ACADEMIA JOURNAL OF BIOLOGY 2024, 46(2): 7–18
DOI: 10.15625/2615-9023/20583

COMBINING MORPHOLOGICAL AND MOLECULAR DATA TO IDENTIFY *Padina* SEAWEED SAMPLES COLLECTED FROM HON THOM, PHU QUOC IN VIETNAM

Huynh Thi Thu Hue[1,2], Vu Dinh Phong[2,3], Nguyen Thi Bich Ngoc[1], Nguyen Thi Thanh Xuan[1], Do Thi Thanh Trung[1], Dam Duc Tien[4], Le Tat Thanh[1,2,*]

[1]Institute of Genome Research, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Ha Noi, Vietnam
[2]Graduate University of Science and Technology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet, Ha Noi, Vietnam
[3]Hoa Binh University, 8 Bui Xuan Phai, My Dinh 2, Nam Tu Liem, Ha Noi, Viet Nam
[4]Institute of Marine Environment and Resources, Vietnam Academy of Science and Technology, 246 Da Nang, Ngo Quyen, Hai Phong City, Vietnam

Received 11 May 2024; accepted 18 June 2024

ABSTRACT

The *Padina* genus has 75 species of which 54 species are classification recognized. The *Padina* genus has been used as nutritional food that supplies vitamins, proteins, and carotenoids by humans for a long time. Recently, several drugs and dietary supplements containing active components extracted from *Padina* have been commercially developed. Species of the *Padina* genus are quietly morphologically similar. Almost all previous studies of the *Padina* genus used the morphological identification method. This study presents the results of correlation analysis between morphological characteristics and the *rbc*L marker in the identification of *Padina* seaweed samples collected at Hon Thom, Phu Quoc, Kien Giang province (HTO), Vietnam. The phylogenetic tree and genetic distance between HTO with references showed the samples belonging to *Padina australis*. Thus, identification methods based on genetic markers and morphology on HTO seaweed samples were consistent.

Keywords*:* Hon Thom, *Padina australis*, morphological, DNA barcoding.

Citation: Huynh Thi Thu Hue, Vu Dinh Phong, Nguyen Thi Bich Ngoc, Nguyen Thi Thanh Xuan, Do Thi Thanh Trung, Dam Duc Tien, Le Tat Thanh, 2024. Combining morphological and molecular data to identify *Padina* seaweed samples collected from Hon Thom, Phu Quoc in Vietnam. *Academia Journal of Biology*, 46(2): 7–18. https://doi.org/10.15625/2615-9023/20583

[*]*Corresponding author email*: thanh.biotech@gmail.com

INTRODUCTION

Phaeophyceae is a macroalgae group that has 4 major genera: *Sargassum, Turbinaria, Dictyota,* and *Padina.* The biomass of Phaeophyceae is higher than other macroalgae groups. Brown algae distributes widely and is dominant in coastal tidal flats of tropical seas. The development of brown algae in deep areas is later than in shallow areas. Their biomass increases significantly from March to June. The *Padina* genus belongs to the Dictyotaceae family (Phaeophyceae: Dictyotales). The *Padina* genus has 75 species in the database www.algaebase.org, in which 54 species are classification recognized (Guiry & Guiry, 2024). Up to this point, in Vietnam, there are 6 species of the *Padina* genus including *Padina boryana* Thivy, *Padina australis* Hauck, *Padina gymnospora, Padina crassa* Yam, *Padina minor* Yamada, *Padina tetrastromica* Hauck (Dam et al., 2020).

Hon Thom island is located in the An Thoi islands, south of Phu Quoc, Vietnam. It is situated 5 kilometers south of Phu Quoc island within the An Thoi archipelago, which also includes Gam Ghi island and Trang island (Trinh et al., 1995). Phu Quoc National Park's marine ecosystem is equally diverse, with 62 types of seaweed, as well as shimmering coral reefs and the presence of hawksbills laying eggs.

The *Padina* genus has been used as nutritional food and vitamins, proteins, minerals, and carotenoid suppliers by humans for a long time. Recently, several drugs containing active components extracted from seaweeds for curing diseases have been commercially developed (Pereira, 2018). Immaculate fucoidan extracted from *Padina tetrastromatica* và *Padina boergesenii* has been used for producing 2% topical ointment which was proved to be effective in a mouse skin wound healing model by vascular, collagen fibers and epidermis formation (Kordjazi et al., 2017). Moreover, halogenated compounds, for example, bromophenol which is not contained in any higher plants, are present in the *Padina* genus. These compounds have pharmacological activities that inner-cell calcium ions break down in the endocrine system (Chung et al., 2003; He et al., 2019; Pandya et al., 1990). Few species of *Padina* genus are researched for potential chemical and pharmacological applications. Biological activity compounds including amino acids, terpene sterol, and polysaccharide sulfate were reported that were found in *Padina.* Compounds extracted from *Padina* have anti-virus, anti-microbial, cytotoxic, immune stimulation, anti-obesity, cardiovascular protection, liver protection activities, and hypoglycemia activity. Metabolites such as bromophenol, xenicane diterpenes, and sterol got attention because of their pharmacological activities (Mohammed et al., 2021). As a result, research on the *Padina* genus has become more and more popular recently.

Padina is a fan-shaped blade or frond, whole, or split into many lobes, with a small petiole at the holdfast, attached by smooth pseudo-roots. The blades have concentric circles, formed by hairlines arranged horizontally, sometimes inlaid with calcium. The blade consists of 2–8 (10) layers of small colorless or light-colored core cells, surrounded by a layer of cells smaller or equal to the inner, pigmented cells (Dam, 2021). Currently, the identification of the *Padina* genus depends on morphological methods. However, researchers may have difficulty identifying seaweed species because of many similar species. Therefore, methods to identify species and relationships among species are essential.

Previous studies using morphological characteristics including the number of cell layers when cross-sectioned, the number of concentric rings on the blade, the distance between the circles, and whether pseudo-roots grow from the holdfast or not for classification and identification (Dam, 2021). There are a few species that are difficult to identify and distinguish. Therefore, additional identification methods are essential to ensure accuracy and efficiency in identifying seaweed species of this genus. Other studies were focused on the relationship between

morphological characteristics and DNA markers in various plants species including Harumanis mango (Yusuf et al., 2020), Cyclamen (Cornea-Cipcigan et al., 2023); tomato (Monika et al., 2018).

Currently, DNA barcoding is widely used in species identification with high accuracy, speed, and automation capabilities (Hebert et al., 2003). It encourages DNA barcoding instead of or in combination with morphological characteristics to effectively and accurately identify *Padina* seaweed. Sanger sequencing not only forms the basis for the newer and automated approaches, but continues to be the most common sequencing approach used in veterinary diagnostic laboratories for sequence verification, and assay monitoring, and as the foundation for many phylogenetic analyses (Crossley et al., 2020).

For DNA sequence verification, the most commonly used database is the Basic Local Alignment Search Tool (BLAST), a bioinformatics tool established by researchers at the National Institute of Health (Altschul et al., 1990). The BLAST algorithm allows comparison of newly generated sequences to a library of sequences (https://blast.ncbi.nlm.nih.gov/Blast.cgi). BLAST detects regions of similarity between biological sequences by comparing nucleotide or protein sequences to sequence databases and calculates the statistical significance of such findings. The software is designed to detect functional and evolutionary relationships between sequences as well as to help identify members of gene families (Crossley et al., 2020).

The phylogenetic analysis includes a multiple sequence alignment step requiring that every sequence utilized has gone through a rigorous quality check and is available as a consensus sequence. Multiple alignment options are available in commercial and free sequencing software programs and web pages (e.g., Geneious, Vector NTI, CLC, BLAST, MEGA, ClustalW, Muscle) (Kumar et al., 2016). The most used structure- and character-based methods are parsimony and maximum-likelihood algorithms. An identity table is often generated and used to analyze the relationship of sequences in terms of percentage identity, from which a number can be used for decision making (Crossley et al., 2020). The choice of algorithm depends on the characteristics of the data and the goals of the analysis. For example, maximum likelihood is often used when the evolutionary model is complex and the data are noisy, while neighbor-joining is often used when the data are relatively clean and the evolutionary model is simple (Azouri et al., 2021).

In this study, we collected the *Padina* samples at Hon Thom, Phu Quoc. The samples were morphologically classified combined with the *rbc*L sequence analysis and compared the molecular characteristics with inter-species and intra-species.

MATERIALS AND METHODS

Materials

The sample of the *Padina* genus was collected at Hon Thom island, Phu Quoc ($9^{o}57'23"N$; $104^{o}00'57"E$) in June 2023 at depths ranging from 2–10 m which is the basis for Phu Quoc tides in June 2023. The sample notation is HTO. Seaweed material was preserved in silica gel for molecular analyses, and each specimen was mounted for a herbarium. Herbarium specimens were deposited in the Marine Biological Museum of the Institute of Marine Environment and Resources, Vietnam Academy of Science and Technology (VAST). Molecular experiments used chemicals and equipment from the Institute of Genome Research, VAST.

Methods

Sampling methods

Normative Act of Committee for Science and Technology of Government State specimens collection during the field survey (1981) (for the tidal zone) and the standard method of English et al. (1997) (for the subtidal zone) were used in the survey. The specimens in the subtidal zone were collected by SCUBA divers equipment, underwater digital camera OLYMPUS TG5 and PT-056 housing (Tokyo, Japan) for collecting samples and taking pictures. The freshly collected

marine macroalgal samples were soaked in a solution of formaldehyde 5%, the specimens were then put on Croki paper, compressed into blotting papers, dried naturally and identified.

Morphological analyses

For morphological observations, thalli were sectioned manually using a razor blade and mounted on glass slides in Karo Syrup/seawater (Vieira et al., 2014). Photographs were taken with an Olympus microscope (Japan) equipped with a ZEISS A-Plan 40X/0, 65 N. A phase contrast 2 objective (Zeiss, Germany) and an Olympus TG 5 digital camera (Japan) (Vieira et al., 2014) at the laboratory of the Marine Botanical Ecology and Resources Department, Institute of Marine Environment and Resources, VAST. The scientific names used follow national and international authors: Taylor (1960), Pham (1969), Nguyen et al. (1993), Tseng (1983).

DNA extraction

DNA was isolated using the protocol by Gautham et al. (2017). Extraction buffer was prepared having the following composition 100 mM Tris-HCl, 20 mM EDTA, 1.5 M NaCl, 1% SDS, 2% PVP and 0.2% β-mercaptoethanol (added fresh just before extraction). Algal tissue (50 mg) was grinded for homogenization in a mortal and pestle with liquid nitrogen and extraction buffer. The ground mixture was centrifuged to remove cell debris at 14,000 rpm; 4 ^{o}C, for 10 min. Absolute ethanol (1/9 volume) and 3 M potassium acetate (1/4 volume, pH 4.8) were added to the supernatant to remove polysaccharide contamination. One volume of chloroform: isoamyl alcohol (24:1) was added to the solution. The tube was vortexted vigorously for a few seconds and incubated at -20 ^{o}C for 20 min with constant mixing. The tube was then centrifuged at 14,000 rpm; 4 ^{o}C for 20 min. RNase A (50 µg) was added to the aqueous phase, and the tube was gently mixed by inversion. The tube was incubated at 37 ^{o}C for 30 min. The tube was centrifuged at 14,000 rpm; 4 ^{o}C for 20 min. An equal volume of isopropanol (1:1), 3 M sodium acetate (1/10) and β-mercaptoethanol (0.2%) were added to the aqueous phase. The tube was kept at -30 ^{o}C for 3 h and centrifuged at 14,000 rpm; 4 ^{o}C for 20 min. The pellet was washed with 1 mL of 70% ethanol and centrifuged at 14,000 rpm, 4 ^{o}C for 10 min. The pellet was air-dried and dissolved in 50 µL of sterile distilled water.

Polymerase chain reaction (PCR) and gel electrophoresis

To evaluate the extracted DNA's eligibility for use in subsequent processes, the polymerase chain reaction (PCR) was conducted using it as a template. The primers Pa *rbc*L forward and Pa *rbc*L reverse were used (Table 1) to amplify the product of 736 base pairs (bp). The PCR was performed in a volume of 25 µL with components including 1 µL Taq Dream polymerase 1 u/µL, 2.5 µL Taq polymerase buffer (10x), 1 µL dNTPs (10 mM), 2 µL genomic DNA (50–100 ng), 1 µL forward primer (10 ppm), 1 µL reverse primer (10 ppm), and nuclease-free water up to 25 µL. The amplification was carried out using the following reaction conditions: initial denaturation of 94 ^{o}C for 3 min; followed by 30 cycles of 94 ^{o}C for 1 min, 58 ^{o}C for 30 min, and 72 ^{o}C for 45 secs, with a final extension of 72 ^{o}C for 5 min. PCR products were run on an agarose gel (0.8% w/v) and visualized using a gel documentation system (Cleaver, England) after staining with ethidium bromide. The reaction without the template served as a non-template control (NTC).

Table 1. Sequence of primers

Primer	Sequence (5'-3')	Length (bp)	Annealing site	Tm (^{o}C)
Pa *rbc*L forward	TGACATTTATCGAGCAAAAGCC	736	95−116	51.9
Pa *rbc*L reverse	TACCCCTGACATACGCATCC		811–830	53.1

Note: The primers are designed based on *rbc*L sequence of *Padina australis* Hauck (Accession number on GenBank: AB358906).

DNA sequencing and analyzing

PCR products were purified by the GeneJET Gel Extraction kit (Thermo Scientific, United States) and sequenced by the Big Dye Terminator kit (ABI Foster City, USA) on Applied Biosystems™ 3500. Nucleotide sequencing was then aligned using ClustalW with other sequences retrieved from the GenBank database. Genetic distance was identified by MEGAX software (version 10.2). The phylogenetic tree was built using the Maximum likelihood method with a bootstrap value of 1,000.

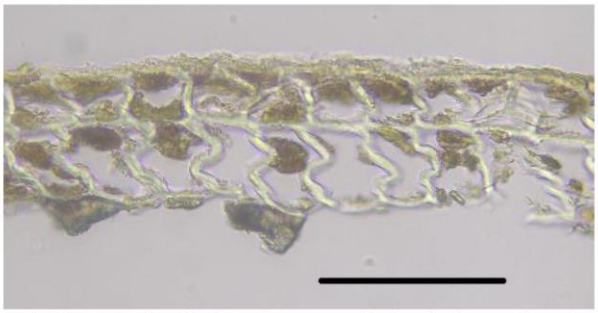
RESULTS AND DISCUSSION

Morphological characteristics

Padina seaweed samples have fan-shaped blades or fronds and split into many lobes. Thallus is about 10 cm and dark brown (Fig. 1B), the elderly part is inlaid with calcium, slightly clear. *Padina australis* Hauck's size is up to 9 cm wide, 7 cm tall and the color is yellowish or dark brown. The distance between hairlines on each surface is 3–4 mm. The arrangement of alternating hairlines between both surfaces is equal (Win et al., 2011). On the blade, there are concentric circles that are formed by hairs arranged horizontally. The cross-sectional layer consists of two cell layers (Fig. 1C). Spore sacs are arranged in concentric rows between two rows of hairlines. The holdfast has a small petiole and is attached by smooth pseudo roots, without pseudo roots growing from the blade. Among *P. australis*, they are distinguished by the arrangement of alternating hairlines at unequal distances, reproductive. *Padina ishigakiensis* is similar to *P. australis* in the thallus structure but differs in the arrangement of alternating hairlines (unequal distance in *P. ishigakiensis* vs equal distance in *P. australis*), in the arrangement of reproductive sori (irregularly spreading between hairlines or far from the hairlines in *P. ishigakiensis* vs close to hairlines distally in a regular distance in *P. australis*) (Win et al., 2011).

A. *Padina australis* at Hon Thom (under water)

B. *Padina australis* (herbarium)



C. Cross-sectional of *Padina australis* (scale bar = 50 µm)

Figure 1. Morphological characteristics of HTO seaweed samples

The morphological classification of *P. australis* Hauck depends on thallus, cell layer number, sporangial sori arrangement, hair band, and reproductive structures. The thallus of the *Padina* species is flabellate-type, with a brown, off-white coloration. It consists of cells that are 2–8 layers thick, fan-shaped with hairs covering its margin. It has a stipe attached to its rhizoidal holdfast with blades conspicuously appearing as several layers of cell thick, with apparent zonations in its thalli producing coextensive rows of hair, distributed in rigid segments. The number of cell layers in the thallus varies among *Padina* species, with *P. australis* having up to 30 cm wide and 25 cm tall thalli (Arraj et al., 2016; Win et al., 2011). The arrangement of sporangial sori relative to hair bands is a key characteristic used to differentiate *Padina* species. In *P. australis*, the sprorangial sori are arranged relative to hair bands in a specific pattern. The presence or absence and degree of calcification of hair bands on the lower thallus surface is another important characteristic used in the morphological classification of *Padina* species. *P. australis* has hair bands on the lower thallus surface. The arrangement of reproductive sori is also a key characteristic used in the morphological classification of *Padina* species. The reproductive sori are arranged in a specific pattern. These morphological characteristics, along with molecular markers, are used to classify and identify *Padina* species, including *P. australis* Hauck (Win et al., 2011; Win et al., 2020; Win et al., 2021).

In conclusion, HTO samples collected at Hon Thom, Phu Quoc have completed morphological similarity with *P. australis* Hauck based on *Padina* classification keys. Then, these samples were used for molecular analysis based on the *rbc*L gene.

*Rbc*L isolating, sequencing and analyzing

In the study, high-quality total DNA would have one high molecular weight and visible clear band (Fig. 2A). The isolated DNA was ensured to be high purity. All samples had an optical density within 1.6–2.0 with high cleanliness that was guaranteed for polymerase chain reaction (PCR). Primers were designed based on the *rbc*L sequence of other seaweed species on GenBank to amplify a 736 bp-length sequence of the *rbc*L gene (Table 1). The PCR product analysis on an agarose gel (0.8% w/v) found the extracted DNA fragments had a size of about 800 bp. The PCR products were purified for sequencing (Fig. 2B).

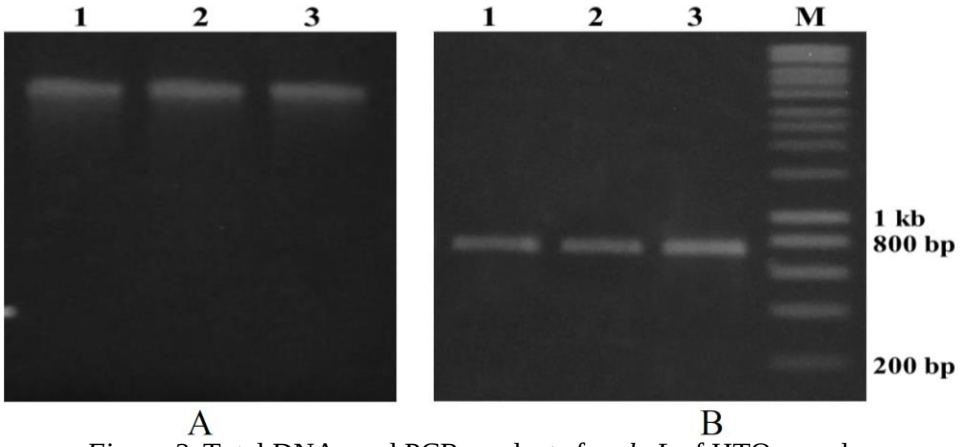


Figure 2. Total DNAs and PCR products for *rbcL* of HTO samples

Note: M: Marker 1kb; 1, 2, 3: PCR product of samples 1, 2, 3

After eliminating interference sequencing fragments, the DNA sequence of the *rbc*L gene was 695 bp for all specimens. This shows that the results of sequencing were

significantly reliable. The HTO *rbc*L sequence has 99,28% identical *P. australis rbc*L sequences. The comparison with GenBank references (LC521691, LC521692, LC521690, LC487933, JQ364052, LC487933, JQ364052, LC487931, EU579959, AB096902, JQ364055, AB489913, AB512532) reaches 98.99% similarity by using BioEdit. The changes were at localities of G642T, G664T, G673T, G693T, A692G and there is an Adenine deletion at the eleventh site (Fig. 3). In the research, the *rbc*L gene sequence had the highest similarity to the species *P. australis* Hauck.

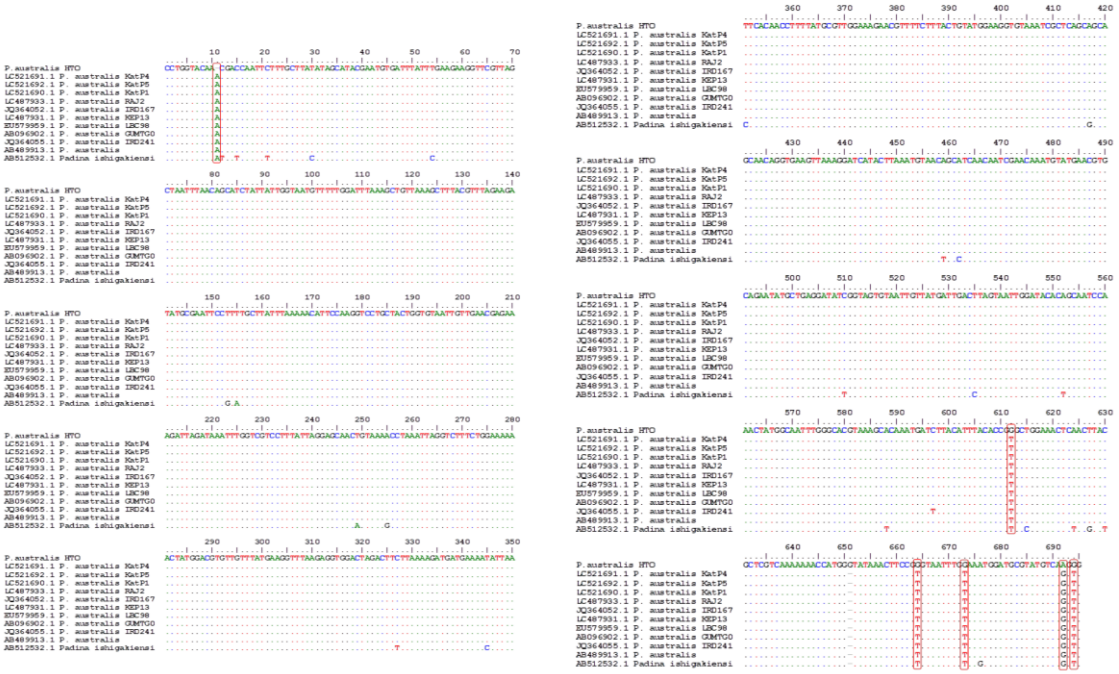


Figure 3. Alignment *rbc*L sequences of *Padina australis* Hauck in Hon Thom with references

The maximum likelihood tree based on *rbc*L sequence of HTO is given in Figure 4. The phylogenetic tree was built based on the *rbc*L sequence of samples and reference sequences from 10 species of *P. australis,* one species of *P. ishigakiensis, Padina japonica, Padina usoehtunis, Padina tetrastromatica, Padina crasse* and 01 outgroup species *Sargassum hemiphyllum.* HTO sequence was displayed at the black dot.

Genetic distance analysis presents that the genetic distance of HTO seaweed samples is low (0.00734) compared with other *P. australis.* However, genetic distance is higher when compared with other species in the *Padina* genus (from 0.04 to 0.05). HTO seaweed samples have a significant genetic distance with *Sargassum hemiphyllum* (0.22) which agreed with inter-species genetic distance comparison (> 20%).

Similarly, *Padina* samples from Myanmar and the Philippines were examined to find that the *Padina* sequences showed low intraspecific variation, ranging from 0 to 0.009% in *rbc*L and 0 to 0.032% in *cox*3 (Win et al., 2021). This indicates the genetic distances between conspecific *Padina* samples are relatively low.

The *rbc*L gene was able to successfully identify *Padina* species, through it failed to differentiate between some closely related species like *Padina antillarum* and *P. tetrastromatica,* or *Padina boergesenii* and *Padina jonesii* (Mohammed et al., 2019). Overall, the *rbc*L gene shows moderate levels of variation within the *Padina* genus, with

around 1–2% divergence between some closely related species, and less than 1% variation within most *Padina* species (Win et al., 2021).

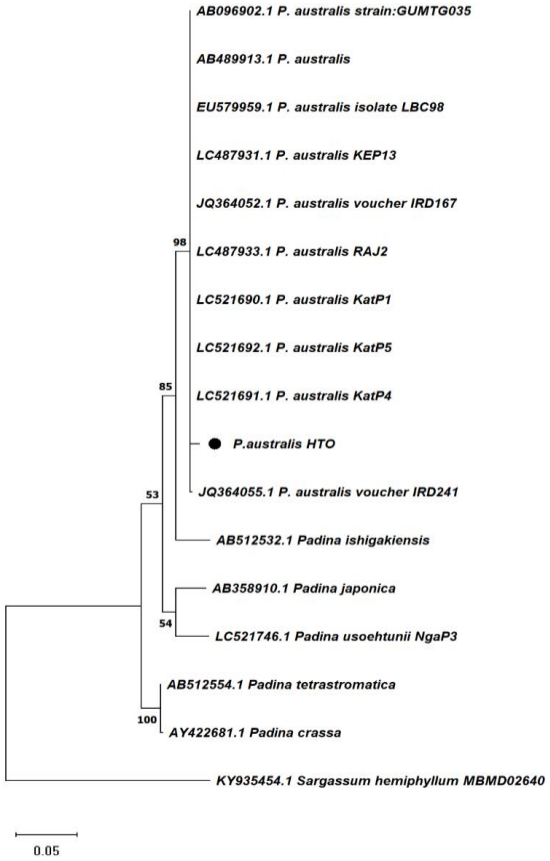


Figure 4. The maximum likelihood tree based on the *rbc*L sequence

The phylogenetic tree was constructed by MEGAX software based on the *rbc*L sequences of HTO samples with sequences published on NCBI. The maximum likelihood method and the Tamura-Nei model analyze evolution. HTO samples were genetically similar to *P. australis* Hauck isolated in Guam, Tagachang Beach (USA) (AB096902), and *P. australis* Hauck isolated in Rakhine State, Thandwe, Ngapali, Kathit (Myanmar) in 2018. Other species form separate branches, totally separate from the branch consisting only of *P. australis*. It concludes that seaweed samples (HTO) collected at Hon Thom island belong to the *P. australis.*

The *rbc*L gene, which encodes the large subunit of the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) enzyme, is a widely used DNA barcode for plant identification and phylogenetic in many studies (Srivastava et al., 2022; Ho et al., 2021; Akash et al., 2018; Mehmet et al., 2022). This means *rbc*L may be better suited for resolving relationships between closely related *Padina* species. Overall, the results indicate that the *rbc*L gene is a reliable and widely used marker for macroalgae phylogenetic analysis, providing robust resolution of relationships, especially among closely related *Padina* species. The combination of *rbc*L and other barcodes like *mat*K can further improve the accuracy of phylogenetic reconstructions.

The phylogenetic tree of *Padina* based on *rbc*L gene researchers reveals insights into the evolutionary relationships of this genus within the brown algae group (Ochrophyta, Phaeophyceae). Research studies have utilized the *rbc*L gene to analyze the genetic sequences of *Padina* species, contributing to a better understanding of their phylogeny and classification within the broader context of brown algae (Narra et al., 2020). The study by Benita et al. (2018) found that the two new *Padina* species they described, *Padina pavonicoides* and *Padina pavonica*, had a 1.67–1.98% divergence in *rbc*L sequences and 5.74–9.05% divergence in *cox*3 sequences. Another study by Silberfeld et al. (2013) examined the species-level diversity of *Padina* using two gene regions, *cox*3 and *rbc*L. They found variation in the species delineation results between the two genes, with the GMYC and statistical parsimony methods sometimes recovering different clusters as separate species. This suggests there can be gene-dependent differences in the genetic distances observed between *Padina* species (Silberfeld et al., 2013).

The relationship between morphological characteristics and DNA markers in the *Padina* genus is an important aspect of its taxonomy and classification. *Padina* species have traditionally been identified and

classified based on their morphological characteristics, such as thallus shape, size, colour, presence or absence of calcification, hairlines, and reproductive structures (Win et al., 2011; Arraj et al., 2016). However, this has been notoriously difficult due to the high morphological plasticity within the genus (Win et al., 2011). Additionally, the molecular data has helped to interpret the evolution of morphological features and the biogeography of *Padina* species. The integration of morphological and molecular approaches has been crucial for improving the taxonomy and classification of the *Padina* genus, as it has allowed researchers to better understand the relationship between the species' physical characteristics and their underlying genetic diversity (Win et al., 2021). The results indicate that the use of these molecular markers, along with morphological data, has allowed for the identification of several *Padina* species, as well as the re-evaluation of taxonomically important morphological characters.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. P.australis_HTO																	
2. LC521691.1_P._australis_KatP4	0,00734																
3. LC521692.1_P._australis_KatP5	0,00734	0,00000															
4. LC521690.1 P. australis KatP1	0,00734	0,00000	0,00000														
5. LC487933.1_P._australis_RAJ2	0,00734	0,00000	0,00000	0,00000													
6. JQ364052.1_P._australis_voucher_IRD167	0,00734	0,00000	0,00000	0,00000	0,00000												
7. LC487931.1_P._australis_KEP13	0,00734	0,00000	0,00000	0,00000	0,00000	0,00000											
8. EU579959.1_P._australis_isolate_LBC98	0,00734	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000										
9. AB096902.1_P._australis_strain:GUMTG035	0,00734	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000									
10. JQ364055.1_P._australis_voucher_IRD241	0,00882	0,00144	0,00144	0,00144	0,00144	0,00144	0,00144	0,00144	0,00144								
11. AB489913.1_P._australis	0,00734	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000	0,00000	0,00144							
12. AB512532.1_Padina_ishigakiensis	0,04504	0,03654	0,03654	0,03654	0,03654	0,03654	0,03654	0,03654	0,03654	0,03811	0,03654						
13. AB358910.1 Padina japonica	0,05989	0,05072	0,05072	0,05072	0,05072	0,05072	0,05072	0,05072	0,05072	0,05237	0,05072	0,06433					
14. LC521746.1_Padina_usoehtunii_NgaP3	0,05769	0,04866	0,04866	0,04866	0,04866	0,04866	0,04866	0,04866	0,04866	0,05029	0,04866	0,06527	0,04865				
15. AB512554.1_Padina_tetrastromatica	0,05773	0,04870	0,04870	0,04870	0,04870	0,04870	0,04870	0,04870	0,04870	0,04870	0,04870	0,06215	0,05222	0,06262			
16. AY422681.1_Padina_crassa	0,05789	0,04883	0,04883	0,04883	0,04883	0,04883	0,04883	0,04883	0,04883	0,04883	0,04883	0,06232	0,05236	0,06279	0,00145		
17. KY935454.1_Sargassum_hemiphyllum_MBMD026	0,22928	0,21929	0,21929	0,21929	0,21929	0,21929	0,21929	0,21929	0,21929	0,21703	0,21929	0,22645	0,22323	0,23509	0,21332	0,21419	

Figure 5. Genetic distance between HTO sample and references based on *rbc*L sequences

CONCLUSION

The characteristics of *Padina* seaweed samples collected at Hon Thom, Phu Quoc were described and identified by morphology method, resulting in *P. australis* Hauck. The phylogenetic tree and genetic distance between HTO with *P. australis* references showed the sample belonging to *P. australis.* By molecular biology methods and bioinformatic tools, the HTO *rbc*L sequence was 695 bp with six variants when comparing intra- and inter-species. Thus, identification methods based on genetic markers and morphology on HTO seaweed samples were consistent. The method will apply to other seaweed species and help to interpret the evolution of morphological features and the biogeography of Vietnam *Padina* species.

REFERENCES

Akash P., Spandan C., Bakhtiyar A. S., Bharat G., Pankaj P., Beena P., 2018. *Rbc*L Marker Based Approach for Molecular Identification of Arthrospira and Dunaliella Isolates from Non-Axenic Cultures. *Journal of Genetics and Genetic Engineering,* 2(2): 2637−5370.

Altschul S. F., Gish W., Miller W., Myers E. W., Lipman D. J., 1990. Basic local alignment search tool. *Journal of Molecular Biology,* 215(3): 403–10.

Arraj H., Mayhoob H., Abbas A., 2016. First records of two *Padina* species (Dictyotales, Phaeophyceae) from the Syrian coast (eastern Mediterranean). *Marine*

Biodiversity Records, 9: 93. https://doi.org/10.1186/s41200-016-0090-x

Azouri D., Abadi S., Mansour Y., Mayrose I., Pupko T., 2021. Harnessing machine learning to guide phylogenetic-tree search algorithms. *Nature Communications*, 12(1): 1983.

Benita M., Dubinsky Z., Iluz D., 2018. *Padina pavonica*: Morphology and Calcification Functions and Mechanism. *American Journal of Plant Sciences,* 09(06): 1156−1168.

Chung H. Y., Ma W. C., Ang Jr P. O., Kim J. S., Chen F., 2003. Seasonal variations of bromophenols in brown algae (*Padina arborescens*, *Sargassum siliquastrum*, and Lobophora variegate) collected in Hong Kong. *Journal of Agricultural and Food Chemistry,* 51: 2619–2624.

Cornea-Cipcigan M., Pamfil D., Sisea C. R., Margaoan R., 2023. Characterization of *Cyclamen* genotypes using morphological descriptors and DNA molecular markers in a multivariate analysis. *Frontiers in Plant Science*, 14: 1100099.

Crossley B. M., Bai J., Glaser A., Maes R., Porter E., Killian M. L., Clement T., Toohey-Kurth K., 2020. Guidelines for Sanger sequencing and molecular assay monitoring. *Journal of Veterinary Diagnostic Investigation*, 32(6): 767−775.

Dam Duc Tien, 2021. Truong Sa archipelago macroalgae. Natural Science and Technology Publishing (In Vietnamese).

Dam Duc Tien, Tran Quoc Toan, Pham Quoc Long, 2020. Popular macroalgae of Vietnam marine area and valuable applications. Natural Science and Technology Publishing (In Vietnamese).

English S., Wilkinson C., and Baker V., 1997. Manual for survey of tropical marine resources. Australian Institute of Marine Science (AIMS), Australia, pp. 390.

Coulson A. R., 1975. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *Journal of Molecular Biology*, 94: 441–448.

Gautham S. R., Anwar A. F., Mohandass R., 2017. A rapid and efficient DNA extraction method suitable for marine macroalgae. *3 Biotech*, 7: 364.

Guiry M. D., Guiry G. M., 2024. AlgaeBase. World-wide electronic publication, University of Galway, Ireland.

He J. H., Chen L. X., Li H., 2019. Progress in the discovery of naturally occurring anti-diabetic drugs and in the identification of their molecular targets. *Fitoterapia*, 134: 270−289.

Hebert P. D., Cywinska A., Ball S. L., de Waard J. R., 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270: 313–321.

Ho V. T., Tran T. K. P., Vu T. T. T., Widiarsih S., 2021. Comparison of matK and rbcL DNA barcodes for genetic classification of jewel orchid accessions in Vietnam. *Journal of Genetic Engineering and Biotechnology*, 19(1): 93.

Kordjazi M., Shabanpour B., Zabihi E., Faramarzi M. A., Ahmadi G. H., Feghhi S. M. A., Hosseini S. A., 2017. Investigation of effects of fucoidan polysaccharides extracted from two species of Padina on the wound-healing process in the rat Turk. *Turkish Journal of Veterinary and Animals Sciences*, 41: 106−117.

Kumar S., Stecher G., Tamura K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution*, 33(7): 1870−4.

Mehmet Y. P., Emre S., İsa B., 2022. DNA barcoding and phylogenetic barcoding and phylogeneticanalysis analysis of endemic Astragalus nezaketiaeand *Vicia alpestris* subsp.hypoleuca (Fabaceae): Evidence from nrDNA ITS and cpDNA and *rbc*L sequences. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 50(3).

Mohammed A. A., Al T. A., Othman A., Abdulrahman A. S. I., Gamal O. and Omar

B., 2019. DNA-barcoding and Species Identification for some Saudi Arabia Seaweeds using *rbc*L Gene. *Journal of Pure and Applied Microbiology*, 13**(**4): 2035−2044.

Mohammed I. R., Iman A. M., Abdel R., Hani S., Eman Z. A., Hashem A., Madkour U., Ramadan A., 2021. A review on the pharmacological potential of the genus *Padina* South African. *Journal of Botany*, 141: 37−48.

Monika S., Vaishali A. K., Singh A. K., Pandey K. D., 2018. Molecular diversity of tomato germplasm (*Lycopersicum esculentum* L.) using lycopene specific markers. *Biocatalysis and Agricultural Biotechnology*, 16: 340−346.

Narra P., Chinnamani P., Annadurai D., Mahendran S., 2020. Identifying seaweeds species of Chlorophyta, Phaeophyta and Rhodophyta using DNA barcodes. *BioRxiv,* 2020-08.

Nguyen Huu Dinh, Huynh Quang Nang, Tran Ngoc But and Nguyen Van Tien, 1993. Marine macroalgae (in the North Vietnam). Science and Technics Publishing House, Hanoi, pp. 364 (In Vietnamese).

Pandya A., Bapodra A., Parekh H., 1990. Brominated phenolic compounds in Indian marine algae. *Planta Medica*, 56: 557.

Pereira L., 2018. Seaweeds as Source of Bioactive Substances and Skin Care Therapy - Cosmeceuticals, Algotheraphy, and Thalassotherapy. *Cosmetics*, 5(4): 68.

Pham Hoang Ho, 1969. Vietnam seaweed (southern part). Learning Resource Center, Saigon, pp. 558 (In Vietnamese).

Silberfeld T., Bittner L., Fernández-García C., Cruaud C., Rousseau F., de Reviers B., Leliaert F., Payri C. E., De Clerck O., 2013. Species Diversity, Phylogeny and Large Scale Biogeographic Patterns of the Genus Padina (Phaeophyceae, Dictyotales). *Journal of Phycology*, 49(1): 130−42.

Srivastava R. P., Saxena G., Singh L., Singh A., Verma P. C., Kaur G., 2022. Interspecific and intraspecific analysis of *Selinum* spp. collected from Indian Himalayas using DNA barcoding. *Journal of Genetic Engineering and Biotechnology*, 20(1): 63.

Taylor W. R., 1960. Marine algae of the Eastern tropical and subtropical coasts of the Americas. The University of Michigan press, Michigan, USA, pp. 870.

Trinh Phung, Trinh The Hieu, Nguyen Van Tac, 1995. Geology of Vietnam continental shelf and adjacent regions. Collection of marine research works, 6: 57−73.

Tseng C. K., 1983. Common Seaweeds of China. Science Press, Beijing, China, pp. 316.

Vieira C., D'hondt S., De Clerck O., Payri C. E., 2014. Toward an inordinate fondness for stars, beetles and Lobophora? Species diversity of the genus Lobophora (Dictyotales, Phaeophyceae) in New Caledonia. *Journal of Phycology,* 50: 1101–1119.

Win N. N., Hanyuda T., Arai S., Uchimura M., Prathep A., Draisma S. G., Phang S. M., Abbott I. A., Millar A. J., Kawai H., 2011. A taxonomic study of the genus *Padina* (Dictyotales, Phaeophyceae) including the descriptions of four new species from Japan, Hawaii, and the Andaman sea. *Journal of Phycology*, 47(5): 1193−209.

Win N. N., Hanyuda T., Kato A., Shimabukuro H., Uchimura M., Kawai H., Tokeshi M., 2020. Global diversity and geographic distributions of Padina species (Dictyotales, Phaeophyceae): new insights based on molecular and morphological analyses. *Journal of Phycology*, 57(2): 454−472.

Win N. N., Mya-Kyawt-Wai, Paul J. L. G., Lawrence M. L., Chaw-Thiri P. P. A., Ni N. M., Takeaki H., Hiroshi K., Mutsunori T., 2021. Taxonomy and species diversity of *Padina* (Dictyotales, Phaeophyceae) from the Indo-Pacific with the description of two new species. *European Journal of Phycology*, 57(1): 1−17.

Yusuf S. N. A., Rahman A. M. A., Zakaria Z., Subbiah V. K., Masnan M. J., Wahab Z., 2020. Morphological Variability Identification of *Harumanis* Mango (*Mangifera indica* L.) Harvested from Different Location and Tree Age. *Tropical Life Science Research*, 31(2): 107−143. doi: 10.21315/tlsr2020.31.2.6