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Photosynthetic pigments of oceanic Chlorophyta belonging to prasinophytes clade VII.

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Abstract

The ecological importance and diversity of pico/nano-planktonic algae remains poorly studied in marine waters, in part because many are tiny and without distinctive morphological features. Amongst green algae, Mamiellophyceae such as *Micromonas* or *Bathycoccus* are dominant in coastal waters while prasinophytes clade VII, yet not formerly described, appear

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to be major players in open oceanic waters. The pigment composition of 14 strains representative of different sub-clades of clade VII was analyzed using a method that improves the separation of loroxanthin and neoxanthin. All the prasinophytes clade VII analyzed here showed a pigment composition similar to that previously reported for RCC287 corresponding to pigment group prasino-2A. However we detected in addition astaxanthin for which it is the first report in prasinophytes. Among the strains analyzed the pigment signature are qualitatively similar within sub-clades A and B. In contrast, RCC3402 from sub-clade C (*Picocystis*) lacks loroxanthin, astaxanthin and antheraxanthin, but contains alloxanthin, diatoxanthin and monadoxanthin, that are usually found in diatoms or cryptophytes. For sub-clades A and B, loroxanthin was lowest at highest light irradiance suggesting a light–harvesting role of this pigment in clade VII as in *Tetraselmis*.

Keywords : phytoplankton, picoplankton, prasinophytes, pigments, HPLC

The paraphyletic group of prasinophytes is an assemblage of free-living unicellular microalgae present in both marine and freshwater habitats (Leliaert et al. 2012). Molecular phylogenetic, ultra-structural, and biochemical approaches have helped taxonomists to re-organize gradually the group into new classes and clades (Guillou et al. 2004, Marin and Melkonian 2010, Subirana et al. 2013, Lemieux et al. 2014a). Currently the prasinophytes are divided into nine groups known as clades I to IX, based on phylogenetic analyses of the nuclear 18S (nuclear-encoded small subunit rRNA) gene (Fawley et al. 2000, Guillou et al. 2004, Viprey et al. 2008). These clades may correspond to true classes, or be composed of a small number of species or of environmental sequences only. For example, Chlorodendrophyceae (Massjuk 2006) known previously as prasinophytes clade IV was recently raised to the class level and added to the "core of chlorophytes" (Fucikova et al. 2014). Clade V corresponds to the order Pycnococaceae with two major species, *Pseudoscourfieldia marina* and *Pycnococcus provasolii* which are probably two forms of a single life cycle (Fawley et al. 1999, Guillou et al. 2004). Clades VIII and IX are composed entirely by environmental sequences without representatives in culture (Viprey et al. 2008). Clade II, previously corresponding to the order Mamiellales, was raised recently to the class level as Mamiellophyceae (Marin and Melkonian 2010) and contains three important genera of marine pico-phytoplankton: *Micromonas* (Butcher 1952), *Bathycoccus* (Eikrem and Throndsen 1990) and *Ostreococcus* (Chrétiennot-Dinet et al. 1995).

In coastal waters, Mamiellophyceae appear largely dominant, especially within the pico-plankton, with the genus *Micromonas* making the highest contribution and followed to a lesser extent by *Bathycoccus* (Throndsen, J. and Kristiansen 1991, Not et al. 2004, Collado-Fabri et al. 2011, Balzano et al. 2012). In contrast in the open ocean, another group of prasinophytes, clade VII, with cell size in the 3 to 5 µm range, has been found to make an important contribution to the pico-plankton community in regions such as the Equatorial Pacific and Mediterranean Sea (Moon-van der Staay et al. 2000, Viprey et al. 2008, Shi et al. 2009). The distribution of clade VII in typically oceanic mesotrophic waters makes this an interesting group. Prasinophyte clade VII contains several cultured strains mostly from tropical and sub-tropical waters but also from temperate regions. Although it has not been described formerly yet. Guillou et al. (2004) divided this group into three well-supported sub-clades, A, B and C, the latter being formed by *Picocystis salinarum*, a small species found in saline lakes (Lewin et al. 2000, Roesler et al. 2002, Krienitz et al. 2012).

Traditionally, pigment signature has been used to determine the taxonomy of algae groups present in the water column (Jeffrey et al. 2011). This approach has been largely superseded by molecular approaches (Liu et al. 2009) but pigments remain an important phenotypic characteristic that allowed to point out the importance of green algae in specific regions of Pacific Ocean, Mediterranean Sea or Arctic Ocean (Obayashi and Tanoue 2002, Miki et al. 2008, Gutiérrez-Rodríguez et al. 2010, Coupel et al. 2014). The study of pigments in different types of prasinophytes has revealed a diversity of photosynthetic signatures in this group. Prasinophytes can be divided into three major groups based on their carotenoid composition (Egeland et al. 1997, Garrido et al. 2009). Group 1 contains the basic set of carotenoids present in Chlorophyceae: neoxanthin, violaxanthin, lutein, zeaxanthin, antheraxanthin and β-β-carotene. Group 2 consists of the basic set of carotenoids plus loroxanthin (2A) and siphonaxanthin (2B). Group 3 contains prasinoxanthin (3A) and uriolide, micromonal, micromonol and dihydrolutein (3B) in addition to the main pigments found in group 1 (Jeffrey et al. 2011).

Within clade VII, only three strains have been analyzed until now: two isolates of *Picocystis salinarum* (subclade C) from saline lakes (Lewin et al. 2000, Roesler et al. 2002) and the marine strain RCC287 (subclade A; Latasa et al. 2004). A large number of clade VII strains are available from the Roscoff Culture Collection (http://roscoff-culture-collection.org/) originating from a range of environment. The aim of this study was to determine the phenotypic characteristics of this important group of marine green algae by analyzing the pigment composition of fourteen strains belonging to the three sub-clades (A, B, C) of prasinophytes clade VII isolated from a range of oceanic location and depths (Table 1). We also assessed the effect of three light irradiances on pigment composition for a subset of these strains.

Twelve strains belonging to clade VII (Table 1) were grown at 22°C in in 25 cm^2 culture flasks with 50 ml of K seawater medium (Keller et al. 1987) under 140 µmol photons $\cdot m^{-2} \cdot s^{-1}$ in continuous light. Two other strains, added later, were grown under the same conditions except for light (100 µmol photons $\cdot m^{-2} \cdot s^{-1}$ in 12:12 Light:Dark cycle). A subset of nine strains was also grown at two other light levels (14 and 65 µmol photons $\cdot$ $m^{-2} \cdot s^{-1}$). All strains were acclimated to the light conditions during at least five generations. Prior to sample collection, cell concentration was determined by flow cytometry using a Becton Dickinson Accuri C6. Approximatively 50 ml of cultures were collected in late exponential or early stationary phase by filtration onto glass fiber GF/F filters (Whatman, Maidstone, UK) without vacuum. Total time for filtration did not exceed 10 min and filters were removed as soon as the passage of liquid trough it was undetectable. Total volume filtered was recorded. Filters were protected from light at all processing stages, immediately frozen in liquid nitrogen and stored at -80°C. Pigments were analyzed within one month. Frozen filters were extracted with 3 mL of 90% acetone in screw cap glass tubes with polytetra-fluoroethylene (PTFE) lined caps, placed in an ice-water bath. After 15 min, filters were homogenized using a stainless steel spatula for filter grinding. Tubes were placed in an ultrasonic bath with water and ice for 5 min. The slurries were then centrifuged 5 minutes at 4.500 r.p.m. and supernatants filtered through 13 mm diameter polypropylene syringe filters (MS PTFE, 0.22 µm pore size) to remove cell and filter debris. Before injection 1 mL of each sample extract was added with 0.4 mL of Milli-Q water to avoid peak distortion. Pigments extracted from clade VII strains were analyzed using a modification of Zapata et al. (2000) method, described by Garrido et al. (2009) to improve the separation of loroxanthin and neoxanthin (Table S1 in the Supporting Information). Pigment extracts of RCC3402 (*Picocystis*) were also analyzed employing a polymeric octadecyl silica column as described by Garrido and Zapata (1997). All graphs and analyses were performed with the R software using the ggplot2 and FactoMineR libraries (R Development Core Team 2013).

Intracellular chlorophyll (Chl) *a* content ranged from 4 to 26 fg per cell in most strains except for RCC996 (VIIA) and RCC3402 (*Picocystis* - clade VIIC) for which it was much higher (Table 2). This range agreed with values previously determined for marine microalgae in the same size range (Simon et al. 1994). More recently, in a field survey, Giovagnetti et al (2013) found 20-60 fg per cell in nanophytoplankton (>3 µm). Brunet et al (2006) estimated a range of 17-168 fg per cell in picoeukaryotes from the DCM and finally, DuRand et al. (2002) as well as Not et al. (2004) reported 25 fg per cell in the picoplanktonic species *Micromonas pusilla*.

All the prasinophytes clade VIIA and B analyzed here showed a very similar pigment composition (Table 2). It did not seem to change drastically between sub-clades A and B, nor with the depth of isolation (Fig. 1). This composition is similar to that reported for RCC287 by Latasa et al. (2004) corresponding to pigment group prasino-2A. We did not observe strong differences (Fig. 1; Table 2) with the data of Latasa et al. (2004): in particular the ratios obtained for zeaxanthin and lutein were very similar in both studies despite the slight difference in light levels (100 *vs*. 140 µmol photons $\cdot$ m^{-2} $\cdot$ s^{-1} in our study): zeaxanthin, 0.042 (w/w) *versus* 0.043 (w/w) and lutein, 0.382 (w/w) *versus* 0.363 (w/w). However their study used a less resolutive method and did not report the presence of loroxanthin and astaxanthin in RCC287. For loroxanthin this is probably due to the co-elution of this pigment with neoxanthin in the analytic method employed by these authors.

In our study, only RCC1124 and RCC1871 (both from sub-clade A) did not contain loroxanthin within strains belonging sub-clades A and B (Table 2). Violaxanthin and lutein were the most abundant carotenoids for sub-clades A and B. Astaxanthin came as third for most other A and B strains except for RCC1871 (sub-clade A) and RCC2339 (sub-clade B) for which it was neoxanthin and β-β-carotene, respectively. *Picocystis* (RCC3402, clade VIIC) had a clearly distinct carotenoid profile compared to sub-clades A and B. It did not contain loroxanthin, astaxanthin and antheraxanthin but instead diatoxanthin, alloxanthin and monadoxanthin (Fig. 1; Table 2). For this strain, β-β-carotene, monadoxanthin and diatoxanthin were the most abundant carotenoids, respectively (Fig. 1; Table 2) and the ratio of accessory pigments to Chl *a* was much lower than in clades VIIA and B (Fig. 1). The presence in *Picocystis* of these pigments usually found in cryptophytes or diatoms (Takaichi 2011), that are parts of the so-called red lineage (by opposition to the green lineage to which clade VII belongs, Falkowski et al. 2004) was also reported by Lewin et al. (2000) and Roesler et al. (2002) as well as found in *Coccomyxa,* a green alga belonging to the Chlorophyceae (Crespo et al. 2009).

We analyzed the influence of irradiance (14, 65 and 140 µmol photons · m^{-2} · s^{-1}) on pigment composition of nine strains of prasinophytes VIIA and B (Fig. 2; Table S2 in the Supporting Information). Accessory chlorophylls and carotenoids involved in light harvesting tend to increase relative to Chl *a* at low light, while photoprotective carotenoids increase at high light (Schlüter et al. 2000, Henriksen et al. 2002, Brunet et al. 2011a). In our study, Chl *b* ratios increased slightly at low light, as expected, except for RCC3376 that showed a very slightly lower ratio at low light than at high light (0.78 *vs.* 0.81; Fig. 2; Table S2). A similar slight decrease was also observed by Garrido et al. (2009) for the green alga *Tetraselmis suecica*.

The increase at low light of neoxanthin, β-ε carotene and loroxanthin points to a light harvesting role for these pigments in most of the strains (Fig. 2; Table S2). The changes can be subtle, as in the case of neoxanthin or drastic, as observed for loroxanthin (Fig. 2). Neoxanthin has been found to be associated with light harvesting complexes in the Mamiellophyceae *Mantoniella squamata* (Wilhelm and Lenarz-Weiler 1987). A major light harvesting role could be suggested for loroxanthin in clades VII A and B in agreement with what observed Garrido et al. (2009) in another Chlorophyta *Tetraselmis*. Interestingly, two strains lacking loroxanthin (RCC1124 and RCC1871) have been isolated from temperate North Atlantic Ocean waters, in contrast to the other strains from subclade A which originate from tropical waters (Table 1).

The increase of astaxanthin (from 2 to 4-fold depending on the strains) with light intensity suggests that this carotenoid has a photoprotective role (Fig. 2), as previously demonstrated in the Chlorophyceae *Haematococcus pluvialis* (Wang et al. 2003, Gao et al. 2012). Among all strains, RCC3374 showed the most impressive accumulation of astaxanthin which contributed up to 42% of the total carotenoid pool under high light conditions (Fig. 2). In comparison, *H. pluvialis* can accumulate 86 - 90% of astaxanthin in the total carotenoid pool after sixteen days cultures of under stress conditions (Sarada et al. 2002).

The photoprotective role attributed to lutein (Jahns and Holzwarth 2012) seems to happen also in these species. Its contribution to total carotenoids increased sharply from low to medium light and stabilized at the highest irradiance (Fig. 2; Table S2). Such increase under high light conditions has been previously reported by Böhme et al. (2002) in the Mamiellophyceae *M. squamata*. These authors suggested that lutein played an important role as intermediate of biosynthesis for light harvesting pigments after light shifts from HL to LL. This role was coherent with its loose binding to the LHC apoprotein, also observed for the violaxanthin cycle (VAZ) carotenoids. However, lutein and loroxanthin are xanthophylls derived from β-ε carotene, and both have also been suggested also to take part in photoprotective mechanisms (non-photochemical quenching, NPQ) to prevent photo-oxidative damage in high light conditions in the green alga *Chlamydomonas reinhardtii* (Niyogi et al. 1997).

As for lutein, the content of the photoprotective xanthophyll cycle involving violaxanthin, antheraxanthin and zeaxanthin (VAZ cycle) relative to Chl *a* increased from low to medium light and then stabilized (Fig. 2; Table S2). However the evolution of individual pigments differed among strains. For example, zeaxanthin did not change much in RCC287 and RCC857 while it increased several-fold in other strains (e.g., RCC719, Fig. 2).

A relationship between strain origin and pigment composition is unlikely according to a Principal Component Analysis (PCA) based on pigments to Chl *a* ratios (Fig. 3). The first two components explained more than 50% (dimension 1 and 2, 33.1 % and 20.4 %, respectively) of the variance. Pigments contributing positively to dimension 1 included some which may have a photoprotective role (lutein, zeaxanthin, antheraxanthin and astaxanthin) while pigments suggested to be involved in light harvesting, such as loroxanthin contributed negatively to this axis. Pigments with moderate response to light, such as Chl *b* and neoxanthin, contributed to dimension 2. Strains distributed along dimension 1 according to the light treatment, irrespectively of their sub-clade, latitude or depth of isolation (surface *vs*. DCM). The use of HPLC data to assess the role of individual pigments as light-harvesting or photoprotective must be considered with caution. Photoacclimation processes operate at different scales (from seconds to several days) and pigment changes are influenced by multiple factors (genetics, ecology, physiology). Despite all this, some common patterns can be found when pigment data are given in terms of their ratios to Chl *a*. Light-harvesting pigments and Chl *a* content increase under low irradiance, and tend to co-vary under variable light conditions. In turn, photoprotective pigments are synthesized under light stress and increase their ratios to Chl *a* in higher light irradiance (Brunet et al. 2011b). The behavior of pigments analyzed in clade VII resembled that expected for light-harvesting or photoprotective ones, but without a more complete dataset (biochemistry, photosynthetic dynamics, etc.) this cannot be stated unambiguously. The discovery of loroxanthin (a putative light harvesting pigment) and astaxanthin (with a suggested photoprotective role) in prasinophytes clades VIIA and B prompts the need to reexamine the pigment composition of other members of this diverse and ancient group using improved analytical protocols.

Recent phylogenetic results pointed clade VII A/B as a sister group of the core of Chlorophyta (Guillou et al. 2004, Leliaert et al. 2012, Lemieux et al. 2014a, 2014b). The presence of astaxanthin as in core Chlorophyta while it is absent in other prasinophytes may reflect another common feature between clade VII and core Chlorophyta. Moreover while Guillou et al. (2004) included *Picocystis* into clade VII based on the phylogenetic analysis of 18S rRNA gene, the recent analysis of chloroplast genomes (Lemieux et al. 2014a) has shown widely divergent traits between *Picocystis* and sub-clade VIIA. The divergent carotenoid composition of *Picocystis* (absence of loroxanthin, astaxanthin, and antheraxanthin, and confirmation of the presence of red lineage pigments such as diatoxanthin and monadoxanthin) reinforce these phylogenetic analyses and point out the interest of pigments as phenotypic markers.

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Table 1. Characteristics of the strains used in this study. RCC refers to the Roscoff Culture Collection (http://www.roscoff-culture-collection.org/).

RCC	Sub-clade	Strain name	Other names	Ocean origin	Region origin	Latitude	Depth Isolation (m)
15	A	CCMP 1205		NA	NA	NA	NA
287	A	NOUM15	NOUM97015	Pacific Ocean	West Equatorial Pacific	0°	120
719	A	IndianOcean_45-8		Indian Ocean	East Equatorial Indian	12°S	76
856	A	Biosope_42 A2	CCMP3325	Pacific Ocean	Marquesas islands	8°S	10
857	A	Biosope_40 A2		Pacific Ocean	Marquesas islands	8°S	10
996	A	Biosope_46 B4S		Pacific Ocean	South East Pacific	9°S	100
998	A	Biosope_46 C3S	NIES2676, CCMP3334	Pacific Ocean	South East Pacific	9°S	100
1124	A	PAP_AD	PAP_Ludwig_AI	Atlantic Ocean	North Atlantic, PAP site	49°N	10
1871	A	RA090205-09		Atlantic Ocean	North Atlantic, English Channel	49°N	0
3374	A	CCMP 2152	A7831	Pacific Ocean	Hawaii	23°N	NA
3376	A	CCMP 2113	A9533	Pacific Ocean	Central Equatorial Pacific	9°N	85
2337	B	JST MH335	MH335, NIES2756	Pacific Ocean	Iki Island	34°N	0
2339	B	JST MH340	MH340, NIES2758, CCMP3360	Pacific Ocean	Iki Island	34°N	0
3402	C	CCMP 1897	SFBB	Pacific Ocean	San Francisco Bay	38°N	0

Remark : Ordered by clade and then RCC number

Table 2. Concentration of Chl *a* per cell, ratios of pigment to Chl *a* concentration and contribution to total carotenoids (in italics) for fourteen strains of prasinophytes clade VII (Chlorophyta) grown at 140 µE.m^{-2}.s^{-1}.

								Carotenoids																			
Strain	sub-clade	Light	fg Chl *a*	Chl *b*	Chlide *a*	Chlide *b*	Sum of carotenoids	Loroxanthin		Neoxanthin		Violaxanthin		Astaxanthin		Antheraxanthin		Zeaxanthin		Lutein *a*		ββ-carotene		βε-carotene		Alloxanthin	
		µE.m^{-2}.s^{-1}	/cell	/Chl *a*	/Chl *a*	/Chl *a*	/Chl *a*	/Chl *a*	%	/Chl *a*	%	/Chl *a*	%	/Chl *a*	%	/Chl *a*	%	/Chl *a*	%	/Chl *a*	%	/Chl *a*	%	/Chl *a*	%	/Chl *a*	%
RCC15	A	140	20.34	0.901	0.066	0.018	1.321	0.058	*4.37*	0.003	*0.20*	0.327	*24.77*	0.165	*12.47*	0.043	*3.25*	0.103	*7.79*	0.376	*28.45*	0.093	*7.03*	0.154	*11.66*	0	*0.00*
RCC287	A	140	4.99	0.986	0.106	0	1.533	0.024	*1.59*	0.167	*10.91*	0.572	*37.29*	0.210	*13.68*	0.024	*1.56*	0.043	*2.79*	0.363	*23.68*	0.079	*5.17*	0.051	*3.32*	0	*0.00*
RCC287[a]	A	*100*	*Nd*	*1.313*	*Nd*	*Nd*	0.759	*Nd*	*Nd*	*0.074*	*9.75*	*0.131*	*17.26*	*Nd*	*Nd*	*0.051*	*6.72*	*0.042*	*5.53*	*0.382*	*50.33*	*0.053*	*6.98*	*0.026*	*3.43*	0	*0.00*
RCC719	A	140	14.89	0.683	0.000	0	1.928	0.035	*1.80*	0.086	*4.44*	0.617	*31.97*	0.264	*13.71*	0.000	*0.00*	0.478	*24.79*	0.193	*10.02*	0.149	*7.73*	0.107	*5.53*	0	*0.00*
RCC856	A	140	23.37	0.860	0.000	0	2.077	0.113	*5.42*	0.079	*3.79*	0.291	*14.02*	0.570	*27.44*	0.042	*2.02*	0.310	*14.94*	0.461	*22.20*	0.136	*6.53*	0.075	*3.63*	0	*0.00*
RCC857	A	140	4.10	1.000	0.000	0	1.399	0.026	*1.89*	0.122	*8.74*	0.534	*38.15*	0.187	*13.38*	0.024	*1.73*	0.094	*6.69*	0.291	*20.83*	0.064	*4.55*	0.056	*4.03*	0	*0.00*
RCC996	A	140	51.57	0.931	0.074	0	1.259	0.043	*3.39*	0.095	*7.52*	0.119	*9.46*	0.191	*15.17*	0.039	*3.06*	0.245	*19.45*	0.364	*28.88*	0.074	*5.85*	0.091	*7.21*	0	*0.00*
RCC998	A	140	26.07	0.783	0.000	0	1.902	0.014	*0.75*	0.117	*6.15*	0.877	*46.10*	0.157	*8.24*	0.058	*3.04*	0.168	*8.84*	0.285	*14.98*	0.131	*6.90*	0.095	*4.99*	0	*0.00*
RCC1124	A	140	8.61	0.960	0.099	0	1.471	0	*0.00*	0.116	*7.87*	0.525	*35.66*	0.148	*10.07*	0.020	*1.39*	0.086	*5.83*	0.399	*27.14*	0.082	*5.59*	0.095	*6.45*	0	*0.00*
RCC1871	A	100	3.30	1.196	0.000	0	1.296	0	*0.00*	0.119	*9.20*	0.460	*35.45*	0.064	*4.92*	0.015	*1.15*	0.091	*7.03*	0.444	*34.28*	0.025	*1.93*	0.078	*6.03*	0	*0.00*
RCC3374	A	140	4.14	0.726	0.229	0	1.841	0.012	*0.67*	0.082	*4.47*	0.272	*14.78*	0.778	*42.25*	0.041	*2.25*	0.118	*6.39*	0.332	*18.06*	0.146	*7.92*	0.059	*3.22*	0	*0.00*
RCC3376	A	140	4.08	0.813	0.105	0	1.526	0.008	*0.53*	0.112	*7.33*	0.572	*37.47*	0.324	*21.26*	0.054	*3.56*	0.129	*8.47*	0.048	*3.14*	0.194	*12.68*	0.085	*5.57*	0	*0.00*
RCC2337	B	140	4.37	0.882	0.394	0.236	2.186	0.031	*1.40*	0.137	*6.27*	0.504	*23.07*	0.302	*13.82*	0.051	*2.34*	0.154	*7.03*	0.706	*32.30*	0.240	*10.97*	0.061	*2.80*	0	*0.00*
RCC2339	B	140	14.58	0.624	0.000	0	1.520	0.026	*1.74*	0.078	*5.13*	0.613	*40.35*	0.045	*2.98*	0.024	*1.56*	0.074	*4.84*	0.302	*19.86*	0.330	*21.72*	0.028	*1.83*	0	*0.00*
RCC3402	C	100	60.40	0.283	0.000	0	0.583	0	*0.00*	0.039	*6.69*	0.035	*6.03*	0	*0.00*	0.003	*0.53*	0.018	*3.17*	0.071	*12.12*	0.129	*22.06*	0.015	*2.59*	0.047	*8.13*

Nd: Not determined

[a]Values reported by Latasa *et al.* 2004

List of supplementary tables

Table S1. Chromatographic retention times and spectral characteristics of the major pigments for strains of prasinophytes clade VII.

Table S2. Ratios of pigment to Chl *a* concentration and contribution to total carotenoids (in italics) for nine strains of prasinophytes clade VII under three light intensities.

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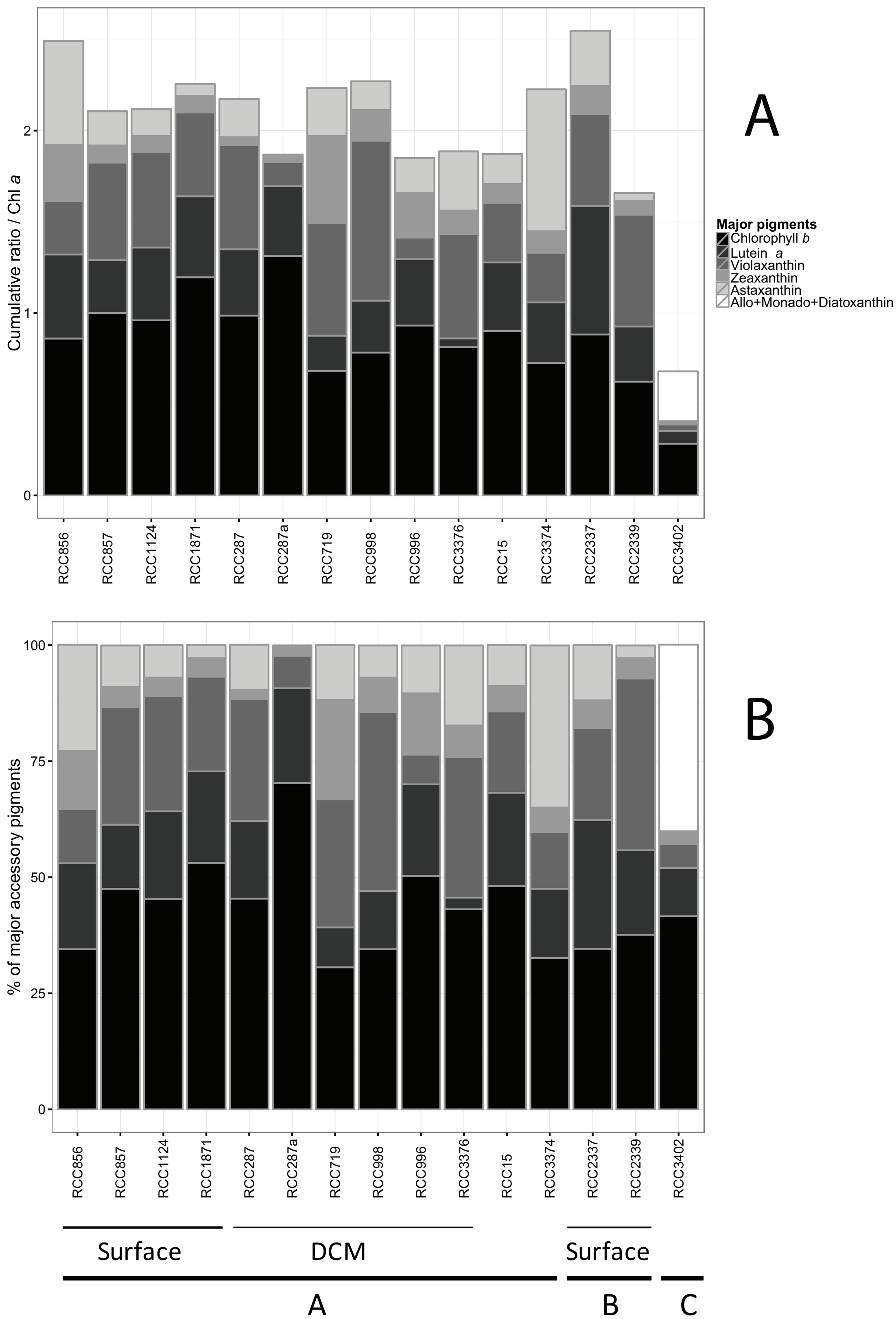
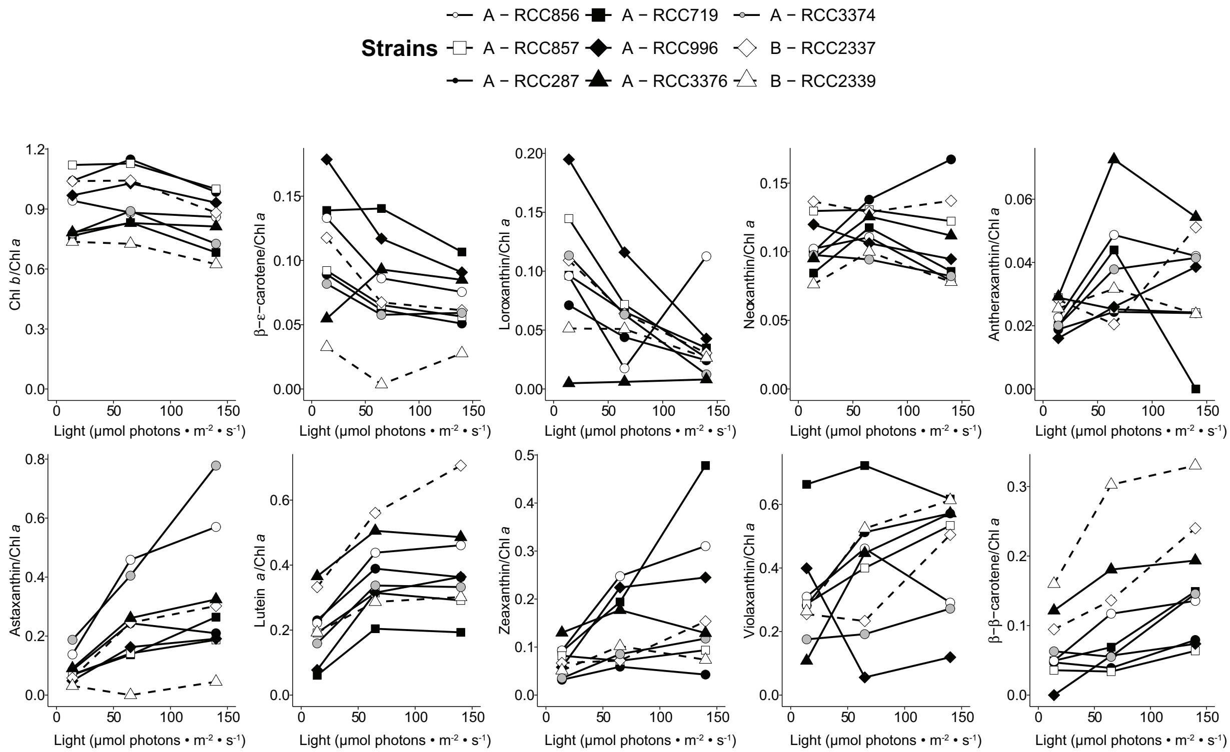
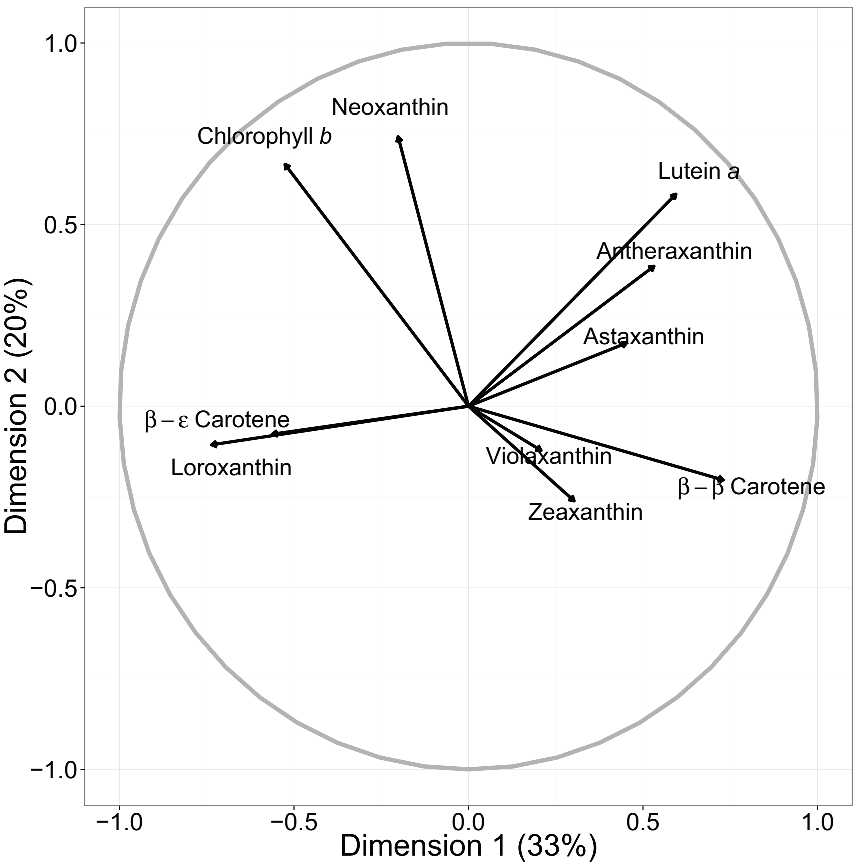
Figure 1. A. Cumulative ratios of Chl *b* and five major carotenoids (lutein *a*, violaxanthin, zeaxanthin, astaxanthin, alloxanthin + monadoxanthin + diatoxanthin) to Chl *a* for 14 strains of prasinophytes clades VII at 140 or 100 µE.m^{-2}.s^{-1} (see Table 2). Strains are ordered by sub-clades (A, B, C) and depth of isolation (surface, deep chlorophyll maximum-DCM). RCC287a correspond to the composition reported by Latasa et al. (2004) for this strain. B. Same as A but with relative abundance of Chl *b* and five major carotenoids.

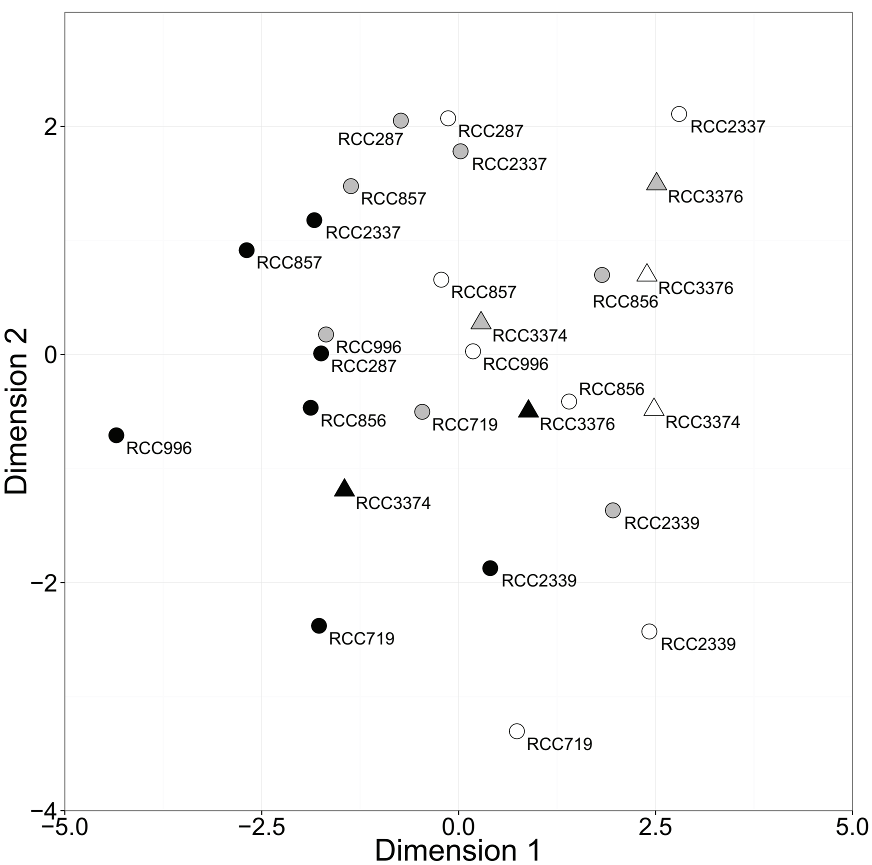
Figure 2. Change in pigment to Chl *a* ratios for Chl *b* and nine major carotenoids in nine strains of prasinophytes clade VII under three light intensities. Solid lines correspond to sub-clade VIIA and dashed lines to VIIB. Open symbols correspond to surface strains, closed ones to DCM strains and grey to unknown depth of isolation.

Figure 3. Principal component analysis using the pigment to Chl *a* ratios as variables for the strains grown at 3 light levels (Table S2). Top. Variables. Bottom. Samples. Circles correspond to clade VIIA and triangles to clade VIIB. Closed symbols correspond to low light, grey symbols to medium light and open symbols to high light.


A
Cumulative ratio / Chl a
0
1
2
Major pigments
Chlorophyll b
Lutein a
Violaxanthin
Zeaxanthin
Astaxanthin
Allo+Monado+Diatoxanthin
RCC856
RCC857
RCC1124
RCC1871
RCC287
RCC287a
RCC719
RCC998
RCC996
RCC3376
RCC15
RCC3374
RCC2337
RCC2339
RCC3402
B
% of major accessory pigments
0
25
50
75
100
Surface
DCM
Surface
A
B
C


Strains
A − RCC856
A − RCC719
A − RCC3374
A − RCC857
A − RCC996
B − RCC2337
A − RCC287
A − RCC3376
B − RCC2339
Chl b/Chl a
β−ε−carotene/Chl a
Loroxanthin/Chl a
Neoxanthin/Chl a
Antheraxanthin/Chl a
Astaxanthin/Chl a
Lutein a/Chl a
Zeaxanthin/Chl a
Violaxanthin/Chl a
β−β−carotene/Chl a
Light (µmol photons • m⁻² • s⁻¹)


Neoxanthin
Chlorophyll b
Lutein a
Antheraxanthin
Astaxanthin
β – ε Carotene
Loroxanthin
Violaxanthin
β – β Carotene
Zeaxanthin
Dimension 2 (20%)
Dimension 1 (33%)
1.0
0.5
0.0
−0.5
−1.0
−1.0
−0.5
0.0
0.5
1.0


RCC287
RCC287
RCC2337
RCC2337
RCC857
RCC3376
RCC2337
RCC857
RCC857
RCC856
RCC3376
RCC3374
RCC996
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RCC996
RCC856
RCC856
RCC719
RCC3376
RCC3374
RCC996
RCC3374
RCC2339
RCC2339
RCC719
RCC2339
RCC719
Dimension 2
Dimension 1
2
0
−2
−4
−5.0
−2.5
0.0
2.5
5.0