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Phylogenetic and Morphological Study of *Desmodesmus* Strains from Can Gio Mangrove Biosphere Reserve

Phuong-Nam Luu[1,2], Ngoc-Nam Trinh[2], Thanh-Cong Nguyen[3], Quoc-Dang Quan[4], Duc-Hoan Huynh[5], Tuong-Lam Le-Nguyen[6], Hoang-Dung Tran[7,*]

[1]Tung Thien Vuong Secondary School, 381 Tung Thien Vuong Street, Ward 12, District 08, 73011, Ho Chi Minh City, Vietnam
[2]Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, 12 Nguyen Van Bao Street, Go Vap District, 71400, Ho Chi Minh City, Vietnam
[3]NTT Hi-Tech Institute, Nguyen Tat Thanh University, 298-300A Nguyen Tat Thanh Street, District 4, 72800, Ho Chi Minh City, Vietnam
[4]University of Science, VNU-HCM, 227 Nguyen Van Cu Street, Ward 04, District 05, 72760, Ho Chi Minh City, Vietnam
[5]Can Gio Mangrove Protection Forest Management Board, Can Gio Mangrove Biosphere Reserve, 1541 Rung Sat Street, An Thoi Dong Commune, Can Gio District, Ho Chi Minh City, 76700, Vietnam
[6]International School Ho Chi Minh City (ISHCMC), 01 Xuan Thuy Street, Thao Dien Ward, Thu Duc City, Ho Chi Minh City, 71107, Vietnam
[7]Faculty of Biology and Environment, Ho Chi Minh City University of Industry and Trade (HUIT), 140 Le Trong Tan Street, Tay Thanh Ward, Tan Phu District, Ho Chi Minh City, 72009, Vietnam

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18S

ABSTRACT

This study focused on five microalgal strains resembling Desmodesmus isolated from the Can Gio Mangrove Biosphere Reserve. The objective was to assess the effectiveness of morphological and molecular methods for algal identification and to evaluate the genetic diversity of the relevant taxa. The five isolated and reference strains were cultured axenically in a BG-11 medium. Both microscopy (at magnifications of 40× and 100×) and molecular techniques (using ITS and 18S rRNA markers) were employed for analysis. Phylogenetic analyses were conducted using Maximum Likelihood and Bayesian inference methods. The results indicated that five of the ten strains were consistently identified using both approaches. Molecular data prompted a taxonomic reassignment for the three remaining strains, while morphological traits were more decisive for two reference strains. Phylogenetic analyses revealed significant genetic diversity within *Desmodesmus*, highlighting the ecological adaptability of genetically distinct variants. This study emphasizes the reliability of molecular tools in algal taxonomy, particularly for differentiating between *Desmodesmus* and *Scenedesmus*-like taxa. It contributes to understanding

* Corresponding author
E-mail: dungth@huit.edu.vn (Hoang-Dung Tran)

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microalgal genetic diversity in the Can Gio Biosphere Reserve and provides a foundation for future ecological and biotechnological applications.

1 Introduction

The Can Gio Mangrove Biosphere Reserve in southern Vietnam is globally recognized for its diverse ecosystems of significant ecological value. This UNESCO-designated site features a unique combination of freshwater and brackish water environments, creating an ideal habitat for microalgae. These organisms are critical for nutrient cycling, water purification, and overall ecosystem stability (Pham 2017; Pham 2019). Beyond their ecological importance, microalgae are increasingly valued for potential applications in biotechnology, renewable energy, and environmental remediation. Despite this significance, systematic studies on microalgal biodiversity and taxonomy in Can Gio remain limited, highlighting the need for focused efforts to explore and conserve these resources (Phung et al. 2019; Kezlya et al. 2023). In response to this need, the Culture Collection of Algae at Ho Chi Minh City (CCAH) was established, amassing over 100 local strains catalogued using morphological and molecular methods (DOST HCMC 2023).

Identifying algae based on morphological traits presents several challenges, primarily because environmental factors such as light, temperature, and nutrient availability can significantly affect cell phenotypes and morphology. These challenges intensify in closely related genera such as *Desmodesmus*, *Tetradesmus*, and *Scenedesmus*, which often exhibit overlapping morphological characteristics (Hegewald 1979; Kessler et al. 1997; Johnson et al. 2007; Mai et al. 2023; Nguyen et al. 2023). To address these issues, molecular markers have become essential for resolving taxonomic ambiguities. The Internal Transcribed Spacer (ITS) region is widely acknowledged for its high resolution in distinguishing closely related species, especially within *Scenedesmus* and *Desmodesmus* (Fawley and Fawley 2020; Kezlya et al. 2023; Nguyen et al. 2023). Other markers, such as the 18S and 23S rRNA genes, serve as complementary approaches, offering broader phylogenetic insights that enhance the robustness of classifications (Shirora 1966; Andersen and Kawachi 2005; Khaw et al. 2020; Kezlya et al. 2023; Nguyen et al. 2023). Choosing an appropriate set of markers with sufficient discriminating power is crucial for producing reliable identifications that supplement or refine morphological data.

This study employed the ITS and 18S markers to verify the identity of algal strains isolated from the Can Gio Biosphere Reserve. These strains displayed morphological characteristics similar to *Scenedesmus*-like *Desmodesmus*, whose overlapping traits with other *Scenedesmus*-like taxa required additional molecular validation. Several well-established *Scenedesmus*-like strains were also included as references. By analyzing both morphological and molecular data, the study aimed to evaluate the chosen markers' effectiveness and the molecular approach's reliability in extending their potential applications to other algal taxa in the region. Additionally, the study assessed the phylogenetic relationships among the isolated strains based on these markers to better understand their genetic diversity, thereby contributing to our knowledge of microalgal biodiversity in Can Gio and paving the way for conservation and future biotechnological applications.

2 Materials and Methods

2.1 Study Subjects

This study focused on *Desmodesmus*-resembling strains CCAH016/2, CCAH020/1, CCAH024/1, CCAH026/1, and CCAH027/1, all isolated from the Can Gio Mangrove Biosphere Reserve. In addition, five well-known *Scenedesmus*-like strains (NIES-94, NIES-97, NIES-685, NIES-2280, and UTEX 2551) were included as references. Details concerning the sampling, isolation, culturing, and morphological identification of the Can Gio isolates are provided in Sections 2.2–2.5.

2.2 Algal Sampling

Microalgal samples were collected in October 2023 from two freshwater locations within the Can Gio Mangrove Biosphere Reserve using a plankton net with a 20–50 µm mesh size. Environmental parameters such as temperature (28–32 °C), salinity (less than 5 ppt), and pH (6.5–7.5) were recorded during the collection. The samples were then transported on ice for further analysis.

2.3 Isolation and Culturing

Single-cell isolation followed micropipette techniques, transferring cells into BG-11 medium. Non-axenic cultures were purified on BG-11 agar plates and then maintained in liquid BG-11 medium under controlled conditions.

2.4 Morphological Identification

Morphological observations were conducted on axenic cultures, focusing on traits such as cell shape, size, chain formation, and wall structure, under a microscope at 40× and 100× magnifications. The classification was based on Shirora's key (Shirora 1966).

2.5 Molecular Identification

Genomic DNA was extracted using the CTAB method (Ajayi et al. 2022). Two genetic markers, ITS and 18S, were amplified by

PCR due to their complementary roles: ITS provides high resolution at the species level, while 18S offers broader phylogenetic insights at the genus level. The annealing temperatures for the PCR were set at 61.6 °C for ITS and 60.5 °C for 18S. The resulting amplicons were approximately 700 bp for ITS and 1200 bp for 18S, as described by White et al. (1990). These amplicons were verified using agarose gel electrophoresis and sequenced using the Sanger method (Sanger et al. 1977). Raw chromatograms were visualized and trimmed with FinchTV, and the sequences were aligned in SeaView to confirm their taxonomic identity against reference databases (Gouy et al. 2010).

2.6 Phylogenetic Analysis

Phylogenetic trees were constructed based on ITS and 18S sequences aligned with MUSCLE (Edgar 2022). Maximum Likelihood and Bayesian Inference methods were performed using IQ-TREE (Minh et al. 2020) and MrBayes (Ronquist et al. 2012), respectively, with branch support assessed through bootstrap (1000 replicates) and posterior probabilities. Phylogenetic trees were visualized with FigTree (Rambault 2018).

3 Results

3.1 Morphological Analysis

A morphological examination of five isolated and five reference strains revealed variability in key features, such as cell shape, size, chain formation, spine presence and length, and chloroplast distribution (Figure 1). Among the isolated strains, CCAH016/2 exhibited elliptical to spindle-shaped cells (approximately 10 µm) that formed chains of 4–8 cells. Each cell had 2–3 µm spines at both ends and evenly distributed chloroplasts, which are

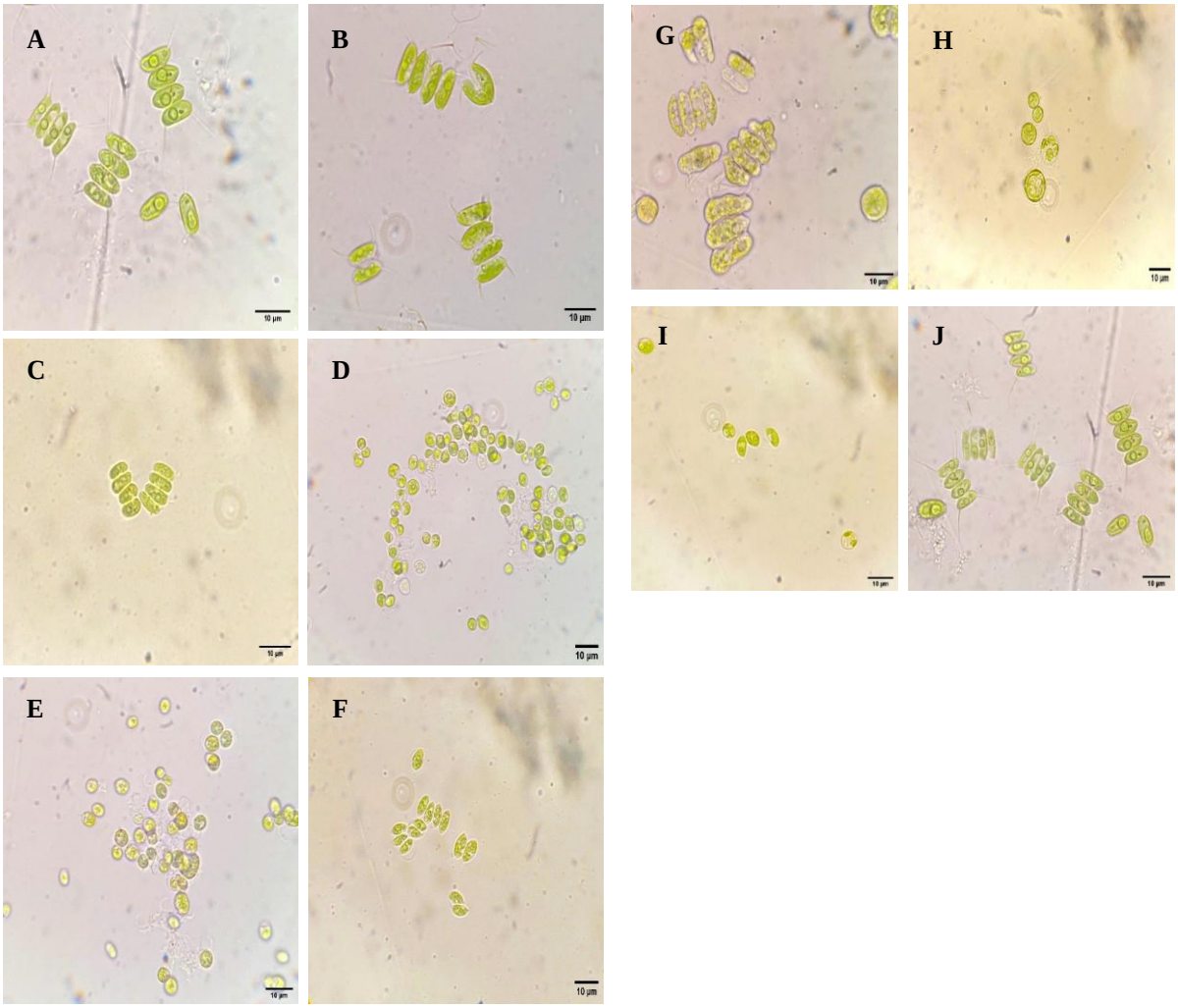

Figure 1 Microscopic observation of the studied samples and reference strains, *(A) CCAH 016/2; (B) CCAH 020/1; (C) CCAH 024/1; (D) CCAH 026/1; (E) CCAH 027/1; (F) NIES-94; (G) NIES-97; (H) NIES-685; (I) NIES-2280; (J) UTEX 2551.*

characteristics consistent with *Desmodesmus armatus*. CCAH020/1 and CCAH024/1 also displayed similar elliptical cells (8–10 µm) but formed shorter chains of 2–4 cells with smaller spines (~2 µm), aligning with *D. armatus*. In contrast, CCAH026/1 consisted of smaller, mostly solitary or paired cells (5–8 µm), ranging from elliptical to spherical, with no prominent spines, suggesting an affinity with *Scenedesmus obliquus*. CCAH027/1 formed short chains (2–4 cells) of elliptical cells (8–10 µm) without spines and contained large, dark-green chloroplasts, indicating its relationship with *Scenedesmus intermedius*.

Among the reference strains, morphological features were characteristic of the *Desmodesmus* and *Scenedesmus* genera. NIES-94 and NIES-2280 were identified as *S. obliquus* due to their lack of spines and round to elliptical cells (5–12 µm). NIES-97 was identified as *D. serratus* because of its chained, cylindrical-to-elliptical cells (6–8 µm) bearing prominent spines. NIES-685 exhibited elliptical cells (8–12 µm) with short spines, consistent with *D. abundans*, while UTEX 2551 presented chained elliptical cells (8–10 µm, with 4–6 cells per chain) and short spines, suggesting similarity to *D. armatus*.

This morphological diversity highlights the distinction between *Desmodesmus*, which typically features chained cells with prominent spines, and *Scenedesmus*, characterized by smoother or variably spined cells. These findings enhance the taxonomy and biodiversity data on Desmodesmus in Vietnam and provide a foundation for future studies of their ecological and biotechnological potential in dynamic environments, such as the Can Gio Biosphere Reserve.

3.2 Molecular Analysis

Molecular analyses of the five studied strains used two genetic markers: ITS and 18S. Examination of the ITS region, which consists of 563 nucleotides, revealed identical sequences between strains CCAH020/1 and CCAH024/1, as well as between CCAH026/1 and CCAH027/1, indicating close genetic relationships. In contrast, strain CCAH016/2 exhibited greater genetic distances (0.1998–0.2040) than the other strains, suggesting distinct genetic divergence. For the 18S region, which is 1029 nucleotides in most strains but 1425 nucleotides in CCAH026/1 and CCAH027/1 due to a unique insertion, the pairwise distances were minimal (0.0009–0.0069), confirming high conservation. Identical sequences were again observed between CCAH020/1 and CCAH024/1, as well as between CCAH026/1 and CCAH027/1. The genetic distances between these groups and CCAH016/2 were slightly higher (0.0427–0.0457), reflecting greater variability than the distances within each group in the 18S region.

BLAST analyses provided additional taxonomic clarification. For strain CCAH016/2, the ITS sequences matched *D. armatus* with 98.93–99.10% similarity, while the 18S region showed 100% identity to the same species. For CCAH020/1, the ITS sequences had 98.64–100% similarity to *D. armatus*, while the 18S region indicated 99.9–100% similarity to either *D. armatus* or *D. communis*. In the case of CCAH026/1, the ITS sequences showed 99.92–100% similarity to *D. intermedius*, while the 18S region demonstrated high similarity to *Desmodesmus* sp. and *Scenedesmus* sp.

Overall, the ITS region provided sufficient variability to distinguish genetic groups, highlighting the divergence of CCAH016/2 from the other strains. Conversely, the highly conserved 18S region confirmed genus-level relationships but offered limited resolution at the species level. These findings underscore the complementary roles of morphological and molecular data in accurately identifying and classifying strains of *Desmodesmus* and *Scenedesmus*.

3.3 Phylogenetic Analysis Based on ITS Sequences

A phylogenetic analysis of the ITS region (Figure 2) was performed to investigate the evolutionary relationships among the studied strains and reference species. The alignment included 68 sequences over 652 nucleotide positions, revealing two primary clusters: Cluster A, which consists of *Desmodesmus*, and *Cluster B*, comprising *Scenedesmus*, *Tetradesmus*, and *Acutodesmus*. High bootstrap values supported both clusters. Within Cluster A, strain CCAH016/2 was placed in Subcluster A5, showing a close relationship with *D. armatus*, but displayed an early divergence pattern, suggesting potential local variation. Strains CCAH020/1 and CCAH024/1 formed a sister clade to *D. armatus* in subcluster A3, indicating that they are genetically distinct yet closely related variants. Meanwhile, strains CCAH026/1 and CCAH027/1 were grouped in subcluster A2, consistent with their classification as *D. intermedius*.

In Cluster B, reference strains NIES-94 and NIES-2280 clustered alongside *S. obliquus*, highlighting a clear evolutionary divergence between *Desmodesmus* and *Scenedesmus*. Notably, NIES-97, initially classified as *Scenedesmus* sp., was reassigned to D. *brasiliensis* in subcluster A1. Additionally, UTEX 2551, originally labelled *Scenedesmus armatus*, was realigned with *D. armatus* in subcluster A5.

These findings support the molecular identifications of the studied strains and clarify taxonomic discrepancies in the reference strains. Specifically, the ITS region has proven highly effective in resolving species-level relationships, illustrating the genetic diversity within *D. armatus* and the distinct evolutionary separation between *Desmodesmus* and related genera.

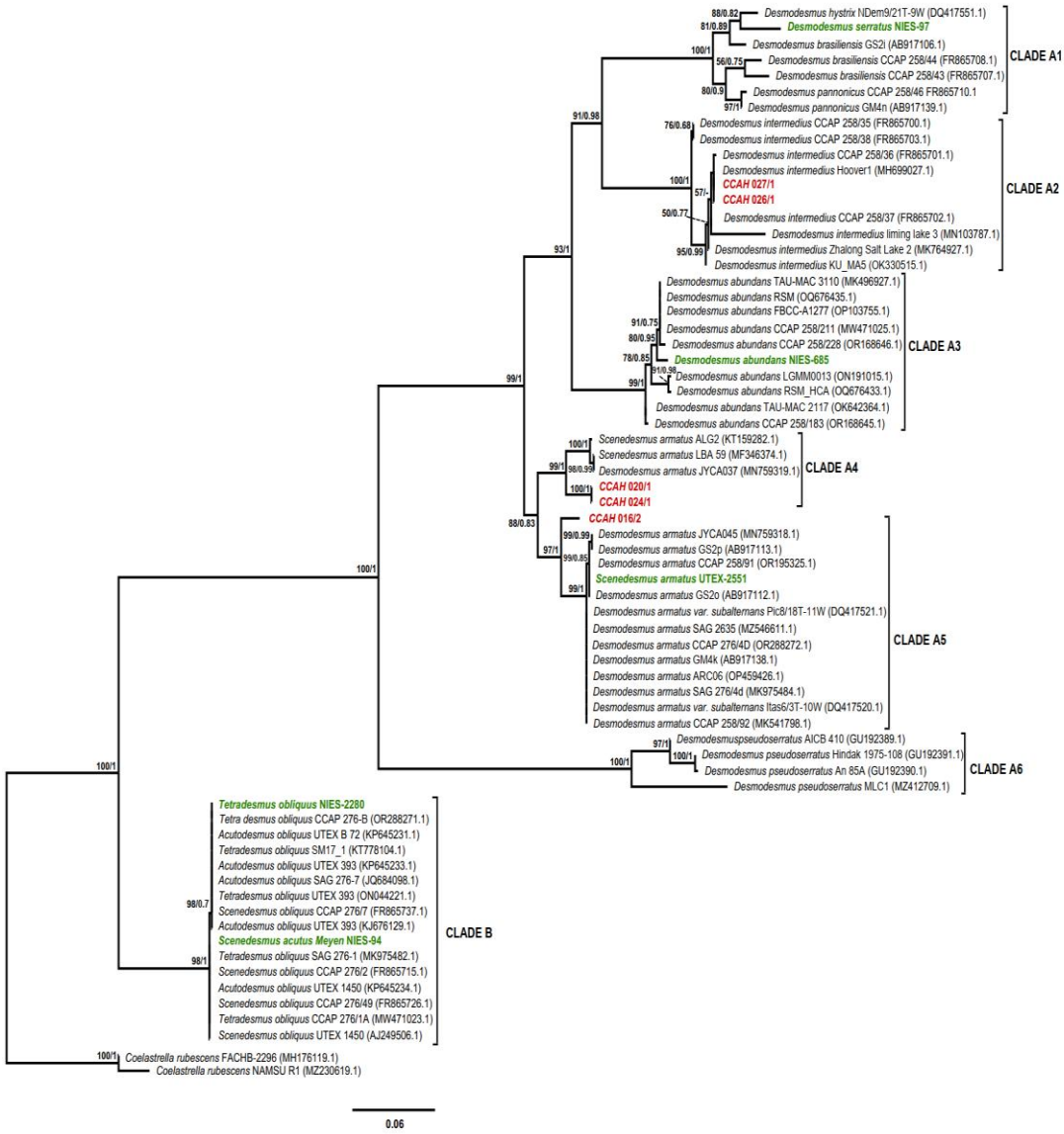

Figure 2 Phylogenetic Tree Based on the ITS Region, 68 ITS sequences, comprising 647 aligned positions, were analyzed to determine the evolutionary relationships among the studied and reference strains. Maximum Likelihood (ML) analysis was performed using IQ-TREE (SYM+G4) with 1,000 bootstrap replicates, while Bayesian Inference (BI) was conducted with MrBayes (SYM+G) for 100 million generations, accompanied by a 25% burn-in period. Branch support values, including ML bootstrap and BI posterior probabilities, are indicated at key nodes. The isolated strains are highlighted in red, and the reference strains are green. The analysis revealed two main clusters (Desmodesmus and Scenedesmus), with distinct subclusters reflecting genetic divergence within the Desmodesmus group.

3.4 Phylogenetic Analysis Based on 18S Sequences

Phylogenetic analysis of the 18S rRNA gene, based on 66 sequences aligned across 1,503 nucleotide positions, revealed two main clusters: Cluster C (*Desmodesmus*) and Cluster D (*Scenedesmus*) (Figure 3). Within Cluster C, the strain CCAH016/2 was grouped in Subcluster C1 alongside *D. armatus*, indicating its early divergence from other strains and highlighting its genetic distinctiveness. The strains CCAH020/1 and CCAH024/1 clustered in C2, confirming their identification as variants of *D. armatus*. In contrast, CCAH026/1 and CCAH027/1 formed a separate group in C5, consistent with *D. intermedius*. Cluster D included reference strains NIES-94 and NIES-2280, aligning with *S. obliquus*. Strain NIES-97 was reassigned from *D. serratus* to *D. pannonicus*, while UTEX 2551 was originally identified as *Scenedesmus armatus* clustered with *D. armatus* in subcluster C3. Overall, this analysis confirmed the identities of the studied strains and highlighted the genetic variability within *D.*

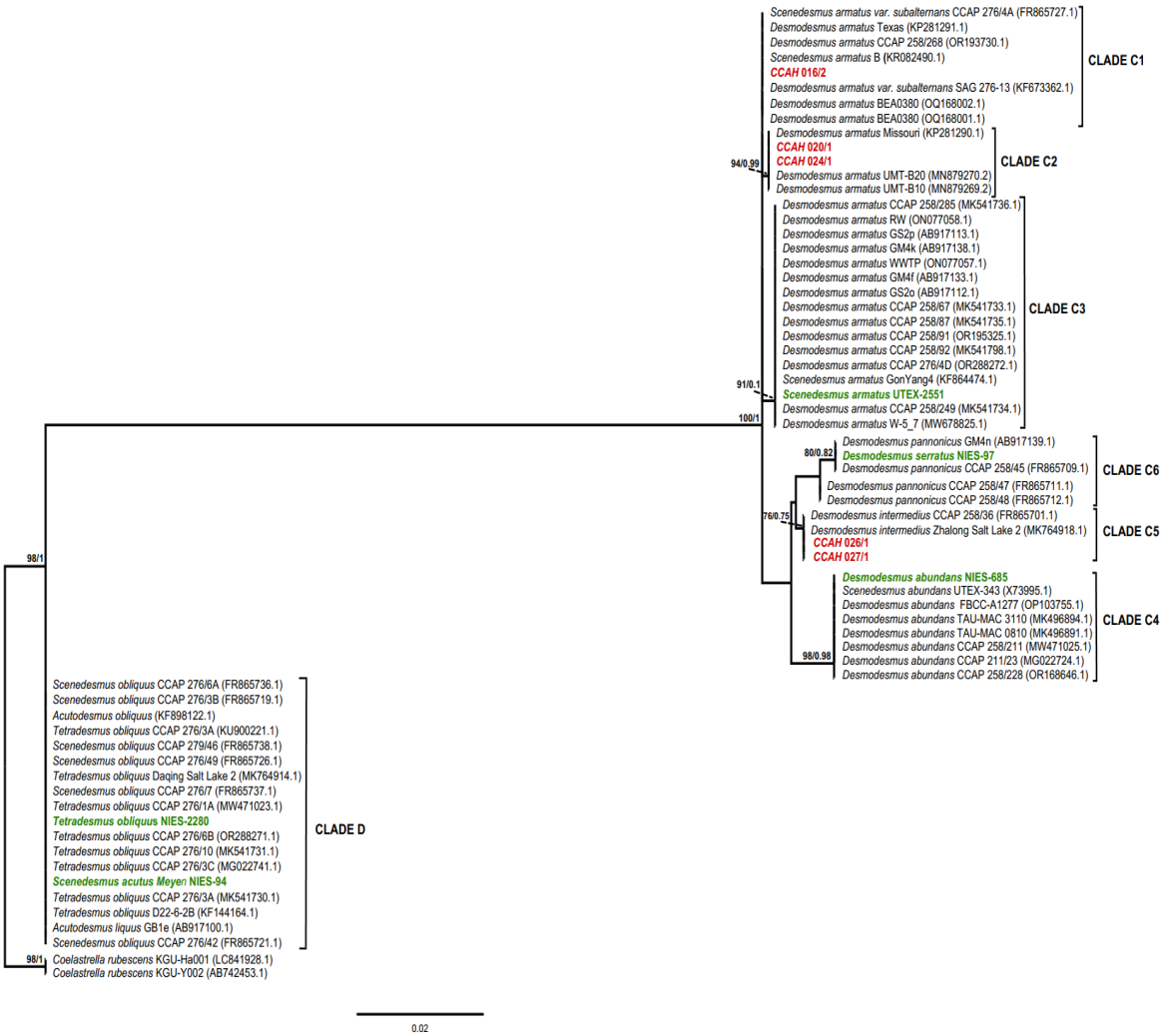

Figure 3 Phylogenetic Tree Based on the 18S Region, A total of 66 sequences, comprising 1503 aligned positions, were analyzed to assess their evolutionary relationships, maximum Likelihood (ML) analysis was conducted using IQ-TREE with the K2P+I model and included 1000 ultrafast bootstrap replicates, Bayesian Inference (BI) was performed in MrBayes using the K80+I model, running for 100 million generations with a 25% burn-in period, sampling every 1000 generations, branch support, indicated by ML bootstrap values and BI posterior probabilities, is shown at major nodes, isolated strains are represented in red, while reference strains are shown in green. The resulting tree reveals two main clusters: Desmodesmus (Cluster C) and Scenedesmus (Cluster D), with subclusters demonstrating genetic divergence within and between the genera.

armatus. However, due to the relatively conserved nature of the 18S rRNA gene, these findings emphasize the necessity for additional ITS data to achieve reliable species-level resolution.

Integrating morphological observations with molecular analyses enabled accurate identifications of the five studied strains. Morphologically, *D. armatus* was suggested for strains CCAH016/2, CCAH020/1, and CCAH024/1. In contrast, strains CCAH026/1 and CCAH027/1 were identified as either *S. obliquus* or *D. intermedius*. Molecular data confirmed these identifications, with ITS and 18S rRNA markers providing high-resolution, species-level classifications. Specifically, CCAH016/2 was confirmed as *D. armatus*, while CCAH020/1 and CCAH024/1 were identified as closely related variants of *D. armatus*. Both CCAH026/1 and CCAH027/1 were consistently identified as *D. intermedius*. Although the 18S rRNA gene effectively established relationships at the genus level, the ITS marker offered superior resolution at the species level, particularly in distinguishing CCAH016/2 from the other strains. Morphological inconsistencies, such as the initial misidentification of CCAH026/1 and CCAH027/1 as Scenedesmus, underscored the necessity for molecular validation. Table 1 summarizes these final identifications, integrating results from morphological, ITS, and 18S rRNA analyses. Overall, the combined approaches confirmed

Table 1 Summary of Morphological and Molecular Identification Results for Isolated and Reference Strains

Sample	Morphological ID	ITS Region ID	18S Region ID	Final Conclusion
CCAH016/2	*D. armatus*	*D. armatus*	*D. armatus*	*D. armatus*
CCAH020/1	*D. abundans*	*D. armatus*	*D. armatus*	*D. armatus*
CCAH024/1	*D. armatus*	*D. armatus*	*D. armatus*	*D. armatus*
CCAH026/1	*S. obliquus*	*D. intermedius*	*D. intermedius*	*D. intermedius*
CCAH027/1	*S. intermedius*	*D. intermedius*	*D. intermedius*	*D. intermedius*
NIES-94	*S. acutus*	*S. obliquus*	Similar to *S. acutus*	*S. obliquus*
NIES-97	*D. serratus*	*D. brasiliensis*	*D. serratus*	*D. serratus*
NIES-685	*D. abundans*	*D. abundans*	*D. abundans*	*D. abundans*
NIES-2280	*S. obliquus*	*S. obliquus*	*S. obliquus*	*S. obliquus*
UTEX 2551	*S. armatus*	*D. armatus*	*D. armatus*	*D. armatus*

the genetic variability within *D. armatus* and the distinct grouping of *D. intermedius*. These findings emphasize the importance of using both morphological and molecular methods for accurately identifying and classifying microalgae, especially in ecologically complex habitats like the Can Gio Biosphere Reserve.

4 Discussion

Combining morphological and molecular analyses was essential for achieving accurate species identification in this study. Although morphological traits, such as cell size, shape, and the presence of spines, initially suggested taxonomic affiliations for *Desmodesmus* and *Scenedesmus*, they ultimately proved insufficient for distinguishing closely related species (Mai et al. 2023; Nguyen et al. 2023). For instance, CCAH026/1 and CCAH027/1, initially identified morphologically as *Scenedesmus* due to their lack of spines, were reclassified as *D. intermedius* based on ITS and 18S data. These findings highlight the crucial role of molecular markers, particularly in ecologically complex and dynamic environments like the Can Gio Biosphere Reserve.

The ITS region was informative, providing high species-level resolution and serving as a common identifier for various algal taxa (Fawley and Fawley 2020; Kezlya et al. 2023; Nguyen et al. 2023). Its effectiveness is demonstrated by its ability to differentiate genetically distinct variants of *D. armatus*, such as CCAH016/2, which diverged early in phylogenetic trees, possibly indicating local adaptation or intraspecific genetic variation. Meanwhile, the highly conserved 18S region supported genus-level identifications and confirmed the relationships between *Desmodesmus* and *Scenedesmus*. Although some prior studies relied mainly on ITS for genus-level classification (Kezlya et al. 2023), the current findings highlight the complementary nature of using ITS and 18S to resolve species-level distinctions in *Desmodesmus*. Therefore, employing multiple molecular markers alongside morphological data is critical, especially for complex or closely related taxa.

Phylogenetic analyses of the ITS and 18S regions also revealed distinct evolutionary boundaries between *Desmodesmus* and *Scenedesmus*, supporting the reclassification of several reference strains. For example, UTEX 2551, initially labelled as *S. armatus*, was reassigned to *D. armatus*, while NIES-97, formerly identified as *S. serratus*, was recognized as *D. pannonicus*. Such taxonomic revisions illustrate the reliability of molecular tools in addressing longstanding classification inconsistencies and emphasize the genetic diversity within *D. armatus* and *D. intermedius*. Identifying *D. intermedius* and genetically distinct variants of *D. armatus* in the Can Gio Biosphere Reserve points to the ecological adaptability of these species. Members of *Desmodesmus* are known to thrive in nutrient-rich, dynamic habitats (Abbas et al. 2024; Lin et al. 2020), a characteristic well suited to the transitional freshwater brackish conditions of Can Gio. The observed genetic variability within *Desmodesmus* could lead to the discovery of strains with specialized properties, such as enhanced lipid production for biofuels or strong stress tolerance for bioremediation. The co-occurrence of multiple *Desmodesmus* species further underscores Can Gio's role as a biodiversity hotspot, reinforcing the need for ongoing research and conservation initiatives. A key limitation of this study is the relatively small number of strains examined. Expanding the genetic dataset for *Desmodesmus* and *Scenedesmus* would refine taxonomic resolution and allow for broader phylogenetic comparisons. Future research should investigate how environmental parameters such as light intensity, nutrient availability, and salinity impact morphological plasticity to enhance traditional identification methods. Moreover, advanced sequencing approaches, such as whole-genome sequencing, could provide additional insights into these genera's evolutionary relationships and adaptive strategies. The implications for

biotechnology and conservation are significant. The documented genetic diversity of *Desmodesmus* strains suggests the potential for novel metabolic capabilities, ranging from increased lipid yields for biofuel production to pollutant degradation for environmental remediation. Additionally, the rich algal biodiversity within the Can Gio Biosphere Reserve highlights its importance as a natural resource, deserving of sustainable management and further investigation for both ecological and economic benefits.

Conclusion

This study integrated morphological and molecular approaches to accurately identify and characterize five microalgal strains isolated from the Can Gio Mangrove Biosphere Reserve. While the morphological observations provided preliminary insights, molecular markers, particularly the ITS region, were essential for achieving species-level identification. This confirmed the presence of *D. armatus* and *D. intermedius* and revealed genetic variability within *D. armatus*. Phylogenetic analyses clarified the evolutionary relationships within and between the genera *Desmodesmus* and *Scenedesmus*, addressing taxonomic ambiguities for several reference strains. These findings enhance our understanding of microalgal diversity in Vietnam and highlight the ecological importance of the Can Gio Biosphere Reserve as a habitat for diverse and resilient microalgae. Additionally, the study emphasizes the potential of *Desmodesmus* strains for future applications in biotechnology and environmental management. Ongoing research and conservation efforts are essential for fully realizing these valuable microorganisms' ecological and economic potential.

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Conflicts of Interest

Authors declare that no conflicts exist.

Author contribution

Phuong-Nam Luu: collected samples, conducted laboratory studies, wrote reports and drafts for publication; Thanh-Cong Nguyen: collected and pre-processed experimental samples; Quoc-Dang Quan: compiled data, edited manuscript before publication; Duc-HoanHuynh: performed preliminary morphological classification; Ngoc-Nam Trinh: adjusted the research and checked the research implementation process; Tuong-Lam Le-Nguyen: conducted laboratory studies, translated manuscript and grammar edited; Hoang-Dung Tran: coordinated the entire research and edited the final manuscript.

References

Abbas, M., Ni, L., & Du, C. (2024). Kinetic modelling and salinity tolerance in *Chlorella vulgaris* and *Desmodesmus communis* (Chlorophyta): insights into differential growth responses. *Aquatic Ecology*, *2024*, 1-14. https://doi.org/10.1007/s10452-024-10153-y.

Ajayi, O.O., Adekanmbi, A., Fagade, O. E., Dianda, M. (2022). Modified Methods for Quick and Safe Extraction of DNA from Microbiological Samples. *Journal of Microbiology and Pathology*, 6 (4), 1-5. DOI: 10.37421/2952-8119.6.158.

Andersen, R. A., & Kawachi, L. (2005). Traditional microalgae isolation techniques. In R. A. Andersen (Ed.), *Algal culturing techniques*, (pp. 83-100). Elsevier Publication.

DOST HCMC (2023). List of New Science and Technology Missions in the year 2023 using the Ho Chi Minh City budget provided by the DOST. Department of Science and Technology in Ho Chi Minh City. Retrieved from https://dost.hochiminhcity.gov.vn/documents/1961/Nhiem_vu_NCKH_moi_2023.xlsx (in Vietnamese).

Fawley, M. W., & Fawley, K. P. (2020). Identification of Eukaryotic Microalgal Strains. *Journal of applied phycology*, *32*(5), 2699–2709. https://doi.org/10.1007/s10811-020-02190-5

Gouy, M., Guindon, S., & Gascuel, O. (2010). SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Molecular Biology and Evolution*, *27*(2), 221–224.

Hegewald, E. (1979). Eine neue Unterteilung der Gattung Scenedesmus Meyen. *Nova Hedwigia*, *30*, 343-376.

Johnson, J.L., Fawley, M.W., & Fawley, K.P. (2007). The diversity of *Scenedesmus* and *Desmodesmus* (Chlorophyceae) in Itasca State Park, Minnesota, USA. *Phycologia*, *46*(2), 214–229.

Kessler, E., Schafer, M., Hummer, C., Kloboucek, A., & Huss, V.A. (1997). Physiological, Biochemical, and Molecular Characters for the Taxonomy of the Subgenera of *Scenedesmus* (Chorococcales, Chlorophyta). *Botanica Acta*, *110*(3), 244-250.

Kezlya, E., Tseplik, N., & Kulikovskiy, M. (2023). Genetic Markers for Metabarcoding of Freshwater Microalgae: Review. *Biology*, *12*, 1038.

Khaw, Y. S., Khong, N. M. H., Shaharuddin, N. A., & Yusoff, F. M. (2020). A simple 18S rDNA approach for the identification of cultured eukaryotic microalgae with an emphasis on primers. *Journal of microbiological methods*, *172*, 105890.

Edgar, R. C. (2022) Muscle5: High-accuracy alignment ensembles enable unbiased assessments of sequence homology and phylogeny. *Nature Communications*, *13*, 6968.

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., Von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular biology and evolution*, *37*(5), 1530-1534.

Lin, W. J., Ho, H. C., Chu, S. C., & Chou, J. Y. (2020). Effects of auxin derivatives on phenotypic plasticity and stress tolerance in five species of the green alga *Desmodesmus* (Chlorophyceae, Chlorophyta). *Peer J*, *8*, e8623.

Mai, X. C., Shen, C. R., Liu, C. L., Trinh, D. M., & Nguyen, M. L. (2023). "DNA signaturing" database construction for *Tetradesmus* species identification and phylogenetic relationships of Scenedesmus-like green microalgae (Scenedesmaceae, Chlorophyta). *Journal of Phycology*, *59*(4), 775-784.

Nguyen, M.L., Mai, X.C., Chu, N.H., Trinh, D.M., Liu, C.L., & Shen, C.R. (2023). DNA signaturing derived from the internal transcribed spacer 2 (ITS2): a novel tool for identifying *Desmodesmus* species (Scenedesmaceae, Chlorophyta). *Fottea Olomouc*, *23*(1), 1-7.

Pham, T. L. (2017). Environmental gradients regulate the spatio-temporal variability of phytoplankton assemblages in the Can Gio Mangrove Biosphere Reserve, Vietnam. O*cean Science Journal*, *52*, 537-547.

Pham, T. L. (2019). Factors governing phytoplankton community in the Can Gio mangrove biosphere reserve, Vietnam. *Vietnam Journal of Marine Science and Technology*, *19*(1), 67–78. https://doi.org/10.15625/1859-3097/19/1/9179

Phung, B.T.M., Thanh, V.D., Chiem, N.H., & Nguyen, C.H. (2019). Diversity of benthic microalgal species in intensive rice cultivation, Cho Moi district, An Giang province Vietnam. *CTU Journal Of Science*, *55*, 53-67.

Rambaut, A. (2018). FigTree v1.4.4. Retrieved from https://github.com/rambaut/figtree

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., et al. (2012). MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic biology*, *61*(3), 539-542.

Sanger, F., Nicklen, S., & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proceedings of the National Academy of Sciences*, *74*(12), 5463–5467.

Shirora, A. (1966).*The plankton of south Viet Nam Fresh Water and Marine Plankton*. Overseas Technical Cooperation Agency Japan.

White, T.J., Bruns, T.D., Lee, S.B., & Taylor, J.W.(1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Protocols: A Guide to Methods and App (pp. 315-322). *Academic Press, New York*.