

Article

Halamphora kolbei (Aleem) Álvarez-Blanco et S. Blanco 2014, A Rare Diatom from the Black Sea: Morphological Observations and Revised Description with Biochemical Composition

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Simple Summary

This study addresses the isolation and taxonomic characterisation of the poorly known diatom *Halamphora kolbei*, integrating fundamental and applied research perspectives. Due to its small cell size and incomplete original description, *H. kolbei* has remained difficult to identify, and published data on the species are extremely scarce. In this work, we summarise the available literature and provide a detailed morphological description of a strain isolated from Sevastopol Bay (Black Sea) using light microscopy, scanning electron microscopy, and phylogenetic analysis. In addition, the strain was evaluated for its productivity and biochemical composition. The results demonstrate that *H. kolbei* is a promising source of eicosapentaenoic acid, an essential omega-3 polyunsaturated fatty acid. This study contributes to the taxonomy and phylogeny of the genus *Halamphora* and highlights the biotechnological potential of this species for the sustainable production of high-value nutrients.

Abstract

The species *Halamphora kolbei* is a rarely reported diatom, and information on its distribution and characteristics is limited. The original taxonomic description of this species is inaccurate and insufficient for identification, as the available literature contains scarce and scattered data. In the present study, we summarised the available data on this species and provided a detailed description of the strain isolated from Sevastopol Bay of the Black Sea. Findings from a phylogenetic analysis of two genes, 18S rRNA and *rbcL*, are presented. The process of isolation, identification and establishment of a monoculture of the *H. kolbei* is described in detail. In addition, the productivity and biochemical characteristics were evaluated to assess the biotechnological potential of the strain. The growth rate, biomass productivity and protein, carbohydrate, lipid and fatty acid composition of this species were determined in this study, under controlled conditions.

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The high content of C14–C16 acids in *H. kolbei* has been revealed to make it a promising source of eicosapentaenoic acid. The detailed description of the species, its photographs and phylogeny, as well as additional morphological keys presented in the paper, will enable scientists around the world to study this species as a promising biological resource.

Keywords: benthic diatom; *Halamphora kolbei*; Black Sea; biochemical composition; bioprospecting; phylogenetic analysis

1. Introduction

For a long time, most dorsiventral diatoms were classified as belonging to the extremely extensive genus *Amphora* C.G. Ehrenberg ex F. T. Kützinger 1844. In fact, *Amphora sensu lato* is broadly defined, encompassing a heterogeneous group of diatoms that includes species from several distinct genera: *Amphora* Ehrenb. ex Kütz. 1844 sensu stricto, *Halamphora* (P. T. Cleve) Levkov [1–3], *Tetramphora* Mereschkowsky [4] and *Seminavis* D. G. Mann [5]. Due to the morphological similarities between species from different genera, high diversity and numerous misinterpretations, an increasing number of studies are being published to clarify their phylogeny [1–3,6–15].

In 2009, Z. Levkov revised the genus *Amphora* and separated the new genus *Halamphora* (P. T. Cleve) Levkov 2009, based on its morphological differences. The author's description of the genus includes: solitary cells, moderately or strongly dorsiventral. Frustules in girdle view are broadly elliptical to rectangular with shortly protracted and broadly rounded to truncate ends, always asymmetrical about the apical plane. The two raphe systems are positioned on the same side of the valve. The valves are linear, semi-lanceolate to semi-elliptical, with a smoothly arched dorsal margin that is straight or constricted in the middle, and a ventral margin that is concave or weakly convex with inflation. The valve ends are variable: extended, shortly subprotracted, broadly rounded, or narrowly rounded and capitate. The ventral mantle is shallow. The striae are uniseriate or biseriate and consist of round, elliptical or transversely elongated areolae. The raphe lies on a raphe ledge and is elevated above the rest of the valve on the dorsal side. The proximal raphe endings are straight or bent ventrally. The distal raphe endings are dorsally bent. The distal raphe endings terminate internally with poorly developed helictoglossae and the proximal endings with fused central helictoglossae [1]. Despite its close resemblance to the genus *Amphora*, two main distinguishing features of the genus *Halamphora* have been identified: (1) the presence of a single H-shaped chloroplast appressed to the ventral girdle and strongly constricted in the middle; (2) the girdle consists of numerous bands that comprise open copulae with one or two rows of round or transversely elongated poroids. In addition, most species of the genus *Halamphora* are brackish and marine, while species of the genus *Amphora* are freshwater or brackish [1]. Furthermore, *Amphora sensu lato*, until recently, was a polyphyletic group that included unrelated genera that are similar in their dorsiventral form. However, molecular phylogenetic studies have confirmed that the species classified as *Halamphora* and *Amphora* form separate, albeit related, clades [3].

Currently, numerous studies describe various species of the genus *Halamphora*, enabling their accurate identification [16–19]. There are many small-celled species within the genus *Halamphora* that are rare in nature and difficult to diagnose. Such species include *Halamphora kolbei* (Aleem) Álvarez-Blanco et S. Blanco 2014. This species was originally described in a work devoted to littoral diatoms of the Eastern Mediterranean (Egyptian coast), where drawings of the species were presented [20]. This species was reported from the coastal waters of the Volo Bay, Greece (Aegean Sea) [21] and in the Mediterranean Sea

in general without a specific location [22]. Later, after Z. Levkov distinguished the genus *Halamphora* [1], *A. kolbei* was reclassified from the genus *Amphora* to this genus on the basis of morphological studies conducted using light and scanning electron microscopy [23]. As *H. kolbei*, the species was reported in Neum Bay of the Adriatic Sea [24]. Overall, the highest number of records for this species is from the seas of the Mediterranean region. Additionally, *H. kolbei* has been found in the Gulf of Mexico (Atlantic Ocean) [25,26] and in the Gulf of California, a marginal sea of the Pacific Ocean [27,28].

Halamphora kolbei, a species described for the Mediterranean Sea, had not been previously documented in the Black Sea. It was recently discovered off the Turkish coast of the Black Sea [29], and later off the Bulgarian coast of the Black Sea [30]. In references describing *H. kolbei*, the morphological description is limited to characteristics such as length, width, and dorsal striae [20–23,29,30]. More fine details, such as girdle bands and poroids, are not provided, and the presence of ventral striae remains unclear. In total, the species is mentioned five times in the literature that includes a description of it [22,23,25,29,30]. Its rarity might result from the challenge of accurately identifying it in natural samples. At the same time, a similar species, *Amphora bigibba* Grunow 1875, is quite widespread in the Black Sea [31–34] and has been found in all climatic zones except the Antarctic [27,28,35–42]. *H. kolbei* may be confused with *A. bigibba* because only a few studies contain complete, detailed morphological descriptions of this species, and no phylogenetic studies have been conducted. The identification of species is a key part of many tasks, including the search for biological resources, ecology, and biodiversity. Morphological descriptions and genetic data are not available for *H. kolbei*. Therefore, a comprehensive study of *H. kolbei*, examining its morphology, genetics, and biochemical composition, is important to analyse its biotechnological potential. All of these are elements of bioprospecting, a process that is extremely important today. The term ‘bioprospecting’ is used to describe the process of searching for bioactive compounds from natural sources, such as plants, fungi, bacteria, and marine organisms. Bioprospecting from natural products involves biological material selection, collection, isolation, identification of natural organisms, bioactivity screening, safety and activity validation, mass production, and commercialisation [43]. The main stage of this process is searching for species. The concept of a species is based on genetic, biochemical and phenotypic similarities. A strain comprises the descendants of a single organism and is classified based on its unique characteristics (ecological, biochemical, functional, etc.). The term strain is commonly used to denote a pure culture [44–46]. Scientific works on the isolation and identification of microorganisms contribute to solving both fundamental and applied global problems. On the one hand, such works are significant in terms of evolution and taxonomy, contributing to our understanding of microbiological ecology and the preservation of biodiversity [47]. On the other hand, this process has historically played a pivotal role in the development of new biotechnologies and drugs, with many therapeutics derived from or inspired by natural products [48].

Marine microalgae represent a valuable reservoir of bioactive molecules, offering significant potential for various biotechnological sectors, including the food, feed, pharmaceutical, cosmetic, and biofuel industries. Research into biological resources is underway, with scientists searching globally for biotechnologically valuable diatoms [49,50]. Bioprospecting has revealed that diatoms can synthesise biologically active compounds such as polyunsaturated fatty acids, carotenoids, fucoxanthin, lipids, and sterols, among others [50–54]. Evidence suggests that species of the genera *Amphora* and *Halamphora* exhibit high lipid content and a unique fatty acid composition [49,55–60]. Isolation and cultivation of microalgal strains of interest are essential steps to laboratory investigations and successful commercial application. This is a stepwise process with each step playing an integral role in the choice of the following [44]. Therefore, obtaining pure

cultures of microalgal strains from these genera to study their morphology, taxonomic affiliation, and biochemical features addresses several urgent scientific issues in both fundamental and applied algology.

The goal of our research was to isolate an *H. kolbei* strain from the Black Sea in pure culture for morphological description, genetic identification, determination of culture growth kinetics, and analysis of biomass composition and specific compound content (fatty acids and fucoxanthin).

2. Materials and Methods

2.1. Isolation and Cultivation

Marine diatoms of epilithon from the Sevastopol Bay of the Black Sea were sampled in September 2021 (44°62'8229" N, 33°52'4878" E). Samples were collected at the water temperature of 20 °C and salinity of 18 psu. For *H. kolbei* isolation, a small volume of this suspension was transferred into Petri dishes and cultured in sterile F/2 medium [61] prepared with filtered seawater. The medium was sterilised by autoclaving at 1 atmosphere and a temperature of 121 °C for 15 min. After approximately one week of enrichment, single living diatom cells were isolated using micropipettes under a Bresser Bio Science Trino (Bresser Optics, Rhede, Germany) light microscope (LM). Diatom cells were transferred into sterile Petri dishes filled with culture medium, labelled and placed under controlled conditions, close to those found in nature (temperature 20 ± 1 °C, irradiation $18.5 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ (4 W m^{-2}), 14 h:10 h L:D cycle) in a KS-200 climate chamber (Russia). The isolated *H. kolbei* strain was registered in the microalgae and cyanobacteria working collection of the Department of Aquaculture and Marine Pharmacology of the A.O. Kovalevsky Institute of Biology of the Southern Seas of RAS in 2022. Strain ID: DIA-A.big.-SevBay.

2.2. Morphological Identification

Images of live cells were obtained using an Axioskop 40 C. Zeiss (Carl Zeiss AG, Oberkochen, Germany) LM at magnifications of 10×20 , 10×40 , and 10×100 . Samples for scanning electron microscopy (SEM), Hitachi SU3500 (Hitachi LTD, Tokyo, Japan) were prepared according to [62]. The diatom suspension was cleaned of organic matter by treating it with KMnO_4 for 24 h, after which HCl was added, and the mixture was heated to remove insoluble salts (e.g., carbonates). The acid was then removed from the samples by rinsing them with distilled water, a process that involved repeated centrifugation. Dried diatom valve preparations were coated with gold-palladium for SEM visualisation.

The terminology of valve ultrastructure follows that of [1,31,32,63–67] and articles [68,69]. Systematics are provided in accordance with [70].

2.3. Molecular Study

Total DNA from the studied strain was extracted using Chelex 100 Chelating Resin, molecular biology grade (Bio-Rad Laboratories, Hercules, CA, USA), according to the manufacturer's protocol. Partial SSU rRNA (378–382 bp, including the highly variable V4 region) and partial *rbcL* plastid genes were amplified using primers D512for (5'-ATTCCAGCTCCAATAGCG-3') and D978rev (5'-GACTACGATGGTATCTAATC-3') from [71] for SSU rDNA fragments, and *rbcL*404+ (5'-GCTTTACGTTTAGAAGATATG-3') and *rbcL*1444- (5'-GCGAAATCAGCTGTATCTGTW G-3') from [72] for *rbcL* fragments. Amplifications were performed using premade polymerase chain reaction (PCR) master mixes (ScreenMix by Evrogen, Moscow, Russia). The SSU rRNA gene was amplified under the following conditions: initial denaturation for 5 min at 95 °C, followed by 35 cycles of 30 s denaturation at 94 °C, 30 s annealing at 52 °C, and 50 s extension at 72 °C,

with a final extension for 10 min at 72 °C. The *rbcL* gene was amplified under the following conditions: initial denaturation for 4 min at 94 °C, followed by 40 cycles of 50 s denaturation at 94 °C, 50 s annealing at 53 °C, and 80 s extension at 72 °C, with a final extension for 7 min at 72 °C. PCR products were visualised by horizontal electrophoresis in 1.0% agarose gel stained with SYBR™ Safe (Life Technologies, Carlsbad, CA, USA). The products were purified with a mixture of FastAP, 10× FastAP Buffer, Exonuclease I (Thermo Fisher Scientific, Waltham, MA, USA), and water. Sequencing was performed by Syntol LLC (Moscow, Russia).

Consensus sequences were assembled and edited from forward and reverse chromatograms using Ridom TraceEdit ver. 1.1.0 (Ridom GmbH, Münster, Germany) and Mega ver. 11 software [73].

For phylogenetic analysis, strains of *Amphora* Ehrenberg ex Kützinger and *Halamphora* (Cleve) Levkov were selected from GenBank for which SSU rRNA and *rbcL* gene sequences were available. Two *Tetramphora* Mereschkowsky strains were chosen as outgroups. The reads of the *H. kolbei* strain were included in the alignments along with corresponding sequences of 42 diatom species downloaded from GenBank (taxa names and accession numbers are given in Table S1).

Nucleotide sequences of the SSU rRNA and *rbcL* genes were aligned separately with the MAFFT ver. 11 software (RIMD, Osaka, Japan) and the E-INS-i model [74]. The resulting alignments were then trimmed by removing the visually identified unpaired sites from the beginning and end of the resulting matrices. For the protein-coding sequences of the *rbcL* gene, it was verified that the beginning of the aligned matrix corresponds to the first position of the codon (triplet). The final alignments had lengths of 450 (SSU rDNA) and 994 (*rbcL*) characters. After removal of the unpaired regions, the aligned SSU rRNA gene sequences were combined with the *rbcL* gene sequences into a single matrix using Mega11 for phylogenetic reconstruction.

Bayesian inference (BI) was used to analyse the dataset with BEAST ver. 1.10.1 software (BEAST Developers, Auckland, New Zealand) [75]. The most appropriate partition-specific substitution models, shape parameter α , and proportion of invariable sites (pinvar) were selected using the Bayesian information criterion (BIC) in jModel-Test ver. 2.1.10 software (Vigo, Spain) [76]. This BIC-based model selection procedure selected the following models, shape parameter α , and proportion of invariable sites (pinvar): NKY + I + G, $\alpha = 0.4170$, and pinvar = 0.6920 for the first codon position of the *rbcL* gene; JC + I + G, $\alpha = 0.4320$, and pinvar = 0.8160 for the second codon position of the *rbcL* gene; GTR + G, $\alpha = 0.5530$ for the third codon position of the *rbcL* gene, NKY + I + G, $\alpha = 0.2820$, and pinvar = 0.5320 for 18S rDNA. A Yule process tree prior was used as the speciation model. Five MCMC analyses were run for seven million generations (burn-in: 1000 million generations). Convergence diagnostics were performed in the Tracer ver. 1.7.1 software (MCMC Trace Analysis Tool, Edinburgh, UK) [75]. The initial 10% trees were discarded, and the remaining trees were used to construct a final chronogram with 90% posterior probabilities. FigTree ver. 1.4.4 (University of Edinburgh, Edinburgh, UK) and Adobe Photoshop CC ver. 19.0 software (Adobe, San Jose, CA, USA) were used for viewing and editing the trees.

2.4. Intensive Cultivation

2.4.1. Cultivation Conditions

The cells of the obtained strain were used as an inoculum at a concentration of 10% (*v/v*) for batch cultivation in one-litre flasks. An intensive culture of *H. kolbei* was grown in 5F nutrient medium under constant temperature conditions of 20 ± 1 °C. The photobioreactor was provided with round-the-clock, one-sided illumination by white PILA LF36W/54-765-2450 lm lamps (Philips, Warszawa, Poland). The average irradiation

of the photobioreactor's working surface was $170 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (33 W m^{-2}). During the growth process, the culture was bubbled with air at a rate of 1.1 litres per litre of culture per minute using a compressor unit. To increase the solubility of atmospheric CO_2 , a dispersant attachment was used in the culture medium. 5F nutrient medium was prepared with sterile seawater supplemented with the following components: $0.75 \text{ g}\cdot\text{L}^{-1} \text{ NaNO}_3$, $0.05 \text{ g}\cdot\text{L}^{-1} \text{ NaH}_2\text{PO}_4 \times \text{H}_2\text{O}$, $0.3 \text{ g}\cdot\text{L}^{-1} \text{ Na}_2\text{SiO}_3 \times 9\text{H}_2\text{O}$, $1\cdot 10^{-4} \text{ g}\cdot\text{L}^{-1} \text{ CuSO}_4 \times 5\text{H}_2\text{O}$, $2.2\cdot 10^{-4} \text{ g}\cdot\text{L}^{-1} \text{ ZnSO}_4 \times 7\text{H}_2\text{O}$, $1\cdot 10^{-4} \text{ g}\cdot\text{L}^{-1} \text{ CoCl}_2 \times 6\text{H}_2\text{O}$, $1.8\cdot 10^{-4} \text{ g}\cdot\text{L}^{-1} \text{ MnCl}_2 \times 4\text{H}_2\text{O}$, $0.63\cdot 10^{-4} \text{ g}\cdot\text{L}^{-1} \text{ Na}_2\text{MoO}_4 \times 2\text{H}_2\text{O}$, $0.0437 \text{ g}\cdot\text{L}^{-1} \text{ Na}_2\text{EDTA}$, $0.0315 \text{ g}\cdot\text{L}^{-1} \text{ FeSO}_4 \times 7\text{H}_2\text{O}$ [77]. The nutrient medium was supplemented with 0.1 g/L of Polysorb MP sorbent (particle size up to $90 \mu\text{m}$) (Polisorb, Kopeisk, Russia), as a microcarrier. The addition of the microcarrier prevented the culture from adhering to the flask walls. The density of the culture at the start of the batch cultivation was 0.2 g of dry weight (DW) per 1 L .

2.4.2. Growth Kinetics and Calculations

Microalgae growth was monitored daily by measuring the wet biomass weight on a Scout SPX222 (OHAUS Ltd., Parsippany, NJ, USA) analytical balance after centrifugation at 1600 g for 5 min at 20°C on an OPN-3 centrifuge (TNK Dastan, Bishkek, Kyrgyzstan). The microalgae biomass was rinsed with distilled water via centrifugation at 6000 g for 15 min at the end of the cultivation. The supernatant was then removed, and the biomass was weighed on an analytical balance with an accuracy of 0.0001 g . Subsequently, the biomass was dried at 105°C in a RA-50/500VZ drying oven (Micron, Shatura, Russia) to constant weight. The water fraction in the wet biomass was determined using the method described in [78]. A coefficient ($k = 0.14$), representing the relationship between dry weight and wet weight and determined experimentally, was used to convert the data to dry weight.

The specific growth rate was determined by measuring changes in culture density during the exponential growth phase using the equation:

$$\mu = \frac{\ln B - \ln B_0}{t - t_0}$$

where μ is the specific growth rate, day^{-1} ; B —is the current culture density at time t , $\text{g}\cdot\text{L}^{-1}$; B_0 is the culture density at the previous time t_0 , $\text{g}\cdot\text{L}^{-1}$.

The biomass productivity during the linear part of the exponential growth phase was calculated using the equation:

$$P = \frac{B - B_0}{t - t_0}$$

where P is the biomass productivity, $\text{g}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$; B is the current culture density at time t , $\text{g}\cdot\text{L}^{-1}$; B_0 is the culture density at the previous time t_0 , $\text{g}\cdot\text{L}^{-1}$.

2.4.3. Analysis of Biomass Composition

Lipid Content and Fatty Acid Composition

Total lipid mass fraction was quantified via the phosphovanillin method [79]. An aliquot (10 mL) of an algal suspension with a known cell concentration was centrifuged at 1600 g for 5 min . The resulting algal pellet was then used for extraction after the removal of the supernatant. This was homogenised with quartz sand using a mechanical homogeniser, then extracted with a Folch mixture (first 5 mL at a $1:1 \text{ v/v}$ ratio, then 5 mL at a $2:1 \text{ v/v}$ ratio). The extraction process was repeated twice to ensure complete lipid recovery. The combined lipid extracts were washed 3–4 times with water to remove non-lipid contaminants. Subsequently, an aliquot of the purified lipid extract was evaporated

and digested in sulfuric acid by heating in a water bath at 100 °C for 20 min. After cooling, the phosphovaniline reagent was added to develop colour. The optical density of the resulting solution was measured at a wavelength of 530 nm using an SF-2000 spectrophotometer (OKB Spektr, Saint Petersburg, Russia). A calibration curve was prepared using standard solutions of olive oil in chloroform as previously described [79].

The lipid productivity over the entire cultivation period was calculated using the following equation:

$$P_L = \frac{C_L \cdot (B_{st} - B_0)}{t}$$

where P_L is the lipid productivity ($\text{mg}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$); C_L is the lipid content in the biomass ($\text{mg}\cdot\text{g}^{-1}$); B_{st} is the culture density in the stationary growth phase ($\text{g}\cdot\text{L}^{-1}$); B_0 is the culture density at the initial time point ($\text{g}\cdot\text{L}^{-1}$); t is the cultivation time (days).

Fatty Acid (FA) Determination

Fatty acid analysis was performed by extracting lipids from the raw material using a Folch mixture. The extracted lipids were washed with distilled water to remove the non-lipid impurities.

A lipid extract aliquot with 3–5 mg of total lipids was evaporated to dryness using a rotary evaporator RV 10 (IKA, Staufen, Germany) at 25 °C. The residue was subjected to saponification by adding 5 mL of an alkaline methanol solution (10 mL of 3 N NaOH with 90 mL of 90% methanol) and heating at 64–65 °C for two hours. After cooling, a few drops of a 1% phenolphthalein solution in ethanol were added. The mixture was extracted three times with 5 mL of hexane to remove unsaponifiable fractions. The aqueous-alcohol phase was acidified by adding 0.3 mL of 6N HCl. Then, fatty acids were extracted three to four times. The combined hexane extracts were evaporated to dryness using a rotary evaporator at 30 °C. To the residue, 5 mL of a 3% (*v/v*) hydrochloric acid in methanol was added for esterification of the FA, and the mixture was refluxed for two hours. After cooling, the resulting fatty acid methyl esters (FAMES) were extracted three times with 5 mL of hexane. The hexane fraction was filtered through an ash-free filter paper and stored at −20 °C for no longer than 24 h prior to analysis.

FAMES were analysed with a Khromatek Crystal 5000.2 (CJS Special Design Bureau “Khromatek”, Yoshkar-Ola, Russia) gas chromatograph equipped with a flame ionisation detector (FID) and a mass spectrometric detector (MSD). The analysis was performed at the Spectrometry and Chromatography core facility in A. O. Kovalevsky Institute of Biology of the Southern Seas of RAS. Separation was achieved using a 60 m BPX5 capillary column (Trajan Scientific Europe Ltd./SGE Analytical Science, Milton Keynes, UK) with an internal diameter of 0.25 cm and a stationary phase thickness of 0.25 μm . The temperature program was as follows: (1) hold at 40 °C for 1 min, (2) increase to 220 °C at 10 °C/min and hold for 6 min, then (3) increase to 280 °C at 10 °C/min and hold for 10 min.

The composition of FA was determined using PID. A mixture of acids, “Supelco 37 component FAME mix” (Sigma-Aldrich, St. Louis, MO, USA) was used as an external standard. FAs not present in the commercial standard mixture were identified by analysing the chromatogram obtained in total ion current mode. Their mass spectra were compared to reference spectra in the NIST 14 library. The mass scanning range was 50–450 *m/z*. Margaric acid was employed as an internal standard [80].

The FA productivity (P_{FA}) over the entire cultivation period was calculated using an equation analogous to that for lipid productivity, substituting the total lipid content with the total FA content in the biomass.

Determination of Total Carbohydrates

The carbohydrate content and mass fraction in the biomass were determined following the Dubois method [81] using crystalline glucose as the standard. A 1 mL sample was taken from a suspension with a predetermined cell concentration and centrifuged for five min at 1600 g. After discarding the supernatant, the resulting algal pellet was rinsed twice with 5 mL of distilled water. Subsequently, 2.5 mL of a phenolic reagent was added to the pellet. After 10 min, 2.5 mL of sulfuric acid was added, and the mixture was placed in a water bath at 100 °C for five min. Following colour development, the optical density of the samples was quantified at a wavelength of 490 nm using an SF-2000 spectrophotometer (OKB Spectr, Saint-Petersburg, Russia). A standard curve was prepared using glucose (0.1 µg·µL⁻¹).

Protein Determination

The protein content was determined by the Lowry method [82], using crystalline human serum albumin as the standard for the calibration curve. A 1 mL aliquot of a suspension with a predetermined cell concentration was centrifuged for five min at 3000 g. The supernatant was removed, and the algal pellet was washed with 5 mL of distilled water. Protein hydrolysis was then carried out in a water bath at 100 °C with 1 N alkali for 10 min. After hydrolysis, the samples were processed according to the Lowry protocol, and their optical density was measured using an SF-2000 spectrophotometer (Russia).

Extraction of Fucoxanthin

Fucoxanthin (Fx) content in the biomass was determined using thin-layer chromatography (TLC) [83]. Fx was extracted from the raw biomass with 96% ethanol using a mechanical homogeniser with quartz sand until the biomass was completely discoloured. An aliquot (0.1 mL) of the extract was applied to chromatographic glass plates coated with a 0.5 mm layer of silica gel. Carotenoid separation was performed using an acetone-hexane (3:7, *v/v*) solvent system. The silica gel zone corresponding to Fx was extracted with ethanol three times until complete decolouration. The combined extracts were centrifuged 3000 g. The Fx concentration was determined by measuring the optical density of the solution at a wavelength of 448 nm and converting it to the dry weight of the algae using the equation:

$$Fx = \frac{10 \cdot V_1 \cdot V_2 \cdot D_{448}}{E \cdot V_3 \cdot W}$$

where Fx is the fucoxanthin concentration in the biomass, mg·g⁻¹; V₁ is the volume of the combined eluate from the TLC plate, mL; V₂ is the total volume of the initial extract, mL; V₃ is the volume of the aliquot applied to the TLC plate, mL; D₄₄₈ is the optical density measured at 448 nm; W is the dry weight of algal sample, g; and E = 1280 mL/(mg·cm) is the specific extinction coefficient for 1% solution of Fx in ethanol [84].

For identification, crystalline fucoxanthin, previously characterised by HPLC, mass spectrometry and NMR spectroscopy, was used as a standard [85].

The Fx productivity (*P_{Fx}*) over the entire cultivation period was calculated using an equation analogous to that for lipid or FA productivity, substituting the total lipid or FA content with the total Fx content in the biomass.

Determination of the Ash Residue

The mass fraction of the ash residue was determined, accounting for the biomass water content. It was calculated from the weight difference before and after sample combustion in a muffle furnace at 550 °C for 4 h.

2.5. Statistical Analysis

For each morphological characteristic (length, width, number of striae, and poroids), 30 measurements were taken. Statistical analyses were performed using Past 4.17 [86]. Data distribution was assessed using the Shapiro–Wilk normality test (W) for each dataset. Since the morphometric results had a non-normal distribution, they are presented as median (med) with interquartile range (IQR: 25th–75th percentile). All analyses of the culture characteristics (dry weight, culture density, productivity, and content of compounds in biomass) were performed in triplicate. The results are presented as mean $\pm$ confidence intervals (CI). CI for the mean were calculated using the Student *t*-distribution at the 95% confidence level using the built-in function in Microsoft Excel. Data plotting was performed using Grapher 3D ver. 3 (Golden Software LLC, Golden, CO, USA).

3. Results

In this research, we present *H. kolbei*, a little-known taxon discovered in the Black Sea epilithon, as a potential candidate for biotechnological applications. Our analysis is based on morphological and genetic data collected from cultured strains. We also explore the similarities and distinctions with morphologically similar species.

3.1. Systematics and Morphology

Class Bacillariophyceae Haeckel 1878 emend. D. G. Mann in Round et al. 1990

Order Naviculales Bessey 1907

Family Catenulaceae Mereschkowsky 1902

Genus *Halamphora* (P.T. Cleve) Levkov 2009

Halamphora kolbei (Aleem) Álvarez-Blanco et S. Blanco 2014

Description (Figures 1 and 2). In natural populations, cells occur singly. In culture, the species forms a film. Chloroplast is H-shaped. Frustules are elliptical and constricted in the middle. The valves are asymmetric with respect to the apical axis but symmetric with respect to the transapical axis. The valve mantle is deeper on the dorsal margin than on the ventral margin. The raphe is located on the ventral margin. Apices are broadly rostrate. The cells are small, 8.6–15 μm in length ($W = 0.894$, $p = 0.006$; med = 13.5, IQR: 12.5–14.0), 3.5–8.5 μm in width at the widest part of the valve ($W = 0.0771$, $p = 0.00002$; med = 4.5, IQR: 4.0–5.1), 2.4–8.1 μm at the constriction ($W = 0.748$, $p = 0.000008$; med = 3.5, IQR: 3.0–4.2) and up to 12 μm in girdle view. Dorsal striae slightly radiate, 24–32 in 10 μm ($W = 0.900$, $p = 0.008$; med = 26, IQR: 26–28), ventral striae 34–38 in 10 μm ($W = 0.880$, $p = 0.003$; med = 36, IQR: 34–38). Girdle bands are numerous (near 10–16), with 36–42 poroids in 10 μm ($W = 0.875$, $p = 0.002$; med = 39, IQR: 37–42). Along the dorsal side of the sternum, there is a row of areolae oriented along the apical axis (indicated by arrows on Figure 2g,h), contrasting with the areolae on the rest of the dorsal surface.

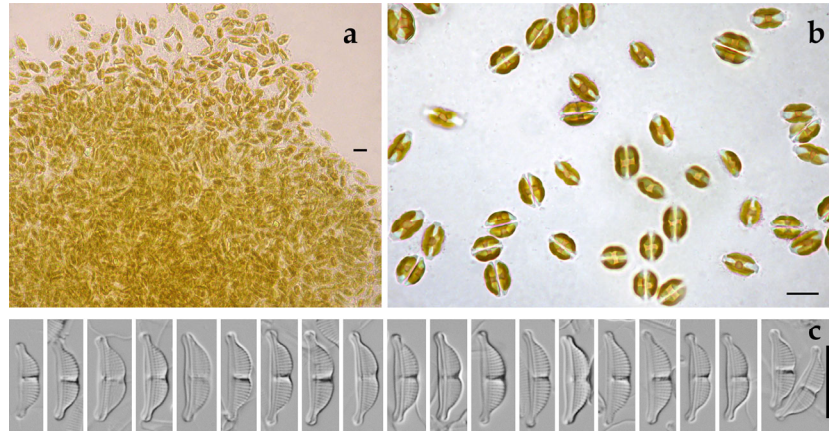


Figure 1. *Halamphora kolbei*, strain DIA-A.big.-SevBay under LM: living cells (a,b); cleaned frustules in valve view (c). Scale bar: 10 μ m.

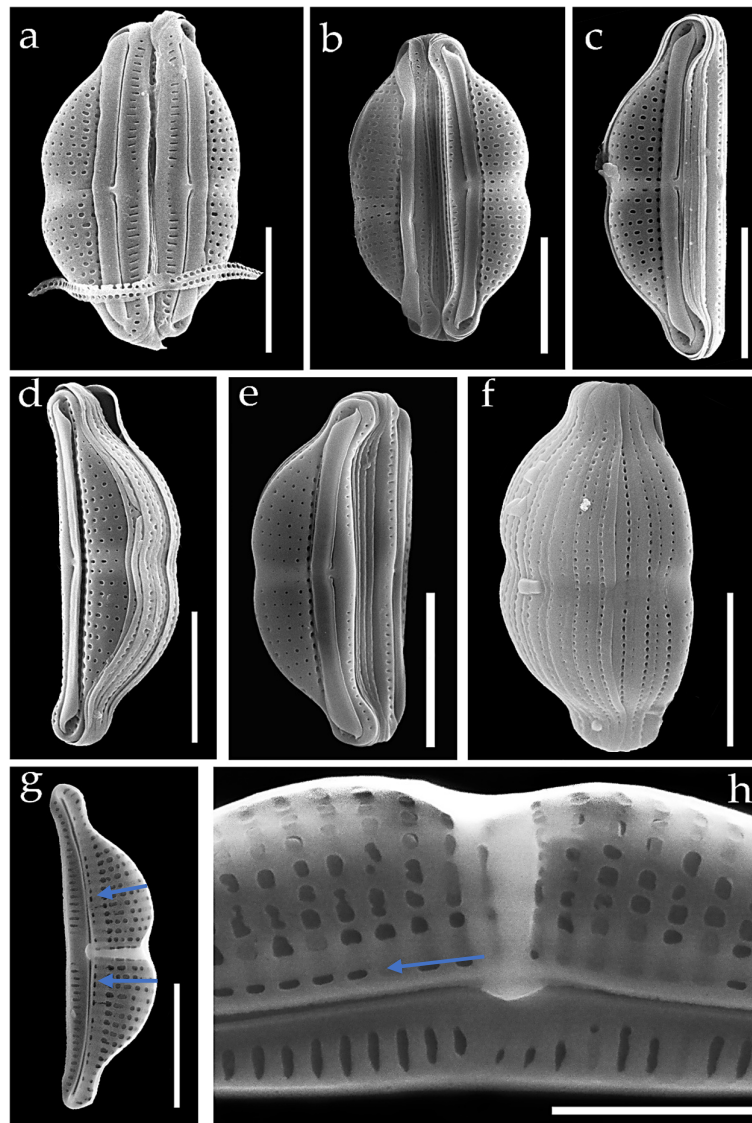


Figure 2. *Halamphora kolbei*, strain DIA-A.big.-SevBay frustules, SEM: in ventral girdle view (a,b); external valve view (c–e); in dorsal girdle view demonstrating details of girdle bands (f); internal

valve view (**g,h**). Scale bar: (**a–g**) 5 μm , (**h**) 2 μm . Blue arrows indicate a row of areolae that oriented along the apical axis.

3.2. Phylogenetic Analysis

Phylogenetic analysis based on the 18S rRNA and *rbcL* genes showed that *Amphora* and *Halamphora* strains form separate clades (Figure 3). The *Halamphora* clade has maximum statistical support (PP = 1), whereas support for the *Amphora* clade is lower (PP = 0.89).

Halamphora strains form two highly supported subclades (PP = 1). The first includes *H. hyalina* and *H. pseudohyalina*, the second includes all other strains of our dataset. In general, the branches within the clade are well supported (PP = 0.73 and higher). The relationships within the *Amphora* clade are well supported (PP 0.74–1) with the exception of branches of some species with weakly statistical support, namely *A. thrumensis*/*A. commutata* (PP 0.57), *A. cf. immarginata*/*A. lativentralis* (PP 0.56), *A. helenensis* (PP 0.63). *Halamphora* strains assigned to a single species (*H. gibba*, *H. subacutiuscula*, *H. americana*, *H. subtropica*) form maximally supported branches (PP = 1). The same is observed among strains of *Amphora* species (e.g., *A. commutata*, *A. abludens*, *A. aliformis*, *A. lativentralis*, *A. copulata*, etc.), indicating the absence of cryptic forms.

The new strain, *H. kolbei* DIA-A.big.-SevBay, is placed within the *Halamphora* clade and forms a separate branch together with the strain *Amphora bigibba* PMFTB0124 with maximum statistical support (PP = 1). The latter strain was isolated from the surface of the sea turtle (*Caretta caretta*) shells from the Adriatic Sea (Crotaria). Probably, this strain is misidentified and belongs to the genus *Halamphora*. Only SEM images of the internal valve side of the strain are publicly available [69], which does not allow for reliable identification. The resulting Maximum Likelihood tree presented in the cited work shows that strain PMFTB0124 (as in our analysis) is placed in a clade containing representatives of *Halamphora*, confirming its affiliation with this genus.

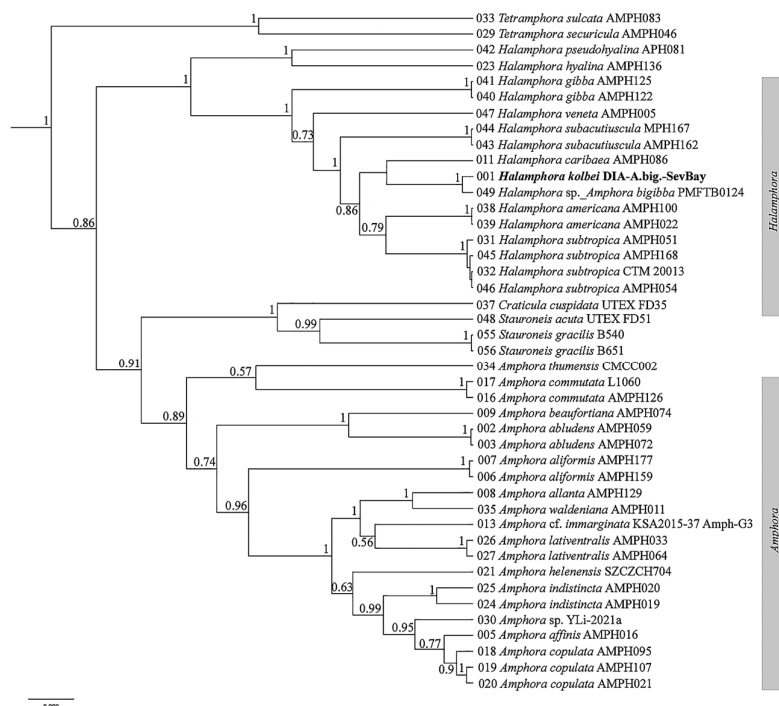


Figure 3. Phylogenetic position of the *Halamphora kolbei*, strain DIA-A.big.-SevBay (indicated in bold) based on Bayesian inference for the partial *rbcL* and 18S rRNA genes. The total length of the

alignment is 1444 characters. Posterior probabilities (PP) from BI are presented on the nodes in order. Only PP above 50 are shown. Strain numbers (if available) are indicated for all sequences.

3.3. Biotechnological Characteristics

3.3.1. Microalgae Growth

The biotechnological characteristics of the *H. kolbei* strain from the Black Sea were assessed through batch cultivation. An intensive culture with a maximum density of 1.44 ± 0.02 g DW·L⁻¹ was used to study biochemical characteristics. The culture reached this maximum density on day 10 (Figure 4).

The growth curve of *H. kolbei* in 5F medium shows an exponential phase with a linear stage of growth. On the first day of cultivation, the lag phase (1) transitioned into the exponential growth phase, during which the culture increased, followed by a straight line, indicating linear growth (2). The highest levels of biomass productivity (0.20 g·L⁻¹·day⁻¹) were seen during the first six days of the experiment. On the seventh day, the culture growth rate decreased (3) from 0.48 day⁻¹ to 0.06 day⁻¹, and the culture entered the stationary phase. The stationary phase was observed from the 10th day of the experiment (4). The specific growth rate is a critical parameter for selecting the production strain, as a high growth rate enables high productivity, reduces contamination risk, and shortens the cultivation time.

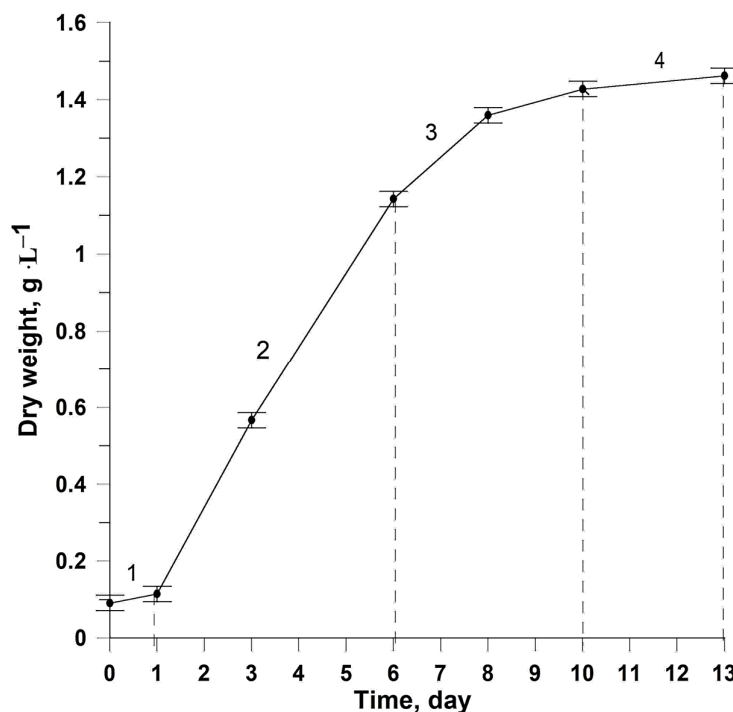


Figure 4. Growth kinetics of *Halamphora kolbei* strain in an intensive batch culture. Numbers indicate growth phases: 1—lag phase, 2—exponential phase, 3—deceleration phase, 4—stationary phase. The blue line indicates the transition to linear growth dynamics.

3.3.2. Biochemical Composition of the Biomass

A biochemical analysis of *H. kolbei* biomass at different growth phases under batch cultivation was carried out to assess the biotechnological potential (Table 1).

Table 1. Biochemical composition of *H. kolbei* biomass during batch cultivation.

Growth Phases	Lipids, %	Proteins, %	Carbohydrates, %	Ash, %	PUFA, mg·g ⁻¹	Fx, mg·g ⁻¹
Exponential						
Culture density B = 1.2 g·L ⁻¹	12 ± 0.1	40 ± 0.4	17.0 ± 0.2	33 ± 0.3	—	7 ± 0.4
Stationary						
Culture density B = 1.44 g·L ⁻¹	22 ± 0.1	35 ± 0.4	8 ± 0.2	37 ± 0.3	42.26 ± 0.5	17 ± 0.4

In *H. kolbei* biomass, the mass fraction of total proteins reached a maximum (40%) during the exponential growth phase. In the stationary phase, under conditions of nutrient depletion, protein synthesis slowed down and its content decreased to 35%. Protein productivity during exponential growth was 6.7 mg·L⁻¹·day⁻¹, and decreased to 2.7 mg·L⁻¹·day⁻¹ in the stationary phase. A similar trend was observed for the carbohydrate content, with a maximum mass fraction (17%) recorded during active growth and a decrease to 8% during the transition to the stationary phase. Carbohydrate productivity during exponential growth was 2.8 mg·L⁻¹·day⁻¹, and decreased to 0.6 mg·L⁻¹·day⁻¹ in the stationary phase. The lipid content in *H. kolbei* biomass doubled to 22% when the culture transitioned to the stationary phase, compared to 12% in the exponential phase. Lipid productivity over the entire cultivation period was 22 mg·L⁻¹·day⁻¹. The ash content in the *H. kolbei* culture averaged 33–37% of dry weight across different growth phases. This indicates that diatom biomass has a relatively low calorific value of approximately 3 kcal·g⁻¹ dry weight, as organic matter accounts for 62–67% of the dry weight.

3.3.3. Fatty Acid Composition

Fatty acids with chain lengths ranging from 14 to 22 carbon atoms were identified in the biomass of the studied *H. kolbei* strain (Table 2).

Table 2. The fatty acid composition and productivity of the *H. kolbei* strain during batch mode cultivation.

Fatty Acid	The Percentage Share of the Total FA, %	Concentration, mg·g ⁻¹ Dry Weight	Productivity, mg·L ⁻¹ ·day ⁻¹
Saturated FA			
Myristic (C14:0)	5.21 ± 0.06	3.99 ± 0.04	0.38
Pentadecanoic (C15:0)	0.54 ± 0.01	0.41 ± 0.05	0.04
Palmitic (C16:0)	12.02 ± 0.56	9.21 ± 0.32	0.88
Stearic (C18:0)	0.99 ± 0.01	0.76 ± 0.03	0.07
Total SFA	18.76	14.37	1.37
Monounsaturated FA			
Palmitoleic (C16:1ω7)	19.78 ± 0.55	15.60 ± 0.53	1.49
Elaidic (E) C18:1 (ω9)	0.77 ± 0.01	0.59 ± 0.01	0.06
Oleic (C18:1ω9)	4.38 ± 0.04	3.36 ± 0.06	0.32
Total MUFA	24.93	19.55	1.87
Polyunsaturated FA			
Hexadecatetraenoic (C16:4ω1)	0.36 ± 0.01	0.28 ± 0.01	0.03
Hexadecatrienoic (C16:3ω4)	3.33 ± 0.03	2.55 ± 0.21	0.24
γ-linolenic (C18:3ω6)	3.71 ± 0.04	2.84 ± 0.05	0.27
Linoleic (C18:2ω6)	1.74 ± 0.05	1.33 ± 0.03	0.13
Arachidonic (C20:4ω6)	1.76 ± 0.1	1.35 ± 0.02	0.13

Eicosotrienoic (C20:3 ω 6)	0.28 $\pm$ 0.01	0.21 $\pm$ 0.01	0.02
Eicosopentaenoic (C20:5 ω 3)	37.77 $\pm$ 0.42	28.95 $\pm$ 0.25	2.77
Docosahexaenoic (C22:6 ω 3)	7.33 $\pm$ 0.07	5.62 $\pm$ 0.05	0.54
Total PUFA	56.28	43.13	4.13
MUFA + PUFA	81.21	62.68	6.00
SFA + MUFA + PUFA	99.97	77.05	7.37
ω 6/ ω 3	0.170	0.17	

Notes: SFA—saturated fatty acids, MUFA—monounsaturated fatty acids, PUFA—polyunsaturated fatty acids.

The *H. kolbei* strain showed an interesting content of essential long-chain PUFAs, with ω -3 FAs being predominant, followed by ω -6 and ω -9 FAs. Among monoenoic fatty acids, palmitoleic acid (C16:1 ω 7) accounted for the highest proportion at 19.78% of total fatty acids. Among polyenoic fatty acids, eicosapentaenoic acid (C20:5 ω 3) was the most abundant at 37.77%, followed by docosahexaenoic acid (C22:6 ω 3) at 7.33%. This suggests that this species could be a valuable source of the rare and valuable eicosapentaenoic acid (EPA). The predominant saturated fatty acid was palmitic acid (C16:0, 12.02%). Overall, polyunsaturated fatty acids (PUFAs) predominated in the studied strain (56.28%), with an ω 6/ ω 3 ratio of 0.17. This ratio indicates that our strain is rich in omega-3 fatty acids. The productivities over the entire cultivation period were 1.37 mg·L⁻¹ day⁻¹ for SFA; 1.87 mg·L⁻¹ day⁻¹ for MUFA; and 4.13 mg·L⁻¹ day⁻¹ for PUFA.

3.3.4. Fucoxanthin

Active accumulation of *Fx* was observed during the deceleration and stationary growth phases. At the beginning of the stationary phase of growth (day 10), the *Fx* content was 10.0 $\pm$ 0.4 mg·g⁻¹ dry weight (culture density 14.4 mg·L⁻¹). By day fourteenth, the *Fx* content reached 17.0 $\pm$ 0.4 mg·g⁻¹ dry weight (culture density 24.99 mg·L⁻¹). Thus, the *Fx* productivity was 2.64 mg·L⁻¹·day⁻¹ during the stationary phase and 1.78 mg·L⁻¹·day⁻¹ over the entire batch cultivation period.

4. Discussion

4.1. Comparative Analysis of Morphological, Phylogenetic and Literature Data on *H. kolbei* and Closely Related Species

Morphological and phylogenetic analysis showed that our strain DIA-A.big.-SevBay from the Black Sea belongs to the genus *Halamphora*. The genus *Halamphora* is known for the following morphological features: a single, H-shaped chloroplast appressed to the ventral girdle and constricted in the middle; a girdle consisting of numerous bands comprising open copulae with one or two rows of round or transversely elongated poroids [1]. Images obtained with LM (Figure 1) and SEM (Figure 2) confirm the results of molecular-genetic analysis (Figure 3), allowing us to identify the studied strain as belonging to the genus *Halamphora*.

Since our strain exhibits a strong morphological affinity with *H. kolbei* and species identified as *A. bigibba* [34,39], we compared their morphological features and reviewed the literature on similar species (Table 3). Despite their bigibboid frustule shape being a common feature of these two species, *A. bigibba* has larger cells than *H. kolbei*, and the latter has more dorsal striae per 10 μ m.

Table 3. Comparison of morphological features of *H. kolbei* and closely related species.

Taxon	Frustule Shape	Valve Apices	Length, μm	Width, μm	Dorsal Striae in 10 μm	Distribution	References
<i>Halamphora kolbei</i> strain (DIA-A.big-SevBay)	elliptical, constricted in the middle	broadly rostrate	12.5–15	3.5–8.5 in valve view/till 12 in gridle view	24–31	The Black Sea	This study
<i>Amphora bigibba</i> from natural populations (most likely <i>H. kolbei</i>)	elliptical, constricted in the middle	broadly rostrate	8.6–12	2.8 (in valve view)	32	The Crimean coast of the Black Sea	[34]
<i>A. bigibba</i> from natural populations (most likely <i>H. kolbei</i>)	elliptical, constricted in the middle	broadly rostrate	11.5–12	3 (in valve view) 11.5 (in gridle view)	26–32	The Israeli sector of the Mediterranean Sea	[39]
<i>A. bigibba</i> (most likely <i>H. kolbei</i>)	elliptical, constricted in the middle	rostrate	13	3.5	30	Culture from the Adriatic Sea. The Croatian National Diatom Collection	[69]
	elliptical, constricted in the middle	broadly rostrate	14–36	10–15	15–20	Sevastopol and Novorossiysk bays of the Black Sea	[31]
	elliptical, constricted in the middle	broadly rostrate	15–25	4–6 (in valve view)	18–22	The Black Sea coasts of the Crimea and the Caucasus	[32]
	elliptical, constricted in the middle	rostrate	18–22	4–5	19–21	The Gulf of California	[37]
<i>A. bigibba</i>	lanceolate with strong constriction in the middle of the dorsal side	rostrate	12–20	4–5	19–20	Micronesia	[68]
	lanceolate with strong constriction in the middle of the dorsal side	broadly rostrate	28–33	valve width—5, frustule width—13–14	22–23	Kuwait	[87]
	lanceolate with strong constriction in the middle	protracted, rostrated	52.5	10 in valve view	17	Volo Bay of the Aegean Sea	[21]
	elliptical, constricted in the middle	broadly rostrate	15	7.5 in gridle view	—		
<i>Halamphora kolbei</i> (Aleem) Álvarez-Blanco et Blanco 2014	semi-lanceolate	rostrate to slightly capitate	12–18	2–4	21–24	The Mediterranean Sea	[22]

	elliptical, constricted in the middle	protracted	11.7–13.3	2.7–3.0	26	The Eastern Mediterranean	[23]
	elliptical, constricted in the middle	protracted, rostrate	13.8–17	3.16–3.40	22	The Turkish coast of the Black Sea	[29]
	elliptical, constricted in the middle	rostrate	15–18	3.2–3.9	23–25	Florida Bay	[25]
	elliptical, constricted in the middle	rostrate	10.5–15.5	2.3–2.4	24–27	The Bulgarian coast of the Black Sea	[30]
<i>Halamphora capitata</i> (R.Hagelstein) Álvarez-Blanco et Blanco 2014	lanceolate with strong constriction in the middle of the dorsal side	protracted	20	4.5	20–22	The Mediterranean Sea	[23]
	elliptical, constricted in the middle	protracted	17	4	24	The Gulf of California	[37]
	lanceolate with strong constriction in the middle of the dorsal side	protracted, rostrate	22.7	4.48	21	The Turkish coast of the Black Sea	[29]
	semi-lanceolate slightly constricted in the middle	subprotracted, subcapitate and ventrally bent	40–65	10–13 (in widest part of the valve)	11–12	The North Sea, The Baltic Sea	[1]

As shown in Table 3, the strain in this study and the species previously found in natural populations in the Black Sea range in length from 8.6 to 12 μm . However, according to [31,32], *A. bigibba* in the Black Sea is significantly longer, ranging from 14 to 36 μm . In [31], the illustration (Table VIII, Figure 19) representing *A. bigibba* shows numerous smooth girdle bands without poroids. The image of the species *A. bigibba* Grunow 1875 presented by the author matches the description in [63] (pl. 25: Figures 74 and 75). Therefore, we suggest that this species was previously misidentified due to a lack of opportunities for detailed morphological and genetic identification of specimens from natural populations [34]. The same is true for our work in the Israeli sector of the Mediterranean Sea [39], where we most likely observed the species *H. kolbei*. It cannot be ruled out that the same error occurred for the strain *A. bigibba* PMFTBO124 from the Adriatic Sea [69]. The phylogenetic tree we constructed (Figure 3) shows that this strain is similar to ours and belongs to the *Halamphora* clade. According to the image in [69], the strain dimensions are $13 \times 3.5 \mu\text{m}$, which is characteristic of *H. kolbei*.

A clear example for comparing *H. kolbei* and *A. bigibba* in terms of size and striae is the work of N. Foged [21], in which both species were found in the same water area of the Volo Bay (Aegean Sea) (Figure 5). The figure clearly shows that the difference in size between the species is more than twofold. *A. bigibba* has large striae that are clearly visible under a light microscope, while those of *H. kolbei* are practically indistinguishable.

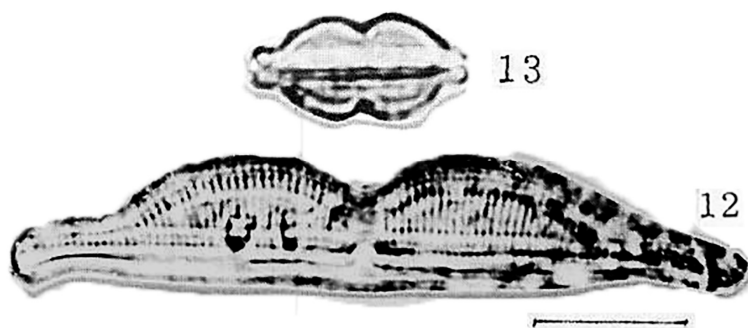


Figure 5. Images of diatoms *Amphora bigibba* (12) and *Halamphora kolbei* (13). Scale bar—10 μ m. Source: [21] (p. 63 Plate XI). Reproduced with permission from the copyright holder, Schweizerbart Science Publishers.

It should be noted that *A. bigibba* is characterised by the presence of two rows of areolae oriented along the apical axis along the dorsal side of the sternum, contrasting with the areolae on the rest of the dorsal surface, as noted by [68]. Such areolae are present in a single row for *H. kolbei* (indicated by arrows in Figure 2g,h).

We have now expanded the description of *H. kolbei* with information on the number of ventral striae in 10 μ m; however, their presence was not previously indicated for this species. For example, I. Alvarez-Blanco and S. Blanco, [23], who transferred *Amphora kolbei* to the genus *Halamphora*, did not note ventral striae in *H. kolbei* under LM [23] (pl. 34, Figures 7–9). However, in [30] it was suggested that the species *Amphora* sp. 4 [23] (pl. 81, Figure 2), which looks identical to the one described by the authors as *H. kolbei* [30] and to our strain. In [30], *H. kolbei* was reported on the Bulgarian coast of the Black Sea and noted that ventral striae are present in the Black Sea population. The authors state that ventral striae may be weakly visible under LM, but depending on the valve position, they may not be visible at all. Additionally, similarities for *Amphora* sp. 4 [23] are visible on the dorsal side of the sternum, where there is a row of areolae oriented along the apical axis (Figure 6), just as in our strain.

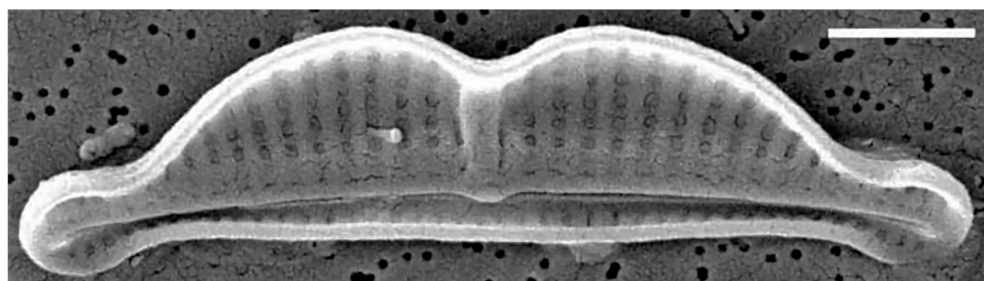


Figure 6. Image of diatom *Amphora* sp. 4. Scale bar—2 μ m. Source: [23] (Pl. 81, Figure 2). Reproduced with permission from the copyright holder, Schweizerbart Science Publishers.

In general, there are relatively few diatoms of the genera *Amphora* and *Halamphora* that have such a unique bigibboid shape; most of them are listed in Table 3. In addition to the above-mentioned *A. bigibba*, which is most similar to *H. kolbei*, we also list the species *H. capitata* and *H. angularis*. *H. capitata* is described as being more constricted in the middle, with ends that are more retracted and capitate than those of *H. kolbei* [23,29,37]. Previously, this species was considered a variety of *A. bigibba* and was identified as a separate species by the same authors as *H. kolbei* [23]. Accordingly, like *A. bigibba*, *H. capitata* is larger and has fewer striae per 10 μ m (Table 3) than *H. kolbei*. *H. angularis* is the largest of all the

species compared and also has very broad striae, the number of which per 10 μm is approximately half that of *H. kolbei* [1].

Thus, based on phylogenetic analysis of the *rbcL* and V4 18S rDNA regions and morphological characteristics, our study strain belongs to *H. kolbei*.

4.2. *H. kolbei* as a Promising Species for Biotechnology

In our study, the maximum productivity of the Black Sea strain *H. kolbei* was 0.20 g DW·L⁻¹·day⁻¹ ($R^2 = 0.98$). It is important to note that data in the literature show that similar species of the genera *Amphora* and *Halamphora* have significantly lower productivity (DW): 0.043 g·L⁻¹ day⁻¹ for *Amphora copulata*, 0.069–0.1 g·L⁻¹·day⁻¹ for *A. subtropica*, and 0.071 g·L⁻¹·day⁻¹ for *H. coffeiformis* [55,88,89]. Thus, *H. kolbei* cultures are characterised by high productivity. An important biotechnological characteristic of *H. kolbei* is its ability to settle quickly to the bottom of a photobioreactor, which can significantly reduce biomass production costs.

The genus *Halamphora* is known as a promising source of protein. Under optimal conditions with high nitrogen concentrations in the culture medium, the protein content in the biomass of diatoms from this genus can reach 30–45% of dry weight, and in some cases even higher [90,91]. In the exponential growth phase, the protein content in the biomass of *H. kolbei* DIA-A.big-SevBay reached its maximum (40%), as during the period of active growth, cells intensively synthesise structural and enzymatic proteins.

During batch cultivation of *H. kolbei*, the total carbohydrate content in the biomass showed wide variation depending on the growth phase, ranging from 8 to 17%. This is consistent with literature data indicating that the total carbohydrate content in *Halamphora* biomass can vary considerably, though under standard conditions it typically ranges from 10 to 28% of dry weight [90,91]. For example, at the onset of the stationary growth phase, *H. coffeiformis* was reported to contain 28 g of carbohydrates per 100 g of dry weight, equivalent to 28% [91]. The observed decrease in carbohydrate content in *H. kolbei* biomass during the stationary phase may be associated with a reduction in the rate of the dark reactions of photosynthesis. This slowdown likely results from limited ATP biosynthesis due to nutrient deprivation in the medium. For instance, phosphorus deficiency slows down ATP biosynthesis, leading to a decreased rate of carbohydrate synthesis in the Calvin cycle [92].

It is interesting to note that the ash content of *H. kolbei* reaches 33–37% of dry weight, which is consistent with the literature data obtained for *H. coffeiformis*, in which the ash content reached 38% DW [60]. In most planktonic diatoms, the ash content is 10–20% DW, while in benthic diatoms, it reaches 30–60% DW [90,93–95]. The higher ash content in benthic diatoms may be related to their denser frustules with a higher silica content.

The *Fx* content in biomass of the studied strain (17 mg·g⁻¹ dry weight) is in the average range characterising the genera *Halamphora* and *Amphora*. For example, in *Halamphora coffeiformis* biomass, *Fx* content reaches 38 mg·g⁻¹ dry weight [96]. *Halamphora suburgida* is a highly productive strain in terms of *Fx* production, reaching a peak concentration of $25.70 \pm 0.03 \mu\text{g}\cdot\text{mL}^{-1}$ under nitrogen-replete conditions ($1.764 \mu\text{M NO}_3^-$) [54]. A less productive strain is *Amphora subtropica*, which yields 15 mg of *Fx* per g of dry biomass [97].

The growing demand for energy, the decline in fuel reserves and the increase in CO₂ and greenhouse gas emissions have all accelerated the transition to renewable and unconventional energy sources [98]. A critical aspect of biodiesel production is the yield of biodiesel per unit area, which reflects the efficiency and viability of different feedstocks. Microalgae emerge as a frontrunner in terms of biodiesel yield, offering a substantially higher output in comparison to conventional oil crops. The biodiesel yield from microalgae is reported to be approximately 20,020 gallons per acre per year [99]. This

remarkable yield underscores the exceptional potential of microalgae as a feedstock for bio-diesel production, reflecting their rapid growth rates, high lipid content, and ability to thrive in diverse environments [100]. The significance of strain selection arises from the need for high biomass productivity and energetic compound content, and the essential process characteristics. Researchers meticulously select strains with rapid growth rates, efficient substrate utilisation, and high biofuel productivity, possessing metabolic pathways conducive to desired bioenergy compound synthesis, ideal for large-scale biofuel production [101]. Efficient feedstock utilisation is another crucial benefit of strain selection. Moreover, certain microbial strains possess the capability to capture and assimilate carbon dioxide during their growth, thereby functioning as carbon sinks [102]. Microalgal biofuels solve the problem of the global energy crisis, noting their capacity to sequester 1.3 kg of CO₂ to produce 1 kg of biomass [103]. Diatoms are a well-known source of lipids. They have a high species richness, with many lipid-rich taxa. This variety leads to a diverse fatty acid composition, making them useful for biodiesel production and as food supplements, among other applications [98]. Diatoms are relatively easy to culture, and some strains can be subjected to environmental stress to increase lipid production [49,104–106]. Lipids are also the main component in diatom species relevant for aquafeeds [107]. The increase in lipid concentration to 22% during the stationary growth phase is likely due to a decrease in irradiance per unit biomass and growth limitation by phosphorus and silicon in the nutrient medium [108,109]. It is known that the lipid content of diatoms can reach 50%: *Nitzschia* sp.—47%, *Cylindrotheca* sp.—37% [98]. The basal lipid content in biomass of representatives of *Halamphora* or *Amphora* corresponds with the literature [110]. However, under nitrogen starvation conditions, the lipid content of certain species can reach up to 45% of dry weight [55,110]. Under our cultivation conditions, the nitrogen content in the nutrient medium was sufficient even in the stationary growth phase, so the lipid content did not exceed 22% of the dry weight. In the study [103], the cost breakdown of microalgal biofuel production was evaluated: 77% of expenses were for cultivation, 12% to harvesting and 7.9% to lipid extraction, while emphasising the potential for simultaneous biomass production in wastewater to reduce economic and environmental costs. Therefore, the development of algal biofuel is expected to be regionally focused [111]. This means it will be essential to cultivate strains that are well-adapted to diverse environmental conditions and inhabit local waters.

The fatty acid composition of the *H. kolbei* strain was determined, revealing certain characteristics. Our findings on the FA composition under the specified culture conditions are consistent with earlier studies on the genus *Halamphora* [59,60,112]. In total, 15 different fatty acids were detected. There are studies where 46 different fatty acids were detected for *Halamphora* cf. *salinicola* [49]. For our *H. kolbei* strain, the highest content among the monoenoic fatty acids was found for palmitoleic acid (C16:1 ω 7, accounting for 19.78% of total fatty acids), and among the polyenoic acids for eicosapentaenoic acid (C20:5 ω 3, accounting for 37.77%) and docosahexaenoic acid (C22:6 ω 3, accounting for 7.33%). For example, the fatty acid profile of thermophilic *Halamphora* spp. strains showed a significant content of two essential fatty acids: eicosapentaenoic acid (EPA, 20:5 ω 3) and arachidonic acid (ARA, 20:4 ω 6). These fatty acids reached approximately 15% and 21%, respectively, of the total fatty acids at 20 °C and 30 °C [59]. The eicosapentaenoic acid content of most diatoms is typically 15–25% [113], which is considerably lower than in our strain. However, the arachidonic acid content in *H. kolbei* is low, whereas in some diatoms it can reach 10–15% [88]. *H. kolbei* has a higher palmitic acid content than many other diatoms, which may indicate a high level of protection against photooxidative stress [88].

Thus, the *H. kolbei* strain we studied has a superior fatty acid composition compared to similar species. This fact is of great practical importance since eicosapentaenoic acid helps prevent cardiovascular disease [114]. Docosahexaenoic acid (DHA) plays an

important role in brain function, vision, and the cardiovascular and immune systems, and reduces the risk of neurodegenerative diseases (Alzheimer's, Parkinson's) [115]. Docosahexaenoic acid (DHA) is a major component of the photoreceptors in the retina of the eye, supporting visual function. It suppresses chronic inflammation by inhibiting pro-inflammatory cytokines (IL-6 and TNF- α) and stimulating the production of anti-inflammatory mediators. It also modulates the immune response by enhancing the activity of T-regulatory cells [115]. Consuming 3–4 g per day of long-chain ω -3 PUFAs has been shown to reduce hypertriglyceridaemia, thrombogenesis, and inflammatory and immune processes [116]. It should also be emphasised that ω -3 from diatoms is absorbed 20–30% better than ω -3 from plant sources [117,118]. As a result, *H. kolbei* biomass, along with products that have a low ω 6/ ω 3 PUFA ratio (ω 6/ ω 3 $\leq$ 1), can be used to prevent diseases such as cardiac arrhythmia, stroke, and myocardial infarction, as well as atherosclerotic changes in blood vessels [116]. It is also a promising source of eicosapentaenoic acid (EPA) for pharmaceutical and dietary supplement production because its bioavailability is higher than that of fish oil. Taking into account essential FAs, the level of ARA and EPA found in *H. kolbei* was even higher than that reported for the fish oils used for aquafeed [107,119]. Therefore, *H. kolbei* biomass can be used as a feed supplement in aquaculture, as its high C14–C16 acid content makes it ideal for fish larvae. Numerous studies on species of the genus *Halamphora* confirm these results [59,60,91,96].

5. Conclusions

A comparative analysis of morphological and phylogenetic data allowed us to identify the isolated Black Sea strain DIA-A.big.-SevBay as *Halamphora kolbei*. The description of the species is supplemented with new data on such morphological characteristics as a description of the girdle part of the frustula, including the number of poroids in 10 μ m, and ventral striae. The biochemical composition of this strain's biomass was found to have a high content of eicosapentaenoic acid (37.77%) and an increased content of palmitoleic acid (19.78%). Thus, studied strain is a valuable biological resource, important for the creation of pharmaceuticals and biologically active supplements. It can also be used as a feed supplement in aquaculture and as a raw material for biodiesel due to its high content of C14–C16 fatty acids.

The resulting phylogenetic sequence will be added to the GenBank, and additional morphological keys will allow scientists around the world to study this species as a promising biological resource. This scientific research is extremely important in terms of evolution and taxonomy. It contributes to our understanding of microbial ecology and the conservation of biodiversity.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/biology15050394/s1>, Table S1. Taxa and the GenBank accession used in phylogenetic analysis.

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Abbreviations

The following abbreviations are used in this manuscript:

18S rRNA	18S ribosomal ribonucleic acid
rbcL	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit
LM	Light microscope
L:D cycle	Light:Dark cycle
SEM	Scanning electron microscope
DNA	Deoxyribonucleic Acid
PCR	Polymerase chain reaction
SSU rDNA	Small subunit ribosomal ribonucleic acid
BI	Bayesian inference
BIC	Bayesian information criterion
MCMC	Markov chain Monte Carlo
DW	Dry weight
FA	Fatty Acid
FAMES	Fatty acid methyl esters
FID	Flame ionisation detector
PID	Photoionisation Detector
Fx	Fucoxanthin
TLC	Thin-layer chromatography
NMR	Nuclear magnetic resonance spectroscopy
med	Median
IQR	Interquartile range
CI	Confidence intervals
PUFA	Polyunsaturated Fatty Acid
SFA	Saturated Fatty Acid
MUFA	Monounsaturated Fatty Acid
ATP	Adenosine Triphosphate
EPA	Eicosapentaenoic acid

References

1. Levkov, Z. *Amphora* sensu lato. In *Diatoms of Europe*; Lange-Bertalot, H., Ed.; A.R.G. Gantner Verlag K.G.: Ruggell, Liechtenstein, 2009; Volume 5; 916p.
2. Stepanek, J.G.; Kociolek, J.P. *Amphora* and *Halamphora* from coastal waters and inland waters of the United States and Japan. *Bibl. Diatomol.* **2018**, *66*, 1–260.
3. Stepanek, J.G.; Kociolek, J.P. Molecular Phylogeny of the Diatom Genera *Amphora* and *Halamphora* (Bacillariophyta) with a Focus on Morphological and Ecological Evolution. *J. Phycol.* **2019**, *55*, 442–456. <https://doi.org/10.1111/jpy.12836>.
4. Stepanek, J.G.; Kociolek, J.P. Re-examination of Mereschkowsky’s genus *Tetramphora* (Bacillariophyta) and its separation from *Amphora*. *Diatom Res.* **2016**, *31*, 123–148.

5. Round, F.E.; Crawford, R.M.; Mann, D.G. *The Diatoms: Biology and Morphology of the Genera*; Cambridge University Press: Cambridge, UK, 1990; 747p.
6. Archibald, R.E.M.; Schoeman, F.R. *Amphora coffeaeformis* (Agardh) Kützinger: A revision of the species under light and electron microscopy. *S. Afr. J. Bot.* **1984**, *3*, 83–102.
7. Clavero, E.; Grimalt, J.O.; Hernández-Maríné, M. The Fine Structure of Two Small *Amphora* Species: *A. tenerrima* Aleem & Hustedt and *A. tenuissima* Hustedt. *Diatom Res.* **2000**, *15*, 195–208.
8. Danielidis, D.B.; Mann, D.G. The systematics of *Seminavis* (Bacillariophyta): The lost identities of *Amphora angusta*, *A. ventricosa* and *A. macilenta*. *Eur. J. Phycol.* **2002**, *37*, 429–448.
9. Sala, S.; Sar, E.; Hinz, F.; Sunesen, I. Studies on *Amphora* subgenus *Halamphora* (Bacillariophyta): The revision of some species described by Hustedt using type material. *Eur. J. Phycol.* **2006**, *41*, 155–168. <https://doi.org/10.1080/09670260600556609>.
10. Garcia, M. *Seminavis atlantica* Garcia, a New Psammic Diatom (Bacillariophyceae) from Southern Brazilian Sandy Beaches. *Braz. J. Biol.* **2007**, *67*, 765–769. <https://doi.org/10.1590/S1519-69842007000400026>.
11. Ács, É.; Ector, L.; Kiss, K.T.; Cserhádi, C.; Morales, E.A.; Levkov, Z. Morphological observations and emended description of *Amphora micrometra* from the Bolivian Altiplano, South America. *Diatom Res.* **2011**, *26*, 199–212.
12. Van de Vijver, B.; Kopalová, K.; Zidarova, R.; Levkov, Z. Revision of the Genus *Halamphora* (Bacillariophyta) in the Antarctic Region. *Plant Ecol. Evol.* **2014**, *147*, 374–391. <https://doi.org/10.5091/plecevo.2014.979>.
13. Stepanek, J.G.; Kociolek, J.P. Several new species of *Amphora* and *Halamphora* from the western USA. *Diatom Res.* **2013**, *28*, 61–75.
14. López-Fuerte, F.O.; Siqueiros-Beltrones, D.A. *Halamphora primus* (Bacillariophyta): A new diatom species from the Gulf of California, Mexico. *Phytotaxa* **2018**, *369*, 278–286. <https://doi.org/10.11646/phytotaxa.369.4.6>.
15. López-Fuerte, F.O.; Sala, S.E.; Lora-Vilchis, M.C.; Murugan, G. *Halamphora siqueirosii* (Bacillariophyta), a new diatom species isolated from a hypersaline evaporation pond in Baja California Peninsula, Mexico. *Phytotaxa* **2020**, *451*, 132–144. <https://doi.org/10.11646/phytotaxa.451.2.3>.
16. You, Q.; Kociolek, J.P.; Wang, Q. Taxonomic studies of the diatom genus *Halamphora* (Bacillariophyceae) from the mountainous regions of southwest China, including the description of two new species. *Phytotaxa* **2015**, *205*, 75–89. <https://doi.org/10.11646/phytotaxa.205.2.1>.
17. Ryabushko, L.I.; Begun, A.A. *Diatoms of Microphytobenthos of the Sea of Japan*; PK KIA: Sevastopol, Ukraine, 2016; Volume 2, 324p.
18. Pane, E.P.; Risjani, Y.; Hamilton, P.B.; Solak, C.N.; Yunianta, Y.; Ertorun, N.; Yilmaz, E. A New Marine Diatom (Bacillariophyceae) Species—*Halamphora lombokensis* sp. nov. and the First Observation for *H. banzuensis* from Kuta Beach Lombok, West Nusa Tenggara, Indonesia. *PhytoKeys* **2024**, *250*, 165–179. <https://doi.org/10.3897/phytokeys.250.132304>.
19. Yilmaz, E.; Gastineau, R.; Solak, C.N.; Górecka, E.; Trobajo, R.; Turmel, M.; Lemieux, C.; Otis, C.; Witkowski, A.; Mann, D.G. Morphological and Molecular Characterization of *Halamphora vantushpaensis* (Bacillariophyceae, Amphipleuraceae), a New Diatom Species Widely Dispersed on the Shores of the Soda Lake Van (Türkiye). *PhytoKeys* **2024**, *249*, 95–114. <https://doi.org/10.3897/phytokeys.249.133205>.
20. Aleem, A.A. Littoral Diatoms from the Eastern Mediterranean. *Acta Horti Gothoburg.* **1950**, *18*, 309–332.
21. Foged, N. Diatoms in the Volvo Bay, Greece. *Bibl. Diatomol.* **1986**, *12*, 1–67. Available online: https://www.borntraeger-cramer.de/journals/bibl_diatom (accessed on 16 July 2025).
22. Witkowski, A.; Lange-Bertalot, H.; Metzeltin, D. *Diatom Flora of Marine Coasts I. In Iconographia Diatomologica, Vol. 7: Diversity—Taxonomy—Identification*; Lange-Bertalot, H., Ed.; A. R. G. Gantner Verlag K. G.: K öningstein, Germany, 2000; 925p.
23. Alvarez-Blanco, I.; Blanco, S. *Benthic Diatoms from Mediterranean Coasts*; Bibliotheca Diatomologica, Volume 60; Gebrüder Borntraeger Verlagbuchhandlung: Stuttgart, Germany, 2014; 409p. Available online: www.borntraeger-cramer.com/publications/detail/isbn/9783443570514/Bibl_Diatomologica_Vol_60 (accessed on 16 July 2025).
24. Hafner, D.; Jasprica, N.; Car, A. Taxonomic Survey of Benthic Diatoms in Neum Bay, Southeastern Adriatic. *Nat. Croat.* **2018**, *27*, 1–26.
25. Wachnicka, A.H.; Gaiser, E.E. Characterization of *Amphora* and *Seminavis* from South Florida, U.S.A. *Diatom Res.* **2007**, *22*, 387–455.
26. López-Fuerte, F.O.; Siqueiros-Beltrones, D.A. A Checklist of Marine Benthic Diatoms (Bacillariophyta) from Mexico. *Phytotaxa* **2016**, *283*, 201–258. <https://doi.org/10.11646/phytotaxa.283.3.1>.
27. Siqueiros-Beltrones, D.A.; Argumedo-Hernández, U.; Murillo-Jiménez, J.M.; Marmolejo-Rodríguez, A.J. Diversidad de diatomeas bentónicas marinas en un ambiente ligeramente enriquecido con elementos potencialmente tóxicos. *Rev. Mex. Biodivers.* **2014**, *85*, 1065–1085. <https://doi.org/10.7550/rmb.43748.s>

28. Siqueiros-Beltrones, D.A.; Martínez, Y.J.; López-Fuerte, F.O. Epiphytic Diatoms from the Central Region of the Gulf of California: Floristics and Biogeographic Remarks. *Diversity* **2023**, *15*, 510. <https://doi.org/10.3390/d15040510>.
29. Kaleli, A.; Kulikovskiy, M.; Solak, C.N. Some New Records for Marine Diatom Flora of Turkey From Akliman, Sinop (Black Sea). *Turk. J. Fish. Aquat. Sci.* **2017**, *17*, 1387–1395.
30. Zidarova, R.; Ivanov, P.; Hristova, O.; Dzhurova, B.; Hineva, E. The Unexpected Diversity in *Amphora* sensu lato (*Bacillariophyta*) at Sozopol Bay, the Western Black Sea. *Phytotaxa* **2022**, *544*, 103–127.
31. Proshchikina-Lavrenko, A.I. *Benthic Diatoms of the Black Sea*; Nauka: Moscow, Russia, 1963; 243p.
32. Guslyakov, N.E.; Zakordonets, O.A.; Gerasimyuk, V.P. *Atlas of Diatoms in the Benthos of the Northwestern Part of the Black Sea and Adjacent Water Bodies*; Naukova Dumka: Kyiv, Ukraine, 1992; 112p.
33. Ryabushko, L.I. *Microphytobenthos of the Black Sea*; EKOSI-Gidrofizica: Sevastopol, Russia, 2013; 416p.
34. Blaginina, A.; Balycheva, D.; Miroshnichenko, E.; Ryabushko, L.; Kapranov, S.; Barinova, S.; Lishaev, D. Does the Elemental Composition of Rock Surfaces Affect Marine Benthic Communities of Diatoms and Cyanobacteria? *J. Mar. Sci. Eng.* **2023**, *11*, 1569. <https://doi.org/10.3390/jmse11081569>.
35. Metzeltin, D.; Witkowski, A. Diatomeen der Bären-Insel. Süßwasser- und marine Arten. *Iconogr. Diatomol.* **1996**, *4*, 3–232.
36. Caraus, I. Algae of Romania. A distributional checklist of actual algae. Version 2.4. *Stud. Cercet. Biol.* **2017**, *7*, 1–1002.
37. López-Fuerte, F.O.; Siqueiros Beltrones, D.A.; Altamirano-Cerecedo, M.d.C. Species Composition and New Records of Diatom Taxa on *Phyllocladon pulcherrimum* (Chlorophyceae) from the Gulf of California. *Diversity* **2020**, *12*, 339. <https://doi.org/10.3390/d12090339>.
38. Moreno, J.L.; Licea, S.; Santoyo, H. *Diatomeas del Golfo de California*; Universidad Autonoma de Baja California Sur: La Paz, Mexico, 1996; 273p.
39. Barinova, S.; Ryabushko, L.; Balycheva, D.; Blaginina, A.; Chiornyavsky, E.; Shiroyan, A. Benthic diatoms on macrophytes of the Israeli Mediterranean coast. *Diversity* **2024**, *16*, 338. <https://doi.org/10.3390/d16060338>.
40. Cholnoky, B.J. Die Diatomeenassoziationen der Santa-Lucía-Lagune in Natal (Südafrika). *Bot. Mar.* **1968**, *11*, 1–121.
41. Giffen, M.H. A checklist of marine littoral diatoms from Namaqualand, South Africa. *Bacillaria* **1984**, *7*, 179–200.
42. McCarthy, P.M. Census of Australian Marine Diatoms. Australian Biological Resources Study, Canberra. Available online: https://www.anbg.gov.au/abrs/Marine_Diatoms/index.html (accessed on 16 July 2025).
43. Pereira, R.C.; Ramos, Y.J.; Moreira, D.d.L. The eco-evolutionary raison d'être of natural products as support for sustainable bioprospecting. *Front. Ecol. Evol.* **2025**, *13*, 1658630. <https://doi.org/10.3389/fevo.2025.1658630>.
44. Singh, P.; Gupta, S.K.; Guldhe, A.; Rawat, I.; Bux, F. Microalgae Isolation and Basic Culturing Techniques. In *Handbook of Marine Microalgae*; Kim, S.-K., Ed.; Academic Press: Boston, MA, USA, 2015; pp. 43–54.
45. Orlova, T.Y.; Aizdaicher, N.A.; Stonik, I.V. *Laboratory Cultivation of Marine Microalgae, Including Mycotoxin Producers: Scientific and Methodological Manual*; Dal'nauka: Vladivostok, Russia, 2011; 89p.
46. Liu, W.; Fu, D.; Pan, T.; Singh, R.P. Characterization and Polyculture Analysis of Microalgae Strains Based on Biomass Production and Nutrient Consumption, and Bacterial Community in Municipal Wastewater. *Water* **2021**, *13*, 3190. <https://doi.org/10.3390/w13223190>.
47. Vuong, P.; Chong, S.; Kaur, P. The little things that matter: How bioprospecting microbial biodiversity can build towards the realization of United Nations Sustainable Development Goals. *npj Biodivers.* **2022**, *1*, 4. <https://doi.org/10.1038/s44185-022-00006-y>.
48. Chigozie, V.U.; Ugochukwu, C.G.; Igboji, K.O. Application of artificial intelligence in bioprospecting for natural products for biopharmaceutical purposes. *BMC Artif. Intell.* **2025**, *1*, 4. <https://doi.org/10.1186/s44398-025-00004-7>.
49. Marzec, M.; Dąbek, P.; Witkowski, A.; Monedeiro, F.; Pomastowski, P.; Buszewski, B.; Nowak, I. Lipid Constituents of Diatoms (*Halophora*) as Components for Production of Lipid Nanoparticles. *Pharmaceutics* **2022**, *14*, 1171. <https://doi.org/10.3390/pharmaceutics14061171>.
50. Grubišić, M.; Šantek, B.; Zorić, Z.; Cošić, Z.; Vrana, I.; Gašparović, B.; Což-Rakovac, R.; Ivančić Šantek, M. Bioprospecting of Microalgae Isolated from the Adriatic Sea: Characterization of Biomass, Pigment, Lipid and Fatty Acid Composition, and Antioxidant and Antimicrobial Activity. *Molecules* **2022**, *27*, 1248. <https://doi.org/10.3390/molecules27041248>.
51. Peng, J.; Yuan, J.P.; Wu, C.F.; Wang, J.H. Fucoxanthin, a marine carotenoid presents in brown seaweeds and diatoms: Metabolism and bioactivities relevant to human health. *Mar. Drugs* **2011**, *9*, 1806–1828. <https://doi.org/10.3390/md9101806>.
52. Martín, L.A.; Popovich, C.A.; Martínez, A.M.; Scodelaro Bilbao, P.G.; Damiani, M.C.; Leonardi, P.I. Hybrid Two-Stage Culture of *Halophora coffeaeformis* for Biodiesel Production: Growth Phases, Nutritional Stages and Biorefinery Approach. *Renew. Energy* **2017**, *118*, 984–992. <https://doi.org/10.1016/j.renene.2017.10.086>.

53. Barkia, I.; Saari, N.; Manning, S.R. Microalgae for High-Value Products Towards Human Health and Nutrition. *Mar. Drugs* **2019**, *17*, 304. <https://doi.org/10.3390/md17050304>.
54. Mukherjee, P.; Bose, R.; Pal, R. Abiotic stress-induced changes in nutritive potential of *Halimnobia subturgida* (Hustedt) Levkov and utilization of processed biomass in diets for ornamental gold fish (*Carassius auratus*). *Aquac. Res.* **2021**, *52*, 1177–1184. <https://doi.org/10.1111/are.14975>.
55. Govindan, N.; Maniam, G.P.; Rahim, M.H.A.; Sulaiman, A.Z.; Ajit, A.; Chatsungnoen, T.; Chisti, Y. Production of renewable lipids by the diatom *Amphora copulata*. *Fermentation* **2021**, *7*, 37. <https://doi.org/10.3390/fermentation7010037>.
56. BenMoussa-Dahmen, I.; Chtourou, H.; Rezgui, F.; Sayadi, S.; Dhouib, A. Salinity stress increases lipid, secondary metabolites and enzyme activity in *Amphora subtropica* and *Dunaliella* sp. for biodiesel production. *Bioresour. Technol.* **2016**, *218*, 816–825. <https://doi.org/10.1016/j.biortech.2016.07.022>.
57. Khumaidi, A.; Muqith, A.; Wafi, A.; Mardiyah, U.; Sandra, L. Phytochemical Screening and Potential Antioxidant of *Amphora* sp. from Different Extraction Methods. *IOP Conf. Ser. Earth Environ. Sci.* **2023**, *1221*, 012056. <https://doi.org/10.1088/1755-1315/1221/1/012056>.
58. Chen, Y.-C. The Biomass and Total Lipid Content and Composition of Twelve Species of Marine Diatoms Cultured under Various Environments. *Food Chem.* **2012**, *13*, 211–219. <https://doi.org/10.1016/j.foodchem.2011.08.062>.
59. Bouzidi, N.; Zili, F.; García-Maroto, F.; López Alonso, D.; Ben Ouada, H. Impact of temperature and growth phases on lipid composition and fatty acid profile of a thermophilic *Bacillariophyta* strain related to the genus *Halimnobia* from north-eastern Tunisia. *J. Mar. Biol. Assoc. UK* **2020**, *100*, 529–536.
60. Baharuddin, N.D.; Mohamed Ramli, N.; Ghazali, N.A.; Tan, H.T.; Khaw, Y.S.; Natrah, I. Evaluation of optimal culture conditions for marine benthic diatom *Halimnobia coffeaeformis* growth and its biochemical properties. *Algal Res.* **2024**, *84*, 103720. <https://doi.org/10.1016/j.algal.2024.103720>.
61. Guillard, R.R.L.; Ryther, J.H. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.* **1962**, *8*, 229–239.
62. Simonsen, R. Untersuchungen zur Systematik und Ökologie der Bodendiatomeen der Westlichen Ostsee. *Int. Rev. Der Gesamten Hydrobiol. Und Hydrogr.* **1962**, *1*, 9–144.
63. Schmidt, A.W.F. *Atlas der Diatomaceen-Kunde*; Series I: Heft 7; Verlag von Ernst Schlegel: Aschersleben, Germany, 1875; 20p.
64. Cox, E.J.; Ross, R. The striae of pennate diatoms. In *Proceedings of the Sixth Symposium on Fossil and Recent Diatoms*; Ross, R., Ed.; Otto Koeltz: Koenigstein, Germany, 1981; pp. 267–278.
65. Nagumo, T. Taxonomic studies of the subgenus *Amphora* Cleve of the genus *Amphora* (*Bacillariophyceae*) in Japan. *Bibl. Diatomol.* **2003**, *49*, 3–265.
66. Gololobova, M.A.; Gogorev, R.M.; Lyakh, A.M.; Dorofeyuk, N.I. The main valve shapes of diatoms: Terminology. I. Valve shapes symmetrical to the apical axis and valve shapes with radial symmetry. *Nov. Sist. Nizshikh Rastenii* **2022**, *56*, 29–54.
67. Spaulding, S.A.; Potapova, M.G.; Bishop, I.W.; Lee, S.S.; Gasperak, T.S.; Jovanoska, E.; Furey, P.C.; Edlund, M.B. Diatoms.org: Supporting taxonomists, connecting communities. *Diatom Res.* **2022**, *36*, 291–304. <https://doi.org/10.1080/0269249X.2021.2006790>. Available online: <https://diatoms.org/genera/amphora/guide> (accessed on 9 July 2025).
68. Lobban, C.S.; Tharngan, B.G. Benthic Marine Diatom Flora (*Bacillariophyta*) of Yap, Micronesia: Preliminary Annotated List, with Some New Mangrove Species. *Diversity* **2025**, *17*, 34. <https://doi.org/10.3390/d17010034>.
69. Ekmanis, T.; Filek, K.; Mucko, M.; Žižek, M.; Kanjer, L.; Matek, A.; Ashworth, M.P.; Gligora Udovič, M.; Ljubešić, Z.; Bosak, S. The Croatian National Diatom Culture Collection: Strains From Biota And Turtlebiome Projects. In *Book of Abstracts, 15th European Diatom Meeting, Ohrid, North Macedonia, 7–10 May 2024*; Macedonian Diatom Society: Skopje, North Macedonia, 2024. Available online: <http://www.turtlebiome.biol.pmf.hr> (accessed on 9 July 2025).
70. Guiry, M.D.; Guiry, G.M. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. Available online: <https://www.algaebase.org> (accessed on 30 October 2025).
71. Zimmermann, J.; Jahn, R.; Gemeinholzer, B. Barcoding diatoms: Evaluation of the V4 subregion on the 18S rRNA gene, including new primers and protocols. *Org. Divers. Evol.* **2011**, *11*, 173–192.
72. Ruck, E.C.; Theriot, E.C. Origin and evolution of the canal raphe system in diatoms. *Protist* **2011**, *162*, 723–737.
73. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874.
74. Katoh, K.; Toh, H. Parallelization of the MAFFT multiple sequence alignment program. *Bioinformatics* **2010**, *26*, 1899–1900.
75. Drummond, A.J.; Rambaut, A. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* **2007**, *7*, 214.

76. Darriba, D.; Taboada, G.L.; Doallo, R.; Posada, D. jModelTest 2: More models, new heuristics and parallel computing. *Nat. Methods* **2012**, *9*, 772.
77. Blaginina, A.A.; Zheleznova, S.N.; Miroshnichenko, E.S.; Gevorgiz, R.G.; Ryabushko, L.I. The Diatom *Nanofrustulum shiloi* As a Promising Species in Modern Biotechnology. *Appl. Biochem. Microbiol.* **2024**, *60*, 483–495. <https://doi.org/10.1134/S0003683824603615>.
78. Sirenko, L.A.; Sakovich, A.I.; Osipov, L.F.; Lukina, L.F.; Kuzmenko, M.I.; Kozitskaya, V.N.; Velichko, I.M.; Myslovich, V.O.; Gavrilenko, M.Y.; Arendarchuk, V.V.; et al. Methods of Physiological and Biochemical Research of Algae. In *Methods of Physiological and Biochemical Research of Algae in Hydrobiological Practice*; Naukova Dumka: Kiev, USSR, 1975; pp. 75–212.
79. Agatova, A.I.; Arzhanova, N.V.; Lapina, N.M.; Naletova, I.A.; Torgunova, N.I. *Rukovodstvo po Sovremennym Biohimicheskim Metodam Issledovaniya Vodnykh Ekosistem, Perspektivnykh dlya Promysla i Marikul'tury*; [Guide to Modern Biochemical Methods for Studying Aquatic Ecosystems Promising for Fisheries and Mariculture]; VNIRO: Moscow, Russia, 2004; 123p.
80. Kates, M. *Techniques of lipidology: Isolation, analysis and identification of lipids*. Elsevier: Amsterdam, The Netherlands; New York, NY, USA; Oxford, UK, 1986; 464p.
81. Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A.; Smith, F. Colorimetric Method for Determination of Sugars and Related Substances. *Anal. Chem.* **1956**, *28*, 350–356.
82. Lowry, O.H.; Rosenbrough, N.J.; Farr, A.L.; Randall, R.J. Protein measurement with the Folin Phenol Reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.
83. Ryabushko, V.I.; Zheleznova, S.N.; Nekhoroshev, M.V. The effect of nitrogen on the accumulation of fucoxanthin by the diatom *Cylindrotheca closterium* (Ehrenb.) Reimann et Lewin. *Algologia* **2017**, *27*, 15–21.
84. Hashimoto, T.; Ozaki, Y.; Taminato, M.; Das, S.K.; Mizuno, M.; Yoshimura, K.; Maoka, T.; Kanazawa, K. The Distribution and Accumulation of Fucoxanthin and Its Metabolites after Oral Administration in Mice. *Br. J. Nutr.* **2009**, *102*, 242–248. <https://doi.org/10.1017/S0007114508199007>.
85. Gevorgiz, R.G.; Gureev, M.A.; Zheleznova, S.N.; Gureeva, E.V.; Nehorošev, M.V. Production of diadinoxanthin in an intensive culture of the diatom *Cylindrotheca closterium* (Ehrenb.) Reimann et Lewin and its pro-apoptotic activity. *Prikl. Biohim. Mikrobiol.* **2022**, *58*, 264–272.
86. Hammer, Ø.; Harper, D.A.T.; Ryan, P.D. PAST: Paleontological Statistics Software Package for Education. *Palaeontol. Electron.* **2001**, *4*, 1–9. Available online: <https://www.nhm.uio.no/english/research/resources/past/> (accessed on 9 February 2026).
87. Al-Yamani, F.Y.; Saburova, M.A. *Illustrated Guide on the Benthic Diatoms of Kuwait's Marine Environment*; Kuwait Institute for Scientific Research: Safat, Kuwait, 2011; 352p.
88. Thongdet, E.; Puanglarp, N.; Piumsomboon, A.; Koeypuksa, W.; Bunnajirakul, S.; Techa, S.; Piyapattanakorn, S.; Aiamsomboon, K. Growth, total lipid content and fatty acid composition of *Amphora* sp. isolated from Gulf of Thailand as alternative lipid source in larviculture. *Agric. Nat. Resour.* **2024**, *58*, 23–32.
89. Rajaram, M.G.; Nagaraj, S.; Manjunath, M.; Boopathy, A.B.; Kurinjimalar, C.; Rengasamy, R.; Jayakumar, T.; Sheu, J.-R.; Li, J.-Y. Biofuel and biochemical analysis of *Amphora coffeaeformis* RR03, a novel marine diatom, cultivated in an open raceway pond. *Energies* **2018**, *11*, 1341.
90. Baharuddin, N.D.; Muthukhrisnan, S.; de Cruz, C.R.; Kassim, Z.; Hasnan, H.H.; Abdullah, M.I.; Khaw, Y.S.; Tan, H.T.; Nazarudin, M.F.; Natrah, I. Evaluation of diatom *Halammphora* sp. and harpacticoid copepod *Amphiascoides neglectus* as live food for black tiger shrimp *Penaeus monodon* postlarvae. *Aquaculture* **2024**, *58*, 740773.
91. Scodelaro Bilbao, P.G.; Martín, L.A.; Popovich, C.A.; Almeyda, M.D.; Chamorro, V.; Leonardi, P.I. Assessment of *Halammphora coffeaeformis* Growth and Biochemical Composition for Aquaculture Purposes. *J. Mar. Sci. Eng.* **2020**, *8*, 282.
92. Huang, W.; Haferkamp, I.; Lepetit, B.; Molchanova, M.; Hou, S.; Jeblick, W.; Bártulos, C.R.; Kroth, P. Reduced Vacuolar β -1,3-Glucan Synthesis Affects Carbohydrate Metabolism as Well as Plastid Homeostasis and Structure in *Phaeodactylum tricornutum*. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 4791–4796. <https://doi.org/10.1073/pnas.1719274115>.
93. Correa-Reyes, J.G.; Sánchez-Saavedra, M.del P.; Viana, M.T.; Flores-Acevedo, N.; Pelaez, C.V. Effect of eight benthic diatoms as feed on the growth of red abalone (*Haliotis rufescens*) postlarvae. *J. Appl. Phycol.* **2009**, *21*, 387–393. <https://doi.org/10.1007/s10811-008-9381-x>.
94. Beekrum, S.; Amonsou, E.O. Nutritional profile, bioactive compounds, and antioxidant activity of microalgal strain, *Amphora* sp., isolated from the Cape coastal waters, South Africa. *Food Sci. Technol.* **2023**, *43*, e76222. <https://doi.org/10.5327/fst.76222>.
95. Affan, A.; Karawita, R.; Jeon, Y.-J.; Lee, J.-B. Growth characteristics and antioxidative status of *Cylindrotheca closterium* (Bacillariophyceae) from Jeju Island, Korea. In *Proceedings of the Asia-Pacific Aquaculture 2007*, Hanoi, Vietnam, 5–8 August 2007; pp. 1–8. Available online: <https://sciwatch.kiost.ac.kr/handle/2020.kiost/30398> (accessed on 30 October 2025).

96. Popovich, C.A.; Pistonesi, M.F.; Vara, M.S.; Buvé, C.L.; Hazeleger, W.C.; Ballesteros-Gomez, A.; Van Loey, A.M.; Hendrickx, M.E.; Levin, M.J.; Damiani, M.C. Potential of the Marine Diatom *Halampahora coffeaeformis* to Simultaneously Produce Omega-3 Fatty Acids, Chrysolaminarin and Fucoxanthin in a Raceway Pond. *Algal Res.* **2020**, *51*, 102030. <https://doi.org/10.1016/j.algal.2020.102030>.
97. Setthamongkola, P.; Siranonthana, N.; Chalermmueanga, R.; Yuyena, Y.; Watanachoteb, J.; Hiransuchalert, R.; Kutoa, M. Antioxidant Activities, Total Phenolic Compounds and Fucoxanthin of Marine Benthic Diatoms *Amphora subtropica* BUUC1502 and *Thalassiosira* sp. *J. Food Health Bioenviron. Sci.* **2021**, *14*, 20–27.
98. Deepika, C. Advancing sustainability: Microalgae biofuels in the circular bioeconomy. In *Microalgal Biofuels*; Woodhead Series in Bioenergy; Jaiswal, K.K., Singh, B., Jaiswal, A.K., Gagnon, Y., Eds.; Woodhead Publishing: Cambridge, UK, 2025; pp. 505–549. <https://doi.org/10.1016/B978-0-443-24110-9.00023-2>.
99. Patle, D.S.; Pandey, A.; Srivastava, S.; Sawarkar, A.N.; Kumar, S. Ultrasound-intensified biodiesel production from algal biomass: A review. *Environ. Chem. Lett.* **2021**, *19*, 209–229. <https://doi.org/10.1007/s10311-020-01080-z>.
100. Osman, M.E.H.; Abo-Shady, A.M.; Gheda, S.F.; Desoki, S.M.; Elshobary, M.E. Unlocking the potential of microalgae cultivated on wastewater combined with salinity stress to improve biodiesel production. *Environ. Sci. Pollut. Res.* **2023**, *30*, 114610–114624. <https://doi.org/10.1007/s11356-023-30370-6>.
101. Deshmukh, S.; Bala, K.; Kumar, R. Selection of microalgae species based on their lipid content, fatty acid profile and apparent fuel properties for biodiesel production. *Environ. Sci. Pollut. Res.* **2019**, *26*, 24462–24473. <https://doi.org/10.1007/s11356-019-05692-z>.
102. Hussain, M.M.; Farooqi, Z.U.R.; Rasheed, F.; Mohy Ud Din, W. Role of Microorganisms as Climate Engineers: Mitigation and Adaptations to Climate Change. In *Climate Change and Microbes*; Apple Academic Press: London, UK, 2022; pp. 1–24.
103. Sarwer, A.; Hamed, S.M.; Osman, A.I.; Jamil, F.; Al-Muhtaseb, A.H.; Alhajeri, N.S.; Rooney, D.W. Algal biomass valorization for biofuel production and carbon sequestration: A review. *Environ. Chem. Lett.* **2022**, *20*, 2797–2851. <https://doi.org/10.1007/s10311-022-01458-1>.
104. D'Ippolito, G.; Sardo, A.; Paris, D.; Vella, F.M.; Adelfi, M.G.; Botte, P.; Gallo, C.; Fontana, A. Potential of Lipid Metabolism in Marine Diatoms for Biofuel Production. *Biotech. Biofuels Bioprod.* **2015**, *8*, 28p.
105. Kaur, R.; Kaur, L.; Gautam, S. Role of lipid droplets in diatoms for biofuel production. *J. Appl. Biol. Biotechnol.* **2021**, *9*, 186–191. <https://doi.org/10.7324/JABB.2021.9525>.
106. Elfituri, A.M.; Rahman, M.A.; Yusoff, F.M.; Banerjee, S.; Dalhum, E. Growth rate, lipid and fatty acids content in some marine and freshwater diatoms. *J. King Abdulaziz Univ. Mar. Sci.* **2025**, *34*, 55–65. <https://doi.org/10.4197/Mar.34-2.5>.
107. De Silva, S.; Francis, D.S.; Tacon, A.G.J. Fish Oil in Aquaculture: In Retrospect. In *Fish Oil Replacement and Alternative Lipid Sources in Aquaculture Feeds*; Turchini, G.M., Ng, W.K., Tocher, D.R., Eds.; CRC Press: Boca Raton, FL, USA, 2011; pp. 1–20.
108. Li, H.-Y.; Lu, Y.; Zheng, J.-W.; Yang, W.-D.; Liu, J.-S. Biochemical and genetic engineering of diatoms for polyunsaturated fatty acid biosynthesis. *Mar. Drugs* **2014**, *12*, 153–166.
109. Yaakob, M.A.; Mohamed, R.M.S.R.; Al-Gheethi, A.; Aswathnarayana Gokare, R.; Ambati, R.R. Influence of nitrogen and phosphorus on microalgal growth, biomass, lipid, and fatty acid production: An overview. *Cells* **2021**, *10*, 393.
110. Navarro, F.E.; Leonardi, P.I.; Damiani, M.C.; Martín, L.; Martínez, A.; Popovich, C. Outdoor Culture of *Halampahora coffeaeformis* in the Semi-Arid Pampa of Argentina: A Comprehensive Analysis of Triacylglycerol Production for Biodiesel. *Algal Res.* **2023**, *74*, 103170. <https://doi.org/10.1016/j.algal.2023.103170>.
111. Hildebrand, M.; Davis, A.K.; Smith, S.R.; Traller, J.C.; Abbriano, R. The Place of Diatoms in the Biofuels Industry. *Biofuels* **2012**, *3*, 221–240.
112. Martín, L.A.; Popovich, C.A.; Martinez, A.M.; Damiani, M.C.; Leonardi, P.I. Oil assessment of *Halampahora coffeaeformis* diatom growing in a hybrid two-stage system for biodiesel production. *Renew. Energy* **2016**, *92*, 127–135.
113. Cheah, Y.T.; Ng, B.W.; Tan, T.L.; Chia, Z.S.; Chan, D.J.C. Biomass and eicosapentaenoic acid production from *Amphora* sp. under different environmental and nutritional conditions. *Biotechnol. Appl. Biochem.* **2023**, *70*, 568–580.
114. Swanson, D.; Block, R.; Mousa, S.A. Omega-3 fatty acids EPA and DHA: Health benefits throughout life. *Adv. Nutr.* **2012**, *3*, 1–7.
115. Banaszak, M.; Dobrzyńska, M.; Kawka, A.; Górna, I.; Woźniak, D.; Przysławski, J.; Drzymała-Czyż, S. Role of Omega-3 fatty acids eicosapentaenoic (EPA) and docosahexaenoic (DHA) as modulatory and anti-inflammatory agents in noncommunicable diet-related diseases—Reports from the last 10 years. *Clin. Nutr. ESPEN* **2024**, *63*, 240–258.

116. Mititelu, M.; Lupuliasa, D.; Neacșu, S.M.; Olteanu, G.; Busnatu, Ș.S.; Mihai, A.; Popovici, V.; Măru, N.; Boroghină, S.C.; Mihai, S.; et al. Polyunsaturated fatty acids and human health: A key to modern nutritional balance in association with polyphenolic compounds from food sources. *Foods* **2025**, *14*, 46.
117. Dalheim, L.; Svenning, J.B.; Olsen, R.L. In vitro intestinal digestion of lipids from the marine diatom *Porosira glacialis* compared to commercial LC n-3 PUFA products. *PLoS ONE* **2021**, *16*, e0252125.
118. Bojko, M.; Olchawa-Pajor, M.; Chyc, M.; Kowalczyk, A.; Latowski, D. Diatoms as a source of polyunsaturated fatty acids essential to the human diet. *New Biotechnol.* **2016**, *33*, S160.
119. Maltsev, Y.I.; Shkurina, N.A.; Kulikovskiy, M.S. The use of diatoms to improve the efficiency of feed for aquaculture. *Issues Mod. Algol.* **2019**, *20*, 303–307. [https://doi.org/10.33624/2311-0147-2019-2\(20\)-303-307](https://doi.org/10.33624/2311-0147-2019-2(20)-303-307).

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