HOKKAIDO UNIVERSITY

Title	Evolution of Green Plants Accompanied Changes in Light-Harvesting Systems
Author(s)	Kunugi, Motoshi; Satoh, Soichirou; Ihara, Kunio et al.
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Fig. 1

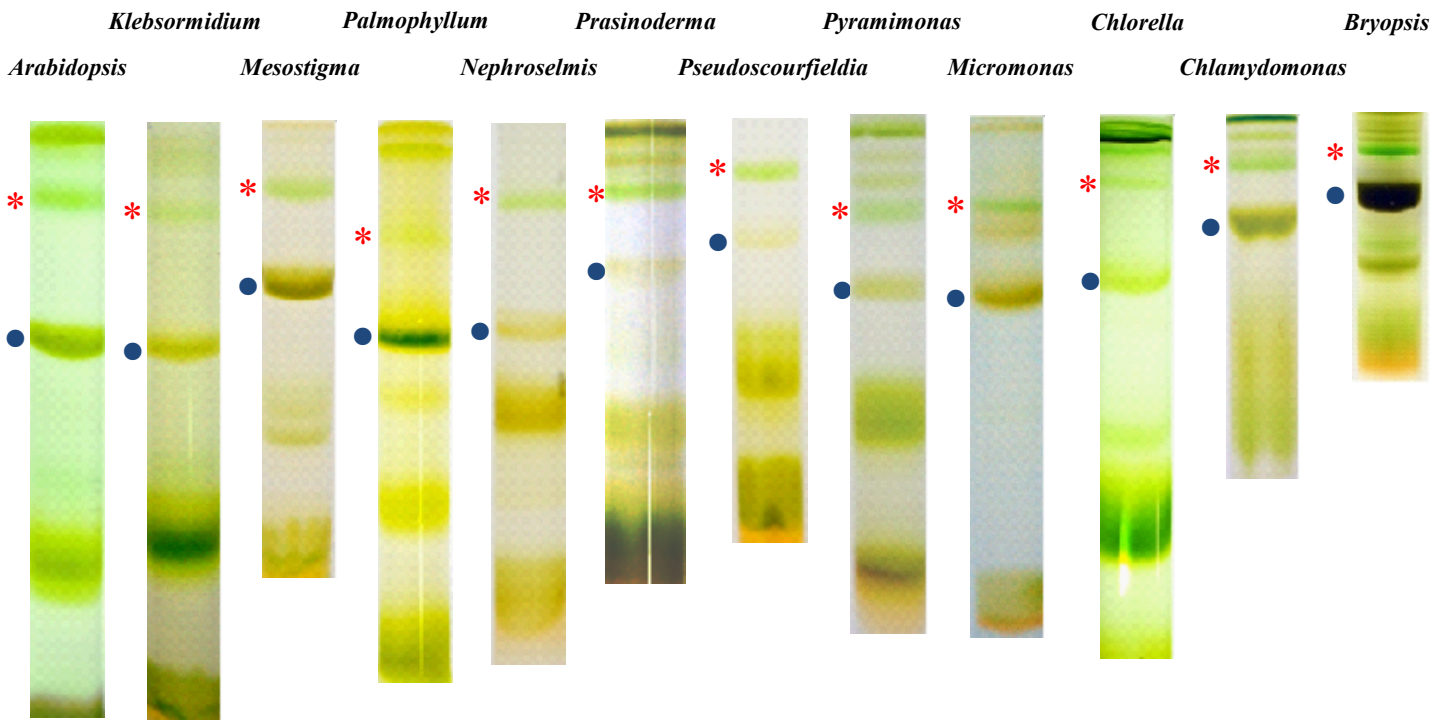

Arabidopsis
Klebsormidium
Mesostigma
Palmophyllum
Nephroselmis
Prasinoderma
Pseudoscourfieldia
Pyramimonas
Micromonas
Chlorella
Chlamydomonas
Bryopsis

Fig. 2

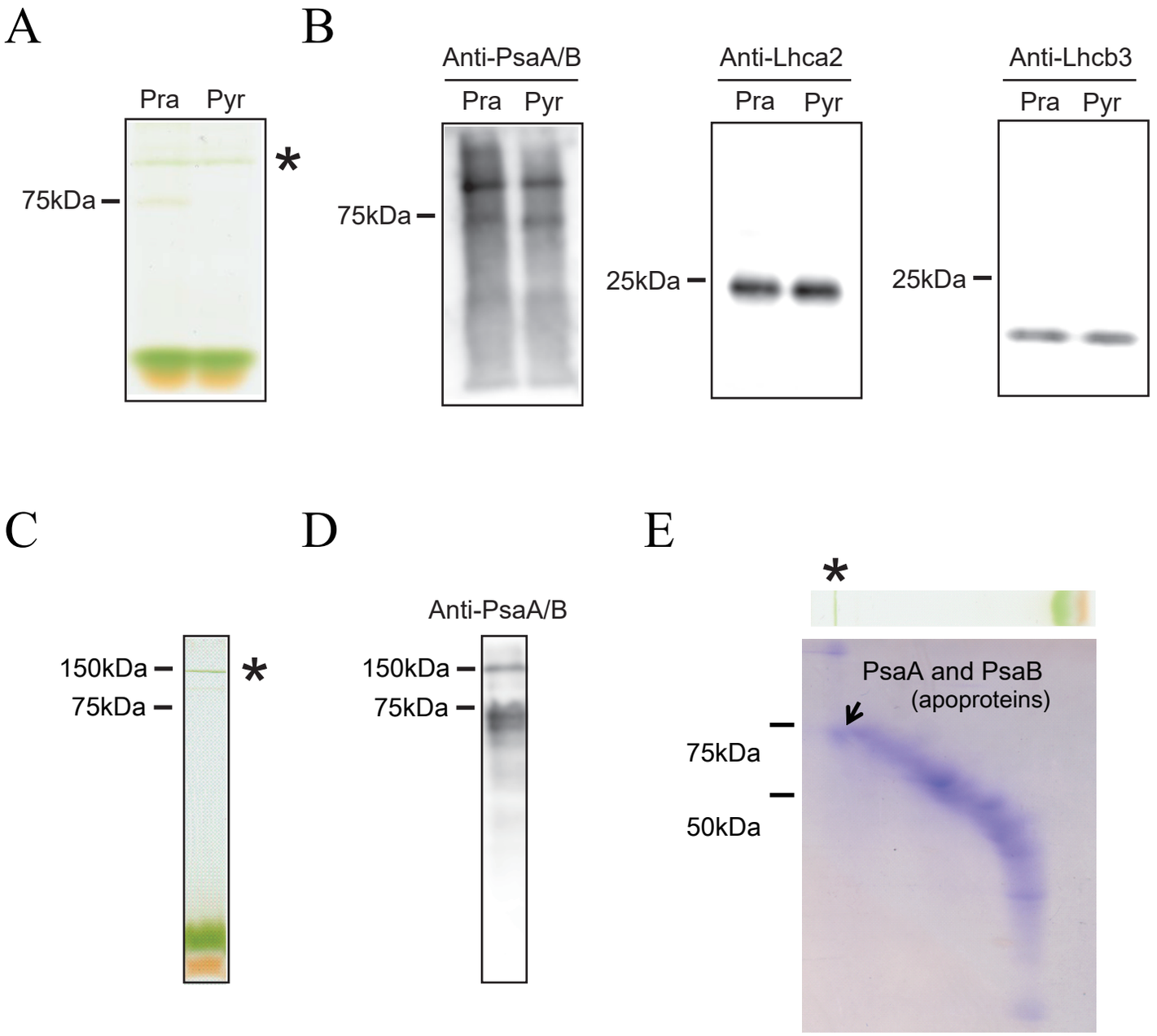

A
Pra
Pyr
75kDa
*
B
Anti-PsaA/B
Pra
Pyr
75kDa
Anti-Lhca2
Pra
Pyr
25kDa
Anti-Lhcb3
Pra
Pyr
25kDa
C
150kDa
75kDa
*
D
Anti-PsaA/B
150kDa
75kDa
E
*
PsaA and PsaB
(apoproteins)
75kDa
50kDa

Fig. 3

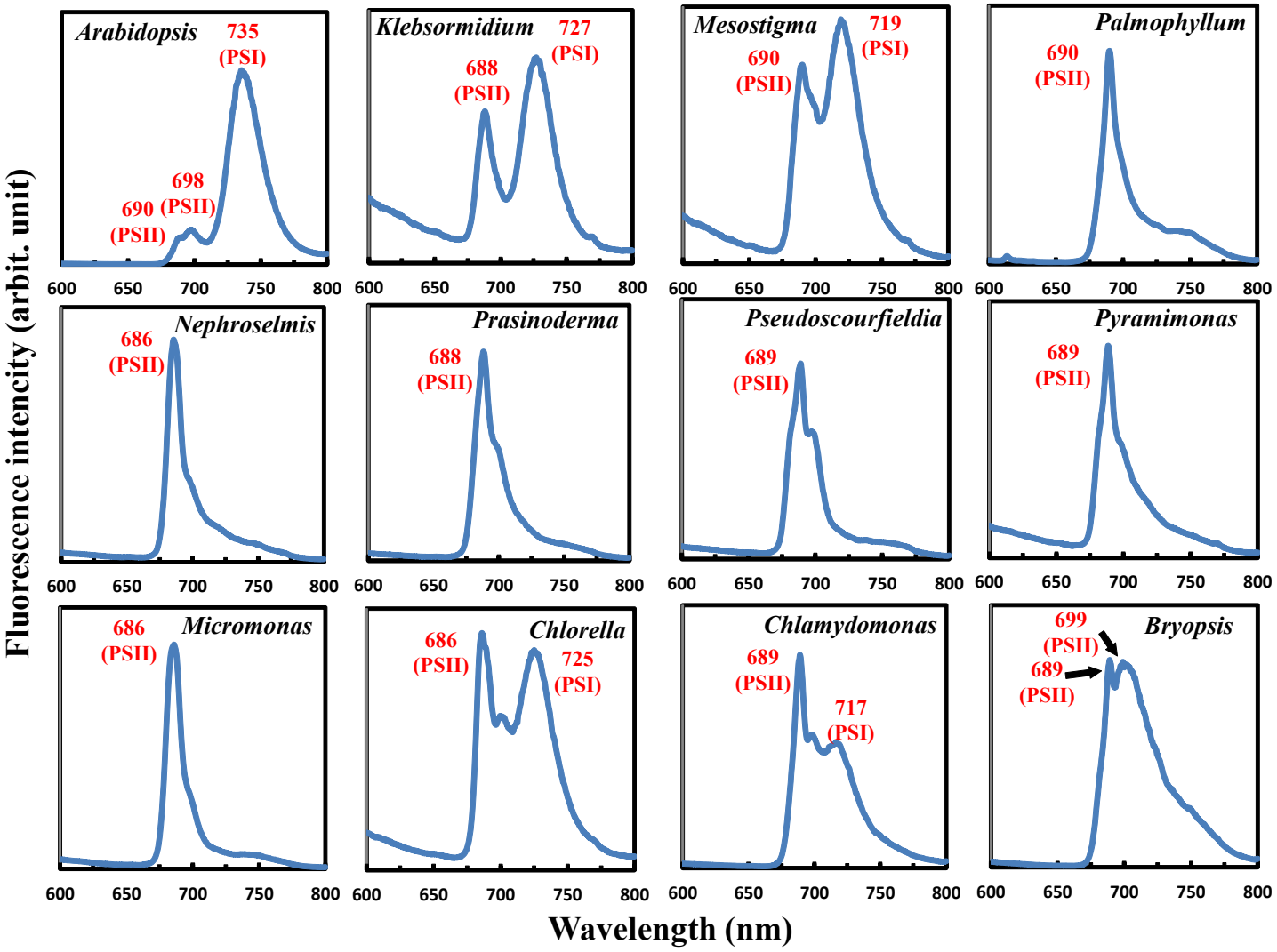

Arabidopsis
735 (PSI)
698 (PSII)
690 (PSII)
Klebsormidium
727 (PSI)
688 (PSII)
Mesostigma
719 (PSI)
690 (PSII)
Palmophyllum
690 (PSII)
Nephroselmis
686 (PSII)
Prasinoderma
688 (PSII)
Pseudoscourfieldia
689 (PSII)
Pyramimonas
689 (PSII)
Micromonas
686 (PSII)
Chlorella
686 (PSII)
725 (PSI)
Chlamydomonas
689 (PSII)
717 (PSI)
Bryopsis
699 (PSII)
689 (PSII)
600 650 700 750 800
Fluorescence intencity (arbit. unit)
Wavelength (nm)

Fig. 4

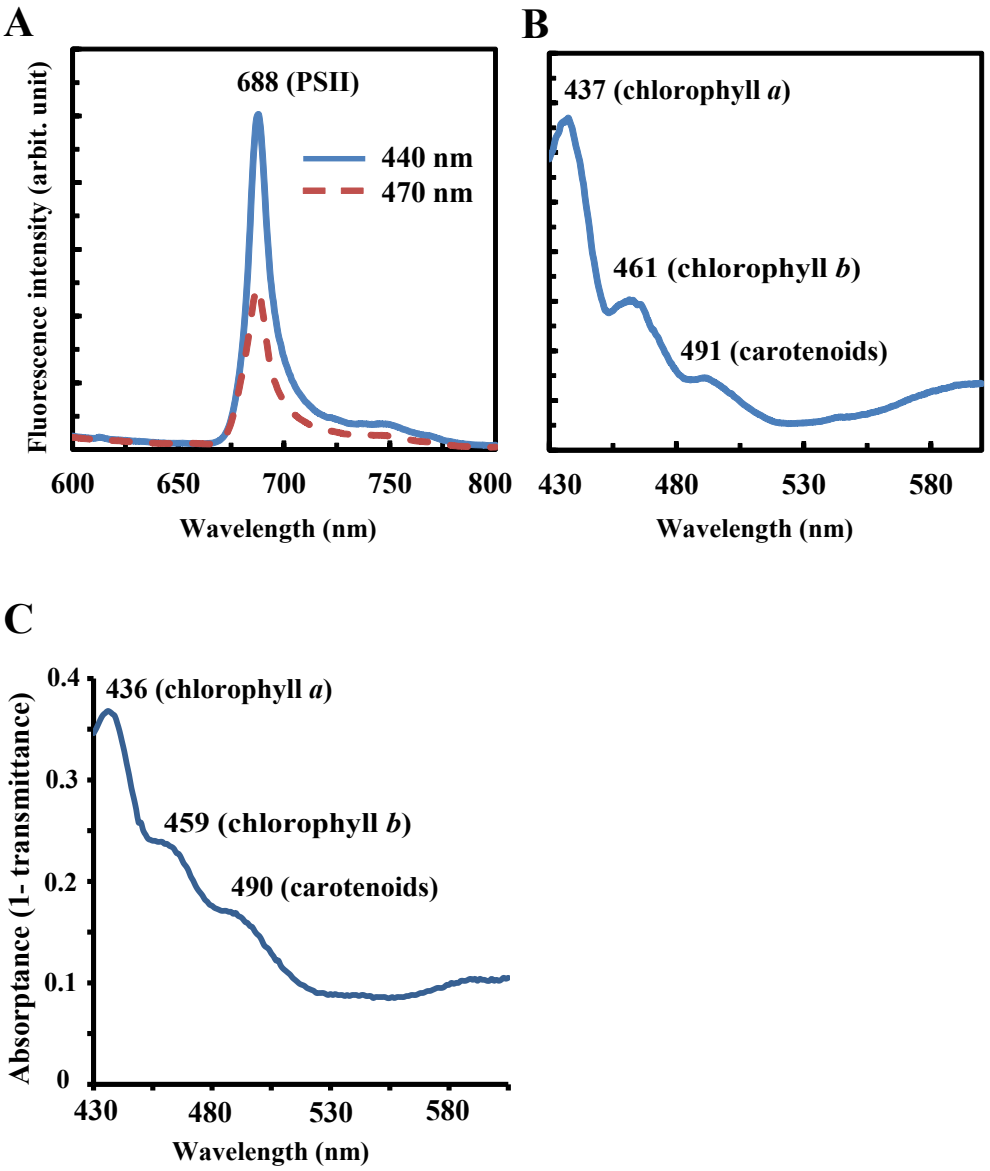

A
688 (PSII)
440 nm
470 nm
Fluorescence intensity (arbit. unit)
600
650
700
750
800
Wavelength (nm)
B
437 (chlorophyll a)
461 (chlorophyll b)
491 (carotenoids)
430
480
530
580
Wavelength (nm)
C
436 (chlorophyll a)
459 (chlorophyll b)
490 (carotenoids)
Absorptance (1- transmittance)
0.4
0.3
0.2
0.1
0
430
480
530
580
Wavelength (nm)

Fig. 5

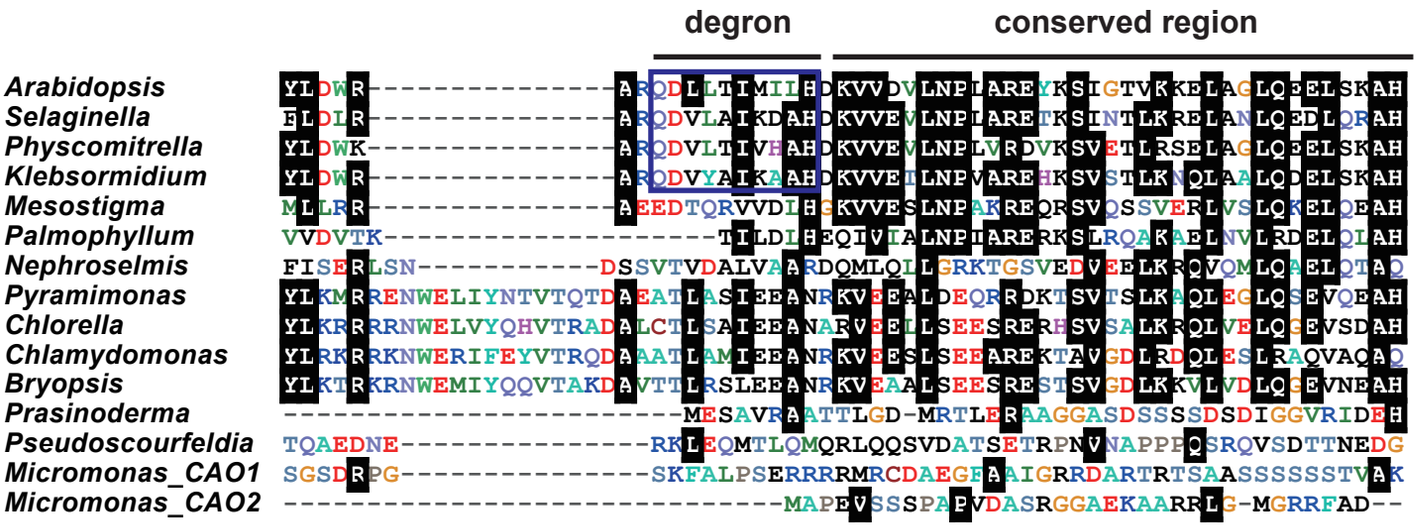
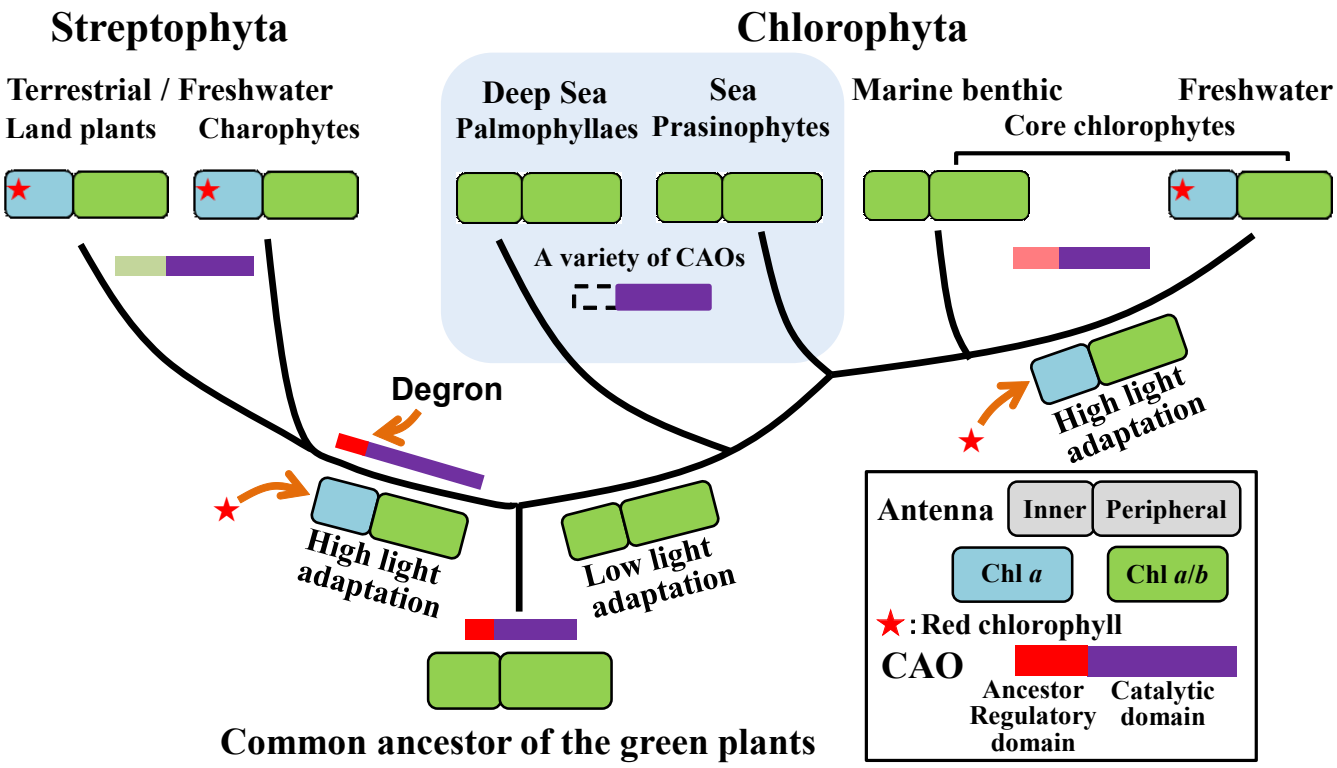
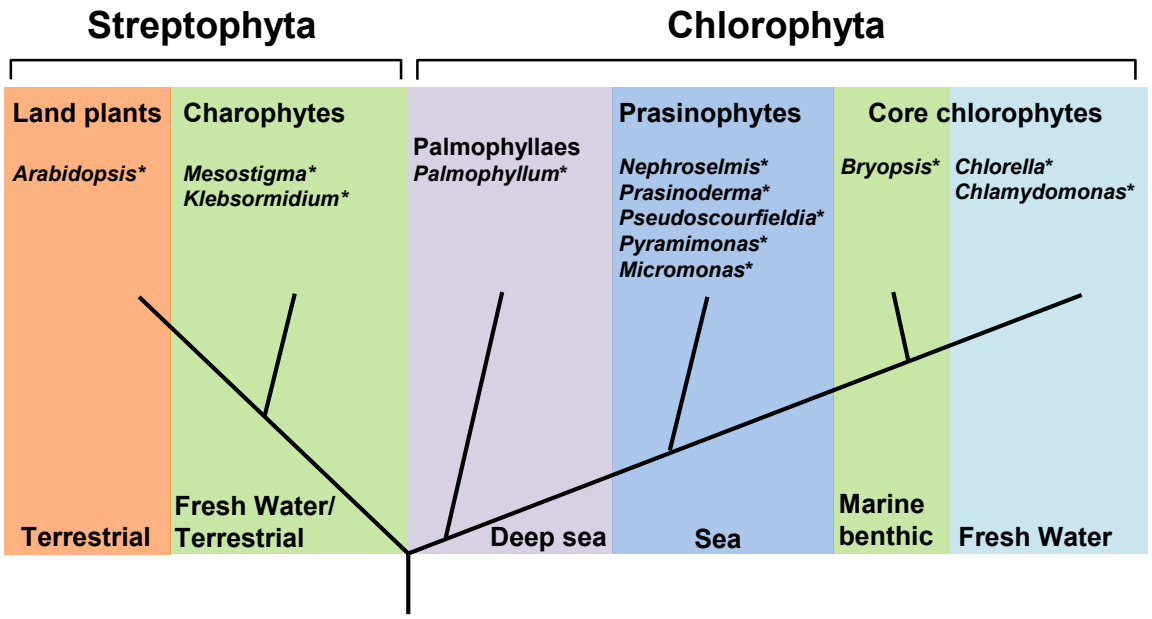

degron
conserved region
Arabidopsis
Selaginella
Physcomitrella
Klebsormidium
Mesostigma
Palmophyllum
Nephroselmis
Pyramimonas
Chlorella
Chlamydomonas
Bryopsis
Prasinoderma
Pseudoscourfeldia
Micromonas_CAO1
Micromonas_CAO2

Fig. 6

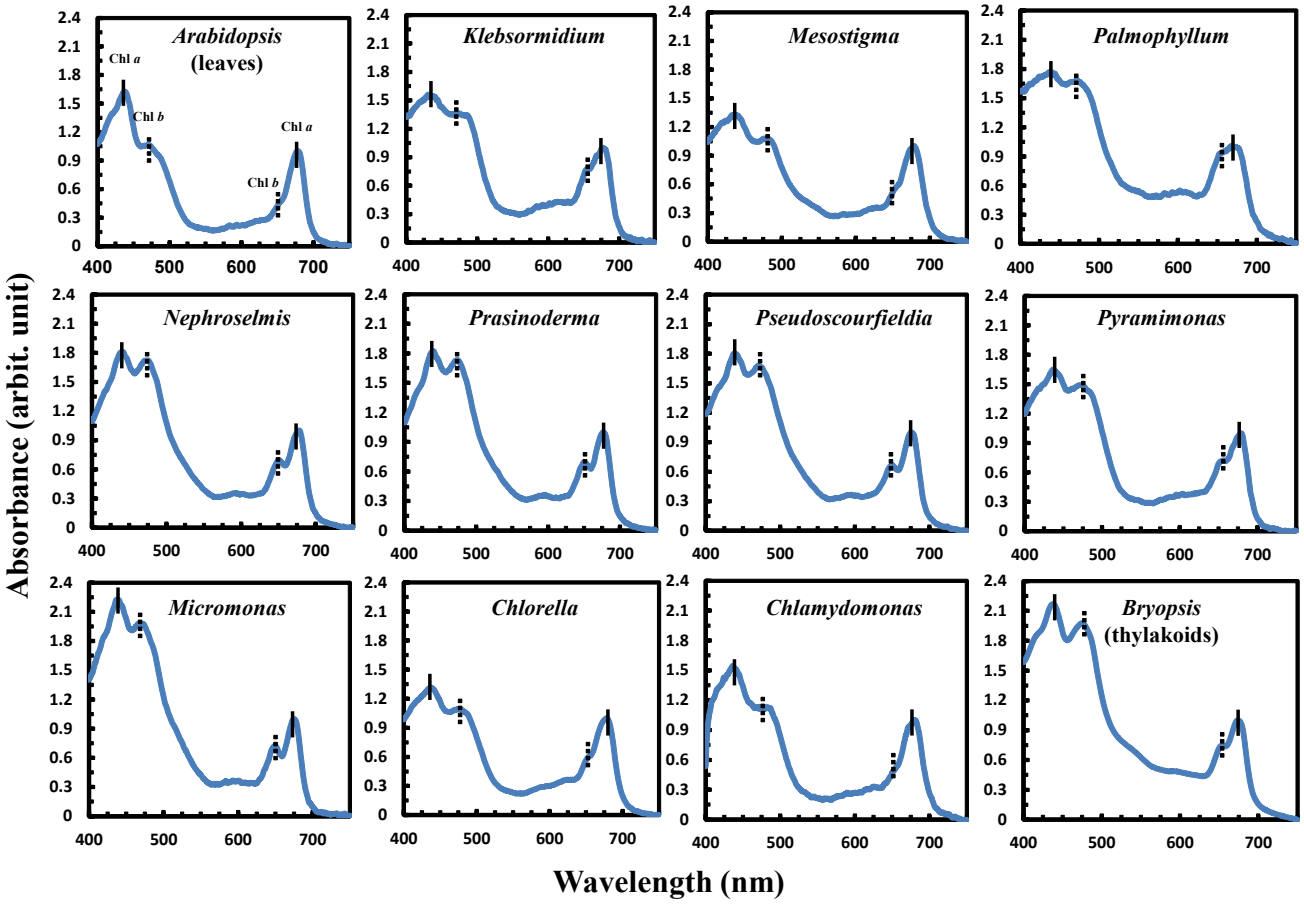

Streptophyta
Chlorophyta
Terrestrial / Freshwater
Land plants
Charophytes
Deep Sea
Palmophyllaes
Sea
Prasinophytes
Marine benthic
Freshwater
Core chlorophytes
A variety of CAOs
Degron
High light adaptation
Low light adaptation
High light adaptation
Common ancestor of the green plants
Antenna
Inner
Peripheral
Chl a
Chl a/b
★: Red chlorophyll
CAO
Ancestor Regulatory domain
Catalytic domain

Supplementary Fig. S1



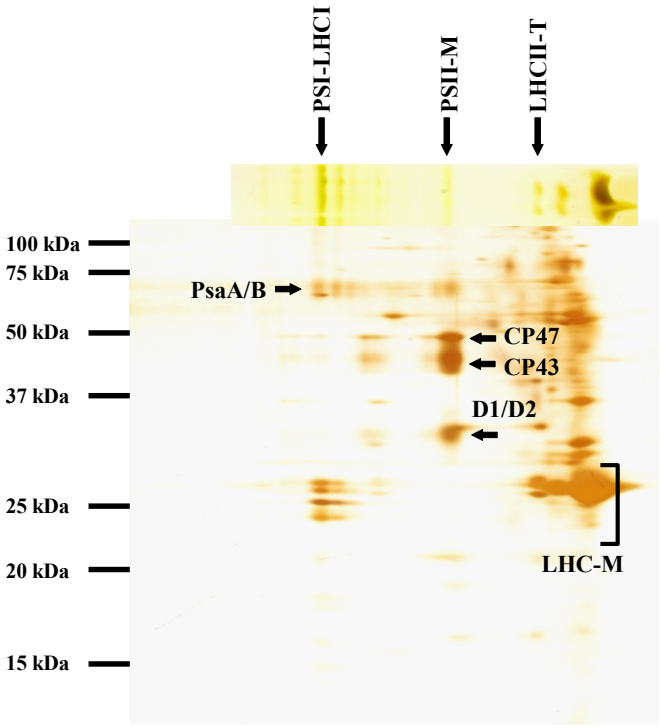
Supplementary Fig. S1 Overview phylogeny of the green lineage. This figure was drawn based on F. Leliaert et al. Species with asterisk were used in this study.

Supplementary Fig. S2



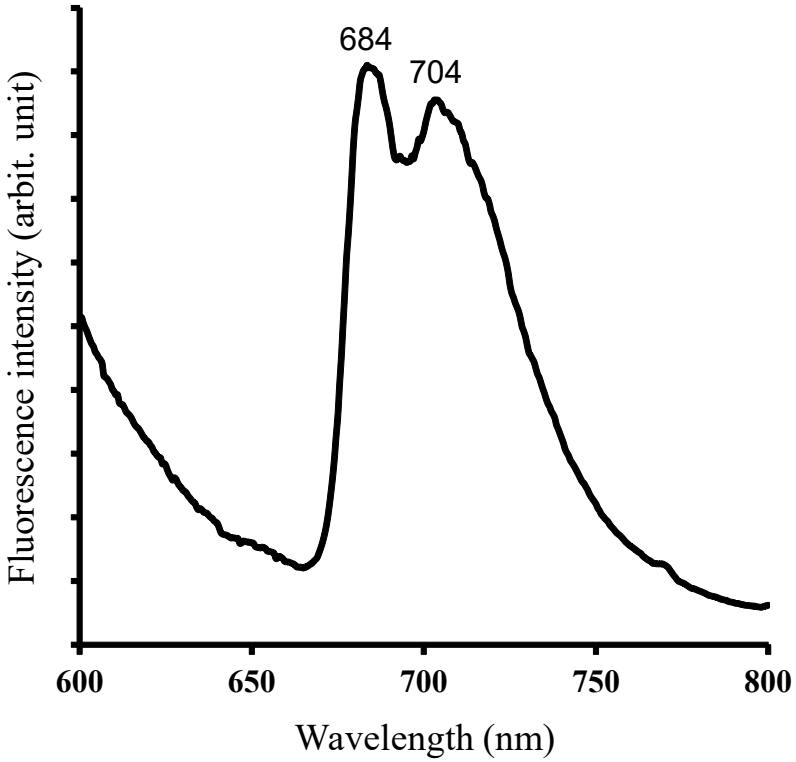
Supplementary Fig. S2 Room temperature absorption spectra of *Arabidopsis* leaves, *Bryopsis* thylakoid membranes, and other green algae cells. Solid vertical line is peak of chl *a*, and the dashed line is the peak of chl *b*.

Supplementary Fig. S3



Supplementary Fig. S3 Separation of chlorophyll-protein complexes from *Nephroselmis*. *Nephroselmis* chlorophyll-protein complexes were analyzed by two-dimensional CN/SDS-PAGE, followed by silver staining. PsaA and PsaB are the apoproteins of PSI core antenna, while CP43 (PsbC) and CP47 (PsbB) are the apoproteins of PSII core antenna.

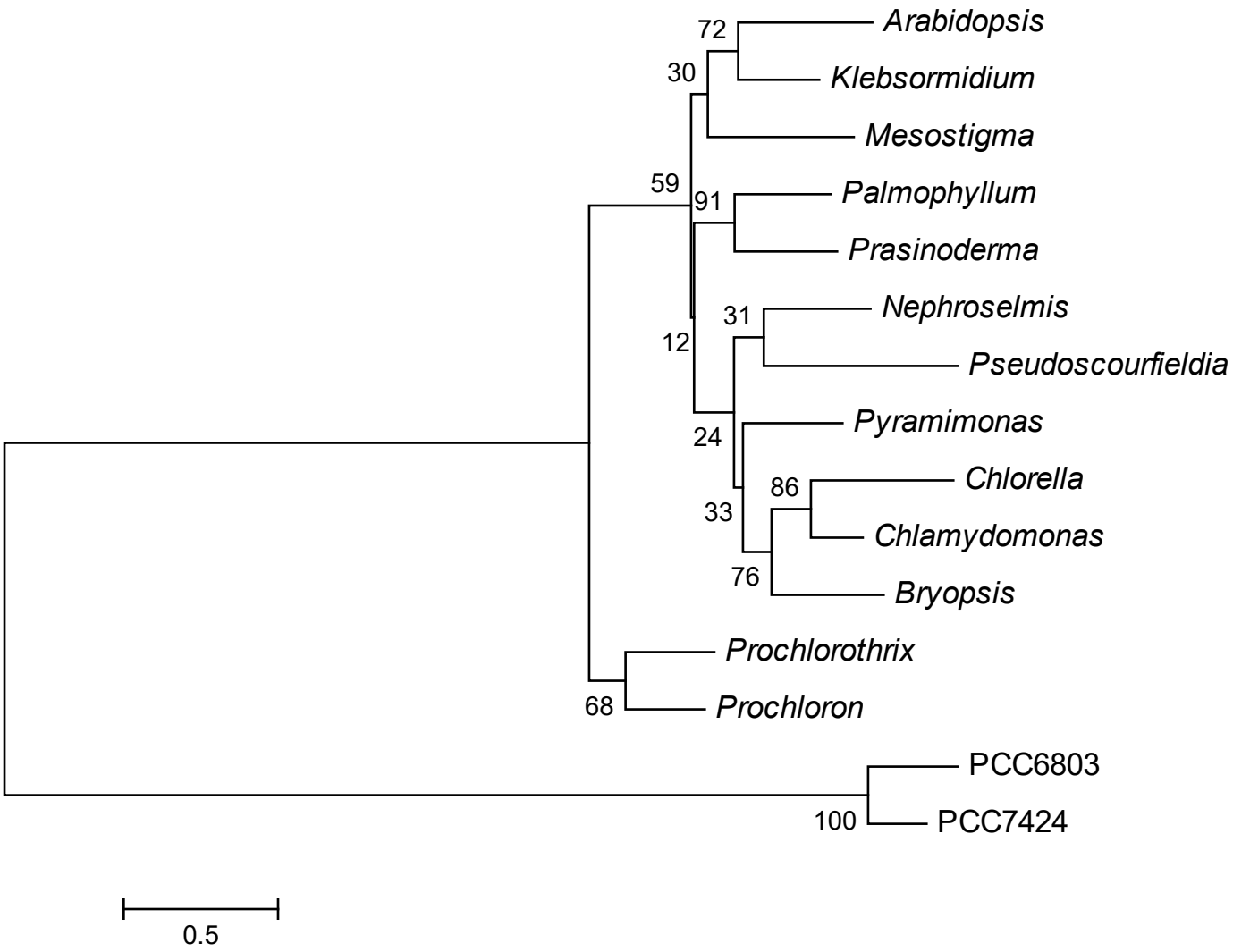
Supplementary Fig. S4



Supplementary Fig. S4 The low-temperature fluorescence emission spectrum of the *Nephroselmis* PSI-LHCI separated by the CN-PAGE. The 684 nm peak might be due to the slight contamination of the PSII dimer, as judged by the two-dimensional SDS-PAGE (Fig. S3).

Supplementary Fig. S5

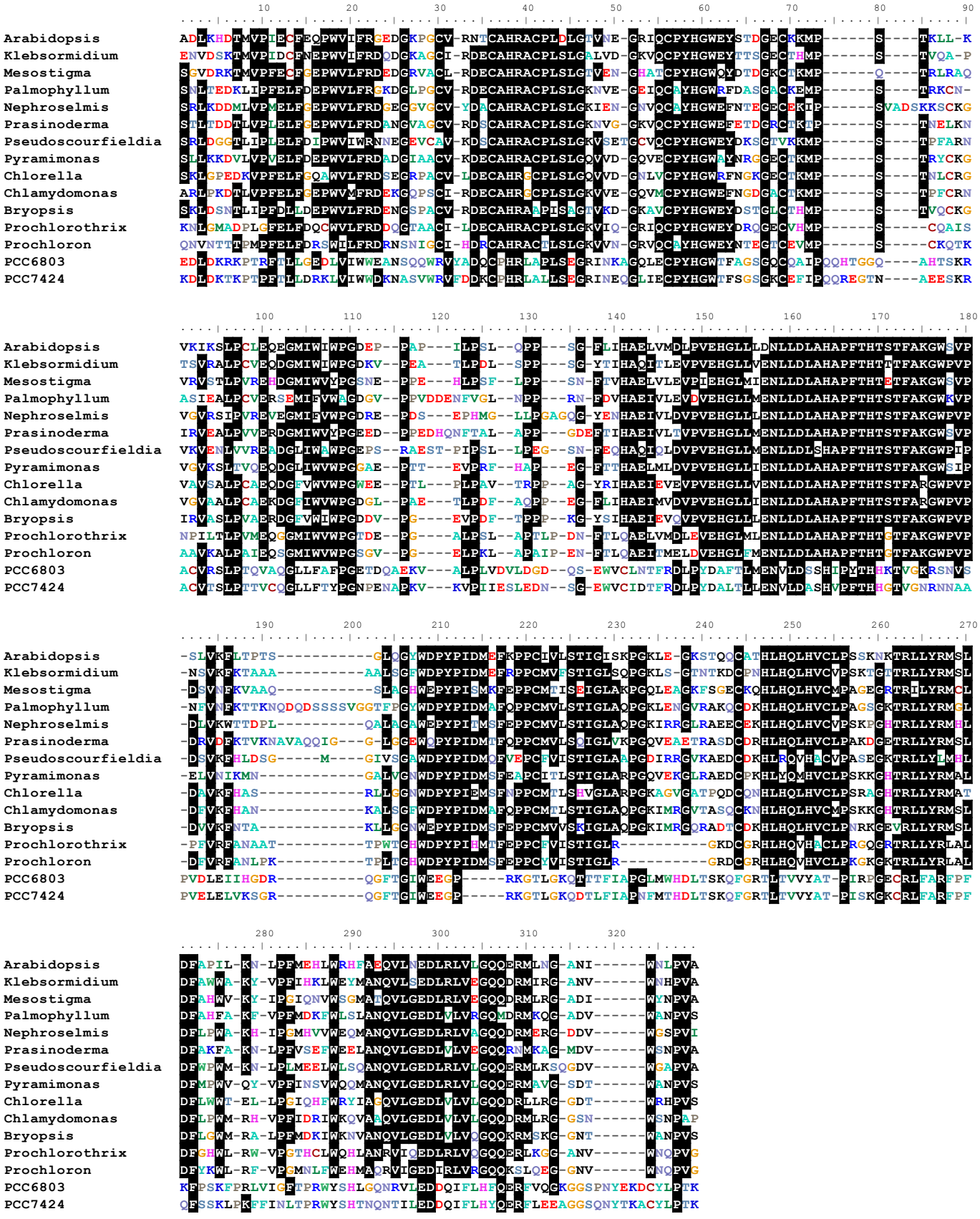
A



Supplementary Fig. S5 Phylogenetic tree of CAO proteins. Maximum likelihood tree (A) was constructed by MEGA v6.1 based on an alignment (B) generated by M-Coffee under the LG model with discrete gamma distribution in eight categories with 2,000 bootstrap replicates. Bootstrap support percentages and accession numbers of the analyzed aligned protein sequences are shown. Two cyanobacterial homologous proteins (*Synechocystis* sp. PCC 6803 (GI: 499174829), *Cyanothece* sp. PCC 7424 (GI: 506434500)) were used for the outgroup.

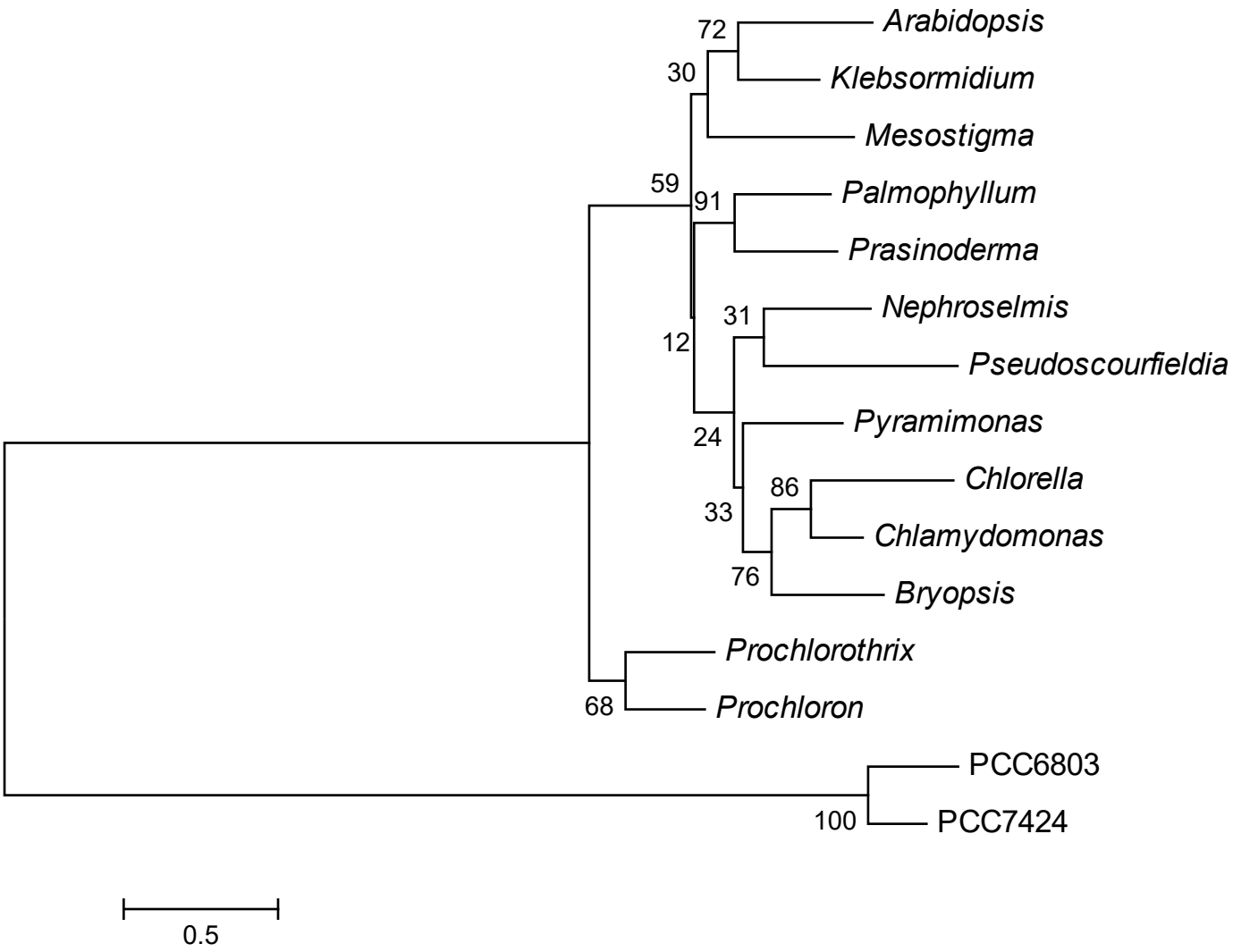
Supplementary Fig. S5

B



Supplementary Fig. S5

A



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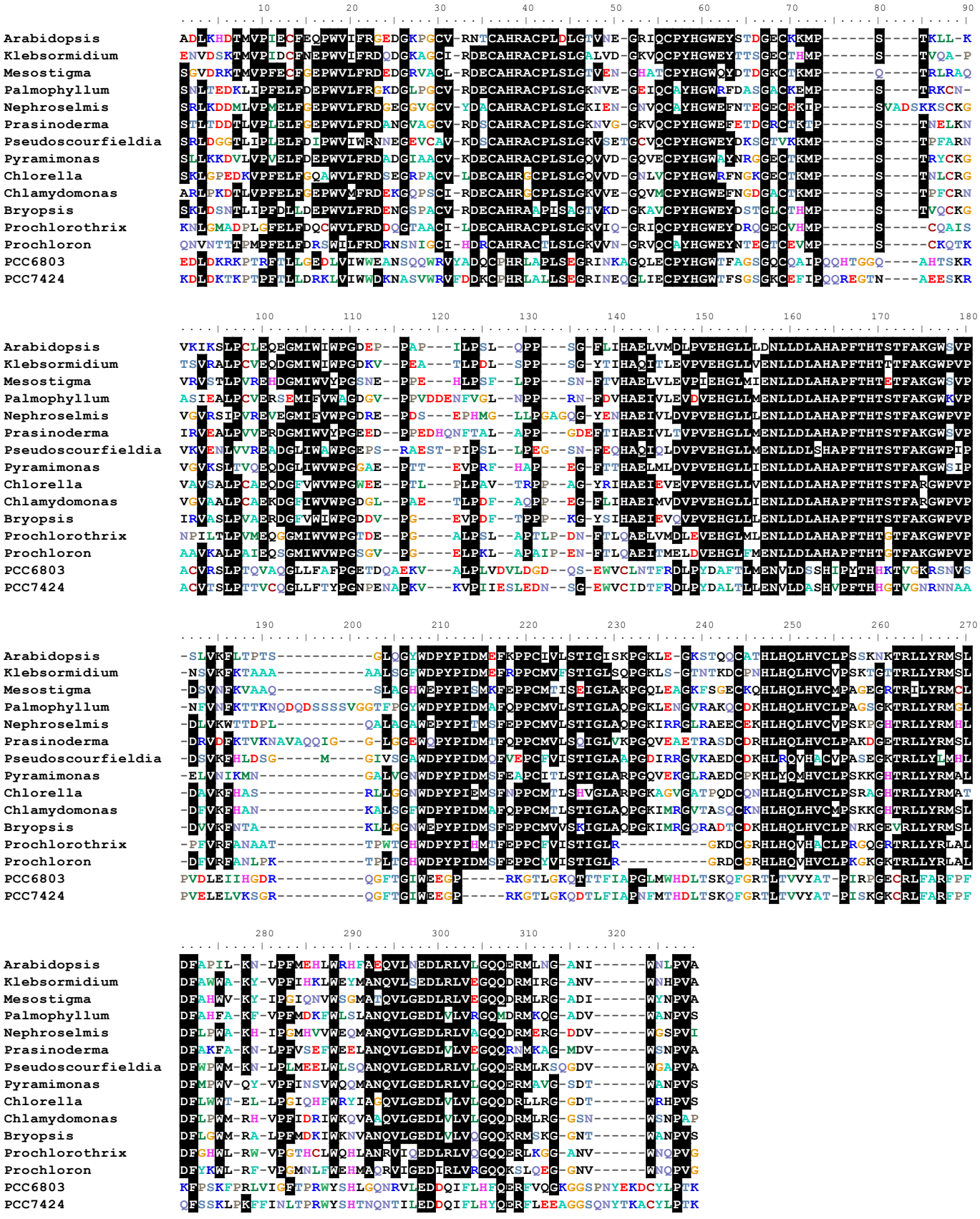
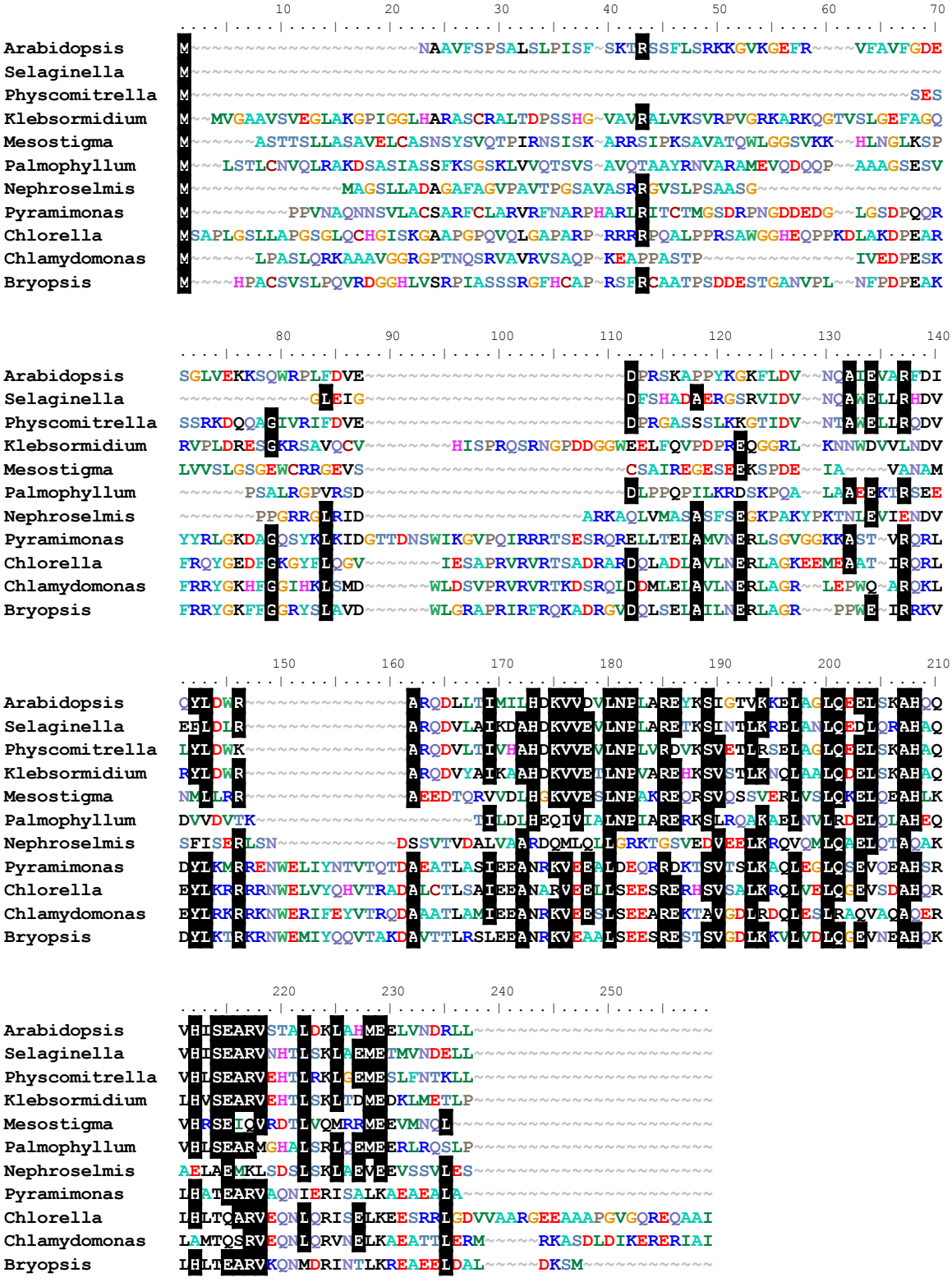
Supplementary Fig. S5

B



Supplementary Fig. S6



Supplementary Fig. S6 Amino acid sequence comparison of CAO regulatory domain among green plants. Multiple sequence alignment was generated by Muscle in MEGA v6.1 with default settings and manual refinement. Black shading indicates the residues conserved in more than half of the aligned CAO sequences. *Prasinoderma*, *Pseudoscourfieldia* and *Micromonas* CAO sequences were omitted, because no significant similarity was found to other CAO seuqences.

Supplementary Table S1 Pigment profiles of *A. thaliana* leaves and green algae total cells.

Classification	Name	DVP	Chl *c* cs170	α-car	β-car	Vio	Neo	Zea	Lut	Dihy	Sip	Pra	Sip B1	Sip B2	Lor
Streptophyta	*Arab*	-	-	-	+	+	+	-	+	-	-	-	-	-	-
	Kleb	-	-	+	+	+	-	-	+	-	-	-	-	-	-
	Meso	-	-	+	+	+	+	-	+	-	+	-	-	-	-
Fresh water chlorophyta	*Chlo*	-	-	+	+	+	+	-	+	-	-	-	-	-	-
	Chla	-	-	+	+	+	+	-	+	-	-	-	-	-	+
	Palm	-	-	+	-	+	+	-	-	+	-	-	-	-	-
Sea water chlorophyta	*Neph*	+	+	+	+	+	+	+	+	-	-	-	+	+	-
	Pras	+	-	+	+	+	+	+	-	+	-	+	-	-	-
	Pseu	+	-	+	+	+	+	+	-	+	-	+	-	-	-
	Pyra	+	-	-	+	+	-	+	+	-	-	-	-	-	-
	Micr	+	-	+	+	+	+	+	-	+	-	+	-	-	-
	Bryo	+	-	+	-	+	+	-	-	-	+	-	-	-	-

Arab, *Arabidopsis*; *Kleb*, *Klebsormidium*; *Meso*, *Mesostigma*; *Chlo*, *Chlorella*; *Chla*, *Chlamydomonas*, *Palm*, *Palmophyllum*; *Neph*, *Nephroselmis*, *Pra*, *Prasinoderma*; *Pseu*, *Pseudoscourfieldia*; *Pyra*, *Pyramimonas*; *Micr*, *Micromonas*; *Bryo*, *Bryopsis*. DVP, 8-vinyl-protochlorophyllide, Chl *c* cs170, Chlorophyll *c* CS170, α-car, α-carotene; β-car, β-carotene, Vio, Violaxanthin; Neo, Neoxanthin; Zea, Zeaxanthin; Lut, Lutein, Dihy, Dihydrolutein; Sip, Siphonaxanthin; Pra, Prasinoxanthin, Sip B1, Siphonaxanthin-derivative B1; Sip B2, Siphonaxanthin-derivative B2. Lor, Loroxanthin.

Supplementary Table S2 The pigment contents of PSI cores and LHCII trimers in seawater chlorophytes.

		Streptophyte	Freshwater chlorophyte	Sea water chlorophytes					
	Sample	*Arab*	*Chla*	*Palm*	*Neph*	*Pras*	*Pseu*	*Pyra*	*Bryo*
α,β-carotene /total Chl	PSI	0.1	0.58	0.09	0.33	0.15	0.17	0.46	0.34
	LHCII	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
(dihyrdo)lutein /total Chl	PSI	0.02	n.d.	n.d.	n.d.	n.d.	0.04	0.03	n.d.
	LHCII	0.47	0.49	0.16	n.d.	0.07	0.32	0.22	n.d.

Arab, *Arabidopsis*; *Chla*, *Chlamydomonas*, *Palm*, *Palmophyllum*; *Neph*, *Nephroselmis*, *Pra*, *Prasinoderma*; *Pseu*, *Pseudoscourfieldia*; *Pyra*, *Pyramimonas*; *Bryo*, *Bryopsis*. PSI cores (heterodimer of PsaA/PsaB) and LHCII trimers (LHCII) were separated by native green gel electrophoresis. The pigments were extracted from the corresponding bands and determined. The relative amounts of the pigments were calculated using the areas of the peaks.

Supplementary Table S3 List of degenerate and race primers used in this study for cloning of *CAO*.

Organism	Primer name	Sequence	Use
Klebsormidium	5’race outer primer	GCGCCAGGTCCAGCAGGTTCTCCAC	5' race PCR
	5’race inner primer	GAGGGTGGCCTCCGGCACTTTGTCC	5' race PCR
Mesostigma	5’race outer primer	AGGTGCTGCTTGCACTCGCCACTGA	5' race PCR
	5’race inner primer	ATCGTCATGCACGGCGGCTCAAACT	5' race PCR
	3’race outer primer	CCTTCCTCCGCCACTGCGGTCAC	3' race PCR
	3’race inner primer	TGAAGGACAAGCCCAACCCGATGGA	3' race PCR
Palmophyllum	CAO Degenerate Fw	GCA***YM***G***I***GC***I***TG***Y***CC**I**CT	Degerate PCR
	CAO Degenerate Rv	CCA***I***C***KI***CGG***T***A***I***CG***I***AC***I***CC	Degerate PCR
	5’race outer primer	GGAATGTGCCACCAACTGAGCTGGAGC	5' race PCR
	5’race inner primer	CGACGTATGCGTAAACGGCGCATGTGCC	5' race PCR
	3’race outer primer	GGCACATGCGCCGTTTACGCATACGTCG	3' race PCR
	3’race inner primer	GCTCCAGCTCAGTTGGTGGCACATTCC	3' race PCR
Nephroselmis	5’race outer primer	CGTGCGCCAGGTCCAGGAGGTTCT	5' race PCR
	5’race inner primer	GCTCCACGGGCACGTCCAGCACTAT	5' race PCR
	3’race outer primer	GGGGAGGGGGGGGTCGGCTGCGTCTA	3' race PCR
	3’race inner primer	CGGCAACGTGCAGTGCGCGTACCACG	3' race PCR
Prasinoderma	5’race outer primer	GCTGAAAGGTCATGTCGATGGGGTAGGG	5' race PCR
	5’race inner primer	GCGACGGCGTTCTTCACGGTCTTG	5' race PCR
Pseudoscourfieldia	5’race outer primer	CGATGCCCATGCCGGAGTCGAGGTGGAAC	5' race PCR
	5’race inner primer	CAGCAGGCCGTGCTCGACGGGAACGTCCAA	5' race PCR
	3’race outer primer	GAGCGGGAACTCGAAACGCCTCCTG	3' race PCR
	3’race inner primer	CTGGCGCAACAACGAAGGCGAGGTA	3' race PCR
Pyramimonas	5’race outer primer	GGGTCCCAGTTTCCAACCAAAGCGCCGTTCA	5' race PCR
	5’race inner primer	GAGTGAATGGAGCATGCGCCAAGTCCAGCA	5' race PCR
	3’race outer primer	GCAGGACGGGTTGATCTGGGTGTGG	3' race PCR
	3’race inner primer	GGACGTGCCTGTGGAGCACGGTCTT	3' race PCR
Bryopsis	CAO Degenerate Fw	GCA***Y***GGCCTGCT***S***AT***K***GAGAA	Degerate PCR
	CAO Degenerate Rv	CA***W***GCACAC***R***TG***S***AGCTGGTG	Degerate PCR
	5'race primer	AGACATGTCGATGGGATAGG	5' race PCR
	3'race primer	GAGAGAGAAGGATCCAAGAGC	3' race PCR

* Italic boldface letters represent the degenerate nucleotides.