

Pigment-based chloroplast types in Dinoflagellates

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ABSTRACT: Most photosynthetic dinoflagellates contain a chloroplast with peridinin as the major carotenoid. Chloroplasts from other algal lineages have been reported, suggesting multiple plastid losses and replacements through endosymbiotic events. The pigment composition of 64 dinoflagellates species (122 strains) was analysed by using high-performance liquid chromatography. In addition to chlorophyll (chl) *a*, both chl *c*₂ and divinyl protochlorophyllide occurred in chl *c*-containing species. Chl *c*₁ co-occurred with *c*₂ in some peridinin-containing (e.g. *Gambierdiscus* spp.) and fucoxanthin-containing dinoflagellates (e.g. *Kryptoperidinium foliaceum*). Chl *c*₃ occurred in dinoflagellates whose plastids contained 19'-acyloxyfucoxanthins (e.g. *Karenia mikimotoi*). Chl *b* was present in green dinoflagellates (*Lepidodinium chlorophorum*). Based on unique combinations of chlorophylls and carotenoids, 6 pigment-based chloroplast types were defined: Type-1: peridinin / dinoxanthin / chl *c*₂ (*Alexandrium minutum*); Type-2: fucoxanthin / 19'-acyloxy fucoxanthins / 4-keto-19'-acyloxy-fucoxanthins / gyroxanthin diesters / chls *c*₂, *c*₃, monogalactosyl-diacylglycerol-chl *c*₂ (*Karenia mikimotoi*); Type-3: fucoxanthin / 19'-acyloxyfucoxanthins / gyroxanthin diesters / chl *c*₂, *c*₃ (*Karlodinium veneficum*); Type-4: fucoxanthin / chl *c*₁ and *c*₂ (*K. foliaceum*); Type-5: alloxanthin / chl *c*₂ / phycobiliproteins (*Dinophysis tripos*); Type-6: neoxanthin / violaxanthin / a major unknown carotenoid / chl *b* (*Lepidodinium chlorophorum*). While plastids with peridinin, and probably those with chl *b*, originated by secondary endosymbiosis, the other chloroplast types were obtained through tertiary endosymbiosis. Chloroplast types corresponded with evolutionary lineages within dinoflagellates. Caution must be observed when only peridinin is used for tracking photosynthetic dinoflagellates in field samples. The additional marker pigments offer oceanographers greater power for detecting dinophytes in mixed populations.

KEY WORDS: Dinophyta · Chlorophyll *c* pigments · Novel fucoxanthin-related pigments · Gyroxanthin diester pigments · Chemotaxonomy · Dinoflagellate chloroplast types · Plastid origin · Oceanography

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INTRODUCTION

Dinoflagellates are a diverse trophic group of alveolate protists with around 50% of autotrophic organisms with a distinct grade of mixotrophy (Jeong et al. 2005). Most photosynthetic species contain a chloroplast surrounded by three membranes with chl c_2 and peridinin as major accessory pigments (Jeffrey et al. 1975).

Several features make autotrophic dinoflagellates unique. First, the presence of a water-soluble light-harvesting protein: the extrinsic peridinin-chl a -protein (PCP) located in the chloroplast thylakoid lumen (Hiller 1999, Hoffman et al. 1996), which occurs in several species together with the ubiquitous intrinsic peridinin-chl a -chl c_2 (CAC) membrane-bound light harvesting protein (Macpherson & Hiller 2003). Second, the presence of a proteobacterial form of the key enzyme in photosynthesis, the ribulose biphosphate carboxylase / oxidase (Rubisco), the so-called form II Rubisco (Morse et al. 1995, Rowan et al. 1996) which is nuclear-encoded. And third, the gain of tertiary plastids in certain autotrophic dinoflagellates (Saldarriaga et al. 2001).

Some photosynthetic dinoflagellates are toxin producers, and the monitoring of these species is the main target of harmful algal bloom (HAB) programs around the world (e.g. GEOHAB: <http://www.geohab.info/>). In consequence, taxonomists and oceanographers have sought procedures for the rapid detection and identification of toxic species (see Hallegraeff et al. 2003). Among these techniques, the use of chloroplast pigments as fingerprints of phytoplankton taxa (Jeffrey & Vesik 1997, Jeffrey et al. 1999) constitutes a useful approach to study the plastid origin and taxonomy of dinoflagellate species. In this context, the presence of peridinin – an allenic trinor carotenoid (i.e., having a C_{37} -skeleton instead of the common C_{40} -xanthophylls) – has been considered a distinctive feature in photosynthetic dinoflagellates (see Bjørnland 1990, Liaen-Jensen 1998). Although the occurrence of peridinin-containing

59 chloroplasts is widespread within autotrophic dinoflagellates, the occurrence of fucoxanthin-
60 containing dinoflagellates was observed in earlier studies (see Jeffrey et al. 1975). Later,
61 several dinoflagellates with different pigment patterns were detected: first, a marine
62 dinoflagellate containing 19'-hexanoyloxyfucoxanthin as the main carotenoid (Tangen &
63 Bjørnland 1981); then a chl *b*-containing "green" dinoflagellate (Watanabe et al. 1987), which
64 was further described as *Lepidodinium viride* (Watanabe et al. 1990), and finally autotrophic
65 *Dinophysis* species with cryptophycean-type phycobiliproteins (see Geider & Gunter 1989,
66 Hewes et al. 1998). Information on chloroplast pigments has usually been reported in new
67 descriptions of dinoflagellates (e.g. Fraga et al. 1995, 2008, 2011, Elbrächter & Schnepf 1996,
68 Bolch et al. 1999, Daugbjerg et al. 2000, Montresor et al. 2003, de Salas et al. 2003, 2004,
69 2005, Garcés et al. 2006, Tillmann et al. 2009, Sampedro et al. 2011).

70 Methodological improvements in high-performance liquid chromatography (HPLC)
71 pigment analysis (revised by Jeffrey et al. 1999, Garrido & Zapata 2006) have enabled us to
72 detect new chl *c* pigments and fucoxanthin acyloxy derivatives and to obtain a more accurate
73 distribution pattern of known pigments. An example of this is the description of 8 pigmentary
74 types in Haptophyta (Zapata et al. 2004).

75 Here we report the pigment composition of 64 dinoflagellate species (122 strains)
76 obtained by HPLC. Six pigment-based chloroplast types are described and compared with
77 other algal lineages. Pigment diversity found in photosynthetic dinoflagellates provides clues
78 both for inferring phylogenetic relationships and tracing the distribution and abundance of
79 dinoflagellates in coastal and open-ocean waters.

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MATERIALS AND METHODS

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Algal cultures. Dinoflagellate cultures were obtained from the Culture Collection of
Instituto Español de Oceanografía (CCVIEO, Vigo, Spain), the Provasoli-Guillard National

84 Center for Marine Algae and Microbiota (NCMA -formerly CCMP-, Bigelow Laboratory for
85 Ocean Sciences, West Boothbay Harbor, Maine, U.S.A.), and the CSIRO Collection of Living
86 Microalgae (CCLM, Hobart, Australia). Most cultures were grown in L1 medium (Guillard &
87 Hargraves 1993) with the exception of the CSIRO strains which were grown in GSe medium
88 (Blackburn et al. 1989). The species, strain numbers, collection site and isolator are listed in
89 Table S1 (in the supplement). Light irradiances were 60-90 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ on 12:12 h
90 light:dark cycles for all strains except *Dinophysis* (150 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$). Cultures of
91 *Dinophysis* species (*D. acuminata*, *D. acuta*, *D. caudata* and *D. tripos*) were established in
92 diluted (1/20) L1-Si medium by feeding them the phototrophic ciliate *Mesodinium rubrum* (= *Myrionecta rubra*), fed with the cryptophyte *Teleaulax* sp. (Park et al. 2006).

94 **Sample preparation.** Cultures were examined by light microscopy before HPLC
95 pigment analysis to ensure proper identification and that the cells were healthy and without
96 malformations. Cells were harvested 2-4 h into the light cycle from cultures actively growing
97 a few days after being transferred. Due to the great diversity of species being analyzed,
98 variable volumes of culture (typically 10 ml) depending on the species, were filtered onto
99 Whatman GF/F filters under reduced pressure until some color was observed on the filter.
100 Filters were frozen immediately at -20°C .

101 **Pigment extraction.** Frozen filters were extracted under low light in
102 polytetrafluoroethylene (PTFE)-lined screw capped tubes with 5 ml 90% acetone using a
103 stainless steel spatula for filter grinding. The tubes were chilled in a beaker of ice and
104 sonicated for 5 minutes in an ultrasonic bath. Extracts were then filtered through 25 mm
105 diameter syringe filters (MFS HP020, 25 mm, and 0.20 μm pore size, hydrophilic PTFE) to
106 remove cell and filter debris. An aliquot (0.5 ml) of acetone extract was mixed with 0.2 ml of
107 water and 200 μl were immediately injected. This procedure avoids peak distortion of early

108 eluting peaks (Zapata & Garrido 1991) and prevents the loss of non-polar pigments prior to
109 injection (Latasa et al. 2001).

110 **HPLC analysis.** Pigments were separated using a Waters Alliance HPLC System
111 (Waters Corporation, Milford, MA) consisting of a 2695 separations module, a Waters 996
112 diode-array detector (1.2 nm optical resolution) and a Waters 474 scanning fluorescence
113 detector. Pigment separation was performed using the HPLC method of Zapata et al. (2000),
114 with a reformulated mobile phase A. The column was a C₈ Waters Symmetry (150 x 4.6 mm,
115 3.5 µm particle-size, 100 Å pore size). Eluent A was methanol: acetonitrile: 0.025 M aqueous
116 pyridine (50:25:25 by vol.). Eluent B was methanol: acetonitrile: acetone (20:60:20 by vol.).
117 Elution gradient was as follow: (time(min): %B) t₀: 0%, t₂₂: 40%, t₂₈: 95%, t₃₇: 95%, t₄₀: 0%.
118 Flow rate was 1.0 ml min⁻¹ and column temperature was fixed at 25° C using a Peltier-column
119 thermostat (Jet-Stream Plus). Solvents were HPLC grade (Romil-*SpS*TM), pyridine was
120 reagent grade (Merck, Darmstadt, Germany). CSIRO strains were analysed following the
121 procedure described by Zapata et al. (2004).

122 **Pigment identification.** Pigments were identified either by co-chromatography with
123 authentic standards obtained from SCOR reference cultures (Jeffrey et al. 1997) and diode-
124 array spectroscopy (see Zapata et al. 2000). After checking for peak purity, spectral
125 information was compared with a library of chlorophyll and carotenoid spectra from pigments
126 prepared from phytoplankton cultures. Compounds such as 4-keto-19'-
127 hexanoyloxyfucoxanthin and chl *c*₁-like *Kryptoperidinium*-type were isolated as described
128 previously (Garrido & Zapata 1998, Egeland et al. 2000, Zapata et al. 2006). Novel
129 carotenoids from *Karenia mikimotoi* were tentatively identified (Garrido et al. in preparation).
130 Pigment nomenclature and abbreviations were as suggested by SCOR WG 78 (Jeffrey &
131 Mantoura 1997). For monogalactosyl-diacylglycerol (MGDG)-chl *c*-like pigments whose
132 molecular structures have been elucidated (Garrido et al. 2000), the nomenclature was

133 MGDG-chl c_2 -*Chrysochromulina polylepis*-type (Zapata et al. 2001). For chlorophylls whose
134 molecular structure is unknown, the pigment name includes a reference to the most likely chl
135 c chromophore (chl c_1 - or c_2 -like), and a mention of the species in which the pigment was
136 initially detected (e.g. chl c_2 -like *Pavlova gyrans*-type, chl c_1 -like *Exanthemachrysis*-type).
137 For tentative identification of unknown pigments, the chromatographic behaviour was studied
138 using 2 HPLC systems: the polymeric C_{18} method of Garrido & Zapata (1997) and the C_8
139 method of Zapata et al. (2000) were compared.

140 **Pigment quantification.** HPLC calibration by external standards was performed using
141 chlorophyll and carotenoid standards isolated from microalgal cultures (see Zapata et al.
142 2000), as well as pigments supplied by DHI (Denmark). The molar extinction coefficients (ϵ ;
143 $l \text{ mol}^{-1} \text{ cm}^{-1}$) provided by Jeffrey (1997) were used for pigment quantification. For chl c -like
144 pigments whose molar extinction coefficients are not available (i.e. chl c_3 , chl c_1 -like
145 *Exanthemachrysis*-type and chl c_2 -like *Pavlova. gyrans*-type) the mean of the extinction
146 coefficients for chl c_1 and c_2 at the blue absorption band (see Jeffrey et al. 1997) was used.
147 The MGDG-chl c_2 were quantified by using the molar extinction coefficient of the chl c_2
148 chromophore. For fucoxanthin-related compounds (i.e. acyloxy and keto derivatives) the
149 molar extinction coefficient for fucoxanthin was used, following the recommendations of
150 Jeffrey et al. (1997), even though the absorption spectra of fucoxanthin-derivatives differ
151 slightly from those of the parent compounds. Thus pigment to chl a ratios are expressed on a
152 molar base ($\text{mol} \cdot \text{mol}^{-1}$).

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RESULTS

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Chromatographic resolution and pigment identities

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Pigment composition: chlorophylls

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The peak number of the pigments together with the retention time and their visible absorption maxima in eluent is shown in Table 1. Of the 63 pigments, 44 were well-known chlorophylls and carotenoids previously compiled by Jeffrey et al. (1997). Structures of chl *c* pigments not included in the above review may be found in Helfrich et al. (1999) and Zapata et al. (2006), and structures of algal carotenoids in Bjørnland et al. (2000) and Egeland et al. (2000). The rest of pigments were unknown chl *c*-like compounds and carotenoids present in trace amount.

Thirteen chlorophylls: chls *a*, *b* and eleven chl *c*-type pigments (7 polar chls *c* and 4 non-polar chl *c*₂-like) were detected. Chl *c*₂ (peak 9) together with divinyl protochlorophyllide (MgDVP; peak 8) were always present in chl *c*-containing dinoflagellates; other chl *c* pigments showed a heterogeneous distribution in some cases at trace levels. Chl *c*₁ (peak 10) was detected in several peridinin-containing dinoflagellates (e.g. *Peridinium aciculiferum*, *Gambierdiscus excentricus*), as well as in the fucoxanthin-containing dinoflagellates *Durinskia baltica* and *Kryptoperidinium foliaceum*. Chl *c*₃ (peak 4) was only present in the genera *Karenia*, *Karlodinium* and *Takayama* (Family Kareniaceae). The MGDG-chl *c*₂-*Chrysochromulina polylepis*-type (peak 56) was a minor peak in *Karenia* spp., some *Karlodinium* species (*K. armiger*, *K. decipiens*) and *Takayama helix*. Two novel non-polar chl *c*₂-like pigments were also detected, the first (peak 51) in *Karlodinium armiger* eluted just before the MGDG-chl *c*₂ *Emiliana huxleyi*-type (peak 53), and the second (peak 60) in *Takayama helix* at a higher retention time than the MGDG-chl *c*₂-*C polylepis*-type (peak 56). Red-shifted chls *c*₁ (peak 5) and chl *c*₂ (peak 3), having absorption spectra and

179 chromatographic behaviour similar to those of chl c_1 -like from *Exanthemachrysis gayraliae*
180 (Van Lenning et al. 2003) and chl c_2 -like from *Pavlova gyraus* (Fawley 1989), occurred in *D.*
181 *baltica* and *K. foliaceum*. Blue-shifted chl c_2 -like pigment (peak 6, λ_{\max} =450 nm) was
182 detected as traces in several peridinin-containing dinoflagellates. Chlorophyll *b* (peak 50) was
183 restricted to *Lepidodinium* (=Gymnodinium) *chlorophorum*.

184 **Table 1 around here**

185 **Carotenoid composition**

186 Among the forty-seven carotenoids detected in the dinoflagellates studied (see Table 1),
187 31 were well-known compounds and 16 were unknown compounds (most of them occurring
188 at minor or trace levels, probably optical or geometrical isomers of known carotenoids).
189 Results from electron visible absorption and chromatographic behaviour in two HPLC
190 systems with distinct selectivity: the C₈ HPLC and the polymeric C₁₈ method of Garrido and
191 Zapata (1997) were taken into account for tentative identification (data not shown).

192 Peridininol (peak 1), the deacetylated derivative of peridinin (peak 11), was the most
193 polar carotenoid detected, it co-occurred with peridinin as well as a minor peridinin-like
194 pigment (peak 12) eluting just after peridinin. Diadinochrome (peak 27), the 5, 8-epoxide
195 rearrangement of diadinoxanthin, diadinoxanthin (peak 28), dinoxanthin (peak 29) and
196 diatoxanthin (peak 37) completed the major carotenoids detected on peridinin-containing
197 dinoflagellates. Other carotenoids that were less abundant or restricted to a few species were:
198 a diadinoxanthin-like pigment (peak 15) detected in *Coolia canariensis* (VGO775),
199 astaxanthin (peak 21), violaxanthin (peak 24), pyrrhoxanthin (peak 25), *trans*-neoxanthin
200 (peak 17) and zeaxanthin (peak 39).

201 Carotenoids of the genus *Karenia* included three novel compounds: peaks 13, 14 and
202 22. The visible absorption spectra of these carotenoids isolated from *Karenia mikimotoi*
203 (CCMP429) are shown in Fig. 1; for comparative purposes visible spectra of 19'-

204 butanoyloxyfucoxanthin (peak 16), 19'-hexanoyloxyfucoxanthin (peak 26), and 4-keto-19'-
205 hexanoyloxyfucoxanthin (peak 23) are also depicted. A major gyroxanthin diester carotenoid
206 (peak 45) occurred in *Karenia* and *Karlodinium* species; in addition, a second compound
207 (peak 46) with a similar absorption spectrum was detected in *Karlodinium veneficum* strains
208 at variable proportions. A third gyroxanthin diester-like pigment (peak 43), less retained than
209 the previous ones, as well as less abundant, was detected in *Karenia mikimotoi*.

210 Alloxanthin (peak 35) and crocoxanthin (peak 47) were detected in *Dinophysis*
211 *acuminata*, *D. acuta*, *D. caudata* and *D. tripos*, monadoxanthin was absent. Carotenoids of
212 the chl *b*-containing dinoflagellate, *Lepidodinium chlorophorum*, included both forms of
213 neoxanthin (*all-trans* neoxanthin (peak 17) and *9-cis* neoxanthin (peak 20)), violaxanthin
214 (peak 24), antheraxanthin (peak 32), zeaxanthin (peak 39) and a major unknown carotenoid
215 (peak 40).

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217 **Pigment-based chloroplast types in dinoflagellates**

218 According to the pigment composition of the dinoflagellates analysed, 6 pigment-based
219 chloroplast types (hereafter 'chloroplast types') were defined, and representative
220 chromatograms are shown in Fig. 2. Chlorophyll and carotenoid composition of the 6
221 chloroplast types are summarised in Table 2.

222 Most dinoflagellates species were peridinin-containing organisms labeled as chloroplast
223 Type-1 (71% of species, 51% of strains), with chl *c*₂ (peak 9) as the major accessory
224 chlorophyll and traces of MgDVP (peak 8). Chl *c*₁ (peak 10) was present in some cases at
225 lower concentration than chl *c*₂ (e.g. *Gambierdiscus excentricus* VGO790, *Peridinium*
226 *aciculiferum* PAER-2, *Protoceratium reticulatum* CCMP1720), although chl *c*₁ was the major
227 chl *c* pigment in *Gyrodinium uncatenum* CS-289/3. The carotenoids peridinin (peak 11) and
228 dinoxanthin (peak 29) were specific to chloroplast Type-1 dinoflagellates. Other carotenoids

229 were diadinoxanthin (peak 28), diadinochrome (peak 27), diatoxanthin (peak 37) and $\beta\beta$ -car
230 (peak 62). Overall, eleven genera, including 44 species (83 strains) of the dinoflagellates
231 studied belonged to pigment Type-1.

232 **Fig 2 around here**

233 Chloroplast Type-2 grouped dinoflagellates with Fuco (peak 19), 19'-
234 acyloxyfucoxanthins and their keto derivatives (up to 6 fucoxanthin-related compounds:
235 peaks 13, 14, 16, 22, 23 and 26), and gyroxanthin diesters (up to three compounds: peaks 43,
236 45 and 46). The array of chl *c* included: chl *c*₂ (peak 9), chl *c*₃ (peak 4), MgDVP (peak 8) and
237 MGDG-chl *c*₂-*C. polylepis*-type (peak 56). Only the genus *Karenia* (5 species, 8 strains)
238 belonged to chloroplast Type-2. Fucoxanthin (peak 19) was the major carotenoid in *K. brevis*,
239 *K. mikimotoi*, and *K. selliformis*, but Hex-fuco (peak 26) was the most abundant carotenoid in
240 *K. papilionacea* and *K. umbella*. Different forms of gyroxanthin diester were tentatively
241 identified: the C_{12:0} (peak 45), the major form described by Bjørnland et al. (2003), and two
242 minor components tentatively assigned as the C_{14:0} (peak 46), and a less retained gyroxanthin
243 diester-like pigment (peak 43). The fingerprint of chloroplast Type-2 dinoflagellates with six
244 19'-acyloxyderivatives is unique for the group of 5 *Karenia* species (8 strains) analysed.

245 The pigment Type-3 resembled Type-2, but it lacked the diverse fucoxanthin pool
246 observed in *Karenia* species. But-fuco and Hex-fuco were the major acyloxyderivatives
247 detected in *Karlodinium* spp. with 19'-acyloxyfucoxanthin-like (peak 33) at trace levels
248 (Acyl-fuco-like/chl *a* = 0.02) and no keto-19'-acyloxyderivatives. The major form of
249 gyroxanthin diester was the C_{12:0} acyl ester at C-19 (peak 45) but the C_{14:0} (peak 46) was also
250 abundant in *K. armiger*, *K. decipiens* and *K. veneficum*. However, *Takayama* cf. *helix*
251 (VGO341), which was also included in chloroplast Type-3, lacked gyroxanthin diesters. Two
252 genera, 4 species (11 strains), of the dinoflagellates studied belonged to chloroplast Type-3.

253 **Table 2 around here**

254 Chloroplast Type-4, comprising fucoxanthin-containing dinoflagellates with no
255 acyloxyderivatives, included two species of different genera: *Durinskia baltica* (1 strain) and
256 *Kryptoperidinium foliaceum* (2 strains) A characteristic feature is the occurrence of β,Ψ -
257 carotene (peak 58). Chl c_1 (peak 10) was the dominant chl c -type pigment, with chl c_2 (peak
258 9) second in importance. Two minor chls c : viz. chl c_1 -like *Exanthemachrysis gayraetae*-type
259 (peak 5) and chl c_2 -like *Pavlova gyraetae*-type (peak 3) were also detected.

260 Chloroplast Type-5 included dinoflagellates from the genus *Dinophysis* (4 species and 4
261 strains) with cryptophyte-like chloroplasts. Acetylenic alloxanthin (peak 35) was the major
262 carotenoid, together with crocoxanthin (peak 47) and chl c_2 (peak 9) and MgDVP (peak 8) as
263 the accessory chlorophylls.

264 Chloroplast Type-6 has green algae-like chloroplast pigments: i.e. the accessory chl b
265 (peak 50), 9'-*cis* neoxanthin (peak 20), *all-trans* neoxanthin isomer (peak 17) and
266 violaxanthin (peak 24). Neither lutein nor free-loroxanthin, siphonaxanthin or its esters were
267 detected. The major carotenoid was an unknown compound (peak 40, λ_{\max} : 420, 443, 475 nm;
268 band ratio (%III/II) = 87) eluting after zeaxanthin (peak 39, see Table 1). Using another
269 HPLC method to improve carotenoid separation in *Tetrasetelmis* species (Garrido et al. 2009)
270 peak 40 eluted before a lutein standard (data not shown). The four strains of *Lepidodinium*
271 *chlorophorum* analysed (BAHME100, RCC1488, RCC1489 and Dino16EUH) showed
272 similar carotenoid profile.

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274 **Quantitative pigment data: chlorophyll pigments**

275 The abundance of chlorophyll and carotenoid pigments expressed as molar ratios with respect
276 to chl a are shown in Tables 3 to 5 (corresponding mass (w:w) ratios are in Tables S2-S4).
277 Chlorophyll c_2 was the most abundant chl c pigment in chloroplast Types-1, -2, -3 and -5. Chl
278 c_2 to chl a ratio in peridinin-containing dinoflagellates (Table 3) ranged from 0.03

279 (*Prorocentrum levis*) to 0.57 (*Amphidinium carterae*). The occurrence of MgDVP in trace
280 amounts was a general trait in chl *c*-containing species (ratios not shown in tables). Chl *c*₁
281 occurred in several peridinin-containing dinoflagellates (Type-1), usually in traces.
282 Intermediate values were observed in *Gambierdiscus excentricus* (chl *c*₁/chl *a* = 0.06) and
283 *Peridinium aciculiferum* (0.16), and the highest in *Gyrodinium uncatenum* (chl *c*₁/chl *a* = 0.41)
284 where chl *c*₁ was the major chl *c* pigment (chl *c*₁/*c*₂ = 1.46). Chloroplast Types-2 and -3
285 (Table 4) showed chls *c*₂ and *c*₃ with chl *c*₃/chl *c*₂ ratios varying from 0.29 to 0.58.
286 Chloroplast Type-4 (Table 5) contained higher values of chl *c*₁ than chl *c*₂ (chl *c*₁/chl *c*₂ =
287 1.38-2.25). Chl *c*₁-like *Exanthemachrysis*-type and chl *c*₂-like *Pavlova*-type were minor
288 pigments in *K. foliaceum* and *D. baltica*, respectively, representing ~6% of the chl *c* pool.
289 MGDG-chl *c*₂ *C. polylepis*-type (peak 56) occurred as a minor pigment in pigment Type-2
290 (MGDG-chl *c*₂- *C. polylepis*-type/chl *a* ≤ 0.005) and certain *Karlodinium* species (e.g. *K.*
291 *armiger* and *K. decipiens*) included in chloroplast Type-3.

292 Finally, chl *b*:chl *a* ratio (Type-6, Table 5) varied among the *Lepidodinium*
293 *chlorophorum* strains. The ratio was low (0.08) for the North Sea strain (BAHME100), while
294 the Nervion River isolate (Dino16EUH) showed the highest chl *b*:chl *a* ratio (0.74).

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296 **Quantitative pigment data: carotenoids**

297 Peridinin was the major carotenoid in almost all peridinin-containing dinoflagellates (Table
298 3). Only in a few cases Diadino/chl *a* ratio surpass Peri/chl *a* ratios, reflecting a high
299 xanthophyll cycle activity. The peri:chl *a* ratio (Table 3) ranged from 0.54 in *Barrufeta*
300 *bravensis* (VGO864) to 2.06 in *Coolia canariensis* VGO787. If normalised to chl *c*₂, the
301 lower values correspond to Gymnodiniales (Peri/chl *c*₂ = 1.60-2.91) and Peridinales (Peri/chl
302 *c*₂ = 1.39-4.87). Prorocentrales showed higher ratios (Peri/chl *c*₂ = 3.51-38.92), especially the
303 benthic, symmetric species of *Prorocentrum*. Diadinoxanthin was usually the second major

304 carotenoid, with ratios Diadino/chl *a* ranging from 0.28, both in *Alexandrium*
305 *pseudogonyaulax* VGO706 and in *A. catenella* AL96, to 1.09 in *Prorocentrum lima* PL2V.
306 Dinoxanthin:chl *a* ratios were rather constant in most strains (mean \pm SD = 0.18 \pm 0.07)
307 although the ratio ranged from 0.01 (*Neoceratium furca* Nfurca1) to 0.42 (*Barrufeta bravensis*
308 VGO860).

309 **Table 3 around here**

310 In chloroplast Type-2 (Table 4), Fuco was the major carotenoid in *Karenia brevis*, *K.*
311 *mikimotoi* and *K. selliformis* (Fuco /chl *a* = 0.24-0.34) whereas Hex-fuco was dominant in *K.*
312 *papilonacea* (Hex-fuco/chl *a* = 0.29) and *K. umbella* (Hex-fuco/chl *a* = 0.32) (Table 4). The
313 contribution of acyloxyfucoxanthins (6 compounds) to the fucoxanthin total pool varied
314 among strains. The Hex-fuco derivatives were always more abundant than the But-fuco
315 pigments. The fucoxanthin pool in chloroplast Type-3 was formed by fuco, But-fuco and
316 Hex-fuco with no keto-derivatives. Fuco was the major carotenoid (Table 4) in five out of six
317 *Karlodinium veneficum* strains (Fuco/chl *a* = 0.30-0.75) and Hex-fuco was the major
318 carotenoid in *K. armiger*, *K. decipiens* and *K. veneficum* VGO870 (Hex-Fuco/chl *a* = 0.21-
319 0.45). *Takayama cf. helix* showed Fuco as major carotenoid (Fuco/chl *a* = 1.18) with only a
320 minor contribution of Hex-fuco (Hex-fuco/chl *a* = 0.06).

321 **Table 4 around here**

322 Gyroxanthin diester pigments were present in chloroplast Types-2 and -3 except in
323 *Takayama cf. helix* (Table 4). In *Karenia* species, the pigment ratio of the major form (peak
324 45: GyrE C12:0) was rather constant (GyrE/chl *a* = 0.11-0.17), a more polar gyroxanthin-like
325 compound (peak 43) was the second most abundant form (GyrE1-like/chl *a* = 0.01-0.03). In
326 *Karlodinium* species GyrE C12:0 (peak 45) was also the major compound (GyrE / chl *a*
327 =0.11-0.24), with variable contribution of the more retained GyrE C14:0 (peak 46, GyrE / chl
328 *a* = 0.02-0.09).

329

330 Fucoxanthin was the major carotenoid in Type-4 chloroplasts (0.52 to 0.75), while $\beta\psi$ -
331 carotene could only be quantified in *K. foliaceum* VGO556 (0.052). Pigment ratios in Type-5
332 (*Dinophysis* species) were rather constant with Allo/chl *a* = 1.32-1.62, Croco/chl *a* = 0.05-
333 0.08 and $\beta\epsilon$ -car/chl *a* = 0.15-0.20.

334 **Table 5 around here**

335 Chloroplast Type-6, represented by four strains of *Lepidodinium chlorophorum*, showed
336 marked differences in carotenoids and chl *b* pigment ratios (Table 5). The North Sea strain
337 (BAH100ME, the type culture) showed violaxanthin as major carotenoid (Viola/chl *a* = 0.32),
338 with an unknown carotenoid (peak 46) as the second most important carotenoid (Unk car/chl
339 *a* = 0.18). In both British Channel (RCC1488, RCC 1489) and Nervion River (Dino16EUH)
340 strains, the unknown carotenoid was the major carotenoid (Unk car/chl *a* = 0.14-0.17)
341 followed by violaxanthin (Viola/chl *a* = 0.08-0.32). Lutein was detected as traces in the four
342 strains. $\beta\epsilon$ -car was the major carotene in BAH100ME and Dino16EUH strains ($\beta\epsilon$ -car/chl*a*:
343 0.03-0.11).

344

345

346 **Occurrence of pigment-based chloroplast types across dinoflagellate taxa**

347 The distribution patterns of pigment-based chloroplast types across dinoflagellate taxa are
348 summarised in Table 6. These examples showed the different specificity of the pigment types
349 across Dinophyta. All orders, except Dinophysiales, include some genera with chloroplast
350 Type-1 chloroplast. Besides representatives of Type-1, Gymnodiniales include other genera of
351 chloroplast Types -2, -3 and -6. Finally, the order Peridinales encompasses representatives of
352 pigment Type-4 as well as the already mentioned Type-1.

353 **Table 6 around here**

354 Three chloroplast types contain a single genus (Type-2 associated to *Karenia*, Type-5
355 to *Dinophysis* and Type-6 to *Lepidodinium*), while pigment Type-3 is linked to two genera
356 (*Karlodinium* and *Takayama*) and Type-4 is confined to three genera (*Durinskia*, *Galeidinium*
357 and *Kryptoperidinium*).

358

359

DISCUSSION

360 Comparison with previous surveys of Dinophyta

361 The first comprehensive study of chloroplast pigments in dinoflagellates used thin layer
362 chromatography to describe the chlorophyll and carotenoid composition of 22 species
363 belonging to the orders Gymnodiniales, Gonyaulacales, Peridinales and Prorocentrales
364 (Jeffrey et al. 1975). Peridinin was the major carotenoid in 19 of the species, while
365 fucoxanthin was the major carotenoid in 3 peridinin-lacking peridinales. Since then,
366 dinoflagellates with other pigment composition have been reported (chl *b*, Watanabe et al.
367 1990; alloxanthin, Meyer-Harms & Pollehne 1998; and acyloxyfucoxanthins, Bjørnland &
368 Tangen 1979, Tengs et al. 2000).

369 We applied HPLC to review the pigment composition of dinoflagellates belonging to
370 six orders of the division Dinophyta (Table S1). Members of the Gonyaulacales,
371 Prorocentrales and Thoracosphaerales analysed contained exclusively the peridinin-containing
372 Type-1 chloroplast (Table 6), which included around two-thirds of the studied species.

373 Recently, an exception to the common chloroplast Type-1 distribution in Gonyaulacales
374 was observed in *Amylax buxus* and *Amylax triacantha*, where anucleated cryptophyte vestiges
375 (probably *Teleaulax*-related) were detected (Koike & Takishita 2008). The order
376 Dinophysiales comprises mostly heterotrophic dinoflagellates with only a reduced number of
377 autotrophic species belonging to the genus *Dinophysis*. In this case the cryptophyte-Type-5
378 chloroplast was present, although the presence of a haptophyte-type plastid in *Dinophysis*

379 *mitra* has been claimed (Koike et al. 2005). Such an important exception should be supported
380 by evidences from HPLC pigment analysis, which has not been yet performed. Peridinin-
381 containing species have never been reported in Dinophysiales. The order Peridinales includes
382 both peridinin-containing chloroplast Type-1 and the fucoxanthin-containing Type-4. The
383 major pigment-based chloroplast diversity was observed in the Gymnodiniales, with 4 out of
384 6 chloroplast Types defined here (Types-1, -2, -3 and -6). In addition, a few Gymnodiniales
385 species have been reported to harbour endosymbiotic algae belonging to chloroplast Type-4
386 (*Gymnodinium quadrilobatum*, Horiguchi & Pienaar 1994) and Type-5 (*Amphidinium latum*,
387 Horiguchi & Pienaar 1992) chloroplasts, but pigment analyses were not detailed in these
388 studies. These findings support the description of Gymnodiniales as a heterogeneous order, as
389 previously indicated by Saldarriaga et al. (2001)

390

391 **Pigment-based chloroplast types and dinophyte phylogeny**

392 Pigmentary groups in phytoplankton have been traditionally based on the occurrence of
393 certain marker pigments (Jeffrey et al. 1999). However, in the present study we define
394 pigment-based chloroplast types to illustrate the fact that dinoflagellates include not only
395 secondary but also tertiary plastids from different algal lineages. Dinoflagellates have
396 acquired and lost their chloroplasts multiple times during their evolutionary history. The
397 ability to acquire and maintain other eukaryotic plastids has led to the diversity of
398 dinoflagellate plastids (Koike et al. 2005). Peridinin-pigmented dinoflagellates contain
399 secondary plastids that appear to have undergone more plastid genome reduction than other
400 eukaryotes. It is generally accepted that peridinin-containing dinoflagellate plastids are
401 derived from red algae (Zhang et al. 1999), but whether they are secondary plastids equivalent
402 to plastids of stramenopiles, haptophytes, or cryptophytes, or they are tertiary plastids derived
403 from one of the other secondary plastids, has not yet been completely resolved (Wang et al.

404 2008). The number of endosymbiotic events in dinoflagellates probably exceeds those in other
405 known eukaryotes (Takishita et al. 2002).

406 Saldarriaga et al. (2004) proposed a scheme of the evolutionary history of
407 dinoflagellates based on molecular trees of concatenated nuclear genes, morphological and
408 palaeontological information. If we superimpose on that scheme the pigment-based
409 chloroplast types (Fig. 3), it appears that most of the pigment diversity in dinoflagellates
410 occurs on a certain group of dinoflagellate orders (Peridinales, Dinophysiales,
411 Gymnodiniales, Thoracosphaerales) sharing a common ancestor.

412 Peridinales occupy a central position in the evolution of dinoflagellates and probably
413 gave rise to other thecate taxa and also to Thoracosphaerales and Blastodinales. Montresor et
414 al. (2003) reported that Suessiales contain peridinin. *Karenia* and *Karlodinium* occupy an
415 early divergent branch in many molecular studies and would represent also the first deviation
416 of the Chloroplast Type-1 (peridinin) among dinoflagellates.

417 **Fig 3 around here**

418 **Tertiary plastids in dinoflagellates**

419 ***Karenia* – pigment Type-2 chloroplast.**

420 Chloroplast Type-2 are considered tertiary plastids related to Type-7 haptophytes (Zapata et
421 al. 2004): both contain chls c_2 , c_3 and MGDG-chl c_2 (14:0/14:0) *Chrysochromulina*-type.
422 However, the carotenoid composition of *Karenia* species detected in our study does not match
423 any of the haptophyte pigment types described to date. In addition to Fuco, Hex-fuco and 4-
424 keto-Hex-fuco, three novel pigments were detected. One of these (peak 15) was tentatively
425 identified as 4-keto-But-fuco, the other two pigments (peaks 13 and 22) shared both
426 absorption spectra with the above mentioned 4-keto forms (Garrido et al. in preparation).
427 Pigment Type-2 embraces well-known HAB species belonging to the genera *Karenia* (i.e., *K.*

428 *brevis*, *K. mikimotoi*, *K. selliformis*). It is noteworthy that the particular fingerprint profile of
429 *Karenia* species could be useful to detect their presence in monitoring programmes (Garrido
430 et al. in preparation).

431 Gyroxanthin diester was considered a marker pigment for *Karlodinium veneficum*
432 (= *Gymnodinium galatheanum*) (see Bjørnland et al. 2000) and also for *Karenia brevis*
433 (Bjørnland et al. 2003). However, a pigment with similar chromatographic properties has been
434 also detected in the pelagophyceans *Pelagomonas calceolata* (Bjørnland et al. 2003), and
435 tentatively in *Aureococcus anophageferens*, *Pelagococcus subviridis* (Zapata 2005) and
436 several Haptophytes (Zapata 2005). The occurrence of gyroxanthin in *Karlodinium* and
437 *Karenia* is noticeable for its quantitative relevance, but it is not taxon-specific (Garcés et al.
438 2006). The genus *Karenia* currently embraces 13 species (Guiry & Guiry 2010). Thus, the
439 homogeneity in the pigment composition observed in the 4 species here analysed should be
440 confirmed by the analysis of 9 additional species whose original or further description (see
441 Table S5 in the supplement) did not include pigment data.

442

443 ***Karlodinium* and *Takayama* –pigment Type-3 chloroplasts**

444 Chloroplast Type-3 lacks keto forms of acyloxyfucoxanthin-derivatives, and the occurrence of
445 both MGDG-chl c_2 and gyroxanthin diester is not a general trait. Pigment Type-3 groups the
446 well-known HAB species *Karlodinium veneficum* (= *K. micrum*, *Gymnodinium galatheanum*).
447 The genus *Karlodinium* currently includes 10 species (de Salas et al. 2008). However, the
448 pigment composition of five of them has not yet been analysed (see Table S5). HPLC pigment
449 analysis of *K. australe* (de Salas et al. 2005) indicates that this species contains Fuco and
450 Hex-fuco as the main carotenoid pigments, with But-fuco present in trace amounts. *K.*
451 *australe* did not produce gyroxanthin-diester, a pigment typical of *Karlodinium veneficum* (*K.*
452 *micrum*, Bjørnland et al. 2000) and *Karenia* species (de Salas et al. 2004).

453 In a recent study Bachvaroff et al. (2009) analysed pigment variability between
454 *Karlodinium veneficum* strains and detected two GyrE compounds sharing its absorption
455 spectra and molecular weight. They were noted as GyrE and *cis* GyrE isomers, not as esters
456 with different fatty acid residues.

457 The marine dinoflagellate genus *Takayama* currently comprises 6 species (Table S5).
458 *Takayama cf. helix* (VGO-341) contains a MGDG-chl c_2 pigment previously detected in
459 *Emiliana huxleyi* (peak 53) and a second one also detected in *Prymnesium faveolatum* (peak
460 60). A different pigment profile was observed in *T. tasmanica* which contains a GyrE-like
461 pigment more polar than that detected in *Karenia umbella* (de Salas et al. 2004). In addition,
462 *Takayama tasmanica* and *T. helix* contain a MGDG-chl c_2 , closely eluting to zeaxanthin,
463 detected also in *Haptolina* (= *Chrysochromulina*) *hirta* and other *Chrysochromulina* species
464 (Zapata et al. 2001, Seoane et al. 2009) recently reassigned to the new genus *Haptolina*
465 (Edwardsen et al. 2011). However, the 2 *Takayama* species differ in the occurrence of GyrE
466 restricted to *T. tasmanica*, and Hex-fuco only detected in *T. helix* (de Salas et al. 2003). The
467 other four *Takayama* species have not yet been analysed for pigment composition: *T.*
468 *acrotrocha*, *T. cladochroma*, *T. pulchella* (de Salas et al. 2003) and *T. tuberculata* (de Salas et
469 al. 2008).

470

471 ***Durinskia* and *Kryptoperidinium* – pigment Type-4 chloroplasts**

472 The dinoflagellate species included in this chloroplast type correspond with those described as
473 bearing diatom endosymbionts: *Durinskia baltica* (= *Peridinium balticum*) (Withers et al.
474 1977, Carty and Cox 1986) and *Kryptoperidinium foliaceum* (Jeffrey & Vesk 1976, Kite et al.
475 1988). Chlorophyll c_1 is the dominant chl c -pigment in *K. foliaceum* and *D. baltica*, such a
476 feature is also characteristic of *Pavlova* spp. (Van Lenning et al. 2003, Zapata et al. 2004) but
477 it is unusual in diatoms (Stauber & Jeffrey 1988).

478 In addition to fucoxanthin and chls c_1 and c_2 , Type 4-dinoflagellates contain minor chl
479 c -like pigments first detected, respectively, in the Pavlovophyceans *Pavlova gyrans* (Fawley
480 1989) and *Exanthemachrysis gayraliae* (Van Lenning et al. 2003). These pigments occur also
481 in several chrysophytes (e.g. synurophyceans and chrysophyceans, Jeffrey & Wright 2006,
482 Zapata 2005). Previous HPLC analysis of *K. foliaceum* (Kempton et al. 2002, McEwan &
483 Keeling 2004) did not detect these pigments. The presence of β,Ψ -carotene in *K. foliaceum*
484 and *D. baltica* is a singular feature with no correspondence in Bacillariophyceae and
485 Pavlovophyceans (haptophytes pigment Type-2). However, β,Ψ -car is a light-sensitive
486 pigment so its potential role as marker pigment is restricted to high light environments.
487 Molecular analyses have traced the chloroplast origin of *Peridinium balticum* (= *Durinskia*
488 *baltica*) and *Kryptoperidinium foliaceum* to a pennate diatom (Chesnick et al. 1997).

489 According to Imanian et al (2010) the endosymbiont in these dinoflagellate species
490 would be closely related with the pennate diatom genus *Nitzschia*. However *K. foliaceum*
491 seems to have incorporated two exogenous plasmids. These authors proposed the term
492 “dinotom” to refer to the complex cell derived from this tertiary endosymbiosis. The nature of
493 the endosymbiont is similar in *Durinskia cappensis* (Pienaar et al. 2007). In the
494 dinoflagellates *Galeidinium rugatum* (Tamura et al. 2005) and *Peridinium quinquecorne*
495 (Horiguchi & Takano 2006), the diatom plastid seems to be from a centric diatom (Takano et
496 al. 2008) obtained by serial replacement of diatom endosymbionts. Whether this different
497 chloroplast (centric vs. pennate diatom) source is reflected in the pigment composition of *G.*
498 *rugatum* and *P. quinquecorne* is still under debate.

499

500 ***Dinophysis* – pigment Type-5 chloroplasts**

501 The genus *Dinophysis* includes both photosynthetic and non-photosynthetic (heterotrophic)
502 species, and the former are known to possess cryptophyte-type plastids that contain the
503 phycobilin pigment phycoerythrin (Hewes et al. 1998, Vesik et al. 1996, Hackett et al. 2003).

504 The origin of the *Dinophysis* spp. chloroplast was traced to *Teleaulax amphioxeia* (Janson
505 2004) and closely related to *Geminiphora cryophyla* in *Dinophysis* spp. from Greenland Sea
506 (Janson 2004, Minnhagen & Janson 2006). Most species belonging to the genus *Dinophysis*
507 harbor chloroplasts of cryptophyte origin. Whether these chloroplasts are temporarily
508 sequestered from the prey (kleptoplastids) or permanent is controversial. Considering both
509 molecular and ultrastructural evidence, Garcia-Cuetos et al. (2010) indicated that the plastids
510 in *D. acuminata* are permanent and originate from *Teleaulax* or another closely related
511 cryptophyte genus. Koike et al. (2005) suggested the presence of haptophyte-type plastid in
512 *Dinophysis mitra* though HPLC pigment data were not reported. If this is confirmed, it would
513 be the first case of an armoured dinoflagellate containing a haptophyte-type chloroplast. The
514 difficulty in culturing *Dinophysis* was a bottleneck for many decades to advance in basic
515 studies on its biology (Park et al. 2006). At present, distinct species of *Dinophysis* have been
516 cultured by different laboratories (*Dinophysis acuminata* (Park et al. 2006), *D. fortii* (Nagai
517 2008), *D. caudata* (Nishitani et al. 2008a), *D. infundibulus* (Nishitani et al. 2008b), *D. tripos*
518 (Rodríguez et al. 2012)). However, our study represents the first HPLC pigment data from
519 cultured *Dinophysis* species.

520

521 ***Lepidodinium chlorophorum* – pigment Type-6 chloroplasts**

522 The genus *Lepidodinium* was recently revised (Hansen et al. 2007), and the previously named
523 *Gymnodinium chlorophorum* was renamed as *L. chlorophorum*. The extant plastid in the
524 dinoflagellate *Lepidodinium viride* is most probably acquired by plastid replacement via
525 tertiary endosymbiosis (reviewed by Delwiche 2007). This dinoflagellate possesses a green-

526 pigmented plastid surrounded by four membranes. First evidence on pigment composition (Dr
527 W.W.C. Gieskes, personal communication to Elbrächter & Schnepf (1996)) pointed out the
528 presence of prasinoxanthin; as a consequence, the prasinophyte-origin of the chloroplast was
529 assumed. Recent results indicate that the green plastids in *Lepidodinium* are derived from an
530 alga belonging to core-chlorophytes (Matsumoto et al. 2011, Minge et al. 2010), distinct from
531 the ancient prasinophyceans. Our results show clearly that *L. chlorophorum* lacks
532 prasinoxanthin. Moreover, the results obtained (see Table 2) differ also from other pigment
533 patterns so far observed within the prasinophycean algae (Egeland et al. 1995, Garrido et al.
534 2009, Latasa et al. 2004, Yoshi 2006).

535 The absence (or traces) of lutein it is noticeable, as is the occurrence of an unknown
536 carotenoid (peak 42) with similar retention time as lutein in the HPLC system employed. Both
537 differences in UV-vis spectrum (Table 1) and chromatographic retention using a different
538 HPLC method (Garrido et al. 2009) allowed distinction of the unknown pigment from lutein
539 and other major pigments detected in Chlorophyta. The structural elucidation of this
540 carotenoid (peak 42) is currently under investigation (Zapata et al. in preparation).

541

542 **Implications of dinoflagellate pigment-types in biological oceanography and** 543 **chemotaxonomy.**

544 The use of HPLC pigment analysis for inferring phytoplankton assemblages through marker
545 pigments increased the interest for phytoplankton pigments in oceanography (revised by
546 Jeffrey et al. 1997, 1999, Jeffrey & Wright 2006). The use of peridinin to map the
547 contribution of dinoflagellates to total chl *a* is generally accepted due to the prevalence of
548 peridinin-containing chloroplasts in photoautotrophic dinoflagellates. In fact, our study seems
549 to validate this general approach, as peridinin was the characteristic marker pigment in two-
550 thirds of the analysed species. However, this approach is prone to error if a single pigment

551 algorithm is employed. For example, the contribution of dinoflagellates to total chl *a* has been
552 usually obtained by using a fixed equation: [chl *a*] DINO = 1.5 [Peri] obtained from a single
553 *Amphidinium* sp. isolate (see Letelier et al. 1993), which corresponds to Peri/chl *a* ratios of =
554 0.67 (mass ratio) or 0.65 (molar ratio, where molar ratio is MW chl *a* /MW Peri = 0.97 x
555 mass ratio). The contribution of dinoflagellates to total chl *a* assuming such a fixed factor will
556 produce either overestimation or subestimation, assuming the Peri/chl *a* range obtained in the
557 present paper (0.54-2.06 molar ratio, Table 3). This range for Peri/chl *a* ratios agrees well
558 with that obtained in field studies using chemical taxonomy programme CHEMTAX (from
559 0.52 to 1.51, Table S6). CHEMTAX (see Mackey et al. 1996) has demonstrated its capacity
560 for reconstructing phytoplankton assemblages from HPLC pigment data (Wright et al. 1996,
561 Mackey et al. 1998, Higgins & Mackey 2000, Wright & van den Enden 2000, Rodríguez et al.
562 2002, 2003, 2006). This program relies upon: (i) a general information of major algal groups
563 present in the study area (ii) an initial pigment to chl *a* ratio matrix basically obtained from
564 the literature for relevant phytoplankton groups (*algal classes*), or even user-defined *algal*
565 *pigment classes* (Rodríguez et al. 2003). In this sense the values shown in Tables 3 to 5
566 represent improved pigment ratios and define new “*chemotaxonomic categories*” (i.e.: *Dinos*
567 *Types-1 to -6*) which may be incorporated into CHEMTAX analysis.

568

569 The use of peridinin as a single marker pigment for dinoflagellates ignores the potential
570 contribution of species from chloroplast types 2-6, whose contribution to chl *a* would be
571 assigned to other algal groups with similar pigment composition (Table S7). For example, the
572 contribution of Hex-fuco-containing dinoflagellates to total chl *a* might be underestimated due
573 to the presence of haptophytes sharing similar pigment composition. Some bloom-forming
574 dinoflagellates (*Karenia brevis*, *K. mikimotoi*, *Karlodinium veneficum*) share several pigment
575 markers with bloom-forming haptophytes (*Chrysochromulina* spp., *Emiliania huxleyi*,

576 *Phaeocystis* spp.), but the specificity of chloroplast Type-2 allows us to discriminate *Karenia*
577 species from hex-fuco-containing haptophytes. Comparing molar pigment ratios of
578 haptophytes from pigmentary groups-6, 7 and 8 (sensu Zapata et al. 2004) with Hex-fuco-
579 containing dinoflagellates, the values chl c_3 /chl a , chl c_2 /chl a , chl c_3 /chl c_2 and total chls
580 c /total fuco are consistently higher in haptophytes relative to dinoflagellates of the Types 2-
581 and -3 (see Tables 3 and 5 in Zapata et al. 2004). The index MGDG-chl c_2 / chl a in Type-2
582 and -3 dinoflagellates is an order of magnitude lower than the values obtained within Types-6,
583 7 and 8 haptophytes (see Zapata et al. 2001, 2004, Seoane et al. 2009).

584 Type-4 dinoflagellates show a pigment profile closely related to some diatoms (Zapata
585 2005) and pavlovophyceans Type-2 (e. g. *Pavlova gyrans*: chl c_2 -like *P. gyrans* type). In
586 addition, *Durinskia baltica* and *Kryptoperidinium foliaceum* contain $\beta\psi$ -car which was
587 claimed as the differential pigment in *Kryptoperidinium foliaceum* (Kempton et al. 2002).
588 However, $\beta\psi$ -car contents have been shown to be dependent of light intensity in *Tetraselmis*
589 *suecica* (Garrido et al. 2009), so the marker quality of this pigment in other species needs
590 confirmation under different light regimes. Type-5 dinoflagellates share the pigment profile
591 with autotrophic cryptophytes. In consequence, it is not possible to discriminate both algal
592 groups in natural samples. The occurrence of green-dinoflagellates is easily detected by
593 pigment analysis, as both the presence of chl b and the singular carotenoid profile are very
594 characteristic. No other chl b -containing organism with similar pigment signature has been
595 reported so far.

596 The importance of dinoflagellates in the picophytoplankton fraction, which have not
597 been cultured yet, has been evidenced. For example, Latasa & Bidigare (1998) found that
598 often more than 50% (and up to 75%) of peridinin appeared in the $< 2 \mu\text{m}$ fraction, whereas
599 Wright et al. (2009), on the basis of peridinin concentration, estimated that dinoflagellates
600 accounted for 0–11% of picoplanktonic chl a in Antarctic waters. In addition, molecular

601 sequences from many unknown dinoflagellates have been retrieved in open ocean samples
602 (Moon-van der Staay et al. 2001, Lin et al. 2006). All of these facts underscore the necessity
603 to isolate and cultivate new species of small dinoflagellates so that their pigment composition
604 can be characterized, to advance in our knowledge about the diversity of photosynthetic
605 dinoflagellates in natural samples.

606

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613

614

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959

FIGURE LEGENDS

960 Fig. 1. (A) HPLC chromatogram from a massive culture of *Karenia mikimotoi* (CCMP429).

961 (B) Enlarged section showing the visible absorption spectra of three novel compounds (peaks
962 13, 14 and 22). For comparative purposes visible spectra of But-fuco (peak 16), Hex-fuco
963 (peak 26) and 4k-Hex-fuco (peak 23) are also depicted. Pigment proportions cannot be
964 compared with those in Fig. 2 because growth conditions were different in massive cultures (4
965 liters) due to self-shading and longer harvesting periods (10-15 days).

966 Fig. 2. HPLC chromatograms of dinoflagellates belonging to major pigment types. Type 1:
967 *Alexandrium margalefi* (VGO661), Type 2: *Karenia mikimotoi* (CCMP429), Type 3:
968 *Karlodinium veneficum* (CCMP415), Type 4: *Kryptoperidinium foliaceum* (VGO556), Type
969 5: *Dinophysis caudata* (VGO1064) and Type 6: *Lepidodinium chlorophorum* (RCC1489).
970 Detection by absorbance at 440 nm. Peak identifications as in Table 1.

971 Fig. 3. Pigment-type chloroplasts and evolutionary history of dinoflagellates. Modified from
972 Saldarriaga et al. (2004). Molecular data and the evolutionary history of dinoflagellates.

973 Pigment-type chloroplasts (1-6) and evolutionary history of dinoflagellates based on molecular data.

974

Table 1. Elution order and visible absorption characteristics of pigments in eluent from dinophyte cultures. Wavelengths given in parenthesis denote shoulders. Occurrence across the six chloroplast-pigment types (T1-T6) is indicated.

Peak no.	Pigment /present in chloroplast pigment Type(s)	Abbreviation	Time (min)	λ maxima in eluent (nm)		
	(Solvent front)					
1	Peridininol / T1	Perid-ol	5.66	477		
2	Unk peridinin-like λ_{\max} 465 nm / T1	Unk-car465	7.28	465		
3	Chlorophyll c_2 -like <i>Pavlova gyrans</i> -type / T4	Chl c_2 -like <i>Pg</i>	7.69	457	586	634
4	Chlorophyll c_3 / T2-3	Chl c_3	8.02	458	591	(629)
5	Chlorophyll c_1 -like <i>Exanthemachrysis</i> -type / T4	Chl c_1 -like <i>Eg</i>	8.31	453	585	635
6	Unk chlorophyll c_2 -like / T1	Chl c_2 -like 450	8.86	450	583	631
7	Chlorophyllide <i>a</i> / T1-6	Chlide <i>a</i>	10.47	430	619	663
8	Divinyl Protochlorophyllide <i>a</i> / T1-6	MgDVP	10.83	440	574	628
9	Chlorophyll c_2 / T1-5	Chl c_2	11.75	453	585	634
10	Chlorophyll c_1 / T1, T4	Chl c_1	12.34	448	583	632
11	Peridinin / T1	Perid	13.85	475		
12	Peridinin-like / T1	Perid-like	14.33	478		
13	Keto-19'-butanoyloxyfucoxanthin-like / T2	But-fuco-like-1	15.68	448	471	
14	4-keto-19'-butanoyloxyfucoxanthin / T2	But-fuco-like-2	16.29	448	470	
15	Unk carotenoid λ_{\max} 447 / T1	Unk-car447 <i>Cc</i>	16.39	(424)	447	477
16	19'-butanoyloxyfucoxanthin / T2-3	But-fuco	17.21	447 470		
17	<i>all-trans</i> neoxanthin / T6	<i>t</i> -neo	17.52	416	442	470
18	Heteroxanthin-like / T3	Het-like <i>Th</i>	17.77	419	443	471
19	Fucoxanthin / T2-4	Fuco	18.27	451		
20	9'- <i>cis</i> neoxanthin / T6	Neo	19.46	413	439	466
21	Astaxanthin / T1	Asta	19.58	480		
22	Keto-hexanoyloxyfucoxanthin-like / T 2	4k-hex-fuco-like	19.61	448	472	
23	4-keto-19'-hexanoyloxyfucoxanthin / T2	4k-hex-fuco	20.34	448	472	
24	Violaxanthin / T1, T6	Viola	20.80	415	441	470
25	Pyrrhoxanthin / T1	Pyrrho	21.12	471		
26	19'-hexanoyloxyfucoxanthin / T2-3	Hex-fuco	21.22	447 471		
27	Diadinochrome / T1	Diadchr	22.90	(410)	430	458
28	Diadinoxanthin / T1-4	Diadino	23.77	(422)	448	477
29	Dinoxanthin / T1	Dino	24.93	418	442	471
30	Unk carotenoid λ_{\max} 463 / T4	Unk-car463- <i>Kf</i>	24.12	463		
31	<i>cis</i> -fucoxanthin / T2-4	<i>c</i> -fuco	24.61	442		
32	Antheraxanthin / T6	Anth	24.77	(422)	447	475
33	19'-acyloxyfucoxanthin-like / T3	Acyl-fuco-like	25.26	448 471		
34	Unk carotenoid λ_{\max} 453 / T4	Unk-car453- <i>Kf</i>	25.42	(428)	453	(482)
35	Alloxanthin / T5	Allo	26.06	(428)	454	483
36	Lycopene-like / T1	Lycy-like-1	26.30	451	476	509
37	Diatoxanthin / T1-4	Diato	26.62	(426)	453	481
38	Unk carotenoid λ_{\max} 447 / T4	Unk-car447- <i>Kf</i>	27.10	(420)	447	(472)
39	Zeaxanthin / T1-4, T6	Zea	27.45	(429)	454	480
40	Unk carotenoid from <i>L. chlorophorum</i> / T6	Unk-car- <i>Lc</i>	27.65	420	443	472
41	Lycopene-like / T1,2	lyco-like-2	28.00	(452)	476	507
42	Unk carotenoid from <i>L. chlorophorum</i> / T6	Unk-car- <i>Lc</i>	28.12	420	443	472
43	Gyroxanthin diester-like / T2-3	GyrE-like	28.58	(418)	445	472
44	Canthaxanthin / T1, T3	Cantha	29.30	472		
45	Gyroxanthin diester-2 / T2-3	GyrE (12:0)	29.56	(418)	445	472

46	Gyroxanthin diester-3 / T2-3	GyrE (14:0)	30.46	(419)	445	471
47	Crocoxanthin / T5	Croco	30.93	(431)	447	476
48	Hydroxyl echinenone / T4	Hydro-echin	31.26		468	
49	β -cryptoxanthin / T1	β -crypto	31.41	(426)	454	480
50	Chlorophyll <i>b</i> / T6	Chl <i>b</i>	31.67	461	597	647
51	Chlorophyll <i>c</i> ₂ MGDG from <i>K. armiger</i> / T3	MGDG-chl <i>c</i> ₂ - <i>Ka</i>	32.17	454	584	634
52	Chlorophyll <i>a</i> allomer / T1-6	Chl <i>a</i> allomer	32.74	420	615	662
53	Chlorophyll <i>c</i> ₂ MGDG from <i>E. huxleyi</i> / T3	MGDG-chl <i>c</i> ₂ - <i>Eh</i>	32.98	454	584	634
54	Chlorophyll <i>a</i> / T1-6	Chl <i>a</i>	33.29	431	617	662
55	Chlorophyll <i>a</i> epimer / T1-6	Chl <i>a</i> '	33.51	431	617	662
56	Chlorophyll <i>c</i> ₂ MGDG from <i>C. polylepis</i> / T2-3	MGDG-chl <i>c</i> ₂ - <i>Cp</i>	33.74	454	584	634
57	β,ψ -carotene-like / T4	$\beta\psi$ -car-like	34.28	(437)	463	492
58	β,ψ -carotene / T4	$\beta\psi$ -car	34.48	(437)	463	493
59	Pheophytin <i>a</i> / T1-6	Pheo <i>a</i>	35.32	409	609	665
60	Chlorophyll <i>c</i> ₂ MGDG from <i>T. helix</i> / T3	MGDG-chl <i>c</i> ₂ - <i>Th</i>	35.45	455	583	634
61	β,ϵ -carotene / T2-6	$\beta\epsilon$ -car	35.49	(422)	447	475
62	β,β -carotene / T1-5	$\beta\beta$ -car	35.67	(426)	454	480
63	<i>cis</i> - β,β -carotene / T4	<i>c</i> - $\beta\beta$ -car	35.94	(426)	452	478

Table 2. Distribution of chlorophylls and carotenoids among pigment-based chloroplast types in Dinophyta. \pm = occasional occurrence.

Pigments	Pigment-based chloroplast types					
	1	2	3	4	5	6
Chlorophylls		Chl <i>c</i> -containing				Chl <i>b</i> -containing
Chl <i>c</i> ₂ -like <i>P. gyrans</i> -type				•		
Chl <i>c</i> ₁ -like <i>K. foliaceum</i> -type				•		
Chl <i>c</i> ₃		•	•			
MgDVP	•	•	•	•	•	•
Chl <i>c</i> ₂	•	•	•	•	•	
Chl <i>c</i> ₁	\pm			•		
Chl <i>b</i>						•
Chl <i>a</i>	•	•	•	•	•	•
MGDG-chl <i>c</i> ₂		•	\pm			
Carotenoids	Per-type	Fuco-type		Allo-type	Vio/Neo-type	
Peridininol	•					
Unk 1/Unk 2	•					
Peridinin	•					
<i>cis</i> -Peridinin	•					
Keto-19'-but-fuco-like		•				
4-keto-19'-but-fuco		•				
19'-but-fuco		•	•			
Fucoxanthin		•	•	•		
Keto-19'-hex-fuco-like		•				
4-keto-19'-hex-fuco		•	\pm			
19'-hex-fuco		•	•			
Violaxanthin				\pm		•
Diadinochrome	•					
Diadinoxanthin	•	•	•	•	•	

Dinoxanthin	•					
Alloxanthin					•	
Diatoxanthin	±	±	±	±	±	
Zeaxanthin	±	±	±	±		±
Unknown carotenoid <i>Lc</i>						•
Lycopene-like	±	±	±	±		±
Gyroxanthin diester 1		•	•			
Gyroxanthin diester 2		•	•			
Gyroxanthin diester 3		•	•			
βΨ-carotene				•		•
β,ε-carotene		•	•	•	•	•
β,β-carotene	•	•	•	•	±	•

Table 3. Molar pigment ratios to chl *a* and its variability in pigment type-1.

Order, species	Strain code	Perid/ chl <i>c</i> ₂	Perid/ chl <i>a</i>	Chl <i>c</i> ₂ /chl <i>a</i>	Chl <i>c</i> ₁ / chl <i>a</i>	Diadino/ chl <i>a</i>	Dino/ chl <i>a</i>
GONYAULACALES							
<i>Alexandrium affine</i>	PA2V	4.08	1.08	0.27	0.00	0.52	0.22
<i>A. andersonii</i>	CCMP1718	3.36	1.14	0.34	0.00	0.60	0.17
<i>A. andersonii</i>	VGO664	4.48	1.39	0.31	0.00	0.64	0.09
<i>A. andersonii</i>	SZN-12	3.19	0.61	0.19	0.00	0.41	0.18
<i>A. catenella</i>	AT02	3.05	0.85	0.28	0.00	0.63	0.24
<i>A. catenella</i>	VGO609	3.00	0.84	0.28	0.00	0.43	0.39
<i>A. catenella</i>	AL96	2.90	0.64	0.22	0.00	0.28	0.14
<i>A. insuetum</i>	ICMB218	3.16	1.02	0.32	0.00	0.38	0.11
<i>A. margalefii</i>	ICMB	3.47	1.11	0.32	0.00	0.41	0.14
<i>A. margalefii</i>	VGO661	2.94	0.90	0.31	0.00	0.30	0.15
<i>A. minutum</i>	AL1V	5.05	1.34	0.27	0.00	0.40	0.17
<i>A. minutum</i>	AMAD01	3.31	1.36	0.41	0.00	0.46	0.17
<i>A. minutum</i>	CLONE4	2.68	1.07	0.40	0.00	0.43	0.20
<i>A. minutum</i>	VGO577	3.14	1.11	0.35	0.00	0.47	0.16
<i>A. ostenfeldii</i>	AOTV-A1A	3.29	0.98	0.30	0.02	0.84	0.20
<i>A. ostenfeldii</i>	AOTV-A4A	3.04	0.89	0.29	0.01	0.80	0.16
<i>A. peruvianum</i>	AM10C	4.94	1.24	0.25	0.00	0.63	0.14
<i>A. pseudogonyaulax</i>	VGO706	3.41	1.28	0.38	0.00	0.28	0.07
<i>A. tamarense</i>	CCMP1493	3.24	0.95	0.29	0.00	0.40	0.17
<i>A. tamarense</i>	MDQ1096	3.30	1.14	0.34	0.00	0.50	0.18
<i>A. tamarense</i>	PE1V	2.81	0.95	0.34	0.00	0.38	0.16
<i>A. tamarense</i>	VGO553	3.59	1.11	0.31	0.00	0.44	0.16
<i>A. tamutum</i>	VGO617	3.30	1.21	0.37	0.00	0.59	0.17
<i>A. taylori</i>	AM8V	2.16	0.55	0.27	0.00	0.67	0.25
<i>A. taylori</i>	VGO703	2.36	0.63	0.27	0.00	0.37	0.23
<i>Coolia canariensis</i>	VGO775	4.11	1.56	0.38	0.00	0.63	0.15

<i>C. canariensis</i>	VGO787	4.00	2.06	0.52	0.00	0.57	0.13
<i>C. monotis</i>	CM2V	3.14	1.02	0.33	0.00	0.58	0.16
<i>C. monotis</i>	CM6V	3.01	1.11	0.37	0.00	0.65	0.17
<i>C. monotis</i>	RIKZ4	2.49	1.08	0.43	0.00	0.59	0.18
<i>C. monotis</i>	CCMP1345	2.65	1.15	0.43	0.00	0.47	0.19
<i>Coolia</i> sp.	VGO923	3.00	1.05	0.35	0.00	0.42	0.13
<i>Coolia tropicalis</i>	CCMP1744	2.23	1.14	0.52	0.07	0.60	0.17
<i>Fragilidium</i> sp.	VGO692	3.05	0.96	0.31	0.00	0.68	0.19
<i>Fragilidium</i> sp.	IO 91-01	2.84	1.03	0.36	0.00	0.52	0.20
<i>Gambierdiscus excentricus</i>	VGO790	3.39	1.52	0.45	0.06	0.62	0.20
<i>Gambierdiscus</i> sp.	VGO920	2.72	0.91	0.33	0.04	0.53	0.18
<i>Gambierdiscus</i> sp.	KC81G1	2.72	1.10	0.41	0.07	0.62	0.16
<i>Lingulodinium polyedrum</i>	LP4V	3.85	1.11	0.29	0.00	0.37	0.20
<i>L. polyedrium</i>	LP9V	4.12	1.10	0.27	0.00	0.37	0.19
<i>Neoceratium furca</i>	Nfurca1	2.81	0.72	0.26	0.00	0.38	0.01
<i>Ostreopsis ovata</i>	OS01BR	3.00	1.19	0.40	0.00	0.76	0.14
<i>O. cf. ovata</i>	VGO611	2.95	1.18	0.40	0.00	0.47	0.13
<i>Ostreopsis cf. siamensis</i>	OS3V	3.42	1.43	0.42	0.00	1.01	0.12
<i>O. cf. siamensis</i>	VGO613	3.49	1.37	0.39	0.00	0.74	0.14
<i>O. cf. siamensis</i>	VGO883	3.35	1.22	0.37	0.00	0.60	0.14
<i>Protoceratium reticulatum</i>	GG1AM	2.84	1.02	0.36	0.01	0.40	0.16
<i>P. reticulatum</i>	CCMP404	3.88	0.94	0.24	0.04	0.62	0.19
<i>P. reticulatum</i>	CCMP1720	4.26	1.02	0.24	0.03	0.64	0.15
GYMNODINIALES							
<i>Akashiwo sanguinea</i>	VGO138	2.20	0.68	0.31	0.00	0.68	0.20
<i>A. sanguinea</i>	VGO626	2.91	0.75	0.25	0.00	0.41	0.20
<i>Amphidinium carterae</i>	A01BR	2.04	1.17	0.57	0.00	0.90	0.10
<i>A. carterae</i>	ACMK03	1.60	0.87	0.54	0.00	0.77	0.19

Order, species	Strain code	Perid/ chl <i>c</i> ₂	Perid/ Chl <i>a</i>	Chl <i>c</i> ₂ /chl <i>a</i>	Chl <i>c</i> ₁ / chl <i>a</i>	Diadino/ chl <i>a</i>	Dino/ chl <i>a</i>
<i>A. carterae</i>	ACRN02	1.81	1.09	0.60	0.00	0.70	0.07
<i>A. cf. carterae</i>	A1V	2.29	0.94	0.41	0.00	0.56	0.09
<i>Barrufeta bravensis</i>	VGO859	1.85	0.61	0.34	0.00	0.75	0.18
<i>B. bravensis</i>	VGO860	1.93	0.79	0.41	0.00	0.81	0.42
<i>B. bravensis</i>	VGO864	1.92	0.54	0.29	0.00	0.89	0.32
<i>Gymnodinium catenatum</i>	GC11V	2.40	0.72	0.30	0.00	0.62	0.28
<i>G. catenatum</i>	GC31AM	1.93	0.58	0.30	0.00	0.51	0.28
<i>G. catenatum</i>	CS-302	1.84	0.82	0.44	0.00	0.64	0.24
<i>G. impudicum</i>	GY1VA	1.94	0.66	0.35	0.00	0.59	0.29
<i>G. instriatum</i>	VGO642	2.51	0.84	0.34	0.00	0.73	0.15
<i>G. cf. microreticulatum</i>	VGO581	1.93	0.59	0.30	0.02	0.55	0.20
<i>G. nolleri</i>	DK5	2.89	1.02	0.35	0.00	0.42	0.32
<i>Gyrodinium uncatenum</i>	CS289-3	2.54	0.71	0.28	0.41	0.75	0.12
PERIDINIALES							
<i>Heterocapsa niei</i>	VGO399	1.39	0.79	0.56	0.12	0.52	0.18
<i>Heterocapsa triquetra</i>	VGO1053	1.40	0.70	0.50	0.00	0.73	0.16
<i>Peridinium aciculiferum</i>	PAER-1	2.62	0.81	0.31	0.14	0.43	0.10
<i>Peridinium aciculiferum</i>	PAER-2	2.75	0.88	0.32	0.16	0.44	0.10
<i>Scrippsiella hangoei</i>	STHV-1	1.95	0.89	0.45	0.02	0.51	0.11
<i>Scrippsiella hangoei</i>	STHV-2	1.94	0.92	0.46	0.02	0.53	0.10
<i>Scrippsiella hangoei</i>	STHV-5	2.04	0.81	0.38	0.02	0.44	0.09
<i>Scrippsiella hangoei</i>	STHV-6	1.96	0.85	0.43	0.03	0.52	0.11
<i>Scrippsiella</i> sp.	S3V	4.87	1.18	0.24	0.00	0.54	0.10
PROROCENTRALES							
<i>P. arenarium</i>	VGO776	7.17	0.95	0.13	0.03	0.59	0.27
<i>P. belizeanum</i>	PBMA01	19.11	1.65	0.09	0.00	0.80	0.25
<i>P. belizeanum</i>	VGO867	24.54	1.43	0.06	0.01	0.75	0.22
<i>P. compressum</i>	VGO621	4.59	1.17	0.26	0.00	0.45	0.12

<i>P. cf. faustiae</i>	VGO894	7.13	1.16	0.16	0.00	0.50	0.26
<i>P. levis</i>	VGO777	38.92	1.20	0.03	0.01	0.62	0.24
<i>P. levis</i>	VGO957	15.04	0.97	0.07	0.00	0.61	0.30
<i>P. lima</i>	PL2V	11.66	1.54	0.13	0.00	1.09	0.30
<i>P. cf. lima</i>	VGO620	3.68	1.32	0.36	0.01	0.82	0.30
<i>P. micans</i>	PM1V	7.66	1.24	0.16	0.00	0.62	0.13
<i>P. minimum</i>	VGO365	4.59	1.21	0.27	0.00	0.53	0.07
<i>P. minimum</i>	VGO367	10.31	1.65	0.16	0.00	0.43	0.08
<i>P. nux</i>	UTEX1008	3.51	1.51	0.43	0.00	0.73	0.11
<i>P. rathymum</i>	VGO893	9.94	1.59	0.16	0.00	0.64	0.16
<i>P. rostratum</i>	PR1V	18.00	1.69	0.09	0.00	0.46	0.12
<i>P. triestinum</i>	PT2V	4.13	1.35	0.33	0.00	0.64	0.14
THORACOSPHAERALES							
<i>Thoracosphaera heimii</i>	CCMP1069	2.35	1.13	0.48	0.00	0.89	0.05
Mean		4.57	1.07	0.33	0.01	0.57	0.18
SD		5.35	0.29	0.12	0.02	0.16	0.07
N		90	90	90	21	90	90

Table 4. Molar pigment ratios (accessory chls/chl *a*) in pigment types-2 & 3. MGDG-chl *c* stands for monogalactosyl-diacylglycerol-chl *c* compounds.

Pigment type-2	Chl <i>c</i> ₃ / chl <i>a</i>	Chl <i>c</i> ₂ / chl <i>a</i>	MGDG-chl <i>c</i> ₂ /chl <i>a</i> Peak 51	MGDG-chl <i>c</i> ₂ /chl <i>a</i> Peak 56	MGDG-chl <i>c</i> ₂ /chl <i>a</i> Peak 60	Chl <i>c</i> ₃ /chl <i>c</i> ₂	ΣChls <i>c</i> /chl <i>a</i>
<i>Karenia brevis</i> CCMP718	0.07	0.12	–	0.005	–	0.58	0.20
<i>K. brevis</i> CCMP2281	0.07	0.15	–	0.004	–	0.47	0.22
<i>Karenia mikimotoi</i> CCMP429	0.08	0.16	–	0.002	–	0.47	0.24
<i>K. papilonacea</i> VGO679	0.05	0.13	–	0.005	–	0.44	0.19
<i>K. selliformis</i> VGO875	0.08	0.16	–	0.003	–	0.51	0.24
<i>K. umbella</i> Gy2DE	0.07	0.16	–	0.002	–	0.58	0.23
Pigment type-3							
<i>Karlodinium armiger</i> GC-7	0.08	0.15	0.010	0.010	0.002	0.55	0.25
<i>K. decipiens</i> Nervión34	0.07	0.22	–	0.002	–	0.33	0.29
<i>K. veneficum</i> CCMP415	0.08	0.22	–	–	–	0.38	0.30
<i>K. veneficum</i> CCMP1974	0.08	0.22	–	–	–	0.35	0.30
<i>K. veneficum</i> CS-310	0.10	0.23	–	–	–	0.38	0.33
<i>K. veneficum</i> GC-4	0.06	0.18	–	–	–	0.29	0.30
<i>K. veneficum</i> VGO691	0.07	0.19	–	–	–	0.38	0.26
<i>K. veneficum</i> VGO870	0.07	0.21	–	–	–	0.31	0.28
<i>Takayama cf. helix</i> VGO341	0.07	0.21	0.005	0.013	0.007	0.33	0.31

Table 4 (cont.) Molar pigment ratios (carotenoids/chl *a*) in pigment types-2 & 3.

Pigment type-2	But-fuco like-1	But-fuco like-2	But-fuco	Fuco	4k-Hex-fuco-like	4k-Hex-fuco	Hex-fuco	GyrE1-like	GyrE2 C12:0	GyrE3 C14:0
Species and strain code										
<i>Karenia brevis</i> CCMP718	0.04	0.07	0.08	0.34	0.05	0.11	0.07	–	0.11	–
<i>K. brevis</i> CCMP2281	0.06	0.06	0.08	0.24	0.09	0.10	0.09	–	0.14	–
<i>K. mikimotoi</i> CCMP429	0.07	0.07	0.10	0.33	0.11	0.11	0.09	0.02	0.11	–
<i>K. papilonacea</i> VGO679	0.02	0.04	0.13	0.14	0.09	0.09	0.29	0.03	0.17	0.01
<i>K. selliformis</i> VGO875	0.08	0.09	0.08	0.27	0.10	0.12	0.08	0.01	0.12	0.01
<i>K. umbella</i> Gy2DE	0.01	0.01	0.13	0.27	0.10	0.08	0.32	–	0.16	–
Pigment type-3										
<i>Karlodinium armiger</i> GC-7	–	–	0.01	0.34	–	–	0.45	–	0.11	0.05
<i>K. decipiens</i> Nervión34	–	–	0.25	0.22	–	–	0.44	–	0.14	0.09
<i>K. veneficum</i> CCMP 415	–	–	0.13	0.56	–	–	0.31	–	0.24	0.02
<i>K. veneficum</i> CCMP 1974	–	–	0.16	0.44	–	–	0.28	–	0.16	0.09
<i>K. veneficum</i> CS-310	–	–	0.18	0.75	–	–	0.33	–	0.20	0.04
<i>K. veneficum</i> GC-4	–	–	0.08	0.72	–	–	0.21	–	0.19	0.04
<i>K. veneficum</i> VGO691	–	–	0.10	0.58	–	–	0.25	–	0.20	0.03
<i>K. veneficum</i> VGO870	–	–	0.19	0.30	–	–	0.39	–	0.20	0.02
<i>Takayama cf. helix</i> VGO341	–	–	–	1.18	–	–	0.06	–	–	–

Table 5. Molar pigment ratios to chl *a* in pigment types-4, 5 & 6. (tr.: trace amounts).

Pigment type 4	Chl <i>c</i> ₂	Chl <i>c</i> ₁ -like <i>Eg.</i>	MgDVP	Chl <i>c</i> ₁	Fuco	β,ψ-car		
<i>Peridinium balticum</i> CS-33	0.04	0.005	-	0.09	0.54	tr.		
<i>Kryptoperidinium foliaceum</i> CS-37	0.06	0.009	0.002	0.11	0.52	tr.		
<i>K. foliaceum</i> VGO556	0.08	0.01	0.004	0.11	0.75	0.05		
Pigment type 5	Chl <i>c</i> ₂	Alloxanthin	Crocoxanthin	β,ε-car				
<i>Dinophysis acuminata</i> VGO1063	0.07	1.62	0.08	0.20				
<i>Dinophysis acuta</i> VGO1065	0.09	1.35	0.07	0.18				
<i>Dinophysis caudata</i> VGO1064	0.07	1.38	0.05	0.19				
<i>Dinophysis tripos</i> VGO1062	0.09	1.32	0.05	0.15				
Pigment type 6	Neo	Viola	Anth	Zea	Unk443	βε-car	ββ-car	chl <i>b</i>
<i>L. chlorophorum</i> Dino16EUH	0.09	0.08	0.01	0.01	0.17	0.04	0.04	0.73
<i>L. chlorophorum</i> RCC1488	0.14	0.16	0.01	0.05	0.16	0.03	0.05	0.56
<i>L. chlorophorum</i> RCC1489	0.14	0.12	0.02	0.06	0.14	0.03	0.05	0.57
<i>L. chlorophorum</i> BAH100ME	0.09	0.32	0.03	0.02	0.18	0.11	0.05	0.08

Table 6. Distribution of pigment-based chloroplast types across Dinophyta taxa. (*Tamura et al. 2005)

CLASS DINOPHYCEAE		Pigment types					
Order	Representative species	1	2	3	4	5	6
Dinophysiales	<i>Dinophysis acuminata</i>					●	
Gonyaulacales	<i>Alexandrium minutum</i>	●					
Gymnodiniales	<i>Gymnodinium catenatum</i>	●					
	<i>Karenia mikimotoi</i>		●				
	<i>Karlodinium veneficum</i>						
	<i>Takayama helix</i>			●			
	<i>Lepidodinium chlorophorum</i>						●
Peridinales	<i>Heterocapsa</i> sp.	●					
	<i>Durinskia baltica</i>						
	<i>Galeidinium rugatum</i> * <i>Kryptoperidinium foliaceum</i>					●	
Prorocentrales	<i>Prorocentrum lima</i>	●					
Thoracosphaerales	<i>Thoracosphaera heimii</i>	●					

Table S1. Cultures used in this study. (Na: not available).

Order, species	Strain code	Origin/isolation year	Isolator
DINOPHYSALES			
<i>Dinophysis acuminata</i> Claparède et Lachmann	VGO1063	Ría de Vigo, NW Spain, 2009	F. Rodríguez
<i>D. acuta</i> Ehrenberger	VGO1065	Ría de Pontevedra, Spain, 2010	F. Rodríguez
<i>D. caudata</i> Saville-Kent	VGO1064	Ría de Pontevedra, Spain, 2010	F. Rodríguez
<i>Dinophysis tripos</i> Gourret	VGO1062	Ría de Vigo, NW Spain, 2009	F. Rodríguez
GONYAULACALES			
<i>Alexandrium affine</i> (Inoue et Fukuyo) Balech	PA2V	Ría de Vigo, NW Spain, 1985	I. Bravo
<i>A. andersonii</i> Balech	CCMP1718	Town Cove, Eastham, MA, USA, 1987	S. Marquis
<i>A. andersonii</i>	SZN-12	Tyrrhenian Sea, Naples, Italy, na	M. Montresor
<i>A. andersonii</i>	VGO664	Saronikos Gulf, Greece, Mediterranean Sea, 2003	S. Fraga
<i>A. catenella</i> (Whedon et Kofoid) Balech	AT02	Thau Lagoon, France, Mediterranean Sea, 1998	D. Kulis
<i>A. catenella</i>	VGO609	Tarragona, Mediterranean Sea, 2002	S. Fraga
<i>A. catenella</i>	AL96	Monterey Bay, California, Pacific Ocean	R. Gester
<i>A. insuetum</i> Balech	ICMB218	Puerto Arenys de Mar, Mediterranean Sea, 2006	N. Sampedro
<i>A. margalefi</i> Balech	VGO661	Els Alfacs, Tarragona, Mediterranean Sea, 2003	I. Bravo
<i>A. minutum</i> Halim	AL1V	Ría de Vigo, NW Spain, 1987	I. Bravo
<i>A. minutum</i>	AMAD01	Port River, S. Australia, Ocean Pacific, 1988	S. Blackburn
<i>A. minutum</i>	GHmin04	Denmark, Atlantic Ocean, 2001	G. Hansen
<i>A. minutum</i>	VGO577	La Fosca, Girona, Mediterranean Sea, 2002	S. Fraga
<i>A. ostenfeldii</i> (Paulsen) Balech et Tangen	AOTV-A1A	Föglö/Åland, Finland, Baltic Sea, 2004	A. Kemp
<i>A. ostenfeldii</i>	AOTV-A4A	Föglö/Åland, Finland, Baltic Sea, 2004	A. Kemp
<i>A. peruvianum</i> (Balech & Mendiola) Balech et Tangen	AM10C	Costa Brava, Mediterranean Sea, 2002	I. Bravo
<i>A. pseudogonyaulax</i> (Biecheler) Horoguchi	VGO706	Alfacs Bay, Ebro Delta, Mediterranean Sea, 2003	I. Bravo
<i>A. tamarense</i> (Lebour) Balech	CCMP1493	Da Yia Bay, China, Pacific Ocean, 1991	G. Usup
<i>A. tamarense</i>	MDQ1096	Mar del Plata, Argentina, Atlantic Ocean, 1996	I. Carreto
<i>A. tamarense</i>	PE1V	Ría de Vigo, NW Spain, 1984	I. Bravo
<i>A. tamarense</i>	VGO553	Kavala Harbor, Greece, Mediterranean Sea, 2002	S. Fraga
<i>A. tamutum</i> Montresor, Beran et John	VGO617	Els Alfacs, Tarragona, Mediterranean Sea, 2002	I. Bravo
<i>A. taylori</i> Balech	AM8V	Costa Brava, Mediterranean Sea, 1994	S. Fraga
<i>A. taylori</i>	VGO703	Alfacs Bay, Ebro Delta, Mediterranean Sea, 2003	I. Bravo
<i>Coolia canariensis</i> Fraga	VGO775	Tenerife, Canary Islands, Spain, 2004	S. Fraga
<i>C. canariensis</i>	VGO787	Tenerife, Canary Islands, Spain, 2004	S. Fraga

<i>C. monotis</i> Meunier	CM2V	Ría de Vigo, NW Spain, 1985	I. Bravo
<i>C. monotis</i>	CM6V	Almería, Spain, Mediterranean Sea, 1999	S. Fraga
<i>C. monotis</i>	RIKZ4	Yerseke, The Netherlands, Atlantic Ocean, 2000	L. Peperzak
<i>C. monotis</i>	SZN43	Naples, Italy, Mediterranean Sea, 1991	M. Montresor
<i>C. cf. monotis</i>	CCMP1345	Florida, USA, 1986	J. Bomber
<i>C. tropicalis</i> Faust	CCMP1744	Twin Cay, Belize, 1994	S. L. Morton
<i>C. cf. tropicalis</i>	VGO923	Manado, Indonesia, 2007	S. Fraga
<i>Fragilidium</i> sp.	IO 91-01	Cascais, Portugal, 2007	S. Fraga
<i>Fragilidium</i> sp.	VGO692	Elefsis Bay, Saronikos Gulf, Grece, 2003	S. Fraga
<i>Gambierdiscus excentricus</i> Fraga	VGO790	Tenerife, Canary Islands, Spain, 2004	S. Fraga
<i>Gambierdiscus</i> sp.	VGO920	Manado, Indonesia, Pacific Ocean, 2007	S. Fraga
<i>Gambierdiscus</i> sp.	KC81G1	Crete, Greece, Mediterranean Sea, 2007	K. Aligizaki
<i>Lingulodinium polyedrum</i> (Stein) Dodge	LP4V	Ría de Lorbé, NW Spain, 2000	S. Fraga
<i>L. polyedrum</i>	LP9V	Ría de Ares, NW Spain, 2002	S. Fraga
<i>Neoceratium furca</i> (Ehrenberg) Gómez, Moreira et Lopez-García	Nfurca1	Baiona, Ría de Vigo, NW Spain, 2009	F. Rodríguez
<i>Ostreopsis cf. ovata</i> Fukuyo	OS01BR	Rio de Janeiro, Brazil, Atlantic Ocean, 2000	S. Fraga
<i>O. cf. ovata</i>	VGO883	Lanzarote, Canary Islands, Atlantic Ocean, 2006	S. Fraga
<i>O. cf. ovata</i>	VGO613	Bahía de Abra, Isl. Madeira, Atlantic Ocean, 2002	S. Fraga
<i>O. cf. siamensis</i> Schmidt	OS3V	Almería, Spain, Mediterranean Sea, 1999	S. Fraga
<i>Protoceratium reticulatum</i> (Claparède et Lachmann) Bütschli	GG1AM	La Atunara, Cadiz, Mediterranean Sea, 1999	S. Fraga
<i>P. reticulatum</i>	CCMP404	Salton Lake, CA, USA, 1966	A. Dodson
<i>P. reticulatum</i>	CCMP1720	Biscayne Bay, Miami, USA, 1994	P. Hargraves
GYMNODINIALES			
<i>Akashiwo sanguinea</i> (Hirasaka) Hansen et Moestrup	VGO138 (=G.spl)	Catalonian Coast, Mediterranean Sea, na	M. Delgado
<i>A. sanguinea</i>	VGO626	Greece, Mediterranean Sea, 2002	S. Fraga
<i>Amphidinium carterae</i> Hulburt	A01BR	Brazil, Atlantic Ocean, 2000	S. O. Lourenço
<i>A. carterae</i>	ACMK03	Maurice Isl., Indian Ocean, 1996	J. Diogène
<i>A. carterae</i>	ACRN02	Reunion Isl., Indian Ocean, 1992	J. Diogène
<i>A. carterae</i>	CS-212	Falmouth, Massachusetts, Atlantic Ocean, 1954	R. Gillard
<i>A. cf. carterae</i>	A1V	Ría de Pontevedra, NW Spain, 1985	I. Bravo
<i>Barrufeta bravensis</i> Sampedro et Fraga	VGO859	La Fosca, Girona, Mediterranean Sea, 2005	S. Fraga
<i>B. bravensis</i>	VGO860	La Fosca, Girona, Mediterranean Sea, 2005	S. Fraga
<i>B. bravensis</i>	VGO864	La Fosca, Girona, Mediterranean Sea, 2005	S. Fraga
<i>Gymnodinium catenatum</i> Graham	GC11V	Ría de Vigo, Spain, NW Spain, 1993	I. Bravo

<i>G. catenatum</i>	GC31AM	La Atunara, Cádiz, Spain, 1999	S. Fraga
<i>G. catenatum</i>	CS-302	Hastings Bay, Tasmania, Australia, 1990	
<i>G. impudicum</i> (Fraga et Bravo) Hansen et Moestrup	GY1VA	Valencia, Mediterranean Sea, 1992	I. Bravo
<i>G. instriatum</i> (Freudenthal et Lee) Coats	VGO642	Vilanova, Barcelona, Mediterranean Sea, 2003	S. Fraga
<i>G. litoralis</i> René			
<i>G. cf. microreticulatum</i> Bolch et Hallegraeff	VGO581	La Fosca, Girona, Mediterranean Sea, 2002	S. Fraga
<i>G. nolleri</i> Ellegaard et Moestrup	DK5	Øresund, Denmark, Atlantic Ocean, 1998	M. Ellegaard
<i>Gyrodinium uncatenum</i> Hulburt	CS-289/3	Bathurst Harbor, Tasmania, Australia, 1988	S. Blackburn
<i>Karenia brevis</i> (Davis) Hansen et Moestrup	CCMP718	Florida, USA, North Atlantic Ocean, 1960	Wilson
<i>K. brevis</i>	CCMP2281	Pensacola Beach, Florida, North Atlantic, 2003	J. Rogers
<i>K. mikimotoi</i> (Miyake et Kominami ex Oda) Hansen et Moestrup	CCMP429	Sutton Harbour, Plymouth, Atlantic Ocean, 1980	D. Harbor
<i>K. papilonacea</i> Haywood et Steidinger	VGO679	Sant Cast, Brittany, Atlantic Ocean, 2003	S. Fraga
<i>K. selliformis</i> Haywood, Steidinger et MacKenzie	VGO876	Bougrara, Tunisia, Mediterranean Sea, 2006	S. Fraga
<i>K. selliformis</i>	VGO877	Bougrara, Tunisia, Mediterranean Sea, 2006	S. Fraga
<i>K. selliformis</i>	VGO901	Bougrara, Tunisia, Mediterranean Sea, 2006	S. Fraga
<i>K. umbella</i> de Salas, Bolch et Hallegraeff	Gy2DE	Derwent River, Tasmania, Australia, 1997	C. Bolch
<i>Karlodinium armiger</i> Bergholtz, Daugberg et. Moestrup	GC-2 IRTA	Alfacs Bay, Ebro Delta, Mediterranean Sea, 2000	M. Fernández
<i>K. armiger</i>	GC-3 IRTA	Alfacs Bay, Ebro Delta, Mediterranean Sea, 2000	M. Fernández
<i>K. armiger</i>	VGO260	Delta del Ebro, Tarragona, Mediterranean Sea, na	M. Delgado
<i>K. decipiens</i> de Salas, Bolch et Hallegraeff	Nervión34	Nervión Estuary, Biscay Bay, 2004	A. Laza-Martinez
<i>K. veneficum</i> (Ballantine) Larsen	CCMP415	59.3, 10.36E, Norway, Atlantic Ocean, NA, 1976	W. Thomas
<i>K. veneficum</i>	CCMP1974	Chesapeake Bay, USA, Atlantic Ocean, 1995	L. Aishao
<i>K. veneficum</i>	CS-310	Illawarra Lake, NSW, Australia, na	C. Bolch
<i>K. veneficum</i>	GC-4 IRTA	Alfacs Bay, Ebro Delta, Mediterranean Sea, 2000	M. Fernández
<i>K. veneficum</i>	VGO691	Bretagne, France, Atlantic Ocean, 2003	S. Fraga
<i>K. veneficum</i>	VGO870	Bougrara, Tunes, Mediterranean Sea, 2006	S. Fraga
<i>Lepidodinium chlorophorum</i> (Elbrächter et Schnepf) Hansen, Botes et de Salas	BAHME100	List / Sylt 55°01.30'N, 08°27.10'E, 1990	M. Elbraechter
<i>L. chlorophorum</i>	Dino16EUH	Nervion River, Biscay Bay, Atlantic Ocean, 2003	A. Laza-Martinez
<i>L. chlorophorum</i>	RCC1488	English Channel, Normandy Coast, Atlantic Ocean	I. Probert
<i>L. chlorophorum</i>	RCC1489	English Channel, Normandy Coast, Atlantic Ocean	I. Probert
<i>Takayama cf. helix</i> de Salas, Bolch et Hallegraeff	VGO 341	Ría de Vigo, NW Spain, 2001	S. Fraga
PERIDINIALES			
<i>Durinskia baltica</i> (=Peridinium balticum Levis) Lemm	CS-38	Salton Sea, CA, USA, na	A. Loeblich
<i>Heterocapsa niei</i> (Loeblich III, 1968) Morrill & Loeblich III	VGO399	Ría de Lorbé, NW Spain, 2002	S. Fraga

<i>H. triquetra</i> (Ehrenberg) Stein	VGO1053	Bahía de Fangar, Ebro Delta, Mediterranean Sea, 2005	I. Bravo
<i>Kryptoperidinium foliaceum</i> (Stein) Lindemann	CS-37	La Parguera, Puerto Rico, Atlantic Ocean, 1965	P. R. Burkholder
<i>K. foliaceum</i>	VGO556	Catoira, Ría de Arousa, NW Spain, 2002	F. Rodríguez
<i>Peridinium aciculiferum</i> Lemmermann	PAER-1	Lake Erken, Sweden, 1995	K. Rengefors
<i>P. aciculiferum</i>	PAER-2	Lake Erken, Sweden, 2004	K. Rengefors
<i>Scrippsiella hangoei</i> (Schiller) Larsen	STHV-1	Baltic Sea, near Tvarminne, Finland, 2002	A. Kremp
<i>S. hangoei</i>	STHV-4	Baltic Sea, near Tvarminne, Finland, 2002	A. Kremp
<i>S. hangoei</i>	STHV-6	Baltic Sea, near Tvarminne, Finland, 2002	A. Kremp
<i>Scrippsiella</i> sp.	S3V	Ría de Vigo, NW Spain, 1989	S. Fraga
PROROCENTRALES			
<i>Prorocentrum arenarium</i> Faust	VGO776	Tenerife, Canary Islands, Spain, 2004	S. Fraga
<i>P. belizeanum</i> Faust	PBMA01	Mayotte Island, Indian Ocean, 1992	J. Diogène
<i>P. belizeanum</i>	VGO867	La Gomera, Canary Islands, Spain, 2005	S. Fraga
<i>P. compressum</i> (Bailey) Abé ex Dodge	VGO621	Greece, Mediterranean Sea, 2002	S. Fraga
<i>P. cf. faustiae</i> Morton	VGO894	Malaysia, na	N. Noor
<i>P. levis</i> Faust, Kibler, Vandersea, Tester et Litaker	VGO777	Tenerife, Canary Islands, Spain, 2004	S. Fraga
<i>P. levis</i>	VGO957	Ebro Delta, Mediterranean Sea, na	J. Diogène
<i>P. lima</i> (Ehrenberg) Dodge	PL2V	Isl. Cies, Ría de Vigo, NW Spain, 1985	I. Bravo
<i>P. lima</i>	VGO620	Torre Mora, Spain, Mediterranean Sea, 2002	S. Fraga
<i>P. micans</i> Ehrenberg	PM1V	Ría de Vigo, NW Spain, 1985	I. Bravo
<i>P. minimum</i> (Pavillard) Schiller	VGO365	Ría de Vigo, NW Spain, 2001	S. Fraga
<i>P. minimum</i>	VGO367	Ría de Vigo, NW Spain, 2001	S. Fraga
<i>P. nux</i> Puigserver et Zingone	UTEX1008	England, North Sea, Atlantic Ocean, 1957	I. Adams
<i>Prorocentrum rathymum</i> Loeblich, Shirley et Schmidt	VGO893	Malaysia, na	Norma
<i>P. rostratum</i> Stein	PR1V	Ría de Vigo, NW Spain, 1990	I. Bravo
<i>P. triestinum</i> Schiller	PT2V	Ría de Vigo, NW Spain, 1989	S. Fraga
THORACOSPHAERALES			
<i>Thoracosphaera heimii</i> (Lohm.) Kamptner	CCMP1069	23°48.9'N89°45.7'W, Gulf of Mexico, 1989	L. Brand

Table S2. Mass (w:w) pigment ratios to chl *a* and its variability in pigment type-1.

Order, species	Strain code	Perid/ chl <i>c</i> ₂	Perid/ chl <i>a</i>	Chl <i>c</i> ₂ /chl <i>a</i>	Chl <i>c</i> ₁ / chl <i>a</i>	Diadino/ chl <i>a</i>	Dino/ chl <i>a</i>
GONYAULACALES							
<i>Alexandrium affine</i>	PA2V	4.23	0.76	0.18	0.00	0.34	0.16
<i>A. andersonii</i>	CCMP1718	3.48	0.80	0.23	0.00	0.39	0.12
<i>A. andersonii</i>	VGO664	4.64	0.98	0.21	0.00	0.42	0.06
<i>A. andersonii</i>	SZN-12	3.30	0.43	0.13	0.00	0.27	0.13
<i>A. catenella</i>	AT02	3.16	0.60	0.19	0.00	0.41	0.17
<i>A. catenella</i>	VGO609	3.11	0.59	0.19	0.00	0.28	0.28
<i>A. catenella</i>	AL96	3.00	0.45	0.15	0.00	0.18	0.10
<i>A. insuetum</i>	ICMB218	3.27	0.72	0.22	0.00	0.25	0.08
<i>A. margalefii</i>	ICMB	3.59	0.78	0.22	0.00	0.27	0.10
<i>A. margalefii</i>	VGO661	3.05	0.64	0.21	0.00	0.20	0.11
<i>A. minutum</i>	AL1V	5.23	0.95	0.18	0.00	0.26	0.12
<i>A. minutum</i>	AMAD01	3.43	0.96	0.28	0.00	0.30	0.12
<i>A. minutum</i>	CLONE4	2.78	0.76	0.27	0.00	0.28	0.14
<i>A. minutum</i>	VGO577	3.25	0.78	0.24	0.00	0.31	0.12
<i>A. ostenfeldii</i>	AOTV-A1A	3.41	0.69	0.20	0.01	0.55	0.14
<i>A. ostenfeldii</i>	AOTV-A4A	3.15	0.63	0.20	0.01	0.52	0.12
<i>A. peruvianum</i>	AM10C	5.12	0.88	0.17	0.00	0.41	0.10
<i>A. pseudogonyaulax</i>	VGO706	3.53	0.90	0.26	0.00	0.18	0.05
<i>A. tamarense</i>	CCMP1493	3.36	0.67	0.20	0.00	0.26	0.12
<i>A. tamarense</i>	MDQ1096	3.42	0.80	0.23	0.00	0.33	0.13
<i>A. tamarense</i>	PE1V	2.91	0.67	0.23	0.00	0.25	0.12
<i>A. tamarense</i>	VGO553	3.72	0.78	0.21	0.00	0.29	0.12
<i>A. tamutum</i>	VGO617	3.42	0.85	0.25	0.00	0.38	0.12
<i>A. taylori</i>	AM8V	2.24	0.39	0.18	0.00	0.44	0.18
<i>A. taylori</i>	VGO703	2.44	0.44	0.18	0.00	0.24	0.17

<i>Coolia canariensis</i>	VGO775	4.26	1.10	0.26	0.00	0.41	0.11
<i>C. canariensis</i>	VGO787	4.14	1.45	0.35	0.00	0.37	0.09
<i>C. monotis</i>	CM2V	3.25	0.72	0.22	0.00	0.38	0.12
<i>C. monotis</i>	CM6V	3.12	0.78	0.25	0.00	0.42	0.12
<i>C. monotis</i>	RIKZ4	2.58	0.76	0.29	0.00	0.38	0.13
<i>C. monotis</i>	CCMP1345	2.75	0.81	0.29	0.00	0.31	0.14
<i>Coolia</i> sp.	VGO923	3.11	0.74	0.24	0.00	0.27	0.09
<i>Coolia tropicalis</i>	CCMP1744	2.31	0.80	0.35	0.05	0.39	0.12
<i>Fragilidium</i> sp.	VGO692	3.16	0.68	0.21	0.00	0.44	0.14
<i>Fragilidium</i> sp.	IO 91-01	2.94	0.73	0.25	0.00	0.34	0.14
<i>Gambierdiscus excentricus</i>	VGO790	3.51	1.07	0.31	0.04	0.40	0.14
<i>Gambierdiscus</i> sp.	VGO920	2.82	0.64	0.22	0.03	0.35	0.13
<i>Gambierdiscus</i> sp.	KC81G1	2.82	0.78	0.28	0.05	0.40	0.12
<i>Lingulodinium polyedrum</i>	LP4V	3.99	0.78	0.20	0.00	0.24	0.14
<i>L. polyedrium</i>	LP9V	4.27	0.78	0.18	0.00	0.24	0.14
<i>Neoceratium furca</i>	Nfurca1	2.91	0.51	0.18	0.00	0.25	0.01
<i>Ostreopsis ovata</i>	OS01BR	3.11	0.84	0.27	0.00	0.50	0.10
<i>O. cf. ovata</i>	VGO611	3.06	0.83	0.27	0.00	0.31	0.09
<i>Ostreopsis</i> cf. <i>siamensis</i>	OS3V	3.54	1.01	0.29	0.00	0.66	0.09
<i>O. cf. siamensis</i>	VGO613	3.62	0.97	0.27	0.00	0.48	0.10
<i>O. cf. siamensis</i>	VGO883	3.47	0.86	0.25	0.00	0.39	0.10
<i>Protoceratium reticulatum</i>	GG1AM	2.94	0.72	0.25	0.01	0.26	0.12
<i>P. reticulatum</i>	CCMP404	4.02	0.66	0.16	0.03	0.40	0.14
<i>P. reticulatum</i>	CCMP1720	4.41	0.72	0.16	0.02	0.42	0.11
GYMNODINIALES							
<i>Akashiwo sanguinea</i>	VGO138	2.28	0.48	0.21	0.00	0.44	0.14
<i>A. sanguinea</i>	VGO626	3.01	0.53	0.17	0.00	0.27	0.14
<i>Amphidinium carterae</i>	A01BR	2.11	0.83	0.39	0.00	0.59	0.07
<i>A. carterae</i>	ACMK03	1.66	0.61	0.37	0.00	0.50	0.14

Order, species	Strain code	Perid/ chl <i>c</i> ₂	Perid/ Chl <i>a</i>	Chl <i>c</i> ₂ /chl <i>a</i>	Chl <i>c</i> ₁ / chl <i>a</i>	Diadino/ chl <i>a</i>	Dino/ chl <i>a</i>
<i>A. carterae</i>	ACRN02	1.88	0.77	0.41	0.00	0.46	0.05
<i>A. cf. carterae</i>	A1V	2.37	0.66	0.28	0.00	0.37	0.06
<i>Barrufeta bravensis</i>	VGO859	1.92	0.43	0.23	0.00	0.49	0.13
<i>B. bravensis</i>	VGO860	2.00	0.56	0.28	0.00	0.53	0.30
<i>B. bravensis</i>	VGO864	1.99	0.38	0.20	0.00	0.58	0.23
<i>Gymnodinium catenatum</i>	GC11V	2.49	0.51	0.20	0.00	0.40	0.20
<i>G. catenatum</i>	GC31AM	2.00	0.41	0.20	0.00	0.33	0.20
<i>G. catenatum</i>	CS-302	1.91	0.58	0.30	0.00	0.42	0.17
<i>G. impudicum</i>	GY1VA	2.01	0.47	0.24	0.00	0.38	0.21
<i>G. instriatum</i>	VGO642	2.60	0.59	0.23	0.00	0.48	0.11
<i>G. cf. microreticulatum</i>	VGO581	2.00	0.42	0.20	0.01	0.36	0.14
<i>G. nolleri</i>	DK5	2.99	0.72	0.24	0.00	0.27	0.23
<i>Gyrodinium uncatenum</i>	CS289-3	2.63	0.50	0.19	0.28	0.49	0.09
PERIDINIALES							
<i>Heterocapsa niei</i>	VGO399	1.44	0.56	0.38	0.08	0.34	0.13
<i>Heterocapsa triquetra</i>	VGO1053	1.45	0.49	0.34	0.00	0.48	0.12
<i>Peridinium aciculiferum</i>	PAER-1	2.71	0.57	0.21	0.10	0.28	0.07
<i>Peridinium aciculiferum</i>	PAER-2	2.85	0.62	0.22	0.11	0.29	0.07
<i>Scrippsiella hangoei</i>	STHV-1	2.02	0.63	0.31	0.01	0.33	0.08
<i>Scrippsiella hangoei</i>	STHV-2	2.01	0.65	0.31	0.01	0.35	0.07
<i>Scrippsiella hangoei</i>	STHV-5	2.11	0.57	0.26	0.01	0.29	0.06
<i>Scrippsiella hangoei</i>	STHV-6	2.03	0.60	0.29	0.02	0.34	0.08
<i>Scrippsiella</i> sp.	S3V	5.04	0.83	0.16	0.00	0.35	0.07
PROROCENTRALES							
<i>P. arenarium</i>	VGO776	7.43	0.67	0.09	0.02	0.38	0.19
<i>P. belizeanum</i>	PBMA01	19.80	1.16	0.06	0.00	0.52	0.18
<i>P. belizeanum</i>	VGO867	25.42	1.01	0.04	0.01	0.49	0.16

<i>P. compressum</i>	VGO621	4.75	0.83	0.18	0.00	0.29	0.09
<i>P. cf. faustiae</i>	VGO894	7.39	0.82	0.11	0.00	0.33	0.19
<i>P. levis</i>	VGO777	40.32	0.85	0.02	0.01	0.40	0.17
<i>P. levis</i>	VGO957	15.58	0.68	0.05	0.00	0.40	0.22
<i>P. lima</i>	PL2V	12.08	1.09	0.09	0.00	0.71	0.22
<i>P. cf. lima</i>	VGO620	3.81	0.93	0.25	0.01	0.53	0.22
<i>P. micans</i>	PM1V	7.94	0.88	0.11	0.00	0.40	0.09
<i>P. minimum</i>	VGO365	4.75	0.85	0.18	0.00	0.35	0.05
<i>P. minimum</i>	VGO367	10.68	1.16	0.11	0.00	0.28	0.06
<i>P. nux</i>	UTEX1008	3.64	1.07	0.29	0.00	0.48	0.08
<i>P. rathymum</i>	VGO893	10.30	1.12	0.11	0.00	0.42	0.12
<i>P. rostratum</i>	PR1V	18.65	1.19	0.06	0.00	0.30	0.09
<i>P. triestinum</i>	PT2V	4.28	0.95	0.22	0.00	0.42	0.10
THORACOSPHAERALES							
<i>Thoracosphaera heimii</i>	CCMP1069	2.43	0.80	0.33	0.00	0.58	0.04
Mean		4.52	0.73	0.22	0.01	0.37	0.12
SD		5.39	0.23	0.08	0.03	0.12	0.05
N		90	90	90	21	90	90

Table S3. Mass pigment ratios (w:w, accessory chls/chl *a*) in pigment types-2 and 3.

Pigment type-2	Chl <i>c</i> ₃ / chl <i>a</i>	Chl <i>c</i> ₂ / chl <i>a</i>	MGDG-chl <i>c</i> ₂ /chl <i>a</i> Peak 51	MGDG-chl <i>c</i> ₂ /chl <i>a</i> Peak 56	MGDG-chl <i>c</i> ₂ /chl <i>a</i> Peak 60	Chl <i>c</i> ₃ /chl <i>c</i> ₂	∑Chls <i>c</i> /chl <i>a</i>
<i>Karenia brevis</i> CCMP718	0.05	0.08	0.00	0.01	0.00	0.62	0.14
<i>K. brevis</i> CCMP2281	0.05	0.10	0.00	0.01	0.00	0.50	0.16
<i>Karenia mikimotoi</i> CCMP429	0.06	0.11	0.00	0.00	0.00	0.50	0.17
<i>K. papilonacea</i> VGO679	0.04	0.09	0.00	0.01	0.00	0.47	0.13
<i>K. selliformis</i> VGO875	0.06	0.11	0.00	0.00	0.00	0.55	0.17
<i>K. umbella</i> Gy2DE	0.05	0.11	0.00	0.00	0.00	0.62	0.16
Pigment type-3							
<i>Karlodinium armiger</i> GC-7	0.06	0.10	0.01	0.01	0.00	0.59	0.19
<i>K. decipiens</i> Nervión34	0.05	0.15	0.00	0.00	0.00	0.35	0.20
<i>K. veneficum</i> CCMP415	0.06	0.15	0.00	0.00	0.00	0.41	0.21
<i>K. veneficum</i> CCMP1974	0.06	0.15	0.00	0.00	0.00	0.38	0.21
<i>K. veneficum</i> CS-310	0.07	0.16	0.00	0.00	0.00	0.41	0.23
<i>K. veneficum</i> GC-4	0.04	0.12	0.00	0.00	0.00	0.31	0.17
<i>K. veneficum</i> VGO691	0.05	0.13	0.00	0.00	0.00	0.41	0.18
<i>K. veneficum</i> VGO870	0.05	0.14	0.00	0.00	0.00	0.33	0.19
<i>Takayama cf. helix</i> VGO341	0.05	0.14	0.01	0.02	0.01	0.35	0.23

Table S3 (cont.). Mass pigment ratios (w:w, carotenoids/chl *a*) in pigment types-2 and 3.

Pigment type-2	But-fuco like-1	But-fuco like-2	But-fuco	Fuco	4k-Hex-fuco -like	4k-Hex-fuco	Hex-fuco	GyrE1 -like	GyrE2 C12:0	GyrE3 C14:0
Species and strain code										
<i>Karenia brevis</i> CCMP718	0.03	0.06	0.07	0.25	0.04	0.10	0.06	0.00	0.10	0.00
<i>K. brevis</i> CCMP2281	0.05	0.05	0.07	0.18	0.08	0.09	0.08	0.00	0.13	0.00
<i>K. mikimotoi</i> CCMP429	0.06	0.06	0.08	0.24	0.10	0.10	0.08	0.02	0.10	0.00
<i>K. papilonacea</i> VGO679	0.02	0.03	0.11	0.10	0.08	0.08	0.25	0.03	0.16	0.01
<i>K. selliformis</i> VGO875	0.07	0.08	0.07	0.20	0.09	0.11	0.07	0.01	0.11	0.01
<i>K. umbella</i> Gy2DE	0.01	0.01	0.11	0.20	0.09	0.07	0.28	0.00	0.15	0.00
Pigment type-3										
<i>Karlodinium armiger</i> GC-7	0.00	0.00	0.01	0.25	0.00	0.00	0.39	0.00	0.10	0.05
<i>K. decipiens</i> Nervión34	0.00	0.00	0.21	0.16	0.00	0.00	0.38	0.00	0.13	0.09
<i>K. veneficum</i> CCMP 415	0.00	0.00	0.11	0.41	0.00	0.00	0.27	0.00	0.23	0.02
<i>K. veneficum</i> CCMP 1974	0.00	0.00	0.13	0.32	0.00	0.00	0.24	0.00	0.15	0.09
<i>K. veneficum</i> CS-310	0.00	0.00	0.15	0.55	0.00	0.00	0.29	0.00	0.19	0.04
<i>K. veneficum</i> GC-4	0.00	0.00	0.07	0.53	0.00	0.00	0.18	0.00	0.18	0.04
<i>K. veneficum</i> VGO691	0.00	0.00	0.08	0.43	0.00	0.00	0.22	0.00	0.19	0.03
<i>K. veneficum</i> VGO870	0.00	0.00	0.16	0.22	0.00	0.00	0.34	0.00	0.19	0.02
<i>Takayama cf. helix</i> VGO341	0.00	0.00	0.00	0.87	0.00	0.00	0.05	0.00	0.00	0.00

Table S4. Mass pigment ratios to chl *a* (w:w) in pigment types-4, 5 and 6. (tr.: trace amounts).

Pigment type 4	Chl <i>c</i> ₂	Chl <i>c</i> ₁ -like <i>Eg.</i>	MgDVP	Chl <i>c</i> ₁	Fuco	β,ψ-car		
<i>Peridinium balticum</i> CS-33	0.03	0.003	0.000	0.06	0.40	tr.		
<i>Kryptoperidinium foliaceum</i> CS-37	0.04	0.006	0.001	0.07	0.38	tr.		
<i>K. foliaceum</i> VGO556	0.05	0.007	0.003	0.08	0.55	0.03		
Pigment type 5	Chl <i>c</i> ₂	Alloxanthin	Crocoxanthin	β,ε-car				
<i>Dinophysis acuminata</i> VGO1063	0.05	1.02	0.05	0.12				
<i>Dinophysis acuta</i> VGO1065	0.06	0.85	0.04	0.11				
<i>Dinophysis caudata</i> VGO1064	0.05	0.87	0.03	0.12				
<i>Dinophysis tripos</i> VGO1062	0.06	0.84	0.03	0.09				
Pigment type 6	Neo	Viola	Anth	Zea	Unk443	βε-car	ββ-car	chl <i>b</i>
<i>L. chlorophorum</i> Dino16EUH	0.06	0.05	0.007	0.006	0.11	0.02	0.02	0.74
<i>L. chlorophorum</i> RCC1488	0.09	0.11	0.007	0.03	0.10	0.02	0.03	0.58
<i>L. chlorophorum</i> RCC1489	0.09	0.08	0.01	0.04	0.09	0.02	0.03	0.57
<i>L. chlorophorum</i> BAH100ME	0.06	0.22	0.02	0.01	0.12	0.07	0.03	0.08

Table S5. Species of *Karenia*, *Karlodinium* and *Takayama* so far described and the pigment information available

<i>Karenia</i> spp.	Species description	HPLC pigment data
<i>K. asterichroma</i>	de Salas et al. 2004b	–
<i>K. bicuneiformis</i>	Botes et al. 2003	–
<i>K. bidigitata</i>	Haywood et al. 2004	–
<i>K. brevis</i>	(C.C. Davis) Hansen & Moestrup, in Daugbjerg et al. 2000	Millie et al. 1997, Örnólfsdóttir et al. 2003
<i>K. brevisulcata</i>	Chang & Ryan, 2004	–
<i>K. concordia</i>	Chang & Ryan, 2004	–
<i>K. cristata</i>	Botes et al. 2003	–
<i>K. digitata</i>	Yang et al. 2000	–
<i>K. longicanalis</i>	Yang et al. 2001	–
<i>K. mikimotoi</i>	(Miyake et Kominami ex Oda) Hansen & Moestrup, in Daugbjerg et al. 2000	Suzuki & Ishimaru 1992, Johnsen & Sakshaug 1993, this paper
<i>K. papilonaceae</i>	Haywood et al. 2004	Laza et al. 2007
<i>K. selliformis</i>	Haywood et al. 2004	This paper

<i>K. umbella</i>	de Salas et al. 2004a	de Salas et al. 2004a, this paper
<i>Karlodinium</i> spp.		
<i>K. antarcticum</i>	de Salas et al. 2008	–
<i>K. armiger</i>	Bergholtz et al. 2006	Bergholtz et al. 2006, Garcés et al. 2006, this paper
<i>K. australe</i>	de Salas et al. 2005	de Salas et al. 2005
<i>K. ballatinum</i>	de Salas et al. 2008	–
<i>K. conicum</i>	de Salas et al. 2008	–
<i>K. corrugatum</i>	de Salas et al. 2008	–
<i>K. corsicum</i>	Siano et al. 2009	–
<i>K. decipiens</i>	de Salas et al. 2008	Laza et al. 2007, this paper
<i>K. veneficum</i>	(Ballantine) Larsen, in Daugbjerg et al. 2000	Johnsen & Sakshaug 1993, Kempton et al. 2002, Bergholtz et al. 2006, Garcés et al. 2006, Bachvaroff et al. 2009, this paper
<i>Takayama</i> spp.		
<i>T. acrotrocha</i>	Siano et al. 2009	–

<i>T. cladochroma</i>	(Larsen) de Salas, Bolch & Hallegraeff, in de Salas et al. 2003	–
<i>Takayama helix</i>	de Salas et al. 2003	de Salas et al. 2003, this paper
<i>T. tasmanica</i>	de Salas et al. 2003	de Salas et al. 2003
<i>T. tuberculata</i>	de Salas et al. 2008	–
<i>T. pulchella</i>	Steidinger et al. 1998	–

Table S6. Peridinin to chl *a* molar ratios used in CHEMTAX analysis of pigment data. Original data were in mass (w:w) ratios; present data are showed into molar ratio by multiplying the ratio of chl *a* MW (894) to MW of peridinin (631) = 1.42

Peridinin to chl <i>a</i> ratio (mole: mole)	Reference
0.75	Mackey et al. 1996
0.75	Wright et al. 1996
0.86-1.42	Mackey et al. 1998
0.61, 0.94	Schlüter et al. 2000
1.51	Wright & van den Enden 2000
0.71; 0.94	Descy et al. 2000
0.77, 1.09	Suzuki et al. 2002
1.51	Ishikawa et al. 2002
0.85	DiTullio et al. 2003
0.76	Örnólfssdóttir et al. 2003
0.76	Rodríguez et al. 2003
0.71	Buchaca et al. 2005

1.51	Llewellyn et al. 2005
1.12	Lewitus et al. 2005
0.88	DiTullio et al. 2005
0.56-0.65	Rodríguez et al. 2006b
0.52-0.88	Schlüter et al. 2006
0.60	Develi et al. 2008
1.10	Lionard et al. 2008
0.92	Wright et al. 2009
1.16	Wright et al. 2010
0.59-0.63	Lauridsen et al. 2011
0.56-0.62	Laviale & Neveux 2011
0.50-0.89	Ruivo et al. 2011
0.99-1.06	Schlüter et al. 2011
0.75	Suzuki et al. 2011

Table S7. Pigment-based chloroplast types in Dinophyceae and its distribution in other algal taxa.

Dinophyceae pigment type	Representative species	Algal taxa with similar pigment-based chloroplast	Representative species
Type-1	<i>Alexandrium minutum</i>	–	–
Type-2	<i>Karenia mikimotoi</i>	–	–
Type-3	<i>Karlodinium veneficum</i>	Haptophyta Type-8*	<i>Phaeocystis</i> spp.?
Type-4	<i>Durinskia baltica</i> , <i>Kryptoperidinium foliaceum</i>	Bacillariophyceae/ Haptophyta-Type 2*	<i>Pseudo-nitzschia australis</i> / <i>Pavlova gyrams</i>
Type-5	<i>Dinophysis acuminata</i>	Cryptophyceae	<i>Teleaulax</i> spp.
Type-6	<i>Lepidodinium chlorophorum</i>	Chlorophyceae/Ulvophyceae Trebouxiophyceae	–

*Haptophyte pigment types as defined in Zapata et al. (2004)

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IN MEMORIAM

While this paper was being reviewed, our colleague and friend Manuel Zapata passed away. He contributed greatly to the present state of knowledge of algal pigments (on their structures, methods of analysis, taxonomical distribution and use as signatures for characterizing natural phytoplankton communities). His HPLC method for algal pigments [Zapata, M; Rodríguez, F., Garrido JL (2000) *Mar Ecol Prog Ser* 195: 29-45] has become a standard procedure in marine laboratories along the world. “Zapa”, as his family and friends called him affectionately, left us an important body of knowledge and a special way to observe nature. We will always remember him.

Fig. 1

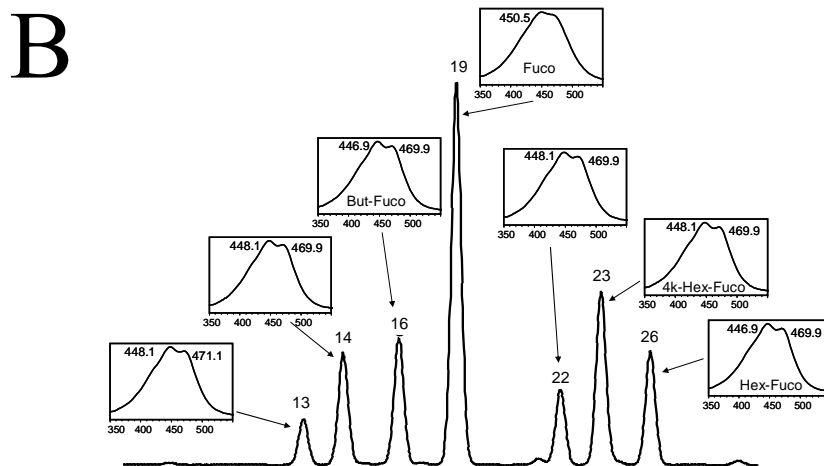
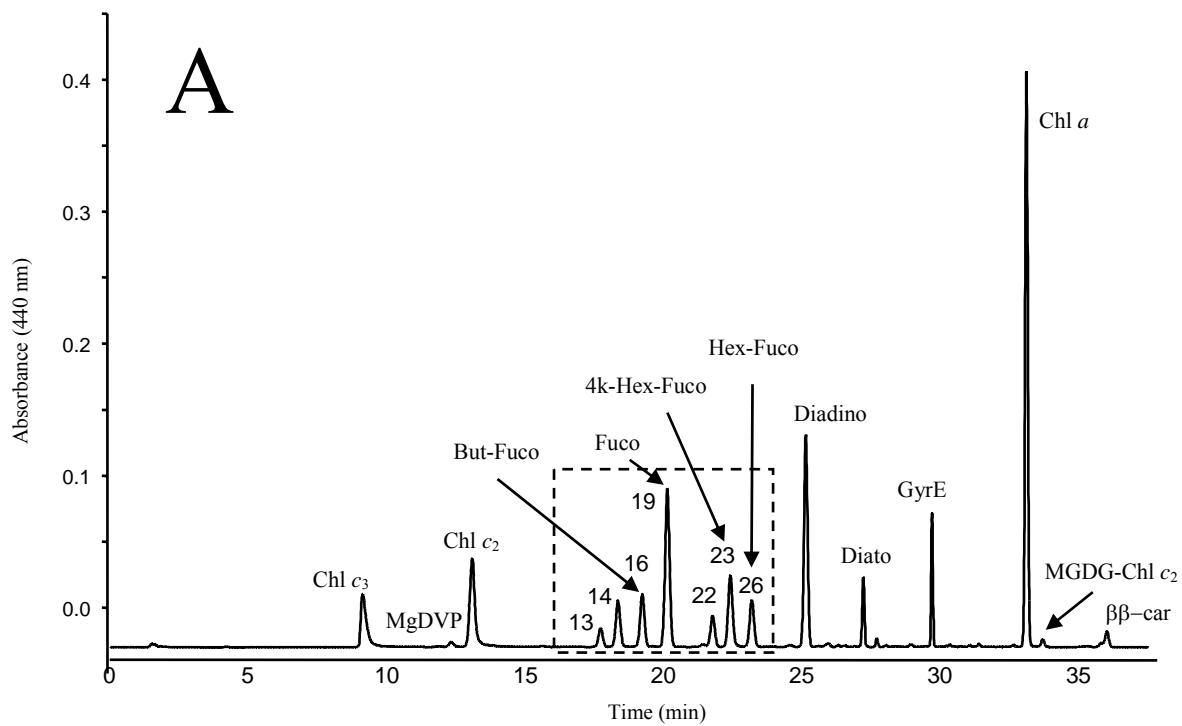


Fig. 2

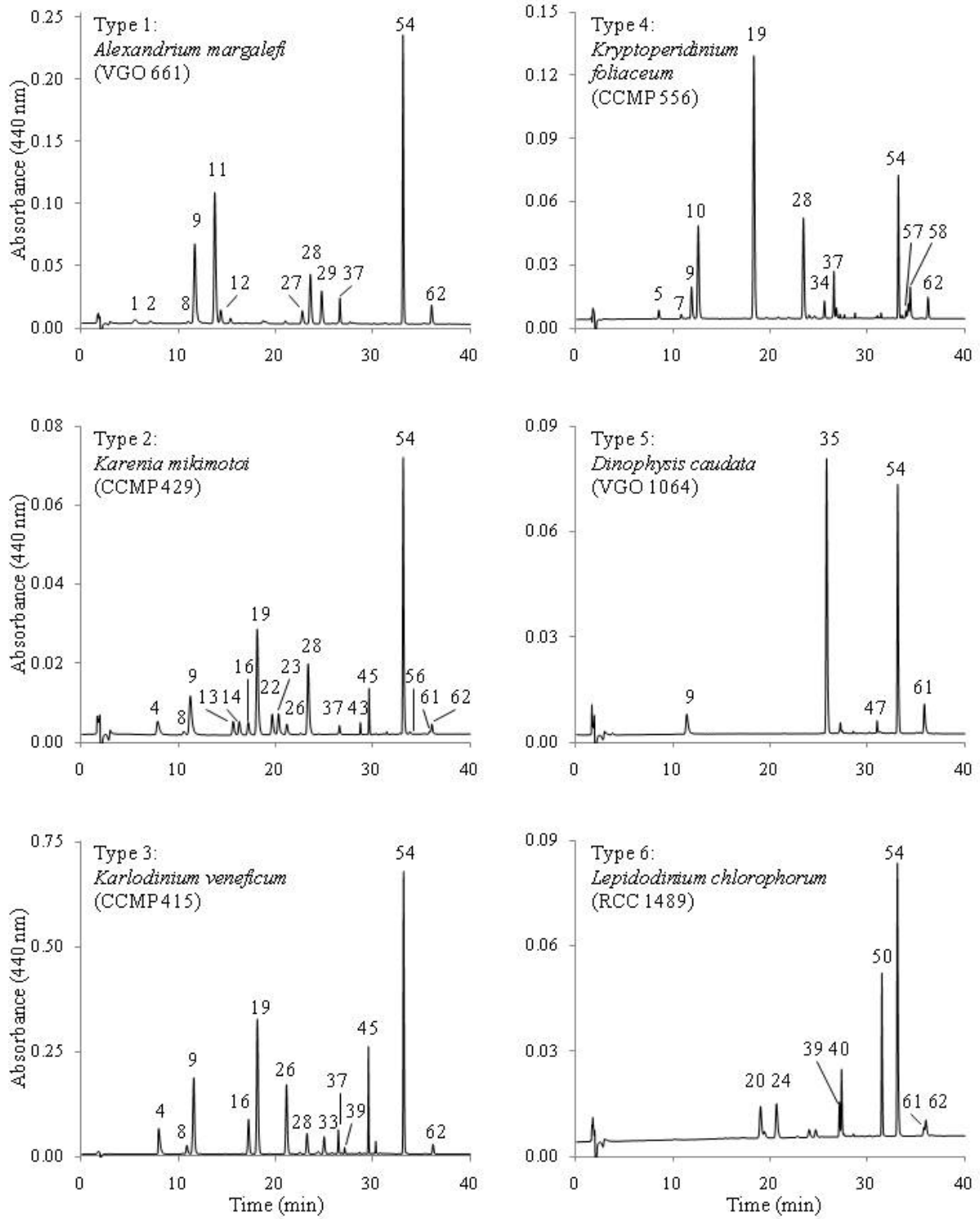
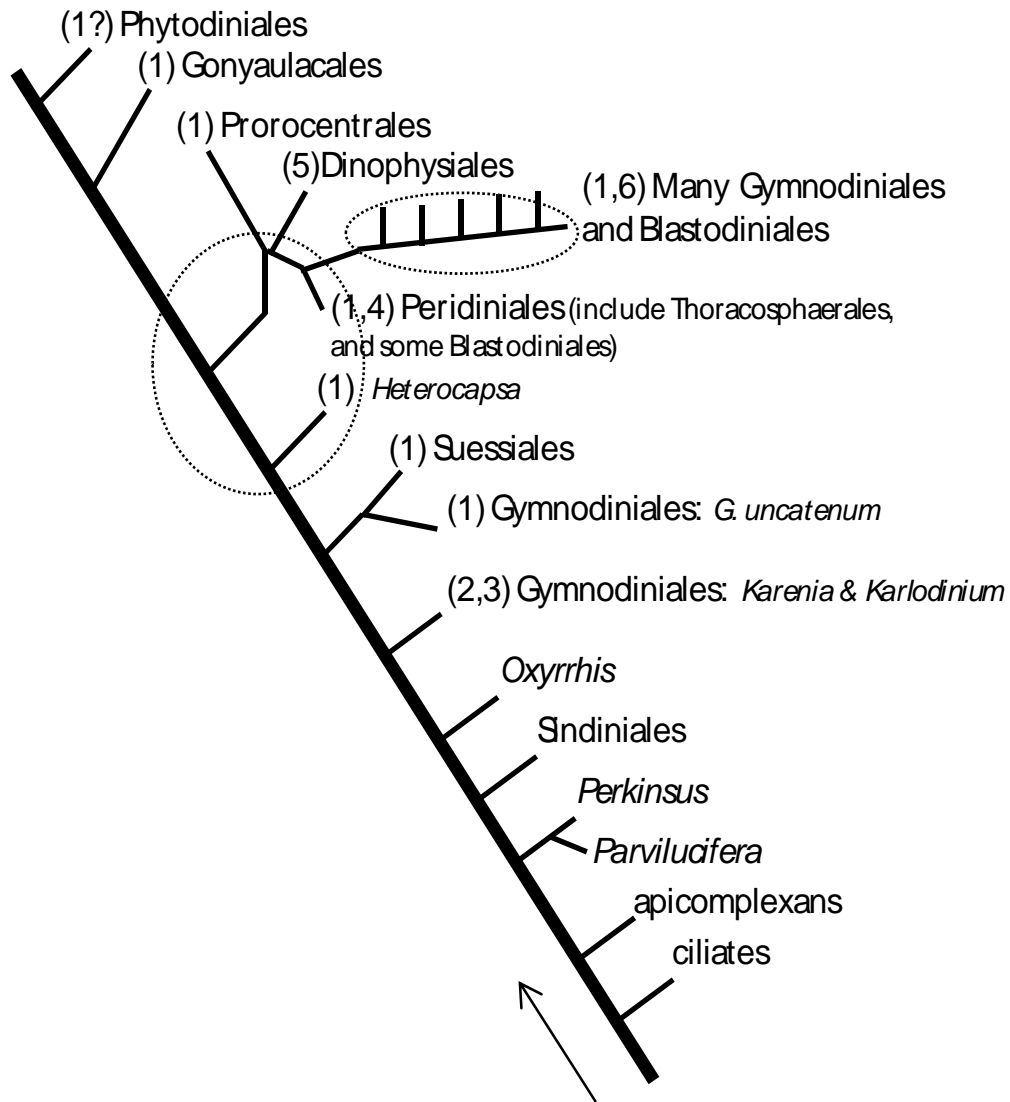


Fig. 3



Pigment-based chloroplast types in dinoflagellates

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Supplement. This supplement includes basic information about the strains used in the present study, and mass pigment to chl *a* ratios

Table S1. Cultures used in this study; na: strain not available

Order and species	Strain code	Origin and isolation year	Isolator
DINOPHYSALES			
<i>Dinophysis acuminata</i> Claparède et Lachmann	VGO1063	Ría de Vigo, NW Spain, 2009	F. Rodríguez
<i>D. acuta</i> Ehrenberger	VGO1065	Ría de Pontevedra, Spain, 2010	F. Rodríguez
<i>D. caudata</i> Saville-Kent	VGO1064	Ría de Pontevedra, Spain, 2010	F. Rodríguez
<i>Dinophysis tripos</i> Gourret	VGO1062	Ría de Vigo, NW Spain, 2009	F. Rodríguez
GONYAULACALES			
<i>Alexandrium affine</i> (Inoue et Fukuyo) Balech	PA2V	Ría de Vigo, NW Spain, 1985	I. Bravo
<i>A. andersonii</i> Balech	CCMP1718	Town Cove, Eastham, MA, USA, 1987	S. Marquis
<i>A. andersonii</i>	SZN-12	Tyrrhenian Sea, Naples, Italy, na	M. Montesor
<i>A. andersonii</i>	VGO664	Saronikos Gulf, Greece, Mediterranean Sea, 2003	S. Fraga
<i>A. catenella</i> (Whedon et Kofoid) Balech	AT02	Thau Lagoon, France, Mediterranean Sea, 1998	D. Kulis
<i>A. catenella</i>	VGO609	Tarragona, Mediterranean Sea, 2002	S. Fraga
<i>A. catenella</i>	AL96	Monterey Bay, CA, USA, Pacific Ocean, year unknown	R. Gester
<i>A. insuetum</i> Balech	ICMB218	Puerto Arenys de Mar, Mediterranean Sea, 2006	N. Sampedro
<i>A. margalefi</i> Balech	VGO661	Els Alfacs, Tarragona, Mediterranean Sea, 2003	I. Bravo
<i>A. minutum</i> Halim	AL1V	Ría de Vigo, NW Spain, 1987	I. Bravo
<i>A. minutum</i>	AMAD01	Port River, S. Australia, Pacific Ocean, 1988	S. Blackburn
<i>A. minutum</i>	GHmin04	Denmark, Atlantic Ocean, 2001	G. Hansen
<i>A. minutum</i>	VGO577	La Fosca, Girona, Mediterranean Sea, 2002	S. Fraga

<i>A. ostensfeldii</i> (Paulsen) Balech et Tangen	AOTV-A1A	Föglö/Åland, Finland, Baltic Sea, 2004	A. Kemp
<i>A. ostensfeldii</i>	AOTV-A4A	Föglö/Åland, Finland, Baltic Sea, 2004	A. Kemp
<i>A. peruvianum</i> (Balech & Mendiola) Balech et Tangen	AM10C	Costa Brava, Mediterranean Sea, 2002	I. Bravo
<i>A. pseudogonyaulax</i> (Biecheler) Horoguchi	VGO706	Alfacs Bay, Ebro Delta, Mediterranean Sea, 2003	I. Bravo
<i>A. tamarense</i> (Lebour) Balech	CCMP1493	Da Yia Bay, China, Pacific Ocean, 1991	G. Usup
<i>A. tamarense</i>	MDQ1096	Mar del Plata, Argentina, Atlantic Ocean, 1996	I. Carreto
<i>A. tamarense</i>	PE1V	Ría de Vigo, NW Spain, 1984	I. Bravo
<i>A. tamarense</i>	VGO553	Kavala Harbour, Greece, Mediterranean Sea, 2002	S. Fraga
<i>A. tamutum</i> Montresor, Beran et John	VGO617	Els Alfacs, Tarragona, Mediterranean Sea, 2002	I. Bravo
<i>A. taylori</i> Balech	AM8V	Costa Brava, Mediterranean Sea, 1994	S. Fraga
<i>A. taylori</i>	VGO703	Alfacs Bay, Ebro Delta, Mediterranean Sea, 2003	I. Bravo
<i>Coolia canariensis</i> Fraga	VGO775	Tenerife, Canary Islands, Spain, 2004	S. Fraga
<i>C. canariensis</i>	VGO787	Tenerife, Canary Islands, Spain, 2004	S. Fraga
<i>C. monotis</i> Meunier	CM2V	Ría de Vigo, NW Spain, 1985	I. Bravo
<i>C. monotis</i>	CM6V	Almería, Spain, Mediterranean Sea, 1999	S. Fraga
<i>C. monotis</i>	RIKZ4	Yerseke, The Netherlands, Atlantic Ocean, 2000	L. Peperzak
<i>C. monotis</i>	SZN43	Naples, Italy, Mediterranean Sea, 1991	M. Montresor
<i>C. cf. monotis</i>	CCMP1345	Florida, USA, 1986	J. Bomber
<i>C. tropicalis</i> Faust	CCMP1744	Twin Cay, Belize, 1994	S. L. Morton
<i>C. cf. tropicalis</i>	VGO923	Manado, Indonesia, 2007	S. Fraga
<i>Fragilidium</i> sp.	IO 91-01	Cascais, Portugal, 2007	S. Fraga
<i>Fragilidium</i> sp.	VGO692	Elefsis Bay, Saronikos Gulf, Greece, 2003	S. Fraga
<i>Gambierdiscus excentricus</i> Fraga	VGO790	Tenerife, Canary Islands, Spain, 2004	S. Fraga
<i>Gambierdiscus</i> sp.	VGO920	Manado, Indonesia, Pacific Ocean, 2007	S. Fraga
<i>Gambierdiscus</i> sp.	KC81G1	Crete, Greece, Mediterranean Sea, 2007	K. Aligizaki
<i>Lingulodinium polyedrum</i> (Stein) Dodge	LP4V	Ría de Lorbé, NW Spain, 2000	S. Fraga
<i>L. polyedrum</i>	LP9V	Ría de Ares, NW Spain, 2002	S. Fraga
<i>Neoceratium furca</i> (Ehrenberg) Gómez, Moreira et Lopez-García	Nfurca1	Baiona, Ría de Vigo, NW Spain, 2009	F. Rodríguez
<i>Ostreopsis cf. ovata</i> Fukuyo	OS01BR	Rio de Janeiro, Brazil, Atlantic Ocean, 2000	S. Fraga
<i>O. cf. ovata</i>	VGO883	Lanzarote, Canary Islands, Atlantic Ocean, 2006	S. Fraga
<i>O. cf. ovata</i>	VGO613	Bahía de Abra, Madeira, Atlantic Ocean, 2002	S. Fraga
<i>O. cf. siamensis</i> Schmidt	OS3V	Almería, Spain, Mediterranean Sea, 1999	S. Fraga
<i>Protoceratium reticulatum</i> (Claparède et Lachmann) Bütschli	GG1AM	La Atunara, Cadiz, Mediterranean Sea, 1999	S. Fraga
<i>P. reticulatum</i>	CCMP404	Salton Lake, CA, USA, 1966	A. Dodson
<i>P. reticulatum</i>	CCMP1720	Biscayne Bay, Miami, FL, USA, 1994	P. Hargraves
GYMNODINIALES			
<i>Akashiwo sanguinea</i> (Hirasaka) Hansen et Moestrup	VGO138	Catalonian Coast, Mediterranean Sea, na	M. Delgado
<i>A. sanguinea</i>	VGO626	Greece, Mediterranean Sea, 2002	S. Fraga

<i>Amphidinium carterae</i> Hulburt	A01BR	Brazil, Atlantic Ocean, 2000	S. O. Lourenço
<i>A. carterae</i>	ACMK03	Maurice Island, Indian Ocean, 1996	J. Diogène
<i>A. carterae</i>	ACRN02	Reunion Island, Indian Ocean, 1992	J. Diogène
<i>A. carterae</i>	CS-212	Falmouth, MA, USA, Atlantic Ocean, 1954	R. Gillard
<i>A. cf. carterae</i>	A1V	Ría de Pontevedra, NW Spain, 1985	I. Bravo
<i>Barrufeta bravensis</i> Sampedro et Fraga	VGO859	La Fosca, Girona, Mediterranean Sea, 2005	S. Fraga
<i>B. bravensis</i>	VGO860	La Fosca, Girona, Mediterranean Sea, 2005	S. Fraga
<i>B. bravensis</i>	VGO864	La Fosca, Girona, Mediterranean Sea, 2005	S. Fraga
<i>Gymnodinium catenatum</i> Graham	GC11V	Ría de Vigo, Spain, NW Spain, 1993	I. Bravo
<i>G. catenatum</i>	GC31AM	La Atunara, Cádiz, Spain, 1999	S. Fraga
<i>G. catenatum</i>	CS-302	Hastings Bay, Tasmania, Australia, 1990	
<i>G. impudicum</i> (Fraga et Bravo) Hansen et Moestrup	GY1VA	Valencia, Mediterranean Sea, 1992	I. Bravo
<i>G. instriatum</i> (Freudenthal et Lee) Coats	VGO642	Vilanova, Barcelona, Mediterranean Sea, 2003	S. Fraga
<i>G. litoralis</i> René			
<i>G. cf. microreticulatum</i> Bolch et Hallegraeff	VGO581	La Fosca, Girona, Mediterranean Sea, 2002	S. Fraga
<i>G. nolleri</i> Ellegaard et Moestrup	DK5	Øresund, Denmark, Atlantic Ocean, 1998	M. Ellegaard
<i>Gyrodinium uncatenum</i> Hulburt	CS-289/3	Bathurst Harbor, Tasmania, Australia, 1988	S. Blackburn
<i>Karenia brevis</i> (Davis) Hansen et Moestrup	CCMP718	Florida, USA, North Atlantic Ocean, 1960	W. B. Wilson
<i>K. brevis</i>	CCMP2281	Pensacola Beach, FL, USA, North Atlantic, 2003	J. Rogers
<i>K. mikimotoi</i> (Miyake et Kominami ex Oda) Hansen et Moestrup	CCMP429	Sutton Harbour, Plymouth, UK, Atlantic Ocean, 1980	D. Harbor
<i>K. papilonacea</i> Haywood et Steidinger	VGO679	Sant Cast, Brittany, Atlantic Ocean, 2003	S. Fraga
<i>K. selliformis</i> Haywood, Steidinger et MacKenzie	VGO876	Boughrara, Tunisia, Mediterranean Sea, 2006	S. Fraga
<i>K. selliformis</i>	VGO877	Boughrara, Tunisia, Mediterranean Sea, 2006	S. Fraga
<i>K. selliformis</i>	VGO901	Boughrara, Tunisia, Mediterranean Sea, 2006	S. Fraga
<i>K. umbella</i> de Salas, Bolch et Hallegraeff	Gy2DE	Derwent River, Tasmania, Australia, 1997	C. Bolch
<i>Karlodinium armiger</i> Bergholtz, Daugberg et Moestrup	GC-2 IRTA	Alfacs Bay, Ebro Delta, Mediterranean Sea, 2000	M. Fernández
<i>K. armiger</i>	GC-3 IRTA	Alfacs Bay, Ebro Delta, Mediterranean Sea, 2000	M. Fernández
<i>K. armiger</i>	VGO260	Delta del Ebro, Tarragona, Mediterranean Sea, na	M. Delgado
<i>K. decipiens</i> de Salas, Bolch et Hallegraeff	Nervi34	Nervi34 Estuary, Biscay Bay, 2004	A. Laza-Martinez
<i>K. veneficum</i> (Ballantine) Larsen	CCMP415	59.3° N, 10.36° E, Norway, Atlantic Ocean, 1976	W. Thomas
<i>K. veneficum</i>	CCMP1974	Chesapeake Bay, USA, Atlantic Ocean, 1995	L. Aishao
<i>K. veneficum</i>	CS-310	Illawarra Lake, NSW, Australia, na	C. Bolch
<i>K. veneficum</i>	GC-4 IRTA	Alfacs Bay, Ebro Delta, Mediterranean Sea, 2000	M. Fernández
<i>K. veneficum</i>	VGO691	Bretagne, France, Atlantic Ocean, 2003	S. Fraga
<i>K. veneficum</i>	VGO870	Boughrara, Tunes, Mediterranean Sea, 2006	S. Fraga
<i>Lepidodinium chlorophorum</i> (Elbrächter et Schnepf) Hansen, Botes et de Salas	BAHME100	List / Sylt 55° 01.30' N, 08° 27.10' E, 1990	M. Elbrächter
<i>L. chlorophorum</i>	Dino16EUH	Nervion River, Biscay Bay, Atlantic Ocean, 2003	A. Laza-Martinez

<i>L. chlorophorum</i>	RCC1488	English Channel, Normandy Coast, Atlantic Ocean, year unknown	I. Probert
<i>L. chlorophorum</i>	RCC1489	English Channel, Normandy Coast, Atlantic Ocean, year unknown	I. Probert
<i>Takayama cf. helix</i> de Salas, Bolch et Hallegraeff	VGO 341	Ría de Vigo, NW Spain, 2001	S. Fraga
PERIDINIALES			
<i>Durinskia baltica</i> (= <i>Peridinium balticum</i> Levis) Lemm	CS-38	Salton Sea, CA, USA, na	A. Loeblich
<i>Heterocapsa niei</i> (Loeblich III, 1968) Morrill & Loeblich III	VGO399	Ría de Lorbé, NW Spain, 2002	S. Fraga
<i>H. triquetra</i> (Ehrenberg) Stein	VGO1053	Bahía de Fangar, Ebro Delta, Mediterranean Sea, 2005	I. Bravo
<i>Kryptoperidinium foliaceum</i> (Stein) Lindemann	CS-37	La Parguera, Puerto Rico, Atlantic Ocean, 1965	P. R. Burkholder
<i>K. foliaceum</i>	VGO556	Catoira, Ría de Arousa, NW Spain, 2002	F. Rodríguez
<i>Peridinium aciculiferum</i> Lemmermann	PAER-1	Lake Erken, Sweden, 1995	K. Rengefors
<i>P. aciculiferum</i>	PAER-2	Lake Erken, Sweden, 2004	K. Rengefors
<i>Scrippsiella hangoei</i> (Schiller) Larsen	STHV-1	Baltic Sea, near Tvarminne, Finland, 2002	A. Kremp
<i>S. hangoei</i>	STHV-4	Baltic Sea, near Tvarminne, Finland, 2002	A. Kremp
<i>S. hangoei</i>	STHV-6	Baltic Sea, near Tvarminne, Finland, 2002	A. Kremp
<i>Scrippsiella</i> sp.	S3V	Ría de Vigo, NW Spain, 1989	S. Fraga
PROROCENTRALES			
<i>Prorocentrum arenarium</i> Faust	VGO776	Tenerife, Canary Islands, Spain, 2004	S. Fraga
<i>P. belizeanum</i> Faust	PBMA01	Mayotte Island, Indian Ocean, 1992	J. Diogène
<i>P. belizeanum</i>	VGO867	La Gomera, Canary Islands, Spain, 2005	S. Fraga
<i>P. compressum</i> (Bailey) Abé ex Dodge	VGO621	Greece, Mediterranean Sea, 2002	S. Fraga
<i>P. cf. faustiae</i> Morton	VGO894	Malaysia, na	N. Noor
<i>P. levis</i> Faust, Kibler, Vandersea, Tester et Litaker	VGO777	Tenerife, Canary Islands, Spain, 2004	S. Fraga
<i>P. levis</i>	VGO957	Ebro Delta, Mediterranean Sea, na	J. Diogène
<i>P. lima</i> (Ehrenberg) Dodge	PL2V	Isl. Cies, Ría de Vigo, NW Spain, 1985	I. Bravo
<i>P. lima</i>	VGO620	Torre Mora, Spain, Mediterranean Sea, 2002	S. Fraga
<i>P. micans</i> Ehrenberg	PM1V	Ría de Vigo, NW Spain, 1985	I. Bravo
<i>P. minimum</i> (Pavillard) Schliller	VGO365	Ría de Vigo, NW Spain, 2001	S. Fraga
<i>P. minimum</i>	VGO367	Ría de Vigo, NW Spain, 2001	S. Fraga
<i>P. nux</i> Puigserver et Zingone	UTEX1008	England, North Sea, Atlantic Ocean, 1957	I. Adams
<i>P. rathymum</i> Loeblich, Shirley et Schmidt	VGO893	Malaysia, na	Norma
<i>P. rostratum</i> Stein	PR1V	Ría de Vigo, NW Spain, 1990	I. Bravo
<i>P. triestinum</i> Schliller	PT2V	Ría de Vigo, NW Spain, 1989	S. Fraga
THORACOSPHAERALES			
<i>Thoracosphaera heimii</i> (Lohm.) Kamptner	CCMP1069	23° 48.9' N, 89° 45.7' W, Gulf of Mexico, 1989	L. Brand

Table S2. Mass (w:w) pigment to chlorophyll (chl) *a* ratios and their variability in pigment-based chloroplast Type 1. Abbreviations as in Table 1 of the main text

Order and species	Strain code	Peri:chl <i>c</i> ₂	Peri:chl <i>a</i>	Chl <i>c</i> ₂ :chl <i>a</i>	Chl <i>c</i> ₁ :chl <i>a</i>	Diadino:chl <i>a</i>	Dino:chl <i>a</i>
GONYAULACALES							
<i>Alexandrium affine</i>	PA2V	4.23	0.76	0.18	0.00	0.34	0.16
<i>A. andersonii</i>	CCMP1718	3.48	0.80	0.23	0.00	0.39	0.12
<i>A. andersonii</i>	VGO664	4.64	0.98	0.21	0.00	0.42	0.06
<i>A. andersonii</i>	SZN-12	3.30	0.43	0.13	0.00	0.27	0.13
<i>A. catenella</i>	AT02	3.16	0.60	0.19	0.00	0.41	0.17
<i>A. catenella</i>	VGO609	3.11	0.59	0.19	0.00	0.28	0.28
<i>A. catenella</i>	AL96	3.00	0.45	0.15	0.00	0.18	0.10
<i>A. insuetum</i>	ICMB218	3.27	0.72	0.22	0.00	0.25	0.08
<i>A. margalefii</i>	ICMB	3.59	0.78	0.22	0.00	0.27	0.10
<i>A. margalefii</i>	VGO661	3.05	0.64	0.21	0.00	0.20	0.11
<i>A. minutum</i>	AL1V	5.23	0.95	0.18	0.00	0.26	0.12
<i>A. minutum</i>	AMAD01	3.43	0.96	0.28	0.00	0.30	0.12
<i>A. minutum</i>	CLONE4	2.78	0.76	0.27	0.00	0.28	0.14
<i>A. minutum</i>	VGO577	3.25	0.78	0.24	0.00	0.31	0.12
<i>A. ostenfeldii</i>	AOTV-A1A	3.41	0.69	0.20	0.01	0.55	0.14
<i>A. ostenfeldii</i>	AOTV-A4A	3.15	0.63	0.20	0.01	0.52	0.12
<i>A. peruvianum</i>	AM10C	5.12	0.88	0.17	0.00	0.41	0.10
<i>A. pseudogonyaulax</i>	VGO706	3.53	0.90	0.26	0.00	0.18	0.05
<i>A. tamarense</i>	CCMP1493	3.36	0.67	0.20	0.00	0.26	0.12
<i>A. tamarense</i>	MDQ1096	3.42	0.80	0.23	0.00	0.33	0.13
<i>A. tamarense</i>	PE1V	2.91	0.67	0.23	0.00	0.25	0.12
<i>A. tamarense</i>	VGO553	3.72	0.78	0.21	0.00	0.29	0.12
<i>A. tamutum</i>	VGO617	3.42	0.85	0.25	0.00	0.38	0.12
<i>A. taylori</i>	AM8V	2.24	0.39	0.18	0.00	0.44	0.18
<i>A. taylori</i>	VGO703	2.44	0.44	0.18	0.00	0.24	0.17
<i>Coolia canariensis</i>	VGO775	4.26	1.10	0.26	0.00	0.41	0.11
<i>C. canariensis</i>	VGO787	4.14	1.45	0.35	0.00	0.37	0.09
<i>C. monotis</i>	CM2V	3.25	0.72	0.22	0.00	0.38	0.12
<i>C. monotis</i>	CM6V	3.12	0.78	0.25	0.00	0.42	0.12
<i>C. monotis</i>	RIKZ4	2.58	0.76	0.29	0.00	0.38	0.13
<i>C. monotis</i>	CCMP1345	2.75	0.81	0.29	0.00	0.31	0.14
<i>Coolia</i> sp.	VGO923	3.11	0.74	0.24	0.00	0.27	0.09
<i>C. tropicalis</i>	CCMP1744	2.31	0.80	0.35	0.05	0.39	0.12

<i>Fragilidium</i> sp.	VGO692	3.16	0.68	0.21	0.00	0.44	0.14
<i>Fragilidium</i> sp.	IO 91-01	2.94	0.73	0.25	0.00	0.34	0.14
<i>Gambierdiscus excentricus</i>	VGO790	3.51	1.07	0.31	0.04	0.40	0.14
<i>Gambierdiscus</i> sp.	VGO920	2.82	0.64	0.22	0.03	0.35	0.13
<i>Gambierdiscus</i> sp.	KC81G1	2.82	0.78	0.28	0.05	0.40	0.12
<i>Lingulodinium polyedrum</i>	LP4V	3.99	0.78	0.20	0.00	0.24	0.14
<i>L. polyedrium</i>	LP9V	4.27	0.78	0.18	0.00	0.24	0.14
<i>Neoceratium furca</i>	Nfurca1	2.91	0.51	0.18	0.00	0.25	0.01
<i>Ostreopsis ovata</i>	OS01BR	3.11	0.84	0.27	0.00	0.50	0.10
<i>O. cf. ovata</i>	VGO611	3.06	0.83	0.27	0.00	0.31	0.09
<i>O. cf. siamensis</i>	OS3V	3.54	1.01	0.29	0.00	0.66	0.09
<i>O. cf. siamensis</i>	VGO613	3.62	0.97	0.27	0.00	0.48	0.10
<i>O. cf. siamensis</i>	VGO883	3.47	0.86	0.25	0.00	0.39	0.10
<i>Protoceratium reticulatum</i>	GG1AM	2.94	0.72	0.25	0.01	0.26	0.12
<i>P. reticulatum</i>	CCMP404	4.02	0.66	0.16	0.03	0.40	0.14
<i>P. reticulatum</i>	CCMP1720	4.41	0.72	0.16	0.02	0.42	0.11
GYMNODINIALES							
<i>Akashiwo sanguinea</i>	VGO138	2.28	0.48	0.21	0.00	0.44	0.14
<i>A. sanguinea</i>	VGO626	3.01	0.53	0.17	0.00	0.27	0.14
<i>Amphidinium carterae</i>	A01BR	2.11	0.83	0.39	0.00	0.59	0.07
<i>A. carterae</i>	ACMK03	1.66	0.61	0.37	0.00	0.50	0.14
<i>A. carterae</i>	ACRN02	1.88	0.77	0.41	0.00	0.46	0.05
<i>A. cf. carterae</i>	A1V	2.37	0.66	0.28	0.00	0.37	0.06
<i>Barrufeta bravensis</i>	VGO859	1.92	0.43	0.23	0.00	0.49	0.13
<i>B. bravensis</i>	VGO860	2.00	0.56	0.28	0.00	0.53	0.30
<i>B. bravensis</i>	VGO864	1.99	0.38	0.20	0.00	0.58	0.23
<i>Gymnodinium catenatum</i>	GC11V	2.49	0.51	0.20	0.00	0.40	0.20
<i>G. catenatum</i>	GC31AM	2.00	0.41	0.20	0.00	0.33	0.20
<i>G. catenatum</i>	CS-302	1.91	0.58	0.30	0.00	0.42	0.17
<i>G. impudicum</i>	GY1VA	2.01	0.47	0.24	0.00	0.38	0.21
<i>G. instriatum</i>	VGO642	2.60	0.59	0.23	0.00	0.48	0.11
<i>G. cf. microreticulatum</i>	VGO581	2.00	0.42	0.20	0.01	0.36	0.14
<i>G. nolleri</i>	DK5	2.99	0.72	0.24	0.00	0.27	0.23
<i>Gyrodinium uncatenum</i>	CS289-3	2.63	0.50	0.19	0.28	0.49	0.09
PERIDINIALES							
<i>Heterocapsa niei</i>	VGO399	1.44	0.56	0.38	0.08	0.34	0.13
<i>H. triquetra</i>	VGO1053	1.45	0.49	0.34	0.00	0.48	0.12
<i>Peridinium aciculiferum</i>	PAER-1	2.71	0.57	0.21	0.10	0.28	0.07

<i>P. aciculiferum</i>	PAER-2	2.85	0.62	0.22	0.11	0.29	0.07
<i>Scrippsiella hangoei</i>	STHV-1	2.02	0.63	0.31	0.01	0.33	0.08
<i>S. hangoei</i>	STHV-2	2.01	0.65	0.31	0.01	0.35	0.07
<i>S. hangoei</i>	STHV-5	2.11	0.57	0.26	0.01	0.29	0.06
<i>S. hangoei</i>	STHV-6	2.03	0.60	0.29	0.02	0.34	0.08
<i>Scrippsiella</i> sp.	S3V	5.04	0.83	0.16	0.00	0.35	0.07
PROROCENTRALES							
<i>Prorocentrum arenarium</i>	VGO776	7.43	0.67	0.09	0.02	0.38	0.19
<i>P. belizeanum</i>	PBMA01	19.80	1.16	0.06	0.00	0.52	0.18
<i>P. belizeanum</i>	VGO867	25.42	1.01	0.04	0.01	0.49	0.16
<i>P. compressum</i>	VGO621	4.75	0.83	0.18	0.00	0.29	0.09
<i>P. cf. faustiae</i>	VGO894	7.39	0.82	0.11	0.00	0.33	0.19
<i>P. levis</i>	VGO777	40.32	0.85	0.02	0.01	0.40	0.17
<i>P. levis</i>	VGO957	15.58	0.68	0.05	0.00	0.40	0.22
<i>P. lima</i>	PL2V	12.08	1.09	0.09	0.00	0.71	0.22
<i>P. cf. lima</i>	VGO620	3.81	0.93	0.25	0.01	0.53	0.22
<i>P. micans</i>	PM1V	7.94	0.88	0.11	0.00	0.40	0.09
<i>P. minimum</i>	VGO365	4.75	0.85	0.18	0.00	0.35	0.05
<i>P. minimum</i>	VGO367	10.68	1.16	0.11	0.00	0.28	0.06
<i>P. nux</i>	UTEX1008	3.64	1.07	0.29	0.00	0.48	0.08
<i>P. rathymum</i>	VGO893	10.30	1.12	0.11	0.00	0.42	0.12
<i>P. rostratum</i>	PR1V	18.65	1.19	0.06	0.00	0.30	0.09
<i>P. triestinum</i>	PT2V	4.28	0.95	0.22	0.00	0.42	0.10
THORACOSPHAERALES							
<i>Thoracosphaera heimii</i>	CCMP1069	2.43	0.80	0.33	0.00	0.58	0.04
Mean		4.52	0.73	0.22	0.01	0.37	0.12
SD		5.39	0.23	0.08	0.03	0.12	0.05
N		90	90	90	21	90	90

Table S3. Mass pigment ratios (w:w, accessory chls:chl *a*) in pigment-based chloroplast Types 2 and 3. Abbreviations as in Table 1 of the main text

Chloroplast Type 2 Species and strain code	Chl <i>c</i> ₃ :chl <i>a</i>	Chl <i>c</i> ₂ :chl <i>a</i>	MGDG-chl <i>c</i> ₂ :chl <i>a</i> Peak 51	MGDG-chl <i>c</i> ₂ :chl <i>a</i> Peak 56	MGDG-chl <i>c</i> ₂ :chl <i>a</i> Peak 60	Chl <i>c</i> ₃ :chl <i>c</i> ₂	Σchl <i>c</i> :chl <i>a</i>
<i>Karenia brevis</i> CCMP718	0.05	0.08	0.00	0.01	0.00	0.62	0.14
<i>K. brevis</i> CCMP2281	0.05	0.10	0.00	0.01	0.00	0.50	0.16
<i>K. mikimotoi</i> CCMP429	0.06	0.11	0.00	0.00	0.00	0.50	0.17
<i>K. papilionacea</i> VGO679	0.04	0.09	0.00	0.01	0.00	0.47	0.13
<i>K. selliformis</i> VGO875	0.06	0.11	0.00	0.00	0.00	0.55	0.17
<i>K. umbella</i> Gy2DE	0.05	0.11	0.00	0.00	0.00	0.62	0.16
Chloroplast Type 3							
<i>Karlodinium armiger</i> GC-7	0.06	0.10	0.01	0.01	0.00	0.59	0.19
<i>K. decipiens</i> Nervión34	0.05	0.15	0.00	0.00	0.00	0.35	0.20
<i>K. veneficum</i> CCMP415	0.06	0.15	0.00	0.00	0.00	0.41	0.21
<i>K. veneficum</i> CCMP1974	0.06	0.15	0.00	0.00	0.00	0.38	0.21
<i>K. veneficum</i> CS-310	0.07	0.16	0.00	0.00	0.00	0.41	0.23
<i>K. veneficum</i> GC-4	0.04	0.12	0.00	0.00	0.00	0.31	0.17
<i>K. veneficum</i> VGO691	0.05	0.13	0.00	0.00	0.00	0.41	0.18
<i>K. veneficum</i> VGO870	0.05	0.14	0.00	0.00	0.00	0.33	0.19
<i>Takayama cf. helix</i> VGO341	0.05	0.14	0.01	0.02	0.01	0.35	0.23

Table S3 (cont.)

Chloroplast Type 2 Species and strain code	But-fuco like-1	But-fuco like-2	But- fuco	Fuco	4k-hex- fuco-like	4k-hex- fuco	Hex- fuco	GyrE1 -like	GyrE2 C12:0	GyrE3 C14:0
<i>Karenia brevis</i> CCMP718	0.03	0.06	0.07	0.25	0.04	0.10	0.06	0.00	0.10	0.00
<i>K. brevis</i> CCMP2281	0.05	0.05	0.07	0.18	0.08	0.09	0.08	0.00	0.13	0.00
<i>K. mikimotoi</i> CCMP429	0.06	0.06	0.08	0.24	0.10	0.10	0.08	0.02	0.10	0.00
<i>K. papilionacea</i> VGO679	0.02	0.03	0.11	0.10	0.08	0.08	0.25	0.03	0.16	0.01
<i>K. selliformis</i> VGO875	0.07	0.08	0.07	0.20	0.09	0.11	0.07	0.01	0.11	0.01
<i>K. umbella</i> Gy2DE	0.01	0.01	0.11	0.20	0.09	0.07	0.28	0.00	0.15	0.00
Chloroplast Type 3										
<i>Karlodinium armiger</i> GC-7	0.00	0.00	0.01	0.25	0.00	0.00	0.39	0.00	0.10	0.05
<i>K. decipiens</i> Nervión34	0.00	0.00	0.21	0.16	0.00	0.00	0.38	0.00	0.13	0.09
<i>K. veneficum</i> CCMP 415	0.00	0.00	0.11	0.41	0.00	0.00	0.27	0.00	0.23	0.02
<i>K. veneficum</i> CCMP 1974	0.00	0.00	0.13	0.32	0.00	0.00	0.24	0.00	0.15	0.09
<i>K. veneficum</i> CS-310	0.00	0.00	0.15	0.55	0.00	0.00	0.29	0.00	0.19	0.04
<i>K. veneficum</i> GC-4	0.00	0.00	0.07	0.53	0.00	0.00	0.18	0.00	0.18	0.04
<i>K. veneficum</i> VGO691	0.00	0.00	0.08	0.43	0.00	0.00	0.22	0.00	0.19	0.03
<i>K. veneficum</i> VGO870	0.00	0.00	0.16	0.22	0.00	0.00	0.34	0.00	0.19	0.02
<i>Takayama cf. helix</i> VGO341	0.00	0.00	0.00	0.87	0.00	0.00	0.05	0.00	0.00	0.00

Table S4. Mass pigment to chlorophyll (chl) *a* ratios (w:w) in pigment-based chloroplast Types 4, 5 and 6; tr.: trace amounts; other abbreviations as in Table 1 of the main text

Chloroplast Type 4	Chl <i>c</i> ₂	Chl <i>c</i> ₁ -like <i>Eg.</i>	MgDVP	Chl <i>c</i> ₁	Fuco	βψ-car		
<i>Peridinium balticum</i> CS-33	0.03	0.003	0.000	0.06	0.40	tr.		
<i>Kryptoperidinium foliaceum</i> CS-37	0.04	0.006	0.001	0.07	0.38	tr.		
<i>K. foliaceum</i> VGO556	0.05	0.007	0.003	0.08	0.55	0.03		
Chloroplast Type 5	Chl <i>c</i> ₂	Alloxanthin	Crocoxanthin	βε-car				
<i>Dinophysis acuminata</i> VGO1063	0.05	1.02	0.05	0.12				
<i>D. acuta</i> VGO1065	0.06	0.85	0.04	0.11				
<i>D. caudata</i> VGO1064	0.05	0.87	0.03	0.12				
<i>D. tripos</i> VGO1062	0.06	0.84	0.03	0.09				
Chloroplast Type 6	Neo	Viola	Anth	Zea	Unk443	βε-car	ββ-car	Chl <i>b</i>
<i>Lepidodinium chlorophorum</i> Dino16EUH	0.06	0.05	0.007	0.006	0.11	0.02	0.02	0.74
<i>L. chlorophorum</i> RCC1488	0.09	0.11	0.007	0.03	0.10	0.02	0.03	0.58
<i>L. chlorophorum</i> RCC1489	0.09	0.08	0.01	0.04	0.09	0.02	0.03	0.57
<i>L. chlorophorum</i> BAH100ME	0.06	0.22	0.02	0.01	0.12	0.07	0.03	0.08

Table S5. Species of *Karenia*, *Karlodinium* and *Takayama* described to date and the available pigment information

<i>Karenia</i> spp.	Species description	HPLC pigment data
<i>K. asterichroma</i>	de Salas et al. (2004b)	–
<i>K. bicuneiformis</i>	Botes et al. (2003)	–
<i>K. bidigitata</i>	Haywood et al. (2004)	–
<i>K. brevis</i>	(C.C. Davis) Hansen & Moestrup, in Daugbjerg et al. (2000)	Millie et al. (1997), Örnólfssdóttir et al. (2003)
<i>K. brevisulcata</i>	Chang & Ryan (2004)	–
<i>K. concordia</i>	Chang & Ryan (2004)	–
<i>K. cristata</i>	Botes et al. (2003)	–
<i>K. digitata</i>	Yang et al. (2000)	–
<i>K. longicanalis</i>	Yang et al. 2001	–
<i>K. mikimotoi</i>	(Miyake et Kominami ex Oda) Hansen & Moestrup, in Daugbjerg et al. (2000)	Suzuki & Ishimaru (1992), Johnsen & Sakshaug (1993), this study
<i>K. papilonaceae</i>	Haywood et al. (2004)	Laza-Martinez et al. (2007)
<i>K. selliformis</i>	Haywood et al. (2004)	This study
<i>K. umbella</i>	de Salas et al. (2004a)	de Salas et al. (2004a), this study
<i>Karlodinium</i> spp.		
<i>K. antarcticum</i>	de Salas et al. (2008)	–
<i>K. armiger</i>	Bergholtz et al. (2006)	Bergholtz et al. (2006), Garcés et al. (2006), this study
<i>K. australe</i>	de Salas et al. (2005)	de Salas et al. (2005)
<i>K. ballantinum</i>	de Salas et al. (2008)	–
<i>K. conicum</i>	de Salas et al. (2008)	–
<i>K. corrugatum</i>	de Salas et al. (2008)	–
<i>K. corsicum</i>	Siano et al. (2009)	–
<i>K. decipiens</i>	de Salas et al. (2008)	Laza-Martinez et al. (2007), this study
<i>K. veneficum</i>	(Ballantine) Larsen, in Daugbjerg et al. (2000)	Johnsen & Sakshaug (1993), Kempton et al. (2002), Bergholtz et al. (2006), Garcés et al. (2006), Bachvaroff et al. (2009), this study
<i>Takayama</i> spp.		
<i>T. acrotrocha</i>	Siano et al. (2009)	–
<i>T. cladochroma</i>	(Larsen) de Salas, Bolch & Hallegraeff, in de Salas et al. (2003)	–
<i>T. helix</i>	de Salas et al. (2003)	de Salas et al. (2003), this study
<i>T. tasmanica</i>	de Salas et al. (2003)	de Salas et al. (2003)
<i>T. tuberculata</i>	de Salas et al. (2008)	–
<i>T. pulchella</i>	Steidinger et al. (1998)	–

Table S6. Peridinin to chlorophyll (chl) *a* molar ratios used in CHEMTAX analysis of pigment data. Original data were in mass (w:w) ratios; here data are shown as molar ratios by multiplying the ratio of chl *a* MW (894) to MW of peridinin (631) = 1.42

Peridinin to chl <i>a</i> ratio (mole:mole)	Source
0.75	Mackey et al. (1996)
0.75	Wright et al. (1996)
0.86–1.42	Mackey et al. (1998)
0.61, 0.94	Schlüter et al. (2000)
1.51	Wright & van den Enden (2000)
0.71; 0.94	Descy et al. (2000)
0.77, 1.09	Suzuki et al. (2002)
1.51	Ishikawa et al. (2002)
0.85	DiTullio et al. (2003)
0.76	Örnólfssdóttir et al. (2003)
0.76	Rodríguez et al. (2003)
0.71	Buchaca et al. (2005)
1.51	Llewellyn et al. (2005)
1.12	Lewitus et al. (2005)
0.88	DiTullio et al. (2005)
0.56–0.65	Rodríguez et al. (2006)
0.52–0.88	Schlüter et al. (2006)
0.60	Eker-Develi et al. (2008)
1.10	Lionard et al. (2008)
0.92	Wright et al. (2009)
1.16	Wright et al. (2010)
0.59–0.63	Lauridsen et al. (2011)
0.56–0.62	Laviale & Neveux (2011)
0.50–0.89	Ruivo et al. (2011)
0.99–1.06	Schlüter et al. (2011)
0.75	Suzuki et al. (2011)

Table S7. Pigment-based chloroplast types in Dinophyceae and their distribution in other algal taxa

Dinophyceae chloroplast type	Representative species	Algal taxa with similar pigment-based chloroplast	Representative species
Type 1	<i>Alexandrium minutum</i>	–	–
Type 2	<i>Karenia mikimotoi</i>	–	–
Type 3	<i>Karlodinium veneficum</i>	Haptophyta Type-8 ^a	<i>Phaeocystis</i> spp.?
Type 4	<i>Durinskia baltica</i> , <i>Kryptoperidinium foliaceum</i>	Bacillariophyceae/ Haptophyta-Type 2 ^a	<i>Pseudo-nitzschia australis</i> / <i>Pavlova gyrans</i>
Type 5	<i>Dinophysis acuminata</i>	Cryptophyceae	<i>Teleaulax</i> spp.
Type 6	<i>Lepidodinium chlorophorum</i>	Chlorophyceae/Ulvophyceae Trebouxiophyceae	–

^aHaptophyte pigment types as defined in Zapata et al. (2004)

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