.73 ± 0.12	0.31 ± 0.03	1.04 ± 0.20	0.59 ± 0.16	1.61 ± 0.30	2.64 ± 0.32	2.27 ± 0.27	3.85 ± 0.40
11	18:3n-6	0.44 ± 0.10	0.32 ± 0.03	0.30 ± 0.04	0.23 ± 0.03	0.23 ± 0.02	0.17 ± 0.02	0.18 ± 0.02	0.19 ± 0.03	0.21 ± 0.03	0.68 ± 0.07	0.47 ± 0.04	1.09 ± 0.10
12	18:3n-3	0.25 ± 0.03	0.24 ± 0.02	0.19 ± 0.02	0.22 ± 0.01	0.21 ± 0.02	0.12 ± 0.01	0.12 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.00
13	18:4n-3	6.64 ± 0.71	5.82 ± 2.31	4.01 ± 1.70	3.46 ± 1.12	4.04 ± 0.73	4.43 ± 1.60	3.91 ± 0.94	4.71 ± 1.32	4.91 ± 1.24	4.53 ± 1.54	5.74 ± 1.91	8.18±2.40
14	20:0	2.73 ± 0.50	3.29 ± 0.48	2.45 ± 0.26	2.19 ± 0.31	3.27 ± 0.64	3.67 ± 0.59	2.71 ± 0.44	4.14 ± 0.65	3.56 ± 0.46	2.36 ± 0.29	3.02 ± 0.54	2.71 ± 0.38
15	20:1n-9	0.99 ± 0.05	0.82 ± 0.15	0.59 ± 0.04	0.44 ± 0.04	0.31 ± 0.02	0.54 ± 0.04	0.28 ± 0.02	0.65 ± 0.09	0.47 ± 0.08	0.25 ± 0.09	0.87 ± 0.10	0.45 ± 0.04
16	20:4n-6	1.27 ± 0.38	0.76 ± 0.20	1.25 ± 0.24	0.90 ± 0.09	1.10 ± 0.22	0.28 ± 0.06	0.47 ± 0.10	0.91 ± 0.30	0.71 ± 0.20	3.45 ± 0.60	1.97 ± 0.26	2.53 ± 0.22
17	20:4n-3	0.20 ± 0.01	0.15 ± 0.01	0.11 ± 0.01	0.16 ± 0.02	0.09 ± 0.01	0.19 ± 0.02	0.08 ± 0.01	0.10 ± 0.01	0.03 ± 0.01	0.19 ± 0.02	0.20 ± 0.02	0.18 ± 0.03
18	20:5n-3	2.71 ± 0.60	0.71 ± 0.20	1.09 ± 0.14	1.61 ± 0.20	0.38 ± 0.06	0.27 ± 0.04	0.39 ± 0.07	0.64 ± 0.13	0.50 ± 0.10	6.44 ± 0.80	9.07 ± 0.95	7.02 ± 0.70
19	22:0	0.24 ± 0.06	0.28 ± 0.07	0.22 ± 0.06	0.15 ± 0.02	0.29 ± 0.09	0.24 ± 0.05	0.20 ± 0.04	0.34 ± 0.07	0.31 ± 0.04	0.23 ± 0.04	0.05 ± 0.01	0.16 ± 0.02
20	22:1	0.31 ± 0.07	0.42 ± 0.04	0.38 ± 0.07	0.17 ± 0.03	0.03 ± 0.01	0.26 ± 0.05	0.13 ± 0.01	0.38 ± 0.06	0.38 ± 0.03	0.00	0.14 ± 0.01	0.22 ± 0.04
21	22:4n-6	3.12 ± 0.50	2.90 ± 0.46	3.52 ± 0.27	3.42 ± 0.33	3.01 ± 0.36	3.69 ± 0.25	3.14 ± 0.11	4.60 ± 0.28	3.35 ± 0.25	3.22 ± 0.26	4.13 ± 0.43	4.31 ± 0.45
22	22:5n-6	8.16 ± 0.91	7.69 ± 0.90	8.27 ± 0.63	8.23 ± 0.62	8.48 ± 0.91	9.51 ± 0.60	7.94 ± 0.61	11.90±0.78	10.10±0.76	7.99 ± 0.78	10.27±0.93	10.86±0.92
23	22:5n-3	0.66 ± 0.06	0.69 ± 0.11	0.90 ± 0.17	0.87 ± 0.13	0.53 ± 0.12	0.55 ± 0.15	0.65 ± 0.06	0.64 ± 0.10	0.33 ± 0.05	0.24 ± 0.08	0.56 ± 0.12	0.37 ± 0.05
24	22:6n-3	24.51 ± 2.42	32.23 ± 8.01	35.60 ± 5.34	31.14 ± 4.80	33.02 ± 8.53	28.41 ± 7.01	32.90 ± 7.42	30.81 ± 5.90	27.14 ± 5.24	19.33 ± 8.03	17.92 ± 6.52	15.20 ± 3.61

	SFA	**39.81**	**41.10**	**38.22**	**39.50**	**48.06**	**44.15**	**44.17**	**40.34**	**46.64**	**39.39**	**36.06**	**30.08**
	MUFA	**7.45**	**5.27**	**5.60**	**5.12**	**2.04**	**3.47**	**2.17**	**3.49**	**2.92**	**13.35**	**12.93**	**10.85**
	PUFA	**51.96**	**52.97**	**55.41**	**54.61**	**49.02**	**51.28**	**52.74**	**55.12**	**49.36**	**44.59**	**48.21**	**56.03**
	n-3	**37.43**	**39.89**	**39.96**	**40.54**	**35.47**	**37.32**	**39.96**	**36.93**	**33.39**	**26.61**	**29.09**	**33.38**
	n-6	**14.53**	**13.08**	**15.46**	**14.07**	**13.55**	**13.97**	**12.77**	**18,19**	**15.97**	**17.98**	**19.12**	**22.65**
	n-9	**3.11**	**4.24**	**4.31**	**3.37**	**1.61**	**3.01**	**1.77**	**2.70**	**2.03**	**2.95**	**3.01**	**3.37**
	n3/n6	**2.58**	**3.05**	**2.58**	**2.88**	**2.62**	**2.67**	**3.13**	**2.03**	**2.09**	**1.48**	**1.52**	**1.47**
	PUFA/SFA	**1.31**	**1.29**	**1.45**	**1.38**	**1.02**	**1.16**	**1.19**	**1.37**	**1.06**	**1.13**	**1.34**	**1.86**
	T (^{o}C)	**25.3**	**25.3**	**28**	**28.3**	**28.1**	**28.5**	**28.4**	**28.5**	**28.3**	**26.5**	**25.7**	**25.4**

Table 3.7. Composition and content (%) of fatty acids in total lipids over 12 months of M.platyphylla species

TT	**Fatty acids**	**Mp1**	**Mp2**	**Mp3**	**Mp4**	**Mp5**	**Mp6**	**Mp7**	**Mp8**	**Mp9**	**Mp10**	**Mp11**	**Mp12**
1	12:0	0.10 ±0.02	0.11 ±0.06	0.13 ±0.05	0.06 ±0.02	0.07 ±0.01	0.05 ±0.01	0.05 ±0.02	0.06 ±0.01	0.05 ±0.01	0.07 ±0.01	0.07 ±0.01	0.10 ±0.01
2	14:0	2.40 ±0.60	2.07 ±0.40	2.08 ±0.20	1.89 ±0.20	2.31 ±0.50	2.35 ±0.60	2.05 ±0.90	2.11 ±0.10	3.43 ±0.20	5.44 ±0.40	1.77 ±0.10	1.86 ±0.10
3	DMA	0.38 ±0.06	0.17 ±0.01	0.28 ±0.01	0.12 ±0.01	0.12 ±0.01	0.09 ±0.02	0.10 ±0.02	0.10 ±0.02	6.68 ±0.60	0.13 ±0.01	0.14 ±0.01	0.16 ±0.01
4	16:0	17.18 ±0.25	17.84 ±0.24	18.76 ±0.15	19.07 ±0.02	18.35 ±0.12	21.19 ±0.15	22.55 ±0.11	20.12 ±0.21	6.74 ±0.03	10.90 ±0.02	18.97 ±0.09	19.77 ±0.18
5	16:1n-7	2.90 ±0.03	0.12 ±0.01	0.12 ±0.01	0.25 ±0.02	0.29 ±0.01	0.12 ±0.01	0.06 ±0.02	0.39 ±0.02	0.14 ±0.02	1.63 ±0.01	0.44 ±0.02	0.44 ±0.02
6	17:1	0.87 ±0.04	0.85 ±0.01	0.65 ±0.04	0.86 ±0.02	0.66 ±0.06	0.78 ±0.02	0.75 ±0.06	0.83 ±0.05	0.93 ±0.05	1.48 ±0.02	1.53 ±0.02	1.39 ±0.03
7	DMA	1.51 ±0.05	1.48 ±0.02	1.76 ±0.03	1.45 ±0.05	1.32 ±0.02	0.97 ±0.08	0.45 ±0.01	0.36 ±0.02	0.79 ±0.05	0.95 ±0.04	0.38 ±0.02	1.20 ±0.01
8	18:0	12.82 ±0.61	13.95 ±0.23	16.27 ±0.12	16.59 ±0.05	15.78 ±0.33	16.63 ±0.64	18.74 ±0.51	15.67 ±0.82	17.01 ±0.45	10.13 ±0.50	12.29 ±0.21	14.16 ±0.52
9	18:1n-9	3.15 ±0.15	3.50 ±0.08	3.16 ±0.06	2.81 ±0.02	2.48 ±0.02	3.25 ±0.02	3.14 ±0.08	3.13 ±0.02	2.68 ±0.01	2.46 ±0.02	2.25 ±0.02	3.46 ±0.01
10	18:2n-6	1.74 ±0.03	0.71 ±0.08	0.35 ±0.02	0.57 ±0.05	0.66 ±0.04	0.94 ±0.05	0.38 ±0.02	0.66 ±0.01	0.56 ±0.05	1.24 ±0.01	1.55 ±0.01	0.91 ±0.05
11	18:3n-6	0.25 ±0.02	0.07 ±0.00	0.11 ±0.02	0.17 ±0.01	0.13 ±0.01	0.20 ±0.03	0.06 ±0.01	0.13 ±0.03	0.18 ±0.02	0.25 ±0.01	0.12 ±0.02	0.21 ±0.01
12	18:3n-3	0.23 ±0.02	0.12 ±0.01	0.20 ±0.02	0.08 ±0.01	0.10 ±0.01	0.18 ±0.01	0.09 ±0.01	0.10 ±0.02	0.08 ±0.00	0.19 ±0.01	0.17 ±0.01	0.24 ±0.01
13	18:4n-3	3.60 ±0.02	3.34 ±0.04	2.35 ±0.03	2.03 ±0.03	1.62 ±0.01	1.56 ±0.03	1.52 ±0.00	1.75 ±0.01	1.53 ±0.01	2.40 ±0.01	3.56 ±0.02	3.93 ±0.03

14	20:0	2.08 ±0.06	2.23 ±0.05	2.32 ±0.03	2.17 ±0.09	1.81 ±0.01	2.40 ±0.01	2.52 ±0.03	2.19 ±0.02	2.26 ±0.02	1.45 ±0.03	2.29 ±0.00	3.32 ±0.03
15	20:1n-9	1.58 ±0.01	1.52 ±0.02	0.80 ±0.05	0.64 ±0.00	0.71 ±0.02	0.50 ±0.03	0.36 ±0.02	0.38 ±0.02	0.52 ±0.03	0.81 ±0.03	0.91 ±0.03	1.55 ±0.02
16	20:4n-6	0.62 ±0.03	0.39 ±0.02	0.54 ±0.04	0.93 ±0.03	0.69 ±0.05	1.26 ±0.01	0.61 ±0.02	0.85 ±0.05	1.14 ±0.08	1.11 ±0.05	0.54 ±0.02	0.34 ±0.02
17	20:4n-3	0.10 ±0.01	0.10 ±0.01	0.08 ±0.00	0.08 ±0.00	0.08 ±0.01	0.09 ±0.01	0.08 ±0.01	0.08 ±0.01	0.09 ±0.01	0.14 ±0.01	0.07 ±0.01	0.07 ±0.01
18	20:5n-3	2.20 ±0.03	1.57 ±0.01	0.96 ±0.05	1.26 ±0.02	1.18 ±0.02	0.77 ±0.05	0.70 ±0.03	0.94 ±0.02	0.83 ±0.02	2.98 ±0.01	1.58 ±0.05	1.21 ±0.05
19	22:4n-6	3.57 ±0.02	3.68 ±0.03	3.52 ±0.01	3.30 ±0.04	2.85 ±0.02	3.07 ±0.03	2.67 ±0.01	2.99 ±0.01	3.01 ±0.03	3.38 ±0.04	3.94 ±0.01	4.55 ±0.01
20	22:5n-6	7.50 ±0.12	7.03 ±0.13	6.55 ±0.12	6.65 ±0.16	6.22 ±0.18	6.13 ±0.15	5.59 ±0.29	5.78 ±0.38	6.01 ±0.12	6.99 ±0.24	8.33 ±0.27	8.39 ±0.04
21	22:5n-3	0.75 ±0.05	0.91 ±0.08	1.10 ±0.08	1.08 ±0.05	0.96 ±0.06	0.92 ±0.05	0.91 ±0.05	1.09 ±0.09	0.96 ±0.04	0.82 ±0.003	0.70 ±0.03	0.81 ±0.03
22	22:6n-3	34.06 ±0.13	35.45 ±0.12	36.45 ±0.85	36.52 ±0.34	40.70 ±0.25	36.18 ±0.37	36.60 ±0.54	38.42 ±0.15	36.91 ±0.68	36.84 ±0.14	34.56 ±0.55	30.21 ±0.24
	SFA	**34.58**	**36.20**	**39.56**	**39.78**	**38.32**	**42.62**	**45.91**	**40.15**	**29.49**	**27.99**	**35.39**	**39.21**
	MUFA	**7.63**	**5.14**	**4.08**	**3.70**	**3.48**	**3.87**	**3.56**	**3.90**	**3.34**	**4.90**	**3.60**	**5.45**
	PUFA	**54.62**	**53.37**	**52.21**	**52.67**	**55.19**	**51.30**	**49.21**	**52.79**	**51.30**	**56.34**	**55.12**	**50.87**
	n-3	**40.94**	**41.49**	**41.14**	**41.05**	**44.64**	**39.70**	**39.90**	**42.38**	**40.40**	**43.37**	**40.64**	**36.47**
	n-6	**13.68**	**11.88**	**11.07**	**11.62**	**10.55**	**11.60**	**9.31**	**10.41**	**10.90**	**12.97**	**14.48**	**14.40**
	n-9	**4.73**	**5.02**	**3.96**	**3.45**	**3.19**	**3.75**	**3.50**	**3.51**	**3.20**	**3.27**	**3.16**	**5.01**
	n3/n6	**2.99**	**3.49**	**3.72**	**3.53**	**4.23**	**3.42**	**4.29**	**4.07**	**3.71**	**3.34**	**2.81**	**2.53**
	PUFA/SFA	**1.58**	**1.47**	**1.32**	**1.32**	**1.44**	**1.20**	**1.07**	**1.31**	**1.74**	**2.01**	**1.56**	**1.30**
	T (°C)	25.3	25.3	28	28.3	28.1	28.5	28.4	28.5	28.3	26.5	25.7	25.4

3.2. Determination of molecular species in polar phospholipid class

Proceed studying and determining the molecular species in the polar phospholipid layer in two species of hydrocoral *M.dichotoma* and *M.platyphylla* , we identified 155 molecular species, of which 77 molecular species in the polar phospholipid layer for *M.dichotoma* belong to the glycerophospholipid and sphingophospholipid groups: PA (2), PE (16), PC (18), PS (10), PI (20), CAEP (11); 78 molecular species for *M.platyphylla* belong to the groups PA (2), PE (18), PC (16), PS (9), PI (21), CAEP (12).

The results of the identification of molecular species in polar phospholipid are presented in the following tables:

Table 3.8. Molecular species of PA identified in Md and Mp samples

Symbol	**Molecular species**	**[M-H]⁻ *m/z***		**Δ m/z ppm**	**Molecular fomular**	**Content %**	
		Actual measurement	Calculate			**Md**	**Mp**
PA 42:4	20:0/22:4	779.5561	780.5669	-3.5	$C_{45}H_{81}O_8P$	99,386	-
PA 38:5	18:5e/20:0	707.5046	708.5094	-2.5	$C_{41}H_{75}O_7P$	0.001	-

Table 3.9. Molecular species of PE identified in Md and Mp samples

Symbol	**Molecular species**	**[M-H]⁻ *m/z***		**Δ m/z ppm**	**Molecular fomular**	**Content (%)**	
		Actual measurement	Calculate			**Md**	**Mp**
PE 35:2	18:1e/17:1	714.5442	714.5443	-0.1	$C_{40}H_{78}NO_7P$	-	2.46
PE 36:2	17:1/19:1	742.5371	742.5392	-2.1	$C_{41}H_{78}NO_8P$	-	2.22
PE 38:6	16:1e/22:5	748.5270	748.5286	-1.6	$C_{43}H_{76}NO_7P$	20.82	7.54
PE 38:5	16:0e/22:5	750.5405	750.5443	-3.8	$C_{43}H_{78}NO_7P$	7.27	3.43
PE 39:6	17:1e/22:5	762.5413	762.5443	-3.0	$C_{44}H_{78}NO_7P$	1.29	1.48
PE 40:7	18:1e/22:6	774.5410	774.5443	-3.3	$C_{45}H_{78}NO_7P$	3.76	1.80
PE 40:6	18:1e/22:5	776.5552	777.5672	-4.8	$C_{45}H_{80}NO_7P$	37.19	39.07
PE 41:6	19:1e/22:5	790.5720	790.5756	-3.6	$C_{46}H_{82}NO_7P$	0.48	2.95
PE 40:6	18:1/22:5	790.5370	790.5392	-2.2	$C_{45}H_{78}NO_8P$	1.03	1.78
PE 40:6	18:1/22:5	790.5344	790.5392	-4.8	$C_{45}H_{78}NO_8P$	6.15	0.66
PE 40:6	18:0/22:6	790.5422	790.5392	3.0	$C_{45}H_{78}NO_8P$	0.49	0.45
PE 40:5	18:0/22:5	792.5549	792.5549	0.0	$C_{45}H_{80}NO_8P$	0.33	1.01
PE 40:4	18:0/22:4	794.5659	794.5705	-4.6	$C_{45}H_{82}NO_8P$	2.39	1.50
PE 41:6	19:1/22:5	804.5518	804.5549	-3.1	$C_{46}H_{80}NO_8P$	9.59	18.56
PE 42:6	20:1/22:5	818.5663	818.5705	-4.2	$C_{47}H_{82}NO_8P$	6.10	13.60
PE 42:4	20:0/22:4	822.5940	822.6018	-7.8	$C_{47}H_{86}NO_8P$	3.11	1.49
Total						*100.0*	*100.0*

LPE 22:5	lyso 22:5//	526.2905	526.2939	-3.4	$C_{27}H_{46}NO_7P$	75.89	96.00
LPE 22:6	lyso 22:6//	524.2730	524.2783	-5.3	$C_{27}H_{44}NO_7P$	4.92	4.00
Not determined						19.19	-
Total						*100.0*	*100.0*

Table 3.10. Molecular species of PC identified in Md and Mp samples

Symbol	Molecular species	[M+H] + *m/z*		Δ *m/z* ppm	Molecular fomular	Content (%)	
		Actual measurement	Calculate			Md	Mp
PC 37:6	15:1e/22:5	778.5818	778.5745	7.3	$C_{45}H_{80}NO_7P$	0.94	0.29
PC 38:6	16:0e/22:6	792.5894	793.5985	-0.8	$C_{46}H_{82}NO_7P$	31.89	31.91
	16:1e/22:5						-
PC 38:5	16:0e/22:5	794.6051	794.6060	-0.9	$C_{46}H_{84}NO_7P$	24.65	35.52
PC 38:6	16:0/22:6	806.5693	806.5695	-0.2	$C_{46}H_{80}NO_8P$	10.64	10.09
PC 38:5	16:0/22:5	808.5829	808.5851	-2.2	$C_{46}H_{82}NO_8P$	5.43	6.19
PC 40:6	18:0e/22:6	820.6146	820.6215	-6.9	$C_{48}H_{86}NO_7P$	7.79	4.43
	18:1e/22:5						-
PC 40:5	18:0e/22:5	822.6338	822.6371	-3.3	$C_{48}H_{88}NO_7P$	12.37	8.44
PC 40:7	18:1/22:6	832.5787	832.5851	-6.4	$C_{48}H_{82}NO_8P$	1.23	0.88
PC 40:6	18:0/22:6	834.5922	834.6008	-8.6	$C_{48}H_{84}NO_8P$	5.06	2.26
Total						*100.00*	*100.0*
LPC 16:0	lyso 16:0e//	482.3598	482.3605	-0.7	$C_{24}H_{52}NO_6P$	54.53	56.51
LPC 16:0	lyso 16:0//	496.3424	496.3398	2.6	$C_{24}H_{50}NO_7P$	3.24	1.90
LPC 18:0	lyso 18:0e//	510.3906	510.3918	-1.2	$C_{26}H_{56}NO_6P$	15.19	17.32
LPC 18:0	lyso 18:0//	524.3681	524.3711	-3.0	$C_{26}H_{54}NO_7P$	3.02	2.29
LPC 20:0	lyso 20:0//	552.3997	552.4024	-2.7	$C_{28}H_{58}NO_7P$	16.10	6.24
LPC 22:6	lyso 22:6//	568.3387	568.3398	-1.1	$C_{30}H_{50}NO_7P$	3.45	4.40
LPC 22:5	lyso 22:5//	570.3569	570.3554	1.5	$C_{30}H_{52}NO_7P$	4.47	11.34
Total						*100.00*	*100.0*

Table 3.11. Molecular species of PS identified in Md and Mp samples

Symbol	Molecular species	[M-H] - *m/z*		Δ *m/z* ppm	Molecular fomular	Content (%)	
		Actual measurement	Calculate			Md	Mp
PS 40:5	18:0/22:5	836.5369	836.5447	-7.8	$C_{46}H_{80}NO_{10}P$	0.63	-
PS 40:4	18:0/22:4	838.5530	838.5603	-7.3	$C_{46}H_{82}NO_{10}P$	4.57	6.40
PS 41:4	19:0/22:4	852.5719	852.5760	-4.1	$C_{47}H_{84}NO_{10}P$	1.98	2.09
PS 42:5	20:0/22:5	864.5698	864.5760	-6.2	$C_{48}H_{84}NO_{10}P$	11.26	16.00
PS 42:4	20:0/22:4	866.5862	867.5991	-1.0	$C_{48}H_{86}NO_{10}P$	79.03	72.97

PS 43:4	21:0/22:4	880.6031	880.6073	-4.2	$C_{49}H_{88}NO_{10}P$	2.54	2.54
Total						*100.00*	
LPS 18:0	Lyso 18:0//	524.2972	524.2994	-2.2	$C_{24}H_{48}NO_{9}P$	1.54	5.88
LPS 19:0	Lyso 19:0//	538.3081	538.3150	-6.9	$C_{25}H_{50}NO_{9}P$	0.34	2.34
LPS 20:0	Lyso 20:0//	552.3261	552.3307	-4.6	$C_{26}H_{52}NO_{9}P$	95.73	85.04
LPS 21:0	Lyso 21:0//	566.3442	566.3463	-2.1	$C_{27}H_{54}NO_{9}P$	2.39	6.74
Total						*100.00*	*100.00*

Table 3.12. Molecular species of PI identified in Md and Mp samples

Symbol	**Molecular species**	**[M-H]$^-$ *m/z***		**Δ *m/z* ppm**	**Molecular fomular**	**Content (%)**	
		Actual measurement	Calculate			**Md**	**Mp**
PI 32:0	16:0e/16:0	795.5340	795.5393	-5.3	$C_{41}H_{81}O_{12}P$	0.27	0.48
PI 33:1	16:0e/17:1	807.5343	807.5393	-5.0	$C_{42}H_{81}O_{12}P$	0.13	0.57
PI 34:0	18:0e/16:0	823.5662	823.5706	-4.4	$C_{43}H_{85}O_{12}P$	0.38	-
PI 34:2	16:0/18:2	833.5138	833.5185	-4.7	$C_{43}H_{79}O_{13}P$	0.07	0.39
PI 35:1	18:0e/17:1	835.5691	835.5706	-1.5	$C_{44}H_{85}O_{12}P$	1.17	1.94
PI 36:4	16:0/20:4	857.5154	857.5181	-2.7	$C_{44}H_{85}O_{12}P$	-	0.30
PI 38:5	16:0e/22:5	869.5544	869.5549	-0.5	$C_{47}H_{83}O_{12}P$	1.40	1.94
PI 38:6	16:0/22:6	881.5176	881.5185	-0.9	$C_{47}H_{79}O_{13}P$	6.58	3.48
PI 38:5	18:0/20:5	883.5282	883.5342	-6.0	$C_{47}H_{81}O_{13}P$	-	1.10
PI 38:4	18:0/20:4	885.5450	885.5498	-4.8	$C_{47}H_{83}O_{13}P$	-	1.00
PI 40:5	18:0e/22:5	897.5815	897.5862	-4.7	$C_{49}H_{87}O_{12}P$	1.84	1.53
PI 40:4	18:0e/22:4	899.5961	899.6019	-5.8	$C_{49}H_{89}O_{12}P$	0.99	-
PI 40:7	18:1/22:6	907.5277	907.5342	-6.5	$C_{49}H_{81}O_{13}P$	0.70	0.36
PI 40:6	18:0/22:6	909.5436	909.5498	-6.2	$C_{49}H_{83}O_{13}P$	5.31	3.72
PI 40:5	18:0//22:5	911.5589	911.5655	-6.6	$C_{49}H_{85}O_{13}P$	3.19	3.52
PI 40:4	18:0/22:4	913.5772	913.5811	-3.9	$C_{49}H_{87}O_{13}P$	8.55	14.66
PI 41:4	19:0/22:4	927.5918	927.5968	-5.0	$C_{50}H_{89}O_{13}P$	2.88	4.18
PI 42:6	20:0/22:6	937.5736	937.5811	-7.5	$C_{51}H_{87}O_{13}P$	0.40	0.53
PI 42:5	20:0/22:5	939.5932	939.5968	-3.6	$C_{51}H_{89}O_{13}P$	5.68	5.39
PI 42:4	20:0/22:4	941.6092	941.6133	-4.1	$C_{51}H_{91}O_{13}P$	58.97	53.80
PI 43:4	21:0/22:4	955.6212	955.6281	-6.9	$C_{52}H_{93}O_{13}P$	1.39	0.96
Not determined						0.10	
Total						*100*	
LPI 20:0	Lyso 20:0//	627.3415	627.3515	-10.0	$C_{29}H_{57}O_{12}P$	84.87	85.50
LPI 18:0	Lyso 18:0//	599.3140	599.3202	-6.2	$C_{27}H_{53}O_{12}P$	15.13	14.50
Total						*100*	*100*

Table 3.13. Molecular species of CAEP identified in Md and Mp samples

Symbol	**Molecular species**	**[M-H]$^-$ *m/z***		**Δ *m/z* ppm**	**Molecular fomular**	**Content (%)**	
		Actual measurement	Calculate			**Md**	**Mp**

CAEP 33:2	18:2b/15:0	627.4842	627.4871	-2.9	$C_{35}H_{69}N_2O_5P$	1.90	0.99
CAEP 33:0	18:0b/15:0	631.5170	631.5184	-1.4	$C_{35}H_{73}N_2O_5P$	1.11	0.29
CAEP 34:2	18:2b/16:0	641.4962	641.5028	-6.6	$C_{36}H_{71}N_2O_5P$	38.62	40.04
CAEP 34:1	18:1b/16:0	643.5115	643.5184	-6.9	$C_{36}H_{73}N_2O_5P$	31.84	40.13
CAEP 34:0	18:0b/16:0	645.5311	645.5341	-3.0	$C_{36}H_{75}N_2O_5P$	14.52	8.63
CAEP 35:3	19:3b/16:0	653.4944	653.5028	-8.4	$C_{37}H_{71}N_2O_5P$	0.56	0.99
CAEP 35:2	18:2b/17:0	655.5098	655.5184	-8.6	$C_{37}H_{73}N_2O_5P$	2.54	1.96
CAEP 35:1	19:1b/16:0	657.5281	657.5341	-6.0	$C_{37}H_{75}N_2O_5P$	4.34	3.69
CAEP 35:0	18:0b/17:0	659.5460	659.5497	-3.7	$C_{37}H_{77}N_2O_5P$	1.46	0.52
CAEP 36:1	18:1b/18:0	671.5466	671.5497	-3.1	$C_{38}H_{77}N_2O_5P$	0.72	0.47
CAEP 38:2	18:2b/20:0	697.5621	697.5654	-3.3	$C_{40}H_{79}N_2O_5P$	1.34	0.28
Not determined						1.05	0.96
Total						*100*	

The results of phospholipid molecular species identification also showed that in the two hydrocoral species *M.dichotoma* and *M.platyphylla* , the GPL forms 1- *O* -Alkyl-2-acyl, 1- *O* -alkenyl-2-acyl (plasmalogen) and 1,2-diacyl were present. The proportions of these lipid types varied greatly depending on each phospholipid group. When comparing the acyl/alkylglycerol group composition of the GPL class pairs, we found a close similarity between the molecular species of the PS and PI groups (Figure 3.2). In addition, there was no similarity between the PS and PC molecular species in terms of acyl/alkylglycerol group composition found in both *Millepora* species . The data also did not clarify the relationship for other GPL class pairs.

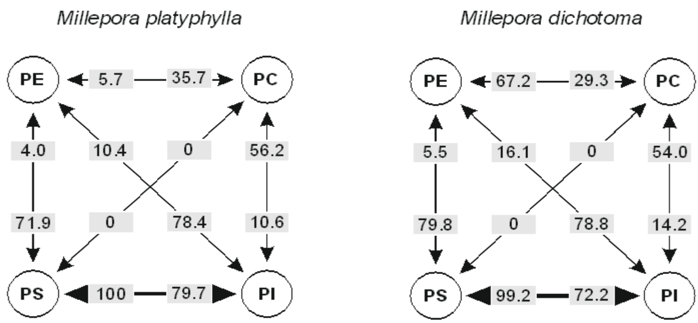

Figure 3.2. Similarity of acyl/alkylglycerol group composition of glycerophospholipid layer pairs in M.dichotoma and M.platyphylla species

3.3. Chlorophyll A content in Millepora samples over 12 months

Two species of hydrocoral *Millepora dichotoma* and *Millepora platyphylla* were analyzed for chlorophyll A content using the method of Parsons et al. (1984). The results are presented in Table 3.14.

Table 3.14. Chlorophyll A content in Millepora samples in 12 months

Month	*M. dichotoma*		*M. platypylla*	
	Chl Md	**T ºC**	**Chl Mp**	**T ºC**
1	3.91	24.9	4.56	25.3
2	3.82	25.4	3.17	25.3
3	3.59	25.3	2.84	28.0
4	4.41	27.3	3.76	28.3
5	2.21	28.7	2.64	28.1
6	2.77	28.8	2.73	28.5
7	1.92	28.4	3.15	28.4
8	1.36	28.5	2.45	28.5
9	2.54	28.4	2.60	28.3
10	3.49	27.7	2.98	26.5
11	6.76	26.8	6.14	25.7
12	4.02	25.4	2.67	25.4

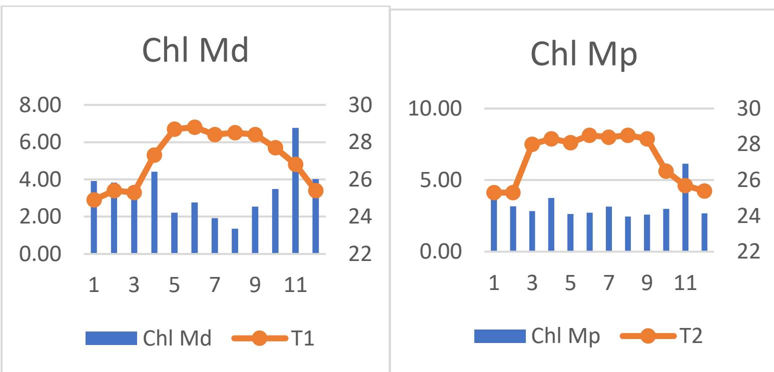

Figure 3.3. Chlorophyll A content in M. dichotoma and M. platypylla in 12 months

Many members of the phylum Cnidaria, including hard corals, soft corals, sea anemones, and hydrocorals, are hosts to unicellular endosymbionts (i.e., zooxanthellae) of the family *Symbiodiniaceae* . These symbioses are often obligate and are important to coral reef ecosystems because they promote the growth of reef- forming calcifying corals. Zooxanthellae contribute up to 95% of the energy requirements of scleractinian corals, providing calcium for calcification, while the host coral supplies nutrients from food sources to its symbiont. Like scleractinian corals, hydrocorals are heterotrophic on a variety of food sources, primarily on plankton, and rely on symbioses with Symbiodiniaceae algae for autotrophy and calcification.

Chart Figure 3.4 shows that, for the Millepora samples studied, the total content of fatty acids 18:1n-7, 18:1n-9, 20:5n-3, 22:6n-3 decreased in November-December, with the lowest value in December. This demonstrates that the heterotrophic food source from plankton of *Millepora* species decreased during this time of the year studied.

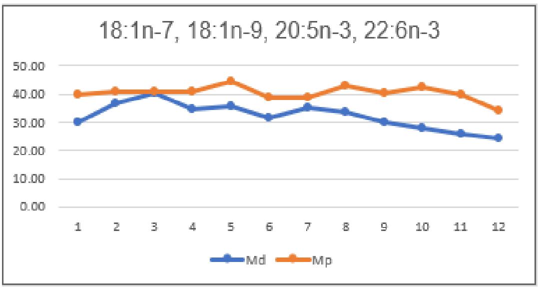

Figure 3.4. Total content of fatty acids 18:1n-9, 18:1n-7, 20:5n-3, 22 :6n-3 in the sample Millepora

From the results, the chlorophyll content in the two samples was similar, the 12-month samples tended to increase from October to April, and increased sharply in October-November. This result is consistent with previous analyses when recording an increase in winter and a decrease in summer of the PL and ST layers, fatty acids 18:4n-3, 18:5n-3 are typical acids for lipids of zooxanthellae algae.

CONCLUSION AND RECOMMENDATIONS

CONCLUSION

1. This is the first comprehensive study on lipidomic of two species of hydrocoral *Millepora dichotoma* and *Millepora platyphylla* collected in Nha Trang sea, Vietnam .

1.1 The total lipid content of two species of hydrocoral *M.dichotoma* and *M.platyphylla* was determined . The total lipid content of the two species *Md* and *Mp* fluctuated between 0.32-0.64% and 0.26 - 0.63%, respectively, compared to dry weight.

1.2. The total lipid composition of the two studied samples was determined to include 6 classes of substances: polar lipids (PoL), sterols (ST); waxy hydrocarbons (HW), triacylglycerols (TAG), monoalkyldiacylglycerols (MADAG) and free fatty acids (FFA). In *M.dichotoma* , the reserve lipid class including TAG (26.96%), MADAG (22.33%), HW (20.93%) had the highest content, followed by the structural lipid class including PoL (21.78%), ST (9.72%) and FFA (2.03%). In *M.platyphylla* , the content of lipid classes was as follows: TAG (31.12%), MADAG (21.12%), HW (22.21%), followed by PoL (15.35%), ST (7.89%) and FFA (1.32%).

1.3. The polar lipid classes in the hydrocoral *Millepora dichotoma* and *Millepora platyphylla* samples have been identified. These include characteristic subclasses of the phylum *Cnidaria*, specifically the phospholipid subclasses: phosphatidic acid (PA), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), and one phosphonolipid, ceramide aminoethylphosphonate (CAEP). In both *Millepora* species, PC and PE were the most abundant, followed by CAEP, while PS and PI had the lowest concentrations.

1.4. The composition and content of fatty acids in two hydrocoral species of the genus Millepora have been identified. Both species contain fatty acids with carbon chains ranging from C12 to C22, including valuable ω3/ω6 polyunsaturated fatty acids (PUFAs) such as 18:4n-3, 20:4n-6, 20:5n-3, 22:5n-6, and 22:6n-3. The presence of 18:4n-3 fatty acid confirms the existence of symbiotic microalgae (Zooxanthellae) in the analyzed Millepora samples. Additionally, the detection of 22:6n-3 in significant amounts suggests that hydrocorals obtain this fatty acid from a heterotrophic food source, specifically planktonic organisms.

2. For the first time, the polar phospholipid molecular species of two species of hydrocoral *M. dichotoma* and *M. platyphylla* were identified in the Nha Trang sea, Vietnam, with 155 polar phospholipid molecular species identified, of which: in M. *dichotoma* , 77 polar phospholipid molecular species were identified, including: PA (2), PE (16), PC (18), PS (10), PI (20), CAEP (11); in *M. platyphylla* , 78 molecular species, including: PA (2), PE (18), PC (16), PS (9), PI (21), CAEP (12). Scientific basis for the close similarity between molecular species of PS - PI groups and dissimilarity between PS - PC group in acyl/alkylglycerol composition in both *Millepora* species has been provided .

3. The first survey of the 12-month variation in lipid composition of two Vietnamese hydrocoral species *M. dichotoma* and *M.platyphylla.* Results:

-The total lipid content and the composition of the lipid classes in the total lipids, including structural lipids and reserve lipids, of the studied samples of *M.dichotoma* species tended to increase in the first months of the year (summer) and decrease in the last months of the year (winter).

-The composition of fatty acids and polar lipid groups varied according to species. For *M. dichotoma* , the content of SFA, PE, PC increased in summer months and decreased in winter months. For M. *platyphylla,* this variation was insignificant.

4. Initial investigation of chlorophyll A content in 2 *Millepora* samples to evaluate the symbiotic relationship between host coral and *zooxanthellea* symbiotic microorganisms.

- Chlorophyll A content fluctuated according to the months of the year in inverse proportion to the fluctuation in total lipid content of the two *Millepora* species studied: decreased in the summer months and increased in the winter months.

- Determined that 18:4n-3 and 18:5n-3 acids are typical fatty acids for lipids of *zooxanthellae* symbiotic microalgae.

RECOMMENDATIONS

Further studies on seasonal variations in coral lipid composition over many years are needed to build a comprehensive database, providing a scientific basis for assessing the relationship between lipid content and overall health and resilience of corals to the effects of marine pollution and climate change.

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

1. **Nguyen Ba Kien** , Trinh Thi Thu Huong, Luu Van Huyen, Nguyen Thanh Vinh, Tran Duy Phong, Dang Thi Minh Tuyet, Nguyen Thi Nga, Dang Thi Phuong Ly, Pham Quoc Long (2019), *Study on total lipid content, lipid class composition of some fire and soft corals collected in Nha Trang, Vietnam* , Vietnam Journal of Marine Science and Technology. 19(1), pp. 87-91.

2. Imbs, AB , Dang, LPT, **Nguyen, KB** . (2019), *Comparative lipidomic analysis of phospholipids of hydrocorals and corals from tropical and cold-water regions* , PLoS One. 14(4), p. e0215759.

3. Imbs, AB , Dang, LPT, **Nguyen, K. B** ., Luu, HV, Pham, LQ (2020). *Annual dynamics of the composition of polar lipids, storage lipids, and fatty acid markers in the hydrocoral Millepora dichotoma Forskål, 1775 from coastal waters of Vietnam* . Russian Journal of Marine Biology, 46(3), pp. 221-225.

4. Dang Thi Phuong Ly, **Nguyen Ba Kien** , Dang Thi Minh Tuyet, Trinh Thi Thu Huong. Pham Quoc Long, Andrey Imbs Borisovich (2021), *Yearly dynamics of the content and composition of total lipid and lipid classes in the hydrocoral Millepora platyphylla from coastal water of Nha Trang, Khanh Hoa, Vietnam*, Vietnam Journal of Marine Science and Technology. 21(4), pp. 533-541