

Fatty Acid, Lipid Class, and Phospholipid Molecular Species Composition of the Soft Coral *Xenia* sp. (Nha Trang Bay, the South China Sea, Vietnam)

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Abstract The soft corals of the genus *Xenia* are common for Indo-Pacific reef ecosystems. Lipid class, fatty acid (FA), phospho- and phosphonolipid molecular species compositions were identified for the first time in the soft coral *Xenia* sp. from Vietnam. Total lipids consisted predominantly of waxes, monoalkyl diacylglycerols, triacylglycerols, sterols, and polar lipids (21.4, 7.7, 14.2, 10.5, and 36.7 %, respectively). Sesquiterpene alcohol, valerenenol, was found. Acids 16:0, 18:3n-6, 20:4n-6, and 20:5n-3 dominated in total FA. The markers of zooxanthellae (18:4n-3 and 18:5n-3) and octocorals (24:5n-6 and 24:6n-3) were detected. Acids 18:5n-3, 20:4n-6, 22:4n-6, and 24:5n-6 concentrated in FA of polar lipids, whereas 14:0, 16:0, 16:1n-7, 18:2n-6, and 18:3n-6 were the major FA of neutral lipids. ChoGpl, EtnGpl, SerGpl, CAEP, PtdIns, and lyso ChoGpl constituted 39.5, 20.8, 20.5, 9.7, 4.3, and 5.3 %, respectively, of the sum of phospho- and phosphonolipids. Thirty-two molecular species of phospholipids and ceramide aminoethylphosphonate (CAEP) were determined by high resolution tandem mass spectrometry. Lyso 18:0e PakCho (4.1 %), 18:0e/20:4 PakCho (20.5 %), 18:1e/20:4 PlsEtn (18.0 %), 18:0e/24:5 PakSer (14.0 %),

and 16:0 CAEP (9.6 %) were the major molecular species. EtnGpl and PtdIns mainly consisted of alkenyl acyl and diacyl forms, respectively. Alkyl acyl forms predominated in ChoGpl and SerGpl. Acid 24:5n-6 was a principal FA in SerGpl, whereas 20:4n-6 was more abundant in ChoGpl and EtnGpl. PtdIns contained various C_{20–24} PUFA. In the context of chemotaxonomy of corals, *Xenia* sp. has the lipid composition typical for soft corals and the FA profile similar to that of alcyonarians with the high level of 18:3n-6.

Keywords Soft corals · *Xenia* · Fatty acids · Lipid classes · CAEP · Phospholipids · Molecular species · Valerenenol · Chemotaxonomy · High resolution mass spectrometry

Abbreviations

APCI	Atmospheric pressure chemical ionization
CAEP	Ceramide aminoethylphosphonate
ChoGpl	Choline glycerophospholipids
DMA	Dimethylacetals
DMOX4	4-Dimethylloxazoline
ESI	Electrospray ionization
EtnGpl	Ethanolamine glycerophospholipids
FA	Fatty acids
FAME	Fatty acid methyl esters
FFA	Free fatty acids
GC	Gas chromatography
GC–MS	Gas chromatography–mass spectrometry
HRMS	High resolution mass spectrometry
HPLC–HRMS	High performance liquid chromatography–high resolution mass spectrometry
lyso ChoGpl	Lyso choline glycerophospholipids
MADAG	Monoalkyl diacylglycerols
NL	Neutral lipids

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NMR	Nuclear magnetic resonance
PakCho	1- <i>O</i> -Alkyl-2-acyl- <i>sn</i> -glycero-3-phosphocholine
PakEtn	1- <i>O</i> -Alkyl-2-acyl- <i>sn</i> -glycero-3-phosphoethanolamine
PCA	Principal components analysis
PL,	Phospholipids
PlsCho	Choline plasmalogen
PlsEtn	Ethanolamine plasmalogen
PoL	Polar lipids
PtdCho	Phosphatidylcholine
PtdEtn,	Phosphatidylethanolamine
PtdIns	Phosphatidylinositol
PtdSer	Phosphatidylserine
PUFA	Polyunsaturated fatty acids
SerGpl	Serine glycerophospholipids
ST	Sterols
TAG	Triacylglycerols
TL	Total lipids
TLC	Thin-layer chromatography
WE	Wax esters

Introduction

The corals of Vietnam are an essential component of Indo-Pacific reef ecosystems that extend across the Pacific and Indian oceans from the eastern coast of Africa to the western coast of America. Many of the coral species, which occur in Vietnam, live so far away as in Hawaii, the Great Barrier Reef of Australia, Seychelles, and the Red Sea. The abundant *Xenia* species are common in the shallow-waters of Vietnam and other Indo-Pacific regions [1–3]. *Xenia* is the genus of zooxanthellate soft corals resembled a mushroom with “arms” coming out from the top and each of them crowned by many-fingered “hands”. *Xenia* is unique among corals because of the ability to use its “hands” to “pulse” in constant grabbing motion.

The chemical studies of *Xenia* species have mainly focused on low molecule weight secondary metabolites with potential biological activity [4]. For example, the genus *Xenia* was shown to be a rich source of diterpenoids [5]. Detailed information on the fatty acid (FA) and lipid compositions of many soft corals was published [6, 7], whereas only one attempt of the analysis of FA composition of *Xenia umbellata* was made [8]. Except for *X. umbellata*, none of numerous *Xenia* species (52 species according to world register of marine species [9]) was characterized by their lipid compositions.

Total lipids (TL) constitute up to 40 % of the dry biomass of corals [10, 11]. Lipids serve as long-term energy stores in corals [10, 12] and are involved in the majority of biochemical and physiological processes (ref. [13]).

Changes in the lipid composition reflect changes in the ecology, nutrition, and health of these animals. For example, the composition of TL depends on the currently prevailing food of corals [14], light regimes [15], and varies during the annual cycle [16]. The level of stored lipids significantly reduced in tumorous coral tissue [17]. Lipids and their FA constitute a part of organic carbon translocated between symbiotic dinoflagellates (zooxanthellae) and coral tissues in symbiotic coral species [18–20]. The partial or complete loss of zooxanthellae by the coral host (a dangerous phenomenon termed coral bleaching) is accompanied by significant variations in total lipid content and lipid class proportions in coral colonies [13, 21–23]. FA play an important role in the regulation of coral metabolism [24] and stress resistance [25]. Total lipid composition can be used as a chemotaxonomic indicator of the coral class or subclass, while FA profiles may serve as chemotaxonomic markers of particular coral families or genera [7, 26, 27].

To extend our knowledge in the field of ecology and biochemistry of corals, we examined total lipid, lipid class, phospholipid, and FA composition of the soft coral *Xenia* sp. collected in the shallow waters of Vietnam. In order to clarify the chemotaxonomic relationships in corals, lipid and FA profiles of *Xenia* sp. and other corals from Vietnam were compared. To our knowledge, there are no data on lipid molecular species of corals. We therefore analyzed the structure and content of molecular species of phospholipids of *Xenia* sp. by tandem mass spectrometry. The accurate quantification of individual molecular species and detection of characteristic fragment ions are necessary to begin bottom-up lipidomics analysis of corals.

Materials and Methods

Study Site and Sampling

The colonies of the soft coral *Xenia* sp. (Anthozoa, Octocorallia, Alcyonacea, Xenidiidae) were collected in July 2013 at a depth of 1.5–2 m on the southeast reefs of Rua Island, Nha Trang Bay, the South China Sea, Vietnam (12°17′15″N, 109°14′29″E). The samples were transported in seawater, sheltered to prevent overexposure to solar radiation and maintain temperatures relatively constant, and brought to the laboratory within 1 h after collecting. On arrival, TL of the corals were prepared immediately. To analyze lipids, four different colonies were taken.

Instrumental Equipment

The GC analysis was carried out on a Shimadzu GC-2010 chromatograph (Kyoto, Japan) with a flame ionization detector on a SUPELCO WAX 10 (Supelco, Bellefonte, PA,

USA) capillary column (30 m × 0.25 mm ID). Carrier gas was He at 30 cm/s.

The GC–MS analysis was performed with a Shimadzu GCMS-QP5050A instrument (Kyoto, Japan) (electron impact at 70 eV) with a MDN-5s (Supelco, Bellefonte, PA, USA) capillary column (30 m × 0.25 mm ID). Carrier gas was He at 30 cm/s.

High resolution MS (HRMS) spectra were recorded using a Shimadzu LCMS-IT-TOF instrument (Kyoto, Japan) at atmospheric pressure chemical ionization (APCI) conditions. Samples were directly injected in MeOH with 0.1 % AcOH. Ion source temperature was 300 °C, the range of detection was m/z 100–1200, potential in the ion source was 4.5 kV. The drying gas (N_2) pressure was 25 kPa. The nebulizer gas (N_2) flow was 2 l/min.

The HPLC–HRMS analysis of polar lipids was performed with a Shimadzu Prominence liquid chromatograph equipped with two LC-20AD pump units, a high pressure gradient forming module, CTO-20A column oven, SIL-20A auto sampler, CBM-20A communications bus module, DGU-20A₃ degasser, and a Shim-Pack diol column (50 mm × 4.6 mm ID, 5 μm particle size) (Shimadzu, Kyoto, Japan). Lipids were detected by a high resolution tandem ion trap–time of flight mass spectrometry with a Shimadzu LCMS-IT-TOF instrument (Kyoto, Japan) operating both at positive and negative ion mode during each analysis at electrospray ionization (ESI) conditions. Ion source temperature was 200 °C, the range of detection was m/z 100–1200, and potential in the ion source was –3.5 and 4.5 kV for negative and positive modes, respectively. The drying gas (N_2) pressure was 200 kPa. The nebulizer gas (N_2) flow was 1.5 l/min.

¹H and ¹³C NMR spectra were recorded using a Bruker Avance DPX-700 spectrometer (Karlsruhe, Germany) at 700 and 125 MHz, respectively, with tetramethylsilane as an internal standard ($CDCl_3$, 30 °C).

Lipids Extraction and Analysis

The coral colonies were chopped into 1–3 mm pieces and TL were extracted using a modified technique of [28]. Lipids were extracted by intensive homogenization in $CHCl_3$ /MeOH (1:2, by vol) (30 ml per 10 g of coral wet wt). The homogenate obtained was filtered, and the residue was repeatedly extracted (6 h, 4 °C) in $CHCl_3$ /MeOH (2:1, by vol) (2 × 30 ml). The extracts were then mixed and separated into layers by adding 35 ml of H_2O and 30 ml of $CHCl_3$. The lower layer was evaporated, and the TL obtained were dissolved in $CHCl_3$ and stored at –18 °C. The total lipid content (mean ± SE, $n = 4$) in the dry samples of *Xenia* sp. was determined gravimetrically [29].

The TL were divided into neutral and polar lipid fractions by column chromatography on silica gel according

to [30]. Total lipid and polar lipid compositions were analyzed by one-dimensional thin-layer chromatography (TLC) using the precoated silica gel plates (10 cm × 10 cm) Sorbfil PTLC-AF-V (Sorbfil, Krasnodar, Russia). For total lipid analysis, the plates were first developed to their full length with n-hexane/Et₂O/AcOH (70:30:1, by vol) and finally to 25 % length with $CHCl_3$ /MeOH/ C_6H_6 /28 % NH_4OH (65:30:10:6, by vol). The solvent system $CHCl_3$ /MeOH/28 % NH_4OH (65:35:5, by vol) was used for polar lipid analysis. After drying in a stream of air, plates were sprayed with 10 % H_2SO_4 /MeOH and heated at 240 °C for 10 min. The chromatograms were scanned using an image scanner (Epson Perfection 2400 PHOTO) in a grayscale mode. The percentages of lipid contents were based on band intensity using an image analysis program (Sorbfil TLC Videodensitometer, Krasnodar, Russia). Peak areas and lipid class percentages were calculated according to [29].

In addition, polar lipids were separated by two-dimensional silica gel TLC with $CHCl_3$ /MeOH/ C_6H_6 /28 % NH_4OH (65:30:10:6, by vol) in the first direction and $CHCl_3$ /MeOH/AcOH/ CH_3COCH_3 / C_6H_6 / H_2O (70:30:4:5:10:1, by vol) in the second direction. To identify phospholipids on TLC plates, authentic standards and the specific spray reagents used earlier [31] were employed. Using spectrophotometry, the phospholipid content was determined after digestion with perchloric acid [32].

Fatty Acid Analysis

Fatty acid methyl esters (FAME) were obtained by the treatment of the lipids with 2 % H_2SO_4 in MeOH in a screw-capped vial (2 h, 80 °C) under Ar and purified by TLC development in benzene. 4,4-Dimethyloxazoline (DMOX) derivatives of FA were prepared from FAME according to [33]. The GC analysis of FAME was carried out at 210 °C. Injector and detector temperatures were 240 °C. FAME were identified by a comparison with authentic standards and using a table of equivalent chain-lengths [34]. The structures of FA were confirmed by GC–MS of their methyl esters and DMOX derivatives. The GC–MS analysis of FAME was performed at 160 °C with a 2 °C/min ramp to 240 °C that was held for 20 min. Injector and detector temperatures were 250 °C. GC–MS of DMOX derivatives was performed at 210 °C with a 3 °C/min ramp to 270 °C that was held for 40 min. The injector and detector temperatures were 250 °C. Spectra were compared with the NIST library and FA mass spectra archive [35].

Analysis of Molecular Species of Phospholipids

HPLC–HRMS was applied for the analysis of chemical structures and amounts of molecular species of phospholipids.

To study the HRMS fragmentation of phospholipid ions, 1-*O*-hexadecyl-2-oleoyl-*sn*-glycero-3-phosphocholine, 1-*O*-(1Z-octadecenyl)-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (Avanti Polar Lipids, Inc. Alabaster, Alabama, USA), and the mixture of soybean phospholipids (L- α -Lecithin Type II-S from Soybean, phosphatidylcholine content 20 %, Sigma Chemicals Co., St. Louis, MO, USA) were used. Phosphatidylserine (PtdSer) and phosphatidylinositol (PtdIns) were isolated from the soybean PL mixture by preparative TLC as described above. PL standards and PL obtained were studied using HPLC–HRMS at ESI conditions. The HPLC separation of PL was performed at the constant content of Et₃N/AcOH (0.08:1 by vol) in the solvent system [36] that allowed carrying out efficient ionization in ESI conditions and obtaining a stable ion signal by the simultaneous registration of positive and negative ions.

The HPLC separation of polar lipids of the soft coral *Xenia* sp. were performed using the binary solvent gradient consisted of solvent mixture A: *n*-hexane/2-propanol/AcOH/Et₃N (82:17:1:0.08, by vol) and mixture B: 2-propanol/H₂O/AcOH/Et₃N (85:14:1:0.08, by vol). The gradient started at 5 % of mixture B and its percentage was increased to 80 % over 25 min. This composition was maintained for 1 min before being returned to 5 % of mixture B over 10 min and maintained at 5 % for another 4 min (the total run time was 40 min). The flow rate was 0.2 ml/min. Polar lipids were detected by HRMS and identified by a comparison with authentic standards using a Shimadzu LCMS Solution control and processing software (v.3.60.361). The quantification of individual molecular species within each polar lipid class was carried out by calculating the peak areas for the individual extracted ion chromatograms [37].

Valerenenol (1)

The chloroform–methanol extract (60 mg) of *Xenia* sp. were submitted to a low-pressure column chromatography on silica gel (Kieselgel, 230–400 mesh, 60 Å) (Merck, Darmstadt, Germany) by the elution with linear gradient of *n*-hexane/Et₂O from 100:1 to 9:1 (by vol). Fraction composition was analyzed by TLC development in *n*-hexane/Et₂O/AcOH (70:30:1, by vol). Plates were sprayed with 10 % H₂SO₄ in MeOH and heated at 120 °C for 10 min. The fractions with a single pink spot of *R*_f 0.5 were combined to yield 12 mg of valerenenol (1Z)-1-[(3*S*,3*aS*,7*S*,7*aS*)-3,7-dimethyloctahydro-4*H*-inden-4-ylidene]-2-methyl-2-propanol (1). EI–MS *m/z* (relative intensity, %): 204 ([M–H₂O]⁺, 27), 189 (14), 175 (2), 161 (17), 149 (100), 133 (18), 119 (22), 107 (35), 105 (41), 93 (28), 91 (39), 81 (16), 79 (20), 77 (19), 69 (12), 67 (12), 65 (10), 55 (29). HRMS, APCI(+), *m/z* (relative intensity, %): 223.2031 (calculated

223.2056, C₁₅H₂₆O) [M+H]⁺ (1.6), 205.1952 [M+H–H₂O]⁺ (100), 149.1302 [M+H–C₄H₁₀O]⁺ (13). ¹H NMR: δ_{H} (700 MHz, CDCl₃) 0.85 (3 H, d, *J* = 6.5 Hz), 0.93 (3 H, d, *J* = 7.5 Hz), 1.36 (3 H, s), 1.38 (3 H, s), 1.30–1.90 (7 H, m), 1.99 (1 H, ddd, *J* = 14.9, 10.5 and 4.4 Hz), 2.18–2.23 (3 H, m), 3.7 (1 H, t, *J* = 9.5 Hz), 5.42 (1 H, d, *J* = 2 Hz). ¹³C NMR: δ_{C} (125 MHz, CDCl₃) 17.6, 20.1, 24.5, 27.4, 29.3, 31.6, 32.2, 32.7, 35.2, 38.5, 43.4, 45.7, 71.1, 134.1, 140.5.

Statistical Analysis

Differences in the mean of FA concentrations were examined with a one-way ANOVA. The raw data were used following examination of the homogeneity of variances (Levene's test) and normality of the data (Shapiro–Wilk's test). To represent relationships between cnidarians and evaluate the distribution of essential lipid classes and FA in the samples, the data were square root transformed prior to analysis and all variables were included in principal components analysis (PCA). All statistical analyses were performed using STATISTICA 5.1 (StatSoft, Inc., USA).

Results

Total Lipids and Valerenenol

TL constituted 16.9 ± 1.7 % of dry tissue of the corals. Polar lipids (PoL), wax esters (WE), triacylglycerols (TAG), sterols (ST), and monoalkyl diacylglycerols (MADAG) were the major lipid classes of *Xenia* sp. (Table 1). Free fatty acids (FFA) comprise 3.1 % of TL only.

When TLC analysis of the TL was performed, the large spot of a non-polar compound (marked as 1) was observed between TAG and FFA spots on a plate. Compound (1) was co-extracted with the TL and constituted 21.5 % of the total chloroform–methanol extract of *Xenia* sp. Compound (1) was purified and characterized by MS and NMR methods. In a HRMS spectrum, compound (1) formed the

Table 1 Composition of main lipid classes (% of total lipids, mean ± SD, *n* = 4) of the soft coral *Xenia* sp

Lipid class	Content (%)
Wax esters	21.4 ± 1.8
Monoalkyldiacyl glycerols	7.7 ± 4.3
Triacylglycerols	14.2 ± 1.9
Free fatty acids	3.1 ± 0.8
Sterols	10.5 ± 1.2
Polar lipids	36.7 ± 3.9

positive quasi-molecular ion $[M+H]^+$ with m/z 223.2031, corresponding to composition $[C_{15}H_{27}O]^+$ (calculated 223.2056), base peak $[M + H - H_2O]^+$ with m/z 205.1952, and fragment $[M+H-C_4H_{10}O]^+$ with m/z 149.1302. A low-resolution MS spectrum gave fragment $[M-H_2O]^+$ with m/z 204 and base peak $[M-C_4H_9O]^+$ with m/z 149, but the peak of molecular ion was absent. The 1H - and ^{13}C -NMR spectra showed characteristics of *ent*-valerenane type skeleton with typical proton signals at δ_H 2.20 (*t*) and 3.70 (*t*, $J = 9.5$ Hz) and the corresponding carbons at δ_C 45.7 (C-9) and 43.4 (C-4). In addition, an olefinic H was observed at δ_H 5.42 (*d*, $J = 2$ Hz) and δ_C 134.1. A trisubstituted double bond is at δ_C 140.5 (C-5) and a *tert* OH group is at δ_C 71.1 (C-12). Thus, compound (**1**) is considered to be a bicarbocyclic sesquiterpene. Two carbon atoms (C-3 and C-8 at δ_C 38.5 and 31.6, respectively) in the skeleton of compound (**1**) are substituted by two *sec.* CH_3 group at δ_H 0.93 (3 H, *d*, $J = 7.5$ Hz) and 0.85 (3 H, *d*, $J = 6.5$ Hz) with δ_C 17.6 and 20.1, respectively. Besides, two *tert.* CH_3 groups in an alcohol moiety at δ_H 1.38 (3 H, *s*) and 1.36 (3 H, *s*) with δ_C 29.3 and 32.2, respectively, were also shown in 1H and ^{13}C NMR of compound (**1**). The comparison of NMR data of compound (**1**) with those published earlier [38] led to the identification of compound (**1**) as valerenol, sesquiterpene alcohol.

Under heating or long keeping at room temperature, valerenol (**1**) easily transformed into less polar compound (**2**) with a greater mobility on TLC. Compound (**2**) was characterized as dehydrovalerenol by the comparison of its full NMR data (Table S1) with the available NMR data of dehydrovalerenol published earlier [38].

Polar Lipids

TL of *Xenia* sp. contained 36.7 % of polar lipids (Table 1). The polar lipids were composed largely (more than 80 %) of phosphorus-contained lipids. Choline glycerophospholipids (ChoGpl), ethanolamine glycerophospholipids (EtnGpl), serine glycerophospholipids (SerGpl), and ceramide aminoethylphosphonate (CAEP) were the major phospho- and phosphonolipids identified (Table 2). Phosphatidylinositol (PtdIns) and lyso choline glycerophospholipids (lyso ChoGpl) were also found in PL of *Xenia* sp. Except for ChoGpl, trace amounts of lyso forms of other PL classes were detected.

To quantify the proportion of each polar lipid class, two independent methods were applied: (1) a classic spectrophotometry measurement of the phosphorus content after two-dimensional TLC separation of lipids; and (2) a densitometry of plate images after one-dimensional TLC (Table 2). Significant differences in proportions of each lipid class evaluated by these two methods were not found ($P > 0.01$). A gradual heating of one-dimensional

Table 2 Phospho- and phosphonolipid composition (% of total phosphorus-contained lipids, mean \pm SD, $n = 4$) of the soft coral *Xenia* sp. (determined by two independent methods)

Lipid class	Content (%)	
	Spectrophotometry	Densitometry
Choline glycerophospholipids	39.5 \pm 1.2	36.5 \pm 1.2
Ethanolamine glycerophospholipids	20.8 \pm 1.5	22.6 \pm 1.1
Serine glycerophospholipids	20.5 \pm 1.1	20.9 \pm 0.6
Ceramide aminoethylphosphonate	9.7 \pm 2.0	10.9 \pm 0.9
Phosphatidylinositol	4.3 \pm 1.5	5.2 \pm 0.5
Lyso phosphatidylcholine	5.3 \pm 1.2	4.0 \pm 1.8

TLC plates showed that unsaturated lipids were quantified at 120 °C, but the spots of saturated lipids appeared at the temperature over 170 °C. The temperature of 240 °C was found to be required for the reliable quantification both of saturated and unsaturated lipids. Precoated silica gel TLC plates with organic binders, such as polyesters or polyvinyl alcohol, cannot be applied at 240 °C because their background becomes dark at the temperature over 150 °C.

Fatty Acids

Acids 16:0, 18:3n-6, 20:4n-6, and 20:5n-3 were the principal FA of total lipids of *Xenia* sp. (Table 3). Several C_{18} polyunsaturated FA (PUFA), such as 18:2n-6, 18:4n-3, and 18:5n-3, were found in addition to 18:3n-6. Acids 22:4n-6 and 22:6n-3 (3.1 and 5.2 % of total FA, respectively) were the major C_{22} PUFA. TL contained 1.4 % of 24:5n-6 and trace amounts of 24:6n-3 and 24:4.

The mass spectrum of the DMOX derivative of 24:5n-6 gave molecular ion peak at m/z 411 (M^+) and five pairs of fragments at m/z 152 and 166, 194 and 206, 234 and 246, 274 and 286, 314 and 326 that indicated the double bonds localization at 6, 9, 12, 15, and 18 carbon atoms of the original FA [35, 39]. The structure of 24:6n-3 was confirmed by the same procedures. The mass spectra of methyl ester and DMOX of 24:4 showed the molecular ions at m/z 374 and 413, respectively. The GC retention times of the derivatives of 24:4 were close to that of 24:4n-6. At the same time, the mass spectrum of DMOX derivative of 24:4 from *Xenia* sp. gave the pair of fragments at m/z 236 and 250 (14 amu gap) instead of the pair at m/z 236 and 248 (12 amu gap), which was in the mass spectrum of DMOX of 24:4n-6. Unfortunately, we could not determine the positions of all double bounds in 24:4 from *Xenia* sp. because of a small amount of the acid.

Trace amounts of odd-chain and methyl-branched FA were found. The specific zooxanthellae PUFA of

Table 3 Fatty acid and fatty aldehyde composition (% of total, mean \pm SD, $n = 4$) of total, neutral, and polar lipids of the soft coral *Xenia* sp

Fatty acids	Total	Neutral	Polar
14:0*	5.5 \pm 0.8	6.7 \pm 0.2	0.9 \pm 0.6
16:0*	18.1 \pm 2.1	30.5 \pm 1.5	8.7 \pm 1.4
16:1n-9	2.3 \pm 0.2	1.1 \pm 0.9	0.5 \pm 0.1
16:1n-7*	0.5 \pm 0.1	3.1 \pm 0.4	0.5 \pm 0.1
7-Me-16:1n-10	0.3 \pm 0.1	0.8 \pm 0.2	–
18:0	5.5 \pm 2.0	7.0 \pm 1.8	6.9 \pm 2.3
18:1n-9*	2.3 \pm 0.4	4.5 \pm 0.5	0.8 \pm 0.1
18:1n-7	0.3 \pm 0.1	0.3 \pm 0.1	0.1 \pm 0.1
18:2n-6*	1.5 \pm 0.2	2.5 \pm 0.1	0.4 \pm 0.1
18:3n-6*	13.1 \pm 1.8	15.3 \pm 0.4	5.3 \pm 1.1
18:4n-3	4.7 \pm 0.8	2.8 \pm 0.7	5.1 \pm 1.4
20:0	0.7 \pm 0.3	0.7 \pm 0.3	0.6 \pm 0.2
18:5n-3*	0.7 \pm 0.2	0.2 \pm 0.1	2.2 \pm 0.8
20:3n-6	1.2 \pm 0.6	1.1 \pm 0.5	0.6 \pm 0.1
20:4n-6*	16.9 \pm 3.9	2.7 \pm 0.8	27.5 \pm 3.3
20:4n-3	0.5 \pm 0.3	0.5 \pm 0.4	0.1 \pm 0.1
20:5n-3	6.9 \pm 0.7	3.4 \pm 0.7	4.4 \pm 0.8
22:0	0.3 \pm 0.1	0.3 \pm 0.1	0.3 \pm 0.1
22:3n-6	0.9 \pm 0.2	0.6 \pm 0.3	1.3 \pm 0.3
22:4n-6*	3.1 \pm 0.6	0.6 \pm 0.2	9.1 \pm 1.4
22:5n-3	0.3 \pm 0.1	0.2 \pm 0.1	0.4 \pm 0.4
22:6n-3	5.2 \pm 1.3	5.7 \pm 2.4	1.6 \pm 0.7
24:4	0.1 \pm 0.1	0.2 \pm 0.2	0.5 \pm 0.2
24:5n-6*	1.4 \pm 0.2	0.2 \pm 0.1	5.1 \pm 0.7
Octadecanal* ^a	3.3 \pm 1.3	0.3 \pm 0.1	11.3 \pm 3.4
Other ^b	4.5 \pm 1.8	8.0 \pm 2.3	5.2 \pm 2.1

* Significant differences in FA contents between neutral and polar lipids (t test $P < 0.01$)

^a Fatty aldehydes were determined as their dimethylacetal derivatives by GC and GC–MS

^b 12:0, 15:0, 17:0, 19:0, i-17:0, 14:1, 15:1, 17:1, 20:1, 22:1, 16:2, 20:2n-6, 24:6n-3, hexadecanal, octadecanal

alcyonarians (16:3n-4 and 16:4n-1) were not detected in *Xenia* lipids.

Under acid methanolysis of *Xenia* lipids, plasmalogen PL gave long-chain aldehyde dimethylacetals (DMA). Octadecanal dimethylacetal was the main DMA and amounted to 3.3 % in a mixture of FAME and DMA obtained from the TL of *Xenia* sp. (Table 3).

The distribution of FA and fatty aldehydes between polar and neutral lipids (NL) was shown in Table 3. FA of polar lipids contained considerable amount of 18:5n-3, 20:4n-6, 22:4n-6, 24:5n-6, and octadecanal. FA of NL were rich in 14:0, 16:0, 16:1n-7, 18:2n-6, and 18:3n-6. Acid 7-Me-16:1n-10 was found in neutral lipid FA. Acid 24:4 was more abundant in polar lipid FA.

Chemotaxonomic Relationship between *Xenia* sp. and Other Corals

To clarify the relationship between *Xenia* sp. investigated and other Vietnamese zooxanthellate corals described previously [7], principal components analysis (PCA) was performed for TL compositional data of the corals. PoL, ST, TAG, MADAG, and WE were used as variables. The outcome of the analysis was visualized in Fig. 1. Reef-building corals and alcyonarians clearly divided into two regions of the two-dimensional space formed by PC1 and PC2. Four *Xenia* specimens were inside the region of alcyonarians. The partial separation of *Xenia* sp. and other alcyonarian species was mainly generated in PC2 under the influence of most variables except for PoL. Factor loading values (in PC2) were positive for WE (0.46), but negative for ST (–0.43) and TAG (–0.70).

Xenia sp. and other Vietnamese soft corals [7] were compared in PCA based on FA compositional data. Zooxanthellate and azooxanthellate species clearly divided into two regions of the two-dimensional space formed by PC1 and PC2 (Fig. 2). Four *Xenia* specimens were inside the region of zooxanthellate species. In addition, the zooxanthellate species subdivided according to the 18:3n-6/16:2n-7 ratio (Fig. 2). The high level of 18:3n-6 and the low level of 16:2n-7 were found in *Xenia* sp. (Table 3). As seen in Fig. 2, *Xenia* specimens were near the species with 18:3n-6/16:2n-7 ratio >1 . The division of *Xenia* and other zooxanthellate soft corals was generated in PC2 mainly under the influence of 18:3n-6, 16:2n-7, 18:2n-7, 18:2n-6, 20:5n-3, 22:4n-6, and 22:6n-3.

HRMS of Phospholipid Standards

HRMS study of the fragmentation patterns of 1-*O*-hexadecyl-2-oleoyl-*sn*-glycero-3-phosphocholine (16:0e/18:1 PakCho), 1-*O*-octadecyl-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (18:1e/18:1 PlsEtn), and main molecular species of phosphatidylserine (PtdSer) and phosphatidylinositol (PtdIns) from soybeans at ESI (\pm) conditions was performed.

16:0e/18:1 PakCho formed negative acetylated molecular ions $[M+CH_3COO]^-$ at m/z 804.6103 and positive quasi-molecular ions $[M+H]^+$ at m/z 746.6035, corresponding to composition $[C_{44}H_{87}NO_9P]^-$ (calculated 804.6124) and $[C_{42}H_{85}NO_7P]^+$ (calculated 746.6058), respectively. At the MS² stage, the ions at m/z 804.6103 eliminated a molecule of C₃H₆O₂ (methyl acetate) and formed ions at m/z 730.5762, that indicated the quasi-molecular negative ions to be formed by the addition of acetate ion to the lipid molecule (Fig. 3a). At the MS³ stage, the ions at m/z 730.5762 formed ions with m/z 281.2463 ($[C_{18}H_{33}O_2]^-$, calculated 281.2486) corresponding to 18:1 acid anions (Fig. 3b).

18:1e/18:1 PlsEtn formed negative quasi-molecular ions $[M-H]^-$ at m/z 728.5587 (calculated 728.5600) and positive cluster ions $[M+H+(C_2H_5)_3N]^+$ with m/z 831.6927 (calculated 831.6950) that corresponded to compositions $[C_{41}H_{79}NO_7P]^-$ and $[C_{47}H_{96}N_2O_7P]^+$, respectively.

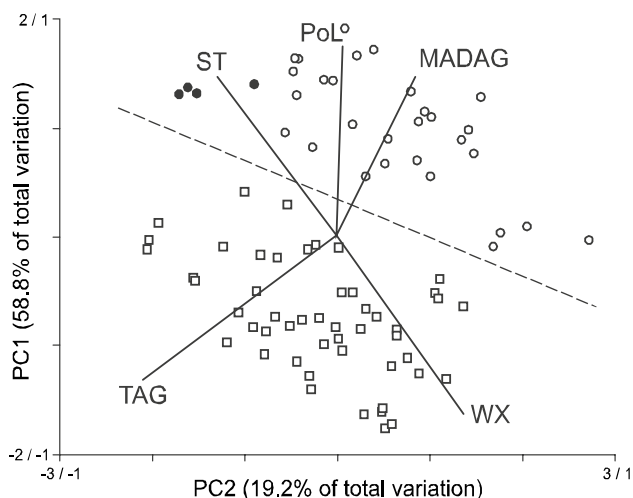
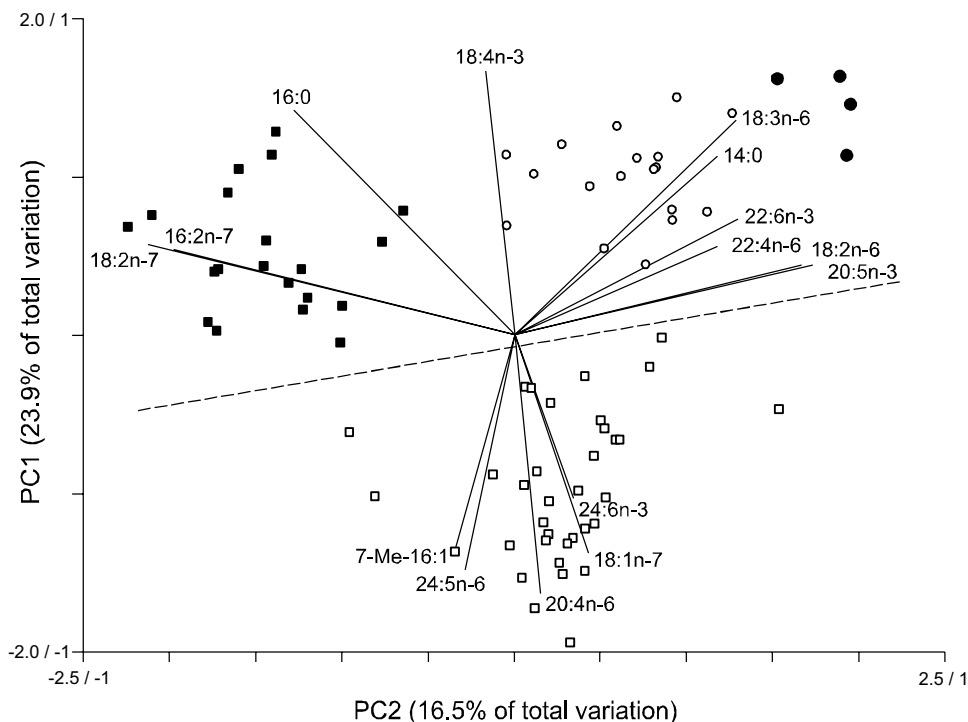


Fig. 1 Principal components analysis (PCA) of transformed lipid class compositional data (see Table 1) of the soft coral *Xenia* sp. (black circles) along with zooxanthellate soft corals (white circles) and reef-building corals (white squares) described previously [7]. The plot with the PC loadings by lipid components (scale from -1 to 1) is superimposed on the plot with the PC scores of the samples (scale from -3 to 3). Dotted line separates soft and reef-building species. PoL polar lipids, ST sterols, TAG triacylglycerols, MADAG monoalkyl diacylglycerols, WE wax esters

Fig. 2 Principal components analysis (PCA) of transformed fatty acid compositional data (see Table 3) of the soft coral *Xenia* sp. (black circles) along with zooxanthellate soft corals with the high level of 18:3n-6 (white circles), zooxanthellate soft corals with the high level of 16:2n-7 (black squares), and azooxanthellate soft corals described previously [7]. The plot with the main PC loadings (>0.5) by FA components (scale from -1 to 1) is superimposed on the plot with the PC scores of the samples (scale from -2.5 to 2.5). Dotted line separates zooxanthellate and azooxanthellate species



MS^2 fragmentation of the negative ions at m/z 728.5587 (Fig. 4) resulted in the formation of anions at m/z 281.2461 ($[C_{18}H_{33}O_2]^-$), corresponding to 18:1 acid anions. The signal at m/z 464.3140 (the loss of 264.2447) was also observed in the MS^2 spectrum. This signal appeared when the quasi-molecular ions $[M-H]^-$ lost a dehydrated molecule of acid 18:1 ($C_{18}H_{32}O$, calculated 264.2453).

The study of ionization of the main soybean PtdSer molecular species showed a predominant formation of negative quasi-molecular ions $[M-H]^-$ at m/z 758.4924. Positive quasi-molecular ions did not form. The elemental composition of the ion at m/z 758.4924 was calculated as $[C_{40}H_{73}NO_{10}P]^-$. MS^2 fragmentation of the PtdSer ion showed a complex spectrum (Fig. 5). The primary ion at m/z 671.4606 was observed in the MS^2 spectrum due to the loss of serine group with monoisotopic mass of 87.0359 ($C_3H_5NO_2$, calculated 87.0320). These were three additional ions at m/z 391.2232 (the loss of 367.2692), 409.2330 (the loss of 349.2594), and 415.2240 (the loss of 343.2684) in the MS^2 spectrum. These signals appeared due to the joint elimination of serine group and acyl fragments from the quasi-molecular ion. Their values corresponded to the ions formed by elimination of three neutral fragments, such as $C_{21}H_{37}NO_4$ ($C_3H_5NO_2 + C_{18}H_{32}O_2$, calculated 367.2723), $C_{21}H_{35}NO_3$ ($C_3H_5NO_2 + C_{18}H_{30}O$, calculated 349.2617), and $C_{19}H_{37}O_4$ ($C_3H_5NO_2 + C_{16}H_{32}O_2$, calculated 343.2723), from the quasi-molecular ions. Consequently, the PtdSer molecular species analyzed was identified as 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphoserine (16:0/18:2 PtdSer).

Fig. 3 HRMS spectra of 1-*O*-hexadecyl-2-oleoyl-*sn*-glycero-3-phosphocholine (16:0e/18:1 PakCho). *a* MS² spectrum of [M+CH₃COO]⁻ at *m/z* 804.6103, *b* MS³ spectrum of precursor ion at *m/z* 730.5762

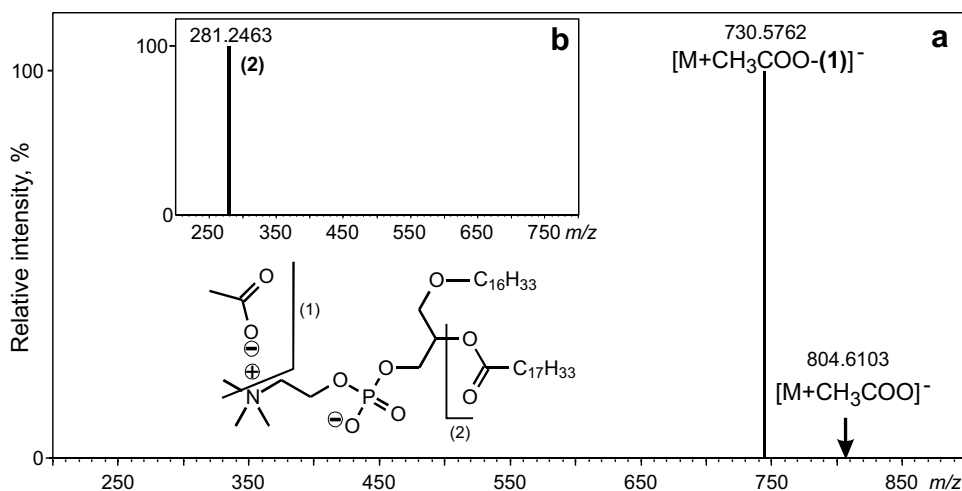


Fig. 4 MS² spectrum of negative quasi-molecular ions ([M-H]⁻ at *m/z* 728.5587) of 1-(1*Z*-octadecenyl)-2-oleoyl-*sn*-glycero-3-phosphoethanolamine (18:1e/18:1 PakEtn)

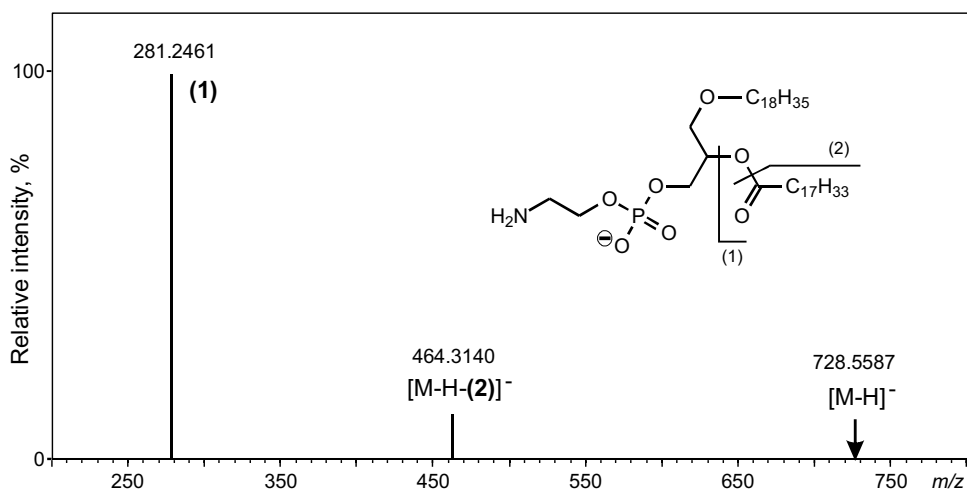
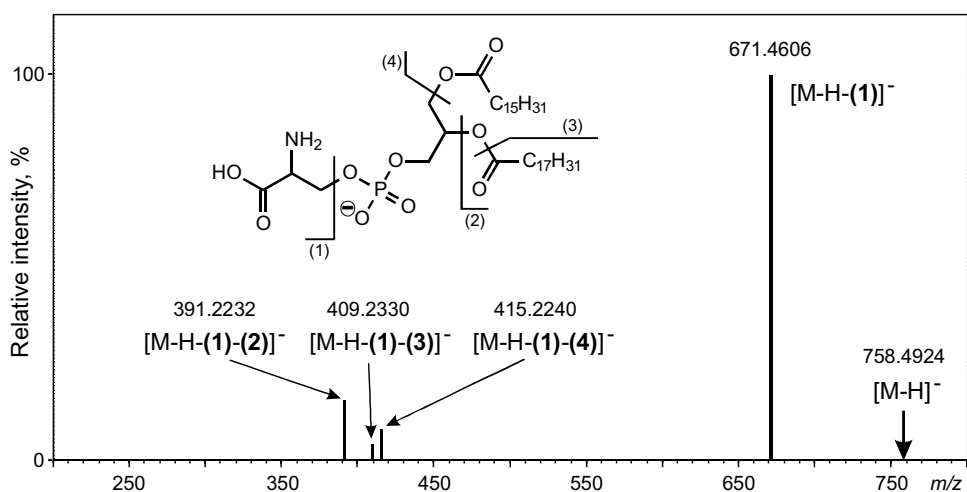


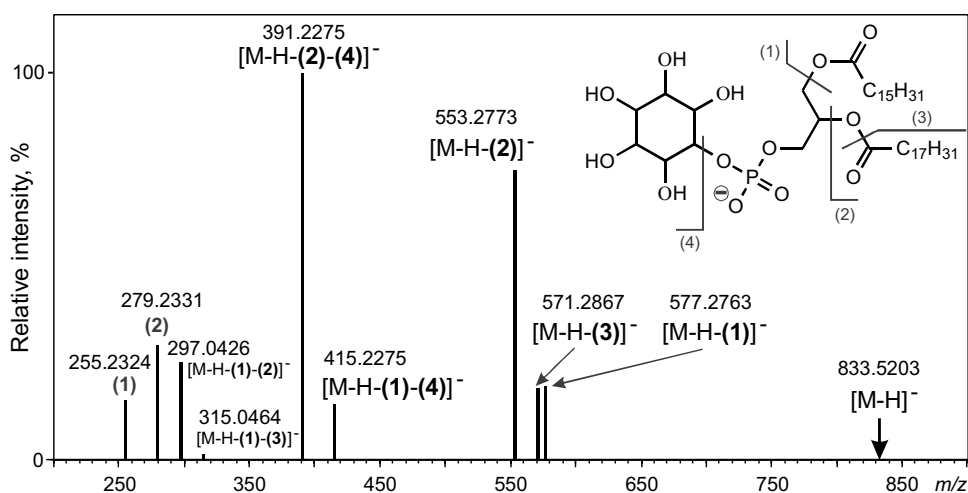
Fig. 5 MS² spectrum of negative quasi-molecular ions ([M-H]⁻ at *m/z* 758.4924) of soybean phosphatidylserine (16:0/18:2 PtdSer)



The main molecular species of soybean PtdIns produced negative quasi-molecular ions [M-H]⁻ at *m/z* 833.5203 corresponding to [C₄₃H₇₈O₁₃P]⁻. Positive

quasi-molecular ions also formed. MS² fragmentation of the ions [M-H]⁻ of PtdIns gave several characteristic ions (Fig. 6). The ions at *m/z* 255.2324 [C₁₆H₃₁O₂]⁻ and

Fig. 6 MS² spectrum of negative quasi-molecular ions ([M–H][–] at *m/z* 833.5203) of soybean phosphatidylinositol (16:0/18:2 PtdIns)



279.2331 [C₁₈H₃₁O₂][–] corresponded to carboxylate anions of 16:0 and 18:2, respectively. The loss of 16:0 and 18:2 gave ions at *m/z* 577.2763 ([M–H–C₁₆H₃₂O₂][–], calculated 577.2783) and 553.2773 ([M–H–C₁₈H₃₂O₂][–], calculated 553.2783). The loss of dehydrated 18:2 led to the formation of ions at *m/z* 571.2867 ([M–H–C₁₈H₃₀O][–], calculated 571.2889). The appearance of ions at *m/z* 297.0426 (calculated 297.0381) was caused by the simultaneous loss of 16:0 and 18:2. The ions at *m/z* 315.0464 (calculated 315.0487) were formed by the simultaneous loss of dehydrated 16:0 and 18:2. The loss of inositol and acyl fragments: [M–H–(C₆H₁₀O₅ + C₁₈H₃₂O₂)][–] (calculated 391.2255), and [M–H–(C₆H₁₀O₅ + C₁₆H₃₂O₂)][–] (calculated 415.2255) gave the ions at *m/z* 391.2275 and 415.2275, respectively, characteristic for PtdIns. Thus, the molecular species was identified as 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphoinositol (16:0/18:2 PtdIns).

Polar Lipid Molecular Species of *Xenia* sp

To determine the structure of molecular species of polar lipids from the soft coral *Xenia* sp., we applied the data on the HRMS fragmentations of PL standards described above (see Figs. 3, 4, 5, 6).

Among choline glycerophospholipids (ChoGpl) of *Xenia* sp., we determined seven components constituted more than 75 % of total ChoGpl (Table S2). Acetylated molecular ions ([M+CH₃COO][–]) of each component lost methyl acetate at the MS² stage. The subsequent fragmentation of components 3–7 formed the anions characterized acyl groups at the MS³ stage. On the base of the fragmentation pathways and the monoisotopic molecular mass values, components 4, 6, and 7 were identified as *O*-alkyl acylglycerophosphocholines, namely 16:0e/18:4 PakCho, 16:0e/20:4 PakCho, and 18:0e/20:4 PakCho, respectively. Component 5 was also identified as *O*-alkyl

acylglycerophosphocholine but there was significant discrepancy between measured (743.5760) and calculated (743.5829) values of its monoisotopic molecular mass. Moreover, the MS³ spectrum of component 5 contained two signals at *m/z* 279.2347 and 251.2019 corresponding to 18:2 and 16:2 anions, respectively. We assumed that component 5 was a mixture of two non-separated isomers, 16:0e/18:2 PakCho and 18:0e/16:2 PakCho.

ChoGpl components 1 and 2 had no fragmentation at the MS³ stage. Based on these data and the elemental composition values, we suggested components 1 and 2 to be mono-alkyl glycerophosphocholines, namely 16:0e lyso PakCho and 18:0e lyso PakCho, respectively.

The presence of arachidonic acid anion in the MS³ spectrum and the value of monoisotopic molecular mass 543.3272 (calculated 543.3325) indicated that component 3 was arachidonoyl glycerophosphocholine (20:4 lyso PtdCho) with a possible *sn*-2 position of acyl group.

Among ethanolamine glycerophospholipids (EtnGpl) of *Xenia* sp., we determined six components constituted more than 91 % of total EtnGpl (Table S3). The signals of positive cluster ions [M+H+Et₃N]⁺ and negative quasi-molecular ions [M–H][–] were observed in the HRMS spectra of all components.

The MS² spectrum of the ions [M–H][–] of component 1 contained the signal at *m/z* 422.2638 formed by the loss of neutral fragment of 234.1976 (C₁₆H₂₆O, calculated 234.1984). This neutral fragment corresponded to dehydrated 16:2 acid. The MS² spectrum also contained the signal of carboxylate anion of 16:2 at *m/z* 251.2036 (calculated 251.2017). According to the elemental composition calculated and the value of monoisotopic molecular mass, component 1 was identified as alkenyl acyl glycerophosphoethanolamine, 15:1e/16:2 PlsEtn (Table S3).

The MS² spectra of quasi-molecular ions of components 2–7 contained signals of three anions: arachidonic

acid ($C_{20}H_{31}O_2$), decarboxylated arachidonic acid ($C_{19}H_{31}$), and $[M-H-C_{20}H_{30}O]^-$. The neutral fragment $C_{20}H_{30}O$ corresponded to dehydrated acid 20:4. Thus, components 2–7 were substituted by arachidonic acid. According to the elemental composition calculated and the value of monoisotopic molecular mass, component 2 was assumed as 20:4 lyso PtdEtn. Components 3, 4, 5, and 6 were identified as 18:1e/20:4 PlsEtn, 19:0e/20:4 PakEtn, 16:1e/20:4 PlsEtn, and 16:0e/20:4 PakEtn, respectively.

Among serine glycerophospholipids (SerGpl) of *Xenia* sp., we determined six components constituted about 80 % of total SerGpl (Table S4). Negative quasi-molecular ions $[M-H]^-$ were in the MS spectrum of each component. The MS² spectrum of $[M-H]^-$ of each component contained a signal of characteristic ion $[M-H-C_3H_5NO_2]^-$ corresponding to the loss of serine group.

The MS² spectra of components 1–3 contained also signals corresponding to the quasi-molecular ion, which lost simultaneously serine and acyl groups. As in the case of soybean PtdSer (Fig. 5), carboxylate anion signals were not observed in the MS² spectra of components 1–3. In contrast to soybean PtdSer, the calculated molecular formulae of components 1–3 contained nine oxygen atoms, and the MS² spectra of quasi-molecular ions of these components contained signals corresponding to the loss of only one acyl group. These are characteristic for alkyl acyl and alkenyl acyl glycerophosphoserines (PakSer and PlsSer). Therefore, components 1, 2, and 3 were identified as 18:1e/24:5 PlsSer, 18:0e/24:5 PakSer, and 19:1e/24:5 PlsSer, respectively.

The calculated molecular formula of component 4 also contained also nine oxygen atoms. The MS² spectrum indicated the presence of tetracosapentaenoic acid ester. The signal at m/z 152.9989 corresponded to the loss of a neutral fragment 445.3175 ($C_3H_5NO_2 + C_{24}H_{38}O_2$, calculated 445.3192). The formula calculated ($[C_3H_6O_5P]^-$) for the ions at m/z 152.9989 corresponded to a fragment of glycerophosphate. Therefore, component 4 was determined as 24:5 lyso PtdSer.

The MS² spectrum of quasi-molecular ions of component 5 showed only one signal at m/z 423.2906 (the loss of serine group). The molecular formula of component 5 ($C_{24}H_{50}NO_8P$) contained eight oxygen atoms. Therefore, this SerGpl molecular species was identified as octadecyl glycerophosphoserine (18:0e lyso PakSer).

Among inositol glycerophospholipids (InsGpl) of *Xenia* sp., we determined twelve components constituted more than 96 % of total InsGpl (Table S5). Both diacyl and alkyl acyl forms of InsGpl were found. Except for components 1, 6, and 10, the MS² spectra of the negative quasi-molecular ions of the components were similar to that of soybean PtdIns described above (Fig. 6). Therefore, components 2, 3, 4, 5, 7, 8, and 9 were easily identified as 16:1/22:6

PtdIns, 16:0/22:6 PtdIns, 18:0/20:4 PtdIns, 18:0/22:4 PtdIns, 18:0/20:3 PtdIns, 18:1/22:6 PtdIns, 18:0/22:6 PtdIns, 18:0/24:6 PtdIns, and 18:0/24:5 PtdIns, respectively (Table S5).

The molecules of components 1 and 6 contained one oxygen atom less than a molecule of PtdIns. The MS² spectra of $[M-H]^-$ of components 1 and 6 contained one signal of a carboxylate anion and three signals of fragments of $[M-H]^-$ after the loss of FA, FA dehydrated, and inositol with FA. Thus, components 1 and 6 were concluded to have only one acyl group. Components 1 and 6 were identified as 18:0e/20:4 PakIns and 18:0e/24:5 PakIns, respectively.

The MS² spectrum of $[M-H]^-$ of component 10 was very complex and had four signals of carboxylate anions, namely 16:0, 18:0, 22:5, and 24:5 (m/z 255.2307 ($[C_{16}H_{31}O_2]^-$, calculated 255.2330), m/z 283.2592 ($[C_{18}H_{35}O_2]^-$, calculated 283.2643), m/z 329.2456 ($[C_{22}H_{33}O_2]^-$, calculated 329.2486), and m/z 357.2778 ($[C_{24}H_{37}O_2]^-$, calculated 357.2799), respectively). Other four signals in the MS² spectrum confirmed that component 10 was a mixture of PtdIns molecular species. These signals at m/z 391.2220, 419.2577, 465.2374, and 493.2697 appeared when the molecular ion lost the fragments amounted to 520.3409, 492.3052, 446.3255, and 418.2932, respectively. Each of these fragments ($C_{30}H_{48}O_7$ ($C_6H_{10}O_5 + C_{24}H_{38}O_2$, calculated 520.3400), $C_{28}H_{44}O_7$ ($C_6H_{10}O_5 + C_{22}H_{34}O_2$, calculated 492.3087), $C_{24}H_{46}O_7$ ($C_6H_{10}O_5 + C_{18}H_{36}O_2$, calculated 446.3244) and $C_{22}H_{42}O_7$ ($C_6H_{10}O_5 + C_{16}H_{32}O_2$, calculated 418.2931)) consisted of inositol and one of FA mentioned above. Only two PtdIns molecular species with the combinations of acyl groups 18:0 + 22:5 and 16:0 + 24:5 had the monoisotopic molecular mass value of 912.5702. Therefore, compound 10 was assumed to be a mixture of 18:0/22:5 PtdIns and 16:0/24:5 PtdIns.

Ceramide aminoethylphosphonate (CAEP) of *Xenia* sp. was represented by one main molecular species. In mass spectra of this molecular species, we observed signals of negative quasi-molecular ions $[M-H]^-$ at m/z 641.5040 ($[C_{36}H_{70}N_2O_5P]^-$, calculated 641.5028), positive quasi-molecular ions $[M+H]^+$ at m/z 643.5199 ($[C_{36}H_{72}N_2O_5P]^+$, calculated 643.5173), and positive cluster ions $[M+H+Et_3N]^+$ at m/z 744.6347 ($[C_{42}H_{87}N_3O_5P]^+$, calculated 744.6378).

The MS² spectrum of $[M-H]^-$ of CAEP showed a specific fragmentation different from the fragmentations of negative quasi-molecular ions of glycerophospholipids. In the MS² spectrum, $[M-H]^-$ lost a neutral fragment amounted to 238.2279 ($C_{16}H_{30}O$, calculated 238.2297) and formed a single ion at m/z 403.2761. This ion eliminated water molecule (18.0107) and formed the ions at m/z 385.2654 in MS³ spectrum.

In the MS² spectrum, a fragmentation of the positive quasi-molecular ion $[M+H]^+$ at m/z 643.5199 was

similar to that described previously [40]. After elimination of 2-aminoethyl phosphonic acid ($C_2H_8NO_3P$), acyl group ($C_{16}H_{30}O$), and water (H_2O) at different combinations, $[M+H]^+$ formed ions at m/z 518.4912 (the loss of 125.0287, $C_2H_8NO_3P$, calculated 125.0242), 500.4804 (the loss of 143.0395, $C_2H_8NO_3P + H_2O$, calculated 143.0347), 280.2637 (the loss of 363.2562, $C_2H_8NO_3P + C_{16}H_{30}O$, calculated 363.2538), and 262.2535 (the loss of 381.2664, $C_2H_8NO_3P + C_{16}H_{30}O + H_2O$, calculated 381.2644). These four ions indicated the presence of long-chain 18:2 base and 16:0 groups in the molecule. The molecular species was attributed to 16:0 CAEP.

On the whole, thirty-three molecular species were identified in polar lipids of the soft coral *Xenia* sp. (Table 4). Alkyl acyl and alkenyl acyl forms of glycerophospholipids predominated in the molecular species determined. Octadecyl glycerophosphocholine (18:0e lyso PakCho, 4.12 %), octadecyl arachidonoyl glycerophosphocholine (18:0e/20:4 PakCho, 20.5 %), octadecenyl arachidonoyl glycerophosphoethanolamine (18:1e/20:4 PlsEnt, 17.97 %), octadecyl tetracosapentanoyl glycerophosphoserine (18:0e/24:5 PakSer, 14.02 %), and hexadecanoyl sphingodienylphosphoethanolamine (16:0 CAEP, 9.6 %) were the major molecular species.

Discussion

Total Lipids and Valerenenol

The qualitative composition of TL of the soft coral *Xenia* sp. (Table 1) was similar to that of Vietnamese corals investigated previously [7]. *Xenia* sp. contained common lipids (WE, TAG, ST, and PL) and MADAG, which constituted a considerable part of lipids of cnidarians. MADAG molecule has highly stable ether bond and its hydrolysis with lipases is hindered. We agree with Joseph [41], who has supposed that MADAG stabilize cnidarians cell membranes exposed to active hydrolytic enzymes of these animals. Generally, the percentage of MADAG in TL of the coral species with zooxanthellae is higher than that of the azooxanthellate coral species [6, 7]. It is possible that symbiotic coral species need MADAG for the optimal functioning of gastrodermal coral cells contained zooxanthellae. To verify this hypothesis, a comparative study of MADAG content in gastrodermal cells and exoderm is required.

The difference in lipid class proportions was the main distinction between *Xenia* (Table 1) and other taxonomic groups of corals from the shallow-waters of Vietnam [7]. PCA of TL compositional data showed a chemotaxonomic resemblance of *Xenia* and other alcyonarians (Fig. 1). The PoL/NL ratio in *Xenia* sp. (1.09) was significantly higher than that in reef-building corals (0.26). This is a reason of

Table 4 The composition of molecular species of phospho- and phosphonolipids from the soft coral *Xenia* sp

Lipid class	Molecular species	Content	
		% of each lipid class	% of total phosphorus-contained lipids ^a
Lyso ChoGpl	16:0e lyso	6.6	0.35
	18:0e lyso	77.8	4.12
	20:4 lyso	1.4	0.07
	Unknown	14.2	0.75
ChoGpl	16:0e/18:4	2.3	0.91
	16:0e/18:2; 18:0e/16:2	6.0	2.37
	16:0e/20:4	15.8	6.24
	18:0e/20:4	51.9	20.50
EtnGpl	Unknown	24.0	9.48
	15:1e/16:2	0.8	0.17
	20:4 lyso	6.5	1.35
	18:1e/20:4	86.4	17.97
SerGpl	19:0e/20:4	2.6	0.54
	16:1e/20:4	2.4	0.50
	16:0e/20:4	0.6	0.12
	Unknown	0.7	0.15
InsGpl	18:1e/24:5	7.6	1.56
	18:0e/24:5	68.4	14.02
	19:1e/24:5	0.6	0.12
	24:5 lyso	0.4	0.08
CAEP	18:0e lyso	1.2	0.25
	Unknown	21.8	4.47
	18:0e/20:4	4.8	0.21
	16:1/22:6	1.5	0.06
	16:0/22:6	13.0	0.56
	18:0/20:4	1.9	0.08
	18:0/22:4	33.1	1.42
	18:0e/24:5	1.7	0.07
	18:0/20:3	0.8	0.03
	18:1/22:6	1.4	0.06
	18:0/22:6	8.0	0.34
	18:0/22:5; 16:0/24:5	8.0	0.34
18:0/24:6	16.1	0.69	
18:0/24:5	6.1	0.26	
Unknown	3.6	0.15	
PakCho	16:0/18:2 base	100.0	9.60
PakEtn		76.0	
PakSer		92.8	
PakIns		76.6	
Alkyl acyl PL		6.5	65.3
Diacyl PL			3.9
lyso PL			6.2
Phosphonolipids			9.6
Unknown			15.0

^a Calculated on the base of the polar lipid class content (Table 2, spectrophotometry data)

the clear chemotaxonomic division of the soft coral *Xenia* sp. and the reef-building corals (Fig. 1). PoL/NL ratios in *Xenia* sp. and other alcyonarians were similar whereas TAG/WE ratios in *Xenia* sp. and other alcyonarians were different. In the average, *Xenia* sp. contained more TAG, but less WE than other zooxanthellate alcyonarians, such as *Sinularia*, *Lobophytum*, and *Sarcophyton* [7].

The resistance of *Xenia* sp., *Sinularia* sp., and *Sarcophyton ehrenbergi* to temperature-induced coral bleaching, a process by which corals lose their zooxanthellae, was compared [42]. *Xenia* sp. was the most vulnerable to high heat stress, whereas the more resistant *S. ehrenbergi* had the same type of zooxanthellae (clade C) as less resistant *Xenia* sp. Thermal resistance of a single coral colony strongly depends on its lipid content. Under thermal stress, corals utilize both TAG and WE, but consumption rates of these storage lipid classes are different [13, 21, 22]. The lower level of WE may be a possible reason of the lower thermal stress resistance of *Xenia* sp. in comparison with other soft coral species.

Valerenenol (1), isovalerenenol, and dehydrovalerenenol (2) were isolated from an unidentified soft coral of the family Xenidiidae [43] and isovalerenenol was then synthesized [38]. Later, several chemical studies described new terpenoids in *Xenia* species but did not mention valerenenol. We found that valerenenol constituted about 1/5 of the substances extracted from *Xenia* tissues by chloroform–methanol. Valerenenol can be suggested as a chemotaxonomic marker but the finding of valerenenol in two *Xenia* species is not enough to attribute this marker to the exact taxonomic level (species, genus, or family). Valerenenol was shown to be unstable and easy converted to dehydrovalerenenol after the loss of a water molecule. A signal of molecule ions was absent in the EI–MS spectrum of valerenenol. Probably, valerenenol instantly lost a water molecule in a GC injector (250 °C) during GC–MS analysis. Therefore, the EI–MS spectrum of dehydrovalerenenol may be obtained instead of that of valerenenol. Furthermore, nobody checked the origin of dehydrovalerenenol in coral extracts. Dehydrovalerenenol is suggested to be a product of non-enzymatic dehydrating of valerenenol or isovalerenenol.

Polar Lipids

ChoGpl, EtnGpl, SerGpl, InsGpl, lyso phospholipids, phosphatidic acid, and CAEP were found in wide-spread Vietnamese soft corals, such as *Sinularia*, *Lobophytum*, and *Sarcophyton* [44, 45]. ChoGpl, EtnGpl, SerGpl were the major phospholipids, and CAEP was a major phospholipid in alcyonarian lipids. Thus, the polar lipid composition of *Xenia* sp. (Table 2) was typical for alcyonarians. Polar lipids of *Xenia* sp. contained PtdIns, which was not described for Caribbean gorgonian corals [46] and the

cold-water soft coral *Gersemia rubiformis* [47]. CAEP is an important feature of lipid composition of corals, jellyfishes, sea anemones [40, 48] and is also present in some other organisms (e.g. mollusks) in substantial quantities.

The soft coral *Xenia* sp. contains endosymbiotic dinoflagellates (*Symbiodinium* group) named zooxanthellae. Therefore, TL extracted from the intact colonies of *Xenia* sp. were a mixture of lipids of symbionts and the host. Zooxanthellae lipids may contribute up to 20 % of the total coral lipids. Zooxanthellae lipids contain 56–67 % of polar lipids [49, 50]. Thus, the polar lipids of zooxanthellae may influence the composition of polar lipids of intact coral colonies. Autotrophic zooxanthellae and the heterotrophic host have different lipid compositions [10, 50]. Glycolipids (typical lipids of photosynthetic membranes) constituted 55 % of polar lipids of *Symbiodinium* strains [51] and were found to be a basic part of non-phosphorus polar lipids of *Xenia* sp. (data not shown). Phosphatidylcholine was the major phospholipid of *Symbiodinium* strains [51]. Hence, among phospholipid classes, the composition of choline glycerophospholipids (ChoGpl) of symbiotic corals is mostly influenced by lipids of zooxanthellae. While 4/5 of total ChoGpl of *Xenia* sp. were alkyl acyl glycerophosphocholines (Table 4) synthesized in coral tissues, a part of other ChoGpl probably originated from zooxanthellae.

Fatty Acids

The first attempt of the analysis of *Xenia* lipids was made for *Xenia umbellata* (the Jeddah coast, Saudi Arabia) [8]. Extremely low lipid content (6.23 µg/g), very high percentages of saturated and monoenoic FA, the absence of tetracosapolyenoic acids, and the presence of only two PUFA (14:3 and 20:4) were found in *X. umbellata*. In our study, the list of FA of *Xenia* sp. (Table 3) was similar to that of other Vietnamese soft corals described previously [6, 7]. Monoenoic FA and PUFA amounted to 5.7 and 56.5 %, respectively, of total fatty acids and aldehydes of *Xenia* sp. Tetracosapolyenoic acids (24:5n-6 and 24:6n-3), the chemotaxonomic markers of octocorals [52], were also found in *Xenia* sp. These FA markers are absent in reef-building corals, hydrocorals and zoanthids [7, 53].

Most of alcyonarian species, as well as *Xenia* sp., contain more 24:5n-6 than 24:6n-3, while 24:6n-3 is the main tetracosapolyenoic acid in some gorgonian species, sea fans, pennatularians, and jellyfishes [6, 7, 52, 54]. If acids 24:5n-6 and 24:6n-3 are synthesized by the same enzyme complex, the 24:5n-6/24:6n-3 ratio can be easily explained by the n-6/n-3 ratio of C_{20–22} PUFA used as a substrate. The composition of C_{20–22} PUFA in cnidarians depends on their food sources, PUFA transfer from symbionts, and own biosynthesis. Furthermore, the selective inhibition of 24:5n-6 synthesis in some gorgonian specimens has been described

[55]. The study of PL molecular species of *Xenia* sp. (Table 4) showed the difference in the distribution of C₂₄ PUFA between different PL classes. The 24:5n-6/24:6n-3 ratio may be an important index for the monitoring of several ecological and biochemical parameters of some cnidarian groups.

C₂₀₋₂₄ PUFA of n-6 series (20:4n-6, 22:4n-6, and 24:5n-6) predominated in polar lipids (PoL) of *Xenia* sp., whereas 18:2n-6 and 18:3n-6 (biosynthetic precursors of n-6 C₂₀₋₂₄ PUFA) were more abundant in neutral lipids (NL) (Table 3). The high level of 20:4n-6 and 24:5n-6 in PoL of corals has been reported previously [6]. The predomination of C₂₀₋₂₄ PUFA of n-6 series in PoL of *Xenia* sp. is explained by the high content of the molecular species of ChoGpl, EtnGpl, and SerGpl substituted by these PUFA (Table 4). Such PL molecular species are probably synthesized in the coral host tissues. Zooxanthellae are known to synthesize essential 18:2n-6 and 18:3n-6 [6, 7] and provide the host with NL included into “lipid droplets” of “lipid bodies” [56]. Therefore, zooxanthellae are a possible source of NL with the high content of 18:2n-6 and 18:3n-6 in *Xenia* sp. Highly unsaturated 18:5n-3 is the main PUFA of mono- and digalactosyl diacylglycerols of zooxanthellae [51, 57]. The inclusion of these glycolipids into a total polar lipid fraction extracted from *Xenia* sp. led to the increasing of 18:5n-3 content in PoL (Table 3). Thus, the distribution of the key PUFA between PoL and NL of *Xenia* sp. reflected both the FA composition of individual lipid classes and the origin of these lipids.

Up to the present study, tetracosatetraenoic acid (24:4) was not found in soft corals. Using the scheme of Sprecher’s pathway [58], the synthesis of 24:5n-6 in *Xenia* sp. could be assumed as follows: 20:4n-6 → 22:4n-6 → 24:4n-6 → 24:5n-6. In this cause, 24:4n-6 must be the intermediate compound. We could not identify 24:4 in *Xenia* sp. as 24:4n-6 and therefore could not confirm Sprecher’s pathway of 24:5n-6 synthesis in this coral species. Acid 22:5n-6 was found in many soft coral species [6]. Hence, we suppose 24:5n-6 in soft corals to be synthesized by further chain elongation of 22:5n-6 (20:4n-6 → 22:4n-6 → 22:5n-6 → 24:5n-6) [7]. The further investigations of the structure and the origin of 24:4 in *Xenia* and special screening of 24:4n-6 in other octocoral genera could help us to determine the pathways of tetraacosapolyenoic acid synthesis in cnidarians.

When total FA are used as PCA variables, the full division of reef-building and soft corals are always observed on PCA plots [7, 53], therefore TL compositional data were used to compare *Xenia* sp., reef-building corals, and soft corals (Fig. 1). The FA compositional data were used to compare *Xenia* sp. and soft corals (Fig. 2). The division of *Xenia* sp. and azooxanthellate soft corals, as well as the location of *Xenia* sp. within zooxanthellate soft

coral species were observed in Fig. 2. This observation highlighted the importance of symbionts in the formation of total FA profile of intact coral colonies. The variables 16:2n-7, 18:2n-7, 18:2n-6, 18:3n-6, 20:5n-3, 22:4n-6, and 22:6n-3 were in charge of the division of *Xenia* and other soft corals (Fig. 2). Some of these variables, namely 18:2n-6, 18:3n-6, 20:5n-3, and 22:6n-3, are characteristic PUFA of symbiotic dinoflagellates [50, 51]. Zooxanthellae probably take part in the synthesis of 16:2n-7 and 18:2n-7 [6]. The transfer of PUFA mentioned above is supposed in coral symbiont-host association [7, 20, 22]. Thus, PUFA of zooxanthellae mainly defined the chemotaxonomic relationship between *Xenia* sp. and the soft coral species with the high level of 18:3n-6 and the low level of C₁₆ PUFA (Fig. 2). The high levels of 18:3n-6 and trace amounts of C₁₆ PUFA were found in FA of reef-building corals [6, 26]. Hence, PUFA patterns in zooxanthellae of *Xenia* sp. are similar to that in reef-building coral species and some soft coral species from the genera *Sarcophyton* and *Sinularia*.

In zooxanthellae of soft corals, 16:3n-4 and 16:4n-1 originate from 16:2n-7 [6]. The inverse correlation between the content of 18:3n-6 and 16:2n-7 was established in most soft coral species [7]. $\Delta 6$ Desaturase converts 18:2n-6 into 18:3n-6 and 16:1n-7 into 16:2n-7. If 18:3n-6 and 16:2n-7 are synthesized by the same enzyme, the substratum (18:2n-6 or 16:1n-7), which is mainly used by $\Delta 6$ desaturase, defines the main product (18:3n-6 or 16:2n-7, respectively) [20]. Probably, we observe the high level of 18:3n-6 and the low level of C₁₆ PUFA in *Xenia* sp. (Table 3) because 18:2n-6 is the main substratum for $\Delta 6$ desaturase.

Molecular Species of Polar Lipids

Each individual lipid class is a mixture of the molecular species differed in their acyl and/or alkyl groups. The analysis of the composition of molecular species of TAG with three acyl groups in a molecule is a nontrivial task. The analysis of molecular species of polar lipids with diacyl or alkyl acyl groups is less complex. LC-MS using tandem high resolution mass spectrometry (HRMS) is one of the most powerful methods of such analyses.

To define characteristic ions and fragmentation pathways of PL molecular species expected in *Xenia* sp., a comparative HRMS of several PL standards was performed. The alkyl acyl glycerophosphocholine was shown to be identified at the MS³ stage (Fig. 3), whereas alkyl acyl glycerophosphoethanolamine gave the ions characterizing FA group at the MS² stage (Fig. 4). The fragmentation of negative quasi-molecular ions of PtdSer and PtdIns allows the identification of its molecular species at the MS² stage (Figs. 5, 6).

For comparison, the fragmentation of a diacyl form of soybean PtdCho and PtdEtn was also defined (data not

shown). Molecular species of these PtdCho and PtdEtn were identified at the MS³ and MS² stages, respectively. At the MS² stage, quasi-molecular ions gave the signals after eliminating of the dehydrated molecules of unsaturated FA. These signals were not observed in the spectra of PtdEtn with saturated FA. The application of (C₂H₅)₃N/AcOH mixture as a reactant resulted in the formation of acetylated negative molecular ions ([M+CH₃COO]⁻) and positive quasi-molecular ions ([M+H]⁺) of PtdCho. At the same time, PtdEtn molecules generated negative quasi-molecular ions [M-H]⁻ and positive cluster ions [M+H+(C₂H₅)₃N]⁺.

We expected alkyl acyl and alkenyl acyl forms in PL of *Xenia* sp. investigated, because the considerable amounts of a plasmalogen form was previously found in some phospholipids (ChoGpl, EtnGpl, and SerGpl) of the soft corals *Lobophytum* and *Sarcophyton* collected in Vietnam [44]. LC-MS approaches have been used for the analysis of molecular species of phospho- and phosphonolipids in marine invertebrates [40, 59] but not in corals. The molecular species profile of polar lipids of the soft coral *Xenia* sp. was elucidated for the first time. Sharp distinctions between individual polar lipid classes were found both in lipid forms and in FA compositions (Table 4). The alkyl acyl form predominated in ChoGpl and SerGpl, but EtnGpl mainly consisted of the alkenyl acyl form (plasmalogens). On the contrary, the diacyl form was mainly presented in PtdIns. A low variety of FA was found in choline, ethanolamine, and serine glycerophospholipids. ChoGpl and EtnGpl were mainly “build” on the base of arachidonic acid (20:4n-6), whereas SerGpl mainly contained tetracosapentaenoic acid (24:5n-6) as acyl group. The main pool of 24:5n-6 (the chemotaxonomic marker of octocorals) was presented by 18:0e/24:5 PakSer. A variety of C_{20–24} PUFA (20:3, 20:4, 22:4, 22:5, 22:6, 24:5, and 24:6) was detected in PtdIns molecular species. Only one molecular species of CAEP with 16:0 acyl group was detected. According to PL chemical structures published previously, alkyl acyl and alkenyl acyl glycerophospholipids of the soft coral *Xenia* sp. are most likely to be 1-*O*-alkyl-2-acyl- and 1-*O*-alkenyl-2-acyl-glycerophospholipids, respectively. However we cannot confirm the positions of alkyl and acyl groups using MS data only.

The application of the tandem HRMS in lipidomics analysis of the soft coral *Xenia* sp. allowed the identification of most PL molecular species and highlighted the peculiar composition of polar lipids of corals. In addition to FA, we assume PL molecular species characteristic for corals to be applied as both trophic markers and environment indicators in coral reef ecosystems.

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