processes

MDPI

Article

A Polyphasic Characterisation of *Tetradesmus almeriensis* sp. nov. (Chlorophyta: Scenedesmaceae)

Sara Turiel [1], Jose Antonio Garrido-Cardenas [2], Cintia Gómez-Serrano [3], Francisco Gabriel Acién [3], Lorenzo Carretero-Paulet [2] and Saúl Blanco [1,4,*]

1 Laboratorio de Diatomología y Calidad de Aguas, Instituto de Investigación de Medio Ambiente, Recursos Naturales y Biodiversidad, La Serna 58, 24007 León, Spain; sturs@unileon.es
2 Department of Biology and Geology, University of Almeria, Cra. Sacramento s/n, 04120 Almeria, Spain; jcardena@ual.es (J.A.G.-C.); lpaulet@ual.es (L.C.-P.)
3 Department of Chemical Engineering, University of Almería, 04120 Almeria, Spain; cinti4201@hotmail.com (C.G.-S.); facien@ual.es (F.G.A.)
4 Departamento de Biodiversidad y Gestión Ambiental, Facultad de Ciencias Biológicas y Ambientales, Universidad de León, Campus de Vegazana s/n, 24071 León, Spain
* Correspondence: sblal@unileon.es

Abstract: The microalga *Tetradesmus almeriensis*, previously known as Scenedesmus almeriensis, has been isolated and cultivated as a highly productive, fast-growing strain known as a natural source of different products of commercial interest, including bioactive compounds such as lutein. This strain produces up to 40 $g \cdot m^{-2} \cdot day^{-1}$ of lutein under optimal conditions and is highly recommendable for outdoor production in temperate and warm climates, showing maximal performance at temperatures up to 35 °C with no photo-inhibition taking place with irradiances greater than 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$. Morphological and molecular data allow its assignment to the Chlorophycean genus *Tetradesmus*. The new species can be distinguished from similar *Tetradesmus* taxa due to its unique combination of features that are seen under light microscopy. We present herein a robust and comprehensive phylogenetic analysis of *T. almeriensis*, together with several additional Scenedesmaceae species, using a combination of maximum likelihood and Bayesian approaches. Our results confirm *T. almeriensis* as a distinct species consistently clustering with other Scenedesmaceae.

Keywords: *Scenedesmus*; Chlorophyceae; new species; bioreactors; *Tetradesmus*; *Acutodesmus*

Citation: Turiel, S.; Garrido-Cardenas, J.A.; Gómez-Serrano, C.; Acién, F.G.; Carretero-Paulet, L.; Blanco, S. A Polyphasic Characterisation of *Tetradesmus almeriensis* sp. nov. (Chlorophyta: Scenedesmaceae). *Processes* **2021**, *9*, 2006. https://doi.org/10.3390/pr9112006

Academic Editor: Fabrizio Bezzo

Received: 31 August 2021
Accepted: 8 November 2021
Published: 10 November 2021

1. Introduction

The Scenedesmaceae family includes colonial coccal Chlorophyceae microalgae that form flat, curved, or three-dimensional coenobia with different cell shapes [1], and its most specious genus is *Scenedesmus*. Since this genus was first introduced by Meyen [2], a large number of taxa have been described morphologically [3], though their actual diversity and taxonomy are controversial [4] due to their large polymorphic, pleomorphic, and phenotypic variability [5]. The proliferation of numerous species, varieties, and forms can largely reflect morphological plasticity within the genus, which demands detailed investigations of the evolutionary diversity of the Scendesmaceae. Due to the large number of species contained in *Scenedesmus*, the genus was historically divided into subgenera and informal groups or subgroups [4,6,7].

Analysis of the small subunit (18S) of nuclear ribosomal RNA genes shows the phylogenetic position of *Scenedesmus* within the Chlorophyceae and its close relationship with coccoid green algae [8], but the sequence analysis of 18S rRNA suggests that the species of *Scenedesmus* sp. can be divided into at least two lineages corresponding to the subgenus *Desmodesmus*, and to another lineage containing the subgenera *Scenedesmus* and Acutudesmus [9,10]. Tsarenko and John [11] established taxonomic relationships between *Acutodesmus* and *Desmodesmus*, and they were later raised to the genus level.

Tetradesmus was originally described by Smith [12] based on the holotype *T. wisconsinensis* G.M. Smith. This species presents spindle-shaped, somewhat curved cells, convex in the centre with short cell tips, with a cosmopolite distribution in the plankton of oligotrophic freshwaters. Chodat [13] soon merged the genus into *Scenedesmus,* but West [14] maintained *Tetradesmus* as a distinct genus. Fott and Komárek [15] also recognised *Tetradesmus* and provided the first key to species and infraspecific taxa. *Tetradesmus* comprises ellipsoidal, spindle-shaped taxa with no or few longitudinal ridges, segregated from *Scenedesmus* s. str. by the presence of acute poles and no mucilage surrounding the coenobia, and unlike *Desmodesmus,* it lacks external spines [16,17]. *Tetradesmus* inhabits freshwater habitats, particularly in nutrient-rich conditions, constituting an important part of the biomass of green algae in many phytoplankton communities, and it is known for its high degree of phenologic plasticity [18]. By transferring *Tetradesmus*' holotype to *Acutodesmus*, Tsarenko and Petlevanny [19] synonymised both genera; however, the generic name *Tetradesmus* has nomenclatural priority over *Acutodesmus*, as noted by Wynne and Hallan [20], who combined existing *Acutodesmus* into *Tetradesmus*. It is noteworthy that in its actual sense, *Tetradesmus* is thought to be polyphyletic [16]. Currently, there are 21 validly published species names of *Tetradesmus* according to AlgaeBase, as well as three infraspecific names [21].

The name Scenedesmus almeriensis has been used informally in the literature since 2007 [22] to name a scenedesmoid species isolated from drying pools in greenhouses, and was first cultivated in the facilities of Las Palmerillas in La Mojonera (Almería, Southeast Spain) for industrial use. During the last decade, S. almeriensis has been commonly used in wastewater treatment studies, as well as in the production of compounds of commercial interest, such as biodiesel [23]. This name, which lacks a formal diagnosis and type designation, should be considered invalid according to International Code of Nomenclature (ICN) rules [3]. This work confirms this name under the most appropriate genus *Tetradesmus*, providing a differential diagnosis against closely related species through a morphological, phylogenetic, and autecological characterisation.

2. Materials and Methods

2.1. Microscopy Examination

Scenedesmus almeriensis strains were provided by the Department of Chemical Engineering at the University of Almeria, Spain. The sample used for the characterisation of the species belongs to a pure culture that came from an inoculate containing 10 mL of the sample, which was preserved in cold, dark conditions throughout the study. For the morphological characterisation, the sample was observed under light (LM), epifluorescence (EM), and scanning electron (SEM) microscopy. LM examination was carried out with a Leica DMR microscope (Leica, UK) equipped with differential interference contrast (DIC) and an Optika digital camera. For this purpose, a subsample was set to sedimentation in distilled water, previously fixed with 5% formaldehyde. Biometry and ultrastructural features were analysed using a total of 50 cells. For the study of the internal structure, a subsample was concentrated, fixed with albumin glycerol, and stained with safranin. The SEM examination was carried out with a high-resolution field emission scanning electron microscope (FESEM; Carl Zeiss MERLIN, Germany), with energy/wavelength dispersive X-ray (EDX/WDX) analytical capability. For this purpose, a subsample was previously fixed with 5% formaldehyde, dehydrated in an increasing ethanol/acetone dilution series, dried at the critical point, and then gold-metallised.

2.2. Sequence Data and Phylogenetic Analysis

We collected *ITS* (*ITS*1/*ITS*4) and *rbc*L-like homologous sequences from a set of 27 species representing the main lineages of the Scenedesmaceae family plus Chlorella vulgaris, belonging to the closely related Chlorellaceae family and conveniently used as the outgroup sequence in our analysis, by scanning the NIH genetic sequence data bank (GenBank) through BLAST using the *ITS* (Sequence ID MF977406) or *rbc*L (Sequence ID MG257492)

sequences from *T. almeriensis*, respectively, as queries (Supplementary Tables S1 and S2). Among the set of sequences obtained after the search, different selection criteria were taken into account. Firstly, the sequences used in previously published phylogenetic analyses were selected. In addition, it was considered that the chosen species was representative of the family, and, when possible, the most complete sequences were chosen.

Nucleotide sequence alignments were constructed using MUSCLE, with settings left at default [24]. Phylogenetic analyses were performed on the basis of the resulting multiple nucleotide sequence alignments using two alternative tree reconstruction methods: maximum likelihood (ML) and Bayesian analysis (BA). Prior to the phylogenetic analysis, the best fit nucleotide substitution model and the associated relevant parameters were inferred for each multiple sequence alignment using jModelTest v2.1 under the AIC test, and further considered to reconstruct ML phylogenies [25]. For *rbc*L sequences, the best fit selected model included +G, i.e., modelling heterogeneity in nucleotide substitution rates across positions in the alignment by means of a Gamma distribution with eight categories and an alpha shape parameter of 0.4810, +I, i.e., with the proportion of invariant sites fixed at 0.45. For *ITS* sequences, the best fit model returned associated parameters as +G, correcting heterogeneity [26] across substitution rates with a gamma distribution with eight categories and an alpha shape parameter of 0.8790, +F, i.e., estimating nucleotide substitution frequencies empirically from the alignment. ML trees were reconstructed by means of PhyMLv3.1 software [26], using the General Time Reversible (GTR) nucleotide substitution model and associated parameters returned for each alignment together with their best fit models. The search for the most likely tree was optimized by selecting "best of NNI & SPR" (nearest-neighbour interchange (NNI) and subtree pruning and regrafting (SPR)) [26], and statistical significance on the retrieved topology was assessed by means of a bootstrap analysis with 1000 replicates [26]. BA trees were obtained using MrBayes v3.2.5 [27], with the setting lset nst = mixed rates = gamma to sample across the GTR substitution model space in the Bayesian MCMC analysis, together with the parameters associated to the best fit models returned in each case by jModelTest v2.1. Searches of the tree space were performed through two independent runs with four Markov chains each, sampling every 100th tree during 1,000,000 generations until convergence between the two runs was reached, determined by the average standard deviation of split sequences below 0.05 (i.e., approaching 0), which reflects the independent tree samples becoming increasingly similar. Finally, the first 25% of the trees were discarded as burn-in, and a majority-rule consensus tree was then constructed to evaluate Bayesian posterior probabilities on the clades. The resulting trees were represented and edited using FigTree v1.4.2.

3. Results and Discussion

3.1. Taxonomy

Class Chlorophyceae
Order Chlorococcales
Family Scenedesmaceae
Genus *Tetradesmus*
Tetradesmus almeriensis sp. nov. (Figures 1 and 2)

Type: Spain. Almeria: water mass of a greenhouse (36°45′08″ N, 2°41′02″ W) (holotype, LEB: fixed cells from original strain mounted on a permanent slide, preserved in a metabolically inactive state). The original strain was deposited in the Algae and Protozoa Culture Collection of the Centre for Hydrology and Ecology, Ambleside, United Kingdom, code CCAP 276/24. Registration: http://phycobank.org/102940 (accessed on 9 November 2021).

Synonym: "Scenedesmus almeriensis", nom. nud.

Etymology: Named after Almeria, the region where the type locality is placed. The original strain was isolated in the Department of Chemical Engineering at the University of Almeria and patented by the University of Almeria and the Cajamar Foundation [28].

Description: Colonies of (2)–4–(8) cells, usually isolated under exponential growth conditions, as seen in other *Tetradesmus* taxa [29]; the individual cells of each coenobium are fusiform-cylindrical with small, polar, conoid-shaped protuberances (Figure 2B); terminal cells equal to central cells (Figure 1A). Cells coplanar, usually displaced vertically from each other (Figure 1). External membrane with a smooth surface, somewhat coarse at high magnifications (Figure 2B), without epistructures such as thorns or spines (Figure 2A). Biometry (range [median values]): cell length: 11.4–19.4 [15.1] µm, cell width: 6.0–9.1 [7.2] µm, and length–width ratio: 2.1 µm. The observation under polarised light revealed no birefringent structures. The cell wall is very resistant (protecting individuals against mechanical stress), composed of two layers, as Chodat and Malinesco showed for *Scenedesmus* [30]. Under LM, the cytoplasm has a granular structure and cells exhibit a more or less central plastid. The nucleus is located in the centre of the cell and the pyrenoid starch layer stained blue reacts with the combination of albumin and Lugol's solution, turning bright red. The pyrenoid is conchoidal, homogeneous, and constituted by 2–3 large starch grains (Figure 1B,C). Autospores, flagellate cells, and sexual reproduction were not observed.

Differential diagnosis: Morphologically, the new species can be placed in the genus *Tetradesmus* based on the criteria proposed by Krienitz and Bock [17], that is, the presence of ellipsoidal cells with acute poles, with no surrounding mucilage and no external spines. Within the group of *Tetradesmus* species with straight cells, *T. almeriensis* exhibits a unique combination of features. The cells in *T. crocinii* are smaller (up to 5.5 µm wide) but larger in *T. cumbricus* (11.0–13.5 µm wide) [15]. Mature cells of *T. adustus* are almost spherical in shape [29]. The similar *T. bajacalifornicus* and *T. deserticola* never constitute colonies, and these taxa show apical protrusions larger than those seen in *T. almeriensis* (compare Figure 2B with Lewis and Flechner [31]). Contrary to what occurs in *T. incrassatulus*, *T. almeriensis* never forms two-row colonies. Finally, the external cells in the colonies of *T. obliquus* are often lunate, a character that is not seen in the species described here. Available SEM images of *T. obliquus* (He et al. [32], Figure 4E) show that this taxon lacks the characteristic apical cone-shaped protuberances that are seen in *T. almeriensis* (Figure 2B).

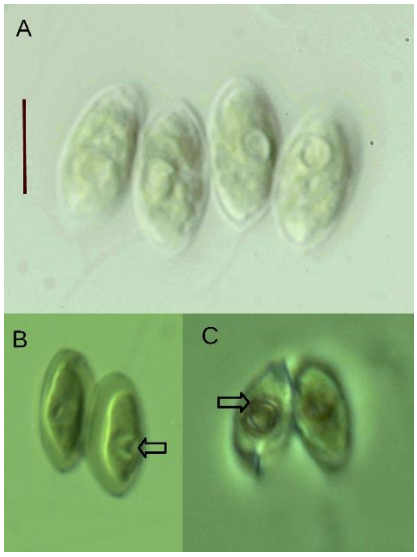

Figure 1. *Tetradesmus almeriensis* sp. nov. specimens from the type material imaged at 1000× DIC LM. (**A**). Habitus. Four-cell coenobium in raw, untreated sample. (**B**). Specimen fixed with haematoxylin to show the trilobated pyrenoid (arrow). (**C**). Cells treated with albumin + Lugol's solution to spot the starch layer surrounding the pyrenoid (arrow). Scale bar = 10 µm.

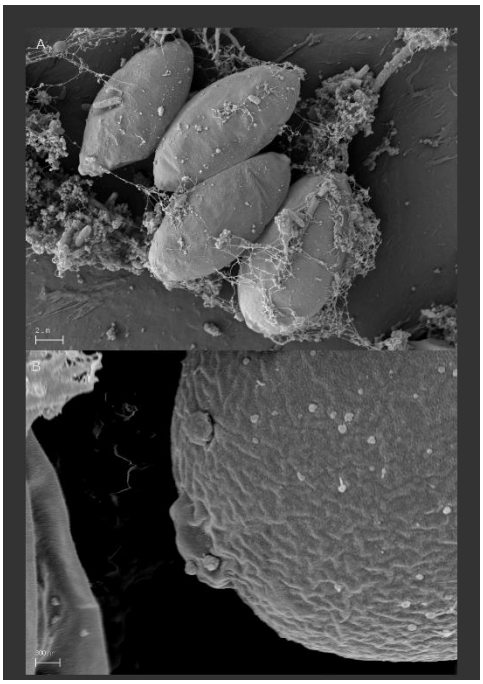

Figure 2. *Tetradesmus almeriensis* sp. nov. Specimens from the type material. SEM. (**A**). Four-cell coenobium. Note the smooth surface and the lack of epistructures. (**B**). Detail of the cell apex showing a small conoid-shaped protuberance.

3.2. Ecology

According to the literature that describes the past occurrences of *T. almeriensis*, this is a euryoic microalga that is able to grow under a wide range of temperatures (from 10 to 48 °C) and pH conditions (from 7.0 to 9.5) and shows strong tolerance to high Cu concentrations (up to 1 ppm) [28], which makes it especially suitable for external production [33], the most suitable growth conditions being a temperature of 30 °C and a pH of 8.0, with no vitamin addition. Regarding conductivity, it is considered to be a freshwater species [34], but it shows a high tolerance to medium concentrations of salt (0.1 M NaCl) [35]. *Tetradesmus almeriensis* shows a very unusual carotenoid content, especially lutein (up to 1% in d.w.) and β-carotene (0.75 mg/g) [32,36]; the starch and fatty acid content are also unusually high, with a yield of 16.7% (similar to that found in *Neochloris oleoabundans*) and 6% (higher than in *Arthrospira platensis*), respectively [33,37–39].

Strains of *T. almeriensis* are distributed worldwide, but no natural occurrences have been reported to date outside the type locality. This *Tetradesmus* has been proven to be a common contaminant in industrial-scale microalgal cultures due to its ability to flourish under a wide range of environmental conditions [40].

3.3. Phylogenetic Analysis and Systematic Position

A preliminary analysis using ribosomal DNA sequencing and subsequent comparison with available similar sequences revealed that the original strain is related to other *Scenedesmus*/*Desmodesmus* species within the class Chlorophyceae. It deviates from the most closely related species such as *S. hindakii* or *S. obtusus*; however, both species are closely related in all published studies (e.g., [16]). Subsequent sequence comparison analyses using *rbc*L sequences confirmed this preliminary analysis. Both sequences were deposited in GenBank under the following accession numbers: MF977406 (*ITS*1-5.8S-*ITS*2) and MG257492 (*rbc*L) [40].

In order to reconstruct the evolutionary relationships of *T. almeriensis* with its close relatives from the Scenedesmaceae family, we compiled two datasets of *rbc*L and *ITS* nucleotide sequences putatively homologous to *T. almeriensis* from 27 Scenedesmaceae species and performed independent ML and BA phylogenetic analyses. For both *rbc*L and

ITS sequences, the ML and BA trees showed nearly identical topologies, with *T. almeriensis* forming a robustly supported clade together with *S. obtusus* and *S. hindakii* (shown in green, Figures 3 and 4), both belonging to the *Scenedesmus* group of species [41]. In *rbc*L-based trees, both ML and BA trees grouped the three-species clade including *T. almeriensis* together with an heterogeneous group integrated by *Scenedesmus*, *Desmodesmus*, and *Tetradesmus* taxa. However, according to the *ITS* trees, the species that are more closely related to the *T. almeriensis* cluster belong to the genus *Tetradesmus*, which evidences the close relationship of the species described here, together with *S. obtusus* and *S. hindakii*, with this genus. Notably, according to Hegewald and Wolf [16], *S. arcuatus*, morphologically identical to *S. hindakii*, clusters with high bootstrap support along with other *Tetradesmus* taxa.

3.4. Culture Conditions

According to Sánchez et al. [33], the lutein production in *T. almeriensis* may vary depending on environmental parameters such as pH, temperature, culture medium, irradiation, and salinity. Over the last few years, several media have been used to cultivate this species, such as Bioprocess [42], Mann and Myers' medium [43], and Hemmerick [44], which were tested with the original *T. almeriensis* strain under standard culture conditions (Iomax: 1100 $\mu E \cdot m^{-2} \cdot s^{-1}$, T: 20 °C, pH: 8, [NaCl]: 0 ppm). Brindley et al. [45] modelised the light regime, thus allowing an optimal growth of *T. almeriensis* in photobioreactors, and they found that a culture at 33 °C in a homogenously illuminated tubular PBR under Io = 3000 $\mu E \cdot m^{-2} \cdot s^{-1}$ gives optimal productivity.

Continuous mode experiments showed that both Bioprocess and Mann and Myers' media are suitable for this strain, resulting in biomass productivities of up to 0.55 $g \cdot L^{-1} \cdot day^{-1}$, decreasing to 0.26 $g \cdot L^{-1} \cdot day^{-1}$ using Hemmerick medium. Cultures can also grow photoautotrophically in bubble column photobioreactors, with the growing conditions that were tested being air bubbling at 0.5 $vol^{-1} \cdot min^{-1}$, circulating heat-treated water through the jacket to maintain the set temperature, and the controlling of pH conditions by injecting CO_2 into the inflowing airstream. In this case, the photobioreactors require artificial illumination to simulate a solar cycle, the irradiance on the reactor surface being controlled by an automated system to provide maximum irradiation ranging from 625 to 1625 $\mu E \cdot m^{-2} \cdot s^{-1}$ at noon. The biomass productivity obtained under such conditions reaches 0.56 $g \cdot L^{-1} \cdot day^{-1}$ [33]. In a solid medium, *T. almerensis* was cultivated successfully in nutritive agar [46] at pH 8 and 26 °C with an 18:6 photoperiod, which led to a productivity of 0.144 $g \cdot L^{-1} \cdot day^{-1}$ [23]. Finally, tris-acetate-phosphate (TAP), a common medium for freshwater green algae, was also used to cultivate this species in a liquid medium. Recently, the influence of culture conditions introduced into the performance of this strain has been studied [36]. Optimal conditions maximising the photosynthesis rate were found to be an irradiance of 200–500 $\mu E \cdot m^{-2} \cdot s^{-1}$, temperature up to 35 °C, pH 8.0, and dissolved oxygen concentration below 20 $mg \cdot L^{-1}$, with no photo-inhibition taking place with irradiances greater than 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$. According to these optimal conditions, this strain is highly recommendable for outdoor production in temperate and warm climates, with biomass productivities greater than 40 $g \cdot m^{-2} \cdot day^{-1}$ being reported in thin-layer reactors, including using wastewaters as the source of nutrients [47].

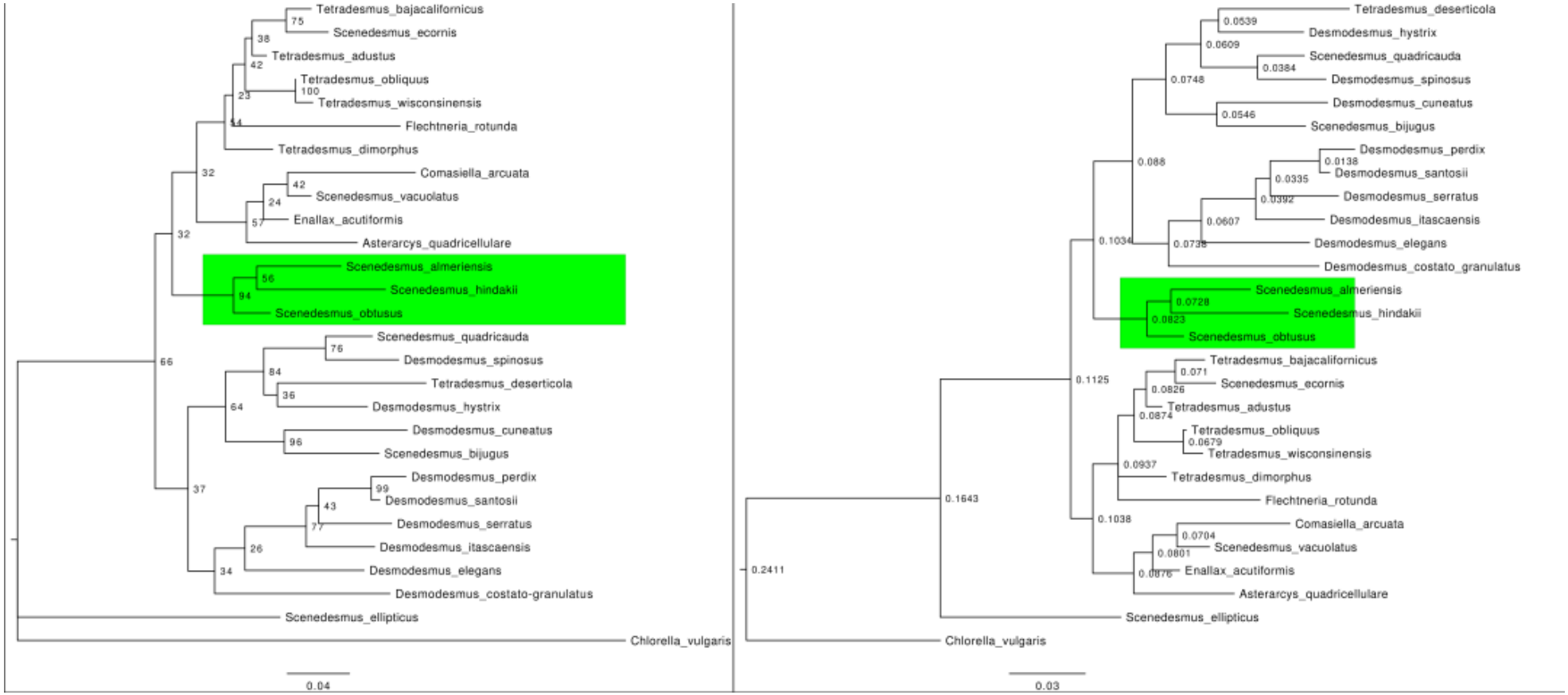

Figure 3. Molecular phylogeny of *rbc*L sequences from *T. almeriensis* and 27 related species. ML and BA trees depicting the evolutionary relationships of *rbc*L sequences from *T. almeriensis* and 27 related species (left and right panels, respectively). Both trees were rooted using *Chlorella vulgaris*. Values next to each node indicate statistical support of clades, i.e., bootstrap support values resulting from an analysis with 1000 replicates or Bayesian posterior probabilities for ML and BA trees, respectively. The tree is drawn to scale, with branch lengths proportional to evolutionary distances between nodes. The scale bar indicates the estimated number of nucleotide substitutions per site.

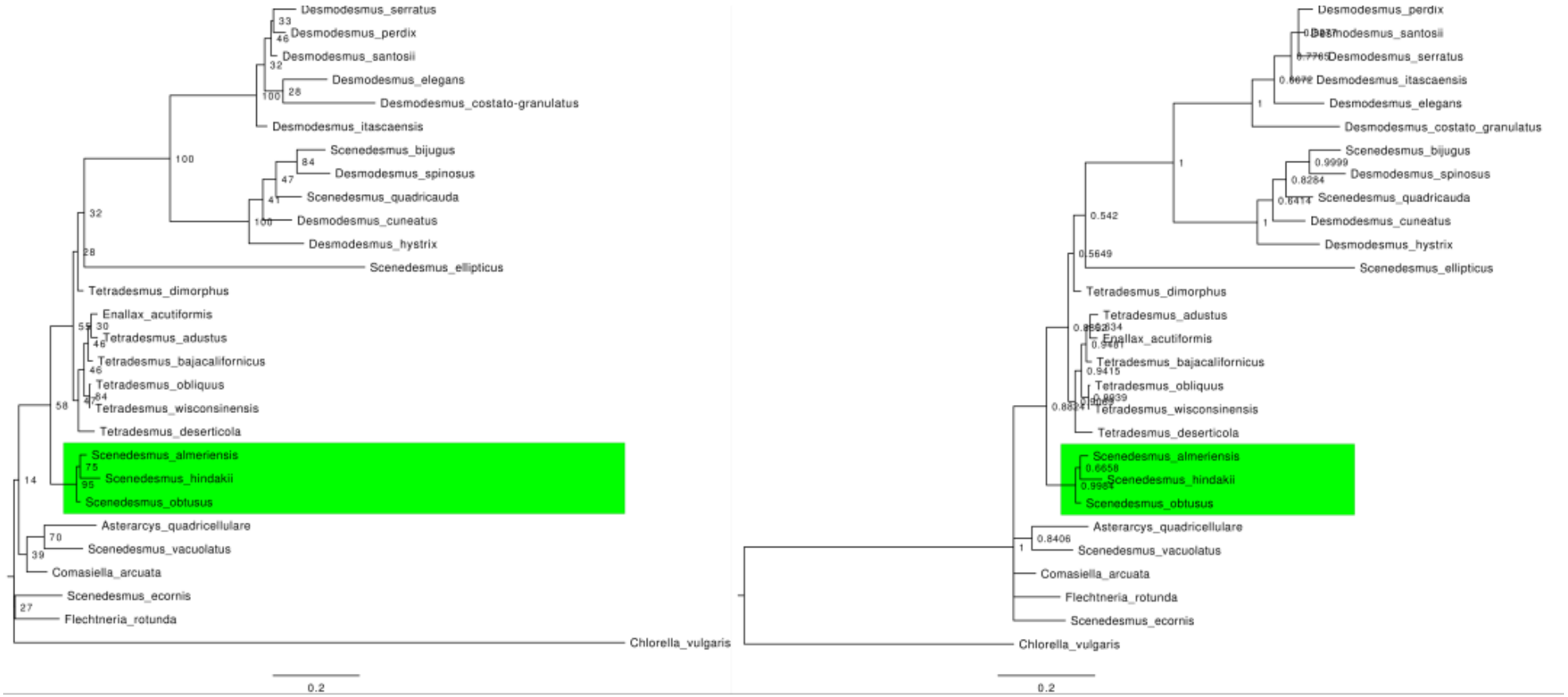

Figure 4. Molecular phylogeny of *ITS* sequences from *T. almeriensis* and 27 related species. ML and BA trees depicting the evolutionary relationships of *ITS* sequences from *T. almeriensis* and 27 related species (left and right panels, respectively). Both trees were rooted using the *Chlorella vulgaris* orthologous sequence. Values next to each node indicate statistical support on clades, i.e., bootstrap support values resulting from an analysis with 1000 replicates or Bayesian posterior probabilities for ML and BA trees, respectively. The tree is drawn to scale, with branch lengths proportional to evolutionary distances between nodes. The scale bar indicates the estimated number of nucleotide substitutions per site.

4. Conclusions

Microalgae growing on photobioreactors are often neither monitored nor controlled in terms of species composition and community structure [48]. Traditionally, in biotechnological studies on novel scenedesmoid microalgae, most isolates are identified as *Scenedesmus* sp. based only on LM [18]. In this regard, molecular taxonomy is particularly important for groups of micro-organisms lacking evident and diversified ultrastructural features [28]. Moreover, the phylogenetic significance of these morphological characters in scenedesmoid taxa is poorly understood [49]. Therefore, the need to investigate not only the phenotypic but also the genotypic characters in a polyphasic approach has been clearly demonstrated. Hence, to the best of our knowledge, researchers all over the world are adopting such multidisciplinary studies whereby knowledge obtained from morphological features is integrated with molecular and ecological data to resolve taxonomic problems within and between scenedesmoid taxa. In this study, we demonstrated *Tetradesmus almeriensis* as a distinct species that contributes to completing a robust phylogenetic dataset on the *Tetradesmus* species for addressing important evolutionary questions within the Scenedesmaceae [29], as well as the biotechnological importance of these taxa in the modern applied industry.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/pr9112006/s1, Table S1: *ITS* sequences extracted from GenBank, NCBI, and used in this study; Table S2: *rbc*L sequences extracted from GenBank, NCBI, and used in this study.

Author Contributions: S.T.: writing—original draft preparation; J.A.G.-C.: visualization, investigation; C.G.-S.: resourcesl F.G.A.: project administration, supervision; L.C.-P.: formal analysis; S.B.: conceptualization, writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research is part of the SABANA project founded by the European Union Horizon 2020 Research and Innovation programme under grant agreement No. 727874.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are available in a publicly accessible repository.

Acknowledgments: SEM images were kindly provided by A. Olenici at the Electronic Microscopy Unit of the University of Jaén (Spain). Technical and human support provided by CICT of Universidad de Jaén (UJA, MINECO, Junta de Andalucía, FEDER) is gratefully acknowledged. R. Álvarez-Nogal helped us in the processing of samples and the acquisition of images.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Hegewald, E.; Wolf, M.; Keller, A.; Friedl, T.; Krienitz, L. *ITS*2 sequence-structure phylogeny in the Scenedesmaceae with special reference to *Coelastrum* (Chlorophyta, Chlorophyceae), including the new genera *Comasiella* and *Pectinodesmus*. *Phycologia* **2010**, *49*, 325–335. [CrossRef]
2. Meyen, F.J.F. Beobachtungen über einige niedere Algenformen. *Nova Acta Phys.-Med. Acad. Caesareae Leopold.-Carol. Nat.* **1830**, *14*, 768–778.
3. Hegewald, E.; Silva, P.C. *Annotated Catalogue of Scenedesmus and Nomenclaturally Related Genera*; J. Cramer: Berlin, Germany, 1988.
4. Komárek, J.; Fott, B.; Huber-Pestalozzi, G. *Das Phytoplankton des Süßwassers*; Systematik und Biologie-Teil 7, 1. Hälfte: Stuttgart, Germany, 1983.
5. Trainor, F.R.; Cain, J.R.; Shubert, L.E. Morphology and nutrition of the colonial green alga *Scenedesmus*: 80 years later. *Bot. Rev.* **1976**, *42*, 5–25. [CrossRef]
6. Uherkovich, G. *Die Scenedesmus-Arten Ungarns*; Akadémiai Kiadó, Verlag der Ungarisch Akad der Wissenschaften: Budapest, Hungary, 1966.
7. Hegewald, E. Vergleichende Beobachtungen an Herbarmaterial und Freilandmaterial von *Scenedesmus*. *Algol. Stud.* **1979**, *24*, 264–286.

8. Lewis, L.A. Diversity and phylogenetic placement of *Bracteacoccus* Tereg (Chlorophyceae, Chlorophyta) based on 18s ribosomal RNA gene sequence data. *J. Phycol.* **1997**, *33*, 279–285. [CrossRef]
9. Kessler, E.; Schäfer, M.; Hümmer, C.; Kloboucek, A.; Huss, V.A.R. Physiological, biochemical, and molecular characters for the taxonomy of the subgenera of *Scenedesmus* (Chlorococcales, Chlorophyta). *Bot. Acta* **1997**, *110*, 244–250. [CrossRef]
10. Hegewald, E.; Hangata, N. Phylogenetic studies on Scenedesmaceae (Chlorophyta). *Algol. Stud.* **2000**, *100*, 29–49. [CrossRef]
11. Tsarenko, P.M.; John, D.M. Phylum Chlorophyta (Green Algae) Order Sphaeropleales. *Freshw. Algal Flora Br. Isles* **2011**, *2*, 461–465.
12. Smith, G.M. *Tetradesmus*, a new four-celled coenobic alga. *Bull. Torrey Bot. Club* **1913**, *40*, 75–87. [CrossRef]
13. Chodat, R. Monographies d'Algues en Culture Pure. *Mat. Crypt. Suisse* **1913**, *4*, 234–241.
14. West, G.S. Algological Notes: XIV-XVII. *J. Bot.* **1915**, *53*, 73–84.
15. Fott, B.; Komárek, J. Die Gattungen *Lauterborniella* Schmidle und *Tetradesmus* GM Smith und ihre Stellung in der Familie der Scenedesmaceae (Chlorococcales). *Preslia* **1974**, *46*, 198–209.
16. Hegewald, E.; Wolf, M. Phylogenetic relationships of *Scenedesmus* and *Acutodesmus* (Chlorophyta, Chlorophyceae) as inferred from 18S rDNA and *ITS*-2 sequence comparisons. *Plant Syst. Evol.* **2003**, *241*, 185–191. [CrossRef]
17. Krienitz, L.; Bock, C. Present state of the systematics of planktonic coccoid green algae of inland waters. *Hydrobiologia* **2012**, *698*, 295–326. [CrossRef]
18. Ismagulova, T.; Chekanov, K.; Gorelova, O.; Baulina, O.; Semenova, L.; Selyakh, I.; Chivkunova, O.; Lobakova, E.; Karpova, O.; Solovchenko, A. A new subarctic strain of *Tetradesmus obliquus*—Part I: Identification and fatty acid profiling. *J. Appl. Phycol.* **2018**, *30*, 2737–2750. [CrossRef]
19. Tsarenko, P.M.; Petlevanny, O.A. Addition to the diversity of algae of Ukraine. *Algol. Suppl.* **2001**, *10*, 1–130.
20. Wynne, M.J.; Hallan, J.K. Reinstatement of *Tetradesmus* GM Smith (Sphaeropleales, Chlorophyta). *Feddes Repert.* **2015**, *126*, 83–86. [CrossRef]
21. Guiry, M.D.; AlgaeBase. World-WIDE Electronic Publication, National University of Ireland, Galway (Taxonomic Information Republished from AlgaeBase with Permission of MD Guiry). Accessed through World Regist. Mar. Species. 2019. Available online: http//www.algaebase.org (accessed on 11 September 2021).
22. Del Campo, J.A.; García-González, M.; Guerrero, M.G. Outdoor cultivation of microalgae for carotenoid production: Current state and perspectives. *Appl. Microbiol. Biotechnol.* **2007**, *74*, 1163–1174. [CrossRef] [PubMed]
23. Dautor, Y.; Chileh, T.; Úbeda Mínguez, P.; Mañas Fernández, A.; García Maroto, F.; López Alonso, D. Microalgas para biodiesel: Desarrollo de un método de transformación genética para Scenedemus almeriensis, una especie con potencial industrial. *Chron. Nat.* **2013**, *3*, 10–17.
24. Edgar, R.C. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* **2004**, *32*, 1792–1797. [CrossRef] [PubMed]
25. Darriba, D.; Taboada, G.L.; Doallo, R.; Posada, D. JModelTest 2: More models, new heuristics and parallel computing. *Nat. Methods* **2012**, *9*, 772. [CrossRef] [PubMed]
26. Guindon, S.; Dufayard, J.-F.; Lefort, V.; Anisimova, M.; Hordijk, W.; Gascuel, O. New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Syst. Biol.* **2010**, *59*, 307–321. [CrossRef] [PubMed]
27. Ronquist, F.; Teslenko, M.; Van Der Mark, P.; Ayres, D.L.; Darling, A.; Höhna, S.; Larget, B.; Liu, L.; Suchard, M.A.; Huelsenbeck, J.P. Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Syst. Biol.* **2012**, *61*, 539–542. [CrossRef] [PubMed]
28. Fernández Sevilla, J.M.; Molina Grima, E.; Pérez Parra, J.; Acién Fernández, F.G.; Magán Cañadas, J.J.; Friedl, T. Nueva Especie de Microalga y su Aplicación Para Consumo Animal, Humano y en la Obtención de Carotenoides. 2016. Available online: https://bit.ly/3kjCy0J (accessed on 9 November 2021).
29. Terlova, E.F.; Lewis, L.A. A new species of *Tetradesmus* (Chlorophyceae, Chlorophyta) isolated from desert soil crust habitats in southwestern North America. *Plant Fungal Syst.* **2019**, *64*, 25–32. [CrossRef]
30. Chodat, R.; Malinesko, O. Sur le polymorphisme de *Scenedesmus acutus*. *Bull. l'Herbier Boissier* **1893**, *1*, 184–190.
31. Lewis, L.A.; Flechtner, V.R. Cryptic species of *Scenedesmus* (Chlorophyta) from desert soil communities of western north america. *J. Phycol.* **2004**, *40*, 1127–1137. [CrossRef]
32. He, M.; Yan, Y.; Pei, F.; Wu, M.; Gebreluel, T.; Zou, S.; Wang, C. Improvement on lipid production by *Scenedesmus obliquus* triggered by low dose exposure to nanoparticles. *Sci. Rep.* **2017**, *7*, 15526. [CrossRef]
33. Sánchez, J.F.; Fernández, J.M.; Acién, F.G.; Rueda, A.; Pérez-Parra, J.; Molina, E. Influence of culture conditions on the productivity and lutein content of the new strain *Scenedesmus almeriensis*. *Process Biochem.* **2008**, *43*, 398–405. [CrossRef]
34. Suyono, E.A.; Haryadi, W.; Zusron, M.; Nuhamunada, M.; Rahayu, S.; Nugroho, A.P. The effect of salinity on growth, dry weight and lipid content of the mixed microalgae culture isolated from Glagah as biodiesel substrate. *J. Life Sci.* **2015**, *9*, 229–233.
35. Benavente-Valdés, J.R.; Aguilar, C.; Contreras-Esquivel, J.C.; Méndez-Zavala, A.; Montañez, J. Strategies to enhance the production of photosynthetic pigments and lipids in chlorophycae species. *Biotechnol. Reports* **2016**, *10*, 117–125. [CrossRef]
36. Macías-Sánchez, M.D.; Fernandez-Sevilla, J.M.; Fernández, F.G.A.; García, M.C.C.; Grima, E.M. Supercritical fluid extraction of carotenoids from *Scenedesmus almeriensis*. *Food Chem.* **2010**, *123*, 928–935. [CrossRef]

37. Fernández-Sevilla, J.M.; Molina Grima, E.; Perez Parra, J.; Acien Fernandez, F.G.; Magan Cañadas, J.J.; Friedl, T. Novel Microalgal Species and Use Thereof for Animal and/or Human Consumption and in the Production of Carotenoids. Spanish Patent P200500374. Available online: https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2006087405 (accessed on 9 November 2021).
38. Bessières, D.; Bazile, J.-P.; Tanh, X.N.T.; García-Cuadra, F.; Acien, F.G. Thermophysical behavior of three algal biodiesels over wide ranges of pressure and temperature. *Fuel* **2018**, *233*, 497–503. [CrossRef]
39. Johnsson, N.; Steuer, F. Bioplastic Material from Microalgae: Extraction of Starch and PHA from Microalgae to Create a Bioplastic Material. 2018. Available online: https://www.diva-portal.org/smash/record.jsf?pid=diva2%3A1228894&dswid=-7903 (accessed on 9 November 2021).
40. Beatrice-Lindner, P.; Garrido-Cardenas, J.A.; Sepulveda, C.; Acien-Fernandez, F.G. A new approach for detection and quantification of microalgae in industrial-scale microalgal cultures. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 8429–8436. [CrossRef]
41. Wang, Q.; Song, H.; Liu, X.; Zhu, H.; Hu, Z.; Liu, G. Deep genomic analysis of *Coelastrella saipanensis* (Scenedesmaceae, Chlorophyta): Comparative chloroplast genomics of Scenedesmaceae. *Eur. J. Phycol.* **2019**, *54*, 52–65. [CrossRef]
42. Harun, R.; Danquah, M.K.; Forde, G.M. Microalgal biomass as a fermentation feedstock for bioethanol production. *J. Chem. Technol. Biotechnol.* **2010**, *85*, 199–203. [CrossRef]
43. Mann, J.E.; Myers, J. On pigments, growth, and photosynthesis of *Phaeodactylum tricornutum*. *J. Phycol.* **1968**, *4*, 349–355. [CrossRef]
44. Stein, E.D. *Isolation and Culture of Algae, Handbook of Phycological Methods*; Culture Methods and Growth Measurements; Cambridge University Press: Cambridge, UK, 1973.
45. Brindley, C.; Jiménez-Ruíz, N.; Acién, F.G.; Fernández-Sevilla, J.M. Light regime optimization in photobioreactors using a dynamic photosynthesis model. *Algal Res.* **2016**, *16*, 399–408. [CrossRef]
46. Zimbro, M.J.; Power, D.A.; Miller, S.M.; Wilson, G.E.; Johnson, J. *Manual of Microbiological Culture Media*; Becton Dickinson: Sparks, MD, USA, 2009.
47. Del Mar Morales-Amaral, M.; Gómez-Serrano, C.; Acién, F.G.; Fernández-Sevilla, J.M.; Molina-Grima, E. Outdoor production of *Scenedesmus* sp. in thin-layer and raceway reactors using centrate from anaerobic digestion as the sole nutrient source. *Algal Res.* **2015**, *12*, 99–108. [CrossRef]
48. Cho, D.-H.; Ramanan, R.; Heo, J.; Kang, Z.; Kim, B.-H.; Ahn, C.-Y.; Oh, H.-M.; Kim, H.-S. Organic carbon, influent microbial diversity and temperature strongly influence algal diversity and biomass in raceway ponds treating raw municipal wastewater. *Bioresour. Technol.* **2015**, *191*, 481–487. [CrossRef]
49. An, S.S.; Friedl, T.; Hegewald, E. Phylogenetic relationships of *Scenedesmus* and *Scenedesmus*-like coccoid green algae as inferred from *ITS*-2 rDNA sequence comparisons. *Plant Biol.* **1999**, *1*, 418–428. [CrossRef]