Phylogenomic Analysis Supports the Monophyly of Cryptophytes and Haptophytes and the Association of Rhizaria with Chromalveolates

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Here we use phylogenomics with expressed sequence tag (EST) data from the ecologically important coccolithophore-forming alga *Emiliania huxleyi* and the plastid-lacking cryptophyte *Goniomonas* cf. *pacifica* to establish their phylogenetic positions in the eukaryotic tree. Haptophytes and cryptophytes are members of the putative eukaryotic supergroup Chromalveolata (chromists [cryptophytes, haptophytes, stramenopiles] and alveolates [apicomplexans, ciliates, and dinoflagellates]). The chromalveolates are postulated to be monophyletic on the basis of plastid pigmentation in photosynthetic members, plastid gene and genome relationships, nuclear "host" phylogenies of some chromalveolate lineages, unique gene duplication and replacements shared by these taxa, and the evolutionary history of components of the plastid import and translocation systems. However the phylogenetic position of cryptophytes and haptophytes and the monophyly of chromalveolates as a whole remain to be substantiated. Here we assess chromalveolate monophyly using a multigene dataset of nuclear genes that includes members of all 6 eukaryotic supergroups. An automated phylogenomics pipeline followed by targeted database searches was used to assemble a 16-protein dataset (6,735 aa) from 46 taxa for tree inference. Maximum likelihood and Bayesian analyses of these data support the monophyly of haptophytes and cryptophytes. This relationship is consistent with a gene replacement via horizontal gene transfer of plastid-encoded *rpl36* that is uniquely shared by these taxa. The haptophytes + cryptophytes are sister to a clade that includes all other chromalveolates and, surprisingly, two members of the Rhizaria, *Reticulomyxa filosa* and *Bigelowiella natans*. The association of the two Rhizaria with chromalveolates is supported by the approximately unbiased (AU)-test and when the fastest evolving amino acid sites are removed from the 16-protein alignment.

Introduction

Multigene phylogenetics and the availability of genome data from protist lineages have provided a major impetus to resolving the eukaryotic tree of life (e.g., Simpson and Roger 2004; Bhattacharya and Katz 2005; Parfrey et al. 2006), leading recently to the hypothesis that eukaryotes comprise 6 putative supergroups (reviewed by Adl et al. 2005; Keeling et al. 2005; Parfrey et al. 2006). These are the protistan roots of all multicellular eukaryotes and comprise the Opisthokonta (e.g., animals, fungi, choanoflagellates), Amoebozoa (e.g., lobose amoebae, slime molds), Archaeplastida or Plantae (red, green [including land plants], and glaucophyte algae), Chromalveolata (e.g., apicomplexans, ciliates, giant kelps), Rhizaria (e.g., cercomonads, foraminifera), and Excavata (e.g., diplomonads, parabasalids). Although within supergroup phylogeny and membership are presently unsettled, in particular for excavates and chromalveolates (Parfrey et al. 2006; Simpson, Inagaki, and Roger 2006), the 6 lineages are a useful tool for testing hypotheses about eukaryote phylogeny and biodiversity. The future addition to phylogenies of uncultured environmental samples or poorly studied organisms such as amoebae or heterotrophic flagellates may expand the number of eukaryotic supergroups or alter their interrelationships. In this study, we generated extensive (6,488 unigenes) expressed sequence tag (EST) data from *Emiliania huxleyi* (Haptophyta) and 496 unique ESTs from the early diverging plastid-lacking *Goniomonas* cf. *pacifica* (Cryptophyta) (Hoef-Emden, Marin, and Melkonian 2002), both of which are chromalveolate protists. *E. huxleyi* is of tremendous importance worldwide because of its dominance in oceanic open waters (Brown and Yoder 1994), its role in cloud production through dimethylsulfide (DMS) release, the effect of *E. huxleyi* "algal blooms" on the optical quality and temperature of oceanic waters, and its role as a major carbon sink in our planet (Buitenhuis et al. 1996).

Studying *E. huxleyi* and *G. pacifica* also advances our understanding of the chromalveolates, a controversial supergroup whose constituent members are yet to be shown to comprise a monophyletic lineage in host (i.e., nuclear) phylogenies. The chromalveolate hypothesis was proposed by Cavalier-Smith (1999) to unite all chlorophyll *c*–containing algae and their nonphotosynthetic sister groups. The chromalveolates originally comprised the chromist (cryptophytes, haptophytes, and stramenopiles; Cavalier-Smith 1986) and alveolate (dinoflagellates, ciliates, and apicomplexans; e.g., Van de Peer and De Wachter 1997) protists. Recent phylogenetic analyses, however, identify two more groups with chromalveolate affinities. The first is the plastid-lacking katablepharid protists that appear as sister to cryptophytes in an analysis of small subunit rDNA (Okamoto and Inouye 2005). The second are telonemids. Analysis of a concatenated dataset of Hsp90 and small subunit rDNA positioned with significant Bayesian support *Telonemia* spp. as sister to cryptophytes in a clade that also included haptophytes (Shalchian-Tabrizi et al. 2006a). Telonemids are widely distributed marine heterotrophic protists that are morphologically distinct from katablepharids. These taxa share characters with different chromalveolates

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including tubular mitochondrial cristae common with alveolates and some chromists and flagellar hairs common with haptophytes and stramenopiles. However cortical alveoli, which are shared not only with alveolates and some stramenopiles but also with glaucophytes in the Plantae, are also found in *T. antarcticum* (Shalchian-Tabrizi et al. 2006a).

The key feature for originally merging chromalveolates is their photosynthetic organelle (plastid [when present]) that is believed to have arisen from a single secondary (red algal) endosymbiosis. Under this hypothesis, some time after its split from other members of the Plantae a red alga was engulfed by the nonphotosynthetic ancestor of the chromalveolates (Bhattacharya, Yoon, and Hackett 2004) and reduced to a 4-membrane-bound plastid that uniquely contained chlorophyll *c*. In chromists, the 4-membrane-bound plastid was retained in all groups except presumably katablepharids and telonemids (Okamoto and Inouye 2005; Shalchian-Tabrizi et al. 2006a). In the alveolates, the dinoflagellates lost one plastid membrane, the apicomplexans lost photosynthesis and retain a remnant organelle (apicoplast) used for other plastid functions such as fatty acid and heme synthesis (Foth and McFadden 2003), and ciliates have apparently lost the plastid. Support for the chromalveolate hypothesis comes from nuclear gene trees that unite stramenopiles and alveolates (Gajadhar et al. 1991; Baldauf et al. 2000; McEwan and Keeling 2004; Nozaki et al. 2004), plastid characters (e.g., storage products) and gene and genome data from this organelle (Yoon et al. 2002, 2004, 2005, 2006a; Bachvaroff, Sanchez Puerta, and Delwiche 2005), the evolutionary history of host-derived plastid-targeted translocators (Weber, Linka, and Bhattacharya 2006), and unique gene duplication and replacement events shared by these taxa (Fast et al. 2001; Harper and Keeling 2003; Patron, Rogers, and Keeling 2004). All chromalveolates except cryptophytes contain a mitochondrion with tubular cristae. Cryptophytes were thought to be ancestral within the chromists, due to the presence of two distinguishing characters, flattened mitochondrial cristae and the remnant red algal nucleus (the nucleomorph; Greenwood 1974) that is found between the second and third plastid membranes Cavalier-Smith 1999). The sister-group relationship of katablepharids and/or telonemids to cryptophytes leaves unclear the ancestral set of chromist characters.

Despite many attempts, the phylogenetic position of cryptophytes and haptophytes remains unresolved. Previous analyses of nuclear genes (often with extensive taxon sampling) show haptophytes to be unaffiliated with any other eukaryotic lineage (e.g., rDNA [Bhattacharya et al. 1995; Van de Peer and De Wachter 1997; Van de Peer et al. 2000], actin [Stibitz, Keeling, and Bhattacharya 2000], HSP90 [Stechmann and Cavalier-Smith 2003]). In contrast, rDNA trees provide moderate support for the grouping of cryptophyte with glaucophyte algae (e.g., Bhattacharya et al. 1995; Van de Peer and De Wachter 1997). The sister-group relationship of cryptophytes and glaucophytes is supported by the presence of flattened cristae in both groups. However a recent analysis using a 143-protein data set supported robustly the monophyly of Plantae and a specific relationship between glaucophytes and green algae/land plants (Rodriguez-Ezpeleta et al. 2005; see also Petersen et al. 2006; Hackett et al. 2007), although haptophytes and cryptophytes were missing from this data set. These results place glaucophytes clearly within Plantae but do not address the position of cryptophytes in the eukaryotic tree. Another phylogenetic treatment (Harper, Waanders, and Keeling 2005) used a HSP90 data set and a concatenated alignment of 6 proteins (actin, alpha- and beta-tubulin, eEF-1alpha, HSP70, and HSP90) to test chromalveolate monophyly. The single-protein HSP90 and 4-protein (actin, alpha- and beta-tubulin, and HSP90) data sets provided weak to moderate bootstrap support for the monophyly of cryptophytes and haptophytes using a variety of phylogenetic approaches. However, bootstrap support for this clade was markedly lowered with the use of the 6-protein data set, and none of the analyses provided evidence for the union of all chromalveolates, suggesting polyphyly of this group (Harper, Waanders, and Keeling 2005). And finally, a recent analysis of plastid genes aimed at determining the extent of horizontal gene transfer (HGT) within the plastid genome uncovered a ribosomal protein gene (*rpl36*) of foreign (likely eubacterial) origin that is uniquely shared by cryptophytes and haptophytes, providing strong evidence for the monophyly of these taxa (Rice and Palmer 2006). Here we assessed the phylogenetic position of haptophytes and cryptophytes using phylogenomics with the EST sequences from *E. huxleyi* and *G. pacifica*. These analyses included complete genome or EST data from other eukaryotes and prokaryotes available in public databases. Phylogenetic analyses of a concatenated 16-protein data set support the monophyly of cryptophytes and haptophytes and their sister-group relationship to a well supported assemblage that includes other chromalveolates and two members of the Rhizaria.

Materials and Methods

Construction of cDNA Libraries and Annotation of ESTs

Construction of the *Emilania huxleyi* (CCMP 371) cDNA library is described in Li et al. (2006). For this paper, we added EST data from the sequencing of a subtracted library from this species that was not presented in Li et al. (2006). The EST sequences from this species are deposited in the dbEST database of GenBank. Total RNA from *Goniomonas* cf. *pacifica* (CCMP 1869) was prepared as in Li et al. (2006), and the cDNA library was constructed using the Stratagene pBluescript II XR cDNA library construction kit (Stratagene), which contains *Xho*I and *Eco*RI digested pBluescript II SK (+) vector. Because the nonphotosynthetic *G. pacifica* was mass cultured (8 L) with bacteria and rice, algal growth (despite the large culture volume) was extremely slow and likely compromised by bacterial competition. For this reason, we initiated library construction with several nanograms of poly(A+) mRNA rather than the 5 μg prescribed by the Stratagene protocol. The low yield of RNA also made difficult efficient size selection for the libraries. However using the available starting material, a starter library was constructed and normalized as described above. The ESTs were sequenced on an ABI 3730 96-channel capillary DNA sequencer (The Roy J. Carver Center for Comparative Genomics, University of Iowa). All ESTs were processed using Phred

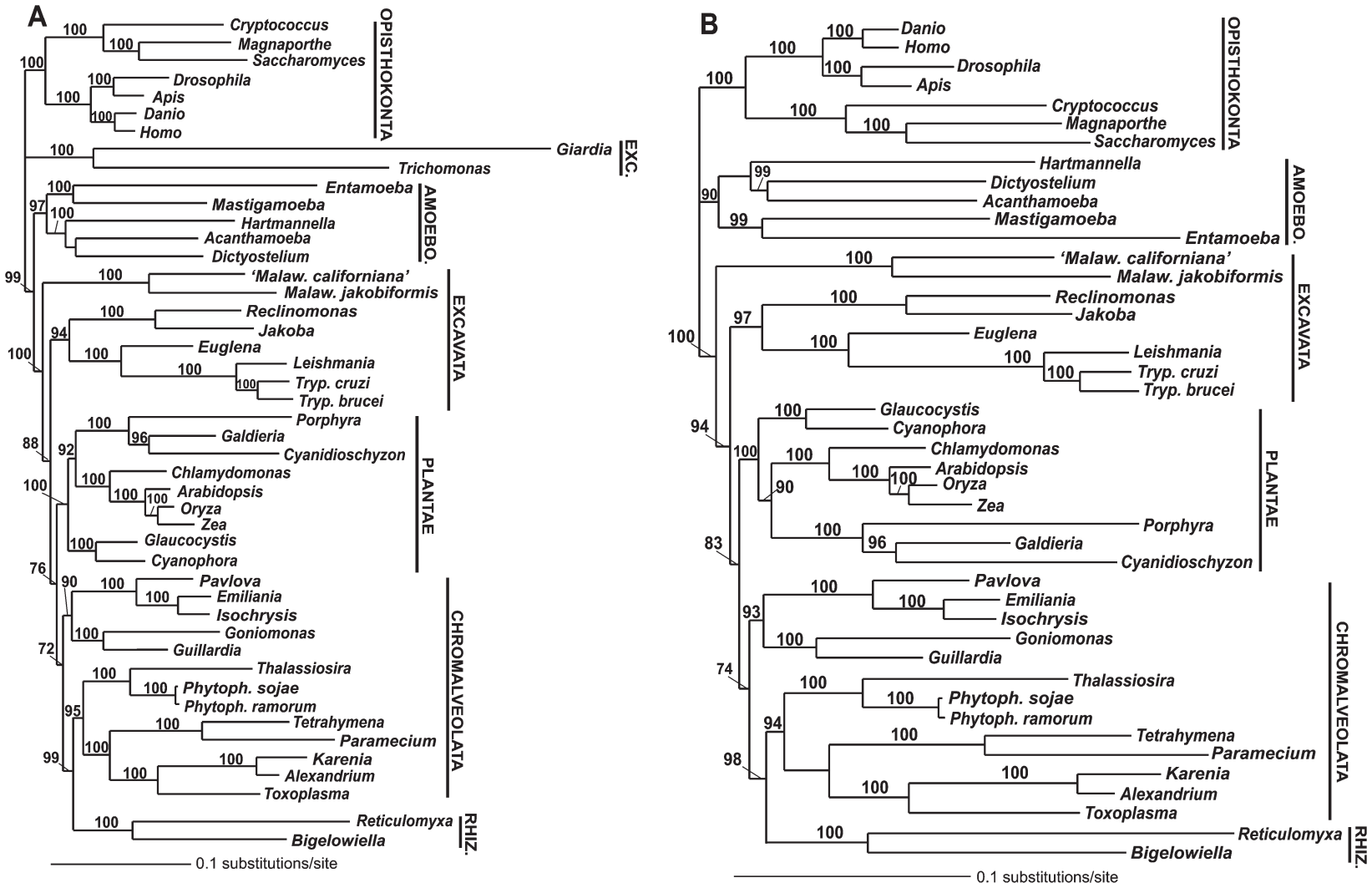

FIG. 2.—Maximum likelihood (RAxML) tree of eukaryotes after the removal of the most divergent class of amino acid sites from the 16-protein data set. (*A*) Phylogeny inferred using the 46-taxon data. (*B*) Phylogeny inferred using the 44-taxon data set after exclusion of the long-branched *G. lamblia* and *T. vaginalis* sequences. The results of bootstrap analyses using PHYML are shown above the branches in these trees. Only bootstrap values >60% are recorded. The branch lengths in the trees are proportional to the number of substitutions per site (see scales in figure). AMOEBO. is Amoebozoa; RHIZ. is Rhizaria; and EXC. Is Excavata.

Table 1
Results of the AU-Test for Alternative Tree Topologies Using the 46-Taxon Phylogeny Shown in Fig. 1.

Clade Moved Rhizaria	Rank	ΔlnL	AU Probability
Best tree	*1*	*-14.0*	*0.962*
Base alveolates	2	14.0	0.108
Base stramenopiles	3	18.3	0.048*
Base chromalveolates	5	26.0	0.007**
Base eugl.+ diplo.+parabas.+jakobids	11	63.3	0.002**
Base Euglenozoa	25	92.3	6e-007**
Base diplo.+parabas.+jakobids	21	86.8	2e-004**
Base malawimonads	19	83.5	7e-005**
Cryptophytes + Haptophytes	**Rank**	**ΔlnL**	**AU Probability**
Best tree	*1*	*-9.9*	*0.863*
Base Plantae	2	9.9	0.389
Base green+glaucophytes	6	27.6	0.167
Base glaucophytes	8	45.5	0.051
Base reds	7	45.0	6e-005**
Base Plantae+chromalveolates	5	25.8	0.013*
Base Amoebozoa	27	185.2	2e-004**
Base Euglenozoa	20	131.5	1e-077**
Base Opisthokonta	24	171.1	2e-005**
Cryptophytes	**Rank**	**ΔlnL**	**AU Probability**
Best tree	*1*	*-8.1*	*0.892*
Base chromalveolates+Rhizaria	2	8.1	0.383
Base Plantae	4	24.2	0.118
Base Plantae+chromalveolates	5	25.2	0.067
Base reds	10	46.6	0.006**
Base Amoebozoa	27	122.6	1e-032**
Base Euglenozoa	20	89.6	8e-009**
Base Opisthokonta	24	117.2	2e-075**
Haptophytes	**Rank**	**ΔlnL**	**AU Probability**
Best tree	*1*	*-8.1*	*0.888*
Base alveolates+Stramen.+ Rhizaria	2	8.1	0.375
Base Plantae	4	24.5	0.106
Base greens+glaucophytes	7	29.4	0.166
Base greens	8	30.8	0.219
Base glaucophytes	10	37.6	0.079
Base reds	11	50.7	2e-005**
Base Plantae+chromalveolates	9	37.5	0.014*
Base Amoebozoa	30	159.3	4e-004**
Base Euglenozoa	24	123.3	4e-009**
Base Opisthokonta	28	146.8	2e-006**

NOTE.—Significantly rejected trees are marked with * when $P < 0.05$ and with ** when $P < 0.01$. Diplo. is diplomonad, eugl. is Euglenozoa, and parabas. is parabasalids.

trees of significantly lower probability. This pattern may be interpreted in at least 3 ways. Under the first scenario, it is possible that the Plantae association is specific to our analysis. Although potentially true, it is a surprising result, given that virtually all other nodes in our tree are highly supported, and that the only alternate nonrejected associations detected by the AU-test are with one clade, the Plantae. Under a second scenario, our results may reflect undetected EGT (either complete or partial, ancient gene replacements) from a Plantae endosymbiont or HGT from these algae that is not evident in any single-protein PHYML bootstrap analysis (except for EF2, which is a long protein [783 aa in our final alignment] with significant phylogenetic signal). A surprising result in this regard is the nature of the Plantae association. Under the chromalveolate hypothesis, the source of EGT is expected to be from the red algal secondary endosymbiont. However, the AU-test strongly rejects the sister-group relationship of cryptophytes, haptophytes, or cryptophytes + haptophytes with red algae ($P < 0.01$). This suggests that, as evident in the EF2 tree (fig. S1) and analyses of nuclear-encoded plastid-targeted proteins in chromalveolates (e.g., Nosenko et al. 2006), nonred sources may also have provided genes to the nucleus of some or all chromalveolates. If this latter hypothesis is correct, then it may explain the great difficulties that are evident when reconstructing chromalveolate interrelationships, in spite of extensive multigene data or a broad taxon sampling (e.g., Harper Waanders, and Keeling 2005; Burki and Pawlowski 2006). Chromalveolate genomes may carry two distinct, conflicting phylogenetic histories, one of their hosts and one of their history of endosymbiotic gene replacements and HGT events, providing a potentially large impediment to multigene analyses of this group. A final possibility is that the cryptophytes and/or haptophyte host cells may be related to the Plantae, as has been suggested by rDNA trees that often support cryptophyte + glaucophyte monophyly (Bhattacharya et al. 1995; Van de Peer and De Wachter 1997; Okamoto and Inouye 2005), which is supported by the presence of flattened mitochondrial cristae in these taxa.

Conclusions

Although it is obvious that much more careful work needs to be done with chromalveolates and Rhizaria to elucidate their phylogeny, our analyses do provide some important insights. First, it is essential that in multigene analyses of chromalveolates each gene is analyzed individually with a broad taxon sample to identify potential cases of EGT or HGT. In the case of EGT, an obvious alternate position is expected with the Plantae. Second, despite the conflict evident within our data set, the overall phylogenetic signal favors two main findings: (1) the association of Rhizaria with chromalveolates, and (2) the monophyly of cryptophytes + haptophytes (see also Patron, Inagaki, and Keeling 2007). This latter result is supported by the independent plastid genome HGT data of Rice and Palmer (2006). One further consideration with regard to the position of the cryptophytes is that the AU-test does not significantly reject the ancestral position of this lineage relative to all other chromalveolates and Rhizaria ($P = 0.383$). This supports the notion of a single loss of the nucleomorph after the cryptophyte divergence, as has been suggested by plastid gene data (e.g., Yoon et al. 2002), but complicates interpretation of the *rpl36* gene replacement. If the cryptophytes are indeed the early divergence in the chromalveolate + Rhizaria clade, then it is possible two copies of *rpl36* may have existed in their common ancestor with independent, differential losses in the cryptophytes and haptophytes (cyanobacterial gene loss) versus other chromalveolates (eubacterial gene loss). Alternatively, and less parsimoniously, there were two

independent *rpl36* gene replacements in cryptophytes and haptophytes. Given these uncertainties, we stress that our results are a working hypothesis that is constrained by the inherent difficulties in inferring "deep" phylogeny due to differences in divergence rates among lineages, covarion substitution processes (e.g., Philippe et al. 2004; Rodriguez-Ezpeleta et al. 2005; Shalchian-Tabrizi et al. 2006b), and potentially confounding EGT. However these results can be tested through the analysis of more sequence data from existing cultured taxa than found in our tree (see EuTree; http://www.biology.uiowa.edu/eu_tree/) and the addition of novel taxa such as katablepharids and telonemids when these turn up in environmental surveys (e.g., picobiliphytes; Not et al. 2007).

Supplementary Material

Supplementary tables and figures are available at *Molecular Biology and Evolution* online (http://www.mbe.oxfordjournals.org/).

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Literature Cited

Abascal F, Zardoya R, Posada D. 2005. ProtTest: Selection of best-fit models of protein evolution. Bioinformatics. 21:2104–2105.

Adl SM, Simpson AG, Farmer MA. (28 co-authors). 2005. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. J Eukaryot Microbiol. 52: 399–451.

Arisue N, Hasegawa M, Hashimoto T. 2005. Root of the Eukaryota tree as inferred from combined maximum likelihood analyses of multiple molecular sequence data. Mol Biol Evol. 22:409–420.

Bachvaroff TR, Sanchez-Puerta MV, Delwiche CF. 2005. Chlorophyll c-containing plastid relationships based on analyses of a multi-gene dataset with all four chromalveolate lineages. Mol Biol Evol. 22:1772–1782.

Baldauf SL, Roger AJ, Wenk-Siefert I, Doolittle WF. 2000. A kingdom-level phylogeny of eukaryotes based on combined protein data. Science. 290:972–977.

Barbier G, Oesterhelt C, Larson MD, Halgren RG, Wilkerson C, Garavito RM, Benning C, Weber AP. 2005. Comparative genomics of two closely related unicellular thermo-acidophilic red algae, *Galdieria sulphuraria* and *Cyanidioschyzon merolae*, reveals the molecular basis of the metabolic flexibility of *Galdieria sulphuraria* and significant differences in carbohydrate metabolism of both algae. Plant Physiol. 137:460–474.

Bhattacharya D, Helmchen T, Bibeau C, Melkonian M. 1995. Comparisons of nuclear-encoded small-subunit ribosomal RNAs reveal the evolutionary position of the Glaucocystophyta. Mol Biol Evol. 12:415–420.

Bhattacharya D, Katz LA. 2005. Frontiers in genomics: Insights into protist evolutionary biology. J Eukaryot Microbiol. University of Iowa, J Eukaryot Microbiol. 52:170–172. May 19–21, 2004

Bhattacharya D, Yoon HS, Hackett JD. 2004. Photosynthetic eukaryotes unite: Endosymbiosis connects the dots. BioEssays. 26:50–60.

Brown CW, Yoder JA. 1994. Coccolithophorid blooms in the global ocean. J. Geophys Res. 99:7467–7482.

Buitenhuis E, van Bleijswijk J, Bakker D, Veldhuis M. 1996. Trends in inorganic and organic carbon in a bloom of *Emiliania huxleyi* in the North Sea. Mar Ecol Prog Ser. 143:271–282.

Burki F, Pawlowski J. 2006. Monophyly of Rhizaria and multigene phylogeny of unicellular bikonts. Mol Biol Evol. 23:1922–1930.

Cavalier-Smith T. 1999. Principles of protein and lipid targeting in secondary symbiogenesis: Euglenoid, Dinoflagellate, and Sporozoan plastid origins and the Eukaryote family tree. J Eukaryot Microbiol. 46:347–366.

Cavalier-Smith T. 1986. The kingdom Chromista: Origin and systematics. In: Round FE, Chapman DJ, eds. Progress in Phycological Research. Bristol, U.K.: Biopress. p. 309–347.

Ciccarelli FD, Doerks T, von Mering C, Creevey CJ, Snel B, Bork P. 2006. Toward automatic reconstruction of a highly resolved tree of life. Science. 311:1283–1287.

Conesa A, Gotz S, Garcia-Gomez JM, Terol T, Talon M, Robles M. 2005. Blast2GO: A universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics. 21:3674–3676.

Driskell AC, Ane C, Burleigh JG, McMahon MM, O'Meara BC, Sanderson MJ. 2004. Prospects for building the tree of life from large sequence databases. Science. 306:1172–1174.

Ewing B, Green P. 1998. Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res. 8:186–194.

Ewing B, Hillier L, Wendl MC, Green P. 1998. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Res. 8:175–185.

Fahrni JF, Bolivar I, Berney C, Nassonova E, Smirnov A, Pawlowski J. 2003. Phylogeny of lobose amoebae based on actin and small-subunit ribosomal RNA genes. Mol Biol Evol. 20:1881–1886.

Fast NM, Kissinger JC, Roos DS, Keeling PJ. 2001. Nuclear-encoded, plastid-targeted genes suggest a single common origin for apicomplexan and dinoflagellate plastids. Mol Biol Evol. 18:418–426.

Foth BJ, McFadden GI. 2003. The apicoplast: A plastid in *Plasmodium falciparum* and other Apicomplexan parasites. Int Rev Cytol. 224:57–110.

Frickey T, Lupas AN. 2004. PhyloGenie: Automated phylome generation and analysis. Nucleic Acids Res. 32:5231–5238.

Gajadhar AA, Marquardt WC, Hall R, Gunderson J, Ariztia-Carmona EV, Sogin ML. 1991. Ribosomal RNA sequences of *Sarcocystis muris*, *Theileria annulata* and *Crypthecodinium cohnii* reveal evolutionary relationships among apicomplexans, dinoflagellates, and ciliates. Mol Biochem Parasitol. 45:147–154.

Greenwood AD. 1974. The Cryptophyta in relation to phylogeny and photosynthesis. In: Saunders JV, Goodchild DJ, eds.

Electron microscopy 1974. Canberra: Australian Academy of Sciences. p. 566–567.
Guindon S, Gascuel O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst Biol. 52:696–704.
Hackett JD, Yoon HS, Butterfield NJ, Sanderson MJ, Bhattacharya D. 2007. Plastid endosymbiosis: Sources and timing of the major events. In: Falkowski P, Knoll A, eds. Evolution of primary producers in the sea. Forthcoming: Elsevier.
Hackett JD, Yoon HS, Soares MB, Bonaldo MF, Casavant TL, Scheetz TE, Nosenko T, Bhattacharya D. 2004. Migration of the plastid genome to the nucleus in a peridinin dinoflagellate. Curr Biol. 14:213–218.
Harper JT, Keeling PJ. 2003. Nucleus-encoded, plastid-targeted glyceraldehyde-3-phosphate dehydrogenase (GAPDH) indicates a single origin for chromalveolate plastids. Mol Biol Evol. 20:1730–1735.
Harper JT, Waanders E, Keeling PJ. 2005. On the monophyly of chromalveolates using a six-protein phylogeny of eukaryotes. Int J Syst Evol Microbiol. 55:487–496.
Hoef-Emden K, Marin B, Melkonian M. 2002. Nuclear and nucleomorph SSU rDNA phylogeny in the Cryptophyta and the evolution of cryptophyte diversity. J Mol Evol. 55: 161–179.
Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics. 17:754–755.
Keeling PJ. 2001. Foraminifera and Cercozoa are related in actin phylogeny: Two orphans find a home? Mol Biol Evol. 18:1551–1557.
Keeling PJ, Burger G, Durnford DG, Lang BF, Lee RW, Pearlman RE, Roger AJ, Gray MW. 2005. The tree of eukaryotes. Trends Ecol Evol. 20:670–676.
Koski LB, Gray MW, Lang BF, Burger G. 2005. AutoFACT: An automatic functional annotation and classification tool. BMC Bioinformatics. 6:151.
Li S, Nosenko T, Hackett JD, Bhattacharya D. 2006. Phylogenomic analysis identifies red algal genes of endosymbiotic origin in the chromalveolates. Mol Biol Evol. 23:663–674.
Longet D, Archibald JM, Keeling PJ, Pawlowski J. 2003. Foraminifera and Cercozoa share a common origin according to RNA polymerase II phylogenies. Int J Syst Evol Microbiol. 53:1735–1739.
Maddison DR, Maddison WP. 2005. MacClade. Sunderland (MA): Sinauer Associates.
Marin B, Nowack EC, Melkonian M. 2005. A plastid in the making: Evidence for a second primary endosymbiosis. Protist. 156:425–432.
Matsuzaki M, Misumi O, Shin IT. (41 co-authors). 2004. Genome sequence of the ultrasmall unicellular red alga *Cyanidioschyzon merolae* 10D. Nature. 428:653–657.
McEwan ML, Keeling PJ. 2004. HSP90, tubulin and actin are retained in the tertiary endosymbiont genome of Kryptoperidinium foliaceum. J Eukaryot Microbiol. 51:651–659.
McFadden GI, van Dooren GG. 2004. Evolution: Red algal genome affirms a common origin of all plastids. Curr Biol. 14:R514–516.
McMahon MM, Sanderson MJ. 2006. Phylogenetic supermatrix analysis of GenBank sequences from 2228 papilionoid legumes. Syst Biol. 55:818–836.
Nikaido I, Asamizu E, Nakajima M, Nakamura Y, Saga N, Tabata S. 2000. Generation of 10,154 expressed sequence tags from a leafy gametophyte of a marine red alga, *Porphyra yezoensis*. DNA Res. 7:223–227.
Nikolaev SI, Berney C, Fahrni JF, Bolivar I, Polet S, Mylnikov AP, Aleshin VV, Petrov NB, Pawlowski J. 2004. The twilight of Heliozoa and rise of Rhizaria, an emerging supergroup of amoeboid eukaryotes. Proc Natl Acad Sci USA. 101:8066–8071.
Nikolaev SI, Berney C, Petrov NB, Mylnikov AP, Fahrni JF, Pawlowski J. 2006. Phylogenetic position of *Multicilia marina* and the evolution of Amoebozoa. Int J Syst Evol Microbiol. 56:1449–1458.
Nosenko T, Lidie KL, Van Dolah FM, Lindquist E, Cheng JF, Bhattacharya D. 2006. Chimeric plastid proteome in the Florida "red tide" dinoflagellate *Karenia brevis*. Mol Biol Evol. 23:2026–2038.
Not F, Valentin K, Romari K, Lovejoy C, Massana T, Tobe K, Vaulot D, Medlin LK. 2007. Picobiliphytes: A marine picoplanktonic algal group with unknown affinities to other eukaryotes. Science. 315:253–255.
Nozaki H, Matsuzaki M, Misumi O, Kuroiwa H, Hasegawa M, Higashiyama T, Shin IT, Kohara Y, Ogasawara N, Kuroiwa T. 2004. Cyanobacterial genes transmitted to the nucleus before divergence of red algae in the Chromista. J Mol Evol. 59:103–113.
Okamoto N, Inouye I. 2005. The katablepharids are a distant sister group of the Cryptophyta: A proposal for Katablepharidophyta Divisio Nova/Kathablepharida Phylum Novum based on SSU rDNA and beta-tubulin phylogeny. Protist. 156:163–179.
Parfrey LW, Barbero E, Lasser E, Dunthorn M, Bhattacharya D, Patterson DJ, Katz LA. 2006. Evaluating support for the current classification of eukaryotic diversity. PLoS Genet. 2:e220.
Patron NJ, Rogers MB, Keeling PJ. 2004. Gene replacement of fructose-1,6-bisphosphate aldolase supports the hypothesis of a single photosynthetic ancestor of chromalveolates. Eukaryot Cell. 3:1169–1175.
Patron NJ, Inagaki Y, Keeling PJ. 2007. Multiple gene phylogenies support the monophyly of cryptomonad and haptophyte host lineages. Curr Biol. 17:1–5.
Petersen J, Teich R, Becker B, Cerff R, Brinkmann H. 2006. The GapA/B gene duplication marks the origin of Streptophyta (charophytes and land plants). Mol Biol Evol. 23:1109–1118.
Philippe H, Snell EA, Bapteste E, Lopez P, Holland PW, Casane D. 2004. Phylogenomics of eukaryotes: Impact of missing data on large alignments. Mol Biol Evol. 21:1740–1752.
Reyes-Prieto A, Yoon HS, Bhattacharya D. 2006. Phylogenomics and its growing impact on algal phylogeny and evolution. Algae. 21:1–10.
Rice DW, Palmer JD. 2006. An exceptional horizontal gene transfer in plastids: Gene replacement by a distant bacterial paralog and evidence that haptophyte and cryptophyte plastids are sisters. BMC Biol. 4:31.
Rodriguez-Ezpeleta N, Brinkmann H, Burey SC, Roure B, Burger G, Loffelhardt W, Bohnert HJ, Philippe H, Lang BF. 2005. Monophyly of primary photosynthetic eukaryotes: Green plants, red algae, and glaucophytes. Curr Biol. 15: 1325–1330.
Rogers MB, Gilson PR, Su V, McFadden GI, Keeling PJ. 2007. The Complete chloroplast genome of the chlorarachniophyte *Bigelowiella natans*: Evidence for independent origins of chlorarachniophyte and euglenid secondary endosymbionts. Mol Biol Evol. 24:54–62.
Scala S, Carels N, Falciatore A, Chiusano ML, Bowler C. 2002. Genome properties of the diatom *Phaeodactylum tricornutum*. Plant Physiol. 129:993–1002.
Schmidt HA, Strimmer K, Vingron M, von Haeseler A. 2002. TREE-PUZZLE: Maximum likelihood phylogenetic analysis using quartets and parallel computing. Bioinformatics. 18:502–504.

Shalchian-Tabrizi K, Eikrem W, Klaveness D. (12 co-authors). 2006a. Telonemia, a new protist phylum with affinity to chromist lineages. Proc Biol Sci. 273:1833–1842.
Shalchian-Tabrizi K, Skanseng M, Ronquist F, Klaveness D, Bachvaroff TR, Delwiche CF, Botnen A, Tengs T, Jakobsen KS. 2006b. Heterotachy processes in rhodophyte-derived secondhand plastid genes: Implications for addressing the origin and evolution of dinoflagellate plastids. Mol Biol Evol. 23:1504–1515.
Shimodaira H. 2002. An approximately unbiased test of phylogenetic tree selection. Syst Biol. 51:492–508.
Shimodaira H, Hasegawa M. 2001. CONSEL: For assessing the confidence of phylogenetic tree selection. Bioinformatics. 17:1246–1247.
Simpson AG. 2003. Cytoskeletal organization, phylogenetic affinities and systematics in the contentious taxon Excavata (Eukaryota). Int J Syst Evol Microbiol. 53:1759–1777.
Simpson AG, Inagaki Y, Roger AJ. 2006. Comprehensive multigene phylogenies of excavate protists reveal the evolutionary positions of "primitive" eukaryotes. Mol Biol Evol. 23:615–625.
Simpson AG, Roger AJ. 2004. The real 'kingdoms' of eukaryotes. Curr Biol. 14:R693–696.
Stamatakis A, Ludwig T, Meier H. 2005. RAxML-III: A fast program for maximum likelihood-based inference of large phylogenetic trees. Bioinformatics. 21:456–463.
Stechmann A, Cavalier-Smith T. 2003. Phylogenetic analysis of eukaryotes using heat-shock protein Hsp90. J Mol Evol. 57:408–419.
Stibitz TB, Keeling PJ, Bhattacharya D. 2000. Symbiotic origin of a novel actin gene in the cryptophyte *Pyrenomonas helgolandii*. Mol Biol Evol. 17:1731–1738.
Van de Peer Y, Baldauf SL, Doolittle WF, Meyer A. 2000. An updated and comprehensive rRNA phylogeny of (crown) eukaryotes based on rate-calibrated evolutionary distances. J Mol Evol. 51:565–576.
Van de Peer Y, De Wachter R. 1997. Evolutionary relationships among the eukaryotic crown taxa taking into account site-to-site rate variation in 18S rRNA. J Mol Evol. 45:619–630.
Walker G, Dacks JB, Martin Embley T. 2006. Ultrastructural description of *Breviata anathema*, n. gen., n. sp., the organism previously studied as "*Mastigamoeba invertens*". J Eukaryot Microbiol. 53:65–78.
Weber AP, Linka M, Bhattacharya D. 2006. Single, ancient origin of a plastid metabolite translocator family in Plantae from an endomembrane-derived ancestor. Eukaryot Cell. 5:609–612.
Wiens JJ. 2003. Missing data, incomplete taxa, and phylogenetic accuracy. Syst Biol. 52:528–538.
Wiens JJ. 2006. Missing data and the design of phylogenetic analyses. J Biomed Inform. 39:34–42.
Yoon HS, Hackett JD, Ciniglia C, Pinto G, Bhattacharya D. 2004. A molecular timeline for the origin of photosynthetic eukaryotes. Mol Biol Evol. 21:809–818.
Yoon HS, Hackett JD, Pinto G, Bhattacharya D. 2002. The single, ancient origin of chromist plastids. Proc Natl Acad Sci USA. 99:15507–15512.
Yoon HS, Hackett JD, Van Dolah FM, Nosenko T, Lidie KL, Bhattacharya D. 2005. Tertiary endosymbiosis driven genome evolution in dinoflagellate algae. Mol Biol Evol. 22:1299–1308.
Yoon HS, Muller KM, Sheath RG, Ott FD, Bhattacharya D. 2006a. Defining the major lineages of red algae (Rhodophyta). J Phycol. 42:482–492.
Yoon HS, Reyes-Prieto A, Melkonian M, Bhattacharya D. 2006b. Minimal plastid genome evolution in the *Paulinella* endosymbiont. Curr Biol. 16:R670–672.

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