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## Photo-protective compounds in red macroalgae from Brittany: Considerable diversity in mycosporine-like amino acids (MAAs)

Lalegerie Fanny <sup>1,\*</sup>, Lajili Sirine <sup>1</sup>, Bedoux Gilles <sup>2</sup>, Taupin Laure <sup>2</sup>, Stiger-Pouvreau Valerie <sup>1</sup>, Connan Solène <sup>1</sup>

<sup>1</sup> Univ Brest, CNRS, IRD, Ifremer, LEMAR, F-29280, Plouzane, France

<sup>2</sup> Marine Biotechnology and Chemistry Laboratory (LBCM, EA 3884), University of South Brittany (UBS), 56017, Vannes, France

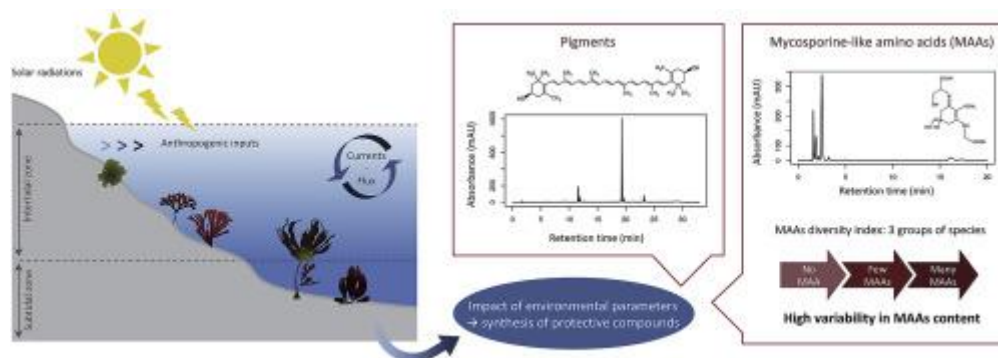
\* Corresponding author : Fanny Lalegerie, email address : [fanny.lalegerie@univ-brest.fr](mailto:fanny.lalegerie@univ-brest.fr)

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### Abstract :

To cope with the biotic and abiotic stresses experienced within their environment, marine macroalgae have developed certain defence mechanisms including the synthesis of photo-protective molecules against light and particularly harmful UV radiation. The aim of this study was to screen selected red algae, a highly diverse phylogenetic group, for the production of photo-protective molecules. The pigment content and composition (i.e. chlorophyll-a, phycobiliproteins and carotenoids) and the composition of mycosporine-like amino acids (MAAs) were studied in 40 species of red macroalgae collected in Brittany (France), at two distinct periods (i.e. February and July 2017). A high inter-specific variability was demonstrated in terms of pigment content and MAA composition. Twenty-three potential MAAs were detected by HPLC, and six were identified by LC-MS (i.e. shinorine, palythine, asterina-330, porphyra-334, usurijene and palythene). This is the first study to report on the composition of pigments and MAAs in a diverse group of red seaweeds from Brittany, including some species for which the MAA composition has never been studied before. Nevertheless, the results suggested that some species of red algae are more likely to cope with high levels of light radiation since those species such as *Bostrychia scorpioides*, *Porphyra dioica*, *Gracilaria vermiculophylla* and *Vertebrata lanosa* are living in environments exposed to higher levels of irradiation, and had various MAAs in addition to their photo-protective pigments.

## Graphical abstract



## Highlights

► Detection of 23 potential MAAs in 40 Rhodophyta species, including 6 already identified (shinorine, palythine, asterina-330, porphyra-334, usurijene, palythene). ► First report on the MAAs composition for some red seaweeds. ► High variability in MAAs content and composition between the different species, with no link with phylogeny, morphology, position on the shore or sampling site. ► A MAAs extraction method using 70% ethanol being less toxic than conventional methanol, and giving potential valorisable extracts.

**Keywords** : Algae, Diversity, HPLC, MAAs, Metabolites, Photo-protection, Pigments, Rhodophyta, Screening

## 25 1. Introduction

26 Macroalgae are known to play a key role in coastal ecosystems as being at the  
27 basis of the food web and providing refugium for many animal species (Lüning, 1990).  
28 In particular, seaweeds produce diverse compounds under natural conditions, which  
29 could potentially be used in health, cosmetic, or food sectors (Holdt and Kraan, 2011;  
30 Stengel et al., 2011; Stiger-Pouvreau and Guérard, 2018; Surget et al., 2017). However,  
31 both the concentration and composition of these bioactive compounds can fluctuate  
32 within individuals, with their habitat and with season. In the context of climate change,  
33 the environmental parameters impacting particularly intertidal seaweeds are in a state of  
34 flux. To cope with changing environmental conditions, seaweeds have developed  
35 particular adaptive, metabolic responses and are thus able to resist and adapt to different  
36 abiotic stresses (Davison and Pearson, 1996; Gantt, 1990).

37  
38 Irradiance is one of the factors which can change over the intertidal range in  
39 coastal areas, in term of quantity and quality because it varies during the day and with  
40 the tide, the turbidity of the water, the climate, the depth or seasons (Sagert et al., 1997).  
41 Light is also very important for seaweeds as algae are photosynthetic organisms (Gantt,  
42 1990; Lobban and Harrison, 1994) with some living in the intertidal zone and thus  
43 exposed directly to the sunlight during emersion phases. One of the consequences of  
44 climate change is the amount of ultraviolet (UV) radiation reaching the Earth's surface  
45 (Bischof et al., 2006; Thomas et al., 2012). UV radiations are notably at the origin of  
46 DNA alterations, the synthesis of reactive oxygen species (ROS) and, in seaweeds, the  
47 photo-inhibition mechanism or the degradation of photosynthetic pigments (Karentz,  
48 2001; Karsten, 2008; Van de Poll et al., 2001). However, due to human activities and

49 ozone depletion, the protective filter that constitutes the atmosphere gradually loses its  
50 effectiveness, which means that the amount of UV to which the organisms are exposed  
51 tends to increase (Carreto et al., 2018; McKenzie et al., 2007). Although some authors  
52 agree that the ozone layer will probably not fully recover before several decades, the  
53 hole in this layer would be resorbing at mid-latitudes due to the implementation of some  
54 policies tending to reduce greenhouse gas emissions, but this would not be the case at  
55 high latitudes and the evolution of the UV level remains complex (McKenzie et al.,  
56 2011; Wilmouth et al., 2018).

57  
58 Different types of macroalgae have developed various strategies to protect  
59 themselves from harmful UV radiation. These include the synthesis of photo-protective  
60 molecules (Bhatia et al., 2011; Le Lann et al. 2016; Rastogi et al., 2010; Sinha et al.,  
61 2007). Among the algal photo-protective compounds, this study focuses on pigments  
62 and mycosporine-like amino acids (MAAs), synthesized by red algae (Rhodophyta).  
63 MAAs were discovered in 1965 in the terrestrial fungus *Ascochyta pisi* (Leach, 1965).  
64 Since then, the presence of MAAs has been demonstrated in a wide range of marine  
65 organisms from bacteria to fish (Bandaranayake, 1998; La Barre et al., 2014; Rosic et  
66 al., 2015), including different species of algae (Bedoux et al., 2014; Sinha et al., 2007).  
67 MAAs are low molecular weight molecules (< 400 Da), soluble in water, with a high  
68 molar extinction coefficient (between 28,000 - 50,000 M<sup>-1</sup>.cm<sup>-1</sup>) and a maximum of  
69 absorption ( $\lambda_{\max}$ ) between 310 - 362 nm (Rastogi et al., 2010). In addition, it has been  
70 demonstrated that red algae, which constitute a well-marked phylogenetic group, have  
71 the greatest diversity and the highest proportion of MAAs (Carreto and Carignan, 2011;  
72 Karentz, 2001; Sinha et al., 2007). Until now, about 20 MAAs have been identified in

73 various red macroalgae (Sinha et al., 2007). However, as some recent studies have  
74 shown (Briani et al., 2018; Hartmann et al., 2016), many MAAs have yet to be  
75 identified. The major role of MAAs in photo-protection against UV radiation has  
76 already been widely demonstrated (Bedoux et al., 2014; Huovinen et al., 2004; Singh et  
77 al., 2008), and functionality could be linked to their individual structures (Wolley et al.,  
78 2018). It appears that some MAAs may also have various functions such as anti-  
79 oxidants (De la Coba et al., 2009; Torres et al., 2018; Wada et al., 2015), osmolytes,  
80 nitrogen storage, or protective agents against desiccation or temperature variations  
81 (Bhatia et al., 2011; Oren and Gunde-Cimerman, 2007).

82

83 Pigments are part of a second significant group of photo-protective compounds  
84 in macroalgae. There are three main families of pigments: chlorophylls, carotenoids and  
85 phycobiliproteins (reviewed in Stengel et al., 2011). They do not all have a role in  
86 photo-protection, such as chlorophylls and phycobiliproteins which harvest light for  
87 photosynthesis. Among the chlorophylls, there is only chlorophyll-*a* in red algae  
88 (Stengel et al., 2011). One characteristic of red algae is the presence of  
89 phycobiliproteins (reviewed by Dumay et al., 2014). These are “secondary pigments”  
90 which also capture solar energy and transfer it to chlorophyll *a* for the photosynthesis;  
91 they are particularly effective under low light conditions (Gantt, 1990; Lobban and  
92 Harrison, 1994). There are 3 main groups of phycobiliproteins in the Rhodophyta:  
93 phycoerythrin (PE,  $\lambda_{\max} = 540\text{-}570$  nm) which provides their red colour, phycocyanin  
94 (PC,  $\lambda_{\max} = 610\text{-}620$  nm) and allophycocyanin (APC,  $\lambda_{\max} = 650\text{-}655$  nm) which both  
95 have a blueish hue (Dumay et al., 2014). Finally, there are two groups of carotenoids:  
96 carotenes ( $\alpha$ -carotene,  $\beta$ -carotene in red algae) and xanthophylls (i.e. zeaxanthin,

97 antheraxanthin, violaxanthin and lutein in red algae) which convey a yellowish  
98 colouration. These last pigments have several physiological roles (Karsten, 2008;  
99 Young and Frank, 1996): they take part in photosynthesis through light harvesting in  
100 thylakoids (Hashimoto et al., 2016) but they also have an important role in photo-  
101 protection (Mimuro and Akimoto, 2003). Indeed, they also may participate in the  
102 dissipation of solar energy (Young et al., 1997) and to the de-activation of reactive  
103 oxygen species (ROS) (Rastogi et al., 2010).

104         According to the nature, quality and quantity of photo-protective compounds,  
105 and also their morphology (blade, filamentous, crustose) and life cycle, different species  
106 of macroalgae are then more or less sensitive to UV radiation (Davison and Pearson,  
107 1996; Roleda et al., 2004). In this context, the aim of the present study was to  
108 investigate the inter-specific variations in red algal photo-protective compounds within  
109 a diverse, representative group of temperate and local red seaweeds. Thus, a screening  
110 was carried out on a large number of red seaweeds sampled from the coast of Brittany  
111 (France). In order to study the maximum number of species, seaweeds were sampled  
112 during two distinctly different seasons, i.e. winter and summer, as some species occur  
113 only during a part of the year. Candidate species for analysis were also collected from  
114 the upper subtidal zone (i.e. emersed only at spring tides for maximum 1 h; Ar Gall and  
115 Le Duff, 2014) and along the intertidal zone (i.e. with different maximum emersion  
116 times from 1 h in the lower intertidal zone to 12 h in the upper intertidal zone during  
117 spring tides; Ar Gall and Le Duff, 2014). A MAA diversity index was determined for  
118 each species, in order to compare and provide hierarchical comparisons within the red  
119 macroalgae collected in this study. Moreover, in order to test the hypotheses of a  
120 relationship between the diversity of MAAs and individual red algal taxonomy, various

121 orders/families of red macroalgae were specifically targeted for collection.

122

## 123 **2. Materials and methods**

### 124 *2.1 Sampling sites*

125 Macroalgae were collected at low tides on four different sites in Brittany (France):

126 Porspoder (48°28'58''N – 4°46'5''W), Portsall (48°33'53''N – 4°42'5''W), Saint-Pabu

127 (48°34'34''N – 4°38'45''W) and Le Faou (48°17'44''N – 4°10'56''W). Portsall,

128 exposed North-West, is a sheltered and mainly rocky site, surrounded by sand and

129 intertidal pools. Despite its western exposure, Porspoder remains sheltered due to the

130 presence of two large rocky over-hangs. The site chosen at Saint-Pabu, was exposed to

131 the North; it is characterized by sandy dunes. Finally, Le Faou differs from the three

132 previous sites because a river flows there into the Bay of Brest, forming a sheltered but

133 muddy estuary at low tide. Two samplings periods were carried out: in February

134 (winter) and July (summer) 2017.

135

### 136 *2.2 Macroalgal samples*

137 Forty species of red macroalgae were collected across the four different sites: 21 species

138 were found both in winter and summer and 19 species were found on the shore only for

139 one season. All species belong to the class of Florideophyceae, except *Porphyra dioica*

140 (Bangiophyceae). These included species from different morpho-anatomical groups (i.e.

141 MAG, according to Steneck and Dethier, 1994): filamentous algae (MAG 2); corticated

142 or polysiphonous filaments algae (MAG 2.5); foliose algae (MAG 3); corticated foliose

143 algae (MAG 3.5); corticated algae (MAG 4); and articulated calcareous algae (MAG 6)

144 (Appendix 1).



145 After collection, algal samples were brought back to the laboratory into sampling bags.  
146 Samples were stored in a fridge and washed within 4 hours of sampling with filtered  
147 seawater to remove residual sediments and salt; visible epiphytes were removed by  
148 hand. Seaweeds were then stored in the freezer before being freeze-dried ( $\beta$ 1-8 LD plus  
149 Christ) and the entire thallus was milled into a fine powder (MM400 Retsch). The dried  
150 powder was kept in darkness at room temperature until analyses.

151

### 152 *2.3 Extraction and assay of chlorophyll-a and carotenoids*

153 Pigments were extracted from 75 mg dry weight (DW) of algal powder in  
154 750  $\mu$ L of 90% acetone, according to Schmid and Stengel (2015). Two successive  
155 extractions of 30 min and 4 h were carried out at 4 °C, under magnetic agitation.  
156 Samples were then centrifuged at 10,000 rpm for 5 min (miniSpin plus, Eppendorf), and  
157 the supernatants combined and filtered for HPLC (High Pressure Liquid  
158 Chromatography) analysis (Dionex Ultimate 3000, ThermoScientific). Pigments were  
159 separated using an ACE C<sub>18</sub> column (150 x 4.6 mm, 3  $\mu$ m) with a guard-column, an  
160 injection volume of 6  $\mu$ L and a run-time of 33 min per sample. Before injection, each  
161 sample was automatically diluted  $\frac{3}{4}$  with ammonium acetate buffer (0.5 M, pH 7.2).  
162 Separation was achieved using a solvent gradient described in Table 1, delivered at a  
163 flow rate of 1.0 mL.min<sup>-1</sup>. A photo-diode array detector (DAD3000, ThermoScientific)  
164 was used for the detection of pigments at 435, 470 and 650 nm. The identification and  
165 quantification of each pigment was based on spectral comparisons and calibration using  
166 commercial standards: chlorophyll *a* (Sigma, USA), and  $\alpha$ -carotene,  $\beta$ -carotene, lutein,  
167 zeaxanthin, antheraxanthin, violaxanthin (DHI, Denmark). Only peaks with an area  
168 larger than 0.4 mAU.min for  $\alpha$ - and  $\beta$ -carotenes and 1 mAU.min for the other pigments

169 were considered in the analysis. HPLC data were collected using Chromeleon 0.7  
170 software (Thermo Scientific Dionex, France).

#### 171 *2.4 Extraction and assay of phycobiliproteins*

172 Tissue concentrations of phycoerythrin (PE) and phycocyanin (PC) were  
173 determined using a method adapted from Sun et al. (2009). Two successive extractions  
174 of 15 min at 4 °C were performed from 75 mg DW of algal powder, with 1.5 mL of  
175 phosphate buffer (0.1 M, pH 6.8). Subsequently, samples were centrifuged for 20 min at  
176 10,000 rpm (miniSpin plus, Eppendorf) and supernatants were used to measure  
177 absorbances at 455, 565, 592, 618 and 645 nm using a spectrophotometer (POLARStar  
178 Omega, BMG LABTECH). The concentrations of PE and PC ( $\text{mg}\cdot\text{g}^{-1}$  DW) were  
179 estimated using the following equations from Beer and Eshel (1985):

$$180 \text{ [PE]} = [(A_{565} - A_{592}) - (A_{455} - A_{592}) \times 0.20] \times 0.12$$

$$181 \text{ [PC]} = [(A_{618} - A_{645}) - (A_{592} - A_{645}) \times 0.51] \times 0.15$$

182

#### 183 *2.5 Mycosporine-like Amino Acids (MAAs) extraction and assay*

184 20 mg DW of finely ground algae were extracted for 2 h with 2 mL of 70%  
185 aqueous ethanol (v/v) at 45 °C with magnetic stirring. After centrifugation (centrifuge  
186 5810, Eppendorf) at 1,500 rpm for 10 min, the supernatant was recovered. The pellet  
187 was re-extracted twice following the same procedure and the combined supernatants  
188 were evaporated to dryness under vacuum (miVac, Genevac, France) at 45 °C. The  
189 evaporated extracts were then stored at -20 °C before analysis.

190 Prior to HPLC analysis, extracts were dissolved in 500  $\mu\text{L}$  of 2.5% aqueous  
191 methanol with 0.1% acetic acid, centrifuged for 5 min at 10,000 rpm (miniSpin plus,

192 Eppendorf), and filtered through 0.2  $\mu\text{m}$ -pore syringe filters. MAAs analyses were  
193 performed using a Dionex Ultimate 3000 HPLC (Thermo Scientific, Germany)  
194 equipped with a diode-array detector (DAD). MAA separation was performed using a  
195 Zorbax Eclipse XDB C<sub>18</sub> column, 5  $\mu\text{m}$ , 4.6  $\times$  250 mm (Agilent, USA) equipped with a  
196 guard-column. For one analysis, 20  $\mu\text{L}$  of extract were injected and the operating  
197 parameters were as follow: 0.1% acetic acid in Milli-Q water as the mobile phase; a  
198 flow rate of 1  $\text{mL}\cdot\text{min}^{-1}$ ; a column temperature of  $25 \pm 1$   $^{\circ}\text{C}$ ; an injector temperature of  
199  $5 \pm 1$   $^{\circ}\text{C}$ ; a run time of 20 min. MAAs were detected at 320, 330, 334 and 360 nm.  
200 HPLC data were collected using Chromeleon 0.7 software (Thermo Scientific Dionex,  
201 France). Individual peaks were identified by online absorption spectra and retention  
202 time (Rt).

203

204 For identification, MAAs extracts were also analyzed by LC-MS using a LC-  
205 ESI-Q-TOF-MS (Dionex, Ultimate 3000, Bruker, micrOTOF-QII) system (Bruker  
206 Daltonik GmbH, Bremen, Germany). The same LC method was used, only the flow was  
207 reduced to 0.5  $\text{mL}\cdot\text{min}^{-1}$ . Source parameters were: positive mode; source temperature,  
208 200  $^{\circ}\text{C}$ ; capillary voltage, 4.5 kV; nebulizer gas ( $\text{N}_2$ ) at 2.8 bars and dry gas ( $\text{N}_2$ ) at 12  
209  $\text{L}\cdot\text{min}^{-1}$ . Mass spectra acquisition was set at 0.5 Hz from  $m/Z$  50 to 1000. MS/MS  
210 analyses were performed with a collision energy of 30 eV and an isolation width of 2  
211  $m/Z$ . All raw data were collected with Compass dataAnalysis Version 4.1. The  
212 quantification of the identified MAAs was accomplished using their molar extinction  
213 coefficients  $\epsilon$  at the wavelengths of maximum absorption, according to Pelillo et al.  
214 (2004), and the molar extinction coefficient of Karsten et al. (1998b). For MAAs whose

215 molecular structure has not yet been elucidated, their quantification was not established.

216 Each MAA was then numbered in the order in which it appeared during the run.

217

218 Finally, the diversity of MAAs within each seaweed species tested was  
219 estimated by the development of a new index. The presence and absence of each MAA  
220 for each species was coded in a table, as 0 or 1 respectively. A distance matrix was then  
221 calculated by comparing species two by two using the following index:

$$Index = \frac{M}{M + N}$$

222 where 'M' is the number of matches between the two species (presence and absence) and  
223 'N' the total number of MAAs which were present in only one of the two species. The  
224 closer the index was to 1, the more similar the two species were in terms of the  
225 composition of their MAAs. A species with many weak indices thus represented a  
226 specific composition. A dendrogram was made from this matrix with the R program (R  
227 development core team, 2008) to represent species that had similar MAA compositions  
228 (hclust with single method, hclust package). In order to highlight any links between the  
229 composition of MAAs by algal species with their phylogeny, a phylogenetic analysis  
230 was performed using *rbcL* and *cox1* genes sequences, as retrieved from GenBank  
231 (Accession numbers for GenBank sequences are listed in Appendix 1 and are available  
232 on <https://www.ncbi.nlm.nih.gov>). Sequences were aligned using the ClustalW  
233 programme in the BioEdit alignment editor (Hall, 1999). A phylogenetic tree was built  
234 using MEGA version 7 (Kumar et al., 2016), based on the Neighbor-Joining clustering  
235 method. Evolutionary distances were computed using the Maximum Composite  
236 Likelihood. The tree was subjected to a bootstrap test (5000 replicates) to estimate  
237 robustness at each branch (pvclust, pvclust package).

238

239 *2.6 Statistical analyses*

240 All data were statistically analyzed with the R program (R development core team,  
241 2008). All extractions were performed in triplicate, and results expressed as average  $\pm$   
242 standard deviation (SD). Comparisons were carried out using a Student's t-test (2  
243 samples) or an ANOVA (more than 2 samples). Beforehand, the conditions of  
244 application were controlled using the Shapiro test for compliance with a normal  
245 distribution, and the F-test or Bartlett's test for the homogeneity of variance (Dytham,  
246 2011). The non-parametric Wilcoxon test (2 samples) or Scheirer-Ray-Hare test (SHR,  
247 more than 2 samples) were used when at least one of the conditions of application was  
248 not met (to replace the t-test or the ANOVA, respectively). If possible, when significant  
249 results were highlighted, *post-hoc* tests (i.e. multiple comparisons) were performed: a  
250 Tukey *post-hoc* test after an ANOVA (TukeyHSD), or a non-parametric *post-hoc* test  
251 after a Scheirer-Ray test (kruskalmc, pirms package) (Dytham, 2011). Principal  
252 Component Analysis (PCA) was carried out (package FactoMineR) to highlight species  
253 which stood out.

254

255 **3. Results**256 *3.1 Pigments: chlorophyll a, carotenoids and phycobiliproteins*

257 The chlorophyll *a* content (Fig. 1) varied significantly among the analyzed red algal  
258 species (SHR test,  $p$ -value  $< 0.0001$ ). In some species, such as *Mastocarpus stellatus* or  
259 *Furcellaria lumbricalis*, chlorophyll *a* concentrations did not exceed  $1 \text{ mg.g}^{-1} \text{ DW}$  -  
260 whatever the season. However, *Callithamnion tetragonum* and *Plumaria plumosa* for  
261 example contained in winter  $8.60 \pm 0.26$  and  $8.37 \pm 0.21 \text{ mg.g}^{-1} \text{ DW}$  of chlorophyll *a*,

262 respectively. Moreover, a seasonal variability was demonstrated with chlorophyll *a*  
263 concentrations decreasing in summer (SHR test, p-value = 0.0040). For example,  
264 *Palmaria palmata* contained  $7.02 \pm 0.31$  mg.g<sup>-1</sup> DW of chlorophyll *a* in February, and  
265  $1.50 \pm 0.27$  mg.g<sup>-1</sup> DW in July. Conversely, *Bostrychia scorpioides* was the only  
266 species with a significantly increased concentration in summer (i.e.  $5.44 \pm 0.43$ , as  
267 compared to  $4.32 \pm 0.09$  mg.g<sup>-1</sup> DW in winter).

268 Similarly, total concentration of carotenoids changed with species (SHR test, p-value <  
269 0.0001) (Fig. 2). For example, *Chondrus crispus* and *F. lumbricalis* contained less than  
270 0.2 mg.g<sup>-1</sup> DW of carotenoids, whereas *Vertebrata lanosa*, *Porphyra dioica* or *B.*  
271 *scorpioides* contained up to 0.78, 0.84 and 0.65 mg.g<sup>-1</sup> DW of total carotenoids,  
272 respectively. A difference in term of composition was also observed (Fig. 2): overall,  
273 lutein was the most common carotenoid in 31 species out of 40. However, the amount  
274 of lutein remained low as compared to chlorophyll *a*, which constituted 90.1% on  
275 average of the total liposoluble pigment content. Moreover, there was no seasonal  
276 variation in the carotenoid composition as a whole, but the concentration was seen to  
277 decrease in summer (SHR test, p-value = 0.0012) (Fig. 2), except for *V. lanosa* in which  
278 the concentration increased (e.g.  $0.78 \pm 0.01$  and  $0.66 \pm 0.04$  mg.g<sup>-1</sup> DW in summer and  
279 winter, respectively). *Gigartina pistillata*, *Metacallophyllis laciniata* and  
280 *Membranoptera alata* did not contain any detectable levels of carotenoids in summer.  
281 However, although there were seasonal variations in chlorophyll *a* and overall  
282 carotenoid amounts, the ratio of carotenoids / chlorophyll *a* appeared to be constant  
283 between both seasons (SHR test, p-value = 0.4919) (data not shown).

284 The different studied red algae contained variable levels of phycobiliproteins  
285 similarly to their chlorophyll *a* or carotenoid amounts (SHR test, p-value < 0.0001)

286 (Fig. 3). For example, *Bornetia secundiflora* and *Membranoptera alata* contained 27.39  
287  $\pm 3.13$  and  $19.67 \pm 0.15$  mg.g<sup>-1</sup> DW of PE in winter, respectively, whereas *C. crispus*,  
288 *Mastocarpus stellatus* or *V. lanosa* contained less than 5.0 mg.g<sup>-1</sup> DW (Fig. 3).  
289 *P. plumosa* was more differentiated from the other species with high levels of PE in  
290 both seasons ( $23.18 \pm 0.49$  mg.g<sup>-1</sup> DW in winter and  $13.58 \pm 1.36$  mg.g<sup>-1</sup> DW in  
291 summer). Moreover, it would appear that, on the whole, the PE content decreased in  
292 summer (SHR test, p-value < 0.0001). *Bostrychia scorpioides* was the only species for  
293 which the PE concentration really increased in summer ( $6.62 \pm 0.22$  mg.g<sup>-1</sup> DW in  
294 summer, compared to  $5.32 \pm 0.50$  mg.g<sup>-1</sup> DW in winter). The results were similar for  
295 phycocyanin (data not shown).

296

297 A Principal Component Analysis (PCA) of all pigments (Fig. 4a, 4b) pointed out  
298 six species: *B. scorpioides*, *Porphyra dioica*, *V. lanosa*, *Gracilaria vermiculophylla*,  
299 *Gracilaria gracilis* and *Plumaria plumosa*. This last species contained the highest  
300 concentration of chlorophyll *a* and phycoerythrin. The five other species were the only  
301 ones containing zeaxanthin with a concentration of 0.5  $\mu$ g.g<sup>-1</sup> DW or greater. *G. gracilis*  
302 also contained antheraxanthin. Finally, the species belonging to similar MAG showed  
303 different pigment content (chlorophyll *a*, carotenoids and phycoerythrin) and  
304 composition. Moreover, no link between pigment composition of the red seaweeds  
305 studied here and their phylogeny, morphology, position on the shore or sampling site  
306 could be demonstrated.

307

308 3.2 *Mycosporine-like Amino Acids*

309 HPLC analyses showed 23 different peaks that may correspond to MAAs (Fig. 5  
310 and Appendix 2), and six (i.e. shinorine, palythine, asterina-330, porphyra-334,  
311 usujirene and palythene) were elucidated by LC-MS. The composition and  
312 concentration of various MAAs showed a difference between the various species of red  
313 macroalgae sampled in this study. These observations were independent of the sampling  
314 season. Amongst the 21 species of algae commonly occurring in both seasons  
315 considered, six did not contain any MAA (these included: *F. lumbricalis*,  
316 *Metacallophyllis laciniata*, *Polyides rotunda*, *Osmundea pinnatifida*, *Membranoptera*  
317 *alata* and *Plumaria plumosa*). For the eight species which could be collected only in  
318 summer, no MAAs were detected in four and for those harvested only in winter, five did  
319 not contain any detectable MAAs. At the same time, *Mastocarpus stellatus* contained a  
320 high concentration of only shinorine (i.e.  $3.12 \pm 0.26$  and  $1.62 \pm 0.07$  mg.g<sup>-1</sup> DW,  
321 respectively in winter and in summer). One should note that three species, i.e. *Palmaria*  
322 *palmata*, *B. scorpioides* and *G. vermiculophylla*, contained eight different MAAs,  
323 visible as separate peaks on the chromatograms (data not shown). A chromatogram of  
324 the MAAs analysed from *P. palmata* (Fig. 6) showed the presence of six identified  
325 MAAs (i.e. shinorine, palythine, asterina-330, porphyra-334, usujirene and palythene)  
326 and two other molecular structures that may correspond to MAAs. Moreover, some  
327 unidentified MAAs were present in only one algal species, such as *B. scorpioides*,  
328 which contained four unidentified (new) MAAs (i.e. MAA\_4, MAA\_13, MAA\_18 and  
329 MAA\_23), *G. pistillata* which had MAA\_14 and MAA\_22, and *V. lanosa* MAA\_1  
330 (Fig. 5).

331



332 Based on HPLC chromatograms, shinorine was the predominant MAA identified  
333 among all sampled red macroalgae in this study. Indeed, with the exception of  
334 *V. lanosa*, shinorine was the only MAA common to all of those species which appeared  
335 able to produce MAAs. Shinorine was found to be in sizeable quantities in *M. stellatus*,  
336 *G. gracilis*, and *Ceramium nodulosum* with  $3.12 \pm 0.26$ ,  $4.00 \pm 0.06$  and  $1.92 \pm 0.11$   
337  $\text{mg.g}^{-1}$  DW, respectively for species harvested in winter (February). These amounts  
338 were approximately halved in July with  $1.62 \pm 0.07$ ,  $1.47 \pm 0.10$  and  $0.86 \pm 0.06 \text{ mg.g}^{-1}$   
339 DW, respectively for the same algae (t test, p-value = 0.0004). The next most common  
340 MAA was palythine which was found in 19 different species.

341 Moreover, no large modifications in the level of MAAs (Wilcoxon test,  
342 p-value = 0.7804) and their composition was observed between both sampling periods  
343 in the different species (Fig. 5). However, some changes were noticed such as in *O.*  
344 *pinnatifida* with the presence of asterina-330, usujirene and palythene in those thalli  
345 collected in winter (February), but not in those collected in summer (July). The same  
346 was observed for *G. pistillata* with the presence of two new MAAs (i.e. MAA\_14 and  
347 MAA\_22) in the winter samples.

348

349 In winter and summer, the analysis of the diversity indices for the MAAs  
350 highlighted three groups of species (Fig. 7A and Appendix 3A): those with no MAA  
351 (Group 1), those with few MAAs (between 1 and 5; Group 2) and those with many  
352 MAAs (6 or more; Group 3). Only *B. scorpioides* seemed to stand out and did not  
353 correspond to any group, even though it contained eight different MAAs. Shinorine was  
354 present in all species of Groups 2 and 3, except in *V. lanosa* which was also the only  
355 species having MAA\_1. Moreover, asterina-330 and palythine were also two MAAs

356 found in some species from Group 2, and in all species from Group 3 (with the  
357 exception of *V. lanosa* and *B. scorpioides*). Finally, there were some MAAs which were  
358 only found in Group 3, notably usujirene and palythene which were present in six of the  
359 nine species of the Group 3 in winter. The high diversity in MAAs did not seem to be  
360 related to phylogeny (Fig. 7B and Appendix 3B), anatomy and morphology, the height  
361 on the shore nor the sampling site (Appendix 1). Hence, species which are close  
362 phylogenetically (i.e. *G. vermiculophylla* and *G. gracilis*, or *Callithamnion tetricum* and  
363 *C. tetragonum* in our study), morphologically (*M. stellatus* and *C. crispus*), or the only  
364 two species harvested from a muddy habitat (i.e. *B. scorpioides* and *G. vermiculophylla*)  
365 did not have the same MAAs profile (see Appendix 1 and Fig. 5). No link was also  
366 found between the MAAs and the fact that a species was introduced or indigenous to  
367 Brittany (data not shown).

368

#### 369 4. Discussion

370 As intertidal macroalgae are organisms directly exposed to solar radiation, they  
371 have developed some protective mechanisms in order to survive, such as the synthesis  
372 of photo-protective compounds. In this sense, the objective of this study was to collect  
373 many different red algal species from a temperate shore in order to have a first overview  
374 of the specific distribution of these compounds, and more particularly of mycosporine-  
375 like amino acids for which data are not actually available for some species.

376

377 Firstly, a high inter-specific variability has been demonstrated in terms of  
378 pigment composition and concentration (Figs 1-3). Some species had a high content of  
379 chlorophyll-*a* (*P. plumosa* or *Callithamnion* sp.) while others had more carotenoids

380 (*P. dioica*, *B. scorpioides*, *V. lanosa*), or others had low concentrations of both pigments  
381 (*C. crispus* or *G. pistillata*). Three groups were observed from the carotenoids  
382 composition, as suggested by Schubert et al. (2006): species rich in lutein, species rich  
383 in zeaxanthin, and those with violaxanthin or antheraxanthin (Fig. 2). Lutein was the  
384 major carotenoid, as already illustrated (Esteban et al., 2009; Marquardt and Hanelt,  
385 2004).

386

387         Furthermore, the quantity of photosynthetic pigment (i.e. chlorophyll-*a* and  
388 phycobiliproteins) decreased in the summer samples (Figs. 1, 3) due to an increase in  
389 irradiance. Indeed, the increase in light energy availability leads species to reduce their  
390 pigment complement to harvest the same quantity of light (Ak and Yücesan, 2012;  
391 Ramus et al., 1976). Another explanation would be linked to the nitrogenous nature of  
392 chlorophyll-*a* and phycobiliproteins (Huovinen et al., 2006; Lüning, 1990; Parjikolaie et  
393 al., 2013). They are used for nitrogen storage in winter and as a nitrogen source when  
394 limitations occur in summer (Barufi et al., 2011; Lapointe and Duke, 1984; Surget et al.,  
395 2017). Stack et al. (2017) demonstrated that the protein content of *Porphyra dioica*  
396 doubled in winter months, perhaps because of phycobiliproteins acting as a nitrogen  
397 storage. In general, the impact of N concentration on pigments has already been widely  
398 demonstrated in many species such as in *Palmaria palmata* (Corey et al., 2013;  
399 Parjikolaei et al., 2013).

400

401         The aim of the second part of this study was to elucidate the MAAs composition  
402 of 40 red macroalgae. The extraction method in 70% ethanol, coupled with the extract  
403 analysis by HPLC, provided reliable data, and had the advantage of being non-toxic,

404 unlike using methanol which is commonly used in the literature (e.g. Briani et al., 2018;  
405 Gröniger et al., 2000; Guihéneuf et al., 2018; Pandey et al., 2017; Yuan et al., 2009).  
406 The use of a less-toxic solvent such as ethanol allowed for the valorization of extracted  
407 MAAs as they have a significant potential for biotechnology applications in for example  
408 human health and cosmetics (Chrapusta et al., 2017; Lawrence et al., 2018; Pangestuti  
409 et al., 2018). For example, Helioguard 365 (Mibelle group, Switzerland) is a  
410 commercial formulation used to protect the skin or hair from UV radiation and based on  
411 the MAAs shinorine and porphyra-334 extracted from the red macroalga *Porphyra*  
412 *umbilicalis* (La Barre et al., 2014; Schmid et al., 2006).

413         The results of the screening revealed a high diversity in MAA composition in  
414 the 40 studied species, including some species for which the MAA composition had not  
415 been reported before, e.g. *Plumaria plumosa*, *Bornetia secundiflora*, *Dilsea carnosa*,  
416 *Gastroclonium ovatum*, *Hypoglossum hypoglossoides*, *Champia parvula* and  
417 *Chylocladia verticillata*. It was already demonstrated that some species of the  
418 Rhodophyta have the capacity to accumulate a high degree of diversity and  
419 concentration of MAAs (e.g. Rastogi et al., 2010; Sinha et al., 2007), but this present  
420 study is the first to report on MAA profiles from as many species of red alga from a  
421 temperate region, here from Brittany. Based on their absorption maxima and retention  
422 time, 23 potential MAAs were identified by HPLC analyses in the 40 red seaweeds  
423 harvested along the Brittany coasts. Shinorine and palythine were found to be the most  
424 common MAAs in this study (Fig. 5), which is in agreement with Karentz (2001). The  
425 composition in MAAs of each species coincided with the literature, even for poorly  
426 studied species such as *Asparagopsis armata* (Figueroa et al., 2008). For example, in  
427 accordance with the bibliography, shinorine and porphyra-334 are found in *P. dioica*

428 (Guihéneuf et al., 2018); shinorine, palythine and porphyra-334 in *C. nodulosum*; or  
429 species contained no MAA such as *Phycodrys rubens* and *F. lumbricalis* (Karsten et al.,  
430 1998b).

431 The development of a diversity index for MAAs allowed for the identification of  
432 three groups of species (Fig. 7A): i.e. species without MAA (e.g. *Plumaria plumosa*,  
433 *H. plumosa*, *M. alata*, *Polyides rotunda* or *F. lumbricalis*), species with a few MAAs  
434 (e.g. *Porphyra dioica*, *Gracilaria gracilis*, *C. tetricum*), and some with more than six  
435 MAAs (e.g. *Palmaria palmata*, *B. scorpioides*, *Gracilaria vermiculophylla*, *V. lanosa*).  
436 These observations suggested a huge diversity of mycosporine-like amino acids among  
437 different members of Rhodophyta, with no apparent link with phylogeny, or  
438 morphology, position on the shore or sampling site. Similarly to our study, the  
439 distribution of various MAAs in other publications is not related to a phylogenetic  
440 pattern (Karentz et al., 1991; McClintock and Karentz, 1997), but more to an  
441 acclimatization to environmental variations (Briani et al., 2018).

442 Furthermore, 23 MAAs of which some potentially new and as yet unidentified  
443 were observed in the 40 species knowing that approximately 20 MAAs have been  
444 reported so far in red algae, suggesting that there may be in this study some MAAs that  
445 have never been yet identified. *B. scorpioides* was the species that stood out the most  
446 because it had four, unique, as yet un-identified MAAs (i.e. MAA\_4, MAA\_13,  
447 MAA\_18 and MAA\_23). This species also did not fit into any of the three groups based  
448 on the diversity index (Fig. 7A). Only six MAAs were identified by LC-MS in the  
449 studied species (i.e. shinorine, palythine, porphyra-334, asterina-330, usurijene,  
450 palythene). In addition, we propose that MAA\_11 corresponded to palythinol by  
451 comparing the results with the literature: indeed, palythinol is a MAA often observed in

452 many red macroalgae in the literature (Sinha et al., 2007) and MAAs\_11 is the fifth  
453 most common MAA in the red algae studied here. MAA\_11 is present for example in  
454 *P. palmata*, which should contain palythanol according to the literature (Gröniger et al.,  
455 2000; Karsten and Wiencke, 1999; Yuan et al., 2009). It is difficult to identify MAAs  
456 based only on the absorbance spectra and retention times of known MAAs in the  
457 bibliography, especially since some MAAs are present in very small quantities which  
458 makes it impossible to detect them in LC-MS. The lack of commercial standards makes  
459 this study particularly complex, in addition to making impossible the quantification of  
460 the different MAAs. In addition, some peaks have similar absorption maxima and  
461 retention times. For example, usujirene and palythene only differ by their cis- or trans-  
462 conformation and less than one minute separated them (Carreto and Carignan, 2011). In  
463 order to identify other MAAs, more biomass for the extractions (if the available  
464 biomass allowed it), or the development of a purification protocol to concentrate each  
465 MAA would therefore be necessary.

466         Moreover, there was no large seasonal difference in terms of the composition  
467 and concentration of MAAs (Fig. 5). This is an unexpected result as the role of photo-  
468 protection by MAAs has been widely demonstrated (e.g. Conde et al., 2000; Singh et  
469 al., 2008). We expected that the concentration of total MAAs would increase in relation  
470 to the intensity of radiation in the marine ecosystem (Karsten et al., 1998a; Torres et al.,  
471 2016). However, it is possible that a possible increase in MAAs content occurred  
472 between the two sampling periods, February and July, indicating that two sampling  
473 periods are not enough to study seasonal variation in MAAs content. This is suggested  
474 by Guihéneuf et al. (2018), who found that the concentration of MAAs increased  
475 between February and May in *P. palmata* and *C. crispus* collected on Irish shores and

476 not during summer as expected. Another explanation is that, as MAAs are nitrogenous  
477 compounds, they are used in summer as a source of nitrogen (Karentz, 2001). In this  
478 sense, a number of studies have already demonstrated the positive effect of nitrogen on  
479 concentrations of MAAs (Figuerola et al., 2014; Korbee et al., 2005; Peinado et al.,  
480 2004).

481 Thus, the variability observed in pigments and MAAs composition suggests that  
482 all species do not seem to have the same protective capabilities against solar radiation.  
483 In an attempt to obtain a first explanation of this diversity, we tried to relate this  
484 composition to phylogeny, or morphology, position on the shore or sampling site, which  
485 was not successful. To date, few studies have examined the link between pigment  
486 composition and phylogeny, and no clear relationship has been found (Vandervelde,  
487 1973; Wang et al., 2018). The MAAs composition would also not be related to a  
488 phylogenetic pattern (Karentz et al., 1991; McClintock and Karentz, 1997). Thus there  
489 is currently no explanation for the composition (presence/absence) in MAAs between  
490 the different species that seems to be random. However, it has been experimentally  
491 demonstrated that an increase in UV radiation could have an impact on the levels of  
492 MAAs, for example in *Palmaria palmata* or *Chondrus crispus* (Hoyer et al., 2002;  
493 Kräbs et al., 2004); or that the MAAs concentration depended on the season (Guihéneuf  
494 et al., 2018), nutrient concentrations and pH (Briani et al., 2018), latitude (Karsten et al.  
495 1998b) or depth (Franklin et al., 1999; Karsten and Wiencke, 1999). For example, the  
496 total MAA concentration in *P. palmata* and *Devalerea ramentaceae* collected at  
497 different depths decrease with sampling depth, although the composition remains the  
498 same (Hoyer et al., 2001; Karsten et al., 1999).  
499

500 In our study, four species stand out due to their diversified compositions of  
501 MAAs: *B. scorpioides*, *G. vermiculophylla*, *V. lanosa* and *P. dioica*, which are the  
502 sampled species the most exposed to solar radiation due to their relatively high position  
503 on the shore. Indeed, *B. scorpioides* lives at the top of muddy estuaries (Sanchez de  
504 Pedro et al., 2014); *G. vermiculophylla* lives at the surface of the intertidal muddy  
505 estuaries (Roleda et al., 2012; Surget et al., 2017); *P. dioica* is located on the upper  
506 intertidal, and *V. lanosa* is an obligate epiphyte on the brown macroalga *Ascophyllum*  
507 *nodosum* (Garbary et al., 2014) which has a wide range of coverage on intertidal  
508 rockyshores at mid-tide level. These four red algal species were characterized by the  
509 presence of several MAAs and a high concentration of carotenoids, in particular  
510 zeaxanthin, a pigment synthesized under higher light conditions (Rmiki et al., 1996;  
511 Schubert and Garcia-Mendoza, 2008). Thus, they stood out from the others on the  
512 Principal Component Analysis of pigments (Fig. 4). Another species, *G. gracilis*, also  
513 stood out the PCA since it contained antheraxanthin, in accordance with Rmiki et al.  
514 (1996) and Schubert et al. (2006). Conversely, *P. plumosa*, with no observed MAA, was  
515 the single species with the highest concentrations of chlorophyll *a* and phycoerythrin.  
516 This alga lives in shaded areas (Yakovleva et al., 1998), which may explain its necessity  
517 to have high concentrations of pigments which absorb light energy, rather than act as  
518 photoprotectants.

519

## 520 5. Conclusions

521 This study highlights the occurrence of numerous MAAs in red macroalgae from  
522 Brittany, with variations in the number and level of MAAs per species. Thus, this  
523 research contributes to reinforce the few existing databases on the MAAs composition



524 of red algae as described by Gröniger et al. (2000) and Sinha et al. (2007), knowing that  
525 the identification of these molecules is difficult due to the lack of commercial standards.  
526 Here we attempted to identify the factors controlling this MAAs diversity: MAAs  
527 composition was no related to a phylogenetic pattern; however, the algal species most  
528 exposed to light radiation were those with a high level and diversity of photoprotective  
529 compounds which could protect their photosynthetic mechanisms against UV-radiation.  
530 Nevertheless, it remains complex to provide conclusions about the actual impact of the  
531 different biotic and abiotic factors on the synthesis of each MAA. Although MAAs  
532 seem to be photo-protective, they may play other and as yet unknown roles in  
533 macroalgal metabolism. Subsequently, it is necessary to select few species and carry out  
534 macroalgal cultures under controlled conditions in order to understand factors  
535 responsible for the production of each MAAs. Thus, the impact of temperature, UV  
536 radiation, nutrients or pH could be tested one by one or in combination, allowing to  
537 highlight a change in the ratio of MAAs for example. This would result in a better  
538 understanding of the synthesis and function of these compounds.

539

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546

#### 547 **Authors' contributions**

548 F.L. performed the analysis of the samples collected in winter, drafted the manuscript  
549 and conducted the statistical analyses. S.L. performed the analysis of the samples  
550 collected in summer and contributed to the writing of the manuscript. G.B. and L.T.  
551 performed the LC-MS analyses. V.S-P designed the diversity index, helped in carrying  
552 out the phylogenetic analyses and contributed to the interpretation of the results. S.C.  
553 designed and supervised the project. All authors reviewed and approved the final  
554 manuscript.

555

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**Table 1.** HPLC solvent gradient. (A) methanol:ammonium acetate buffer 0.5 M (80:20), (B) acetonitrile:milliQ water (87.5:13.5), (C) ethylacetate 100%.

Time (min)	% A	% B	% C
0	90	10	0
1	0	100	0
11	0	78	22
24	0	25	75
26	0	25	75
27	0	100	0
28	90	10	0
33	90	10	0

**Appendix 1.** List of the 40 red macroalgal species collected in February and/or July 2017, with their associated GenBank accession numbers (for the genes *rbcL* and *cox1*), morpho-anatomical group (i.e. MAG; according to Steneck and Dethier, 1994), localisation on the shore, substrate and sampling site. The different MAGs are: (2) filamentous algae; (2.5) corticated or polysiphonous filaments algae; (3) foliose algae; (3.5) corticated foliose algae; (4) corticated algae and (6) articulated calcareous algae.

Species	Accession numbers		MAG	Localisation	Substrate	Sampling sites
	<i>rbcL</i>	<i>cox1</i>				
<i>Ahnfeltiopsis devoniensis</i>	KU640212.1	KF641876.1	4	Lower intertidal	Rocky	Portsall
<i>Asparagopsis armata</i>	GQ337068.1	KJ960343.1	2.5	Lower intertidal	Rocky	Portsall
<i>Bonnemaisonia hamifera</i>	FJ195604.1	KJ960353.1	2.5	Lower intertidal	Rocky	Portsall
<i>Bornetia secundiflora</i>	No data		2.5	Upper subtidal	Rocky	Portsall
<i>Bostrychia scorpioides</i>	AY920825.1	MF094019.1	2.5	Upper intertidal	Muddy	Le Faou
<i>Calliblepharis jubata</i>	KC121138.1	KJ960410.1	4	Lower intertidal	Rocky	Portsall
<i>Callithamnion tetragonum</i>	AF439301.1	MF447481.1	2	Lower intertidal	Rocky	Portsall
<i>Callithamnion tetricum</i>	AF439300.1	KJ960434.1	2	Lower intertidal	Rocky	Portsall
<i>Ceramium echionotum</i>	AF439313.1	KJ960509.1	2.5	Lower intertidal	Rocky	Portsall
<i>Ceramium nodulosum</i>	No data		2.5	Lower intertidal	Rocky	Portsall

<i>Ceramium secundatum</i>	KT250273.1	KT250269.1	2.5	Lower intertidal	Rocky	Portsall
<i>Champia parvula</i>	EF613312.1	HQ422864.1	2.5	Lower intertidal	Rocky	Portsall
<i>Chondracanthus acicularis</i>	KJ202090.1	KJ202081.1	4	Lower intertidal	Rocky	Portsall
<i>Chondrus crispus</i>	KF026483.1	GU645233.1	4	Lower intertidal	Rocky	Portsall
<i>Chylocladia verticillata</i>	No data		2.5	Intertidal	Rocky	Portsall
<i>Corallina</i> sp.	No data		6	Lower intertidal	Rocky	Porspoder <sup>a</sup> ; St-Pabu <sup>b</sup>
<i>Dilsea carnosa</i>	KT310705.1	KY572820.1	3.5	Upper subtidal	Rocky	Portsall
<i>Furcellaria lumbricalis</i>	No data		4	Intertidal	Rocky	Portsall
<i>Gastroclonium ovatum</i>	KU726714.1	KJ960700.1	2.5	Lower intertidal	Rocky	Portsall
<i>Gelidium corneum</i>	HM629821.1	KJ960706.1	4	Lower intertidal	Rocky	Porspoder
<i>Gigartina pistillata</i>	AY294375.1	KJ960717.1	4	Lower intertidal	Rocky	Porspoder <sup>a</sup> ; Portsall <sup>b</sup>
<i>Gracilaria gracilis</i>	AY049400.1	KF714853.1	4	Lower intertidal	Rocky	Portsall
<i>Gracilaria vermiculophylla</i>	JQ768774.1	JQ794759.1	4	Upper intertidal	Muddy	Le Faou
<i>Griffithsia corallinoides</i>	No data		2.5	Lower intertidal	Rocky	Portsall
<i>Heterosiphonia plumosa</i>	AF083379.1	KJ960780.1	2.5	Upper subtidal	Rocky	Portsall <sup>a</sup> ; St-Pabu <sup>b</sup>
<i>Hypoglossum hypoglossoides</i>	AF257368.1	KJ179930.1	3.5	Lower intertidal	Rocky	Portsall

<i>Lomentaria articulata</i>	KU726701.1	KU707860.1	2.5	Lower intertidal	Rocky	Portsall
<i>Mastocarpus stellatus</i>	U02992.1	KY572683.1	4	Lower intertidal	Rocky	Portsall
<i>Membranoptera alata</i>	JQ864359.1	KJ960846.1	3.5	Lower intertidal	Rocky	Portsall
<i>Metacallophyllis laciniata</i>	No data		3.5	Upper subtidal	Rocky	Portsall
<i>Osmundea hybrida</i>	AF281878.1	KX258831.1	4	Lower intertidal	Rocky	Portsall
<i>Osmundea pinnatifida</i>	JX828140.1	KU566536.1	4	Lower intertidal	Rocky	Porspoder <sup>a</sup> ; Portsall <sup>b</sup>
<i>Palmaria palmata</i>	LN999410.1	KY572816.1	3.5	Lower intertidal	Rocky	Porspoder <sup>a</sup> ; Portsall <sup>b</sup>
<i>Phycodrys rubens</i>	JX110932.1	KY572841.1	3.5	Upper subtidal	Rocky	Porspoder
<i>Plocamium cartilagineum</i>	HQ224543.1	JF271583.1	4	Lower intertidal	Rocky	Portsall
<i>Plumaria plumosa</i>	KU381993.1	HQ412551.1	2.5	Lower intertidal	Rocky	Portsall
<i>Polyides rotunda</i>	No data		4	Lower intertidal	Rocky	Portsall
<i>Porphyra dioica</i>	JN703282.1	JN847313.1	3	Upper intertidal	Rocky	Portsall <sup>a</sup> ; St-Pabu <sup>b</sup>
<i>Sphaerococcus coronopifolius</i>	AY294376.1	KJ961109.1	4	Upper subtidal	Rocky	St-Pabu
<i>Vertebrata lanosa</i>	MF120849.1	KX344122.1	2.5	Intertidal	Rocky	Portsall

<sup>a</sup>Samples collected in winter; <sup>b</sup>Samples collected in summer

**Figure legends**

**Figure 1.** Chlorophyll *a* concentrations ( $\text{mg}\cdot\text{g}^{-1}$  DW) (mean  $\pm$  standard deviation,  $n=3$ ) of 40 red macroalgal species, collected from Brittany in February (winter) and July (summer) 2017. Blank spaces indicate that chlorophyll-*a* level was below the limit of detection or quantification.

**Figure 2.** Carotenoid concentrations ( $\text{mg}\cdot\text{g}^{-1}$  DW) (mean,  $n=3$ ) in 40 red macroalgal species, collected from Brittany in February (winter) and July (summer) 2017. Blank spaces indicate that carotenoids level was lower than the limit of detection or quantification.

**Figure 3.** Phycoerythrin concentrations ( $\text{mg}\cdot\text{g}^{-1}$  DW) (mean  $\pm$  standard deviation,  $n=3$ ) in 40 red macroalgal species, collected from Brittany in February (winter) and July (summer) 2017. Blank spaces indicate that phycoerythrin level was lower than the limit of quantification.

**Figure 4.** Principal Component Analysis (PCA) of pigment contents, for 21 species of red macroalgae collected in winter and summer (A: variables; B: samples). Ellipses were drawn around species that had a confidence level of 95% or more. Dimension 1 was principally characterised by chlorophyll *a* level and dimension 2 by zeaxanthin presence and level (correlation coefficient = 0.93 and 0.88, respectively).

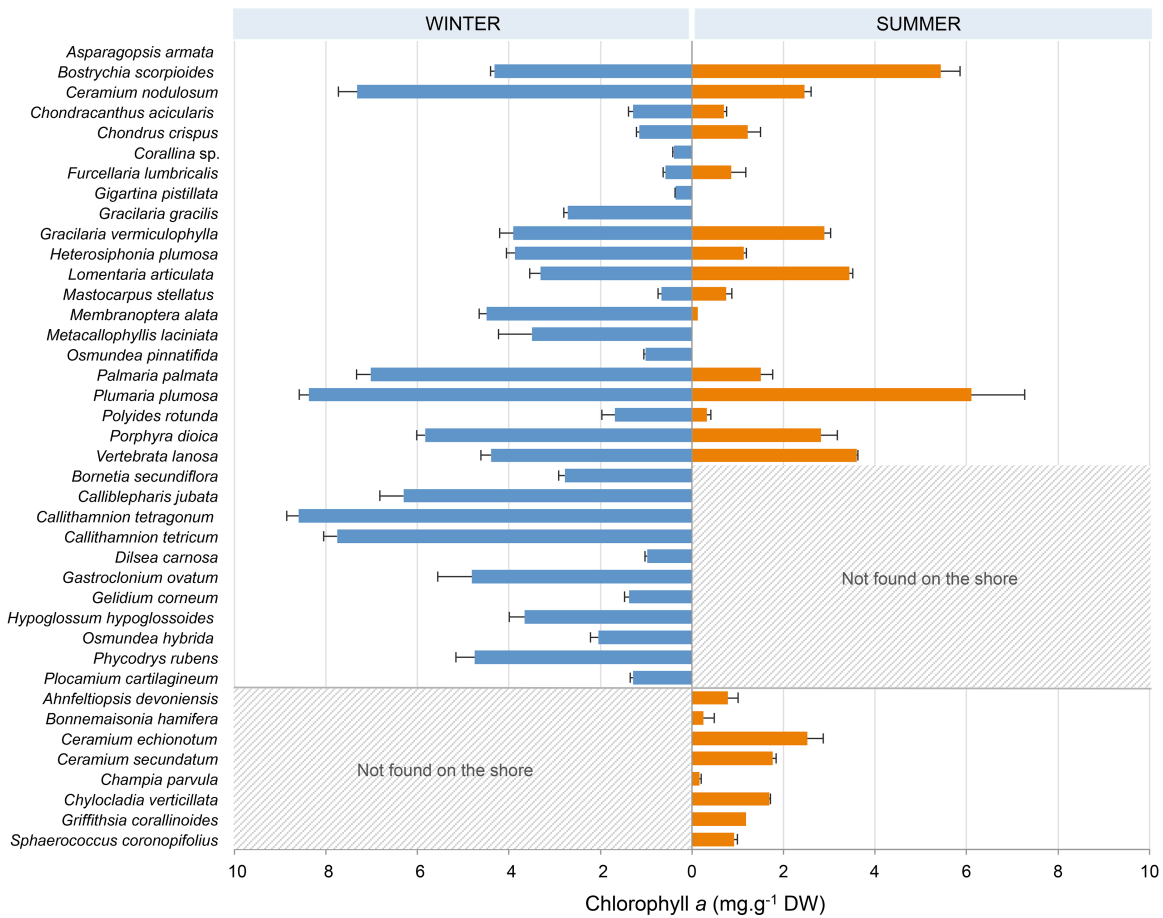
**Figure 5.** Mycosporine-like amino acids composition (mAU.min) (mean,  $n=3$ ) in 40 red macroalgal species, collected from Brittany in February (winter) and July (summer) 2017. Blank spaces indicate that MAAs level was lower than the limit of detection or quantification.

**Figure 6.** Chromatogram at 330 nm of MAAs extracted from the red macroalga *Palmaria palmata* collected in February 2017 (winter) from Porspoder (Brittany, France).

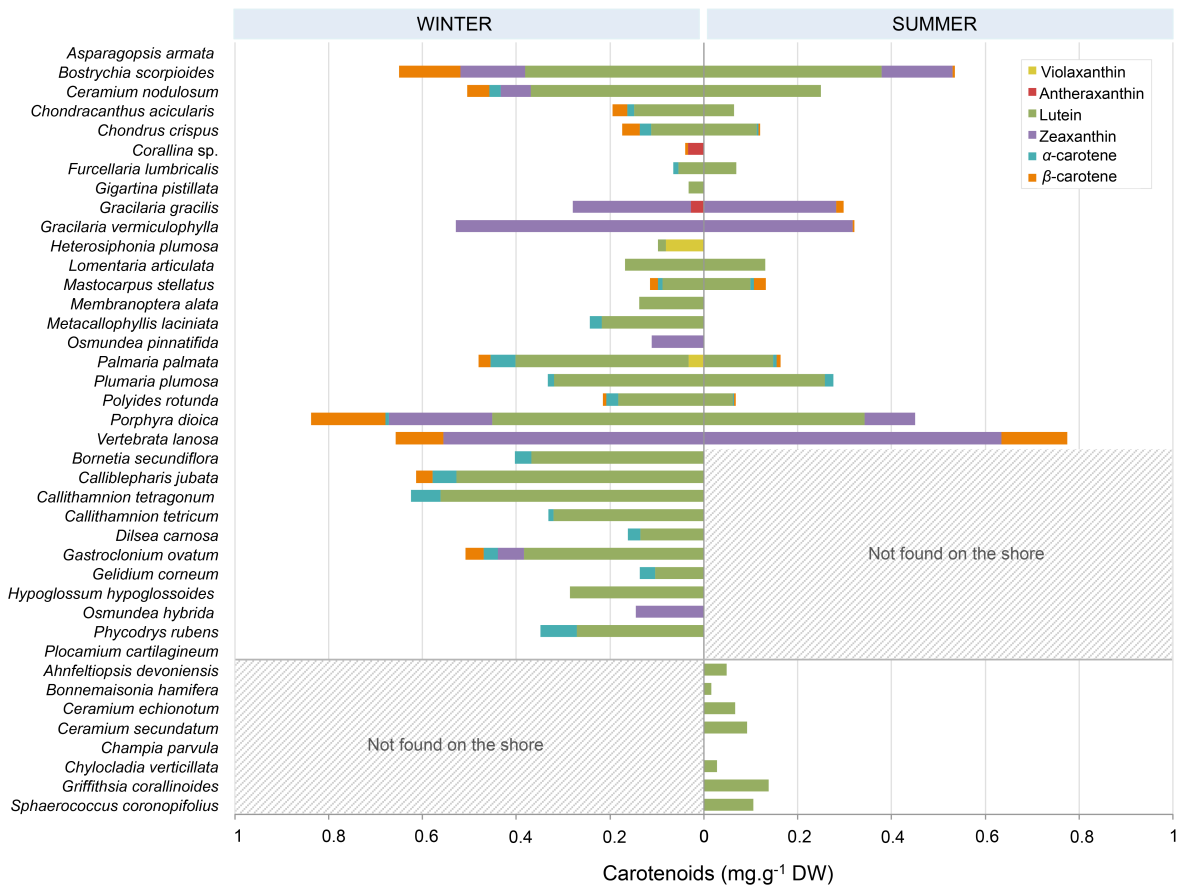
**Figure 7.** (A) Dendrogram based on the diversity indices of MAAs, obtained from hierarchical cluster analysis (single linkage method); (B) Neighbor-joining phylogenetic tree based on *rbcL* and *cox1* genes sequences, for the 32 macroalgal species collected from Brittany during winter. The most relevant boot-strap values are shown next to the branches. Some species are not represented on the phylogenetic tree due to missing sequences on GenBank (see Appendix 1).

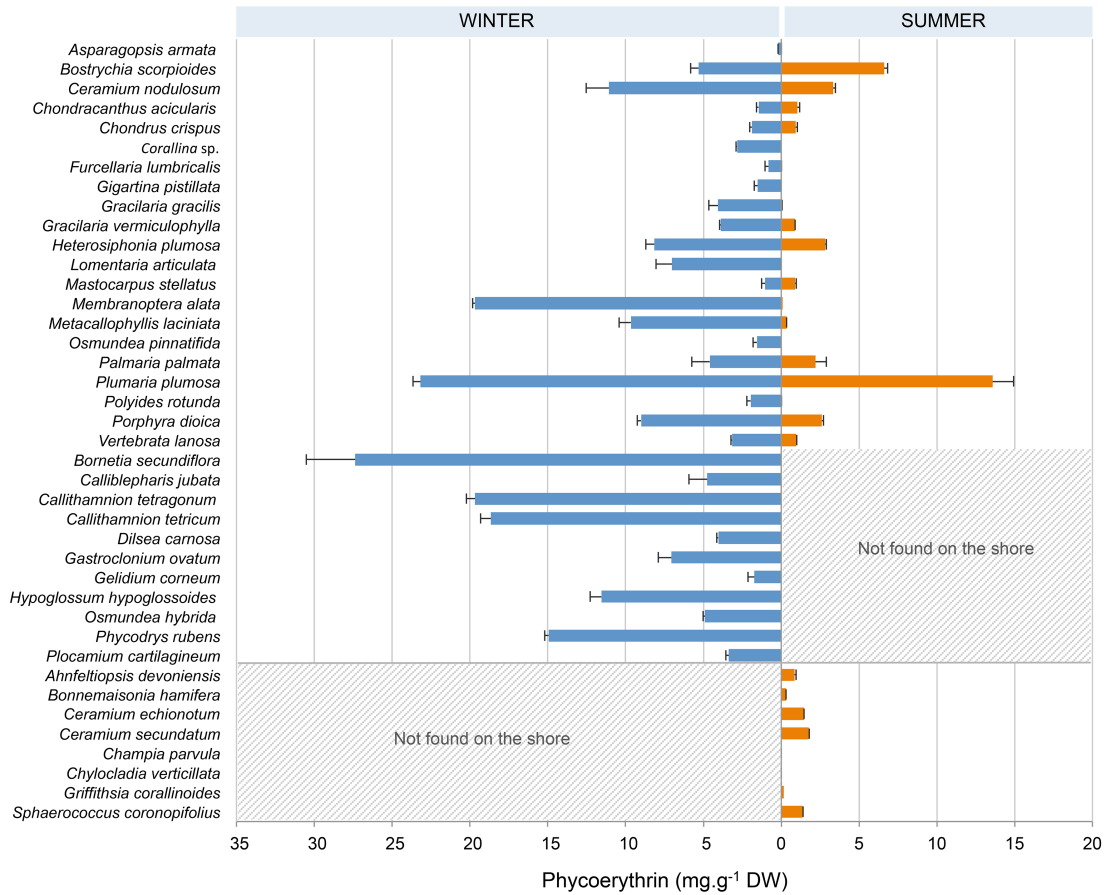
**Appendix 2.** List of the 23 different peaks analysed in 40 red macroalgae species, that may correspond to MAAs, with their absorption spectra,  $\lambda_{\max}$  and their retention time (Rt).

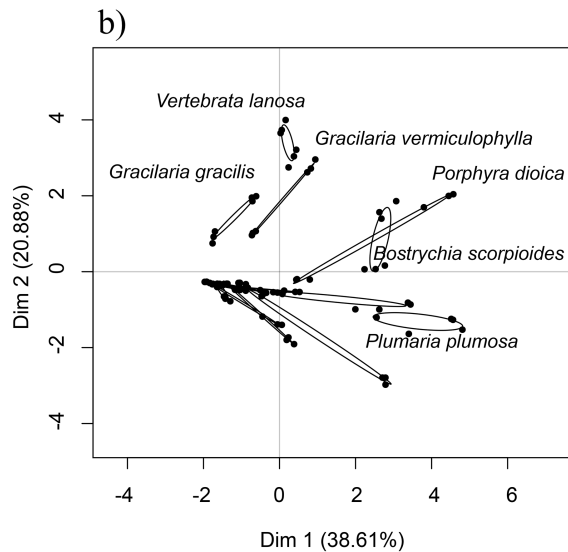
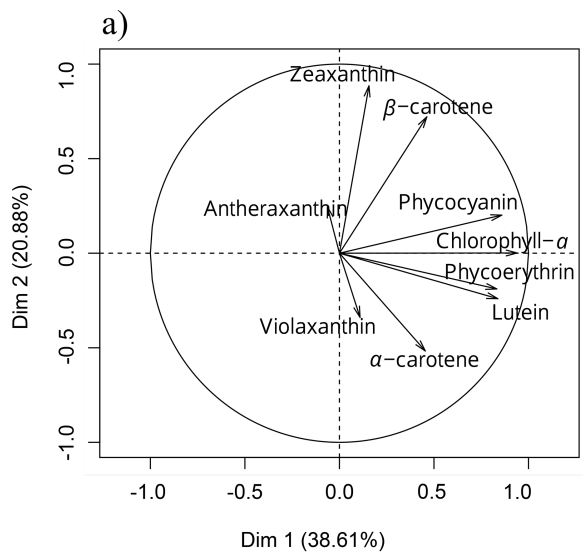
**Appendix 3.** (A) Dendrogram based on the diversity indices of MAAs, obtained from hierarchical cluster analysis (single linkage method); (B) Neighbor-joining phylogenetic tree based on *rbcL* and *cox1* genes sequences, for the 29 macroalgal species collected in summer. The most relevant boot-strap values are shown next to the branches. Some species are not represented on the phylogenetic tree due to missing sequences on GenBank (see Appendix 1).



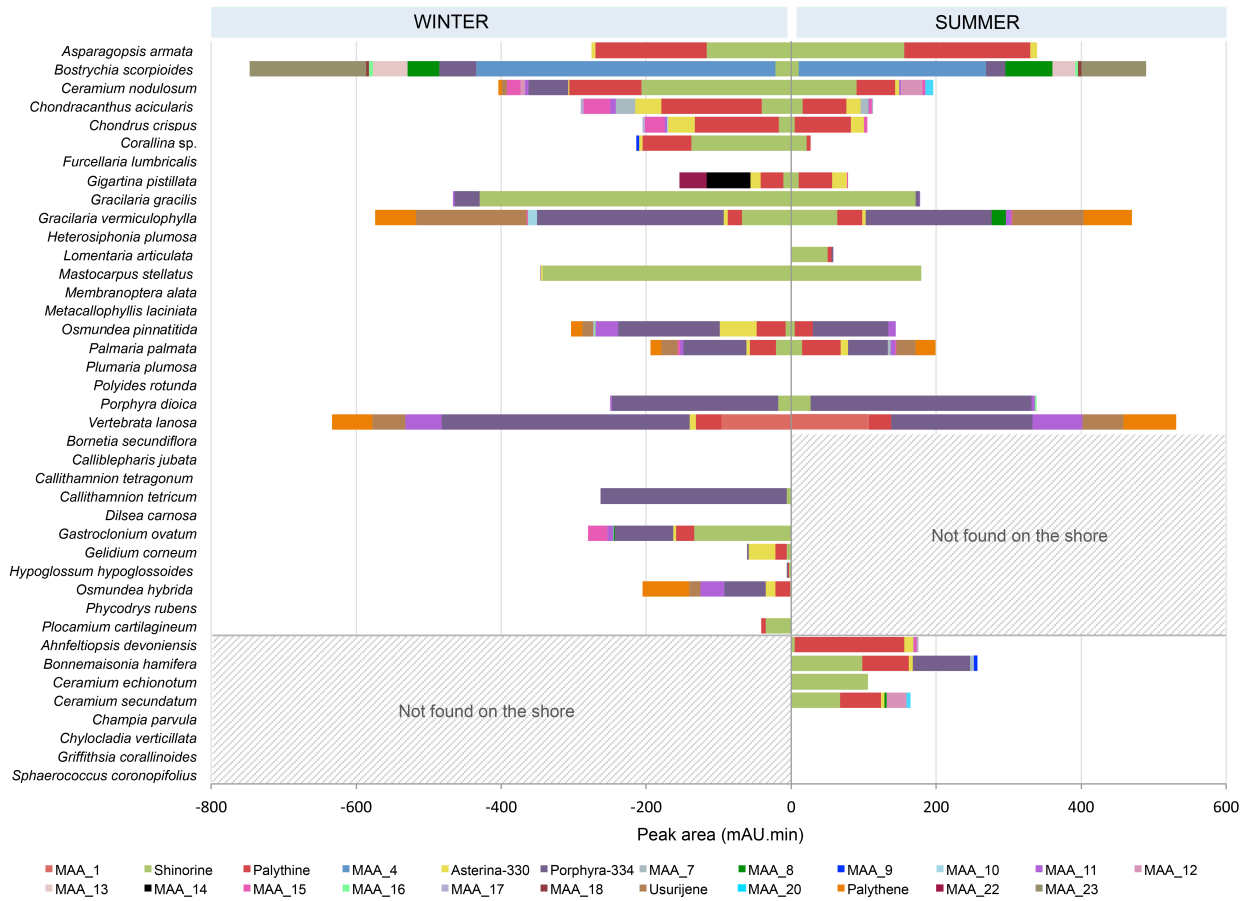


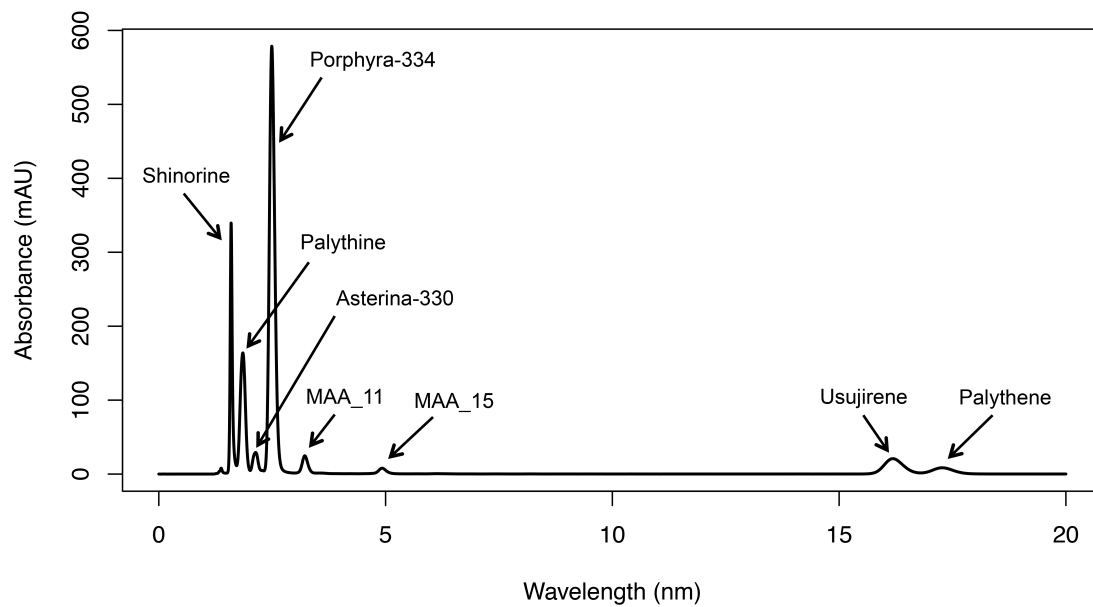






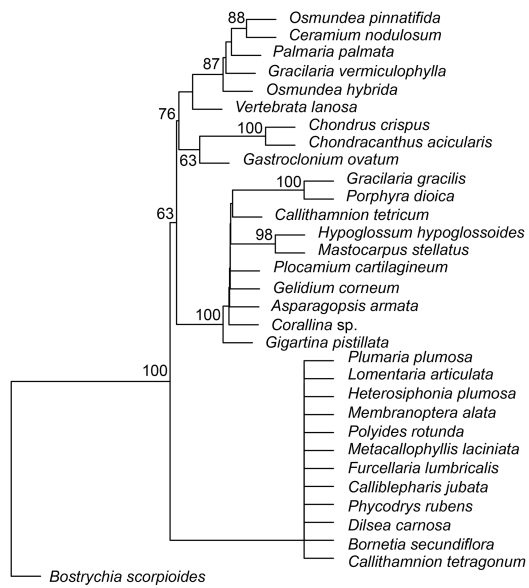
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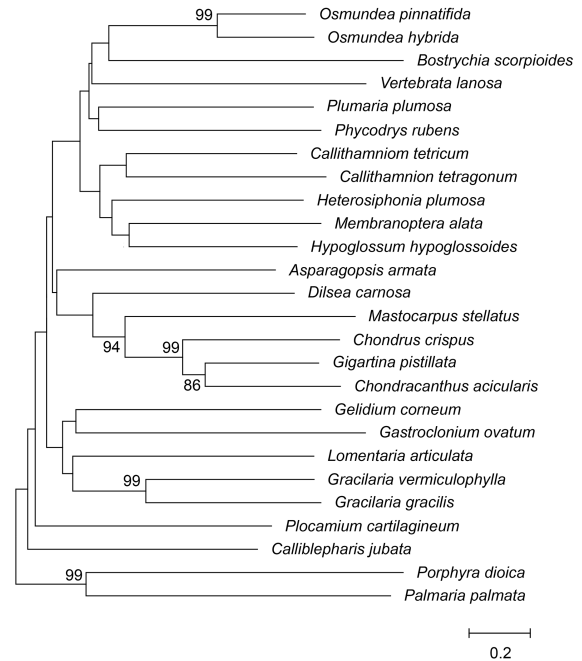


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a)



b)



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## Highlights

- Detection of 23 potential MAAs in 40 Rhodophyta species, including 6 already identified (shinorine, palythine, asterina-330, porphyra-334, usurijene, palythene)
- First report on the MAAs composition for some red seaweeds
- High variability in MAAs content and composition between the different species, with no link with phylogeny, morphology, position on the shore or sampling site
- A MAAs extraction method using 70% ethanol being less toxic than conventional methanol, and giving potential valorisable extracts