Algal Research 77 (2024) 103353

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Lipidomic fingerprinting of the red seaweed *Asparagopsis* spp. evidencing specific profiling in gametophyte and tetrasporophyte life stages

Diana Lopes [a,b,*], Felisa Rey [a,b], Tânia Melo [a,b], Marisa Pinho [a,b], Ana S.P. Moreira [b], Katia Pes [c], Leonardo Mata [c,d], M. Rosário Domingues [a,b]

[a] *CESAM - Centre for Environmental and Marine Studies, Department of Chemistry, University of Aveiro, Santiago University Campus, 3810-193 Aveiro, Portugal*
[b] *Mass Spectrometry Centre, LAQV-REQUIMTE, Department of Chemistry, University of Aveiro, Santiago University Campus, 3810-193 Aveiro, Portugal*
[c] *Marine Plant Ecology Research Group, Centre of Marine Sciences, University of Algarve (CCMAR), 8005-139 Gambelas, Faro, Portugal*
[d] *Greener Grazing, LLC, Greenfield, MA 01301, USA*

ARTICLE INFO

Keywords:
Fatty acids
Lipidomics
Mass spectrometry
Rhodophyta
Seaweed

ABSTRACT

In recent years, there has been a growing interest in seaweed biomass for multiple industrial applications due to their broad spectrum of high-value nutrients and bioactive metabolites. Seaweed lipids, and particularly the polar lipids, are a source of polyunsaturated fatty acids (PUFA), which have been reported to possess various biological activities, and are a sustainable alternative to fish oils. However, the profile of polar lipids in seaweeds is still understudied and therefore it is crucial to expand the knowledge of the lipidome of seaweeds, particularly of those with commercial potential, including the invasive species such as *Asparagopsis* sp. These invasive species produce a large biomass, making it a sustainable strategy for reducing its abundance, which would result in both environmental and economic benefits.

This study aimed to comprehensively characterize the fatty acids and polar lipid profile of the two *Asparagopsis* species (*A. armata* and *A. taxiformis*), across two life stages (gametophyte and tetrasporophyte) and two different origins (wild and cultivated), using highly sensitive mass spectrometry-based approaches.

Our study provides the first detailed characterization of the polar lipidome of *Asparagopsis* species and revealed that the gametophyte stage exhibited a greater number of polar lipid species than the tetrasporophyte stage. The phosphatidylcholine and the sulfoquinovosyl-diacylglycerol classes are the most abundant in all the samples studied. Although the lipidome of *A. armata* and *A. taxiformis* from the gametophyte stage showed similar distributions, variations in the relative abundance of lipid molecular species were observed. The statistical analysis of all *A. armata* specimens revealed that the lipid profile of samples from the same life stage were similar, regardless of their distinct origin (i.e., wild and cultivated). Furthermore, several polar lipid species with reported bioactivity were identified in *Asparagopsis* spp., providing insights into the lipid composition of these invasive species and their potential for further biotechnological applications. However, given the low absolute content of lipids in *Asparagopsis* species, it is unlikely to be viable to use nuisance or farmed biomass as a source of lipids alone.

1. Introduction

Marine macroalgae, also known as seaweed, are getting an increasing interest in western countries as a source of biomass for food and feed, and due to their value-added ingredients for different industrial and biotechnological applications [1]. From the multiple compounds of interest in seaweed biomass, lipids are probably the less studied, comparing to other molecules, due to their low abundance, ranging between 1 and 10 % of dry weight (depending on the species) [2–4]. However, seaweed lipids are a source of omega-3 ($n-3$) and omega-6 ($n-6$) polyunsaturated fatty acids (PUFA), such as alpha-linolenic acid (ALA, 18:3 $n-3$) and linoleic acid (LA, 18:2 $n-6$). Both are essential FA that need to be consumed through the diet, as mammals are unable to synthesize them. Macroalgae are also a source of long-chain $n-3$ and $n-6$ PUFA including eicosapentaenoic acid (EPA, 20:5 $n-3$), docosahexaenoic acid (DHA, 22:6 $n-3$) and arachidonic acid (AA,

---

* Corresponding author at: CESAM - Centre for Environmental and Marine Studies, Department of Chemistry, University of Aveiro, Santiago University Campus, 3810-193, Aveiro, Portugal.

E-mail address: dianasalzedaslopes@ua.pt (D. Lopes).

https://doi.org/10.1016/j.algal.2023.103353
Received 25 July 2023; Received in revised form 23 November 2023; Accepted 2 December 2023

20:4 $n-6$), which play an important role in the prevention and management of non-communicable diseases [5,6]. In addition, seaweed have a beneficial $n-3/n-6$ PUFA ratio, which contributes to their effectiveness as a dietary supplement or as part of a balanced diet [7]. PUFA are found mainly esterified in polar lipids rather than in free form. Polar lipids are widespread in algae, and often represent the most abundant lipid category in the lipid pool [8–10]. They are essential structural lipids, acting as building blocks of seaweed membranes (plastidial and extraplastidial), but are also important signalling molecules, playing vital regulatory roles in several cellular processes essential to cell survival [11]. The polar lipids identified in algae include phospholipids (PL), glycolipids (GL) and betaine lipids (BL) which have been shown to possess various biological activities, including anti-inflammatory, antioxidant, among others. These activities have attracted attention for their potential exploitation in pharmaceutical and nutraceutical applications [12–15]. Therefore, the study of the lipid composition of seaweed species that are (or have the potential to be) commercially available from aquaculture and/or wild harvested (if highly abundant or invasive) is interesting from a commercial application perspective.

The red seaweed genus *Asparagopsis* (Rhodophyta phylum) matches these two criteria in European waters. It has demonstrated capacity to be farmed in the ocean for the extraction of bioactive molecules for the cosmetics industry [16] and also on-land in tanks, acting as a seaweed biofilter for the effluents of fish farms [17]. Currently, the efforts to make *Asparagopsis* spp. a commercially cultivable seaweed are increasing worldwide due to the discovery of their notable anti-methanogenic activity in ruminant animals [18], contributing to the reduction of the livestock impact on climate change [19]. Furthermore, algae of the *Asparagopsis* genus are considered invasive species in many regions in Europe, forming dense assemblages over large areas and impacting local ecosystems [20,21]. Harvesting biomass as part of a managed biomass control plan can represent an opportunity for the valorization of their biomass, including biotechnological applications [22,23].

Asparagopsis spp. have a myriad of macromolecules and secondary metabolites with biotechnological relevance as reviewed by Felix et al. [23], but little is known about the lipids in this genus. The few studies that addressed the analysis of lipids in *Asparagopsis* spp. were focused on screening the FA composition [24–27], and only one of these performed the analysis of lipid classes by thin layer chromatography (TLC) [27]. Furthermore, these studies were focused only on the gametophyte stage. To the best of our knowledge, no studies addressed the detailed characterization of the polar lipid profile of *Asparagopsis* spp. The *Asparagopsis* genus comprises the two morpho species, *A. taxiformis* (Delile) Trevisan de Saint-Léon 1845, and *A. armata* Harvey, 1855, with heteromorphic life stages, comprised by gametophytes exhibiting a feathery appearance and by tetrasporophytes consisting of branched filaments formed by series of four cells [17]. Chemical dissimilarities have been reported, particularly in halogen compounds, between both species and their life stages, as well as between wild and cultivated tetrasporophytes [28–30]. Therefore, addressing the lipidome signature between species and under distinct life stages or from different origins is a key step to understand the nutritional and bioactive potential of lipids of *Asparagopsis* spp. and consequently contribute to the biomass valorization. Mass spectrometry (MS)-based lipidomics approach is a high sensitivity methodology that allows the structural characterization of the huge diversity of lipid species distributed by different lipid classes. Recently, this technique was used to identify the lipidome and add-value of different marine species, including the lipid species composition and FA content of several macroalgae species [2,4,31,32].

The aim of this study was to characterize and compare the lipidome (FA and polar lipid profile) of the two *Asparagopsis* species (*A. armata* and *A. taxiformis*) in the gametophyte stage, as well as two life stages (gametophyte and tetrasporophyte) and wild versus cultured tetrasporophytes of *A. armata* using a MS-based approach.

2. Materials and methods

2.1. Macroalgae samples

The gametophytes of *A. armata* and *A. taxiformis* were collected from rocky substrates between 2 and 5 m deep by snorkeling in Praia da Baleeira, Portugal (37° 00′ 41.0″ N, 8° 55′ 48.8″ W) on 17/04/2020, the time of the year when gametophytes individuals are large and present in abundance in the region. The tetrasporophyte phase of *A. armata* was hand-collected from unattached (drifting) individuals in Praia de Faro, Portugal (36° 59′ 39.0″ N, 7° 58′ 31.6″ W) using a cloth bag on 28/04/2020. Samples of cultured tetrasporophyte of *A. armata* were also collected from laboratory cultures at Centro de Ciências do Mar Algarve, Portugal on 28/04/2020 from individuals collected on Praia dos Arrifes, Portugal (37° 04′ 36.0″ N, 8° 16′ 33.1″ W) on the 01/03/2019 and maintained in tumble culture conditions since then. The wild collected samples were transported to the laboratory in seawater inside a cooling box. In the laboratory, all seaweed samples were rinsed for 5 s with running distilled freshwater, enough time to wash out excess external saltwater and thus minimize external salts differences between samples that could influence the results. Excess freshwater was removed using absorbent paper towel, and finally dried at 30 °C in the dark for 4 days using a VENTI-Line oven (VWR, USA). Dried samples were stored in pre-labelled zip-lock bags and sent to the University of Aveiro for lipidomic analysis.

2.2. Lipid extraction

A modified Bligh and Dyer protocol [33] was used to extract the lipids from *Asparagopsis*'s biomass. The biomass was macerated to thin particles with liquid nitrogen and using a mortar and pestle. After maceration, 250 mg of biomass (five replicates, $n = 5$) were mixed with 3.75 mL of MeOH:CH_2Cl_2 (2:1 v/v) in a glass tube and incubated on ice for 2 h, with periodic mixing. The tubes with the mixture were then centrifuged for 10 min at 2000 rpm, and the organic phase containing the lipid fraction was collected to a new glass tube. The biomass residue was re-extracted with a mixture of 3 mL MeOH:CH_2Cl_2 (2:1 v/v) and 2.3 mL of Milli-Q water was added to the final organic phase (containing the lipids) followed by centrifugation for 10 min at 2000 rpm. This step allowed removing non-lipid substances from the lipid extract. The lower phase was transferred to a new glass tube and evaporated in nitrogen flow. The lipid extracts were weighed and subsequently stored at −20 °C until analysis.

2.3. Fatty acids analysis

The fatty acids analysis was performed by gas chromatography mass spectrometry (GC–MS) after the conversion of fatty acids to fatty acid methyl esters (FAMEs) by mixing 30 μg of lipid extracts with 1 mL of *n*-hexane containing the internal standard methyl nonadecanoate (1.0 μg mL^{-1}) (Sigma, St. Louis, MO, USA) followed by the addition of 200 μL of 2.0 M methanolic solution of potassium hydroxide [3,34]. Samples were vigorously mixed by vortex for 2 min, and 2 mL of a saturated solution of NaCl was added, following by centrifugation at 1000 rpm for 5 min. A volume of 600 μL of the upper phase was transferred to a new glass tube and evaporated in a nitrogen flow. FAMEs were dissolved in *n*-hexane and analyzed on an Agilent Technologies 8860 GC System (Santa Clara, CA, USA) interfaced with an Agilent 5977B Mass Selective Detector (Agilent, Santa Clara, CA, USA) and equipped with a DB-FFAP capillary column (length: 30 m; internal diameter: 0.32 mm and film thickness: 0.25 μm; J&W Scientific, Folsom, CA, USA) according to Monteiro et al. [35]. The Agilent MassHunter Qualitative Analysis 10.0 software was used for fatty acids identification and quantification. The fatty acids profile was expressed in relative percentage (%) calculated by dividing the peak area of each FA by the sum of the peak area of all identified FA.

2.4. Lipidome analysis by mass spectrometry

The lipid extract was analyzed by hydrophilic interaction liquid chromatography (HILIC) on an Ultimate 3000 Dionex (Thermo Fisher Scientific, Bremen, Germany) with an autosampler, coupled to a Q-Exactive hybrid quadrupole mass spectrometer (MS) (Thermo Fisher, Scientific, Bremen, Germany). Solvent gradient and compositions of mobile phase A and B used for this analysis were described by Monteiro et al. [35]. The lipid extract was mixed with the starting eluent and a mixture of standards 1,2-dimyristoyl-sn-glycero-3-phosphocholine (dMPC, PC 14:0/14:0; CAS Number 18194-24-6); 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (dMPE, PE 14:0/14:0; CAS Number 998-07-2); 1,2-dimyristoyl-sn-glycero-3-phospho-(10-rac-)glycerol (dMPG, PG 14:0/14:0; CAS Number 200880-40-6); 1,2-dimyristoyl-sn-glycero-3-phospho-L-serine (dMPS, PS 14:0/14:0; CAS Number 105405-50-3); 1,2-dipalmitoyl-sn-glycero-3-phosphatidylinositol (dPPI, PI 16:0/16:0; CAS Number 34290-57-8); 10,30-bis[1-dimyristoyl-sn-glycero-3-phospho]-glycerol (tMCL, (CL14:0)4; CAS Number 63988-20-5); 1,2-dimyristoyl-sn-glycero-3-phosphate (dMPA, PA 14:0/14:0; CAS Number 80724-31-8); 1-nonadecanoyl-2-hydroxy-sn-glycero-3-phosphocholine (LPC 19:0; CAS Number 95416-27-6); N-heptadecanoyl-D-erythro-sphingosylphosphorylcholine (NPSM, SM d18:1/17:0; CAS Number 121999-64-2); N-heptadecanoyl-D-erythro-sphingosine (Cer (d18:1/17:0; CAS Number 67492-16-4) purchased from Avanti Polar Lipids, Inc. (Alabaster, AL, USA) and introduced into the Ascentis Express HILIC column (length:15 cm; internal diameter: 2.1 mm and particle size: 2.7 μm; Sigma-Aldrich, Darmstadt, Germany). The mass spectrometer equipped with Orbitrap® was operated in simultaneous positive and negative ionization modes utilizing high resolution at 70,000 and AGC target of 1×10^6. For MS/MS analysis, a resolution of 17,500 and AGC target of 1×10^5 was used. Xcalibur (V3.3, Thermo Fisher Scientific, USA) was the software used for data acquisition and analysis. The identification of the polar lipid species was achieved by interpretation of their LC-MS/MS spectra, typical retention time and mass accuracy (error < 5 ppm). The semi-quantification of the identified polar lipids species in *Asparagopsis* spp. was carried out by determining the peak area of each species and dividing it by the area of the standard lipid species. In the case of the lipids form the glycolipids and betaine lipids, due to the absence of internal commercial standards, the semi quantification was performed by dividing it by the area of the standard species with the closest retention time (Cer d18:1/17:0 for MGMG, MGDG, DGMG and DGDG; dMPG for SQMG and SQDG; dmPE for MGTS and DGTS). To determine variations of lipid species within a lipid class, the matrix containing the normalized areas of each class was used to calculate the relative abundance (%) of each lipid species, as previously described [3].

2.5. Statistical analysis

The polar lipidome variations among wild gametophyte *A. armata*, wild tetrasporophyte *A. armata* and cultivated tetrasporophyte *A. armata* were assessed. Metaboanalyst was utilized for statistical analysis of lipidomic data. To address variables equal to 0, they were replaced with a small value, which was assumed to represent the limit of detection (half of the minimum positive value in the original data). A data filtering process using the interquartile range (IQR) was conducted to identify variables with near-constant values across experimental conditions. Subsequently, the normalized data were logarithmically transformed and autoscaled for further analysis. Principal component analysis (PCA) was applied to visualize the general 2D clustering patterns in the dataset.

For comparative analysis of the normalized extracted ion chromatograms (XIC) areas of identified lipid species among different samples, a one-way ANOVA test followed by Tukey's HSD post hoc comparisons was performed. To control for multiple testing, the *p*-values were corrected using the Benjamini–Hochberg false discovery rate (FDR, q values). For generating the hierarchal clustering heatmap, the 25 polar lipids with the lowest q values were utilized. All reported data are presented as means and standard deviation of five replicates (n = 5).

3. Results and discussion

3.1. Adaptation of fatty acids profile of Asparagopsis species related to their life stage and origin

The FA profile of gametophyte and tetrasporophyte life stages of *Asparagopsis* species from wild and cultivated specimens revealed the presence of saturated (SFA) (14:0, 16:0 and 18:0), monounsaturated (MUFA) (16:1 *n*−7 and 18:1), and polyunsaturated (PUFA) (16:2 *n*−6, 16:4 *n*−1, 20:4 *n*−6 and 20:5 *n*−3) fatty acids, but showing different relative abundances depending on the species and life stage (Table 1). The palmitic acid (16:0) was the most abundant FA across the *Asparagopsis* species, regardless the life stages and origin, while FA 16:2 *n*−6 and 16:4 *n*−1 were only present in *A. armata* gametophyte, in accordance with previously reported in other studies with *Asparagopsis* [25,27,36,37]. All *Asparagopsis* samples had a higher SFA proportion compared to PUFA and MUFA.

The gametophyte life stage of *A. armata* and *A. taxiformis* revealed different FA profiles. *Asparagopsis armata* showed higher proportion of MUFA and PUFA and lower proportion of SFA than *A. taxiformis* (23.1 % vs 13.3 % of MUFA; 14.8 % vs 2.9 % of PUFA; 62.1 % vs 84.6 % of SFA, *A. armata* vs *A. taxiformis*, respectively). A low PUFA proportion in *A. taxiformis* was reported in some studies [27,38], while a major PUFA proportion was stated in studies of *A. armata* [26,36].

The comparison of both life stages of *A. armata* pinpoints a dissimilar proportion between SFA and unsaturated FA (sum of MUFA and PUFA). Gametophyte stage has lower proportion in SFA and higher proportion of unsaturated FA than the tetrasporophyte stage. The same trend in the proportion of SFA and unsaturated FA was also observed between gametophyte and tetrasporophyte stages of *Chondrus crispus*, from Rhodophyta phylum [39].

The analysis of tetrasporophyte life stage of *A. armata* from both origins (i.e., wild and cultivated) showed that the wild *A. armata* had lower proportion of SFA and higher proportion of MUFA than cultivated *A. armata* (73.4 % vs 82.5 % of SFA; 19.5 % vs 10.5 % of MUFA, wild vs cultivated, respectively). The proportion of PUFA was similar (7.1 % vs

Table 1

Fatty acid profile of total lipid extract of *Asparagopsis* species determined by GC–MS analysis. Values are expressed in relative abundance (%) as mean of five samples (n = 5) ± standard deviation. Double bond position of 18:1 was not assigned.

Fatty acids	Gametophyte *A. taxiformis* (wild)	Gametophyte *A. armata* (wild)	Tetrasporophyte *A. armata* (wild)	Tetrasporophyte *A. armata* (cultivated)
14:0	10.2 ± 0.7	7.4 ± 0.3	10.9 ± 0.4	5.6 ± 0.4
16:0	50.8 ± 0.7	35.8 ± 0.4	40.6 ± 0.6	49.9 ± 0.8
16:1 *n*−7	8.1 ± 0.9	15.0 ± 0.4	6.5 ± 0.6	nd
16:2 *n*−6	nd	1.3 ± 0.1	nd	nd
16:4 *n*−1	nd	1.3 ± 0.0	nd	nd
18:0	23.8 ± 1.2	18.9 ± 1.1	21.8 ± 1.8	27.0 ± 2.3
18:1	5.2 ± 0.3	8.2 ± 0.3	12.9 ± 0.6	10.5 ± 0.9
20:4 *n*−6	nd	3.0 ± 0.1	2.7 ± 0.4	4.7 ± 1.0
20:5 *n*−3	2.0 ± 0.3	9.3 ± 0.2	4.5 ± 0.9	2.3 ± 0.3
SFA	84.7 ± 1.0	62.1 ± 0.7	73.4 ± 2.0	82.5 ± 2.0
MUFA	13.3 ± 0.8	23.1 ± 0.5	19.5 ± 1.1	10.5 ± 0.9
PUFA	2.0 ± 0.3	14.8 ± 0.3	7.1 ± 1.2	7.0 ± 1.2

Abbreviations: SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; nd: not detected.

7.0 %) between samples of different origin. The lack of studies characterizing the FA profile of tetrasporophyte life stage impairs a critical comparison.

In general, few dissimilar trends in the FA profile were noticed for the same species when compared with results from literature.

Environmental factors and life stages may contribute to variations in the FA profile and composition of collected samples [40]. Marine organisms are known for their remarkable adaptability to environmental shifts, allowing them to fine-tune their metabolism and adapt to variations in their native habitats. This adaptability is evident in the case of macroalgae, which exhibit distinct FA profile when grown in the wild compared to cultivated settings [2], when harvested in different seasons [41] and from different origin [42]. The variations in FA composition are understandably attributed to differences in the growth environments, encompassing natural fluctuations like temperature and light exposure conditions. Similarly, the variations in FA profile between gametophyte and tetrasporophyta life stages are related to a complex interplay of biological factors, including varying nutritional requirements, and underlying genetic influences, which collectively shape the specific metabolic processes and priorities of each life stage [43].

3.2. Polar lipids profile of *Asparagopsis* species

The average lipid content of *Asparagopsis* species was determined through gravimetric analysis of lipid extracts, yielding values of 0.7 ± 0.1 dry weight (DW) for Gametophyte *A. armata* wild, 1.8 ± 0.3 % DW for Gametophyte *A. taxiformis* wild, and 1.9 ± 0.4 % DW for both wild and cultivated Tetrasporophyte *A. armata*.

The polar lipid species identified by high resolution LC-MS in *Asparagopsis* species comprise glycolipids (GL), phospholipids (PL), betaine lipids (BL) and sphingolipids (SL) groups. PL represent the group with the highest number of identified lipid species, distributed along the following classes: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), and the lyso forms (i.e., PL molecular species with one esterified fatty acid), LPC, LPE and LPG. GL was the second lipid group in number of identified species, distributed among the classes digalactosyldiacylglycerol (DGDG), monogalactosyldiacylglycerol (MGDG), sulfoquinovosyldiacylglycerol (SQDG), and their lyso forms digalactosylmonoacylglycerol (DGMG), monogalactosylmonoacylglycerol (MGMG) and sulfoquinovosylmonoacylglycerol (SQMG). BL included diacylglycerol-trimethylhomoserine (DGTS) and monoacylglycerol-trimethylhomoserine (MGTS) classes. Lipid species from the SL class inositol phosphoceramide (PI-Cer) were also detected in *Asparagopsis* samples. The total number of lipid species identified in the distinct groups was summarized in Table 2.

The total number of identified lipid species for gametophyte life stage was 241 and 240 in *A. armata* and *A. taxiformis*, respectively. For tetrasporophyte life stage, a total of 215 lipid species were identified from wild and cultivated *A. armata*. Most lipid species are common across the samples, but their proportion varied between samples. Some of the less abundant species were only detected in specific samples (Table S1).

As PL and GL groups exhibit the highest number of identified lipid species, the relative abundance per lipid class within these two groups was represented in Fig. 1 (and Fig. S1).

Most PL classes occur in extraplastidial membranes, with exception of PG which occur in both extraplastidial and thylakoid membranes. GL are exclusive of photosynthetic organisms, being localized in plastidial and thylakoid membranes. PC, PG and SQDG are the most abundant classes over all *Asparagopsis* samples. Red macroalgae are particularly rich in PC and PG [44–46]. In comparison to other investigated conditions, SQDG in the cultivated tetrasporophyre stage displays a reduced proportion, possibly indicating a regulation of the photosystem. SQDG and PG are anionic lipids, distinguished by their negative charged head groups, and they serve as the primary lipid components in chloroplasts. These lipids are interchangeable, allowing for the maintenance of the overall quantity of anionic lipids within the thylakoid membrane [47]. Notably, photosynthetic activity is significantly affected by irradiance levels, which are different in wild and culture conditions [48]. Therefore, the observed reduction in SQDG proportion in the cultivated tetrasporophyte stage appear to be counterbalanced by a higher proportion of PG within the same sample.

3.2.1. Polar lipid characterization of gametophyte life stage of *A. armata* and *A. taxiformis*

Most of the identified lipid species were common between the *A. armata* and *A. taxiformis* species in gametophyte life stage (Table S1). However, there were variations in their relative abundance across different classes of GL (Fig. 2), BL (Fig. 3) and PL (Fig. 4). The statistical differentiation was not performed because, comparison involving only two different species are inadequate as elucidated by Garland and Adolph [49]. Nevertheless, in-depth comparisons of the lipid signature between few closely related species, when interpreted with proper attention and carefully, can unveil important information on the lipid metabolism adaptation that can then be explored in future larger-scale comparative studies.

The most abundant lipid species of each GL class in *A. armata* were represented by DGDG 32:4, SQDG 30:0, MGDG 32:1 and MGMG 16:0, while in *A. taxiformis* the species DGDG 34:1, SQDG 30:0, MGDG 34:1 and MGMG 16:0 were the most representative (Fig. 2). Despite the differences in the most abundant DGDG and MGDG lipid species, the major lipid species in both seaweed's species have the same number of carbons.

The most abundant BL lipid species were DGTS 32:1 and MGTS 18:1 in *A. armata*, and DGTS 32:4 and MGTS 16:0 in *A. taxiformis* (Fig. 3). However, most of the BL species exhibited similar relative abundance in both classes.

Regarding to the PL group, *A. armata* displayed PC 38:6, PE 34:2, PG 32:1 and LPC 20:5 as most abundant lipid species within each class. *Asparagopsis taxiformis* had PC 34:1, PE 34:1, PG 32:1 and LPC 18:1 as most abundant species. In *A. armata*, the most abundant species in PL group were esterified with PUFA, particularly PC and LPC. On the other hand, in *A. taxiformis* they were esterified with MUFA, resulting in a lower degree of unsaturation, as it was found in the FA analysis.

The lipids of *Asparagopsis* species are scarcely studied, and the published works used traditional methods for their analysis. In this sense, the PL classes PE and PG were previously identified in *A. taxiformis* by TLC [27]. The SQMG class was the only lipid class identified in *A. taxiformis* using a liquid chromatography coupled to mass

Table 2

Number of polar lipid species identified in phospholipids (PL), glycolipids (GL), betaine lipids (BL), and sphingolipids (SL), for gametophyte and tetrasporophyte life stages of *Asparagopsis* spp. samples.

Lipid class	Gametophyte *A. armata* (wild)	Gametophyte *A. taxiformis* (wild)	Tetrasporophyte *A. armata* (wild)	Tetrasporophyte *A. armata* (cultivated)
PL	105	105	102	93
GL	87	86	80	89
BL	47	47	31	31
SL	2	2	2	2
Total number of lipid species	241	240	215	215

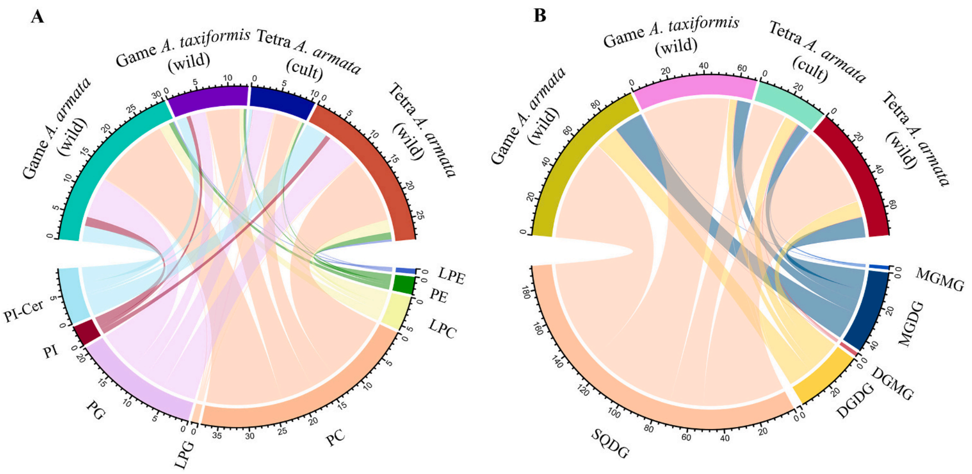

Fig. 1. Chord diagram showing the distribution of phospholipids (A) and glycolipids (B) classes (normalized extracted ion chromatograms (XIC) area) in the lipidome of gametophyte *Asparagopsis armata* and *Asparagopsis taxiformis* from wild and tetrasopophyte *Asparagopsis armata* from wild and cultivated origin.

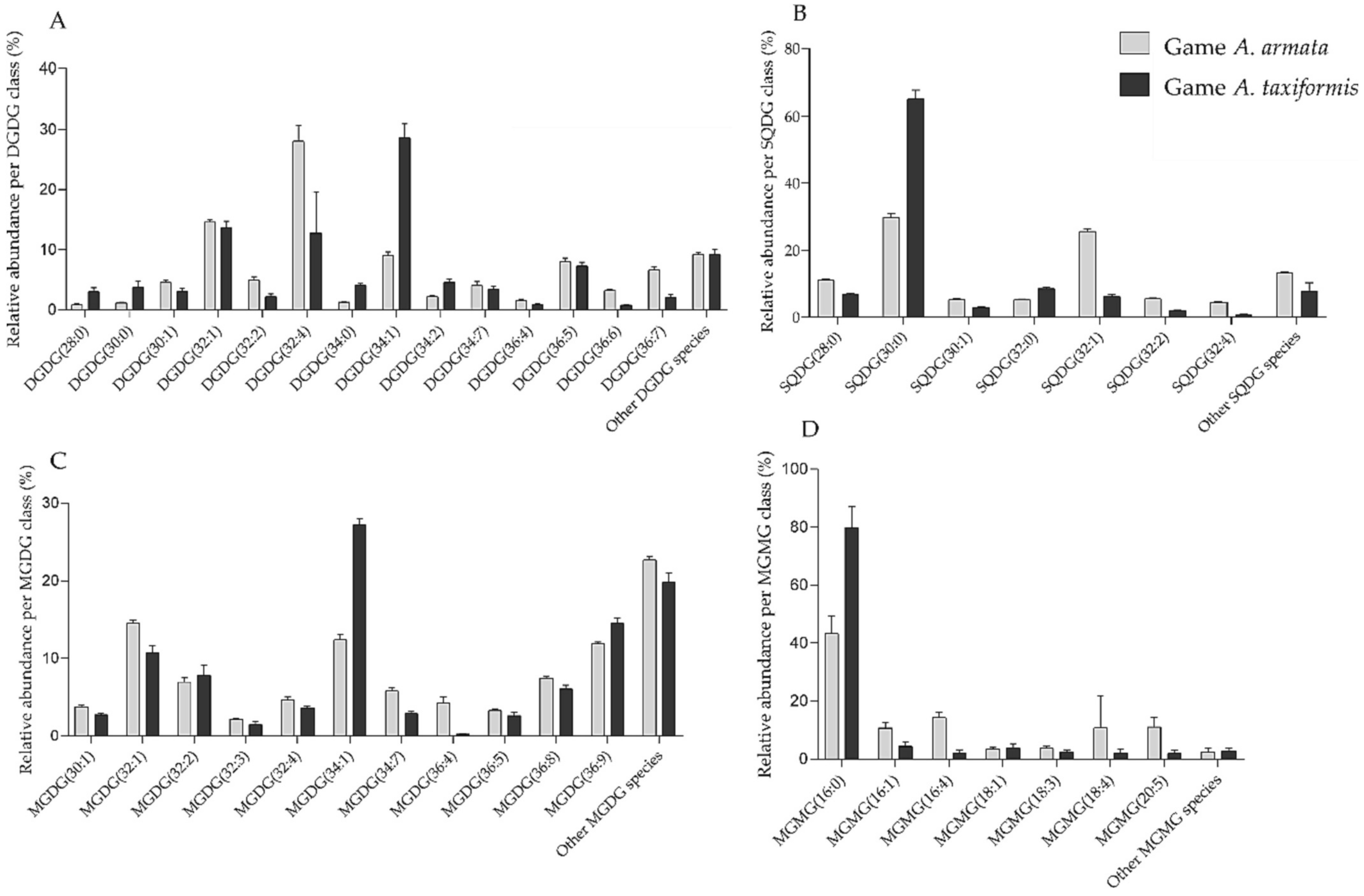

Fig. 2. Relative abundance of the most representative glycolipids classes of *Asparagopsis armata* and *Asparagopsis taxiformis* in the gametophyte stage. (A) digalactosyldiacylglycerol (DGDG); (B) sulfoquinovosildiacylglycerol (SQDG); (C) monogalactosyldiacylglycerol (MGDG) and (D) monogalactosylmonoacylglycerol (MGMG). Values are average of five samples (n = 5) ± standard deviation. Only lipid species with relative abundance >3 % were individually represented. The bars corresponding to other species are the sum of all species with a relative abundance <3 %.

spectrometry (LC-ESI/IT-TOF) approach [38].

This is the first study with detailed characterization of the polar lipidome of two *Asparagosis* species (*A. armata* and *A. taxiformis*), and shows that the lipid species are mostly coincident, with variation in terms of relative abundance. The preservation of lipid species in macroalgae from the same genus was verified in a study that compared the lipidome of three *Ulva* species cultivated under the same conditions [35]. This study revealed the conservation of lipid species throughout the *Ulva* genus, with the identification of 222 common lipid species in the three *Ulva* sp. and only 10 lipid species were specific to one or two of the *Ulva* species [35].

Several lipid species identified in the gametophyte life stage of

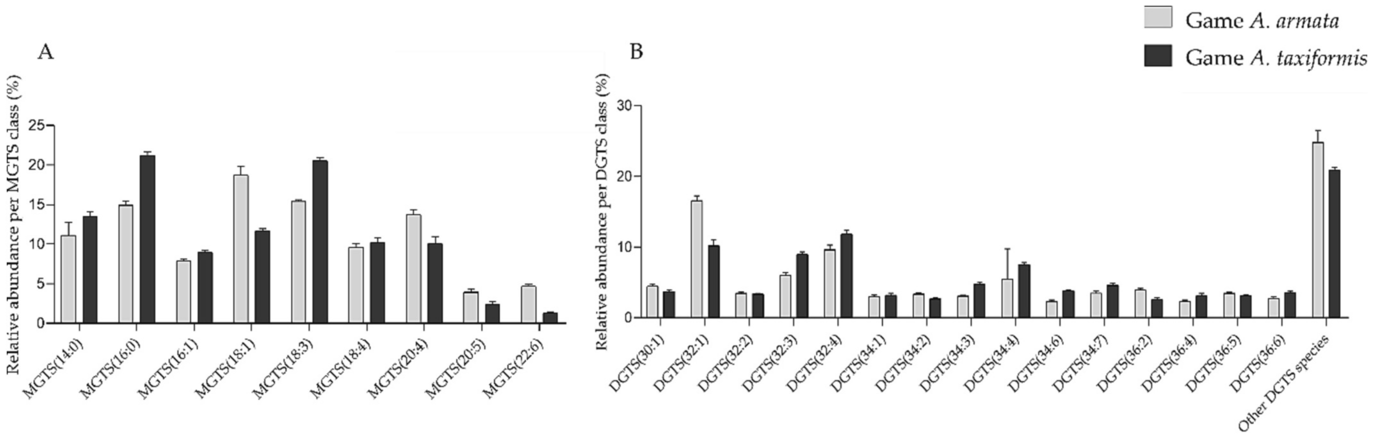

Fig. 3. Relative abundance of identified betaine lipid classes of *Asparagopsis armata* and *Asparagopsis taxiformis* in the gametophyte stage. (A) Monoacylglyceryltrimethylhomoserine (MGTS) and (B) diacylglyceryltrimethylhomoserine (DGTS). Values are average of five samples (n = 5) ± standard deviation. Only lipid species with relative abundance >3 % were individually represented. The bars corresponding to other species are the sum of all species with a relative abundance <3 %.

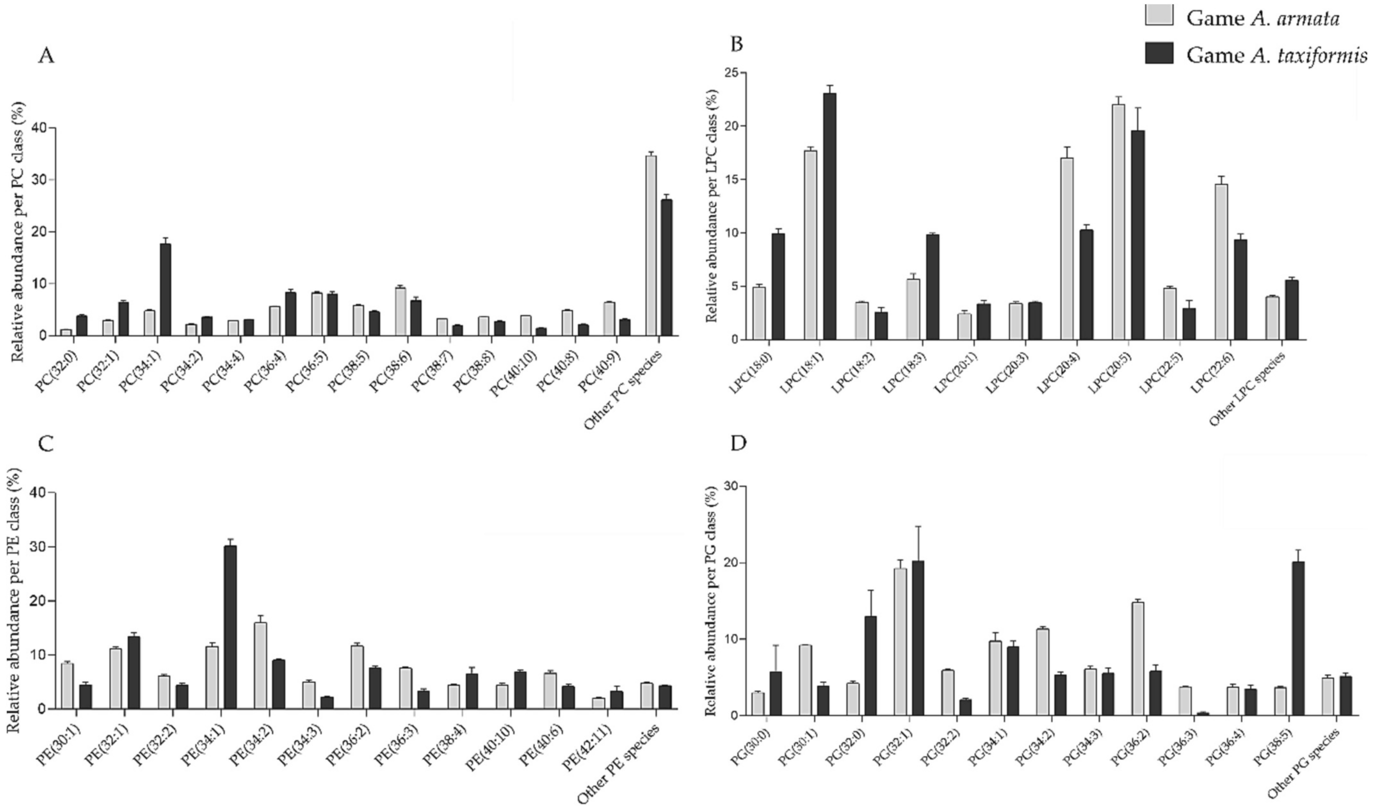

Fig. 4. Relative abundance of identified phospholipids classes of *Asparagopsis armata* and *Asparagopsis taxiformis* in the gametophyte stage. (A) Phosphatidylcholine (PC), (B) lysophosphatidylcholine (LPC), (C) phosphatidylethanolamine (PE) and phosphatidylglicerol (PG). Values are average of five samples (n = 5) ± standard deviation. Only lipid species with relative abundance >3 % were individually represented. The bars corresponding to other species are the sum of all species with a relative abundance <3 %.

A. armata and *A. taxiformis* have previously been associated with bioactivity, namely the MGDG 36:4, DGDG 36:4 and DGDG 36:5 which exhibit anti-inflammatory activity by inhibiting nitric oxide production [50]. The DGDG 36:5 has also been highlighted for its anti-inflammatory properties [50,51], and PG 34:2 has shown selective anti-inflammatory effects [52]. Furthermore, certain lysophospholipids, such as LPC, which were identified in this study, have been associated with beneficial health effects, namely LPC esterified with PUFA are commonly linked to anti-inflammatory effects [53].

3.2.2. *Variation of polar lipidome according to life stage and origin*

The principal component analysis (PCA) with lipid species dataset of wild gametophyte *A. armata*, wild tetrasporophyte *A. armata* and cultivated tetrasporophyte *A. armata* showed a clear separation between the three groups, with the first two principal components explaining 64.7 % (PC1 45 %, PC2 19.7 %) of total variance observed (Fig. 5A).

The heatmap/clustering analysis (Fig. 5B) shows the 50 lipid species that most contributed to differentiation between samples of *A. armata*. The first cluster between the gametophyte and the two samples in

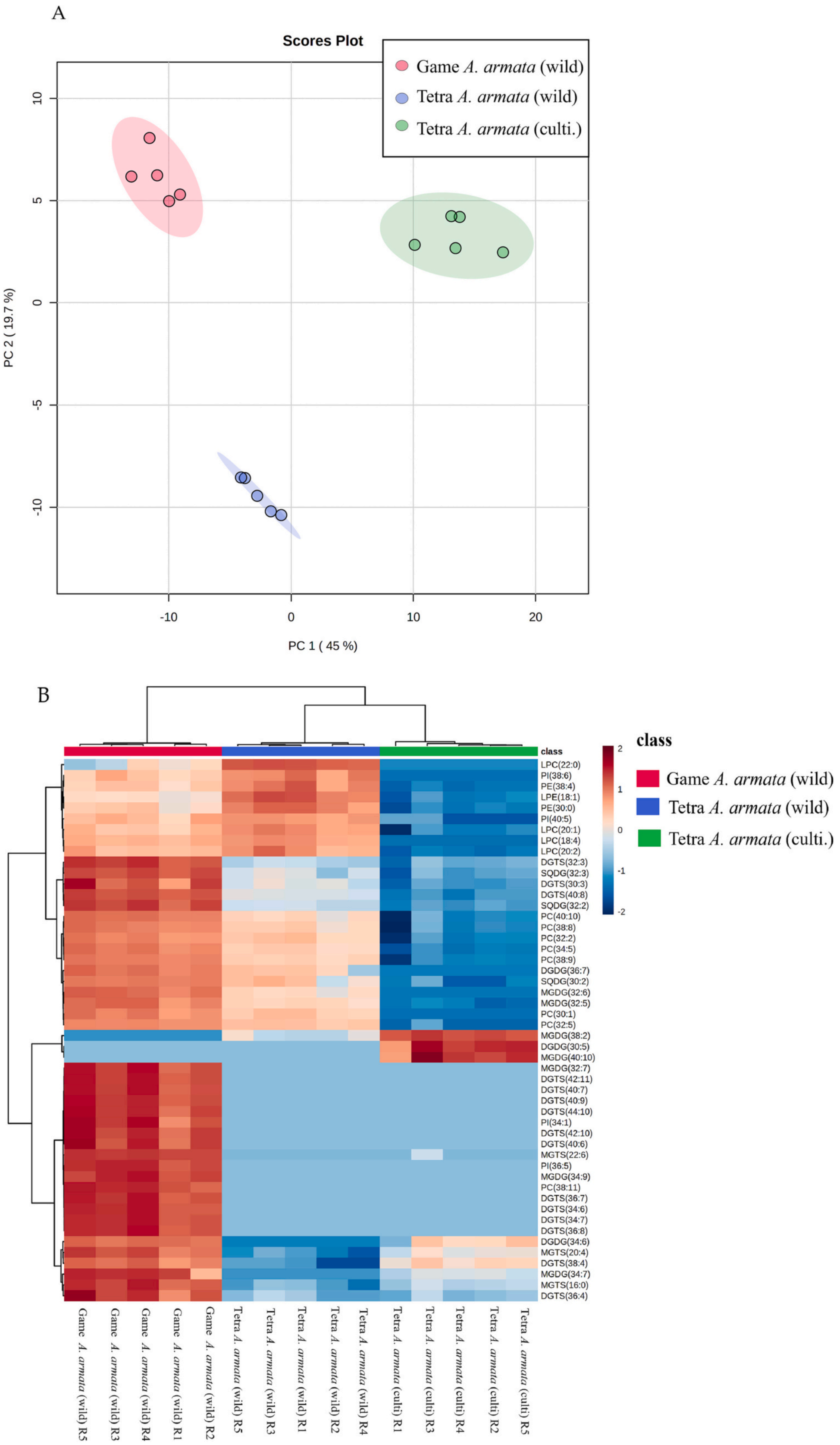

Fig. 5. A) Principal component analysis (PCA) and B) heatmap/cluster analysis of gametophyte and tetrasporophyte life stages of *Asparagopsis armata* cultivated and from wild.

tetrasporophyte stage pinpoints the closeness of life stages over the origin of the samples. The set of lipid species that more contributed to discriminate between wild and cultivated *Asparagopsis*, include lysophospholipids species (LPC, LPE), which are most abundant in wild individuals. The great relative abundance of these lyso species in macroalgae from the wild could be related to stress factors [54]. Lysophospholipids are described as signalling molecules in plants and can respond rapidly to abiotic stress triggers, including those related to salt, temperature stress, and nitrogen and phosphate scarcity [55,56]. These signalling molecules have the capacity to interact with proteins, affecting their activity, or they can influence the recruitment of proteins to cell membranes and participate in phytohormone signalling pathways. Consequently, lyso-phospholipids can impact diverse aspects of plant biology, including growth and development, cellular processes, and adaptation to stress conditions [57].

The lipid species that most discriminate the tetrasporophyte and gametophyte life stages are mostly BL, which are less abundant in tetrasporophyte stage.

Univariate analysis results of the normalized XIC area of the 25 lipid species that more contributed to discrimination between life stages were represented in the Fig. 6, including 11 species of DGTS, 5 species of MGDG, 4 species of PC, 2 species of PI and 1 species of MGTS, 1 LPC and 1 SQDG.

The top 25 lipid species that most contributed to discrimination between gametophyte *A. armata* wild, tetrasporophyte *A. armata* wild and tetrasporophyte *A. armata* cultivated were significantly different mainly between gametophyte and tetrasporophyte life stages, while only 10 out of 25 lipid species presented differences between tetrasporophyte *A. armata* wild and cultivated (LPC 18:4, MGDG 32:6, PC 30:1, DGTS 40:8, SQDG 32:2, MGDG 32:5, PI 38:6, MGDG 38:2, PC 32:5; PC34:5).

There is a great contribution of BL species for samples discrimination, they are high abundant in gametophyte stage, and the low abundant in tetrasopophyte stage.

Other studies, such the comparison of lipidome of *Porphyra dioica* in two different life stages (gametophyte and sporophyte) showed differences in PL profile, namely in number and relative abundance of molecular lipid species [32]. The authors suggest that lipidome of different life stages were more likely related with shifts in lipids of extraplastidial membranes rather than in plastidial membranes [32]. The same hypothesis seems to be valid for *Asparagopsis* life stages, as the DGTS, MGTS and PC species belong mainly to extraplastidial membranes and are those that more contributed for samples discrimination.

The top 25 species that significantly contributed to differentiate between cultivated and wild *A. armata* tetrasporophyte (Fig. S2) include molecular species of the following classes PI (3 lipid species), PC (2 species), LPC (3 species), PE (4 species), PG (1 species), PI-Cer (2 species), MGDG (6 species), DGDG (1 species) and SQDG (3 species). Most of these species exhibited a higher proportion in wild than in cultivated tetrasporophyte, except the SQDG 36:6 and MGDG 32:0, which were more abundant in cultivated than in wild tetrasporophyte.

The invasive nature of the genus *Asparagopsis* raises concerns across

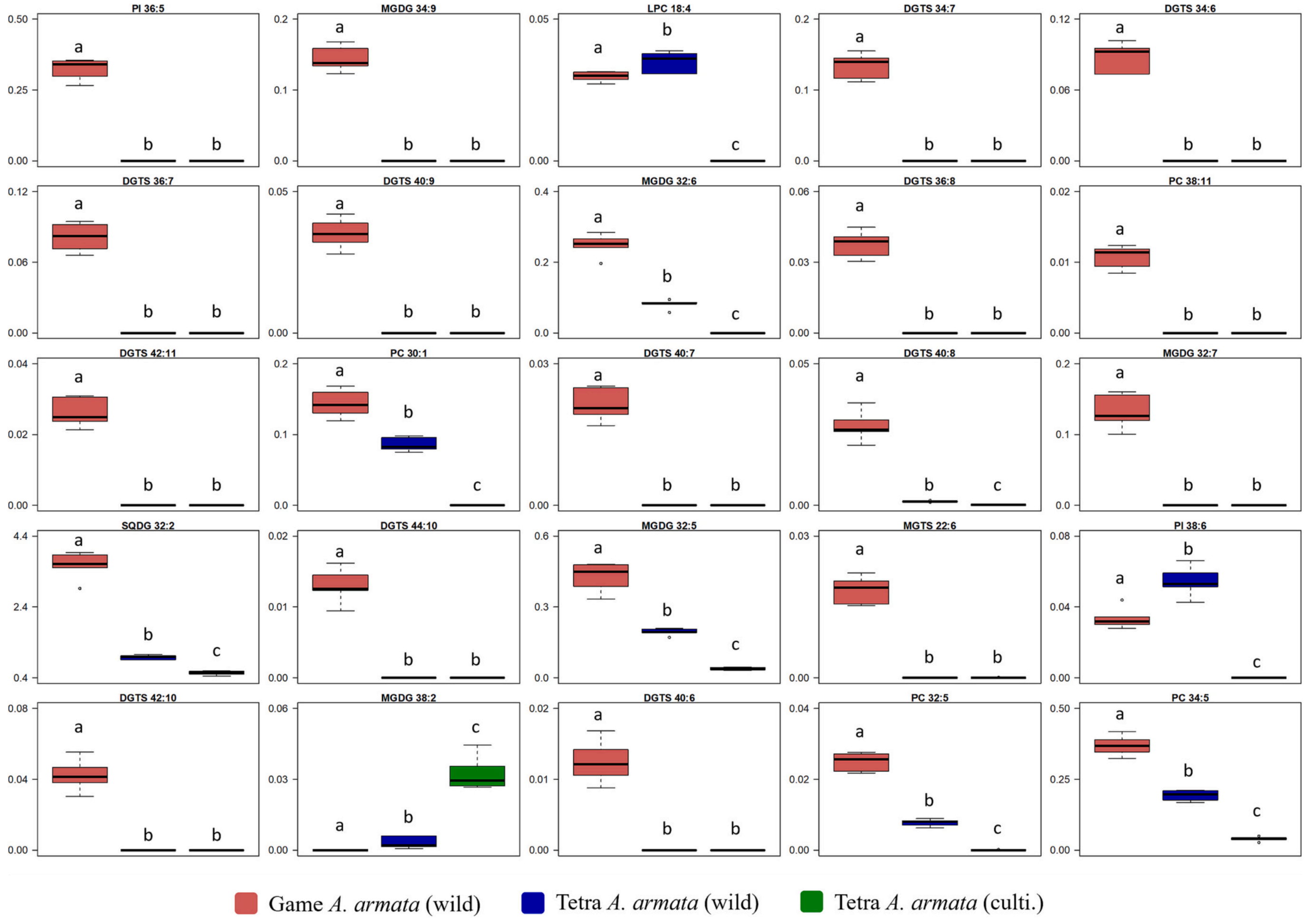

Fig. 6. Boxplots of the top 25 lipid species that most contributed to discrimination between gametophyte *Asparagopsis armata* wild, tetrasporophyte *A. armata* cultivated and tetrasporophyte *A. armata* wild (sorted by the lowest q values). Y-axis represent the normalized extracted ion chromatogram (XIC) area of lipid species. Different letters represent significant differences among samples (Tukey's HSD post hoc analysis, $q < 0.05$).

different regions of the globe. Thus, it is important to find a commercial application for the available large amounts of *Asparagopsis* biomass. Despite the negative impact in marine ecosystems, *Asparagopsis* species display a great biotechnological potential, as supported by the identification of bioactive polar lipids, regardless of the specific species, life stage, and origin. The Asparagopsis biomass used in this study was harvest during the spring. When interpreting the results pertaining to lipid content, the results should be considered with caution as seaweed lipids can exhibit variation influenced by factors such as species, geographic location, season, temperature, salinity, light intensity, and interactions among these factors [46,56,58,59]. In terms of characterizing the polar lipidome, previous research has demonstrated that the composition in lipid species is typically unique to each macroalgae species [44], with variations primarily observed in the abundance of specific lipid species in response to varying abiotic condition, as reported for example for the adaptation of *Ulva rigida* lipidome with seasons [41].

4. Conclusion

In this study, we conducted the first comprehensive characterization of the polar lipidome of two *Asparagopsis* species, encompassing different life stages and origins. Our findings revealed distinct FA profile, depending on the species and life stages examined. Specifically, the gametophyte stage of *A. armata* exhibited higher proportion of PUFA compared to gametophyte stage of *A. taxiformis*, whereas the tetrasporophyte life stage of *A. armata* displayed lower PUFA proportion than the gametophyte life stage. Notably, the most prominent MUFA and PUFA identified across all samples were 18:1 and 20:5 $n{-}3$, respectively.

Our results demonstrate that the gametophyte life stage encompasses higher number of total lipids species compared to tetrasporophyte life stage. However, both stages exhibited a greater relative abundance of PL and GL, with PC and SQDG being the predominant lipid classes. The lipidomic profiles of *A. armata* and *A. taxiformis* in gametophyte life stage displayed similarities, with variations primarily observed in the relative abundance of lipid species. Irrespective of their origin (wild vs cultivated), samples from the same life stage exhibited a similar lipid profile, indicating that the lipidome remains consistent during the first stage.

Furthermore, we identified lipid species in *Asparagopsis* spp. that have been previously associated with bioactive properties. These findings provide valuable insights into the lipid composition of *Asparagopsis* spp. and its potential as a source of bioactive lipids for various applications. However, considering the inherently low absolute lipid content in *Asparagopsis* spp., the feasibility of utilizing nuisance or farmed biomass solely as a lipid source presents limited feasibility. Diversifying the utility of the biomass in a biorefinery approach, including the extraction of valuable compounds beyond lipids holds the potential to significantly enhance their economic viability providing a more comprehensive and resource-efficient approach to unlock the full potential of *Asparagopsis* genus.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2023.103353.

CRediT authorship contribution statement

Diana Lopes: Conceptualization, Formal analysis, Methodology, Writing – original draft. **Felisa Rey:** Data curation, Formal analysis, Methodology, Validation, Writing – review & editing, Software. **Tânia Melo:** Data curation, Methodology, Validation, Writing – review & editing. **Marisa Pinho:** Data curation, Methodology, Writing – review & editing. **Ana S.P. Moreira:** Data curation, Methodology, Writing – review & editing. **Katia Pes:** Resources, Writing – review & editing. **Leonardo Mata:** Conceptualization, Resources, Validation, Visualization, Writing – review & editing. **M. Rosário Domingues:** Conceptualization, Data curation, Methodology, Supervision, Validation, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

The authors are grateful to University of Aveiro, Fundação para a Ciência e Tecnologia (FCT), and Ministério da Ciência Tecnologia e Ensino Superior (MCTES) for the financial support to the research units LAQV/REQUIMTE (UIDP/50006/2020 + UIDB/50006/2020), and CESAM (UIDB/50017/2020 + UIDP/50017/2020 + LA/P/0094/2020), and to Greener Grazing, LLC, for financially supporting the provision of Asparagopsis biomass for the study. Tânia Melo (CEECIND/01578/2020) and Felisa Rey (CEECIND/00580/2017) acknowledge FCT/MCTES for individual funding in the scope of the Individual Call to Scientific Employment Stimulus. Marisa Pinho acknowledges FCT/MCTES her Phd grant (UI/BD/153346/2022). Ana S. P. Moreira acknowledges the research contract funding from the LAQV-REQUIMTE (UIDB/50006/2020) project.

References

[1] N. Trivedi, R. Sharma, A.S. Mondal, D. Dixit, Recent Advances in Biotechnology of Seaweeds: An Overview, Sustainable Global Resources of Seaweeds Volume 1: Bioresources, Cultivation, Trade and Multifarious Applications, 2022, pp. 627–644.

[2] F. Rey, P. Cartaxana, T. Melo, R. Calado, R. Pereira, H. Abreu, P. Domingues, S. Cruz, M.R. Domingues, Domesticated populations of *Codium tomentosum* display lipid extracts with lower seasonal shifts than conspecifics from the wild—relevance for biotechnological applications of this green seaweed, Mar. Drugs 18 (2020) 188.

[3] A.S. Moreira, E. da Costa, T. Melo, D. Lopes, A.C. Pais, S.A. Santos, B. Pitarma, M. Mendes, M.H. Abreu, P.N. Collén, Polar lipids of commercial *Ulva* spp. of different origins: profiling and relevance for seaweed valorization, Foods 10 (2021) 914.

[4] E. Da Costa, T. Melo, A.S.P. Moreira, C. Bernardo, L. Helguero, I. Ferreira, M. T. Cruz, A.M. Rego, P. Domingues, R. Calado, M.H. Abreu, M.R. Domingues, Valorization of lipids from *Gracilaria* sp. through lipidomics and decoding of antiproliferative and anti-inflammatory activity, Mar. Drugs 15 (2017) 62.

[5] P.C. Calder, Very long-chain n−3 fatty acids and human health: fact, fiction and the future, Proc. Nutr. Soc. 77 (2018) 52–72.

[6] I. Djuricic, P.C. Calder, Beneficial outcomes of omega-6 and omega-3 polyunsaturated fatty acids on human health: an update for 2021, Nutrients 13 (2021) 2421.

[7] J. Lunn, H. Theobald, The health effects of dietary unsaturated fatty acids, Nutr. Bull. 31 (2006) 178–224.

[8] M. Kendel, G. Wielgosz-Collin, S. Bertrand, C. Roussakis, N. Bourgougnon, G. Bedoux, Lipid composition, fatty acids and sterols in the seaweeds *Ulva armoricana*, and *Solieria chordalis* from Brittany (France): an analysis from nutritional, chemotaxonomic, and antiproliferative activity perspectives, Mar. Drugs 13 (2015) 5606–5628.

[9] J. Santos, F. Guihéneuf, G. Fleming, F. Chow, D. Stengel, Temporal stability in lipid classes and fatty acid profiles of three seaweed species from the north-eastern coast of Brazil, Algal Res. 41 (2019), 101572.

[10] S. Khotimchenko, Lipids from the marine alga *Gracilaria verrucosa*, Chem. Nat. Compd. 41 (2005) 285–288.

[11] P. Kumari, M. Kumar, C. Reddy, B. Jha, Algal lipids, fatty acids and sterols, in: Functional Ingredients from Algae for Foods and Nutraceuticals, Elsevier, 2013, pp. 87–134.

[12] A.P. Simopoulos, The importance of the ratio of omega-6/omega-3 essential fatty acids, Biomed. Pharmacother. 56 (2002) 365–379.

[13] E. Alves, M. Dias, D. Lopes, A. Almeida, M.R. Domingues, F. Rey, Antimicrobial lipids from plants and marine organisms: an overview of the current state-of-the-art and future prospects, Antibiotics 9 (2020) 441.

[14] L.D. Abd El-Samee, I. El-Wardany, S. Abdel-Fattah, N.A. Abd El-Azeem, M. Elsharkawy, Dietary omega-3 and antioxidants improve long-chain omega-3 and lipid oxidation of broiler meat, Bull. Natl. Res. Centre 43 (2019) 1–9.

[15] D. Lopes, F. Rey, M.C. Leal, A.I. Lillebø, R. Calado, M.R. Domingues, Bioactivities of lipid extracts and complex lipids from seaweeds: current knowledge and future prospects, Mar. Drugs 19 (2021) 686.

[16] S. Kraan, K.A. Barrington, Commercial farming of *Asparagopsis armata* (Bonnemaisoniceae, Rhodophyta) in Ireland, maintenance of an introduced species? J. Appl. Phycol. 17 (2005) 103–110.

[17] A. Schuenhoff, L. Mata, R. Santos, The tetrasporophyte of *Asparagopsis armata* as a novel seaweed biofilter, Aquaculture 252 (2006) 3–11.

[18] M. Zanolla, R. Carmona, L. Mata, J. De la Rosa, A. Sherwood, C.N. Barranco, A. R. Muñoz, M. Altamirano, Concise review of the genus *Asparagopsis* Montagne, 1840, J. Appl. Phycol. (2022) 1–17.

[19] P.J. Gerber, H. Steinfeld, B. Henderson, A. Mottet, C. Opio, J. Dijkman, A. Falcucci, G. Tempio, Tackling Climate Change Through Livestock: A Global Assessment of Emissions and Mitigation Opportunities, Food and Agriculture Organization of the United Nations (FAO), 2013.

[20] B. Schaffelke, C.L. Hewitt, Impacts of Introduced Seaweeds, 2007.

[21] C.O. Silva, M.F. Lemos, R. Gaspar, C. Gonçalves, J.M. Neto, The effects of the invasive seaweed *Asparagopsis armata* on native rock pool communities: evidences from experimental exclusion, Ecol. Indic. 125 (2021), 107463.

[22] P. Ning, G. Yang, L. Hu, J. Sun, L. Shi, Y. Zhou, Z. Wang, J. Yang, Recent advances in the valorization of plant biomass, Biotechnol. Biofuels 14 (2021) 102.

[23] R. Félix, P. Dias, C. Félix, T. Cerqueira, P.B. Andrade, P. Valentão, M.F. Lemos, The biotechnological potential of *Asparagopsis armata*: what is known of its chemical composition, bioactivities and current market? Algal Res. 60 (2021), 102534.

[24] G. El-Baroty, M. Moussa, M. Shallan, M. Ali, A. Sabh, E. Shalaby, Contribution to the aroma, biological activities, minerals, protein, pigments and lipid contents of the red alga: *Asparagopsis taxiformis* (Delile) Trevisan, J. Appl. Sci. Res. 3 (2007) 1825–1834.

[25] A. Galindo, D.B. Reis, I. Rodríguez, J.A. Pérez, B. Abdul-Jalbar, R. Zárate, N. Nunes, M.A.P. de Carvalho, N.G. Acosta, C. Rodríguez, Lipid characterization of 14 macroalgal species from Madeira Archipelago: implications for animal and human nutrition, Bot. Mar. 65 (2022) 51–67.

[26] H. Pereira, L. Barreira, F. Figueiredo, L. Custódio, C. Vizetto-Duarte, C. Polo, E. Rešek, A. Engelen, J. Varela, Polyunsaturated fatty acids of marine macroalgae: potential for nutritional and pharmaceutical applications, Mar. Drugs 10 (2012) 1920–1935.

[27] N. Nunes, G.P. Rosa, S. Ferraz, M.C. Barreto, M.P. de Carvalho, Fatty acid composition, TLC screening, ATR-FTIR analysis, anti-cholinesterase activity, and in vitro cytotoxicity to A549 tumor cell line of extracts of 3 macroalgae collected in Madeira, J. Appl. Phycol. (2019) 1–13.

[28] N.A. Paul, R. de Nys, P. Steinberg, Chemical defence against bacteria in the red alga *Asparagopsis armata*: linking structure with function, Mar. Ecol. Prog. Ser. 306 (2006) 87–101.

[29] A. Vergés, N.A. Paul, P.D. Steinberg, Sex and life-history stage alter herbivore responses to a chemically defended red alga, Ecology 89 (2008) 1334–1343.

[30] N. Andreakis, G. Procaccini, C. Maggs, W.H. Kooistra, Phylogeography of the invasive seaweed *Asparagopsis* (Bonnemaisoniales, Rhodophyta) reveals cryptic diversity, Mol. Ecol. 16 (2007) 2285–2299.

[31] D. Lopes, A.S. Moreira, F. Rey, E. da Costa, T. Melo, E. Maciel, A. Rego, M. H. Abreu, P. Domingues, R. Calado, Lipidomic signature of the green macroalgae *Ulva rigida* farmed in a sustainable integrated multi-trophic aquaculture, J. Appl. Phycol. 31 (2019) 1369–1381.

[32] E. Da Costa, V. Azevedo, T. Melo, A.M. Rego, D.V. Evtuguin, P. Domingues, R. Calado, R. Pereira, M.H. Abreu, M.R. Domingues, High-resolution lipidomics of the early life stages of the red seaweed *Porphyra dioica*, Molecules 23 (2018) 187.

[33] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917.

[34] S. Aued-Pimentel, J.H.G. Lago, M.H. Chaves, E.E. Kumagai, Evaluation of a methylation procedure to determine cyclopropenoids fatty acids from Sterculia striata St. Hil. Et Nauds seed oil, J. Chromatogr. A 1054 (2004) 235–239.

[35] J.P. Monteiro, E. Costa, T. Melo, P. Domingues, A. Fort, M.R. Domingues, R. Sulpice, Lipidome in-depth characterization highlights the nutritional value and species-specific idiosyncrasies of different *Ulva* species, Algal Res. 64 (2022), 102694.

[36] C.P. Rocha, D. Pacheco, J. Cotas, J.C. Marques, L. Pereira, A.M. Gonçalves, Seaweeds as valuable sources of essential fatty acids for human nutrition, Int. J. Environ. Res. Public Health 18 (2021) 4968.

[37] D.C. Pinto, M.L. Lesenfants, G.P. Rosa, M.C. Barreto, A.M. Silva, A.M. Seca, GC- and UHPLC-MS profiles as a tool to valorize the red alga *Asparagopsis armata*, Appl. Sci. 12 (2022) 892.

[38] C. Ragonese, L. Tedone, M. Beccaria, G. Torre, F. Cichello, F. Cacciola, P. Dugo, L. Mondello, Characterisation of lipid fraction of marine macroalgae by means of chromatography techniques coupled to mass spectrometry, Food Chem. 145 (2014) 932–940.

[39] M. Tasende, Fatty acid and sterol composition of gametophytes and sporophytes of *Chondrus crispus* (Gigartinaceae, Rhodophyta), Sci. Mar. 64 (2000) 421–426.

[40] S. Polat, Y. Ozogul, Seasonal proximate and fatty acid variations of some seaweeds from the northeastern Mediterranean coast, Oceanologia 55 (2013) 375–391.

[41] A.S. Moreira, E. da Costa, T. Melo, R. Sulpice, S.M. Cardoso, B. Pitarma, R. Pereira, M.H. Abreu, P. Domingues, R. Calado, Seasonal plasticity of the polar lipidome of *Ulva rigida* cultivated in a sustainable integrated multi-trophic aquaculture, Algal Res. 49 (2020), 101958.

[42] E. da Costa, F. Ricardo, T. Melo, R. Mamede, M.H. Abreu, P. Domingues, M. R. Domingues, R. Calado, Site-specific lipidomic signatures of sea lettuce (*Ulva* spp., *chlorophyta*) hold the potential to trace their geographic origin, Biomolecules 10 (2020) 489.

[43] J.S. Hughes, S.P. Otto, Ecology and the evolution of biphasic life cycles, Am. Nat. 154 (1999) 306–320.

[44] D. Lopes, T. Melo, F. Rey, E. Costa, A.S.P. Moreira, M.H. Abreu, P. Domingues, A. I. Lillebø, R. Calado, M. Rosário Domingues, Insights of species-specific polar lipidome signatures of seaweeds fostering their valorization in the blue bioeconomy, Algal Res. 55 (2021), 102242.

[45] E. Peralta-García, E. Caamal-Fuentes, D. Robledo, E. Hernández-Núñez, Y. Freile-Pelegrín, Lipid characterization of red alga *Rhodymenia pseudopalmata* (*R. hodymeniales*, *R. hodophyta*), Phycol. Res. 65 (2017) 58–68.

[46] S.V. Khotimchenko, I.M. Yakovleva, Lipid composition of the red alga *Tichocarpus crinitus* exposed to different levels of photon irradiance, Phytochemistry 66 (2005) 73–79.

[47] K. Kobayashi, Role of membrane glycerolipids in photosynthesis, thylakoid biogenesis and chloroplast development, J. Plant Res. 129 (2016) 565–580.

[48] Y. Nakajima, Y. Umena, R. Nagao, K. Endo, K. Kobayashi, F. Akita, M. Suga, H. Wada, T. Noguchi, J.-R. Shen, Thylakoid membrane lipid sulfoquinovosyl-diacylglycerol (SQDG) is required for full functioning of photosystem II in *Thermosynechococcus elongatus*, J. Biol. Chem. 293 (2018) 14786–14797.

[49] T. Garland Jr., S.C. Adolph, Why not to do two-species comparative studies: limitations on inferring adaptation, Physiol. Zool. 67 (1994) 797–828.

[50] Arjun H. Banskota, Roumiana Stefanova, Sandra Sperker, Santosh Lall, James S. Craigie, Jeff T. Hafting, Lipids isolated from the cultivated red alga *Chondrus crispus* inhibit nitric oxide production, J. Appl. Phycol. 26 (2014) 1565–1571.

[51] A.H. Banskota, R. Stefanova, P. Gallant, P.J. McGinn, Mono- and digalactosyldiacylglycerols: potent nitric oxide inhibitors from the marine microalga *Nannochloropsis granulata*, J. Appl. Phycol. 25 (2013) 349–357.

[52] A. Bruno, C. Rossi, G. Marcolongo, A. Di Lena, A. Venzo, C.P. Berrie, D. Corda, Selective in vivo anti-inflammatory action of the galactolipid monogalactosyldiacylglycerol, Eur. J. Pharmacol. 524 (2005) 159–168.

[53] S.T. Tan, T. Ramesh, X.R. Toh, L.N. Nguyen, Emerging roles of lysophospholipids in health and disease, Prog. Lipid Res. 80 (2020), 101068.

[54] X. Liu, D. Ma, Z. Zhang, S. Wang, S. Du, X. Deng, L. Yin, Plant lipid remodeling in response to abiotic stresses, Environ. Exp. Bot. 165 (2019) 174–184.

[55] E. Susanto, A.S. Fahmi, M. Hosokawa, K. Miyashita, Variation in lipid components from 15 species of tropical and temperate seaweeds, Mar. Drugs 17 (2019) 630.

[56] T.C. Hopkins, E.J.S. Graham, J. Schwilling, S. Ingram, S.M. Gómez, A.J. Schuler, Effects of salinity and nitrogen source on growth and lipid production for a wild algal polyculture in produced water media, Algal Res. 38 (2019), 101406.

[57] Q. Hou, G. Ufer, D. Bartels, Lipid signalling in plant responses to abiotic stress, Plant Cell Environ. 39 (2016) 1029–1048.

[58] E. Susanto, A.S. Fahmi, M. Abe, M. Hosokawa, K. Miyashita, Lipids, fatty acids, and fucoxanthin content from temperate and tropical brown seaweeds, Aquat. Proc. 7 (2016) 66–75.

[59] M. Nomura, H. Kamogawa, E. Susanto, C. Kawagoe, H. Yasui, N. Saga, M. Hosokawa, K. Miyashita, Seasonal variations of total lipids, fatty acid composition, and fucoxanthin contents of *Sargassum horneri* (Turner) and *Cystoseira hakodatensis* (Yendo) from the northern seashore of Japan, J. Appl. Phycol. 25 (2013) 1159–1169.