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

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# Abstract

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

# Introduction

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

To enable functional studies of brown algae, strain Ec32 of the small filamentous alga *Ectocarpus* sp. has been established as a genetic and genomic model (Peters *et al.* 2004; Heesch *et al.* 2010; Cock *et al.* 2010). This strain was formerly described as *Ectocarpus siliculosus*, but has since been shown to belong to an independent clade by molecular methods (Stache-Crain *et al.* 1997; Peters *et al.* 2015). More recently, three additional brown algal genomes, that of the kelp species *Saccharina japonica* (Ye *et al.* 2015), that of *Cladosiphon okamuranus* (Nishitsuji *et al.* 2016), and that of *Nemacystus decipiens* (Nishitsuji *et al.* 2019), have been characterized. Comparisons between these four genomes have allowed researchers to obtain a first overview of the unique genomic features of brown algae, as well as a glimpse of the genetic diversity within this group. However, given the evolutionary distance between these algae, it is difficult to link genomic differences to physiological differences and possible adaptations to their lifestyle. To be able to generate more accurate hypotheses on the role of particular genes and genomic features for adaptive traits, a common strategy is to compare closely related strains and species that differ only in a few genomic features. The genus *Ectocarpus* is particularly well suited for such comparative studies because it comprises a wide range of morphologically similar but genetically distinct strains and species that have adapted to different marine and brackish water environments (Kützing 1843; Harvey 1848; Stache-Crain *et al.* 1997; Montecinos *et al.* 2017). One species within this group, *Ectocarpus subulatus* Kützing (Peters *et al.* 2015), comprises isolates highly resistant to elevated temperature (Bolton 1983) and low salinity. A strain of this species was even isolated from freshwater (West and Kraft 1996), constituting one of the handful of known marine-freshwater transitions in brown algae (Dittami *et al.* 2017).

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

# Results

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

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

## Gene prediction and annotation

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

## Repeated elements

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

## Organellar genomes

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

## Global comparison of predicted proteomes

### Metabolic network-based comparisons

Similar to the network previously obtained for *Ectocarpus* sp. Ec32 (Prigent *et al.* 2014), the *E. subulatus* Bft15b metabolic network comprised 2,074 metabolic reactions and 2,173 metabolites in 464 pathways, which can be browsed at <http://gem-aureme.irisa.fr/sububftgem>. In total, 2,445 genes associated with at least one metabolic reaction, and 215 pathways were complete (Figure 2). Comparisons between both networks were carried out on a pathway level (Supporting Information Text S1, Section “Metabolic network-based comparisons”), but no pathways were found to be truly specific to either Ec32 and/or Bf15b.

### Genes under positive selection

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

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

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

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

The large majority of *Ectocarpus* sp. Ec32-specific proteins (511) also corresponded to proteins of unknown function without matches in public databases. Ninety-seven proteins were part of the *E. siliculosus* virus-1 (EsV-1) inserted into the Ec32 genome and the remaining 81 proteins were poorly annotated, usually only via the presence of a domain. Examples are ankyrin repeat-containing domain proteins (12), Zinc finger domain proteins (6), proteins containing wall sensing component (WSC) domains (3), protein kinase-like proteins (3), and Notch domain proteins (2).

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

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

### Correlation with gene expression patterns

To assess whether genomic adaptations in *E. subulatus* Bft15b were located preferentially in genes that are known to be responsive to salinity stress, we compared expanded gene families to previously available expression data obtained for a freshwater strain of *E. subulatus* grownin freshwater vs seawater (Dittami *et al.* 2012). This analysis revealed that genes that were down-regulated in response to low salinity were significantly over-represented among the gene families expanded in *Ectocarpus* sp. Ec32 or reduced in *E. subulatus* Bft15b, (42% of genes vs 26% for all genes; Fischer exact test p=0.0002), while genes that were upregulated in response to low salinity were significantly under-represented (25% vs 33%; Fischer exact test p=0.006; Figure 3, Supporting Information Table S6). This indicates that *E. subulatus* Bft15b has mainly lost members of gene families that were generally down-regulated in low salinities, and conserved those that were upregulated in this condition.

## Targeted manual annotation of specific pathways

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

Regarding gene families reduced in *E. subulatus* Bft15b or expanded in *Ectocarpus* sp. Ec32, the *E. subulatus* genome encoded only 320 WSC-domain containing proteins, vs. 444 in *Ectocarpus* sp.. Many of these genes were down-regulated in response to low salinity, (61% of the WSC domain containing genes with available expression data; Fischer exact test, p=0.0004) while only 7% were upregulated (Fischer exact test, p-value=0.0036). In yeast, WSC domain proteins may act as cell surface mechanosensors and activate the intracellular cell wall integrity signalling cascade in response to hypo-osmotic shock (Gualtieri *et al.* 2004). Whether or not they have similar functions in brown algae, however, remains to be established. Furthermore, we found fewer aryl sulfotransferase, tyrosinases, potential bromoperoxidases, and thyroid peroxidases in the *E. subulatus* genome compared to *Ectocarpus* sp., and it entirely lacks haloalkane dehalogenases (Supporting Information Text S1). All of these enzymes are involved in the production of polyphenols and halogenated defence compounds, suggesting that *E. subulatus* may be investing less energy in defence, although a potential bias induced by differences in the assembly completeness cannot be excluded here.

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

# Discussion

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

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

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

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

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

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

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

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

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

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

The vast majority of genomic differences between the two investigated species of *Ectocarpus,* however, corresponds to proteins of entirely unknown functions. All of the 11 gene pairs under positive selection were unknown genes taxonomically restricted to brown algae. Of the 1,611 *E. subulatus* Bft15b-specific genes, 88% were unknown. Most of these genes were expressed and are thus likely to correspond to true genes; their absence from the *Ectocarpus* sp. Ec32 genome was also confirmed at the nucleotide level. A large part of the mechanisms that underlie the adaptation to different ecological niches in *Ectocarpus* may, therefore, lie in these genes of unknown function. This can be partly explained by the fact that still only few brown algal genomes have been sequenced, and that currently most of our knowledge on the function of their proteins is based on studies in model plants, animals, yeast, or bacteria, which have evolved independently from stramenopiles for over 1 billion years (Yoon *et al.* 2004). They differ from land plants even in otherwise highly conserved aspects, for instance in their life cycles, cell walls, and primary metabolism (Charrier *et al.* 2008). Substantial contributions of lineage-specific genes to the evolution of organisms and the development of innovations have also been described for animal models (Tautz and Domazet-Lošo 2011), and studies in basal metazoans furthermore indicate that they are essential for species-specific adaptive processes (Khalturin *et al.* 2009).

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

## Conclusion and future work

We have shown that since the separation of *E. subulatus* and *Ectocarpus sp.* Ec32, both genomes have been shaped partially by the activity of viruses and transposons, particularly large retrotransposons. Over this period of time, *E. subulatus* has adapted to environments with high abiotic variability including brackish water and even freshwater. We have identified a few genes that likely contribute to this adaptation, including HSPs, CBPs, a reduction of genes involved in halogenated defence compounds, or some changes in cell wall polysaccharide-modifying enzymes. However, the majority of genes that differ between the two examined *Ectocarpus* species or that may be under positive selection encode proteins of unknown function. This underlines the fundamental differences that exist between brown algae and terrestrial plants or other lineages of algae. Studies as the present one, *i.e.* without strong *a priori* assumptions about the mechanisms involved in adaptation, are therefore essential to start elucidating the specificities of this lineage as well as the various functions of the unknown genes.

# Methods

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

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

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

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

Redundancy of mate-pairs (MPs) was reduced to mitigate the negative effect of redundant chimeric MPs during scaffolding. To this means, mate-pair reads were aligned with bwa-0.6.1 to a preliminary *E. subulatus* Bft15b draft assembly calculated from paired-end data only. Mate-pairs that did not map with both reads were removed, and for the remaining pairs, read-starts were obtained by parsing the cigar string using Samtools and a custom Pearl script. Mate-pairs with redundant mapping coordinates were removed for the final **assembly**, which was carried out using SOAPDenovo2 (Luo *et al.* 2012). Scaffolding was then carried out using SSPACE basic 2.0 (Boetzer *et al.* 2011) (trim length up to 5 bases, minimum 3 links to scaffold contigs, minimum 15 reads to call a base during an extension) followed by a run of GapCloser (part of the SOAPDenovo package, default settings). A dot plot of syntenic regions between *E. subulatus* Bft15b and *Ectocarpus* sp. Ec32 was generated using D-Genies 1.2.0 (Cabanettes and Klopp 2018). Given the high degree of synteny observed (Supporting Information Text S1), additional scaffolding was carried out using MeDuSa and the *Ectocarpus* sp. Ec32 genome as reference (Bosi *et al.* 2015). This super-scaffolding method assumes that both genome structures are be similar. Annotations were generated first for version 1 of the Bft15b genome and then transferred to the new scaffolds of version 2 using the ALLMAPS (Tang *et al.* 2015) liftover function. Both the assemblies with (V2) and without (V1) MeDuSa scaffolding have been made available. RNA-seq reads were cleaned using Trimmomatic (default settings), and a second Bft15b genome-guided assembly was performed with Tophat2 and with Cufflinks. Sequencing coverage was calculated based on mapped algal reads only, and for mate-pair libraries the genomic coverage was calculated as number of unique algal mate-pairs \* span size / assembly size.

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

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

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

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

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

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

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

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

The search for **genes under positive selection** was based ona previous analysis in other brown algae (Lipinska *et al.* 2019). Therefore, Orthofinder analyses were expanded to include also *Macrocystis pyrifera*, *Scytosiphon lomentaria* (Lipinska *et al.* 2019), and *Cladosiphon okamuranus* (Nishitsuji *et al.* 2016). Rates of non-synonymous to synonymous substitution (ω=dN/dS) were searched for in clusters of single-copy orthologs. Protein sequences were aligned with Tcoffee (Di Tommaso *et al.* 2011) (M-Coffee mode), translated back to nucleotide using Pal2Nal (Suyama *et al.* 2006), and curated with Gblocks (Talavera and Castresana 2007) (-t c -b4 20) or manually when necessary. Sequences that produced a gapless alignment that exceeded 100bp were retained for pairwise dN/dS analysis between *Ectocarpus* strains using CodeML (F3x4 model of codon frequencies, runmode = −2) of the PAML4 suite (Yang 2007). Orthogroups for which the pairwise dN/dS ratio between *Ectocarpus* species exceeded 0.5, which were not saturated (dS < 1), and which contained single-copy orthologs in at least two other species were used to perform positive selection analysis with CodeML (PAML4, F3x4 model of codon frequencies): branch-site models were used to estimate dN/dS values by site and among branches in the species tree generated for each orthogroup. The branch leading to the genus *Ectocarpus* was selected as a ‘foreground branch’, allowing different values of dN/dS among sites in contrast to the remaining branches that shared the same distribution of ω. Two alternative models were tested for the foreground branch: H1 allowing the dN/dS to exceed 1 for a proportion of sites (positive selection), and H0 constraining dN/dS<1 for all sites (neutral and purifying selection). A likelihood ratio test was then performed for the two models (LRT=2×(lnLH1-lnLH0)) and genes for which H1 fitted the data significantly better (p<0.05) were identified as evolving under positive selection.

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

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# Author contributions

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

# Additional Information

## Competing interests

The authors declare no competing interest.

## Data availablility

Sequence data (genomic and transcriptomic reads) were submitted to the European Nucleotide Archive (ENA) under project accession number PRJEB25230 using the EMBLmyGFF3 script (Dainat and Gourlé 2018). A JBrowse (Skinner *et al.* 2009) instance comprising the most recent annotations is available via the server of the Station Biologique de Roscoff (<http://mmo.sb-roscoff.fr/jbrowseEsu>). The reconstructed metabolic network of *E. subulatus* is available at <http://gem-aureme.irisa.fr/sububftgem>. Additional resources and annotations including a blast server are available at <http://application.sb-roscoff.fr/project/subulatus/index.html>. The complete set of manual annotations is provided in Supporting Information Table S7.

# References

**Aite M, Chevallier M, Frioux C, *et al.*** **2018**. Traceability, reproducibility and wiki-exploration for “à-la-carte” reconstructions of genome-scale metabolic models. *PLOS Computational Biology* **14**: e1006146.

**Amtmann A**. **2009**. Learning from evolution: *Thellungiella* generates new knowledge on essential and critical components of abiotic stress tolerance in plants. *Molecular plant* **2**: 3–12.

**Archibald JM**. **2009**. The puzzle of plastid evolution. *Current biology* **19**: R81-8.

**Avia K, Coelho SM, Montecinos GJ, *et al.*** **2017**. High-density genetic map and identification of QTLs for responses to temperature and salinity stresses in the model brown alga *Ectocarpus*. *Scientific reports* **7**: 43241.

**Le Bail A, Dittami SM, de Franco PO, *et al.*** **2008**. Normalisation genes for expression analyses in the brown alga model *Ectocarpus siliculosus*. *BMC Molecular Biology* **9**: 75.

**Bernt M, Donath A, Jühling F, *et al.*** **2013**. MITOS: improved de novo metazoan mitochondrial genome annotation. *Molecular Phylogenetics and Evolution* **69**: 313–9.

**de Boer JG, Yazawa R, Davidson WS, Koop BF**. **2007**. Bursts and horizontal evolution of DNA transposons in the speciation of pseudotetraploid salmonids. *BMC Genomics* **8**: 422.

**Boetzer M, Henkel C V, Jansen HJ, Butler D, Pirovano W**. **2011**. Scaffolding pre-assembled contigs using SSPACE. *Bioinformatics (Oxford, England)* **27**: 578–9.

**Bolton JJ**. **1983**. Ecoclinal variation in *Ectocarpus siliculosus* (Phaeophyceae) with respect to temperature growth optima and survival limits. *Marine Biology* **73**: 131–138.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

**Emms DM, Kelly S**. **2015**. OrthoFinder: solving fundamental biases in whole genome comparisons dramatically improves orthogroup inference accuracy. *Genome Biology* **16**: 157.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

**Nishitsuji K, Arimoto A, Iwai K, *et al.*** **2016**. A draft genome of the brown alga, *Cladosiphon okamuranus* , S-strain: a platform for future studies of ‘mozuku’ biology. *DNA Research*: dsw039.

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

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

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

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

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

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

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

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

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

**Rival A, Ilbert P, Labeyrie A, *et al.*** **2013**. Variations in genomic DNA methylation during the long-term in vitro proliferation of oil palm embryogenic suspension cultures. *Plant Cell Reports* **32**: 359–368.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

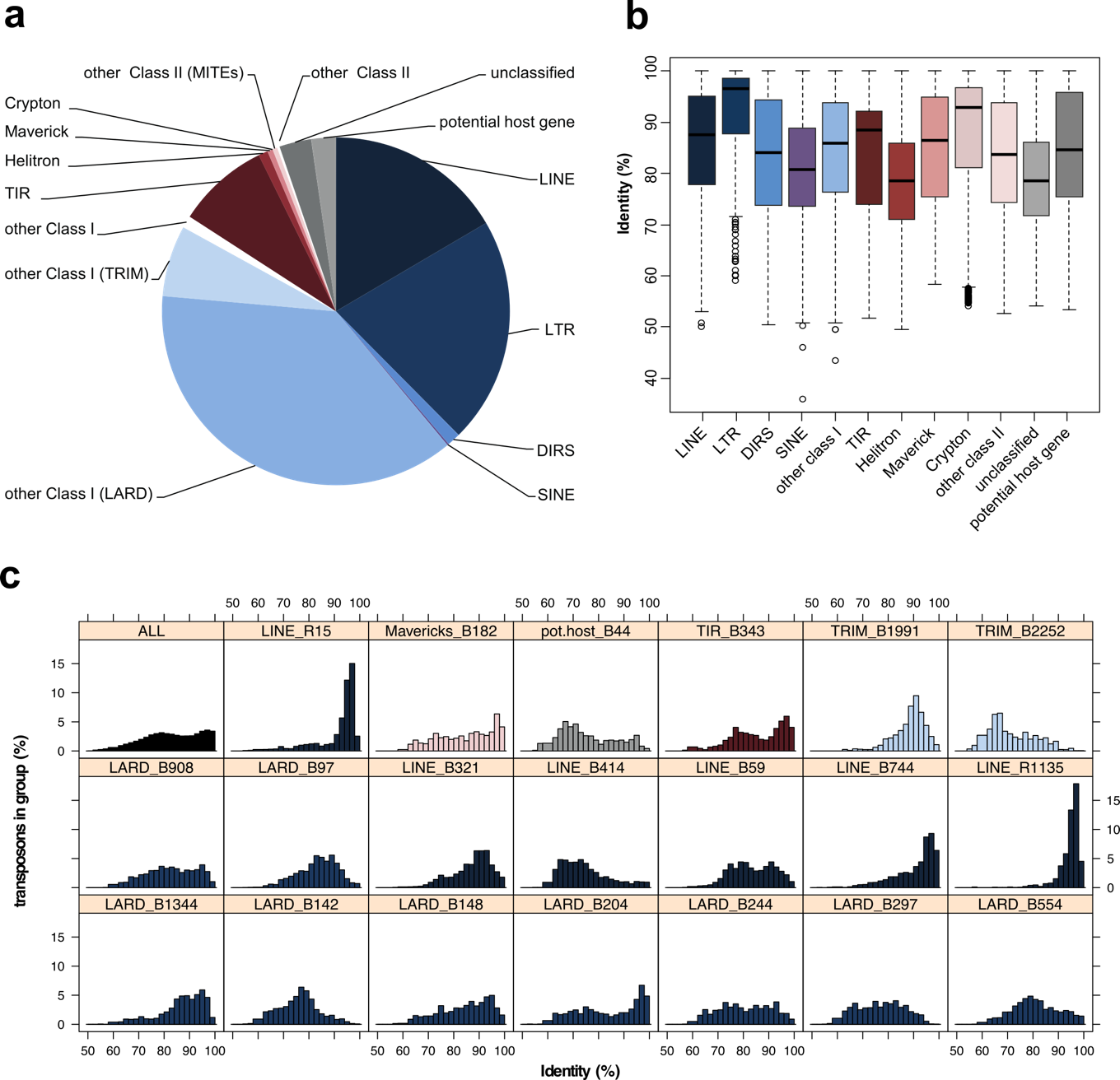
**Ye N, Zhang X, Miao M, *et al.*** **2015**. *Saccharina* genomes provide novel insight into kelp biology. *Nature communications* **6**: 6986.

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

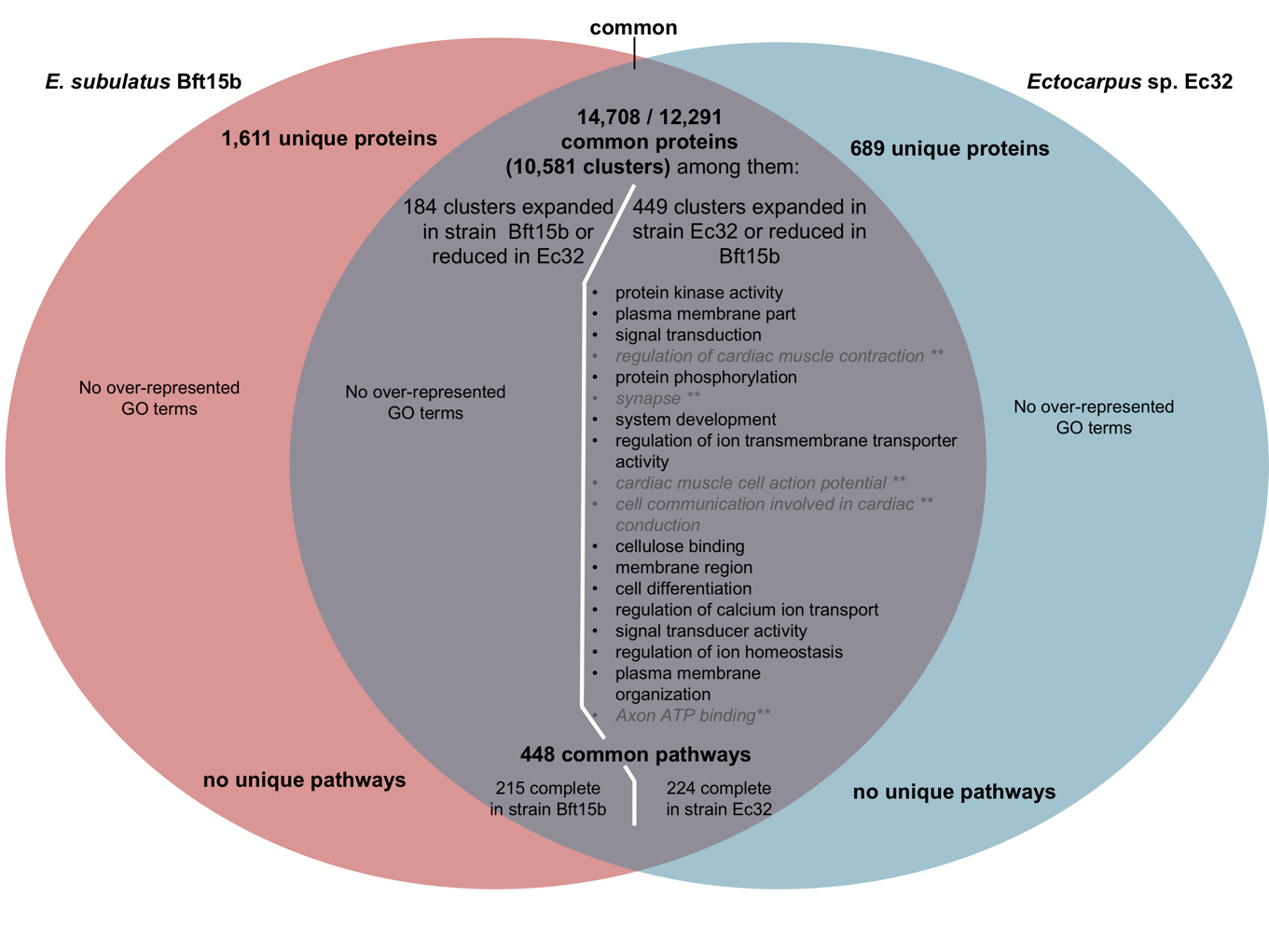
**Zeng X, Long H, Wang Z, *et al.*** **2015**. The draft genome of Tibetan hulless barley reveals adaptive patterns to the high stressful Tibetan Plateau. *Proceedings of the National Academy of Sciences of the United States of America* **112**: 1095–100.

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

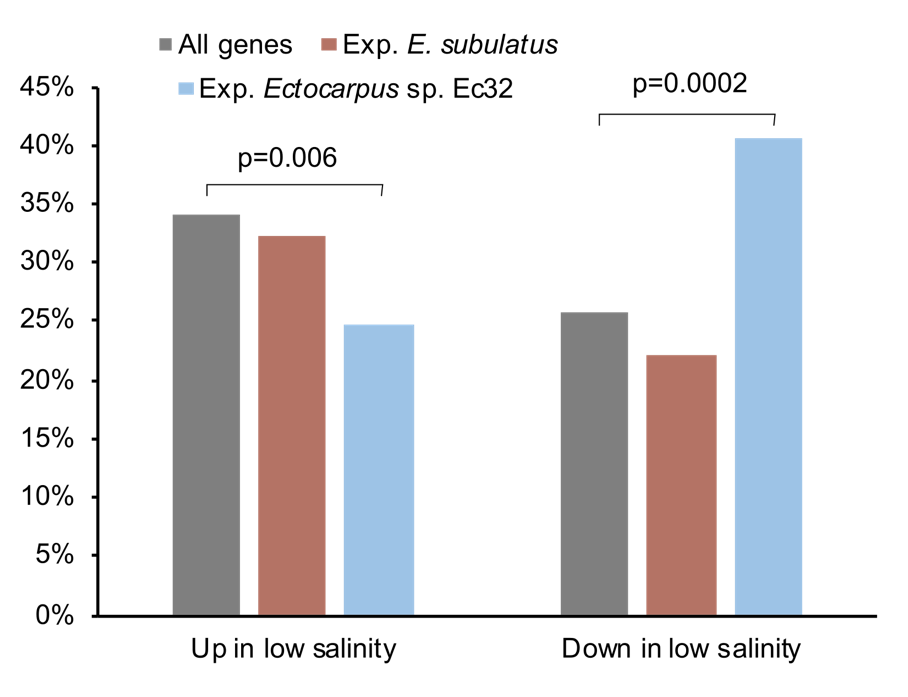
# Figures



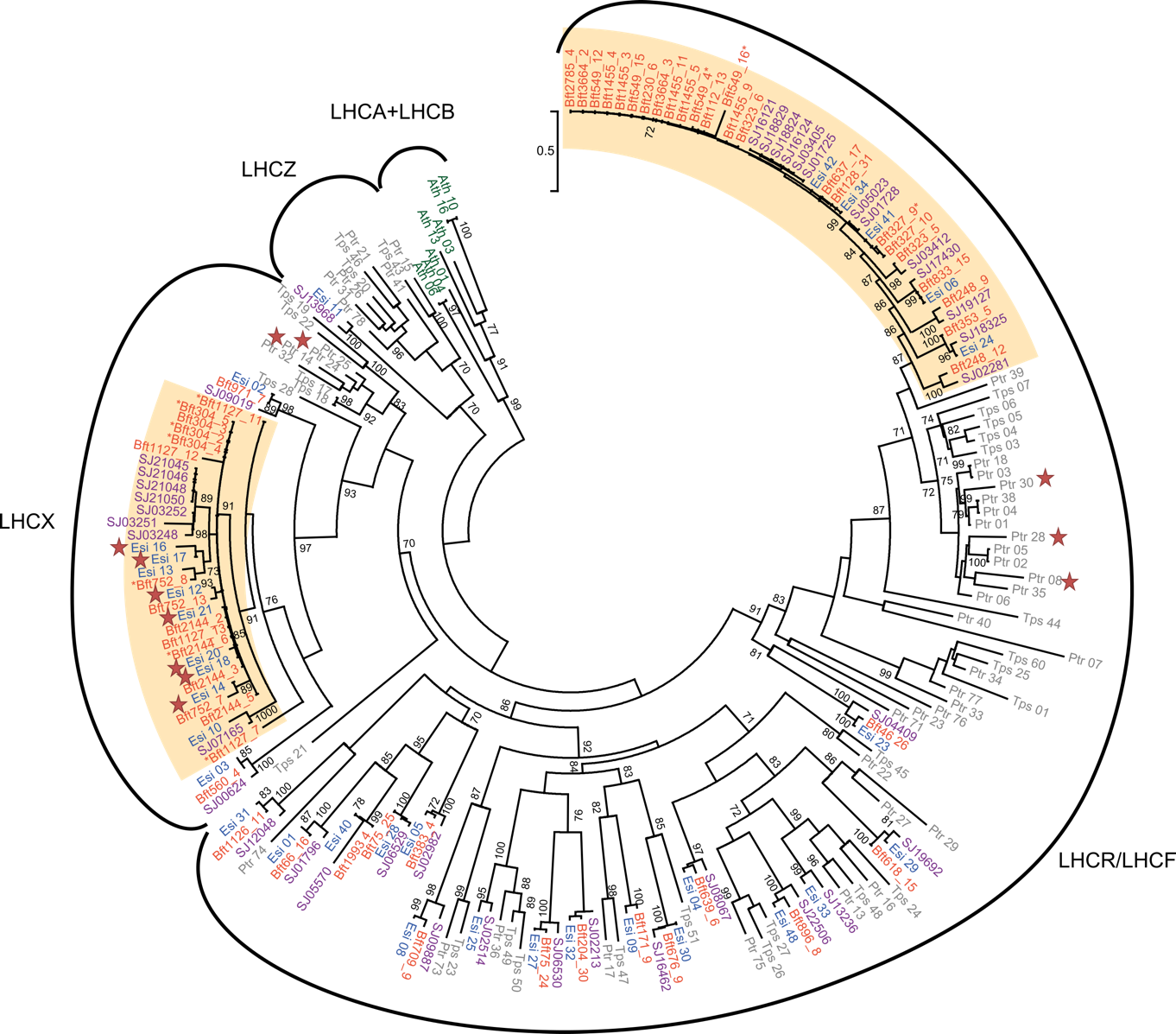
**Figure 1**: Repeated elements identified within the genome of *E. subulatus* Bft15b. A) Number of transposons detected in the different superfamilies; B) Boxplot of sequence identity levels for the detected superfamilies; and C) Distribution of sequence identities in all and the 20 most abundant transposon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

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



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



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

# Tables

**Table 1:** Assembly statistics of available brown algal genomes. PE = paired-end, MP = mate-pair, n.d. = not determined

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | *E. subulatus* Bft15b | *Ectocarpus* sp. Ec32 (Cock *et al.* 2010) | *S. japonica* (Ye *et al.* 2015) | *C. okamuranus* (Nishitsuji *et al.* 2016) | *N. decipiens* (Nishitsuji *et al.* 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 *et al.* 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% |

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

\*# not considering proteins absent from all three brown algal genomes.

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

|  |  |  |  |
| --- | --- | --- | --- |
| Cluster(s) | # Ec32 | # Bft15b | Putative annotation or functional domain |
| *Cell-wall related proteins* | | | |
| 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) |
| *Transcriptional regulation and translation* | | | |
| 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 |
| *Cell-cell communication and signaling* | | | |
| 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 |
| *Others* | | | |
| 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 |
|  |  |  |  |