Current bioeconomical interest in stramenopilic Eustigmatophyceae: a review

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ABSTRACT
Today's global problems and challenges have given rise to a new field of interest – bioeconomics. It is strongly related to phycoprospecting, or searching for specific algal strains of commercial importance. There has been growing interest in the small algal class Eustigmatophyceae in recent years. These microscopic stramenopilic algae, which have all the advantages of microalgal cultivation, have proved to be promising commercial sources of valuable compounds (e.g. carotenoids, unsaturated fatty acids, amino acids) in aquaculture, agriculture, biofuels production, medicine, pharmaceutics, cosmetics, wastewater treatment, environmental control, etc. The present review shows the main genera and strains of commercial importance, outlines their main fields of application and some gaps in our knowledge in this aspect. Today, the great promising bioeconomical potential of these algae has generally been recognized, but in the present state of its infancy, it is far from being fully exploited.

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Introduction
Many problems and challenges in today's world, and the shortage of natural resources in particular, have led to the emergence of a new field of interest that seeks to integrate and bridge economics and biology: bioeconomics [1]. Despite that its exact definition is yet disputable, bioeconomics is enlarging its scope owing to the persistent transfer of ideas from economics to biology and vice versa. It already includes 'renewable biological resources and their conversion into food, feed, bio-based products and bio-energy' [2]. The search for specific algal strains of commercial importance, or phycoprospecting [3] (sometimes referred to by the broader term bioprospecting [4]), has become one of the most modern branches of recent studies related to the increasing interest in the search for sustainable technologies and mitigating the economic costs. Microscopic algae (microalgae), most of which are highly adaptable phototrophs, do not compete with other sources, like plants which are used for food [5, 6], and produce high amounts of valuable compounds, thus attracting considerable interest for biotechnological production of a broad range of products from fuel to pharmaceuticals, functional foods, nutraceuticals, pigments, etc. [7, 8]. Initially, the focus was on the best-known groups: blue-green algae (Cyanobacteria/Cyanoprokaryota) and green algae (Chlorophyta and Streptophyta) and they were much better explored. Although the bioeconomical interest in the small stramenopilic algal class Eustigmatophyceae has been growing in recent years, data have not been summarized. The present review shows the main genera and strains of commercial importance, outlines their main fields of application and some gaps in our knowledge in this aspect.

Carotenoids of Eustigmatophyceae
The bioeconomical interest in the microscopic algae of the small stramenopilic class Eustigmatophyceae raised with the discovery of their potential for high extraplastidal carotenoid production. It could be traced back to the paper by Antia and Cheng [9], who studied the marine Nannochloropsis oculata (Droop)

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Hibberd and provided ‘the first documented evidence of eustigmatophycean production of astaxanthin (free and monoesterified) and astacene in significant amounts, indicating the capacity of this algal type to synthesize the highest oxidation level of four keto-carotenoids known from the algal kingdom, and hitherto found only in the Chlorophyceae, Euglenophyceae and Dinophyceae’ [9, p.47]. This finding was extremely important because all mentioned algal classes contained generally different esterified forms of astaxanthin (AsX) [e.g. 10].

Further studies showed that the interest in *Nannochloropsis* (and in the derived genus *Microchloropsis*) lay in the availability of a range of valuable pigments (e.g. chlorophyll a, canthaxanthin, zeaxanthin and AsX) in contrast with the green genus *Haematococcus*, commercially cultured for its capacity to accumulate AsX [11]. Afterwards, AsX was found in *Vischeria* (Syn. *Eustigmatos*) ([12] and references therein). In two *Vischeria* strains AsX reached 9 and 13% of total carotenoid content showing their commercial potential [12]. Algae are the primary source of this red-coloured pigment (long popular as ‘haematochrom’) in the aquatic food chains and its invaluable role as a dietary supplement and food or feed additive intended for human, animal and aquaculture consumption is known worldwide. Its primary use today is as an animal feed additive to impart colouration, including farm-raised salmon, shrimps, crabs and chicken egg yolks [e.g. 13–15]. In the European Union (EU), AsX is considered a food dye with the E number E161e but it is not yet approved for use as a food additive in the EU or the USA [24, 27]. ViX has been approved only for use in the EU [24], where in animal regulations it is specifically allowed for cats and dogs [16]. In the USA, as a food colouring (or colour additive) it has been approved only in five strains of *Vischeria* in particular achieved a status of generally recognized as safe (GRAS), meaning that it can be sold as a dietary supplement [19]. Its main benefits explored in medicine are due to its strong antioxidant activity, which gives it its anti-inflammatory and anti-cancer properties together with skin and eye care potential and possibility to enhance the immune response [14, 20, 21].

The rich palette of carotenoid pigments in different genera of Eustigmatophyta [12] logically led to the recent studies of their commercial biotechnological potential. Six species of genus *Vischeria* were reported as possible novel sources of natural β-carotene due to their considerable production in bubble column and flat panel photobioreactors (100 and 470.2 mg L⁻¹, respectively) [22, 23]. β-Carotene is best known for its provitamin A activity function and strong stimulatory effect on the immune system. It is now of increasing demand and has extensive applications [22, 23]. As AsX, it is broadly used as a colorant and feed additive (E160a [24]), antioxidant, heart-preventive and anti-cancer agent in the food and aquaculture, pharmaceutical and cosmetics industries [25]. Presently, the primary algal source of β-carotene is the green microalga *Dunaliella salina*. However, researchers have yet to achieve high β-carotene concentration in the total low biomass yield (1.5–2 g L⁻¹) under harsh conditions of high salinity, high light intensity (>500 μmol photons m⁻² s⁻¹) and low temperature [22]. To date, the β-carotene content in the studied *Vischeria* strains is lower than that in *Dunaliella* (up to 7% of the dry weight vs. 10%, respectively), although Li et al. [22] believed that it could be increased with improvement of the culture conditions. Further, some eustigmatophyceans (and particularly *Vischeria stellata*) accumulate higher biomass than *Dunaliella* and therefore, considering their high intracellular β-carotene content, may be more promising as future natural sources of this pigment. Similar results for *V. stellata* were obtained by Gao et al. [26].

Apart from the above-mentioned carotenoids, eustigmatophyceans contain violaxanthin (ViX) as one of their primary photosynthetic pigments (for details see [12]). Up to now this red-coloured pigment is sometimes used as a food colourant under the general E number E161e but it is not yet approved for use as a food additive in the EU or the USA [24, 27]. ViX has demonstrated strong radical scavenging activity, valid inhibition of lipid peroxidation and red blood cell hemolysis, anti-proliferative, anti-inflammatory and proapoptotic activity against human cancer cell lines in vitro, which indicated that this pigment has great potential to be widely applied in healthcare and medical products [28, 29].

Lutein (Lut) is another carotenoid pigment which was relatively recently found in Eustigmatophyta [12]. It accounts for 13–25% of the total carotenoid content in five strains of *Vischeria*. Therefore, the authors suggested their possible role as its commercial sources considering that this pigment is a high-value product with extensive applications in feed, food, nutraceutical and pharmaceutical industries [12]. Due to its yellow-red colour, it has primarily been used as a colourant in food and supplement manufacturing. As a food additive under the E number E161b, Lut is approved for use in the EU [24], where in animal regulations it is specifically allowed for cats and dogs [16]. In the USA, it was firstly restricted to animal feed, especially for chicken, where it shows up in the colour of the skin and egg yolks, but more recently, crystalline Lut
achieved GRAS-status and was allowed for use as an ingredient in milk-based meal replacements [30]. Humans and animals cannot synthesize Lut and can naturally obtain it by ingesting plants. Despite controversial opinions on its exact role and even underestimation and underappreciation by clinicians and vision researchers, recent studies show that dietary supplements containing Lut reduce progression of age-related macular degeneration and cataract formation, enhance curation of other ocular diseases and support the functions of normal eyes [31–37]. Moreover, it was extensively reported that consumption of food rich in Lut is associated with lower incidence of cancer and cardiovascular diseases [37].

Lut is isomeric with zeaxanthin (ZeX), which is commonly synthesized in higher plants, but has been documented also in algae and in Eustigmatophyta in particular [12]. It amounted to 8–11% of the total carotenoid content of five Vischeria strains [12]. Since the concentration of ZeX in the macula, together with its functions and effects, are quite similar to those of Lut [32, 37], it could be supposed that eustigmatophyte algae have commercial potential in future dietary, health-care and medical products. At present, ZeX is being obtained from other plant sources and from the aquatic blue-green algal genus Spirulina [38]. Therefore, it sounds very promising to seek for novel ZeX sources, especially among aeroterrestrial stramenopilic algae. The great potential of eustigmatophyceans as commercial sources of a wide range of carotenoids has been already outlined [12].

The valuable eustigmatophycean carotenoids include also canthaxanthin (CaX) [12], which was firstly isolated from the edible mushroom Cantharellus cibarius. This pigment is known mainly as a food additive for farmed salmon in environments where AsX sources are not available, or is used in combination with AsX [39]. CaX gives farmed salmon a colour similar to pink/red species of wild salmon and could be consequently transferred to humans. Ingested in the human body, despite its subsequent low concentrations, CaX can serve as a carotenoid source alternative to the use of synthetic dietary supplements [40]. In the USA, it is approved as food additive for solid, semisolid and liquid food, and for feed for salmonids and broiler chicken as well [18]. In the EU, under E number E161g [16, 24], it is allowed as additive to trout, salmon and poultry feed and is used in broiler chicken feed to enhance the yellow colour of chicken skin. CaX affected positively the diet of representatives of 12 trout families and the commercial diet of the shrimp Penaeus monodon, enhanced by adding cholesterol [41–43]. CaX serves as a potent lipid-soluble antioxidant in animal tissues, including broiler meat and the chick embryo [44–46]. In the egg, the pigment is allocated from the yolk to the developing embryo probably to serve as protection against oxidative damage, especially during the sensitive periods of hatching and early posthatch life [45, 46]. The supplementation of broiler breeder diets with CaX improved the hatchability rate, fertility, and reduced the thiobarbituric reactive substances in eggs and embryo mortality [47]. Despite some controversial data on its role in human chronic diseases, CaX has received more attention and has been extensively studied as a component in tanning pills and creams, for its anti-cancer, anti-tumor and anti-dermatosis properties and its very strong modifying effect with respect to the dynamic and structural properties of lipid membranes [48–50]. CaX and other carotenoids could be utilized as chemosensitisers, especially as adjuvants in chemotherapy [51].

Chlorophylls and chlorophyllins in Eustigmatophyceae

Chlorophyll a is the main photosynthetic pigment of Eustigmatophyceae. Although its high amounts and combinations with different chlorophyllins are well-documented [12, 52], its application in human activities is still poorly investigated. The health benefits of chlorophyll like detoxication, antioxidant effects, boosting of the immune system, wound healing, weight loss, skin healing and anti-cancer activity are well-known [53, 54], and many products of blue-green and green algae (e.g. Aphanizomenon, Spirulina and Chlorella) are broadly used due to its high content and have already achieved high public awareness. Since chlorophyll is registered as a food additive (colourant) under E number E140 [24], its usage from fast-growing microscopic eustigmatophycean algae could be strongly proposed. Chlorophyll is used as food and beverage colouration [55, 56].

Vitamins and use of Eustigmatophyceae as food for humans and in aquacultures

Tocopherols (vitamin E) and especially their most abundant form - α-tocopherol - are among the most valuable healthcare products due to their strong antioxidant activity in vivo and their capability to prevent light-induced pathologies of skin and eyes, or degenerative disorders like the socially significant atherosclerosis, cardiovascular diseases and cancer [57]. All tocopherols are synthesized only by photosynthetic
organisms. Therefore, seeking for low-cost plant sources of tocopherols is of primary importance. Originally, tocopherols were considered as dietary factor in animal breeding with a positive effect on the reproduction and survival due to improved resistance to stress and diseases [57]. Later, owing to its positive effects, α-tocopherol, under E number E307, was approved as a dietary supplement for humans [24]. Research has demonstrated the significant potential of the marine eustigmatophyte Nannochloropsis oculata (fondly named ‘marine chlorella’) as an α-tocopherol rich source of lower production costs for mariculture [57]. In this species, the bioaccessibility of tocopherols was higher than those of β-carotene and lycopene; it was also higher in comparison with their availability from the diatom Chaetoceros [58]. The experiments under different culture conditions depending on nitrogen source, concentration and growth phase showed the potential to increase the tocopherol content in the studied microalgae, thus proving their potential for large-scale production [57].

In addition to vitamin E, eustigmatophyceans are rich in vitamins B, C, D, and K. This, in combination with their small dimensions and absence of tough cell wall makes eustigmatophyceans easily assimilated by larval animals [59]. The same authors proposed a strain of Monodus subterraneus, grown at relatively high temperature (25–30°C) as a commercial planktonic feed for tropical aquacultures. Microalgae are commonly required for larval nutrition during a brief period, either for direct consumption in the case of molluscs and peneid shrimp or indirectly as food for the live prey fed to small fish larvae. Despite the advantages of live microalgae in aquaculture, there was a trend to avoid using them due to their high cost and the difficulty in producing, concentrating and storing them [60]. However, the marine Nannochloropsis has been one of the commonly used microalgae in aquacultures [61–64]. Microchloropsis gaditana (Syn. Nannochloropsis gaditana) was considered to be a ‘premium’ food for rotifers [65]. Similar results on the growth rate and composition of 12 marine species from different taxonomic groups as food for Brachionus plicatilis, subsequently used as food for marine fish (mainly Pagrus major), indicated Nannochloropsis sp. (KMMCC-33) as ‘the best microalgal species for the mass culture of the rotifer’ [66]. An alternative method of culturing N. oculata by using green water from red tilapia (Oreochromis sp.) culture system as a fertilizer instead of conventional mediums and fertilizer has been proposed [67].

An effective approach for transferring cDNA of the fish growth hormone (GH) into N. oculata was developed by Chen et al. [68]. The transgenic microalga were given as food to artemia, which was subsequently used as food for red-tilapia larvae. The results on significantly greater growth of larvae fed by artemia incubated with transgenic microalgae (316% in weight gain and 217% in body length increase versus 104% and 146% respectively in larvae fed by artemia incubated with nontransgenic microalgae) proved the species as a potential good bioreactor material for producing foreign protein, which could be of benefit for both pharmaceutical and agricultural industries [68]. The authors strongly suggested that, for humans, the consumption of nontransgenic fish fed temporarily on GH-transgenic algae is much safer than direct consumption of GH-transgenic fish, especially when transgenic algae are applied under controlled conditions outdoors.

Lipids (fatty acids and sterols) of Eustigmatophyceae

Considering the broad use of N. oculata as basic food in aquacultures, Patterson et al. [69] studied the sterols and fatty composition of five other marine eustigmatophyceans with the idea to find better algal diet for improved productivity of oyster fisheries. This study was conducted 20 years after the first report of lipids (mainly sterols) in the eustigmatophyte Monodus subterraneus by Mercer et al. [70]. The authors proved the presence of free sterols (mainly cholesterol, and 24-ethylcholesterol and isofucosterol in smaller amounts) in all studied strains, the palmitic acid (16:0) as a major fatty acid in four of the strains and omega-3 eicosapentaenoic acid (EPA; 20:5n-3) as the major fatty acid in two of the strains. The strain Sticho-0-18, rich in EPA as a major fatty acid and in sterols (25.77% of the dry weight, or 0.86 pg/cell), was proposed as the best eustigmatophyte strain for oyster food [69]. A recent study of the sterol content of N. oculata indicated that it might be useful as a potential source of natural anti-inflammatory and anti-cancer compounds [71].

Before the work by Patterson et al. [69], half of the strains studied by them had been proved as rich in long-chain polyunsaturated fatty acids (PUFAs) [72, 73]. PUFAs contain essential fatty acids (EFAs) and participate in many metabolic processes, playing an important role in the life and death of cardiac cells. PUFAs also reduce the blood cholesterol, thus reducing the morbidity risks of coronary heart-diseases and helping in the prevention of hypertension, diabetes.
Type II, many ocular diseases, arthritis and cystic fibrosis [74–79]. Low EFAs levels, or wrong balance of types among them, could be a factor in a number of illnesses, including osteoporosis [80]. Globally, the primary source for PUFAs and EFAs are fish. However, fish cannot synthesize them but obtain them via the aquatic food chains with algae at the basis. Yet, the application of fish oil as food additive is limited due to problems associated with its typical fishy smell, unpleasant taste and poor oxidative stability, together with inapplicability for certain purposes because of the presence of mixed fatty acids [60]. In addition, it has to be stressed that many fish also accumulate pollutants which change the odour and composition of the extracted oils and that fish are considered declining resources [81]. Therefore, considering the increased interest in these fatty acids for human consumption, in order to meet the demands of the expanding market, it is essential to search for novel algal sources. Studies have shown a high content of EPA in marine Nannochloropsis (up to 44% of the total fatty acid content - TFA) and its potential application for human diet [66, 82–86]. Research has vastly concentrated on testing the optimal growth conditions, of environmental conditions and on the effects of nutrients and nutrient starvation for obtaining the maximum EPA yield in different types of reactors [59, 84, 85, 87–105]. Most reports show that higher EPA yield could be achieved via the so-called ‘physiological forcing’, which is based on the development of a set of conditions to maximize the production of a desired biochemical [106]. Successful manipulation of the PUFA and EPA content in the same alga is possible using other techniques (random mutagenesis, ultraviolet mutagenesis, etc.) [8, 107–111].

Apart from the marine species, high content of EPA has been demonstrated in the freshwater and aeroterrrestrial eustigmatophyceans of genera Monodus (up to 49.3% TFA), Vischeria, Ellipsoidion (18.8% TFA) and Trachydiscus [26, 59, 90, 93, 112–121]. Trachydiscus minutus was considered as nearly a top-producer of EPA among microalgae with its 10–36% d.w. content and high productivity of 88 mg L⁻¹ per day (for details see [81]). Similar to the studies of EPA in marine algae, most of the authors applied different culture designs and conditions for obtaining a maximum EPA yield from the freshwater and aeroterrrestrial species (e.g. [26, 112]).

In this review, the fatty acids found in smaller amounts are not discussed except the mentioning of docosahexaenoic acid (DHA, 22:6n-3) as recorded in the genera Nannochloropsis and Vischeria, and the unusually high content of myristic (14:0) acid (20–54%) in Trachydiscus [66, 81, 94, 114, 115, 119–125]. The general similarity of PUFA composition in both marine and freshwater eustigmatophyceans has led to the suggestion that freshwater species show potential for use in aquacultures [115]. The freshwater Nannochloropsis limnetica had significantly higher long-chain PUFA content in comparison with green freshwater planktonic algae [122]. Like in other studies, the content of PUFA was highly variable depending on culture conditions with highest concentrations found in non-aerated suspension cultures rich in phosphates. Therefore, N. limnetica was recommended as a high-quality food resource in aquacultures, where it could be used as direct food for rotifers or as an alternative supplement to replace the traditional fish-based food in aquacultures [122]. The same authors stressed on the possibility to use N. limnetica also as an alternative to fish oil produced from fatty fish, which is widely used in human medicine to reduce the risk of myocardial illnesses.

### Eustigmatophyceae as potential source for biofuels

The fatty acids and other lipids of Eustigmatophyceae have become a focus of studies due to increased interest in biofuel production from next generation alternative sources [126]. In this respect, Pilatova [81] provided the first review on fatty acid distribution in Eustigmatophyta alongside with the fatty acid profiles and the above-mentioned PUFAs. The studies were oriented towards the finding of prospective oleaginous species, rich mainly in neutral lipids (and triacylglycerol, or TAG, in particular) and the evaluation of the effects of culture growth conditions on the lipid productivity and fatty acid composition [81, 126, 127]. The study of the neutral lipids in marine Nannochloropsis [73, 128, 129] led to the discovery of unusual C₃₀ – C₃₂ 1, 15 alkyl-diols and monosaturated C₃₂ 1, 15 diol. These long-chain alcohols are significant constituents of the lipids of marine Eustigmatophyceae [129]. Therefore, their identification in three freshwater species of the genus Vischeria is also of interest [129]. In addition to these unusual diols and n-alcohols in marine Nannochloropsis, Gelin et al. [130] detected small amounts of C₂₈ – C₃₄ mono-hydroxy fatty acids both in free and bounded form, which suggests that these microalgae can be potential sources of such fatty acids. Besides the already mentioned EPAs, palmitic and palmitoleic acid, eustigmatophyceans also contain other fatty acids in lesser
amounts, for example, arachidonic acid (20:4n-6) and various C₁₈ acids, amongst which oleic acid (18:1) – for example [73, 126, 131]. Apart from the most studied aquatic halotolerant genus Nannochloropsis [132–134], Zhang et al. [135] found a significantly high lipid content (60.59% d.w.) in the soil eustigmatophyte Vischeria polyphem (Syn. Eustigmatus polyphem) and proposed it as a novel oleaginous alga for biodiesel production due to its high growth and biomass rate. Another novel oleaginous alga, also promising for biodiesel production is Vischeria stellata with total lipid content of about 56% with 52% neutral lipids [26]. Despite all widely known ‘theoretical’ advantages, generally, based on the maturity of current technology, the true potential of microalgae biofuel towards energy security and its feasibility for commercialization are still questionable. A lot of research is required to bring current microalgae biofuel research to a new dimension and consequently, to revolutionize the entire microalgae biofuel industry towards long-term sustainability [136].

A promising line of research into microalgae biofuel could be marine microalgae. For example, screening of 96 strains of marine microalgae from different groups, outlined the eustigmatous genus Nannochloropsis as the most promising for biodiesel feedstock [137]. Similar results have been obtained after screening of 175 microalgal strains from 21 classes of 7 phyla [7]. Some studies have suggested Nannochloropsis for large-scale biodiesel production [138, 139]. Nannochloropsis has become recognized as a model organism to obtain lipids owing to the combination of easy growth at a large scale, high lipid content, new genomic information and innovative genetic transformation techniques [127, 140]. As summarized by [8], to exploit the ability of Nannochloropsis to generate special lipids, a great deal of effort has been put into omics studies like genome sequencing, transcriptomic and lipidomic analyses [141–146]. The development of molecular biology tools that enable the generation of Nannochloropsis transformants is increasing [147–149]. The coordination of omics data and functional analyses of key genes related to lipid biosynthesis would yield important information on algal lipid metabolism that is expected to form the basis for future metabolic engineering techniques (for details see [8]).

**Eustigmatophyceae for cosmetics, medicine, pharmaceutics and healthcare products**

In some cases, whole biomass and residual ‘cake’ after oil extraction may be further utilized as sources of other end products of interest, for example protein, essential amino acids and other nutrients for animal feed. Microalgae have long been recognized as a potential protein source for nutrition applications, but only a few microalgae (e.g. Spirulina, Chlorella and Dunaliella) have been commercially exploited. The interest in the total nutrient content of microalgae is rising due to their richness in macronutrients as a potential future food source preserving natural resources [86, 150]. This applies to Eustigmatophyceae as well, whose total nutrient biomass profiles, nutrient bioavailability and safety are subject of investigations (e.g. 84, 86, 150). Thus algae-rich diets (including those based on Nannochloropsis) are well accepted, well tolerated and suitable for the maintenance of body weight and normal organ function at least in experimental animals (for details see [86]). Another study [150] showed that N. granulata has high content of essential amino acids and of leucine, lysine and tryptophan in particular, non-essential amino acids and crude protein (ca 33% in the whole alga and ca. 40% in lipid extracted algae) together with rich elemental composition of different minerals and trace elements (with highest content of Zn). Studies of total nutrients have also become oriented towards the effects of enriched growth media and cultivation time on nutritional composition (e.g. 85).

Microalgae are a source of vitamins, pigments, proteins and other substances beneficial for the skin, but only a few microalgae species are consolidated in the skin care market, wherein the principal ones are the green Chlorella and blue-green Spirulina [151]. However, currently Nannochloropsis is enlisted together with them as ‘traditionally used’ in skin-protection care [152]. The application of Nannochloropsis in cosmetics and cosmeceutics rapidly increases due to its lipid (and especially high PUFA) content and due to the tanning effects of canthaxanthin [49, 50]. Extracts of N. oculata and Microchloropsis gaditana could act as an optimum protective sheath against oxidative stress and positively influence collagen synthesis [151, 153]. An ingredient of N. oculata with excellent skin elasticity properties and skin-tightening effects (short and long-term) was launched by Pentapharm (Basel, Switzerland) [50, 60, 151, 154]. In future, broader use of Eustigmatophyceae as part of the composition of face and skin care products with anti-ageing, refreshing/regenerating, thickening and anti-irritant properties is to be expected. The comparative study of antioxidant properties and total phenolic content of various extracts of a
diatom, *Chaetoceros* sp., and *Nannochloropsis* sp. showed a higher antioxidant capacity of the diatom in comparison with the eustigmatophyte extracts [155]. Different solvent extracts contained different antioxidant capacities in terms of reducing and radical-scavenging power and the correlation between the antioxidant properties and the total phenolic content was not significant, indicating that phenolic compounds might not be a major source of the antioxidant properties found in these two microalgae [155].

A recent study reported a new freshwater eustigmatophyte Forest Park Isolate 5 (FP5), which is able to shift the absorption of chlorophyll *a* to >705 nm and to grow under solely far-red light [156]. A new case of natural engineering with a novel lineage of endosymbiotic bacteria in eustigmatophyceans was discovered [157]. These authors also found a six-gene operon that possibly encodes a pathway which involves metabolites from both organisms resulting in an isoprenoid–cyclitol-derived compound, probably with antimicrobial or other protective activity. Such new findings give reason for new views and future prospects for the commercial applicability of Eustigmatophyceae.

Considering the increasingly recognized role of microalgae in health and disease prevention [158], the advancement of omics technologies in personalized medicine has spanned over to Eustigmatophyceae as well. They have been used in the improvement of protein extraction protocols, allowing even deeper mining of the proteomes [159, 160]. This, along with the lack of toxic records from Eustigmatophyceae (e.g. [86, 161]), highlights that this field of potential applications deserves more attention, apparently having been underestimated in comparison with the studies on biofuel and carotenoid production.

**Eustigmatophyceae in environmental control**

In an environmental aspect, *Nannochloropsis* was enlisted among promising biocontrol agents of mosquitoes [162, 163] and marine algae tested for CO₂ sequestration [152, 164, 165]. Eustigmatophyceae could also be applicable in water purification and wastewater treatment according to some successful experiments on their cultivation using different types of wastewaters [67, 165–167].

**Summary of topics and time-span development of the studies related to the bioeconomical potential of eustigmatophyceae**

During work on this review, we found 109 scientific papers concerning valuable compounds and different applications of Eustigmatophyceae in human affairs (Figures 1 and 2). There is a general trend towards increasing the number of publications on this topic in the last four decades (Figure 1). More than 50% of the works were published after 2011, with a twofold increase in the number of papers published in the period 2011–2018 in comparison with the previous decade (59 and 24 papers, respectively). A closer look at the main focus of these papers reveals that most of them are related to studies on lipid content and biofuels (Figure 2), while other topics have received much less attention so far. The least exploited topic is that of the application of eustigmatophycean phytohormones, which had been studied only in *Nannochloropsis* with finding of abscisic acid and cytokinin as promising for biotechnological purposes [168].

**Conclusions**

Eustigmatophyceae have all the microalgal advantages of cultivation as a superior feedstock (effective
land and CO₂ utilization, hundred times higher growth rate in comparison with terrestrial plants and possibility to double their biomass in a day, self-purification if coupled with wastewater treatment) in combination with exceptionally high adaptability. They do not compete with other sources and contain a great palette of valuable bioactive compounds, which could be used by humans separately, or combined in different products. Still, many aspects of the applied research on eustigmatophyceans remain underexplored or yet awaiting to be exploited (e.g. their potential as biofertilizers, as producers of antibiotic metabolites in the pharmaceutical industry, or as compounds in molecular cooking). Therefore, we have to state that the great promising bioeconomical potential of these stramenopilic microalgae has generally been recognized, but in the present state of its infancy is far from being realized to its full potential.

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