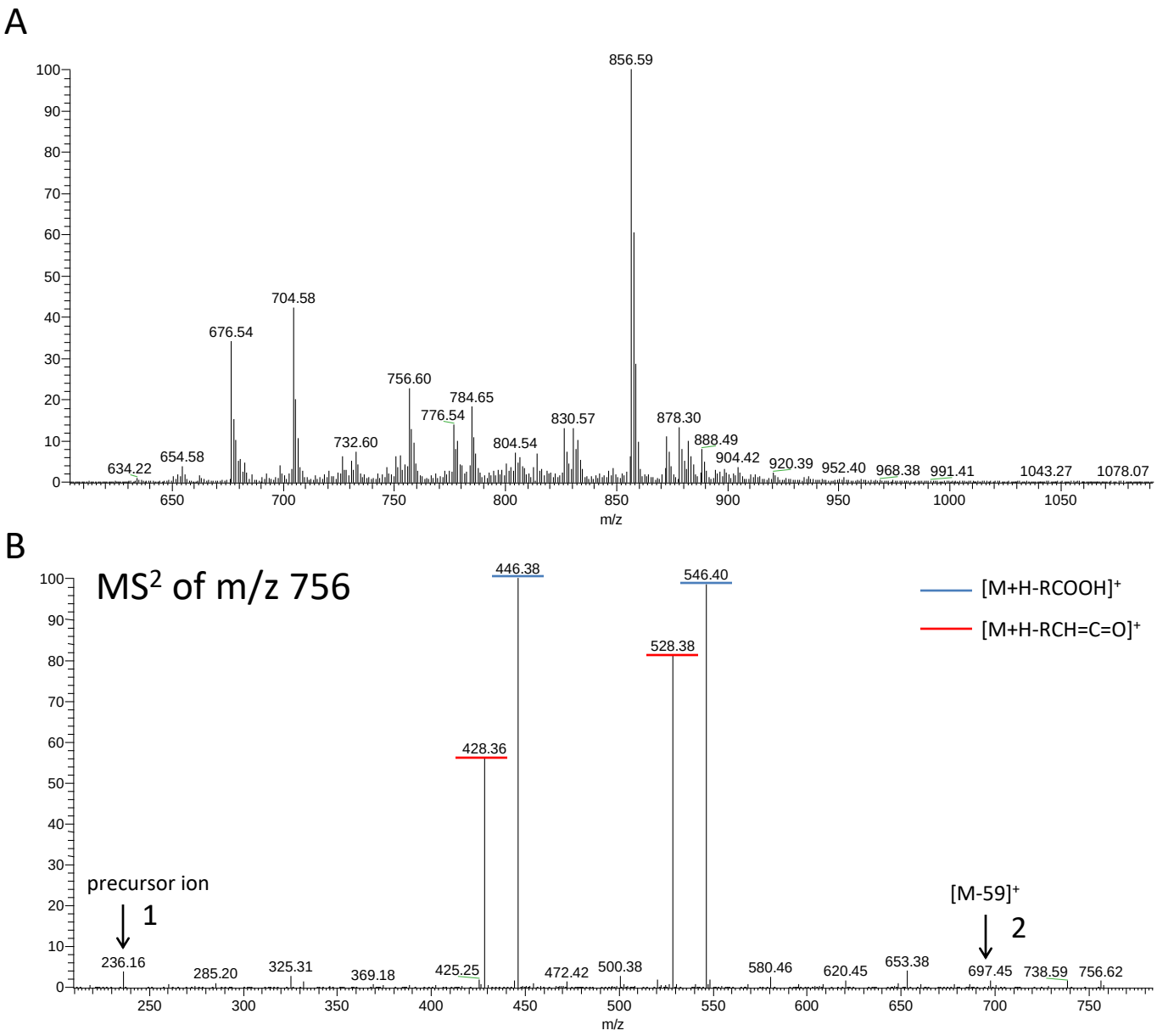
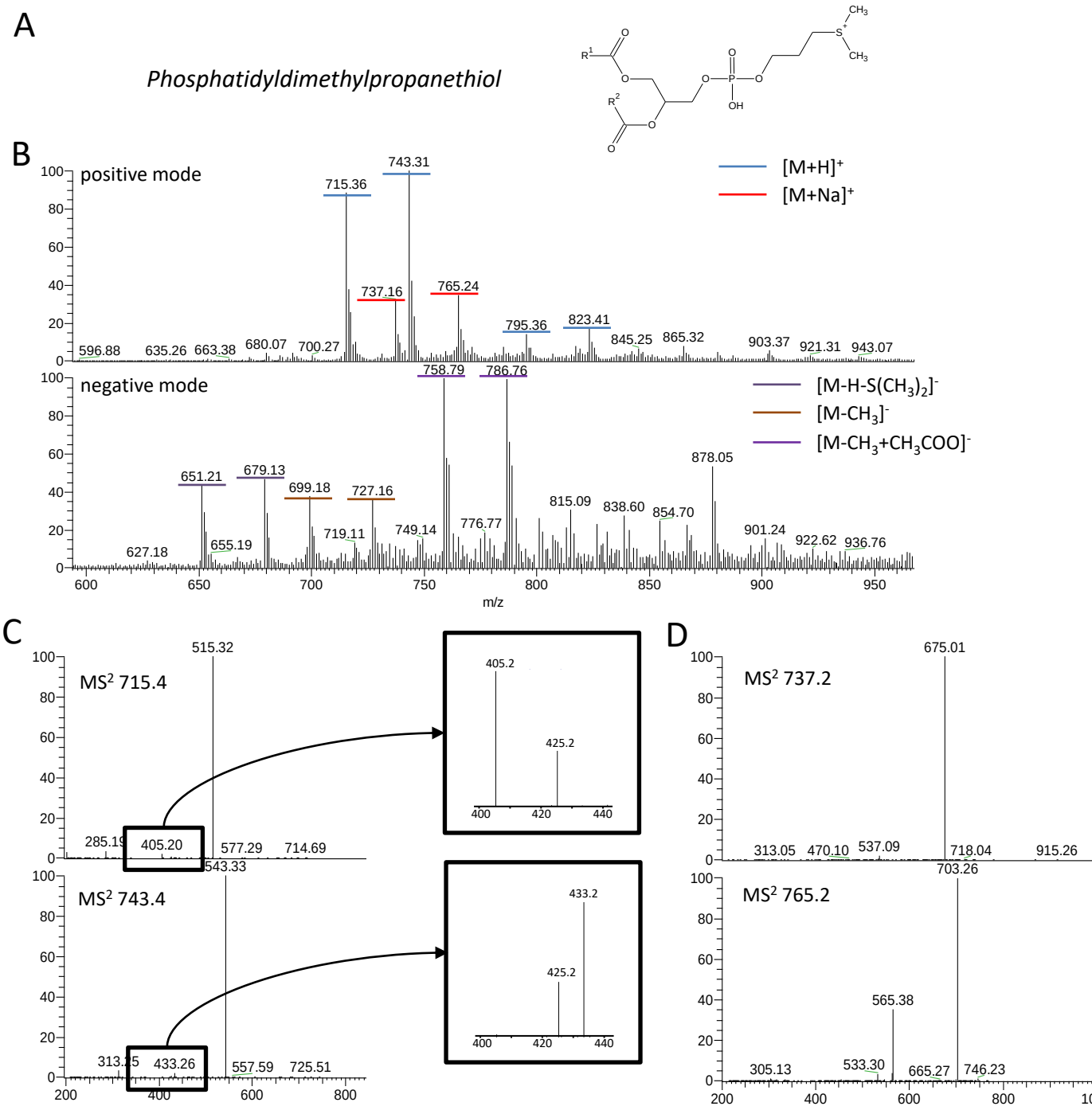


Supplemental figure 1. Identification of DGTA by mass spectrometry.
A. Full scan spectrum in positive mode of the 2D-TLC spot called DGTA in Figure 3. B. MS^2 analysis of the m/z 756. Arrow heads indicate fragments corresponding to the DGTA signature:
1: precursor m/z 236 corresponds to the polar head mass of either DGTA or DGTS 2: the fragment 697 results from a neutral loss of 59 from m/z 756 that is characteristic of DGTA and DGTS. 3: absence of a m/z 669 corresponding to a neutral loss of 87 characteristic to DGTS (Armada et al., 2013)



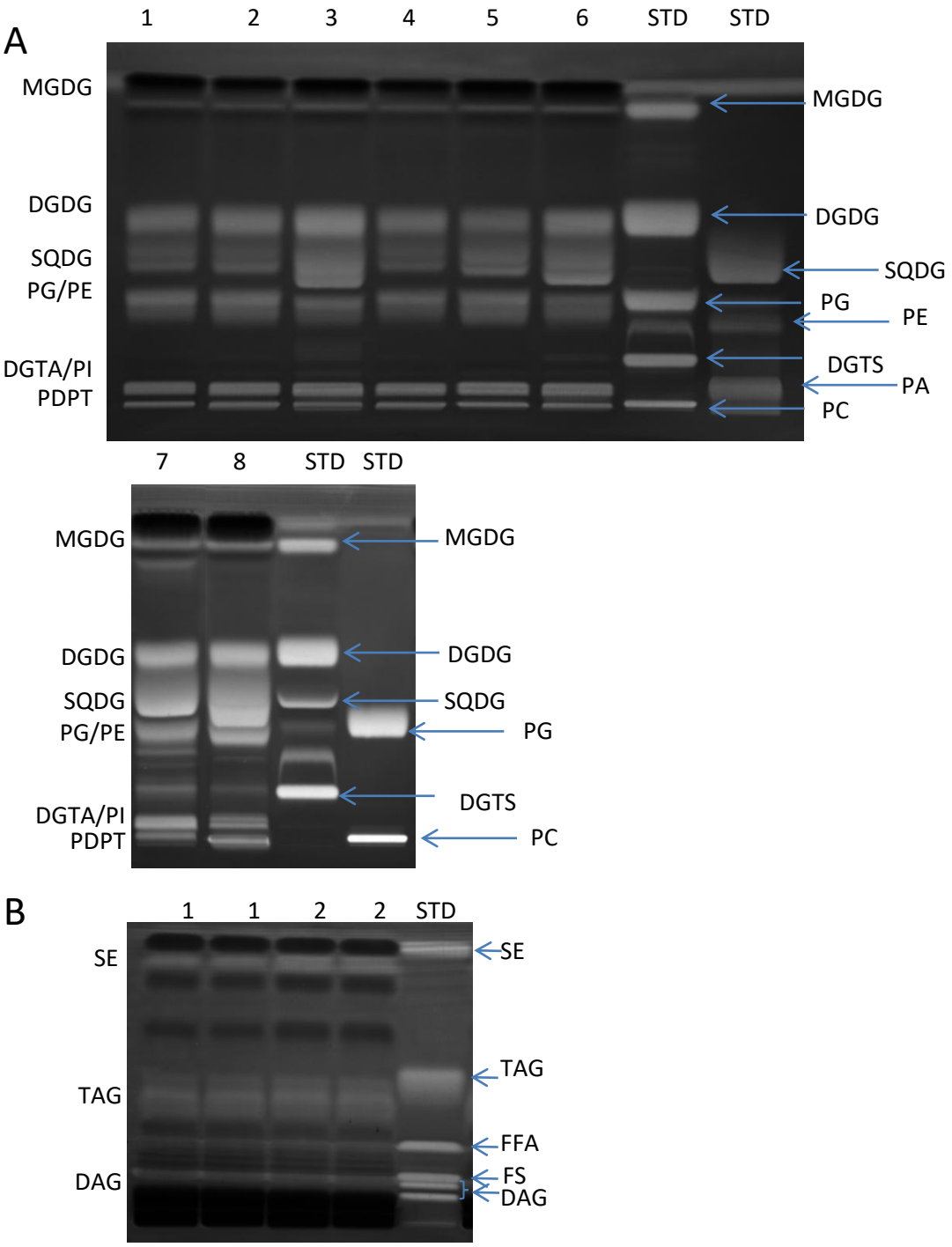
Supplemental figure 2. Identification of phosphatidyldimethylpropanethiol in O. tauri

A. Structure of the sulfonolipid phosphatidyldimethylpropanethiol

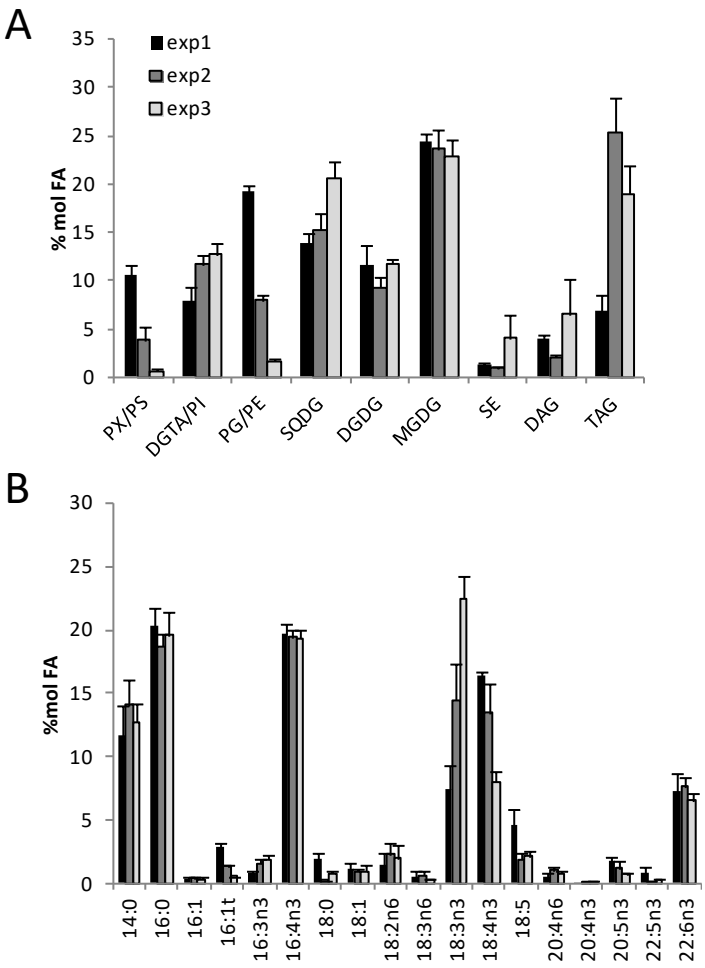
B- Fullscan analysis in positive and negative mode of the PX spot from Figure 3. Two adducts ($[M+H]^+$ and $[M+Na]^+$) are visible in positive mode. The $[M+H]^+$ adducts 715.4 and 743.4 in negative mode give rise to 3 adducts [M-15], [M+44] and [M-63] corresponding to $[M-CH_3]^-$ (respectively m/z 699.2 and 727.2), to $[M-CH_3+CH_3COO]^-$ (respectively m/z 758.8 and 786.8) by analogy with PC and to $[M-H-S(CH_3)_2]^-$ (respectively m/z 651.2 and 679.1).

C. MS^2 analysis of the $[M+H]^+$ adducts 715.4 and 743.4. They both give rise to a neutral loss of 200 corresponding to the polar head of PDPT as described in Fulton et al., 2014. In the insert a zoom of the 400 m/z region show the fragments $[M+H-S(CH_3)_2\text{-}RCOOH]^+$ showing that m/z 715 and 743 correspond to the molecule 14:0/16:4 and 16:0/16:4 respectively.

D. MS^2 analysis of the $[M+Na]^+$ adducts 737.2 and 765.2. They both give rise to a neutral loss of 62 corresponding to the loss of $S(CH_3)_2$ probably the same way the sodium adduct of PC is losing $N(CH_3)_3$ (m/z 59).The isolated m/z 765.2 $[M+Na]^+$ adduct is probably contaminated with an $[M+H]^+$ adduct that will give the neutral loss of 200.

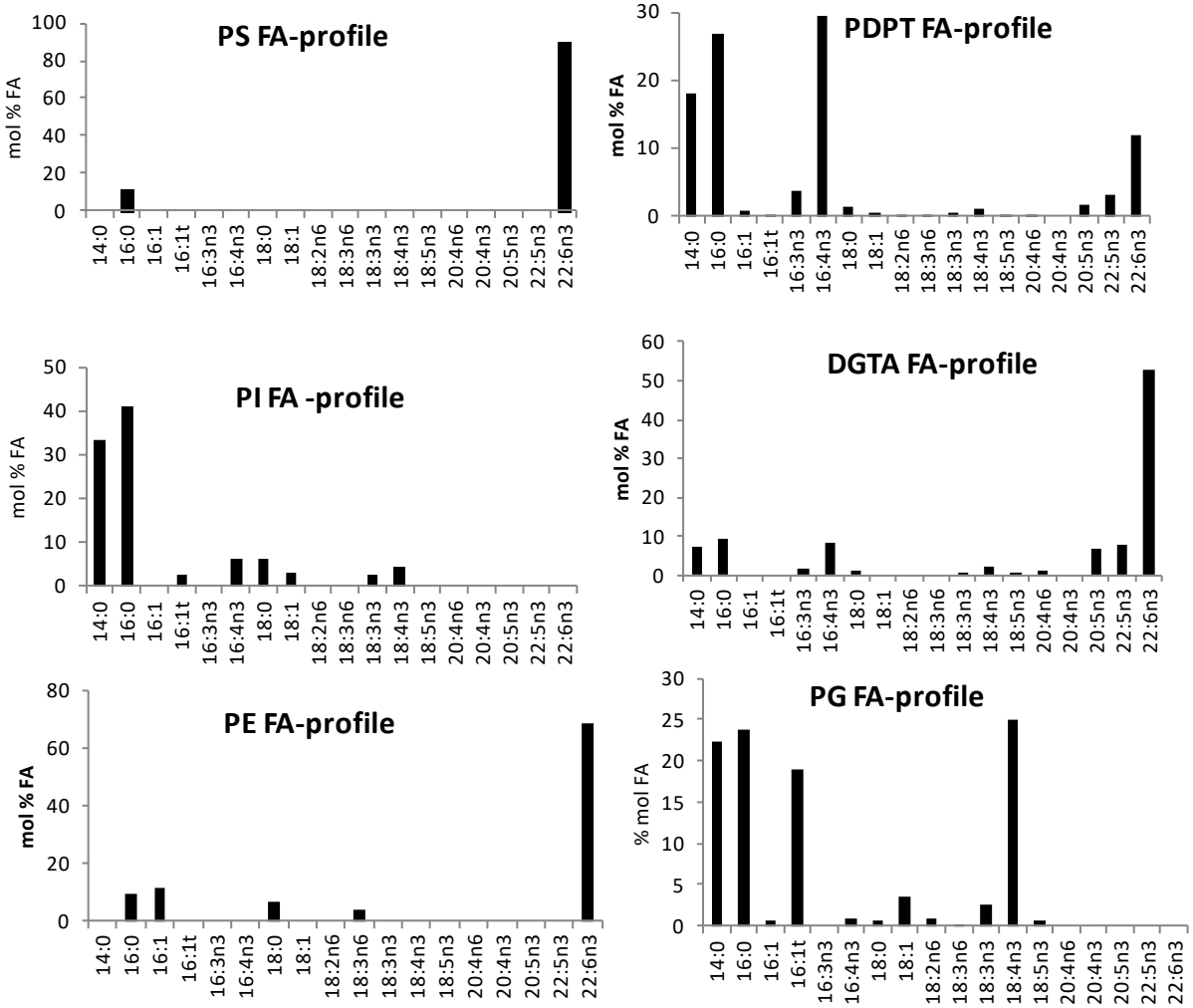


Supplemental figure 3. 1D-HPTLC developments of polar and neutral lipids of Mamiellales species. The polar solvent used was that described by Mock and Kroon (2002) and consisted in a mixture of methyl acetate/isopropanol/chloroform/methanol/KCl 0.25% (25:25:25:10:4 v/v/v/v). (A). Neutral lipids were separated in hexane/diethyl-ether/glacial-acetic-acid (60:10:1.22 v/v/v) (B). Lipids fraction deduced from standard migration (STD right from the figure) and MS-MS analysis are indicated on the left. Standard (STD) are indicated on the right. Top labels correspond to species: 1. O. sp. RCC788, 2. O. mediterraneus. 3. O. lucimarinus RCC802, 4. O. sp. RCC808, 5. O. tauri, 6. O. lucimarinus RCC3401 7. Micromonas pusilla 8. Bathycoccus prasinos

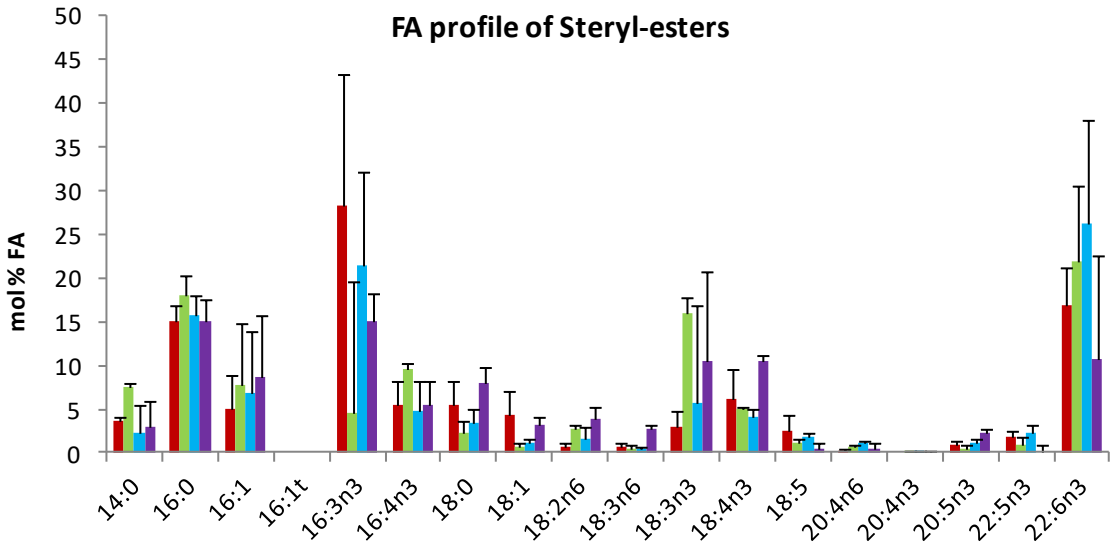


Supplemental figure 4. Variation of lipid- and FA- profiles in the genus Ostreococcus according to the experimental set.

The 4 different species of Ostreococcus have been averaged for each experiment. Error bars are SEM. Note that variations are reduced compared to the variations observed for a given species between the three experiments: differences were greater between experimental sets than between species. TAGs were accumulated in experiment 2 and 3 and phospholipids were decreased while non-phosphorus lipids were increased (SQDG, DGTA). ALA was increased at the expense of SDA in experiment 2 and 3. These changes were shown to be the result of phosphorus limitation in *O. tauri* (see below). It is therefore likely that the lots of natural sea water used as culture media basis in these experiments might have contained more or less phosphorus. Though not intended, the variations observed allowed further to deduce that the glycerolipidome from the distinct *Ostreococcus* clades varied in a similar way depending on the nutrient enrichment of the culture media.



Supplemental figure 5. FA composition of O. tauri phospholipids and DGTA.
Phospholipids and DGTA were resolved by 2D-developments and analyzed by FAMES-GC-FID.

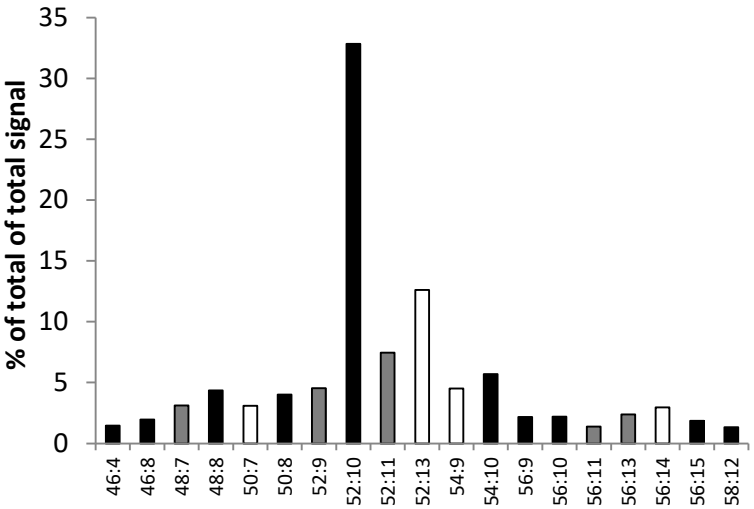


Supplemental figure 6. FA composition of steryls esters of *Ostreococcus* mediterranean species.

mol sp	sn-1/sn-2	PG	SQDG	MGDG	DGDG	PI	PS	PE	PDPT	DGTA
28:0	14:0/14:0	12	4		2	35				
28:1	14:0/14:1									1
30:0	14:0/16:0 - 16:0/14:0	9	13		4	54				
30:1	14:0/16:1 (S); 16:0/14:1 (B)		2							1
30:3	14:0-16:3								9	3
30:4	14:0/16:4								35	9
32:0	16:0/16:0				4	10			=	
32:1	16:1/16:0	4								
32:2	18:2/14:0				3					
32:3	18:3/14:0 (S,M,D) - 16:3/16:0 (S) 16:0/16:3 (T,B)		5	2	15				9	3
32:4	18:4/14:0 (S,D) - 16:4/16:0 (S) - 16:0/16:4 (P,T,B)		7	2	16				36	11
32:5	18:5/14:0 (D)		1	2	5					
32:6	na		4	1						
32:7	16:3/16:4			**1**						
32:8	16:4/16:4			2						
34:1	18:1-16:0 (P)	6	1							
34:2	18:2/16:0 (S)	4	5		3					
34:3	18:3/16:0	9	29		12					
34:4	18:4/16:0 (P, S, M,D)- 16:0/18:4 ;*14:0/20:4; 18:0-16:4* (B)	25	32	2	5					2
34:5	18:4/16:1 (P) ; 14:0-20:5 (B)	32	1	3						1
34:6	18:3-16:3 ; 18:2-16:4			5						
34:7	18:3/16:4 (M, D) ; 18:4/16:3 (D)			26	11					
34:8	18:4/16:4			40	12					
34:9	18:5/16:4			16	8					
36:5	14:0/22:5									3
36:6	14:0/22:6								5	6
36:7	22:6/14:1									1
36:8	20:4/16:4									2
36:9	20:5/16:4									2
38:5	16:0/22:5									2
38:6	16:0/22:6								6	5
38:9	22:6/16:3, 22:5/16:4, 20:5/18:4									3
38:10	22:6/16:4									4
40:9	18:3/22:6									2
40:10	20:5-20:5 ; 18:4/22:6									2
42:10	22:6/20:4 ; 22:5/20:5									2
42:11	22:6/20:5									3
44:11	22:5/22:6									8
44:12	22:6/22:6						100	100		26

Supplemental figure 7. Positionnal analysis of FA in polar glycerolipids.
Molecular species (mol sp) and the corresponding *sn-1/sn-2* combinations are shown in column 1 and 2. The letters in brackets refers to the lipid class in which the FA combination was detected. (P: PG S: SQDG, D:DGDG, M: MGDG, B: DGTA T: PDPT). The percentage of the total signal for each lipid class analyzed is indicated and corresponding bars are scaled according to the highest percentage detected in each lipid class. na: not analyzed.

A



B

mol sp	*sn-1,3/sn-2/sn-1,3*
46:4	14:0/14:0/18:4
46:8	16:4/14:0/16:4
48:8	16:4/14:0/18:4
50:8	16:4/16:0/18:4
52:10	16:4/14:0/22:6
54:10	16:4/16:0/22:6
	18:4/14:0/22:6
56:9	18:3/16:0/22:6
	18:4/16:0/22:5
56:10	18:4/16:0/22:6
56:15	18:5/16:4/22:6
58:12	22:6/14:0/22:6

C

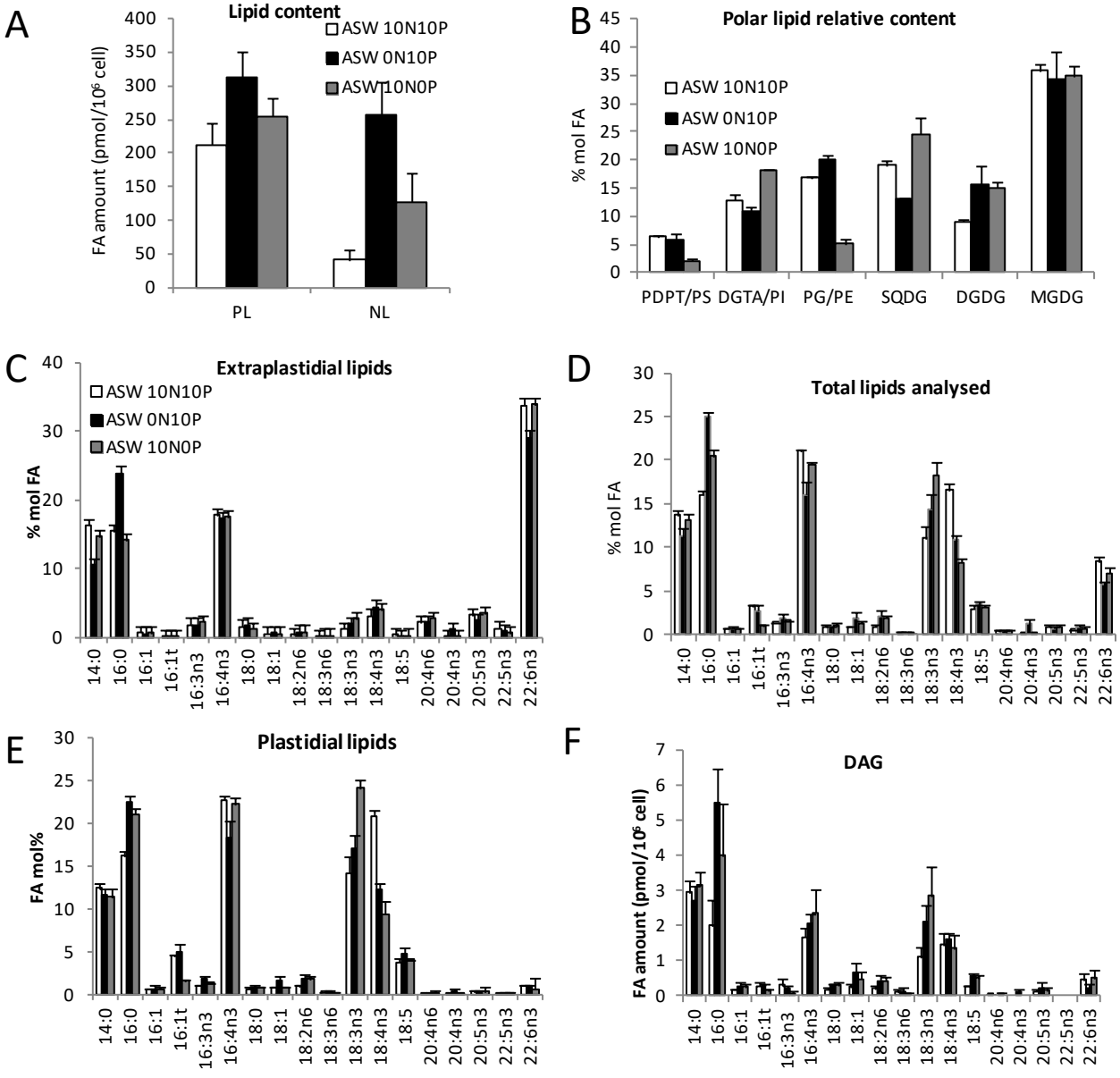
mol sp	*sn-1,3/sn-2/sn-1,3*
48:7	16:3/14:0/18:4
	16:4/14:0/18:3
52:9	16:3/14:0/22:6
	16:4/14:0/22:5
	18:4/14:0/20:5
	16:4/16:0/20:5
52:11	18:3/16:4/18:4
	18:4/16:3/18:4
56:11	16:4/18:1/22:6
	16:3/18:3/22:5
	18:3/18:3/20:5
	18:5/18:1/20:5
56:13	16:3/18:4/22:6
	16:4/18:3/22:6
	16:4/18:4/22:5

D

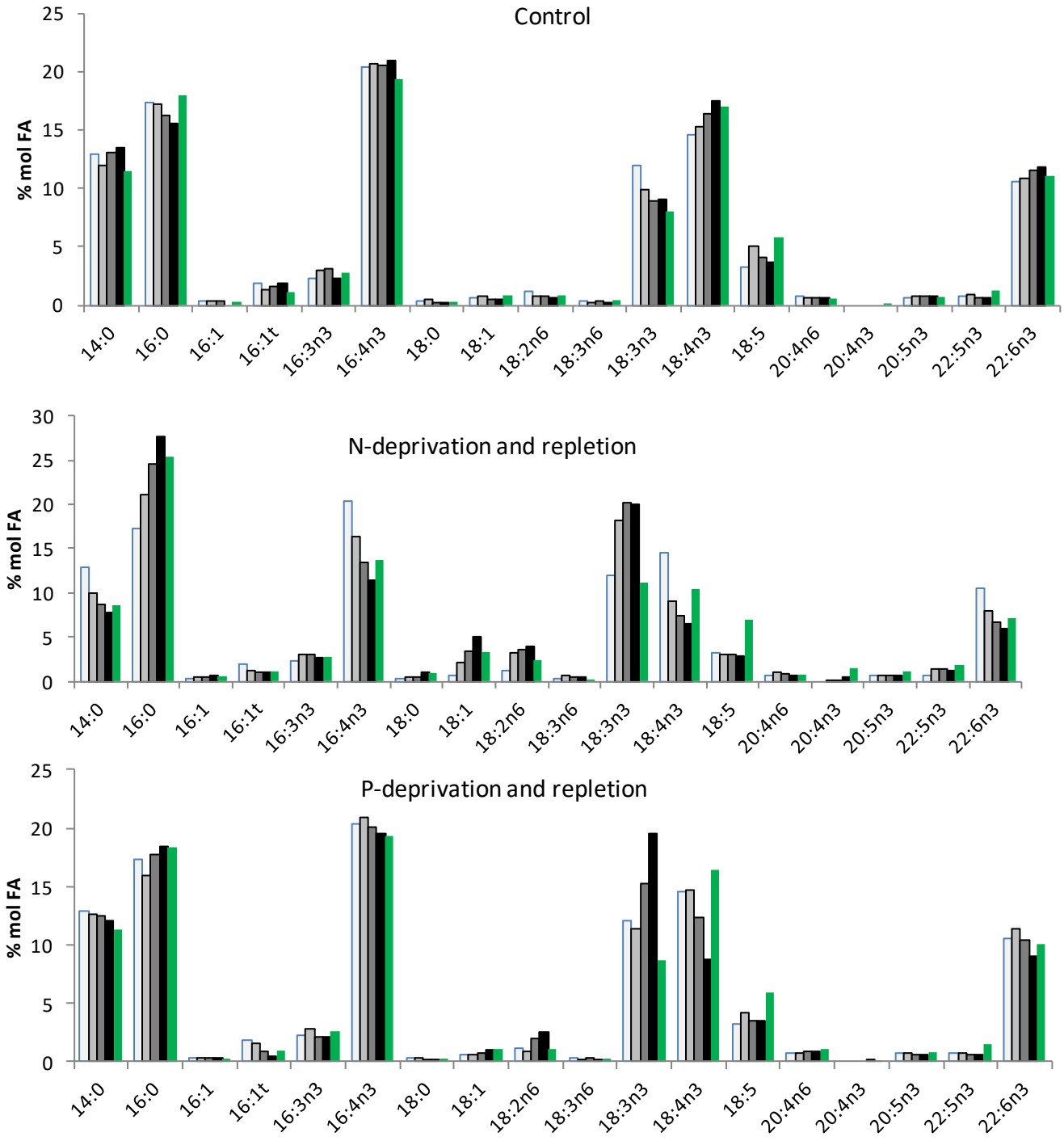
mol sp	*sn-1,3/sn-2/sn-1,3*
46:4	14:0/16:0/16:4
50:7	16:4/16:0/18:3
	16:3/16:0/18:4
	18:3/14:0/18:4
52:13	18:4/16:4/18:5
	16:4/16:4/20:5
54:9	18:4/16:0/20:5
	16:3/16:0/22:6
	16:4/16:0/22:5
	18:3/14:0/22:6
	18:4/14:0/22:5
56:14	16:4/18:4/22:6
	18:5/16:3/22:6
	18:5/16:4/22:5

Supplemental figure 8. FA positionnal analysis in TAGs.

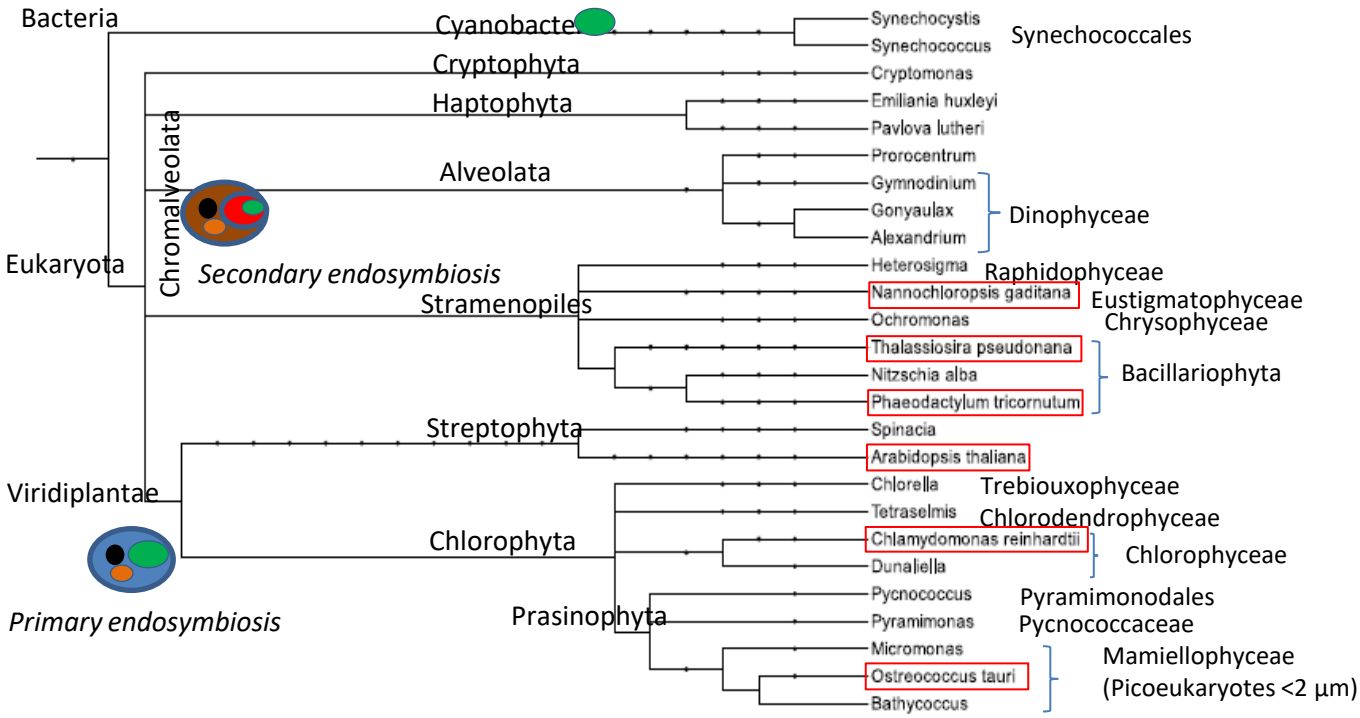
A. Relative abundance of detected molecular species (mol sp) indicated in absciss expressed as percentage of the total signal from analyzed peaks. Colour of the bars reflect the degree of confidence in the corresponding FA-positional analysis : black correspond to certainty, grey to likely position that would require MS^3 to be ascertained; white correspond to mix of molecular species whose FA-position could not be determined. (B-C) FA-positionnal analysis of TAGs shown in (A). B. TAGs Molecular species for which the FA-position is determined. C. TAGs species for which the FA-position is the most likely but would require MS3 nalysis to be ascerytained. D. TAGs molecular species of undertermined FA position.



Supplemental figure 9. Effect of N and P deprivation on lipid and FA-profiles of various lipid pools.
Cells were starved for three days. Means of three independent experiments are shown. Bars are SEM. A. Cellular amounts of polar and neutral lipids. B. Content of structural glycerolipids relative to the total of FA in polar lipids. A to E. Relative FA composition variations in various lipid pools upon N and P deprivation. PDPT/PS, DGTA/PI were assigned to the pool of non plastidial lipids in (A), while PG, SQDG, MGDG, DGDG were assigned to the pool of plastidial lipids in (B). F. FA amounts in DAGs.



Suplemental figure 10. FA composition changes upon a kinetics of nutrient depletion and repletion. Global FA profile of control cells, N deprived cells and P deprived cells are shown at the start of the experiment (white bars) , 1 day (light grey bars), 2 days (grey bars) 3 days (black bars) after depletion and 17hrs after medium repletion (green bars). Repletion was performed adding 10N and 10 P to N and P starved culture respectively, and adding 10N 10P to control cultures.



Suplemental figure 11. Phylogenetic Tree representing of chosen photosynthetic organisms. The tree has been designed by PyloT (http://phylot.biobyte.de/) (Letunic and Bork 2016). Super-group, division, and when relevant, class are indicated. Genus /species represented correspond to organisms that were informative with respect to lipid and/or FA composition for the present work. Red boxes indicates the availability of a complete molecular tool-box. Primary and secondary endosymbiotic events are schematized : the engulfment of cyanobacteria by an ancestral non photosynthetic eukaryote (black nucleus, orange mitochondria) gave rise primary chloroplast (two membranes); the engulfement of photosynthetic eukaryote, a red microalga in the case of chromalveolates, gave rise to secondary plast which display additional membranes.