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Title: Pigment compositions are linked to the habitat types in dinoflagellates

Running title: Habitat-related Pigments in Dinoflagellates

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Abstract

Compared to planktonic species, benthic dinoflagellates living in sandy beach or seafloor are a little known about ecology, physiology, even their existences. In a previous study, we discovered 13^2,17^3-cyclopheophorbide *a* enol (cPPB-*a*E) from sand-dwelling benthic dinoflagellates. This enol had never been detected in phytoplankton despite it is a chlorophyll *a* catabolite. We speculated from this discovery that habitat selection might be linked to pigment compositions in dinoflagellates. To test the hypothesis of habitat selection linking to pigment compositions, we conducted extensive analysis of pigments with high performance liquid chromatography (HPLC) for 40 species using 45 strains of dinoflagellates including three habitat types; sand-dwelling benthic forms, tidal pool inhabitants and planktonic species. Those 40 dinoflagellates are also able to distinguished into two types based on their chloroplast origins; red alga-derived secondary chloroplasts and diatom-derived tertiary ones. By plotting the pigments profiles onto three habitats, we noticed that twelve pigments including cPPB-*a*E were found to occur only in benthic sand-dwelling species of red alga-derived type. The similar tendency was also observed in dinoflagellates with diatom-derived chloroplasts, i.e. additional sixteen pigments including chl c_3 were found only in sand-dwelling forms. This is the first report of the occurrence of chl c_3 in dinoflagellate

with diatom-derived chloroplasts. These results clarify the far greater diversity of pigments are produced by the dinoflagellates living in sand regardless of chloroplast types relative to those of planktonic and tidal pool forms. Dinoflagellates seem to produce a part of their pigments in response to their habitats.

Key words; benthic, diatom, dinoflagellate, HPLC, planktonic, pigment

Abbreviation; cPPB-*a*E: 13^2,17^3-cyclopheophorbide *a* enol, PCDi: pigments common to all dinotoms, PCPe: pigments common to all peridinin-type dinoflagellates, UC: unknown carotenoid

Introduction

Dinoflagellates are unicellular eukaryotes mainly known as marine primary producers. About 77% of species are marine planktonic which drift near the surface of the sea. On the other hand, although in the minority, the benthic marine dinoflagellates account for about 8% of living species (Taylor et al. 2008, Hoppenrath et al. 2014). They have been recorded mainly from sandy beaches (e.g. Herdman 1922), intertidal flats (e.g. Hoppenrath et al. 2007), tidal pools in rocky shores (e.g. Horiguchi and Chihara 1988), sandy sea floors (e.g. Yamada

et al. 2013), and the surface of either seaweeds (e.g. Parsons and Preskitt 2007) or of sediments (e.g. Faust et al. 2008). Some benthic representatives swim in the interstitial spaces between sand grains, while others attach themselves to the substrata, existing as non-motile vegetative cells or temporary cysts (e.g. Horiguchi and Chihara 1987, Murray and Patterson 2002, Saburova et al. 2009).

Tidal pools, considered as part of benthic habitats, (Hoppenrath et al. 2014) were one of our focal habitats because dinoflagellates here show an intermediate life cycle between benthic and planktonic organisms (Horiguchi and Chihara 1988). They are planktonic cells when the tide is low, but when the pool is flooded at high tide, they become benthic by forming temporary cysts attaching to the rock surfaces. Because of this intermediary behavioral pattern, we regarded tidal pool dinoflagellates should be distinguished from sand-dwelling dinoflagellates or planktonic forms.

Dinoflagellates can be further distinguished by chloroplast types. More than 95% of photosynthetic dinoflagellates possess a red algal derived secondary chloroplast (Zhang et al. 1999, Taylor et al. 2008), which contains chlorophyll a/c_2 with peridinin, a xanthophyll unique to dinoflagellates. This typical chloroplast type in dinoflagellate called ‘peridinin-type’. In contrast, some groups of dinoflagellates have replaced their

peridinin-type chloroplasts with one from other microalgae, belonging to Haptophyta (Tengs et al. 2000, Zapata et al. 2012), Chlorophyta (Watanabe et al. 1990, Matsumoto et al. 2012) or Bacillariophyta (diatoms) (Riley and Wilson 1967, Chesnick et al. 1997, Takano et al. 2008, Zhang et al. 2011, Zhang et al. 2014).

In a previous study, we discovered 13^2,17^3-cyclopheophorbide *a* enol (cPPB-*a*E) for the first time in photosynthetic organisms (Yamada et al. 2014). Through our pigment analytical survey, we discovered that the enol was only detected in sand-dwelling benthic dinoflagellates (Yamada et al. 2014). From this result, we came to an idea that habitat selection might be linked to pigment compositions. Although recently Zapata et al. (2012) reported pigment compositions of 64 dinoflagellate species, including wide taxonomic groups, little attention was made to relate pigment profiles and habitat types. Therefore, to date, no comprehensive studies relating pigment composition to habitat types have not been available.

Here we report on the pigment profiles of 40 species (using 45 strains) of dinoflagellates by HPLC. We distinguished them into three habitat types; 29 species (31 strains) of sand-dwelling benthic forms, three tidal pool inhabitants (five strains), and eight planktonic species (nine strains). In this study, a hierarchical cluster analysis was employed to classify HPLC pigments data into habitat groups and to assess the effects of environmental factors on

pigment compositions.

These 40 dinoflagellates can be divided into one of two chloroplast types: of red alga-derived (peridinin-type) secondary chloroplast and diatom-derived tertiary one. We treated 34 species (35 strains) of peridinin-type species and six species (ten strains) of dinoflagellates having diatom-derived chloroplasts; the latter dinoflagellates are collectively called ‘dinotoms’ (Imanian et al. 2010). Dinotoms are known to possess chlorophylls c_1 and c_2 with fucoxanthin as the major xanthophyll (Mandelli 1968, Jeffery et al. 1975, Withers et al. 1977, Tamura et al. 2005). The endosymbiont diatoms originated from four species belonging to different four genera: *Chaetoceros* (Horiguchi and Takano 2006), *Cyclotella* (Zhang et al. 2014), *Discostella* (Takano et al. 2008, Zhang et al. 2011), and *Nitzschia* (Chesnick et al. 1997, Tamura et al. 2005). Six dinotoms analyzed in this study can be distinguished to two diatom species derived types, i.e. possessing a *Nitzschia*-type endosymbiont (Tamura et al. 2005, Pienaar et al. 2007) (represented by five species, nine strains), and *Discostella*-type (Zhang et al. 2011) (represented by one species, two strains). If our hypothesis that pigment profiles are related to habitat were valid, it would be expected that, regardless of chloroplast types, similar tendencies could be seen between habitat types.

In addition to dinoflagellates, we included pigment data from the three free-living diatoms;

Cylindrotheca closterium (Ehrenberg) Reimann & JC Lewin, *Nitzschia* sp. and *Tabularia* sp. A comparison of pigment composition between endosymbiont diatoms and free-living one would provide insight into whether pigment modifications occurred after endosymbiotic event (s) or not.

Materials and methods

Cultures and species identifications

All culture strains used here were isolated from sandy beaches, sandy sea floors, tidal pools or the surface of seawater or freshwater (STable 1). Each sand sample was placed in a plastic cup and enriched with Daigo's IMK medium (marine, Nihon Pharmaceutical Co., Ltd., Tokyo) or with URO medium (freshwater, Kimura and Ishida 1985), and cultured at 15, 20 or 25ºC, which were close to water temperature at sampling site of each species, with an illumination of 60 µmol·photons·m^{-2}·s^{-1} under a 16:8 h light:dark cycle without suspension. Dinoflagellate cells that appeared in the cup were isolated using capillary pipettes with several rinses in sterilized medium under an inverted microscope and subsequently clonal cultures were established. The culture strains were maintained in petri dishes in IMK, URO or f/2 medium (marine, Guillard and Ryther 1962) using the same conditions indicated above.

In these petri dishes, planktonic species can swim freely without agitation, while benthic species can attach to the inside wall of petri dishes or swim near surface of substrata and spend benthic life cycle. All culture strains were identified morphologically using the light microscope (Figs. S1 and S2) and verified by analysis of molecular data (Figs. S3 and S4, Table S2).

Pigment extraction and HPLC analysis

After being cultured for 1 to 4 months, each culture was centrifuged at 10000g for 5 min and the cells were pelleted. For the species firmly attaching to the inside wall of petri dish, the cells were collected by gentle sweeping by clean paint brush. Then the cells were centrifuged as above. The pelleted cells were suspended in 100% acetone and homogenized by stainless beads (5 mm in diameter, TCS0-0100, Bio medical science, Tokyo) for 1 min using a ShakeMaster grinding apparatus (BioMedical Science, Tokyo). The homogenates were centrifuged for 15 min at 22000g. The pigments in the supernatant were separated on a Symmetry C_8 column (150 X 4.6 mm, Waters, Milford) according to a method reported previously (Zapata et al. 2000). The elution profiles (Fig. 1) were monitored by measuring the absorbance at 450 nm (SPD-M10A, Shimadzu, Kyoto), and the pigments were identified

by their retention times and spectral patterns.

Hierarchical clustering analysis

The distributions of detected pigments were analyzed by software R with the Ward method, which clusters orderly two objects with smaller differences. For binary similarity measure, the Sokal and Michener index (Sokal and Michener 1958) was used because we regarded both states of pigment existence, i.e. presence or absence of pigments, are important. This index considers equally both attributes, presence or absence, between two objects (Choi et al. 2010).

Results

The chloroplasts of dinoflagellates analyzed originated from phylogenetically different algae. Therefore, the results for peridinin-type dinoflagellates with red algal-derived chloroplasts and for dinotoms with diatom-derived chloroplasts are considered separately.

[Peridinin-type dinoflagellates]

Pigment compositions

Forty-two pigments were detected in 35 strains (representing 34 species) of peridinin-type

dinoflagellates (Fig. S5). Five of them were chlorophylls and related molecules, i.e. chlorophyll c_2 (chl c_2, peak 4), chlorophyll c_1 (chl c_1, peak 5), 13^2,17^3-cyclopheophorbide *a* enol (cPPB-*a*E, peak 36), chlorophyll *a* (chl *a*, peak 49) and pheophythin *a* (peak 53) (in order of retention time). Chl c_2, chl *a* and pheophythin *a* were detected in all samples. cPPB-*a*E is thought to be degradation product of pheophythin *a* (Ma and Dolphin 1999, Louda et al. 2000).

Thirty-seven carotenoids were detected in total of which only twelve were identifiable, peridininol (peak 1), peridinin (peak 6), a peridinin-like carotenoid (peak 7), violaxanthin (peak 14), diadinochrome (peak 18), diadinoxanthin (peak 19), dinoxanthin (peak 22), diatoxanthin (peak 24), a diadinochrome-like carotenoid (peak 25), zeaxanthin (peak 27), a lutein-like carotenoid (peak 28) and β-carotene (peak 54) (in order of retention time). The other twenty-five pigments were unknown carotenoids (UCs). All the dinoflagellates analyzed commonly had the following ten carotenoids; peridininol, peak 2 UC, peridinin, a peridinin-like carotenoid, peak 11 UC, diadinoxanthin, dinoxanthin, zeaxanthin, lutein-like carotenoid (except in *Stylodinium littorale* Horiguchi & Chihara, Analysis Number 23), β-carotene.

The pigments (chlorophylls and carotenoids) detected in all peridinin-type dinoflagellates

are here designated as PCPe (Pigments common to all peridinin-type dinoflagellates) (Fig. S5).

Habitat-type distribution of pigment profiles

We grouped the peridinin-type dinoflagellates into three habitat categories; benthic, sand-dwellers (25 species, 26 strains), tidal pool inhabitants (two species, two strains) and planktonic forms from sea surface (seven species, seven strains). The cluster analysis based on HPLC pigments categorized them into 4 groups (Fig. 2). These clusters represent the pigments only detected from sand-dwellers (cluster A), from tidal pool inhabitants and sand-dwellers (cluster B), from planktonic species and sand-dwellers (cluster C), and from all habitat type dinoflagellates (cluster D), respectively.

Cluster A is consisted of pigments detected only from sand-dwelling dinoflagellates. The presence of twelve pigments, i.e. cPPB-*a*E, peak 16, 17, 33, 37, 39, 40, 42, 43, 44, 51 and 52 UCs, was restricted to this habitat. However, these pigments are not universally shared by all sand-dwellers. In particular, each of peak 33, 37, 39, 40, 42, 43 and 44 UCs were only detected in one respective dinoflagellate (Fig. S5). The remaining restricted pigments were detected in two (peak 16 and 17 UCs), three (peak 52 UC), six (cPPB-*a*E) or seven species

(peak 51 UC) of sand-dwelling dinoflagellates.

Cluster B contained two pigments, i.e. peak 12 UC and chl c_1. The former was specific to tidal pool member, while the latter was found in the species from both tidal pool and sand. There were no pigments limited in distribution to planktonic species. Pigments from planktonic species were restricted to clusters C and D, and these pigments were also found in other habitat type species. On the contrary, sand-dwelling species possessed most diverse pigments, i.e. found in all clusters 1 - 4, and all pigments detected in planktonic and tidal pool dinoflagellates were found in at least one sand-dwelling species except for peak 12 UC which is tidal pool specific.

Phylogenetic distribution of pigment profiles

Based on morphology and molecular phylogeny inferred from SSU rDNA data, we identified the dinoflagellate species (Figs. S1 and S3). The species analyzed in this study consisted of phylogenetically wide range of groups. Figure S6 shows the similarity of pigment composition among species by clustering analysis. In some cases, closely related species, i.e. congeneric species, show similar type of pigment profiles (boxed groups in Fig. S6), while in other instances little similarities have been detected even between the species

within the same genus. Correlations between the phylogenetic positions and pigment compositions were not strongly supported.

[Dinotoms]

Pigment compositions

Thirty-eight pigments were detected from ten strains, representing six species of dinotoms (Fig. S7). Five chlorophylls and its derivatives were detected, in order of retention time, chlorophyll c_3 (chl c_3, peak 3), chlorophyll c_2 (chl c_2, peak 4), chlorophyll c_1 (chl c_1, peak 5), chlorophyll *a* (chl *a*, peak 49) and pheophythin *a* (peak 53). cPPB-*a*E was not detected. Additionally, thirty-three carotenoids were detected in dinotoms, eight of which were identified as a fucoxanthin-like carotenoid (peak 9), fucoxanthin (peak 10), diadinoxanthin (peak 19), diatoxanthin (peak 24), zeaxanthin (peak 27), a lutein-like carotenoid (peak 28), β-ψ carotene (peak 50) and β-carotene (peak 54). The remaining twenty-five pigments were UCs. Ten pigments were detected from all dinotoms (PCDi: Pigments common to all dinotoms) include chl c_2, chl c_1, fucoxanthin, diadinoxanthin, peak 21 UC, zeaxanthin, chl *a*, β-ψ carotene, pheophythin *a*, β-carotene (in order of retention time, Fig. S7).

Habitat type / Phylogenetic distribution of pigment profiles

The molecular phylogenetic analyses supported a monophyletic dinotom grouping (Figs. S3 and S4). Our strains were divided into four main clades within the monophyletic group, the *Durinskia* cf. *baltica* (Levander) Carty & Cox clade (No.36 and 37), the *Durinskia* sp. clade (No.38, 39 and 40), the *Galeidinium* spp. clade (No.41, 42 and 43) and the *Peridiniopsis* cf. *kevei* Grigorszky clade (No.44 and 45) (Fig. S4). These analyzed dinotoms were also categorized to three habitats as in the case for the peridinin-type dinoflagellates; four benthic sand-dwelling species (*D.* cf. *baltica* and three species of *Galeidinium* spp. clade), one tidal pool inhabitant (*Durinskia* sp.) and one planktonic species (*P.* cf. *kevei*). Figure 3 shows that all detected pigments plotted on habitat types were grouped to mainly four types by cluster analysis; pigments detected only from sand-dwellers (cluster A), from tidal pool inhabitants (cluster B), mainly from planktonic species (cluster C) and common pigments for all habitat type dinotoms (cluster D).

Cluster A was consisted of specific pigments for sand-dwelling four species (five strains). These pigments could be separated to three pigment groups. The first group contained nine pigments, peak 23, 26, 33, 34, 35, 39, 41, 43 and 44 UCs, and these were shared by *Galeidinium rugatum* Tamura & Horiguchi (No.41) and Unidentified coccoid dinotom 1

(No.42). The second group consisted of three pigments, peak 16, 45 and 46 UCs, which were shared by three species in *Galeidinium* spp. clade (No.41, 42 and 43). The third group included four pigments, chl c_3, peak 29, 47 and 48 UCs, that were shared by all sand-dwelling dinotoms. The number of shared pigments seems to reflect phylogenetic closeness between them based on phylogenetic analysis of SSU rDNA data (Fig. S4); the highest number of shared specific pigments, were detected in the closest species, *G. rugatum* (No.41) and Unidentified coccoid dinotom 1 (No.42).

We analyzed only one species as representative of tidal pool (three strains) and planktonic habitats (two strains), respectively. In cluster B, peak 40 UC was shown to be specific for tidal pool species, *Durinskia* sp. (No.38, 39 and 40). Pigments in cluster C were mainly from freshwater planktonic species, *P.* cf. *kevei* (No.44 and 45), and could be separated to two categories; peak 13 and 52 UCs which were only detected in planktonic species, and peak 12 and 15 UCs which were shared by planktonic and sand-dwelling species. PCDi and the pigments detected from all habitat type dinotoms were included in cluster D.

Some pigments appeared in the positions that were difficult to interpret. A fucoxanthin-like carotenoid, a lutein-like carotenoid, peak 30, 32 and 38 UCs were detected, but showed no clear correlations with habitat types.

Comparison between pigment profiles of Nitzschia*-type dinotoms and free-living diatoms*

It is known that the species of the genus *Durinskia* and members of the *Galeidinium* spp. clade possess a *Nitzschia*-type diatom endosymbiont (Tamura et al. 2005, Pienaar et al. 2007 and unpublished data). To compare the pigment compositions of dinotoms to those of free-living diatoms, we added three diatoms, *Cylindrotheca closterium* (No.46), *Nitzschia* sp. (No.47) and *Tabularia* sp. (No.48) as representatives for HPLC analyses (Fig. 4 and Fig. S7).

Free-living diatoms shared all PCDi with the dinotoms with the exception of β-ψ carotene (Fig. S7). Other detected pigments from these diatoms were also detected in *Nitzschia*-type dinotoms. Moreover *Nitzschia*-type dinotoms possessed many pigments that have not been detected in free-living diatoms (Fig. 4). Additionally, the pigment compositions were different among the *Nitzschia*-type dinotoms. Sand-dwelling three species in *Galeidinium* spp. clade produced the highest number of specific pigments for dinotoms.

DISCUSSION

Validity of detected pigments

Recently Zapata et al. (2012) reported pigment compositions of 64 dinoflagellate species

(122 strains). This is the only comprehensive research for pigments using a HPLC that dealt with both peridinin-type dinoflagellates and dinotoms, which allows us to compare to our results. Most of common pigments of both dinoflagellate types in this study (PCPe and PCDi) are corresponding with detected pigments in Zapata et al. (2012). However, the number of detected pigments in this study greatly exceeds that of Zapata et al. (2012). Most of such additional pigments reported here, including cPPB-*a*E, chl c_3 and many UCs, have never been detected from peridinin-type dinoflagellates and dinotoms.

In the interpretation of pigment compositions, it should also be considered the culture age because the strains used in this study had been cultured for periods ranging from 1-4 month(s). Because each species shows different cell division rate, it was necessary to use different culture ages for optimal harvesting. Cultures of all strains were checked for cell density and cell morphology using the inverted microscope for suitability for the HPLC analysis. The physiological conditions of cultures should also be considered whether the differences of culture age affect cellular condition or not.. Therefore, we measured the photosynthetic activities of three species cultured for different periods, i.e. one and three/four month(s). Fv/Fm ratio of three benthic species showed no significant differences in photosynthetic activities were found between different culture periods by t-test (Table S3).

We suggest, therefore, that the all strains used for pigment analyses were in healthy state and detected pigments including many unknown carotenoids were not formed by stress condition.

Why these minor pigments have never been mentioned in the previous pigment studies in dinoflagellates? One possible reason might be that the previous studies simply ignored most unknown pigments. Minor peaks have been neglected in most of the previous reports because these reports aimed to identify the type of chloroplast of a target dinoflagellate. However, these minor pigments are equally-characteristic for dinoflagellates because they cannot be detected in other microalgae, such as cyanobacteria, the chlorophyte *Chlamydomonas reinhardtii* (data not shown), and diatoms (Fig. 4).

Are pigment profiles related to phylogenetic positions?

In this study, we analyzed 34 peridinin-type species and six dinotom species. In some closely related species of peridinin-type dinoflagellates, it was shown that their pigment profiles are similar to each other (Fig. S6). In the dinotoms, it was also indicated their pigment profiles were somewhat reflected in the phylogenetic positions; within sand-dwelling four species, the more closely related species based on molecular phylogeny shared higher number of pigments (Fig.4 and Fig. S4). Thus it seems that pigment

compositions relate with phylogenetic positions, but we noticed that these closely related dinoflagellates are, at the same time, all sand-dwelling dinoflagellates. Such observations lead to the hypothesis that the habitat has a role to play in determining to produce the specific pigments; we think that some closely related species are living in similar environment, and thus, spending similar life cycle, and these conditions affect on their pigment profiles.

Pigment profiles are related to habitat

We noticed cPPB-*a*E and eleven unknown carotenoids were restricted to benthic sand-dwelling species in peridinin-type dinoflagellates, although seven of them were from one each representative species. The distributions of peak 12 UC and chl c_1 were also restricted. The former was found in only a tidal pool dinoflagellate, while the latter has been detected in eight sand-dwelling and one tidal pool species. On the other hand, no specific pigments were identifiable for planktonic dinoflagellates.

In dinotoms, chl c_3 and fifteen unknown carotenoids were only detected in sand-dwelling forms. The tidal pool dinotom had also the unique peak 40 UC. Contrary to peridinin-type dinoflagellates in which no specific pigments were found, the freshwater planktonic dinotom uniquely has 13 and 52 UCs.

These results highlighted that the far greater diversity of pigments produced by both chloroplast type dinoflagellates living in sand relative to those of planktonic and tidal pool forms. We suggest that dinoflagellates respond to their habitat in the production of their pigments.

The results of pigment analysis relative to three free-living diatoms reinforce the idea that diverse pigment of benthos is a response brought about by the host dinoflagellates. The number of pigments in all *Nitzschia*-type dinotoms is in general, greater than that in free-living diatoms. Further, the sand-dwelling species of the *Galeidinium* spp. clade even show a greater diversity of pigment profile even compared to those of other *Nitzschia*-type dinotoms.

Interestingly, all of specific pigments for sand-dwelling dinoflagellates both chloroplast types are minor pigments, and most are unknown carotenoids. These unknown carotenoids possess almost the same absorbance spectra (data not shown), so it is difficult to contemplate that they have more than one function. Most of these UCs are suggested as intermediates or degradation products of one or several novel carotenoid(s) (Takaichi personal communication). However, the identity of the main carotenoid(s) remains obscure, and therefore, the further studies are needed to determine the structure and functions of these

individual UCs. Only cPPB-*a*E and chl c_3 were specifically identified and the presence of chl c_3 is the first report from dinotoms.

All specific pigments are rare one, and such pigments are detected only from a part of sand-dwelling species, indicated to us that these pigments are produced not constantly but occasionally depending on their physiological conditions. Similar situation can be found in the case of chl c_1 production. Although the biosynthetic pathway of chlorophyll *c* is still unknown, one pathway that chl c_1 and c_3 are synthesized from chl c_2 independently (Beale 1999, Green 2011) has been proposed. Chl c_1 is thought to be synthesized by the reduction of the 8-vinyl group of chl c_2 (Green 2011). Recently it was suggested that DVR (3,8-divinyl chlorophyllide reductase), an enzyme that functions as a chlorophyll *a*-synthesizing enzyme, has low specificity for substrate and can also work to produce chl c_1 from chl c_2 (Ito and Tanaka 2014). We suggest that the production of chl c_1 takes place whenever chl c_2 exists, although that the amount of chl c_1 produced is often too small to detect. Actually, although it is generally known that peridinin-type dinoflagellates have chl *a* and c_2, chl c_1 has been detected in quite a few of dinoflagellates from both planktonic and benthic species in previous studies (e.g. Carreto et al. 2001, Fraga et al. 2011, Wakahama et al. 2012, Zapata et al. 2012) and these examples indicate the presence of above mechanism (Ito and Tanaka

2014). Like chl c_1, most of these minor pigments detected in this study must be produced by the mechanism not under control as intermediates or degradation products.

Why, then, do the only sand-dwelling benthic dinoflagellates produce these specific extra pigments? At this moment, we have no answer for this question. To answer this question, further photosynthetic studies, such as the determination of the structure and roles of the unknown carotenoids, the synthetic pathway, enzymes of these pigments and ecological responses within the microhabitat, are needed.

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FIGURES

Fig. 1 HPLC chromatograms of the detected pigments of analyzed dinoflagellates (a) Peridinin-type dinoflagellate, (b) Dinotom. Pigments were extracted from *Pyrocystis* sp., No. 20; planktonic (a1), *Alexandrium hiranoi*, No.1; tidal pool living (a2), *Bispinodinium angelaceum*, No.11; sand-dwelling (a3), *Peridiniopsis* cf. *kevei*, No.45; planktonic (b1), *Durinskia* sp., No.39; tidal pool living (b2), Unidentified coccoid dinoflagellate 1, No.42; sand-dwelling (b3). The absorbance at 450 nm monitored. Black circles indicate typical pigments for dinoflagellates. Double black circles indicate characteristic pigments for each

chloroplast type. Peak numbers are same to Figs. S5 and S7. Peak 4 = chl c_2, peak 6 = peridinin, peak 10 = fucoxanthin, peak 19 = diadinoxanthin, peak 22 = dinoxanthin, peak 24 = diatoxanthin, peak 27 = zeaxanthin, peak 49 = chl *a*, peak 54 = β-carotene.

Fig. 2 Result of clustering analysis of pigment distribution according to habitat-type in peridinin-type dinoflagellates: sand-dwelling benthic, tidal pool inhabiting, planktonic The binary similarity and dissimilarity measure is from Sokal and Michener index. Cluster A; pigments only detected from sand-dwellers, Cluster B; from tidal pool inhabiters and sand-dwellers, Cluster C; from planktonic species and sand-dwellers, Cluster D; from all habitat type dinoflagellates. Black circles indicate PCPe. Numbers indicates peak numbers of unknown carotenoids.

Fig. 3 Result of clustering analysis of pigment distribution according to habitat-type in dinotoms: sand-dwelling benthic, tidal pool inhabiting, planktonic The binary similarity and dissimilarity measure is from Sokal and Michener index. Cluster A; only from sand-dwellers, Cluster B; from tidal pool inhabiters, Cluster C; mainly from planktonic

species, and Cluster D; common pigments for all habitats. Black circles indicate PCDi. Numbers indicates peak numbers of unknown carotenoids.

Fig. 4 Comparisons of pigment profiles between endosymbiont *Nitzschia*-type diatom in dinotoms and free-living diatoms related to *Nitzschia* PCDi is excepted. FLC = a fucoxanthin-like carotenoid, DT = diatoxanthin, LLC = a lutein-like carotenoid. Numbers indicates peak numbers of unknown carotenoids.

Title: Pigment compositions are linked to the habitat types in dinoflagellates

Journal name: Journal of plant research

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Supplementary materials and methods

DNA extraction and PCR amplification

DNA extractions were performed by using the benzyl chloride method (Zhu et al. 1993) or by using the QuickExtract FFPE RNA Extraction Kit (Epicentre, Wisconsin) from the same culture strains that used for HPLC analyses. For the latter method, several dinoflagellate cells (1 to 10 cells) were isolated using capillary pipettes under an inverted microscope and transferred into 10μl of QuickExtract FFPE solution. The sample was then heated at 56 ºC for 1 hour and then 98 ºC for 2 min. The solution was used as template DNA. The PCR amplification process consisted of 1 initial cycle of denaturation at 94 ºC for 5 min, followed by 40 cycles of denaturation at 94 ºC for 30 s, annealing at 55 ºC for 30 s, and extension at 72ºC for 1 min. The final extension cycle was at 72 ºC for 7 min. The primer combinations are as follows; SR1b (F) - SR3 (R); SR2spin (F) - SR7 (R); SR4 (F) - SR9p(R); SR6 (F) - SR11(R); SR8 (F) - SR12b (R) (Nakayama et al. 1996, Yamaguchi and Horiguchi 2005, Yamada et al. 2014). For *Peridiniopsis* cf. *kevei*, the following newly-designed primers were used in stead of SR7; SR7PP (R): TAACGACCTCCAATCTCTAG (the pair primer is SR2spin), and in stead of SR6-SR11 pair; SR6PP (F): GCATTCGTATTTAACTGTCA-SR11PP (R): CATCACGATGCGTTTTAAC. The PCR products were purified and sequenced using an ABI PRISM Big Dye Terminator (Applied Biosystems, Foster City). The sequence reactions were run on a DNA autosequencer ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City). Both forward and reverse strands were sequenced.

Sequence analysis

The accession numbers of sequences included in the alignments are shown in Supplemental Table 2. The SSU rDNA sequences were aligned manually, based on the published secondary structure of the SSU rRNA molecule, using the alveolate taxa available at the rRNA server (http://www.psb.ugent.be/rRNA) (database no longer available). As outgroups, the apicomplexan organisms, *Sarcocystis muris* (Blancbard) Alexieff and *Toxoplasma gondii* Nicolle et Manceaux, were used for peridinin-type dinoflagellates, while the dinoflagellates *Ceratium fusus* Ehrenberg and *Gonyaulax spinifera* (Claparède & Lachmann) Diesing were designated for dinotoms. The aligned sequences were analyzed by Bayesian method using the MrBayes 3.1.2. (Huelsenbeck and Ronquist 2001). The GTR+I+G model was selected by MrModeltest 2.2 (Nylander et al. 2004) as a suitable evolutionary model. Markov chain Monte Carlo iterations were carried out until 50,000,000 generations were attained, when the average standard deviations of split frequencies fall bellows 0.01 indicating convergence of the iterations.

Photosynthetic activity of culture strains.

Chlorophyll fluorescence was determined by a PAM fluorometer (PAM 101/102/103, Heinz Waltz, Effeltrich, Germany). Strains of cultured for one, three or four month(s) under continuous light (60 μmol·photons ·m^{-2}·s^{-1}), respectively were dark-adapted for 20 min.

Supplementary tables

Table S1 The list of dinoflagellate strains, sampling stations and culture conditions (next page)

[1]Habitat-type: P = Planktonic, S = Sand-dwelling and T = Tidal pool. Unit of culture temperature = degrees C, All samples were cultured under the 60 μmol· photons / $m^2 \cdot s^{-1}$, light: dark = 16:8hours. Some of the dinoflagellates were treated as undescribed, novel species (No.8, 12, 17, 19, 27 and the dinoflagellates included in unidentified group) or currently-unidentifiable to the species rank (No.1, 2, 20, 21, 24 and 25) because for precise identification, SEM observation is needed.

Analysis No.	Strain No.	Species name	Habitat-type[1]	Sampling location	Temperature
Peridinin-type dinoflagellates					
1	HG3	*Alexandrium hiranoi*	T	Miura Beach, Kanagawa, Japan (J)	20
2	HG222	*Alexandrium* sp. 1	P	Salt Rock, South Africa (SA)	25
3	NY008	*Alexandrium* sp. 2	P	Otaru Bay, Hokkaido, J	25
4	HG149	*Amphidinium cupulatisquama*	S	Ikei Island, Okinawa, J	25
5	NY004	*Amphidinium gibbosum*	S	Off Mageshima Islands, Kagoshima, J	25
6	HG214	*Amphidinium steinii*	S	Off Mageshima Islands, Kagoshima, J	25
7	HG220	*Amphidinium steinii*	S	Off Mageshima Islands, Kagoshima, J	25
8	HG213	*Amphidinium* sp.	S	Off Mageshima Islands, Kagoshima, J	25
9	HG156	*Amphidiniella sedentaria*	S	Odo Beach, Okinawa, J	25
10	Cx7	*Biecheleria natalensis*	T	Mission Rocks, SA	20
11	HG236	*Bispinodinium angelaceum*	S	Off Mageshima Islands, Kagoshima, J	25
12	NY002	*Ceratocorys* sp.	S	Off Mageshima Islands, Kagoshima, J	25
13	HG154	*Gymnodinium dorsalisulcum*	S	Odo Beach, Okinawa, J	25
14	HG2	*Halostylodinium arenarium*	S	Ishigaki Island, Okinawa, J	20
15	HG17	*Heterocapsa circularisquama*	P	Ago Bay, Mie, J	25
16	TM43	*Heterocapsa psammophilla*	S	Tami-no Beach, Hiroshima, J	20
17	HG228	*Moestrupia* sp.	S	Off Mageshima Islands, Kagoshima, J	25
18	HG225	*Plagiodinium belizeanum*	S	Off Mageshima Islands, Kagoshima, J	25
19	HG177	*Plagiodinium* sp.	S	Off Mageshima Islands, Kagoshima, J	25
20	NY007	*Pyrocystis* sp.	P	Minami-Daito Island, Okinawa, J	20
21	NY012	*Scrippsiella* sp.	P	Uchiura Bay (Muroran City), Hokkaido, J	20
22	TM57	*Spiniferodinium galeiforme*	S	Tami-no Beach, Hiroshima, J	20
23	NY017	*Stylodinium littorale*	S	Off Mageshima Islands, Kagoshima, J	25
24	HG-193	*Symbiodinium* sp. 1	S	Salt Rock, SA	20
25	NY010	*Symbiodinium* sp. 2	S	Tokashiki Island, Okinawa, J	25
26	HG163	*Testudodinium corrugatum*	S	Odo Beach, Okinawa, J	25
27	HG230	*Testudodinium* sp.	S	Off Mageshima Islands, Kagoshima, J	25
28	HG252	*Thoracosphaera heimii*	P	Off Nansei Islands, J	25
29	NY005	Unidentified athecate dinoflagellate 1	S	Off Mageshima Islands, Kagoshima, J	25
30	HG167	Unidentified athecate dinoflagellate 2	S	Off Mageshima Islands, Kagoshima, J	25
31	NY003	Unidentified athecate dinoflagellate 3	S	Off Mageshima Islands, Kagoshima, J	25
32	NY011	Unidentified thecate dinoflagellate 1	S	Off Mageshima Island, Kagoshima, J	25
33	NY013	Unidentified thecate dinoflagellate 2	P	Otaru Bay, Hokkaido, J	20
34	HG151	Unidentified thecate dinoflagellate 3	S	Odo Beach, Okinawa, J	25
35	NY014	Unidentified thecate dinoflagellate 4	S	Off Mageshima Islands, Kagoshima, J	25
Dinotoms					
36	HG171	*Durinskia* cf. *baltica*	S	Tokashiki Island, Okinawa, J	25
37	HG265	*Durinskia* cf. *baltica*	S	Odo Beach, Okinawa, J	25
38	HG181	*Durinskia* sp.	T	Marina Beach, South Africa	20
39	Cx18	*Durinskia* sp.	T	Marina Beach, South Africa	20
40	Cx22	*Durinskia* sp.	T	Marina Beach, South Africa	20
41	HG249	*Galeidinium rugatum*	S	Mecherchar Island, Palau	25
42	HG180	Unidentified coccoid dinotom 1	S	Hanashiro, Okinawa, J	25
43	HG204	Unidentified coccoid dinotom 2	S	Marina Beach, SA	20
44	DA08	*Peridiniopsis* cf. *kevei**	P	Shikotsu Lake, Hokkaido, J	20
45	HG327	*Peridiniopsis* cf. *kevei**	P	Biwa Lake, Shiga, J	20
Free-living diatoms					
46	NY018	*Cylindrotheca closterium*	P	Oshoro Bay, Hokkaido, J	15
47	NY060	*Nitzschia sp.*	S	Off Mageshima Islands, Kagoshima, J	20
48	NY059	*Tabularia sp.*	S	Hanasaki, Hokkaido, J	20

Table S2 Genbank accession numbers of samples used in this study

Species Name	Strain	Accession Number
Adenoides eludens (Herdman) Balech		EF492484
Akashiwo sanguinea (Hirasaka) Hansen & Moestrup		U41085
Alexandrium hiranoi Kita & Fukuyo		AY641564
Alexandrium hiranoi Kita & Fukuyo	HG3	LC056070
Alexandrium insuetum Balech		AB088298
Alexandrium minutum Halim		U27499
Alexandrium ostenfeldii (Paulsen) Balech & Tangen		AJ535384
Alexandrium pseudogonyaulax (Biecheler) Horiguchi ex Kita & Fukuyo		AB088302
Alexandrium tamarense (Lebour) Balech		AF022191
Alexandrium tamutum Montresor, Beran & John		AJ535378
Alexandrium taylori Balech		AJ535385
Alexandrium sp. 1	HG222	LC056069
Alexandrium sp. 2	NY008	LC056068
Amphidiniella sedentaria Horiguchi		AB212091
Amphidiniella sedentaria Horiguchi	HG156	LC057317
Amphidiniopsis dragescoi (Balech) Hoppenrath, Selina, Yamaguchi & Leander		AY238479
Amphidinium carterae Hulburt		AF009217
Amphidinium cupulatisquama Tamura & Horiguchi	HG149	LC056067
Amphidinium gibbosum (Maranda & Shimizu) Jørgensen & Murray		L13719
Amphidinium gibbosum (Maranda & Shimizu) Jørgensen & Murray	NY004	AB863027
Amphidinium massartii Biecheler		AF274255

Amphidinium operculatum Claparède & Lachmann		AB704006
Amphidinium cf. *rhynchocephalum* Anissimowa		AY443012
Amphidinium steinii Lemmermann	HG214	LC054920
Amphidinium steinii Lemmermann	HG220	LC054921
Amphidinium sp.		AB626895
Amphidinium sp.	HG115	AB477347
Amphidinium sp.	HG213	LC054922
Archaeperidinium minutum (Kofoid) Jørgensen		AB564308
Azadinium spinosum Elbrächter & Tillmann		JN680857
Biecheleria baltica Moestrup, Lindberg, & Daugbjerg		EF058252
Biecheleria natalensis (Horiguchi & Pienaar) Moestrup	Cx7	LC054923
Bispinodinium angelaceum Yamada & Horiguchi	HG236	AB762397
Borghiella tenuissima (Lauterborn) Moestrup, Hansen & Daugbjerg		AY443025
Ceratium fusus Ehrenberg		AF022153
Ceratocorys sp.	NY002	LC054924
Cochlodinium polykrikoides Margalef		AY421781
Dinophysis acuta Ehrenberg		AJ506973
Dinophysis norvegica Claparède & Lachmann		AJ506974
Diplopsalis lenticula Bergh		AB716909
Durinskia agilis (Kofoid & Swezy) Saburova, Chomérat & Hoppenrath		JF514516
Durinskia baltica (Levander) Carty & Cox		AF231803
Durinskia cf. *baltica* (Levander) Carty & Cox	HG171	LC054925
Durinskia cf. *baltica* (Levander) Carty & Cox	HG265	LC054926
Durinskia capensis Pienaar, Sakai & Horiguchi		AB271107

Durinskia sp.	HG181	LC054927
Durinskia sp.	Cx18	LC054928
Durinskia sp.	Cx22	LC054929
Galeidinium rugatum Tamura & Horiguchi	HG249	AB195668
Gonyaulax spinifera (Claparède & Lachmann) Diesing		AF022155
Gymnodinium catenatum Graham		AF022193
Gymnodinium dorsalisulcum (Hulbert, McLaughlin & Zahl) Murray, de Salas & Hallegraeff		DQ837534
Gymnodinium dorsalisulcum (Hulbert, McLaughlin & Zahl) Murray, de Salas & Hallegraeff	HG154	LC054930
Gymnodinium fuscum Stein		AF022194
Gymnodinium impudicum (Fraga & Bravo) Hansen & Moestrup		AF022197
Halostylodinium arenarium Horiguchi & Yoshizawa-Ebata	HG2	LC054931
Herdmania litoralis Dodge		AB564300
Heterocapsa circularisquama Horiguchi	HG17	LC054932
Heterocapsa niei (Loeblich III) Morrill & Loeblich III		AF274265
Heterocapsa psammophilla Tamura, Iwataki & Horiguchi	TM43	LC054933
Heterocapsa pygmaea Loeblich III, Schmidt & Sherley		AF274266
Heterocapsa rotundata (Lohmann) Hansen		AF274267
Heterocapsa triquetra (Ehrenberg) Stein		AF022198
Jadwigia applanata Moestrup, Lindberg & Daugbjerg		EF058240
Karenia brevis (Davis) Hansen & Moestrup		AF172714
Karlodinium micrum (Leadbeater & Dodge) Larsen		AF172712
Kryptoperidinium foliaceum (Stein) Lindemann		AF274268
Lepidodinium viride Watanabe, Suda, Inouye, Sawaguchi & Chihara		AF022199
Moestrupia sp.	HG228	LC054934

Nematodinium sp.		FJ947039
Paragymnodinium shiwhaense Kang, Jeong, Moestrup & Shin		AM408889
Pfiesteria piscicida Steidinger & Burkholder		AY112746
Pentapharsodinium tyrrhenicum (Balech) Montressor, Zingone & Marino		AF022201
Peridiniopsis borgei Lemmermann		EF058241
Peridiniopsis cf. *kevei* Grigorszky		AB353770
Peridiniopsis cf. *kevei* Grigorszky	DA08	LC054935
Peridiniopsis cf. *kevei* Grigorszky	HG327	LC054936
Peridiniopsis niei Liu & Hu		HM596542
Peridiniopsis penardii (Lemmermann) Bourrelly		AB353771
Peridiniopsis polonicum (Woloszynska) Bourrelly		AY443017
Peridinium aciculiferum Lemmermann		AY970653
Peridinium cinctum (Müller) Ehrenberg		DQ166209
Peridinium quinquecorne Abé		AB246744
Peridinium willei Huitfeldt-Kaas		AF274272
Plagiodinium belizeanum Faust & Balech	HG225	LC054937
Plagiodinium sp.	HG177	LC054938
Polarella glacialis Montresor, Procaccini & Stoecker		AF099183
Prorocentrum lima (Ehrenberg) Stein		Y16235
Prorocentrum maculosum Faust		Y16236
Prorocentrum micans Ehrenberg		M14649
Prorocentrum minimum (Pavillard) Schiller		AY421791
Pseudopfiesteria shumwayae (Glasgow & Burkholder) Litaker, Steidinger, Mason, Shields & Tester		AF080098
Pyrocystis lunula (Schütt) Schütt		AF274274

Pyrocystis noctiluca Murray ex Haeckel		AF022156
Pyrocystis sp.	NY007	LC054939
Sabulodinium undulatum Saunders & Dodge		DQ975474
Scrippsiella hangoei (Schiller) Larsen		AY970662
Scrippsiella precaria Montresor & Zingone		DQ847435
Scrippsiella sweeneyae Loeblich III		AF274276
Scrippsiella trochoidea (Stein) Balech ex Loeblich III		AF274277
Scrippsiella sp.	NY012	LC054940
Spiniferodinium galeiforme Horiguchi & Chihara		GU295203
Spiniferodinium galeiforme Horiguchi & Chihara	TM57	LC054941
Stylodinium littorale Horiguchi & Chihara	NY017	LC054942
Symbiodinium californium Banaszak, Iglesias-Prieto & Trench		AF225965
Symbiodinium corculorum Trench		L13717
Symbiodinium goreaui Trench & Blank		EF036539
Symbiodinium microadriaticum Freudenthal		M88521
Symbiodinium sp. 1	HG193	AB863030
Symbiodinium sp. 2	NY010	AB863031
Testudodinium corrugatum (Larsen & Patterson) Horiguchi, Tamura & Yamaguchi	HG163	AB704004
Testudodinium corrugatum (Larsen & Patterson) Horiguchi, Tamura & Yamaguchi	TM-85	AB704003
Testudodinium maedaense Katsumata & Horiguchi	MAE-18	AB704005
Testudodinium testudo (Herdman) Horiguchi, Tamura, Katsumata & Yamaguchi	KOM-30	AB704002
Testudodinium sp.	HG230	LC054943
Thecadinium petasatum Kofoid & Skogsberg		GU295204
Thoracosphaera heimii (Lohmann) Kamptner		AF274278

Thoracosphaera heimii (Lohmann) Kamptner	HG252	LC054944
Togula britannica (Herdman) Jørgensen, Murray & Daugbjerg		AY443010
Togula jolla Jørgensen, Murray & Daugbjerg		AF274252
Unidentified athecate dinoflagellate 1	NY005	AB863028
Unidentified athecate dinoflagellate 2	HG167	AB863029
Unidentified athecate dinoflagellate 3	NY003	LC054945
Unidentified coccoid dinotom 1	HG180	LC054946
Unidentified coccoid dinotom 2	HG204	LC054947
Unidentified thecate dinoflagellate 1	NY011	LC054948
Unidentified thecate dinoflagellate 2	NY013	LC054949
Unidentified thecate dinoflagellate 3	HG151	LC054950
Unidentified thecate dinoflagellate 4	NY014	LC054951
Outgroup		
Sarcocystis muris		M64244
Toxoplasma gondii		L24381
Diatom		
Cylindrotheca closterium	NY018	LC054954
Nitzschia sp.	NY060	LC054952
Tabularia sp.	NY059	LC054953

Table S3 The Fv/Fm ratio using PAM method of three dinoflagellates which were cultured in one, three or four months The Fv/Fm ratio by PAM fluorometer were measured in three times for each strain and the averages of these values were used for t-test with two-tailed test (p<0.05). In *Durinskia* sp. (No.38), three months cultured strain was used because cells of this species become the deformation after maintained in four months. One month = cultured from 1 April 2015 to 1 May 2015. Three months = cultured from 7 January 2015 to 6 April 2015. Four months = cultured from 3 December 2014 to 6 April 2015.

Culture period	1	2	3	Average	
***Amphidinium gibossum* (No. 5) Sand-dwelling, Peridinin-type**					
One month	0.556	0.458	0.583	0.532	
Four months	0.472	0.420	0.425	0.439	$t(4)=2.253$, $p=0.087$
Unidentified coccoid dinotom 1 (No.42) Sand-dwelling, Dinotom					
One month	0.512	0.517	0.565	0.531	
Four months	0.442	0.556	0.549	0.516	$t(4)=0.386$, $p=0.719$
***Durinskia* sp. (No.38) Tidal pool living, Dinotom**					
One month	0.480	0.480	0.458	0.473	
Three months	0.467	0.515	0.500	0.494	$t(4)=1.337$, $p=0.252$

Supplementary figures

Fig. S1 Light micrographs of peridinin-type dinoflagellates used in this study (next page) (1) *Alexandrium hiranoi*; analysis No.1 (2) *Alexandrium* sp. 1; No.2 (3) *Alexandrium* sp. 2; No. 3 (4) *Amphidinium cupulatisquama*; No.4 (5) *Amphidinium gibbosum*; No.5 (6) *Amphidinium steinii*; No. 6 (7) *Amphidinium steinii*; No. 7 (8) *Amphidinium* sp.; No.8 (9) *Amphidiniella sedentaria*; No.9 (10) *Biecheleria natalensis*; No.10 (11) *Bispinodinium angelaceum*; No.11 (12) *Ceratocorys* sp.; No. 12 (13) *Gymnodinium dorsalisulcum*; No.13 (14) *Halostylodinium arenarium*; No.14 (15) *Heterocapsa circularisquama*; No.15 (16) *Heterocapsa psammophilla*; No.16 (17) *Moestrupia* sp.; No.17 (18) *Plagiodinium belizeanum*; No.18 (19) *Plagiodinium* sp.; No.19 (20) *Pyrocystis* sp.; No. 20 (21) *Scrippsiella* sp.; No.21 (22) *Spiniferodinium galeiforme*; No.22 (23) *Stylodinium littorale*; No.23 (24) *Symbiodinium* sp. 1; No.24 (25) *Symbiodinium* sp. 2; No.25 (26) *Testudodinium corrugatum*; No.26 (27) *Testudodinium* sp.; No.27 (28) *Thoracosphaera heimii*; No.28 (29) Unidentified athecate dinoflagellate1; No.29 (30) Unidentified athecate dinoflagellate 2; No.30 (31) Unidentified athecate dinoflagellate 3; No.31 (32) Unidentified thecate dinoflagellate1; No.32 (33) Unidentified thecate dinoflagellate 2; No.33 (34) Unidentified thecate dinoflagellate 3; No.34 (35) Unidentified thecate dinoflagellate 4; No.35. The scale bar = 10μm.

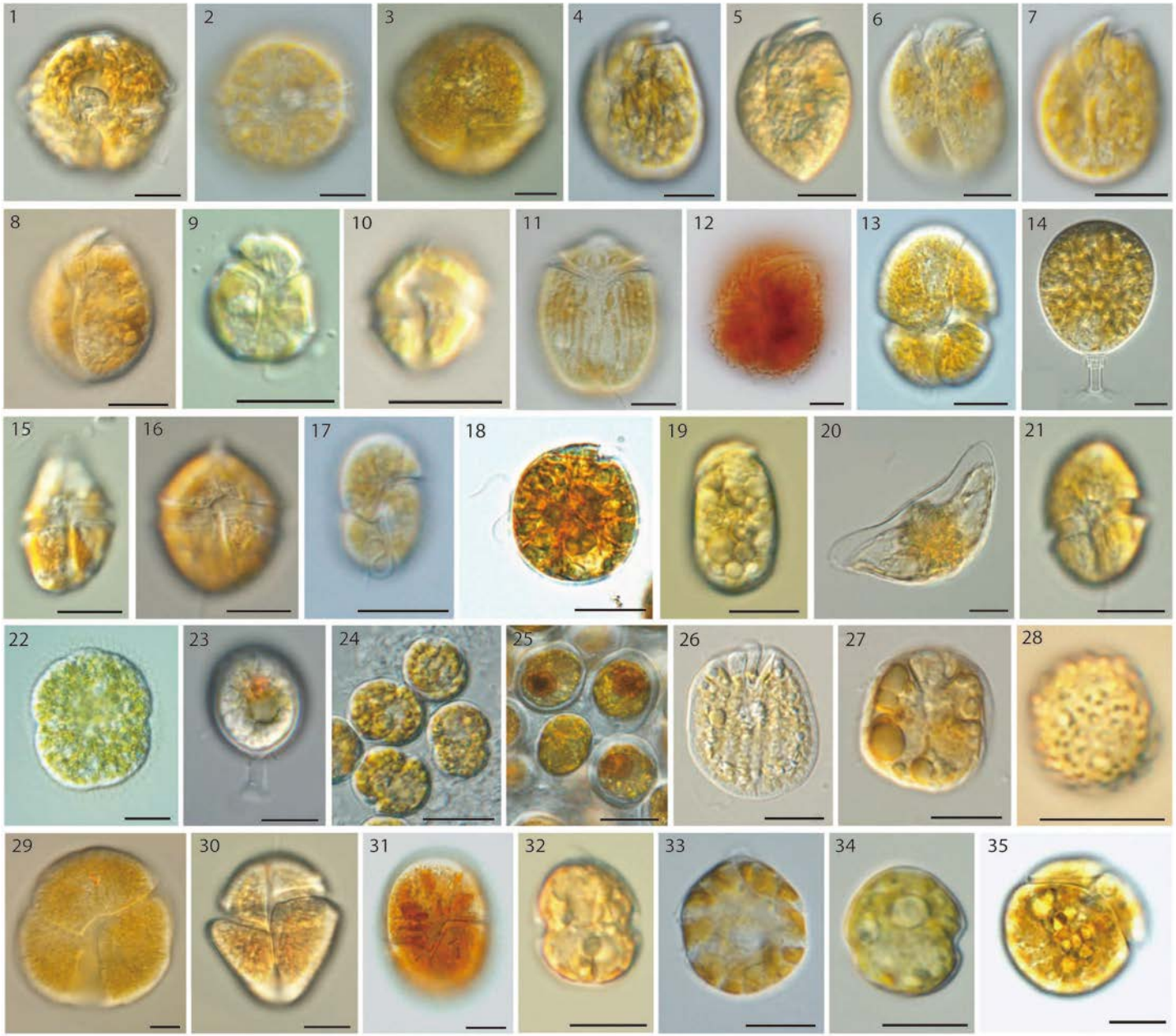
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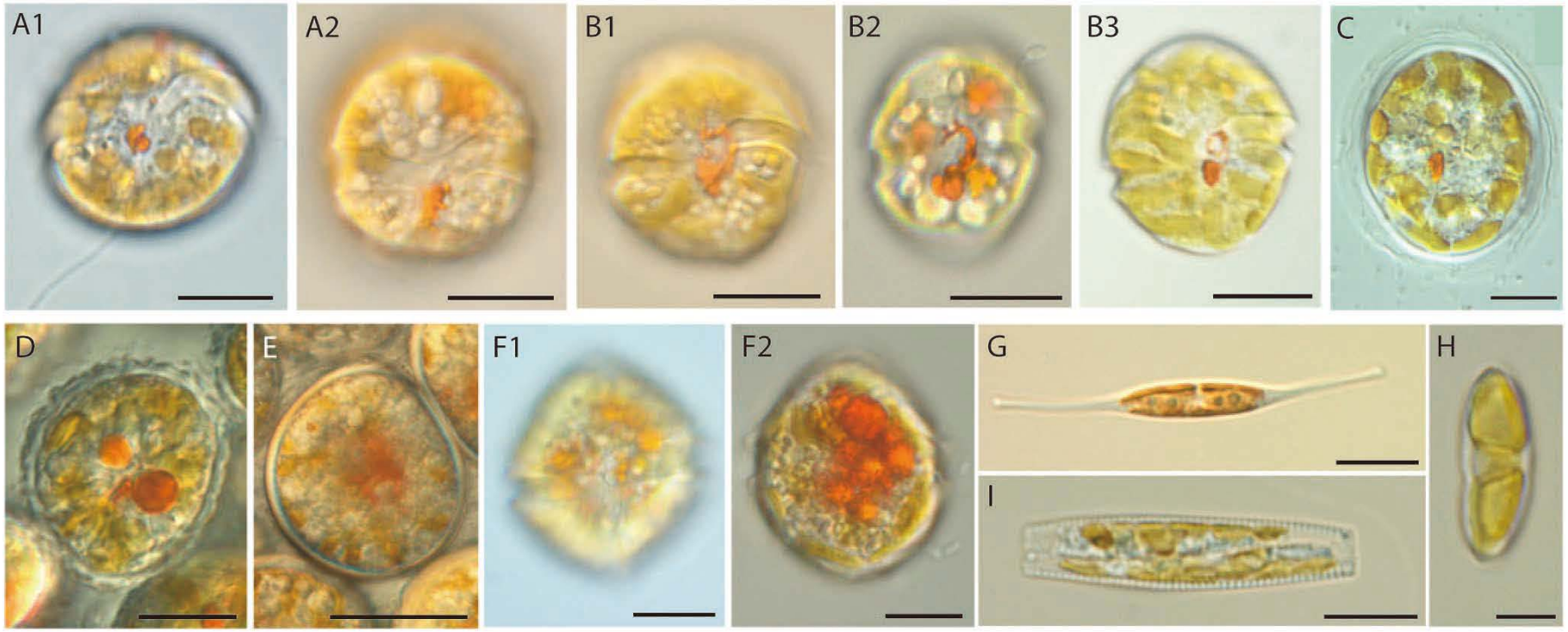
Fig. S2 Light micrographs of dinotoms and free-living diatoms used in this study (next page) (A) *Durinskia* cf. *baltica* (A1) analysis number 36, collected from Tokashiki Islands, Okinawa, Japan (A2) No.37, collected from Odo Beach, Okinawa, Japan (B) *Durinskia* sp. (B1) No.38, collected from Marina Beach, South Africa (B2) No.39, collected from Marina Beach, South Africa (B3) No.40, collected from Marina Beach, South Africa (C) *Galeidinium rugatum*; No.41. Non-motile cell. (D) Unidentified coccoid dinotom 1; No.42. Non-motile cell. (E) Unidentified coccoid dinotom 2; No.43. Non-motile cell. (F) *Peridiniopsis* cf. *kevei* (F1) No.44, sampling from Shikotu-Lake, Hokkaido, Japan (F2) No.45, sampling from Biwa-Lake, Shiga, Japan. (G) *Cylindrotheca closterium*, free-living diatom close to endosymbiont diatom *Nitzschia* of (A) to (E); No.46. (H) *Nitzschia* sp. free-living diatom; No.47. (I) *Tabularia* sp. free-living diatom; No.48. The scale bar = 5μm. The other the scale bar = 10μm.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A2
B1
B2
B3
C
D
E
F1
F2
G
I
H

Fig. S3 The Bayesian molecular phylogeny inferred from SSU rDNA of Dinophyta including peridinin-type dinoflagellates and dinotoms Numbers on the major nodes represent posterior probability. Only PP>0.5 is shown. Analyzed strains and numbers are shown by bold type. Gray boxes with asterisk indicate dinotoms.

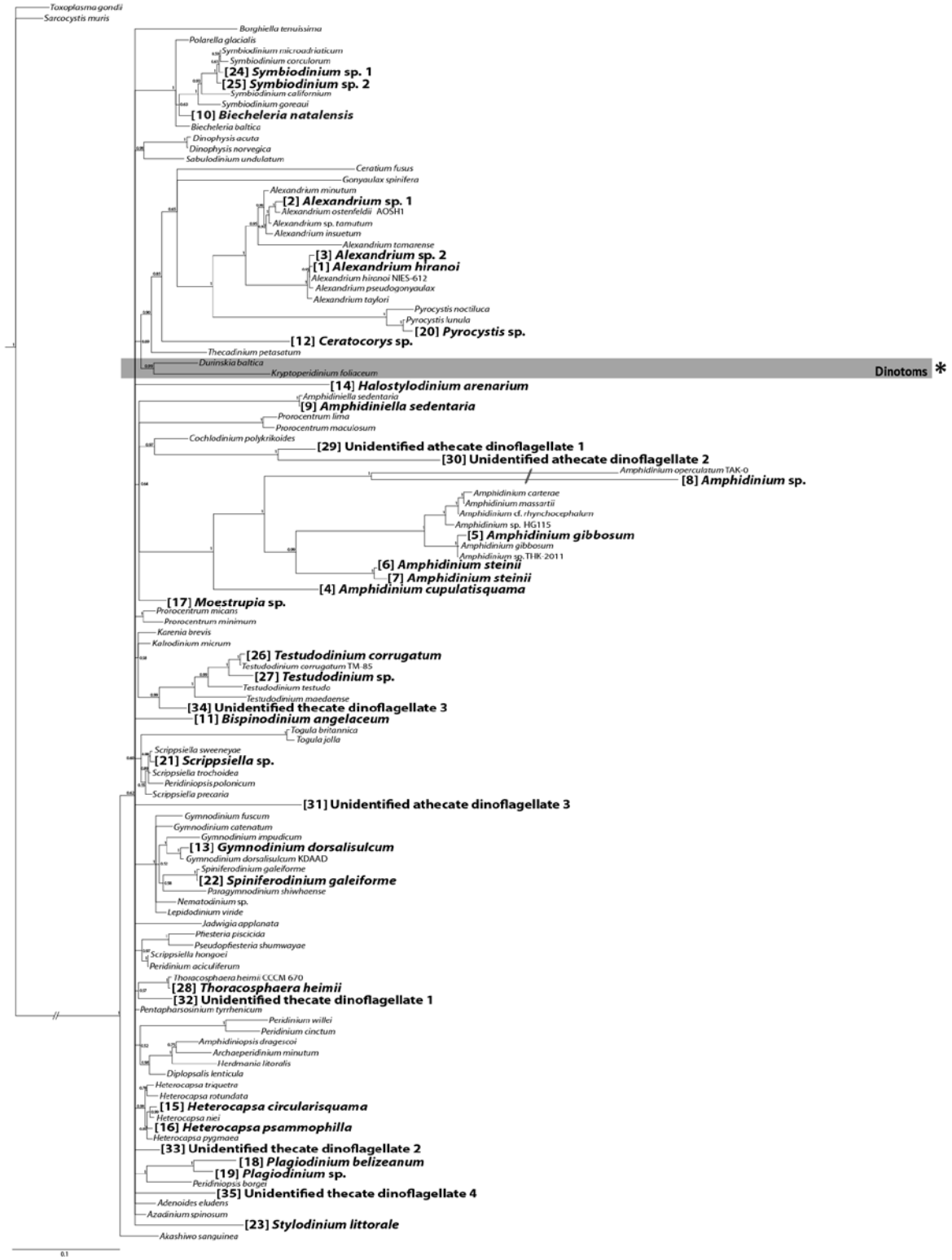

Fig. S4

The Bayesian molecular phylogeny inferred from host dinoflagellate SSU rDNA of dinotoms Bold species with analysis number were HPLC samples. Numbers on the major nodes represent posterior probability. Only PP>0.5 is shown. Dark-gray boxes indicate dinotoms with *Nitzschia*-type endosymbiont diatom (*Durinskia* cf. *baltica* clade, *Durinskia* sp. clade and *Galeidinium* spp. clade). Light-gray box indicate dinotoms with *Discostella*-type endosymbiont diatom (*Peridiniopsis* cf. *kevei* clade).

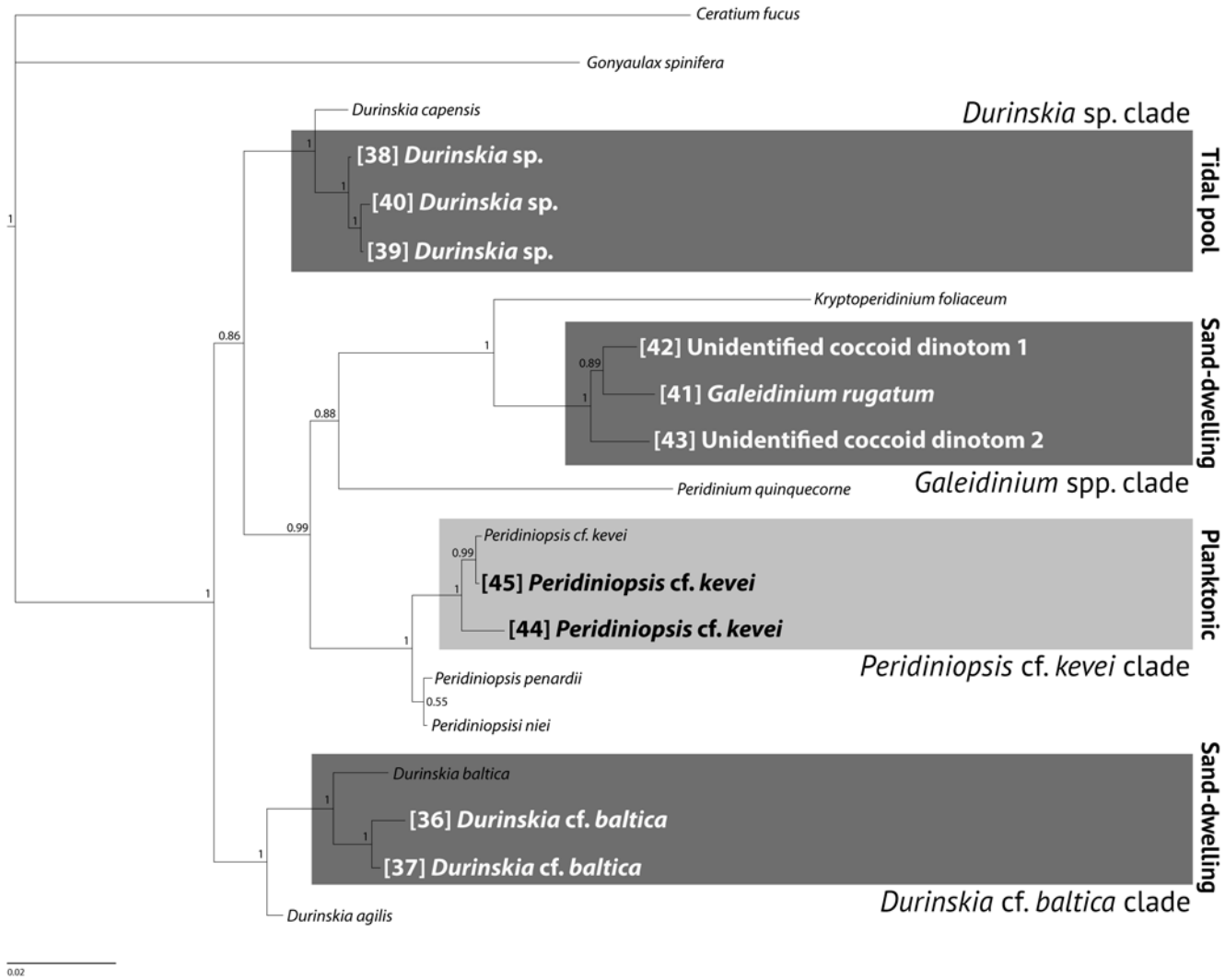

Fig. S5 Elution order and visible absorption characteristics of pigments in eluent from peridinin-type dinoflagellates (next page) The gray column indicated pigments common to all peridinin-type dinoflagellates (PCPe). The lutein-like carotenoid has the same absorbance spectrum and retention time as lutein by HPLC. Therefore the pigment should be identified as lutein, however, it is known that diatoms do not possess the enzyme gene for synthesizing lutein (Bertrand 2010). Also, lutein has never been detected from dinoflagellate, but only found from chlorophytes including land plants, multi-cellular red algae and chlorarachniophytes among phototrophic organisms (Takaichi 2011). In this study, this pigment was detected from all peridinin-type dinoflagellates (except for *Stylodinium littorale*, No.23), three strains of dinotoms and even free-living diatom, *Cylindrotheca closterium*. Therefore, we decided to call it lutein-like carotenoid instead of lutein.

Peak No.	Pigment	Time (min)	λmax (nm)	Analysis No. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
1-1	Peridininol	4.9	480	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
1-2	Peridininol	5.2	480	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
2	Unknown carotenoid	7.0	466	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
4	Chlorophyll *c*2	10.0	452/633/585	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
5	Chlorophyll *c*1	10.3	449/632/583	○				○			○														○	○	○	○					○					○
6	Peridinin	12.0	474.4	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
7	Peridinin-like carotenoid	12.5	475.8	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
8	Unknown carotenoid	13.6	465	○	○	○	○	○		○		○			○	○		○	○		○	○	○	○	○		○	○	○	○	○	○	○	○	○	○	○	○
11-1	Unknown carotenoid	15.5	463	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
11-2	Unknown carotenoid	15.5	465	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
12	Unknown carotenoid	15.9	447/479	○																																		
13	Unknown carotenoid	16.7	475	○				○	○		○	○	○	○		○	○	○	○	○			○		○	○	○	○	○	○	○	○	○	○	○	○	○	
14	Violaxanthin	16.8	441/470/415			○	○														○			○													○
15	Unknown carotenoid	17.2	474									○						○											○	○		○		○	○			
16	Unknown carotenoid	17.3	484																							○										○		
17	Unknown carotenoid	17.9	458						○										○																			
18	Diadinochrome	17.6	429/457	○	○	○	○	○				○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○			○	○	○	○	○	○	○	○
19	Diadinoxanthin	17.9	447/476	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
20	Unknown carotenoid	18.1	463								○							○													○			○				
22	Dinoxanthin	18.3	441/470/417	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
24	Diatoxanthin	18.7	452/480	○		○					○							○	○																		○	
25	Diadinochrome-like carotenoid	18.8	424/449	○				○	○			○		○		○	○	○							○			○							○	○		
27	Zeaxanthin	19.2	442/468	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
28	Lutein-like carotenoid	19.3	443/472	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○		○	○	○	○	○	○	○	○	○	○	○	○
30	Unknown carotenoid	19.6	454	○										○	○		○	○			○					○				○	○		○		○	○	
31	Unknown carotenoid	19.9	435/451	○		○						○			○	○			○		○	○								○		○		○				○
32	Unknown carotenoid	20.2	445/475								○							○	○																		
33	Unknown carotenoid	20.2	465																														○				
36	cPPB-*a*E	20.5	425/451/685/626					○						○													○	○			○	○					
37	Unknown carotenoid	20.5	429/456													○																					
38	Unknown carotenoid	20.6	471									○					○								○	○			○		○			○	○	○	○	
39	Unknown carotenoid	20.7	468																							○											
40	Unknown carotenoid	20.9	451/478													○																					
41	Unknown carotenoid	21.0	464				○																								○						
42	Unknown carotenoid	21.2	466/494											○																							
43	Unknown carotenoid	21.3	470													○																					
44	Unknown carotenoid	21.7	469																																	○	
47	Unknown carotenoid	22.5	477									○	○																						○	○	
48	Unknown carotenoid	22.9	456																															○		○	○	
49	Chlorophyll *a*	24.2	430/662/619	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
51	Unknown carotenoid	25.1	471						○	○	○						○									○						○	○	○				
52	Unknown carotenoid	25.8	446																													○	○	○				
53	Pheophythin *a*	25.9	409/664/608	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
54	*β*-carotene	26.4	453/478	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○

Fig. S6 Result of clustering analysis of pigment distribution according to species in peridinin-type dinoflagellates (next page) The binary similarity and dissimilarity measure is from Sokal and Michener index. Gray columns indicated the species possessing similar pigment compositions each other between close related species. Numbers indicates peak numbers of unknown carotenoids.

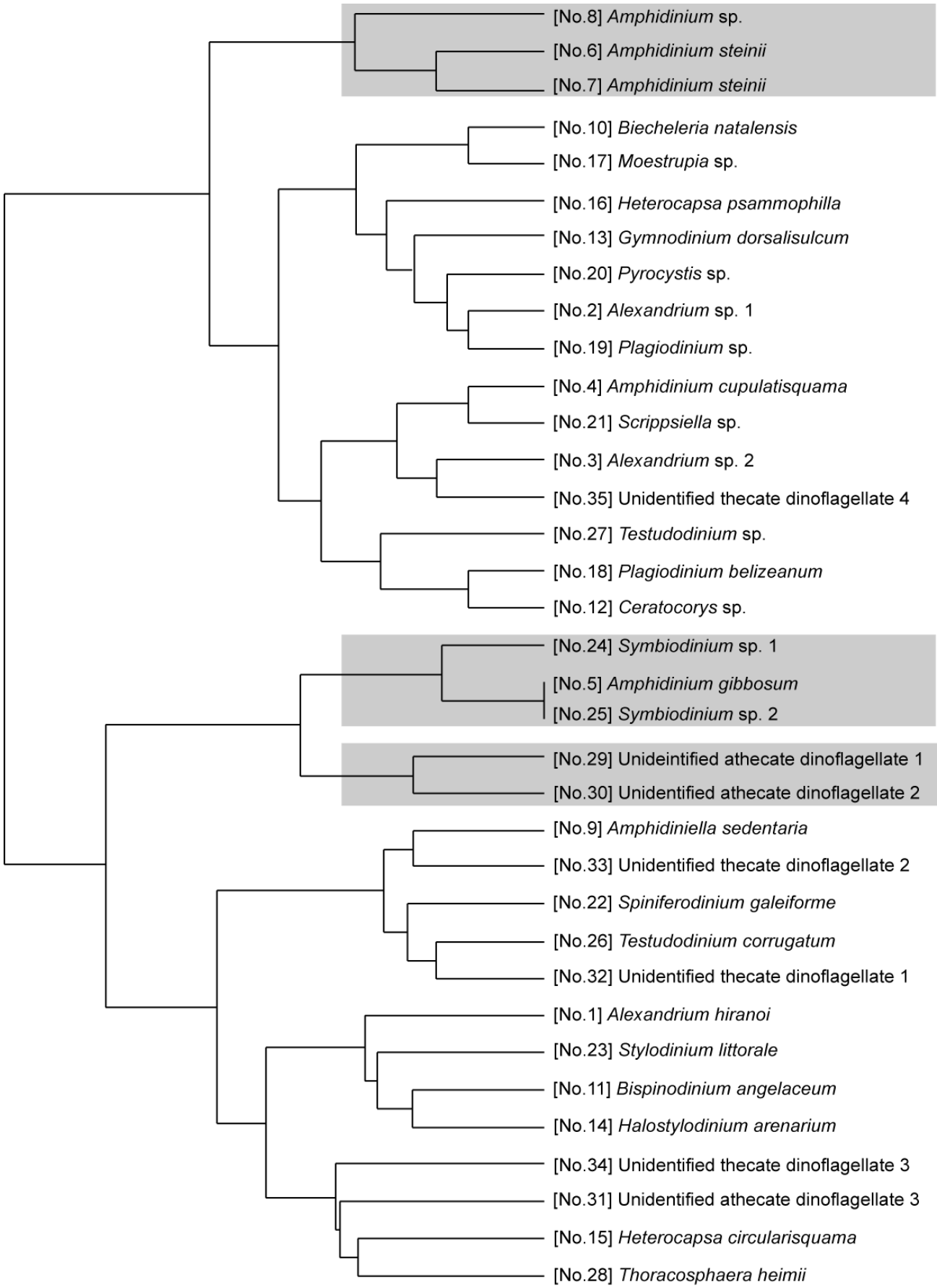
[No.8] Amphidinium sp.
[No.6] Amphidinium steinii
[No.7] Amphidinium steinii
[No.10] Biecheleria natalensis
[No.17] Moestrupia sp.
[No.16] Heterocapsa psammophilla
[No.13] Gymnodinium dorsalisulcum
[No.20] Pyrocystis sp.
[No.2] Alexandrium sp. 1
[No.19] Plagiodinium sp.
[No.4] Amphidinium cupulatisquama
[No.21] Scrippsiella sp.
[No.3] Alexandrium sp. 2
[No.35] Unidentified thecate dinoflagellate 4
[No.27] Testudodinium sp.
[No.18] Plagiodinium belizeanum
[No.12] Ceratocorys sp.
[No.24] Symbiodinium sp. 1
[No.5] Amphidinium gibbosum
[No.25] Symbiodinium sp. 2
[No.29] Unideintified athecate dinoflagellate 1
[No.30] Unidentified athecate dinoflagellate 2
[No.9] Amphidiniella sedentaria
[No.33] Unidentified thecate dinoflagellate 2
[No.22] Spiniferodinium galeiforme
[No.26] Testudodinium corrugatum
[No.32] Unidentified thecate dinoflagellate 1
[No.1] Alexandrium hiranoi
[No.23] Stylodinium littorale
[No.11] Bispinodinium angelaceum
[No.14] Halostylodinium arenarium
[No.34] Unidentified thecate dinoflagellate 3
[No.31] Unidentified athecate dinoflagellate 3
[No.15] Heterocapsa circularisquama
[No.28] Thoracosphaera heimii

Fig. S7 Elution order and visible absorption characteristics of pigments in eluent from dinotoms and free-living diatoms The gray column indicated pigments common to all dinotoms (PCDi). Diatom 1 is *Cylindrotheca closterium*, No.46. Diatom 2 is *Nitzschia* sp., No.47. Diatom 3 is *Tabularia* sp., No.48.

Peak No.	Pigment	Time (min)	λmax (nm)	Analysis No.												
				36	37	38	39	40	41	42	43	44	45	Diatom1	Diatom2	Diatom3
3	Chlorophyll *c*3	7.9	456/590	○	○				○	○	○				○	
4	Chlorophyll *c*2	10.0	452/633/585	○	○	○	○	○	○	○	○	○	○	○	○	○
5	Chlorophyll *c*1	10.3	449/632/583	○	○	○	○	○	○	○	○	○	○	○	○	○
9	Fucoxanthin-like carotenoid	14.1	451			○	○	○	○				○			
10	Fucoxanthin	14.6	449	○	○	○	○	○	○	○	○	○	○	○	○	○
12	Unknown carotenoid	15.9	447/479						○				○			
13	Unknown carotenoid	16.7	475										○			
15	Unknown carotenoid	17.2	474						○				○			
16	Unknown carotenoid	17.3	484						○	○	○					
19	Diadinoxanthin	17.9	447/476	○	○	○	○	○	○	○	○	○	○	○	○	○
20	Unknown carotenoid	18.1	463	○	○	○			○	○	○	○	○			
21	Unknown carotenoid	18.2	444	○	○	○	○	○	○	○	○	○	○	○	○	○
23	Unknown carotenoid	18.6	463						○	○						
24	Diatoxanthin	18.7	452/480	○	○	○	○	○			○	○	○		○	
26	Unknown carotenoid	18.9	481						○	○						
27	Zeaxanthin	19.2	442/468	○	○	○	○	○	○	○	○	○	○	○	○	○
28	Lutein-like carotenoid	19.3	443/472			○						○	○	○		
29	Unknown carotenoid	19.3	445	○					○		○					
30	Unknown carotenoid	19.6	454	○				○	○	○	○		○	○		
32	Unknown carotenoid	20.2	445/475				○	○				○	○	○		
33	Unknown carotenoid	20.2	465						○	○						
34	Unknown carotenoid	20.4	470						○	○						
35	Unknown carotenoid	20.5	464						○	○						
38	Unknown carotenoid	20.6	471	○	○				○	○	○	○	○			
39	Unknown carotenoid	20.7	468						○	○						
40	Unknown carotenoid	20.9	451/478				○	○								
41	Unknown carotenoid	21.0	464						○	○						
43	Unknown carotenoid	21.3	470						○	○						
44	Unknown carotenoid	21.7	469						○	○						
45	Unknown carotenoid	22.2	466						○	○	○					
46	Unknown carotenoid	22.3	468						○	○	○					
47	Unknown carotenoid	22.5	477	○					○	○						
48	Unknown carotenoid	22.9	456	○							○			○		
49	Chlorophyll *a*	24.2	430/662/619	○	○	○	○	○	○	○	○	○	○	○	○	○
50	β-Ψcarotene	24.9	462/491	○	○	○	○	○	○	○	○	○	○			
52	Unknown carotenoid	25.8	446										○			
53	pheophythin *a*	25.9	409/664/608	○	○	○	○	○	○	○	○	○	○	○	○	○
54	β-carotene	26.4	453/478	○	○	○	○	○	○	○	○	○	○	○	○	○

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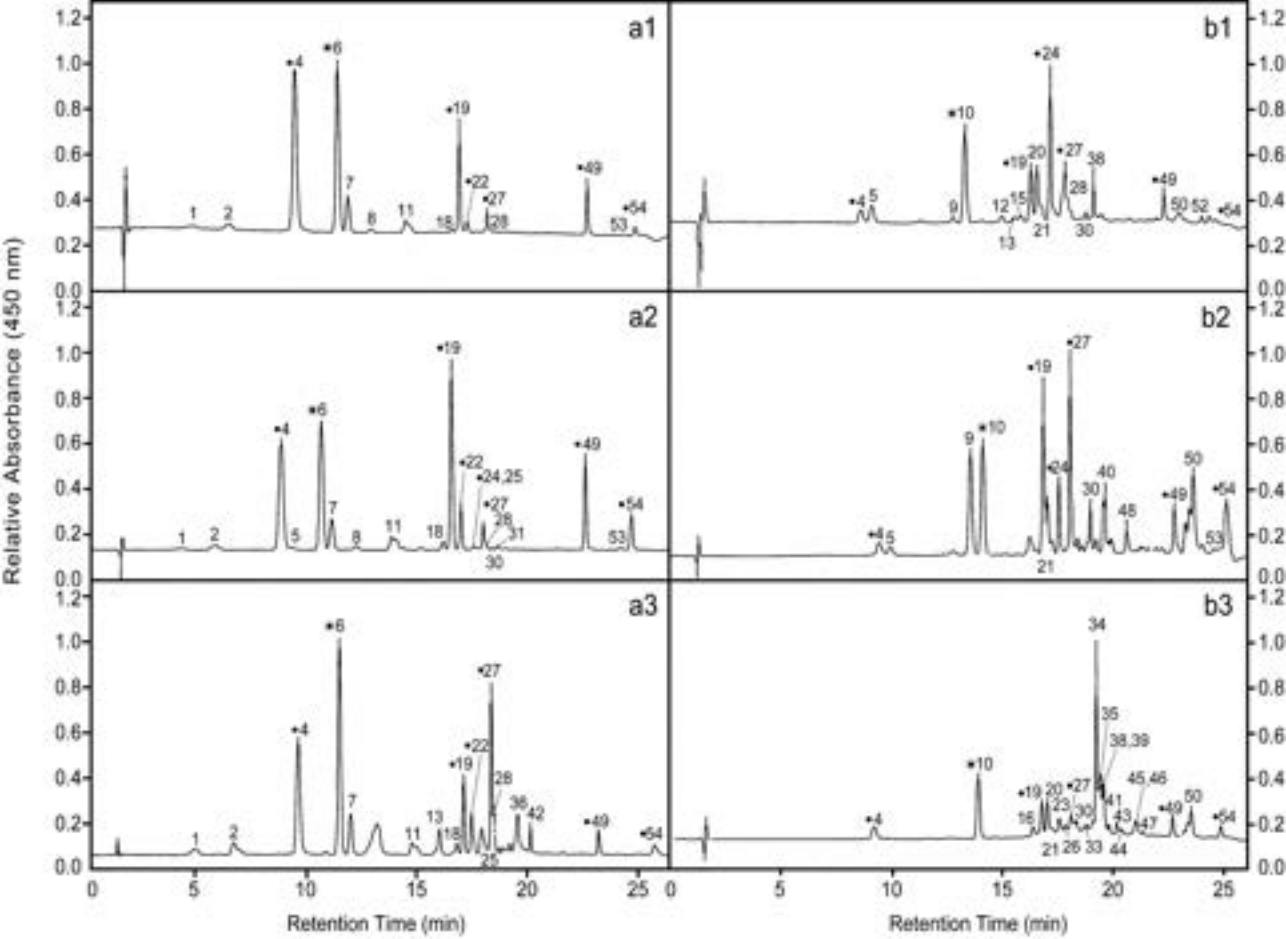
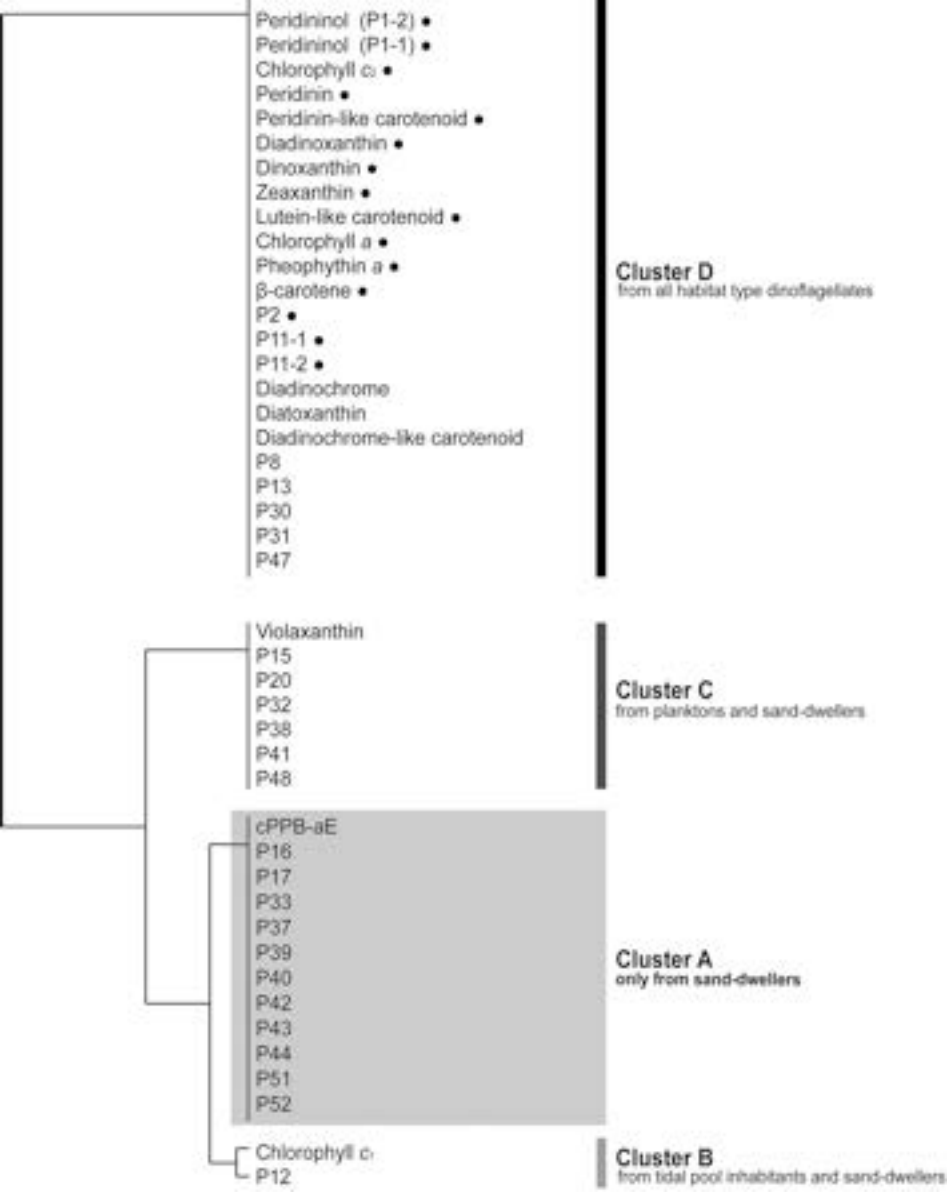
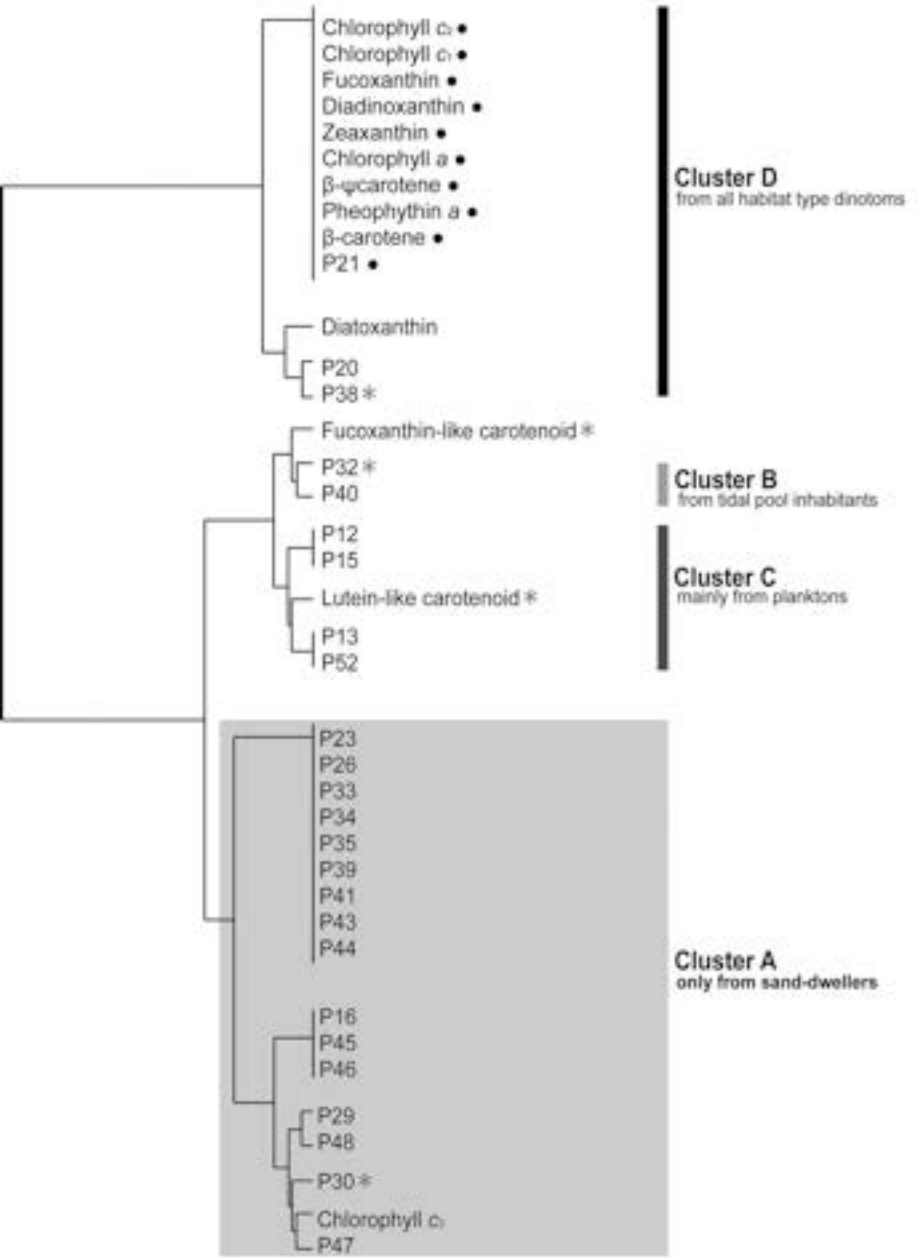

a1
a2
a3
b1
b2
b3
Relative Absorbance (450 nm)
Retention Time (min)
Retention Time (min)


Peridininol (P1-2) •
Peridininol (P1-1) •
Chlorophyll c_3 •
Peridinin •
Peridinin-like carotenoid •
Diadinoxanthin •
Dinoxanthin •
Zeaxanthin •
Lutein-like carotenoid •
Chlorophyll *a* •
Pheophythin *a* •
β-carotene •
P2 •
P11-1 •
P11-2 •
Diadinochrome
Diatoxanthin
Diadinochrome-like carotenoid
P8
P13
P30
P31
P47
Cluster D
from all habitat type dinoflagellates
Violaxanthin
P15
P20
P32
P38
P41
P48
Cluster C
from planktons and sand-dwellers
cPPB-aE
P16
P17
P33
P37
P39
P40
P42
P43
P44
P51
P52
Cluster A
only from sand-dwellers
Chlorophyll c_1
P12
Cluster B
from tidal pool inhabitants and sand-dwellers


Chlorophyll c_3 •
Chlorophyll c_1 •
Fucoxanthin •
Diadinoxanthin •
Zeaxanthin •
Chlorophyll *a* •
β-ψcarotene •
Pheophythin *a* •
β-carotene •
P21 •
Diatoxanthin
P20
P38 *
Fucoxanthin-like carotenoid *
P32 *
P40
P12
P15
Lutein-like carotenoid *
P13
P52
P23
P26
P33
P34
P35
P39
P41
P43
P44
P16
P45
P46
P29
P48
P30 *
Chlorophyll c_2
P47
Cluster D
from all habitat type dinotoms
Cluster B
from tidal pool inhabitants
Cluster C
mainly from planktons
Cluster A
only from sand-dwellers

Analysis No.	Species Name	Habitat-type	Pigment																										
			Chl *c*	FLC	DT	LLC	12	15	16	20	23	26	29	30	32	33	34	35	38	39	40	41	43	44	45	46	47	48
Nitzschia-type dinotoms																													
36	*Durinskia* cf. *baltica*	Sand-dwelling	Chl *c*₂		DT					20			29	30					38								47	48
37	*Durinskia* cf. *baltica*		Chl *c*₂		DT					20									38									
38	*Durinskia* sp.	Tidal pool		FCL	DT	LLC				20																		
39	*Durinskia* sp.			FCL	DT										32						40							
40	*Durinskia* sp.			FCL	DT									30	32						40							
41	*Galeidinium rugatum*		Chl *c*₂	FCL			12	15	16	20	23	26	29	30		33	34	35	38	39		41	43	44	45	46	47	
42	Unidentified coccoid dinotom 1	Sand-dwelling	Chl *c*₂						16	20	23	26		30		33	34	35	38	39		41	43	44	45	46	47	
43	Unidentified coccoid dinotom 2		Chl *c*₂		DT				16	20			29	30					38						45	46		48
Free-living diatoms																													
46	*Cylindrotheca closterium*	Planktonic				LLC								30	32													48
47	*Nitzschia* sp.	Sand-dwelling	Chl *c*₂		DT																							
48	*Tabularia* sp.																											