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Original Article

Integrated Morphological and DNA Barcoding Classification of Select Edible Seaweed Species in Vietnam

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Abstract: This study applied an integrative taxonomic approach combining morphological observations with DNA barcoding to identify three economically important seaweed species from Vietnam: *Gracilaria eucheumatoides*, *Ulva lactuca*, and *Gelidiella acerosa*. The mitochondrial *cox*1 and plastid *rbc*L markers successfully generated species-level molecular data, with sequence identities of 100% compared to authenticated reference sequences available in GenBank. Phylogenetic analyses consistently recovered all examined specimens within well-supported monophyletic clades corresponding to their respective taxa, while barcode gap analyses revealed low intraspecific divergence and clear interspecific separation. Morphological characteristics were congruent with molecular evidence for all examined specimens. Rather than introducing DNA barcoding as a novel methodology in Vietnam, this study contributes additional molecular and morphological reference data for three commercially important seaweed taxa that remain insufficiently documented in several coastal regions of the Vietnam East Sea. The generated datasets provide supplementary baseline information that may support future studies on species authentication, biodiversity assessment, and biological traceability associated with sustainable seaweed resource management and international market requirements.

Keywords: Anatomy, DNA barcode, Edible seaweed, Morphology, Phylogeny.

1. Introduction

Marine macroalgae, commonly known as seaweeds, are among the most ecologically important photosynthetic organisms in marine ecosystems, underpinning marine biodiversity and generating substantial socio-economic value for coastal communities worldwide. Red algae (Rhodophyta) and green algae (Chlorophyta) in particular provide nutrient-dense food sources and serve as raw materials for the industrial production of agar, carrageenan, and bioactive compounds with pharmaceutical and nutraceutical applications

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[1]. The global seaweed aquaculture industry has grown into a multi-billion-dollar sector, supporting livelihoods for millions of coastal inhabitants and contributing to food security and marine resource diversification [2].

In Vietnam, national research priorities emphasize the sustainable utilization and value-added development of these marine resources [3]. Recent assessments highlight a strategic necessity for improved resource management and product traceability. Within this context, *Gracilaria, Ulva*, and *Gelidiella* have attracted significant industrial interest due to their multifaceted applications in hydrocolloid extraction and bioresource industries [4]. Among the 890 seaweed species recorded in Vietnam to date [5–7], *Gracilaria eucheumatoides* Harvey 1860, *Ulva lactuca* Linnaeus 1753, and *Gelidiella acerosa* (Forsskål) Feldmann & Hamel 1934 represent high-value taxa widely distributed along the coastline. However, these species exhibit significant phenotypic plasticity; conventional morphology-based classification often proves inadequate when confronted with variations induced by environmental fluctuations and habitat-specific adaptations [8, 9]. Such taxonomic uncertainty constrains biodiversity studies and poses obstacles to supply chain traceability.

Demand for precise biological traceability has intensified alongside tightening global market standards. The European Union's 2017 "yellow card" on Vietnam's fisheries under Illegal, Unreported, and Unregulated (IUU) regulations, for example, mandated greater transparency [10]. These requirements now encompass the rapidly expanding seaweed sector, demanding rigorous documentation of species authenticity and sustainable sourcing to ensure international market access [11].

To meet these standards, the scientific identification of Vietnam's marine flora has evolved through the adoption of integrative taxonomy. Over the past decade, Vietnamese researchers have transitioned from purely morphological descriptions to molecular-based approaches. Foundational works pioneered by research groups within the Vietnam Academy of Science and Technology (VAST), including the Institute of Biology (formerly Institute of Biotechnology), the Institute of Oceanography (Nha Trang), and the Institute of Marine Environment and Resources (now the Institute of Oceanography – Hai Phong Branch) have generated a substantial body of molecular data on regional biodiversity. This includes phylogenetic relationships of the Scytosiphonaceae [12] and Bangiales [13], genetic diversity assessments of *Kappaphycus* and *Eucheuma* [14], and population analyses of *Gracilaria* [15], taxonomy and classification of *Meristotheca* [16], *Newhousia* [17] and *Lobophora* [6].

The present study builds on *this body of work, focusing on G. eucheumatoides, U. lactuca,* and *G. acerosa*. These three species were specifically selected due to their dominant roles in local coastal economies and their significant, yet under-documented, export potential. This investigation employs an integrative framework combining detailed morphological characterization with DNA barcoding, primarily utilizing the *cox*1 and *rbc*L genetic markers for their proven reliability in marine algal systematics [18]. The research objectives encompass the generation of robust molecular datasets to bridge geographic gaps in Southeast Asian biodiversity databases while establishing a foundational framework for future traceability initiatives and sustainable resource management in the Vietnam East Sea region.

2. Experimental

2.1. Sampling

A total of eleven specimens, representing three ecologically and economically important macroalgal taxa: *Gracilaria eucheumatoides* (n = 3), *Ulva lactuca* (n = 5), and *Gelidiella acerosa* (n = 3) were collected from five representative coastal sites spanning the northern, central, and southern regions of Vietnam between 2022 and 2024. Sampling was conducted by direct hand collection during SCUBA assisted dives at depths suitable for the natural distribution of the target species.

All sampling procedures strictly adhered to established ethical guidelines and biodiversity conservation protocols, minimizing disturbance to natural habitats. Geographic coordinates and site specific information for each collection locality are mapped in Figure 1.

Immediately after collection, fresh materials were gently rinsed with ambient seawater to remove epiphytes, sediments, and associated fauna. For anatomy and morphological analyses, cleaned thalli were fixed in 5% formalin–seawater solution and stored in labeled plastic vials. Additional herbarium specimens were prepared by carefully spreading fresh thalli on roki paper, overlaying them with absorbent newsprint sheets, and pressing under constant weight within a ventilated desiccation chamber until completely dried.

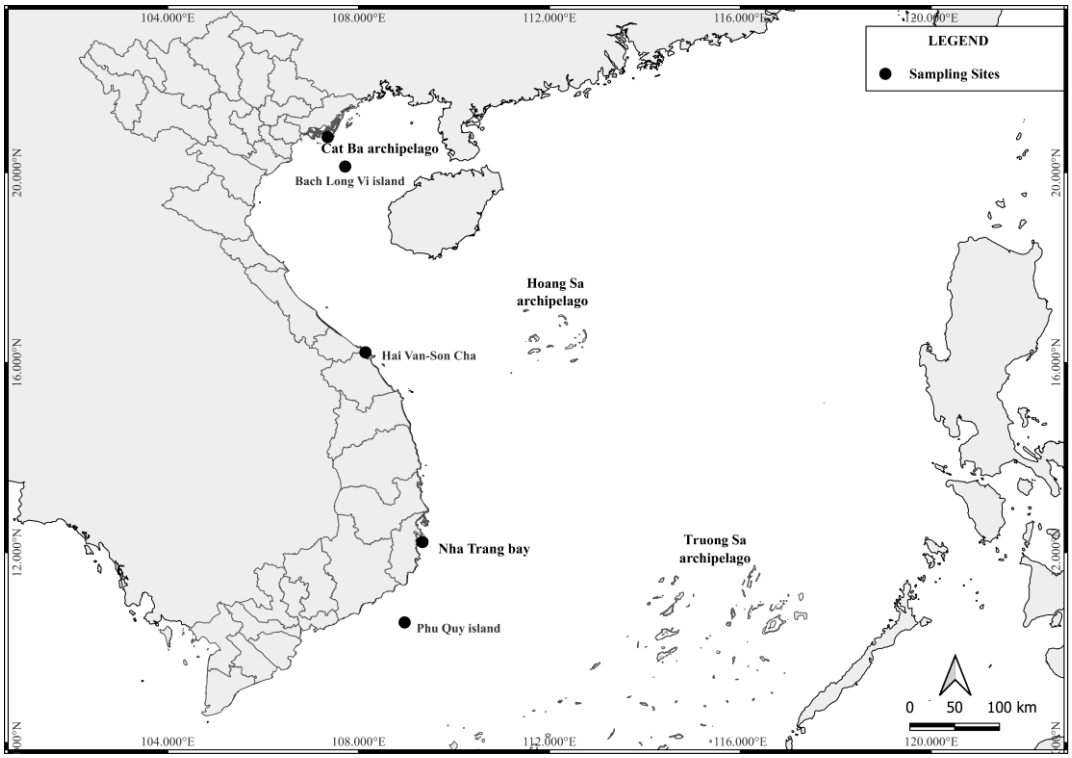

Figure 1. Map of sampling data.

For molecular analyses, a subset of fresh material was blotted dry, trimmed into small fragments, and preserved in 2-mL cryovials containing silica gel (0.2 mm particle size) to ensure rapid desiccation. Samples were subsequently stored at –10 °C until DNA extraction.

2.2. Morphological Identification

All specimens were examined at the Laboratory of Marine Biological Resources and Applications, Institute of Oceanography – Hai Phong Branch, using an integrative taxonomic approach based on external morphology and cellular anatomy. Morphological characterization followed classical phycological taxonomic standards [19, 20], with diagnostic features evaluated from both macroscopic thallus morphology and microscopic anatomical observations. Thin hand-cut sections were prepared and examined using a Nikon ECLIPSE NI-U compound microscope at magnifications ranging from ×10 to ×40. Key diagnostic characters, including medullary cell arrangement, cortical layering, reproductive structures, and pigmentation, were documented. Microscopic

observations were performed by Trang-Anh Thi Nguyen (VNU) and independently cross-checked by Mr. Linh Manh Nguyen.

Species identification proceeded through a two-step process. First, identification was guided by both national and international taxonomic references, including the foundational works of Pham Hoang Ho (1969) [19], Tseng (1983) [21], Nguyen Huu Dinh et al., (1993) [20], Nguyen Van Tien (2007) [22], and more recent revisions such as Hayden et al., (2003) [23], Jamas et al. (2017) [24] and Nguyen Manh Linh et al., (2022) [25]. Second, nomenclature and species validation were cross-checked against the global algal taxonomic repository AlgaeBase (www.algaebase.org), with additional notes made on synonymy, biology, ecology, distribution, voucher specimens, and potential economic value when available. This integrative approach ensured consistency with current international taxonomic frameworks while retaining regional specificity in identification.

2.3. DNA Extraction, Amplification, and Sequencing

Genomic DNA was extracted from silica gel–preserved tissue samples using the MagPurix® Plant DNA Extraction Kit v1.3 (ZP02014; Zinexts Life Science Corporation, New Taipei City, Taiwan) following the manufacturer's instructions. Fresh algal tissues were incubated in PLA buffer for 4 h at 60 °C with agitation (Provocell incubator, ESCO, Singapore) prior to automated extraction on the MagPurix 12 A platform (Zinexts Life Science Corporation). Final DNA extracts were eluted in 100 µL and stored at –20 °C for downstream applications.

Polymerase chain reaction (PCR) amplification was performed in 20 µL reaction volumes using either MG 2× Taq PreMix (MGmed, Seoul, Korea) or AccuPower® Taq PCR Premix (Bioneer Corp., Korea), depending on experimental conditions. Reactions were carried out using Kyratec SC300G-R2 (Kyratec, Mansfield, Australia) and AllInOneCycler (A-2041-1N; Bioneer Corp., Korea) thermal cyclers. Gene markers were selected based on their established utility in DNA barcoding studies of macroalgae. The mitochondrial *cox*1 locus was amplified using primers GazF1 (5′-TCAACAAATCATAAAGATATTGG-3′) and GazR1 (5′-ACTTCTGGATGTCCAAAAAAYCA-3′) [26], generating expected amplicons of approximately 850–1000 bp, while the plastid *rbc*L locus was amplified using primers rbcLF57 (5′-GTAATTCCATATGCTAAAATGGG-3′) and rbcLrevNEW (5′-ACATTTGCTGTTGGAGTYTC-3′) [27], yielding fragments of approximately 1,200–1,500 bp. PCR products exhibited high specificity and were verified for integrity by electrophoresis on 1.5% agarose gels stained with GelRed. Successfully amplified fragments were purified and submitted to Macrogen for bidirectional Sanger sequencing.

2.4. DNA Barcoding and Phylogenetic Analyses

The obtained DNA sequences underwent thoroughly edited and trimmed using Chromas (Technelysium Pty Ltd.) and manually curated in BioEdit v7.2 [28] and AliView [29]. Consensus sequences were subjected to BLAST searches against the NCBI GenBank database (http://www.ncbi.nlm.nih.gov/BLAST/) to confirm taxonomic identity. Sequence alignment was performed with ClustalW [30].

For phylogenetic reconstruction, aligned sequences were analyzed together with reference sequences retrieved from GenBank. Maximum likelihood phylogenetic inferences were performed using Seaview [31] with the GTR+Gamma substitution model. Mega X was used independently to estimate pairwise genetic distances [32]. Results were visualized as heatmaps with hierarchical clustering using the pheatmap package [33] in R version 4.4.1 [34]. These analyses provided a comprehensive overview of intra- and interspecific sequence similarities, thereby complementing the phylogenetic reconstructions. Branch support was assessed using ultrafast bootstrapping (1000 replicates) [35]. *Padina* species were

included as outgroup taxa to root the trees and visually delineate the ingroup taxa from distantly related lineages; this choice reflects their phylogenetically distant position and does not imply close evolutionary relationships.

3. Results and Discussion

3.1. Morphological and Taxonomic Descriptions

Ulva lactuca (Linnaeus 1753) – Rong cải biển nhăn.

Synonyms: - *Ulva fasciata* Delile 1813.

Description: Thallus of *U. lactuca* is thin, delicate, and leaf-like, typically forming broad, flattened sheets or lobes with a soft texture. Individual blades usually measure 10–15 cm in length and 5–10 cm in width, with a thickness ranging from 30–80 μm. Margins of the thallus are often undulated or irregularly folded, with rounded or blunt apices, and the overall coloration varies from light green to deep green depending on age and habitat conditions. Surface observations reveal polygonal cells with rounded corners, each cell occupying an average area of 100–140 μm². Transverse sections demonstrate a bilayered cell arrangement composed of quadrate to rectangular cells (13–25 μm in width and 20–35 μm in length), with relatively thickened cell walls. Each chloroplast typically contains two pyrenoids (Figure 2).

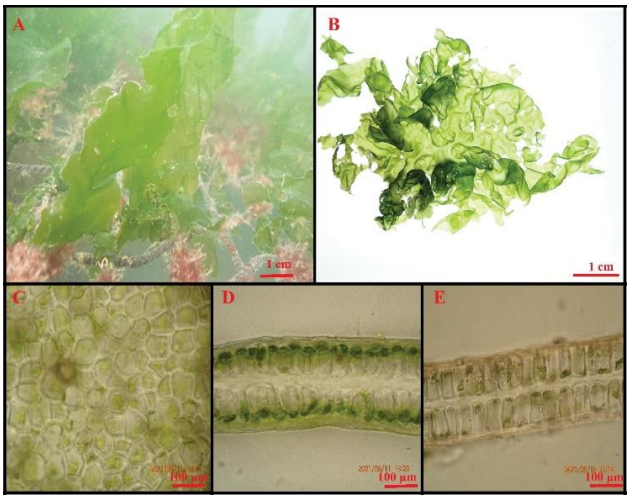

Figure 2. Morphological and anatomical characteristics of *Ulva lactuca*.

Notes: (A) In situ habitat photograph showing U. lactuca growing on fishnet at Cat Ba Island, Hai Phong City, Vietnam, at a depth of approximately 2 m; voucher specimen IMER22011; scale bar = 1 cm. (B) Freshly collected specimen from Nha Trang, Khanh Hoa Province, Vietnam; voucher specimen IMER23100; scale bar = 1 cm. (C) Surface view of vegetative cells. (D) Transverse section of the thallus, voucher specimen IMER22011. (E) Longitudinal section of the thallus, voucher specimen IMER22011. Scale bars: (C–E) = 100 μm.

Habitat: This species is commonly distributed in intertidal to shallow subtidal zones, typically attaching to rocks, pebbles, mollusk shells, submerged wood, or saltpan substrates at elevations of 2.0–2.5 m above chart datum. Seasonal growth patterns are pronounced: *U. lactuca* thrives from January to July, with peak development observed in March–April, when thalli often exceed 20 cm in length and may occasionally reach up to 30 cm. In contrast, thalli deteriorate rapidly during the monsoon season (July–September), reflecting

the species' sensitivity to hydrodynamic stress and salinity fluctuations [36].

Economic values: *U. lactuca* is an economically and pharmacologically valuable species widely used as food, fertilizer, and in traditional medicine for the treatment of helminthic infections, burns, goiter, and detoxification [37]. Its extracts have demonstrated antibacterial, antiviral, and antimicrobial activities. Biochemical analyses reveal a rich composition of amino acids (e.g., alanine α, β, arginine, asparagine, cysteine, cystine, glutamine, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, tryptophan, and valine), carbohydrates (glucuronic acid, glucose, rhamnose, glucan, starch), lipids and fatty acids, and essential minerals (B, Ca, P, Fe, Na, N, K, I, Mg, Mn, Cu). Pigment composition includes carotene, lutein, chlorophyll a and b, siphonaxanthin, and zeaxanthin. Additional metabolites include tocopherols, terpenes, and methane-yielding precursors [38]. Notably, *U. lactuca* has been proposed as a bioindicator for cadmium contamination in marine environments [39].

Gracilaria eucheumatoides (Harvey 1860) – Rong câu chân vịt.

Synonyms: - *Hydropuntia eucheumatoides* (Harvey) Gurgel and Fredericq 2004.

Description*:* Thallus of *G. eucheumatoides* is cartilaginous and flattened, measuring 5–15 cm in length and 3–5 mm in width, occasionally wider. Branching is irregular to pinnate, with some branches growing adventitiously. Branches are distinctly flattened, lacking cylindrical axes, and often bear short spines or conical protuberances (1–1.5 mm) along the margins. Additional protuberant outgrowths are observed both as lateral connections between adjacent branches and as attachment processes on the ventral surface. In transverse section, the cortex is composed of 3–4 layers of cells: the outermost layer consists of elongate cells aligned longitudinally (10–25 μm in length), beneath which lie rounded cortical cells with diameters of 5–10 μm. Medulla is formed by 10–15 layers of spherical to oval cells, gradually increasing in size from the periphery inward, ranging from 35–300 μm in diameter. Reproductive structures are well-differentiated. Tetrasporangia are elongated to clavate, measuring 50 × 15 μm, lacking cruciate division, and surrounded by elongated cortical cells. Spermatangial structures are branched, measuring approximately 130 × 40 μm, and typically arise from the fusion of 3–6 smaller units. Cystocarps are globose, protruding on the branch surface, with diameters of 1–2 mm. The pericarp is relatively thick (400–500 μm), consisting of 25–30 cell layers: the outermost cylindrical cells (20–25 × 10–13 μm) are arranged perpendicularly, whereas the inner layers are composed of uniformly distributed rounded cells. Carpospores are spherical, 15–20 μm in diameter (Figure 3).

Habitat: This species typically forms dense mats, creeping among crevices of rocks and dead coral substrates from the lower intertidal zone to depths of 1–2 m. Phenological observations indicate recruitment beginning in December–January, with optimal growth occurring from April to July. In contrast, growth is markedly reduced during the rainy season (August–October). Vegetative propagation appears to be the primary mode of regeneration, supporting its persistence under fluctuating environmental conditions [40].

Economic values: *G. eucheumatoides* is an important economic species widely exploited for multiple purposes. It serves as a food source and a raw material for agar production, while also yielding bioactive compounds such as fatty acids, lipids, minerals, plant growth regulators, and pigments. Polysaccharides including galactans, funoran, and furcellaran, as well as starch, proteins, and simple sugars, are abundant. Beyond its nutritional and industrial values, this species has been traditionally used as fishing bait, organic fertilizer, and even as a natural pesticide. Pharmacological studies further highlight its potential as a source of medicinal compounds [41].

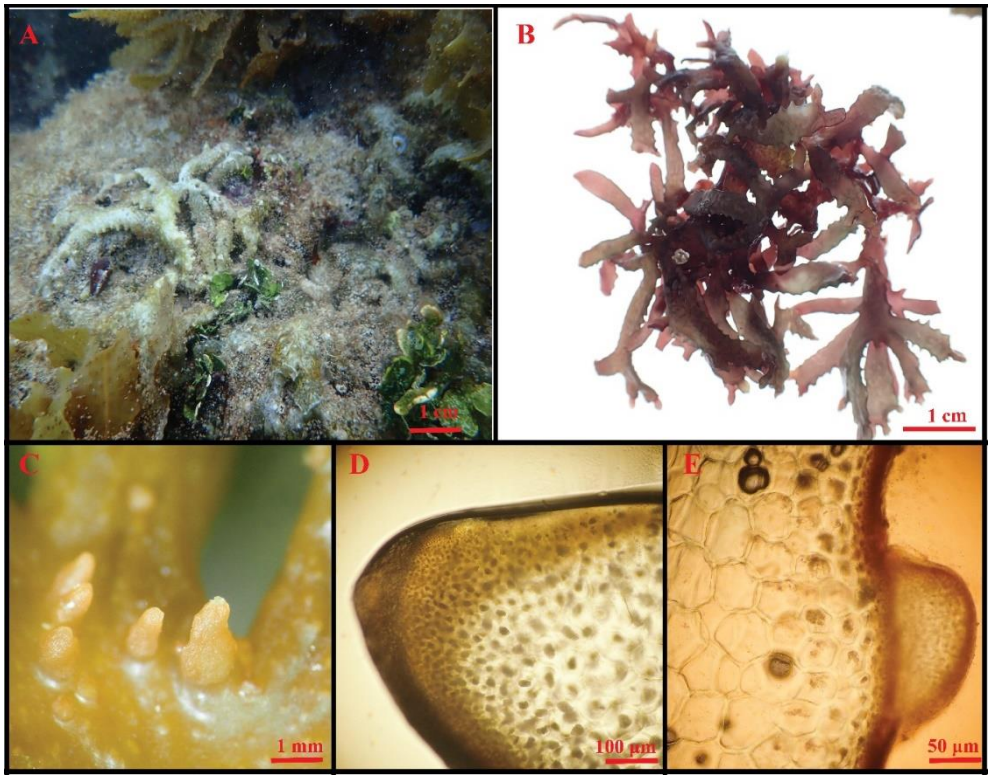

Figure 3. Morphological and anatomical characteristics of *Gracilaria eucheumatoides*.

Notes: (A) In situ habitat photograph showing the specimen growing in its natural habitat at Hai Van–Son Cha, Hue City, Vietnam, at a depth of approximately 5 m; voucher specimen IMER23144; scale bar = 1 cm. (B) Freshly collected specimen from Hai Van–Son Cha, Hue City, Vietnam; voucher specimen IMER23144; scale bar = 1 cm. (C) Surface view of vegetative cells. (D) Transverse section of the thallus, voucher specimen IMER23144. (E) Longitudinal section of the thallus, voucher specimen IMER23144. Scale bars: (C) = 1 mm; (D) = 100 µm; (E) = 50 µm.

Gelidiella acerosa (Feldmann and Hamel 1934) - Rong đá cong.

Description: Thallus of *G. acerosa* is delicate and filamentous, often resembling barbed wire in texture. It typically reaches 2–10 cm in height, though some specimens may extend to 15 cm. The main axis, cylindrical to slightly compressed, measures 0.5–1.5 mm in diameter and may grow prostrate or erect. From the primary axes, lateral branches arise irregularly or in opposite pairs, tapering to sharp, stiff apices that give the thallus its characteristic acerose (needle-like) appearance. Holdfast system is well-developed, consisting of discoid pads or stoloniferous extensions that anchor the alga firmly to hard substrates such as rocks, dead corals, and other calcareous surfaces. The thallus surface is smooth and covered by a compact outer cortical layer composed of small, elliptical cells. Beneath this, multiple layers of polygonal to ovoid cortical cells rich in pigments form a dense sheath. The central medulla is composed of large, rounded to oval cells with thickened walls, loosely arranged compared to the cortex. In longitudinal section, medullary cells appear elongated along the branch axis and are loosely interconnected, whereas cortical cells are smaller and radially arranged towards the thallus periphery. Reproductive structures are distinctive. Tetrasporangia are typically aligned in V-shaped rows or distributed longitudinally

along the branches. Cystocarps are globose and borne on short lateral branches, lacking a conspicuous ostiole. Carposporangia within the cystocarps are spherical and embedded in a thickened pericarp (Figure 4).

Habitat: *G. acerosa* typically inhabits the mid- to lower-intertidal zones and shallow subtidal environments, growing on rocky substrates, boulders, dead corals, or other hard surfaces. It thrives in areas with moderate to strong wave action, good water circulation, and high-water clarity [20]. Ecologically, this species plays a crucial role as a primary producer in coral reef and rocky shore ecosystems, contributing both as a food source and as structural habitat for small marine invertebrates.

Economic values: This species holds significant ecological and economic importance. It is harvested as a raw material for agar production and is locally consumed as food [42]. Additionally, it has been used as livestock feed and as an organic fertilizer. Recent studies highlight its pharmacological potential, with extracts demonstrating antioxidant, antibacterial, and anti-inflammatory activities, suggesting promising applications in nutraceuticals and marine-derived pharmaceuticals [43].

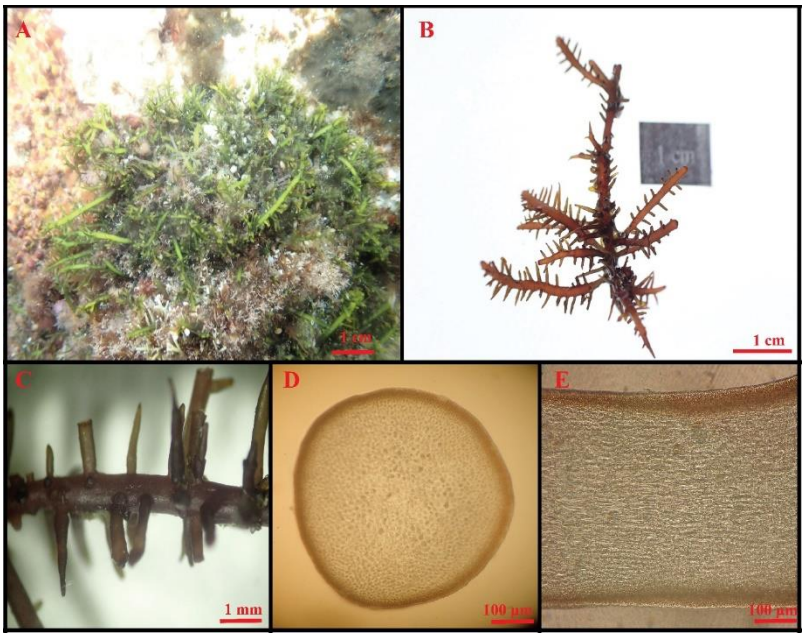

Figure 4. Morphological and anatomical characteristics of *Gelidiella acerosa*.

Notes: (A) In situ habitat photograph showing the specimen growing in its natural habitat at Hai Van–Son Cha, Hue City, Vietnam, at a depth of approximately 5 m; voucher specimen IMER24129; scale bar = 1 cm. (B) Freshly collected specimen from Hai Van–Son Cha, Hue City, Vietnam; voucher specimen IMER24129; scale bar = 1 cm. (C) Surface view of vegetative cells. (D) Transverse section of the thallus, voucher specimen IMER24200. (E) Longitudinal section of the thallus, voucher specimen IMER24200. Scale bars: (C) = 1 mm; (D–E) = 100 µm.

3.2. DNA Extraction and Quality Assessment

Genomic DNA extraction from all 11 seaweed specimens yielded high-quality nucleic acid preparations characterized by robust molecular weights and consistently optimal concentrations across all taxonomic groups examined. Spectrophotometric analysis revealed DNA purity ratios (A_{260}/A_{280}) consistently ranging between 1.8-2.0, indicating minimal protein and RNA contamination and

thus high DNA purity. Gel electrophoresis visualization further confirmed the presence of high molecular weight genomic DNA with minimal degradation, indicating good nucleic acid integrity and demonstrating the efficacy of the cryopreservation protocol and extraction methodology. The successful extraction outcomes across all three target species (*Gracilaria eucheumatoides*, *Ulva lactuca*, and *Gelidiella acerosa*) established a solid foundation for subsequent molecular barcoding analyses, with DNA concentrations ranging from 50-200 ng/μL, well within the optimal range for PCR amplification protocols.

3.3. PCR Amplification and DNA Sequencing

The efficacy of DNA barcoding applications fundamentally depends upon the reliability of PCR amplification protocols and the specificity of primer systems employed for target gene regions. In this comprehensive molecular investigation, both *cox*1 and *rbc*L barcode markers demonstrated exceptional amplification success, achieving a 100% amplification rate across all 11 specimens examined. This remarkable amplification consistency underscores the robust primer design and optimized PCR conditions employed in the current study. PCR amplification products exhibited high specificity and generated amplicons of expected molecular sizes, with *cox*1 products ranging from 861-945 base pairs and *rbc*L fragments spanning 1.200-1.369 base pairs, conforming precisely to predicted amplicon lengths based on primer positioning and target gene architecture. Agarose gel electrophoresis revealed clean, single-band amplification products with no evidence of non-specific amplification or primer-dimer artifacts, confirming the high specificity of the molecular protocols employed.

Subsequently, all amplification products were successfully processed through automated DNA sequencing protocols, achieving a comprehensive 100% sequencing success rate. The resulting chromatograms demonstrated high signal intensity, minimal background noise, and excellent base-calling accuracy throughout the entire length of each sequence, facilitating confident nucleotide identification and ensuring high-quality sequence data for downstream phylogenetic analyses. The complete dataset, comprising 22 high-quality DNA sequences (11 *cox*1 and 11 *rbc*L sequences), was compiled into standardized FASTA format files and subjected to comprehensive multiple sequence alignment protocols using ClustalW algorithms, generating robust sequence alignments that facilitated detailed comparative genomic analysis of the genetic information obtained from all study specimens.

3.4. DNA Barcoding Analysis and Species Identification

Definitive species identification was achieved through an integrative analysis of morphological traits and DNA barcoding. BLAST searches of *cox*1 and *rbc*L sequences yielded high taxonomic congruence, with all specimens showing 100% sequence identity to authenticated NCBI GenBank reference sequences (Table 1). Both markers demonstrated exceptional discriminatory power and 100% identification accuracy at the species level, as further validated by neighbor-joining and maximum likelihood phylogenetic distance analyses.

3.5. Phylogenetic Analysis and Evolutionary Relationships

The phylogenetic reconstructions elucidate the evolutionary relationships and ancestral lineage patterns among the target seaweed species, employing both *cox*1 and *rbc*L molecular markers to construct comprehensive phylogenetic frameworks. Maximum Likelihood (ML) phylogenetic trees were constructed for each primer pair utilizing the complete dataset of 11 sequences from Vietnamese seaweed specimens, supplemented with carefully selected reference sequences retrieved from GenBank to provide robust comparative context and evolutionary perspective (Figure 5).

Both *cox*1 (Figure 5A) and *rbc*L (Figure 5B) phylogenetic reconstructions demonstrate unequivocal monophyletic clustering and distinct evolutionary separation of the three economically significant seaweed species investigated throughout Vietnamese coastal waters: *Ulva lactuca*, *Gracilaria eucheumatoides*, and *Gelidiella acerosa*. The congruent topological patterns observed across both molecular markers provide compelling evidence for the taxonomic validity and evolutionary distinctness of these commercially important taxa.

The *cox*1 phylogenetic analysis reveals exceptionally robust species-level resolution with pronounced genetic differentiation among target taxa. All *U. lactuca* specimens collected from diverse geographic localities across Vietnam's extensive coastline form a highly supported monophyletic cluster, exhibiting perfect congruence with international reference sequences and demonstrating remarkable intraspecific genetic cohesion despite broad geographic distribution. This clustering pattern reflects strong evolutionary conservation within the species and indicates stable genetic boundaries that transcend geographic isolation. Similarly, *G. acerosa* specimens display tight phylogenetic clustering with their corresponding reference sequences, forming a distinct and well-supported clade that maintains complete evolutionary separation from congeners and related species within the genus. The clear demarcation of this lineage underscores the taxonomic integrity of *G. acerosa* and validates its distinct evolutionary trajectory. Concurrently, *G. eucheumatoides* specimens establish their own exclusive phylogenetic clade, demonstrating perfect concordance with BLAST identification results and providing robust molecular support for morphology-based taxonomic assignments. Particularly noteworthy is the pronounced branch length variation observed within the *cox*1 phylogenetic tree, which reveals exceptional phylogenetic resolution and clearly defined DNA barcoding gaps between species-level taxa. These substantial interspecific genetic distances, coupled with minimal intraspecific variation, confirm the superior discriminatory power of the *cox*1 marker for species identification and phylogenetic inference among closely related marine macroalgal taxa.

Table 1. Taxonomic and molecular identification of seaweed through BLAST analysis using cox1 and rbcL barcodes, along with respective GenBank numbers

Vouchers	**Morphology**	**Scientific name**	**BLAST top hit *cox*1**	**BLAST top hit *rbc*L**	**Identity (%)**
VNA24129	*Gelidiella acerosa*	*Gelidiella acerosa*	KX184337	KX184389	100
IMER24200	*Gelidiella acerosa*	*Gelidiella acerosa*	KX184336	KX184389	100
IMER24201	*Gelidiella acerosa*	*Gelidiella acerosa*	KX184337	KX184389	100
VNA23144	*Gracilaria eucheumatoides*	*Gracilaria eucheumatoides*	NC085259	OP669593	100
IMER24100	*Gracilaria eucheumatoides*	*Gracilaria eucheumatoides*	NC085259	OP669593	100
IMER24300	*Gracilaria eucheumatoides*	*Gracilaria eucheumatoides*	NC085259	OP669592	100
IMER22102	*Ulva lactuca*	*Ulva lactuca*	MH763013	OQ687093	100
IMER22011	*Ulva lactuca*	*Ulva lactuca*	MH763013	OQ687093	100
VNA24579	*Ulva lactuca*	*Ulva lactuca*	MH763013	OQ687093	100
IMER23100	*Ulva lactuca*	*Ulva lactuca*	MH763013	OQ687093	100
IMER23279	*Ulva lactuca*	*Ulva lactuca*	MH763013	OQ687093	100

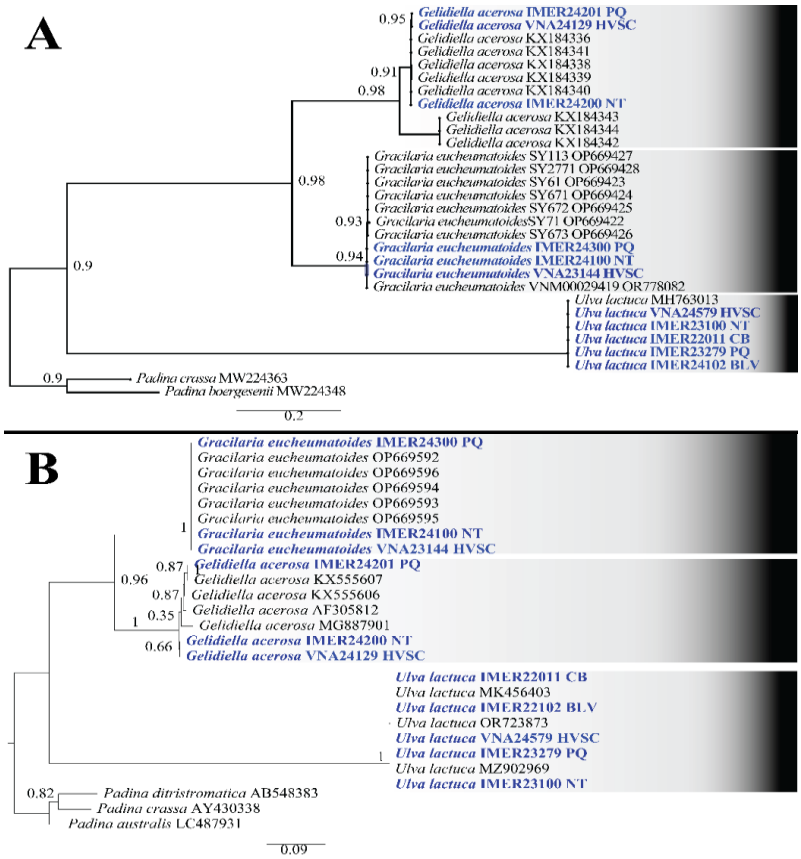

Figure 5. Maximum likelihood phylogenetic trees of economically important Vietnamese seaweed species based on DNA barcoding markers.

Notes: A) *cox*1-based phylogeny showing species-level relationships and genetic distances among *Ulva lactuca*, *Gracilaria eucheumatoides*, and *Gelidiella acerosa* specimens collected from Vietnamese coastal waters, with reference sequences from GenBank. B) *rbc*L-based phylogenetic reconstruction demonstrating genus-level relationships and taxonomic validation. Bootstrap support values >70% are shown at major nodes. Padina species serve as outgroup taxa. Scale bars represent substitutions per site.

The *rbc*L based phylogenetic reconstruction similarly demonstrates strong monophyletic support for all three study species, although interspecific genetic distances appear more compressed relative to *cox*1 analysis. Despite these reduced phylogenetic distances, the *rbc*L marker maintains consistent branching patterns and provides reliable genus-level identification accuracy, confirming its valuable complementary role in taxonomic verification and assessment of deeper evolutionary relationships within marine algal lineages. The structural stability and topological consistency observed in *rbc*L phylogenetic trees validate the marker's utility as a secondary barcoding tool, particularly for confirming species identifications and evaluating broader phylogenetic relationships that extend beyond immediate species boundaries. The convergent results between *cox*1 and *rbc*L analyses strengthen confidence in taxonomic assignments and provide robust molecular evidence for species delimitation

The strategic incorporation of well-characterized outgroup taxa (*Padina australis*, *P. distromatica*, *P. crassa*, and *P. borgeana*)

significantly enhances the analytical rigor and interpretive reliability of the phylogenetic reconstructions. These carefully selected outgroup representatives effectively root the phylogenetic trees and provide essential evolutionary context that clearly delineates the target species from distantly related lineages. The pronounced phylogenetic separation between ingroup and outgroup taxa validates the monophyletic integrity of each target species while simultaneously confirming the overall reliability and accuracy of the reconstructed evolutionary relationships.

3.6. Pairwise Nucleotide Similarity Matrix Analysis

The nucleotide similarity matrix, visualized as a heatmap with dendrogram clustering (Figure 6), illustrates the genetic relationships within and among the three Vietnamese seaweed species examined in this study. This hierarchical clustering approach, interpreted alongside the phylogenetic results, clarifies species boundaries and intraspecific genetic diversity across the target taxa.

The nucleotide matrix provided critical insights into the intra- and interspecific variability across the studied taxa. The Ulva lactuca cluster exhibited an almost perfect level of sequence similarity (≈1.00), underscoring its remarkable genetic stability and the strong integrity of this species across its distributional range in Vietnam. Similarly, the Gelidiella acerosa group formed a highly homogeneous cluster with pairwise similarity values ranging from ~0.95 to 0.99. Such high concordance is indicative of low intraspecific variability, further corroborating its monophyletic status, as consistently observed in the phylogenetic reconstruction. In contrast, Gracilaria eucheumatoides displayed a much broader spectrum of intraspecific similarity (≈0.62–0.93). This marked heterogeneity suggests the existence of diverse haplotypes or potential population subdivision across geographic regions. The observed variation could be linked to environmental heterogeneity, localized adaptation, or historical demographic events, and highlights the need for further population-level studies to elucidate the evolutionary dynamics and biogeographic structuring of this species in Vietnam.

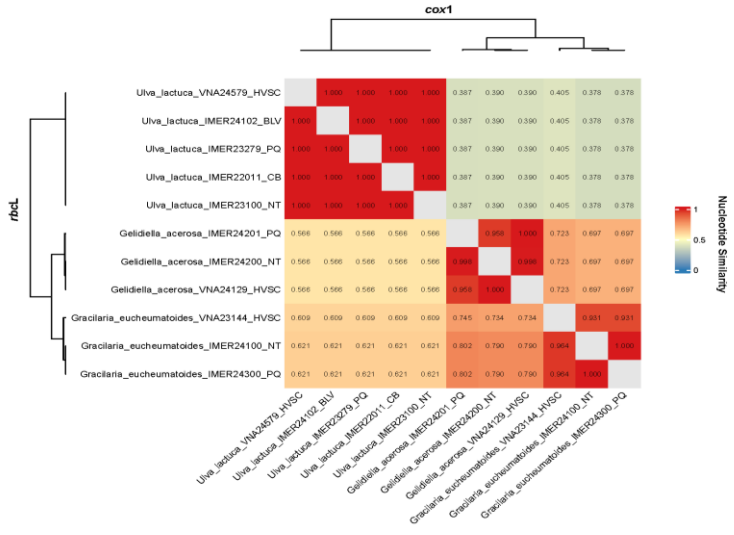

Figure 6. Heatmap of nucleotide similarity matrix based on *rbc*L and *cox*1.

Notes: The color-coded similarity matrix clearly delineates distinct molecular clusters corresponding to each species. Red intensities indicate high levels of nucleotide similarity (approaching 1.0), while lighter shades represent lower similarity values between the taxa analyzed.

3.7. Discussion

This study demonstrates the value of integrating traditional morphology with DNA barcoding for the identification of commercially important seaweeds along the Vietnamese coast. While morphological traits remain important for field recognition, their plasticity under environmental influences necessitates molecular corroboration for taxonomic precision.

Morphological observations confirmed that the three focal taxa *Ulva lactuca, Gracilaria eucheumatoides*, and *Gelidiella acerosa* exhibited diagnostic traits consistent with previous descriptions [24, 25, 44]. Seaweed morphological plasticity, induced by light intensity, salinity, and hydrodynamic conditions, has historically caused misidentifications, particularly among convergent taxa such as *Ulva* [45–47] and *Gracilaria* [48]. The difficulties in distinguishing cryptic or pseudocryptic species underscore the importance of supplementing morphology with molecular markers.

The integration of two universal markers, *cox*1 and *rbc*L, provided robust molecular evidence that strengthened morphological identifications. BLAST analyses revealed 100% sequence identity with reference sequences in GenBank, thereby eliminating the possibility of mislabeling or cross-contamination. The presence of a well-defined barcode gap, with low intraspecific distances (0.0–0.01) and substantially higher interspecific divergences (0.045–0.089), further validated the effectiveness of these markers, aligning with the criteria for an ideal DNA barcode [49, 50]. The superior discriminatory power of *cox*1 is consistent with Saunders (2005) [26], who developed novel primers for red algae to amplify the 5′ *cox*1 barcode region and first demonstrated its promise for species identification in Rhodophyta, and with Robba et al., (2006) [51], who confirmed *cox*1 as the primary barcoding locus across multiple red algal orders including Florideophyceae and Bangiales.

For *Ulva lactuca*, the near-uniform intraspecific similarity (≈1.00) observed in the present study aligns with findings by Saunders and Kucera (2010) [27], who evaluated *rbc*L, *tuf*A, UPA, LSU, and ITS as barcode markers for marine green macroalgae and demonstrated that *tuf*A and *rbc*L are effective for discriminating *Ulva* species, while noting that foliose *Ulva* species exhibit considerable morphological plasticity requiring molecular confirmation. The genetic homogeneity recorded for *U. lactuca* in the present study suggests a single cohesive population in the study area, contrasting with the cryptic diversity reported in *Ulva* from other Indo-Pacific regions.

Analyses of the pairwise nucleotide matrix provided additional evidence for species level resolution. The intraspecific similarity of *Gelidiella acerosa* (0.95–0.99) recorded here is consistent with findings by Iha et al., (2015) [52], who applied a combined COI-5P, UPA, and *rbc*L barcoding approach to Brazilian Gelidiales and confirmed *G. acerosa* as a genetically distinct species. Further corroborating evidence comes from Boo et al. (2016) [53], who used *cox*1 and *rbc*L to resolve phylogenetic relationships within Gelidiales and established the utility of these markers for generic and species-level delimitation. By contrast, *Gracilaria eucheumatoides* exhibited broader intraspecific variation (0.62–0.93), suggesting the existence of diverse haplotypes or population subdivision across geographic regions. This pattern is comparable to findings in closely related *Gracilaria* species across Southeast Asia: Yang et al., (2013) [54] applied COI barcoding to *G. salicornia* from the Philippines, Malaysia, Thailand, China, and Japan, identifying eight distinct haplotypes with intraspecific nucleotide divergences of 0.0–1.3%, demonstrating that COI effectively reveals biogeographic structuring within the genus. Similarly, Yow et al., (2013) [55] used *cox*1 and the *cox*2-3 spacer to assess seven distinct haplotypes in *Gracilaria changii* across 11 geographically distinct populations from Peninsular Malaysia, confirming that mitochondrial markers are particularly powerful

tools for detecting population-level differentiation in *Gracilaria*. In Vietnam specifically, Le et al., (2020) [56] used *rbc*L and COI-5P sequences to distinguish *Gracilaria phuquocensis* previously misidentified as *G. mammillaris* from the southern coast of Vietnam, with *rbc*L divergence of 0–0.5% among specimens from Malaysia, Philippines, Thailand, and Vietnam confirming broad regional distribution of Vietnamese *Gracilaria* haplotypes. Such patterns may reflect biogeographic structuring, localized adaptation, or cryptic evolutionary lineages, a phenomenon already documented in several *Gracilaria* species across Asia-Pacific, underscoring the need for expanded COI and *rbc*L sampling in Vietnamese coastal waters. These findings support the value of DNA barcoding for species identification and its potential as a foundation for population genetics and phylogeographic research in Vietnamese seaweeds.

These findings are directly relevant to fisheries, aquaculture, and resource management. In Southeast Asia, the two-marker system of *cox*1 + *rbc*L has been highlighted as cost-efficient for species identification and phylogenetic studies, particularly where research funding may be limited. As Vietnam faces increasing demands for traceability and transparency in seafood supply chains [57], building a reliable molecular reference system is essential. While prior domestic molecular work on Vietnamese seaweeds has addressed species discovery and morpho-molecular taxonomy notably Le et al., (2020) [56] on *Gracilaria phuquocensis* and the broader inventories documented by Nguyen et al., (2023) [5] standardized barcode datasets integrating both *cox*1 and *rbc*L for commercially traded species such as *G. eucheumatoides*, *U. lactuca*, and *G. acerosa* remain limited for Vietnamese coastal waters. The molecular records generated here therefore contribute to filling this gap, enriching global databases such as GenBank and BOLD with georeferenced sequence data for three economically important species. This reduces risks of mislabeling, fraudulent trade, or species substitution, while simultaneously providing a scientific foundation for sustainable exploitation, genetic conservation, and biodiversity monitoring.

Despite the robustness of the results, challenges remain. First, while both *cox*1 and *rbc*L proved effective, the global reference databases remain incomplete, especially for Southeast Asian seaweeds, and the reliability of DNA barcoding depends heavily on the comprehensiveness and accuracy of these reference libraries [58, 59]. Second, the high morphological plasticity and potential intraspecific divergence observed in groups like *Gracilaria* highlight the need for voucher-linked reference specimens to ensure traceability and reproducibility across studies. Future research should integrate additional genetic markers (e.g., ITS, *tuf*A, *psb*A, *cox*3), expand geographic sampling along the Vietnamese coastline and across adjacent Southeast Asian waters, and apply population-level analyses to uncover hidden diversity and evolutionary dynamics within these commercially important genera.

4. Conclusion

This study successfully applied an integrative taxonomic approach to provide definitive identification for three economically important seaweed species in Vietnam: *Gracilaria eucheumatoides*, *Ulva lactuca*, and *Gelidiella acerosa*. Morphological characterizations were consistently supported by molecular data, with *cox*1 and *rbc*L sequences showing 100% sequence identity to authenticated reference sequences in GenBank. Phylogenetic reconstructions confirmed that these specimens clustered within well-supported monophyletic clades corresponding to their respective taxa. The analyzed barcode gap, characterized by low intraspecific distances and clear interspecific divergence, validates the reliability of these markers for the species-level discrimination of the studied genera.

These results provide a molecular baseline for three of Vietnam's commercially important seaweed species. By filling geographic gaps in global sequence repositories, this work supports species authentication and biological traceability efforts. Such data are increasingly important for aligning Vietnam's seaweed industry with international market standards and supply chain transparency requirements.

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