Carotenoids in five aeroterrestrial strains from *Vischeria/Eustigmatos* group: updating the pigment pattern of Eustigmatophyceae

Maya Stoyneva-Gärtner\(^a\), Petya Stoykova\(^b\), Blagoy Uzunova\(^a\), Ivayla Dincheva\(^c\), Ivan Atanassov\(^d\), Petya Draganova\(^a\), Cvetanka Borisova\(^a\) and Georg Gärtner\(^e\)

\(^a\)Department of Botany, Faculty of Biology, Sofia University “St Kliment Ohridski”, Sofia, Bulgaria; \(^b\)Functional Genetics Legumes Group, AgroBioInstitute, Agricultural Academy, Sofia, Bulgaria; \(^c\)Plant Genetic Resources Group, AgroBioInstitute, Agricultural Academy, Sofia, Bulgaria; \(^d\)Molecular Genetics Group, AgroBioInstitute, Agricultural Academy, Sofia, Bulgaria; \(^e\)Institute of Botany, Faculty of Biology, University of Innsbruck, Innsbruck, Austria

**ABSTRACT**

Carotenoids have received particular attention both for their importance in algal systematics and hydrobiology and for their health benefits for humans, along with other applications in various industries. Here, we provide new data on the carotenoid content of five *Vischeria/Eustigmatos* strains isolated from aeroterrestrial habitats in Bulgaria and kept in the Algal Collection of Sofia University (ACUS). The obtained pigment pattern with nine carotenoids and generalization of literature data allowed us to update the knowledge on the chemotaxonomic characteristics of Eustigmatophyceae with a total of 47 pigments reported for the group, out of which 37 are carotenoids. The important photosynthetic pigment lutein, considered also a high-value product with extensive applications in feed, food, nutraceutical and pharmaceutical industries, was proved as a novel carotenoid for the group. The results also confirmed the presence of luteoxanthin, which is rarely reported in algae. All strains had a high content of the commercially valuable health-promoting xanthophyll astaxanthin. There were also different amounts of beta-carotene, violaxanthin, vaucheriaxanthin, canthaxanthin, zeaxanthin and antheraxanthin. Based on the differences in the pigment composition of the members of the ecological groups of aquatic and aeroterrestrial species, we propose the existence of two main pigment types in the class Eustigmatophyceae, with the aquatic type further divided into freshwater and marine pigment subtypes.

**INTRODUCTION**

Carotenoids are natural compounds, commonly known as plant pigments responsible for red, yellow and orange colours of fruits and vegetables. They are isoprenoids and contain a 40-carbon polyene chain backbone with cyclic groups \[1,2\]. Carotenoids are typically divided into two types: carotenes, or hydrocarbon carotenoids, which only have carbon and hydrogen atoms (e.g. \(\alpha\)-carotene, \(\beta\)-carotene), and xanthophylls, which are oxidized carotenoid derivatives. In them, oxygen may be present as OH groups (e.g. lutein), as oxo-groups (e.g. canthaxanthin), or in a combination of both (e.g. astaxanthin) \[3\].

Besides in plants, carotenoids are widely distributed in photosynthetic bacteria, algae, archaea, fungi and animals \[2,4,5\]. Due to their photochemical properties \[6\] carotenoids play an important role in plant and algal photosynthesis and, being specifically distributed in algal classes, have been successfully used in chemosystematics \[7–10\]. As a result of these chemotaxonomic implications, at present, an unknown microalga may be assigned to a particular algal class or phylum on the basis of its detailed carotenoid composition. The extensive use of carotenoids in phylogenetic and taxonomic studies of photosynthetic organisms is easily explainable with their wide distribution, structure variability and relatively easy identification \[11\]. Since the end of the twentieth-century and the beginning of the twenty-first century, carotenoids, together with other photosynthetic pigments, have been widely applied in high-performance liquid chromatography (HPLC)-identification and quantification of aquatic algae \[12–15\]. In addition to their importance for algal systematics and hydrobiology, carotenoids are strong
antioxidants, scavenging agents and compounds essential to vision, growth and development. Carotenoids have strong health benefits for humans in decreasing the risk of cancer, stimulating the immune system, cardiovascular disease prevention, antiadipetric and antiobesity effects, skin health, neuroprotective effects, positive impact on pregnancy and lactation, etc. [16–22]. Carotenoids have also received particular attention for their applications in food and drinks, aquaculture, cosmetic and pharmaceutical industries as colourants and feed additives [23].

Owing to the increasing demand, these health-promoting compounds logically have become the focus of many studies related to human health and biotechnologies. Nowadays more than 750 structurally defined carotenoids are reported from nature [4,5]; out of these, about 200 from algal sources [24]. Nevertheless, in spite of general recognition of main pigment patterns in microalgal phyla (divisions) and classes, and the growing interest in the exploitation of novel sources of commercially valuable algal carotenoids [25], there are virtually no detailed analyses for the vast majority of freshwater and aeroterrestrial species [26]. The small monophyletic class Eustigmatophyceae of peculiar phototrophic eukaryotes which contain only chlorophyll a and carotenoids as photosynthetic pigments, is not an exception although some of its members have been recognized as important biotechnological sources, sparking a renewed interest in this group. For details, see [27]. However, due to lack of a recent monograph, all information is scattered in different sources and is difficult to track down, especially in cases of controversial pigment data.

The aim of the present study was to provide new data on the carotenoid content of five strains of the Vischeria/Eustigmatos group, isolated from aeroterrestrial habitats of Bulgaria and kept in the Algal Collection of Sofia University (ACUS) [28] under the following accession numbers: ACUS 00002 [KD]; ACUS 00010, ACUS 00024 and ACUS 00025 [PM]; and ACUS 00104 [BS]. To grow sufficient biomass for pigment analyses, the strains were incubated in a bottle-type bioreactor with stirring and aeration (controlled aeration rate and CO₂ concentration) in liquid BBM. This was done in the cultivation room of AgroBiologInstitute (ABI) of Bulgarian Agriculture Academy at 22–23 °C and a 16/8 h light/dark photoperiod with cool white fluorescent lamps.

Identification of carotenoids
Carotenoids were identified by liquid chromatography/electrospray ionization tandem mass spectrometry (LC-ESI-MS) analysis [35,36] with high-performance liquid chromatography (HPLC). The extracts were diluted 1:6 in 50% methanol, 0.1% formic acid. Two microliters of sample were introduced into the electrospray ionization (ESI) source of the mass-spectrometer (Thermo Scientific™ Orbitrap Elite™ Hybrid Ion Trap-Orbitrap) via direct infusion at a speed of the mobile phase (50% methanol, 0.1% formic acid) of 5 s min⁻¹. Full MS spectra were collected in the FT mode in a normal mass range from 500 to 650 m/z (mass-to-charge) range at a resolution of 30,000 and 50 ms ion injection time. The ion source was operated at 2.5 kV ion spray voltage and capillary temperature of 250 °C. In the MS/MS analysis, precursor ions were selected with an isolation window of 1 and subjected to CID at either 20 V or 40 V. Fragment spectra (MS/MS) were collected at the same settings as described for MS except that the resolution was increased to 60,000. Data were processed using Xcalibur 2.2 SP1.48 software (Thermo Fisher Scientific Inc., Waltham, MA).

Identification and peak assignment of carotenoids were based on the comparison of their retention times, full scan mass spectra, and MS/MS fragmentation patterns with those of authentic standards analyzed under
identical conditions [37–48]. Transitions to distinguish between compounds were monitored considering the m/z value of protonated molecules as precursor ions, and the m/z value of the product ions, which produce a fingerprint pattern specific to the compound under investigation. Mass spectral data were standardly collected in a positive ionization mode.

Results and discussion

Carotenoid composition

The carotenoid composition in the pigment extracts from the algal cells of the five examined aeroterrestrial strains of Vischeria/Eustigmatos group are presented in Table 1. The compounds are listed in the order of the observed sequence of chromatographic peaks, which reflects the decreasing polarity of eluted components.

Fragmentation of the pigment with m/z 617 [M + H]^+ yielded dehydrated product ion at m/z 599 [M + H-H2O]^+. The mass spectrum at m/z 601 and MS2 fragmentation at m/z 583 and was identified as violaxanthin [39]. The mass spectrum displayed fragments at m/z 583 [M + H-H2O]^+, 565 [M+H-18-18H]^+, which corresponded to the loss of one water molecule and two water molecules respectively, 509 [M + H-92]^+, 491 [M + H-18-18H]^+, 352, 221, 181. The ion at m/z 221 corresponds to the oxo-ring fused to the 3-hydroxy-β-ring. This fragment is produced by cleavage between carbons 10 and 11. The fragments at m/z 352, 221 and 181 indicated epoxy substituents located in β-rings with hydroxyl groups, with cleavage between C12 and C13, C10 and C11, and C8 and C9, respectively. Compound (3) with a [M + H]^+ at m/z 601 was identified as luteoxanthin. This 5,8-epoxy xanthophyll was characterized by the major product ion at m/z 583 [M + H-H2O]^+ and at m/z 221, resulting from the cleavage of C10-C11 bond in the polyene chain from the epoxy end group. The diagnostic ion m/z 221 indicated the presence of an epoxy substituent in a β-ring with a hydroxyl group [39].

The mass spectra of 5,6-epoxyxanthophylls were very similar to those of 5,8-epoxyxanthophylls: the molecular ions and protonated molecules, and the fragmentation profile were the same. The mass spectra of compound 4, astaxanthin, obtained in the positive ion mode showed the protonated molecule at m/z 597 [M + H]^+ and fragment ions in the MS/MS at m/z 579 [M + H-H2O]^+ and m/z 561 [M+H-18-18H]^+, corresponding to the loss of one and two hydroxyl groups, respectively [40], and a fragment at m/z 505 [M + H-H2O]^+ and fragment ions MS2 at m/z 537 and fragment ions MS2 (m/z 444, 333, 177), and was referred as β-carotene [48].

Analysis of literature data on the pigment composition of Eustigmatophyceae

Eustigmatophyceae is a small monophyletic class (sometimes ranked at a phylum level – Eustigmatophyta [49–54]) in the yellow-brown (ochrophyte) evolutionary line of algae with different flagella in the large phylum Heterokontophyta, or Ochrophyta [27,55–61], or in the
Table 1. HPLC-ESI<sup>+−</sup>-MS/MS characteristics of carotenoids in aeroterrestrial eustigmatophycean strains of *Vischeria/Eustigmatos* group, isolated from soil in the gorge Kresnensko defile (KD), from alpine soils in Pirin Mt (PM) and from the rock surface of Belogradchishki skali (BS) in Bulgaria.

| No. | Pigment | Formula | RT | Parent ion (m/z) | MS<sup>2</sup> product ions (m/z) | ACUS 00002 (KD) | ACUS 00010 (PM) | ACUS 00024 (PM) | ACUS 00025 (PM) | ACUS 00104 (BS) |
|-----|---------|---------|----|-----------------|----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1   | Vaucherixanthin<sup>b</sup> | C<sub>40</sub>H<sub>56</sub>O<sub>3</sub> | 2.52 | [M + H]<sup>+</sup> 617.4631 | [M + H-18]<sup>+</sup> 599.4139, [M + H-18-18]<sup>+</sup> 581.4149 | 7 | 8 | 8 | 7 | 3 |
| 2   | Violaxanthin<sup>c</sup> | C<sub>40</sub>H<sub>56</sub>O<sub>4</sub> | 2.61 | [M + H]<sup>+</sup> 601.4190 | [M + H-18]<sup>+</sup> 583.4164, [M + H-18-18]<sup>+</sup> 565.4041, [M + H-92]<sup>+</sup> 509.3975, [M + H-18-92]<sup>+</sup> 491.4027, 221.1536 | nd | nd | nd | 32 | 15 |
| 3   | Luteoxanthin<sup>b</sup> | C<sub>40</sub>H<sub>52</sub>O<sub>4</sub> | 2.97 | [M + H]<sup>+</sup> 601.4758 | [M + H-18]<sup>+</sup> 583.4136, 221.1819, 181.1663 | 13 | 15 | 14 | nd | nd |
| 4   | Astaxanthin<sup>b</sup> | C<sub>40</sub>H<sub>52</sub>O<sub>4</sub> | 3.28 | [M + H]<sup>+</sup> 597.3939 | [M + H-18]<sup>+</sup> 579.4391, [M + H-18-18]<sup>+</sup> 561.4243, [M + H-92]<sup>+</sup> 505.4028 | 13 | 6 | 6 | 5 | 9 |
| 5   | Antheraxanthin<sup>c</sup> | C<sub>40</sub>H<sub>56</sub>O<sub>3</sub> | 3.31 | [M + H]<sup>+</sup> 585.4139 | [M + H-18]<sup>+</sup> 567.4202, [M + H-18-18]<sup>+</sup> 549.4096, [M + H-92]<sup>+</sup> 505.4028 | 16 | 12 | 15 | 19 | 19 |
| 6   | Lutein<sup>b</sup> | C<sub>40</sub>H<sub>56</sub>O<sub>2</sub> | 3.55 | [M + H]<sup>+</sup> 569.4257 | [M + H-18]<sup>+</sup> 551.4166, [M + H-18-18]<sup>+</sup> 533.4137, [M + H-92]<sup>+</sup> 477.4032, [M + H-106]<sup>+</sup> 463.3975, [M + H-153]<sup>+</sup> 416.4017 | 15 | 17 | 19 | 25 | 13 |
| 7   | Zeaxanthin<sup>a</sup> | C<sub>40</sub>H<sub>56</sub>O<sub>2</sub> | 3.76 | [M + H]<sup>+</sup> 569.4173 | [M + H-18]<sup>+</sup> 551.4072, [M + H-18-18]<sup>+</sup> 533.4019, 463.4103 | 8 | 10 | 9 | 8 | 11 |
| 8   | Cantaxanthin<sup>b</sup> | C<sub>40</sub>H<sub>52</sub>O<sub>2</sub> | 3.89 | [M + H]<sup>+</sup> 565.4008 | [M + H-18]<sup>+</sup> 547.4733, [M + H-92]<sup>+</sup> 473.4114, 363.3941, 203.3891 | 4 | 3 | nd | 4 | 7 |
| 9   | β-Carotene<sup>c</sup> | C<sub>40</sub>H<sub>56</sub> | 13.98 | [M + H]<sup>+</sup> 537.3735 | [M + H-92]<sup>+</sup> 444.3885 | 24 | 29 | 29 | nd | 23 |

<sup>a</sup>The numbers in the first column reflect the order of the chromatographic peaks. RT, retention time, values for each strain are presented as per cent from the total carotenoid content.

<sup>b</sup>Identification by comparison with literature data.

<sup>c</sup>Identification by comparison with MS spectrum of the standard compound.
Kingdom Chromista of Cavalier-Smith [62,63]. Due to the peculiar, straw-like structure of flagellar hairs, eustigmatophyceans are assigned also to the broader group of organisms – stramenopiles [64], as they were informally named by Patterson [65]. Forty years after the description, the class contained about 10 genera and 18 species [66], but more recently it was enriched by new genera and a clade [67–71], thus recognized as accommodating about 15 genera and 30 species [27] with a trend for increase in the members [27,59].

**Chlorophylls in Eustigmatophyceae**

Class Eustigmatophyceae has a unique combination of photosynthetic pigments, notably primarily for the absence of secondary chlorophylls b and c, and lack of the xanthophyll fucoxanthin, typical of most classes in the ochrophyte line of algal evolution [19]. The lack of c-type chlorophylls (or their precursors chlorophyllides c) was amply underlined as one of the main peculiarities of the class [72], by which it is similar to the relatively recently discovered heterokont classis Aurearenophyceae [73]. Recently, small amounts of chlorophyll c (ca. 0.18% of the dry weight) were found spectrophotometrically in a strain of the freshwater eustigmatophycean species *Trachydiscus minutus* (Bourrelly) Ettl. [74], which in the authors’ opinion, needs to be confirmed chromatographically.

Detailed discussion on chlorophyll types and their content in Eustigmatophyceae is beyond the scope of the present study, although we would like to remind about the opinion that it is possible to find small amounts of chlorophyll c in the group [54]. This is noteworthy, due to the immense role of David Hibberd as one of the two outstanding experts who discovered the peculiarities of the group of eustigmatophycean algae and advocated their separation from other algal classes and phyla, and from yellow-green algae (class Xanthophyceae = Tribophyceae) in particular [75–80]. Similarly, possible presence of c-type chlorophyll was indicated for all eustigmatophycean algae, which were fondly called eustigs [51]. A summary of chlorophylls and their derivatives identified in research papers is provided in Table 2.

**Carotenoids in Eustigmatophyceae**

Following [81], the presence of chlorophyll a in combination with violaxanthin as major xanthophyll was highlighted as ‘an important diagnostic character which is significant enough to be detected even in the total spectrum of extracted pigments’ [54, p. 327]. The same two pigments were also outlined as indicative of the group also in [52,53]. Violaxanthin accounted for up to 60% of the carotenoid composition of eustigs in a study which discovered its light-harvesting (LH) function [82]. A subsequent study reported that violaxanthin apparently did not play the LH role in other phosynthetic organisms [55]. However, another report indicated that violaxanthin-Chl a/c1/c2-protein complex could act as a second LH complex which occurred in lesser amounts to the major fucoxanthin-Chl a/c1-protein LH in some brown algae [83]. Thus, violaxanthin, which was already known from higher plants and some other algal classes [84], became broadly accepted as the main accessory pigment of Eustigmatophyceae (which absorbs light to drive photosynthesis in addition to chlorophyll [85]). The predominance of violaxanthin in the members of the class was underlined also in [86,87]. Later, the eustigmatophycean violaxanthin-chlorophyll α-protein complex was proved as the first example of an LH complex binding only non-carbonyl carotenoids with carotenoid-to-chlorophyll energy transfer efficiency of over 90% [88]. The earlier evidence accumulated in [89,90] indicated also the unique primary structure of the eustigmatophycean LH apoprotein, which differed from that of analogous proteins from non-green algae, green algae or higher plants. More recently, the high violaxanthin content and very low chl a-to-violaxanthin ratio were used as markers of Eustigmatophyceae in HPLC studies [91]. In addition, violaxanthin as a dominant xanthophyll could play an important role in the photoprotective xanthophyll cycle of eustigmatophyceans [27,92–94]. This cycle appears to be an important protection mechanism of plant cells from photooxidative cell damage [95,96]. Only a few studies do not report violaxanthin in the unsaponified pigment extract of *Vischeria* sp. [97,98] and there is one report of small amounts of violaxanthin in the bizarre eustigmatophycean alga *Pseudostaurastrum limneticum*, which has no stigma in the zoospores in contrast to other members of the group [99].

Besides violaxanthin, other important photosynthetic pigments in Eustigmatophyceae are beta-carotene and vaucherixanthin (in a free form and/or in an ester form) [25,67,82,88,89,92,99–123] – Table 2. Lubián and Montero [92] highlighted the high content of beta-carotene, accounting for over 60% of total carotenoids, in *Vischeria helvetica* but did not even mention vaucherixanthin for the studied strain (Table 2). It was supposed that similar to the green algae of genus *Dunaliella*, rich in beta-carotene, eustigs synthesized more beta-carotene to protect
## Table 2. Photosynthetic pigments reported in specialized papers on photosynthetic pigments of aeroterrestrial, freshwater and marine Eustigmatophyceae.

| Taxon/Ecological group | Photosynthetic pigments | NP | References |
|------------------------|--------------------------|----|------------|
| **Aeroterrestrial algae** |                          |    |            |
| Eustigmatos magnus (J. B. Petersen) D. J. Hibberd (Syn. Pleurochloris magnus J. B. Petersen; incl. strain 860-2) | Chl a; beta-carotene; AnX, AsX, CaX, CrX, ME, LuX, ME, NX, NX L, VNax E, VaX E, ViX, ZeX, Other/minor | 18 | 7: [25, 81, 100, 101, 103, 134], TS |
| Eustigmatos polyphem (Pitschmann) D. J. Hibberd | Chl a; beta-carotene; AnX, LuX, VIX, VaX, ZeX, Other/minor | 8 | 2: [25, 122] |
| Eustigmatos vischeri D. J. Hibberd | Chl a; beta-carotene; AnX, LuX, VIX, VaX, ZeX, Other/minor | 8 | 1: [25] |
| Monodus subterraneus Boye-Petersen (incl. strain 848/1) | Chl a; Unidentified chlorophyll; beta-carotene; AnX, AuX, CaX, CrX, ME, LuX, NX, NX L, V-Nax E U (II), V-Nax E V (II), N-Vax E U (II), N-Vax E V (II), NX L, NX, Vax E, VIX, ZeX, Other/minor | 22 | 4: [81, 100–102] |
| Pleurochloris commutata Pascher (incl. strain 860-1) | Chl a; beta-carotene; AnX, AsX, CaX, CrX, ME, LuX, NX, NX L, V-Nax E U (II), V-Nax E V (II), N-Vax E U (II), N-Vix V (II), NX L, NX, Vax E, VIX, ZeX, Other/minor | 16 | 7: [25, 81, 93, 98, 100, 101, 139] |
| Visheria helvetica (Vischer et Pascher) D. J. Hibberd (Syn. Polyedriella helvetica Vischer et Pascher; incl. strain 861/1) | Chl a; beta-carotene; AnX, LuX, VIX, ZeX, Other/minor | 10 | 2: [25, 116] |
| Visheria punctata Vischer | Chl a; beta-carotene; AnX, LuX, Vax E, VIx, ZeX, Other/minor | 15 | 7: [25, 81, 100, 101, 123, 124], TS |
| Visheria sp. (Chodat) Pascher (incl. strain 887/2) | Chl a; beta-carotene; AnX, LuX, Vax E, ViX, Zex, Other/minor | 9 | 1: T5 |
| Visheria sp. Strain ACUS 00025 | Beta-carotene; AnX, LuX, Vax E, ViX, ZeX | 7 | 1: T5 |
| Visheria sp. Strain ACUS 00024 | Beta-carotene; AnX, LuX, Vax E, ViX, ZeX | 7 | 1: T5 |
| **Aquatic algae: freshwater** |                          |    |            |
| Botryochloropsis similis H. R. Preisig et C. Wilhelm | Chl a; beta-carotene; Vax E, ViX | 4 | 1: [115] |
| Chlorobotrys regularis (West) Bohlin | Chl a; beta-carotene; NX (?), VaX E, ViX | 5 | 1: [106] |
| Pseudostaurastrum limneticum (Borge) Cout et Rousselin | Chl a; beta-carotene; AnX, CrX, ME, Vax E (II), Vix, ZeX | 7 | 1: [99] |
| Trachydiscus minutus (Bourrelly) Ettl | Chl a; Beta-carotene; AnX, AsX, CaX, CrX, ME, LuX, NX, LX L, Vax E, Vix, ZeX, Other/minor | 9 | 6: [77, 74] |
| Eustigmatophycean strain (clone) Tunis | Beta-carotene; AnX, AsX, CaX, CrX, ME, LuX, NX L, VaX E, ViX, ZeX, Other/minor | 7 | 1: TS |
| Microchloropsis gaditana (L. M. Lubian) M.W. Fawley, I. Jameson & K.P. Fawley (Syn. Nannochloropsis gaditana L. M. Lubian) | Chl a; beta-carotene; AnX, AsX, CaX, Vax E, Vix, ZeX | 9 | 6: [92, 108–112] |
| Microchloropsis salina (D.J. Hibberd) M.W. Fawley, I. Jameson & K.P. Fawley (Syn. Monalanthus salina Bourrelly, Nannochloropsis salina) | Chl a; beta-carotene; AnX, AsX, CaX, Vax E, Vix, ZeX, Other/minor | 15 | 7: [82, 104, 107, 112, 114, 117, 118] |
| Microchloropsis salina – SCB sticho strain | Beta-carotene; AnX, AsX, CaX, Vax E, Vix, ZeX, Other/minor | 7 | 1: TS |
| Nannochloropsis granulata B. Karlsen et D. Potter | Chl a; beta-carotene derivative; CaX, Vax L, Vix | 5 | 2: [103, 134] |
| Nannochloropsis limnetica L.Krienitz, M. Hepperle, H.-B.Stich & W.Weiler (incl. three Baical strains) | Chl a; beta-carotene; AnX, AsX, CaX, Vax E, Vix, ZeX, Other/minor | 14 | 2: [119, 121] |
| Nannochloropsis oculata (Droop) J. D. Hibberd (incl. CS179) | Chl a; beta-carotene; AnX, AsX, CaX, CrX, ME, LuX, NX L, VaX E, ViX, ZeX, Other/minor | 24 | 5: [104, 107, 112, 117, 118] |
| Nannochloropsis oceanica (Suda et Miyashita) | Chl a; beta-carotene; AnX, AsX, CaX, CrX, ME, LuX, NX L, VaX E, ViX, ZeX, Other/minor | 4 | 1: [105] |
| Nannochloropsis sp. (close to N. limnetica) | Chl a; beta-carotene; AnX, AsX, CaX, CrX, ME, LuX, NX L, VaX E, ViX, ZeX, Other/minor | 8 | 2: [88, 120] |
| Nannochloropsis sp. (Strain CAR A2) | Chl a; beta-carotene; AnX, AsX, CaX, CrX, ME, LuX, NX L, VaX E, ViX, ZeX, Other/minor | 9 | 1: [82] |
| Nannochloropsis sp. | Chl a; beta-carotene; AnX, AsX, CaX, CrX, ME, LuX, NX L, VaX E, ViX, ZeX, Other/minor | 5 | 1: [89] |

Xanthophylls are listed in alphabetical order. Abbreviations used: AnX, antheraxanthin; AsX, astaxanthin; AuX, auroxanthin; AuX, auroxanthin; CaX, canthaxanthin; Chl, chlorophyll; CrX, cryptoxanthin; DE, diepoxide; DFE, difuranoepoxide; E, ester; Ech, echinenone; Ep, epimer; F, furanoid; Fe, furanoepoxide; I, isomer; KC, ketocarotenoids; L, like; LuX, luteoxanthin; ME, monoepoxide; N, neo-; NC, neochrome; NP, number of pigments; NX, neoxanthin; TS, this study; U and V in the end, or 1 and 2, identification letters or numbers given by the authors; VaX, vaucheriaxanthin; ViX, violaxanthin; ZeX, zeaxanthin.
themselves from the damage caused by ageing or environmental stresses, such as nutrient depletion and high light irradiance [25]. The amount of beta-carotene significantly varies in different studies, but its high relative concentration is commonly emphasized [97,124]. The predominance of beta-carotene over the xanthophylls firstly was outlined in [97,124] as a unique feature of the studied algae but then was confirmed for most eustigmatophycean species analyzed afterward. The highest published value showed that beta-carotene could account for more than 85% of the total carotenoid content and over 5% of the dry weight of *Eustigmatos polyphem* [25,122].

The main pigment composition (chlorophyll *a*, beta-carotene, violaxanthin and vaucheriaxanthin, free or in ester form) or at least the main accessory role of violaxanthin is repeatedly indicated for the whole class in phycological and protistan handbooks, as well as in different review papers [5,27,57–61,87,125,126]. Some references [77,126] emphasize the replacement of anteraxanthin by violaxanthin as a significant difference with yellow-green algae (Xanthophyceae) from which Eustigmatophyceae were taxonomically derived [75]. However, further studies proved the presence of both pigments (Table 2) and this is a possible explanation for the opinion that ‘minor details in pigmentation’ distinguish eustigs from yellow-green algae [127]. This was supported by statements that ‘in the pigmentation of their plastids, eustigs are indeed very much like the true yellow-green xanthophytes…’ [41, p. 198] and that in Eustigmatophyceae the ‘accessory pigment (carotenoid) composition is similar to Xanthophyceae except for the absence of heteroxanthin’ [128, p. 492]. For Xanthophyceae, the same authors mentioned that ‘the principal accessory pigments are beta-carotene, vaucheriaxanthin, dioxanxanthin, and dioxanthins may also be present’ [41, p. 198].

Violaxanthin is commonly present; epoxaxanthin, diadinoxanthin, and diatoxanthins may also be present [81,131] or had them in minute concentrations at the biosynthetic oxidation level of canthaxanthin [103,132]. After the first report of ‘ketocarotenoids’ accounting for 5% and 4% of the total carotenoid content [104], a number of studies showed the presence of canthaxanthin [82,93,105,107,109–112,118,119,121]. The content of canthaxanthin tends to increase during growth of the cultures even when violaxanthin and vaucheriaxanthan remain the major xanthophylls [107,110,111,125]. However, canthaxanthin and other ketocarotenoids never achieved ageing associated predominance in the total carotenoid mixture [107]. Parallel to the increase in ketocarotenoid amounts, the dynamics of relative pigment content in Eustigmatophyceae during growth show an increase in the total chlorophyll *a*/carotenoid ratio. This is visible as a change from the initially green colour of cultures to brown and finally bright orange, when cultures become older and nutrients are depleted [110,112,125]. The change in the amount of pigments
reflected in culture colour is considered a good indicator of physiological stage and response of pigment concentrations (and thus of the photosynthetic efficiency) to factors like light intensity and nutrient concentrations [110]. Variations in cellular pigmentation in response to growth conditions were also shown in [93,133]. Orange colouration of cultures in the process of ageing was noted in [25,122] but was explained with the increase in intracellular beta-carotene content together with the decrease in the concentrations of other carotenoids and chlorophylls. Li et al. [25] suggested that further improving of culture conditions could raise the amount of this valuable pigment. Similar gradual increase in the beta-carotene content by prolongation of the culture time resulting in its exclusive production at the end of cultivation was reported in [123].

In Eustigmatophyceae, the first documentation of \textit{in vivo} ‘production of astaxanthin (free plus monoesterified) and astacene in significant amounts, indicating the capacity of this algal type to synthesize the highest oxidation level of 4-keto carotenoids known from the algal kingdom and hitherto found only in the Chlorophyceae, Euglenophyceae and Dinophyceae...’ was in [107, p. 47]. Later other studies showed the presence of the ketocarotenoid astaxanthin in a few eustigmatophycean species [93,109,112]. Strain KGU-Y001 of \textit{Vischeria helvetica} could utilize both the photoprotection activity of the violaxanthin cycle and the antioxidant activity of astaxanthin to resist high-light stress [93]. Aburai et al. [93] believed that its pathway for astaxanthin biosynthesis was different from those known for green algae and from the six eustigs (incl. \textit{V. helvetica}) investigated in [25]. The ‘unknown keto-carotenoids A, B, C and D’ were detected and were suggested to be intermediates in the biosynthesis of astaxanthin and its ester [107]. In addition, Ishida and Hara [143] reported the ‘unknown ketocarotenoid E’ with a possible relationship with astacene (Table 2).

Echinonene is an intermediate in the biosynthesis of canthaxanthin. It was found in \textit{Nannochloropsis oculata} and \textit{Microchloropsis salina} but was obtained in too small a quantity to be identified with certainty, and its spectrum suggested that it could be a cis-isomer [107]. Despite missing from previous lists, the pigment was expected by the same authors to occur in the strains investigated earlier [103,126], which contained low amounts of canthaxanthin. Echinonene was reported much later in \textit{Nannochloropsis limnetica} and in its three Baltic strains [119,121] – Table 2.

Epoxycarotenoids – xanthophylls like antheraxanthin, neoxanthin and zeaxanthin – as minor components in Eustigmatophyceae were first proved in [102] – Table 2. Another study [134] found a monoepoxide and a diepoxide, but the used chromatographic technique could not fully resolve the xanthophylls (Table 2). Afterward, the reporting of zeaxanthin and antheraxanthin could be traced back to [25,92,107,112,122] – Table 2. Some records indicate only zeaxanthin [67,81,82,99,117–121], and in addition, a zeaxanthin-like pigment [119]. Zeaxanthin, inseparable from lutein, was listed in [93]. Antheraxanthin was reported in [98,102] and in [107], where its furanoid form was also found, and in [114, p. 437] with a note that the ‘experimental procedure did not allow a distinction between vaucheriaxanthin and antheraxanthin’. Later, antheraxanthin was found in [25,92,112,116] – Table 2. The presence of zeaxanthin in algae which contain violaxanthin was underlined in [102]. Considering the LH role of violaxanthin [82], Stransky and Hager [102,135] suggested that there is a light-induced de-epoxidation cycle between violaxanthin, antheraxanthin and zeaxanthin in eustigmatophyceans. This cycle, named \textit{Viola cycle}, is similar to that in the majority of land plants, brown and green algae and some species of red algae [14,136–138]. The same interconversion between violaxanthin, antheraxanthin and zeaxanthin in the xanthophyll cycle was later confirmed for eustigs [101]. Experimental evidence demonstrated that it operates under high-irradiance–induced stress conditions, showing that under proper conditions of high irradiance (2000 \(\mu\)mol m\(^{-2}\) s\(^{-1}\)) and temperature (40 °C), up to 78% of violaxanthin was de-epoxidated in a short time-interval to render zeaxanthin [92]. In subsequent experiments [112], the violaxanthin content was restored (without any cell damage) after 24 hours exposure to dim light and lower temperatures (25 °C). The same study also proved the major role of violaxanthin and vaucheriaxanthin with dynamics in their content in culture conditions: the decrease in violaxanthin correlated with the increase in zeaxanthin and antheraxanthin, while the decrease in vaucheriaxanthin correlated with the increase in the ketocarotenoids canthaxanthin and astaxanthin. An earlier report demonstrated the combined effect of light intensity and nitrate concentration on the cellular content of canthaxanthin in eustigs [111].

The epoxycarotenoid neoxanthin (allenic epoxide), an intermediate produced from violaxanthin, is rarely reported in Eustigmatophyceae [67,100–102,104,107,114,118,139] – Table 2. A study mentions 9-cis-neoxanthin and esterified neoxanthin among the ‘other carotenoids’ without more explanations [25].
Neoxanthin-like xanthophylls are indicated only in a few reports [81,100,101,116] – Table 2.

Another epoxycarotenoid, the xanthophyll luteoxanthin, is even less documented in pigment studies (Table 2), always reported as a minor pigment [25,81,82,107,122]. It was called ‘anomalous (half epoxide, half furanoid)’ pigment derived from its ‘diepoxide parent, violaxanthin’ [107, p. 46]. The furanised luteoxanthin – auroxanthin – was recorded as a minor pigment only in *Monodus subterraneus* and *Pleurochloris commutata* [81] – Table 2. A minor xanthophyll supposed to be cryptoxanthin was found in *Vischeria punctata* [98] but was not confirmed due to trace amounts available and ‘possibly cryptoxanthin’ was indicated for *V. stellata* [81], while other studies detected cryptoxanthin epoxides [81,100,101,107] – Table 2. Neochrome has been reported only in *Nannochloropsis oculata* [107] – Table 2.

Whittle [106] reported that almost all eustigmatophycean species contained beta-carotene, whereas alpha-carotene was detected only in the authentic strain of *Microchloris gaditana* and served as a biochemical ground for distinguishing from *Nannochloropsis* species (Table 2). Afterward however, alpha-carotene was not discovered in other strains of the same species [120].

In addition to the pigments certainly identified in Eustigmatophyceae, different studies have reported some other minor carotenoids (Table 2).Such were generally considered to be of minor importance [5,14,130,140], was found in all five strains included in alphabetical order, are antheraxanthin, astaxanthin, beta-carotene, canthaxanthin, vaucheriaxanthin, vaucheriauxanthinester, violaxanthin and zeaxanthin, in addition to the main chlorophyll *a*, as well as some unidentified minor pigments and possibly neoxanthin (which was proved in aeroterrestrial algae, but was only speculated to occur in the aquatic forms) – Table 3. However, even these common pigments, were not found in all studied species (Table 2) and have been recorded in different amounts. So far, auroxanthin, lutein, luteoxanthin, cryptoxanthin, cryptoxanthin monoeoxide, neo-violaxanthin isomers U and V, neoxanthin-like xanthophyll as well as three isomers of neo-vaucheriaxanthin esters and two unknown epoxides together with phaeophytin *a* and unidentified chlorophyll have been found only in aeroterrestrial forms (Table 3). Only aquatic species reportedly contain alpha-carotene, furanoid form of antheraxanthin, astacene, astaxanthinester, canthaxanthin-like xanthophyll, cryptoxanthin furaneoxide and cryptoxanthin difuranooepoxide, echinenone, violaxanthin-like pigments 1 and 2, neochrome, isomer of vaucheriaxanthin ester, vaucheriaxanthin-like and zeaxanthin-like xanthophylls, as well as some other minor carotenoids together with various forms of chlorophyll *a* (derivatives, epimers, etc.) – Table 3. Similar to the pigments in aeroterrestrial species, those in different aquatic algae have various distribution (Table 2) in different amounts. Moreover, in aquatic algae, the number of pigments found in the marine species (30) is higher, in contrast to fewer pigments (10) reported in the freshwater eustigs (Table 3). The only pigments common for these two ecological subgroups are beta-carotene, canthaxanthin, zeaxanthin, vaucheriaxanthin, vaucheriauxanthinester and violaxanthin (Table 3).

The results obtained in this study generally revealed that: (1) vaucheriaxanthin, although present in all studied strains, was not their major xanthophyll; (2) beta-carotene and violanxanthin, which were commonly accepted as the major photosynthetic pigments in combination with chlorophyll *a*, were with different distribution in the members of Eustigmatophyceae (Table 1). The minor role of beta-carotene was briefly noted also in [120] and the decrease in alpha + beta-carotene content during the ageing of cultures was shown in [110].

By contrast, the ketocarotenoid astaxanthin, which has been quite rarely reported before for marine Eustigmatophyceae (Table 2) and was assigned mainly to green algae, as well as to a few dinoflagellate species and to some cyanoprokaryotes/cyanobacteria [5,14,130,140], was found in all five strains included in

**General pigment pattern of Eustigmatophyceae**

The qualitative pigment pattern of Eustigmatophyceae generalized on the basis of reviewed literature data and our results, is presented in Table 3.

The accumulated data on the pigment composition in Eustigmatophyceae, as summarized in Tables 1–3, show that out of 47 pigments reported in total (incl. the group of ‘other/minor’), 37 are carotenoids. The highest number (34) comes from studies on aquatic (mostly marine and rarely freshwater) species. Fewer pigments (25) are known from the studies on the aeroterrestrial representatives of the group. The pigments common to both ecological groups, in
Astaxanthin accounted for 5–6% of their total carotenoid content, but in two of the strains it reached 9% and 13% (Table 1). Considering the broad usage of this lipid-soluble pigment as a safe dietary supplement [141], its detection in our aeroterrestrial strains could be of commercial interest. Our finding is in conformity with previous reports of its presence in a strain of the aerophytic *Vischeria helvetica* [93].

Another ketocarotenoid, canthaxanthin, was detected in four of our strains but always in lower amounts than astaxanthin (Table 1). Canthaxanthin is also recorded as a minor or trace pigment in some
species of cyanoprokaryotes, dinoflagellates, diatoms, chlorophytes and prymnesiophytes [24]. Viologlanxin shows oscillating dynamics in its content and can convert reversibly into astaxanthin and canthaxanthin during the algal growth [92,102,111,112]. Hence, we believe that our results are in agreement with these findings, not necessarily indicating a minor role of violaxanthin. There is need for further more detailed studies to clarify the reason for these observations.

In our study, we were not able to identify echinone, the carotenoid intermediate of the biosynthesis of canthaxanthin. This pigment, discovered so far only in three aquatic eustigmatophycean species (Table 2), had been considered more typical of prokaryotic algae (phylum Cyanoprokarya) and quite rare in eukar-
yotes, where, besides in eustigs, it was found in a few species of the phyla Euglenophyta, Haptophyta and Pyrrhophyta [14,24]. The results from our analysis did not show the presence of some other carotenoids reported rarely by earlier authors, like alpha-carotene, neoxanthin, cryptoxanthin and its esters, auroxanthin, neochrome, astacene or intermediates of astaxanthin and its ester (Tables 1 and 2).

Another rarely reported xanthophyll, the epoxycarotenoid luteoxanthin, was found in a relatively high content (13–15%) in three of the five studied strains (Tables 1 and 2). These findings confirm its previous records in marine and aeroterrestrial eustigmatophy-
cean species [25,81,82,107,122]. This is noteworthy, since luteoxanthin has been extremely rarely reported from algae at all (e.g. chlorophyte Dunaliella tertiolecta) with a note in [24] that it could undergo rearrange-
ment with violaxanthin in weak acid solutions.

Two other epoxycarotenoids, antheraxanthin and zeaxanthin, known as minor xanthophylls in some eustigmatophyceans (Table 2), were found in all the strains studied by us, with constant higher amounts of antheraxanthin (Table 1). The relative amount of antheraxanthin in the total carotenoid content in our strains was considerably higher in comparison with its content in other strains of the same genera obtained in [25]. Antheraxanthin is involved in the Viola xantho-
phyll cycle typical of land plants, brown and green algae [137]. It is known as one of the minor pigments in the small peculiar phylum Chlorarachniophyta with plastids derived from green algae [e.g. 61] and in some red and golden algae as well [14,24]. Zeaxanthin is commonly synthesized in higher plants. It was long believed to be a relatively rare xanthophyll in algae, generally referred to their prokaryotic counterparts (Cyanoprokaryota) as one of the dominant pigments, even among the prokaryotes containing chlorophyll b (prochlorophytes) [14,24,58] and in the few cyanoprokaryotes with chlorophyll f [130]. However, zeaxanthin is also found as important pigment in the eukaryotic phyla Glaucocystophyta, Rhodophyta, Pyrrhophyta (=Dinophyta), Chlorarachniophyta, in the green algal classes Chlorophyceae and Prasinophyceae, and as being ubiquitously spread in the ochrophyte classes Dictyochophyceae, Pinguiophyceae, Pelagophyceae, Phaeophyceae and Raphidophyceae but disjunctively in some other ochrophytes from the classes Bacillariophyceae and Chrysophyceae [14,130]. It occurs in the novel ochrophyte class Aurearenophyceae [70,130]. Once zeaxanthin was indicated even as a dominant pigment in Eustigmatophyceae, as well as in ‘cyanobacteria, chrysophytes, pelagophytes, rhodo-
phytes and dinoflagellates Pigment Type 5; minor in pinguioths, raphidophytes, chlorarachniophytes, chlorophytes, prasinophytes and trebouxioths; and occasional in dictyochophytes and dinoflagellates Pigment Type 3’ [24, p. 810]. Later however, the same author placed zeaxanthin among the accompanying pigments of eustigs [130].

Zeaxanthin has an isomer lutein, which differs just by the shift of a single double bond [2]. Similarly to zeax-
anthin, lutein was normally assigned to higher plants and was thought to be less commonly distributed among algae [4,84,114], where it was usually considered the most important carotenoid of the eukaryotic green algae [14,21,51,55,61,83,87,102,103,130,142]. Lutein has been found also in the phyla Chlorarachniophyta [14,55,56,143,144] and Rhodophyta [14,21,51,55,61,130,145,146]. In Rhodophyta, most algae contain either zeaxanthin or lutein as the sole dihydroxy-xanthophyll [14]. Egeland [130] summarized the occurrence of lutein in dinoflagellates with endosymbiotic origin from green algae. The presence of lutein in Xanthophyceae (in ‘Tribronom aequale, Heterococcus fuoreresis and Ophiocytium majus’) was underlined in [97] as discovered by Jamikorn [147] but, to our knowledge, was not even discussed in the later works. Besides this finding, in the large ochrophyte/heterokont algal evolutionary line, lutein was detected in the marine silicoflagellates, or Dictyochophyceae s.str. [55] and was assigned to the class Raphidophyceae as lutein or lutein monooxide [148,149]. These two groups, despite possessing chloro-
phyll c, were thought to be relatively close phylogeneti-
cally to the Eustigmatophyceae [57,58,61,87,126,134,150–154]. According to [98], yellow-green algae (to which eustigs belonged at that time before being assigned as a novel class) could synthesize lutein epox-
ide. This ability however, was mostly ignored in later eustigs studies. To date, lutein is not indicated in the
pigment studies of Eustigmatophyceae except its first mentioning in [25], where it was included as a table footnote with four more pigments among the ‘other carotenoids’ without any explanation, and its later inseparable inclusion together with zeaxanthin in [93], also without discussion. The lack of lutein in the studied eustigs was underlined in [97,102,113,114] and was proposed as an explanation for the major role of violaxanthin [114]. In our study, lutein was detected in all investigated strains of the Vischeria/Eustigmatos group (Table 1). Moreover, in all of them it accounted for a considerable part (13–25%) to the total carotenoid content. This shows that it is necessary to update Eustigmatophyceae in a chemotaxonomical aspect. It also shows that the isolated strains could be further explored as potential commercial sources of lutein, which is a high-value product with extensive applications in feed, food, nutraceutical and pharmaceutical industries [21].

The updated general pigment pattern of Eustigmatophyceae (Table 3) shows the chemotaxonomic diversity of carotenoids found in the class. However, no pigment was commonly found in all studied strains (Table 2). Naturally, the knowledge on the photosynthetic pigments and their diversity have generally increased with the advancement of analytical techniques and the accumulation of more intensive studies on a given species (Tables 2 and 3). The results obtained by us allow to retool the general eustigmatophycean pigment pattern by proving the presence of the important pigment lutein and of luteoxanthin, which is rarely reported from algae. The data generalized in Tables 2 and 3 open a discussion of the differences in the carotenoid composition among the ecological groups of aeroterrestrial and aquatic algae, and even of the difference between freshwater and marine species. The pigment distribution strongly suggests the possibility to propose at least two main pigment types in Eustigmatophyceae (with further division of aquatic type into two subtypes) following the models accepted for other algal groups (e.g. cyanoprokaryotes with five pigment types (PT), hapto-phytes with eight PT, pyrrophophytes with five PT, chlorophytes with two PT and prasinophytes with five PT) [14,130,155,156]. The pigment pattern of each type/subtype provided in Table 3 is logically explainable with differences in the light composition and intensity in the habitats. However, since the analytical methods applied up to now have different resolution and the cellular pigmentation varies in response to growth conditions [107,110,111,132], there is need of further more unified quantitative studies with more taxa investigated from all ecological groups to prove the two proposed PT in Eustigmatophyceae. At present, besides chlorophyll a, only beta-carotene, canthaxanthin, zeaxanthin, vaucher ixanthin, vaucher ixanthin ester and violaxanthin could be tentatively outlined as common for eustigs from all ecological groups, despite the strong variability in their quantities.

At the same time, all accumulated data in our study and previous ones point to the need of further analyses of these pigments and their eustigmatophycean bearers as potential valuable natural sources due to their high antioxidant capacity and high scavenging activity [157]. Our results also support the opinion of Lubián et al. [112], who described Eustigmatophyceae as novel sources of valuable pigments related both to capacity for single pigment accumulation and to the availability of a broad range of carotenoids. Last but not least, we would like to stress the importance of further studies on eustigs regarding the trend towards increasing their number of members [27,59]. Another direction of future research is based on the fundamental particular roles that algal pigments play in the process of photosynthesis and photoprotection thereby determining algal biodiversity under a changing environmental and climatic scenario, and in the associated biogeochemical cycles [10,158].

**Conclusions**

The results from our study, with nine identified carotenoids, together with the generalization of literature data, allowed us to update the knowledge on the pigment pattern of Eustigmatophyceae with a total of 47 pigments, of which 37 are carotenoids. Lutein was proved as a novel pigment for this class. Based on the differences in the pigment composition of the members of the ecological groups of aquatic and aeroterrestrial species, we propose the existence of two main pigment types in the class Eustigmatophyceae, with the aquatic type further divided into freshwater and marine pigment subtypes. Besides this chemotaxonomic result, our data confirm the high relative amount and diverse combinations of commercially important pigments in the studied aeroterrestrial strains of the Vischeria/Eustigmatos group. Apart of the well-known and largely explored beta-carotene, they contain carotenoids (e.g. lutein, luteoxanthin, astaxanthin, canthaxanthin) considered as high-value functional products with extensive applications in human affairs. Their finding in the originally isolated strains from protected areas in Bulgaria proves the need of further phycoprospecting in the country and future
studies on the eustigmatophycean microalgae as important commercial sources.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This work was supported by the America for Bulgaria Foundation under Grant number AGR.0050.20160121.

**References**

[1] Croteau R, Kutchan TM, Lewis NG. Natural products (secondary metabolites). In: Buchanan BB, Gruissem W, Jones RL, editors. Biochemistry and molecular biology of plants. Rockville (MD): American Society of Plant Physiologists; 2000. p. 1250–1317.

[2] Barredo J-L, editor. Microbial carotenoids from bacteria and microalgae. methods and protocols. New York (NY): Humana Press Springer; 2012.

[3] Liaaen-Jensen S. Basic carotenoid chemistry. In: Mayne ST, Krinsky NI, Sies H, editors. Carotenoids in health and disease. New York (NY): Marcel Dekker; 2004. p. 1–30.

[4] Britton G, Liaaen-Jensen S, Pfander H, editors. Carotenoids handbook. Basel (Switzerland): Birkhäuser; 2004.

[5] Takaichi S. Carotenoids in algae: distributions, biosyntheses and functions. Mar Drugs. 2011;9:1101–1118.

[6] Britton G. Structure and properties of carotenoids in relation to function. Faseb J. 1995;9:1551–1558.

[7] Björnland T, Liaaen-Jensen S. Distribution patterns of carotenoids in relation to chromophyte phylogeny and systematics. In Green JC, Leadbeater BSC, Diver WI, editors. The chromophyte algae: problems and systematics. In Green JC, Leadbeater BSC, Diver WI, editors. The chromophyte algae: problems and systematics. In Green JC, Leadbeater BSC, Diver WI, editors. The chromophyte algae: problems and systematics. In Green JC, Leadbeater BSC, Diver WI, editors. The chromophyte algae: problems and systematics. In Green JC, Leadbeater BSC, Diver WI, editors. The chromophyte algae: problems and systematics. In Green JC, Leadbeater BSC, Diver WI, editors. The chromophyte algae: problems and systematics. In Green JC, Leadbeater BSC, Diver WI, editors. The chromophyte algae: problems and systematics.

[8] Rowan KS. Photosynthetic pigments of algae. Cambridge (UK): Cambridge University Press; 1989.

[9] Liaaen-Jensen S. Carotenoids in chemosystematics. In: Britton G, Liaaen-Jensen S, Pfander H, editors. Carotenoids: biosynthesis and metabolism. Basel (Switzerland): Birkhäuser; 1998. p. 217–247.

[10] Roy S, Llewellyn CA, Egeland ES, editors. Phytoplankton pigments: characterization, chemotaxonomy and applications in oceanography. Cambridge (UK): Cambridge University Press; 2011.

[11] Ragan MA, Chapman DJ. A biochemical phylogeny of the protists. New York (NY): Academic Press; 1978.

[12] Mackey MD, Mackey DJ, Higgins HW. CHEMTAX-a program for estimating class abundances from chemical markers: application to HPLC measurements of phytoplankton. Mar Ecol Prog Ser. 1996;144;265–283.

[13] Jeffrey SW, Mantoura RFC, Wright SW. Phytoplankton pigments in oceanography. Paris (France): UNESCO Publishing; 1997.

[14] Jeffrey SW, Wright SW, Zapata M, et al. Microalgal classes and their signature pigments. In: Roy S, Llewellyn CA, Egeland ES, editors. Phytoplankton pigments: characterization, chemotaxonomy and applications in oceanography. Cambridge (UK): Cambridge University Press; 2011. p. 3–77.

[15] Descy J-P, Sarmento H, Higgins HW. Variability of phytoplankton pigment ratios across aquatic environments. Eur J Phycol. 2009;44:319–330.

[16] Paiva SA, Russell RM. Beta-carotene and other carotenoids as antioxidants. J Am Coll Nutr. 1999;18:426–433.

[17] Johnson EJ. The role of carotenoids in human health. Nutr Clin Care. 2002;5:56–65.

[18] Chew BP, Park JS. Carotenoid action on the immune response. J Nutr. 2004;134:257–261.

[19] Krinsky NI, Johnson EJ. Carotenoid actions and their relation to health and disease. Mol Aspects Med. 2005;26:459–516.

[20] Zhang J, Sun Z, Sun P, et al. Microalgal carotenoids: beneficial effects and potential in human health. Food Funct. 2014;5:413–425.

[21] Sun Z, Li T, Zhou ZG, et al. Microalgae as a source of lutein: chemistry, biosynthesis, and carotenogenesis. Adv Biochem Eng Biotechnol. 2016;153:37–58.

[22] Zieлиnska MA, Wesołowska A, Pawlus B, et al. Health effects of carotenoids during pregnancy and lactation. Nutrients. 2017;9:838.

[23] Prieto A, Pedro Canavate JP, García-González M. Assessment of carotenoid production by Dunaliella salina in different culture systems and operation regimes. J Biotechnol. 2011;151:180–185.

[24] Egeland ES. Data sheets aiding identification of phytoplankton carotenoids and chlorophylls. In: Roy S, Llewellyn CA, Egeland ES, et al., editors. Phytoplankton pigments: characterization, chemotaxonomy and applications in oceanography. Cambridge (UK): Cambridge University Press; 2011. p. 675–822.

[25] Li Z, Sun M, Li Q, et al. Profiling of carotenoids in six microalgae (Eustigmatophyceae) and assessment of their β-carotene productions in bubble column photobioreactor. Biotechnol Lett. 2012;34:2049–2053.

[26] Schagerl M, Pichler C, Donabaum K. Patterns of major photosynthetic pigments in freshwater algae. 2. Dinophyta, Euglenophyta, Chlorophyceae and Charales. Ann Limnol - Int J Lim. 2003;39:49–62.

[27] Eliás M, Amaral R, Fawley KP, et al. Eustigmatophyceae. In: Archibald JM, Simpson AJB, Slamovits CH, editors. Handbook of the protists. Berlin (Germany): Springer; 2016. p. 367–407.

[28] Petrova A, editor. VII National Botanical Conference; St Kliment Ohridski [dissertation]. Innsbruck (Austria): University of Innsbruck; 2009.

[29] Uzunov B, Stoyneva M, Mancheva A, et al. [ACUS – the new collection of living aeroterrestrial alga of the University of Sofia “St Kliment Ohridski”]. In: Petrova A, editor. VII National Botanical Conference; 2011 Sept 29–30; Sofia (Bulgaria): Bulg. Bot. Soc. 2012. p. 271–274. Bulgarian.

[30] Uzunov BA. Aeroterrestrial algae from Pirin Mountain (Bulgaria) [dissertation]. Innsbruck (Austria): University of Innsbruck; 2009.

[31] Stoyneva MP. Contribution to the knowledge on the biodiversity of hydro- and aerobiontic prokaryotic
and eukaryotic algae in Bulgaria [D.Sc. thesis]. Sofia (Bulgaria): Sofia University “St Kliment Ohridski”; 2014. Bulgarian.

[31] Gärtner G, Stoyneva MP, Mancheva AD, et al. A new method in collection and cultivation of aerophytic and endolithic algae. Ber Nat Med Verrein Innsbruck. 2010;96:27–34.

[32] Mancheva AD. [Investigation of aerophytic algae from the natural landmark Belogradchik rocks] [dissertation]. Sofia (Bulgaria): Sofia University “St Kliment Ohridski”; 2013. Bulgarian.

[33] Stoykova P, Stoyneva-Gärtner M, Draganova P, et al. Morphological characterisation and phylogenetic analysis of aeroterrestrial Vischeria/Eustigmatos strains with industrial potential. Biotechnol Biotechnol Equip. Forthcoming.

[34] Stoyneva-Gärtner MP, Isheva T, Ivanov P, et al. Red list of Bulgarian algae. II. Microalgae. Ann Sofia Univ, Book 2 – Botany 2016;100:15–55.

[35] Fenn JB, Mann M, Meng CK, et al. Electrospray ionization for mass spectrometry of large biomolecules. Science. 1989;246:64–71.

[36] Cappiello A, Famiglini G, Mangani F, et al. A simple approach for coupling liquid chromatography and electron ionization mass spectrometry. J Am Soc Mass Spectrom. 2002;13:265–273.

[37] Nitsche H. The structure of vaucheriaxanthin. Z Naturforsch C. 1973;28:641–645.

[38] Ren D, Zhang S. Separation and identification of the yellow carotenoids in Potamogeton crispus L. Food Chem. 2008;106:410–414.

[39] de Rosso VV, Mercadante AZ. Identification and quantification of carotenoids, by HPLC-PDA-MS/MS, from Amazonian fruits . J Agric Food Chem. 2007;55:5062–5072.

[40] Rivera S, Vilaro F, Canela R. Determination of carotenoids by liquid chromatography/mass spectrometry: effect of several dopants. Anal Bioanal Chem. 2011;400:1339–1346.

[41] Holtin K, Kuehnle M, Rehbein J, et al. Determination of astaxanthin and astaxanthin esters in the microalgae Haematococcus pluvialis by LC-(APCI)MS and characterization of predominant carotenoid isomers by NMR spectroscopy. Anal Bioanal Chem. 2009;395:1613–1622.

[42] van Breemen RB, Dong LL, Pajkovic ND. Atmospheric pressure chemical ionization tandem mass spectrometry of carotenoids. Int J Mass Spectrom. 2012;312:163–172.

[43] Crupi P, Milella RA, Antonacci D. Simultaneous HPLC-DAD-MS (ESI+) determination of structural and geometrical isomers of carotenoids in mature grapes. J Mass Spectrom. 2010;45:971–980.

[44] Rivera SM, Canela-Garayoa R. Analytical tools for the analysis of carotenoids in diverse materials. J Chromatogr A. 2012;1224:1–10.

[45] de Azevedo-Meleiro CH, Rodriguez-Amaya DB. Qualitative and quantitative differences in the carotenoid composition of yellow and red peppers determined by HPLC-DAD-MS. J Sep Sci. 2009;32:3652–3658.

[46] Neto FC, Guaratini T, Colepicolo P, et al. Effect of charge generation in ESI source on the neutral aromatic elimination mechanism in xanthophylls. Revista Semioses. 2016;10:70–76.

[47] Rivera SM, Christou P, Canela-Garayoa R. Identification of carotenoids using mass spectrometry. Mass Spectrom Rev. 2014;33:353–372.

[48] de Quirós ARB, Frecha-Ferreiro S, Vidal-Pérez AM, et al. Antioxidant compounds in edible brown seaweeds. Eur Food Res Technol. 2010;231:495–498.

[49] van den HC, Algen JHM. Einführung in die Phykologie. Stuttgart (Germany): Thieme; 1978. German.

[50] Loeblich AR, III, Loeblich LA. Division Eustigmatophyta. In: Laskin AI, Lechavalier HA, editors. Handbook of microbiology, Vol. II. Cleveland (OH): C. R. C. Press; 1978. p. 481–487.

[51] Margulis L, Chapman A. Kingdoms and domains: an illustrated guide to the phyla of life on earth. 4th ed. Amsterdam (Netherlands): Elsevier; 2009.

[52] John DM. Phylum Eustigmatophyta. In: John DM, Whitton BA, Brook AJ, editors. The freshwater algal flora of the British Isles. An identification guide to freshwater and terrestrial algae. Cambridge (UK): Cambridge University Press; 2002. p. 271–272.

[53] John DM. Phylum Eustigmatophyta. In: John DM, Whitton BA, Brook AJ, editors. The freshwater algal flora of the British Isles. An identification guide to freshwater and terrestrial algae. 2nd ed. Cambridge (UK): Cambridge University Press; 2011. p. 346–347.

[54] Hibberd D. J. Phylum Eustigmatophyta. In: Margulis L, Corliss JO, Melkonian M, et al., editors. Handbook of Protocista. Boston (MA): Jones and Bartlett Publishers; 1990. p. 326–333.

[55] van den HC, Mann DG. Algae JHM. An introduction to phycology. Cambridge (UK): Cambridge University Press; 1995.

[56] de Reviers B. Biologie et phylogénie des algues. Tome 2 [Biology and phylogeny of algae. Vol. 2]. Paris (France): Belin; 2003. French.

[57] Andersen RA. Biology and systematics of heterokont algae and haptophyte algae. Am J Bot. 2004;91:1508–1522.

[58] Temniskova DN, Stoyneva MP. Algology. Vols. 1 and 2. Sofia (Bulgaria): Pensoft Publishers; 2011. Bulgarian.

[59] Hofbauer KW. Class Eustigmatophyceae. In: Frey W, editor. Syllabus of plant families, 13th ed, A. Engler’s Syllabus der Pflanzenfamilien, 2/1 Phototrophic eukaryotic algae. Stuttgart (Germany): Gebr. Borntraeger Verlagsbuchhandlung; 2015. p. 109–117.

[60] Stoyneva-Gärtner MP, Uzunov BA. [Bases of the systematics of algae and fungi]. Sofia (Bulgaria): Dzhey Ey Em Dzhi Books; 2017. Bulgarian.

[61] Lee RE. Phycology. 5th ed. Cambridge (UK): Cambridge University Press; 2018.

[62] Cavalier-Smith T. The kingdom Chromista. In: Round FE, Chapman DJ, editors. Progress in phycological research. Vol. 4. Bristol (UK): Biopress Ltd.; 1986. p. 309–347.

[63] Cavalier-Smith T. The kingdom Chromista. In: Green JC, Leadbeater BSC, Diver WL, editors. The
chromophyte algae: problems and perspectives. Oxford (UK): Clarendon Press; 1989. p. 379–405.

[64] Derelle R, López-García P, Timpano H, et al. A phylogenomic framework to study the diversity and evolution of stramenopiles (=heterokonts). Mol Biol Evol. 2016;33:2890–2898.

[65] Patterson DJ. Stramenopiles: chromophytes from a protistan perspective. In: Green JC, Leadbeater BSC, Diver WL, editors. The chromophyte algae: problems and perspectives. Oxford (UK): Clarendon Press; 1989. p. 357–379.

[66] Gärtner G, Stoyneva MP, Uzunov BA, et al. Ultraceutre of vegetative cells and autosporoles of aeroplyric strain of Vischeria stellata (Chodat ex Poulton) Pascher (Eustigmatophyceae) from Bulgaria. Fottea. 2012;12:273–280.

[67] Pribyl P, Eliáš M, Cupak V, et al. Zoosporogenesis, morphology, ultrastructure, pigment composition, and phylogenetic position of Trachydiscus minitus (Eustigmatophyceae, Heterokontophyta). J Phycol. 2012;48:231–242.

[68] Fawley M, Eliáš M, Fawley M. The diversity and phylogeny of commercially important algal class Eustigmatophyceae, including the new clade Goniochloridales. J Appl Phycol. 2014;26:1773–1782.

[69] Fawley MW, Jameson I, Fawley KP. The phylogeny of the genus Nannochloropsis (Monodopsidaceae, Eustigmatophyceae), with descriptions of N. australis sp. nov. and Microchloropsis gen. nov. Phycolgia. 2015;54:545–552.

[70] Fawley MW, Fawley KP. Rediscovery of Tetraedriella subglobosa Pascher, a member of the Eustigmatophyceae. Fottea. 2017;17:96–102.

[71] Nakayama T, Nakamura A, Yokoyama A, et al. Taxonomic study of a new eustigmatophycean alga, Vaucoulliniride crystallifera gen. et sp. nov. J Plant Res. 2015;28:249–257.

[72] Turner MF, Gowan RJ. Some aspects of the nutrition and taxonomy of fourteen small green and yellow-green algae. Bot Mar. 1984;27:249–255.

[73] Kai A, Yoshii Y, Nakayama T, et al. Aurearenophyceae classis nova, a new class of Heterokontophyta based on a new marine unicellular alga Aurearen cruciata gen. et sp. nov. inhabiting sandy beaches. Protist. 2008;159:435–457.

[74] Iliiev I, Petkov G, Lukavsky J, et al. The alga Trachydiscus minitus (Pseudostaurotrum minutum): growth and composition. Gen Appl Plant Physiol. 2010;36:222–231.

[75] Hibberd DJ, Leedale GF. Eustigmatophyceae—a new algal class with unique organization of the motile cell. Nature. 1970;225:758–760.

[76] Hibberd DJ, Leedale GF. A new algal class—the Eustigmatophyceae. Taxon. 1971;20:523–525.

[77] Hibberd DJ, Leedale GF. Observations on the cytology and ultrastructure of the new algal class, Eustigmatophyceae. Ann Bot. 1972;36:49–71.

[78] Hibberd DJ. Eustigmatophyceae. In: Cox ER, editor. Phytoflagellates: form and function. New York (NY): Elsevier; 1980. p. 319–334.

[79] Hibberd DJ. Notes on taxonomy and nomenclature on the algal classes Eustigmatophyceae and Tribophyceae (synonym Xanthophyceae). Bot J Linn Soc. 1981;82:93–119.

[80] Hibberd DJ. Eustigmatophyceae: introduction and bibliography. In: Rosowski JR, Parker BC, editors. Selected papers in phycology II. Lawrence (KS): Phycological Society of America; 1982. p. 728–730.

[81] Whittle SJ, Casselton PJ. The chloroplast pigments of the algal classes Eustigmatophyceae and Xanthophyceae. I. Eustigmatophyceae. Br Phycol J. 1975;10:179–191.

[82] Owens TG, Gallagher JC, Alberte RS. Photosynthetic lightharvesting function of violaxanthin in Nannochloropsis spp. (Eustigmatophyceae). J Phycol. 2007;23:79–85.

[83] Anderson JM, Barrett J. Light-harvesting pigment-protein complexes of algae. In: Staehelin LA, Arntzen CJ, editors. Photosynthesis III. Photosynthetic membranes and light-harvesting systems. Berlin (Germany): Springer-Verlag; 1986. p. 269–285.

[84] Goodwin TW. The biochemistry of the carotenoids. In. Plants. 2nd ed. Vol. 1. New York (NY): Chapman and Hall; 1980.

[85] Gantt E. Phycobilisomes. In: Staehelin LA, Arntzen CJ, editors. Photosynthesis III. Photosynthetic membranes and light harvesting systems. Encyclopedia of plant physiology. Vol 19. Berlin (Germany): Springer-Verlag; 1986. p. 260–268.

[86] South RG, Whittick A. Introduction to phycology. Oxford (UK): Blackwell Scientific Publications; 1987.

[87] Graham LE, Graham MJ, Wilcox LW. Algae. 2nd ed. San Francisco (CA): Benjamin Cummings; 2009.

[88] Keşan G, Litvin R, Bina D, et al. Efficient light-harvesting using non-carbonyl carotenoids: energy transfer dynamics in the VCP complex from Nannochloropsis oceanica. Biochim Biophys Acta. 2016;1857:370–379.

[89] Sukení A, Livne A, Neori A, et al. Purification and characterization of a light-harvesting chlorophyll-protein complex from the marine eustigmatophyte Nannochloropsis sp. Plant Cell Physiol. 1992;33:1041–1048.

[90] Sukení A, Livne A, Apt KE, et al. Characterisation of a gene encoding the light-harvesting violaxanthin-chlorophyll protein of Nannochloropsis sp. (Eustigmatophyceae). J Phycol. 2000;36:563–570.

[91] Fietz S, Nicklisch A. An HPLC analysis of the summer xanthophyll cycle-dependent photoprotection in Nannochloropsis gaditana (Eustigmatophyceae): effects of exposure time and temperature. Phycologia. 1998;37:16–23.

[92] Carbonera D, Agostini A, Di Valentin M, et al. Regulation and function of a binding protein (VCP) from Nannochloropsis gaditana (Eustigmatophyceae). Biochim Biophys Acta. 2014;1837:1235–1246.

[93] Lubián LM, Montero O. Excess light-induced violaxanthin cycle activity in Nannochloropsis gaditana (Eustigmatophyceae): effects of exposure time and temperature. Phycolologia. 1998;37:16–23.

[94] Aburai N, Okhubo S, Miyashita H, et al. Composition of carotenoids and identification of aerial microalgae isolated from the surface of rocks in mountainous districts of Japan. Algal Res. 2013;2:237–247.

[95] Goss R, Jakob T. Regulation and function of xanthophyll cycle-dependent photoprotection in algae. Photosyn Res. 2010;106:103–122.
[126] Lee RE. Phycolology. 4th ed. Cambridge (UK): Cambridge University Press; 2008.

[127] Christensen T. Algae. A taxonomic survey. Fasc. 1. Odensel: AOTrTryk as; 1980.

[128] Ott DW, Oldham-Ott CK, Rybalka N, et al. Xanthophyte, eustigmatophyte and raphidophyte algae. In: Wehr JD, Sheath RG, Kociolek P., editors. Freshwater algae of North America. Ecolog and classification. 2nd ed. Amsterdam (Netherlands): Elsevier; 2015. p. 485–530.

[129] Egeland ES. Algal carotenoider og kjemystematikk [Algal carotenoids and chemosystematics] [dissertation]. Trondheim: Norwegian University of Science and Technology; 1996. Norwegian.

[130] Egeland ES. Carotenoids. In: Borowitzka MA, Beardall J, Raven JA, editors. Physiology of microalgae. Heidelberg (Germany): Springer; 2016. p. 507–564.

[131] Liaaen-Jensen S. Algal carotenoids and chemosystematics. In: Faulkner DJ, Fenical WH, editors. Marine natural products chemistry, NATO conference series IV, marine sciences. Vol. 1. New York (NY): Plenum; 1977. p. 239–259.

[132] Hommersand MH, Huang Y-S. Cytological and biochemical studies on Monodus from the treated sewage ponds in Morehead City. In: Kuenzler EJ, Chestnut AF, Weiss CM, editors. Structure and functioning of estuarine ecosystems exposed to treated sea water and wastes, Ill. Nat. Sci. Found. Ann. Rept. 1972–1973. Sea Grant Publication; 1973; UNC-SG-73–10. p.15–32.

[133] Sukenik A, Carmeli Y, Berner T. Regulation of fatty acid composition by irradiance level in the eustigmatophyte Nanochloropsis sp. J. Phycol. 1989;25:686–692.

[134] Guillard RRL, Lorenzen CJ. Yellow-green algae with chlorophyllide c. J Phycol. 1972;8:10–73.

[135] Stransky H, Hager A. Das Carotinoidmuster und die Verbreitung des lichtinduzierten Xanthophyllcyclus in verschiedenen Algenklassen VI. Chemosystematische Betrachtung. [The carotenoid pattern and the occurrence of the light induced xanthophyll cycle in various classes of algae. VI. Chemosystematic Study]. Arch Microbiol. 1970;73:315–323. German.

[136] García-Plazaola JI, Matsubara S, Osmond CB. The lutein epoxide cycle in higher plants: its relationships to other xanthophyll cycles and possible functions. Funct Plant Biol. 2007;34:759–773.

[137] Brunet C, Johnsen G, Lavaud J, et al. Pigments and photoacclimation processes. In: Roy S, Llewellyn CA, Egeland ES, et al. editors. Phytoplankton pigments: characterization, chemotaxonomy and applications in oceangraphy. Cambridge (UK): Cambridge University Press; 2011. p. 445–471.

[138] Larkum A. Photosynthesis and light harvesting in algae. In: Borowitzka MA, Beardall J, Raven JA, editors. Physiology of microalgae. Heidelberg (Germany): Springer; 2016. p. 67–88.

[139] Chrystal J, Larkum AWD. Pigment-protein complexes and light harvesting in eustigmatophyte algae. In: Biggem J, editor. Progress in photosynthesis research, Vol. II. Dordrecht (Netherlands): Martinus Nijhoff Publishers; 1987. p. 189–192.

[140] Steiger S, Sandmann G. Cloning of two carotenoid ketolase genes from Nostoc punctiforme for the heterologous production of canthaxanthin and astaxanthin. Biotechnol Lett. 2004;26:813–817.

[141] Ambati RR, Siew-Moi P, Sarada R, et al. Astaxanthin: sources, extraction, stability, biological activities and its commercial applications—a review. Mar Drugs. 2014;12:128–152.

de Reviers B. Biologie et phylogénie des algues. Tome 1 [Biology and phylogeny of algae. Vol.1]. Paris (France): Belin; 2002.

[142] Ishida K-L, Hara Y. Taxonomic studies on the Chlorarachniophyta. I. Chlorarachniion globosum sp. nov. Phycologia. 1994;33:351–358.

[143] Kawai H, Nakayama T. Chlorarachniophyta. In: Frey W., editor. Syllabus of plant families. A. Engler’s Syllabus der Pflanzenfamilien. 13th ed. 2/1. Stuttgart (Germany): Gebr. Borntraeger Verlagsbuchhandlung; 2015. p. 178–181.

[144] Gabrielson PW, Garbary DJ, Sommerfield MR, et al. Phylum Rhodophyta. In: Margulis L, Corliss JO, Melkonian M, et al. editors. Handbook of Protocista. Boston (MA): Jones and Bartlett Publishers; 1990. p. 102–118.

[145] Yoon HS, Nelson W, Lindstrom S, et al. Rhodophyta. In: Archibald JM, Simpson AJB, Slamovits CH, editors, Handbook of the Protists. New York (NY): Springer; 2016. p. 89–134.

[146] Jamikorn M. 1954. Carotenogenesis in Mycobacterium phlei and other microorganisms [dissertation]. Liverpool (UK): University of Liverpool; 1954.

[147] Heywood P. Phylum Raphidophyta. In: Margulis L, Corliss JO, Melkonian M, et al., editors. Handbook of Protocista. Boston (MA): Jones and Bartlett Publishers; 1990. p. 318–325.

[148] Chapman DJ, Haxo FT. Chloroplast pigments of CHLOROMONADOPHYCEAE. J Phycol. 1966;2:89–91.

[149] Cavalier-Smith T, Chao EE, Allsopp TEP. Ribosomal RNA evidence for chloroplast loss within Heterokonta: pedinellid relationships and a revised classification of Ochristan algae. Arch Protistenknd. 1995;145:209–220.

[150] Daugbjerg N, Andersen R. A molecular phylogeny of the raphidophytes Heterosigma carterae and Chattonella antiqua using 16S rRNA sequences. Biochem Syst Ecol. 1996;24:221–235.

[151] Tynell JV, Bergquist PR, Gray RD, et al. Phylogeny of the raphidophytes Heterosigma carterae and Chattonella antiqua using 16S rDNA sequences. J Phycol. 1997;33:103–1041.

[152] Noguchi F, Tanifuji G, Brown MW, et al. Complex evolution of two types of cardioline synthase in the eukaryotic lineage stramenopiles. Mol Phylogenetics Evol. 2016;101:133–141.
Zapata M, Jeffrey SW, Wright SW, et al. Photosynthetic pigments in 37 species (65 strains) of Haptophyta: implications for oceanography and hemotaxonomy. Mar Ecol Prog Ser. 2004;270:83–102.

Jeffrey SW, Wright SW. Photosynthetic pigments in marine microalgae: insights from cultures and the sea. In: Subba Rao DV, editor. Algal cultures, analogues of blooms and applications. Enfield (UK): Science Publishers; 2006. p. 33–90.

Assunção MF, Amaral R, Martins CB, et al. Screening microalgae as potential sources of antioxidants. J Appl Phycol. 2017;29:865–877.

Llewellyn CA, Roy S, Johnsen G, et al. Perspectives on future directions. In: Roy S, Llewellyn CA, Egeland ES, et al. editors. Phytoplankton pigments: characterization, chemotaxonomy and applications in oceanography. Cambridge (UK): Cambridge University Press; 2011. p. 605–625.