CHAPTER TWO

Diversity and Evolution of Algae: Primary Endosymbiosis

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Abstract

Oxygenic photosynthesis, the chemical process whereby light energy powers the conversion of carbon dioxide into organic compounds and oxygen is released as a waste product, evolved in the anoxygenic ancestors of Cyanobacteria. Although there is still uncertainty about when precisely and how this came about, the gradual oxygenation of the Proterozoic oceans and atmosphere opened the path for aerobic organisms and ultimately eukaryotic cells to evolve. There is a general consensus that photosynthesis was acquired by eukaryotes through endosymbiosis, resulting in the enslavement of a cyanobacterium to become a plastid. Here, we give an update of the current understanding of the primary endosymbiotic event that gave rise to the Archaeplastida. In addition, we provide an overview of the diversity in the Rhodophyta, Glaucophyta and the Viridiplantae (excluding the Embryophyta) and highlight how genomic data are enabling us to understand the relationships and characteristics of algae emerging from this primary endosymbiotic event.

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1. INTRODUCTION

1.1. Early Evolution of Oxygenic Photosynthesis

The origin of oxygenic photosynthesis has changed the face of our planet in all aspects. The first organisms that developed oxygenic photosynthesis are thought to have been the anoxygenic ancestors of Cyanobacteria (Allen & Martin, 2007), but when and how this came about remains a matter of debate (Farquhar, Zerkle, & Bekker, 2011; Hohmann-Marriott & Blankenship, 2011). Estimates based on geological and geochemical evidence and molecular phylogenetic analyses calibrated with the fossil record agree on a minimum age of 2.3 billion years ago (Tomitani, Knoll, Cavanaugh, & Ohno, 2006), but the origin of oxygenic photosynthesis may date back to 3.4 or even 3.8 billion years ago (Buick, 2008; Russell & Hall, 2006) (Fig. 2.1E). Because oxygenic photosynthesis involves the photolysis of water into electrons, protons and free oxygen, Cyanobacteria are singularly responsible for oxygenating the atmosphere and transforming a once reducing environment into an oxidising one (Holland, 2006).

With oxygen becoming gradually available as a very potent electron acceptor, the path lay open for aerobic organisms to evolve. Aerobes soon managed to maintain much more productive ecosystems as more energy per electron transfer could be harvested. Consequently, oceanic primary production increased an order of magnitude (Canfield, Rosing, & Bjerrum, 2006), permitting the evolution of more complex life forms (Catling, Glein, Zahnle, & McKay, 2005) and adapted or novel biochemical pathways (Falkowski, 2006; Raymond & Segré, 2006). The rising atmospheric oxygen is thought to have directly triggered cellular compartmentalization and eukaryogenesis (Fig. 2.1E). Atmospheric oxygen is thought to have constrained the topology of ancient transmembrane proteins by limiting the size and number of the external domains of transmembrane proteins (Acquisti, Kleffe, & Collins, 2007). When oxygen levels rose, the constraint likely decreased, permitting larger and more communication–related transmembrane proteins opening the door for subsequent compartmentalization. Alternatively, rising oxygen levels is speculated to have promoted cellular compartmentalization in order to protect the metabolic activities of the plasma membrane from rising levels of reactive oxygen species in the cellular environment (Gross & Bhattacharya, 2010). The fossil record (Javaux, 2011; Knoll, Javaux, Hewitt, & Cohen, 2006) and time-calibrated phylogenies (Hedges, Blair, Venturi, & Shoe, 2004; Parfrey, Lahr, Knoll, & Katz, 2011)
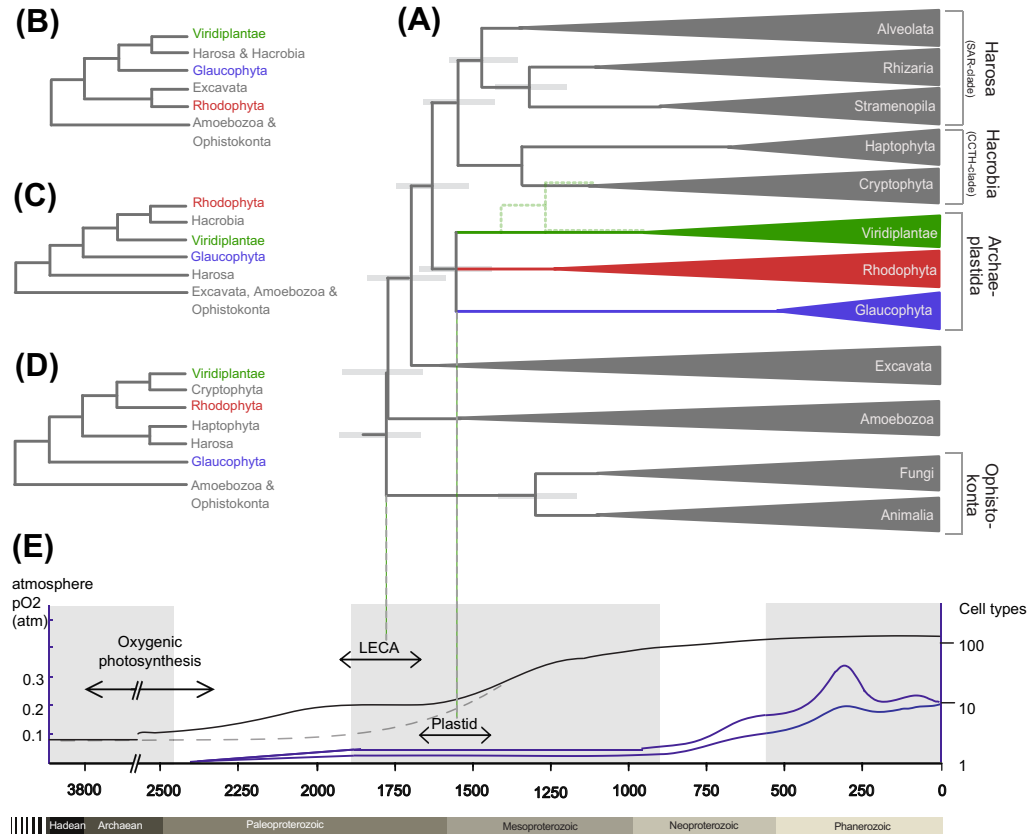
Figure 2.1 Relationships of Archaeplastida with main eukaryotic lineages and correlation between the rise in atmospheric oxygen and the evolution of organismal complexity. (A) Time-calibrated tree of extant eukaryotes (after Parfrey et al., 2011). The tree topology is adjusted for the current uncertainty with respect to the branching order within the Archaeplastida. The dotted green line denotes the sister relationship between Viridiplantae and Cryptophyta in the analysis of Parfrey et al. (2011). Nodes are at mean divergence times and gray bars represent 95% highest probability density of node age. (B–D) Alternative topologies suggested by, respectively, Nozaki et al. (2009), Hampl et al. (2009) and Baurain et al. (2010). (E) Atmospheric partial oxygen pressure (blue lines) and cellular complexity (black line) (after Holland, 2006, and Hedges et al., 2004). Blue lines denote maximum and minimum estimates of atmospheric O_2 partial pressure, respectively. Cellular complexity is defined as number of cell types. The black dashed line shows a more conservative interpretation of cellular complexity in the Proterozoic. The alternation of gray and white periods denotes the five different stages in oxygenation of the atmosphere according to Holland (2006). LECA: last eukaryotic common ancestor. See the colour plate.

suggest that the major eukaryotic lineages diverged already in the Paleo–proterozoic era (2500–1600 Ma), but that diversity within major extant clades expanded later, beginning about 800 Ma, coinciding with the spread of oxygen through the Neoproterozoic oceans (Fig. 2.1A, E).

1.2. Origin of Plastids: Primary Endosymbiosis

Even though there is still considerable debate regarding the precise mechanisms and sequence of events that resulted in the first eukaryotic cell (de Duve, 2007; Embley & Martin, 2006; Martin & Muller, 1998; Poole & Neumann, 2011; Roger, 1999), there is a general consensus that photosynthetic eukaryotes emerged from a heterotrophic eukaryote which engulfed a cyanobacterium. The cyanobacterium was gradually enslaved and integrated into the cellular machinery as a new organelle: the plastid. This event has been termed primary endosymbiosis. The cyanobacterial origin of plastids is supported by overwhelming genetic evidence and ultrastructural similarities between plastids and their cyanobacterial relatives (Box 2.1). The original cyanobacterial genome underwent a drastic reduction with most genes either lost or transferred to the host nucleus,

BOX 2.1 Ultrastructural and Molecular Similarities Between Plastids and Cyanobacteria

Homology between (envelope) membranes	Presence of galactolipids, β-barrel proteins and occurrence of a peptidoglycan layer in Glaucophyta
Chloroplast DNA	Similarities in structure and gene content of the circular genome, organized into discrete nucleoids
Molecular phylogeny	Phylogenetic studies indicate that chloroplast gene sequences are nested within eubacterial homologs
Nuclear genes of cyanobacterial origin	Ample presence of genes of presumed cyanobacterial origin in the nuclear genome as a consequence of EGT
Ribosomes	Chloroplasts contain ribosomes that are 70S in size similar to prokaryotic ribosomes, as opposed to typical eukaryotic, cytosolic and endoplasmic-reticulum-associated, which are 80S in size; Inhibition by antibiotics (e.g. streptomycin, kanamycin) that affect ribosome function in free-living Eubacteria
Photosynthetic apparatus	The presence of two photosystems (PS I and PS II) in which a central chlorophyll *a* molecule is oxidized; electrolysis of H_2O as electron donor and release of O2; similarities in the electron transport chains; shared phycobilisomes between Cyanobacteria and Glaucophyta and Rhodophyta

termed endosymbiotic gene transfer (EGT). A fraction of the genome is retained within the primary plastid, minimally encoding its own protein synthesizing machinery and a number of genes involved in photosynthesis (Gould, Waller, & McFadden, 2008). Genes that have been transferred to the host nucleus are transcribed and translated in the host cytosol or endoplasmic reticulum and are targeted back to the chloroplast using a protein import system (Bhattacharya, Archibald, Weber, & Reyes-Prieto, 2007). In contrast to what might be intuitively expected, also gene products of host origin can be plastid-targeted and only a subset of cyanobacterial genes takes up a function in the organelle (Deusch et al., 2008; Martin et al., 2002). The overall emerging picture is one of large genomic impact of the symbiont on its host after primary endosymbiosis (Elias & Archibald, 2009), although the exact impact on the genomic content of Archaeplastida remains uncertain. Some phylogenomic analyses estimate the contributed genes to range around 20% of the total gene number when including a correction for the high rate of divergence (Deusch et al., 2008; Martin et al., 2002). Others calculate more modest percentages of chloroplast-derived genes, ranging around 5% while acknowledging these proportions are most likely underestimations due to high sequence divergence (Moustafa & Bhattacharya, 2008; Reyes-Prieto, Hackett, Soares, Bonaldo, & Bhattacharya, 2006). Next to sequence divergence, amelioration and modularity of transferred genes are thought to be additional complicating factors to detect horizontal gene transfer (Chan et al., 2011). Remarkably, some phylogenomic analyses, with the exception of the glaucophyte study of Reyes-Prieto et al. (2006), indicate that more than 50% of the transferred genes have other functions, from metabolism to cell division, instead of being plastid targeted (Deusch et al., 2008; Martin et al., 2002).

Three extant groups of photosynthetic eukaryotes have primary plastids: the green plants, red algae and the glaucophytes. Together they make up the Archaeplastida. Even though the cyanobacterial origin of the plastids in these groups is beyond dispute, the number of endosymbiotic events and the relationships among the three lineages is more contentious (Delwiche, 1999, 2007). For a long time, variation in plastid structure and light-harvesting pigments has given credit to a polyphyletic origin of primary plastids, that is, the hypothesis that primary plastids resulted from multiple independent primary endosymbiotic events. Recent evidence points towards a single origin of primary plastids, which implies a single ancestor of the plastid as well as the monophyly of the three lineages that

make up the Archaeplastida (Keeling, 2010; Rodriguez-Ezpeleta *et al.*, 2005). As pointed out by Larkum, Lockhart, and Howe (2007), however, support for a single origin of plastids should be treated with caution and several lines of evidence, which are predominantly based on phylogenetic tree methods, may not disprove all alternative scenarios of plastid acquisition. There is at least one exception to this rule: *Paulinella chromatophora*, a cercozoan amoeba with photosynthetic inclusion of cyanobacterial origin (Marin, Nowack, & Melkonian, 2005; Nowack *et al.*, 2011; Nowack & Grossman, 2012).

Even though several analyses provide moderate to strong support for a monophyletic Archaeplastida (Burki *et al.*, 2007, 2009; Hackett, Yoon, Li, Reyes-Prieto, Rummele, & Bhattacharya, 2007; Patron, Inagaki, & Keeling, 2007; Rodriguez-Ezpeleta *et al.*, 2005), other studies suggest that the Archaeplastida might be paraphyletic with respect to the Hacrobia (Burki, Okamoto, Pombert, & Keeling, 2012; Hampl *et al.*, 2009) or the entire Chromalveolata (Baurain *et al.*, 2010; Nozaki *et al.*, 2009) (Fig. 2.1A–D). The incongruence between analyses is likely caused by systematic biases including EGT as suggested by the high instability of resultant topologies of photosynthetic clades with varying levels of taxon sampling and missing data (Parfrey *et al.*, 2010). Indeed, gene sampling has been shown to account for at least some of the incongruence among the relationships of primary plastid lineages (Inagaki, Nakajima, Sato, Sakaguchi, & Hashimoto, 2009). The persistent incongruence of large concatenated data sets shows that a solution may not be found by increasing sequence length (Baurain *et al.*, 2010; Burki *et al.*, 2009; Hampl *et al.*, 2009). Instead when relaxing the assumption of vertical gene transfer by abandoning concatenation and choosing for a gene-by-gene approach, Chan *et al.* (2011) and Price *et al.* (2012) provide additional evidence for monophyly of red and green algae.

In the light of the persistent uncertainty on the monophyly of Archaeplastida, it may not come as a surprise that the relationships between green plants, red algae and glaucophytes are still unclear. Traditionally, glaucophytes are thought to have diverged before the red algae and green plants based on similarities of the plastid with cyanobacteria, such as the presence of a peptidoglycan layer surrounding the plastids (originally named 'cyanelles'). Phylogenetic gene analyses are unfortunately not conclusive on the relationships between the major clades of the Archaeplastida (Rodriguez-Ezpeleta *et al.*, 2005; Rodriguez-Ezpeleta, Philippe, Brinkmann, Becker, & Melkonian, 2007). Furthermore, several studies point towards an early diverging red algal

lineage (Burki et al., 2009; Hackett et al., 2007; Patron et al., 2007), although this result might be biased by the inclusion of clades with secondary plastids (Deschamps & Moreira, 2009). Therefore, analyses concentrating on EGT genes of cyanobacterial origin only might be more trustworthy. Even so, phylogenetic analyses are ambiguous either suggesting the glaucophytes (Reyes-Prieto & Bhattacharya, 2007) or green lineage (Deschamps & Moreira, 2009) as earliest diverging lineage within the Archaeplastida.

Under the assumption of a single origin of primary plastids, the question remains what kind of cyanobacterium participated in the origin of plastids. Unfortunately, due to the large divergence times and the considerable extent of horizontal gene transfer between cyanobacteria (Deusch et al. 2008), the phylogenetic signal of these relationships is seriously eroded. Some studies suggest a rather deep origin of plastids, predating diversification of most extant cyanobacterial lineages (Criscuolo & Gribaldo, 2011; Reyes-Prieto et al., 2010; Rodriguez-Ezpeleta et al., 2005; Sato, Wise, & Hoober, 2006), other studies suggest that plastids are more closely related to one of the contemporary clades such as N-fixing subsection I (Deschamps et al., 2008; Falcon, Magallon, & Castillo, 2010) or filamentous heterocyst-forming subsection IV (Deusch et al., 2008). In addition, it is difficult to determine when this primary endosymbiosis occurred. Estimates based on fossil evidence and biomarkers are widely divergent (Knoll, 1992). A recent calibrated phylogeny of Parfrey et al. (2011) corroborates earlier studies with an estimated ages of the clade containing Viridiplantae, red algae and glaucophytes around 1.5–1.6 billion years ago (Hedges et al., 2004; Yoon, Hackett, Ciniglia, Pinto, & Bhattacharya, 2004) (Fig. 2.1E). Following the origin of Archaeplastida, photosynthesis spread widely among diverse eukaryotic groups via secondary and tertiary endosymbiotic events (Archibald, 2009; Gould et al., 2008; Keeling, 2010). Overviews of the intricate histories of plastid acquisition are provided in the next chapter of this volume (Archibald, 2012).

2. RED ALGAE

2.1. Red Algae Defined

The red algae or Rhodophyta are a distinct lineage of eukaryotic algae, containing about 5000–6000 species of mostly multicellular marine algae. The red algae are distinguishable among eukaryotic lineages by a combination of biochemical and ultrastructural features, some of which they share with Glaucophyta and Cyanobacteria. First, red algal plastids lack

chlorophyll accessory pigments. Instead light energy is directed to the reaction centre by phycobiliproteins (phycocyanin, allophycocyanin and phycoerythrin). Light-harvesting antennae pigments are grouped in hemi-spherical protein complexes, phycobilisomes, anchored to the thylakoids. These are not stacked in grana like in the Viridiplantae but lie singly and more or less equidistant in the plastid stroma. One of the most distinctive characters of the red algae is the complete absence of flagella and centrioles in all life stages, which affects mitosis and, at least in some groups, their life cycles (Graham, Graham, & Wilcox, 2009; Maggs, Verbruggen, & De Clerck, 2007; Saunders & Hommersand, 2004; van den Hoek, Mann, & Jahns, 1995; Yoon, Müller, Sheath, Ott, & Bhattacharya, 2006b; Yoon, Zuccarello, & Bhattacharya, 2010).

From the early twentieth century until very recently, red algae were classified in two distinct groups, most commonly treated as classes, Bangio-phyceae and Florideophyceae, within a single phylum, Rhodophyta. This dichotomy in the classification is reflected in the morphological complexity that characterizes the red algae, with the Bangiophyceae uniting the morphologically simple forms (unicells or undifferentiated filaments and blades) and the Florideophyceae containing the more complex growth forms. Growth in the Florideophyceae is essentially filamentous, but individual filaments may aggregate to form a pseudoparenchymatous tissue. Growth forms include filaments, blades, elaborately branched thalli as well as calcified crusts (coralline algae). A wealth of molecular and ultrastructural data, however, made clear that this traditional classification did not reflect the antiquity and diversity of the rhodophytes (Müller, Lynch, & Sheath, 2010; Müller *et al.*, 2001; Oliveira & Bhattacharya, 2000). The structurally simple Bangiophyceae is composed of a series of radiations that define the ancestral lineages of the red algae, and part of the traditionally circumscribed Bangio-phyceae is more closely related to the Florideophyceae. Hence, a new classi-fication was originally proposed by Saunders and Hommersand (2004) and subsequently refined by Yoon *et al.* (2006b). The phylum Rhodophyta is now subdivided into two subphyla, Cyanidiophytina and Rhodophytina, and seven classes, Cyanidiophyceae, Bangiophyceae, Compsopogonophyceae, Florideophyceae, Porphyridiophyceae, Rhodellophyceae and Stylonemato-phyceae (Fig. 2.2). The diversity contained in the Compsopogonophyceae, Porphyridiophyceae, Rhodellophyceae and Stylonematophyceae is still ill-defined as can be witnessed by the many unnamed lineages that typically adorn phylogenetic trees (Scott *et al.*, 2008; West, Zuccarello, Scott, West, & Karsten, 2007; Yang *et al.*, 2010; Yokoyama *et al.*, 2009; Zuccarello, Kikuchi, & West,

2010; Zuccarello, Oellermann, West, & De Clerck, 2009; Zuccarello, West, & Kikuchi, 2008; Zuccarello et al., 2011).

Red algae are an ancient lineage (Xiao, Zhang, & Knoll, 1998; Yoon et al., 2004). A 1.2-billion-year-old fossil, *Bangiomorpha pubescens* (Butterfield, 2000), which bears a lot of resemblance to extant *Bangia* species, is regarded as the oldest taxonomically resolved eukaryotic fossil. The taxonomic affinity of *Bangiomorpha* was long contested, with some authors (e.g. Cavalier-Smith, 2006) advocating that *Bangiomorpha* is a blue-green alga or a mixture of at least two species of blue-green algae, possibly related to the Stigonematales. *Bangiomorpha* being a eukaryotic fossil would indicate a Mesoproterozoic origin of red algae and by extension all major lineages of the eukaryotes, which contradicts the hypothesis of Cavalier-Smith that places eukaryogenesis at 850 Ma (Cavalier-Smith, 2010). A red algal nature of *Bangiomorpha*, however, is not in conflict with the most recent timing of eukaryotic diversification using calibrated phylogenies (Parfrey et al., 2011) or the interpretation of several microfossils that place the origin of the eukaryotes at around 1800 Ma (Knoll et al., 2006). In addition to the fossils indicative of a Mesoproterozoic origin of red algae, the remarkably well-preserved multicellular red algae from the Doushantuo Formation in southern China (ca. 600 Ma) are clear proof that red algae had already radiated prior to the Precambrian radiation.

2.2. Cyanidiophytes

The updated classification of the red algae also better reflects the ultrastructural and ecological diversity of the group. Of special interest are the Cyanidiophyceae, a group of unicellular and presumably asexual algae, which live in thermoacidophilic conditions that are detrimental to most eukaryotic live on earth (Barbier et al., 2005; Ciniglia, Yoon, Pollio, Pinto, & Bhattacharya, 2004; Matsuzaki et al., 2004; Yoon et al., 2006a). Yoon et al. (2004) suggested that the Cyanidiophyceae diverged from the remaining red algae prior to the secondary endosymbiotic event that gave rise to the Chromalveolata. Even though a reinterpretation of this event is necessary now that the chromalveolate hypothesis is being increasingly challenged (Archibald, 2012; Baurain et al., 2010), it does vouch for the antiquity of the divergences that separate the deep red algal lineages. Because the Cyanidiophyceae are one of few eukaryotic groups that thrive in environments that are otherwise dominated by Archaea and Bacteria, their enzymes are of special interest to the biotechnology and pharmaceutical industry. It is,

therefore, not surprising that *Cyanidioschyzon merolae* was the first eukaryotic alga for which a full genome sequence was available (Matsuzaki *et al.*, 2004; Misumi *et al.*, 2005) and the genome of the related *Galdieria sulphuraria* is currently being sequenced (Barbier *et al.*, 2005). The small and compact genome of *C. merolae*, combined with an extremely reduced cell architecture (one nucleus, mitochondrion and chloroplast and the absence of a cell wall), makes it an ideal organisms to address the synchronization and mechanisms of the organellar division during cytokinesis (Kuroiwa, 1998). Only seven Cyanidiophyceae species are currently described, but this number severely underestimates the diversity. Applying environmental sequencing from a single locality in Italy, Ciniglia *et al.* (2004) unveiled considerable cryptic diversity and within-lineage (often 'intraspecific') sequence divergence that is comparable, for example, to between-order divergences in the non-Cyanidiales red algae.

2.3. Of Nori and Red Seaweed

The great majority of red algae are multicellular, marine seaweeds, with an enormous range of morphologies and complex haplodiploid life histories, which involve additional zygote amplification stages resulting in large numbers of spores from a single fertilization (Verbruggen *et al.*, 2010). Red seaweeds belong nearly exclusively to two classes, Bangiophyceae (in the narrow sense of the new classification) and Florideophyceae (Fig. 2.2). Traditionally, two genera belonging to a single family, Bangiaceae, have been recognized in the Bangiophyceae. Unbranched uniseriate to multiseriate filaments have been placed in the genus *Bangia* and blades in the genus *Porphyra* (Sutherland *et al.*, 2011). The latter genus is commonly known as 'nori', which is cultivated as one of the most profitable mariculture crops in the north-western Pacific (Niwa *et al.*, 2009). Molecular phylogenetic analyses resulted in the recognition of 15 genera, 7 filamentous and 8 foliose, which are only separable on molecular rather than morphological grounds. Regrettably, this taxonomic vigour also resulted in the fact that the *Porphyra*, which has wrapped sushi for decades, has now become a *Pyropia* (Zuccarello, 2011).

The near-morphological stasis that characterizes the Bangiophyceae, contrasts sharply with the wealth of growth forms that is encountered in its sister taxon, the Florideophyceae. DNA sequence data have progressively refined an ordinal classification (e.g. Choi, Kraft, Lee, & Saunders, 2002; Harper & Saunders, 2001; Le Gall & Saunders, 2007; Saunders, Chiovitti, & Kraft, 2004), which was originally entirely based on morphological
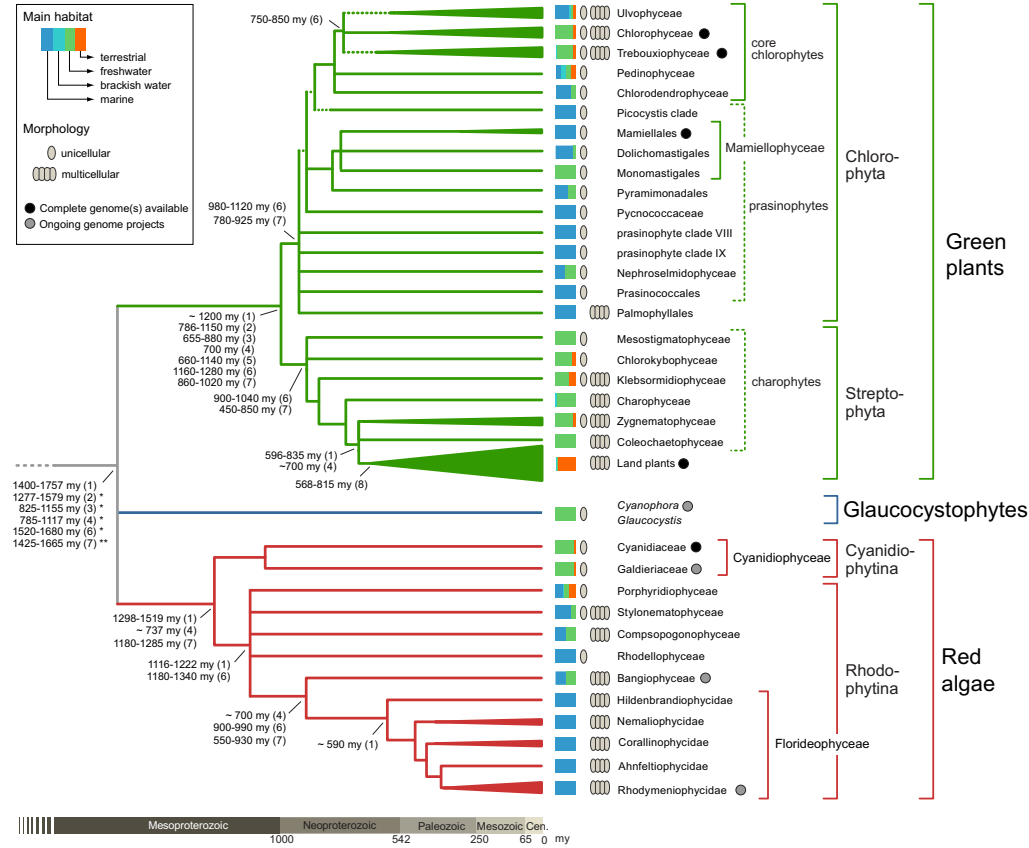
Figure 2.2 Relationships among major groups of Archaeplastida based on published molecular phylogenetic data (Leliaert *et al.*, 2012; Yoon *et al.*, 2010) with indication of main habitats and morphology. Divergence times are based on molecular clock studies calibrated with the fossil record: (1) Yoon *et al.* (2004), (2) Hedges *et al.* (2004), (3) Douzery, Snell, Bapteste, Delsuc, and Philippe (2004), (4) Berney and Pawlowski (2006), (5) Roger and Hug (2006) (r8s-PL method), (6) Herron *et al.* (2009) and (7) Parfrey *et al.* (2011). *excluding glaucophytes; **including cryptomonads. See the colour plate.

and reproductive features (Maggs *et al.*, 2007). The Florideophyceae now includes some 25 orders grouped into five subclasses, Hildenbrandiophycidae, Nemaliophycidae, Ahnfeltiophycidae, Rhodymeniophycidae and Corallinophycidae (Le Gall & Saunders, 2007; Maggs *et al.*, 2007; Saunders & Hommersand, 2004), which are reasonably well supported by ultrastructural characters. Ordinal relationships, however, remain at least partly unresolved (Verbruggen *et al.*, 2010) and form the motivation of a Red Tree of Life project (http://dblab.rutgers.edu/redtol/).

Genomic data of red seaweeds are currently limited to a number of organelle genomes and expressed sequence tag (EST) libraries of commercially important species such as *Chondrus*, *Gracilaria* and *Porphyra* (Asamizu *et al.*, 2003; Collen *et al.*, 2006; Hagopian, Reis, Kitajima, Bhattacharya, & De Oliveira, 2004; Nikaido *et al.*, 2000) and the coralline red alga *Calliarthron tuberculosum* (Chan *et al.*, 2011), but this may change soon with whole genome project of the carragenophyte *Chondrus crispus* and *Porphyra umbilicalis* in progress.

3. GREEN PLANTS (VIRIDIPLANTAE)

3.1. Green Plants Defined

The green plant clade (Viridiplantae) includes green algae and embryophytic land plants and is one of the main groups of photosynthetic eukaryotes. Green plants are diverse in terms of species number, morphology, biochemistry and ecology. Monophyly of the group is well established based on ultrastructural, biochemical and molecular data (Leliaert *et al.*, 2012; Lewis & McCourt, 2004).

Green plants share a number of unique characteristics. The chloroplasts are surrounded by a double membrane, have thylakoids grouped in lamellae and contain chlorophyll *a* and *b* along with some accessory pigments including carotenoids and xanthophylls. Pyrenoids (when present) are embedded within the chloroplast and are surrounded by starch, which is the main reserve polysaccharide. Cell walls (when present) are generally composed of cellulose. Many green algae are flagellates or have flagellate cells in some stage of the life cycle. The flagella (generally two or four on a cell) are isokont, which means that they are similar in structure, although they may differ in length. The region between the flagellar axoneme and the basal body is characterized by a stellate structure (Graham *et al.*, 2009; van den Hoek *et al.*, 1995).

Apart from these unifying ultrastructural and biochemical features, green plants are extremely diverse morphologically. They range from unicells with

sizes comparable to bacteria to large and complex multicellular or siphonal life forms. Although the described species diversity of land plants (including over 250,000 species) exceeds that of green algae (about 15,000 named species), green algae encompass a greater cytomorphological, biochemical and reproductive diversity, which reflect their old evolutionary age (Leliaert et al., 2012). The progenitor of green plants was likely a unicellular flagellate or at least had flagellate stages in its life cycle. Colonial and multicellular forms have evolved multiple times in several lineages, including the Streptophyta, Ulvophyceae, Chlorophyceae, Trebouxiophyceae and Palmophyllales (Fig. 2.2).

Green plants are also ecologically very diverse. They are especially abundant in freshwater (most charophytes, Chlorophyceae and Trebouxiophyceae) and marine environments (Ulvophyceae and prasinophytes; Not et al., 2012), but some have adapted to specific habitats, such as dry land (Lewis & Lewis, 2005; López-Bautista, Rindi, & Guiry, 2006), arctic (De Wever et al., 2009) and marine deep water environments (Zechman et al., 2010). Several members of the core chlorophytes live in symbiosis with a diverse array of eukaryotes (Friedl & Bhattacharya, 2002; Kerney et al., 2011; Lewis & Muller-Parker, 2004) or have adopted a heterotrophic life style as parasites (Joubert & Rijkenberg, 1971; Sudman, 1974). Embryophytes have dominated terrestrial habitats for millions of years; some land plants have adapted secondarily to freshwater or marine environments.

3.2. Evolutionary History of Green Plants

Green plants have played a significant ecological role for millions of years (Leliaert, Verbruggen, & Zechman, 2011; O'Kelly, 2007). The ecological importance of green algae has been mainly in marine and freshwater environments. The origin of land plants from a green algal ancestor was a key event in the evolution of life on earth. This event initiated the development of the entire terrestrial ecosystem and has led to environmental changes on a global scale (Kenrick & Crane, 1997). Time-calibrated phylogenies, calibrated with the scarce fossil record, have estimated the origin of the green plant lineage somewhere between 700 and 1500 Ma (Berney & Pawlowski, 2006; Hedges et al., 2004; Herron, Hackett, Aylward, & Michod, 2009; Yoon et al., 2004). An early split in the evolution of green plants gave rise to two main clades: the Chlorophyta and Streptophyta (Leliaert et al., 2012; Lemieux, Otis, & Turmel, 2007; Rodriguez-Ezpeleta et al., 2007) (Fig. 2.2).

The Chlorophyta probably diversified as unicellular algae in the Meso- and Neoproterozoic. These green algae were dominant in the oceanic

phytoplankton of the Paleozoic as evidenced by fossil deposits of resistant outer walls of prasinophytic cysts, known as phycomata (Colbath, 1983; Knoll, 1992; O'Kelly, 2007; Tappan, 1980). This early radiation of Chlorophyta was important to the eukaryotic greening that shaped the geochemistry of our planet (Worden *et al.*, 2009). During the Mesozoic, the dominance of marine green algae in the phytoplankton gradually decreased as they were largely displaced by the red–plastid–containing dinoflagellates, coccolithophores and diatoms (Falkowski *et al.*, 2004; Leliaert *et al.*, 2011; O'Kelly, 2007; Simon, Cras, Foulon, & Lemee, 2009). These ancestral green unicells gave rise to modern prasinophytes and the core Chlorophyta that diversified as unicellular and multicellular organism in marine, freshwater and terrestrial habitats.

The Streptophyta probably originated in the Neoproterozoic and diversified as unicellular algae in freshwater environments (Becker & Marin, 2009). Two important groups of multicellular charophytes diversified during the Paleozoic: the conjugating green algae (Zygnematophyceae) and stoneworts (Charophyceae) (Becker & Marin, 2009). Similar to the situation in marine environments, red–plastid–containing dinoflagellates, diatoms and chrysophytes gradually took over the green dominance in Early Cretaceous and Cenozoic freshwater ecosystems (Becker & Marin, 2009). Ancestral charophytes invaded the land during the mid-Ordovician and early Silurian (480–430 million years ago), giving rise to the land plants (Delaux, Nanda, Mathé, Sejalon-Delmas, & Dunand, 2012; McCourt, Delwiche, & Karol, 2004).

Molecular phylogenetic studies have drastically reshaped our views of green plant evolution and continue to do so (Leliaert *et al.*, 2012; Marin, 2012; Timme, Bachvaroff, & Delwiche, 2012). However, many uncertainties remain, especially about the deepest branches of the green plants. One of the main goals of the Green Algal Tree of Life Project (http://alleyn.eeb.uconn.edu/gratol/) is to resolve relationships among the main green algal lineages. Phylogenetic hypotheses are critical in providing an evolutionary framework for comparative genomic studies. In the following section, we give a brief overview of the major green plant lineages and their relationships.

3.3. Chlorophyta

The Chlorophyta form a large and morphologically diverse clade of marine, freshwater and terrestrial green algae. The flagellar apparatus in this clade is characterized by a symmetrical cruciate root system wherein rootlets of variable (X) numbers of microtubules alternate with rootlets composed of

two microtubules to form a 'X-2-X-2' arrangement. The orientation of this flagellar root system has been an important character for defining the main groups of Chlorophyta. Molecular data have revealed several major chlorophytan clades. Several early diverging clades of unicellular algae, collectively termed the prasinophytes, form a paraphyletic assemblage at the base of the chlorophytan tree. These clades are relatively species poor compared to the three principal clades of the core Chlorophyta: Ulvophyceae, Trebouxiophyceae and Chlorophyceae (Fig. 2.2).

Prasinophytes form a heterogeneous assemblage of mostly unicellular algae with diverse cell shapes that are naked, covered by walls or organic body scales; flagella are present or absent (Leliaert et al., 2011; Melkonian, 1990; Sym & Pienaar, 1993). Mitotic processes, biochemical features and photosynthetic pigments are equally diverse, reflecting the paraphyletic nature of the group (Fawley, Yun, & Qin, 2000; Guillou et al., 2004; Latasa, Scharek, Le Gall, & Guillou, 2004; Nakayama et al., 1998; Zingone et al., 2002). Prasinophytes are predominantly found in marine environments, although several species also occur in freshwater. About 10 distinct prasinophyte lineages have been identified, but their phylogenetic affinities remain largely unresolved (Leliaert et al., 2011, 2012; Marin & Melkonian, 2010; Turmel, Gagnon, O'Kelly, Otis, & Lemieux, 2009) (Fig. 2.2).

The Nephroselmidophyceae includes flagellates with complex scale covering and is possibly one of the earliest diverging chlorophytan lineages (Turmel et al., 2009). Although Nephroselmis is one of the few prasinophytes where sexual reproduction has been observed (Suda, Watanabe, & Inouye, 2004), genomic evidence, such as the identification of meiosis–related genes, indicates that sexual reproduction is probably more widespread among prasinophytes (Derelle et al., 2006; Worden et al., 2009). Future population genomic studies may enable us to estimate the prevalence of sexual recombination in algae (Toulza, Knoll, Cavanaugh, & Ohno, 2012).

The Mamiellophyceae includes the morphologically and ecologically diverse Mamiellales and two smaller clades, the Monomastigales and Dolichomastigales (Marin & Melkonian, 2010; Nakayama, Kawachi, & Inouye, 2000; Sym & Pienaar, 1993; Turmel et al., 2009; Zingone et al., 2002). The Mamiellales include marine and freshwater flagellates and coccoid forms. Species of Ostreococcus and Micromonas are among the smallest eukaryotes known, with cell sizes of 0.5–2 μm (Derelle et al., 2006; Palenik et al., 2007; Worden et al., 2009) and are important components of marine picoeukaryotic communities (Leliaert et al., 2012; Not et al., 2004;

O'Kelly, Sieracki, Thier, & Hobson, 2003; Vaulot, Eikrem, Viprey, & Moreau, 2008). We refer to Chapter 10 (Toulza *et al.*, 2012) for a review on environmental genomics in the Mamiellales and other microalgae.

The Pyramimonadales includes large flagellates covered by complex body scales found in marine and freshwater environments. Some species are unique among green plants in possessing a food uptake apparatus (Moestrup, Inouye, & Hori, 2003), which has been interpreted as a character that might have been inherited from a phagotrophic ancestor of the green plants (O'Kelly, 2007).

The *Picocystis* clade includes the coccoid *Picocystis* from saline lakes. Together with some undescribed coccoids ('CCMP1205 clade'), these prasinophytes might form the closest sister lineages of the core chlorophytes, although strong support is lacking (Marin & Melkonian, 2010). Several other prasinophytic groups have uncertain phylogenetic affinities. These include the Pycnococcaceae, a clade of marine flagellate and coccoid species (Nakayama, Suda, Kawachi, & Inouye, 2007; Turmel *et al.*, 2009); the Prasinococcales, a clade of marine coccoids (Hasegawa *et al.*, 1996; Sieburth, Keller, Johnson, & Myklestad, 1999) and two clades ('clades VIII and IX') that are known from environmental sequencing only (Lepère, Vaulot, & Scanlan, 2009; Shi, Marie, Jardillier, Scanlan, & Vaulot, 2009; Viprey, Guillou, Ferréol, & Vaulot, 2008).

The Palmophyllales includes green algae from dimly lit benthic marine habitats. These algae feature a unique type of multicellularity, forming well-defined macroscopic bodies composed of small spherical cells embedded in a firm gelatinous matrix. Phylogenetic analysis either places the Palmophyllales as the sister clade to all other Chlorophyta or allies it with the Prasinococcales (Leliaert *et al.*, 2011; Zechman *et al.*, 2010).

The core Chlorophyta evolved from one of the ancestral prasinophytic lineages probably somewhere in the Neoproterozoic (Herron *et al.*, 2009). The core Chlorophyta includes the species-poor and early-diverging Pedinophyceae (marine and freshwater uniflagellates) and Chlorodendrophyceae (marine and freshwater quadriflagellates), and the large and diverse clades, Trebouxiophyceae, Ulvophyceae and Chlorophyceae (TUC) (Leliaert *et al.*, 2012; Marin, 2012). The TUC clades include a wide variety of morphological forms and eco-physiological features. Unlike the prasinophytes, where sexual reproduction has rarely been observed, the core chlorophytes encompass a large diversity of life cycle strategies, many of which involve sexual reproduction. Marine members of the Ulvophyceae generally have life cycles involving an alternation between two free-living multicellular phases

(a haploid gametophyte and diploid sporophyte). Many freshwater Chlorophyceae and Trebouxiophyceae have a haploid vegetative phase and a single-celled, often dormant zygote as the diploid phase. Conversely, terrestrial members of the core chlorophytes are mainly asexual (Rindi, 2011). We refer to Chapter 6 (Umen and Olson, 2012) for a review on the evolution of sex in the chlorophycean green algae *Chlamydomonas* and *Volvox*.

A new mode of cell division likely evolved in the clade uniting the Chlorodendrophyceae and the TUC clade and was subsequently lost in the Ulvophyceae (Leliaert *et al.*, 2012). This type of cell division is mediated by a phycoplast, which is an array of microtubules oriented parallel to the plane of cell division, determining the formation of a new cell wall (Graham *et al.*, 2009; van den Hoek *et al.*, 1995). Morphological and eco-physiological adaptations probably allowed successful radiation of the Trebouxiophyceae and Chlorophyceae in freshwater and terrestrial habitats and diversification of the Ulvophyceae along marine shorelines (Becker & Marin, 2009; Cocquyt, Verbruggen, Leliaert, & De Clerck, 2010; Leliaert *et al.*, 2012).

The relationships among the core chlorophytan lineages are difficult to resolve, probably as a result of their antiquity and the short time span of diversification (Cocquyt *et al.*, 2010; O'Kelly, 2007). Furthermore, some phylogenetic studies showed that at least the Trebouxiophyceae and Ulvophyceae might not be monophyletic (e.g. Lü *et al.*, 2011; Turmel, Otis, & Lemieux, 2009; Zuccarello *et al.*, 2009) (but see Marin, 2012; Cocquyt *et al.*, 2010).

The Trebouxiophyceae includes flagellates, coccoids, colonies and multicellular filaments and blades. The group is predominantly freshwater or terrestrial; some members occur in brackish or marine habitats. Many species are photosynthetic symbionts with lichen fungi, various protists, invertebrates and plants; others have evolved a free-living or parasitic heterotrophic life style (Friedl & Rybalka, 2011; Leliaert *et al.*, 2012). Analysis of the complete genome of *Chlorella variabilis* NC64A (an endosymbiont of the ciliate *Paramecium bursaria*) has provided insights into the genetic facilitation of an endosymbiotic lifestyle (Blanc *et al.*, 2010). In particular, expansion of protein families containing protein–protein interaction domains and adhesion domains could have been involved in adaptation to symbiosis. Although *Chlorella* (and many other members of Trebouxiophyceae) has been assumed to be asexual and non-motile, meiosis- and flagella-specific proteins have been found in its genome, suggestive of cryptic sex and involvement of a flagella-derived structure in sexual reproduction (Blanc *et al.*, 2010).

The Chlorophyceae includes flagellates, coccoids and various colonial and multicellular forms. The group occurs mainly in freshwater and to a lesser extent in terrestrial habitats; some are marine (Klochkova *et al.*, 2008). Five main lineages have been recognized: the speciose and diverse Sphaeropleales and Chlamydomonadales including some of the most common freshwater phytoplankters, and the smaller clades, Chaetophorales, Oedogoniales and Chaetopeltidales (Leliaert *et al.*, 2012). The unicellular flagellate *Chlamydomonas* has been extensively studied as a model for photosynthesis, chloroplast biogenesis, flagellar assembly and function, cell–cell recognition, circadian rhythm and cell cycle control (Grossman *et al.*, 2003). The colonial *Volvox* has served as a model for the evolution of multicellularity, cell differentiation and colony motility (Herron & Michod, 2008; Kirk, 2003). Analysis of the complete genomes of *Chlamydomonas reinhardtii* and *Volvox carteri* has provided important genetic insights into the evolution of multicellularity and sex (Merchant *et al.*, 2007; Prochnik *et al.*, 2010, Umen and Olson, 2012). The Ulvophyceae includes unicells and multicellular algae as well as giant-celled forms with unique cellular characteristics (Cocquyt *et al.*, 2010; Leliaert *et al.*, 2012). Ulvophytes are generally known as macroalgae growing along marine coasts (green seaweeds). Species in the Ulvales, Bryopsidales and Cladophorales frequently dominate rocky shores, tropical lagoons and reefs. Some species of *Ulva* can form extensive, free-floating blooms, known as green tides (Ye *et al.*, 2011). *Caulerpa* and *Codium* species are notorious for their invasive nature (Williams & Smith, 2007). Several ulvophytes (e.g. *Ulva* and *Cladophora*) have secondarily adapted to freshwater environments. The Trentepohliales is atypical with respect to both morphology and ecology, occurring exclusively in terrestrial habitats (López–Bautista & Chapman, 2003). Some early diverging lineages (Oltmannsiellopsidales and *Ignatius*) include microscopic organisms occurring in freshwater or terrestrial habitats, indicating that the ancestral ulvophytes may have been freshwater or terrestrial unicells (Cocquyt *et al.*, 2010).

3.4. Streptophyta and the Origin of Land Plants

The Streptophyta include a paraphyletic assemblage of green algae (charophytes) and the land plants. Charophytes range in morphology from unicellular to complex multicellular organisms and occur in freshwater or moist terrestrial habitats. Streptophyta share a number of unique traits,

including motile cells (when present) with two subapically inserted flagella and an asymmetrical flagellar apparatus that contains a distinctive multilayered structure and parallel basal bodies; open mitosis with a persistent mitotic spindle and several unique enzymes (Leliaert *et al.*, 2012). There are six main lineages of charophytes: Mesostigmatophyceae, Chlorokybophyceae, Klebsormidiophyceae, Zygnematophyceae, Charophyceae and Coleochaetophyceae (McCourt *et al.*, 2004) (Fig. 2.2). Many phylogenetic studies have aimed to resolve the relationship among these lineages and in particular to determine the origins of land plants (Karol, McCourt, Cimino, & Delwiche, 2001; Lemieux *et al.*, 2007; Rodriguez-Ezpeleta *et al.*, 2007; Timme *et al.*, 2012; Wodniok *et al.*, 2011).

Mesostigma (Mesostigmatophyceae) and *Chlorokybus* (Chlorokybophyceae) form the earliest-diverging streptophytic lineages (Timme *et al.*, 2012) (Fig. 2.2). *Mesostigma* is a flagellate covered with diverse organic scales and is found in freshwater habitats. *Chlorokybus* forms packets of a few non-motile cells and grows in moist terrestrial environments (McCourt *et al.*, 2004). The freshwater or terrestrial filamentous Klebsormidiophyceae diverged after the Mesostigmatophyceae and Chlorokybophyceae.

In contrast to these three early-diverging lineages that undergo cell division by furrowing, the remaining lineages (Charophyceae, Zygnematophyceae, Coleochaetophyceae and the land plants) evolved a new mechanism of cell division involving a phragmoplast, which consists of an array of microtubules oriented perpendicularly to the plane of cell division, determining the formation of the cell plate and new cell wall. Most of these later-diverging streptophytes also have cell walls with plasmodesmata, facilitating cytoplasmic communication between cells and development of complex tissues (Graham, Cook, & Busse, 2000). Species in the three early-diverging lineages have never been observed to reproduce sexually in contrasts to the remaining streptophytes where sex is widespread (McCourt *et al.*, 2004).

The Zygnematophyceae (conjugating green algae) is a species-rich and morphologically diverse clade, including non-motile unicells, filaments and small colonial forms. Sexual reproduction occurs by a unique process of conjugation, involving fusion of non-motile gametes. Flagellate stages are completely absent. The Charophyceae (stoneworts) include freshwater algae with complex macroscopic bodies composed of a main axis with whorled branches. Growth is by an apical meristematic cell. Sexual reproduction is oogamous with oogonia and antheridia surrounded by sterile cells. Charophyceae are well represented in the fossil record, which is a large diversity extending back to the Silurian (McCourt *et al.*, 2004).

The Coleochaetophyceae is a small clade of branched filaments that sometimes form discoid parenchymatous thalli (Graham, 1984). Based on morphological similarities with embryophytes, *Coleochaete* has traditionally been put forward as the closest relative of land plants (Graham, 1984; Graham *et al.*, 2000). For example, some species of *Coleochaete* have corticated zygotes that are retained on the mother plant from which they receive nourishment via placental transfer cells with wall ingrowths. Also, cytokinesis and phragmoplast formation are similar to land plants (Graham *et al.*, 2000).

Identifying the closest living relative of land plants has proven to be a difficult task (Cocquyt *et al.*, 2010; Karol *et al.*, 2001; Lemieux *et al.*, 2007; Rodriguez-Ezpeleta *et al.*, 2007; Turmel *et al.*, 2009; Turmel, Otis, & Lemieux, 2006, 2007). Recent studies based on broad phylogenomic sampling have suggested the Zygnematophyceae, or a clade uniting the Zygnematophyceae and Coleochaetophyceae, as sister lineage of the land plants (Timme *et al.*, 2012; Wodniok *et al.*, 2011; Laurin-Lemay, Brinkmann, & Philippe, 2012). The inferred relationship between Coleochaetophyceae and land plants is in line with earlier morphology-based hypotheses (Graham, 1984; Graham *et al.*, 2000), and the relationship between Zygnematophyceae and land plants is supported by some cellular and molecular features, including similarities in auxin signalling (De Smet *et al.*, 2011) and chloroplast movement (Wada, Kagawa, & Sato, 2003).

4. GLAUCOPHYTES

The Glaucophyta (also known as Glaucocystophyta) is a small and inconspicuous group of unicellular algae found in freshwater and terrestrial environments (Baldauf, 2008; Bhattacharya & Schmidt, 1997; Kies & Kremer, 1990; Schenk, 2001). The importance of the group lies mainly in its critical phylogenetic position, branching deeply within the Archaeplastida (Fig. 2.1) (Bhattacharya, Helmchen, Bibeau, & Melkonian, 1995; Moreira, Le Guyader, & Philippe, 2000; Nozaki *et al.*, 2009; Price *et al.*, 2012; Reyes-Prieto & Bhattacharya, 2007; Rodriguez-Ezpeleta *et al.*, 2005).

Glaucophytes are unique among photosynthetic eukaryotes in that they contain unusual plastids (originally named 'cyanelles') with several characteristics reminiscent of Cyanobacteria: plastids are surrounded by a prokaryote-type peptidoglycan wall (except for *Glaucosphaera vacuolata*) and contain only chlorophyll *a* and phycobilins (Steiner & Löffelhardt, 2002; Steiner, Yusa, Pompe, & Löffelhardt, 2005). Similar to red algae, plastids have unstacked

thylakoids and light–harvesting proteins organized into phycobilisomes. About 13 species have been described in five genera: *Glaucocystis*, including coccoid cells with a cellulosic wall and two short rudimentary flagella; *Cyanophora* and *Peliaina* are wall–less flagellates with two heterokont flagella and *Gloeochaete* and *Cyanoptyche*, including non–motile cells in a gelatinous matrix, repro–ducing by motile or non–motile spores (Schenk, 2001).

To date, the genome of a single glaucophyte has been sequenced (Price et al., 2012; Stirewalt, Michalowski, Löffelhardt, Bohnert, & Bryant, 1995). Genomic data and a better understanding of the phylogenetic position of glaucophytes will provide valuable insights into the endosymbiotic origin and evolution of plastids in eukaryotes.

5. ARCHAEPLASTIDA GENOME STUDIES

Genomic data are rapidly accumulating. To date, about 10 complete genomes have been sequenced, but several other genome projects are ongoing (Tirichine & Bowler, 2011). Whole–genome data provide a great resource for analysis of eukaryotic genome evolution and user friendly online platforms for exploring this genome information is becoming increasingly available (e.g. Pico-Plaza, http://bioinformatics.psb.ugent.be/pico-plaza/; Van Bel et al., 2012).

BOX 2.2 Glossary

Archaeplastida	A group of photosynthetic eukaryotes that are hypothesized to have evolved from a common ancestor with a primary plastid comprising the green plants (Viridiplantae), red algae (Rhodophyta) and the glaucophytes (Glaucophyta)
Biomarker	Organic molecules derived from distinctive cellular components in geological deposits indicative for the existence of certain organisms in specific time periods (e.g. 2-methyl hopanoids as evidence for cyanobacteria or okenone for purple bacteria)
Calibrated phylogeny	Phylogeny in which the branch lengths are proportional to time using geo-paleontological date (e.g. fossils, biomarkers and geological events) or substitution rates as scaling parameters
Chromalveolata	Group consisting of chlorophyll *c* containing phototrophs and some clades that secondarily lost their plastids, hypothesized to have evolved from a common ancestor with a secondary red algal derived plastid, including Alveolata, Stramenopila, Haptophyta and Cryptophyta
Concatenation	Data set in which sequence information of multiple genes is combined and analyzed together assuming all genes share a common history

BOX 2.2 Glossary—cont'd

Endosymbiosis	The term may refer either to one organism living inside another organism or to the process in which an organism is engulfed and subsequently enslaved by a host and thereby progressively integrated in the cellular machinery of the host as an organelle. The latter is often referred to as symbiogenesis
Endosymbiotic gene transfer	The process by which genes are transferred from the endosymbiont into the host genome during and following endosymbiosis
Gene-by-gene approach	Phylogenetic approach in which the evolutionary history of each gene is analyzed separately as opposed to concatenating all genes in a single matrix under the assumption of a single evolutionary history
Hacrobia	Group comprising Haptophyta and Cryptophyta, also known as the CCTH clade
Harosa	Group comprising of Stramenopila, Alveolata and Rhizaria, also known as the SAR clade
Horizontal gene transfer	Inheritance of genes from one organism or species to another that is not its descendent or ancestor
Oxygenic photosynthesis	The chemical process whereby light energy powers the conversion of carbon dioxide into organic compounds and oxygen is released as a waste product
Peptidoglycan layer	Biopolymer, also known as murein, consisting of a sugar of alternating residues of β-(1,4) linked N-acetylglucosamine and N-acetylmuramic acid and crosslinking peptides outside the (inner) plasma membrane of Eubacteria and plastids of Glaucophyta
Plastid	An endosymbiont derived, membrane-bound organelle that carries out photosynthesis in eukaryotes
Primary/secondary/ tertiary plastid	Plastid that is the result of, respectively one, two or three endosymbiotic events
Vertical gene transfer	Inheritance of genes from one organism to its offspring.

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