



Photosynthetic Pigments of oceanic Chlorophyta belonging to prasinophytes clade VII

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3 **Photosynthetic Pigments of oceanic Chlorophyta belonging to prasinophytes clade VII.**

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18 **Abstract**

19
20 The ecological importance and diversity of pico/nano-planktonic algae remains poorly
21 studied in marine waters, in part because many are tiny and without distinctive morphological
22 features. Amongst green algae, Mamiellophyceae such as *Micromonas* or *Bathycoccus* are
23 dominant in coastal waters while prasinophytes clade VII, yet not formerly described, appear to
24 be major players in open oceanic waters. The pigment composition of 13 strains representative of
25 different sub-clades of clade VII was analyzed using a method that improves the separation of
26 loroxanthin and neoxanthin. All the prasinophytes clade VII analyzed here showed a pigment
27 composition similar to that previously reported for RCC287 corresponding to pigment group
28 prasino-2A. However we detected in addition astaxanthin for which it is the first report in
29 prasinophytes. Among the strains analyzed the pigment signature are qualitatively similar within
30 sub-clades A and B. In contrast RCC3402 from sub-clade C (*Picocystis*) lacks loroxanthin,
31 astaxanthin and antheraxanthin. For sub-clades A and B, loroxanthin was lowest at highest light
32 irradiance suggesting a light-harvesting role of this pigment in clade VII as in *Tetraselmis*.

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34 **Keywords :** phytoplankton, picoplankton, prasinophytes, pigments, HPLC

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39 The paraphyletic group of prasinophytes is an assemblage of free-living unicellular
40 microalgae present in both marine and freshwater habitats (Leliaert et al. 2012). Molecular
41 phylogenetic, ultra-structural, and biochemical approaches have helped taxonomists to re-
42 organize gradually the group into new classes and clades (Guillou et al. 2004, Marin and
43 Melkonian 2010, Subirana et al. 2013, Lemieux et al. 2014a). Currently the prasinophytes are
44 divided into nine groups known as clades I to IX, based on phylogenetic analyses of the nuclear
45 18S (nuclear-encoded small subunit rRNA) gene (Fawley et al. 2000, Guillou et al. 2004, Viprey
46 et al. 2008). These clades may correspond to true classes, or be composed of a small number of
47 species or of environmental sequences only. For example, Chlorodendrophyceae (Massjuk 2006)
48 known previously as prasinophytes clade IV was recently raised to the class level and added to
49 the “core of chlorophytes” (Fucikova et al. 2014). Clade V corresponds to the order
50 Pycnococaceae with two major species, *Pseudoscourfieldia marina* and *Pycnococcus provasolii*
51 which are probably two forms of a single life cycle (Fawley et al. 1999, Guillou et al. 2004).
52 Clades VIII and IX are composed entirely by environmental sequences without representatives in
53 culture (Viprey et al. 2008). Clade II, previously corresponding to the order Mamiellales, was
54 raised recently to the class level as Mamiellophyceae (Marin and Melkonian 2010) and contains
55 three important genera of marine pico-phytoplankton: *Micromonas* (Butcher 1952), *Bathycoccus*
56 (Eikrem and Throndsen 1990) and *Ostreococcus* (Chrétiennot-Dinet et al. 1995).

57 In coastal waters, Mamiellophyceae appear largely dominant, especially within the pico-
58 plankton, with the genus *Micromonas* making the highest contribution and followed to a lesser
59 extent by *Bathycoccus* (Throndsen, J. and Kristiansen 1991, Not et al. 2004, Collado-Fabri et al.
60 2011, Balzano et al. 2012). In contrast in the open ocean, another group of prasinophytes, clade
61 VII, with cell size in the 3 to 5 μm range, has been found to make an important contribution to

62 the pico-plankton community in regions such as the Equatorial Pacific and Mediterranean Sea
63 (Moon-van der Staay et al. 2000, Viprey et al. 2008, Shi et al. 2009). The distribution of clade
64 VII in typically oceanic mesotrophic waters makes this an interesting group. Prasinophyte clade
65 VII contains several cultured strains, but it has not been described formerly yet. Guillou *et al.*
66 (2004) divided this group into three well-supported sub-clades, A, B and C, the latter being
67 formed by *Picocystis salinarum*, a small species found in saline lakes (Lewin et al. 2000, Roesler
68 et al. 2002, Krienitz et al. 2012).

69 Traditionally, pigment signature has been used to determine the taxonomy of algae groups
70 present in the water column (Jeffrey et al. 1997). This approach has been largely superseded by
71 molecular approaches (Liu et al. 2009) but pigments remain an important phenotypic
72 characteristic that allowed to point out the importance of green algae in specific regions of Pacific
73 Ocean, Mediterranean Sea or Arctic Ocean (Obayashi and Tanoue 2002, Miki et al. 2008,
74 Gutiérrez-Rodríguez et al. 2010, Coupel et al. 2014). The use of pigments in order to discriminate
75 different types of prasinophytes has proven to be a difficult task since a diversity of
76 photosynthetic signatures can be found in this group. Prasinophytes can be divided into three
77 major groups based on their carotenoid composition (Egeland et al. 1997, Garrido et al. 2009).
78 Group 1 contains the basic set of carotenoids present in Chlorophyceae: neoxanthin, violaxanthin,
79 lutein, zeaxanthin, antheraxanthin, astaxanthin and β - β -carotene. The two later pigments are
80 found to accumulate in *Haematococcus* and *Dunaliella* species under stress conditions (Lemoine
81 and Schoefs 2010). Group 2 consists of the basic set of carotenoids plus loroxanthin (2A) and
82 siphonaxanthin (2B). Group 3 contains prasinoxanthin (3A) and uriolide/micromonal (3B) in
83 addition to the pigments found in group 2 (Jeffrey et al. 2011)

84 Within clade VII, a single strain RCC287 has been analyzed for its pigment composition
85 (Latasa et al. 2004). A large number of clade VII strains are available from the Roscoff Culture
86 Collection (<http://roscoff-culture-collection.org/>) originating from a range of environment. Their
87 genetic diversity is currently analyzed allowing further delineation of new sub-clades (Lopes et
88 al. in preparation) within the two major sub-clades A and B described by Guillou *et al* (2004).
89 Among the 44 strains of clade VII present in the RCC, 39 belong to sub – clade A. The aim of
90 this study was to determine the phenotypic characteristics of this important group of marine green
91 algae by analyzing the pigment composition of thirteen strains belonging to the three sub-clades
92 (A, B, C) of prasinophytes clade VII and isolated from a range of oceanic location and depths
93 (Table 1). We also assessed the effect of three light irradiances on pigment composition for a
94 subset of these strains.

95 Thirteen strains belonging to clade VII (Table 1) were grown at 22°C in K seawater
96 medium (Keller et al. 1987) under $140 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in continuous light. A subset of nine strains was
97 also grown at two other light levels (14 and $65 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Prior to sample collection, cell
98 concentration was determined by flow cytometry using a Becton Dickinson Accuri C6.
99 Approximately 50 ml of cultures were harvested in late exponential or early stationary phase
100 and were filtered onto glass fiber GF/F (Whatman, Maidstone, UK) without vacuum and
101 immediately frozen in liquid nitrogen and stored at -80°C . Pigments were analyzed within one
102 month. Frozen filters were extracted with 3 mL of 90% acetone in screw cap glass tubes with
103 polytetra-fluoroethylene (PTFE) lined caps, placed in an ice-water bath. After 15 minutes, filters
104 were homogenized using a stainless steel spatula for filter grinding. Tubes were placed in an
105 ultrasonic bath with water and ice for 5 minutes. The slurries were then centrifuged 5 minutes at
106 4.500 r.p.m. and supernatants filtered through 13 mm diameter polypropylene syringe filters (MS

107 PTFE, 0.22 μm pore size) to remove cell and filter debris. Before injection 1ml of each sample
108 extract was added with 0.4 ml of Milli-Q water to avoid peak distortion. Pigments extracted from
109 clade VII strains were analyzed using a modification of Zapata *et al.* (2000) method, described by
110 Garrido *et al.* (2009) to improve the separation of loroxanthin and neoxanthin (Table S1). All
111 graphs and analyses were performed with the R software using the ggplot2 and FactoMineR
112 libraries (R Development Core Team 2013).

113 Intracellular chlorophyll (Chl) *a* content ranged from 4 to 26 fg per cell in most strains
114 except for RCC996 (VIIA) and RCC3402 (*Picocystis* - clade VIIC) for which it was much higher
115 (Table 2). This range agreed with values previously determined for marine microalgae in the
116 same size range (Simon et al. 1994).

117 All the prasinophytes clade VIIA and B analyzed here showed a very similar pigment
118 composition (Table 2). It did not seem to change drastically with between sub-clades A and B,
119 nor with the depth of isolation (Figure 1). This composition is very similar to that reported for
120 RCC287 by Latasa *et al.* (2004) corresponding to pigment group prasino-2A. We did not observe
121 strong differences (Figure 1, Table 2) with the data of Latasa *et al.* (2004): in particular the ratios
122 obtained for zeaxanthin and lutein, were very similar in both studies despite the slight difference
123 in light levels (100 vs. 140 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in our study): zeaxanthin, 0.042 (w/w) *versus* 0.043 (w/w)
124 and lutein, 0.382 (w/w) *versus* 0.363 (w/w). However their study used a less resolutive method
125 and did not report the presence of loroxanthin and astaxanthin in RCC287. For loroxanthin this is
126 probably due to the co-elution of this pigment with neoxanthin in the analytic method employed
127 by these authors. For astaxanthin, our strains were grown in continuous light in contrast to Latasa
128 *et al.* (2004) who used L:D cycles. This might have triggered the synthesis and accumulation of

129 this carotenoid, which is known to be synthesized under stressful conditions, such as high light,
130 UV irradiation or nutrient depletion in Chlorophyceae, like *Haematococcus* and *Dunaliella*
131 (Lemoine and Schoefs 2010) as well in some strains of Trebouxiophyceae such as *Picochlorum*
132 (Lubián et al. 2000). In *Haematococcus*, maximal production of astaxanthin has been obtained
133 under to continuous illumination (Domínguez-Bocanegra et al. 2004)

134 In our study, only RCC1124 (sub-clade A) did not contain loroxanthin within strains
135 belonging sub-clades A and B (Table 2). *Picocystis* (RCC3402, clade VIIC) did not contain
136 loroxanthin, astaxanthin and antheraxanthin (Table 1). Violaxanthin and lutein were the most
137 abundant carotenoids for clade VIIA and VIIB. Astaxanthin came as the third more abundant
138 within VIIA and RCC2337 (VIIB), while β - ϵ -carotene came third for RCC2339 (VIIB). For
139 *Picocystis* (clade VIIC), lutein and β - ϵ -carotene were the most abundant carotenoids (Figure 1,
140 Table 2), but the ratio of accessory pigments to Chl *a* was much lower than in clades VIIA and B
141 (Figure 1).

142 We analyzed the influence of irradiance (14, 65 and 140 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) on pigment
143 composition of nine strains of prasinophytes VIIA and B (Figure 2 and S1, and Table S2).
144 Change in light intensities induce opposite trends in the ratios of photosynthetic and
145 photoprotective pigments relative to Chl *a*. Accessory chlorophylls and carotenoids involved in
146 light capture either increase relative to Chl *a* at low light, while photoprotective carotenoids
147 increase at high light (Schlüter et al. 2000, Henriksen et al. 2002). In our study, Chl *b* ratios
148 increased slightly at low light, as expected, except for RCC3376 that showed a very slightly
149 lower ratio at low light than at high light (0.78 vs. 0.81) (Figure 2 and Table S2). A similar slight
150 decrease was also observed by Garrido *et al.* (2009) for the green alga *Tetraselmis suecica*.

151 Neoxanthin, β - β carotene and loroxanthin appeared to have a light harvesting role for
152 most of the strains, except RCC287 (Figure 2 and Table S2), but change could be subtle as in the
153 case of neoxanthin. Neoxanthin has been found to be associated with light harvesting complexes
154 in the Mamiellophyceae *Mantoniella squamata* (Wilhelm and Lenarz-Weiler 1987). The most
155 drastic changes were observed for loroxanthin (Figure 2) suggesting that this pigment has a major
156 light harvesting role in clades VII A and B in agreement with what observed by Garrido *et al.*
157 (2009) with *T. suecica*. One strain, RCC3376 (sub-clade A), had very low ratios of loroxanthin to
158 Chl *a* which did not seem to change with light (Figure 2).

159 The increase of astaxanthin (from 2 to 4-fold depending on the strains) with light intensity
160 suggests that this carotenoid has a photoprotective role for in clade VIIA and B (Figure 2), as
161 previously demonstrated in the Chlorophyceae *Haematococcus pluvialis* (Wang et al. 2003, Gao
162 et al. 2012). Among all strains, RCC3374 showed the most impressive accumulation of
163 astaxanthin which contributed up to 42% of the total carotenoid pool under high light conditions
164 (Figure 2). In comparison, *H. pluvialis* can accumulate 86 - 90% of astaxanthin in the total
165 carotenoid pool after sixteen days cultures of under stress conditions (Sarada et al. 2002).
166 However RCC3374 cell size (3 μm) is about 7 times smaller than *H. pluvialis* resting cells
167 (25 μm , Gu et al. 2013) and maximum cell density is 3.10^6 cell mL^{-1} . A rough computation show
168 that our RCC3374 culture was able to produce only 0.006 mg/L of astaxanthin against 8.3–10.69
169 mg/l found in old cultures of *H. pluvialis* (Sarada et al. 2002). If one could increase the cell
170 concentration about 1000-fold with a different medium and manipulate astaxanthin cell content
171 through stress, then clade VII could be an interesting contender for astaxanthin production.

172 Lutein also showed a photoprotective behavior. Its contribution to total carotenoids
173 increased sharply from low to medium light and the stabilized at the highest light (Figure 2,
174 Table S2). Such increase under high light conditions has been previously reported by Böhme et
175 al. (2002) in the Mamiellophyceae *M. squamata*. These authors suggested that lutein played an
176 important role as intermediate of biosynthesis for light harvesting pigments after light shifts from
177 HL to LL. This role was coherent with its loose binding to the LHC apoprotein, also observed for
178 the violaxanthin cycle (VAZ) carotenoids. However, lutein and luteoxanthin are xanthophylls
179 derived from β - ϵ carotene, and both have also been suggested also to take part in photoprotective
180 mechanisms (non-photochemical quenching, NPQ) to prevent photo-oxidative damage in high
181 light conditions in the green alga *Chlamydomonas reinhardtii* (Niyogi et al. 1997).

182 As for lutein, the content of the photoprotective xanthophyll cycle involving violaxanthin,
183 antheraxanthin and zeaxanthin (VAZ cycle) relative to Chl *a* increased in general sharply from
184 low to medium light and the stabilized at high light (Figure 2 and S1, Table S2). Their specific
185 behavior however differed among strains. For example, zeaxanthin did not change much in
186 RCC287 and RCC857 while it increased several-fold in other strains (e.g. RCC719, Figure 2).

187 In order to assess the visualize any hidden relationship between strain origin and pigment
188 composition, we performed a Principal Component Analysis (PCA) based on pigments to Chl *a*
189 ratios (Figure 3). The first two components explained more than 50% (dimension 1 and 2, 33.1 %
190 and 20.4 %, respectively) of the variance. Pigments contributing positively to dimension 1
191 corresponded to photoprotective pigments (lutein, β - ϵ carotene, zeaxanthin, violaxanthin,
192 antheraxanthin and astaxanthin) while pigments involved in photosynthetic capture, such as
193 luteoxanthin and β - β -carotene, contributed negatively to this axis. Pigments with moderate

194 response to light, such as Chl *b* and neoxanthin, contributed to dimension 2. Strains under
195 medium and high light were separated from those at low light along dimension 1 which reflects
196 that for several pigments the largest changes occurred between low and medium light and then
197 stabilized at high light. This analysis confirmed that pigment composition and change did not
198 seem to be linked to strain sub-clade (A vs. B) or depth of isolation (surface vs. DCM).

199 Recent phylogenetic results pointed clade VII A/B as a sister group of the core of
200 Chlorophyta (Guillou et al. 2004, Leliaert et al. 2012, Lemieux et al. 2014a, 2014b). The
201 presence of astaxanthin as in core Chlorophyta while it is absent with other prasinophytes may
202 reflect another common feature between clade VII and core Chlorophyta. Moreover while
203 Guillou *et al* (2004) included *Picocystis* into clade VII based on the phylogenetic analysis of 18S
204 rRNA gene, the recent analysis of chloroplast genomes (Lemieux et al. 2014a) has shown widely
205 divergent traits between *Picocystis* on one side and sub-clades VIIA and B, on the other side.
206 The divergent carotenoid composition of *Picocystis* (absence of loroxanthin, astaxanthin, and
207 antheraxanthin) reinforce these phylogenetic analyses.

208 Some of the important findings of this study include the discovery of loroxanthin and
209 astaxanthin in prasinophytes clades VIIA and B, which had not been reported before, as well the
210 possible role of the former pigment as a light harvesting accessory pigment. This should prompt
211 the need to reexamine the pigment composition of other members of this diverse and ancient
212 group constituted by prasinophytes using improved analytical protocols.

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217

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340 **List of tables**

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

343 Table 2. Concentration of Chl *a* per cell, ratios of pigment to Chl *a* concentration and
344 contribution to total carotenoids (in italics) for thirteen strains of prasinophytes clade VII
345 (Chlorophyta) grown at 140 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

346

347 **List of supplementary tables**

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

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

352

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354 Figure 1. Cumulative ratios of Chl *b* and of four major carotenoids (lutein *a*,
355 violaxanthin, zeaxanthin, astaxanthin) to Chl *a* for 13 strains of prasinophytes clades VII at 140
356 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Strains are ordered by sub-clades (A, B, C) and depth of isolation (surface, deep
357 chlorophyll maximum-DCM). RCC287a correspond to the composition reported by Latasa *et al.*
358 (2004) for this strain.

359 Figure 2. Change in pigment to Chl *a* ratios for Chl *b* and five major carotenoids in nine
360 strains of prasinophytes clade VII under three light intensities. Top: pigments involved in light
361 capture. Bottom: pigments involved in photoprotection. Solid lines correspond to sub-clade
362 VIIA and dashed lines to VIIB. Open symbols correspond to surface strains, closed ones to
363 DCM strains and grey to unknown depth of isolation.

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

368

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371 Figure S1. Change in pigment to Chl *a* ratios for Chl *b* and four minor carotenoids in nine strains
372 of prasinophytes clade VII under three light intensities. Solid lines correspond to clade VIIA and
373 dashed lines to VIIB. Open symbols correspond to surface strains, closed ones to DCM strains
374 and grey to unknown depth of isolation.

375

376

377

Table 1

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

Remark : Ordered by clade and then RCC number

Table 2

Strain	sub - clade	Light $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$	fg Chl <i>a</i> /cell	Chl <i>b</i> /Chl <i>a</i>	Chlide <i>a</i> /Chl <i>a</i>	Chlide <i>b</i> /Chl <i>a</i>	Sum of carotenoids /Chl <i>a</i>	Carotenoids																	
								Loroxanthin		Neoxanthin		Violaxanthin		Astaxanthin		Antheraxanthin		Zeaxanthin		Lutein <i>a</i>		$\beta\beta$ - carotene		$\beta\epsilon$ - carotene	
								/Chl <i>a</i>	%	/Chl <i>a</i>	%	/Chl <i>a</i>	%	/Chl <i>a</i>	%	/Chl <i>a</i>	%	/Chl <i>a</i>	%	/Chl <i>a</i>	%	/Chl <i>a</i>	%	/Chl <i>a</i>	%
RCC15	A	140	20.3	0.90	0.066	0.018	1.32	0.058	4.4	0.003	0.2	0.327	24.8	0.165	12.5	0.043	3.3	0.103	7.8	0.376	28.4	0.154	11.7	0.093	7.0
RCC287	A	140	5.0	0.99	0.106	0	1.53	0.024	1.6	0.167	10.9	0.572	37.3	0.210	13.7	0.024	1.6	0.043	2.8	0.363	23.7	0.051	3.3	0.079	5.2
RCC719	A	140	14.9	0.68	0.000	0	1.93	0.035	1.8	0.086	4.4	0.617	32.0	0.264	13.7	0.000	0.0	0.478	24.8	0.193	10.0	0.107	5.5	0.149	7.7
RCC856	A	140	23.4	0.86	0.000	0	2.08	0.113	5.4	0.079	3.8	0.291	14.0	0.570	27.4	0.042	2.0	0.310	14.9	0.461	22.2	0.075	3.6	0.136	6.5
RCC857	A	140	4.1	1.00	0.000	0	1.40	0.026	1.9	0.122	8.7	0.534	38.2	0.187	13.4	0.024	1.7	0.094	6.7	0.291	20.8	0.056	4.0	0.064	4.5
RCC996	A	140	51.6	0.93	0.074	0	1.26	0.043	3.4	0.095	7.5	0.119	9.5	0.191	15.2	0.039	3.1	0.245	19.5	0.364	28.9	0.091	7.2	0.074	5.9
RCC998	A	140	26.1	0.78	0.000	0	1.90	0.014	0.8	0.117	6.1	0.877	46.1	0.157	8.2	0.058	3.0	0.168	8.8	0.285	15.0	0.095	5.0	0.131	6.9
RCC1124	A	140	8.6	0.96	0.099	0	1.47	0.000	0.0	0.116	7.9	0.525	35.7	0.148	10.1	0.020	1.4	0.086	5.8	0.399	27.1	0.095	6.5	0.082	5.6
RCC3374	A	140	4.1	0.73	0.229	0	1.84	0.012	0.7	0.082	4.5	0.272	14.8	0.778	42.3	0.041	2.3	0.118	6.4	0.332	18.1	0.059	3.2	0.146	7.9
RCC3376	A	140	4.1	0.81	0.105	0	1.53	0.008	0.5	0.112	7.3	0.572	37.5	0.324	21.3	0.054	3.6	0.129	8.5	0.048	3.1	0.085	5.6	0.194	12.7
RCC2337	B	140	4.4	0.88	0.394	0.236	2.19	0.031	1.4	0.137	6.3	0.504	23.1	0.302	13.8	0.051	2.3	0.154	7.0	0.706	32.3	0.061	2.8	0.240	11.0
RCC2339	B	140	14.6	0.62	0.000	0	1.52	0.026	1.7	0.078	5.1	0.613	40.4	0.045	3.0	0.024	1.6	0.074	4.8	0.302	19.9	0.028	1.8	0.330	21.7
RCC3402	C	140	131.1	0.26	0.016	0	0.54	0.000	0.0	0.048	8.9	0.014	2.7	0.000	0.0	0.000	0.0	0.071	13.1	0.200	37.0	0.016	3.0	0.191	35.4
RCC287^a	A	100	<i>Nd</i>	1.31	<i>Nd</i>	<i>Nl</i>	0.76	<i>Nd</i>	<i>Nd</i>	0.074	9.7	0.131	17.3	<i>Nd</i>	<i>Nd</i>	0.051	6.7	0.042	5.5	0.382	50.3	0.026	3.4	0.053	7.0

nd: Not determined

^aValues reported by Latasa *et al.* 2004

Table S1

Pigment	Abbreviation	Zapata et al. (2000)		Garrido et al. (2009)	
		Rt (min)	Vis maxima (nm)	Rt (min)	Vis maxima (nm)
Chlorophyllide <i>b</i>	Chlide <i>b</i>	6.21	466, 647		
Chlorophyllide <i>a</i>	Chlide <i>a</i>	11.43	431, 665		
Loroxanthin	Lor	20.18	443, 468	4.81	448, 475
Neoxanthin	Neo	20.18	443, 468	5.07	438, 467
Violaxanthin	Viola	21.92	443, 472	5.39	442, 470
Astaxanthin	Asta	22.94	481	5.63	478
Antheraxanthin	Anth	25.76	447, 476		
Zeaxanthin	Zea	27.85	454, 481		
Lutein	Lut	28.03	447, 476		
Chlorophyll <i>b</i>	Chl <i>b</i>	32.04	463, 649		
Chlorophyll <i>a</i>	Chl <i>a</i>	33.6	432, 664		
$\beta\epsilon$ -carotene	$\beta\epsilon$ -car	35.82	449, 476		
$\beta\beta$ -carotene	$\beta\beta$ -car	36.23	454, 479		

Table S2

Strain	sub - clade	Light μE/m ² /s	FCM								Carotenoids														
			Mean FL3-A /cell	fg Chl <i>a</i> / cell	Chl <i>b</i>	Chlide <i>a</i>	Chlide <i>b</i>	Lor	Lor %	Neo	Neo%	Viola	Viol%	Asta	Asta%	Anth	Anth%	Zea	Zea%	Lut	Lut%	ββ - car	ββ - car%	βε - car	βε - car%
RCC287	A	140	97 734	4.99	0.986	0.106	0.000	0.024	1.6	0.167	10.9	0.572	37.3	0.210	13.7	0.024	1.6	0.043	2.8	0.363	23.7	0.051	3.3	0.079	5.2
		65	134 088	36.28	1.148	0.077	0.000	0.044	2.9	0.138	9.1	0.512	33.9	0.243	16.1	0.024	1.6	0.059	3.9	0.389	25.8	0.062	4.1	0.039	2.6
		14	187 046	20.13	1.041	0.000	0.000	0.071	7.4	0.100	10.5	0.282	29.5	0.085	8.9	0.019	2.0	0.032	3.3	0.230	24.1	0.089	9.3	0.047	4.9
RCC719	A	140	90 702	14.89	0.683	0.000	0.000	0.035	1.8	0.086	4.4	0.617	32.0	0.264	13.7	0.000	0.0	0.478	24.8	0.193	10.0	0.107	5.5	0.149	7.7
		65	101 762	22.74	0.832	0.000	0.000	0.066	3.9	0.118	6.9	0.722	42.7	0.137	8.1	0.044	2.6	0.194	11.5	0.204	12.1	0.140	8.3	0.069	4.1
		14	214 898	26.24	0.766	0.000	0.000	0.096	7.6	0.084	6.6	0.663	52.1	0.069	5.4	0.020	1.6	0.091	7.1	0.061	4.8	0.139	10.9	0.049	3.9
RCC856	A	140	145 875	23.37	0.860	0.000	0.000	0.113	5.4	0.079	3.8	0.291	14.0	0.570	27.4	0.042	2.0	0.310	14.9	0.461	22.2	0.075	3.6	0.136	6.5
		65	83 423	58.62	0.882	0.000	0.000	0.018	0.9	0.111	5.6	0.461	23.2	0.459	23.1	0.049	2.5	0.248	12.5	0.438	22.1	0.086	4.3	0.117	5.9
		14	231 009	56.88	0.942	0.000	0.000	0.096	8.2	0.102	8.8	0.310	26.6	0.138	11.8	0.023	1.9	0.093	8.0	0.222	19.0	0.133	11.4	0.049	4.2
RCC857	A	140	104 853	4.10	1.000	0.000	0.000	0.026	1.9	0.122	8.7	0.534	38.2	0.187	13.4	0.024	1.7	0.094	6.7	0.291	20.8	0.056	4.0	0.064	4.5
		65	150 099	11.46	1.128	0.000	0.000	0.072	5.7	0.131	10.4	0.400	31.9	0.141	11.3	0.025	2.0	0.072	5.7	0.314	25.1	0.065	5.2	0.034	2.7
		14	179 776	11.90	1.120	0.000	0.000	0.145	13.6	0.130	12.2	0.287	27.0	0.070	6.6	0.029	2.7	0.082	7.7	0.191	18.0	0.092	8.7	0.036	3.4
RCC996	A	140	253 221	51.57	0.931	0.074	0.000	0.043	3.4	0.095	7.5	0.119	9.5	0.191	15.2	0.039	3.1	0.245	19.5	0.364	28.9	0.091	7.2	0.074	5.9
		65	321 893	120.69	1.028	0.000	0.000	0.116	9.8	0.106	9.0	0.056	4.7	0.163	13.8	0.026	2.2	0.224	19.0	0.315	26.7	0.117	9.9	0.057	4.8
		14	418 516	61.80	0.968	0.000	0.000	0.195	17.8	0.120	11.0	0.399	36.5	0.049	4.4	0.016	1.5	0.058	5.3	0.077	7.1	0.179	16.4	0.000	0.0
RCC3374	A	140	53 612	4.14	0.726	0.229	0.000	0.012	0.7	0.082	4.5	0.272	14.8	0.778	42.3	0.041	2.3	0.118	6.4	0.332	18.1	0.059	3.2	0.146	7.9
		65	105 492	9.87	0.890	0.110	0.000	0.063	4.8	0.094	7.1	0.192	14.5	0.405	30.5	0.038	2.9	0.085	6.4	0.337	25.4	0.058	4.3	0.055	4.1
		14	136 153	11.33	0.777	0.000	0.000	0.113	12.1	0.098	10.5	0.176	18.8	0.187	20.1	0.020	2.1	0.035	3.8	0.159	17.0	0.082	8.8	0.063	6.8
RCC3376	A	140	50 333	4.08	0.813	0.105	0.000	0.008	0.4	0.112	5.7	0.572	29.1	0.324	16.5	0.054	2.7	0.129	6.6	0.486	24.7	0.085	4.3	0.194	9.9
		65	74 109	2.70	0.830	0.142	0.000	0.006	0.3	0.126	6.7	0.447	23.9	0.261	14.0	0.073	3.9	0.177	9.5	0.505	27.1	0.093	5.0	0.181	9.7
		14	101 238	10.08	0.783	0.053	0.000	0.005	0.5	0.095	9.5	0.108	10.8	0.091	9.1	0.029	2.9	0.130	13.0	0.365	36.6	0.055	5.5	0.122	12.2
RCC2337	B	140	84 624	4.37	0.882	0.394	0.236	0.031	1.4	0.137	6.3	0.504	23.1	0.302	13.8	0.051	2.3	0.154	7.0	0.706	32.3	0.061	2.8	0.240	11.0
		65	76 671	64.32	1.043	0.250	0.172	0.064	4.2	0.129	8.4	0.233	15.3	0.245	16.1	0.020	1.3	0.072	4.7	0.560	36.7	0.067	4.4	0.136	8.9
		14	213 378	18.09	1.040	0.158	0.118	0.109	9.1	0.137	11.4	0.255	21.3	0.058	4.8	0.028	2.4	0.067	5.6	0.332	27.7	0.118	9.8	0.095	7.9
RCC2339	B	140	123 995	14.58	0.624	0.000	0.000	0.026	1.7	0.078	5.1	0.613	40.4	0.045	3.0	0.024	1.6	0.074	4.8	0.302	19.9	0.028	1.8	0.330	21.7
		65	178 752	37.66	0.726	0.040	0.000	0.051	3.6	0.100	7.1	0.524	37.4	0.000	0.0	0.032	2.3	0.102	7.3	0.287	20.5	0.004	0.3	0.303	21.6
		14	223 724	75.86	0.736	0.000	0.000	0.051	5.8	0.076	8.6	0.264	29.8	0.031	3.5	0.025	2.9	0.051	5.8	0.193	21.8	0.033	3.7	0.160	18.1

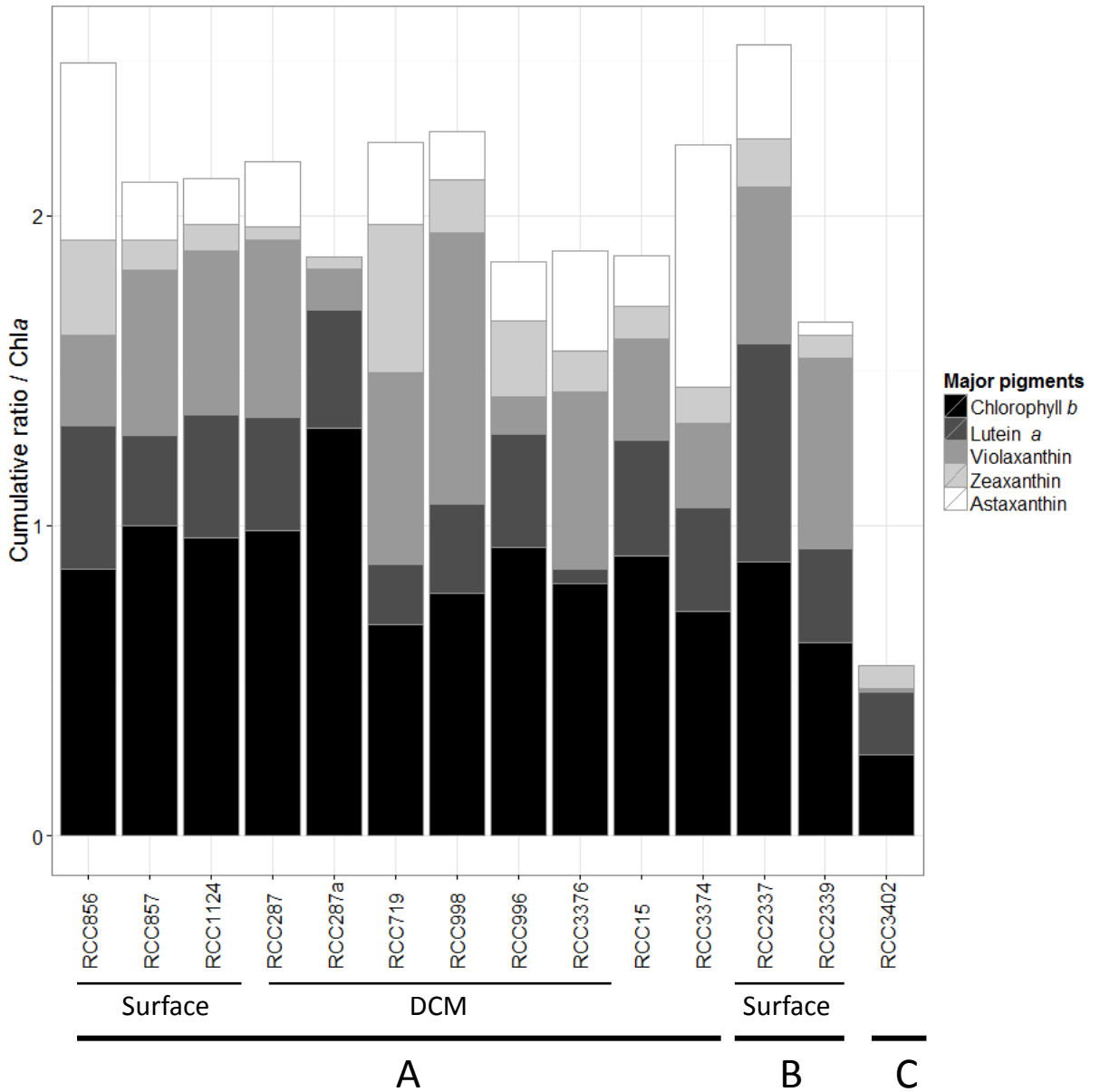


Figure 1

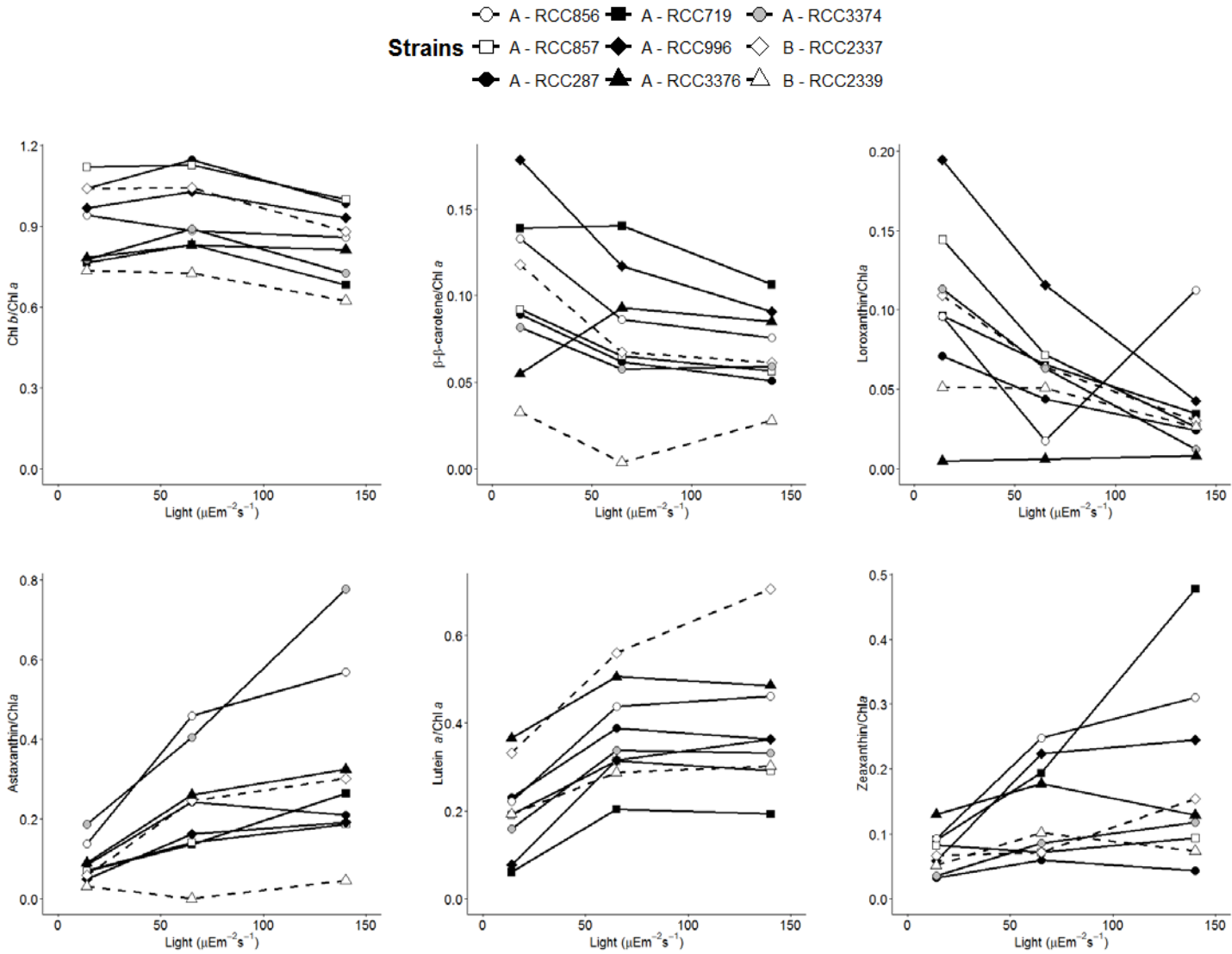


Figure 2

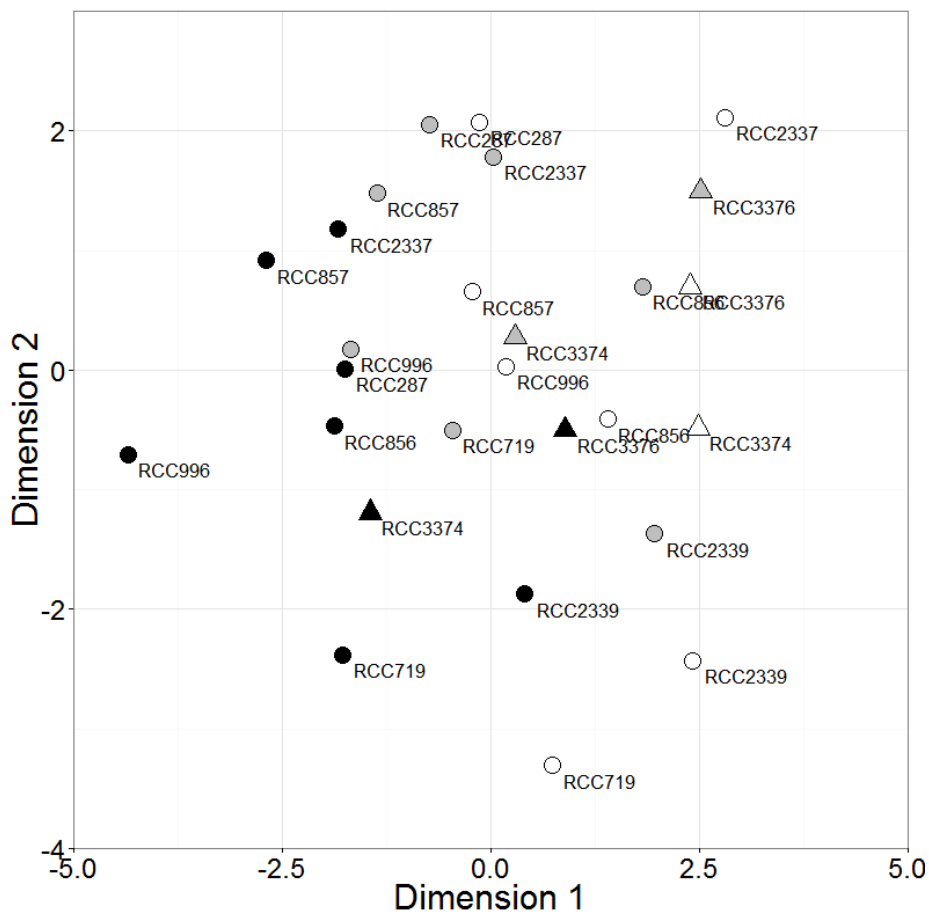
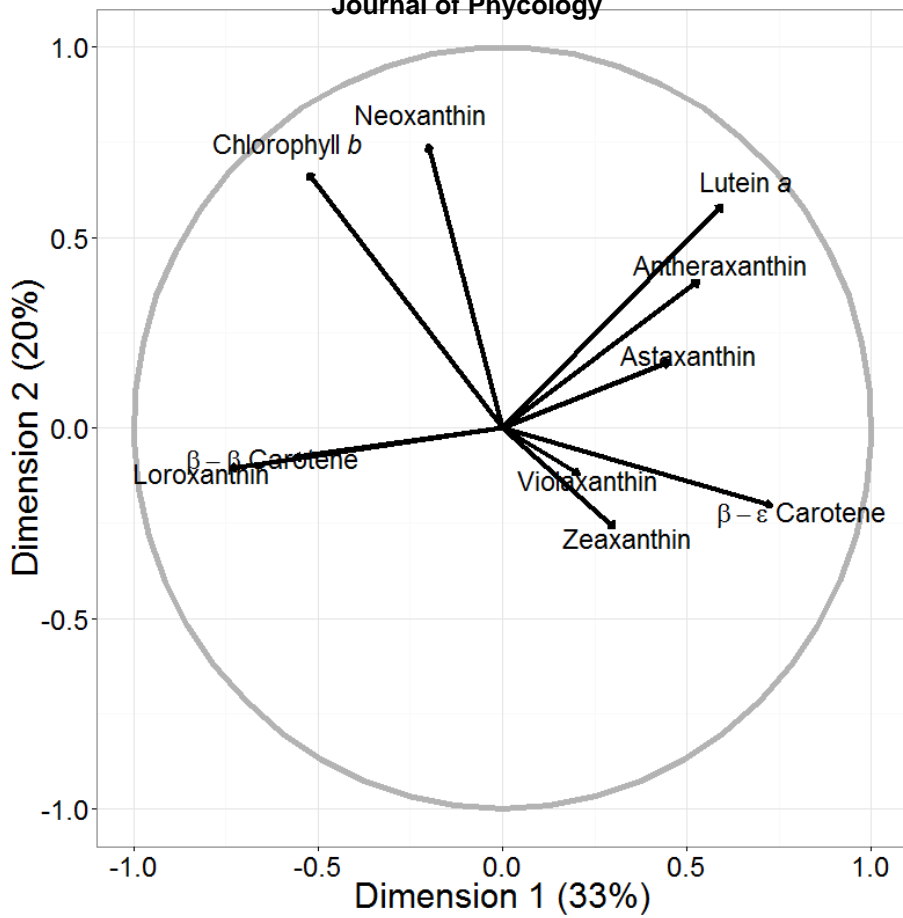


Figure 3

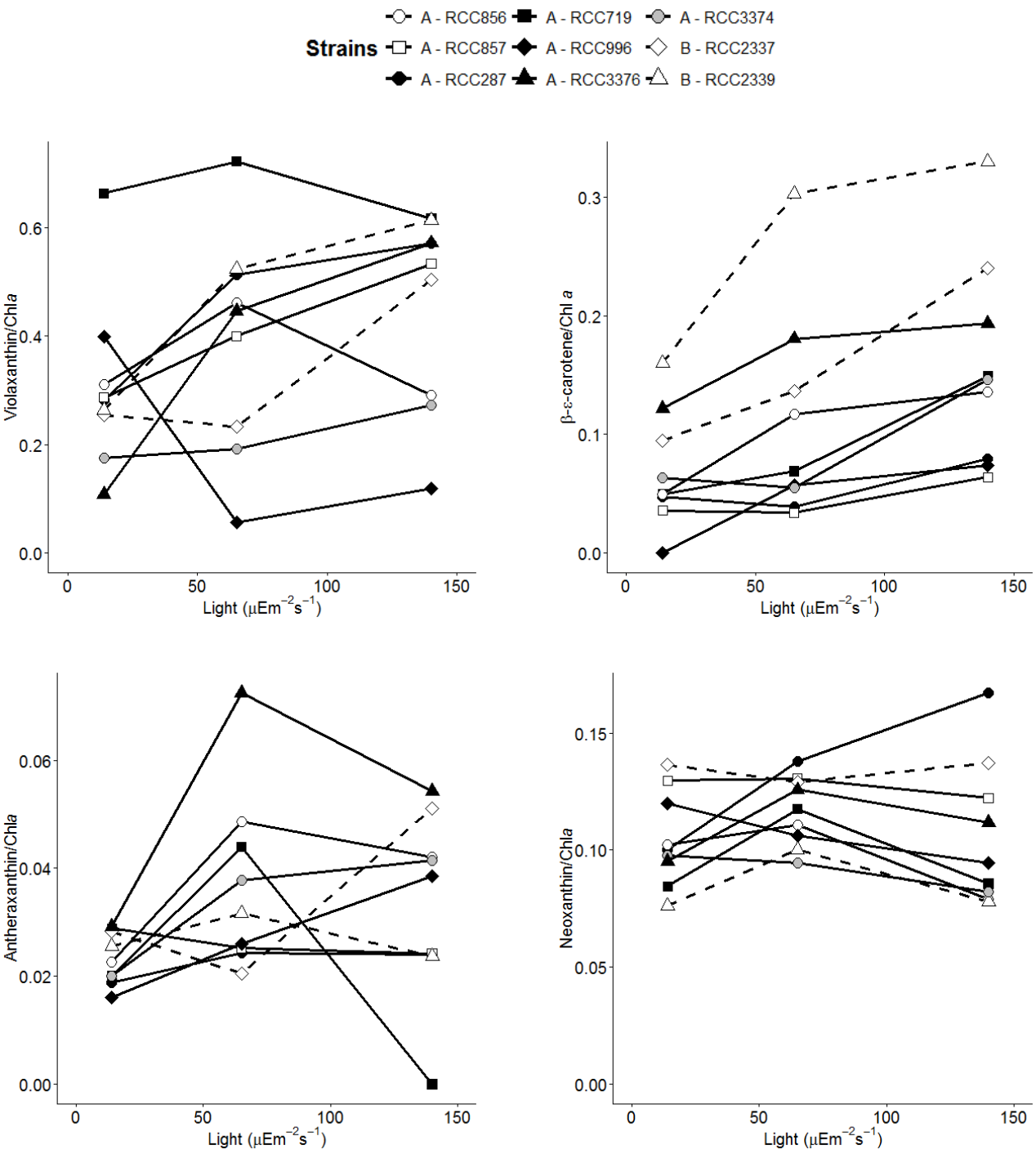


Figure S1.