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Original Article

Morphological and chloroplast haplotype variation of the tropical seagrass *Cymodocea rotundata* from offshore Vietnam: implications for conservation

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ABSTRACT

Cymodocea rotundata, a tropical seagrass abundant in Southeast Asia, exhibits considerable morphological plasticity. However, consistent morphological differences among populations may reflect genetic divergence potentially influenced by local environmental conditions, particularly in geographically isolated systems. We examined populations from two offshore Vietnam islands , Ly Son (more disturbed) and Phu Quy (less impacted), using morphological traits, biomass measurements, nuclear SSR markers, and chloroplast haplotypes (*trn*L–*trn*F). Morphological and biomass analyses revealed clear differences between islands: Phu Quy exhibited significantly longer and wider leaves, greater sheath length, and taller shoots, while Ly Son individuals had thicker rhizomes and higher below-ground biomass, suggesting phenotypic differentiation associated with contrasting environmental conditions. Although the SSR polymorphism was limited, the results indicated moderate differentiation and higher intra-population variability in Ly Son. Chloroplast sequencing of nine individuals identified four haplotypes, including both shared and island-specific lineages, with Ly Son exhibiting higher haplotype diversity. Together, these results support the recognition of Ly Son and Phu Quy as distinct conservation units. Overall, populations from both islands appear vulnerable to coastal development and environmental change, highlighting the need for site-specific conservation strategies to preserve their genetic and morphological diversity.

Keywords: biomass, chloroplast markers, *Cymodocea rotundata*, seagrass genetics, SSR

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Introduction

Seagrasses, comprising approximately 72 species identified globally, are the only group of flowering plants that have fully adapted to life in the marine environment (Short *et al.,* 2011). They play critical ecological roles in coastal ecosystems by stabilizing sediments, improving water quality, storing carbon, and providing habitats and nursery grounds for a wide range of marine species (Duarte *et al.,* 2008; Hemminga & Duarte, 2000). However, under the increasing pressure from climate change and human activities, seagrass meadows worldwide are experiencing severe declines in both extent and ecological function (Short *et al.,* 2011; Waycott *et al.,* 2009). Understanding the biological variation of seagrass populations, both morphologically and genetically, is essential for designing effective conservation and restoration strategies to enhance their adaptability in dynamic environments.

Cymodocea rotundata Ehrenb. & Hempr. ex Aschers (Cymodoceaceae), a dominant tropical seagrass in the Indo-Pacific, thrives across diverse habitats in Southeast Asia, from intertidal flats to subtidal zones up to 10 meters deep, on substrates such as sand or mud, and in salinities of 29 – 35 ‰ (Green & Short, 2003; Ramili *et al.,* 2018; Short *et al.,* 2007). Characterized by linear, flat, and strap-like leaves and extensive rhizome systems, *C. rotundata* supports sediment stabilization and primarily reproduces clonally, though sexual reproduction via seeds occurs under specific conditions, such as water temperatures of 27 - 31 °C (Arriesgado *et al.,* 2016; Larkum *et al.,* 2006; McMillan, 1982). Morphological traits such as leaf size, shoot height, and rhizome diameter often vary between habitats and are influenced by local environmental conditions (Larkum *et al.,* 2006). While such plasticity is well known, consistent morphological differences between populations may reflect underlying genetic divergence or environmentally associated differentiation, especially in geographically isolated systems (Pazzaglia *et al.,* 2021; Procaccini *et al.,* 2007).

Molecular markers are powerful tools to explore the genetic structure, connectivity, and evolutionary history of seagrass populations. Nuclear markers, including microsatellite (SSR), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Internal Transcribed Spacer (ITS), and Single Nucleotide Polymorphism (SNP), have been widely used to evaluate genetic diversity and population structure in seagrasses (Davey *et al.,* 2016; Procaccini *et al.,* 2007). In *C. rotundata,* SSR markers have been employed popularly and revealed significant genetic differentiation and varying clonal richness across Indo-Pacific populations, with varying clonal richness linked to environmental conditions and oceanographic barriers (Arriesgado *et al.,* 2023; 2016). More recently, Start Codon Targeted (ScoT) markers have been applied to *C. rotundata* and *C. serrulata* on India's Palk Bay Coast, showing moderate genetic diversity (h: 0.33 for *C. rotundata*), which highlights habitat degradation as a limiting factor (Dilipan and Arulbalachandran, 2022). Chloroplast markers, such as *mat*K and *rbc*L, have been employed to assess haplotype diversity and historical biogeographic patterns (Nguyen XV *et al.*, 2015). For instance, *mat*K sequences revealed high haplotype diversity in *C. rotundata* populations at Tidore Island, North Maluku, Indonesia (Hd: 0.975), compared to nearby islands, highlighting their potential as conservation units, while *rbc*L showed lower resolution for detecting genetic variation (Ramili *et al.,* 2020). In Vietnam, most genetic studies on seagrasses have focused on species such as *Enhalus acoroides, Thalassia hemprichii*, *Ruppia brevipedunculata,* and *Halophila beccarii*, revealing strong genetic structuring driven by coastal currents and habitat fragmentation (Dierick *et al.*, 2021; Nguyen *et al.,* 2022a; 2021; Phan *et al.,* 2017; Triest *et al.,* 2021). Given the geographic isolation of *C. rotundata* populations in the Vietnam island systems, such populations may harbor unique genetic traits or haplotypes, providing important insights into population differentiation and highlighting the need for site-specific conservation strategies. In addition to molecular data, morphological characteristics, particularly those related to leaves and rhizomes, can provide useful information on phenotypic variation among populations (Hughes *et al.*, 2009; McDonald *et al.*, 2016). Although seagrass morphology is highly plastic and influenced by environmental conditions, consistent morphological differences between island populations may reflect underlying genetic divergence or environmentally associated differentiation.

To address these gaps, this study investigates morphology, biomass, and genetic variation in *C. rotundata* from two offshore islands in Vietnam - Ly Son and Phu Quy. These two islands differ markedly in their levels of anthropogenic disturbance: Phu Quy is more remote (110 km from the mainland), less populated, and minimally affected by tourism or coastal development (Binh Thuan Statistics Office, 2024), whereas Ly Son is closer to the mainland (28 km offshore), heavily populated, and subjected to intense human impacts - including historical coral sand extraction from seagrass beds to support long-established garlic cultivation (Quang Ngai Statistics Office, 2024). Such contrasts in environmental pressure provide an opportunity to examine the morphological and genetic variation of *C. rotundata* under contrasting island environmental contexts. Thus, we hypothesize that populations of *C. rotundata* in Ly Son and Phu Quy exhibit distinct morphological traits, biomass allocation patterns, and genetic structures associated with differing degrees of environmental disturbance and habitat stability. Biomass and morphological traits related to leaves and rhizomes were measured to assess phenotypic variation between island populations. Nuclear SSR markers were first applied

to assess nuclear variation and to guide the selection of representative individuals, followed by chloroplast *trn*L-*trn*F sequencing to evaluate haplotype diversity and phylogenetic relationships. Together, these data provide a foundation for improved conservation and restoration strategies targeting *C. rotundata* in Vietnam's island seagrass ecosystems.

Materials and Methods

Study area

This study was conducted on two offshore islands in Vietnam: Ly Son Island (Quang Ngai Province, Central Vietnam, 15.38°N, 109.12°E) and Phu Quy Island (Lam Dong Province, South-Central Vietnam, 10.52°N, 108.94°E). Both islands feature coarse sandy substrates mixed with coral debris, typical of those found on extinct volcanic islands, supporting extensive *Cymodocea rotundata* meadows in shallow subtidal zones (Nguyen *et al.,* 2009; 2018; Tin *et al.,* 2021). On each island, four sampling sites were selected (LS1 - LS4 on Ly Son in August 2023; PQ1 - PQ4 on Phu Quy in August 2024) based on the continuous presence of *C. rotundata* in shallow subtidal zones (< 2 m depth), strategically distributed around islands to ensure geographic representation (Figure 1).

Sampling and sample processing

At each site, a 50-meter transect was established parallel to the shoreline across *C. rotundata* meadows. Five short shoots were collected at 10-m intervals along the transect for genetic analysis to minimize the probability of sampling the same genet. Samples were rinsed with seawater and kept fresh during transport to the laboratory.

For morphological and biomass assessment, three 0.25-m² quadrats were placed at 25-m intervals along the same transect. All seagrass material within each quadrat was collected and transported to the laboratory for analysis. Within each quadrat, 10–30 short shoots, leaves, and rhizomes were randomly selected for measurement, depending on the abundance of *C. rotundata*. Morphological traits, including leaf length, leaf width, sheath length, horizontal rhizome internode length, and horizontal rhizome diameter, were measured using a 30-cm plastic ruler or digital calipers (precision ± 0.01 mm), selected based on their relevance to seagrass growth and environmental adaptation (Short & Coles, 2001). Above- and below-ground biomass was determined by separating seagrass material into shoots (leaves and sheaths) and rhizomes (including roots), oven-drying at 60 °C for 48 hours until constant weight, and weighing with an electronic balance (± 0.01 g) to calculate dry weight per square meter (Short & Coles, 2001).

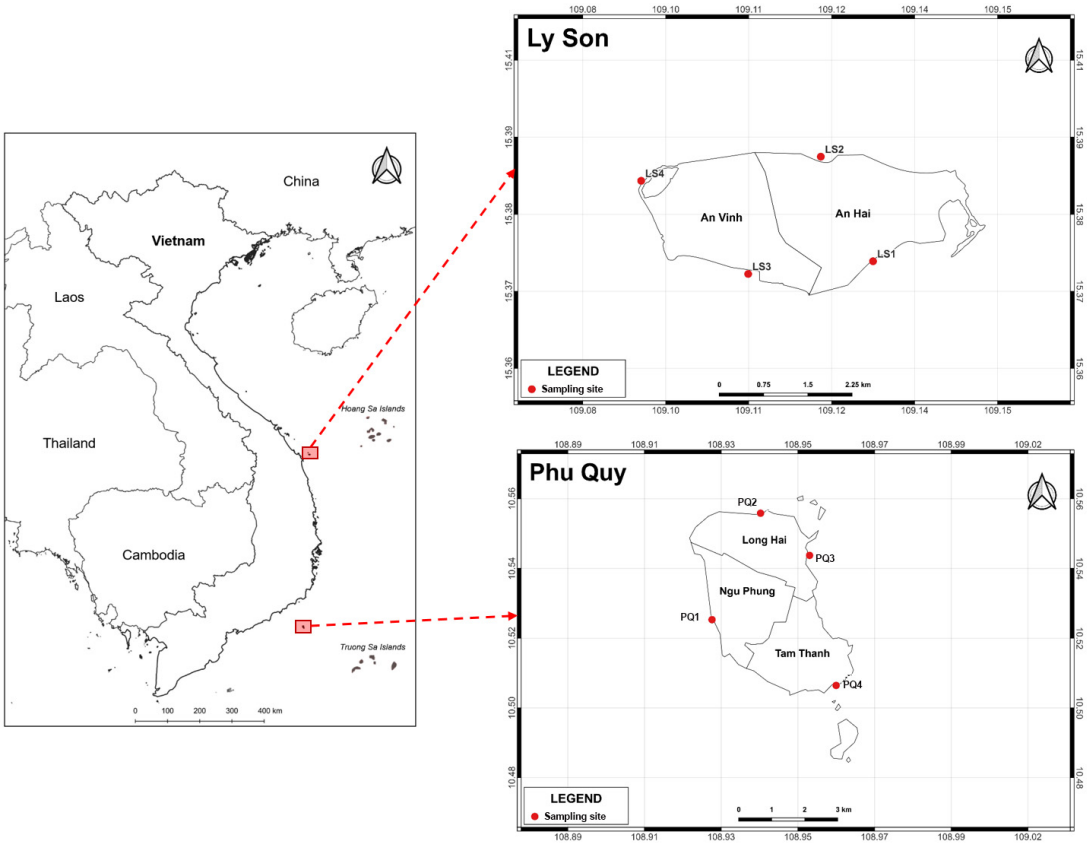

Figure 1. Sampling sites in Ly Son and Phu Quy islands, Vietnam (red dots).

DNA extraction

Genomic DNA was extracted from fresh leaf tissues using the FavorPrep Plant Genomic DNA Extraction Mini Kit (Favorgen Biotech Corp, Taiwan), following the manufacturer's instructions. DNA quality and concentration were assessed using a Nanodrop spectrophotometer and by visualization on 1 % agarose gels. One sample yielded poor DNA quality and was excluded, resulting in 39 *C. rotundata* individuals retained for subsequent genetic analyses.

SSR exploratory analysis

To provide complementary information on nuclear variation and to guide the selection of cpDNA samples, twelve SSR primer pairs (Cymrot040, Cymrot166, Cymrot015, Cymrot004, Cymrot044, Cymrot164, Cymrot006, Cymrot027, Cymrot162, Cymrot003, Cymrot039, and Cymrot153) previously developed for *Cymodocea rotundata* (Arriesgado *et al.*, 2014) were used on 39 individuals. SSR loci were amplified individually, and band presence or absence was recorded as binary data (1 = presence, 0 = absence) (Chaudhary *et al.*, 2018). This exploratory approach enabled a preliminary assessment of genetic variation and guided the selection of divergent individuals for subsequent cpDNA sequencing.

Primers were diluted from 100 µM stock to 10 µM working solutions. Each 20 µL PCR reaction included 10 µL of 2× Go Taq® Green Master Mix (M7502, Promega, USA), 0.5 µL each of forward and reverse primers (10 µM), 1 µL of template DNA, and 8 µL of nuclease-free water. Amplification was carried out in a Bio-Rad T100 thermal cycler under the following conditions: initial denaturation at 95 °C for 15 minutes; 30 cycles of 95 °C for 30 seconds, 57 °C for 30 seconds, and 72 °C for 45 seconds, followed by a final extension at 72 °C for 5 minutes. PCR products were resolved on 2 % agarose gels using a horizontal gel electrophoresis system (Mupid-Exu, Japan). Gels were stained with SafeView Classic Nucleic Acid Stain (abm, Canada) and visualized under UV illumination (Figure 1S). Band sizes of primers were estimated by comparison with the GeneRuler 1 kb DNA Ladder (#SM0313, Thermo Fisher Scientific). All molecular procedures were conducted at the Biotechnology Laboratory, Biology Department, University of Sciences, Hue University.

cpDNA sequencing

Based on the SSR results, nine genetically distinct *C. rotundata* individuals were selected for sequencing of the *trnL-trnF* chloroplast intergenic spacer. The universal primer pair UniC (5'-CGAAATCGGTAGACGCTACG-3') and UniF (5'-ATTTGAACTGGTGACACGAG-3') was used. PCR reactions were performed in a 50 µL volume containing 100 ng of DNA, 20 pmol of each primer, 25 µL of 2× Go Taq® Green Master Mix (M7502, Promega, USA), and nuclease-free water to achieve the final volume. The thermal cycling profile included an initial denaturation at 95 °C for 10 minutes, followed by 30 cycles of denaturation at 95 °C for 1 minute, annealing at 57 °C for 1 minute, and extension at 72 °C for 1 minute, with a final extension at 72 °C for 10 minutes (a Bio-Rad T100). PCR products were checked on 1 % agarose gels stained with SafeView Classic Nucleic Acid Stains (abm, Canada) and compared to a GeneRuler 1 kb DNA Ladder (#SM0313). PCR products were purified and sequenced at DNA Sequencing Company (Can Tho, Vietnam).

Data analysis

Morphological data

Morphological traits were compared between island populations using Mann-Whitney U tests, as the data did not meet the assumptions of normality and homogeneity of variances (tested using Shapiro–Wilk and Levene's tests). In contrast, comparisons of above-ground biomass, below-ground biomass, and above-/below-ground biomass ratio (A/B biomass ratio) were performed using independent-samples t-tests, as these variables met the normality assumption (Shapiro–Wilk test, $p > 0.05$). All statistical analyses were conducted in R v4.3.1, and boxplots of morphological traits and biomass variables were generated using the ggplot2 package.

SSR data

SSR presence/absence data were scored as binary (1 = presence, 0 = absence) and used to compute pairwise genetic distances using the Jaccard coefficient via the 'vegdist()' function in the *vegan* package in R v4.3.1 (R Core Team, 2023). Principal Coordinates Analysis (PCoA) was performed using 'cmdscale()' to visualize genetic relationships among individuals. The first two axes were used to generate biplots, and eigenvalues were extracted to calculate the proportion of explained variance. Visualization of the PCoA results was done using *ggplot2* and *ggrepel* to display sample codes and enhance clarity.

A UPGMA dendrogram was constructed based on the Jaccard distance matrix using the 'hclust()' function with the "average" method. The resulting tree was visualized using the 'fviz_dend()' function in the *factoextra* package, with group rectangles added to highlight clustering patterns among individuals.

cpDNA data from the trnL-trnF region

Raw sequence chromatograms were edited in BioEdit v7.2.5 and aligned using the MUSCLE algorithm implemented in MEGA11 (Tamura *et al.*, 2021). Sequence identity was confirmed through BLAST searches against the NCBI NR-NT database (Altschul *et al.*, 1990). Phylogenetic trees were reconstructed using the Maximum Likelihood method

with IQ-TREE v2.1.3 (Nguyen LT *et al.*, 2015), employing the best-fit nucleotide substitution model determined by ModelFinder and 1,000 ultrafast bootstrap replicates. Tree visualization was performed in Interactive Tree of Life (iTOL) v6 (Letunic & Bork, 2021). Haplotypes and haplotype diversity (Hd) were obtained with the *pegas* package in R v4.3.1. The number of variable sites, haplotypes, and sequence divergence among individuals were summarized to support phylogenetic interpretation.

Results

Morphological variation of *Cymodocea rotundata* between islands

Morphological traits of *Cymodocea rotundata* exhibited significant variation between populations from Ly Son and Phu Quy islands (Figure 2). Mann-Whitney U tests indicated that samples from Phu Quy had significantly greater leaf length (112.5 ± 38.9 mm), shoot height (181.9 ± 64.4 mm), sheath length (45.3 ± 19.9 mm), and leaf width (4.06 ± 0.79 mm) compared to those from Ly Son (83.3 ± 29.0 mm, 138.0 ± 44.3 mm, 38.3 ± 12.2 mm, and 3.98 ± 0.81 mm, respectively; $p < 0.05$ for all comparisons). In contrast, rhizome diameter was significantly larger in Ly Son (2.54 ± 0.45 mm) than in Phu Quy (2.26 ± 0.43 mm; $p < 0.05$). No significant difference was observed in internode length ($p > 0.05$). These morphological differences indicate phenotypic differentiation between island populations and are associated with biomass distribution patterns. The higher below-ground biomass in Ly Son (281.080 g DW/m^2) compared to Phu Quy (148.623 g DW/m^2; $p = 0.009$) aligns with the larger rhizome diameter, indicating a potential investment in below-ground structures. Above-ground biomass showed no significant difference between the two islands (43.15 vs 44.95 g DW m ^{2}; p = 0.876). However, the above-ground/below-ground biomass ratio was higher in Phu Quy (0.305) than in Ly Son (0.153) and approached statistical significance ($p = 0.066$), suggesting a tendency toward greater above-ground allocation in Phu Quy (Figure 3).

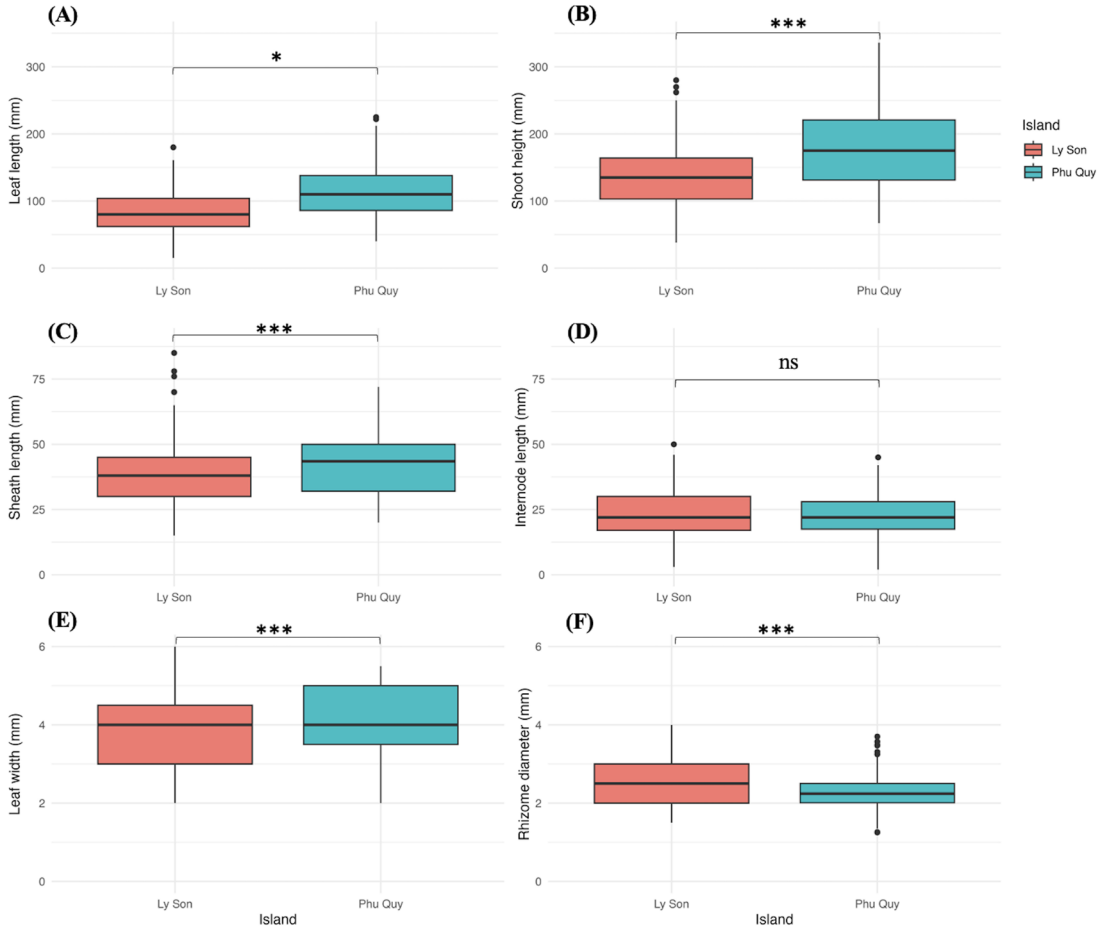

Figure 2. Boxplots showing the variation in six morphological traits of *Cymodocea rotundata* between Ly Son and Phu Quy Islands. (A) Leaf length, (B) Shoot height, (C) Sheath length, (D) Rhizome internode length, (E) Leaf width, and (F) Rhizome diameter. Asterisks indicate significant differences (* : $p < 0.05$; ** : $p < 0.01$; ***: $p < 0.001$), ns: non-significant.

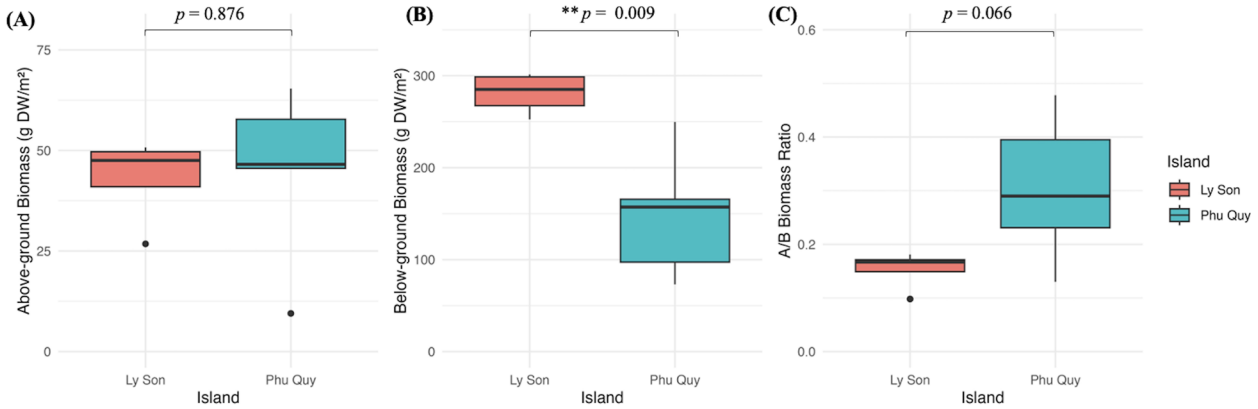

Figure 3. Boxplots showing the variation in biomass allocation of *Cymodocea rotundata* between Ly Son and Phu Quy Islands. (A) Above-ground biomass, (B) Below-ground biomass, (C) Above-/below-ground biomass ratio. *, *p* indicates significant differences.

Genetic variation between island populations based on SSR markers

A total of 39 *C. rotundata* individuals were analyzed using 12 SSR loci. All loci amplified successfully, but polymorphism was limited: the number of alleles per locus ranged from 1 to 2, with only a few loci (e.g, Cymrot044, Cymrot003, Cymrot039) showing variation, while the majority (7/12) were monomorphic (Table S1).

PCoA based on Jaccard distances explained 94.73 % of the total variation (PCoA1 = 73.67 %; PCoA2 = 21.06 %), revealing a clearer clustering of individuals by population (Figure 4). Samples from Phu Quy (PQ1-PQ4) tended to cluster tightly near the origin of the PCoA plot, indicating high genetic similarity among these populations. In contrast, samples from Ly Son (particularly LS1) were distributed along negative values of PCoA1, indicating greater genetic divergence. A few individuals, such as LS1.30 and LS1.50, remained distinct from the main clusters, possibly reflecting localized genetic structure or limited gene flow within the island.

The UPGMA clustering tree based on Jaccard distances supported the PCoA results, with most sample clusters corresponding to their respective islands or populations (Figure 5). The LS1-LS3 group formed a separate clade, while some PQ samples (e.g, PQ191, PQ180) were positioned near the LS cluster, suggesting potential allele sharing or low differentiation among certain individuals. Genetic distances between major clusters ranged from approximately 0.05 to over 0.2, with LS1.30, LS1.40, and LS1.50 forming the most divergent branches.

Overall, the binary SSR dataset provided only limited resolution due to low polymorphism. Nevertheless, these exploratory analyses were informative for assessing moderate differentiation between islands and were also used to guide the selection of representative individuals for subsequent cpDNA sequencing.

Genetic variation based on *trn*L-*trn*F sequences

The *trn*L–*trn*F region was successfully sequenced with high quality, yielding a length of 974 bp (Figure S2). The sequences showed high similarity to the *Cymodocea rotundata* reference sequence from GenBank (accession number: NC_079845), ranging from 99.90 % (LS1.50) to 100 % (LS1.30). Nucleotide sequence alignment among the nine *C. rotundata* samples revealed a high degree of similarity, with variation observed at only three nucleotide positions: position 868 (T deletion in LS2.40, LS3.50, and PQ30), position 909 (T deletion in LS1.50, PQ80, and PQ191), and position 929 (T deletion in LS2.40) (Figure S2).

A total of four haplotypes were identified from the nine sequences (Table 1). Hap_1 consisted of LS1.30, PQ148, and PQ180, which shared identical sequences; Hap_2 included LS1.50, PQ80, and PQ191; Hap_3 corresponded to LS2.40; and Hap_4 comprised LS3.50 and PQ30. The haplotypes differed from each other by only 1-2 nucleotides. Comparison with previously published sequences confirmed high similarity among the study samples and substantial divergence from other seagrass species, particularly those from different genera and families (Cymodoceaceae *vs.* Hydrocharitaceae) (Figure S3).

Phylogenetic analysis showed that all study samples and the reference *C. rotundata* sequence clustered together in a distinct clade, separated from other species (Figure 6). Among the nine samples, LS1.50 exhibited the greatest sequence divergence, showing 99.90 % similarity to *C. rotundata* (NC_079845) and 95.38 % similarity to *Cymodocea nodosa* (OZ188032). No distinct clustering was observed between samples from Ly Son and Phu Quy, as individuals from both islands were intermixed within the phylogenetic tree. This pattern suggests recent historical gene flow from the same source or eventually between these remote islands. For example, LS1.50 and PQ191 shared the same haplotype, while LS1.30 and PQ180 had 100 % sequence identity.

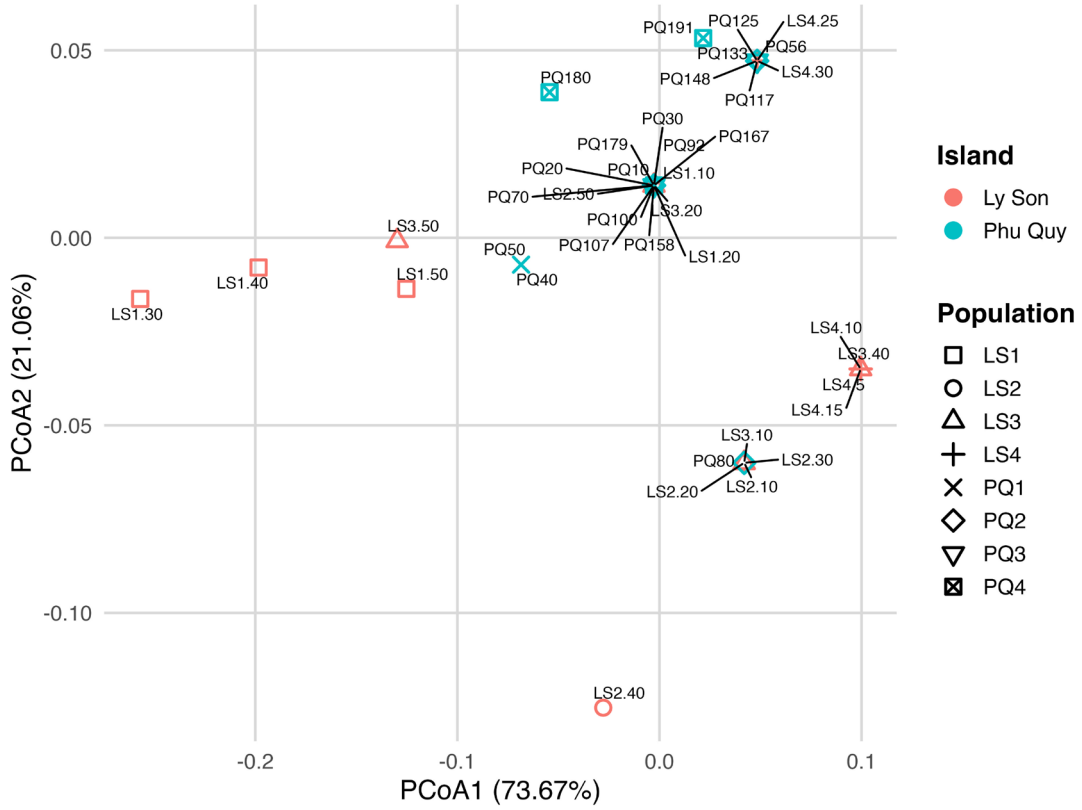

Figure 4. PCoA of *C. rotundata* based on binary SSR data using Jaccard distances. Samples are grouped by population (LS1–LS4: Ly Son Island; PQ1–PQ4: Phu Quy Island).

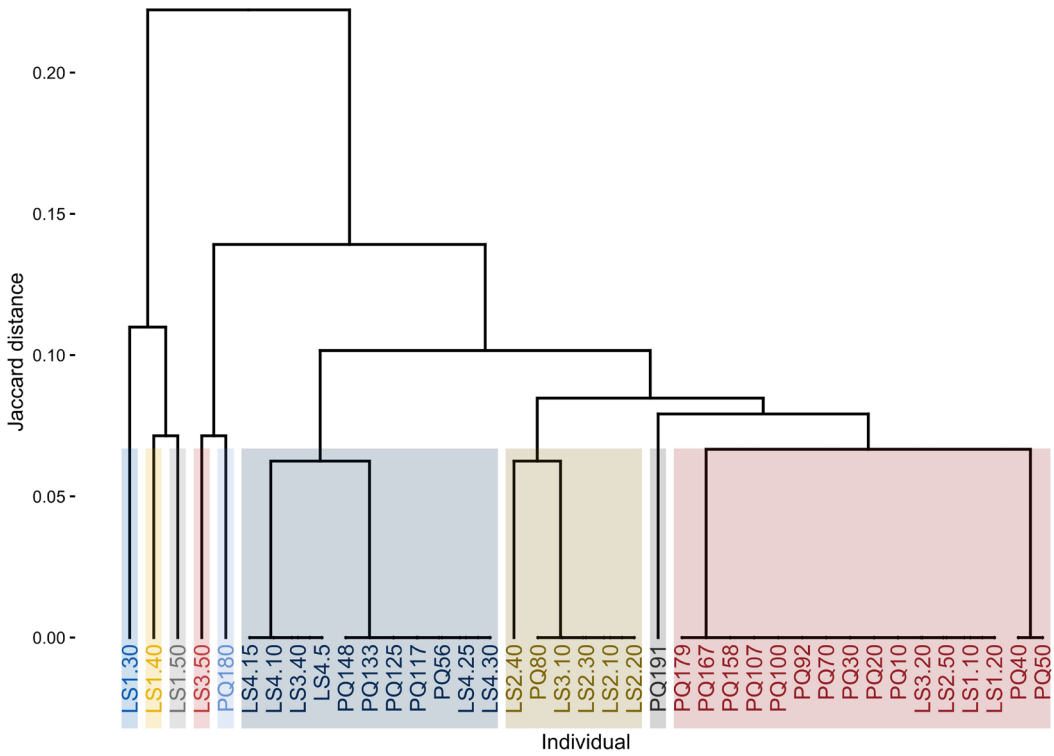

Figure 5. UPGMA dendrogram based on Jaccard distance using SSR data of *C. rotundata*

Table 1. Distribution of haplotypes and haplotype diversity (Hd) of *Cymodocea rotundata* at Ly Son and Phu Quy Islands.

Haplotype	Ly Son (n = 4)	Phu Quy (n = 5)	Total (n=9)	Sample code
Hap_1	1	2	3	LS1.30, PQ148, PQ180
Hap_2	1	2	3	LS1.50, PQ80, PQ191
Hap_3	1	0	1	LS2.40
Hap_4	1	1	2	LS3.50, PQ30
Total of haplotypes	4	3	4	
Haplotype diversity (Hd)	1	0.8	0.81	

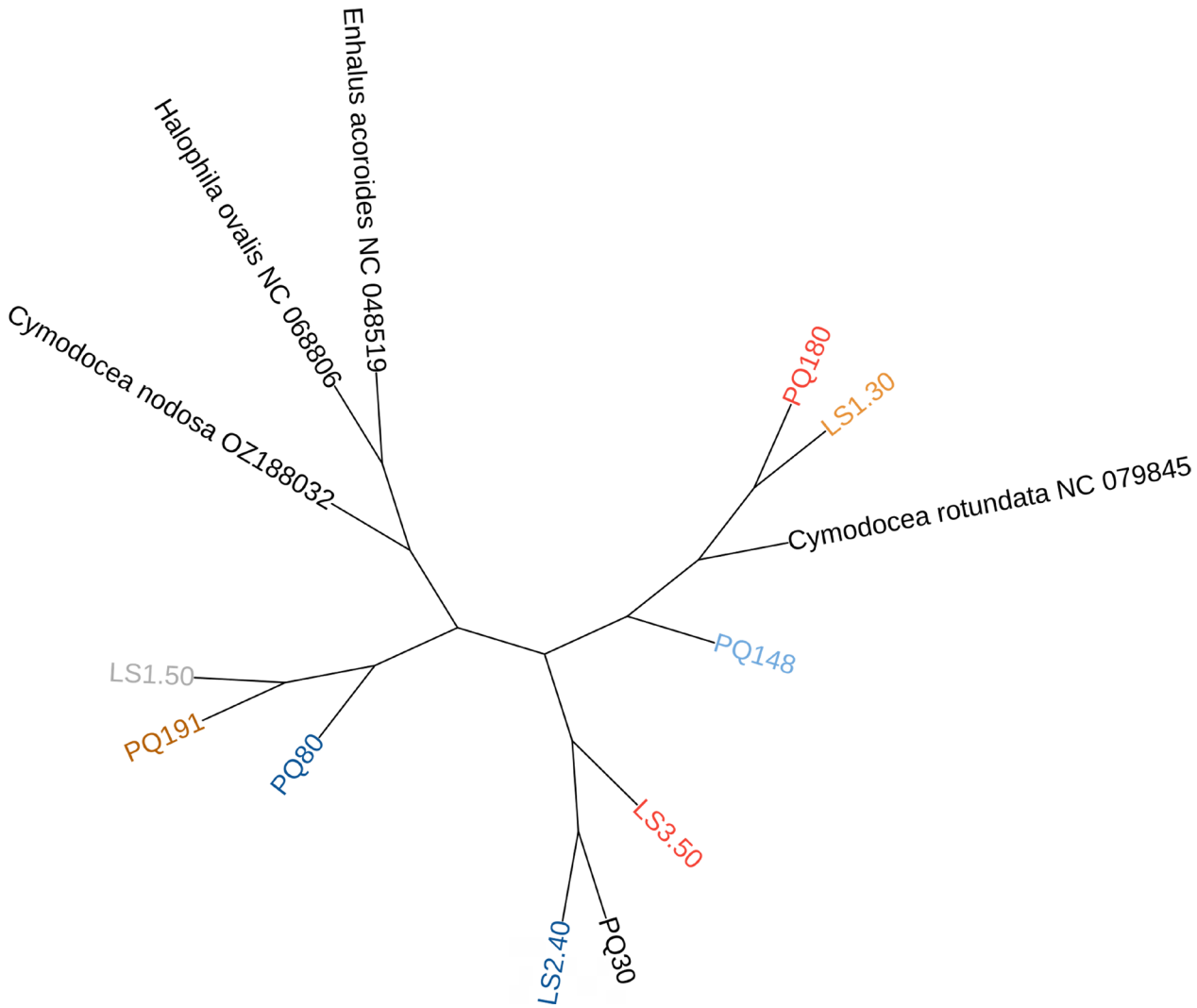

Figure 6. Maximum Likelihood phylogenetic tree of *Cymodocea rotundata* samples from Ly Son (LS) and Phu Quy (PQ) Islands based on *trnL-trnF* sequences, including reference sequences from GenBank.

Discussion

Genetic variation and haplotype diversity across islands

Genetic analysis based on both nuclear (SSR) and chloroplast (*trnL-trnF*) markers revealed moderate genetic variation between *Cymodocea rotundata* populations from Ly Son and Phu Quy Islands. Despite the within-species overlap, individuals from the two islands generally formed somewhat distinct genetic clusters, suggesting restricted gene flow and population differentiation between islands. The exploratory SSR analysis showed that Phu Quy individuals formed a tight genetic cluster, whereas Ly Son individuals, particularly

from site LS1, were more scattered. This pattern points to higher intra-island variability in Ly Son, possibly due to heterogeneous environmental conditions or historical colonization events. A few samples (e.g, LS1.30, LS2.40, PQ180) were separated from their respective island clusters, indicating the presence of unique genotypes. These may reflect localized demographic processes, microhabitat differentiation, or historical admixture. Similar spatial genetic structuring has been reported in *C. rotundata* populations in the Western Pacific, where limited seed dispersal and habitat discontinuity constrain gene flow (Arriesgado *et al.,* 2016).

Although clonal reproduction is known to be a dominant reproductive strategy in many seagrass species (Hughes & Stachowicz, 2009; Sherman *et al.,* 2016), including *C. rotundata* (Arriesgado *et al.,* 2023), the present study was not designed to quantify clonal richness or clone size structure. Shoots were sampled at 10-m intervals to reduce repeated sampling of the same genet for population-level comparison. Given the limited number of individuals per site and the absence of spatially explicit sampling within meadows, robust estimation of clonal structure would require dedicated sampling designs with finer spatial resolution and higher-resolution genotyping approaches.

Chloroplast *trn*L-*trn*F sequence variation further supported this genetic structure by revealing both shared and island-specific haplotypes. Shared haplotypes imply occasional gene flow, possibly mediated by ocean currents as in *Posidonia oceanica* (Micheli *et al.,* 2010) and *Enhalus acoroides* (Nakajima *et al.,* 2014) or by large marine herbivores (Tol *et al.,* 2017). However, the detection of island-specific haplotypes, such as those found in Ly Son highlights the existence of distinct maternal lineages.

The combined evidence from nuclear and chloroplast markers suggests that Ly Son may serve as a regional genetic reservoir. Its higher within-population variability and presence of rare genotypes may contribute to the evolutionary resilience of the population under environmental change (Hughes *et al.,* 2008; Hughes & Stachowicz, 2011, 2004). However, increasing anthropogenic pressures, particularly tourism and agricultural expansion for garlic cultivation, could threaten this diversity through habitat fragmentation (Cao *et al.,* 2012; Nguyen *et al.,* 2022b), as observed in other disturbed seagrass systems (Triest *et al.,* 2021). While SSR markers revealed broad-scale genetic structure and individual divergence, the use of *trn*L-*trn*F sequences provided insights into maternal lineage diversity. Although less commonly used than *mat*K or *rbc*L (Pazzaglia *et al.,* 2021; Ramili *et al.,* 2020), the *trn*L-*trn*F region proved useful in resolving haplotypes in *C. rotundata*. Future studies incorporating more variable chloroplast markers (e.g, *mat*K, *psb*A–*trn*H) or high-resolution nuclear markers such as single-nucleotide polymorphisms (SNPs) could provide finer-scale resolution of gene flow and population history (Pazzaglia *et al.,* 2021; Yu *et al.,* 2023).

Morphological variation and potential links to genetic and environmental factors

Environmental heterogeneity, including variation in light, water depth, sediment type, nutrients, and hydrodynamic conditions, has been widely recognized as a driver of seagrass morphology (Japar Sidik *et al.,* 1999; Kou & den Hartog, 2006; McDonald *et al.,* 2016). The present findings in *C. rotundata* add to the growing body of evidence that morphological plasticity in seagrasses is an important component of their ecological response to local environmental conditions. Significant morphological differences were observed between Ly Son and Phu Quy populations of *C. rotundata*. Individuals from Phu Quy exhibited longer and wider leaves, taller shoots, and longer sheaths, whereas those from Ly Son had significantly thicker rhizomes and greater belowground biomass. These differences likely reflect environmental influences, with potential contributions from genetic variability. In particular, the larger rhizome diameter and higher belowground biomass observed in Ly Son may represent a morphological response associated with sediment disturbance. Seagrass species in high-energy or physically unstable environments often allocate more biomass belowground to enhance anchorage and resistance to burial or erosion (Hemminga & Duarte, 2000; Tanaka & Nakaoka, 2006; Terrados *et al.,* 1998). The long-documented extraction of coral sand from Ly Son seagrass meadows for garlic cultivation and other coastal disturbances (Nguyen *et al.,* 2018; Quang Ngai Statistics Office, 2024) may have caused sediment instability, potentially contributing to the development of thicker rhizomes in *C. rotundata*. A similar morphological differentiation in relation to wave exposure has been observed in *Zostera marina* in the Yellow Sea, China. Individuals at a wave-exposed site were found to develop shorter, narrower leaves and longer roots, which enhance resistance to hydrodynamic stress (Li *et al.,* 2023). In addition, Ly Son exhibited higher haplotype diversity, suggesting the presence of multiple maternal lineages, which may be associated with observed morphological variability. While direct genotype-phenotype associations were not assessed in this study, increased genetic diversity has been linked to broader trait expression and resilience in clonal seagrass populations (Ehlers *et al.,* 2008; Hughes *et al.,* 2009; Reusch *et al.,* 2005).

In contrast, individuals from Phu Quy, which is more isolated and less disturbed, exhibited longer and wider leaves, taller shoots, and thinner rhizomes. This suite of traits is commonly associated with seagrasses growing in more stable environments, where mechanical constraints are reduced, and allocation to photosynthetic tissue is favored (Hemminga & Duarte, 2000; Tanaka & Nakaoka, 2006). The compact genetic structure and more uniform morphology observed in Phu Quy further support the role of local environmental conditions in shaping population-level traits.

Conservation and restoration implications

The genetic and morphological divergence between Ly Son and Phu Quy populations underscores their potential as distinct conservation units. The higher haplotype diversity in Ly Son and unique genotypes (e.g, Hap_3 in LS2.40) suggest that these populations may harbor distinct genetic lineages with conservation relevance, similar to high-diversity Pacific populations of *Z. marina* (Yu *et al.,* 2023). Ly Son, with its high anthropogenic pressure from tourism and coastal development, faces greater risks of habitat degradation (Cao *et al.,* 2012; Nguyen *et al.,* 2022b), as observed in other degraded seagrass systems (Dilipan & Arulbalachandran, 2022). Conservation strategies should prioritize the protection of Ly Son's seagrass meadows from anthropogenic disturbances such as anchoring, pollution, and sediment disruption associated with coastal agriculture (e.g, garlic cultivation), while preserving the relatively undisturbed conditions of Phu Quy to maintain its genetic and morphological diversity. Restoration efforts could leverage observed gene flow between islands to enhance genetic connectivity through seed or propagule transplantation, as suggested for other seagrass species (Jahnke *et al.,* 2015; Pazzaglia *et al.,* 2021).

In summary, this study demonstrates moderate genetic and morphological variation between *Cymodocea rotundata* populations from Ly Son and Phu Quy Islands. Despite some genetic overlap, unique genotypes and significant differences in morphology and biomass suggest these populations may represent separate conservation units. Higher haplotype diversity and below-ground biomass in Ly Son highlight distinct population characteristics under disturbed environmental conditions, whereas Phu Quy represents a comparatively more stable system. Both populations remain vulnerable to coastal development and environmental change, necessitating site-specific conservation strategies to preserve local genetic diversity and prevent homogenization.

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Supplementary Data

The following supplementary materials are available online:

Table S1. Allele frequencies at SSR loci in *Cymodocea rotundata* based on presence/absence data.

Supplementary Figure S1. PCR amplification of 12 SSR loci in *Cymodocea rotundata* (sample PC191) visualized by agarose gel electrophoresis (GeneRuler 1 kb DNA Ladder was used as a size marker).

Figure S2. Multiple sequence alignment of *trnL–trnF* region sequences of *Cymodocea rotundata* from Ly Son and Phu Quy islands. (Each row represents one individual; each column indicates a variable site. Sequence labels correspond to sampling locations: LS = Ly Son, PQ = Phu Quy.)

Figure S3. Multiple sequence alignment of *trnL–trnF* region sequences from nine *Cymodocea rotundata* samples collected from Ly Son (LS) and Phu Quy (PQ) Islands, compared with published sequences of seagrasses from other genera and families.

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Author Contributions

PTTH: Conceptualization; Methodology; Investigation; Formal analysis; Writing – original draft; Visualization; Supervision; Funding acquisition. TTNT: Methodology; Investigation; Data curation. HTQ: Methodology; Data curation. LQD: Investigation; Data curation; Validation. HCT: Methodology, Investigation. NHB: Investigation; Visualization. DNMN: Data curation. TTHT: Investigation; Validation. LT: Review & editing; Supervision.

Conflicts of Interest

The authors confirm that there are no financial or personal conflicts of interest that could have influenced the research presented in this manuscript.

Data Availability

The datasets supporting the findings of this study are available in Zenodo at doi: 10.5281/zenodo.17173059.

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