

Supplementary Information

Chloroplast phylogenomic analyses reveal the deepest-branching lineage of the Chlorophyta, Palmophyllophyceae class. nov.

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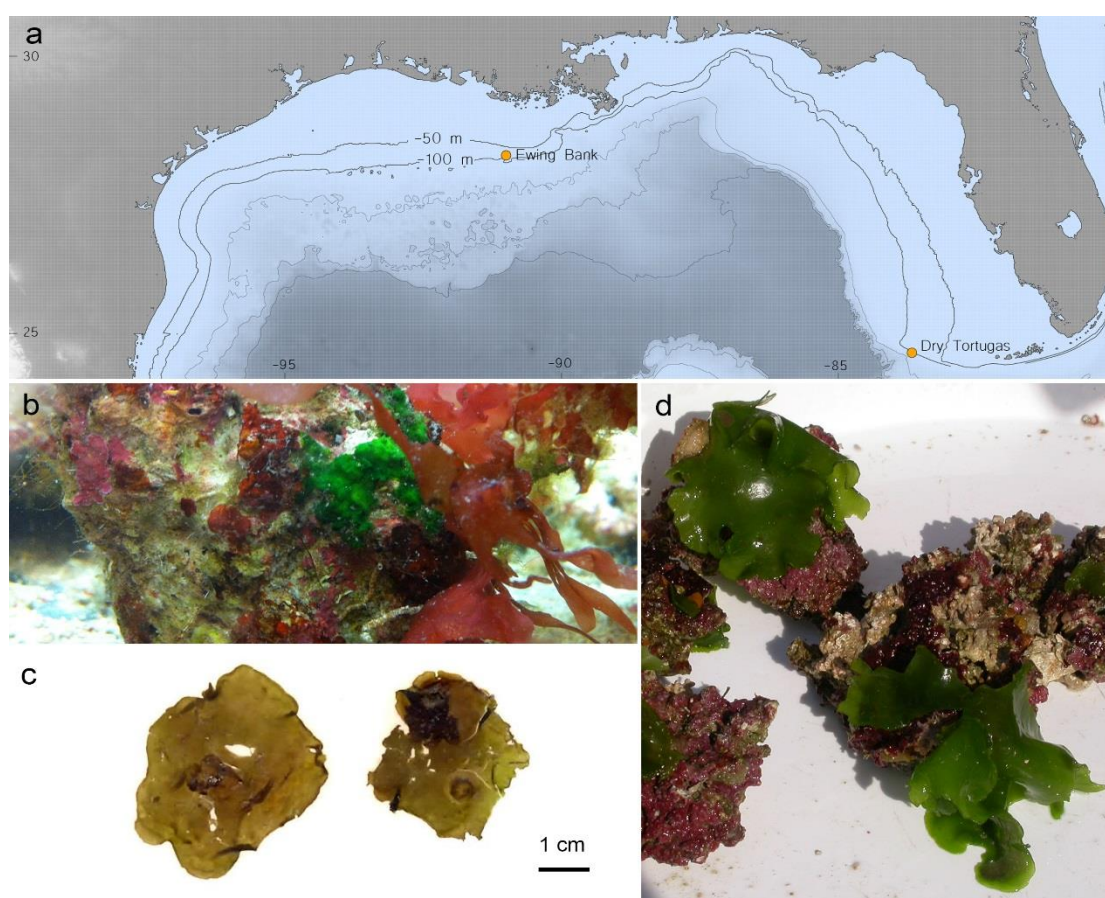


Figure S1. (a) Location of the sequenced specimen of *Verdigellas peltata*: offshore Louisiana, Ewing Bank, NW Gulf of Mexico (box dredge in: 28 05.58'N, 91 01.01'W; box dredge out: 28 05 67'N, 91 01.49'W), at ca. 70 m depth, and the specimen depicted in Fig. S1d. Map created using the R¹ package marmap v. 0.9.5² (<https://cran.r-project.org/web/packages/marmap>) (b) Photograph of live specimen, maintained in laboratory microcosm, growing from surface of rhodolith (LAF-8-26-12-6-1). (c) Photograph of the herbarium voucher of the sequenced specimen (LAF-8-26-12-6-1). (d) *Verdigellas peltata* collected in the vicinity of Dry Tortugas, FL, SE Gulf of Mexico (2 Jun 2004, 24°39.03'N, 83°41.02'W, growing on rhodoliths among red algal crusts, depth between 104 and 94 m, collected with box dredge aboard the R/V Pelican, coll. S. Fredericq (NSF-I-30-1, LAF-6-2-04-3-1).

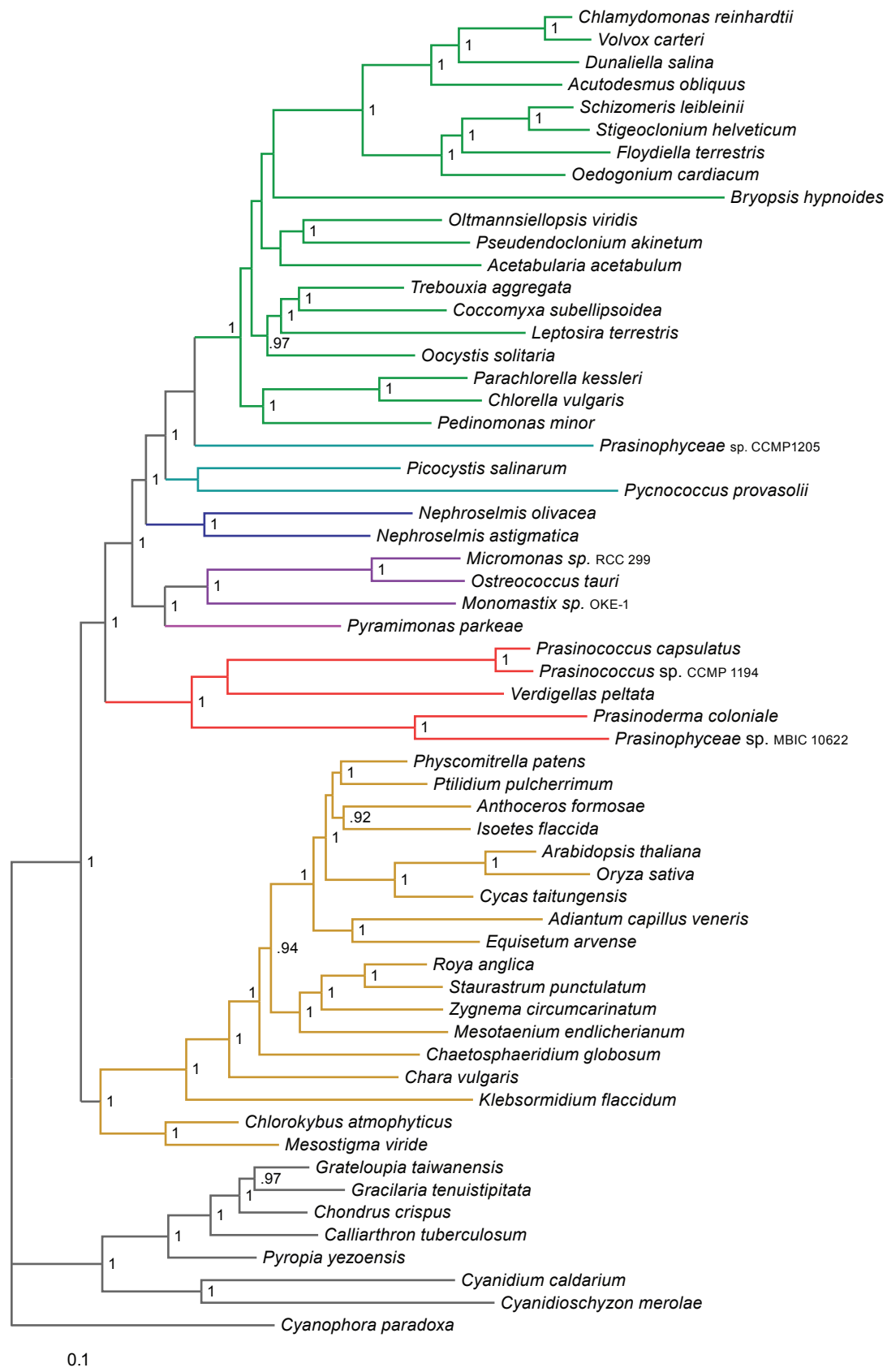


Figure S2. Plastid phylogeny of the green plants obtained by Bayesian inference (MrBayes) of a concatenated protein alignment of 71 chloroplast genes (13,730 amino acid positions) under a cpREV+Γ4+F model. The majority rule consensus tree is shown with node support given as posterior probabilities (values < .90 are not shown).

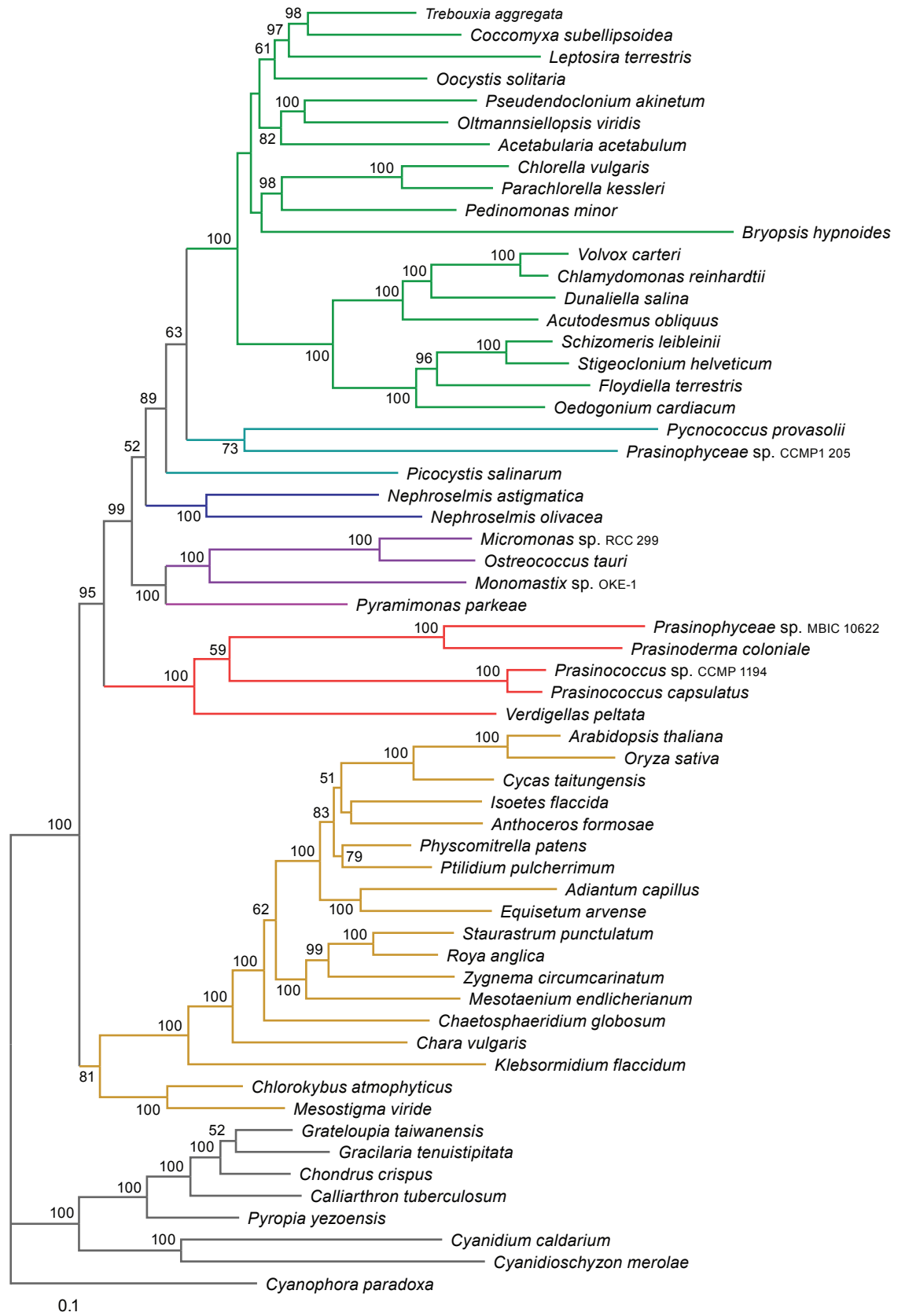


Figure S3. Plastid phylogeny of the green plants obtained by maximum likelihood inference (RAxML) of a concatenated protein alignment of 71 chloroplast genes (13,730 amino acid positions) under a cpREV+Γ4+F model. The maximum likelihood tree is shown with bootstrap support for the nodes (values < 50 are not shown).

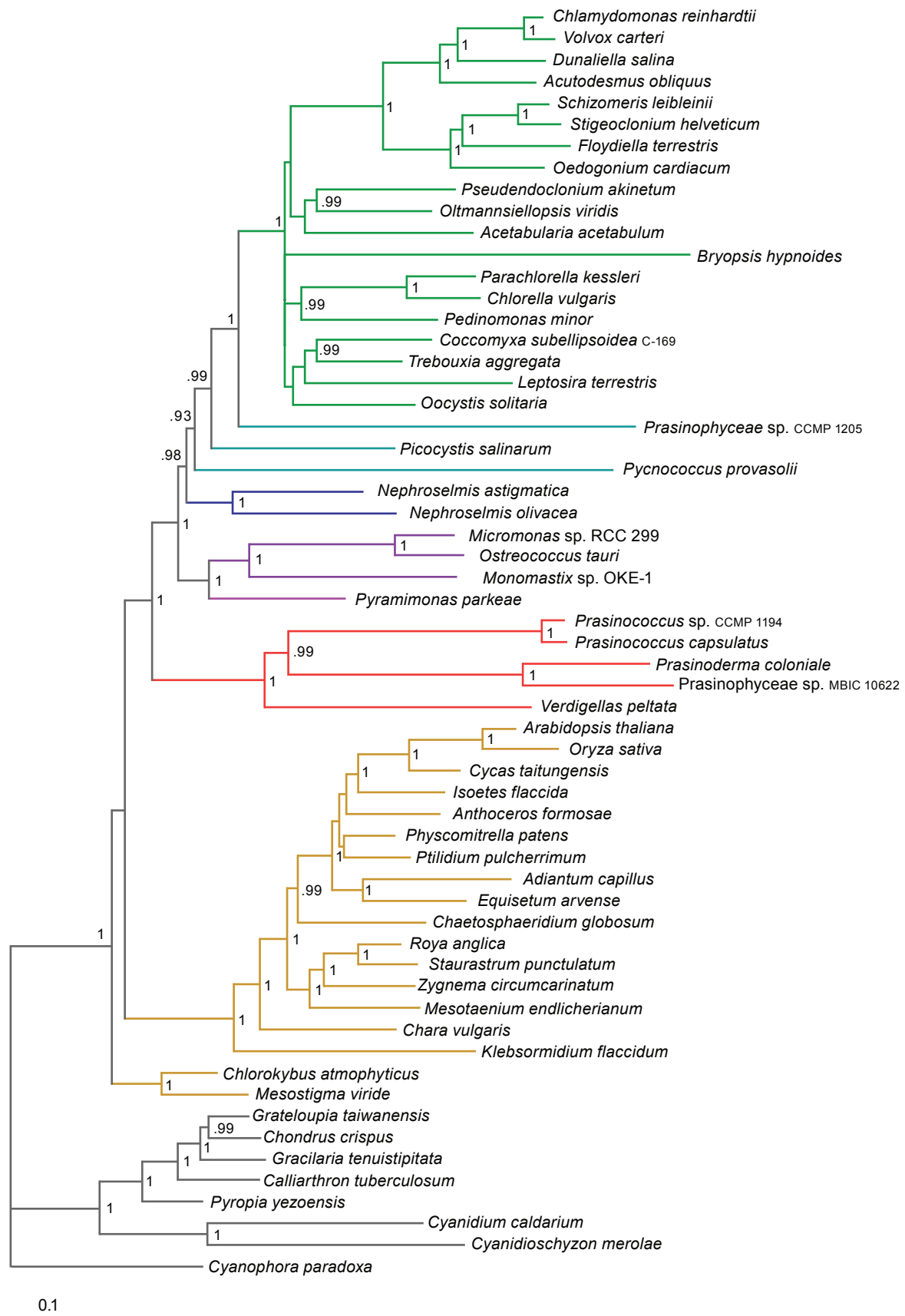


Figure S4. Plastid phylogeny of the green plants obtained by Bayesian inference (Phylobayes) of a concatenated protein alignment of 71 chloroplast genes (13,730 amino acid positions) under a site-heterogeneous CAT+Γ4 model. The consensus tree is shown with node support given as posterior probabilities (values < .90 are not shown).

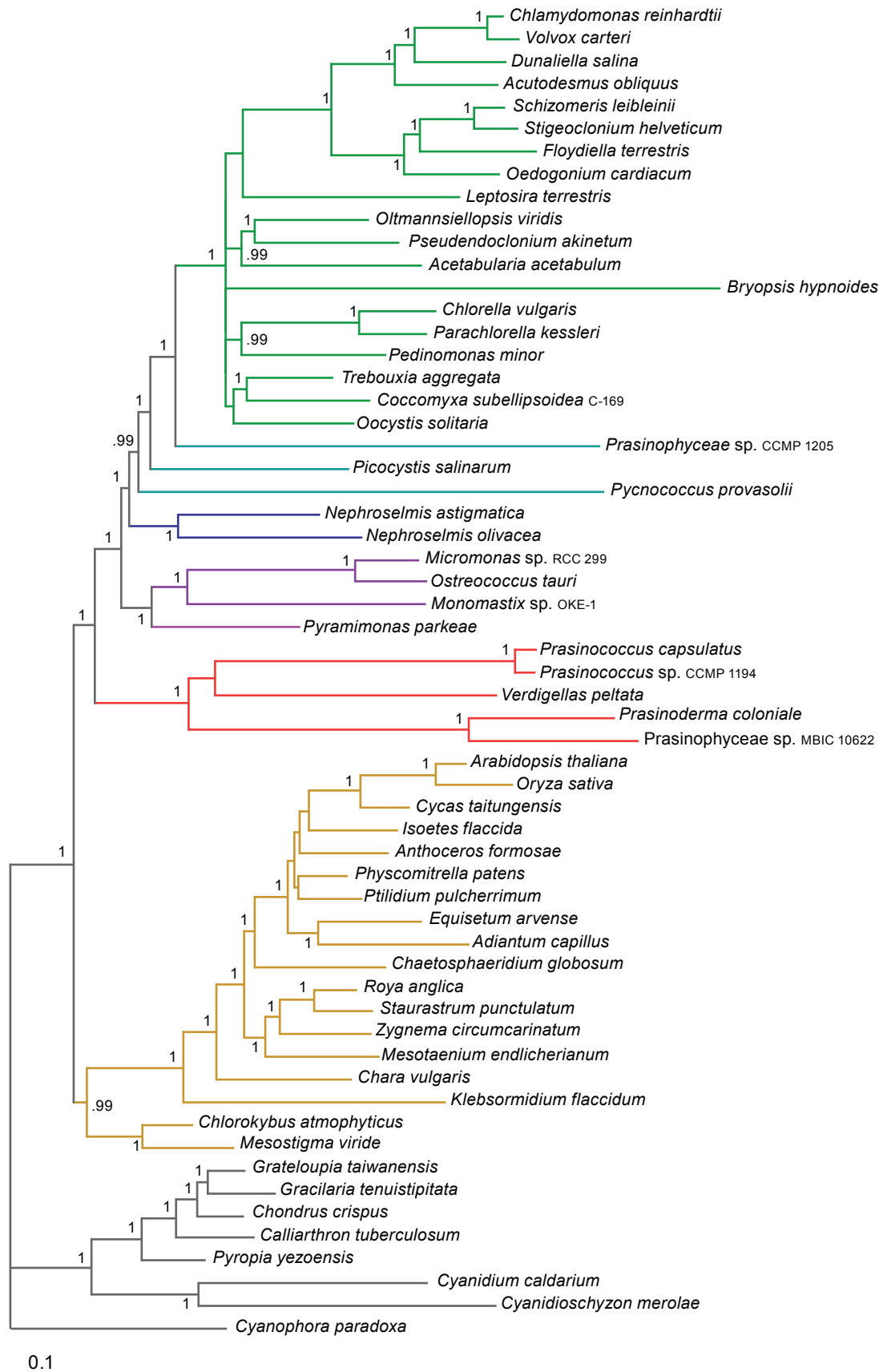


Figure S5. Plastid phylogeny of the green plants obtained by Bayesian inference (Phylobayes) of a concatenated protein alignment of 71 chloroplast genes (13,730 amino acid positions) under a site-heterogeneous CATGTR+ Γ 4 model. The consensus tree is shown with node support given as posterior probabilities (values < .90 are not shown).

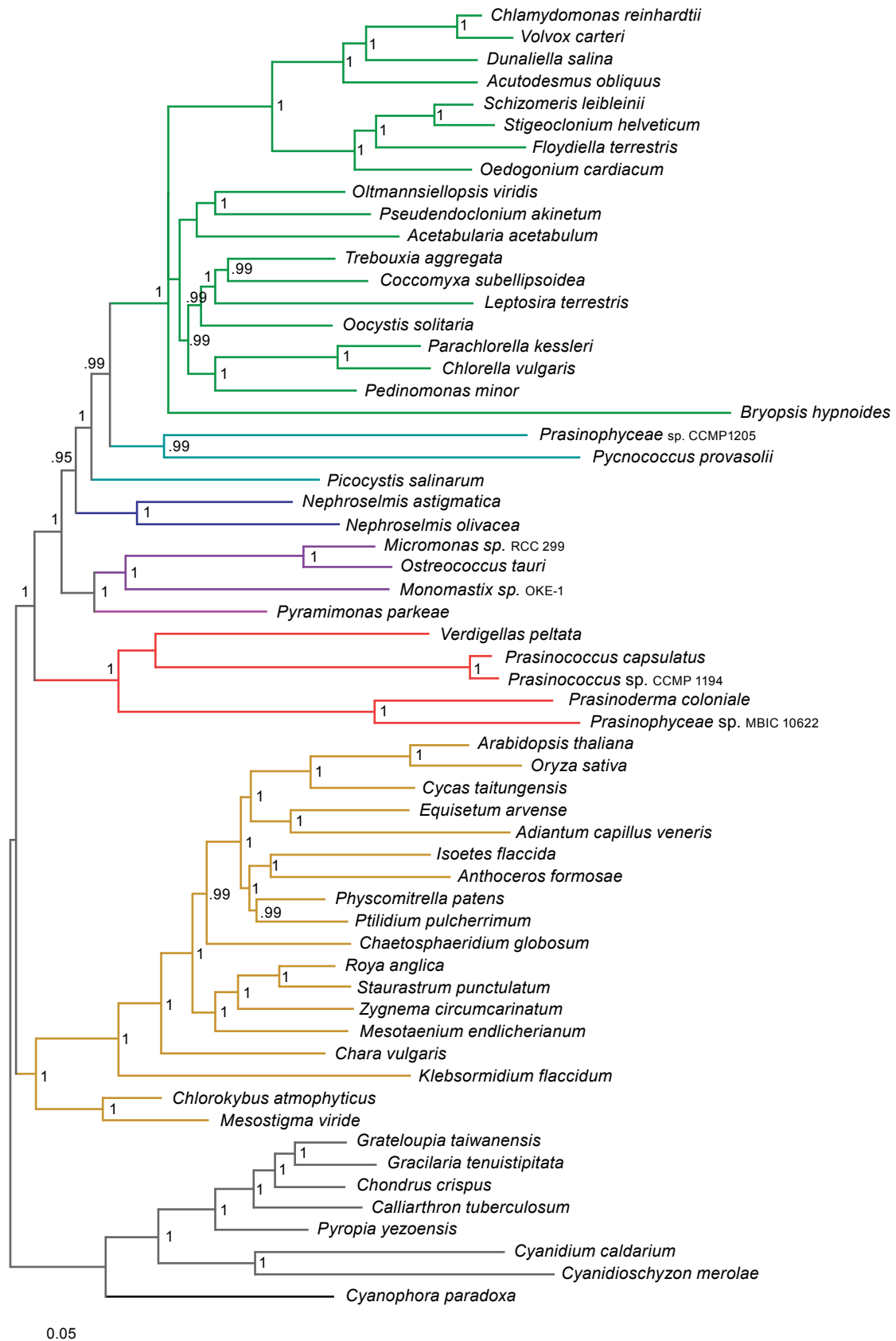


Figure S6. Plastid phylogeny of the green plants obtained by Bayesian inference (Phylobayes) of the concatenated protein alignment of 71 chloroplast genes (13,730 amino acid positions), recoded by the Dayhoff6 recoding scheme, and analysed under a GTR+ Γ 4 model. The consensus tree is shown with node support given as posterior probabilities (values < .90 are not shown)

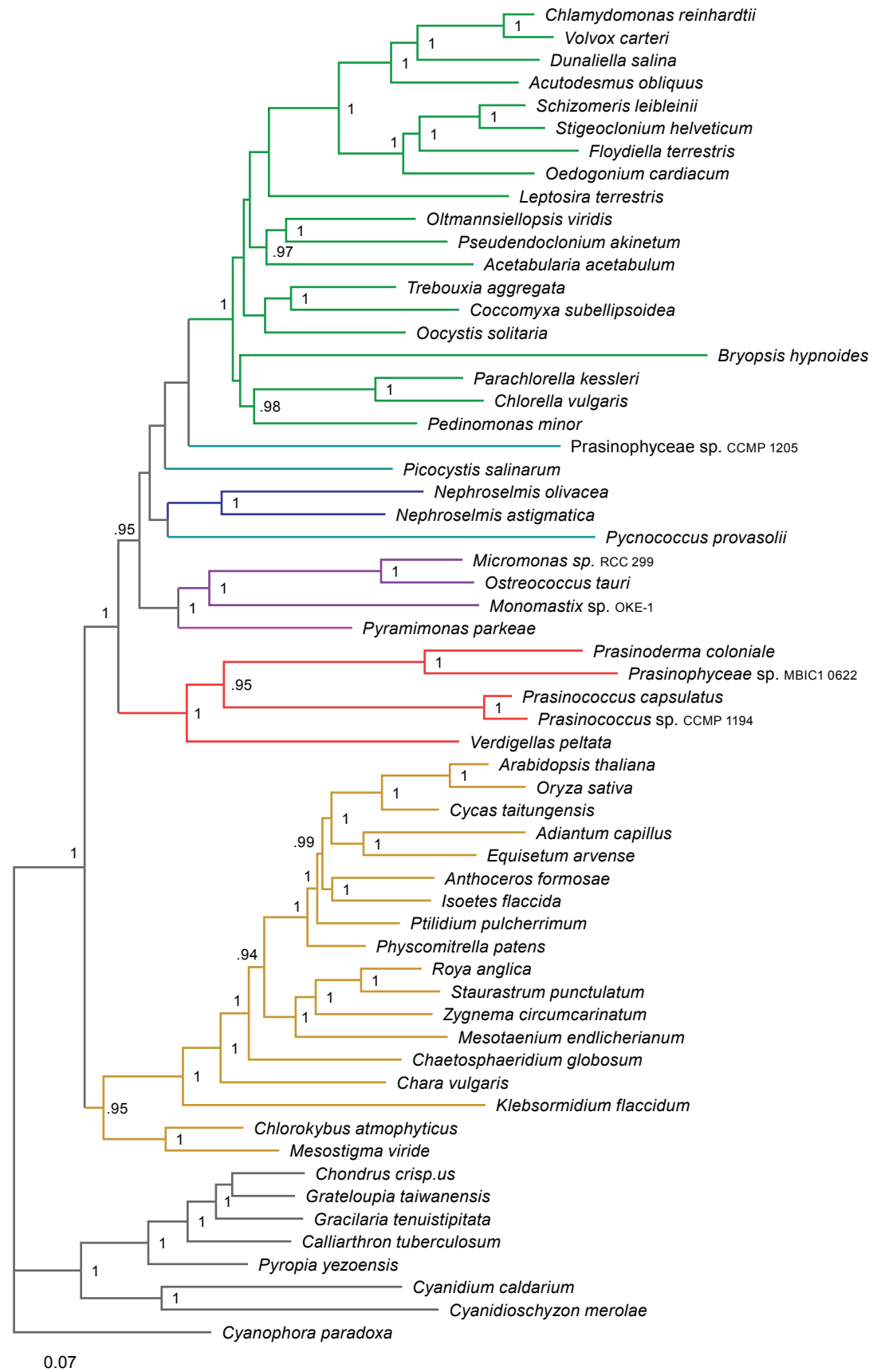


Figure S7. Plastid phylogeny of the green plants obtained by Bayesian inference (MrBayes) of a concatenated nucleotide alignment of 71 chloroplast genes (1st and 2nd codon position: 29,662 positions) under a GTR+ Γ 4+I model and a partitioning strategy in which codon positions were treated separately (2 partitions). The majority rule consensus tree is shown with node support given as posterior probabilities (values < .90 are not shown).

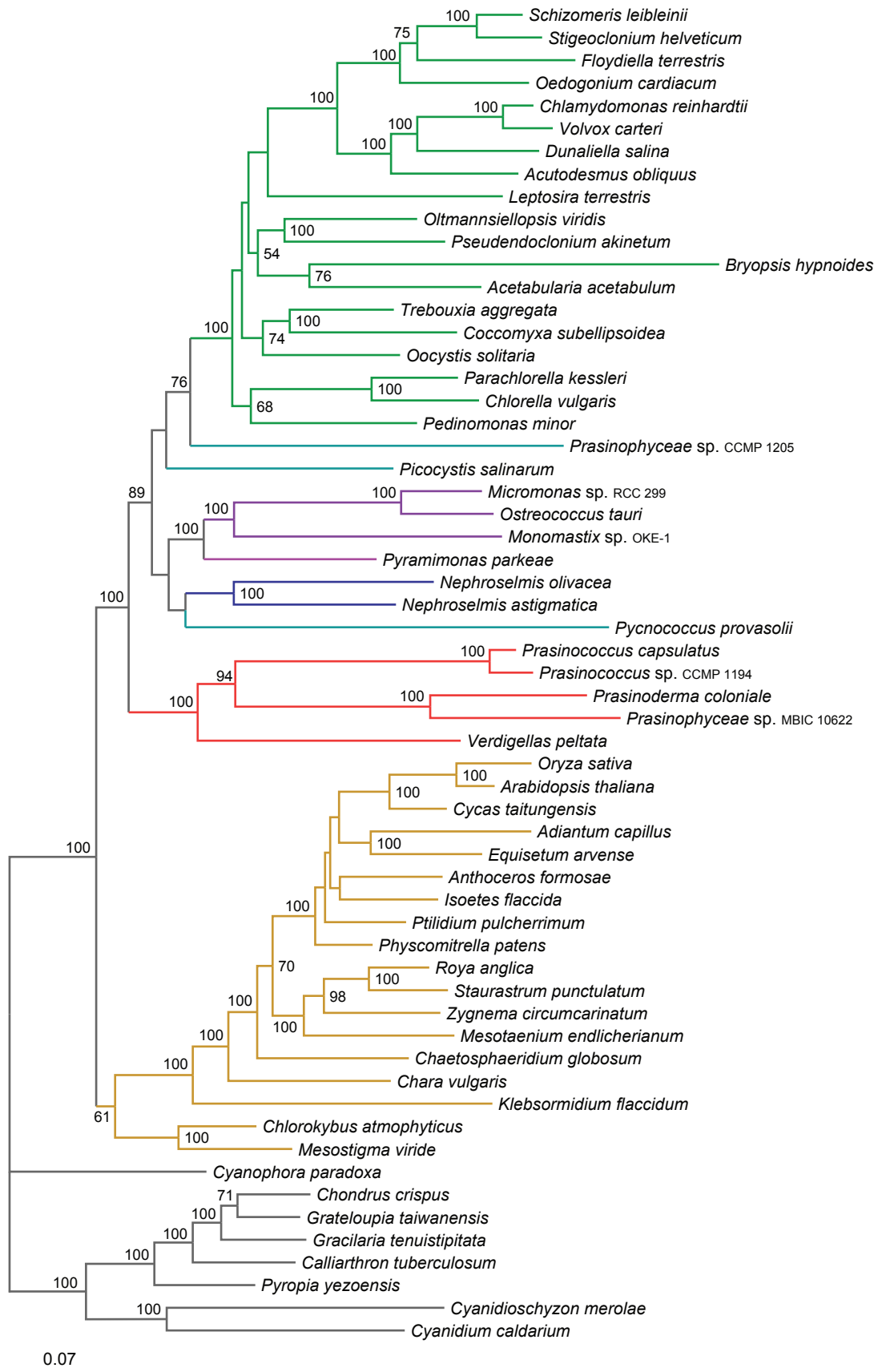


Figure S8. Plastid phylogeny of the green plants obtained by maximum likelihood inference (RAxML) of a concatenated nucleotide alignment of 71 chloroplast genes (1st and 2nd codon position: 29,662 positions) under a GTR+Γ4+I model and a partitioning strategy in which codon positions were treated separately (2 partitions). The maximum likelihood tree is shown with bootstrap support for the nodes (values < 50 are not shown).

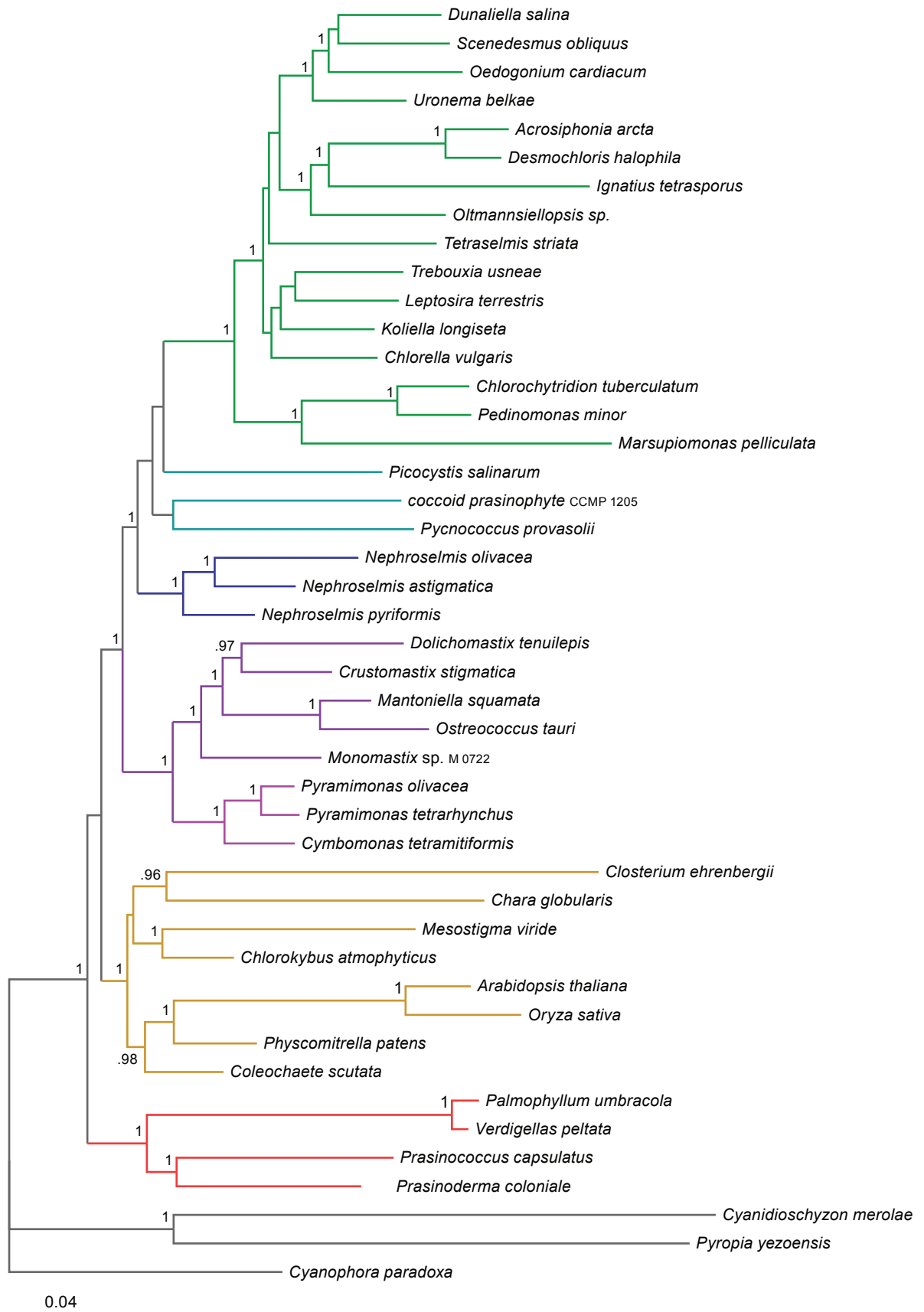


Figure S9. Nuclear rDNA phylogeny of the green plants obtained by Bayesian inference (MrBayes) of a concatenated alignment of 18S and 28S rRNA genes (4,579 positions) using unlinked GTR+ Γ 4+I models for the 18S and 28S partitions. The majority rule consensus tree is shown with node support given as posterior probabilities (values < .90 are not shown).

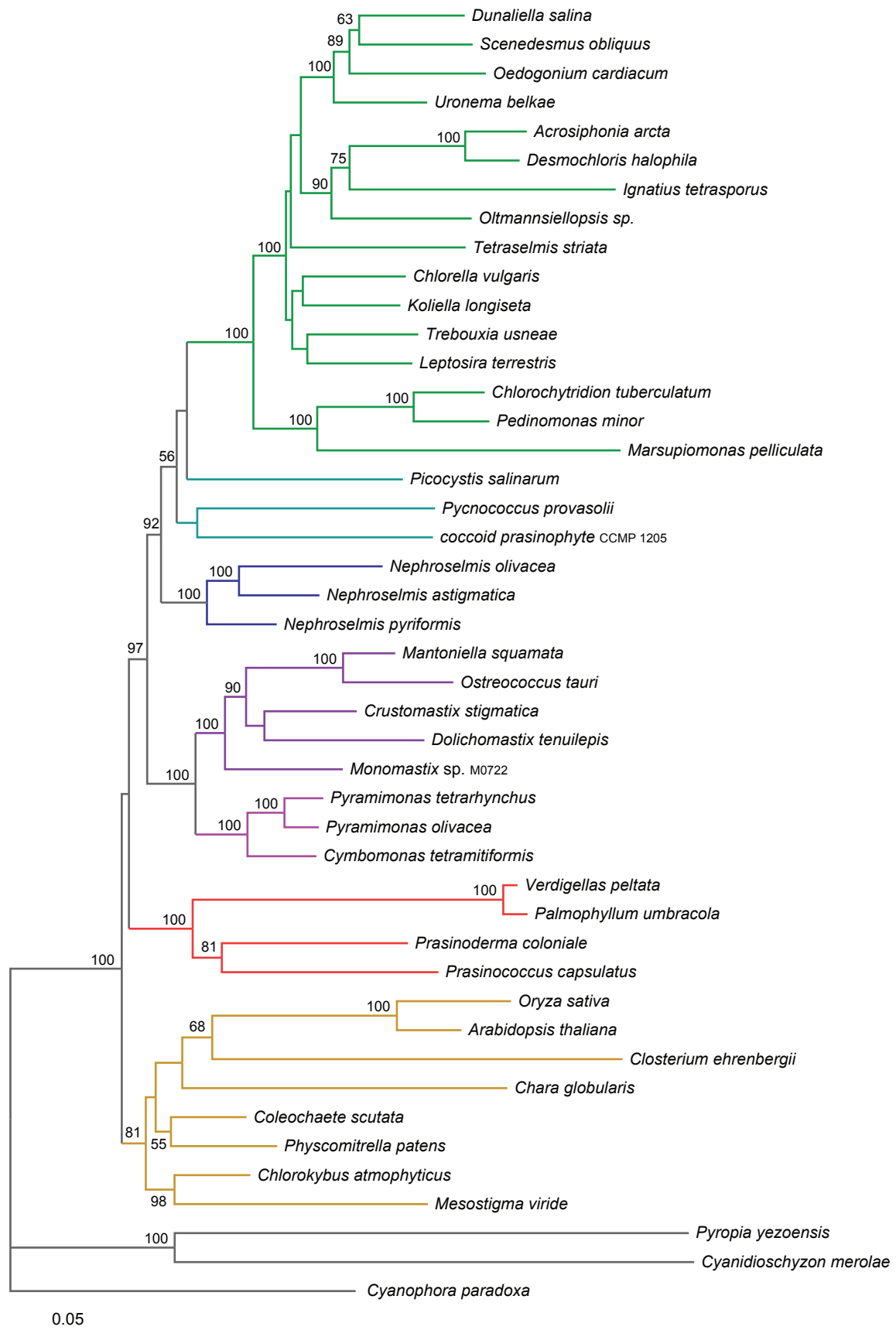


Figure S10. Nuclear rDNA phylogeny of the green plants obtained by maximum likelihood inference (RAxML) of a concatenated alignment of 18S and 28S rRNA genes (4,579 positions) using unlinked GTR+ Γ 4+I models for the 18S and 28S partitions. The maximum likelihood tree is shown with bootstrap support for the nodes (values < 50 are not shown).

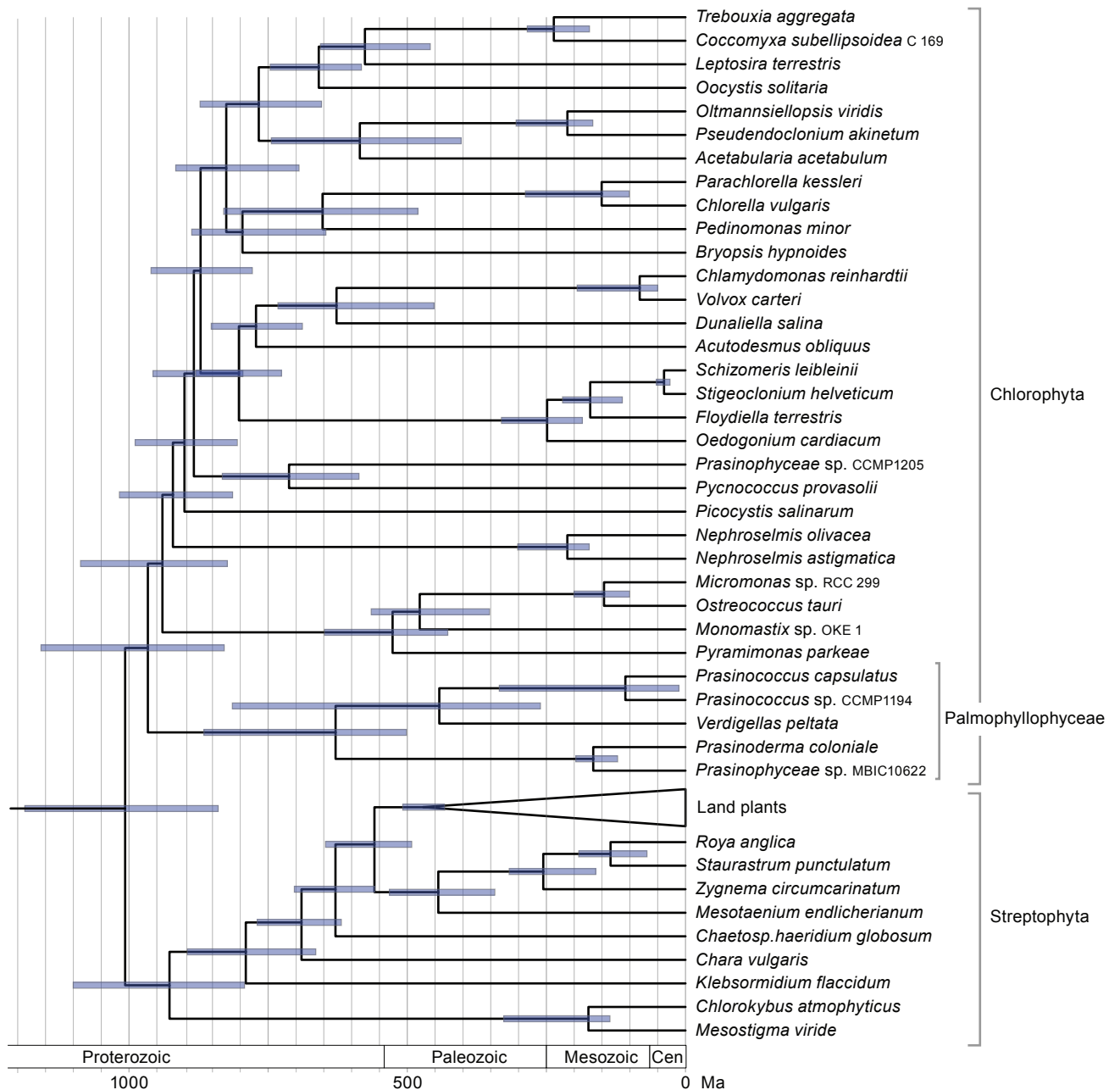


Figure S11. Tentative time-calibrated phylogeny of the green plants obtained by Bayesian inference with BEAST v1.8.2^{3,4} based on a concatenated protein alignment of 71 chloroplast genes (13,730 amino acid positions), using a cpREV+Γ4 model with divergence times estimated under an uncorrelated lognormal relaxed molecular clock model and the birth-death tree prior. The majority rule consensus tree obtained from the MrBayes analysis using a cpREV+Γ4+F model (Figs 4, S2) was used to fix the topology. Two nodes in the tree were constrained in time based on previous molecular clock analyses: The root of the Viridiplantae was constrained using a normal prior with mean 970 Mya, standard deviation 200, and truncated at 655 and 1280 Mya based on previous studies⁵⁻¹¹. The root of the land plants was constrained using a normal prior with mean 475 Mya and standard deviation 20¹². Markov Chain Monte Carlo (MCMC) analyses were run for 10 million generations, with subsampling every 1,000 generations, and summary statistics and trees were generated using the last 5 million generations. Outgroups have been pruned from the tree after the analysis.

Table S1. Comparison of phenotypic features between genera of the Palmophyllophyceae class. nov.

Genus	<i>Prasinococcus</i>	<i>Prasinoderma</i>	<i>Verdigellas</i>	<i>Palmophyllum</i>	<i>Palmoclathrus</i>
Currently accepted species ¹³	<i>P. capsulatu</i>	<i>P. coloniale</i> , <i>P. singularis</i>	<i>V. fimbriata</i> , <i>V. peltata</i> <i>V. nektongamnea</i>	<i>P. crassum</i> , <i>P. umbracola</i>	<i>P. stipitatus</i>
Thallus morphology	Unicellular; cells solitary.	Unicellular; cells solitary (<i>P. singularis</i>) or forming loose colonies (<i>P. coloniale</i>).	Thallus macroscopic, composed of isolated spherical cells in a gelatinous matrix. Thallus with one or several stipes and blades of various form.	Thallus macroscopic, composed of isolated spherical cells in a gelatinous matrix. Thallus irregular crustose lobes.	Thallus macroscopic, composed of isolated spherical cells in a gelatinous matrix. Thallus with a distinctive stipe and a cup-shaped, perforate blade.
Cell morphology	Coccoid, (sub)spherical, 3.5-5.5 (-8) µm long.	Coccoid, spherical, 2.2-5.5 µm in diameter.	Coccoid, (sub)spherical, 3.2-10 µm long.	Coccoid, subspherical, 6-7 µm long.	Coccoid, subspherical, 8-10 µm long.
Cell wall	Thin cell wall surrounded by a thick ellipsoidal gelatinous capsule (including the polysaccharide capsulan).	Thick, multi-layered cell wall, sometimes surrounded by a mucus-like secretion (<i>P. singularis</i>).	Thin cell wall with extended external gelatinous matrix forming the thallus. Cell walls thickened by trilaminar sheets to inner surface.	Thin cell wall with extended external gelatinous matrix forming the thallus.	Thin cell wall with extended external gelatinous matrix forming the thallus.
Subcellular characteristics	Cup-shaped chloroplast enclosing a single mitochondrion, a nucleus, and a large Golgi body. Chloroplast with a large pyrenoid surrounded by a starch sheath; pyrenoid matrix penetrated by a bifurcate extension of the cytoplasm and the mitochondrion. ^a Cell wall at the open end of the cup-shaped chloroplast perforated by a complex structure composed of a round collared lid, with 8 to 14 pores ("decapore structure"). ^b	Cup-shaped chloroplast enclosing a single mitochondrion, a nucleus, and a large Golgi body. Chloroplast with a large pyrenoid surrounded by a starch sheath; pyrenoid matrix penetrated by a bifurcate extension of the cytoplasm and the mitochondrion. ^a Cell wall lacking pores or projecting appendages.	Cup-shaped chloroplast enclosing a nucleus, a large Golgi body, a large vacuole, and an inconspicuous mitochondrion. Chloroplast with scattered starch grains; without pyrenoid. Vacuole with heterogeneous content, including amorphous, fibrillar, and trilaminar components. Cell wall at the open end of the cup-shaped chloroplast perforated by pores. Lumen of the nuclear envelope with spherical glycoprotein inclusions.	Cup-shaped chloroplast enclosing a nucleus, a large Golgi body (sometimes 2 or 3), a large vacuole ("dictyosome-associated membrane-bound body"), and a mitochondrion. Chloroplast with scattered starch grains; without pyrenoid. Cell wall at the open end of the cup-shaped chloroplast perforated by pores ("mucilage secretion organelles").	Cup-shaped chloroplast enclosing a nucleus, a large Golgi body, a large dictyosome-associated vacuole ("membrane bound body"), and a mitochondrion. Chloroplast with scattered starch grains; without pyrenoid.
Cell division	Unequal binary fission in which one of the daughter cells retains the parent wall, while the other is released with a newly produced cell wall.	Unequal binary fission in which one of the daughter cells retains the parent wall, while the other is released with a newly produced cell wall.	Not studied.	Not studied.	Unequal binary fission associated with the segregation of the prominent membrane-bound body into only one of the two daughter cells. Parental cell wall is discarded and incorporated into the gelatinous matrix.
Major pigments	Chlorophylls a and b, prasinoxanthin, MgDVP, uriolide, micromonol; chlorophyll b/a ratio ~ 0.6-1. ^c	Chlorophylls a and b, prasinoxanthin, MgDVP, uriolide, micromonol; chlorophyll b/a ratio ~ 0.6-0.9. ^c	Not studied.	Chlorophylls a and b, carotene and xanthophylls including neoxanthin as well as an unidentified orange pigment; chlorophyll b/a ratio ~ 1.6. ^d	Chlorophylls a and b, carotene, and xanthophylls including lutein, violaxanthin and neoxanthin; chlorophyll b/a ratio ~ 1. ^d
Habitat	Coastal to oceanic waters from surface to 200 m deep.	Coastal to oceanic waters, from surface to - 40 m deep	Benthic, locally abundant on deep-reef slopes, 40-150 m deep.	Benthic, locally abundant in shady habitats, 3-90 m deep.	Benthic, 7-60 m deep.
References	14-19	18-21	22-24	25,26	27-29

^a This feature has also been described in the Pycnococcaceae ³⁰.^b A decapore structure has also been described in *Pycnococcus provasolii*, although not as well developed ³⁰^c Studied using HPLC chromatography: see Latasa et al. ¹⁸ and Jouenne et al. ²¹ for a complete list of pigments.^d Studied using thin layer chromatography

Table S2. Taxon sampling for the plastid phylogeny.

Species	GenBank/EMBL Accession number(s)	Reference	Clade
<i>Acetabularia acetabulum</i>	HG518425-HG518474, HG794360	31	Chlorophyta
<i>Acutodesmus obliquus</i>	NC_008101	32	Chlorophyta
<i>Bryopsis hypnoides</i>	NC_013359	33	Chlorophyta
<i>Chlamydomonas reinhardtii</i>	NC_005353	34	Chlorophyta
<i>Chlorella vulgaris</i>	NC_001865	35	Chlorophyta
<i>Coccomyxa</i> sp.	NC_015084	36	Chlorophyta
<i>Dunaliella salina</i>	NC_016732	37	Chlorophyta
<i>Floydiella terrestris</i>	NC_014346	38	Chlorophyta
<i>Leptosira terrestris</i>	NC_009681	39	Chlorophyta
<i>Micromonas</i> sp. RCC 299	NC_012643	40	Chlorophyta
<i>Monomastix</i> sp. OKE-1	NC_012101	41	Chlorophyta
<i>Nephroselmis astigmatica</i>	KJ746600	42	Chlorophyta
<i>Nephroselmis olivacea</i>	NC_000927	43	Chlorophyta
<i>Oedogonium cardiacum</i>	NC_011031	44	Chlorophyta
<i>Oltmannsiellopsis viridis</i>	NC_008099	45	Chlorophyta
<i>Oocystis solitaria</i>	FJ968739	46	Chlorophyta
<i>Ostreococcus tauri</i>	NC_008289	47	Chlorophyta
<i>Parachlorella kessleri</i>	NC_012978	46	Chlorophyta
<i>Pedinomonas minor</i>	NC_016733	46	Chlorophyta
<i>Picocystis salinarum</i>	KJ746599	42	Chlorophyta
<i>Prasinococcus capsulatus</i>	AB491660, AB561011, AB561019, AB561027, AB561035, AB561044, AB561052, AB561060, AB561068, AB561076, AB561084	48	Chlorophyta
<i>Prasinococcus</i> sp. CCMP 1194	KJ746597	42	Chlorophyta
<i>Prasinoderma coloniale</i>	KJ746598	42	Chlorophyta
<i>Prasinophyceae</i> sp. CCMP 1205	KJ746601	42	Chlorophyta
<i>Prasinophyceae</i> sp. MBIC 10622	KJ746602	42	Chlorophyta
<i>Pseudendoclonium akinetum</i>	NC_008114	49	Chlorophyta
<i>Pycnococcus provasolii</i>	NC_012097	41	Chlorophyta
<i>Pyramimonas parkeae</i>	NC_012099	41	Chlorophyta
<i>Schizomeris leibleinii</i>	NC_015645	50	Chlorophyta
<i>Stigeoclonium helveticum</i>	NC_008372	51	Chlorophyta
<i>Trebouxia aggregata</i>	EU123962-EU124002	52	Chlorophyta
<i>Verdigellas peltata</i>	LT174527	This study	Chlorophyta
<i>Volvox carteri</i>	EU755264-EU755299	53	Chlorophyta
<i>Adiantum capillus-veneris</i>	NC_004766	54	Streptophyta
<i>Anthoceros formosae</i>	NC_004543	55	Streptophyta
<i>Arabidopsis thaliana</i>	NC_000932	56	Streptophyta
<i>Chaetosphaeridium globosum</i>	NC_004115	57	Streptophyta
<i>Chara vulgaris</i>	NC_008097	58	Streptophyta
<i>Chlorokybus atmophyticus</i>	NC_008822	59	Streptophyta

<i>Cycas taitungensis</i>	NC_009618	60	Streptophyta
<i>Equisetum arvense</i>	NC_014699	61	Streptophyta
<i>Isoetes flaccida</i>	NC_014675	61	Streptophyta
<i>Klebsormidium flaccidum</i>	KJ461680	62	Streptophyta
<i>Mesostigma viride</i>	NC_002186	63	Streptophyta
<i>Mesotaenium endlicherianum</i>	KJ461682	62	Streptophyta
<i>Oryza sativa</i>	NC_008155	64	Streptophyta
<i>Physcomitrella patens</i>	NC_005087	65	Streptophyta
<i>Ptilidium pulcherrimum</i>	NC_015402	66	Streptophyta
<i>Roya anglica</i>	KJ461681	62	Streptophyta
<i>Staurostrum punctulatum</i>	NC_008116	67	Streptophyta
<i>Zygnema circumcarinatum</i>	NC_008117	67	Streptophyta
<i>Calliarthron tuberculosum</i>	NC_021075	68	Rhodophyta
<i>Chondrus crispus</i>	NC_020795	69	Rhodophyta
<i>Cyanidioschyzon merolae</i>	NC_004799	70	Rhodophyta
<i>Cyanidium caldarium</i>	NC_001840	71	Rhodophyta
<i>Gracilaria tenuistipitata</i>	NC_006137	72	Rhodophyta
<i>Grateloupia taiwanensis</i>	NC_021618	73	Rhodophyta
<i>Pyropia yezoensis</i>	KC517072	74	Rhodophyta
<i>Cyanophora paradoxa</i>	NC_001675	75	Glaucophyta

Table S3. Taxon sampling for the nuclear rDNA phylogeny.

Species	18S rDNA accession number	28S rDNA accession number	Clade
<i>Acrosiphonia arcta</i>	AY303600	HQ603261	Chlorophyta
<i>Chlorella vulgaris</i>	AB162910	AB237642	Chlorophyta
<i>Chlorochytridium tuberculatum</i>	HE610134	HE610134	Chlorophyta
<i>Crustomastix stigmatica</i>	AJ629844	HE610148	Chlorophyta
<i>Cymbomonas tetramitiformis</i>	FN562438	HE610151	Chlorophyta
<i>Desmochloris halophila</i>	AB049416	HE610118	Chlorophyta
<i>Dolichomastix tenuilepis</i>	FN562449	HE610149	Chlorophyta
<i>Dunaliella salina</i>	DQ447646	DQ015739	Chlorophyta
<i>Ignatius tetrasporus</i>	FN562432	HE610121	Chlorophyta
<i>Koliella longiseta</i>	HE610126	HE610126	Chlorophyta
<i>Leptosira terrestris</i>	Z28973	Z95378	Chlorophyta
<i>Mantoniella squamata</i>	X73999	HE610150	Chlorophyta
<i>Marsupiomonas pelliculata</i>	HE610136	HE610137	Chlorophyta
<i>Monomastix</i> sp. M0722	FN562447	HE610147	Chlorophyta
<i>Nephroselmis astigmatica</i>	FN562433	HE610145	Chlorophyta
<i>Nephroselmis olivacea</i>	FN562436	HE610146	Chlorophyta
<i>Nephroselmis pyriformis</i>	X75565	HE610144	Chlorophyta
<i>Oedogonium cardiacum</i>	U83133	AF183478	Chlorophyta

<i>Oltmannsiellopsis</i> sp.	HE610120	HE610120	Chlorophyta
<i>Ostreococcus tauri</i>	CAID01000012	CAID01000012	Chlorophyta
<i>Palmophyllum umbracola</i>	FJ619275	-	Chlorophyta
<i>Pedinomonas minor</i>	HE610132	HE610132	Chlorophyta
<i>Picocystis salinarum</i>	AF125167	HE610138	Chlorophyta
<i>Prasinococcus capsulatus</i>	AF203401	HE610141	Chlorophyta
<i>Prasinoderma coloniale</i>	FN562437	HE610143	Chlorophyta
<i>prasinophyte CCMP1205</i>	U40921	HE610139	Chlorophyta
<i>Pycnococcus provasolii</i>	X91264	HE610140	Chlorophyta
<i>Pyramimonas olivacea</i>	FN562442	HE610153	Chlorophyta
<i>Pyramimonas tetrarhynchus</i>	FN562441	HE610152	Chlorophyta
<i>Scenedesmus obliquus</i>	AJ249513	AF183482	Chlorophyta
<i>Tetraselmis striata</i>	X70802	HE610129	Chlorophyta
<i>Trebouxia usneae</i>	Z68702	Z95385	Chlorophyta
<i>Uronema belkiae</i>	AF182821	AF183489	Chlorophyta
<i>Verdigellas peltata</i>	LT174528	This study	Chlorophyta
<i>Arabidopsis thaliana</i>	CP002686	AC016828	Streptophyta
<i>Chara globularis</i>	Y16465	AJ271115	Streptophyta
<i>Chlorokybus atmophyticus</i>	M95612	AB491667	Streptophyta
<i>Closterium ehrenbergii</i>	AF115437	AF419992	Streptophyta
<i>Coleochaete scutata</i>	X68825	AB491669	Streptophyta
<i>Mesostigma viride</i>	AF408245	DQ980472	Streptophyta
<i>Oryza sativa</i>	CP012609	AA02002154	Streptophyta
<i>Physcomitrella patens</i>	AF223015	AC158196	Streptophyta
<i>Cyanidioschyzon merolae</i>	AB158483	AB158483	Rhodophyta
<i>Pyropia yezoensis</i>	KJ578747	KJ578748	Rhodophyta
<i>Cyanophora paradoxa</i>	AY823716	AY216932	Glaucophyta

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