THE MODEL SYSTEM ECTOCARPUS: INTEGRATING FUNCTIONAL GENOMICS INTO BROWN ALGAL RESEARCH

J. Mark Cock

THE MODEL SYSTEM *ECTOCARPUS*: INTEGRATING FUNCTIONAL GENOMICS INTO BROWN ALGAL RESEARCH

J. Mark Cock
Algal Genetics Group, UMR 8227, CNRS, Sorbonne Université, UPMC University Paris 06, Paris, France
Integrative Biology of Marine Models, Station Biologique de Roscoff, CS 90074, F-29688 Roscoff, France

Author for correspondence: e-mail: cock@sb-roscoff.fr

Much of the detailed knowledge we have today in biology was obtained through the study of a limited number of model organisms, with often one or a small number of models serving as key reference species for entire phylogenetic lineages (Davis 2004). For example *Escherichia coli, Saccharomyces cerevisiae, Drosophila melanogaster* and *Arabidopsis thaliana* have played extremely important roles in investigating diverse biological features of eubacteria, fungi, animals and land plants, respectively.

As members of the stramenopiles, the brown algae (Phaeophyceae) are very distantly related to the most commonly used eukaryotic model organisms, whether they be from the animal, fungi or land plant lineages, and these classical models are therefore of limited relevance for the study of the many unusual features that are specific to the brown algal lineage. Based on this observation, Peters et al. (2004) proposed the adoption of *Ectocarpus* as a central model organism for brown algal research. This choice was based primarily on the small size of its nuclear genome, the small size of the organism itself and the capacity to complete its sexual life cycle in a relatively short time (about three months) in the laboratory. *Ectocarpus* was also chosen because a considerable body of earlier work, principally from Dieter Müller's laboratory in Konstanz, had demonstrated its potential as a model organism and had already led to several important discoveries including life cycle structure, its sexual pheromone and an inserted viral genome (Müller 1964, 1967, 1991, Müller et al. 1971, Maier 1995, Müller and Eichenberger 1997). This commentary aims both to provide a rapid overview of the resources that have been made available since *Ectocarpus*[1] was proposed as a model system (Fig.) and to encourage brown algae phycologists to integrate this model system into their current research programs, even if those programs are focused on other brown algal species or address questions in scientific domains, such as population genetics for example, where a functional genomics approach may not seem immediately relevant.

The key requirements for a model organism are the availability of a high-quality reference genome sequence together with tools that allow exploration of the function of genomic features, particularly genes. The *Ectocarpus* genome was the first to be characterised for a macroalga (Cock et al. 2010) and the quality of the annotation of the genome has been steadily improved since the release of the first version in 2010, notably with a second, updated version being made available in 2017 (Cormier et al. 2017) and recent improvements to the chromosome-scale assembly based on chromosome contact (Hi-C) data (Baudry et al. 2020). The most recent version of the genome, housed in the Orcae database (Sterck et al. 2012), not only includes protein-coding genes but also various types of non-coding gene, including microRNAs (Tarver et al. 2015) and long non-coding RNAs (Cormier et al. 2017).

Both forward and reverse genetic approaches are now available to explore gene function in *Ectocarpus*. Forward genetics was initially developed using a classical positional cloning (genetic mapping) approach (Macaisne et al. 2017) but a more efficient cloning-by-sequencing method is now available (Godfroy et al. 2017). Quantitative genetics has also been applied in *Ectocarpus*, and

[1] Note that the currently accepted species *Ectocarpus siliculosus* actually consists of several species (Montecinos et al. 2017) and the reference genome strain (strain Ec32) does not appear to be *Ectocarpus siliculosus*. I have therefore used "*Ectocarpus*" here to avoid confusion.

validated by the identification of quantitative trait loci (Avia et al. 2017). The availability of increasingly dense genetic maps has been an important factor both in the development of these genetic approaches and as information that was used to construct the chromosome-scale assembly of the genome (Heesch et al. 2010, Avia et al. 2017, Cormier et al. 2017). These genetic maps provide extensive markers for future mapping projects.

As far as reverse genetics is concerned, RNA interference has been shown to work in *Ectocarpus* (Macaisne et al. 2017, Godfroy et al. 2017), using a method adapted from *Fucus* (Farnham et al. 2013). However, the current method is inefficient, as introduced small interfering RNAs affect only a small percentage of treated individuals and the approach is only suitable when visible phenotypes can be detected early during development. A major recent advance has therefore been the establishment of a CRISPR-Cas9-based protocol that allows the generation of targeted gene knockouts (Badis et al. 2021). In theory this approach now allows any *Ectocarpus* gene to be mutated in order to investigate its function, although, as with any system of this type, lethality or functional redundancy can potentially cause problems for some target genes.

The expression pattern of a gene often provides useful information about is potential function and can be important in selecting genes for further analysis using a reverse genetic approach. Extensive RNA-seq data is now available for *Ectocarpus* and an interactive website has recently been set up to facilitate analysis of this data (https://rnaseqaggregator.sb-roscoff.fr/ectocarpus/), allowing the expression patterns of multiple genes to be investigated across various life cycle and developmental stages and tissues. Proteomic methodologies are also available (Ritter et al. 2014) and stramenopile-adapted bioinformatic tools have been developed to predict the subcellular localisation of proteins (Hectar at https://webtools.sb-roscoff.fr/; ASAFind at https://rocaplab.ocean.washington.edu/tools/asafind/; Gschloessl et al. 2008, Gruber et al. 2015). Chromatin status is also a very useful indicator of the functional state of genome features. *Ectocarpus* DNA is not methylated (Cock et al. 2010), but an inventory of histone post-translational modifications has recently been carried out and a ChIP-seq protocol has been developed to assay the genome-wide distribution of these modifications (Bourdareau et al. 2021, 2022).

With the above methodologies in hand, it is now possible to use *Ectocarpus* as a model system to efficiently explore the functions of brown algal genes in a broad range of contexts including cell biology, development, metabolism, reproduction, interactions with other organisms, resistance to stress, *etc*.. To give just one example, analysis of *Ectocarpus* mutants has led to the identification of two TALE homeodomain proteins that are required for deployment of the sporophyte developmental program, providing new tools to manipulate the life cycle genetically and allowing new insights into the evolution and molecular basis of brown algal life cycles in general (Arun et al. 2019).

In the brown algal community, many laboratories are interested in questions concerning the evolution, diversification and biogeography of brown algal species and populations, and it may not be immediately obvious how a model organism approach might be relevant to these questions. Interestingly, although work on classical model systems in other lineages often initially tended to focus on limited numbers of laboratory strains and to ignore the organisms' ecology and natural variability, it was quickly realised that integrating natural variation into model system research not only provided a means to obtain new insights into gene and genome function but also allowed the tools developed for a model organism to be brought to bear on ecological or population-related questions (Cutter et al. 2009, Weigel 2012, Takou et al. 2019, Haudry et al. 2020, Bai et al. 2022). Work on natural populations of *Ectocarpus* are complicated by the need to analyse field isolates in the laboratory to distinguish *Ectocarpus* from several similar filamentous Ectocarpales species and to distinguish different species within the genus. This requirement somewhat limits the scope for population-based approaches but note that several studies have used large-scale sampling of field populations to address taxonomic or ecological questions (Peters et al. 2010, Couceiro et al. 2015,

Montecinos et al. 2017). However, analysis of natural *Ectocarpus* populations is clearly not relevant to all research questions, which are often focused on major constituents of coastal ecosystems such as Fucales or Laminariales species or on species of economic relevance for aquaculture. An alternative strategy in such cases would be to couple work on the brown alga of interest with targeted functional analyses in *Ectocarpus*. For example, work on another brown algal species that implicates specific sets of genes or specific genetic loci in a particular process could be complimented by an analysis of the functions of *Ectocarpus* orthologues of those genes, or at least a selected subset of the genes. The sets of genes or genetic loci of interest in the study species could be derived from different types of analysis, for example transcriptomic analysis of gene expression under different, environmentally-relevant conditions or from genome scans for genes associated with specific phenotypes or correlated with population structure. Several groups are developing QTL and GWAS approaches for brown algal species and these are expected to identify loci of potential interest for functional analyses. Moreover, approaches that combine population genetics with model-system-enabled functional genomics will be of increasing interest with the growing use of genomic approaches to address biogeographic and evolutionary questions in brown algal research (e.g. Bringloe et al. 2021). One particularly interesting example in this respect is a recent study that used a population genetic approach to identify loci that appear to be linked to the domestication of *Undaria pinnatifida* (Graf et al. 2021). Analysis of the predicted functions of genes within a locus that appeared to be under selection during domestication identified an interesting gene with a role in mannitol metabolism that would be a prime candidate for functional analysis in *Ectocarpus*.

Future development of additional resources for *Ectocarpus* is expected to make this system even more attractive as a model system. For example, CRISPR-Cas9-based gene editing is currently limited to creating mis-repair mutations that knock out gene function but there is scope for improving on the existing methodology to edit genes more precisely to create specific new alleles or proteins tagged with additional domains to allow subcellular localisation or to monitor interactions with other cellular components. Similarly, additional genomic and transcriptomic data would also be very useful. Genomic sequence variation within and between species can provide important insights into the evolutionary histories of genes and allow evaluation of the selection pressures they are under. The Phaeoexplorer project (https://phaeoexplorer.sb-roscoff.fr/home/) is generating complete, annotated genome sequences for 12 additional *Ectocarpus* species but it would also be interesting to have more information about intraspecific sequence variation as a tool to study gene evolution. Similarly, transcriptomic data is available for multiple life cycle and reproductive stages but more precise transcriptomes, perhaps based on single cell analyses, would be extremely useful to dissect genome function during development. But perhaps the most important advances will come not from the development of additional tools and resources but from creative integration of *Ectocarpus* as a model system approach into projects addressing diverse aspects of brown algal biology, including projects concerned with evolutionary, ecological, biogeography or population genetics questions.

References

Arun, A., Coelho, S.M., Peters, A.F., Bourdareau, S., Pérès, L., Scornet, D., Strittmatter, M. Lipinska, A.P., Yao, H., Godfroy, O., Montecinos, G.J., Avia, K., Macaisne, N., Troadec, C., Bendahmane, A. and Cock, J.M. 2019. Convergent recruitment of TALE homeodomain life cycle regulators to direct sporophyte development in land plants and brown algae. *eLife*. 8:e43101.

Avia, K., Coelho, S.M., Montecinos, A.E., Cormier, A., Lerck, F., Mauger, S., Faugeron, S., Valero, M., Cock, J.M. and Boudry, P. 2017. High-density genetic map and identification of QTLs for responses to high temperature and low salinity stresses in the model alga *Ectocarpus* sp. *Sci. Rep.* 7:43241.

Badis, Y., Scornet, D., Harada, M., Caillard, C., Godfroy, O., Raphalen, M., Gachon, C.M.M. , Coelho, S.M., Motomura, T., Nagasato, C. and Cock, J.M. 2021. Targeted CRISPR-Cas9-

based gene knockouts in the model brown alga *Ectocarpus*. *New Phytol.* 231:2077–91.
Bai, F.-Y., Han, D.-Y., Duan, S.-F. & Wang, Q.-M. 2022. The Ecology and Evolution of the Baker's Yeast *Saccharomyces cerevisiae*. *Genes*. 13:230.
Baudry, L., Guiglielmoni, N., Marie-Nelly, H., Cormier, A., Marbouty, M., Avia, K., Mie, Y.L. et al. 2020. instaGRAAL: chromosome-level quality scaffolding of genomes using a proximity ligation-based scaffolder. *Genome Biol.* 21:148.
Bourdareau, S., Godfroy, O., Gueno, J., Scornet, D., Coelho, S.M., Tirichine, L. & Cock, J.M. 2022. An Efficient Chromatin Immunoprecipitation Protocol for the Analysis of Histone Modification Distributions in the Brown Alga *Ectocarpus*. *Methods Protoc.* 5:36.
Bourdareau, S., Tirichine, L., Lombard, B., Loew, D., Scornet, D., Wu, Y., Coelho, S.M. and Cock, J.M. 2021. Histone modifications during the life cycle of the brown alga *Ectocarpus*. *Genome Biol.* 22:12.
Bringloe, T.T., Zaparenkov, D., Starko, S., Grant, W.S., Vieira, C., Kawai, H., Hanyuda, T. et al. 2021. Whole-genome sequencing reveals forgotten lineages and recurrent hybridizations within the kelp genus *Alaria* (Phaeophyceae). *J. Phycol.* 57:1721–38.
Cock, J.M., Sterck, L., Rouzé, P., Scornet, D., Allen, A.E., Amoutzias, G., Anthouard, V. et al. 2010. The *Ectocarpus* genome and the independent evolution of multicellularity in brown algae. *Nature*. 465:617–21.
Cormier, A., Avia, K., Sterck, L., Derrien, T., Wucher, V., Andres, G., Monsoor, M., Godfroy, O, Lipinska, A., Perrineau, M.-M., Van De Peer, Y., Hitte, C., Corre, E., Coelho, S.M. and Cock, J.M. 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 Phytol.* 214:219–32.
Couceiro, L., Le Gac, M., Hunsperger, H.M., Mauger, S., Destombe, C., Cock, J.M., Ahmed, S. et al. 2015. Evolution and maintenance of haploid-diploid life cycles in natural populations: The case of the marine brown alga *Ectocarpus*. *Evolution*. 69:1808–22.
Cutter, A.D., Dey, A. & Murray, R.L. 2009. Evolution of the *Caenorhabditis elegans* genome. *Mol. Biol. Evol.* 26:1199–234.
Davis, R.H. 2004. The age of model organisms. *Nat. Rev. Genet.* 5:69–76.
Dittami, S., Scornet, D., Petit, J., Ségurens, B., Da Silva, C., Corre, E., Dondrup, M. 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 Biol.* 10:R66.
Farnham, G., Strittmatter, M., Coelho, S.M., Cock, J.M. & Brownlee, C. 2013. Gene silencing in *Fucus* embryos: developmental consequences of RNAi-mediated cytoskeletal disruption. *J Phycol*. 49:819–29.
Godfroy, O., Uji, T., Nagasato, C., Lipinska, A.P., Scornet, D., Peters, A.F., Avia, K. et al. 2017. DISTAG/TBCCd1 Is Required for Basal Cell Fate Determination in *Ectocarpus*. *Plant Cell*. 29:3102–22.
Graf, L., Shin, Y., Yang, J.H., Choi, J.W., Hwang, I.K., Nelson, W., Bhattacharya, D. et al. 2021. A genome-wide investigation of the effect of farming and human-mediated introduction on the ubiquitous seaweed *Undaria pinnatifida*. *Nat. Ecol. Evol.*
Gruber, A., Rocap, G., Kroth, P.G., Armbrust, E.V. & Mock, T. 2015. Plastid proteome prediction for diatoms and other algae with secondary plastids of the red lineage. *Plant J.* 81:519–28.
Gschloessl, B., Guermeur, Y. & Cock, J. 2008. HECTAR: a method to predict subcellular targeting in heterokonts. *BMC Bioinf.* 9:393.
Haudry, A., Laurent, S. & Kapun, M. 2020. Population Genomics on the Fly: Recent Advances in *Drosophila*. *Methods Mol. Biol. Clifton NJ*. 2090:357–96.
Heesch, S., Cho, G.Y., Peters, A.F., Le Corguillé, G., Falentin, C., Boutet, G., Coëdel, S., Jubin, C., Samson, G., Corre, E., Coelho, S.M. and Cock, J.M. 2010. A sequence-tagged genetic map for the brown alga *Ectocarpus siliculosus* provides large-scale assembly of the genome sequence. *New Phytol*. 188:42–51.

Macaisne, N., Liu, F., Scornet, D., Peters, A.F., Lipinska, A., Perrineau, M.-M., Henry, A., Strittmatter, M., Coelho, S.M. and Cock, J.M. 2017. The *Ectocarpus IMMEDIATE UPRIGHT* gene encodes a member of a novel family of cysteine-rich proteins with an unusual distribution across the eukaryotes. *Development*. 144:409–18.

Maier, I. 1995. Brown algal pheromones. *In* Round, F. E. & Chapman, D. J. [Eds.] *Progress in Phycological Research*. Biopress, Bristol, pp. 51–102.

Montecinos, A.E., Couceiro, L., Peters, A.F., Desrut, A., Valero, M. & Guillemin, M.-L. 2017. Species delimitation and phylogeographic analyses in the *Ectocarpus* subgroup *siliculosi* (Ectocarpales, Phaeophyceae). *J. Phycol.* 53:17–31.

Müller, D.G. 1964. Life-cycle of *Ectocarpus siliculosus* from Naples, Italy. *Nature*. 26:1402.

Müller, D.G. 1967. Generationswechsel, Kernphasenwechsel und Sexualität der Braunalge *Ectocarpus siliculosus* im Kulturversuch. *Planta*. 75:39–54.

Müller, D.G. 1991. Mendelian segregation of a virus genome during host meiosis in the marine brown alga *Ectocarpus siliculosus*. *J Plant Physiol*. 137:739–43.

Müller, D.G. & Eichenberger, W. 1997. Mendelian genetics in brown algae: inheritance of a lipid defect mutation and sex alleles in *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae). *Phycologia*. 36:79–81.

Müller, D.G., Jaenicke, L., Donike, M. & Akintobi, T. 1971. Sex Attractant in a Brown Alga: Chemical Structure. *Science*. 171:815–7.

Peters, A.F., Mann, A.D., Córdova, C.A., Brodie, J., Correa, J.A., Schroeder, D.C. & Cock, J.M. 2010. Genetic diversity of *Ectocarpus* (Ectocarpales, Phaeophyceae) in Peru and northern Chile, the area of origin of the genome-sequenced strain. *New Phytol.* 188:30–41.

Peters, A.F., Marie, D., Scornet, D., Kloareg, B. & Cock, J.M. 2004. Proposal of *Ectocarpus siliculosus* (Ectocarpales, Phaeophyceae) as a model organism for brown algal genetics and genomics. *J Phycol*. 40:1079–88.

Ritter, A., Dittami, S.M., Goulitquer, S., Correa, J.A., Boyen, C., Potin, P. & Tonon, T. 2014. Transcriptomic and metabolomic analysis of copper stress acclimation in *Ectocarpus siliculosus* highlights signaling and tolerance mechanisms in brown algae. *BMC Plant Biol.* 14:116.

Sterck, L., Billiau, K., Abeel, T., Rouzé, P. & Van de Peer, Y. 2012. ORCAE: online resource for community annotation of eukaryotes. *Nat Methods*. 9:1041.

Takou, M., Wieters, B., Kopriva, S., Coupland, G., Linstädter, A. & De Meaux, J. 2019. Linking genes with ecological strategies in *Arabidopsis thaliana*. *J. Exp. Bot.* 70:1141–51.

Tarver, J.E., Cormier, A., Pinzón, N., Taylor, R.S., Carré, W., Strittmatter, M., Seitz, H., Coelho, S.M. and Cock, J.M. 2015. microRNAs and the evolution of complex multicellularity: identification of a large, diverse complement of microRNAs in the brown alga *Ectocarpus*. *Nucl Acids Res*. 43:6384–98.

Weigel, D. 2012. Natural variation in Arabidopsis: from molecular genetics to ecological genomics. *Plant Physiol.* 158:2–22.

Funding

This research was funded by the Centre National de la Recherche Scientifique and the Agence Nationale de la Recherche project Epicycle (ANR-10- BTBR-04-01).

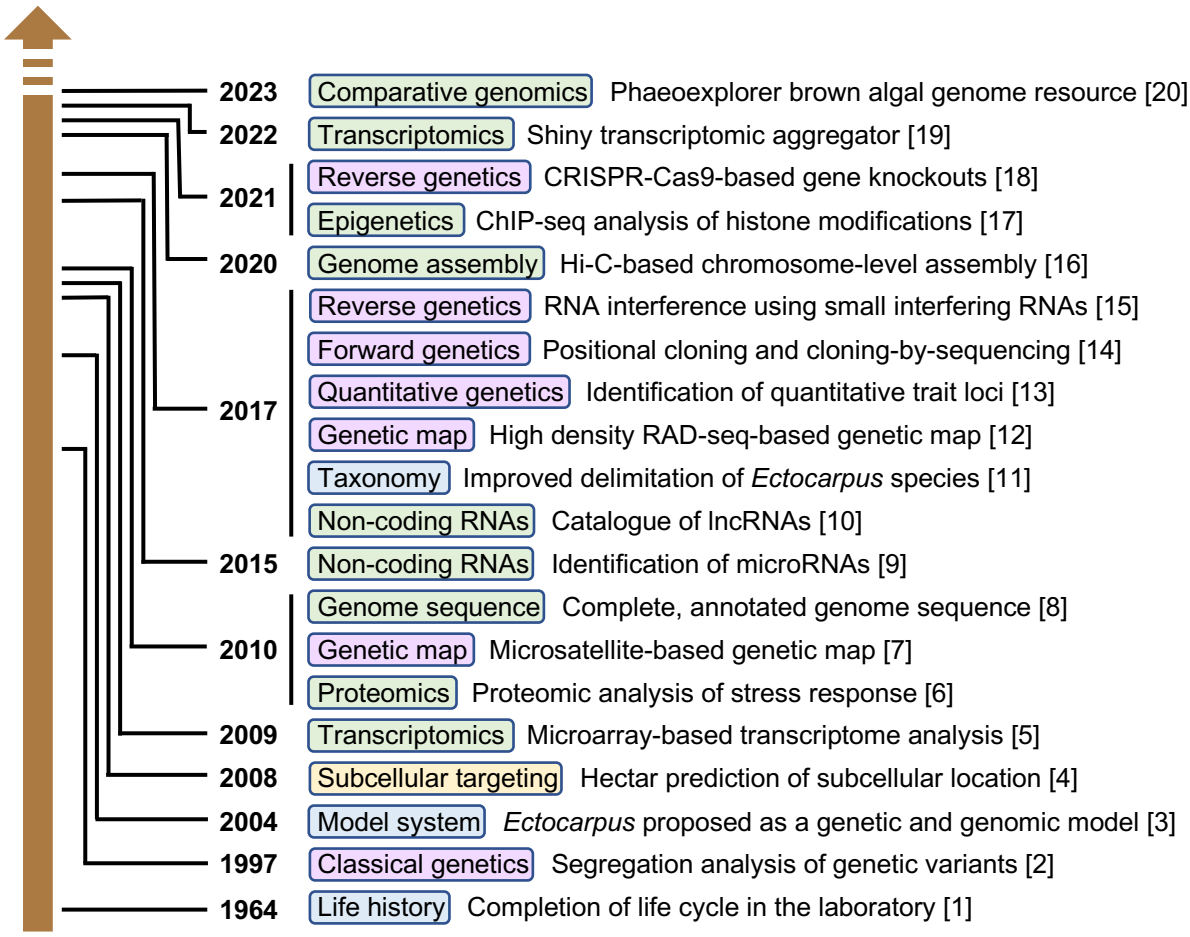
2023
Comparative genomics
Phaeoexplorer brown algal genome resource [20]
2022
Transcriptomics
Shiny transcriptomic aggregator [19]
2021
Reverse genetics
CRISPR-Cas9-based gene knockouts [18]
Epigenetics
ChIP-seq analysis of histone modifications [17]
2020
Genome assembly
Hi-C-based chromosome-level assembly [16]
2017
Reverse genetics
RNA interference using small interfering RNAs [15]
Forward genetics
Positional cloning and cloning-by-sequencing [14]
Quantitative genetics
Identification of quantitative trait loci [13]
Genetic map
High density RAD-seq-based genetic map [12]
Taxonomy
Improved delimitation of Ectocarpus species [11]
Non-coding RNAs
Catalogue of lncRNAs [10]
2015
Non-coding RNAs
Identification of microRNAs [9]
2010
Genome sequence
Complete, annotated genome sequence [8]
Genetic map
Microsatellite-based genetic map [7]
Proteomics
Proteomic analysis of stress response [6]
2009
Transcriptomics
Microarray-based transcriptome analysis [5]
2008
Subcellular targeting
Hectar prediction of subcellular location [4]
2004
Model system
Ectocarpus proposed as a genetic and genomic model [3]
1997
Classical genetics
Segregation analysis of genetic variants [2]
1964
Life history
Completion of life cycle in the laboratory [1]

Figure legend

FIG. Timeline indicating the establishment of tools and resources for the model brown alga *Ectocarpus*. Colours indicate biological (blue), genetic (mauve), genomic (green) and bioinformatic (orange) resources. References are 1, (Müller 1964); 2, (Müller and Eichenberger 1997); 3, (Peters et al. 2004); 4, (Gschloessl et al. 2008, Gruber et al. 2015); 5, (Dittami et al. 2009); 6, (Ritter et al. 2014); 7, (Heesch et al. 2010); 8, (Cock et al. 2010); 9, (Tarver et al. 2015); 10, (Cormier et al. 2017); 11, (Montecinos et al. 2017); 12, (Cormier et al. 2017); 13, (Avia et al. 2017); 14, (Godfroy et al. 2017, Macaisne et al. 2017); 15, (Macaisne et al. 2017); 16, (Baudry et al. 2020); 17, (Bourdareau et al. 2021); 18, (Badis et al. 2021); 19, https://rnaseqaggregator.sb-roscoff.fr/ectocarpus/; 20, https://phaeoexplorer.sb-roscoff.fr/home/.