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Development and physiology of the brown alga *Ectocarpus siliculosus*: two centuries of research

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**Development and physiology of the brown alga *Ectocarpus siliculosus*:
two centuries of research**

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1 **Development and physiology of the brown alga *Ectocarpus siliculosus*: two**
2 **centuries of research**

3

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25 **TOTAL: 10 455**

1 **Summary**

2

3 Brown algae share several important features with land plants, such as their photoautotrophic
4 nature and their cellulose-containing wall, but the two groups are distantly related from an
5 evolutionary point of view. The heterokont phylum, to which the brown algae belong, is a
6 eukaryotic crown group that is phylogenetically distinct not only from the green lineage, but
7 also from the red algae and the opisthokont phylum (fungi and animals). As a result of this
8 independent evolutionary history, the brown algae exhibit many novel features and,
9 moreover, have evolved complex multicellular development independently of the other major
10 groups mentioned above. In 2004, a consortium of laboratories, including the Station
11 Biologique in Roscoff and Genoscope, initiated a project to sequence the genome of
12 *Ectocarpus siliculosus*, a small filamentous brown alga that is found in temperate, coastal
13 environments throughout the globe. The *E. siliculosus* genome, which is currently being
14 annotated, is expected to be the first completely characterised genome of a multicellular alga.
15 In this review we look back over two centuries of work on this brown alga and highlight the
16 advances that have led to the choice of *E. siliculosus* as a genomic and genetic model
17 organism for the brown algae.

18

19 **198 words**

20

1 **Keywords**

2 Brown alga; phaeophyceae; *Ectocarpus*; development; reproduction; pheromone;
3 metabolism; defense

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8 **Abbreviations:**

9 ARA, arachidonic acid; BL, blue light; CE: chloroplast envelope; CER: chloroplast
10 endoplasmic reticulum; Ci, inorganic carbon; EPA, eicosapentaenoic acid; DGDG,
11 digalastosyldiacylglycerol; DPG, diphosphatidylglycerol; DGTA,
12 diacylglycerylhydroxymethyl-*N,N,N*-trimethyl- β -alanine; DGTS,
13 diacylglyceryltrimethylhomoserine; MGDG, monogalactosyldiacylglycerol; PC,
14 phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PG,
15 phosphatidylglycerol; SQGD, sulfoquinovosyldiacylglycerol.

1 I- Introduction

2

3 The brown algae belong to the division Heterokonta and are therefore only very distantly
4 related to the three most intensely studied eukaryotic groups, the animals, fungi and green
5 plants (Baldauf, 2003; Davis, 2004; Fig. 1a). This independent evolutionary history has
6 furnished brown algae with many novel metabolic, physiological, cellular and ecological
7 characteristics including a complex halogen metabolism, cell walls containing many unusual
8 polysaccharides and high resistance to osmotic stress. Developmental processes are
9 particularly interesting in this group, which evolved complex multicellularity independently
10 of the three other major groups listed above. From a more applied point of view, the
11 evolutionary history of the brown algae also underlies the high commercial value of several
12 members of the group in the sense that they have evolved novel biomolecules such as
13 polysaccharides and defence elicitors that have a wide range of applications in industry
14 (Klarzynski *et al.*, 2000; McHugh, 2003).

15 It is important to note, however, that whilst the independent evolutionary history of the
16 brown algae is the source of much of the interest of this group, it can also be seen as a
17 handicap because the well-developed model organisms from the plant and animal lineages are
18 of limited relevance to brown algal biology. Specialised brown algal models have been
19 developed in specific domains, for example members of the fucoids for cell biology
20 approaches (see references in Corellou *et al.*, 2005), but a polyvalent model organism that
21 allows access to a wide range of questions at the molecular level has been lacking. This
22 situation is changing with the development of genomic and genetic tools for the filamentous
23 brown alga *Ectocarpus siliculosus*. The sequencing of the genome of this alga has recently
24 been completed and the sequence is currently being annotated

1 (http://www.cns.fr/externe/English/Projets/Projet_KY/organisme_KY.html). It is therefore an
2 opportune moment to look back at the emergence of *Ectocarpus* as a model organism.

3 Research on *Ectocarpus* began in the 19th century with descriptions of species and
4 taxonomy, followed by studies aimed at unravelling reproduction and life history. Other
5 major aspects that have been studied include the sexual pheromones and infection of
6 *Ectocarpus* by viruses. Research has also been carried out on ultrastructure, photosynthesis
7 and carbon uptake, gamete recognition and resistance to anti-fouling agents. Several
8 eukaryotic parasites of *Ectocarpus* have been described. A proposition to adopt *Ectocarpus* as
9 a general model organism for the brown algae was made in 2004 (Peters *et al.*, 2004a). This
10 proposition was based partly on this alga's long history as an experimental organism but also
11 took into account several features that make *Ectocarpus* an interesting model for genetic and
12 genomic approaches. These features include its small size, the fact that the entire life cycle
13 can be completed in Petri dishes in the laboratory (Müller *et al.*, 1998), its high fertility and
14 rapid growth (the life cycle can be completed in 3 months), the ease with which genetic
15 crosses can be carried out and the relatively small size of the genome (200 Mbp compared
16 with 1095 and 640 Mbp for *Fucus serratus* and *Laminaria digitata* respectively; Le Gall *et*
17 *al.*, 1993 and Peters *et al.*, 2004a).

18 Here we present an overview of the work that has been carried out on *Ectocarpus* over the
19 last two centuries and discuss how the availability of a number of genomic tools, in particular
20 the complete genome sequence, is expected to accelerate research in many domains of brown
21 algal biology in the coming years.

22

1 II– Diversity and taxonomy, distribution and ecology

2 1 - Diversity and taxonomy

3 Dillwyn (1802-1809) published the first valid description of *Ectocarpus* (using the name
4 *Conferva siliculosa*) based on material collected by W.J. Hooker on "rocks in the sea at
5 Cromer and Hastings". These English localities lie in Norfolk and East Sussex, respectively.
6 Type material, collected by Hooker in 1807, is housed at BM (BM000685585 and
7 BM000685588) under the name *C. confervoides*. Lyngbye (1819) described the genus
8 *Ectocarpus* based on material from Denmark and cited *C. siliculosa* Dillwyn as basionym.
9 The correct nomenclature, therefore, is *E. siliculosus* (Dillwyn) Lyngbye (see Silva *et al.*,
10 1996 for further details). *E. siliculosus* is the type species of the order Ectocarpales which
11 includes most of the smaller brown algae. Originally regarded as phylogenetically primitive,
12 molecular systematics has shown the Ectocarpales to belong to a group of brown algal orders
13 that evolved rather recently. They are closely related to the large and highly differentiated
14 Laminariales which are major components of coastal marine floras (Rousseau & de Reviers,
15 1999; Draisma *et al.*, 2003; Cho *et al.*, 2004; Kawai *et al.*, 2007, Fig. 1b). Many species have
16 been described in *Ectocarpus* (www.algaebase.org, April 2007, lists 392 taxa of *Ectocarpus*
17 of whom 98 are flagged as "current"; numerous strains publicly available at the Culture
18 Collection of Algae and Protozoa (CCAP) in Oban, U-K, Gachon *et al.*, 2007a). However,
19 only *E. fasciculatus* Harvey (1841) is currently recognized as a second well-defined species,
20 based on morphology (Russell 1966, 1967a), crossing studies and sequence analyses (Stache-
21 Crain *et al.*, 1997). Crossing experiments have shown that the taxon *E. siliculosus* may
22 represent a species complex (Stache-Crain *et al.*, 1997) and ongoing, refined analyses are
23 expected to resolve this complex, increasing the number of recognised species. Identification
24 of different species of *Ectocarpus* based on morphology is difficult due to the plasticity of the
25 commonly examined features (habit, branching pattern, size of sporangia). In addition, the

1 two generations of a species may differ considerably (Müller, 1972a; Kornmann & Sahling,
2 1977).

3

4 **2 – Distribution**

5 *E. siliculosus* is distributed world-wide in temperate regions, but does not occur in the
6 tropics and south of the Antarctic convergence (Stache, 1990; Wiencke & Clayton, 2002). It
7 occurs in fully marine and in low-salinity habitats (e.g. 5 psu in Finland) and has even been
8 recorded at a fresh-water site in Australia (West & Craft, 1996) and in a salt polluted river in
9 Germany (Geissler, 1983). Records of *E. fasciculatus* are mainly from the North Atlantic but
10 there are some from Korea, Chile, South Georgia and South Africa (www.algaebase.org,
11 2007). On the shore, *Ectocarpus* occurs from high inter-tidal pools to the sub-littoral. It is
12 found on abiotic substrata (rocks, wood, plastic, ship hulls) and epiphytic on macrophytes or
13 free-floating (Russell, 1967a,b; 1983a,b). As a result of its ability to grow on a range of
14 abiotic substrates, *Ectocarpus* is a common fouling alga (Morris & Russell, 1974).

15

16 **3 – Ecology**

17 There have been a limited number of ecophysiological and ecological studies in *Ectocarpus*.
18 Growth rate is dependent on temperature, and there is evidence that temperature also
19 influences the life cycle, at least in some strains (Müller, 1963; Bolton, 1983; see the
20 “sporophyte and gametophyte architecture” section). The thermo-sensitivity of different
21 strains suggests that there is a genetic heterogeneity within the *Ectocarpus* genus (Bolton,
22 1983). A similar result was obtained for osmo-acclimation (Thomas & Kirst, 1991a,b; see the
23 “Abiotic stress” section). In the field, *Ectocarpus* is a short-lived annual which may dominate
24 the ectocarpoid flora on kelps (Russell, 1983a,b). Despite the commonness of *Ectocarpus*,

1 there are few data concerning phenology in the field (mostly to be extracted from floristic
2 works) and nothing precise on seasonality or habitats of the two generations.

3 The availability of the *E. siliculosus* genome sequence is expected to facilitate the analysis
4 of the ecology of this species by providing a basis for the development of molecular markers.
5 Particularly important challenges in this respect will include the identification of the sex locus
6 and of genes specifically expressed in the two generations, as these will provide molecular
7 tools that can be used to investigate several aspects of the life cycle under field conditions.
8 Molecular markers will also allow the exploration of genetic polymorphism amongst
9 *Ectocarpus* species from multiple locations across the globe.

10

11

12 **III- Development**

13

14 ***1 – Life cycle and reproduction***

15 Male and female gametes are morphologically identical in *Ectocarpus* (isogamy) but differ
16 with respect to their physiology and their behaviour: female gametes settle sooner and
17 produce a pheromone whilst male gametes swim for longer and are attracted to the
18 pheromone produced by the female. Studies on the reproduction of *Ectocarpus* began with the
19 observation of sexual fusions involving the attraction of male gametes to settled female
20 gametes from field thalli of *E. siliculosus* at Naples, Italy (Berthold, 1881). These findings
21 were hotly debated until Sauvageau (1896, 1897) and Oltmanns (1899) succeeded in
22 repeating the experiment. Gamete fusions in *Ectocarpus* were later used by Hartmann (1934)
23 to support his erroneous theory of relative sexuality (see Müller, 1976a for details). Knight
24 (1929) identified the young, unilocular sporangium as the site of meiosis, Papenfuss (1935)
25 and Kornmann (1956) published major contributions to the life history of *Ectocarpus*, and

1 Boalch (1961) developed refined culture techniques. The entire life history of *E. siliculosus*
2 from Naples was finally unravelled by Müller (1964, 1966, 1967, 1972b) using clonal cultures
3 and chromosome counts. It is schematised in Fig. 2. The basic life history of *E. siliculosus*
4 involves an alternation between the sporophyte and dioecious gametophytes, and sex
5 determination is genotypic (Müller, 1967). Male and female gametophytes are
6 morphologically indistinguishable. One of the problems for understanding the life history was
7 that sporophytes and gametophytes are difficult to distinguish morphologically. Another
8 problem was that zooids from plurilocular reproductive organs have different functions
9 according to the generation forming them: on sporophytes they contain asexual zoospores that
10 directly reproduce the sporophyte, on gametophytes they contain gametes. Further
11 complications include the parthenogenesis of unfused gametes, which develop into haploid
12 parthenogenetic sporophytes morphologically indistinguishable from diploid sporophytes, and
13 heteroblasty (different fates) of spores from unilocular sporangia, developing either into
14 gametophytes or into sporophytes. Furthermore, life cycle generation is not determined
15 rigidly by ploidy (Müller, 1967).

16 An important challenge for the future will be the characterisation of the genetic mechanisms
17 that control life cycle progression in *Ectocarpus*. This will require the development of
18 methodologies for positional cloning of mutated loci and of genome-wide methods to analyse
19 gene expression throughout the life cycle. Work is currently ongoing in several groups to
20 develop these techniques.

21

22 ***2 – Sporophyte and gametophyte architecture***

23 *E. siliculosus* is a small filamentous alga that grows to about 30 cm in length in nature, but
24 it may become fertile in the laboratory at 1-3 cm.

1 Sporophyte development is initiated with the germination of the zygote. The first division
2 produces two cells of identical developmental fate (Peters *et al.*, 2004b). Subsequent mitoses
3 lead to the formation of a basal (or prostrate) filamentous structure, defining the early
4 sporophyte (Fig. 3a). Phaeophycean hairs, *i.e.* hyaline filaments devoid of plastids developing
5 from a basal meristem, are absent in *Ectocarpus* but present in the sister genus *Kuckuckia*.
6 However, in *Ectocarpus* the distal end of filaments, or of plurilocular sporangia, may be less
7 pigmented and resemble a hair; such structures may be referred to as pseudo-hairs (Cardinal,
8 1964; Pedersen, 1989). If the growth conditions are favourable (Ravanko, 1970), erect
9 filaments (called "upright" filaments) emerge after a few days, contributing to the
10 establishment of an overall filamentous architecture (Fig. 3c).

11 The typical structure of a vegetative cell is illustrated in Fig. 4. Features common to all
12 brown algal cells include a chloroplast surrounded by four membranes, arranged as two
13 double-membraned envelopes (chloroplast envelope, CE). The second envelope is loosely
14 associated with the chloroplast and forms part of the chloroplast endoplasmic reticulum
15 (CER). The lamellae of the chloroplast are composed of three thylakoids, which are absent
16 from the pyrenoid space (Bouck, 1965; Oliveira & Bisalputra, 1973). In *Ectocarpus* the
17 chloroplast is ribbon-shaped. The size and number of chloroplasts may vary within the same
18 organism (Ravanko, 1970). Other typical features of *Ectocarpus* include several prominent
19 and pedunculated pyrenoids on the inner face of the chloroplast, which are used as a
20 taxonomic marker for the Ectocarpales (Evans, 1966; Rousseau & de Reviere 1999). CER and
21 CE envelopes also surround pyrenoids, this time being tightly adjacent. A third external
22 envelope, called the pyrenoid sac, surrounds the pyrenoid but has no connection with the
23 reticulum system (Bouck, 1965). The nuclear envelope is continuous with the CER, which,
24 itself is in close vicinity to the Golgi apparatus (Bouck, 1965; Oliveira & Bisalputra, 1973). It
25 has been hypothesised that these connections create a complex network of membranes

1 allowing photosynthates to be efficiently transferred from the chloroplast to the Golgi
2 apparatus, the latter being also in direct contact with the CER (Oliveira & Bisalputra, 1973).
3 Cytoplasmic ER is dispersed throughout the cytoplasm and is mainly rough (Oliveira &
4 Bisalputra, 1973). Osmiophilic bodies (OSB) which are thought to contain lipids, are
5 dispersed throughout the cytoplasm and probably originate from the CER (Oliveira &
6 Bisalputra, 1973). They have been observed within the cell wall, and also external to it.
7 Vacuoles can be either large structures occupying peripheral locations (Oliveira & Bisalputra,
8 1973) in fixed material, or most of the cellular space (as in land plant cells; Knight, 1929,
9 confirmed by data from our laboratory, after staining with cresyl blue or neutral red, on
10 sporophytic filaments). The nuclear region encompasses two centrioles, which are considered
11 as a microtubule organising centre (MTOC; Katsaros *et al.*, 1991). The chromosome number
12 in the haploid nucleus is estimated to be ca 25 (Peters *et al.*, 2004a). Mitochondria are
13 preferentially peripherally located. They are maternally inherited (Peters *et al.*, 2004b). The
14 cell wall consists of a fibrillar matrix (see the “Photosynthesis and carbohydrate metabolism”
15 section) with several plasmodesmata distributed uniformly along the cross walls. Upon
16 ageing, cell wall ingrowths occur, accompanied by a reduction in the size of the nucleus,
17 mitochondria, ER and Golgi, followed by the disintegration of the chloroplasts and finally by
18 autolysis of the cytoplasm (Oliveira & Bisalputra, 1977a,b).

19
20 Most of the reproductive organs are carried by upright filaments (Müller, 1964). Two types
21 of reproductive organs are produced by the sporophyte: plurilocular and unilocular sporangia.
22 Plurilocular sporangia are cone-shaped three-dimensional structures of variable size (Knight,
23 1929), composed of a large number of locules with different shapes (Baker & Evans, 1973b;
24 Fig. 3e). These locules are generated by several successive mitoses and each gives rise to a
25 single zoospore, which is released through the apex of the sporangium. The mito-spores,

1 which are bi-flagellate and competent for swimming shortly after their release (Müller, 1980),
2 are a means of vegetative reproduction. Unilocular sporangia are born on the side of branches
3 (Fig. 3f, Baker & Evans, 1973a). A single meiosis occurs within the single thick-walled locule
4 (Baker & Evans, 1973a) and this is followed by several mitoses, which generate about a
5 hundred meio-spores, half of which are female and half male (Müller, 1980). Müller (1963)
6 and Ravanko (1970) reported that plurilocular sporangia were produced when the external
7 temperature was relatively high (~20°C or summer), whereas unilocular sporangia were
8 produced when the temperature was lowered to 13°C (mimicking winter conditions).
9 However, this temperature dependence is not observed in many strains of *Ectocarpus* (A.F.
10 Peters, unpublished results). Meio-spores germinate to produce haploid, dioecious
11 gametophytes (Fig. 3b,d). These are filamentous organisms similar to the sporophyte, but with
12 two important differences. Firstly, the meio-spore germinates asymmetrically to produce a
13 rhizoid and an upright filament, so no prostrate structure forms (A.F. Peters, unpublished
14 results). Secondly, the thallus is more ramified than that of the sporophyte (Müller, 1980).
15 Gametophytes produce only plurilocular gametangia and these are similar structurally to
16 plurilocular sporangia on sporophytes. Gametes resemble meio-spores in terms of their size
17 and motility.

18 The developmental patterning varies greatly across the *Ectocarpus* species complex and is
19 also dependent on the environment, growth conditions and even the age of the algae for some
20 features (Ravanko, 1970). This plasticity is observed for the branching frequency and the
21 number, shape, structure and positioning of the reproductive organs on filaments (Knight,
22 1929; Müller, 1980; Kim & Lee, 1992). Phytohormones, especially cytokinins, have also been
23 reported to influence the development of *Ectocarpus* sporophytes (Pedersén, 1968, 1973).

24

1 As mentioned above, brown algae are interesting for developmental studies because they
2 have independently evolved complex multicellularity. *Ectocarpus* is a relevant model to
3 address this problem as it is closely related to complex algae such as the Laminariales and the
4 Fucales. However, developmental processes in *Ectocarpus* are clearly simplified compared
5 with its morphogenetically more complex sister families, which provides an advantage for the
6 detailed dissection of these developmental processes. In particular the simple growth pattern
7 of the uniseriate, branched filaments represents an ideal system for the combined application
8 of genetic and mathematical modelling approaches to understanding developmental patterning
9 and then addressing the issue of the evolution of development and multicellularity.

10

11 **3 – Gametes and spores**

12 Several electron microscopy studies of the motile cells (zoids) of *Ectocarpus* have been
13 reported (Baker & Evans 1973a,b; Lofthouse & Capon, 1975; Maier, 1997a,b). The typical
14 structure of an *Ectocarpus* zoid is illustrated fig. 5.

15 *Ectocarpus* zoids correspond to the “primitive” type of brown algal zoid according to Kawai
16 (1992). Gametes and spores typically contain a single chloroplast with a pyrenoid (Baker &
17 Evans, 1973a; Lofthouse & Clayton, 1975; Maier, 1997a). As in the vegetative cells, lamella
18 are composed of three thylakoids and the nuclear envelope is in continuity with the
19 chloroplast endoplasmic reticulum (Maier, 1997a). The nucleus of the male gamete is rich in
20 heterochromatin. Several dictyosomes are present in gametes and spores (Baker & Evans,
21 1973b; Maier, 1997a). The Golgi apparatus of mito-spores is very active both before and after
22 release (Baker & Evans, 1973b). This secretory activity may have important functions in the
23 biosynthesis of the adhesive required for gamete adhesion and for the synthesis of new cell
24 wall compounds during germination.

1 *Ectocarpus* gametes and spores are characterized by two flagella with lateral insertion. One
2 is oriented forward and equipped with mastigonemes (hairs), and propels the cell with
3 meandering beats. The second is oriented obliquely backwards, and has no mastigonemes.
4 Most of the time this posterior flagella is passively dragged but occasional lateral beats induce
5 abrupt changes in direction of up to 180° (Geller & Müller, 1981). Gametes and spores have a
6 concave depression at the level of the eyespot into which the swelling of the proximal part of
7 the posterior flagellum fits (Baker & Evans, 1973b; Kreimer *et al.*, 1991; Maier, 1997a). The
8 possible function of the eyespot is to reflect and focus incident light onto the site of
9 photoreception (Kawai *et al.*, 1990; Kreimer *et al.*, 1991). Zoids are capable of positive
10 phototaxis and their posterior flagellum shows strong autofluorescence when irradiated by
11 blue light (450 nm). The photoreceptor pigment is a flavoprotein, which is periodically
12 shaded by a carotenoid stigma (Müller *et al.*, 1987; Kawai, 1988). The acronema (the
13 whiplash tip) is extremely sensitive to mechanical stress and plays an important role in
14 establishing the initial sexual contact between gametes. The details of the flagellar apparatus
15 of both female and male gametes have been studied by electron microscopy (Müller & Falk,
16 1973; Maier, 1997b). Despite their different behaviours, no difference in their fine structure
17 has been detected (Müller & Falk, 1973).

18 The pheromone that attracts male gametes to female gametes is an unsaturated hydrocarbon.
19 The substance initially identified was ectocarpene (all-cis-1-(cycloheptadiene-2',5'-yl)-
20 butene-1) (Müller *et al.*, 1971; Müller, 1976b; Müller, 1978; Müller & Schmid, 1988) but
21 more recently Boland *et al.* (1995) have shown that a thermally labile cyclopropyl precursor,
22 pre-ectocarpene, is more active by three orders of magnitude and is thus the actual
23 pheromone.

24 There is evidence that cell-to-cell recognition between *Ectocarpus* gametes is mediated by
25 N-acetyl glucosamine residues exposed on the plasma membrane of the female gametes and

1 that these residues are specifically recognized by a receptor on the male gamete (Schmid,
2 1993; Schmid *et al.*, 1994a). In addition, the lectins concanavalin A (Con A) and *Aleuria*
3 *aurantia* agglutinin (AAA) bind specifically to the anterior flagella of *Ectocarpus* gametes
4 and the molecules with which they interact also may be involved in the gamete recognition
5 process (Maier & Schmid, 1995).

6 Zoids from plurilocular and unilocular *Ectocarpus* sporangia share similar overall
7 intracellular structures, but there are some important differences: plurilocular zoids are
8 smaller and swim faster with more rapid changes of direction (Baker & Evans, 1973a). Zoids
9 from unilocular sporangia are strikingly different from the other *Ectocarpus* cell types (both
10 plurilocular zoids and vegetative cells) in that the nucleus is physically separated from the
11 chloroplast. Moreover, secretory activity is lower than in zoids from plurilocular sporangia
12 (Baker & Evans, 1973b).

13 Gametes of *Ectocarpus* have also been used to study chemotaxis (Boland *et al.*, 1983),
14 receptor modelling (Boland *et al.*, 1989) and phototaxis (Kawai *et al.*, 1990; Kreimer *et al.*,
15 1991). Protocols for isolation and biochemical characterisation of plasma membrane and CER
16 membrane have also been developed (Schmid *et al.*, 1992). Together with more recently
17 developed molecular tools, these methods now offer access to a range of interesting
18 biochemical events that take place during gamete interaction at fertilization such as
19 chemoreception, cell-cell recognition and fusion processes.

20

21

22 **IV- Metabolism**

23

24 ***1- Photosynthesis and carbohydrate metabolism***

1 Marine environment constrains several aspects of photosynthesis in brown algae. First,
2 carbon dioxide is not the main source of inorganic carbon (Ci). Indeed, seawater in
3 equilibrium with air contains only 13 μM CO_2 , but more than 2 mM anionic carbon, mainly in
4 the form of HCO_3^- (Beer, 1994). Secondly, when seaweeds are immersed they do not receive
5 the full light spectrum. Light absorption increases with water depth and varies according to
6 the wavelength: red-light (650 nm) is first absorbed followed by purple (400 nm) and yellow-
7 (550 nm) lights. In contrast green (500 nm) and blue (450 nm) lights display a strong
8 penetration: at 10 m depth red light is almost fully absorbed whereas the absorption of green-
9 and blue lights is not significant.

10 Adapted to these conditions, brown algae differ from land plants by the pigment
11 composition of their light harvesting complexes (LHCs). Chlorophyll c (chl c) and the
12 carotenoid fucoxanthin are indeed the main light-harvesting pigments of brown algae. Their
13 presence broadens the absorption spectrum toward the green light relative to the chlorophytes.
14 Brown algae features two different LHCs associated to photosystems I and II (PSI and PSII),
15 but their pigment composition is controversial. Barrett and Anderson (1980) described a
16 fucoxanthin-chl a/c protein and a violaxanthin enriched-chl a/c protein. Conversely Alberte *et*
17 *al.* (1981) reported a chl a/c protein devoid of fucoxanthin and a second LHC containing chl a
18 and fucoxanthin but not chlorophyll c. More recent analyses showed that the LHCs associated
19 to PSI and PSII are in fact virtually identical with respect to their pigmentation and peptide
20 composition. All complexes bound chl a, chl c and fucoxanthin in the proportion 6 : 2 : 7, but
21 the LHC associated to PSI is significantly enriched in violaxanthin (De Martino *et al.*, 2000).
22 As in plants, these accessory pigments transfer energy to chlorophyll a within the
23 photosynthetic reaction centres (Grossman *et al.*, 1995). Violaxanthin does not participate in
24 light harvesting but is involved in photoprotection (Demmig-Adams & Adams, 1992;
25 Lemoine *et al.*, 1995).

1

2 Under saturating red light, photosynthesis of *E. siliculosus* follows a circadian rhythm
3 with maxima at about noon and can be stimulated by a pulse of blue light (BL) (Schmid &
4 Dring, 1992). This stimulation is also observed in other Phaeophytes, but mostly absent in
5 green or red algae (Schmid *et al.*, 1994b). BL induces several responses: an acidification at
6 the surface of *E. siliculosus* (Schmid & Dring, 1993), accompanied by bicarbonate uptake
7 (Schmid, 1998). A plasma membrane H⁺-ATPase is thought to be activated, and the resulting
8 acidification to increase the conversion of HCO₃⁻ to CO₂ in the extracellular space (Schmid &
9 Dring, 1993). Recently, such BL-induced H⁺-ATPases have been identified in the brown alga
10 *Laminaria digitata* (Klenell *et al.*, 2002). BL also triggers the mobilization of an internal
11 carbon source, since photosynthesis is stimulated even in the absence of external Ci (Schmid
12 & Dring, 1996). As a result, a C₄-like metabolism was initially proposed to exist in *E.*
13 *siliculosus* (Schmid & Dring, 1996) but not all the enzymes necessary for a C₄ cycle were
14 detected (Bush & Schmid, 2001) and the pool of intermediates seems to be too small to act as
15 an organic carbon stock (Hillrichs & Schmid, 2001). The sequestration of a pool of inorganic
16 carbon in the vacuole and its movement to the cytosol in response to BL is now the favoured
17 hypothesis (Schmid & Hillrichs, 2001). Analysis of the sequence of the *E. siliculosus* genome
18 will help to confirm whether or not a C₄ pathway exists in the brown algae.

19 In contrast to the *Plantae* (Moreira *et al.*, 2000), the *Phaeophyceae* do not store the carbon
20 assimilated by photosynthesis as insoluble starch granules, but instead as the soluble 1,3-β-
21 glucan polymer (laminarin) localised in the cytosol (Craigie, 1974), and as mannitol, involved
22 in osmo-acclimation (Davis *et al.*, 2003; see the “Abiotic stresses” section). Brown algae also
23 produce complex polysaccharides which constitute their cell wall. They synthesize some
24 neutral polysaccharides in common with land plants, such as cellulose (Carpita & McCann,
25 2000), but also unique anionic polysaccharides, such as alginates and sulphated fucans

1 (Kloareg & Quatrano, 1988). *Ectocarpus* has not been well studied in this respect, but
2 preliminary analyses in our laboratory confirm that all of the polysaccharides typical of brown
3 algae are present in this genus (Estelle Deniaud, pers. comm.). The biosynthetic pathways of
4 these brown algal polysaccharides are essentially unknown and the genome of *E. siliculosus*
5 will be a much anticipated asset to investigate these crucial metabolisms.

6

7 **2- Lipid metabolism**

8 Worldwide, at least 50 species of brown algae are used as human food. Their lipid content
9 has therefore attracted considerable attention from the viewpoint of both nutrition and
10 pharmacology. A number of studies have been conducted to profile and quantify the fatty
11 acids and the different classes of lipids in these organisms, and to investigate whether the
12 various lipid patterns correlate with the taxonomic position or any other characteristic of the
13 brown algae. Brown algal polar lipids include several common glycolipids (MGDG, DGDG,
14 SQGD, see list of abbreviations) and phospholipids (PC, PE, PI, PG and DPG). Interestingly,
15 several reports have highlighted the high proportion of long chain polyunsaturated fatty acids
16 (LC-PUFAs) (Eichenberger *et al.*, 1993). Moreover, most of the Phaeophyceae contain the
17 betaine lipids DGTA and DGTS, and either contain the common phosphatidylcholine
18 phospholipids in surprising low amounts or do not produce them at all (Eichenberger *et al.*,
19 1993). All Ectocarpales contain phosphatidylcholine, but exhibit interspecific variation in
20 their DGTA content: *E. fasciculatus* strains contain this lipid while *E. siliculosus* strains do
21 not (Müller & Eichenberger, 1995; Müller, 1995). DGTA may therefore be used as a
22 taxonomic marker (Müller, 1995), although an *E. fasciculatus* strain deficient in DGTA
23 biosynthesis has been described. Genetic analysis of such a strain identified an autosomal
24 locus necessary for the biosynthesis of this lipid (Müller & Eichenberger, 1997).

1 A novel phosphoglyceride, designated PX, was first isolated from *E. siliculosus*. It was
2 shown to account for 2-4% of total lipids (Schmid *et al.*, 1994c), and to accumulate mostly in
3 the plasma membrane of gametes. Since PX is rich in 20:4n-6 (ARA) and 20:5n-3 (EPA), it
4 has been suggested that it could represent a potential reservoir for pheromone precursors. PX
5 was subsequently detected in other brown algae (Ectocarpales, Fucales and Sphacelariales;
6 Schmid *et al.*, 1994c).

7 Lipid metabolism plays also major roles in the control of defense mechanisms. Therefore,
8 together with the genome sequence, it is likely that the efforts to screen mutants with altered
9 resistance to pathogen attacks, will lead to the phenotyping of plants impaired in lipid or fatty
10 acid metabolic pathways, thereby potentially revealing novel specific traits of this metabolism
11 in brown algae.

14 **V- Interactions with the environment**

16 ***I- Ectocarpus pathogens***

17 Despite of their small size and ephemeral life stages, filamentous brown algae have been
18 frequently reported to be plagued by various pathogens, including viruses (Müller *et al.*,
19 1998) and eukaryotic parasites of different phylogenetic lineages: oomycetes, chytrids and
20 hyphochytrids (Andrews 1976; Küpper & Müller 1999; Müller *et al.* 1999) and by parasites
21 related to the Plasmodiophorea (Karling, 1944; Maier *et al.*, 2000). In addition, numerous
22 historical records described ectocarpoids with abnormal sporangia or vegetative cells
23 suspected to contain unknown parasites (Ratray, 1885; Müller *et al.*, 1998).

24 The oomycete *Eurychasma dicksonii* has been described mainly in wild populations of
25 *Pylaiella littoralis* (Küpper & Müller, 1999) but it displays a broad host range and infects

1 various brown algae including *Ectocarpus* (Müller *et al.*, 1999) in which it was initially
2 described by Wright (1879). There is a current effort to set up a defined pathosystem using *E.*
3 *siliculosus* and *E. dicksonii*, and *Ectocarpus* strains have been shown to exhibit differential
4 susceptibility to a defined *Eurychasma* strain. Conversely, several *Eurychasma* strains exhibit
5 different host specificities, suggesting co-evolution of the two species (Gachon *et al.*, 2007b).
6 The molecular bases of resistance and virulence are under investigation.

7 Chytrids were described earlier by Petersen (1905) and the hyphochytrid *Anisolpidium*
8 *ectocarpii* was described by Karling (1943) and Johnson (1957). Like *E. dicksonii*,
9 *Chytridium polysiphoniae* (Chytridiomycota) is ubiquitous and can infect many hosts,
10 including *E. siliculosus* and *E. fasciculatus* (Müller *et al.*, 1999). Interestingly, its negative
11 effects on photosynthesis of its host was described at the cellular level in the related
12 ectocarpoid *P. littoralis* using fluorescence kinetic microscopy (Gachon *et al.*, 2006).
13 Recently, the 18S rRNA genes of *Chytridium polysiphoniae* and *Eurychasma dicksonii* were
14 sequenced and used to clarify their phylogenetic affiliations (Küpper *et al.*, 2006). The
15 plasmodiophorean *Maullinia ectocarpii* is an obligate intracellular parasite of *Ectocarpus* spp.
16 (Maier *et al.*, 2000). However, the extent to which this infection occurs in nature and its effect
17 on algal fitness are presently unknown.

18 Viral infections represent by far the most studied phenomenon in *E. siliculosus* (Müller,
19 1996; Müller & Knippers, 2001). Until the late eighties most reports of virus infections in
20 brown algal tissues were based on electron microscopy studies, which sporadically described
21 “virus-like particules” (VLP). Viruses were obtained in culture for the first time from a New
22 Zealand strain of *E. siliculosus* after lysis of host cells allowing evaluation of their infection
23 potential (Müller *et al.*, 1990; Müller, 1991). Virus infections were found in about 50% of the
24 individuals of a given natural population (Dixon *et al.*, 2000; Müller *et al.*, 2000) and were

1 shown to occur worldwide in correlation with the cosmopolitan distribution of *E. siliculosus*
2 (Müller, 1991; Sengco *et al.*, 1996).

3 The viruses that infect different ectocarpoid algae exhibit considerable variability in size
4 and diameter and, in general, they display a high level of host specificity (Müller *et al.*, 1998).
5 However, several instances of trans-specific infection have been described, for example
6 between EsV-1 (*Ectocarpus siliculosus* virus-1) and *Kuckuckia kylinii* (Müller, 1992; Müller
7 & Schmid, 1996) and also between EfasV-1 (*Ectocarpus fasciculatus* virus-1) and *E.*
8 *siliculosus* (Müller *et al.*, 1996; Sengco *et al.*, 1996). Interestingly, EsV-1 and EfasV-1 are the
9 most similar of the brown algal viruses in terms of their genome size (Müller *et al.*, 1996).

10 The EsV-1 virus specifically infects the single-celled gametes or spores, *i.e.* the only cells in
11 the life history that lack a cell wall (Maier & Müller, 1998). Following infection, a single
12 copy of the viral DNA appears to integrate into the host genome (Delaroque *et al.*, 1999). The
13 viral DNA is then transmitted, *via* mitotic divisions, to all the cells of the developing alga.
14 This has been confirmed by regenerating algae from protoplasts derived from virus-infected
15 gametophytes (Kuhlenkamp & Müller, 1994). Despite the fact that they carry the integrated
16 virus, vegetative cells do not produce viral particles (Müller *et al.*, 1998). Viral particles are
17 only produced in reproductive organs (sporangia and gametangia) of mature algae from where
18 they are released to infect a new generation of zoids. In addition to these cycles of re-
19 infection, the viral genome can be also transmitted to progeny through meiosis, in which case
20 it segregates as a Mendelian factor and is inherited by half of the progeny (Müller, 1991;
21 Bräutigam *et al.*, 1995). The pathogenic character of viral infections has been unambiguously
22 confirmed, but this association's main impact is on reproductive success. Plant sterility varies
23 from partial (Müller *et al.*, 1990) to total (Müller & Frenzer, 1993) but no significant
24 difference in photosynthesis, respiration and growth rate were observed in infected

1 gametophytes or sporophytes (Del Campo *et al.*, 1997). This contrasts with the reduced
2 photosynthetic performance of *Feldmannia* species infected with FsV (Robledo *et al.*, 1994).

3 The EsV-1 genome is a circular DNA molecule of a relatively large size (335 Kbp) for a
4 phycodnavirus (Van Etten & Meints, 1999; Van Etten *et al.*, 2002) with double-stranded
5 regions interrupted by single-stranded regions (Lanka *et al.*, 1993; Klein *et al.*, 1994). Both
6 EsV-1 and the related *Feldmannia irregularis* virus (FirrV-1) have been sequenced (Delaroque
7 *et al.*, 2001; Delaroque *et al.*, 2003). EsV-1 contains approximately 231 genes with a wide
8 range of predicted functions including DNA metabolism, signalling, transposition, DNA
9 integration and polysaccharide metabolism (Delaroque *et al.*, 2000a,b; Delaroque *et al.*,
10 2003). It has also been proposed that the ability of the virus to integrate into its host's genome
11 could be exploited to develop a transformation vector for a wide range of brown algae,
12 including *E. siliculosus* (Henry & Meints, 1994, Delaroque *et al.*, 1999). However, the
13 complex integration pattern of the virus into the algal genome will considerably complicate
14 this task (N. Delaroque, pers. comm.). A microarray has been constructed to analyse EsV-1
15 gene expression (Declan Schroeder, pers. comm.) and it will be particularly interesting in the
16 future to couple the analysis of viral and genome-wide host gene expression during viral
17 infection.

18 The development of genomic tools provides a new context to investigate the possible
19 genetic basis of the co-evolution between some pathogens and brown algae. The search for
20 inducers of defense responses and resistance against parasites is also still opened as, in
21 contrary with kelps, *Ectocarpus* does not react with an oxidative burst upon recognition of
22 alginic fragments (Küpper *et al.*, 2002a).

23

24

25 **2- Abiotic stresses**

1 *E. siliculosus* is able to exploit a wide range of habitats and environmental conditions (see
2 the “Distribution” section). This feature seems likely to be based at least as much as on a high
3 intrinsic genetic variability as on a general physiological toughness, as illustrated by work
4 carried out on copper and saline stress responses.

5 Interspecific variations in copper tolerance have been observed between different
6 strains of *E. fasciculatus* and *E. siliculosus*, with the latter being the most tolerant (Morris,
7 1974). Differences have also been observed among *E. siliculosus* strains that are differently
8 exposed to copper in their natural habitat (Russell & Morris, 1970; Hall, 1981). Cu^{2+}
9 interferes with the general process of photosynthesis in brown algae, and particularly in *E.*
10 *siliculosus*, by competing with magnesium for metal binding sites in the chlorophyll
11 molecules (Küpper *et al.*, 2002b). A study of the mechanism of tolerance to copper and other
12 heavy metals suggested a co-tolerance to copper, cobalt and zinc, and provided evidence for
13 an exclusion mechanism to explain the particularly low sensitivity of *E. siliculosus* copper
14 tolerant strains (Hall *et al.*, 1979; Hall, 1980, 1981). However, as yet there is no clear
15 explanation for the intra-specific variation with respect to this trait within this species.

16 Ability of some *E. siliculosus* strains to tolerate copper has been considered to
17 develop bioassays in which this alga is used for monitoring marine antifouling characteristics
18 of copper-based materials (Hall and Baker, 1985, 1986). In addition, copper chloride was
19 used to inhibit *E. siliculosus* infestations in tank cultures of *Gracilaria gracilis* (Van Heerden
20 *et al.*, 1997).

21
22 Russell and Bolton (1975) reported the occurrence of salinity ecotypes within *E. siliculosus*.
23 This study was extended by Thomas and Kirst (1991a,b) who showed that large differences in
24 photosynthesis, accumulation of osmotically active compounds (mannitol; Davis *et al.*, 2003)
25 and vitality occur between *E. siliculosus* isolates from different geographic locations

1 following changes in salinity. They also observed that sporophytes were more salt tolerant
2 than gametophytes, irrespective of their level of ploidy.

3 Detailed investigations are necessary to decipher the physiological and cellular bases of salt
4 and heavy metal tolerance in *E. siliculosus*. Mutagenesis and transcriptomic approaches will
5 thus help to better understand the mechanisms involved in osmotic and oxidative adaptation,
6 and to explain how these algae can cope with such a wide range of environmental conditions.

7

8

9 **VI- Conclusion**

10

11 Taken together, the above sections illustrate the broad range of phenomena that have been
12 studied in *Ectocarpus* and provide an indication of the domains that could be further explored
13 in the future. Notably, a large proportion of this past work, covering many diverse aspects of
14 *Ectocarpus* biology, was carried out in Dieter Müller's laboratory in Konstanz, and the efforts
15 of this group have therefore laid the foundations for the development of *Ectocarpus* as a
16 model organism.

17 The *Ectocarpus* genome project has federated a consortium of laboratories with an interest
18 in this organism and these laboratories are currently developing several molecular tools.
19 These include mutant screens, genetic transformation and genome-scale analysis of gene
20 expression. Several developmental mutants have been isolated and positional cloning of some
21 of the affected genes should be feasible in the near future. The availability of the genome
22 sequence together with the ability to analyse gene function by forward and reverse genetic
23 approaches will make it possible to address additional questions, many of which have been
24 evoked in this review. Examples include the biosynthesis of diverse, brown-algal-specific
25 metabolites, such as lipids and complex cell wall components, and the genetic basis of

1 resistance to biotic and abiotic aggression. The genome will also be an invaluable aide for the
2 study of the ecology of *Ectocarpus*, by serving as the base for the development of neutral and
3 selected molecular markers for the analysis of field isolates. In conclusion, the *Ectocarpus*
4 genome sequence and the tool development associated with this project are providing access
5 to a relatively unexplored branch of the eukaryotic tree and some exciting discoveries can be
6 expected in this domain in the coming years.

7

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9

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16
17 **Figure legends:**

18
19 **Figure 1: Phylogeny of brown algae and Ectocarpales.**

20 **a:** Position of brown algae within the eukaryotes (adapted from Baldauf, 2003). Brown algae
21 belong to the heterokont phylum, which is phylogenetically distant from land plants and the
22 green and red algae. Photosynthetic organisms are framed. **b:** Position of the Ectocarpales (in
23 bold) within the brown algae (adapted from Kawai *et al.*, 2007).

24

1 **Figure 2: Life cycle of *Ectocarpus siliculosus*.**

2 Diploid sporophytes produce meio-spores (by meiosis) in unilocular sporangia (UL). Meio-
 3 spores grow into male or female gametophytes (dioecism). Gametophytes produce gametes in
 4 plurilocular gametangia (PL). Fusion of gametes produces a zygote that grows into a diploid
 5 sporophyte, completing the sexual cycle. Unfused gametes may grow parthenogenetically and
 6 form a parthenosporophyte, which is indistinguishable from the diploid sporophyte. Both
 7 sporophytes and parthenosporophytes can reproduce themselves asexually by the production
 8 of mito-spores in plurilocular sporangia.

9

10 **Figure 3: Morphology of *Ectocarpus siliculosus*.**

11 Photographies of one week old vegetative sporophyte (a) and gametophyte (b) and schemes
 12 representing the whole body of the mature sporophyte (c) and gametophyte (d) after six
 13 weeks of growth, are shown. e: Plurilocular sporangium or gametangium (occurring on the
 14 sporophyte and the gametophyte, respectively) before (left) and after (right) release of zoids.
 15 f: Unilocular sporangium from sporophyte. Sporangia and gametangia can be either sessile or
 16 pedicellate (Kim & Lee, 1992).

17

18 **Figure 4: General ultrastructure of a vegetative cell of *Ectocarpus siliculosus*.**

19 The general ultrastructure of a vegetative cell is similar in both prostrate and erect filaments
 20 (Oliveira & Bisalputra, 1973). The different compartments of the cell are illustrated (see text
 21 for details). Lines represent membranes and define sub-cellular compartments, except for
 22 thylakoids, drawn as a thick black line. Depending on their type and age, vegetative cell size
 23 varies from 10 to 35 μm in length, and 5 to 15 μm in width (in laboratory culture conditions).

24

25 **Figure 5: General intracellular structure of *Ectocarpus siliculosus* zoids.**

1 A scheme representing the different compartments of an *Ectocarpus siliculosus* zoid cell from
2 a plurilocular sporangium or gametangium is presented (see text for details). Legends are the
3 same as in fig. 4.

4

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7 communicate unpublished information about viral integration, and to Declan Schroeder
8 (Marine Biological Association in Plymouth) for authorisation to mention the microarray for
9 the EsV-1 virus.

Figure 1

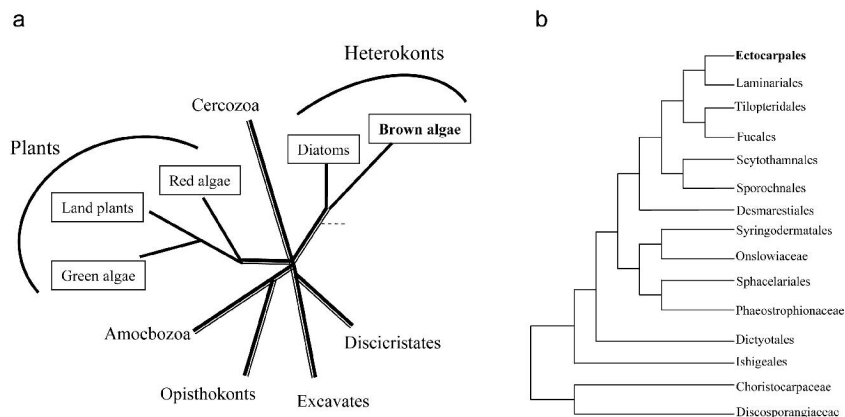


Figure 1: Phylogeny of brown algae and Ectocarpales. a: Position of brown algae within the eukaryotes (adapted from Baldauf, 2003). Brown algae belong to the heterokont phylum, which is phylogenetically distant from land plants and the green and red algae. Photosynthetic organisms are framed. b: Position of the Ectocarpales (in bold) within the brown algae (adapted from Kawai et al., 2007).

275x190mm (300 x 300 DPI)

Figure 2

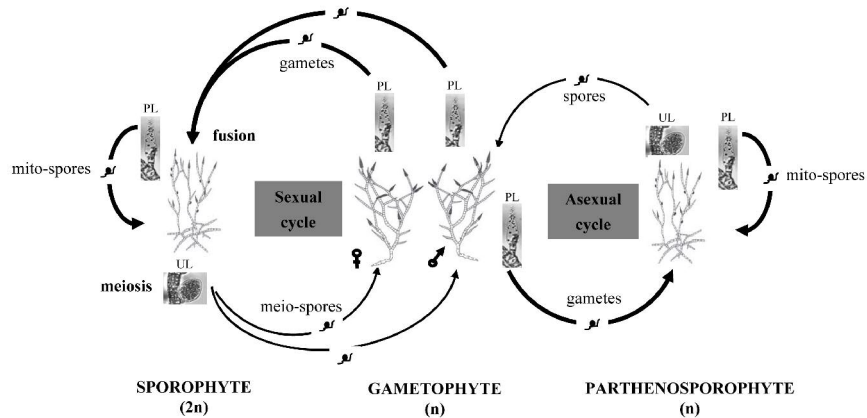


Figure 2: Life cycle of *Ectocarpus siliculosus*. Diploid sporophytes produce meio-spores (by meiosis) in unilocular sporangia (UL). Meio-spores grow into male or female gametophytes (dioecism). Gametophytes produce gametes in plurilocular gametangia (PL). Fusion of gametes produces a zygote that grows into a diploid sporophyte, completing the sexual cycle. Unfused gametes may grow parthenogenetically and form a parthenosporophyte, which is indistinguishable from the diploid sporophyte. Both sporophytes and parthenosporophytes can reproduce themselves asexually by the production of mito-spores in plurilocular sporangia.

254x190mm (300 x 300 DPI)

Figure 3

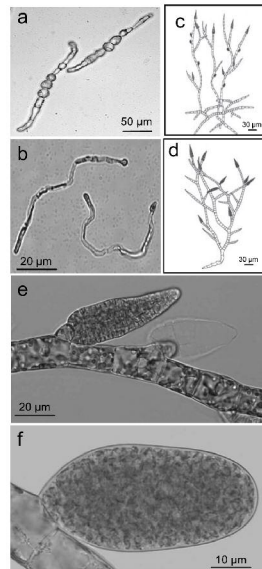


Figure 3: Morphology of *Ectocarpus siliculosus*. Photographies of one week old vegetative sporophyte (a) and gametophyte (b) and schemes representing the whole body of the mature sporophyte (c) and gametophyte (d) after six weeks of growth, are shown. e: Plurilocular sporangium or gametangium (occurring on the sporophyte and the gametophyte, respectively) before (left) and after (right) release of zoids. f: Unilocular sporangium from sporophyte. Sporangia and gametangia can be either sessile or pedicellate (Kim & Lee, 1992).

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Figure 4

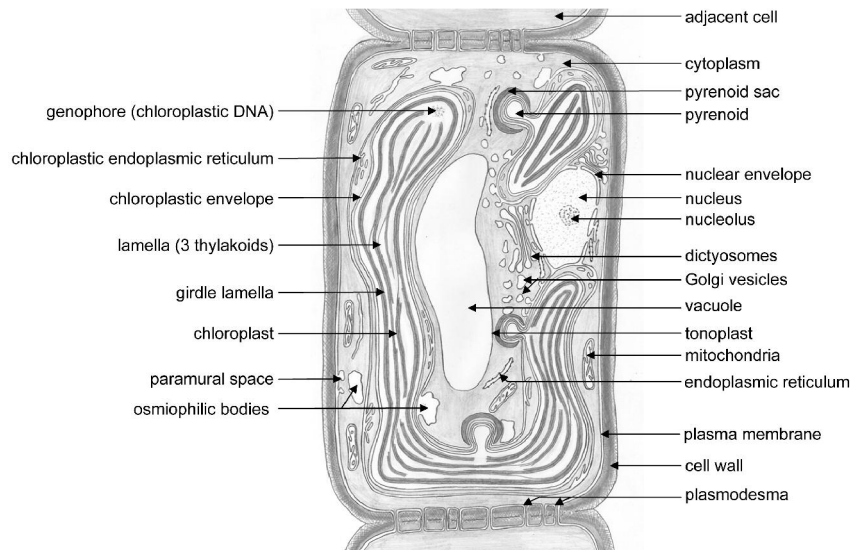


Figure 4: General ultrastructure of a vegetative cell of *Ectocarpus siliculosus*. The general ultrastructure of a vegetative cell is similar in both prostrate and erect filaments (Oliveira & Bisalputra, 1973). The different compartments of the cell are illustrated (see text for details). Lines represent membranes and define sub-cellular compartments, except for thylakoids, drawn as a thick black line. Depending on their type and age, vegetative cell size varies from 10 to 35 μ m in length, and 5 to 15 μ m in width (in laboratory culture conditions).

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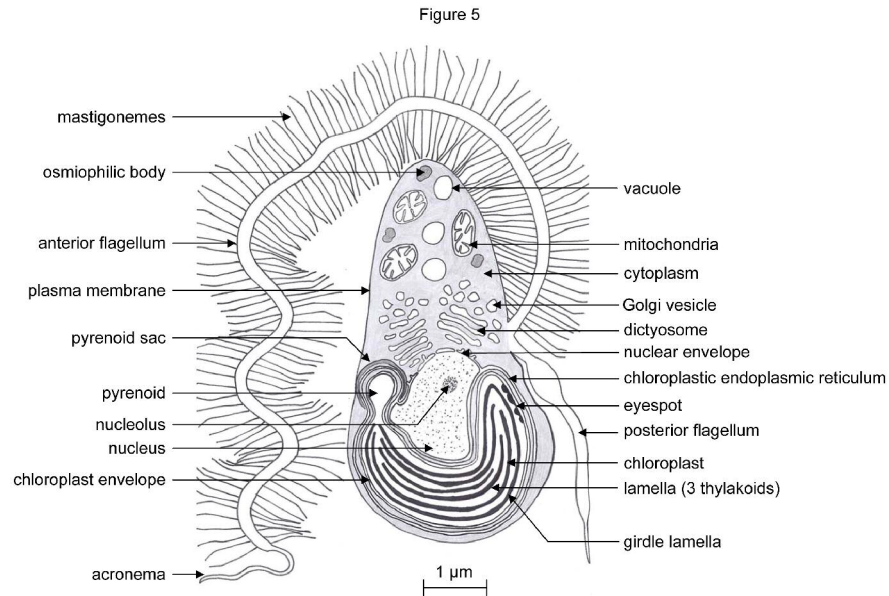


Figure 5: General intracellular structure of *Ectocarpus siliculosus* zoids. A scheme representing the different compartments of an *Ectocarpus siliculosus* zoid cell from a plurilocular sporangium or gametangium is presented (see text for details). Legends are the same as in Fig. 4.

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