Red macroalgae in the genomic era

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Summary

Rhodophyta (or red algae) are a diverse and species-rich group that forms one of three major lineages in the Archaeplastida, a eukaryotic supergroup whose plastids arose from a single primary endosymbiosis. Red algae are united by several features, such as relatively small intron-poor genomes and a lack of cytoskeletal structures associated with motility like flagella and centrioles, as well as a highly efficient photosynthetic capacity. Multicellular red algae (or macroalgae) are one of the earliest diverging eukaryotic lineages to have evolved complex multicellularity, yet despite their ecological, evolutionary, and commercial importance, they have remained a largely understudied group of organisms. Considering the increasing availability of red algal genome sequences, we present a broad overview of fundamental aspects of red macroalgal biology and posit on how this is expected to accelerate research in many domains of red algal biology in the coming years.

I. Introduction

The red algae (Rhodophyta) are a key group of photosynthetic eukaryotes that inhabit coastal ecosystems around the globe. From an evolutionary perspective, red algae have had a profound impact on the evolution of other life forms, having donated plastids to several other eukaryotic supergroups via secondary and tertiary endosymbiosis (Archibald, 2012). Fossil evidence suggests that red

algae may have been the first eukaryotic lineage to have evolved complex multicellularity (Strassert *et al.*, 2021), with modern-day red algae exhibiting a broad range of complexity in both form and developmental patterning. Between the 1950s and 1990s, the advent of electron and fluorescence microscopy led to studies of red algal cell biology (Dawes *et al.*, 1960; Neushul, 1971; Garbary *et al.*, 1992; Karyophyllis *et al.*, 2000), while mutant approaches were also attempted to study their underlying genetics (van der Meer, 1979; Zhang & van der Meer, 1988). Owing to the lack of genetic and genomic tools and the rather small community of phycologists at the time, these areas remained understudied. Consequently, a deep understanding of the molecular machinery underlying development and reproduction in red macroalgae has lagged behind that of green (Umen & Olson, 2012; De Clerck *et al.*, 2018; Umen, 2020; Coelho & Umen, 2021) and brown algae (Coelho *et al.*, 2011; Godfroy *et al.*, 2017, 2023; Coelho & Cock, 2020). However, the increasing availability of genetic and genomic tools (Collén *et al.*, 2013; Brawley *et al.*, 2017; Lipinska *et al.*, 2020) and their application in red macroalgae promises to invigorate new discoveries in this largely overlooked lineage of eukaryotes.

Here, we review landmark studies on the red algae that have addressed their evolutionary origins and phylogenetic relationships, their cell and reproductive biology, their complex life cycles, as well as emerging genomic resources that are furthering our understanding of eukaryotic evolution. Because model organisms are critical for detailed mechanistic analysis, we also discuss some species of red macroalgae as potential developmental model systems. As the biology of unicellular red algae has been reviewed recently (Miyagishima & Tanaka, 2021), we mainly focus our review on multicellular red algae.

II. The nature and origin of red algae

The phylum Rhodophyta is characterised by an extraordinary diversity of morphological complexity, ranging from unicellular to multicellular species that vary from a few micrometres to many centimetres in length. Red macroalgae are primarily marine organisms although *c.* 3% live solely in freshwater habitats (Sheath, 1984). A small number of extremophilic unicellular species also occur in hot springs, soil, and caves, while some multicellular species belonging to the Stylonematophyceae have even been found in sloth hair (Sheath, 1984; Cole & Sheath, 1990). No multicellular species have been reported on dry land, which is believed to be due to an evolutionary bottleneck experienced by their last common ancestor where *c.* 25% of core genes were lost, likely limiting their evolutionary plasticity (Collén *et al.*, 2013).

Red algae are thought to have arisen between the late Mesoproterozoic to early Neoproterozoic era (1.3–0.9 billion years ago; Sánchez-Baracaldo *et al.*, 2017), although an earlier appearance in the late Paleoproterozoic has also been proposed (Blank, 2013; Yang *et al.*, 2016). The oldest examples of multicellular red algae in the fossil record are that of the *c.* 1.2 billion-year-old filamentous alga *Bangiomorpha* (Butterfield, 2000) and the *c.* 1.6 billion-year-old filamentous algae *Rafatazmia* and *Denaricion* (Bengtson *et al.*, 2017), suggesting that the red algal crown group is likely to have originated well before the Paleoproterozoic era.

The first photosynthetic eukaryotes emerged through a primary endosymbiosis between a cyanobacterium and a heterotrophic eukaryotic host. The resulting primary plastids are evident in three main eukaryotic lineages – the red algae, the green algae (and their land plant relatives), and the glaucophyte algae, which together form the Archaeplastida Supergroup. Red algal-derived plastids subsequently spread to other groups through a cascade of secondary and even tertiary endosymbioses via the capture of algae with primary or secondary plastids by heterotrophic hosts, respectively (Keeling, 2010). Complex plastids with multiple membranes have arisen through these events, some of which have retained remnants of the endosymbiont nucleus (called the nucleomorph; Curtis *et al.*, 2012). This led to the diversification of several photosynthetic organisms of global ecological importance, such as diatoms, dinoflagellates, and brown algae (Keeling, 2010). Thus, ancestral red algae have contributed both nuclear and plastid genes that have profoundly impacted the evolution and diversification of several key eukaryotic lineages (Archibald, 2015a,b; Gawryluk *et al.*, 2019; Strassert *et al.*, 2021).

III. Phylogeny of red algae

The red algae are currently comprised of *c.* 7500 described species (Guiry & Guiry, 2023), the vast majority of which are multicellular and marine. Red macroalgae were classically divided into two major subdivisions based on their morphological features – the Bangiophyceae and the Florideophyceae (Fig. 1; Cole & Sheath, 1990). The Florideophyceae have complex branched thalli that are often pseudoparenchymatous with distinctive reproductive structures (discussed below), whereas the Bangiophyceae have a much simpler organisation, lacking distinct morphological features that rendered their classification more challenging. Molecular phylogenies have confirmed this traditional taxonomic separation and have grouped these two multicellular classes together in the subphyllum Eurhodophytina (Fig. 1), with the Bangiophyceae assemblage appearing to have paraphyletic origins (Yoon *et al.*, 2006; Verbruggen *et al.*, 2010; Qiu, 2016; Muñoz-Gómez *et al.*, 2017; Van Beveren *et al.*, 2022). The other two Rhodophyta subphyla correspond to divergent ancient lineages that arose at the beginning of the Mesoproterozoic (*c.* 1500 million years ago (Ma)): the Cyanidiophytina, which contain thermoacidophilic unicellular red algae (Janouškovec *et al.*, 2013), and the Proteorhodophytina, which are mainly unicellular but sometimes exhibit simple multicellular forms (Yoon *et al.*, 2006).

The Florideophyceae is the largest class comprising over 94% of currently described red algal taxa (Guiry & Guiry, 2023), with multigenic phylogenies revealing the existence of five subclasses (Le Gall & Saunders, 2007; Yang *et al.*, 2016). The earliest diverging subclass, the Hildenbrandiophycidae, contains *c.* 20 species that diverged early on during florideophyceaen evolution around the middle of the Neoproterozoic era (Yang *et al.*, 2016). The Hildenbrandiophycidae differ markedly from other florideophytes having crustose-forming thalli with no obvious report of sexual reproduction (meiosis and fertilisation) in culture thus far,

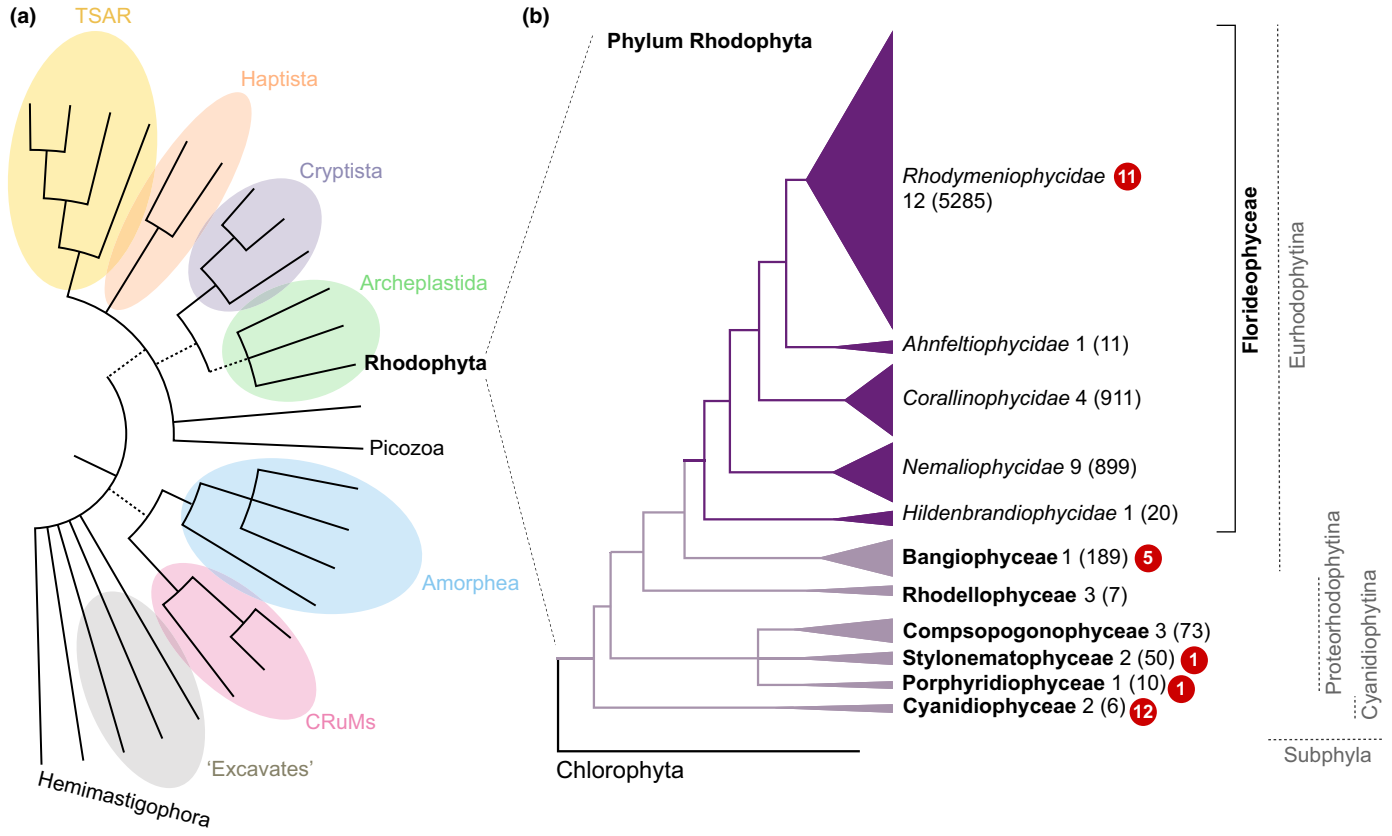

Fig. 1 (a) The Eukaryotic Tree of Life (based on Burki *et al*., 2020) with the position of Rhodophyta marked in bold. (b) Phylogeny of the Rhodophyta (based on Yang *et al*., 2016). Classes are shown in bold, subclasses are in italic, numbers correspond to the number of orders, and the number of specie is given in brackets (from Guiry & Guiry, 2023). The number of available genome sequences per class are shown in red circles.

although this may change with the discovery of spermatia in *Hildenbrandia* (Pueschel, 2022). In the other four remaining subclasses (Nemaliophycidae 899 spp., Corallinophycidae 911 spp., Ahnfeltiophycidae 11 spp., and Rhodymeniophycidae 5285 spp.; Guiry & Guiry, 2023), thallus morphology is more complex, and in most species, sexual reproduction involves zygotic amplification in the carposorophyte (described below). The development and morphology of the carposporophyte is increasingly complex in the more recently diverged subclasses of the Florideophyceae, suggesting that its innovation and elaboration could have played a key role in the success and radiation of the Florideophyceae (Yang *et al*., 2016).

IV. Red algal cell biology

One of the earliest features noted about red algal cells was the lack of flagella and centrioles at any point in their life cycle (Cole & Sheath, 1990), a fact that has been confirmed in the genomic era by the lack of flagella-related genes in the red algal genomes sequenced thus far (Collén *et al*., 2013; Qiu *et al*., 2015; Brawley *et al*., 2017). Other aspects of the cytoskeletal machinery also appear to differ in red algae, with no obvious genes encoding dynein- and myosin-dependent motor proteins that are normally common in animals and plants (Brawley *et al*., 2017). Other interesting absences extend to several other pathways normally essential in other eukaryotes, including GPI-anchor biosynthesis, autophagy, and phytochrome-dependent photoreception (Collén *et al*., 2013; Brawley *et al*., 2017).

Red algae are notably diverse in their pigment composition, with many macroalgae being valuable sources of phycobiliproteins (including phycoerythrins, phycocyanin, and allophycocyanin), carotenes (carotenoids and xanthophylls), and Chl*a* (Cole & Sheath, 1990). Although the precise pathways involved in pigment metabolism are unclear, several pigment mutants were isolated during pioneering genetic studies in the florideophyte *Gracilaria* (e.g. van der Meer, 1979), which sadly remain unidentified given the lack of genetic and genomic tools at the time. More recently, patterns of colour inheritance and the association between pigment genotype and fitness in natural populations of *Gracilaria* were revealed in a series of studies associating genetic crosses and classical genetic analysis (van der Meer, 1979; Zhang & van der Meer, 1988; Guimarães *et al*., 2003; Marchi & Plastino, 2020). Another notable feature of red algal cells is the lack of grana in the chloroplast (a common feature in 'higher' plants) such that thylakoids membranes are unstacked while chloroplasts lack an external endoplasmic reticulum (Gibbs, 1962; Bouzon *et al*., 2014). Studies on chloroplast protein import systems in *Cyanidioschyzon merolae* suggest that protein translocation across the inner and outer envelope appears to be conserved and unique when compared to plants, respectively (Baek *et al*., 2022).

Another key feature of red algal plastids is the presence of phycobilisomes on the surface of the chloroplast thylakoids, which

are composed of pigmented phycobiliproteins that give red algae their striking and distinctive colour. Phycobilisomes are highly efficient light-harvesting antennae that allow red algae to photosynthesise in very dim environments at much greater depth than other species of algae (Adir *et al.*, 2020). Recent cryo-EM studies in *C. merolae*, *Porphyridium purpureum*, and *Griffithsia pacifica* have revealed fascinating insight into the structure of phycobilisomes. Specialised classes of phycobilisome linker proteins, which have greatly expanded during red algal evolution (Lee *et al.*, 2019), interact with phycobiliproteins to fine-tune the efficient unidirectional transfer of energy from the phycobilisome over to photosystem I and II during photosynthesis (Zhang *et al.*, 2017; Pi *et al.*, 2018; Ma *et al.*, 2020; Li *et al.*, 2021). The dynamics of energy transfer also appear to differ among different species adapted to differing light environments (Xie *et al.*, 2021). In addition to acting as light-harvesting antennae, phycobilisomes also function as nitrogen stores in red algae and have been shown to undergo dynamic alteration in response to nitrogen stress (Zhao *et al.*, 2016).

The unique cell walls of red algae are a key economically valuable feature of these organisms. Like plants, the red algal cell wall is primarily composed of cellulose microfibrils that are embedded in a matrix composed of polysaccharides, and to a lesser extent, proteins and aromatic substances (Carpita & McCann, 2002). However, red algal cell walls differ in that they deposit unique polysaccharides called sulfated galactans that have special linear backbones constituted of alternating (1→3)-β-D-galactopyranose units (A-unit) and (1→4)-α-galactopyranose residues (B-unit; Ficko-Blean *et al.*, 2015). These sulfated galactans contribute to the mechanical strength and elasticity of the red algal cell wall and include polysaccharides with major industrial applications, such as agar, agarose, and carrageenans. The cell wall of several red algae has been characterised using biochemical methods over the last decades (Hardouin *et al.*, 2022). Transcriptomic analysis of life cycle stages in *Chondrus crispus* has revealed differential expression of genes encoding several carrageenan-related cell wall enzymes, particularly those encoding galactose-sulfurylases, carbohydrate-sulfotransferases, glycosyltransferases, and glycoside hydrolases (Lipinska *et al.*, 2020); Carrageenan-related pathways appear to differ between the gametophytes and tetrasporophytes, but also between male and female gametophytes, suggesting that carrageenan metabolism is altered to specify differential physiology between the 'isomorphic' life cycle stages of *C. crispus* (Lipinska *et al.*, 2020).

Vegetative cell fusions that produce a multinucleate syncytium are rare in land plants but are one of the more remarkable features of red algae. Red algal cells may fuse as part of a wound-repair process (Waaland & Cleland, 1974) but can also occur during normal vegetative morphogenesis (L'Hardy-Halos, 1970). Cell fusions occur frequently during thallus development in multi-axial pseudoparenchymatous species (Pueschel, 2021). The female reproductive apparatus in many florideophytes may also exhibit highly specific cell fusions, which might play key roles during fertilisation (Cole & Sheath, 1990). The mechanisms and regulatory events underlying syncytium formation remain poorly understood, as is the role of this pervasive process in developmentally complex red algae. It has been proposed that cell fusions enhance transport because they provide flexibility in moving photosynthates to developmental or reproductive sinks and/or to aid in carbon storage (Pueschel, 2021).

Multicellular eukaryotes have evolved ways to communicate between cells and this is thought to be a prerequisite for complex multicellularity. Gap junctions in animals or plasmodesmata in land plants are used as intercellular routes for cytoplasmic substances and signalling molecules between adjacent cells (Lucas & Lee, 2004; Evans & Lymbery, 2020). Plasmodesmata have been well documented in land plants, some green algae, and analogous structures also exist in brown algae (Lucas & Lee, 2004; Nagasato *et al.*, 2017). Red algae differ from other multicellular plants and algae in that they lack structures resembling plasmodesmata. Instead, most red algae have pit connections between cells that are formed between two daughter cells by an incomplete centripetal unfurrowing of the plasmalemma (Kim *et al.*, 2022). Cytokinesis is therefore incomplete and leaves a pore or connection that is subsequently 'plugged' with mostly proteinaceous materials to form a pit plug (reviewed in Raven, 1997). Pit plugs were thought to prevent cytoplasmic continuity between cells as the pit plug tightly occludes the connection between adjacent cells. However, recent studies suggest that despite their occlusion, pit plugs can act as size-specific conduits for macromolecules that could allow intercellular communication between the vegetative cells of red algae (Kim *et al.*, 2022). Future endeavours combining both genomics and genetic in model organisms will hope to shed light on the assembly, dynamics and function of pit connections in red algae.

V. Emergence of multicellularity in the red algae

Together with brown macroalgae, red macroalgae represent two of only five eukaryotic groups that have independently evolved complex multicellularity. However, very little is known about the molecular mechanisms underlying multicellular development in these two macroalgal groups, particularly when compared with animals and plants. Red algae are thought to be one of the first eukaryotic groups to have acquired complex multicellularity given that fossilised filamentous structures with cells connected by structures resembling pit connections and pit plugs were found in *c.* 1.6 billion-year-old strata (Bengtson *et al.*, 2017). The fossils had a morphology similar to members of the extant genus *Bangia* and exhibited different cell types, including reproductive structures, suggesting that sexual reproduction potentially occurred (Butterfield, 2000).

Multicellularity appears to have evolved at least twice within the red algae – in the ancestor of the clade that includes the Stylonematophyaceae and Compsopogonophyceae, and in the ancestor of the Florideophyceae and Bangiophyceae (Fig. 1). On the contrary, the phylogeny of Yoon *et al.* (2010) suggests at least three independent transitions to multicellularity. Using the broad definition of Cock & Collén (2015) and Coelho & Cock (2022), complex multicellularity is likely to have emerged only once in the red algae, namely within the Florideophyceae. In the other red algal groups, only branched or unbranched filaments and more simple sheet-like structures are found (Coelho & Cock, 2022), reinforcing the idea that complex multicellularity is an evolutionarily rare trait.

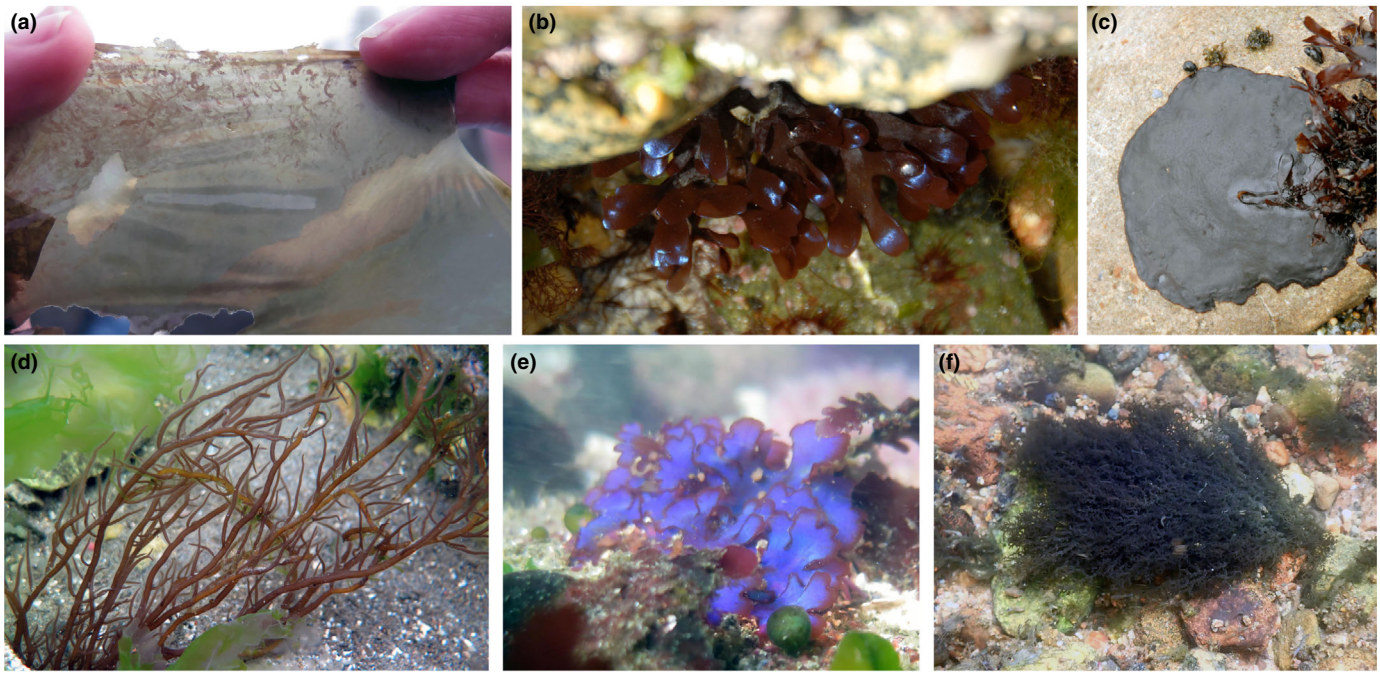

Fig. 2 (a) *Pyropia* sp. bearing reproductive structures at Botanical Beach, Vancouver Island, BC, Canada. (b) *Chondrus crispus* gametophyte showing characteristic iridescence at Pointe de Primel, Brittany, France. (c) *Mastocarpus papillatus* crustose tetrasporophyte at a site near Cayucos, CA, USA. (d) *Gracilaria vermiculophylla* fixed to hard substratum at Hakodate, near Shinori, Japan. (e) *Goiocladia laciniata* at Botanical Beach. (f) *Sheathia involuta*, a freshwater red alga, in a stream near Waupaca, WI. Photo credits: S.A. Krueger-Hadfield.

In recent years, insights into the evolution of complex multicellularity in the brown and red macroalgae have been obtained using genomic approaches, in particular through the analysis of the complete genome sequences for multicellular species in comparison with closely related unicellular species sister to these groups (Nakamura-Gouvea *et al.*, 2022). Despite these endeavours, the molecular basis of developmental patterning in the red algae is not commensurate with what is known in all the other multicellular groups (Cock & Collén, 2015). Recent efforts using *Ectocarpus* as a comparative model have increased our knowledge on the genetic frameworks involved in development processes (Coelho *et al.*, 2020). Multicellular red algae comprise a myriad of species with different types of thallus architecture, ranging from simple filaments to blades or pseudoparenchymatous tissue in the most complex species (Fig. 2; Cole & Sheath, 1990), providing an excellent opportunity to investigate gene conservation or novelty across multicellular eukaryotes. The availability of genomic and genetic tools for model red algae (see section below) promises to further our understanding of the molecular basis of developmental processes in this group of eukaryotes.

VI. Reproductive biology

1. Sex has deep origins in red algae

A defining feature of sexual reproduction is a change in chromosome number, or ploidy, at meiosis and fertilisation (Coelho *et al.*, 2007). Meiosis of diploid ($2n$) cells will give rise to haploid ($1n$) cells containing one set of chromosomes, which eventually re-unite at fertilisation once two haploid cells (or gametes) fuse to form a diploid zygote. Sexual life cycles are broadly grouped into three major types depending on the ploidy where multicellularity and/or mitotic divisions occur. Animals have a life cycle where the multicellular stage is diploid, and the haploid phase is restricted to unicellular gametes (a diploid life cycle henceforth). At the other extreme is the haplontic life cycle (haploid henceforth) where only the zygote is diploid, which immediately undergoes meiosis to produce haploid spores that may divide mitotically, in some cases to form a multicellular individual (Coelho *et al.*, 2007). This life cycle is found in most fungi, charophytes, dinoflagellates, alveolates, and some unicellular green algae. By contrast, the life cycle of plants and most macroalgae is haploid–diploid as multicellular development occurs in both the haploid and diploid phases, resulting in distinct life forms that can be free-living and ecologically distinct (Bell, 1982; Valero *et al.*, 1992; Hughes & Otto, 1999; Coelho *et al.*, 2007). This strategy, called the alternation of generations (Drew, 1955; John, 1994), has arisen independently in at least three eukaryotic lineages – the brown algae, green algae and their land plant relatives, and the red algae (Bourdareau *et al.*, 2017). The haploid phase is called the gametophyte as it produces gametes and differentiates specialised cell types that support fertilisation. The diploid phase is called the sporophyte as it produces haploid spores at meiosis that will eventually grow and develop into a gametophyte to complete the life cycle (Coelho *et al.*, 2007). Note that the gametophyte and sporophyte generations are often uncoupled from ploidy, for example in the parthenogenetic cycle of many brown algae where unfused gametes develop into haploid sporophytes ('parthenosporophytes'; Coelho *et al.*, 2011; Bourdareau *et al.*, 2017). Indeed, during studies on algal reproduction,

Drew (1955) distinguished nuclear phases with different ploidy (haploid or diploid) from somatic phases characterised by different morphologies.

The life cycles of red algae are some of the most complex and diverse, with most alternating between gametophytic and sporophytic generations (Fig. 3). Although the earliest diverging clades of unicellular red algae were long thought to reproduce asexually, recent evidence has emerged for sexual reproduction in the unicellular red alga *Galdieria*, a member of the Cyanidophyceae class. The *Galdieria* life cycle alternates between diploid cells with a cell wall and haploid cells without a cell wall, although both cell types proliferate asexually via mitosis (Hirooka *et al.*, 2022). Upon acetic acid stress, haploid cells can undergo self-diploidisation or they can fuse with other haploid cells to generate heterozygous diploids through isogamous fertilisation (Hirooka *et al.*, 2022). Interestingly, BELL/KNOX TALE-HD and MADS-box transcription factors (TF) are differentially expressed between the haploid and diploid cells in *Galdieria* (Hirooka *et al.*, 2022). Knockouts for each of these TFs block self-diploidisation and confirm that they play an essential role in the haploid-to-diploid transition. This is strikingly reminiscent of the role in haploid-to-diploid transitions of BELL/KNOX homologues in plants (Lee *et al.*, 2008; Sakakibara *et al.*, 2013; Dierschke *et al.*, 2021; Hisanaga *et al.*, 2021) and ORO/SAM in brown algae (Coelho *et al.*, 2011; Arun *et al.*, 2019), highlighting the deep origins of this system in the evolution of sexual reproduction in eukaryotes.

2. The Bangiophyceae undergo a heteromorphic alternation of generations

Species in the Bangiophyceae, the second largest class of red macroalgae, are characterised by a life cycle with a dominant gametophyte generation, as is typical of bryophytes and many green algae. Bangiophyceaen species, such as *Bangia*, *Porphyra* and their close *Pyropia* relatives, alternate between macroscopic, gametophytic leafy blades and microscopic, sporophytic filaments called the conchocelis. Once mature, the conchocelis will form conchosporangia that produce and release individual diploid spores called conchospores. The time of reductive divisions appears to vary among different Bangiophyceaen species, with some undergoing meiosis within the conchosporangium while others undergo meiosis after the release of conchospores (Burzycki & Waaland, 1987; Tseng & Sun, 1989). It is likely to be a mixture of both with environmental factors also potentially impacting the precise timing of events (Guiry, 1987). The latter strategy is characteristic of *Pyropia yezoensis*, which appears to establish gametophytic identity within the conchosporangium before the onset of meiosis which, when mechanically isolated from the conchosporangium, can develop directly into gametophytes (Mikami *et al.*, 2019). This period of apospory has led to the proposal that *P. yezoensis* undergoes a 'triphasic' life cycle (discussed below), with the conchosporangium proposed as a distinct diploid phase in addition to the conchocelis (Mikami *et al.*, 2019). However, caution should be exercised here since the strict

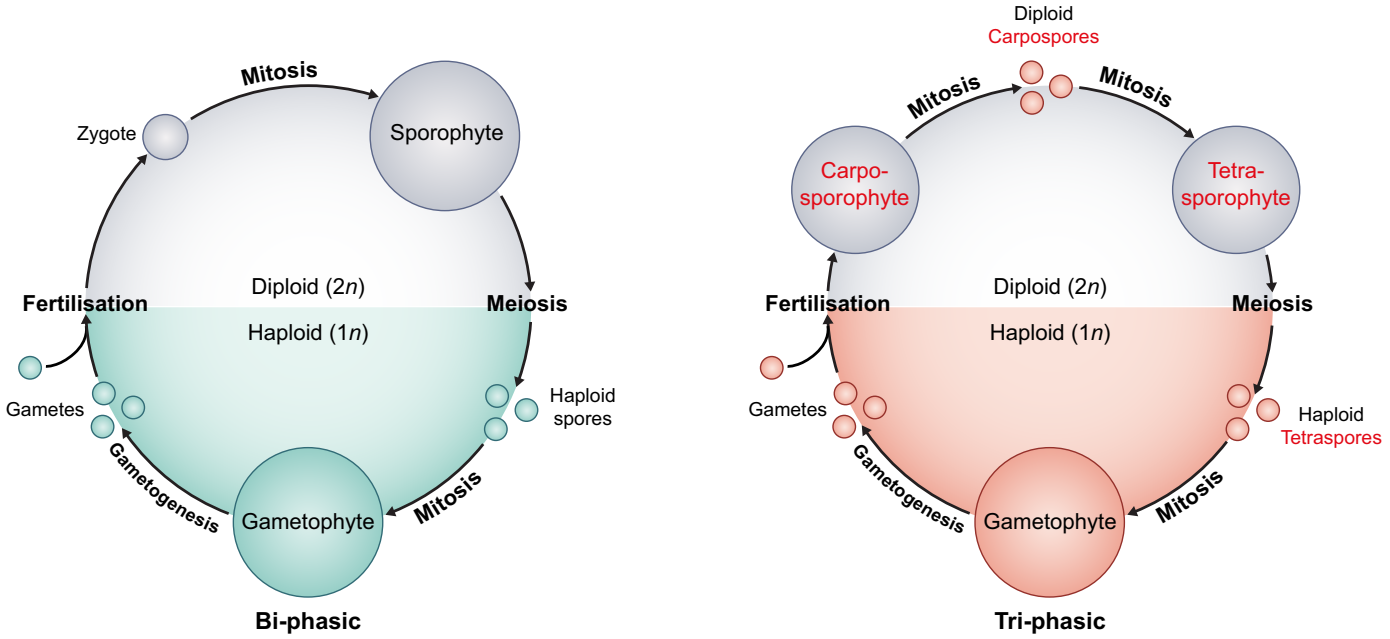

Fig. 3 Red algal life cycle compared with land plant life cycle. Both schemes represent haploid–diploid life cycles where mitotic divisions occur in both the diploid zygote and haploid meiotic spores to produce distinct and sometimes free-living sporophytes and gametophytes, respectively. Green algae, some red algae, most brown algae, and all land plants have a 'biphasic' alternation between gametophytes and sporophytes (left). In most of the Florideophyte red algae, these alternations are called 'triphasic' since the gametophytes and so-called tetrasporophyte generations are bridged by an additional diploid stage called the carposporophyte (Feldmann, 1972). The carposporophyte arises after fertilisation of the carpogonium by spermatia and will remain on the female gametophyte. Once mature, the multicellular (diploid) carposporophyte will release (diploid) carpospores that will germinate into a free-living diploid tetrasporophyte. Tetrasporophytes go through meiosis and produce (haploid) tetraspores which then germinate into either a male or female gametophyte, completing the 'triphasic' life cycle.

definition of an independent multicellular generation is one that should start with a single reproductive cell (or spore) and end with another type of spore (Bell & Koufopanou, 1991). In the case of *P. yezoensis*, the conchosporangium is differentiated from existing diploid cells of the conchocelis and not from a diploid spore. Thus, we agree with previous interpretations that the life cycle of all Bangiophyceaean species, including *P. yezoensis*, involves typical biphasic alternations. Interestingly, transcriptomic analysis in *P. yezoensis* has identified a KNOX-like TALE-HD TF that is predominately expressed in the diploid conchocelis (Mikami *et al.*, 2019). Although its function in life cycle control has yet-to-be established, the sporophyte-enriched expression of this KNOX-like TF suggests that it could play a role in the haploid-to-diploid transition in a similar manner to its distant unicellular ancestor *Galdieria* (Hirooka *et al.*, 2022).

3. The complex 'triphasic' life cycle of the Florideophyceae

Unique to most of the Florideophyceae is a sexual life cycle in which two, often free-living, generations – the diploid tetrasporophyte and haploid gametophytes – are bridged by a third (multicellular) stage called the carposporophyte (Fig. 3). Male gametophytes release nonmotile spermatia while female gametophytes produce a carpogonium (the egg cell) that is retained on the female gametophytic thallus (Fig. 4). After fertilisation (discussed below), the carposporophyte develops and remains attached to the female gametophytic thallus within a structure called the cystocarp, which consists of diploid carposporophytic tissue that is surrounded by haploid maternal tissue (Fig. 4; Fredericq & Hommersand, 1989). The carposporophyte has been considered to represent a distinct 'phase' in this life cycle since it too produces spores as a result of amplifying the zygote into genetically identical diploid carpospores. The carpospores are eventually released from the cystocarp and germinate into a free-living diploid tetrasporophyte that produces haploid spores through meiosis. These haploid tetraspores then germinate into either a male or female gametophyte, completing the life cycle. Given that three distinct stages typify this life cycle strategy, it has classically been termed a 'triphasic' life cycle in the literature (Fredericq & Hommersand, 1989), with each of the three so-called 'phases' representing distinct stages of development rather than a change in ploidy.

Zygotic amplification in the carposporophyte has been proposed to mitigate inefficient fertilisation by nonmotile spermatia and the lack of dispersal of the carpogonia (Searles, 1980). However, Engel *et al.* (1999) and Krueger-Hadfield *et al.* (2015) demonstrated high fertilisation success in which multiple males fertilised each female, raising questions about Searles' (1980) hypothesis. Maggs and colleagues also demonstrated high fertilisation success

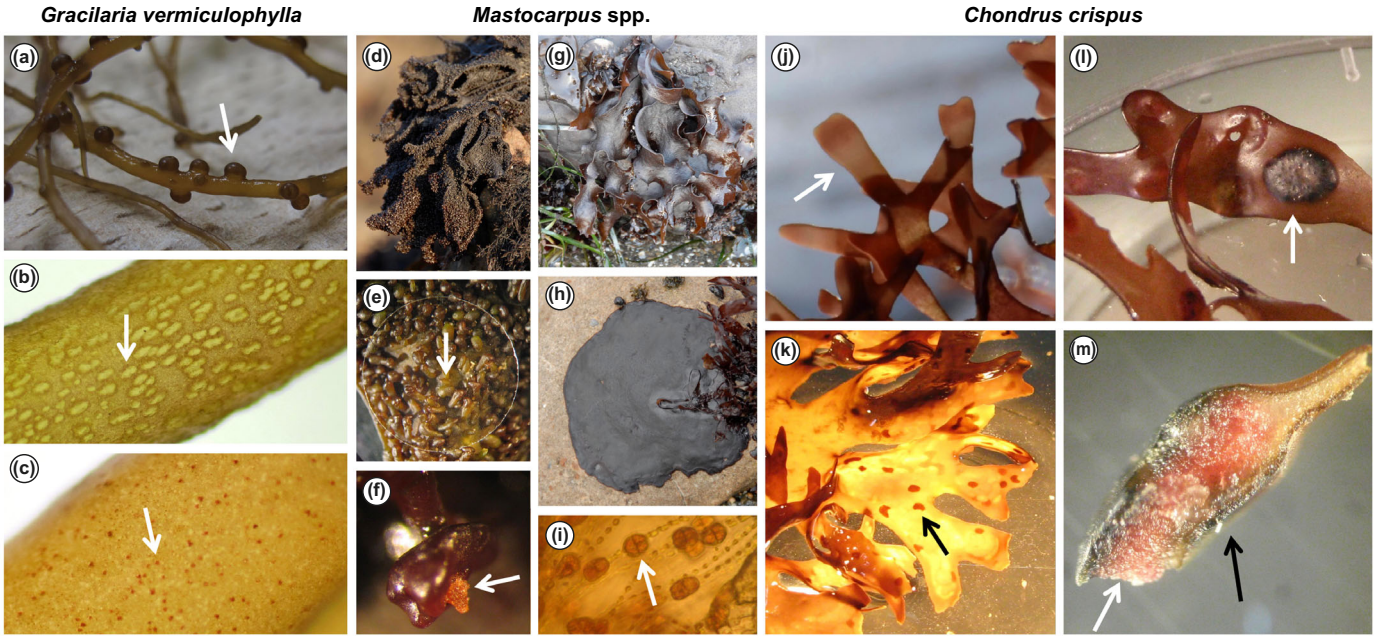

Fig. 4 Assortment of reproductive structures for three red macroalgae: *Gracilaria vermiuclophylla*, *Mastocarpus* spp., and *Chondrus crispus*. (a) Female *G. vermiuclophylla* gametophyte-bearing cystocarps, the hemispherical swellings along the thallus shown with the white arrow. (b) Male *G. vermiuclophylla* gametophyte bearing spermatangial sori, the white, oblong structures on the thallus shown with the white arrow (×40). (c) *Gracilaria vermiuclophylla* tetraporophyte bearing tetrasporangial sori, the pink spots along the thallus shown with the white arrow (×40). (d) Female *Mastocarpus* sp. gametophyte-bearing cystocarps called papillae shown with the white arrow. (e) *Mastocarpus* sp. papillae or cystocarps under a 12 mm diameter round coverslip, one papilla is noted shown with the white arrow (×35). (f) *Mastocarpus* sp. cystocarp extruding carpospores shown with the white arrow (×40). (g) Male *Mastocarpus* sp. gametophyte. (h) Crustose *Mastocarpus* sp. tetrasporohyte. (i) *Mastocarpus* sp. cruciate tetraspores in filaments shown with the white arrow (×400). (j) Male *C. crispus* gametophyte showing distinctive white bands just below the apices, noted by the white arrow. (k) *C. crispus* tetrasporophyte bearing tetrasporangial sori, the reddish patches on the thallus near the apices noted by the black arrow. (l) Female *C. crispus* gametophyte with a large cystocarp, noted by the white arrow. (m) Cross section of the cystocarp shown in 11 excised from the female gametophyte. The brownish tissue is the pericarp, noted by the black arrow, and the reddish tissue is the carposporophyte, wherein the carpospores are produced following fertilisation, noted by the white arrow (Images © SA Krueger-Hadfield).

in *Neosiphonia harveyi* in which successful and unsuccessful fertilisation is easily measured by morphological observations (Maggs *et al.*, 2011). 'Pollination' mechanisms might also explain high fertilisation success and could be common among red algae since isopods have been shown to increase the rates of fertilisation in *Gracilaria gracilis* (Lavaut *et al.*, 2022). Moreover, genetic studies in natural populations revealed very few repeated tetrasporophytic genotypes suggesting that zygotic amplification has a negligible effect on population structure and that fertilisation success is by no means inefficient (Engel *et al.*, 2004; Krueger-Hadfield *et al.*, 2015). It has been suggested that the evolution of the carposporophyte was a major innovation, playing a central role in the success and explosive diversification of florideophytes (Lee *et al.*, 2016). Although the evolutionary significance of this third phase in the life cycle is still unclear, it is likely that this phase enhances reproductive success. Various studies suggest that the carposporophyte, whose development depends on the female gametophyte, may mediate sperm competition through female choice (Richerd *et al.*, 1993; Engel *et al.*, 1999). Indeed, Fredericq & Hommersand (1989) suggest that the carposporophyte may play a role in rejecting incompatible or disharmonious fertilisations.

The 'triphasic' life cycle is predicted to have arisen *c.* 750 Ma as the Nemaliophycidae split from the Hildenbrandiophycidae (Yang *et al.*, 2016). Much has been postulated about its evolutionary origins, with early proponents suggesting that the carposporophyte originated from a once free-living, independent generation (Umezaki, 1989, and references therein). In this evolutionary scenario, the zygote would have been retained on the female gametophyte and then divided to form a carposporophyte that lived 'parasitically' on the female gametophyte. An appraisal by Guiry (1987) rejected this idea and suggested that the carposporophyte evolved *in situ* as a means to amplify the progeny arising from a single fertilisation event. The ancestral pattern of red algal life cycles still remains difficult to ascertain, but it is possible that haploid–diploid life cycles evolved independently multiple times in different red algal lineages (Gabrielson *et al.*, 1986). For example, given their distinct phylogenetic origins, the Bangiophyceaean life cycle likely arose independently from the earlier diverging subclasses of the Florideophyceae. The earliest diverging of these subclasses, the Hildenbrandiophycidae, is a group of crustose-forming algae that produce tetrasporangia but no carposporophyte (Pueschel, 1982; Saunders & Bailey, 1999; Sherwood & Sheath, 2003) while the sister Nemaliophycidae subclass is characterised by a wide range of life cycles, including heteromorphic alternations that lack a free-living tetrasporophyte (Stegenga, 1978), gametophytes that remain attached to the tetrasporophyte (Yoshizaki, 2019; Nan *et al.*, 2020), and a short-lived carposporophyte that produces just a handful of carpospores (Lee & Kurogi, 1983). In the Batrachospermales, there are heteromorphic macroscopic gametophytes and microscopic sporophytes called 'chantransia' that are physically connected to one another (Sheath, 1984). Vegetative meiosis occurs on the chantransia in which only one of the four meiotic products results in a gametophyte while the other three are lost (Sheath, 1984). In the Nemaliophycidae, there are also biphasic alternations as seen in the Palmariales and some Acrochaetiales species (Stegenga, 1978) although it is unclear whether this represents the ancestral pattern of the Florideophyceae or whether it is derived from the 'triphasic' life cycle.

4. Gamete recognition and fertilisation mechanisms

The elaboration of the Florideophyte life cycle has resulted in a mode of sexual reproduction that rivals the sophistication of that seen in land plants. A typical feature of red algae is the glaring absence of flagella such that rapid sperm motility was lost in this lineage. Although spermatia and spores of some red algae can move and glide with the support of mucilaginous appendages (Pickett-Heaps *et al.*, 2001), it is unlikely that these processes alone explain the efficient rates of fertilisation observed in natural populations (Engel *et al.*, 1999; Krueger-Hadfield *et al.*, 2015; Lavaut *et al.*, 2022). In many species, the union of gametes is facilitated by the trichogyne, a thin hair-like projection from the carpogonium that receives and delivers the male nucleus for fertilisation (Pickett-Heaps & West, 1998; Han *et al.*, 2012). It is worth noting that trichogynes are not easily observed in all species, which is likely due to their diminutive nature in many species (Xu *et al.*, 2008) or is perhaps suggestive of other mechanisms involved in red algal fertilisation. The spermatia of many red algal species are 'sticky' and can be decorated by various types of appendages that help them attach to the trichogyne, neighbouring vegetative tissue, and/or onto sea pollinators that facilitate their transfer over large distances through the water column (Broadwater & Scott, 1982; Magruder, 1984; Kim & Fritz, 1993). An extreme example is found in the alga *Antithamnion nipponicum*, which bears two long extracellular appendages that can extend up to 10 times (30 μm) the length of the spermatial head (2–4 μm in diameter; Kim & Fritz, 1993). The limited movement of spermatia in some species may also help optimise their site of fusion with the trichogyne or when first attached to neighbouring vegetative tissue (Pickett-Heaps & West, 1998). For example, the spermatia of *Aglaothamnion negletum* have fimbriate cone-shaped appendages that sometimes first attach to vegetative hair cells and later bind to a nearby trichogyne once it makes contact through water motion (Magruder, 1984).

In most cases, however, spermatia do not tend to bind to any other vegetative tissue of the gametophyte and tetrasporophyte, indicating that cell-to-cell recognition mechanisms mediate specific binding to the trichogyne. The tip of the trichogyne in *Melanothamnus harveyi* (formerly *Polysiphonia harveyi*) appears to release substances by exocytosis that might play a role in the formation of an extracellular coat that aids spermatial adherence (Broadwater & Scott, 1982). The most direct evidence for a gamete recognition system has been garnered from experiments with different *Antithamnion* species (Kim & Fritz, 1993; Kim & Kim, 1999). Preincubation of spermatia and trichogynes with various lectins and carbohydrates indicates that gamete recognition involves a double-docking system, with spermatia carrying L-fucose lectin receptors and the trichogyne a-methyl D-mannose receptors that each bind complementarily to glycoproteins on the opposing gamete (Kim & Fritz, 1993; Kim & Kim, 1999). Affinity chromatography in *Aglaothamnion oosumiense* has identified a GalNAc-binding lectin that is highly expressed in female

gametophytes as a candidate that could mediate the binding of spermatia onto the trichogyne (Han *et al.*, 2012). A clearer picture of the genes encoding these cell surface receptors and their conservation in other red algal lineages remains an important open question.

Once spermatia adhere to the trichogyne, a microtubule-mediated mitosis is triggered to form a binucleate spermatium that coincides with an influx of Ca^{2+} ions (Mine & Tatewaki, 1994; Shim *et al.*, 2020). Nuclear divisions of spermatia may be a common theme among many red algae as they have been reported in several Florideophyceae species (Cole & Sheath, 1990; Mine & Tatewaki, 1994; Pickett-Heaps & West, 1998; Shim *et al.*, 2020). Their biological significance is less clear but careful observations in *Bostrychia moritziana* have demonstrated that only one spermatium will fertilise the carpogonial nucleus (Pickett-Heaps & West, 1998; Shim *et al.*, 2020). Although both nuclei enter the trichogyne, sometimes together with nuclei from other plasmogamy events, the first spermatium to enter will engage with the transport system to travel down to the carpogonium. The time of entry thus appears to be critical in determining which spermatium will undergo fertilisation. Once a spermatium enters the carpogonium, a vacuole contraction draws the sperm nucleus into contact with the egg nucleus and pinches off the carpogonium cytoplasm to separate it from the trichogyne, thus preventing multiple fertilisation events (Broadwater & Scott, 1982). The movement of spermatia is mediated by actin microfilaments that wrap around the male nucleus as it is transported down the trichogyne, which eventually degenerate after karyogamy has taken place (Shim *et al.*, 2020). The interplay between reactive oxygen species (ROS) and Ca^{2+} signalling also plays a role in communication between the spermatium and trichogyne (Shim *et al.*, 2022), which might be a common signalling mechanism in other red algal developmental processes (Moon *et al.*, 2022). Once the spermatium is attached, ROS diffuses across the cell membranes in the form of H_2O_2 and triggers more ROS production in the carpogonium and associated cells (Shim *et al.*, 2022). Inhibition of calcium-mediated H_2O_2 signalling perturbs the ability of spermatia to fuse to the trichogyne but also blocks carposporophyte development, demonstrating its key role during both pre- and postfertilisation events. Further investigation into these intricate cell signalling events will hope to reveal how ROS patterns and potentially other signalling pathways modulate downstream events during fertilisation and early carposporophyte development.

VII. Red algae as models for understanding the evolution of sex

The alternation of ploidy levels constitutes a major genomic change that directly influences phenotypic plasticity (Bell, 1994). Genetic models often predict the evolutionary stability of a single free-living stage, such as that seen in the life cycle of animals (diploid) or charophytes (haploid) (reviewed in Valero *et al.*, 1992; Mable & Otto, 1998). By contrast, ecological models suggest that more than one free-living stage can be favoured through niche partitioning (Ebenman, 1992; Moran, 1994; Podolsky & Moran, 2006), although most ecological predictions have focused on vertebrates or invertebrates that undergo metamorphosis (Albecker *et al.*, 2021). While there are huge morphological changes from larvae to adults during metamorphosis, these changes occur in the same diploid genome formed following fertilisation. However, this is not the case across all eukaryotic taxa since many lineages, including algae, have life cycles that alternate between distinct free-living stages (Coelho *et al.*, 2007; Coelho & Cock, 2020). A recent model (Rescan *et al.*, 2016) has explored the interplay between genetic and ecological parameters in the evolution and stability of life cycles. Across the three lineages of macroalgae, life cycles are highly diverse and encapsulate the diversity observed across eukaryotes (Bell, 1994), positioning macroalgal taxa as useful models to resolve the conflict between genetic and ecological predictions (Mable & Otto, 1998).

Strongly heteromorphic gametophytes and tetrasporophytes do not pose a problem for ecological predictions in which phases must differ in form and function (e.g. Moran, 1994). Lubchenco & Cubit (1980) demonstrated differences in herbivory between the crustose and foliose stages of several macroalgae, including *Mastocarpus stellatus*. However, this is more challenging when explaining the stability of 'isomorphic' alternations as morphologically similar gametophtyes and tetrasporophytes are expected to be ecologically similar. Hughes & Otto (1999) used phenotypic data generated by (Destombe *et al.*, 1989) to generate theoretical models and demonstrated that subtle ecological differences between stages are sufficient to stabilise 'isomorphic' alternations on evolutionary timescales. The importance of subtle differences in survival, breakage, or growth between isomorphic gametophytes and tetrasporophytes has also been demonstrated (da Silva Vieira & Pimenta Santos, 2010). Despite these theoretical predictions, more empirical work is necessary across red algal taxa with isomorphic alternations. Recent studies in brown and green algae have shown differences in associated microbial fungi (Bonthond *et al.*, 2022), survival (Krueger-Hadfield & Ryan, 2020), chemical defences (Vergés *et al.*, 2008), thallus strength (Lees *et al.*, 2018), growth rates (Guillemin *et al.*, 2013), fecundity rates, light and temperature optima, and/or herbivore selectivity (Lubchenco & Cubit, 1980). Many of these studies have focused on species that are invasive, ecologically important or of economic interest, so further studies in a broader range of red algal taxa have the potential to broaden our understanding of the selective advantage of alternations in ploidy levels.

In addition to studies focused on the life cycle, red algae also have the potential to expand our understanding of reproductive system variation. Reproductive systems describe the relative rates of sexual vs asexual reproduction in a population (Barrett, 2011), which in turn govern the distribution of genetic diversity within and among populations. To date, most studies have focused on angiosperms and the sexual axis of variation between outcrossing and selfing, although Otto & Marks (1996) hypothesised that reproductive systems are likely to be correlated with life cycle types. Outcrossing should be associated with diploid-dominant life cycles, while selfing and clonality should correlate with a prolonged haploid stage as deleterious mutations are exposed to selection and can be more easily purged. In the case of haploid–diploid taxa, intragametophytic selfing in monoicous taxa, where sperm and

eggs are produced on the same gametophyte, results in instantaneous, genome-wide homozygosity since gametes are mitotically-produced (Klekowski, 1973). The work of Sessa *et al.* (2016) in ferns suggests that the evolutionary consequences of this immediate loss of genetic diversity across an entire genome has been important for the evolution of haploid–diploid taxa. Freshwater red algae, in which many species also have monoicous gametophytes (Sheath, 1984), would be an excellent taxa with which to document reproductive system variation.

Selfing can also occur when fertilisation takes place between separate male and female gametophytes that share the same sporophytic parent. Such intergametophytic selfing is analogous to selfing in monoecious angiosperms and hermaphroditic animals (Klekowski, 1973). Importantly, this means that separate sexes cannot be used as a proxy for outcrossing, as is often done in angiosperm and animal taxa, but must instead empirically quantify the reproductive mode and selfing rate (Krueger-Hadfield, 2020; Krueger-Hadfield *et al.*, 2021a). Nevertheless, reproductive system variation remains poorly characterised across eukaryotes, preventing the systematic investigation of these correlations (Olsen *et al.*, 2020). Existing data suggest a remarkable variation in red algal reproductive systems (reviewed in Krueger-Hadfield *et al.*, 2021a). For example, *G. gracilis* was found to be an obligate outcrosser (Engel *et al.*, 2004) in contrast to the highly selfing *C. crispus* (Krueger-Hadfield *et al.*, 2013, 2015), even though both species have isomorphic gametophytes and tetrasporophytes and occupy the same mid-intertidal zone in the eastern North Atlantic. Thus, red algae can illuminate the convergence of life cycle-related (e.g. Hughes & Otto, 1999) and reproductive system-related hypotheses (e.g. Otto & Marks, 1996), which will ultimately expand our understanding of the evolution of sex. Note that intraspecific variation has been observed in most orders of the Florideophyceae, including simultaneous occurrence of gametangia and tetrasporangia on the same individual (mixed phase reproduction), bisexuality, as well as direct development of tetrasporophytes from tetraspores or gametophytes from carposporophytes (e.g. Maggs, 1988; Kain & Destombe, 1995). Life cycle variants constitute an exceptional opportunity for deciphering the complex phenomena involved in sexual reproduction in red algae.

VIII. Red algal genomics

A deeper knowledge of genes encoded within red algal genomes is key to increasing our understanding of red algal biology. While several uni- and multicellular red algae from different environments and taxonomic positions have been sequenced, the number of species analysed still remains low, particularly in relation to other major eukaryotic groups (Table 1). In general, red algal genome sizes vary between 8.8 Mbp (*Cyanidium caldarium*) and 108 Mbp (*Neopyropia* (*Pyropia*) *yezoensis*) and contain between 4808 (*C. merolae*) and 13 125 (*Porphyra umbilicalis*) genes. These numbers are relatively low when compared to other eukaryotes of similar complexity and lifestyle, such as brown and green seaweeds. One explanation to the relatively limited size of red algal genomes could be the absence of known polyploidisation events (Bhattacharya *et al.*, 2018) or a hypothesised ancient genome reduction (to be described later). However, it remains premature to make generalisations about red algal genome characteristics given the few genomes available, with the Florideophyceae being particularly under-sampled, despite most red algae being classified there. For example, the unpublished genome of *Kappaphycus alvarezii* suggests a substantial increase in genome size (Jia *et al.*, 2020), while estimates using microspectrophotometry indicate genome sizes of up to 3 pg DNA per diploid cell in certain red algae, suggesting that genome sizes could be > 1000 Mbp (Kapraun, 2005). These observations suggest that more complex and larger red algal genomes may be found in future studies.

Detailed genomic knowledge has led to major and sometimes unexpected insights into the evolution, biochemistry, physiology, and ecology of different organisms. For example, nucleomorph chromosome sequences from the cryptomonad *Guillardia theta* have revealed that a remnant nuclear genome was acquired through a secondary endosymbiosis of a red algal symbiont (Douglas *et al.*, 2001). The genome subsequently underwent a substantial reduction in size and contains only 44 genes with very few introns. The first full genome sequence of a red alga was from the unicellular extremophile *C. merolae* (Matsuzaki *et al.*, 2004), which was shown to have a very small genome containing a limited number of genes with almost no introns, which is unsurprising considering its small size and extreme habitat. The first red macroalgae with an available genome sequence were from the florideophyte *C. crispus* (Collén *et al.*, 2013), which, rather surprisingly, had a relatively low number of genes with very little introns and only a modest increase in gene family diversification.

The sequencing of more red algal genomes led to the conclusion that several genome reduction events occurred during red algal evolution (Table 1). An in-depth phylogenetic analysis indicated that at least two episodes of genes loss and genome contraction occurred that vastly reduced the number of genes and introns among the red algae (Qiu *et al.*, 2015). The first event is believed to have been ancestral, having occurred after the split from the Viridiplantae and Glaucophyta lineage, while the second occurred specifically within the Cyanidiophyceae (Fig. 1). These ancestral genome purging events likely explain why red algae lack flagella, which can be considered counter-intuitive given the aquatic nature of the lineage. In addition to flagella, other pathways were also lost, including pyrimidine degradation, phytochrome-based light sensing, autophagy-related genes, sulfatases as well as GPI-anchor-related proteins (Collén *et al.*, 2013; Qiu *et al.*, 2015). The loss of light-sensing mechanisms is probably secondary to the loss of flagella since light perception would likely no longer have been required given the lack of flagella-based locomotion. Further puzzling is the lack of autophagy genes, which is normally essential in most multicellular eukaryotes (Shemi *et al.*, 2015), as well as pyrimidine degradation and sulfatases, all of which are required to re-utilise nutrients during development and starvation stress. These essential metabolic pathways could be complemented by bacterial endosymbionts or perhaps suggest that analogous pathways could have emerged in red algae that remain to be described.

The reason for the loss of genomic material in red algae is unclear. One hypothesis is that this was a result of ancestral red algae

Table 1 Sequenced red algal (*sensu lato*) species with their organisation, phylogenetic position, and principal habitat.

Species	Organisation	Class/order	Environment	Reference
Agarophyton vermiculophyllum (Ohmi) Gurgel, Norris & Fredericq 2018	Multicellular	Florideophyceae Gracilariales	Marine	Flanagan *et al*. (2021)
Asparagopsis taxiformis (Delile) Trevisan 1845	Multicellular	Florideophyceae Bonnemaisoniales	Marine	Thapa *et al*. (2020)
Calliarthron tuberculosum (Postels & Ruprecht) Dawson 1964	Multicellular	Florideophyceae Corallinales	Marine	Chan *et al*. (2011)
Chondria armata (Kützing) Okamura 1907	Multicellular	Florideophyceae Ceramiales	Marine	Steele *et al*. (2022)
Chondrus crispus Stackhouse 1797	Multicellular	Florideophyceae Gigartinales	Marine	Collén *et al*. (2013)
Digenea simplex (Wulfen) Agardh 1822	Multicellular	Florideophyceae Ceramiales	Marine	Chekan *et al*. (2019)
Gracilariopsis chorda (Holmes) Ohmi 1958	Multicellular	Florideophyceae Gracilariales	Marine	Lee *et al*. (2018)
Gracilaria changii (Xia & Abbott) Abbott, Zhang & Xia 1991	Multicellular	Florideophyceae Gracilariales	Marine	Ho *et al*. (2018)
Gracilaria domingensis (Kützing) Sonder ex Dickie 1874	Multicellular	Florideophyceae Gracilariales	Marine	Nakamura-Gouvea *et al*. (2022)
Gracilariopsis lemaneiformis (Bory) Dawson, Acleto & Foldvik 1964	Multicellular	Florideophyceae Gracilariales	Marine	Zhou *et al*. (2013)
Kappaphycus alvarezii (Doty) Liao 1996	Multicellular	Florideophyceae Gracilariales	Marine	Jia *et al*. (2020)[1]
Neoporphyra (*Pyropia*) *haitanensis* (Chang & Zheng) Brodie & Yang 2020	Multicellular	Bangiophyceae Bangiales	Marine	Cao *et al*. (2020)
Neoporphyra haitanensis (Chang & Zheng) Brodie & Yang 2020	Multicellular	Bangiophyceae Bangiales	Marine	Chen *et al*. (2022)
Neopyropia (*Pyropia*) *yezoensis* (Ueda) Yang & Brodie 2020	Multicellular	Bangiophyceae Bangiales	Marine	Nakamura *et al*. (2013)
Neopyropia (*Pyropia*) *yezoensis* (Ueda) Yang & Brodie 2020	Multicellular	Bangiophyceae Bangiales	Marine	Wang *et al*. (2020)
Palmaria palmata (Linnaeus) Weber & Mohr 1805	Multicellular	Florideophyceae Palmariales	Marine	Chekan *et al*. (2019)
Porphyra umbilicalis Kützing 1843	Multicellular	Bangiophyceae Bangiales	Marine	Brawley *et al*. (2017)
Porphyridium purpureum (Bory) Drew & Ross 1965	Unicellular	Porphyridiophyceae Porphyridiales	Freshwater, marine, brackish	Bhattacharya *et al*. (2013)
Rhodosorus marinus Geitler 1930	Unicellular, oligocellular	Stylonematophyceae Stylonematales	Marine	GenBank: JAMWBK000000000.1[2]
Cyanidiococcus yangmingshanensis Liu, Chiang, Yoon & Fu 2020	Unicellular	Cyanidiophyceae Cyanidioschyzonales	Acidic hot springs	Liu *et al*. (2020)
Cyanidioschyzon merolae De Luca, Taddei & Varano 1978	Unicellular	Cyanidiophyceae Cyanidioschyzonales	Acidic hot springs	Matsuzaki *et al*. (2004)
Cyanidioschyzon merolae De Luca, Taddei & Varano 1978	Unicellular	Cyanidiophyceae Cyanidioschyzonales	Acidic hot springs	Nozaki *et al*. (2007)
Cyanidioschyzon merolae De Luca, Taddei & Varano 1978	Unicellular	Cyanidiophyceae Cyanidioschyzonales	Acidic hot springs	Rossoni *et al*. (2019)
Cyanidium caldarium (Tilden) Geitler 1933	Unicellular	Cyanidiophyceae Cyanidiales	Acidic hot springs	Cho *et al*. (2023)
Cyanidiococcus yangmingshanensis Liu, Chiang, Yoon & Fu 2020	Unicellular	Cyanidiophyceae Cyanidioschyzonales	Acidic hot springs	Cho *et al*. (2023)
Galdieria (*sulphuraria*) *yellowstonensis* Yoon, Park & McDermott 2023	Unicellular	Cyanidiophyceae Galdieriales	Acidic hot springs	Cho *et al*. (2023)
Galdieria sulphuraria (Galdieri) Merola 1982	Unicellular	Cyanidiophyceae Galdieriales	Acidic hot springs	Schönknecht *et al*. (2013)
Galdieria sulphuraria – 8 strains (Galdieri) Merola 1982	Unicellular	Cyanidiophyceae Galdieriales	Acidic hot springs	Rossoni *et al*. (2019)
Galdieria partita Sentsova, nom. inval. 1991	Unicellular	Cyanidiophyceae Galdieriales	Acidic hot springs	Hirooka *et al*. (2022)
Galdieria phlegrea Yoon, Park & Ciniglia 2023	Unicellular	Cyanidiophyceae Galdieriales	Acidic hot springs	Qiu *et al*. (2015)

Table 1 (Continued)

Species	Organisation	Class/order	Environment	Reference
Galdieria phlegrea Yoon, Park & Ciniglia 2023	Unicellular	Cyanidiophyceae Galdieriales	Acidic hot springs	Rossoni *et al*. (2019)
Picozoa spp. Seenivasan, Sausen, Medlin & Melkonian 2013	Unicellular, nonphotosynthetic	Picomonadea Picomonadida	Marine, brackish	Schön *et al*. (2021)
Rhodelphis limneticus Tikhonenkov, Gawryluk, Mylnikov & Keeling 2019	Unicellular, nonphotosynthetic	Rhodelphea Rhodelphida	Freshwater	Gawryluk *et al*. (2019)

[1]Preprint only.
[2]Unpublished.

experiencing an evolutionary bottleneck in their extreme early environment (Bhattacharya *et al*., 2013; Collén *et al*., 2013). The only extant red algae known to live and even thrive in such extreme environments have a highly reduced gene content, which might be evolutionary advantageous by being less constraining on their survival and ensuring more efficient nutrient use (Cho *et al*., 2023). However, this 'hot start' hypothesis of red algal evolution assumes that primordial red algae were extremophiles or that all mesophilic red algae later became extinct (Bhattacharya *et al*., 2018). Because of the possible constraints of a reduced genomic repertoire, horizontal gene transfer might have been beneficial in their subsequent adaptation to mesophilic environments. Indeed, many candidate genes believed to have been acquired by horizontal gene transfer appear to originate from bacteria and are likely to be involved in stress-related functions (Bhattacharya *et al*., 2018). By contrast, the regain of complex eukaryotic functions through the acquisition of multiple genes, as would be required to regain flagella for example, would have been highly unlikely as such genes are not normally clustered by function within eukaryotic genomes.

Recent advances in red algal genomics include the availability of genomic information from Picozoa (Schön *et al*., 2021) and *Rhodelphis* (Gawryluk *et al*., 2019), two early diverging non-photosynthetic taxa within the red algal group. Picozoa is believed to have radiated before *Rhodelphis*, with the latter having the remnants of a plastid unlike Picozoa. Interestingly, both these taxa are comprised of flagellated organisms with Rhodelphis having a relatively larger genome and a substantial degree of intron-containing genes. These two taxa are thus likely to have separated from the red algal lineage before the first genome reduction event and suggest that the ancestor of the red algae was a flagellated heterotrophic protist. Moreover, because Picozoa lacks remnants of plastids, it is possible that the red algae diverged before the first endosymbiotic event, highlighting the potential of more than one primary endosymbiosis occurring during Archaeplastida evolution. Alternatively, all traces of the plastid were lost in the Picozoa.

The analysis of red algal genomes has also given insights into the physiology of red algae, including inorganic carbon uptake (Wang *et al*., 2020), osmotic regulation (Chen *et al*., 2022), carbohydrate metabolism (Manat *et al*., 2022), and agar and secondary metabolites (Nakamura-Gouvea *et al*., 2022). The currently sequenced red algal genomes have been mostly species of economical relevance, such as *Pyropia/Porphyra* or nori (Brawley *et al*., 2017) and *Gracilaria* spp. (Lipinska *et al*., 2023), and species with potential biochemical application, such as the Cyanidales. A deeper knowledge of red algal diversity at the genomic level is therefore lacking and would require more genomes from the Florideophyceae, the largest group of algae, and Proteorhodophytina, a group with large evolutionary diversity (Fig. 1). The increasing number of published red algal genome sequences have prompted the creation of the Rhodoexplorer Red Algal Genome Database (https://rhodoexplorer.sb-roscoff.fr), a genome repository and interface that will facilitate data accessibility and comparative genomic analyses in red algae (Lipinska *et al*., 2023). This platform aims to host red macroalgal genomes as they become increasingly available and will represent a valuable resource to the growing community of researchers interested in several aspects of red algal biology.

IX. Moving towards a red macroalgal model system

A key strength of traditional phycology has been the broad interest in characterising numerous algal species, which have provided detailed descriptions of life cycle diversity, phenology, morphological diversity, ecology, and evolution. Although this has helped provide a very broad overview of fundamental aspects of red algal biology, a lack of a concerted effort in *bonafide* model organisms has hampered deeper insight, particularly at the molecular level. The application of genetic and genomic approaches in model organisms has been a major factor underlying advances into the molecular basis of a wide range of biological processes across eukaryotes. The brown alga *Ectocarpus* is a key example where combined effort from labs around the world has led to a range of genetic tools and genomic resources that have facilitated novel discoveries in brown algal biology (e.g. Tarver *et al*., 2015; Avia *et al*., 2017; Baudry *et al*., 2020; Müller *et al*., 2021; Gueno *et al*., 2022).

Unicellular species have clear advantages as red algal model systems since they grow fast, have small genome sizes while CRIPSR-Cas9 and genetic manipulation systems already exist. However, unicellular models obviously lack the complexity of multicellular species and are unable to enlighten our understanding of red algal reproduction and development, so there is a pressing need to develop similar model organisms for the red macroalgae. With this in mind, we discuss some species that have been subject to investigation in recent years that could be further developed as red macroalgal model organisms to help address the outstanding questions this review has raised.

The *Porphyra* and *Pyropia* genera are distributed world-wide with over a hundred different species described, which includes the important aquaculture crop *nori* that is commonly cultivated in Asia. In addition to their obvious commercial benefits in the food industry, these bangiophyceaen species are interesting model species for the red algae given the availability of several high-quality reference genomes (Table 1), well-established transformation systems (Kong *et al.*, 2017), and a biphasic life cycle that can be easily controlled in the laboratory (Saga & Kitade, 2002; Gantt *et al.*, 2010).

Chondrus crispus, or Irish moss, is a common red alga found on rocky shores in the Northern Atlantic and adjacent waters such as the North Sea (Collén *et al.*, 2014). It has attracted attention because it is the historical source of carrageenan, which is used in the food industry as a thickening agent. Because of the ecological and economic interest in this species, an abundant scientific literature exists for *C. crispus* compared with other seaweeds in addition to a reference genome sequence (Collén *et al.*, 2013). *Chondrus crispus* has a typical red algal life cycle and is one of the few red algae for which detailed reproductive system data are available, including both direct (i.e. paternity analyses, Krueger-Hadfield *et al.*, 2015) and indirect estimates of selfing (i.e. F_{IS} values, Krueger-Hadfield *et al.*, 2013). One major hurdle for using *C. crispus* as a model for genetic studies is the fact that the life cycle in laboratory conditions is very long (up to 2 yr; Chen & McLachlan, 1972).

Gracilaria species have been classically used for genetics and inheritance studies since the 1970's (e.g. van der Meer, 1979; Zhang & van der Meer, 1988). Genomic resources, including high-quality genomes (Lipinska *et al.*, 2023), are now available for several species of *Gracilaria*. A particularly interesting species is *G. caudata*, whose life cycle can be completed within 5 months in laboratory conditions, which has already allowed for the characterisation of mutants (Marchi & Plastino, 2020). We also note that *G. gracilis* (Engel *et al.*, 2004), *G. chilensis* (Guillemin *et al.*, 2012; Huanel *et al.*, 2022), and *G. vermiculophylla* (Krueger-Hadfield *et al.*, 2017, 2021a,b; Flanagan *et al.*, 2021) have had their reproductive systems and genetic structure thoroughly documented.

Other members of the Florideophyceae have been investigated more recently that could make interesting model organisms. For example, species within the *Griffithsia* genus appear to be highly attractive for red algal cell biology, having been used to study phycobilisomes, photosynthesis, intercellular transport, and cellular repair (Zhang *et al.*, 2017; Pi *et al.*, 2018; Ma *et al.*, 2020; Li *et al.*, 2021; Kim *et al.*, 2022). Genetic diversity, speciation, and ecology have been documented within the *Bostrychia* genus (Karsten *et al.*, 1993; Zuccarello & West, 2011) where aspects of reproduction have also been investigated, including fertilisation (e.g. Pickett-Heaps & West, 1998; Shim *et al.*, 2020) and haploid sex determination (Shim *et al.*, 2021). Finally, species within (or formerly attributed) to the *Polysiphonia* genus have been subjected to extensive electron microscopic studies in the past (Scott *et al.*, 1980; Delivopoulos & Kugrens, 1984) and are perhaps the most classic 'textbook' examples of red algae that are used to describe the 'triphasic' life cycle. However, a major caveat for these species is that reference genome sequences remain unavailable.

X. Conclusion

Our review has summarised several decades of research into red macroalgal biology that have addressed a broad range of phenomena, including the evolutionary origin and phylogenetic relationship of red algae, their unique cell biology, complex reproduction, and elaborate life cycles. The Rhodoexplorer project has brought together a consortium of laboratories interested in red macroalgae that are actively developing new molecular resources and tools in this field of research. The increasing availability of genomic resources for several red macroalgae will be key to enable future exploration into the molecular mechanisms underlying many aspects of their fascinating biology. Reference genome sequences will be invaluable for studying the ecology of natural populations of red macroalgae, while the development of model systems will allow for the application of genetic and molecular biology approaches in a concerted manner by laboratories across the world. The coming years thus promise to deliver new and exciting discoveries into the ecology, physiology, metabolism, and development of red algae, which will ultimately enlighten our understanding of this relatively understudied red branch in the eukaryotic tree of life.

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