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### The genome of *Ectocarpus subulatus* –

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# 1 The genome of *Ectocarpus subulatus* – a 2 highly stress-tolerant brown alga

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33

## 34 **Abstract**

35 Brown algae are multicellular photosynthetic stramenopiles that colonize marine rocky shores  
36 worldwide. *Ectocarpus* sp. Ec32 has been established as a genomic model for brown algae. Here we  
37 present the genome and metabolic network of the closely related species, *Ectocarpus subulatus*  
38 Kützinger, which is characterized by high abiotic stress tolerance. Since their separation, both strains  
39 show new traces of viral sequences and the activity of large retrotransposons, which may also be  
40 related to the expansion of a family of chlorophyll-binding proteins. Further features suspected to  
41 contribute to stress tolerance include an expanded family of heat shock proteins, the reduction of genes  
42 involved in the production of halogenated defence compounds, and the presence of fewer cell wall  
43 polysaccharide-modifying enzymes. Overall, *E. subulatus* has mainly lost members of gene families  
44 down-regulated in low salinities, and conserved those that were up-regulated in the same condition.  
45 However, 96% of genes that differed between the two examined *Ectocarpus* species, as well as all  
46 genes under positive selection, were found to encode proteins of unknown function. This underlines  
47 the uniqueness of brown algal stress tolerance mechanisms as well as the significance of establishing  
48 *E. subulatus* as a comparative model for future functional studies.

49

## 50 **Introduction**

51 Brown algae (Phaeophyceae) are multicellular photosynthetic organisms that are successful colonizers  
52 of rocky shores in the world's oceans. In many places they constitute the dominant vegetation in the  
53 intertidal zone, where they have adapted to multiple stressors including strong variations in  
54 temperature, salinity, irradiation, and mechanical stress (wave action) over the tidal cycle (Davison  
55 and Pearson 1996). In the subtidal environment, brown algae form kelp forests that harbor highly  
56 diverse communities (Steneck *et al.* 2002). They are also harvested as food or for industrial purposes,  
57 such as the extraction of alginates (McHugh 2003). The worldwide annual harvest of brown algae has  
58 reached 10 million tons in 2014 and is constantly growing (Food and Agriculture Organization of the  
59 United Nations 2016). Brown algae share some basic photosynthetic machinery with land plants, but  
60 their plastids derived from a secondary or tertiary endosymbiosis event with a red alga, and they belong  
61 to an independent lineage of eukaryotes, the stramenopiles (Archibald 2009). This phylogenetic  
62 background, together with their distinct habitat, contributes to the fact that brown algae have evolved  
63 numerous unique metabolic pathways, life cycle features, and stress tolerance mechanisms.

64 To enable functional studies of brown algae, strain Ec32 of the small filamentous alga *Ectocarpus* sp.  
65 has been established as a genetic and genomic model (Peters *et al.* 2004; Heesch *et al.* 2010; Cock *et*  
66 *al.* 2010). This strain was formerly described as *Ectocarpus siliculosus*, but has since been shown to  
67 belong to an independent clade by molecular methods (Stache-Crain *et al.* 1997; Peters *et al.* 2015).  
68 More recently, three additional brown algal genomes, that of the kelp species *Saccharina japonica*  
69 (Ye *et al.* 2015), that of *Cladosiphon okamuranus* (Nishitsuji *et al.* 2016), and that of *Nemacystus*  
70 *decipiens* (Nishitsuji *et al.* 2019), have been characterized. Comparisons between these four genomes  
71 have allowed researchers to obtain a first overview of the unique genomic features of brown algae, as  
72 well as a glimpse of the genetic diversity within this group. However, given the evolutionary distance  
73 between these algae, it is difficult to link genomic differences to physiological differences and possible  
74 adaptations to their lifestyle. To be able to generate more accurate hypotheses on the role of particular

75 genes and genomic features for adaptive traits, a common strategy is to compare closely related strains  
76 and species that differ only in a few genomic features. The genus *Ectocarpus* is particularly well suited  
77 for such comparative studies because it comprises a wide range of morphologically similar but  
78 genetically distinct strains and species that have adapted to different marine and brackish water  
79 environments (Kützing 1843; Harvey 1848; Stache-Crain *et al.* 1997; Montecinos *et al.* 2017). One  
80 species within this group, *Ectocarpus subulatus* Kützing (Peters *et al.* 2015), comprises isolates highly  
81 resistant to elevated temperature (Bolton 1983) and low salinity. A strain of this species was even  
82 isolated from freshwater (West and Kraft 1996), constituting one of the handful of known marine-  
83 freshwater transitions in brown algae (Dittami *et al.* 2017).

84 Here we present the draft genome and metabolic network of a strain of *E. subulatus*, establishing the  
85 genomic basis for its use as a comparative model to study stress tolerance mechanisms, and in  
86 particular low salinity tolerance, in brown algae. Similar strategies have been successfully employed  
87 in terrestrial plants, where “extremophile” relatives of model- or economically relevant species have  
88 been sequenced to explore new stress tolerance mechanisms in the green lineage (Amtmann 2009;  
89 Dassanayake *et al.* 2011; Oh *et al.* 2012; Dittami and Tonon 2012; Ma *et al.* 2013; Zeng *et al.* 2015).  
90 The study of the *E. subulatus* genome, and subsequent comparative analysis with other brown algal  
91 genomes, in particular that of *Ectocarpus* sp. Ec32, provides insights into the dynamics of *Ectocarpus*  
92 genome evolution and divergence, and highlights important adaptive processes, such as a potentially  
93 retrotransposon driven expansion of the family of chlorophyll-binding proteins with subsequent  
94 diversification. Most importantly, our analyses underline that most of the observed differences  
95 between the examined species of *Ectocarpus* correspond to proteins with yet unknown functions.

## 96 **Results**

### 97 **Sequencing and assembly of the *E. subulatus* genome**

98 A total of 34.7Gb of paired-end read data and of 28.8Gb of mate-pair reads (corresponding to 45  
99 million non-redundant mate-pairs) were acquired (Supporting Information Table S1). The final  
100 genome assembly size of strain Bft15b was 227Mb (Table 1), and we also obtained 123Mb of bacterial  
101 contigs corresponding predominantly to *Alphaproteobacteria* (50%, with the dominant genera  
102 *Roseobacter* 8% and *Hyphomonas* 5%), followed by *Gammaproteobacteria* (18%), and *Flavobacteria*  
103 (13%). The mean sequencing coverage of mapped reads was 67X for the paired-end library, and the  
104 genomic coverage was 6.9, 14.4, and 30.4X for the 3kb, 5kb, and 10kb mate-pair libraries,  
105 respectively. RNA-seq experiments yielded 8.8Gb of RNA-seq data, of which 96.6% (Bft15b strain  
106 in seawater), 87.6% (freshwater strain in seawater), and 85.3% (freshwater strain in freshwater)  
107 aligned with the final genome assembly of the Bft15b strain.

### 108 **Gene prediction and annotation**

109 The number of predicted proteins in *E. subulatus* was 60% higher than that predicted for Ec32 (Table  
110 1), mainly due to the presence of mono-exonic genes, many of which corresponded to transposases,  
111 which were not removed from our predictions, but had been manually removed from the Ec32 genome.  
112 Ninety-eight percent of the gene models were supported by at least one associated RNA-seq read, and  
113 92% were supported by at least ten reads, with lowly-expressed (<10 reads) genes being generally  
114 shorter (882 vs 1,403 bases), and containing fewer introns (2.6 vs 5.7). In 7.3% of all predicted proteins  
115 we detected a signal peptide, and 3.7% additionally contained an 'ASAFAP'-motif (Supporting  
116 Information Table S2) indicating that they are likely targeted to the plastid(Gruber *et al.* 2015). Overall  
117 the BUSCO (Simão *et al.* 2015) analyses indicate that the *E. subulatus* genome is 86% complete  
118 (complete and fragmented genes) and 91% when not considering proteins also absent from all other  
119 currently sequenced brown algae (Table 1).

## 120 **Repeated elements**

121 Thirty percent of the *E. subulatus* genome consisted of repeated elements. The most abundant groups  
122 of repeated elements were large retrotransposon derivatives (LARDs), followed by long terminal  
123 repeats (LTRs, predominantly Copia and Gypsy), and long and short interspersed nuclear elements  
124 (LINEs, Figure 1A). The overall distribution of sequence identity levels within superfamilies showed  
125 two peaks, one at an identity level of 78-80%, and one at 96-100% (Figure 1C). An examination of  
126 transposon conservation at the level of individual families revealed a few families that follow this  
127 global bimodal distribution (*e.g.* TIR B343 or LARD B204), while the majority exhibited a unimodal  
128 distribution with peaks either at high (*e.g.* LINE R15) or at lower identity levels (*e.g.* LARD B554)  
129 (Figure 1C). Terminal repeat retrotransposons in miniature (TRIM) and LARDs, both non-  
130 autonomous groups of retrotransposons, were among the most conserved families. A detailed list of  
131 transposons is provided in Supporting Information Table S3. In line with previous observations carried  
132 out in *Ectocarpus* sp. Ec32, no methylation was detected in the *E. subulatus* genomic DNA.

## 133 **Organellar genomes**

134 Plastid and mitochondrial genomes from *E. subulatus* have 95.5% and 91.5% sequence identity with  
135 their *Ectocarpus* sp. Ec32 counterparts in the conserved regions respectively. Only minor structural  
136 differences were observed between organellar genomes of both *Ectocarpus* genomes, as detailed in  
137 Supporting Information Text S1.

## 138 **Global comparison of predicted proteomes**

### 139 **Metabolic network-based comparisons**

140 Similar to the network previously obtained for *Ectocarpus* sp. Ec32 (Prigent *et al.* 2014), the *E.*  
141 *subulatus* Bft15b metabolic network comprised 2,074 metabolic reactions and 2,173 metabolites in  
142 464 pathways, which can be browsed at <http://gem-aureme.irisa.fr/sububftgem>. In total, 2,445 genes  
143 associated with at least one metabolic reaction, and 215 pathways were complete (Figure 2).



144 Comparisons between both networks were carried out on a pathway level (Supporting Information  
145 Text S1, Section “Metabolic network-based comparisons”), but no pathways were found to be truly  
146 specific to either Ec32 and/or Bf15b.

#### 147 **Genes under positive selection**

148 Out of the 2,311 orthogroups with single-copy orthologs that produced high quality alignments, 172  
149 gene pairs (7.4%) exhibited dN/dS ratios  $> 0.5$  (Supporting Information Table S4). Among these, only  
150 eleven (6.4%) were found to fit significantly better with the model allowing for positive selection in  
151 the *Ectocarpus* branch. These genes are likely to have been under positive selection, and two of them  
152 contained a signal peptide targeting the plastid. All of them are genes specific to the brown algal  
153 lineage with unknown function, and only two genes contained protein domains related to a  
154 biochemical function (one oxidoreductase-like domain, and one protein prenyltransferase, alpha  
155 subunit). However, all of them were expressed at least in *E. subulatus* Bft15b. There was no trend for  
156 these genes to be located in specific regions of the genome (all except two for *Ectocarpus* sp. Ec32  
157 were on different scaffolds) and none of the genes were located in the pseudoautosomal region of the  
158 sex chromosome.

#### 159 **Genes specific to either *Ectocarpus* genome, and expanded genes and gene families**

160 After manual curation based on tblastn searches to eliminate artefacts arising from differences in the  
161 gene predictions, 184 expanded gene clusters and 1,611 predicted proteins were found to be specific  
162 to *E. subulatus* compared to *Ectocarpus* sp., while 449 clusters were expanded and 689 proteins were  
163 found specifically in the latter (Figure 2, Supporting Information Table S5). This is far less than the  
164 2,878 and 1,093 unique clusters found for a recent comparison of *N. decipiens* and *C. okamuranus*  
165 (Nishitsuji *et al.* 2019). Gene set enrichment analyses revealed no GO categories to be significantly  
166 over-represented among the genes unique to or expanded in *E. subulatus* Bft15b, but several categories  
167 were over-represented among the genes and gene families specific to or expanded in the *Ectocarpus*  
168 sp. Ec32 strain. Many were related either to signalling pathways or to the membrane and transporters

169 (Figure 2), but it is difficult to distinguish between the effects of a potentially incomplete genome  
170 assembly and true gene losses in Bft15b. In the manual analyses we therefore focussed on the genes  
171 specific to and expanded in *E. subulatus*.

172 Among the 1,611 *E. subulatus*-specific genes, 1,436 genes had no homologs (e-value < 1e-5) in the  
173 UniProt database as of May 20<sup>th</sup> 2016: they could thus, at this point in time, be considered lineage-  
174 specific and had no function associated to them. Among the remaining 175 genes, 145 had hits (e-  
175 value < 1e-5) in *Ectocarpus* sp. Ec32, *i.e.* they likely correspond to multi-copy genes that had diverged  
176 prior to the separation of *Ectocarpus* and *S. japonica*, and for which the *Ectocarpus* sp. Ec32 and *S.*  
177 *japonica* orthologs were lost. Thirteen genes had homology only with uncharacterized proteins or were  
178 too dissimilar from characterized proteins to deduce hypothetical functions; another eight probably  
179 corresponded to short viral sequences integrated into the algal genome (EsuBft1730\_2, EsuBft4066\_3,  
180 EsuBft4066\_2, EsuBft284\_15, EsuBft43\_11, EsuBft551\_12, EsuBft1883\_2, EsuBft4066\_4), and one  
181 (EsuBft543\_9) was related to a retrotransposon. Two adjacent genes (EsuBft1157\_4, EsuBft1157\_5)  
182 were also found in diatoms and may be related to the degradation of cellobiose and the transport of  
183 the corresponding sugars. Two genes, EsuBft1440\_3 and EsuBft1337\_8, contained conserved motifs  
184 (IPR023307 and SSF56973) typically found in toxin families. Two more (EsuBft1006\_6 and  
185 EsuBft308\_11) exhibited low similarities to animal and fungal transcription factors, and the last  
186 (EsuBft36\_20 and EsuBft440\_20) consisted almost exclusively of short repeated sequences of  
187 unknown function (“ALEW” and “GAAASGVAGGAVVVNG”, respectively). In total, 1.7%  
188 contained a signal peptide targeting the plastid, *i.e.* significantly less than the 3.7% in the entire dataset  
189 (Fisher exact test, p<0.0001).

190 The large majority of *Ectocarpus* sp. Ec32-specific proteins (511) also corresponded to proteins of  
191 unknown function without matches in public databases. Ninety-seven proteins were part of the *E.*  
192 *siliculosus* virus-1 (EsV-1) inserted into the Ec32 genome and the remaining 81 proteins were poorly  
193 annotated, usually only via the presence of a domain. Examples are ankyrin repeat-containing domain

194 proteins (12), Zinc finger domain proteins (6), proteins containing wall sensing component (WSC)  
195 domains (3), protein kinase-like proteins (3), and Notch domain proteins (2).

196 Regarding the 184 clusters of expanded genes in *E. subulatus*, 139 (1,064 proteins) corresponded to  
197 proteins with unknown function, 98% of which were found only in *Ectocarpus*. Furthermore, nine  
198 clusters (202 proteins) represented sequences related to transposons predicted in both genomes, and  
199 eight clusters (31 proteins) were similar to known viral sequences. Only 28 clusters (135 proteins)  
200 could be roughly assigned to biological functions (Table 2). They comprised proteins potentially  
201 involved in modification of the cell-wall structure (including sulfation), in transcriptional regulation  
202 and translation, in cell-cell communication and signalling, as well as a few stress response proteins,  
203 notably a set of HSP20s, and several proteins of the light-harvesting complex (LHC) potentially  
204 involved in non-photochemical quenching. Only 0.6% of all genes expanded in Bft15b contained a  
205 signal peptide targeting the plastid, i.e. significantly less than the 3.7% in the entire dataset (Fisher  
206 exact test,  $p < 0.0001$ ).

207 Striking examples of likely expansions in *Ectocarpus* sp. Ec32 or reduction in *E. subulatus* Bft15b  
208 were different families of serine-threonine protein kinase domain proteins present in 16 to 25 copies  
209 in Ec32 compared to only 5 or 6 in Bft15b, Kinesin light chain-like proteins (34 vs. 13 copies), two  
210 clusters of Notch region containing proteins (11 and 8 vs. 2 and 1 copies), a family of unknown WSC  
211 domain containing proteins (8 copies vs. 1), putative regulators of G-protein signalling (11 vs. 4  
212 copies), as well as several expanded clusters of unknown and viral proteins. However, these results  
213 need to be taken with caution because the *E. subulatus* Bft15b genome was less complete than that of  
214 *Ectocarpus* sp. Ec32.

### 215 **Correlation with gene expression patterns**

216 To assess whether genomic adaptations in *E. subulatus* Bft15b were located preferentially in genes  
217 that are known to be responsive to salinity stress, we compared expanded gene families to previously

218 available expression data obtained for a freshwater strain of *E. subulatus* grown in freshwater vs  
219 seawater (Dittami *et al.* 2012). This analysis revealed that genes that were down-regulated in response  
220 to low salinity were significantly over-represented among the gene families expanded in *Ectocarpus*  
221 sp. Ec32 or reduced in *E. subulatus* Bft15b, (42% of genes vs 26% for all genes; Fischer exact test  
222  $p=0.0002$ ), while genes that were upregulated in response to low salinity were significantly under-  
223 represented (25% vs 33%; Fischer exact test  $p=0.006$ ; Figure 3, Supporting Information Table S6).  
224 This indicates that *E. subulatus* Bft15b has mainly lost members of gene families that were generally  
225 down-regulated in low salinities, and conserved those that were upregulated in this condition.

### 226 Targeted manual annotation of specific pathways

227 In addition to the global analyses carried out above, genes related to cell wall metabolism, sterol  
228 metabolism, polyamine and central carbon metabolism, algal defence metabolites, transporters, and  
229 abiotic stress response were manually examined and annotated, because, based on literature studies,  
230 these functions could be expected to explain the physiological differences between *E. subulatus*  
231 Bft15b and *Ectocarpus* sp. Ec32. Overall the differences between both *Ectocarpus* strains with respect  
232 to these genes were minor; a detailed description of these results is available in Supporting Information  
233 Text S1 and Supporting Information Table S7, and a brief overview of the main differences is  
234 presented below.

235 Regarding gene families reduced in *E. subulatus* Bft15b or expanded in *Ectocarpus* sp. Ec32, the *E.*  
236 *subulatus* genome encoded only 320 WSC-domain containing proteins, vs. 444 in *Ectocarpus* sp..  
237 Many of these genes were down-regulated in response to low salinity, (61% of the WSC domain  
238 containing genes with available expression data; Fischer exact test,  $p=0.0004$ ) while only 7% were  
239 upregulated (Fischer exact test,  $p\text{-value}=0.0036$ ). In yeast, WSC domain proteins may act as cell  
240 surface mechanosensors and activate the intracellular cell wall integrity signalling cascade in response  
241 to hypo-osmotic shock (Gualtieri *et al.* 2004). Whether or not they have similar functions in brown  
242 algae, however, remains to be established. Furthermore, we found fewer aryl sulfotransferase,

243 tyrosinases, potential bromoperoxidases, and thyroid peroxidases in the *E. subulatus* genome  
244 compared to *Ectocarpus* sp., and it entirely lacks haloalkane dehalogenases (Supporting Information  
245 Text S1). All of these enzymes are involved in the production of polyphenols and halogenated defence  
246 compounds, suggesting that *E. subulatus* may be investing less energy in defence, although a potential  
247 bias induced by differences in the assembly completeness cannot be excluded here.

248 Regarding gene families expanded in *E. subulatus* Bft15b or reduced in *Ectocarpus* sp. Ec32, we  
249 detected differences with respect to a few “classical” stress response genes. Notably an HSP20 protein  
250 was present in three copies in the genome of *E. subulatus* and only one copy in *Ectocarpus* sp.. We  
251 also found a small group of LHCX-family chlorophyll-binding proteins (CBPs) as well as a larger  
252 group belonging to the LHCF/LHCR family that have probably undergone a recent expansion in *E.*  
253 *subulatus* (Figure 4). Some of the proteins appeared to be truncated (marked with asterisks), but all of  
254 them were associated with RNA-seq reads, suggesting that they may be functional. A number of these  
255 proteins were also flanked by LTR-like sequences. CBPs have been reported to be up-regulated in  
256 response to abiotic stress in stramenopiles (Zhu and Green 2010; Dong *et al.* 2016), including  
257 *Ectocarpus* (Dittami *et al.* 2009), probably as a way to deal with excess light energy when  
258 photosynthesis is affected.

## 259 **Discussion**

260 Here we present the draft genome and metabolic network of *E. subulatus* strain Bft15b, a brown alga  
261 which, compared to *Ectocarpus* sp. Ec32, is characterized by high abiotic stress tolerance (Bolton  
262 1983; Peters *et al.* 2015). Based on time-calibrated molecular trees, both species separated roughly 16  
263 Mya (Dittami *et al.* 2012), *i.e.* slightly before the split between *Arabidopsis thaliana* and *Thellungiella*  
264 *salsuginea* (7-12 Mya) (Wu *et al.* 2012). This split was probably followed by an adaptation of *E.*  
265 *subulatus* to highly fluctuating and low salinity habitats (Dittami *et al.* 2017).

## 266 **Traces of recent transposon activity and integration of viral sequences**

267 The *E. subulatus* Bft15b genome is only approximately 6% (flow cytometry) to 23% (genome  
268 assembly) larger than that of *Ectocarpus* sp. Ec32, and no major genomic rearrangements or  
269 duplications were detected. However, we observed traces of recent transposon activity, especially from  
270 LTR transposons, which is in line with the absence of DNA methylation. Bursts in transposon activity  
271 have been identified as one potential driver of local adaptation and speciation in other model systems  
272 such as salmon (de Boer *et al.* 2007) or land plants (Hu *et al.* 2011; Wu *et al.* 2012). Furthermore,  
273 LTRs are known to mediate the retrotransposition of individual genes, leading to the duplication of  
274 the latter (Tan *et al.* 2016). In *E. subulatus* Bft15b, only a few expansions of gene families were  
275 observed since the separation from *Ectocarpus* sp. Ec32, and only in the case of the recent expansion  
276 of the LHCR family were genes flanked by a pair of LTR-like sequences. These elements lacked both  
277 the group antigen (GAG) and reverse transcriptase (POL) proteins, which implies that, if retro-  
278 transposition was the mechanism underlying the expansion of this group of proteins, it would have  
279 depended on other active transposable elements to provide these activities.

280 A second factor that has shaped the *Ectocarpus* genomes were viruses. Viral infections are a common  
281 phenomenon in Ectocarpales (Müller *et al.* 1998), and a well-studied example is the *Ectocarpus*  
282 *siliculosus* virus-1 (EsV-1) (Delaroque *et al.* 2001). It was found to be present latently in several strains  
283 of *Ectocarpus* sp. closely related to strain Ec32, and has also been found integrated in the genome of  
284 the latter, although it is not expressed (Cock *et al.* 2010). As previously indicated by comparative  
285 genome hybridization experiments (Dittami *et al.* 2011), the *E. subulatus* Bft15b genome does not  
286 contain a complete EsV-1 like insertion, although a few shorter EsV-1-like proteins were found. Thus,  
287 the EsV-1 integration observed in *Ectocarpus* sp. Ec32 has likely occurred after the split with *E.*  
288 *subulatus*, and the biological consequences of this insertion remain to be explored.

## 289 **Few classical stress response genes but no transporters involved in adaptation**

290 One aim of this study was to identify genes that may potentially be responsible for the high abiotic  
291 stress and salinity tolerance of *E. subulatus*. Similar studies on genomic adaptation to changes in  
292 salinity or to drought in terrestrial plants have previously highlighted genes generally involved in stress  
293 tolerance to be expanded in “extremophile” organisms. Examples are the expansion of catalase,  
294 glutathione reductase, and heat shock protein families in desert poplar (Ma *et al.* 2013), arginine  
295 metabolism in jujube (Liu *et al.* 2014), or genes related to cation transport, abscisic acid signalling,  
296 and wax production in *T. salsuginea* (Wu *et al.* 2012). In our study, we found that gene families  
297 reduced in *E. subulatus* Bft15b compared to the marine *Ectocarpus* sp. Ec32 model have previously  
298 been shown to be repressed in response to stress, whereas gene families up-regulated in response to  
299 stress had a higher probability of being conserved. However, there are only few signs of known stress  
300 response gene families among them, notably the two additional HSP20 proteins and an expanded  
301 family of CBPs. *E. subulatus* Bft15b also has a slightly reduced set of genes involved in the production  
302 of halogenated defence compounds that may be related to its habitat preference: it is frequently found  
303 in brackish and even freshwater environments with low availability of halogens. It also specializes in  
304 habitats with high levels of abiotic stress compared to most other brown algae, and may thus invest  
305 less energy in defence against biotic stressors.

306 Another anticipated adaptation to life in varying salinities lies in modifications of the cell wall.  
307 Notably, the content of sulfated polysaccharides is expected to play a crucial role as these compounds  
308 are present in all marine plants and algae, but absent in their freshwater relatives (Kloareg and  
309 Quatrano 1988; Popper *et al.* 2011). The fact that we found only small differences in the number of  
310 encoded sulfatases and sulfotransferases indicates that the absence of sulfated cell-wall  
311 polysaccharides previously observed in *E. subulatus* in low salinities (Torode *et al.* 2015) is probably  
312 a regulatory effect or simply related to the lack of sulfate in low salinity. This is also coherent with the  
313 wide distribution of *E. subulatus* in marine, brackish water, and freshwater environments.

314 Finally, transporters have previously been described as a key element in plant adaptation to different  
315 salinities (Rao *et al.* 2016). Similar results have also been obtained for *Ectocarpus* in a study of  
316 quantitative trait loci (QTLs) associated with salinity and temperature tolerance (Avia *et al.* 2017). In  
317 our study, however, we found no indication of genomic differences related to transporters between the  
318 two species. This observation corresponds to previous physiological experiments indicating that  
319 *Ectocarpus*, unlike many terrestrial plants, responds to strong changes in salinity as an osmoconformer  
320 rather than an osmoregulator, *i.e.* it allows the intracellular salt concentration to adjust to values close  
321 to the external medium rather than keeping the intracellular ion composition constant (Dittami *et al.*  
322 2009).

### 323 **Species-specific genes of unknown function are likely to play a dominant role in** 324 **adaptation**

325 In addition to genes that may be directly involved in the adaptation to the environment, we found  
326 several gene clusters containing domains potentially involved in cell-cell signalling that were  
327 expanded in the *Ectocarpus* sp. Ec32 genome (Table 2), *e.g.* a family of ankyrin repeat-containing  
328 domain proteins (Mosavi *et al.* 2004). These observed differences may be, in part, responsible for the  
329 existing pre-zygotic reproductive barrier between the two examined species of *Ectocarpus* (Lipinska  
330 *et al.* 2016).

331 The vast majority of genomic differences between the two investigated species of *Ectocarpus*,  
332 however, corresponds to proteins of entirely unknown functions. All of the 11 gene pairs under  
333 positive selection were unknown genes taxonomically restricted to brown algae. Of the 1,611 *E.*  
334 *subulatus* Bft15b-specific genes, 88% were unknown. Most of these genes were expressed and are  
335 thus likely to correspond to true genes; their absence from the *Ectocarpus* sp. Ec32 genome was also  
336 confirmed at the nucleotide level. A large part of the mechanisms that underlie the adaptation to  
337 different ecological niches in *Ectocarpus* may, therefore, lie in these genes of unknown function. This



338 can be partly explained by the fact that still only few brown algal genomes have been sequenced, and  
339 that currently most of our knowledge on the function of their proteins is based on studies in model  
340 plants, animals, yeast, or bacteria, which have evolved independently from stramenopiles for over 1  
341 billion years (Yoon *et al.* 2004). They differ from land plants even in otherwise highly conserved  
342 aspects, for instance in their life cycles, cell walls, and primary metabolism (Charrier *et al.* 2008).  
343 Substantial contributions of lineage-specific genes to the evolution of organisms and the development  
344 of innovations have also been described for animal models (Tautz and Domazet-Lošo 2011), and  
345 studies in basal metazoans furthermore indicate that they are essential for species-specific adaptive  
346 processes (Khalturin *et al.* 2009).

347 Despite the probable importance of these unknown genes for local adaptation, *Ectocarpus* may still  
348 heavily rely on classical stress response genes for abiotic stress tolerance. Many of the gene families  
349 known to be related to stress response in land plants (including transporters and genes involved in cell  
350 wall modification), and for which no significant differences in gene contents were observed, have  
351 previously been reported to be strongly regulated in response to environmental stress in *Ectocarpus*  
352 (Dittami *et al.* 2009, 2012; Ritter *et al.* 2014). This high transcriptomic plasticity is probably one of  
353 the features that allow *Ectocarpus* to thrive in a wide range of environments, and may form the basis  
354 for its capacity to further adapt to “extreme environments” such as freshwater (West and Kraft 1996).

## 355 **Conclusion and future work**

356 We have shown that since the separation of *E. subulatus* and *Ectocarpus sp.* Ec32, both genomes have  
357 been shaped partially by the activity of viruses and transposons, particularly large retrotransposons.  
358 Over this period of time, *E. subulatus* has adapted to environments with high abiotic variability  
359 including brackish water and even freshwater. We have identified a few genes that likely contribute  
360 to this adaptation, including HSPs, CBPs, a reduction of genes involved in halogenated defence  
361 compounds, or some changes in cell wall polysaccharide-modifying enzymes. However, the majority  
362 of genes that differ between the two examined *Ectocarpus* species or that may be under positive

363 selection encode proteins of unknown function. This underlines the fundamental differences that exist  
364 between brown algae and terrestrial plants or other lineages of algae. Studies as the present one, *i.e.*  
365 without strong *a priori* assumptions about the mechanisms involved in adaptation, are therefore  
366 essential to start elucidating the specificities of this lineage as well as the various functions of the  
367 unknown genes.

## 368 **Methods**

369 **Biological material.** Haploid male parthenosporophytes of *E. subulatus* strain Bft15b (Culture  
370 Collection of Algae and Protozoa CCAP accession 1310/34), isolated in 1978 (exact collection date  
371 unknown) by Dieter G. Müller from an intertidal mudflat in vicinity of Duke Marine Station  
372 (34.716561,-76.6744956), Beaufort, North Carolina, USA, were grown in 14 cm (100 ml) Petri Dishes  
373 in Provasoli-enriched seawater (Starr and Zeikus 1993) under a 14/10 daylight cycle at 14°C. Strains  
374 were examined by light microscopy (800X magnification, phase contrast) to ensure that they were  
375 free of contaminating eukaryotes, but did still contain some alga-associated bacteria. Approximately  
376 1 g fresh weight of algal culture was dried on a paper towel and immediately frozen in liquid nitrogen.  
377 For RNA-seq experiments, in addition to Bft15b, a second strain of *E. subulatus*, the diploid freshwater  
378 strain CCAP 1310/196 isolated from Hopkins River Falls, Australia (West and Kraft 1996), was  
379 included. One culture was grown as described above for Bft15b, and for a second culture, seawater  
380 was diluted 20-fold with distilled water prior to the addition of Provasoli nutrients (Dittami *et al.* 2012)  
381 (culture condition referred to as freshwater).

382 **Flow cytometry** experiments to measure nuclear DNA contents were carried out as previously  
383 described (Bothwell *et al.* 2010), except that young sporophyte tissue was used instead of gametes.  
384 Samples of the genome-sequenced *Ectocarpus* sp. strain Ec32 (CCAP accession 1310/4 from San Juan  
385 de Marcona, Peru) were run in parallel as a size reference.

386 **DNA and RNA** were extracted using a phenol-chloroform-based protocol (Le Bail *et al.* 2008). For  
387 **DNA sequencing**, four Illumina libraries were prepared and sequenced on a HiSeq2000: one paired-  
388 end library (Illumina TruSeq DNA PCR-free LT Sample Prep kit #15036187, sequenced with 2x100  
389 bp read length), and three mate-pair libraries with span sizes of 3kb, 5kb, and 10kb respectively  
390 (Nextera Mate Pair Sample Preparation Kit; sequenced with 2x50bp read length). One poly-A enriched  
391 RNA-seq library was generated for each of the three aforementioned cultures according to the Illumina  
392 TruSeq Stranded mRNA Sample Prep kit #15031047 protocol and sequenced with 2x50 bp read  
393 length.

394 The degree of **DNA methylation** was examined by HPLC on CsCl-gradient purified DNA (Le Bail *et*  
395 *al.* 2008) from three independent cultures per strain as previously described (Rival *et al.* 2013).

396 Redundancy of mate-pairs (MPs) was reduced to mitigate the negative effect of redundant chimeric  
397 MPs during scaffolding. To this means, mate-pair reads were aligned with bwa-0.6.1 to a preliminary  
398 *E. subulatus* Bft15b draft assembly calculated from paired-end data only. Mate-pairs that did not map  
399 with both reads were removed, and for the remaining pairs, read-starts were obtained by parsing the  
400 cigar string using Samtools and a custom Pearl script. Mate-pairs with redundant mapping coordinates  
401 were removed for the final **assembly**, which was carried out using SOAPDenovo2 (Luo *et al.* 2012).  
402 Scaffolding was then carried out using SSPACE basic 2.0 (Boetzer *et al.* 2011) (trim length up to 5  
403 bases, minimum 3 links to scaffold contigs, minimum 15 reads to call a base during an extension)  
404 followed by a run of GapCloser (part of the SOAPDenovo package, default settings). A dot plot of  
405 syntenic regions between *E. subulatus* Bft15b and *Ectocarpus* sp. Ec32 was generated using D-Genies  
406 1.2.0 (Cabanettes and Klopp 2018). Given the high degree of synteny observed (Supporting  
407 Information Text S1), additional scaffolding was carried out using MeDuSa and the *Ectocarpus* sp.  
408 Ec32 genome as reference (Bosi *et al.* 2015). This super-scaffolding method assumes that both genome  
409 structures are be similar. Annotations were generated first for version 1 of the Bft15b genome and then  
410 transferred to the new scaffolds of version 2 using the ALLMAPS (Tang *et al.* 2015) liftover function.

411 Both the assemblies with (V2) and without (V1) MeDuSa scaffolding have been made available. RNA-  
412 seq reads were cleaned using Trimmomatic (default settings), and a second Bft15b genome-guided  
413 assembly was performed with Tophat2 and with Cufflinks. Sequencing coverage was calculated based  
414 on mapped algal reads only, and for mate-pair libraries the genomic coverage was calculated as  
415 number of unique algal mate-pairs \* span size / assembly size.

416 As cultures were not treated with antibiotics prior to DNA extraction, **bacterial scaffolds were**  
417 **removed** from the final assembly using the taxoblast pipeline (Dittami and Corre 2017). Every  
418 scaffold was cut into fragments of 500 bp, and these fragments were aligned (blastn, e-value cutoff  
419 0.01) against the GenBank non-redundant nucleotide (nt) database. Scaffolds for which more than  
420 90% of the alignments were with bacterial sequences were removed from the assembly (varying this  
421 threshold between 30 and 95% resulted in only very minor differences in the final assembly). Finally,  
422 we ran the Anvi'o v5 pipeline to identify any remaining contaminant bins (both bacterial and  
423 eukaryote) based on G/C and kmer contents as well as coverage (Eren *et al.* 2015). "Contaminant"  
424 scaffolds were submitted to the MG-Rast server to obtain an overview of the taxa present in the sample  
425 (Meyer *et al.* 2008). They are available at [http://application.sb-](http://application.sb-roscoff.fr/blast/subulatus/download.html)  
426 [roscoff.fr/blast/subulatus/download.html](http://application.sb-roscoff.fr/blast/subulatus/download.html).

427 **Repeated elements** were searched for *de novo* using TEdenovo and annotated using TEannot with  
428 default parameters. LTR-like sequences were predicted by the LTR-harvest pipeline (Ellinghaus *et al.*  
429 2008). These tools are part of the REPET pipeline (Flutre *et al.* 2011), of which version 2.5 was used  
430 for our dataset.

431 **BUSCO** 2.0 analyses (Simão *et al.* 2015) were run on the servers of the IPlant Collaborative (Goff *et*  
432 *al.* 2011) with the general eukaryote database as a reference and default parameters and the predicted  
433 proteins as input.

434 **Plastid and mitochondrial genomes** of *E. subulatus* Bft15b, were manually assembled based on  
435 scaffolds 416 and 858 respectively, using the published organellar genomes of *Ectocarpus* sp. Ec32  
436 (accessions NC\_013498.1, NC\_030223.1) as a guide (Le Corguillé *et al.* 2009; Cock *et al.* 2010;  
437 Delage *et al.* 2011). Genes were manually annotated based on the result of homology searches with  
438 *Ectocarpus* sp. Ec32 using a bacterial genetic code (11) and based on ORF predictions using ORF  
439 finder. Ribosomal RNA sequences were identified by RNAmmer (Lagesen *et al.* 2007) for the plastid  
440 and MITOS (Bernt *et al.* 2013) for the plastid, and tRNAs or other small RNAs were identified using  
441 ARAGORN (Laslett and Canback 2004) and tRNAscan-SE (Schattner *et al.* 2005). In the case of the  
442 mitochondrial genome, the correctness of the manual assembly was verified by PCR where manual  
443 and automatic assemblies diverged.

444 Putative **protein-coding sequences** were identified using Eugene 4.1c (Foissac *et al.* 2008).  
445 Assembled RNA-seq reads were mapped against the assembled genome using GenomeThreader 1.6.5,  
446 and all available proteins from the Swiss-Prot database as well as predicted proteins from the  
447 *Ectocarpus* sp. Ec32 genome (Cock *et al.* 2010) were aligned to the genome using KLAST (Nguyen  
448 and Lavenier 2009). Both aligned *de novo*-assembled transcripts and proteins were provided to Eugene  
449 for gene prediction, which was run with the parameter set previously optimized for the *Ectocarpus* sp.  
450 Ec32 genome (Cock *et al.* 2010). The subcellular localization of the proteins was predicted using  
451 SignalP version 4.1(Nielsen 2017) and the ASAFIND software version 1.1.5 (Gruber *et al.* 2015).

452 **For functional annotation**, predicted proteins were submitted to InterProScan and compared to the  
453 Swiss-Prot database by BlastP search (e-value cutoff 1e-5), and the results imported to Blast2GO  
454 (Götz *et al.* 2008) The genome and all automatic annotations were imported into Apollo (Lee *et al.*  
455 2013; Dunn *et al.* 2017) for manual curation. During manual curation sequences were aligned with  
456 characterized reference sequences from suitable databases (e.g. CAZYME, TCDB, SwissProt) using  
457 BLAST, and the presence of InterProScan domains necessary for the predicted enzymatic function  
458 was manually verified.

459 The *E. subulatus* Bft15b **genome-scale metabolic model** reconstruction was carried out as previously  
460 described (Prigent *et al.* 2014) by merging an annotation-based reconstruction obtained with Pathway  
461 Tools (Karp *et al.* 2016) and an orthology-based reconstruction based on the *Arabidopsis thaliana*  
462 metabolic network AraGEM (de Oliveira Dal’Molin *et al.* 2010) using Pantograph (Loira *et al.* 2015).  
463 A final step of gap-filling was then carried out using the Meneco tool (Prigent *et al.* 2017). The entire  
464 reconstruction pipeline is available via the AuReMe workspace (Aite *et al.* 2018). For pathway-based  
465 analyses, pathways that contained only a single reaction or that were less than 50% complete were not  
466 considered.

467 **Functional comparisons of gene contents** were based primarily on orthologous clusters of genes  
468 shared with version 2 of the *Ectocarpus* sp. Ec32 genome (Cormier *et al.* 2017) as well as the *S.*  
469 *japonica* (Areschoug) genome (Ye *et al.* 2015). They were determined by the OrthoFinder software  
470 version 0.7.1 (Emms and Kelly 2015). To identify genes specific to either of the *Ectocarpus* genomes,  
471 we examined all proteins that were not part of a multi-species cluster and verified their absence in the  
472 other genome by tblastn searches (threshold e-value of 1e-10). Only genes without tblastn hit that  
473 encoded proteins of at least 50 amino acids were further examined. A second approach consisted in  
474 identifying clusters of genes that were expanded or reduced in either of the two *Ectocarpus* genomes  
475 based on the Orthofinder results. Blast2GO 3.1 (Götz *et al.* 2008) was then used to identify  
476 significantly enriched GO terms among the genes specific to either *Ectocarpus* genome or the  
477 expanded/reduced gene families (Fischer’s exact test with FDR correction FDR<0.05). These different  
478 sets of genes were also examined manually for function, genetic context, GC content, and EST  
479 coverage (to ensure the absence of contaminants).

480 The search for **genes under positive selection** was based on a previous analysis in other brown algae  
481 (Lipinska *et al.* 2019). Therefore, Orthofinder analyses were expanded to include also *Macrocystis*  
482 *pyrifera*, *Scytosiphon lomentaria* (Lipinska *et al.* 2019), and *Cladosiphon okamuranus* (Nishitsuji *et*  
483 *al.* 2016). Rates of non-synonymous to synonymous substitution ( $\omega$ =dN/dS) were searched for in

484 clusters of single-copy orthologs. Protein sequences were aligned with Tcoffee (Di Tommaso *et al.*  
485 2011) (M-Coffee mode), translated back to nucleotide using Pal2Nal (Suyama *et al.* 2006), and curated  
486 with Gblocks (Talavera and Castresana 2007) (-t c -b4 20) or manually when necessary. Sequences  
487 that produced a gapless alignment that exceeded 100bp were retained for pairwise dN/dS analysis  
488 between *Ectocarpus* strains using CodeML (F3x4 model of codon frequencies, runmode = -2) of the  
489 PAML4 suite (Yang 2007). Orthogroups for which the pairwise dN/dS ratio between *Ectocarpus*  
490 species exceeded 0.5, which were not saturated (dS < 1), and which contained single-copy orthologs  
491 in at least two other species were used to perform positive selection analysis with CodeML (PAML4,  
492 F3x4 model of codon frequencies): branch-site models were used to estimate dN/dS values by site and  
493 among branches in the species tree generated for each orthogroup. The branch leading to the genus  
494 *Ectocarpus* was selected as a ‘foreground branch’, allowing different values of dN/dS among sites in  
495 contrast to the remaining branches that shared the same distribution of  $\omega$ . Two alternative models were  
496 tested for the foreground branch: H1 allowing the dN/dS to exceed 1 for a proportion of sites (positive  
497 selection), and H0 constraining dN/dS < 1 for all sites (neutral and purifying selection). A likelihood  
498 ratio test was then performed for the two models (LRT=2×(lnLH1-lnLH0)) and genes for which H1  
499 fitted the data significantly better (p<0.05) were identified as evolving under positive selection.

500 **Phylogenetic analyses** were carried out for gene families of particular interest. For chlorophyll-  
501 binding proteins (CBPs), reference sequences were obtained from a previous study (Dittami *et al.*  
502 2010), and aligned together with *E. subulatus* Bft15b and *S. japonica* CBPs using MAFFT (G-INS-i)  
503 (Katoh *et al.* 2002). Alignments were then manually curated, conserved positions selected in Jalview  
504 (Waterhouse *et al.* 2009), and maximum likelihood analyses carried out using PhyML 3.0 (Guindon  
505 and Gascuel 2003), the LG substitution model, 1000 bootstrap replicates, and an estimation of the  
506 gamma distribution parameter. The resulting phylogenetic tree was visualized using MEGA7 (Kumar  
507 *et al.* 2016).

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## 516 **Author contributions**

517 Conceived the study: SMD, AP, AS, HH, CB, TT. Provided materials: AFP, APL. Performed  
518 experiments: SMD, SD, IGN, DM, MMP. Analysed data: SMD, APL, EC, LBG, NP, MA, KA, CHC,  
519 JC, AC, LD, SD, CF, AGo, AGr, CH, DJ, HK, XL, GVM, AEM, MM, PP, MMP, ASim, CT, HSY,  
520 TT. Wrote the manuscript: SMD, KA, APL, JC, LD, CH, Ago, AGr, GVM, ASim, TT. Revised and  
521 approved of the final manuscript: all authors.

## 522 **Additional Information**

### 523 **Competing interests**

524 The authors declare no competing interest.

### 525 **Data availability**

526 Sequence data (genomic and transcriptomic reads) were submitted to the European Nucleotide  
527 Archive (ENA) under project accession number PRJEB25230. Assembled scaffolds were deposited  
528 under accession numbers LR740778-LR742460 (Biosample ERS4125292). A JBrowse (Skinner *et al.*  
529 2009) instance comprising the most recent annotations is available via the server of the Station



530 Biologique de Roscoff (<http://mmo.sb-roscoff.fr/jbrowseEsu>). The reconstructed metabolic network  
531 of *E. subulatus* is available at <http://gem-aureme.irisa.fr/sububftgem>. Additional resources and  
532 annotations including a blast server are available at [http://application.sb-  
533 roscoff.fr/project/subulatus/index.html](http://application.sb-roscoff.fr/project/subulatus/index.html). The complete set of manual annotations is provided in  
534 Supporting Information Table S7.

## 535 **References**

- 536 **Aite M, Chevallier M, Frioux C, et al. 2018.** Traceability, reproducibility and wiki-exploration for  
537 “à-la-carte” reconstructions of genome-scale metabolic models. *PLOS Computational Biology* **14**:  
538 e1006146.
- 539 **Amtmann A. 2009.** Learning from evolution: *Thellungiella* generates new knowledge on essential  
540 and critical components of abiotic stress tolerance in plants. *Molecular plant* **2**: 3–12.
- 541 **Archibald JM. 2009.** The puzzle of plastid evolution. *Current biology* **19**: R81-8.
- 542 **Avia K, Coelho SM, Montecinos GJ, et al. 2017.** High-density genetic map and identification of  
543 QTLs for responses to temperature and salinity stresses in the model brown alga *Ectocarpus*.  
544 *Scientific reports* **7**: 43241.
- 545 **Le Bail A, Dittami SM, de Franco PO, et al. 2008.** Normalisation genes for expression analyses in  
546 the brown alga model *Ectocarpus siliculosus*. *BMC Molecular Biology* **9**: 75.
- 547 **Bernt M, Donath A, Jühling F, et al. 2013.** MITOS: improved de novo metazoan mitochondrial  
548 genome annotation. *Molecular Phylogenetics and Evolution* **69**: 313–9.
- 549 **de Boer JG, Yazawa R, Davidson WS, Koop BF. 2007.** Bursts and horizontal evolution of DNA  
550 transposons in the speciation of pseudotetraploid salmonids. *BMC Genomics* **8**: 422.
- 551 **Boetzer M, Henkel C V, Jansen HJ, Butler D, Pirovano W. 2011.** Scaffolding pre-assembled  
552 contigs using SSPACE. *Bioinformatics (Oxford, England)* **27**: 578–9.
- 553 **Bolton JJ. 1983.** Ecoclinal variation in *Ectocarpus siliculosus* (Phaeophyceae) with respect to

554 temperature growth optima and survival limits. *Marine Biology* **73**: 131–138.

555 **Bosi E, Donati B, Galardini M, et al. 2015.** MeDuSa: a multi-draft based scaffolder. *Bioinformatics*  
556 **31**: 2443–2451.

557 **Bothwell JH, Marie D, Peters AF, Cock JM, Coelho SM. 2010.** Role of endoreduplication and  
558 apomeiosis during parthenogenetic reproduction in the model brown alga *Ectocarpus*. *New*  
559 *phytologist* **188**: 111–21.

560 **Cabanettes F, Klopp C. 2018.** D-GENIES: dot plot large genomes in an interactive, efficient and  
561 simple way. *PeerJ* **6**: e4958.

562 **Charrier B, Coelho SM, Le Bail A, et al. 2008.** Development and physiology of the brown alga  
563 *Ectocarpus siliculosus*: two centuries of research. *New phytologist* **177**: 319–32.

564 **Cock JM, Sterck L, Rouzé P, et al. 2010.** The *Ectocarpus* genome and the independent evolution  
565 of multicellularity in brown algae. *Nature* **465**: 617–21.

566 **Le Corguillé G, Pearson G, Valente M, et al. 2009.** Plastid genomes of two brown algae,  
567 *Ectocarpus siliculosus* and *Fucus vesiculosus*: further insights on the evolution of red-algal derived  
568 plastids. *BMC Evolutionary Biology* **9**: 253.

569 **Cormier A, Avia K, Sterck L, et al. 2017.** Re-annotation, improved large-scale assembly and  
570 establishment of a catalogue of noncoding loci for the genome of the model brown alga *Ectocarpus*.  
571 *New Phytologist* **214**: 219–232.

572 **Dainat J, Gourelé H. 2018.** NBISweden/EMBLmyGFF3: EMBLmyGFF3-1.2.2. *Zenodo*.

573 **Dassanayake M, Oh D-H, Haas JS, et al. 2011.** The genome of the extremophile crucifer  
574 *Theellungiella parvula*. *Nature Genetics* **43**: 913–918.

575 **Davison IR, Pearson GA. 1996.** Stress tolerance in intertidal seaweeds. *Journal of Phycology* **32**:  
576 197–211.

577 **Delage L, Leblanc C, Nyvall Collén P, et al. 2011.** In silico survey of the mitochondrial protein  
578 uptake and maturation systems in the brown alga *Ectocarpus siliculosus* (E Newbigin, Ed.). *PLoS*  
579 *ONE* **6**: e19540.

580 **Delaroque N, Müller DG, Bothe G, Pohl T, Knippers R, Boland W. 2001.** The complete DNA  
581 sequence of the *Ectocarpus siliculosus* virus EsV-1 genome. *Virology* **287**: 112–132.

582 **Dittami SM, Corre E. 2017.** Detection of bacterial contaminants and hybrid sequences in the  
583 genome of the kelp *Saccharina japonica* using Taxoblast. *PeerJ* **5**: e4073.

584 **Dittami SM, Gravot A, Goulitquer S, et al. 2012.** Towards deciphering dynamic changes and  
585 evolutionary mechanisms involved in the adaptation to low salinities in *Ectocarpus* (brown algae).  
586 *Plant Journal* **71**: 366–377.

587 **Dittami SM, Heesch S, Olsen JL, Collén J. 2017.** Transitions between marine and freshwater  
588 environments provide new clues about the origins of multicellular plants and algae. *Journal of*  
589 *Phycology* **53**: 731–745.

590 **Dittami SM, Michel G, Collén J, Boyen C, Tonon T. 2010.** Chlorophyll-binding proteins  
591 revisited--a multigenic family of light-harvesting and stress proteins from a brown algal perspective.  
592 *BMC Evolutionary Biology* **10**: 365.

593 **Dittami SM, Proux C, Rousvoal S, et al. 2011.** Microarray estimation of genomic inter-strain  
594 variability in the genus *Ectocarpus* (Phaeophyceae). *BMC Molecular Biology* **12**: 2.

595 **Dittami SM, Scornet D, Petit J-L, et al. 2009.** Global expression analysis of the brown alga  
596 *Ectocarpus siliculosus* (Phaeophyceae) reveals large-scale reprogramming of the transcriptome in  
597 response to abiotic stress. *Genome biology* **10**: R66.

598 **Dittami SM, Tonon T. 2012.** Genomes of extremophile crucifers: new platforms for comparative  
599 genomics and beyond. *Genome biology* **13**: 166.

600 **Dong H-P, Dong Y-L, Cui L, et al. 2016.** High light stress triggers distinct proteomic responses in  
601 the marine diatom *Thalassiosira pseudonana*. *BMC Genomics* **17**: 994.

602 **Dunn N, Diesh C, Deepak, et al. 2017.** GMOD/Apollo: Apollo2.0.8(JB#d3827c). *Zenodo*.

603 **Ellinghaus D, Kurtz S, Willhoeft U. 2008.** LTRharvest, an efficient and flexible software for de  
604 novo detection of LTR retrotransposons. *BMC Bioinformatics* **9**: 18.

605 **Emms DM, Kelly S. 2015.** OrthoFinder: solving fundamental biases in whole genome comparisons

606 dramatically improves orthogroup inference accuracy. *Genome Biology* **16**: 157.

607 **Eren AM, Esen ÖC, Quince C, et al. 2015.** Anvi'o: an advanced analysis and visualization  
608 platform for 'omics data. *PeerJ* **3**: e1319.

609 **Flutre T, Duprat E, Feuillet C, Quesneville H. 2011.** Considering transposable element  
610 diversification in de novo annotation approaches (Y Xu, Ed.). *PLoS ONE* **6**: e16526.

611 **Foissac S, Gouzy J, Rombauts S, et al. 2008.** Genome annotation in plants and fungi: EuGene as a  
612 model platform. *Current Bioinformatics* **3**: 11.

613 **Food and Agriculture Organization of the United Nations F. 2016.** *Global production statistics*  
614 *1950-2014*. <http://www.fao.org/fishery/statistics/global-production/en>. 16 Sep. 2016.

615 **Goff SA, Vaughn M, McKay S, et al. 2011.** The iPlant collaborative: cyberinfrastructure for plant  
616 biology. *Frontiers in Plant Science* **2**: 34.

617 **Götz S, García-Gómez JM, Terol J, et al. 2008.** High-throughput functional annotation and data  
618 mining with the Blast2GO suite. *Nucleic Acids Research* **36**: 3420–35.

619 **Gruber A, Rocap G, Kroth PG, Armbrust EV, Mock T. 2015.** Plastid proteome prediction for  
620 diatoms and other algae with secondary plastids of the red lineage. *Plant Journal* **81**: 519–28.

621 **Gualtieri T, Ragni E, Mizzi L, Fascio U, Popolo L. 2004.** The cell wall sensor Wsc1p is involved  
622 in reorganization of actin cytoskeleton in response to hypo-osmotic shock in *Saccharomyces*  
623 *cerevisiae*. *Yeast* **21**: 1107–1120.

624 **Guindon S, Gascuel O. 2003.** A simple, fast, and accurate algorithm to estimate large phylogenies  
625 by maximum likelihood. *Systematic biology* **52**: 696–704.

626 **Harvey WH. 1848.** *Phycologia britannica, or, a history of British sea-weeds: containing coloured*  
627 *figures, generic and specific characters, synonymes, and descriptions of all the species of algae*  
628 *inhabiting the shores of the British Islands*. London: Reeve & Benham.

629 **Heesch S, Cho GY, Peters AF, et al. 2010.** A sequence-tagged genetic map for the brown alga  
630 *Ectocarpus siliculosus* provides large-scale assembly of the genome sequence. *New phytologist* **188**:  
631 42–51.

632 **Hu TT, Pattyn P, Bakker EG, et al. 2011.** The *Arabidopsis lyrata* genome sequence and the basis  
633 of rapid genome size change. *Nature genetics* **43**: 476–81.

634 **Karp PD, Latendresse M, Paley SM, et al. 2016.** Pathway Tools version 19.0 update: software for  
635 pathway/genome informatics and systems biology. *Briefings in Bioinformatics* **17**: 877–890.

636 **Katoh K, Misawa K, Kuma K, Miyata T. 2002.** MAFFT: a novel method for rapid multiple  
637 sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**: 3059–66.

638 **Khalturin K, Hemmrich G, Fraune S, Augustin R, Bosch TCG. 2009.** More than just orphans:  
639 are taxonomically-restricted genes important in evolution? *Trends in Genetics* **25**: 404–413.

640 **Kloareg B, Quatrano RS. 1988.** Structure of the cell-walls of marine-algae and ecophysiological  
641 functions of the matrix polysaccharides. *Oceanogr Mar Biol* **26**: 259–315.

642 **Kumar S, Stecher G, Tamura K. 2016.** MEGA7: Molecular Evolutionary Genetics Analysis  
643 Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* **33**: 1870–1874.

644 **Kützing FT. 1843.** *Phycologia generalis oder Anatomie, Physiologie und Systemkunde der Tange.*  
645 Leipzig: F.A. Brockhaus.

646 **Lagesen K, Hallin P, Rødland EA, Staerfeldt H-H, Rognes T, Ussery DW. 2007.** RNAmmer:  
647 consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Research* **35**: 3100–8.

648 **Laslett D, Canback B. 2004.** ARAGORN, a program to detect tRNA genes and tmRNA genes in  
649 nucleotide sequences. *Nucleic Acids Research* **32**: 11–6.

650 **Lee E, Helt GA, Reese JT, et al. 2013.** Web Apollo: a web-based genomic annotation editing  
651 platform. *Genome biology* **14**: R93.

652 **Lipinska AP, Van Damme EJM, De Clerck O. 2016.** Molecular evolution of candidate male  
653 reproductive genes in the brown algal model *Ectocarpus*. *BMC evolutionary biology* **16**: 5.

654 **Lipinska AP, Serrano-Serrano ML, Cormier A, et al. 2019.** Rapid turnover of life-cycle-related  
655 genes in the brown algae. *Genome Biology* **20**: 35.

656 **Liu M-J, Zhao J, Cai Q-L, et al. 2014.** The complex jujube genome provides insights into fruit tree  
657 biology. *Nature Communications* **5**: 5315.

658 **Loira N, Zhukova A, Sherman DJ. 2015.** Pantograph: A template-based method for genome-scale  
659 metabolic model reconstruction. *Journal of Bioinformatics and Computational Biology* **13**: 1550006.

660 **Luo R, Liu B, Xie Y, et al. 2012.** SOAPdenovo2: an empirically improved memory-efficient short-  
661 read de novo assembler. *GigaScience* **1**: 18.

662 **Ma T, Wang Junyi, Zhou G, et al. 2013.** Genomic insights into salt adaptation in a desert poplar.  
663 *Nature communications* **4**: 2797.

664 **McHugh DJ. 2003.** A guide to the seaweed industry. *FAO Fisheries Technical Paper (FAO, Rome,*  
665 *Italy).*

666 **Meyer F, Paarmann D, D'Souza M, et al. 2008.** The metagenomics RAST server - a public  
667 resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC*  
668 *bioinformatics* **9**: 386.

669 **Montecinos AE, Couceiro L, Peters AF, Desrut A, Valero M, Guillemin M-L. 2017.** Species  
670 delimitation and phylogeographic analyses in the *Ectocarpus* subgroup *siliculosi* (Ectocarpales,  
671 Phaeophyceae) (M Cock, Ed.). *Journal of Phycology* **53**: 17–31.

672 **Mosavi LK, Cammett TJ, Desrosiers DC, Peng Z. 2004.** The ankyrin repeat as molecular  
673 architecture for protein recognition. *Protein Science* **13**: 1435–1448.

674 **Müller DG, Kapp M, Knippers R. 1998.** Viruses in marine brown algae In: Academic Press, 49–  
675 67.

676 **Nguyen VH, Lavenier D. 2009.** PLAST: parallel local alignment search tool for database  
677 comparison. *BMC Bioinformatics* **10**: 329.

678 **Nielsen H. 2017.** Predicting Secretory Proteins with SignalP In: Daisuke Kihara, ed. *Protein*  
679 *Function Prediction*. Berlin, Heidelberg: Springer, 59–73.

680 **Nishitsuji K, Arimoto A, Higa Y, et al. 2019.** Draft genome of the brown alga, *Nemacystus*  
681 *decipiens*, Onna-1 strain: Fusion of genes involved in the sulfated fucan biosynthesis pathway.  
682 *Scientific Reports* **9**: 4607.

683 **Nishitsuji K, Arimoto A, Iwai K, et al. 2016.** A draft genome of the brown alga, *Cladosiphon*

684 *okamuranus*, S-strain: a platform for future studies of ‘mozuku’ biology. *DNA Research*: dsw039.

685 **Oh D-H, Dassanayake M, Bohnert HJ, Cheeseman JM. 2012.** Life at the extreme: lessons from  
686 the genome. *Genome biology* **13**: 241.

687 **de Oliveira Dal’Molin CG, Quek L-E, Palfreyman RW, Brumbley SM, Nielsen LK. 2010.**  
688 AraGEM, a genome-scale reconstruction of the primary metabolic network in *Arabidopsis*. *Plant*  
689 *physiology* **152**: 579–89.

690 **Peters AF, Coucerio L, Tsiamis K, Küpper FC, Valero M. 2015.** Barcoding of cryptic stages of  
691 marine brown algae isolated from incubated substratum reveals high diversity. *Cryptogamie,*  
692 *Algologie* **36**: 3–29.

693 **Peters AF, Marie D, Scornet D, Kloareg B, Cock JM. 2004.** Proposal of *Ectocarpus siliculosus*  
694 (Ectocarpales, Phaeophyceae) as a model organism for brown algal genetics and genomics. *Journal*  
695 *of Phycology* **40**: 1079–1088.

696 **Popper ZA, Michel G, Hervé C, et al. 2011.** Evolution and diversity of plant cell walls: from algae  
697 to flowering plants. *Annual review of plant biology* **62**: 567–90.

698 **Prigent S, Collet G, Dittami SM, et al. 2014.** The genome-scale metabolic network of *Ectocarpus*  
699 *siliculosus* (EctoGEM): a resource to study brown algal physiology and beyond. *Plant Journal* **80**:  
700 367–381.

701 **Prigent S, Frioux C, Dittami SM, et al. 2017.** Meneco, a Topology-Based Gap-Filling Tool  
702 Applicable to Degraded Genome-Wide Metabolic Networks (C Kaleta, Ed.). *PLOS Computational*  
703 *Biology* **13**: e1005276.

704 **Rao AQ, ud Din S, Akhtar S, et al. 2016.** Genomics of salinity tolerance in plants In:  
705 Abdurakhmonov IY, ed. *Plant Genomics*. InTech, 273–299.

706 **Ritter A, Dittami SM, Goulitquer S, et al. 2014.** Transcriptomic and metabolomic analysis of  
707 copper stress acclimation in *Ectocarpus siliculosus* highlights signaling and tolerance mechanisms in  
708 brown algae. *BMC Plant Biology* **14**: 116.

709 **Rival A, Ilbert P, Labeyrie A, et al. 2013.** Variations in genomic DNA methylation during the

710 long-term in vitro proliferation of oil palm embryogenic suspension cultures. *Plant Cell Reports* **32**:  
711 359–368.

712 **Schattner P, Brooks AN, Lowe TM. 2005.** The tRNAscan-SE, snoscan and snoGPS web servers  
713 for the detection of tRNAs and snoRNAs. *Nucleic Acids Research* **33**: W686-9.

714 **Simão FA, Waterhouse RM, Ioannidis P, Kriventseva E V., Zdobnov EM. 2015.** BUSCO:  
715 assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*  
716 **31**: 3210–3212.

717 **Skinner ME, Uzilov A V, Stein LD, Mungall CJ, Holmes IH. 2009.** JBrowse: a next-generation  
718 genome browser. *Genome research* **19**: 1630–8.

719 **Stache-Crain B, Müller DG, Goff LJ. 1997.** Molecular systematics of *Ectocarpus* and *Kuckuckia*  
720 (Ectocarpales, Phaeophyceae) inferred from phylogenetic analysis of nuclear- and plastid-encoded  
721 DNA sequences. *Journal of Phycology* **33**: 152–168.

722 **Starr RC, Zeikus JA. 1993.** UTEX - the culture collection of algae at the University of Texas at  
723 Austin: 1993 list of cultures. *Journal of Phycology* **29**: 1–106.

724 **Steneck RS, Graham MH, Bourque BJ, et al. 2002.** Kelp forest ecosystems: Biodiversity,  
725 stability, resilience and future. *Environmental Conservation* **29**: 436–459.

726 **Suyama M, Torrents D, Bork P. 2006.** PAL2NAL: robust conversion of protein sequence  
727 alignments into the corresponding codon alignments. *Nucleic acids research* **34**: W609-12.

728 **Talavera G, Castresana J. 2007.** Improvement of phylogenies after removing divergent and  
729 ambiguously aligned blocks from protein sequence alignments. *Systematic biology* **56**: 564–77.

730 **Tan S, Cardoso-Moreira M, Shi W, et al. 2016.** LTR-mediated retroposition as a mechanism of  
731 RNA-based duplication in metazoans. *Genome research* **26**: 1663–1675.

732 **Tang H, Zhang X, Miao C, et al. 2015.** ALLMAPS: robust scaffold ordering based on multiple  
733 maps. *Genome Biology* **16**: 3.

734 **Tautz D, Domazet-Lošo T. 2011.** The evolutionary origin of orphan genes. *Nature Reviews*  
735 *Genetics* **12**: 692–702.



736 **Di Tommaso P, Moretti S, Xenarios I, et al. 2011.** T-Coffee: a web server for the multiple  
737 sequence alignment of protein and RNA sequences using structural information and homology  
738 extension. *Nucleic acids research* **39**: W13-7.

739 **Torode TA, Marcus SE, Jam M, et al. 2015.** Monoclonal antibodies directed to fucoidan  
740 preparations from brown algae. *PLoS One* **10**: e0118366.

741 **Waterhouse AM, Procter JB, Martin DMA, Clamp M, Barton GJ. 2009.** Jalview Version 2--a  
742 multiple sequence alignment editor and analysis workbench. *Bioinformatics (Oxford, England)* **25**:  
743 1189–91.

744 **West J, Kraft G. 1996.** *Ectocarpus siliculosus* (Dillwyn) Lyngb. from Hopkins River Falls, Victoria  
745 - the first record of a freshwater brown alga in Australia. *Muelleria* **9**: 29–33.

746 **Wu H-J, Zhang Z, Wang J-Y, et al. 2012.** Insights into salt tolerance from the genome of  
747 *Thellungiella salsuginea*. *Proceedings of the National Academy of Sciences of the United States of*  
748 *America* **109**: 12219–24.

749 **Yang Z. 2007.** PAML 4: phylogenetic analysis by maximum likelihood. *Molecular biology and*  
750 *evolution* **24**: 1586–91.

751 **Ye N, Zhang X, Miao M, et al. 2015.** *Saccharina* genomes provide novel insight into kelp biology.  
752 *Nature communications* **6**: 6986.

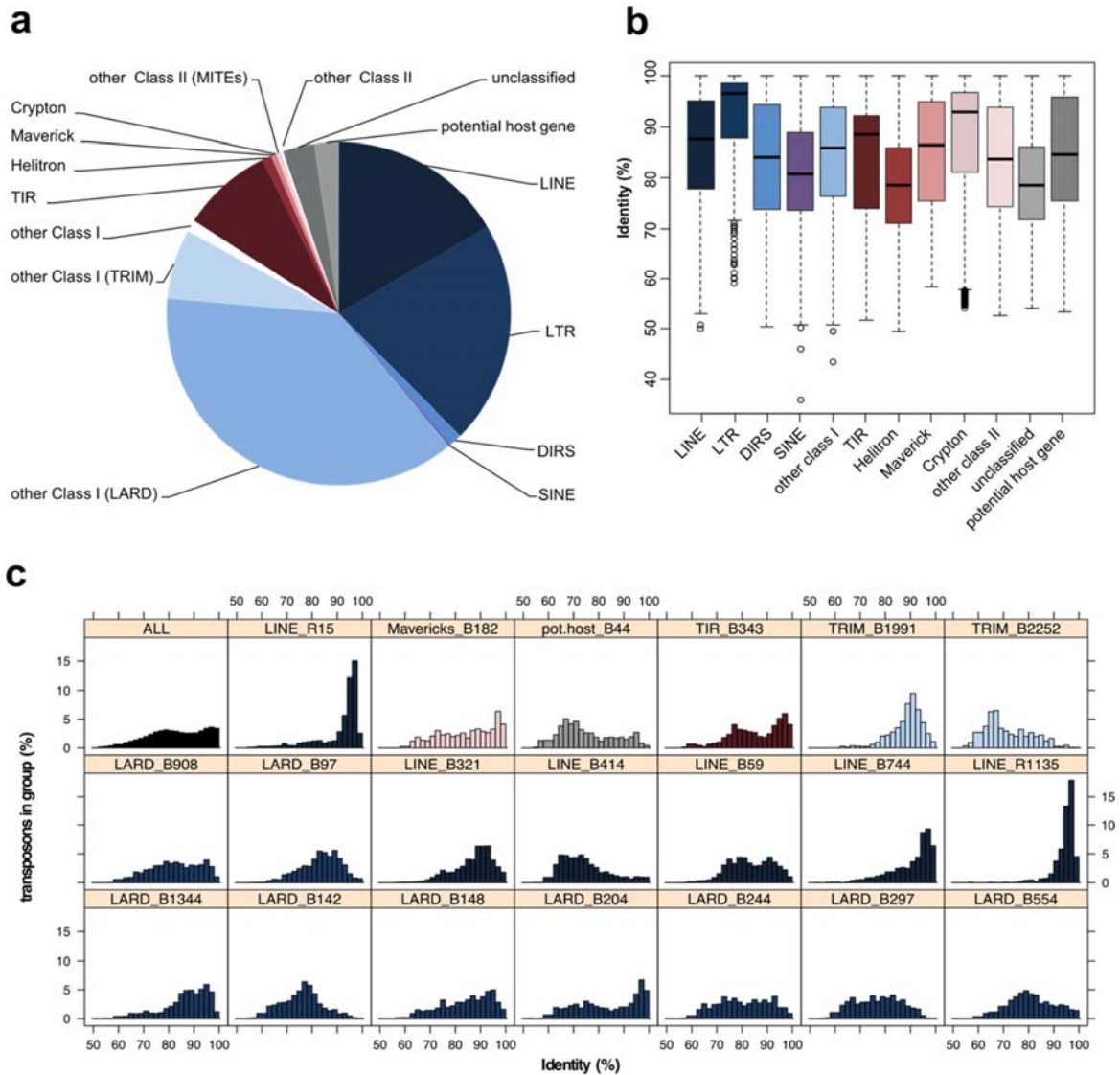
753 **Yoon HS, Hackett JD, Ciniglia C, Pinto G, Bhattacharya D. 2004.** A molecular timeline for the  
754 origin of photosynthetic eukaryotes. *Molecular biology and evolution* **21**: 809–18.

755 **Zeng X, Long H, Wang Z, et al. 2015.** The draft genome of Tibetan hulless barley reveals adaptive  
756 patterns to the high stressful Tibetan Plateau. *Proceedings of the National Academy of Sciences of*  
757 *the United States of America* **112**: 1095–100.

758 **Zhu S-H, Green BR. 2010.** Photoprotection in the diatom *Thalassiosira pseudonana*: Role of  
759 LI818-like proteins in response to high light stress. *Biochimica et Biophysica Acta (BBA) -*  
760 *Bioenergetics* **1797**: 1449–1457.

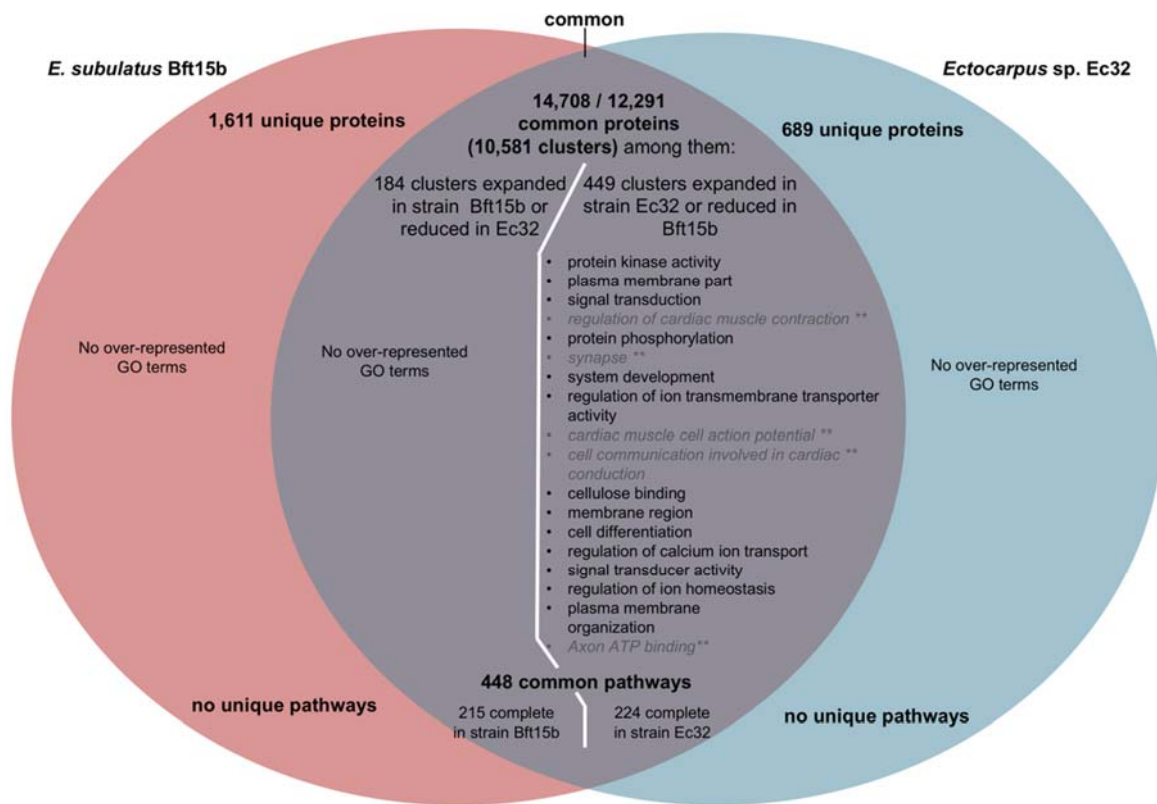
761

762 **Figures**



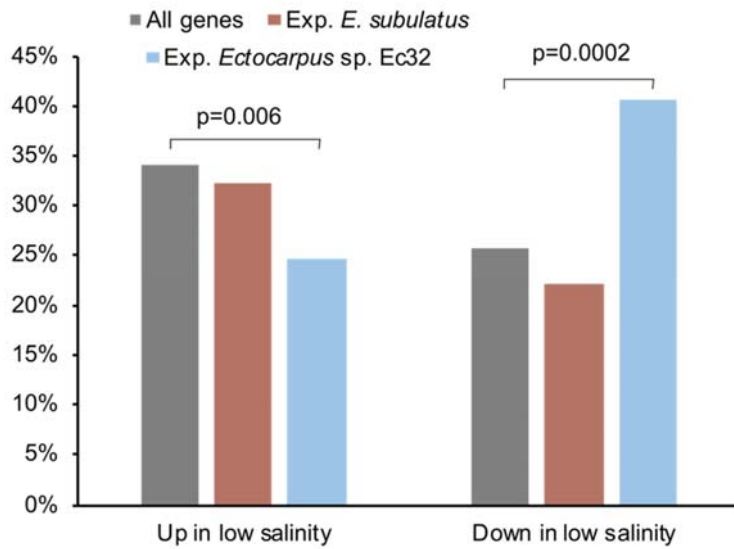
763

764 **Figure 1:** Repeated elements identified within the genome of *E. subulatus* Bft15b. A) Number of  
 765 transposons detected in the different superfamilies; B) Boxplot of sequence identity levels for the  
 766 detected superfamilies; and C) Distribution of sequence identities in all and the 20 most abundant  
 767 transposon families.



769

770 **Figure 2:** Comparison of gene content and metabolic capacities of *E. subulatus* Bft15b and *Ectocarpus*  
 771 sp. Ec32. The top part of the Venn diagram displays the number of predicted proteins and protein  
 772 clusters unique and common to both genomes in the OrthoFinder analysis. The middle part shows GO  
 773 annotations significantly enriched ( $FDR \leq 0.05$ ) among these proteins. For the common clusters, the  
 774 diagram also contains the results of gene set enrichment analyses for annotations found among clusters  
 775 expanded in *E. subulatus* Bft15b and those expanded in *Ectocarpus* sp. Ec32. Functional annotations  
 776 not directly relevant to the functioning of *Ectocarpus* or shown to be false positives are shown in grey  
 777 and italics. The bottom part shows the comparison of both genomes in terms of their metabolic  
 778 pathways.



779

780 **Figure 3:** Percentage of significantly (FDR<0.05) up- and down-regulated genes in *E. subulatus* in  
 781 response to low salinity (5% seawater). Grey bars are values obtained for all genes with expression  
 782 data (n=6,492), while brown and blue bars include only genes belonging to gene families expanded in  
 783 *E. subulatus* Bft15b (n=99) or *Ectocarpus* sp. Ec32 (n=202), respectively (“Exp.” stands for  
 784 expanded). P-values correspond to the result of a Fisher exact test. Gene expression data were obtained  
 785 from previous microarray experiments(Dittami *et al.* 2012). Please refer to Supporting information  
 786 Table S6 for additional data.



796 names indicate incomplete proteins. Probable expansions in *E. subulatus* Bft15b are indicated by an  
 797 ocher background.

## 798 Tables

799 **Table 1:** Assembly statistics of available brown algal genomes. PE = paired-end, MP = mate-pair, n.d.

800 = not determined

|   | <i>E. subulatus</i> Bft15b | <i>Ectocarpus</i> sp. Ec32 (Cock <i>et al.</i> 2010) | <i>S. japonica</i> (Ye <i>et al.</i> 2015) | <i>C. okamuranus</i> (Nishitsuji <i>et al.</i> 2016) | <i>N. decipiens</i> (Nishitsuji <i>et al.</i> 2019) |
|---|----------------------------|--|--|--|---|
| Sequencing strategy                             | Illumina (PE+MP)           | Sanger+Bac libraries                                 | Illumina PE+PacBio                         | Illumina (PE+MP)                                     | Illumina (PE+MP)                                    |
| Genome size estimate (flow cytometry)           | 226                        | 214(Peters <i>et al.</i> 2004)*                      | 545  | 140  | n. d.   |
| Genome size (assembled)                         | 242 Mb                     | 196 Mb   | 537 Mb                                     | 130 Mb   | 154 Mb  |
| Genomic Coverage                                | 119 X                      | 11 X <sup>#</sup>                                    | 178 X                                      | 100 X  | 420 X   |
| G/C contents                                    | 54%                        | 53%  | 50%  | 54%  | 56%   |
| Number of scaffolds >2kb                        | 1,757                      | 1,561  | 6,985                                      | 541  | 685   |
| Scaffold N50 (kb)                               | 510 kb                     | 497 kb   | 254 kb                                     | 416 kb   | 1,863 kb  |
| Number of predicted genes                       | 25,893                     | 17,418   | 18,733                                     | 13,640   | 15,156  |
| Mean number of exons per gene                   | 5.4                        | 8.0  | 6.5  | 9.3  | 11.2  |
| Repetitive elements                             | 30%                        | 30% <sup>##</sup>                                    | 40%  | 4.1%   | 8.8%  |
| BUSCO genome completeness (complete+fragmented) | 86% (91% <sup>*#</sup> )   | 94% (99% <sup>*#</sup> )                             | 91% (96% <sup>*#</sup> )                   | 88% (93% <sup>*#</sup> )                             | 92% (97% <sup>*#</sup> )                            |
| BUSCO Fragmented proteins                       | 13.5%                      | 7.4%   | 14.2%                                      | 11.9%  | 5.6%  |

801 <sup>##</sup> 23% according to (Cock *et al.* 2010), but 30% when re-run with the current version (2.5) of the

802 REPET pipeline.

803 <sup>\*#</sup> not considering proteins absent from all three brown algal genomes.

804

805 **Table 2:** Clusters of orthologous genes identified by OrthoFinder as expanded in the genome of *E.*  
806 *subulatus* Bft15b or reduced in *Ectocarpus* sp. Ec32, after manual identification of false positives, and  
807 removal of clusters without functional annotation or related to transposon or viral sequences.

| Cluster(s)  | # Ec32 | # Bft15b | Putative annotation or functional domain         |
|---|--------|----------|--|
| <i>Cell-wall related proteins</i>                 |        |          |  |
| OG0000597   | 1      | 3        | Peptidoglycan-binding domain                     |
| OG0000284, -782, -118                             | 6      | 12       | Carbohydrate-binding WSC domain                  |
| OG0000889   | 1      | 2        | Cysteine desulfuration protein                   |
| OG0000431   | 1      | 3        | Galactose-3-O-sulfotransferase (partial)         |
| <i>Transcriptional regulation and translation</i> |        |          |  |
| OG0000785   | 1      | 2        | AN1-type zinc finger protein                     |
| OG0000059   | 4      | 10       | C2H2 zinc finger protein                         |
| OG0000884   | 1      | 2        | Zinc finger domain                               |
| OG0000766   | 1      | 2        | DNA-binding SAP domain                           |
| OG0000853   | 1      | 2        | RNA binding motif protein                        |
| OG0000171   | 1      | 6        | Helicase   |
| OG0000819   | 1      | 2        | Fungal transcriptional regulatory protein domain |
| OG0000723   | 1      | 2        | Translation initiation factor eIF2B              |
| OG0000364   | 2      | 3        | Ribosomal protein S15                            |
| OG0000834   | 1      | 2        | Ribosomal protein S13                            |
| <i>Cell-cell communication and signaling</i>      |        |          |  |
| OG0000967   | 1      | 2        | Ankyrin repeat-containing domain                 |
| OG0000357   | 2      | 3        | Regulator of G protein signaling domain          |
| OG0000335   | 2      | 3        | Serine/threonine kinase domain                   |
| OG0000291   | 2      | 3        | Protein kinase                                   |
| OG0000185   | 3      | 4        | Octicosapeptide/Phox/Bem1p domain                |
| <i>Others</i>                                     |        |          |  |
| OG0000726   | 1      | 3        | HSP20  |
| OG0000104   | 1      | 9        | Light harvesting complex protein                 |
| OG0000277   | 3      | 3        | Major facilitator superfamily transporter        |
| OG0000210   | 2      | 4        | Cyclin-like domain                               |
| OG0000721   | 1      | 2        | Myo-inositol 2-dehydrogenase                     |
| OG0000703   | 1      | 2        | Short-chain dehydrogenase                        |
| OG0000749   | 1      | 2        | Putative Immunophilin                            |
| OG0000463   | 1      | 3        | Zinc-dependent metalloprotease with notch domain |

808