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Origin and evolution of sex-determination systems in the brown algae

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Summary

Sexual reproduction is a nearly universal feature of eukaryotic organisms. Meiosis appears to have had a single ancient origin but the mechanisms underlying male or female sex determination are diverse and have emerged repeatedly and independently in the different eukaryotic groups. The brown algae are a group of multicellular photosynthetic eukaryotes that have a distinct evolutionary history compared with animals and plants, as they have been evolving independently for over a billion years. Here, we review recent work using the brown alga *Ectocarpus* as a model organism to study haploid sex chromosomes, and highlight how the diversity of reproductive and life cycle features of the brown algae offer unique opportunities to characterise the evolutionary forces and the mechanisms underlying the evolution of sex determination.

Introduction

Brown algal sexual reproduction has been studied for many years and it has long been known, for example, that female gametes attract males by chemical signals consisting of species-specific pheromones (Müller *et al.*, 1971). However, although early work indicated that sex was determined by genetic factor(s) during meiosis (Müller, 1975), the mechanisms of sex determination and differentiation in this major group of complex multicellular eukaryotes have only recently started to be understood. In this insight article, we highlight recent advances on how brown algae are contributing to increase our knowledge about the molecular process and evolutionary forces involved in sex determination.

Unusual features of UV sex chromosome systems

Whilst meiotic sex is believed to be very ancient and to have a single evolutionary origin (Speijer *et al.*, 2015), sexual systems involving fusion of gametes produced by separate sexes have emerged several times independently across the diverse eukaryote supergroups (Bachtrog *et al.*, 2011). For many years, our understanding of the mechanisms underlying sex determination was derived from research on X and Y chromosomes in humans and other animal model species, leading to the impression that sex determination mechanisms are old and conserved. In recent years,

33 however, the taxonomic breadth of study organisms has been considerably expanded, and an influx
34 of new genomic data has shown that sexes in eukaryotes are determined by a remarkable diversity
35 of mechanisms, ranging from purely epigenetic to purely genetic, and that these mechanisms may
36 evolve extremely rapidly in many taxa (Beukeboom & Perrin, 2015).

37 The sex of an organism can either be determined by environmental (or developmental) cues
38 or can be determined genetically by a sex locus or sex chromosome. For systems where sex is
39 determined genetically, one important factor that influences the nature of sex determination is the
40 stage of the life cycle when sex is determined. In animals and flowering plants, XX/XY or ZW/ZZ
41 systems determine sex during the diploid phase of the life cycle, but in many other eukaryotes,
42 including bryophytes and most brown, red and green algae, sex is determined during the haploid
43 phase, i.e., during the gametophyte generation (Coelho *et al.*, 2018). In these haploid phase sex
44 determination systems, sex is determined by U and V sex chromosomes, which are present in the
45 female and male gametophytes respectively (Figure 1A).

46 Analysis of XY/XX systems led to the proposition of a model for sex chromosome evolution
47 that begins with the acquisition of a sex-determining locus by an autosome to create a proto-Y and
48 proto-X chromosome pair (reviewed in Charlesworth *et al.*, 2005; Bachtrog *et al.*, 2011;
49 Charlesworth, 2015; Wright *et al.*, 2016). When sexually antagonistic genes (i.e. genes with alleles
50 that are advantageous to one sex but deleterious to the other) arise on the proto-Y chromosome,
51 this is thought to favour the suppression of recombination between male-beneficial alleles of these
52 genes and the sex-determining locus to ensure that these alleles are always inherited by males. This
53 loss of recombination is expected to lead to degeneration of the non-recombining region due to
54 Hill-Robertson effects. X chromosomes, on the other hand, are able to recombine in XX females and
55 therefore do not exhibit the same pattern of degeneration as the Y. ZW/ZZ systems are thought to
56 evolve in a similar manner to XY/XX systems, with the W chromosome having a similar evolutionary
57 fate to the Y.

58 U/V systems are expected to exhibit unique evolutionary dynamics compared to XY/XX or
59 ZW/ZZ systems because of their specific mode of inheritance (Coelho *et al.*, 2018). Contrary to XY
60 and ZW systems, where males are XY and females ZW, there is no heterogametic sex in UV systems,
61 because the U and V chromosomes function in independent individuals, the female and male, which
62 are both haploid. The U and V are expected to be under similar evolutionary pressures because 1)
63 they function independently in different haploid individuals and therefore there is no masking of
64 defective alleles and 2) because the sex-determining regions (SDRs) of the U and the V never (or
65 only very rarely) recombine (whereas X and Z chromosomes can recombine in females and males
66 respectively; Bull, 1978; Immler & Otto, 2015). Consequently, U and V SDRs are predicted to evolve
67 in a symmetrical manner and to degenerate more slowly than the SDRs of XY or ZW systems (Figure
68 1B). Note that deleterious mutations in SDR genes can still be masked if the genes function only

69 during the diploid sporophyte phase and this may allow both the U and V chromosomes to
70 degenerate to some extent (Lewis, 1961; Immler & Otto, 2015).

71 When the different types of brown algal sexual system are mapped onto a phylogenetic tree,
72 the distribution pattern indicates that there has been considerable switching between sexual
73 systems during evolution (Silberfeld *et al.*, 2010)(Figure 1A). Dioicy (i.e., separate sexes during the
74 haploid phase of the life cycle) appears to have been the ancestral state but many extant species
75 have co-sexual haploid sex determination systems (i.e. are monoicous) and there has been a
76 transition to diploid sex determination in the order Fucales, which includes both dioecious and
77 monoecious species. This broad range of sex determination systems makes the brown algae a very
78 interesting group to investigate the origin and evolution of sexual systems.

79 The UV sex chromosomes of *Ectocarpus* sp.

80 In recent years, the brown alga *Ectocarpus* sp. has emerged as a model organism to test
81 longstanding theoretical predictions about the evolution of haploid UV systems (Ahmed *et al.*, 2014;
82 Luthringer *et al.*, 2015a). The *Ectocarpus* sp. U and V SDRs are non-recombining regions of similar
83 size and structure (each contains about 20 genes; Figure 1B) and, overall, SDR genes tend to be
84 expressed at lower levels than autosomal genes. The SDR regions also exhibit accumulation of
85 transposable elements (TEs), lower optimal codon usage and low DNA diversity (Ahmed *et al.*, 2014;
86 Avia *et al.*, 2018). Evolutionary analyses have indicated that many of the SDR genes are evolving
87 rapidly, and that this is due to relaxed purifying selection (Lipinska *et al.*, 2017). Together, these
88 features indicate that there is a certain amount of genetic degeneration of the *Ectocarpus* SDR, even
89 if less marked than in diploid systems. It is not surprising that a certain amount of degeneration is
90 observed, considering that these regions stopped recombining at least 100 Mya (Ahmed *et al.*,
91 2014).

92 Work on *Ectocarpus* has also revealed some interesting features of the pseudoautosomal
93 regions (PAR) of the sex chromosomes, i.e. the recombining regions of the chromosome that flank
94 the SDR (Luthringer *et al.*, 2015b). Although these regions recombine at a similar rate to the
95 autosomal regions of the genome, they exhibit many genomic characteristics typical of non-
96 recombining regions, such as TE enrichment and low gene density. Remarkably, these regions also
97 contain clusters of genes that are significantly upregulated in the sporophyte. A modelling-based
98 approach indicated that the evolutionary trajectory of the PAR in haploid systems can be impacted
99 by sporophyte-beneficial alleles that are under differential levels of sexual selection (Luthringer *et al.*
100 *et al.*, 2015b). The PAR of *Ectocarpus* exhibits surprisingly high levels of neutral diversity and several
101 lines of evidence indicate that genes in this region may be under balancing selection. It will also be
102 of interest to determine if the enrichment of life-cycle regulated genes in the PAR observed for
103 *Ectocarpus* is also a feature of other UV sex chromosome systems.

104 Evolution of U and V sex chromosomes

105 The brown algae exhibit a bewildering variety of sexual systems and types of life cycle,
106 implying a considerable amount of switching between variants of these features on an evolutionary
107 timescale (Figure 1A). This diversity is potentially of great interest to test theoretical predictions
108 about the evolution of sexual systems. For example, following Bull's suggestion that haploid
109 purifying selection influences the evolution of UV sex chromosomes (Bull, 1978), it has been
110 suggested that the relatively low level of degeneration of the *Ectocarpus* non-recombining SDRs
111 compared with well-studied X/Y and Z/W systems could be due to the former experiencing purifying
112 selection during the haploid phase of the life cycle (Ahmed *et al.*, 2014). The Y chromosome of the
113 angiosperm *Silene latifolia* also appears to be degenerating slowly and this has similarly has been
114 attributed to the fact that plants have a (short) haploid phase during which haploid purifying
115 selection can act to maintain Y gene functionality (Lenormand & Dutheil, 2005; Chibalina & Filatov,
116 2011; Bergero *et al.*, 2015; Krasovec *et al.*, 2018). The haploid, gametophyte generation is dominant
117 in some brown algae but highly reduced in others, and it would be interesting to investigate whether
118 there is a general correlation between haploid phase complexity and the level of SDR degeneration.

119 In several sexual systems the non-recombining, sex-determining regions of the sex
120 chromosomes have been shown to expand over time, resulting in an SDR made up of sub-regions of
121 different ages, so-called 'strata' (Hughes *et al.*, 2012). SDR expansion is thought to be driven by the
122 inclusion of sexually antagonist genes as inclusion of such genes in the SDR helps ensure that these
123 genes are inherited by the sex in which they are advantageous (Charlesworth, 2017). Note, however,
124 that other forces such as meiotic drive, genetic drift and heterozygous advantage may also be
125 involved (Branco *et al.*, 2017; Ponnikas *et al.*, 2018). *Ectocarpus* male and female gametophytes are
126 morphologically similar (Lipinska *et al.*, 2015), and they produce only slightly dimorphic gametes,
127 limiting the scope for sexual conflict, and the relatively small size of the *Ectocarpus* SDR (about
128 1Mbp) may therefore reflect a low level of sexual antagonism. This low level of dimorphism may
129 explain why strata have not been detected in the *Ectocarpus* SDR (Ahmed *et al.*, 2014). In this
130 context, the range of sexual dimorphism across brown algal species could be exploited to investigate
131 this proposed role for sexual dimorphism in determining sex chromosome structure.

132 Gene content of the sex-determining region

133 Recent studies have shown that the sex chromosomes of several, diverse brown algal species
134 are derived from a common, >100 MY ancestral sex chromosome (Lipinska *et al.*, 2017).
135 Comparisons of sex chromosomes across these species have indicated that there has been a high
136 rate of turnover of SDR genes over evolutionary time, with a remarkably high level of gene
137 movement in and out the SDR (Lipinska *et al.*, 2017). Consistent with theoretical expectations (Bull,
138 1978) genes that were acquired from the autosomes appear to have been retained in the SDR

139 because they confer a sex-specific role in reproduction. Concerning the genes that have moved out
140 of the SDR, this process could be a means for these genes to "escape" the degenerating effect of
141 location within the non-recombining SDR, as has been suggested for Y-derived retrogenes in XY
142 sexual systems (Hughes *et al.*, 2015; Lipinska *et al.*, 2017).

143 Despite the high rate of SDR gene turnover, a conserved subset of genes has been stably
144 maintained in the SDR over >100 MY of evolution. One of these conserved loci, which encodes a
145 HMG domain protein, is the only sex-specific gene that has been found in the male SDR of all brown
146 algae studied so far. Moreover, the *Ectocarpus* orthologue is strongly upregulated in sexually mature
147 male gametophytes (Lipinska *et al.*, 2017). This gene is therefore, currently, the best candidate for
148 the male sex-determining gene in these species. The regulators of gender determination in many
149 animals and fungi (Graves & Peichel, 2010; Li *et al.*, 2013) also encode HMG domain proteins.
150 Consequently, if a role for the brown algal HMG genes in sex-determination is confirmed this would
151 open up very interesting questions about deep homology or independent re-use of master sex-
152 determining genes during eukaryote evolution.

153 Gene networks downstream of the sex determination locus

154 In organisms with genetic sex determination and separate sexes, the SDR (or, more
155 specifically, the sex-determining gene) is at the top of a cascade of sex differentiation loci, with most
156 of the genes involved in building a male or a female individual being located on autosomes (Parsch
157 & Ellegren, 2013). Transcriptional profiling allows gene expression to be compared between females
158 and males on a genome-wide scale, providing information about the genes that act downstream of
159 the sex-determining gene. Such studies have often identified large numbers of genes with sex-
160 biased expression patterns, although the number of sex-biased genes detected may vary greatly
161 between tissues or developmental stages (Grath & Parsch, 2016). Overall, *Ectocarpus* exhibits a low
162 level of differential gene expression (i.e. few sex-biased genes) reflecting the low level of sexual
163 dimorphism at the morphological level. In diploid systems, male-biased genes evolve faster than
164 female-biased genes, reflecting different selection levels in males and females (Meiklejohn *et al.*,
165 2003; Parsch & Ellegren, 2013). In *Ectocarpus*, in contrast, female- and male-biased genes evolve at
166 similar rates and both evolve more rapidly than unbiased genes. The similar evolutionary rates
167 found for male and female-biased genes are consistent with the fact that *Ectocarpus* present a low
168 level of sexual dimorphism, providing little scope for differences in sexual selection. A proportion of
169 the *Ectocarpus* sex-biased genes have been shown to be evolving under positive selection
170 suggesting that their more rapid rate of evolution may be driven partially by adaptive evolution.

171 Similar numbers of male- and female-biased genes were identified in *Ectocarpus* (Lipinska *et al.*
172 *et al.*, 2015). In *Fucus vesiculosus*, however, which is dioecious and exhibits diploid sex determination
173 (Figure 1A) more male- than female-biased genes were observed (Martins *et al.*, 2013). This excess

174 of male-biased genes has been interpreted as an indication that there may be a male heterogametic
175 (XY) sex chromosome system in this species.

176 Transitions between sexual systems in the brown algae

177 Brown algae have a broad range of sexual systems including both haploid and diploid sexual
178 systems, and in both cases species can either have separate sexes or be co-sexual (Figure 1A).
179 Transitions to monoicy (co-sexuality in the haploid stage of the life cycle) have occurred frequently
180 (Silberfeld *et al.*, 2010), but the proximate mechanisms, evolutionary drivers and consequences of
181 these transitions remain obscure. Insights into these questions could be obtained by studying life
182 cycle variants isolated in the field or by genetic manipulation of laboratory strains, using for example
183 RNAi (Farnham *et al.*, 2013) or by developing novel methodologies such as CRISPR/Cas9.
184 Manipulation of the U/V sex chromosome ratio in *Ectocarpus* has suggested that female identity
185 may be the default pathway (Ahmed *et al.*, 2014), activated only in the absence of the male sex-
186 determination gene. Interestingly, some genetically male individuals of the kelp *Undaria pinnatifida*
187 have been shown to produce oogonia in addition to antheridia (i.e. they are monoicous)(Li *et al.*,
188 2014). Similarly, several male *Laminaria pallida* lines from a South African population possess
189 unusual reproductive structures resembling small eggs, which are capable of parthenogenesis (Ingo
190 Maier, pers. commun.). The ability of individuals that carry a V chromosome to become monoicous
191 (i.e. produce both male and female structures) suggests that female identity may be locally activated
192 (or de-repressed) when the male-determining system is not fully active. The frontier between
193 epigenetic (or developmental) and genetic sex determination therefore appears to be fuzzy, as has
194 been suggested for the green lineage (Pannell, 2017). Closely related strains exhibiting dioicy and
195 monoicy will be valuable to access the (epigenetic) mechanisms underlying the transition to
196 monoicy. In addition, field population studies are required to understand the relevance of these
197 processes in nature, for example transitions to monoicy could allow some populations to adapt to
198 conditions of low density or fragmented habitats.

199 Brown algae also represent interesting systems to investigate transitions from haploid to
200 diploid sex determination because such a transition occurred relatively recently in the lineage
201 leading to the Fucales (Silberfeld *et al.*, 2010); Figure 1). Such transitions are expected to require an
202 intermediate stage with epigenetic sex determination because the genetic mechanism of UV
203 chromosome sex determination is very different to that of XY and ZW systems (Beukeboom & Perrin,
204 2015).

205 In conclusion, the brown algae are providing important insights into the evolution of sexual
206 systems. Future work, combining the experimental advantages of model systems such as *Ectocarpus*
207 and the broad scope of sexual system variations across the brown algae, is expected to bring
208 additional advances in the coming years.

209

210 **Figure legend**

211

212 Figure 1. Diversity of sexual systems in the brown algae. A. Evolutionary tree of the brown
 213 algae showing the major orders. Type of sexual system is indicated for representative species in
 214 each order. Sex is expressed during the diploid phase in the Fucales but during the haploid phase in
 215 all the other orders. Representative species are shown for the four main types of sexual system.
 216 White bars indicate sex chromosomes and grey regions the SDR. The sex chromosomes are circled
 217 in red during the phase where they determine sex. Note that the diploid sex determination system
 218 in *F. vesiculosus* is represented as a XX/XY system, but it could be also a ZW/WW system. B.
 219 Schematic representation of the U and V sex chromosomes of *Ectocarpus* together with a summary
 220 of how the selective pressures acting on UV chromosomes are expected to differ from those that
 221 act on XY and ZW chromosomes (Bull, 1978; Immler & Otto, 2015). The sex-determining region (SDR)
 222 and the pseudoautosomal (PAR) regions are drawn to scale based on the current version of the
 223 *Ectocarpus* genome assembly (Cormier *et al.*, 2017 and unpublished data).

224

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225

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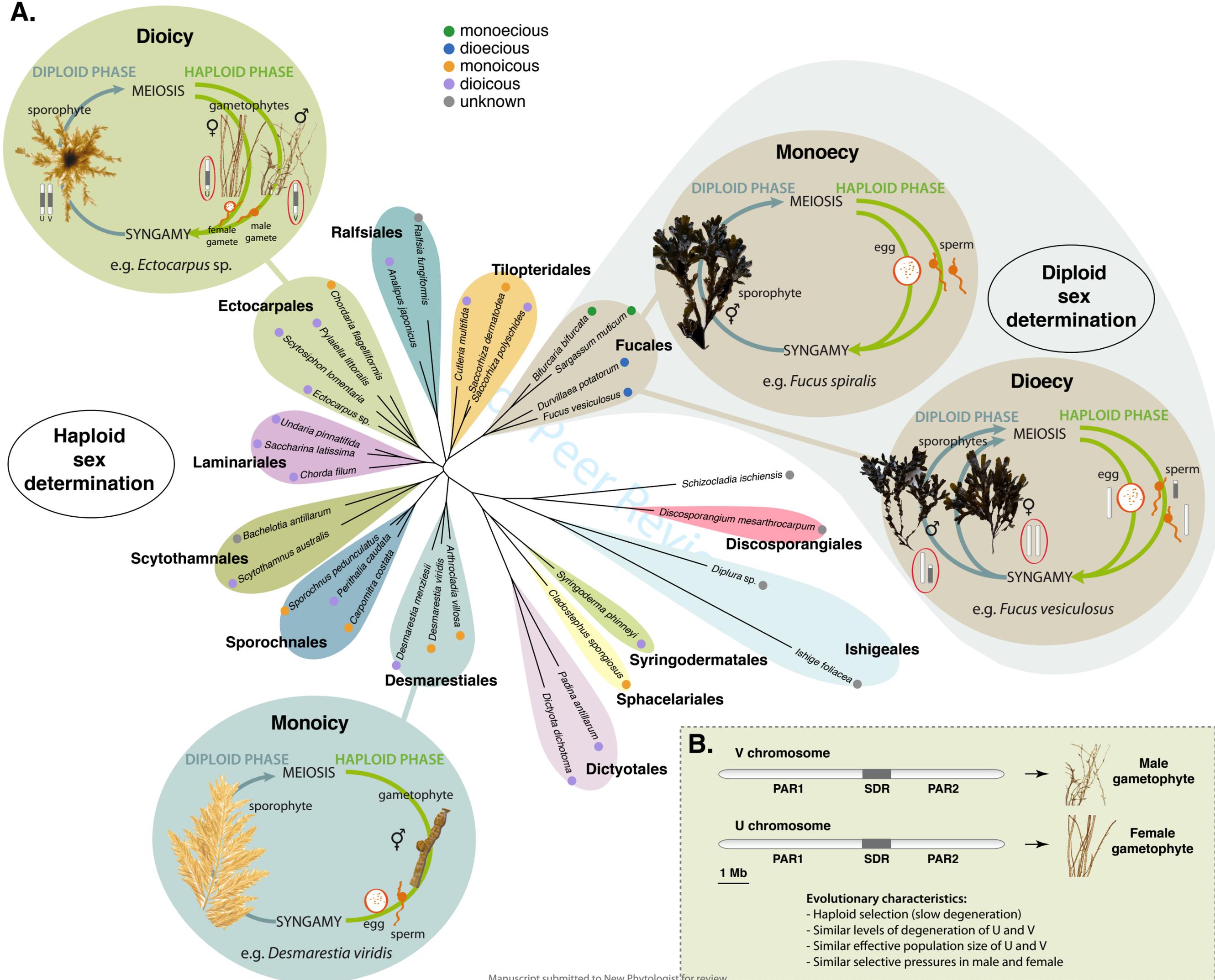
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318

For Peer Review

A.



B.

