Division Glaucocystophyta

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in

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1 Introduction

The Glaucocystophyta (synonym Glaucophyta, Skuja 1954) defines a small (approx. 9 genera and 13 species) group of photosynthetic protists that includes flagellate, palmelloid and coccoid cells whose unique set of characters has led to their classification into a distinct division or phylum (Kies, 1979; Kies & Kremer, 1986, 1990). The evolutionary relationship of glaucocystophytes to other eukaryotes is unknown though the existence of a cruciate flagellar root system with multilayered structures (MLS) has suggested a relationship to prasinophyte green algae (Melkonian, 1983) whereas plastid characters have been used to position glaucocystophytes as a sister group to red algae (Biliphyta, Cavalier-Smith 1982, 1987. *Cyanophora paradoxa*, the best-studied glaucocystophyte, has also been included in the Cryptophyta on the basis of cell morphology (Bourrelly, 1970; Gillott, 1990).

Glaucocystophyta and other cyanelle-containing taxa (e.g., *Paulinella chromatophora*, Filosea) have been closely associated with the theory of endosymbiosis since they are characterized by such 'primitive' plastid characters as the existence of only chlorophyll-*a* and phycobilins, phycobilisomes, carboxysomes and concentric (unstacked) thylakoids. *Paulinella c.* was the first protist to be identified as containing a "cyanobacterial-like endosymbiont" as its photosynthetic organelle (Lauterborn 1895). Cyanelle characters within glaucocystophytes have been interpreted as supporting a relatively recent endosymbiosis between a modified coccoid cyanobacterium and a nonphotosynthetic eukaryote (Geitler, 1959; Hall & Claus, 1963). The existence of a peptidoglycan wall surrounding the cyanelles of all glaucocystophytes (and the photosynthetic organelle of *Paulinella c.*), except for *Glaucosphaera vacuolata* (Kies & Kremer, 1990; Kraus *et al.*, 1990), further suggested that cyanelles must be of a recent origin. The cyanelles of some glaucocystophytes (i.e., *Cyanophora*, *Glaucocystis nostochinearum*) were even raised to the rank of cyanobacterial taxa to reflect the distinctive ultrastructure and putative polyphyletic origin of these organelles (Hall & Claus, 1963, 1967). This latter view regarding *Cyanophora* is no longer accepted since analyses of the cyanelle genome size in this taxon show it to be comparable to other plastids (Herdman & Stanier 1977; Löffelhardt *et al.* this book). Analyses of gene content, gene order and phylogeny (e.g., small subunit ribosomal DNA [SSU rDNA], rpoC1) of the *Cyanophora* cyanelle show this organelle to be more closely related to extant plastids than to cyanobacteria (Bohnert *et al.*, 1982; Giovannoni *et al.*, 1988; Douglas & Turner, 1991; Palenik & Haselkorn, 1992).

In order to delineate the phylogeny of the glaucocystophytes (*sensu* Kies & Kremer 1986) and gain insights into the origin of the characters that define this algal division, the nuclear- and plastid-encoded SSU rDNA sequences have been determined from *Cyanophora paradoxa* Korsh. (Kies strain, Sammlung von Algenkulturen Göttingen, SAG B 45.84 [Schlösser 1984]), *Glaucocystis nostochinearum* Itzigs. (SAG 45.88), *Glaucosphaera vacuolata* Korsh. (SAG B 13.82) and *Gloeochaete wittrockiana* Lagerheim (SAG B46.84 [see also Bhattacharya & Medlin 1995; Helmchen *et al.* 1995]). The nuclear- and plastid-encoded SSU rDNA nucleotide sequences, respectively, of the glaucocystophyte taxa have been released to the Genbank/EMBL/DDBJ databases with the following accession numbers; *Cyanophora* (X68483, X81840), *Glaucocystis* (X70803, X82496), *Glaucosphaera* (X81902, X81903), *Gloeochaete* (X81901, X82495). All other rDNA sequences analyzed in this study are available from these sequence databases.

The single copy genomic actin gene of *Cyanophora* has also been characterized to position this taxon in the actin phylogeny (Bhattacharya and Weber in preparation). The *Cyanophora* actin sequence was found to contain five spliceosomal introns (four at novel positions in the actin intron catalogue, Weber & Kabsch 1994). Actin is a multiple copy gene family in many eukaryotes (e.g., angiosperms, animals) but exists as a single copy in several protist lineages (e.g., green algae, red algae, ciliates) and in the fungi (Bhattacharya *et al.*, 1991, 1995). When in multiple copies, the actin gene is often a valuable tool for studying concerted gene evolution, gene function diversification and the evolutionary relationships of eukaryotic lineages (for review see Sheterline & Sparrow 1994). This last hypothesis is supported by the observation that all actin genes (whether in single or multiple copy) form lineage-specific

monophyletic groups in phylogenies suggesting that actin gene duplications have occurred after the divergence of eukaryotic lineages (Bhattacharya *et al.*, 1995). That the archezoan, *Giardia lamblia*, contains a single-copy actin gene (Drouin *et al.*, 1995) is consistent with the hypothesis that all actin coding regions are descendants of a single sequence found in the ancestor of this primitive eukaryote. When in single copies actin may be used as a phylogenetic marker. Actin coding regions used in the phylogenetic analyses, with the exception of the *Cyanophora p.*, *Coleochaete scutata* and *Scherffelia dubia* sequences (Bhattacharya unpublished data), are available from the Genbank/EMBL/DDBJ databases.

2 The Glaucocystophyte Nuclear Lineage

2.1 SSU rDNA

The position of the glaucocystophyte host cell within the SSU rDNA phylogeny was determined using the maximum likelihood (pfastDNAml V 2.1, Schmidt *et al.* unpublished data), maximum parsimony (PAUP V3.1.1, Swofford 1993) and LogDet (Lockhart *et al.*, 1994) methods. Bootstrap resampling was done with each phylogenetic method (except the LogDet analysis) to test the stability of monophyletic groups within the trees. The phylogenies inferred from these methods are summarized in Fig. 1A, B.

The SSU rDNA analyses provide evidence for an origin of the glaucocystophyte host cell within the eukaryote crown group radiation. The bootstrap analyses with the rDNA data set support the monophyly of the Glaucocystophyta as does the relatively long common branch length that unites them in the maximum likelihood (Fig. 1A) and LogDet analyses (Fig. 1B). All major algal groups (except euglenophytes) trace their origins to this radiation that has primarily been identified with SSU rDNA analyses (Sogin *et al.*, 1986). The phylogenetic analyses are also consistent with a sister group relationship between glaucocystophytes and cryptophyte algae (see also Marin *et al.* 1996).

The monophyly of the glaucocystophyte/cryptophyte lineage was previously tested with user-defined tree analyses with the maximum likelihood method (Bhattacharya & Medlin, 1995). Topologies were created with the RETREE program of PHYLIP that address three alternate hypotheses regarding the evolutionary position of the glaucocystophytes, 1) as an independent lineage within the crown group radiation, 2) on the branch uniting them with the rhodophytes and, 3) on the branch uniting them with the chlorophytes. The log-likelihoods of these alternate trees and that of the 'best' tree (i.e., as a sister group to the cryptophytes and see Fig. 1A in Bhattacharya & Medlin 1995) were compared with the likelihood ratio test (LRT, Kishino & Hasegawa 1989). The user-defined tree analyses were consistent with a monophyletic origin of the glaucocystophytes/cryptophytes since the disruption of this clade resulted in a significantly 'worse' tree as did the positioning of the glaucocystophytes with either the red or green algae.

A sister group relationship between glaucocystophytes and cryptophytes is surprising since these protists do not share obvious morphological/ultrastructural characters (apart from the possession of flattened mitochondrial cristae, a character that is shared with red and green algae, animals/fungi). This relationship is taken as a preliminary result and must be further tested with protein sequence comparisons (e.g., actin, Bhattacharya work in progress). There are no host cell characters that unequivocally position the glaucocystophytes as a sister group to any other eukaryotic lineage (and, therefore, the confusing taxonomy). The crown group radiation is characterized by the divergence of many (presently) morphologically distinct groups; resolving the interrelationships of these lineages poses one of the most important challenges in molecular evolution. Our analyses also suggest that the existence of MLS and cortical alveoli (a system of vesicles underlying the plasma membrane), found in all glaucocystophytes, do not alone distinguish this algal group. Both characters are presumably of a primitive origin and were found in the common ancestor of the crown group eukaryotes; MLS are also found in the zooflagellate Jakoba sp. and in dinoflagellates (O'Kelly, 1992) and cortical alveoli are found in the dinoflagellates (included in the alveolates in Fig. 1A). There is no evidence for a specific relationship between glaucocystophytes

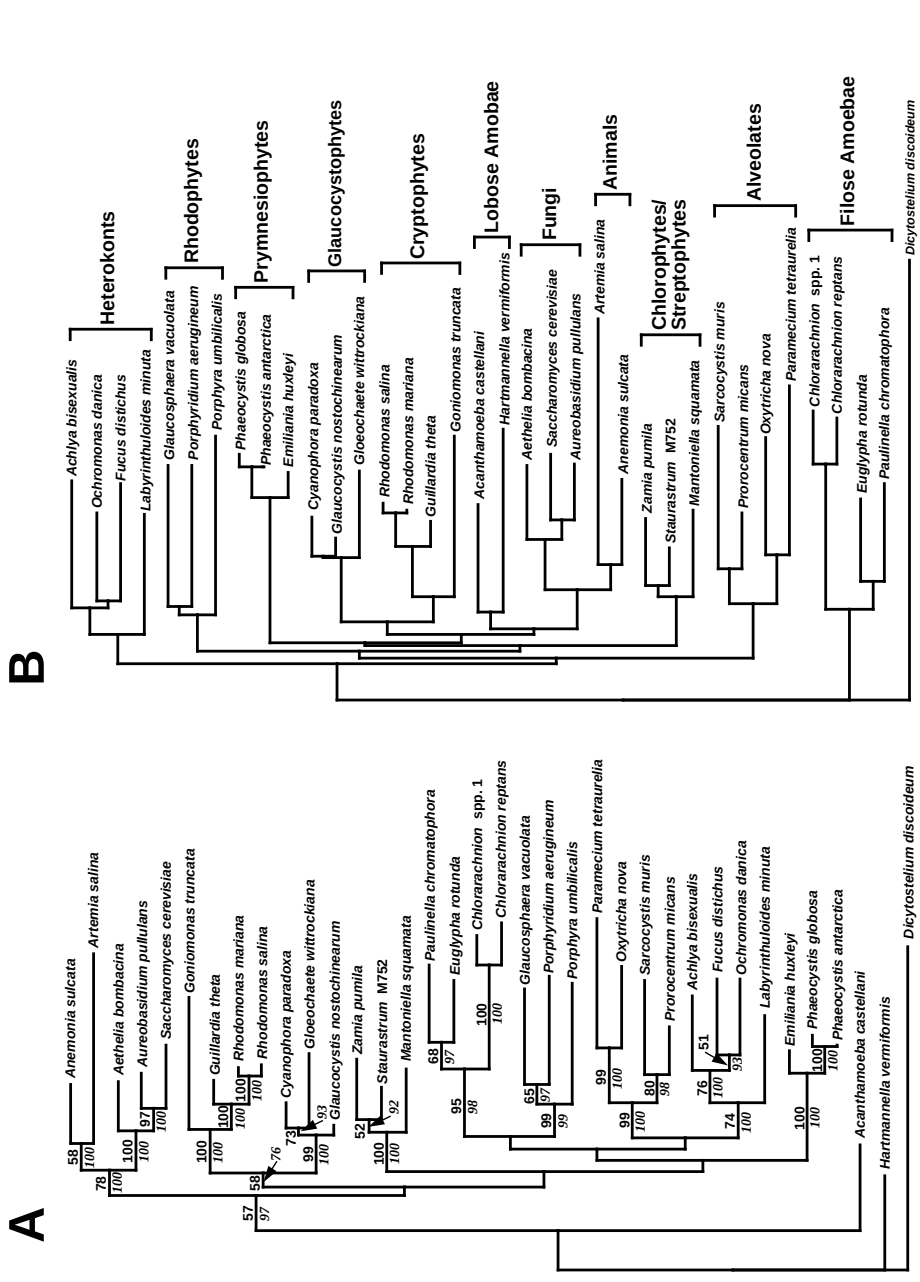

Figure 1:
Phylogeny of eukaryotes based on nuclear-encoded SSU rDNA sequence comparisons. A) Tree inferred with the parallel maximum likelihood computer program (pfastDNAml V 2.1, Schmidt *et al.* unpublished data) using 1565 aligned nucleotides. The bootstrap (Felsenstein, 1985) values (100 replications) above the internal nodes are inferred from the maximum likelihood analysis whereas the bootstrap values (200 replications) shown below the internal nodes are inferred from a weighted (RC index over the interval $1 - 1000$) maximum parsimony analysis (PAUP V3.1.1, Swofford 1993 using a heuristic search procedure with a branch-swapping algorithm (TBR, tree bisection-reconnection). The maximum parsimony tree has a consistency index$= 0.648$. Arrows are used to show bootstrap values where these do not fit on the branches. Only bootstrap values above 50% are shown. B) Tree inferred with the neighbor-joining method (Saitou & Nei, 1987) using a LogDet matrix (Lockhart *et al.*, 1994) as input. Only parsimony sites (485 nt) were included in the LogDet analysis, gaps were excluded. These phylogenies are rooted within the branch leading to *Dictyostelium discoideum*.

and alveolates in the rDNA analyses.

The phylogenetic analyses do not position *Glaucosphaera* within the Glaucocystophyta but instead show that this taxon is a red alga. This is an important result since *Glaucosphaera* was hypothesized to be an evolutionary 'link' between the glaucocystophytes and the red algae on the basis of plastid characters (i.e., with loss of the peptidoglycan wall in the *Glaucosphaera* plastid, Cavalier-Smith 1982, 1987); rhodoplasts also have two envelope membranes and contain phycobilisomes and unstacked thylakoids. The positioning of *Glaucosphaera* in Fig. 1A, 1B as a member of the red algae is supported, however, by other morphological and biochemical data (e.g., presence of R- phycocyanin in the *Glaucosphaera* plastid [the glaucocystophyte cyanelles contain C-phycocyanin, Richardson & Brown 1970] and the complete absence of flagellae and basal bodies). On the basis of electron microscopic studies, McCracken & Nadakavukaren (1980) described only one deeply lobed rhodoplast without a pyrenoid instead of several lens shaped cyanelles (Korshikov, 1930) in *Glaucosphaera*. These authors suggested placing *Glaucosphaera* in the red algal order, Porphyridiales. Our phylogenetic analyses support the hypothesis of McCracken & Nadakavukaren (1980) and suggest that the following characters, together, may be used to characterize the Glaucocystophyta; presence of flattened microtubule-associated cortical vesicles [lacunae] under the plasma membrane, cruciate flagellar roots with associated MLS and a cyanelle bound by a peptidoglycan wall.

2.2 Actin

The actin phylogeny was inferred with the maximum likelihood and maximum parsimony methods (Fig. 2). The apicomplexan (*Plasmodium falciparum*, *Toxosplasma gondii*) actin sequences are used to root this phylogeny of crown group eukaryotes since this protist lineage represents an early divergence in actin phylogenies which use the actin-related proteins (products of actin gene duplications in the common ancestor of all eukaryotes) as the outgroup root (Bhattacharya unpublished data). As shown above with the SSU rDNA sequence comparisons, phylogenetic analyses of actin coding regions are also consistent with the origin of many eukaryotes within a near simultaneous divergence (Bhattacharya *et al.*, 1991; Bhattacharya & Ehlting, 1995; Drouin *et al.*, 1995). Phylogenetic analyses of the *Cyanophora* single-copy actin coding region positions this sequence within this crown group radiation (Fig. 2). The sister-group relationship between the red and green algae in Fig. 2 has some bootstrap support in the maximum parsimony analysis (69%). This is an interesting result since the red and green algae possess so- called 'simple' plastids with 2-bounding membranes (see Bhattacharya & Medlin 1995). These plastids, along with the cyanelles, form a paraphyletic radiation of closely related lineages in SSU rDNA phylogenies (see below). That the host cells of the rhodophyte and chlorophyte plastids are also closely related in the actin trees is consistent with a monophyletic origin of the plastids in these groups. Further actin sequences from glaucocystophytes, red and green algae and cryptophytes are required to test this interesting finding.

3 Phylogeny of the Glaucocystophyte Cyanelles

The phylogeny of the glaucocystophyte cyanelle SSU rDNA coding regions was determined using maximum likelihood, maximum parsimony and LogDet methods. These analyses are consistent with a monophyletic origin of the cyanelle in the common ancestor of the glaucocystophytes, *Cyanophora*, *Glaucocystis* and *Gloeochaete* (Fig. 3A, B). Variation in cyanelle size and shape and ultrastructure (e.g., carboxysome shape, Hall & Claus 1967) within these taxa is not an indication of a polyphyletic origin. The positioning of the *Glaucosphaera* plastid SSU rDNA as a sister group to rhodoplasts is also consistent with the host cell phylogeny. The positioning of the glaucocystophyte cyanelles at the base of the plastid lineage in the maximum likelihood and LogDet phylogenetic analyses is consistent with a single loss of the peptidoglycan wall surrounding glaucocystophyte plastids (see Bhattacharya & Medlin 1995; Helmchen *et al.* 1995, Delwiche and Palmer [this book] for further discussion).

The 'primitive' characters of the glaucocystophyte cyanelles were likely found in the cyanobac-

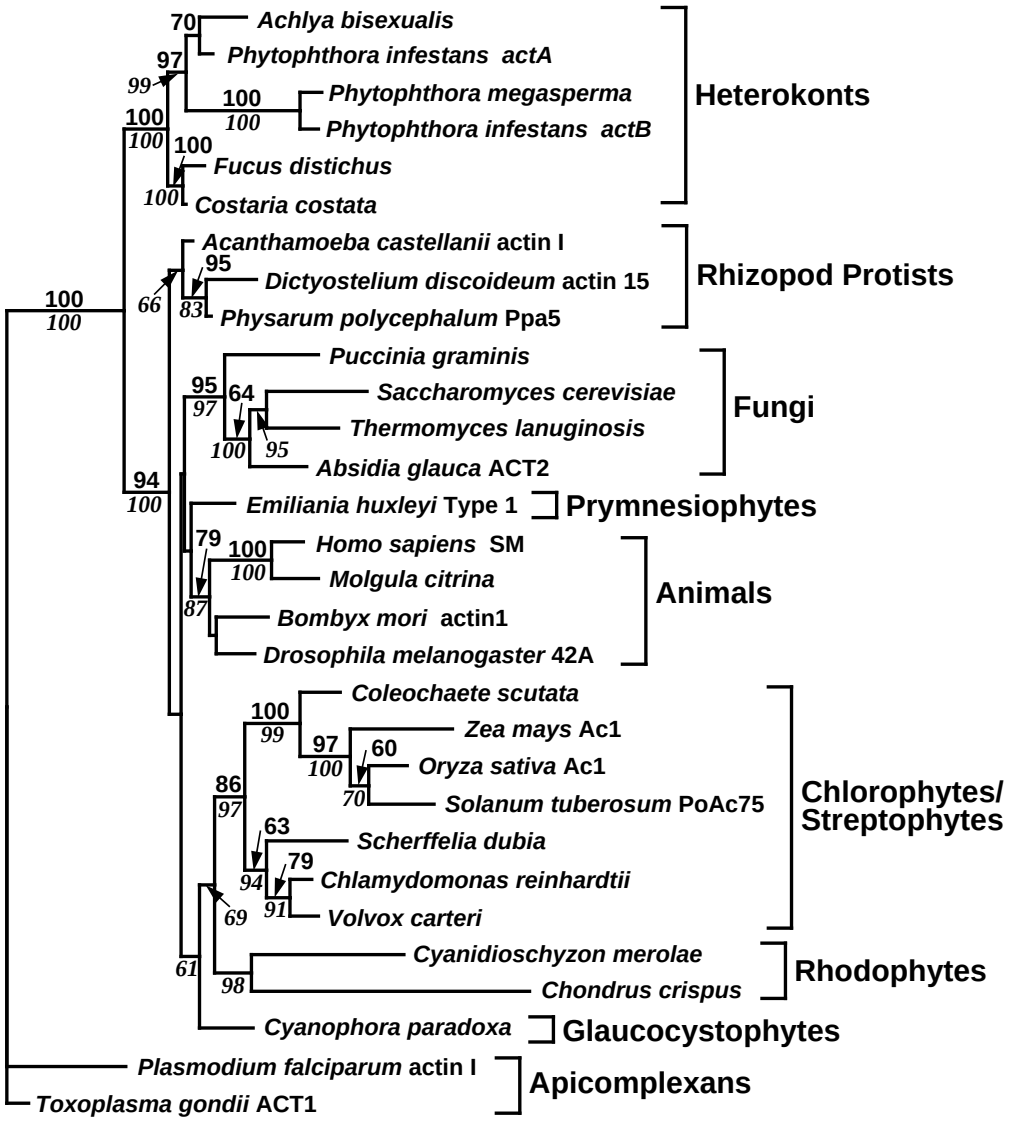

Figure 2:
Phylogeny of eukaryotes based on actin coding region sequence comparisons. Tree inferred with the parallel maximum likelihood computer program pfastDNAml (V 2.1, Schmidt *et al.* unpublished data) using 744 aligned nucleotides. The bootstrap (Felsenstein, 1985) values (100 replications) above the internal nodes are inferred from the maximum likelihood analysis whereas the bootstrap values (200 replications) shown below the internal nodes are inferred from a weighted (RC index over the interval $1 - 1000$) maximum parsimony analysis (PAUP V3.1.1, Swofford 1993) using a heuristic search procedure with a branch-swapping algorithm (TBR, tree bisection-reconnection). Only bootstrap values above 60% are shown. The maximum parsimony bootstrap consensus phylogeny has a consistency index= 0.736. Arrows are used to show bootstrap values where these do not fit on the branch.

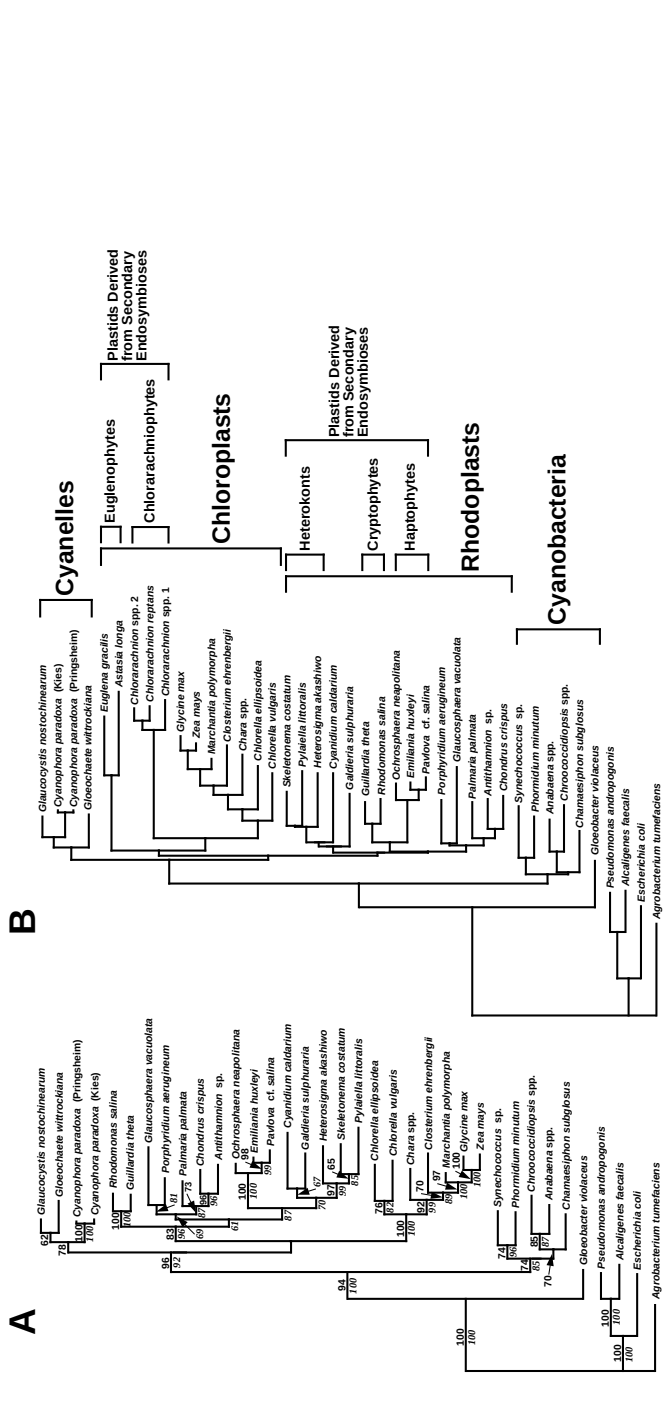

Figure 3:
Phylogeny of plastids based on SSU rDNA sequence comparisons. A) Tree inferred with the parallel maximum likelihood computer program pfastDNAml (V 2.1, Schmidt *et al.* unpublished data) using 1393 aligned nucleotides. The bootstrap (Felsenstein, 1985) values (100 replications) above the internal nodes are inferred from the maximum likelihood analysis whereas the bootstrap values (200 replications) shown below the internal nodes are inferred from a weighted (RC index over the interval $1 - 1000$) maximum parsimony analysis (PAUP V3.1.1, Swofford 1993) using a heuristic search procedure with a branch-swapping algorithm (TBR, tree bisection-reconnection). Only bootstrap values above 60% are shown. The maximum parsimony bootstrap consensus phylogeny has a consistency index= 0.632. Arrows are used to show bootstrap values where these do not fit on the branch. B) Tree inferred with the neighbor-joining method (Saitou & Nei, 1987) using a LogDet matrix (Lockhart *et al.*, 1994) as input. Only parsimony sites (518 nt) were included in the LogDet analysis, gaps were excluded. These phylogenies are rooted within the branch leading to *Agrobacterium tumefaciens*.

terium that gave rise to these plastids and do not support a common ancestry of photosynthetic eukaryotes containing only chlorophyll-a, phycobilins, phycobilisomes and concentric thylakoids (i.e., glaucocystophytes, red algae, *Paulinella c.*). If the sister group relationship between glaucocystophytes and cryptophytes is correct than it may be hypothesized that the common ancestor of this lineage contained a cyanelle that was lost and later replaced by an eukaryotic endosymbiont within the cryptophytes (see Maier [this book] for details). The finding of nuclear-encoded cyanelle proteins within an early- diverging nonphotosynthetic cryptophyte such as *Goniomonas truncata* would lend credence to this hypothesis and provide further insights into the dynamics of plastid endosymbiosis.

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