

Study of the morphology and genetics of the seagrass *Halophila ovalis* in the Wallace Line coastal waters on the Minahasa Peninsula, Indonesia

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Abstract. This study aimed to quantify the morphological characters of *Halophila ovalis* and provide baseline information on the species' genetic characters (DNA chloroplast). The morphological data analyses employed the Excel 2016 Program combined with XL-STAT 2015 for the principal components analysis (PCA). The sequence data were analyzed using MEGA-X and version 6 DnaSP Program and compared with specimens of the Genbank. These were then used to construct the phylogenetic topology, nucleotide base composition, nucleotide base sequence, polymorphic sites, and haplotype. Seagrasses belonging to *Halophila* are typically small and narrow, less than 1 cm wide, and have a distinctive "strap-like" shape with a pointed tip. The PCA identified four morphometric variables (leaf width, leaf length, petiole length, and rhizome diameter) related to PC1, which explains 51% of the observed variation. Species confirmation with the nBLAST technique found that the specimens were *H. ovalis* with the highest score and identity reaching 100%. The phylogenetic topology showed that *H. ovalis* from different coastal waters in Minahasa Peninsula occurred in a single clade with the GenBank metadata. Samples from various sites in Indonesia (including Bali) were identical to the recently studied *H. ovalis* reflecting low variability in nucleotide base sequences. Hence, *H. ovalis* specimens from the sites in this study showed high genetic connectivity.

Key Words: DNA, genetics, morphology, phylogeny, seagrass.

Introduction. Several terminologies are frequently used interchangeably with seagrass, including seagrass, sea grass, weeds, and hiamun (Wagey 2013). However, the name "seagrass" is used in this publication. Seagrass research has long been neglected in Indonesia, even though these plants may be found in nearly all of the country's marine water. They provide crucial habitats for numerous animals, including large commercial and recreational fisheries. They are also an important food source for mega-herbivores, such as green sea turtles, dugongs, and manatees. Seagrasses contribute to the stabilization of sediment and produce much organic carbon. However, anthropogenic effects directly threaten these plants and their environments, and there is still a long way to go before answering the major research issues and putting them into management plans (Short et al 2016; Thangaradjou & Bhatt 2018).

As a megadiverse country, Indonesia hosts extraordinary biological richness, encompassing a wide array of terrestrial and marine life (Myers et al 2000). In order to document and monitor this diversity and to help offset the accelerating loss of species and habitats, ongoing efforts in data collection on organismal variety are essential both for research imperatives and practical conservation needs (Myers et al 2000). Historically, seagrass species identification has relied primarily on morphological characteristics (Wagey 2017). However, in tropical regions with high species richness and morphological complexity, identification based only on vegetative traits can be challenging due to significant phenotypic plasticity and overlapping morphological features among species (Wagey 2017). Most tropical seagrass research has focused on ecological parameters such as species distribution, shoot density, and meadow extent rather than detailed taxonomic resolution (Orth & Heck 2023).

Several studies on seagrass genetic diversity and genetic population have been conducted in spatially widespread coastal seascapes in the South China Sea (Hernawan 2018), the Western Indian Ocean (Jahnke et al 2019), English archipelago (Alotaibi et al 2019), and Western Australia (Sinclair et al 2020). The investigations included both tropical and temperate seagrass. Southeast Asia has the greatest diversity of seagrass with 12-15 species (Fortes et al 2018; Thangaradjou & Bhatt 2018). Molecular investigations like genetic diversity assessment, genetic population analysis, and seagrass phylogenetics are critical to understanding seagrass distribution, evolution, and conservation (Jahnke et al 2019; Lamit & Tanaka 2019; Clarito et al 2020).

DNA barcodes have practical applications such as ecological surveys and sample verification. Translating these applications into usable DNA barcodes requires selecting one or more loci that can be routinely and accurately sequenced to identify large, taxonomically diverse samples and to facilitate interspecific comparisons; this selection is guided by three main criteria: primer universality, discriminatory power between species, and ease of amplification or recoverability of the sequence (Yang et al 2018). Considering these criteria, this study investigated the genetic diversity of the seagrass *Halophila ovalis* in the coastal waters of the Minahasa Peninsula. Limited taxonomic knowledge often leads to nomenclatural errors and species misidentifications, which have been documented among *Halophila* species (Guiry & Guiry 2023). Indonesia, as one of the world's megadiverse countries, urgently needs to validate and document species within its jurisdiction. Members of the genus *Halophila* have a relatively wide distribution except at high latitudes (Hemminga & Duarte 2000). Therefore, this study focuses on the morphological and genetic characterization of *H. ovalis* in North Sulawesi, Indonesia.

Material and Method

Study sites. Five study sites representing different coastal zones of the Minahasa Peninsula, North Sulawesi, Indonesia, were selected: 1) Arakan/Rap-rap (ARA) – northern part; 2) Malalayang (MAL) – western part, Sulawesi Sea; 3) Bahoi–Likupang (BAH) – eastern part, Molucca Sea; 4) Tanjung Merah (TjM) – southern part; and 5) Ratatotok–Belang (RAT) – southeastern part, Molucca Sea (Figure 1).

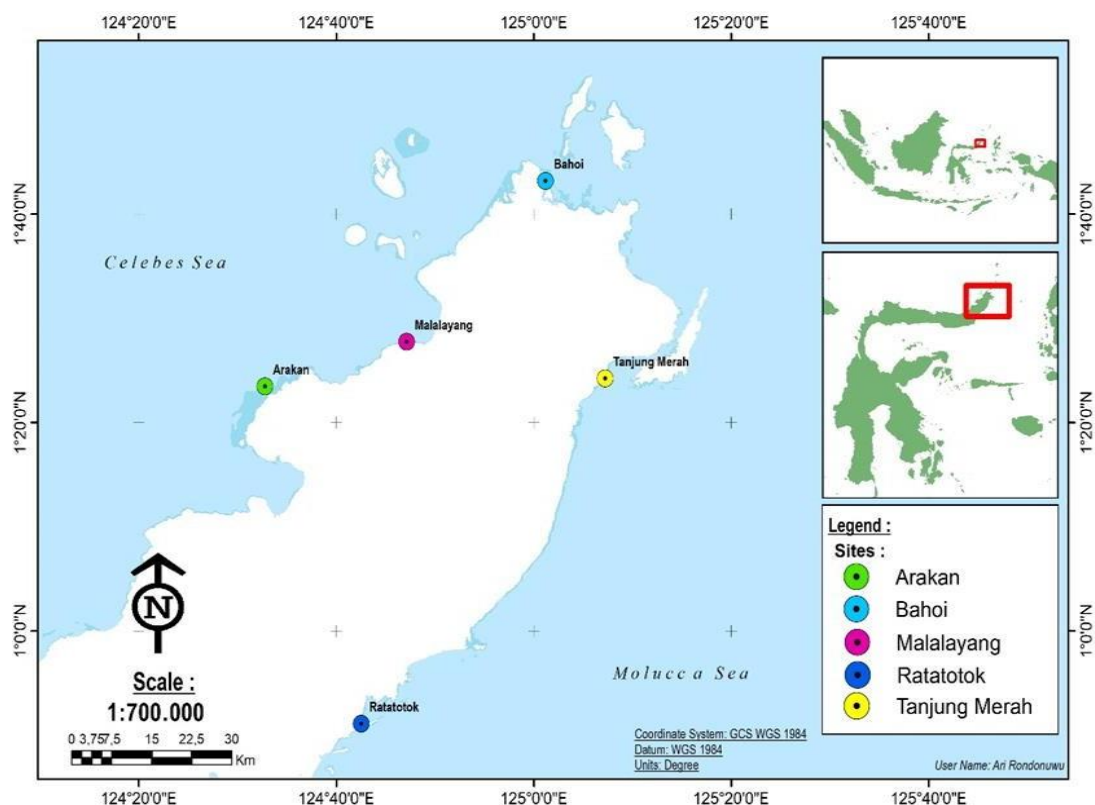


Figure 1. The location of location of the sampling sites in North Sulawesi, Indonesia.

Sampling was conducted between March and June 2022 at depths of 3 m and 5 m under bright weather conditions. Each sampling site was georeferenced using a Global Positioning System (GPS) device. Site coordinates, sampling dates, and measured environmental parameters are presented in Table 1. The data presented in Table 1 should be considered as descriptive only due to limited data collection of in situ parameters. Although there are differences in the environmental parameters, for example, Arakan has warmer water temperature in the shallow (3 meters) but also lowest temperature at 5 meter depth. The rest of the variables, however, such as salinity were similar across sites.

Table 1

Environmental condition of the study sites

Parameters	Site				
	ARA	MAL	BAH	TjM	RAT
Date	19-3-2022	19-3-2022	19-3-022	21-3-2022	28-6-2022
Latitude (N)	1°23'28.50"	1°27'46.22"	1°43'13.06"	1°24'14.41"	0°51'05.57"
Longitude (E)	124°32'26"	124°47'08.49"	125°01'12.53"	125°07'14.05"	124°42'31.66"
Weather	Bright	Bright	Bright	Bright	Bright
Water temp., 3 m (°C)	30.80	31.90	29.80	30.90	29.50
Water temp., 5 m (°C)	24.10	28.10	26.10	27.01	27.00
Salinity, 3 m (‰)	25.00	25.00	25.00	25.00	25.00
Salinity, 5 m (‰)	23.00	23.00	23.00	23.00	23.00
pH, 3 m	6.15	5.88	6.30	6.55	No data
pH, 5 m	5.20	5.50	6.05	6.23	No data
Substrate type (3 m)	Sandy	Sandy	Sand mixed with coral shards	Sandy	Sandy mud
Substrate type (5 m)	Sandy	Sandy	Sand mixed with coral shards	Sandy	Sandy mud
Associated biota (3 m)	No data	Anemones, <i>Chaetodon</i>	<i>Echinothrix</i> & <i>Diadema setosum</i>	No data	Fish, molluscs, echinoderms
Associated biota (5 m)	No data	Anemones, <i>Chaetodon</i>	<i>Diadema setosum</i>	No data	Fish, molluscs, echinoderms
Habitat type (3 m)	Sand-coral reef	Sand-coral reef	Sand-coral reef	No data	Seagrass meadow, coral reef, Anemone, soft coral

Note: ARA = Arakan; MALA = Malalayang; BAH = Bahoi; TjM = Tanjung Merah; RAT = Ratatotok.

Environmental parameters. Water parameters were measured in situ at each site. Temperature, pH, and dissolved oxygen were recorded using a HORIBA LAQUA PD-220K handheld pH/ORP/DO/Temperature meter, calibrated with standard buffer solutions (pH 4.0, 7.0, and 10.0) before use. Water temperature ranged from 24.1 to 31.9°C, and pH varied between 5.20 and 6.55. Salinity was determined using a handheld refractometer, ranging from 23 to 25‰. Substrate type was determined by direct underwater observation and categorized as sandy, sandy mud, or sand mixed with coral fragments. The habitats were primarily sand-coral reef environments, with Ratatotok–Belang also characterized by seagrass meadows, soft corals, and anemones. Associated benthic organisms included sea anemones (Anthozoa), butterflyfish (*Chaetodon* spp.), sea urchins (*Echinothrix* and *Diadema setosum*), molluscs, and reef fishes.

Sample handling and morphological identification. Seagrass samples of *H. ovalis* were collected by SCUBA divers from each study site (ARA, MAL, BAH, TjM, and RAT). Samples were rinsed with freshwater to remove sediment and epiphytes, then transported to the Integrated Laboratory, Faculty of Fisheries and Marine Sciences, Sam Ratulangi University, for analysis.

A total of 30 samples (n = 30) were analyzed for each morphological variable: leaf width (LW), leaf length (LL), petiole length (PL), internode (IN), rhizome diameter (DoR), number of left cross veins (NCVL), number of right cross veins (NCVR), and number of

leaves (NL). Measurements were taken using a digital caliper for quantitative traits. The presence of reproductive structures such as fruits, flowers, and scales (scutes) was also recorded. Species identification followed diagnostic features described by Waycott et al (2004), El Shaffai (2011), and Wagey (2013).

Molecular work. Observations and measurements of morphological characters were conducted both in the field and at the Integrated Laboratory of the Faculty of Fisheries and Marine Sciences, Sam Ratulangi University. DNA extraction was carried out at the Biotechnology Service Unit, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado. Polymerase chain reaction (PCR) amplification was performed using 5× FIREPol PCR Master Mix in a total reaction volume of 50 µL, containing 20 pmol of each primer and template DNA. The primer pairs used were: rbcLaF (forward): 5′-ATG TCA CCA CAA ACA GAG ACT AAA GC-3′ and rbcLaR (reverse): 5′-CTT CTG CTA CAA ATA AGA ATC GAT CTC-3′. PCR thermal cycling conditions were as follows: initial denaturation at 95°C for 2 min, followed by 40 cycles of 95°C for 40 s, 55°C for 40 s, and 72°C for 50 s, with a final extension at 72°C for 10 min. The PCR products were separated using 1% agarose gel electrophoresis in 1× TBE buffer and visualized under UV illumination. Amplified products were sent, along with the primers, to 1st BASE Malaysia for sequencing.

Data analysis. Morphological data of *H. ovalis* (DoR, IN, LL, LW, NCVL, NCVR, NL, and PL) obtained from five sampling sites at two depths (3 m and 5 m) were first organized and tabulated in Microsoft Excel. These data were then subjected to Principal Component Analysis (PCA) using XLSTAT 2015 to examine patterns of morphological variation among samples and to determine which traits contributed most to overall variability. Prior to analysis, the data were standardized to remove the effect of differing measurement scales among parameters.

The PCA produced eight principal components (F1-F8) that summarized the relationships among morphological traits. The first two components (F1 and F2) explained the largest proportion of the total variance and were used to visualize grouping patterns among sites and depths. The loading values of each variable on the PCA axes were interpreted to identify the traits that contributed most strongly to each component. High positive loadings indicated parameters that increased together, while negative loadings suggested inverse relationships.

In this study, F1 typically represented the overall leaf dimension traits (LL, LW, PL, and NL), capturing variation related to leaf size and growth form, whereas F2 was mainly associated with structural traits such as internode length (IN) and rhizome diameter (DoR), reflecting differences in horizontal growth and substrate adaptation. The remaining components (F3-F8) explained minor variations and were considered less influential in discriminating site and depth effects. The PCA biplot was used to visualize the clustering of sampling sites and to interpret how morphological traits contributed to variation among shallow and deep populations of *H. ovalis*. Following the PCA, a Pearson correlation matrix of the morphological variables was computed to evaluate the strength and direction of linear relationships among traits, providing additional insights into interdependence between morphological parameters.

Molecular analysis. The DNA sequences obtained were edited and aligned using the MEGA-X program (Kumar et al 2018). Sequence similarity was verified by comparison with reference sequences in the GenBank database using BLAST and the Barcode of Life Data System (BOLD). Phylogenetic trees were reconstructed in MEGA-X using default parameters with 1,000 bootstrap replications. Polymorphism analyses - including nucleotide composition, polymorphic sites, transitions, transversions, indels (insertions/deletions), and haplotypes - were conducted using DnaSP version 6.12.03 (Rozas et al 2017).

Results

Morphological variations of seagrass *H. ovalis*. The results of measurements (mean) and observations obtained showed that *H. ovalis* at all locations had the characteristics of oval/or-shaped leaves, smooth and slippery surfaces; LW 10.59-14.18 mm (3 m depth) and 7.82-14.38 mm (5 m depth); LL 19.14-29.92 mm (3 m depth) and 14.10-28.46 mm (5 m depth); 15-26 pairs of leaf veins, and this species is found in sandy substrate mixed with coral rubble. The representative samples of *H. ovalis* are shown in Figure 2.

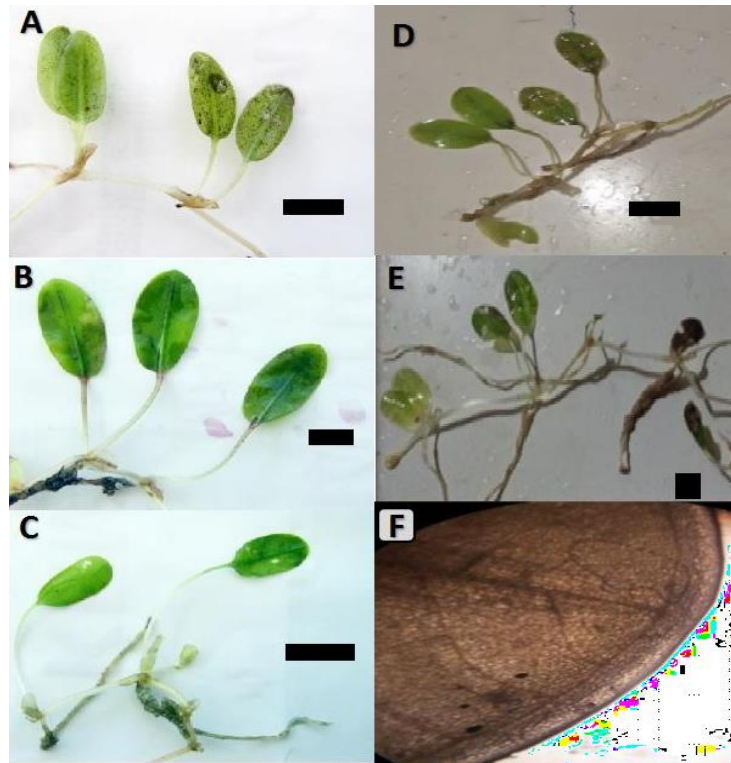


Figure 2. Leaf shape patterns of *H. ovalis* from the sampling sites. A, Arakan/Rap-rap; B, Bahoi; C, Tanjung Merah, D, Malayang; E, Rataatok; and F, sample photomicrograph (40x) of the edge of *H. ovalis* leaf from Rataatok. Scale bars = 10 mm.

Variations in each morphological trait are presented as barplots in Figure 3. At both depths, *H. ovalis* exhibited significant variation in morphological parameters across the five sampling sites. In the shallow sites (A), leaf-related traits such as LL, LW, and PL showed the highest values, particularly at Rataatok and Tanjung Merah, indicating favorable growth conditions. DoR and IN remained relatively consistent across sites, with only slight increases in deeper sediments. At greater depth (B), a general reduction in most parameters was observed except for PL and NL, which remained comparatively high at Rataatok. The number of cross veins (NCVL and NCVR) exhibited moderate variability among sites and depths, suggesting morphological plasticity in response to environmental gradients such as light availability and substrate type. Overall, *H. ovalis* displayed site-dependent morphological differences rather than depth-dependent differences, reflecting its adaptability to varying coastal conditions in North Sulawesi.

Based on the PCA in Figure 4, the resulting eigenvalues showed that the variance explained by the first two principal components (axes 1 and 2) accounted for 66.21% of the total variability in the dataset (Figure 5; Table 2). This indicates that most of the variation in the morphological data can be effectively represented along these two axes. The remaining 33.79% of the variance was explained by components 3 to 8. Overall, the analysis accounted for 78.69% of the total variation, suggesting that the PCA provided a reliable summary of the morphological characteristics of *H. ovalis*.

To interpret the contribution of each variable to the model, the position of the variables along axis 1 and axis 2 was examined (Figures 4 and 5). Among the eight

variables, LL and LD were positioned closer to axis 1, indicating a strong influence on the first principal component. In contrast, NL was positioned nearer to axis 2, suggesting a smaller effect on the first axis. Variables that point in the same direction - such as IN, DoR, and PL - were positively correlated, and their proximity to the correlation circle indicates a significant contribution to overall morphological variation.

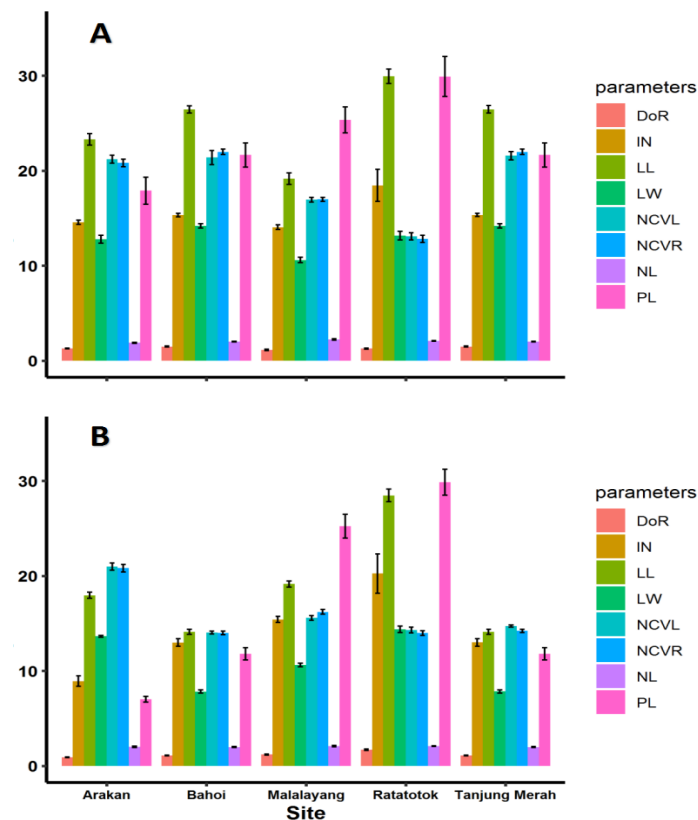


Figure 3. Morphological parameters of the seagrass *H. ovalis* from the sampling locations at two depths: A, shallow (3 m) and B, deep (5 m). Error bars are the standard error of the means (n = 30). LW = leaf width (mm), LL = leaf length (mm), PL = petiole length (mm), IN = internode (mm), DoR = rhizome diameter (mm), NCVL = number of left cross vein, NCVR = number of right cross vein, NL = number of leaves.

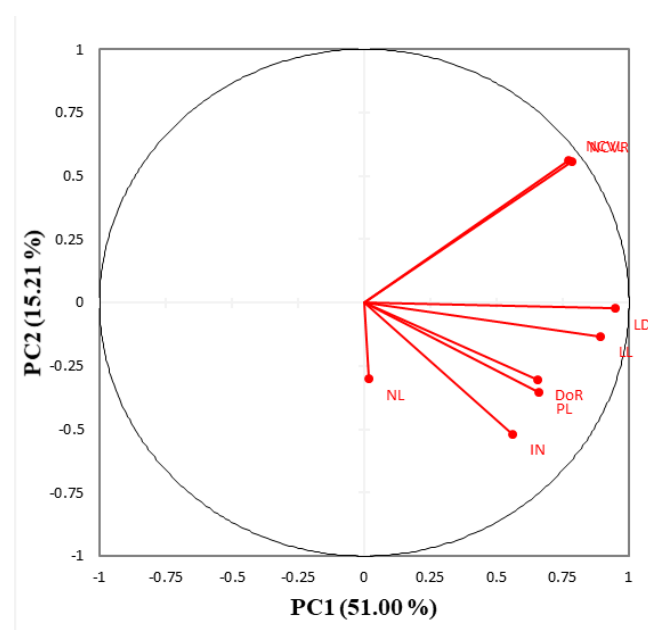


Figure 4. PCA biplot of seagrass morphometric variables.

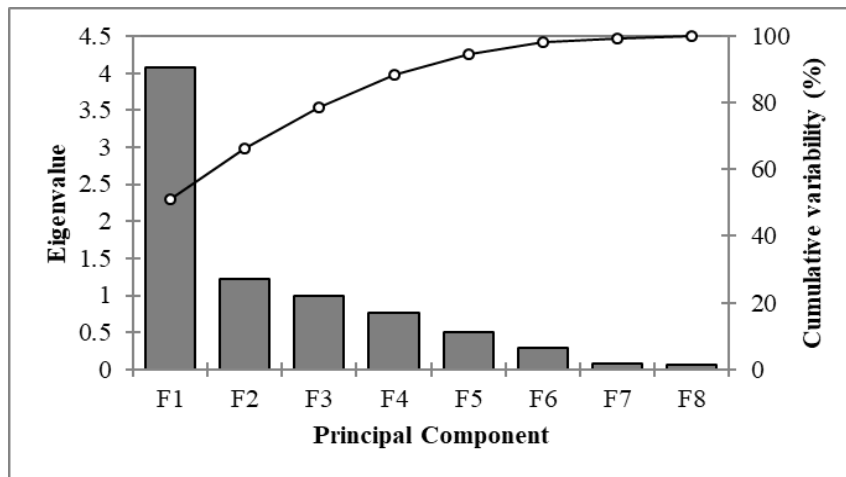


Figure 5. Plot of cumulative variable value of root characteristics. PC 1 and 2 explain > 70% of the variability. Note: F1-8 represents PC components.

Table 2

Summary of principal component analysis (PCA) results for the eight morphological variables of *Halophila ovalis*, showing eigenvalues, percentage of variance, and cumulative variance explained by each principal component (F1–F8).

PCA summary	F1	F2	F3	F4	F5	F6	F7	F8
Eigenvalue	4.08	1.22	0.99	0.76	0.51	0.29	0.08	0.06
Variability (%)	51.00	15.21	12.48	9.52	6.36	3.61	1.04	0.79
Cumulative %	51.00	66.21	78.69	88.20	94.56	98.17	99.21	100.00

Note: F1-8 represents PC components

The Pearson correlation matrix (Table 3) reveals strong positive correlations among several morphological traits of *H. ovalis*. LW and LL are highly correlated ($r = 0.903$), indicating that larger leaves tend to be both longer and wider. PL also shows moderate to high correlations with LW ($r = 0.616$) and LL ($r = 0.659$), suggesting that leaf size and petiole elongation increase together. DoR is moderately correlated with LW ($r = 0.573$) and LL ($r = 0.513$), implying that thicker rhizomes are associated with larger leaf structures. The NCVL and NCVR sides exhibit a very strong correlation ($r = 0.929$), reflecting bilateral vein symmetry in the leaf morphology. In contrast, the NL shows very weak or negligible correlations with all other traits, suggesting that leaf count per shoot is largely independent of individual leaf dimensions or rhizome thickness. Overall, these correlations highlight coordinated growth patterns among vegetative structures but limited association with leaf number in *H. ovalis*.

Table 3

Pearson correlation matrix of the morphological variables

Variables	LW	LL	PL	IN	DoR	NCVL	NCVR	NL
LW	1	0.903	0.616	0.493	0.573	0.666	0.695	-0.008
LL	0.903	1	0.659	0.470	0.513	0.547	0.564	-0.019
PL	0.616	0.659	1	0.341	0.321	0.282	0.297	0.084
IN	0.493	0.470	0.341	1	0.461	0.194	0.205	0.004
DoR	0.573	0.513	0.321	0.461	1	0.358	0.340	0.070
NCVL	0.666	0.547	0.282	0.194	0.358	1	0.929	-0.004
NCVR	0.695	0.564	0.297	0.205	0.340	0.929	1	-0.023
NL	-0.008	-0.019	0.084	0.004	0.070	-0.004	-0.023	1

Note: LW = leaf width, LL = leaf length, PL = petiole length, IN = internode, DoR = rhizome diameter, NCVL = number of left cross vein, NCVR = number of right cross vein, NL = number of leaves, n = 30 for each variable.

Genetic confirmation of seagrass *H. ovalis*. After obtaining a DNA band that matches the length of the target gene, then sequencing was carried out to confirm the amplification of the *rbcl* gene from each sample (Lin et al 2021). The sequencing results were confirmed with Basic Local Alignment Search Tools (BLAST) to determine whether the gene sequence obtained was the target gene of seagrass *H. ovalis*. This result can be proven. Confirmation was achieved by calculating the percent homology between the obtained *rbcl* sequence and reference *rbcl* sequences. Query from Genbank showed a high match rate of 100%. The high match indicates that the target gene obtained is the true *H. ovalis* *rbcl* gene (Stevanus & Pharmawati 2021).

Seagrass *H. ovalis* was identified at Arakan/Raprap, Malalayang–Manado, Bahoi–Likupang, Tanjung Merah, Ratatotok–Belang, and Bali (Wagey et al 2016) based on the results of the alignment of the *rbcl* gene sequences that was 564 bp. Alignment was conducted to determine the nucleotide base-sequence homology level of the *rbcl* gene sequence obtained between samples from different locations. The composition of the nucleotide base frequency of the seagrass *H. ovalis* based on 7 nucleotide sequences is A = 28.01%, T = 28.27%, C = 21.02% and G = 22.70% with the dominant bases being T and A. Table 4 shows only one nucleotide base substitution at position 564 of the nucleotide sequence.

Table 4

H. ovalis nucleotide base sequence showing substitution at position 564 from different sampling locations

Position	Nucleotide base variation by location						
	Bali	Arakan	Malalayang	Bahoi	Tanjung Merah	Ratatatok-1	Ratatatok-2
541	G	-	-	-	-	-	-
542	T	-	-	-	-	-	-
543	T	-	-	-	-	-	-
544	T	-	-	-	-	-	-
545	A	-	-	-	-	-	-
546	T	-	-	-	-	-	-
547	G	-	-	-	-	-	-
548	A	-	-	-	-	-	-
549	A	-	-	-	-	-	-
550	T	-	-	-	-	-	-
551	G	-	-	-	-	-	-
552	T	-	-	-	-	-	-
553	C	-	-	-	-	-	-
554	T	-	-	-	-	-	-
555	A	-	-	-	-	-	-
556	C	-	-	-	-	-	-
557	G	-	-	-	-	-	-
558	T	-	-	-	-	-	-
559	G	-	-	-	-	-	-
560	G	-	-	-	-	-	-
561	T	-	-	-	-	-	-
562	G	-	-	-	-	-	-
563	G	-	-	-	-	-	-
564	A	-	-	C	-	C	C

Only position 564 was polymorphic (T ↔ C); all other alignment positions (1-563) were identical across samples; no indels detected. Haplotype assignment: Hap_1 = T (Bali, Arakan/Rap-rap, Tanjung Merah); Hap_2 = C (Malalayang, Bahoi, Ratatotok-1, Ratatotok-2); h = 2; Hd = 0.5714.

The nucleotide base substitution is a transition, not a transversion. In addition, there was no indel (insert = insertion and deletion = deletion) of nucleotide bases. The transition point is the substitution of nucleotide bases from bases A and G (purines) or between

bases T and C (pyrimidines), while transversion is the substitution between a purine base and a pyrimidine base (Lyons & Lauring 2017).

The results of the phylogenetic reconstruction of *H. ovalis* sequences from different locations in the Minahasa peninsula are presented in Figure 6. Wagey et al (2016) also reported genetically identical *H. ovalis* in Arakan, Bali and Tanjung Merah. In comparison, the results of DNA polymorphism analysis did not have a clear haplotype. That indicates the 7 *H. ovalis* sequences from these locations have the same ancestor.

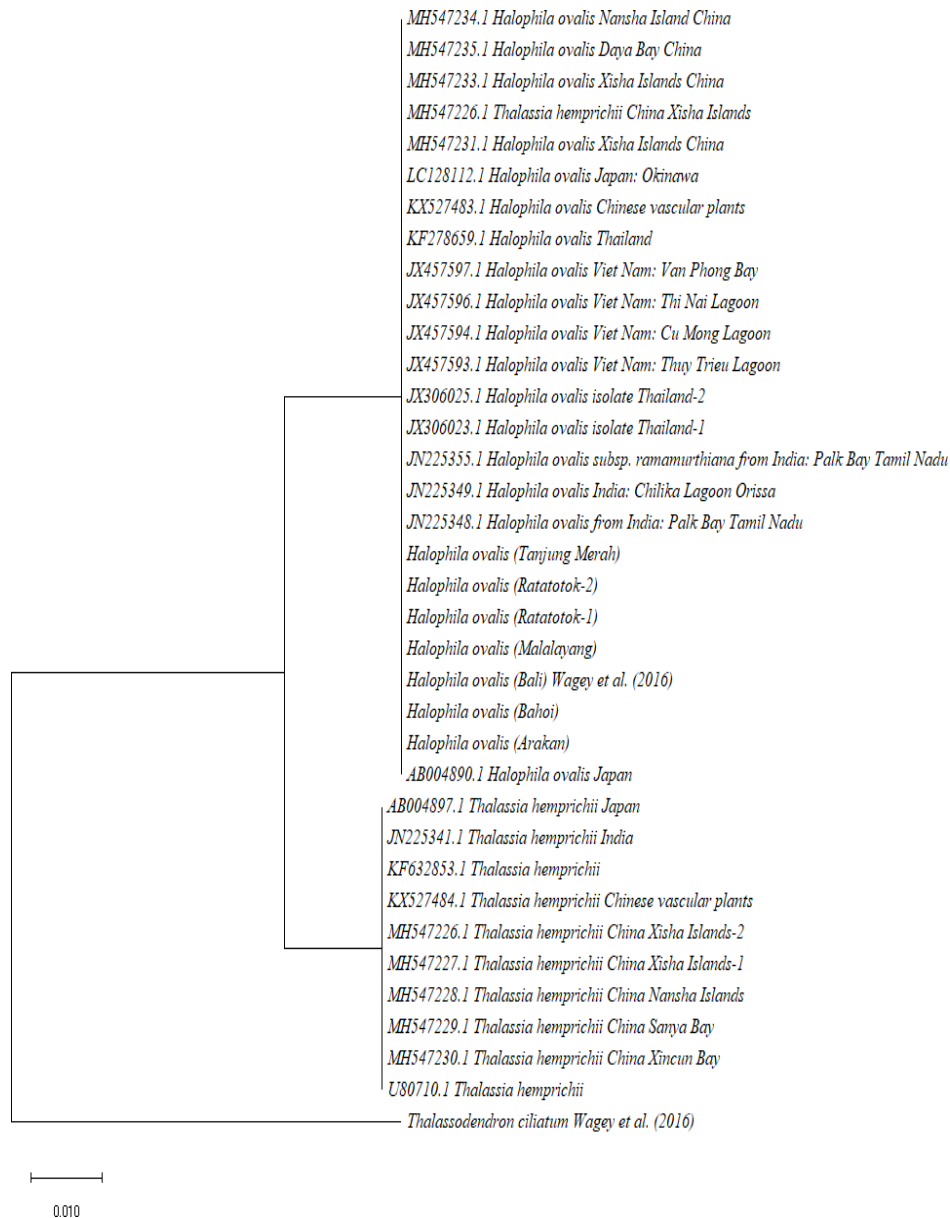


Figure 6. Phylogenetic reconstruction (Neighbor-Joining method – MEGA X).

Haplotype distribution can be described as follows:

Number of haplotypes, h : 2

Haplotype diversity, Hd : 0.5714

Hap_1: 3 [1-2 5]

Hap_2: 4 [3-4 6-7]

Hap_1: 3 [*H. ovalis* (Bali)-*H. ovalis* (Arakan/Raprap) *H. ovalis* (Tanjung Merah)]

Hap_2: 4 [*H. ovalis* (Malalayang)-*H. ovalis* (Bahoi) *H. ovalis* (Ratatotok-1)-*H. ovalis* (Ratatotok-2)]

Discussion. Although seagrass productivity increases with increasing temperature, at temperatures above 45°C seagrass will experience stress and ultimately death (Strydom et al 2020). Salinity is relatively uniform at 30 and 23 ‰ and is in the range suitable for seagrass life of 10-40‰. The degree of acidity (pH) is beyond the tolerance of seagrass, namely 7-8.5. So temperature and salinity can support seagrass life at the research site, but the high pH can inhibit metabolism and respiration (Berg et al 2019). The pH of water strongly influences aquatic organisms and is therefore used as an indicator of water quality. Seagrass leaves serve as shelters and attachment sites for animals, providing protection from sunlight. The elongated, ribbon-like leaves form hanging structures that act as pathways for macrozoobenthos migrating from the sediment to the seagrass blades. The rhizomes are submerged and extend horizontally, with segments that produce short, upright stems bearing leaves and flowers. These rhizomes are buried in the substrate and play a crucial role in the vegetative reproduction of *H. ovalis* (not considered as a parameter in this study). The number of leaf veins was used as a morphological determinant for this species.

The substrate characteristics at the research site consist of sand, mud, and sand mixed with coral fragments, with sand being the predominant substrate. Seagrasses can thrive in a wide range of sediment types, from fine mud to sand mixed with coral rubble (Ambo-Rappe 2022). The primary requirement for a suitable seagrass substrate is sufficient sediment depth (Lanuru 2011). Deep sediments provide two key advantages: they stabilize the seabed and ensure an adequate nutrient supply for seagrass growth. In Indonesia, seagrass beds are classified into several categories based on substrate type, including mud, sandy mud, sand, and sand mixed with coral fragments. As both a growth medium and nutrient source, the substrate plays a crucial role in the growth and survival of seagrass (Yunitha et al 2014).

Seagrass is a flowering aquatic plant that lives in seawater, has leaves, has roots, and reproduces by seeds and shoots. The seagrass roots store oxygen for photosynthesis, which flows from the leaf's epidermal layer through diffusion along the lacunal system (air). Morphologically, leaves are easily recognized from shape, tip, and presence or absence of ligules. Ligula supports and protects the young leaves that are just growing so they don't get watered easily. Leaves are generally elongated except for the genus *Halophila*. The genus *Halophila* has a petiolate (oval) leaf shape and does not have a midrib (Kuo 2007). Seagrass leaves elongate like ribbons hanging down which acts as a path for macrozoobenthos that migrates from sediments to seagrass leaves. Rhizomes are immersed in a substrate that plays an important role in vegetative reproduction (Short et al 2016).

The leaf shape of *H. ovalis* and the number of leaves were used as a morphological determinant of this species. The morphological characters used were leaf width, leaf length, petiole length, rhizome diameter, internode, number of leaf bones (left and right), and number of leaves. These morphological parameter data were analyzed by PCA using the XLSTAT 2015 application; the correlation coefficient calculation method used the classical/Pearson method. The basic concept of PCA is group analysis; the same characters are grouped into one group, while different characters are separated into different groups. It was observed that *H. ovalis* from different locations in the Minahasa Peninsula is in the same clade (first) with *Halophila* deposited in the GenBank database. The second clade is a group of the genus *Thalassia*, within the same family with *Halophila*, namely the Hydrocharitaceae. The third clade is an outgroup specimen as a member of the family Cymodoceaceae, clearly separated from the first and second clades. Based on the research results limited to the problems and objectives, several conclusions can be made with some caveats. First, the environmental conditions of *H. ovalis* in North Sulawesi at sampling locations (Arakan/Raprap, Malalayang, Bahoi, Tanjung Merah, and Ratatotok) at two depth strata (3 and 5 meters) did not differ significantly, but sampling on environmental variables need to be quantified and monitored. The measured and observed environmental parameters were within the tolerable limits of the seagrass *Halophila*. At the same time, quantitative morphological characters or vegetative morphology of *H. ovalis* (leaf width, leaf length, petiole length, internode, rhizome diameter, number of left and right leaf veins, number of leaves) as

well as the qualitative morphological characters (leaf shape, leaf surface, leaf margins, number of pairs of leaves) conform to the known diagnostic characteristics of *H. ovalis*.

The genetic characteristics of *H. ovalis* from five research sites along the coastal waters of the Minahasa Peninsula, North Sulawesi, were found to be identical, including when compared with specimens from outside the region. To advance research on the biology of *H. ovalis*, the following actions are recommended:

a) explore additional *Halophila* specimens beyond the focal taxon and compare them with members of the *Halophila* section, a group known for its taxonomic complexity;

b) develop identification keys based on locally occurring species to support the sustainable use of fisheries resources;

c) conduct further analyses using additional genetic markers, such as microsatellites, to assess population-level genetic diversity.

Conclusions. The environmental conditions supporting the growth of *Halophila ovalis* were recorded along the Minahasa Peninsula, North Sulawesi, at five research sites: Arakan/Raprap, Malalayang, Bahoi, Tanjung Merah, and Ratatotok. Data were collected at depths of 3 and 5 meters under sunny conditions. The measured environmental parameters were within the optimal range for seagrass growth, indicating that the studied habitats provide suitable conditions for the survival and development of *H. ovalis*.

Qualitative morphological analyses confirmed traits characteristic of *H. ovalis*. The leaves were observed to be oval and asymmetrical, with smooth surfaces and entire (non-serrated) margins. The occurrence of a single pair of leaves per shoot was also identified as a defining feature of the species.

Genetic analyses revealed that *H. ovalis* populations from the five research sites in the coastal waters of the Minahasa Peninsula exhibited identical genetic characteristics, including when compared with specimens from Bali (outgroup). Nucleotide sequence analysis using the MEGA-X program and BLAST comparison with the GenBank database showed a 100% similarity. Phylogenetic tree reconstruction further confirmed that *H. ovalis* specimens from the Minahasa Peninsula form a clade with those from Bali and GenBank reference sequences based on the *rbcl* gene. Additionally, analysis of polymorphic nucleotide sites using DnaSP indicated that these populations likely share a common ancestor.

Acknowledgements. We would like to thank Ministry of Research, Technology, and Higher Education for a research grant (Fundamental Research 2024) that fully funded this study. We extend our sincere appreciation to the Institute for Research & Community Service, Sam Ratulangi University for its strong support.

Conflict of interest. The authors declare that there is no conflict of interest.

References

- Alotaibi N. M., Kenyon E. J., Cook K. J., Börger L., Bull J. C., 2019 Low genotypic diversity and long-term ecological decline in a spatially structured seagrass population. *Scientific Reports* 9(1):18387.
- Ambo-Rappe R., 2022 The success of seagrass restoration using *Enhalus acoroides* seeds is correlated with substrate and hydrodynamic conditions. *Journal of Environmental Management* 310:114692.
- Berg P., Delgard M. L., Polsenaere P., McGlathery K. J., Doney S. C., Berger A. C., 2019 Dynamics of benthic metabolism, O₂, and pCO₂ in a temperate seagrass meadow. *Limnology and Oceanography* 64(6):2586-2604.
- Clarito Q. Y., Suarte N. O., Bontia E. C., Clarito I. M., 2020 Determining seagrass community structure using the Braun "Blanquet" technique in the intertidal zones of Islas de Gigantes, Philippines. *Sustinere Journal of Environment and Sustainability* 4(1):1-54.

- El Shaffai A., 2011 Field guide to seagrasses of the Red Sea. 1st edition. International Union for the Conservation of Nature, Gland, Switzerland, 56 pp.
- Fortes M., Ooi J. L. S., Tan Y. M., Prathep A., Bujang J. S., Yaakub S. M., 2018 Seagrass in Southeast Asia: a review of status and knowledge gaps, and a road map for conservation. *Botanica Marina* 61(3):269-288.
- Guiry M. D., Guiry G. M., 2023 AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available at: <http://marinespecies.org/aphia.php?p=taxdetails&id=208930>. Accessed: October, 2023.
- Hemminga M. A., Duarte C. M., 2000 Seagrass ecology. Cambridge University Press, Cambridge, United Kingdom, 298 pp.
- Hernawan U. E., 2018 Seagrass population connectivity in the South China Sea. *Marine Research Indonesia* 43(2):87-96.
- Jahnke M., Gullström M., Larsson J., Asplund M. E., Mgeleka S., Silas M. O., Hoamby A., Mahafina J., Nordlund L. M., 2019 Population genetic structure and connectivity of the seagrass *Thalassia hemprichii* in the Western Indian Ocean is influenced by predominant ocean currents. *Ecology and Evolution* 9(16):8953-8954.
- Kumar S., Stecher G., Li M., Knyaz C., Tamura K., 2018 MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* 35(6):1547-1549.
- Kuo J., 2007 New monoecious seagrass of *Halophila sulawesii* (Hydrocharitaceae) from Indonesia. *Aquatic Botany* 87(2):171-175.
- Lamit N., Tanaka Y., 2019 Species-specific distribution of intertidal seagrasses along environmental gradients in a tropical estuary (Brunei Bay, Borneo). *Regional Studies in Marine Science* 29:100671.
- Lanuru M., 2011 Bottom sediment characteristics affecting the success of seagrass (*Enhalus acoroides*) transplantation in the west coast of South Sulawesi (Indonesia). 3rd International Conference on Chemical, Biological and Environmental Engineering. International Proceedings of Chemical Biological and Environmental Engineering 20:97-102.
- Lin X., Dong J., Yang Q., Zhou W., Wang Y., Zhang Y., Ahmad M., Sun Y., Wang Y., Ling J., 2021 Identification of three seagrass species in coral reef ecosystem by using multiple genes of DNA barcoding. *Ecotoxicology* 30(5):919-928.
- Lyons D. M., Luring A. S., 2017 Evidence for the selective basis of transition-to-transversion substitution bias in two RNA viruses. *Molecular Biology and Evolution* 34(12):3205-3215
- Myers N., Mittermeier R. A., Mittermeier C. G., da Fonseca G. A., Kent J., 2000 Biodiversity hotspots for conservation priorities. *Nature* 403(6772):853-858.
- Orth R. J., Heck Jr. K. L., 2023 The dynamics of seagrass ecosystems: history, past accomplishments, and future prospects. *Estuaries and Coasts* 46(6):1653-1676.
- Rozas J., Ferrer-Mata A., Sanchez-DelBarrio J. C., Guirao-Rico S., Librado P., Ramos-Onsins S. E., Sanchez-Gracia A., 2017 DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Molecular Biology and Evolution* 34(12):3299-3302.
- Short F. T., Short C. A., Novak A. B., 2016 Seagrasses. In: *The wetland book*. Finlayson C., Milton G., Prentice R., Davidson N. (eds), Springer, Dordrecht, pp. 1-19.
- Sinclair E. A., Edgeloe J. M., Anthony J. M., Statton J., Breed M. F., Kendrick G. A., 2020 Variation in reproductive effort, genetic diversity and mating systems across *Posidonia australis* seagrass meadows in Western Australia. *AoB PLANTS* 12(4):plaa038.
- Stevanus, Pharmawati M., 2021 Biodiversity and phylogenetic analyses using DNA barcoding rbcL gene of seagrass from Sekotong, West Lombok, Indonesia. *Biodiversitas* 22(1):50-57.
- Strydom S., Murray K., Wilson S., Huntley B., Rule M., Heithaus M., Bessey C., Kendrick G. A., Burkholder D., Fraser M. W., Zdunic K., 2020 Too hot to handle: unprecedented seagrass death driven by marine heatwave in a world heritage area. *Global Change Biology* 26(6):3525-3538.

- Thangaradjou T., Bhatt J. R., 2018 Status of seagrass ecosystems in India. *Ocean and Coastal Management* 159:7-15.
- Wagey B. T., 2013 [Seagrass]. Unsrat Press, Manado, 129 pp. [in Indonesian]
- Wagey B. T., 2017 Morphometric analysis of congeneric seagrasses (*Cymodocea rotundata* and *Cymodocea serrulata*) in the coastal areas of Bunaken National Park, North Sulawesi, Indonesia. *AAFL Bioflux* 10(6):1638-1646.
- Wagey B. T., Lasabuda R., Tilaar F., Katuuk V., 2016 Spatial morphological variability of the seagrass *Halophila ovalis* (R.Br.) Hooker f in Minahasa Peninsula waters. *Asian Journal of Biodiversity* 7(1):56-78.
- Waycott M., McMahon J., Mellors J., Calladine A., Kleine D., 2004 A guide to tropical seagrasses of the Indo-West Pacific. James Cook University, Townsville, 72 pp.
- Yang F., Ding F., Chen H., He M., Zhu S., Ma X., Jiang L., Li H., 2018 DNA barcoding for the identification and authentication of animal species in traditional medicine. *Evidence-Based Complementary and Alternative Medicine* 2018:5160254.
- Yunitha A., Wardiatno Y., Yulianda F., 2014. Diameter substrat dan spesies lamun di Pantai Baho, Minahasa Utara: analisis korelasi. *Jurnal Ilmu Pertanian Indonesia* 19(3):130-135.

Received: 02 September 2025. Accepted: 12 October 2025. Published online: 16 November 2025.

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How to cite this article:

Wagey B. T., Paruntu C. P., Tilaar F. F., Kepel R. C., 2025 Study of the morphology and genetics of the seagrass *Halophila ovalis* in the Wallace Line coastal waters on the Minahasa Peninsula, Indonesia. *AAFL Bioflux* 18(6):2475-2487.