

**Divergent copies of the large inverted repeat in the chloroplast  
genomes of ulvophycean green algae**

**(Supplementary Information)**

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Taxon	Technology	Sequencing reads (no.)		Fold coverage of chloroplast genome sequences						
		Total	cpDNA	Average	IR-SC1	IR-SC2	IR-SC3	IR-SC4	IR-SC5	IR-SC6
<i>Ignatius tetrasporus</i>	Roche 454	49,055	41,372	60	43	32	44	32	-	-
<i>Pseudocharacium americanum</i>	Roche 454	40,038	37,504	58	45	58	43	32	-	-
<i>Dangermannia microcystis</i>	Roche 454	80,710	48,067	108	117	75	36	48	-	-
<i>Pseudoneochloris marina</i>	Roche 454	82,351	59,134	156	161	149	152	113	-	-
<i>Chamaetrichon capsulatum</i>	Illumina	1,997,570	96,903	154	125	105	119	93	173	123
<i>Trichosarcina mucosa</i>	Illumina	4,067,674	275,477	361	320	218	167	323	-	-

**Supplementary Table S1. Summary statistics of the chloroplast genome sequencing and assemblies.** The sequence coverage values observed at the junctions between IR and single-copy regions (IR-SC1 to IR-SC6) are provided in the order these junctions appear in the GenBank accessions of the chloroplast genomes.

Taxon	ORF <sup>a</sup>	Coordinates <sup>b</sup>	Conserved domain or similar protein
<i>Ignatius tetrasporus</i>	437	22433-21120	HNH endonuclease (pfam01844) with RRXRR domain (pfam14239)
	112	46900-46562	HNH endonuclease with RRXRR domain (pfam14239)
	100	158515-158213	HNH endonuclease
	128	158980-158594	HNH endonuclease (pfam01844)
	106	159328-159008	HNH endonuclease with RRXRR domain (pfam14239)
	98	220328-220624	Transposase
<i>Dangermannia microcystis</i>	246	123637-122897	Group II intron maturase (pfam08388) and HNH (pfam01844) domains
	114	124647-124303	N-terminal domain of reverse transcriptase (pfam13655)
<i>Chamaetrichon capsulatum</i>	680	84264-86306	Hypothetical protein MVEG-06986 of <i>Mortierella verticillata</i>
	206	121843-121223	Hypothetical protein PsakCp041 of <i>Pseudendozonium akinetum</i> (orf239)
<i>Trichosarcina mucosa</i>	190	67813-68064	DNA breaking-rejoining enzyme, phage integrase family (pfam00589)
	121	86662-87027	Hypothetical protein PsakCp022 of <i>Pseudendozonium akinetum</i> (orf156)
	233	154383-155084	DNA breaking-rejoining enzyme, phage integrase family (pfam00589)
	178	155855-155319	Hypothetical chloroplast-encoded protein of <i>Prasiola crispa</i> and <i>Mankyua chejuensis</i>

**Supplementary Table S2. Non-canonical genes identified as freestanding ORFs in the ulvophycean chloroplast genomes examined in this study.** <sup>a</sup>Reported here are the freestanding ORFs larger than 100 codons that revealed similarity (*E*-value threshold of 1e-06) with proteins of known functions and/or recognized protein domains in BlastP searches. Each ORF is identified by the number of amino acid residues in the encoded protein. <sup>b</sup>Genomic coordinates of the ORFs in the GenBank accessions.

Number	Sequence	Strand	Genomic position (3' end)
<i>Ignatius tetrasporus</i>			
1	GATGTATCAACGTCAAATCACG	+	183754
2	GATCTAGGGCTTGCTTTGCATTC	-	186657
3	TTAAAGCGATTGAGCGAAGCAG	+	188149
4	TAGAGGTATCCGATTTGCTCAG	-	192460
5	GTGACTTTAACCATGCGATGAG	-	451
6	AAGGAGTGTTGCGACTACAAAC	+	237146
7	TGCAAATAGTACTCAAAGCGTC	-	235837
8	TAAAGCTGATGGAAGACGTTTC	+	231801
<i>Pseudoneochloris marina</i>			
1	CAACCTACACAATCTTCCGTAC	-	103470
2	CTCGAAAGCTTGAACGTTGAAC	+	95139
3	GTAGCACCAGCTATTCAACCTC	+	128692
4	GAGTCGCACGAAAACAAGAGTC	-	419
5	AACAGGTTACTGCCTAAGGACG	+	129287
5	AACAGGTTACTGCCTAAGGACG	-	102782
6	GATGCTTTCAGCGATTATCCAC	+	95917
6	GATGCTTTCAGCGATTATCCAC	-	134412

**Supplementary Table S3. Sequences of the PCR primers used to investigate flip-flop recombination in the *Ignatius* and *Pseudoneochloris* chloroplast genomes.**

Taxon	Collection	GenBank accession(s)
<b>Streptophytes</b>		
<i>Mesostigma viride</i>	NIES 296	NC 002186
<i>Chlorokybus atmophyticus</i>	SAG 48.80	NC 008822
<b>Prasinophytes</b>		
<i>Prasinococcus</i> sp. CCMP 1194	CCMP 1194	KJ746597
<i>Prasinophyceae</i> sp. MBIC 10622	NBRC 102842	KJ746602
<i>Prasinoderma coloniale</i>	CCMP 1220	NC 024817
<i>Pyramimonas parkeae</i>	CCMP 726	FJ493499
<i>Monomastix</i> sp. OKE-1	PC O'Kelly	NC 012101
<i>Ostreococcus tauri</i>	OTTH0595	NC 008289
<i>Micromonas commoda</i>	RCC 299	NC 012575
<i>Pycnococcus provasolii</i>	CCMP 1203	NC 012097
<i>Nephroselmis olivacea</i>	NIES 484	NC 000927
<i>Nephroselmis astigmatica</i>	NIES 252	NC 024829
<i>Picocystis salinarum</i>	CCMP 1897	NC 024828
<i>Prasinophyceae</i> sp. CCMP 1205	CCMP 1205	KJ746601
<b>Chlorodendrophyceae</b>		
<i>Scherffellia dubia</i>	SAG 17.86	NC 029807
<i>Tetraselmis</i> sp. CCMP 881	CCMP 881	KU167097
<b>Pedinophyceae</b>		
<i>Marsupiomonas</i> sp. NIES 1824	NIES 1824	KM462870
<i>Pedinomonas tuberculata</i>	SAG 42.84	NC 025530
<i>Pedinomonas minor</i>	UTEX LB 1350	NC 016733
<b>Chlorellales</b>		
<i>Parachlorella kessleri</i>	SAG 211-11g	NC 012978
<i>Dicloster acuatus</i>	SAG 41.98	NC 025546
<i>Marvania geminata</i>	SAG 12.88	NC 025549
<i>Pseudochloris wilhelmii</i>	SAG 1.80	NC 025547
<i>Chlorella vulgaris</i>		NC 001865
<i>Chlorella variabilis</i>	NC 64A	NC 015359
<b>Core Trebouxiophyceae</b>		
<i>Oocystis solitaria</i>	SAG 83.80	FJ968739
<i>Planctonema lauterbornii</i>	SAG 68.94	NC 025541
<i>Koliella corcontica</i>	SAG 24.84	NC 025536
<i>Geminella terricola</i>	SAG 20.91	NC 025542
<i>Geminella minor</i>	SAG 22.88	NC 025544
<i>Gloeotilopsis sterilis</i>	UTEX 1704	NC 025538
<i>Pleurostrosarcina brevispinosa</i>	UTEX 1176	KM462875
<i>Parietochloris pseudoalveolaris</i>	UTEX 975	NC 025532
<i>Neocystis brevis</i>	CAUP D802	NC 025535
<i>Stichococcus bacillaris</i>	UTEX 176	NC 025527
<i>Prasiolopsis</i> sp. SAG 84.81	SAG 84.81	KM462862
<i>Chlorella mirabilis</i>	SAG 38.88	NC 025528
<i>Koliella longiseta</i>	UTEX 339	NC 025531
<i>Pabia signiensis</i>	SAG 7.90	NC 025529
<i>Leptospira terrestris</i>	UTEX 333	NC 009681
<i>Xylochloris irregularis</i>	CAUP H7801	NC 025534
<i>Trebouxia aggregata</i>	SAG 219-1D	EU123962-EU124002
<i>Myrmecia israelensis</i>	UTEX 1181	NC 025525
<i>Characium perforatum</i>	SAG 28.85	NC 025543
<i>Microthamnion kuetzingianum</i>	UTEX 318	NC 025537
<i>Lobosphaera incisa</i>	SAG 2007	NC 025533
<i>Watanabea reniformis</i>	SAG 211-9b	NC 025526
<i>Dictyochloropsis reticulata</i>	SAG 2150	NC 025524
<i>Choricystis minor</i>	SAG 17.98	NC 025539
<i>Botryococcus braunii</i>	SAG 807-1	NC 025545
<i>Elliptochloris bilobata</i>	CAUP H7103	NC 025548
<i>Trebouxiophyceae</i> sp. MX-AZ01	MX-AZ01	NC 018569

<i>Coccomyxa subellipsoidea</i>	NIES 2166	NC 015084
<i>Paradoxia multiseta</i>	SAG 18.84	NC 025540
<b>Bryopsidales</b>		
<i>Tydemania expeditionis</i>	FL1151	NC 026796
<i>Bryopsis plumosa</i>	West4718	NC 026795
<i>Bryopsis hypnoides</i>		NC 013359
<b>Ignatiales</b>		
<i>Pseudocharacium americanum</i>	UTEX 2112	KY407658
<i>Ignatius tetrasporus</i>	UTEX 2012	KY407659
<b>Oltmannsiellopsidales</b>		
<i>Dangemannia microcystis</i>	SAG 2022	KY407660
<i>Oltmannsiellopsis viridis</i>	NIES 360	NC 008099
<b>Ulvales</b>		
<i>Pseudoneochloris marina</i>	UTEX 1445	KY407657
<i>Ulva fasciata</i>		NC 029040
<i>Ulva</i> sp. UNA	UNA00071828	KP720616
<b>Ulotrichales</b>		
<i>Chamaetrichon capsulatum</i>	UTEX 1918	KY407661
<i>Pseudoclonium akinetum</i>	UTEX 1912	NC 008114
<i>Trichosarcina mucosa</i>	SAG 4.90	KY407656
<i>Gloeotilopsis planctonica</i>	SAG 29.93	KX306824
<i>Gloeotilopsis sarcinoidea</i>	UTEX 1710	KX306821
<b>Chlorophyceae</b>		
<i>Floydiella terrestris</i>	UTEX 1709	NC 014346
<i>Oedogonium cardiacum</i>	SAG 575-1b	NC 011031
<i>Stigeoclonium helveticum</i>	UTEX 441	NC 008372
<i>Schizomeris leibleinii</i>	UTEX LB 1228	NC 015645
<i>Treubaria triappendiculata</i>	SAG 38.83	NC 028578
<i>Golenkinia longispicula</i>	SAG 73.80	KT625092 - KT625150
<i>Jenufa minuta</i>	CAUP H8102	NC 028582
<i>Jenufa perforata</i>	CAUP H 8101	NC 028581
<i>Bracteacoccus giganteus</i>	UTEX 1251	NC 028586
<i>Mychonastes jurisii</i>	SAG 37.98	NC 028579
<i>Acutodesmus obliquus</i>	UTEX 393	NC 008101
<i>Hafniomonas laevis</i>	NIES 257	NC 028583
<i>Carteria cerasiformis</i>	NIES 425	NC 028585
<i>Carteria crucifera</i>	UTEX 432	KT624870 - KT624932
<i>Carteria</i> sp. SAG 8-5	SAG 8-5	KT625419
<i>Chloromonas typhlos</i>	UTEX LB 1969	KT624630 - KT624716
<i>Chloromonas radiata</i>	UTEX 966	KT625008 - KT625084
<i>Oogamochlamys gigantea</i>	SAG 44.91	NC 028580
<i>Lobochlamys segnis</i>	SAG 9.83	KT624806 - KT624869
<i>Lobochlamys culleus</i>	SAG 19.72	KT625151 - KT625204
<i>Chlamydomonas asymmetrica</i>	SAG 70.72	KT624933 - KT625007
<i>Volvox carteri</i> f. nagariensis	UTEX 2908	GU084820
<i>Chlamydomonas reinhardtii</i>		NC 005353
<i>Phacotus lenticularis</i>	SAG 61-1	NC 028587
<i>Microglena monadina</i>	SAG 31.72	KT624717 - KT624805
<i>Chlamydomonas moewusii</i>	UTEX 97	EF587443-EF587503
<i>Characiochloris acuminata</i>	SAG 31.95	NC 028584
<i>Dunaliella salina</i>	CCAP 19/18	NC 016732
<i>Chlamydomonas applanata</i>	SAG 11-9	KT625417
<i>Stephanosphaera pluvialis</i>	SAG 78-1a	KT625299 - KT625409
<i>Chloromonas perforata</i>	SAG 11-43	KT625416
<i>Haematococcus lacustris</i>	SAG 34-1b	KT625205 - KT625298
<i>Chlorogonium capillatum</i>	UTEX 11	KT625085 - KT625091

**Supplementary Table S4. GenBank accession codes of the chloroplast genome sequences used to infer phylogenomic trees.**

**Supplementary Figure S1. Gene map of the *Ignatius tetrasporus* chloroplast**

**genome.** Filled boxes represent genes, with colors denoting gene categories as indicated in the legend. Genes on the outside are transcribed counterclockwise; those on the inside are transcribed clockwise.

**Supplementary Figure S2. Gene map of the *Pseudocharacium americanum***

**chloroplast genome.** Filled boxes represent genes, with colors denoting gene categories as indicated in the legend. Genes on the outside are transcribed counterclockwise; those on the inside are transcribed clockwise.

**Supplementary Figure S3. Gene map of the *Dangemannia microcystis* chloroplast**

**genome.** Filled boxes represent genes, with colors denoting gene categories as indicated in the legend. Genes on the outside are transcribed counterclockwise; those on the inside are transcribed clockwise.

**Supplementary Figure S4. Gene map of the *Pseudoneochloris marina* chloroplast**

**genome.** Filled boxes represent genes, with colors denoting gene categories as indicated in the legend. Genes on the outside are transcribed counterclockwise; those on the inside are transcribed clockwise.

**Supplementary Figure S5. Gene map of the *Chamaetrichon capsulatum* chloroplast**

**genome.** Filled boxes represent genes, with colors denoting gene categories as indicated in the legend. Genes on the outside map are transcribed counterclockwise; those on the inside are transcribed clockwise.

**Supplementary Figure S6. Gene map of the *Trichosarcina mucosa* chloroplast**

**genome.** Filled boxes represent genes, with colors denoting gene categories as indicated in the legend. Genes on the outside are transcribed counterclockwise; those on the inside are transcribed clockwise.

**Supplementary Figure S7. Gene locations of the rapidly-evolving sites that were removed from the PCG-AA (1,303 sites) and PCG12RNA (4,492 sites) data sets by filtration with TIGER.**

**Supplementary Figure S8. Phylogenomic trees inferred from the PCG12RNA data set using RAxML.** Bootstrap support values are reported on the nodes. A black dot indicates that the corresponding branch received a value of 100%. The scale bar denotes the estimated number of substitutions per site.

**Supplementary Figure S9. Phylogenomic trees inferred from the PCG12RNA data set using PhyloBayes.** Posterior probability values are reported on the nodes. A black dot indicates that the corresponding branch received a value of 1.0. The scale bar denotes the estimated number of substitutions per site.

**Supplementary Figure S10. Phylogenomic trees inferred from the PCG-AA data set using RAxML.** Bootstrap support values are reported on the nodes. A black dot indicates that the corresponding branch received a value of 100%. The scale bar denotes the estimated number of substitutions per site.

**Supplementary Figure S11. Phylogenomic trees inferred from the PCG-AA data set using PhyloBayes.** Posterior probability values are reported on the nodes. A black dot

indicates that the corresponding branch received a value of 1.0. The scale bar denotes the estimated number of substitutions per site.

### **Supplementary Figure S12. Repertoires of canonical genes in the ulvophycean**

**chloroplast genomes compared in this study.** Only the genes missing in one or more genomes are indicated. The presence of genes and pseudogenes is denoted by blue and light blue boxes, respectively. Protein-coding genes that were lost only once are mapped on the cladogram. A total of 95 genes are shared by all compared genomes: *accD*, *atpA*, *B*, *E*, *F*, *H*, *I*, *ccsA*, *cemA*, *chlI*, *clpP*, *ftsH*, *infA*, *petA*, *B*, *D*, *G*, *L*, *psaA*, *B*, *C*, *I*, *J*, *M*, *psbA*, *B*, *C*, *D*, *E*, *F*, *H*, *I*, *J*, *K*, *L*, *M*, *N*, *T*, *Z*, *rbcL*, *rpl2*, *5*, *14*, *16*, *19*, *20*, *23*, *32*, *36*, *rpoA*, *C2*, *rps2*, *3*, *4*, *7*, *8*, *9*, *11*, *12*, *14*, *18*, *19*, *rrf*, *rrl*, *rrs*, *tufA*, *ycf1*, *3*, *4*, *12*, *trnA*(*ugc*), *C*(*gca*), *D*(*guc*), *E*(*uuc*), *F*(*gaa*), *G*(*gcc*), *G*(*ucc*), *H*(*gug*), *I*(*gau*), *K*(*uuu*), *L*(*uaa*), *L*(*uag*), *Me*(*cau*), *Mf*(*cau*), *N*(*guu*), *P*(*ugg*), *Q*(*uug*), *R*(*acg*), *R*(*ucu*), *S*(*gcu*), *S*(*uga*), *T*(*ugu*), *V*(*uac*), *W*(*cca*), *Y*(*gua*).

## **References**

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- 2 Turmel, M., Otis, C. & Lemieux, C. Mitochondrion-to-chloroplast DNA transfers and intragenomic proliferation of chloroplast group II introns in *Gloeotilopsis* green algae (Ulotrichales, Ulvophyceae). *Genome Biol Evol* **8**, 2789-2805, doi:10.1093/gbe/evw190 (2016).



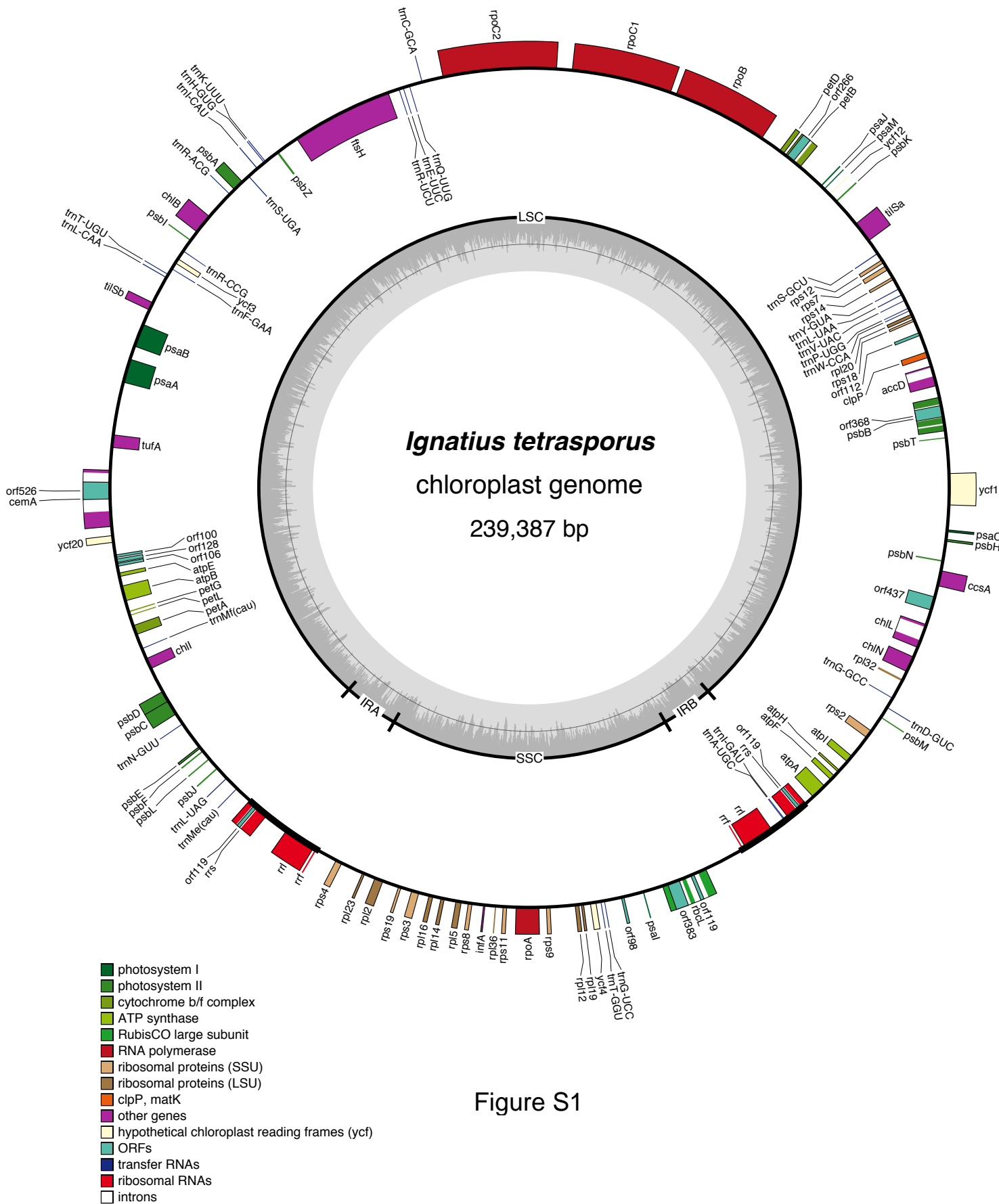
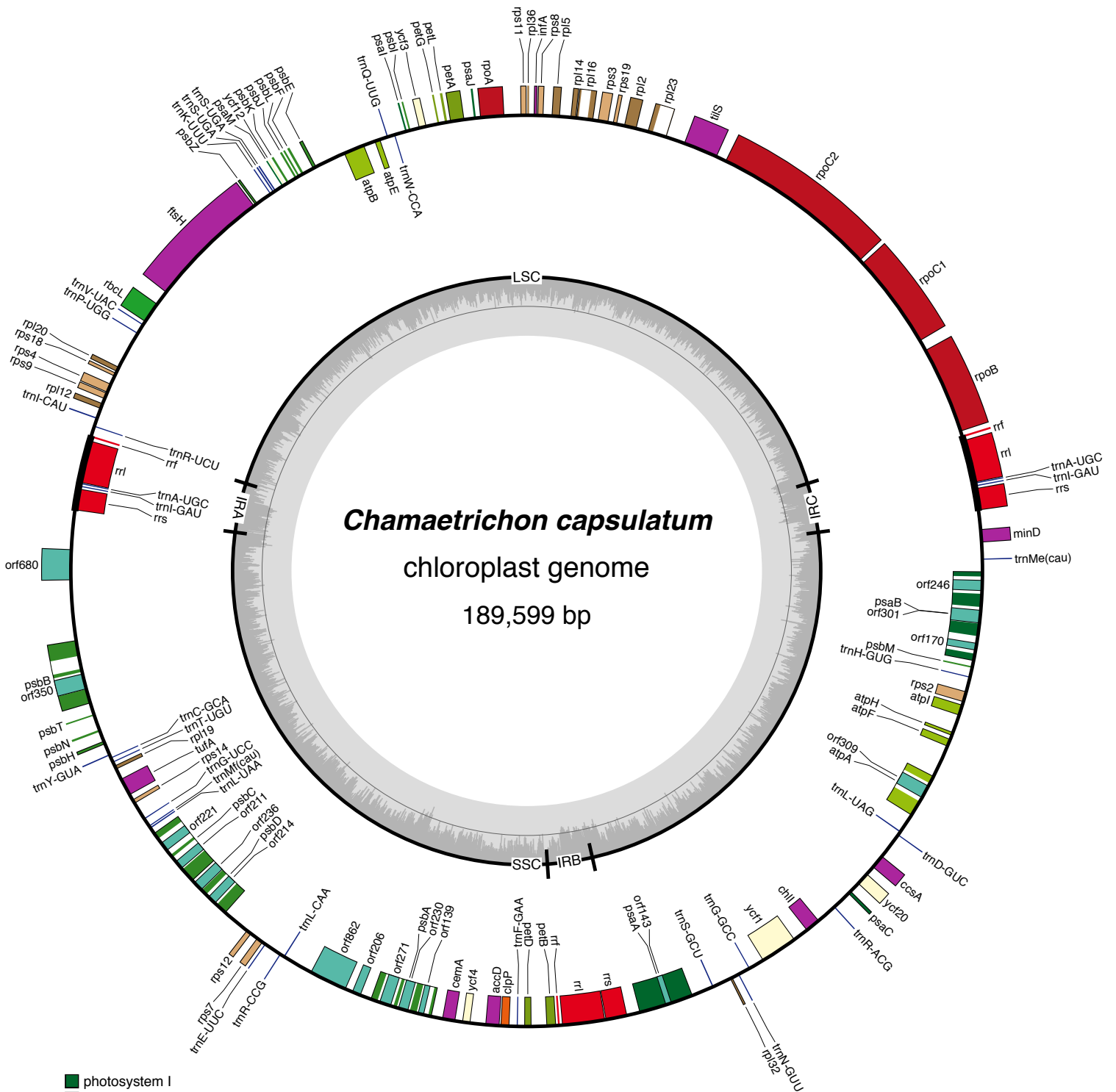


Figure S1









- photosystem I
- photosystem II
- cytochrome b/f complex
- ATP synthase
- RubisCO large subunit
- RNA polymerase
- ribosomal proteins (SSU)
- ribosomal proteins (LSU)
- clpP, matK
- other genes
- hypothetical chloroplast reading frames (ycf)
- ORFs
- transfer RNAs
- ribosomal RNAs
- introns

Figure S5



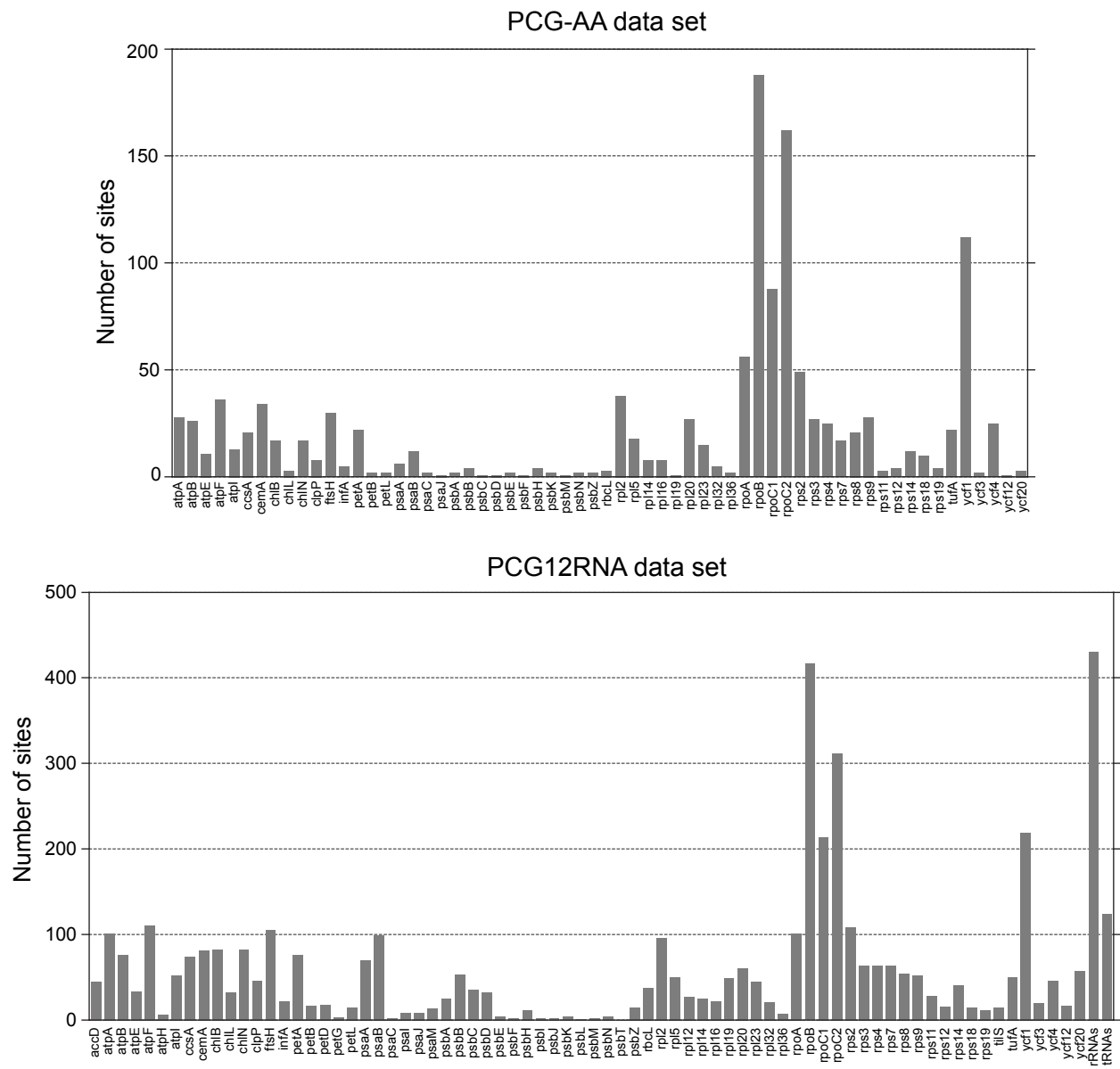


Figure S7

PCG12RNA  
RAXML GTR

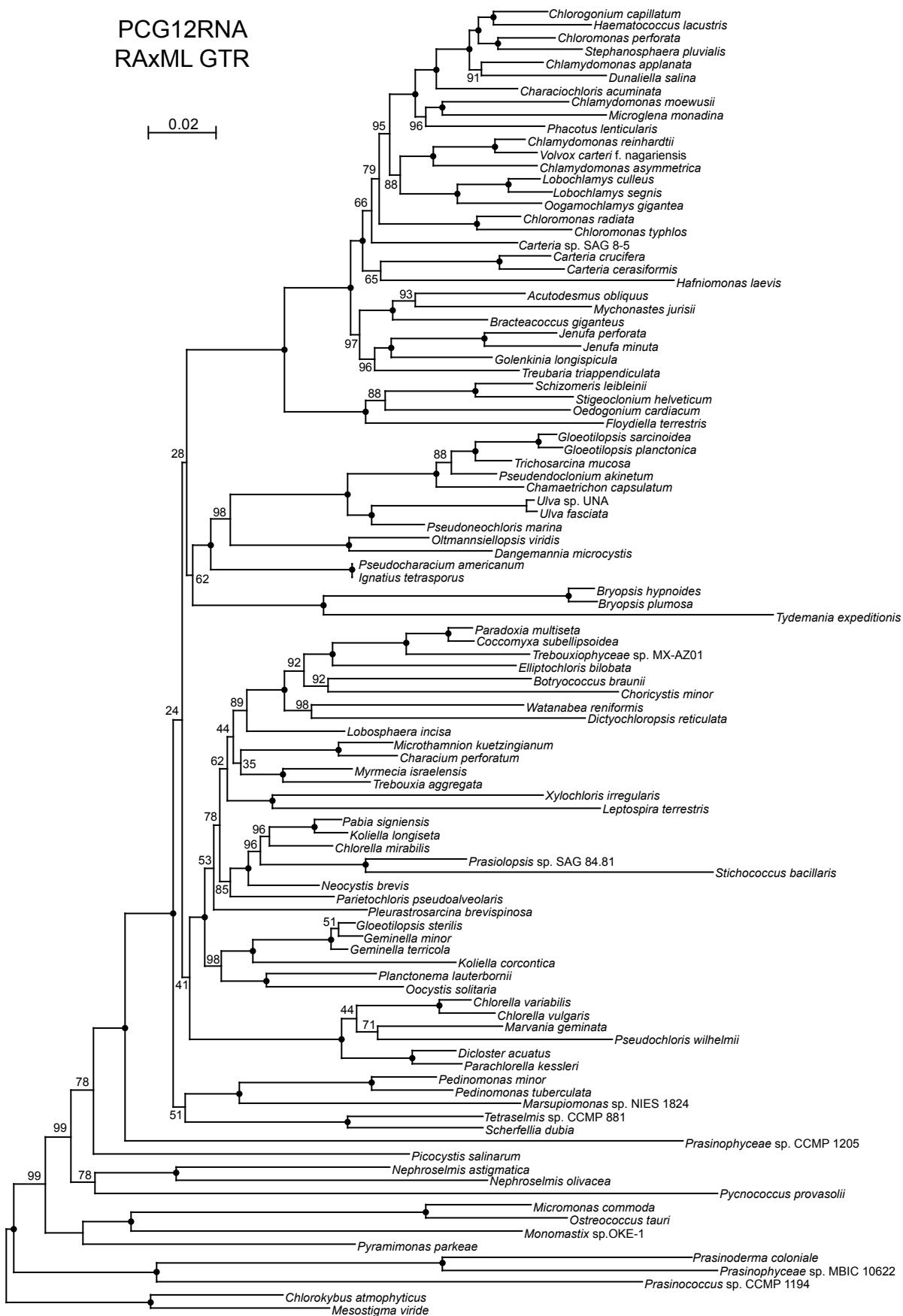


Figure S8





PCG-AA  
RAXML GTR

0.1

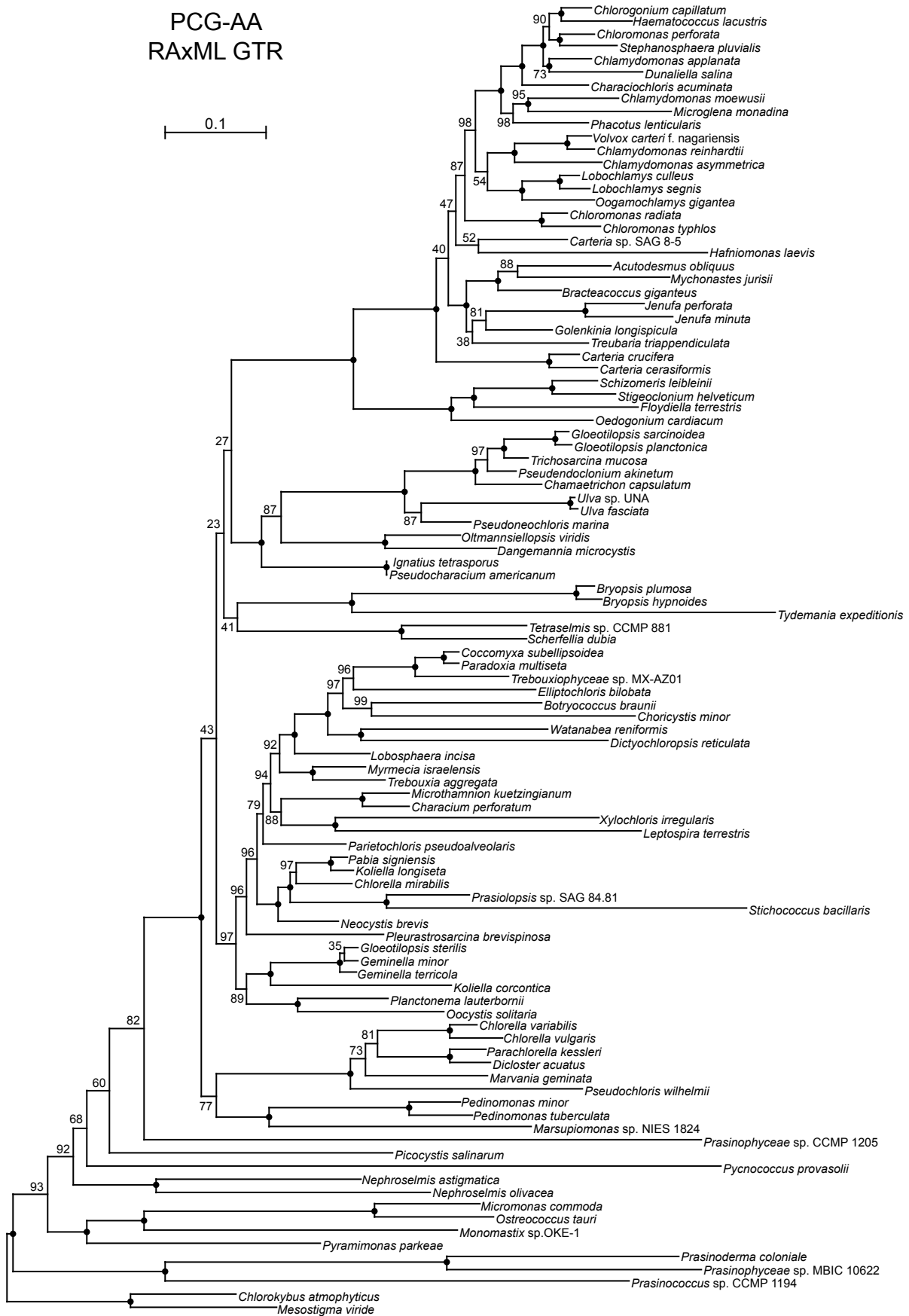


Figure S10

PCG-AA  
PhyloBayes CAT-GTR

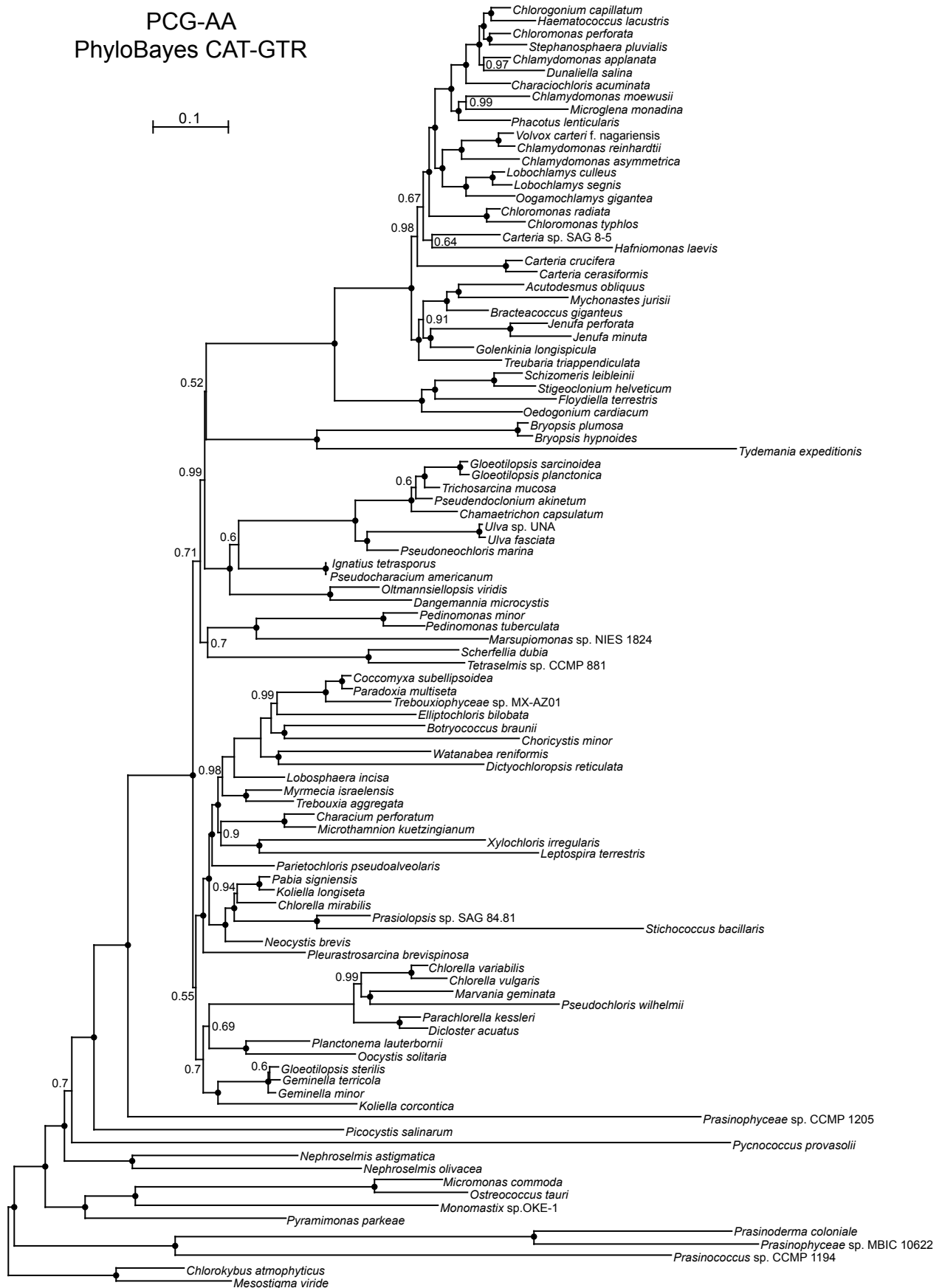


Figure S11

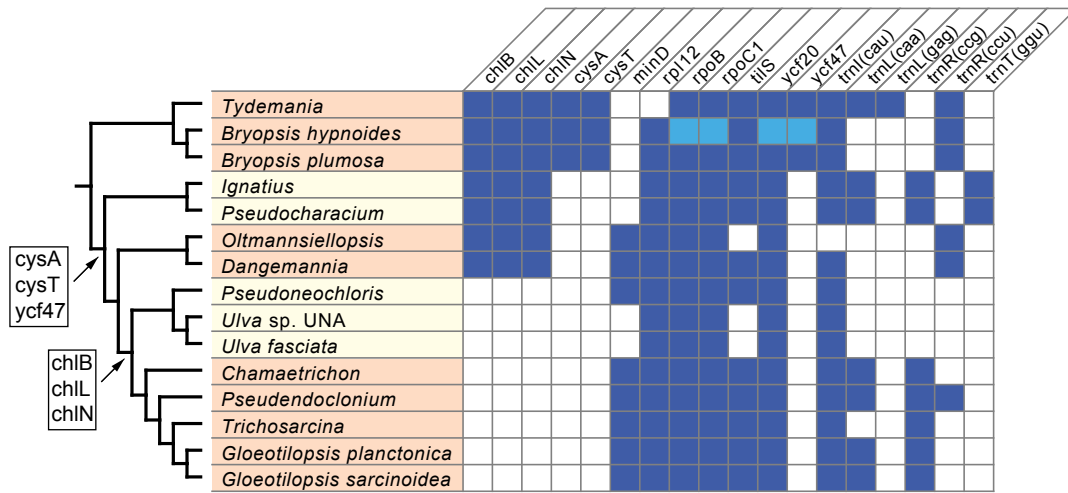


Figure S12