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

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32

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 **Organelar 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 20th 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 al.*
395 *et 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.sbroscoff.fr/blast/subulatus/download.html)
426 [roscoff.fr/blast/subulatus/download.html](http://application.sbroscoff.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 ω . 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.

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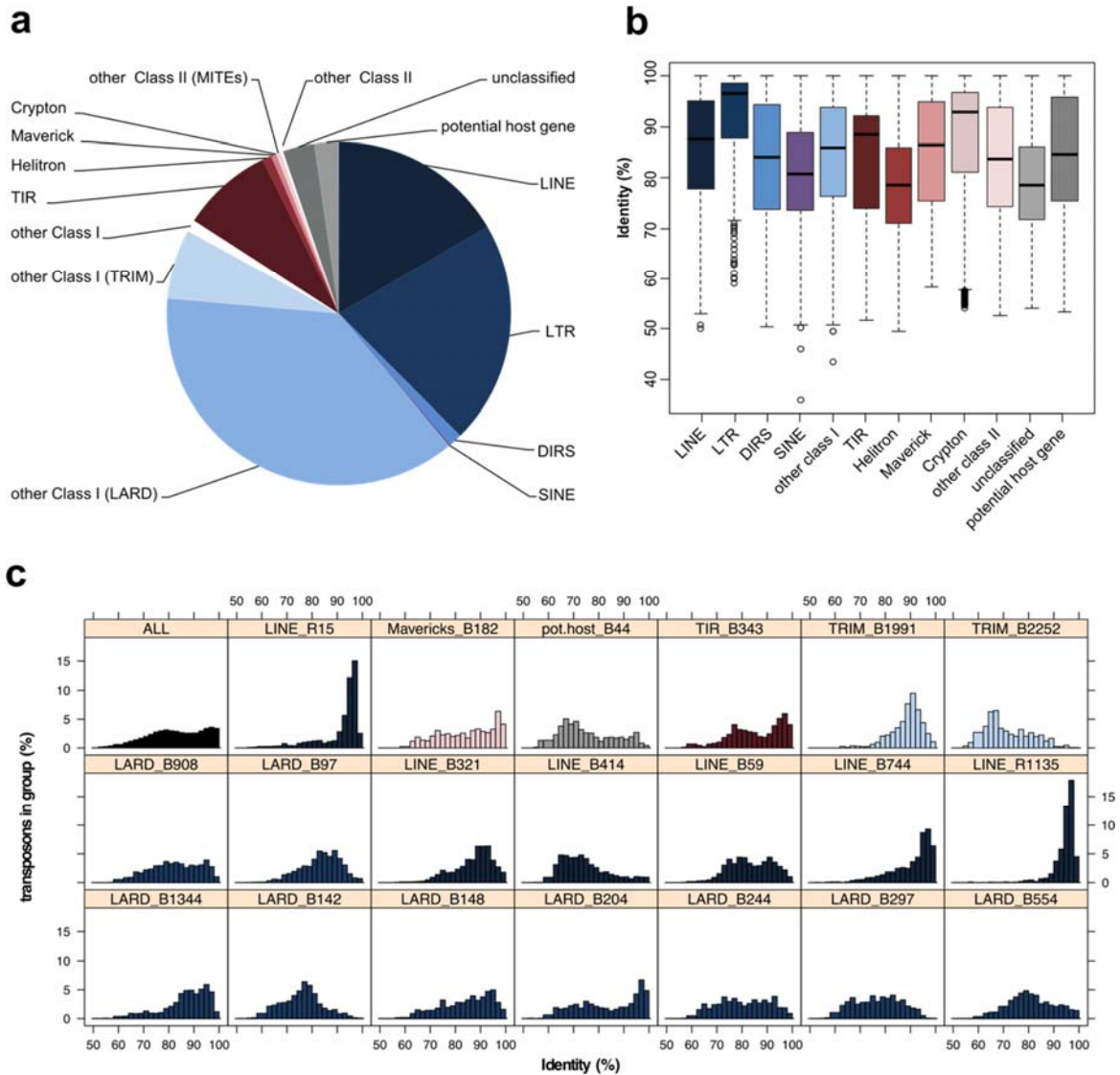
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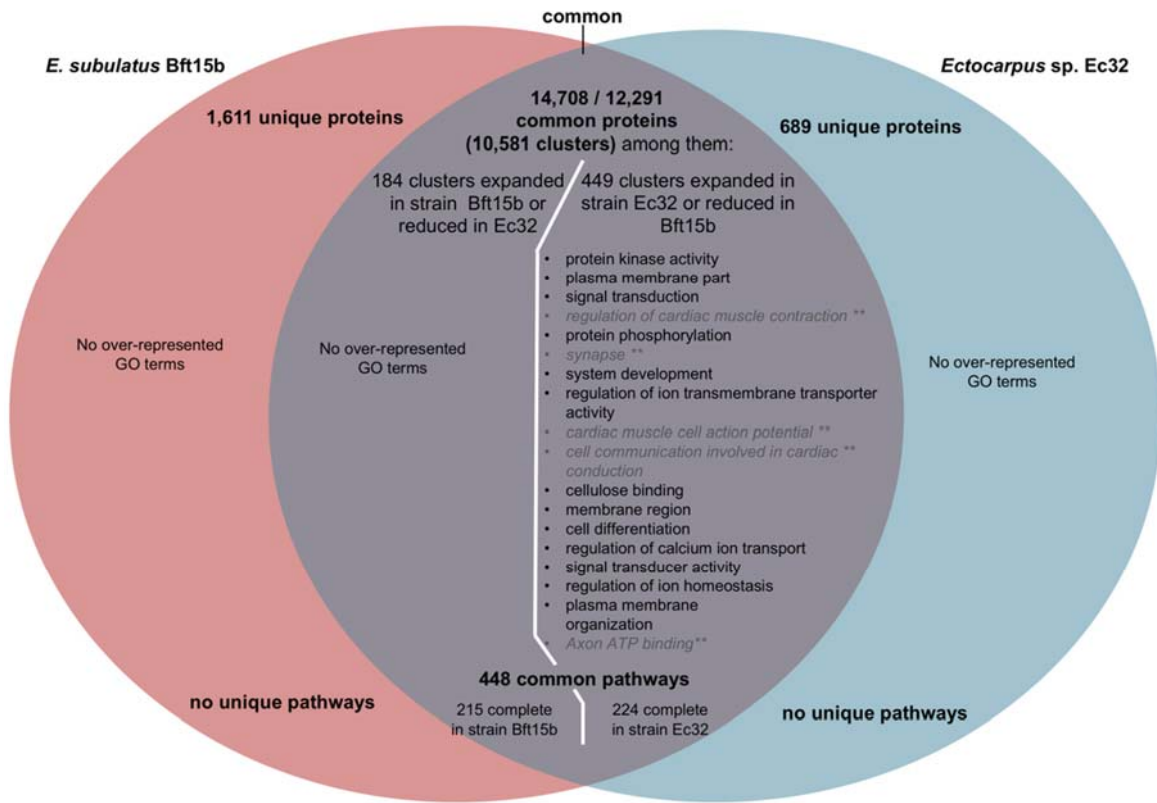
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762 **Figures**



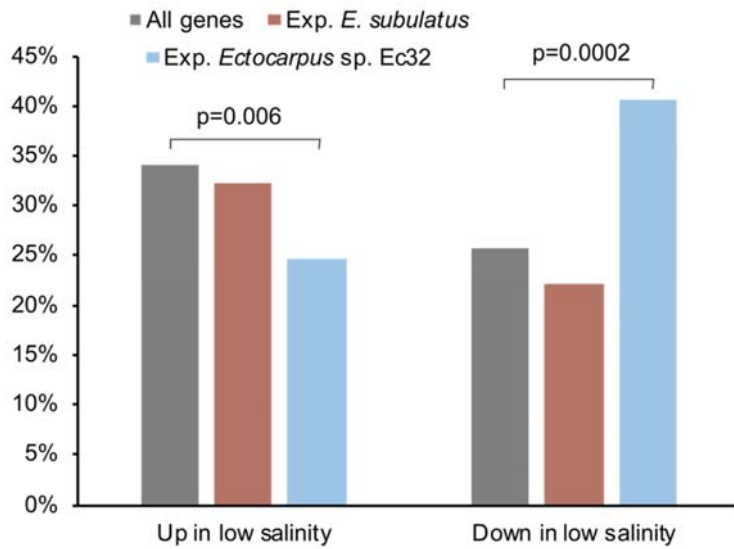
763

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.



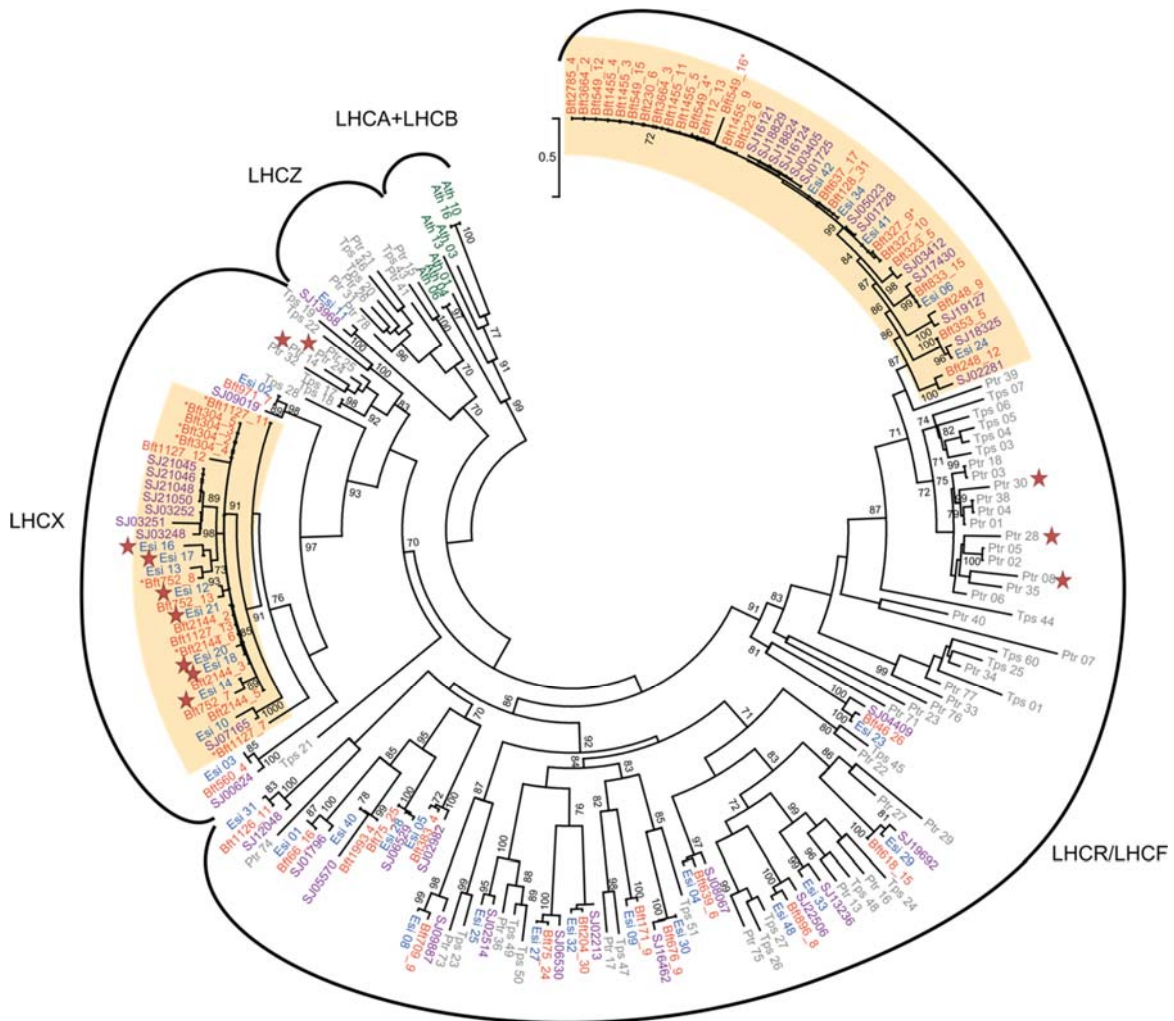
769

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.



789 **Figure 4:** Maximum likelihood tree of chlorophyll binding proteins (CBPs) sequences in *E. subulatus*
 790 Bft15b (orange) *Ectocarpus* sp. Ec32 (blue), *S. japonica* (purple), and diatoms (*Thalassiosira*
 791 *pseudonana* and *Phaeodactylum tricornutum*, grey). Support values correspond to the percentage of
 792 bootstrap support from 1000 replicate runs, only values $\geq 70\%$ are shown. *A. thaliana* sequences
 793 (green) were added as outgroup. Accessions for *E. subulatus* Bft15b are given without the Esu prefix;
 794 for *Ectocarpus* sp. Ec32, diatoms and *A. thaliana*, see (Dittami *et al.* 2010). Stars indicate genes that
 795 have been previously shown to be stress-induced (Dittami *et al.* 2010), asterisks next to the protein

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 [#]	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% ^{##}	40%	4.1%	8.8%
BUSCO genome completeness (complete+fragmented)	86% (91% ^{*#})	94% (99% ^{*#})	91% (96% ^{*#})	88% (93% ^{*#})	92% (97% ^{*#})
BUSCO Fragmented proteins	13.5%	7.4%	14.2%	11.9%	5.6%

801 ^{##} 23% according to (Cock *et al.* 2010), but 30% when re-run with the current version (2.5) of the

802 REPET pipeline.

803 ^{*#} 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