# The Fundamental Physiological Processes

Photosynthesis provides the major energy supply and carbon input to the biosystems of the Earth. It may also be almost as old as life itself, dating back at least 3.7 billion years (Ga). Only two major primary photosynthetic pigments are recognized, **chlorophyll** (Chl) and **bacterio-chlorophyll** (BChl). There are only eight major light-harvesting systems. The photosynthetic systems of Cyanobacteria, photosynthetic protists (algae) and land plants are built around chlorophyll *a* (Chl *a*), although now chlorophyll *d* (Chl *d*) may also be considered as a major pigment. These organisms are all capable of splitting water and forming oxygen in the process, in **oxygenic photosynthesis** (Fig. 1). This is all the more remarkablebecause anoxygenic photosynthesis carried out by anoxygenic photosynthetic bacteria employs a number of BChls (although *Heliobacteria* employ γ-bacteriochlorophyll which, while it is a bacterio-chlorophyll – i.e. it possesses a bacterio-chlorin ring – is close to chlorophyll in structure. It might be concluded from this logic that BChl preceded Chl on the early Earth. However, this is by no means certain and has been challenged; it is quite possible that the early photosynthetic organisms possessed Chl, or perhaps Chl + BChl and had many similarities to cyanobacteria. A possible scenario sees the pro-cyanobacterial organisms developing towards oxygenic photosynthesis over the billion years up to the Great Oxidation Event (GOE) at 2.45 Ga, alongside anoxygenic photosynthetic bacteria. Then, as water splitting became ubiquitous, anoxygenic photosynthetic bacteria were outcompeted by their more efficient relatives and were pushed to the borders of habitable ecosystems, where they were forced to exist on low light, much of it relegated to the infrared region. It was at about this time, viz. the GOE, that Cyanobacteria would first be recognizable, although

procyanobacteria would have existed long before this. It is therefore just as remarkable that these two great realms of the photosynthetic world do not share any similar light-harvesting systems, despite the fact that both groups have developed a wide, but not fully comprehensive, set of such pigment systems, to absorb energy from sunlight. In the Cyano -bacteria, algae and land plants, the light-harvesting systems are mainly based on the chlorophylls, with the notable exception of the phycobiliproteins. The proteins interacting with the chlorophylls are surprisingly few: the CAB proteins, the relatives of the innerantennae complex (CP43 and CP47), and the novel peridinin chlorophyll complex (PCP) (see Fig.2).

The cyanobacteria, red algae and cryptophyte algae also possess phycobiliproteins. In addition, the carotenoids have a large role to play in all photosynthetic systems, although again there is little overlap between the carotenoids found in anoxygenic and oxygenic photosynthesis. For other reviews in this area the reader is referred to Larkum and Barrett ( 1983 ), Larkum and Howe( 1997 ), Larkum ( 2003 ), and Falkowski and Raven ( 2007 ).

## The Photosynthetic Pigments of Cyanobacteria and Algae

We now take a closer look at those pigments which defi ne the Cyanobacteria and algae (and the land plants, evolving as they did from streptophyte green algae). For convenience the reader is referred to Fig. 5 for an explanation of how the various algal phyla are related.

### Chlorophylls

Chl *a* , and Chl *b* were isolated and their chemical structure determined in the early twentieth century, Chl *c* was although Chl *d* was not fully recognized until its rediscovery in the cyanobacterium, *Acaryochloris marina* 1 (Fig. 3 ).

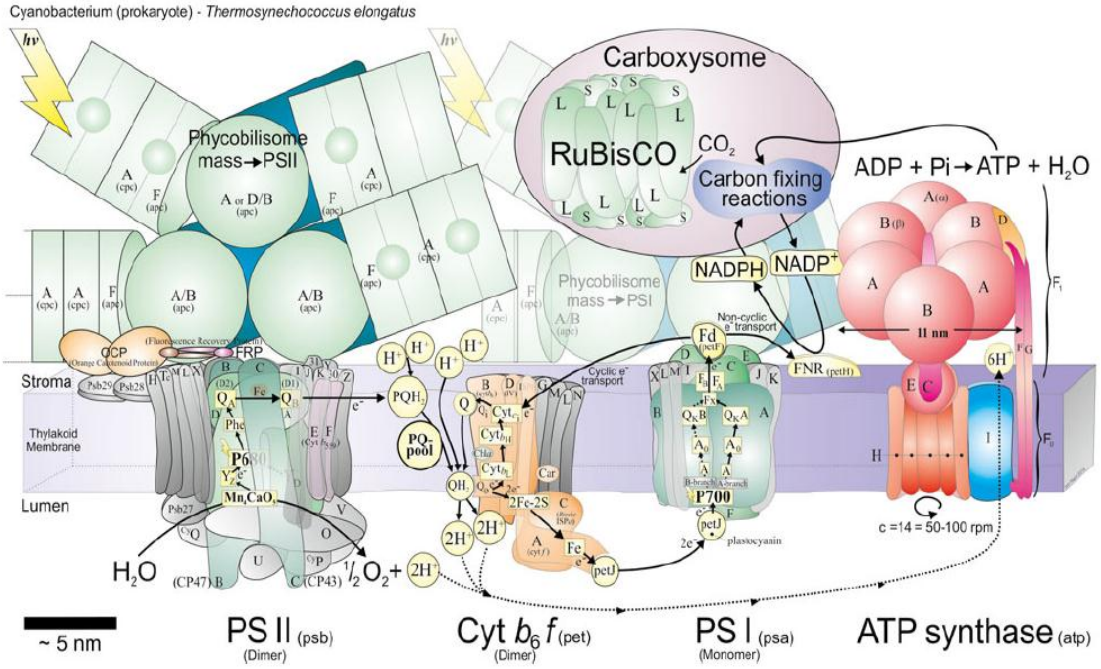



**Fig. 1** A diagram of the thylakoid membrane showing the major supercomplexes (Reproduced from www.scienceopen.com and John Nield with

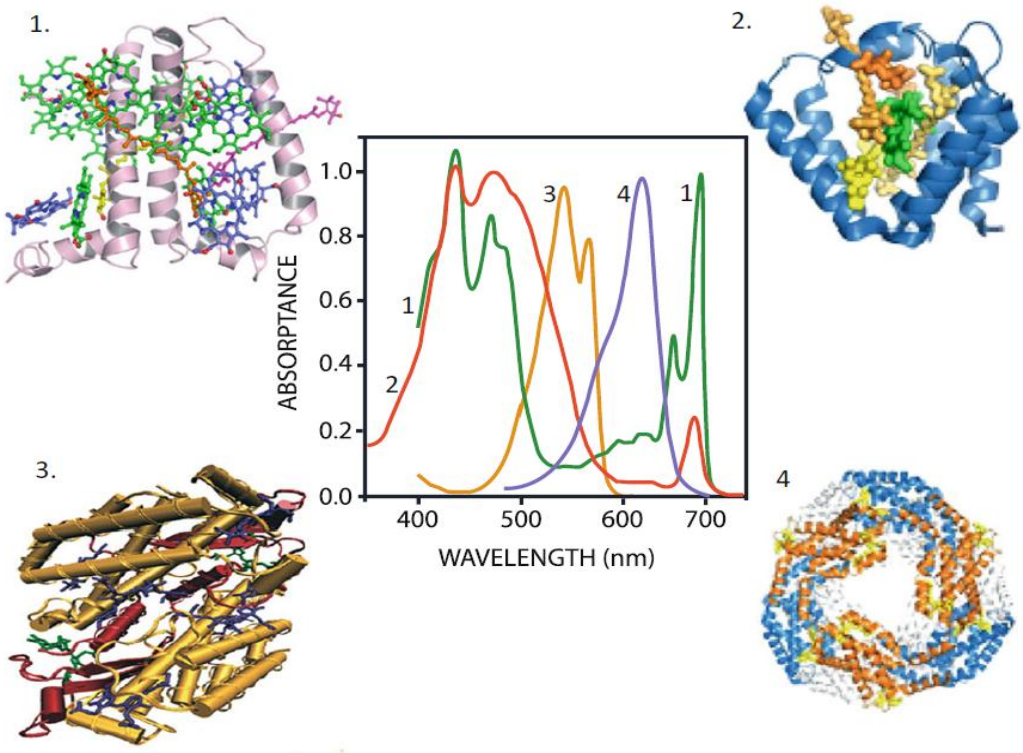
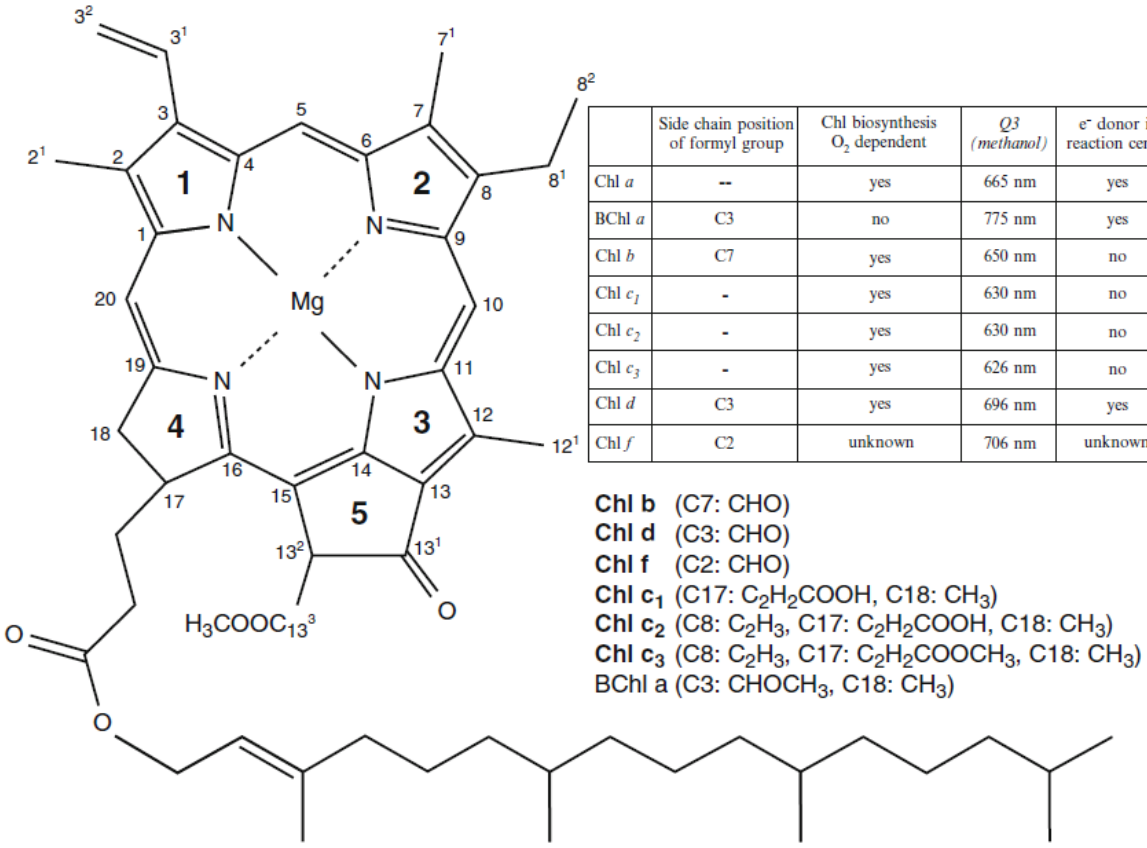



**Fig. 2** Space filling diagrams of the major light harvesting complexes of eukaryotic algae and land plants. (*1*) LHCII; (*2*) Peridinin chlorophyll complex; (*3*) Phycobiliprotein subunit; (*4*) Novel phycoerythrin from *Chroomonas* (Reproduced from www.scienceopen.com with permission)




| | Side chain position of formyl group | Chl biosynthesis $O_2$ dependent | *Q3 (methanol)* | $e^-$ donor in reaction centre |
|---|---|---|---|---|
| Chl *a* | -- | yes | 665 nm | yes |
| BChl *a* | C3 | no | 775 nm | yes |
| Chl *b* | C7 | yes | 650 nm | no |
| Chl $c_1$ | - | yes | 630 nm | no |
| Chl $c_2$ | - | yes | 630 nm | no |
| Chl $c_3$ | - | yes | 626 nm | no |
| Chl *d* | C3 | yes | 696 nm | yes |
| Chl *f* | C2 | unknown | 706 nm | unknown |

**Fig. 3** Chemical structures and in vivo absorption spectra of Chls *a*, *b*, *c*, *d* and *f*.

Chls *a* and *b* are found in higher plants, including agricultural crops, and were an obvious target. However, Chl *b* is also found in a variety of algae, most notably the Chlorophyceae (but also Euglenophyceae, chlorarachniophytes, and in the cyanobacteria, *Prochlorococcus* , *Prochlorothrix* and *Prochloron* ). Chl *c* is found only in chromophytic algae (also called Chromista and alveolates); all of which occur only in algae with secondary plastids (see Sect. 3.2 ); this bizarre pigment, for which no real function has been agreed, is divided into Chl *c 1* and Chl *c 2*. Most algae which possess Chl *c* possess both *c 1* and *c 2* , but two phyla, the Dinophyceae and the Cryptophyceae possess only *c 2* , while a small group, mainly of prymnesiophytes, possess a third type, Chl *c 3*. In addition, the biosynthetic intermediate, Mg-2,4 divinyl phaeoporphyrin dimethyl ester (MgDVP), is found in some chromophytes, in the primitive green algae Monadophyceae and in

*Prochlorococcus* and*Prochloron*. Of all these Chls, Chl*a* has ranked supreme until recently, taking on the photochemical role in both Photosystem I (PSI) and Photosystem II (PSII). Chl *a* has a Soret peak at 436 nm (in vivo) and Q Y peaks at 680–700 nm (in vivo) and is a good photosynthetic pigment for visible light, but needs augmentation in the blue, green and orange and near-infra red regions of the spectrum. However, the supremacy of Chl *a* has recently been challenged by Chl *d* which appears to replace Chl *a* in a photochemical role in PSI and PSII of *Acaryochloris marina*. The only role known for Chl *a* in the photosystems of *A. marina* is the apparent need for one molecule of Chl *a* in PSII where it is converted to pheophytin *a* and acts as the primary acceptor. Nevertheless, Chl *a* probably dominates in most oxygenic photosynthetic situations, while Chl *d* most likely ekes out a most restricted role where mainly near-infra red (NIR) light (700–750 nm) predominates (note also that this is a region where some BChls allow for anoxygenic photosynthetic activity). Recently a fi fth major Chl was discovered (Chl *f* ). With a peak in vivo of 735 nm this Chl can absorb light further into the NIR than Chl *d*. However, it does not take on any photochemical role and so far has only been found in small amounts when the algae that contain it are grown under NIR.

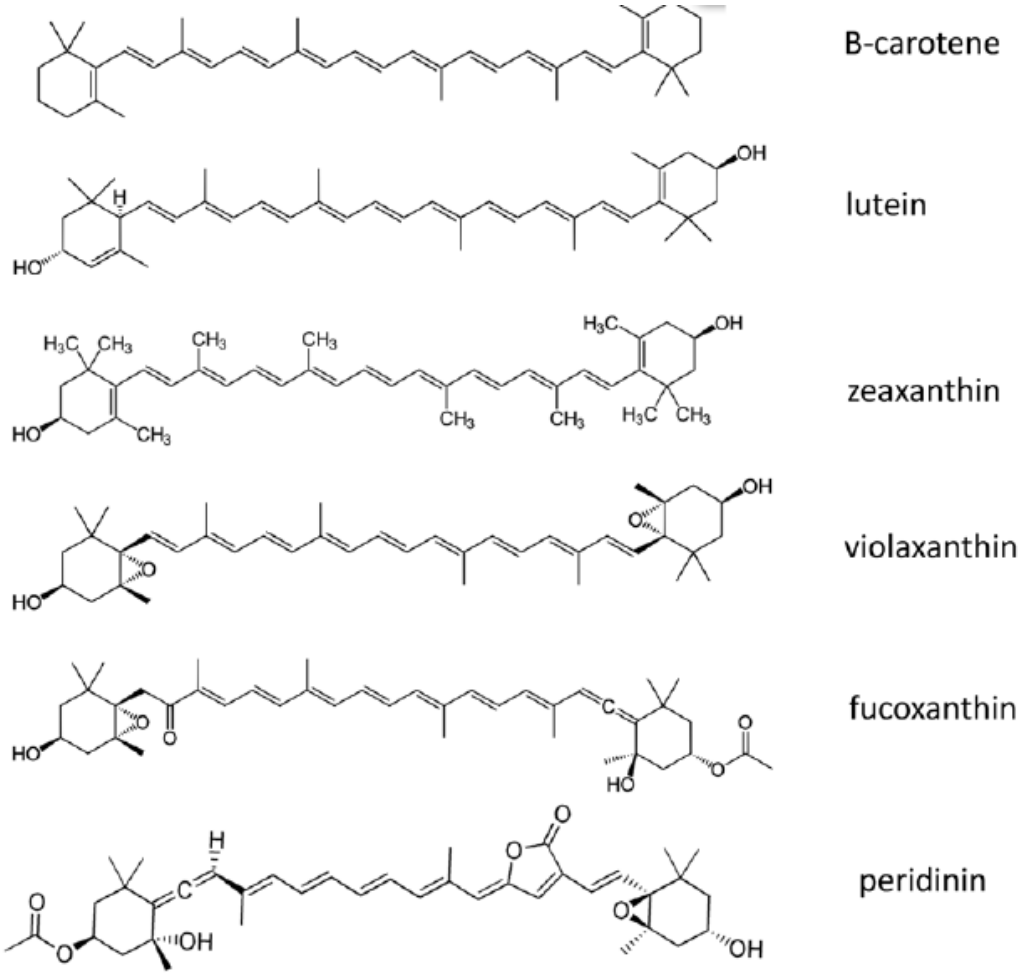



**Fig. 4** Chemical structures of some of the more important carotenoids (**a**) β-carotene, (**b**) Violaxanthin, (**c**) Zeaxanthin, (**d**) Fucoxanthin, (**e**) Peridinin, (**f**) Siphonaxanthin

## Carotenoids

The carotenoids in algae are very diverse (Fig. 4) and very different from those in anoxygenic bacteria, partly because they have to exist in a highly oxidising environment, but where anoxygenic photosynthetic bacteria exist in aerobic environments the carotenoids are very different again. With the carotenes there is less room for differences, except in chain length, and alpha- and beta-carotenes play a similar role, but nevertheless they are often quite specific in their distribution. With xanthophylls, there are almost endless variants that are found in photosynthesis. Many of these play a light harvesting role, such as fucoxanthin, peridinin and vaucherioxanthin, which harvest light in the

green region of the spectrum (500–550 nm) (Fig. 4 ). However there are other xanthophylls, which play other roles. For example, zeaxanthin + violaxanthin (green algae and land plants) and diatoxanthin + diadinoxanthin (chromophytic algae) take part in non-photochemical quenching in the xanthophyll cycle (see Sect. 7.1). The water-soluble keto-carotenoid 3′-hydroxyechinenone is the major carotenoid in the orange carotenoid protein (Sect. 7.3).

**Phycobiliproteins**

Phycobiliprotein (PBP) plays an exceptional role in light harvesting in Cyanobacteria and a small number of algal classes, notably Rhodophyceae, Glaucophyceae and Cryptophyceae. PBPs harvest light in the region of 490–650 nm where the "so-called" green window is a region where Chl and carotenoid pigments are poorly absorbing. In fact PBPs play such a dominant role in Cyanobacteria that it is often assumed that they evolved before light-harvesting Chls, an assumption that is likely to be wrong (see above). It is much more likely that at least some Chls apart from Chl *a* evolved before the evolution of PBPs. Larkum (2006) advanced the idea that phycobilisomes only evolved as a shading response to other algae. This has the merit that PBP is an "expensive" molecule (in terms of nitrogen), is not a membrane intrinsic molecule and is specifi cally tailored for absorption of light where the Chl and, generally, carotenoids have poor absorption properties. PBPs are built into phycobilisomes and from which the energy is funneled into the thylakoid membrane via a specifi c stalk of additional molecules (see Fig. 1). It has recently been shown that passage of energy down this stalk is modulated in many cyanobacteria by a caroteno-protein, the orange carotenoid protein (OCP).

# 3 The Evolution of Protists with Plastids (Algae)

## 3.1 Algae with Primary Plastids

Current evidence suggests that endosymbiosis of a cyanobacterium and an early protist organism gave rise to the ancestral line of plastids over 1 Gaago (Fig. 5); there has even been some speculation as to from which group of Cyanobacteria the first endosymbionts came. A single endosymbiosis is generally held to be the most likely, because it is held to be a very unlikely event; this is called the **monophyletic hypothesis**. This hypothesis is embodied in the concept of the Archaeplastida, in which it is stated that this single endosymbiosis gave rise to the modern Glaucophyta, Rhodophyta and Chlorophyta. These three phyla all have primary plastids, i.e. plastids with only twoenvelope membranes, in contradistinction to the secondary plastids, which have three or four envelope membranes. Since today the primary plastids contain only a few genes (usually much less than 250) it is assumed that the original nucleus lost most of its genes, some to the host nucleus and others altogether. An alternative **polyphyletic hypothesis** states that there were several endosymbioses at that time (1–2 Ga), but that reticulate evolution, occurring over many Ma, gave rise to the three modern classes with primary plastids, i.e. the Glaucophyceae, the Rhodophyceae and the Chlorophyceae. This is the **shopping bag model**. It has gained support recently from evidence that cyanobacterial endosymbioses have occurred much more recently than the period when primary plastids evolved, and have given rise to endosymbionts where some genes have been transferred to the host nucleus, e.g. *Paulinella* and other examples. It was also supported by an analysis of slowly evolving genes. A further point to notice is that the protistan organism(s) into which this endosymbiosis

occurred, already possessed a mitochondrial endosymbiont, since all protists have been shown to possess at least a relict mitochondrion and likely a chlamydial endosymbiont too. Thus a key feature of modern research is to identify thecyanobacterial organism(s) and the genes, which were transferredto the protistan host(s), and those that have survived (either in the plastid or the host DNA).

### 3.1.1 Glaucophytes

Glaucophyceae are protist organisms (eukaryotic algae), whose affi nities are not clear. There are about a dozen species, none of which is common. *Cyanophora paradoxa* is the most studied and consists of a motile cell without a cell wall. Another species, *Glaucocystis* is immotile, although it retains vestigial flagella. It has a cellulose cell wall. *Gloeochaete* has both motile and immotile stages and it appears that its cell wall is not composed of cellulose. The structure of the fl agellum root and the presence of two unequal flagella suggest links with Chlorophyta, although the presence of cortical alveoli is a difficulty with such a suggestion. The primary plastid, known as a cyanelle, has by definition two outer membranes, but in addition there is a vestigial cell wall, a peptidoglycan layer lying between the two bounding membranes. The latter layer could be the vestige of the cell wall of the cyanobacterium, which gave rise to the symbiosis. The photosynthetic machinery of the cyanelles is the most similar to cyanobacteria of all the plastids. Only Chl *a* is present and the major light harvesting proteins are phycobiliproteins, which lie in the plastid matrix and funnel energy down to the thylakoid membrane via a stalk. There are no LH proteins in the CAB family present (Fig. 9); but, as would be expected however endosymbiosis took place there are proteins, which are relatives of the HLIP protein of

Cyanobacteria (see Sect. 9), and which have one alpha- helix and the Stress Enhanced Proteins (SEP), with two helices (see Sect. 9), which occur in Glaucophyceae, Rhodophyceae and diatoms.

The plastid genome of *Cyanophora* has a size of 139 Kb and holds 192 genes. If the concept of Archaeplastida is upheld then Glaucophyceae, Rhodophyceae and Chlorophyceae share acommon origin. Recently sequencing of the whole genome of *Cyanophora paradoxa* was accomplished. While this work indicated many shared characteristics between these three phyla there are many differences, which still cannot be explained. For example the lack of any LHC genes is not easy to explain on a shared origin.

### 3.1.2 Rhodophyta

The Rhodophyta is a group of non-fl agellate protists with affi nities with Amoebozoa. The presence of two outer membranes places their plastids with the primary plastids. However, there are many features which separate them from the plastids of glaucophytes. Firstly, their phycobiliproteins show considerable evolution as compared with cyanobacteria and glaucophytes. For example, many rhodophytes have an extra pigment group, the gamma phycobiliproteins. They also have a CAB protein (see Fig. 9 ) with affi nities with the CAB proteins of green algae and many Chl *c* -containing algae, although their only chlorophyll is Chl *a* . They also have a special form of starch known as fl oridean starch. Like most Cyanobacteria, the thylakoids are non-appressed and bear phycobilisomes on their outer surface. Two whole genomes of rhodophyte algae have been sequenced: the primitive hot springs alga, *Cyanidioschyzon merolae* and the advanced red alga (Floridiophyceae) *Porphyridium purpureum*. In addition to this, six plastid genomes are

available; from *Cyanidioschyzon merulae*, from two bangiophyte algae and from three advanced red algae.

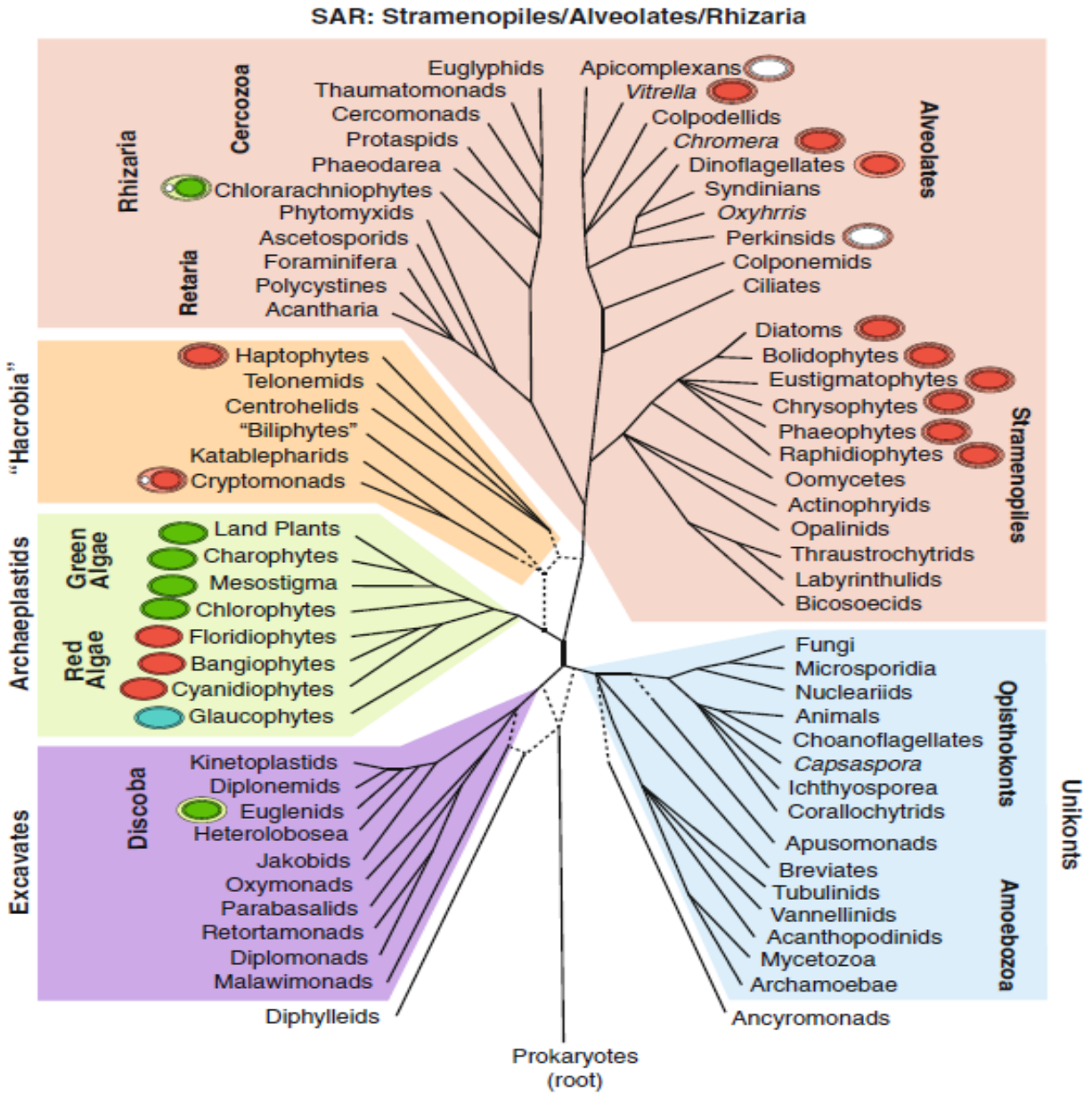



**Fig. 5** The photosynthetic eukaryotic algae arranged phylogenetically, based on the arrangement of Keeling (2013) (Reproduced from Annual Review of Plant Biology with permission)

It should therefore be possible to trace the evolutionary inheritance of the cyanobacterial symbiont that gave rise to the red algal plastid. However, this has not been achieved to date (see Li *et al.* 2014). The plastid genome, although varying between the different red algae, shows generally similar features in having a size of 150–190 Kb and a protein coding gene number of 193–234. This is a much larger size that is found in green plastids.