

Intraspecific Variation of *Sargassum polycystum* C. Agardh from Sundaland, Indonesia, Based on Morphological Characters

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Abstract

Sargassum polycystum C. Agardh is a keystone species in tropical waters' ecosystems and is characterized by high morphological plasticity. The Sundaland region, which consists of a wide range of oceanographic conditions, offers diverse microhabitats that may promote complex patterns of phenotypic variation. However, studies on intraspecific variation of *S. polycystum* in this region are still limited. Therefore, this study aimed to examine the morphological variation of *S. polycystum* at ten locations in Sundaland and identify key diagnostic characters that drive population differentiation. A total of 200 individuals were analyzed using 37 morphological characters. Phenotypic relationships were mapped using UPGMA cluster analysis with Gower Similarity Index and Principal Component Analysis (PCA). The results showed relatively homogeneous water chemistry parameters across sites, whereas physical characteristics varied among locations. Quantitative traits were uniform across populations ($p = 0.99$), suggesting growth stability associated with nutritional homogeneity. UPGMA analysis separated populations into two groups based on habitat typology. PCA showed that phylloid margin shape and vesicle morphology were the main diagnostic characters. Intertidal populations were characterized by serrated phylloids and densely spined primary branches, which likely function as drag-reducing mechanisms, whereas subtidal populations developed winged vesicles to enhance buoyancy. These findings suggest a segregation mechanism, where morphological variation in *S. polycystum* is driven by phenotypic plasticity to environmental physical constraints, regardless of water chemistry. A comprehensive understanding of these microhabitat-based ecological adaptation patterns provides important fundamental information for resolving macroalgal taxonomy, while simultaneously supporting sustainable coastal resource management and conservation strategies in tropical waters.

Keywords: intraspecific, phenotypic plasticity, *Sargassum polycystum*, Sundaland, morphological variation

Introduction

Sargassum polycystum C. Agardh is a brown macroalgal species widely distributed in tropical and subtropical waters, including the Sundaland region of Indonesia. It plays a crucial role in coastal ecosystems as a keystone species by providing habitat, contributing to nutrient cycling, and enhancing ecosystem stability (Kadi, 2005; Phillips *et al.*, 2005). Morphologically, *S. polycystum* is known to have a high level of polymorphism and phenotypic plasticity. These characteristics allow modifications to the thallus structure, including attachment apparatus, axis, branches, phylloids, vesicles, and receptacles, as an adaptive response to dynamic environmental pressures (Mattio and Payri, 2011).

Morphological variation within the genus *Sargassum*, particularly in *S. polycystum*, is strongly influenced by abiotic environmental factors such as habitat type, hydrodynamic conditions (currents and

wave action), and substrate structure. These factors can alter thallus shape and size without underlying genetic changes, a phenomenon referred to as phenotypic plasticity (Mattio and Payri, 2011; Kim *et al.*, 2022). Previous studies in the Indo-Pacific region have reported interpopulation variation in leaf length, branching density, vesicle dimensions, and thallus texture (Camacho *et al.*, 2015). These findings suggest that morphological variation is an adaptive strategy to maximize survival (Mattio *et al.*, 2013; González-Nieto *et al.*, 2020).

Sundaland, a major center of marine biodiversity characterized by diverse environmental conditions, offers a wide range of microhabitats, from sheltered waters to areas exposed to strong currents. This heterogeneity in physical conditions has the potential to drive complex phenotypic variation in *S. polycystum*. However, comprehensive studies of intraspecific variation of this species in Sundaland are still limited. A thorough understanding of morphological variation patterns in response to

environmental factors is important for solving complex taxonomic problems and sustainable coastal resource management. Therefore, this study aimed to examine intraspecific morphological variation of *S. polycystum* in Sundaland and identify key diagnostic characters that contribute to differentiation between populations.

Materials and Methods

Study area and sample collection

This study was conducted at ten sites in the Sundaland region of Indonesia, including the Seribu Islands (2 sites), Karimunjawa (2 sites), Lampung (2 sites), Bali (2 sites), West Kalimantan (1 site), and Bangka (1 site) (Figure 1). Site selection was carried out purposively to represent diverse habitat typologies and natural populations of *Sargassum polycystum* (González-Nieto *et al.*, 2020). A total of 200 complete individuals (N=20 per site) were randomly collected in the subtidal or intertidal zones (Noormohammadi *et al.*, 2011; González-Nieto *et al.*, 2020; Sargazi, 2021). Collected specimens were immediately cleaned of epiphytes and debris, fixed in Formaldehyde Acetic Acid (FAA) solution, and preserved as a wet herbarium collection for further observation.

Environmental parameters measurement

Habitat characterization included in situ measurements of substrate type and zonation. Habitat zonation was determined based on tidal exposure, categorizing sampling sites into the intertidal zone, which is periodically exposed during low tide, and the shallow subtidal zone, which remains continuously submerged at a depth of 0.5-1.5 m. Substrate types within these zones were assessed using in situ visual estimation, categorizing the benthic composition into distinct types such as fine sand, mud, coral rubble, and hard rocky substrates. Furthermore, chemical parameters such as pH, salinity, total nitrate, and total phosphate concentrations were analyzed in the laboratory using spectrophotometry.

Morphological characterization

A total of 37 phenotypic characters (11 quantitative, 26 qualitative) were measured using a ruler and digital caliper with an accuracy of 0.01 mm. Specimens were described using standard *Sargassum* terminology (Cheang *et al.*, 2008; Oo *et al.*, 2015; Aouissi *et al.*, 2018; Yip *et al.*, 2018; Sargazi, 2021) (Supplementary Material Table S1). Qualitative characters were converted to binary (0/1) for presence/absence traits or multistate (1–n) data

for shape and texture variations to facilitate statistical computation.

Data analysis

Statistical analyses were performed using PAST software version 4.0. The quantitative variation was tested using one-way PERMANOVA (9999 permutations) on normalized data. The phenetic relationships were mapped using UPGMA cluster analysis based on the Gower Similarity Index. The Gower Index was used because of its robustness in analyzing mixed-type datasets without requiring preliminary data transformations that could potentially introduce bias in the analysis results. The Gower similarity (S_{ij}) between individuals is calculated as the average of trait similarities:

$$S_{ij} = \frac{\sum w_k s_k}{\sum w_k}$$

For quantitative traits, the similarity is calculated as

$$s_k = 1 - \frac{|x_i - x_j|}{R_k}$$

where the absolute difference is divided by the trait's total range (R_k). This intrinsically standardizes all variables to a 0-1 scale, preventing numerical bias. Principal Component Analysis (PCA) was applied to visualize population ordination and identify key diagnostic characters that trigger intraspecific differentiation (Noormohammadi *et al.*, 2011).

Results and Discussion

Environmental conditions at the ten sampling locations showed differences between chemical and physical parameters. Based on chemical parameters (Table 1), parameters at all locations were relatively homogeneous and stable. This finding was confirmed by a one-factor PERMANOVA multivariate analysis, which showed no significant differences in chemical parameters among locations ($F = 0.00165$; $P\text{-value} = 1.0$). Nutrient concentrations were in the low range, with Total Nitrogen content ranging from 0.12 ± 0.001 mg.L⁻¹ to 0.18 ± 0.001 mg.L⁻¹, and Total Phosphate between 0.012 and 0.347 ppm. Furthermore, water acidity (pH) was classified as alkaline, ranging from 7.63–8.49, and salt content ranging from 2.68 ± 0.003 to 3.093 ± 0.001

In contrast to chemical conditions, physical parameters showed variation in habitat zoning and substrate types. Based on zoning, habitats were classified into two primary typologies: intertidal and subtidal zones (Figure 2). Habitats in the intertidal zone are exposed at the lowest tide (Menyawakan Island/KRMY, Kelagian Kecil Island/LPKK, Pahawang

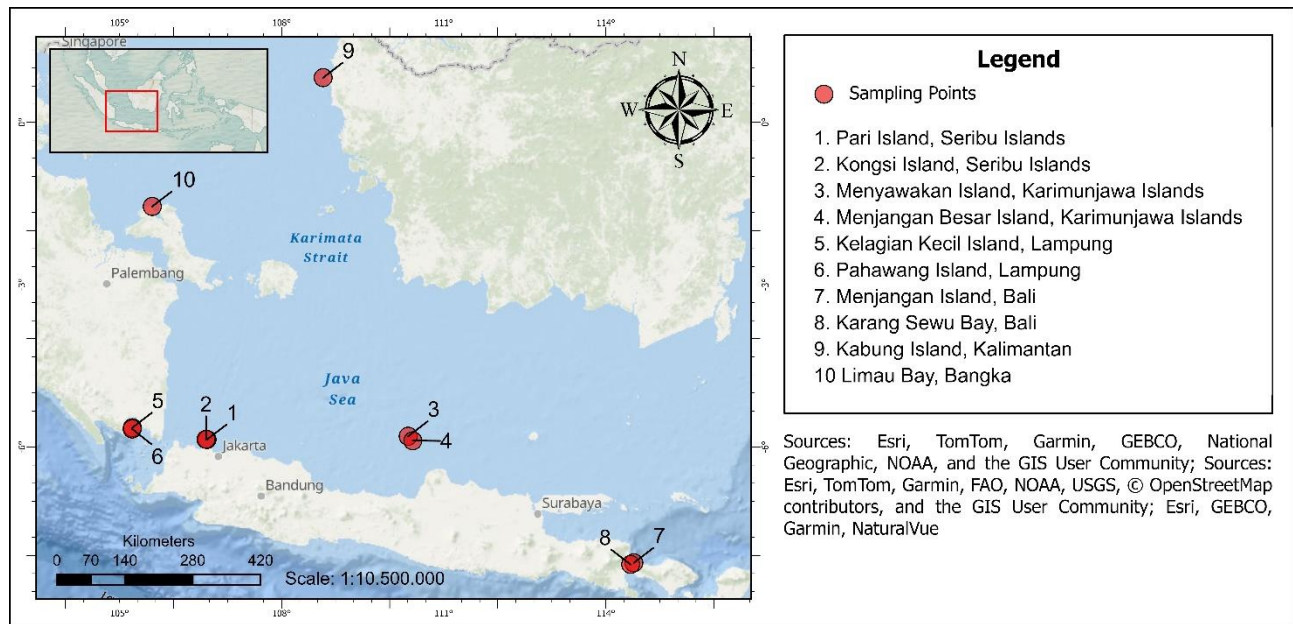


Figure 1. Map of the study area showing the distribution of ten *Sargassum polycystum* sampling sites across the Sundaland region, Indonesia. The inset map (bottom center) shows the relative position of the study area within the Indonesian archipelago. Numbered colored circles correspond to specific collection sites: (1) Pari Island and (2) Kongsii Island in Seribu Islands; (3) Menyawakan Island and (4) Menjangan Besar Island in Karimunjawa; (5) Kelagian Kecil Island and (6) Pahawang Island in Lampung; (7) Menjangan Island and (8) Karangsewu Bay in Bali; (9) Kabung Island in West Kalimantan; and (10) Limau Bay in Bangka.

Island/LPPH, Menjangan Island/BLMJ, and Limau Bay/BKTL), while the subtidal zone is always inundated with a depth of >1.0 m (Kongsii Island/KSKG, Pari Island/KSPR, and Kabung Island/KLKB). Variations in the bottom substrate were observed, ranging from the dominance of fine sand in Limau Bay/BKTL, dead coral beds on Menjangan Island/BLMJ, to a mixed substrate of sand and coral rubble on Kongsii Island/KSKG (Table 1).

The environmental differences directly affect the morphological patterns of *Sargassum polycystum* populations. Morphometric analysis showed a high level of uniformity in quantitative traits ($p= 0.99$), showing the presence of bottom-up environmental control mechanisms (Hurd *et al.*, 2014). Growth rates and biomass accumulation were assumed to be comparable across populations because Total Nitrogen and Total Phosphate concentrations were homogeneous throughout the sampling area and did not constitute limiting factors. This pattern supports the occurrence of a separation mechanism, whereby quantitative traits remain stable under constant water chemistry conditions, while qualitative traits exhibit greater plasticity in response to physical environmental stressors. This finding is in line with a report (Widyartini *et al.*, 2017), which stated that the stability of chemical parameters directly affects the uniformity of *Sargassum* vegetative growth metrics.

Supporting these observations, UPGMA cluster analysis of qualitative traits partitioned the *S. polycystum* populations into two distinct groups at a Gower distance of 0.28 (Figure 3). This grouping was statistically robust with a bootstrap value of 100% on the main branch, showing high character stability (Felsenstein, 1985). The cophenetic correlation coefficient value of $r= 0.7699$ also validated the quality of the dendrogram (Rohlf, 1998; Sokal, 1986). The 28% divergence reflects pronounced phenotypic plasticity in response to habitat pressures, while the remaining 72% intraspecific similarity preserves the taxonomic integrity of *S. polycystum*.

Population separation in the cluster analysis reflects a clear division of ecotypes based on zonation type. Cluster A generally represents the intertidal zone morphotype exposed/dry during the lowest maximum tide, including the KRMV, LPKK, LPPH, BLMJ, and BKTL populations. Conversely, cluster B represents the subtidal morphotype that is always inundated. Cluster B consists of the KSKG, KSPR, and KLKB populations. This pattern shows that the morphological divergence of *S. polycystum* in Sundaland is driven not by geographic distance (*i.e.*, isolation by distance) but by similarities in physical habitat typology. Strong evidence of this is observed in populations from Bali and Karimunjawa. Although both populations are geographically proximate, the

Karangsewu Bay (BLKS) subtidal population is distinct from the Menjangan Island (BLMJ) intertidal population due to differences in habitat zonation.

Similarly, the Menjangan Besar Island (KRMB) (subtidal) population is separated from the Menyawakan Island (KRMY) (intertidal) population.

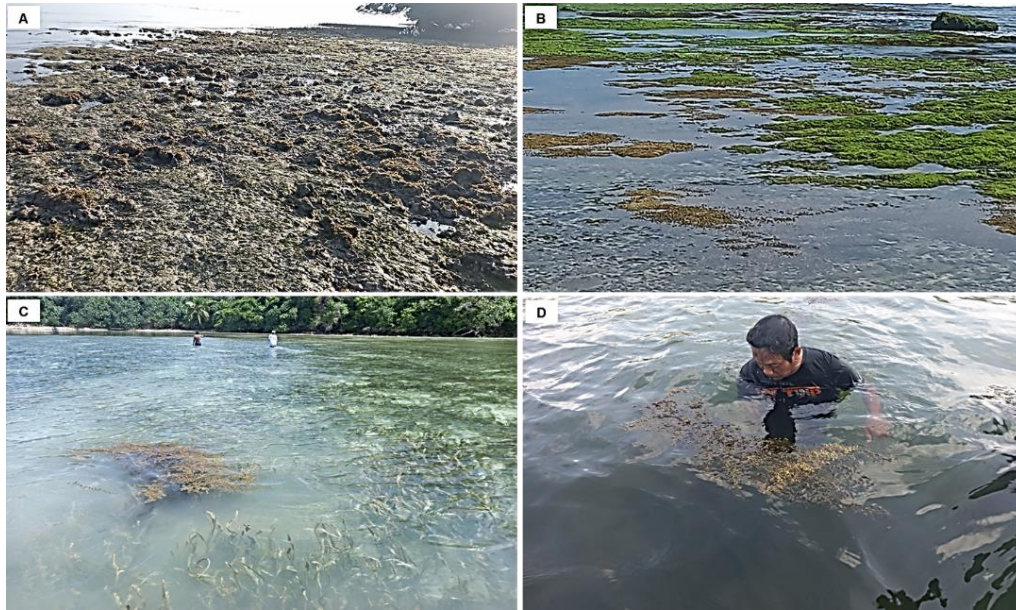


Figure 2. Habitat of *S. polycystum* in the Sundaland Region. intertidal coral reef flats affected by daily tides with substrates of sand, mud, and dead coral fragments (A and B) and subtidal locations that are always inundated with seawater with varying substrates (C and D).

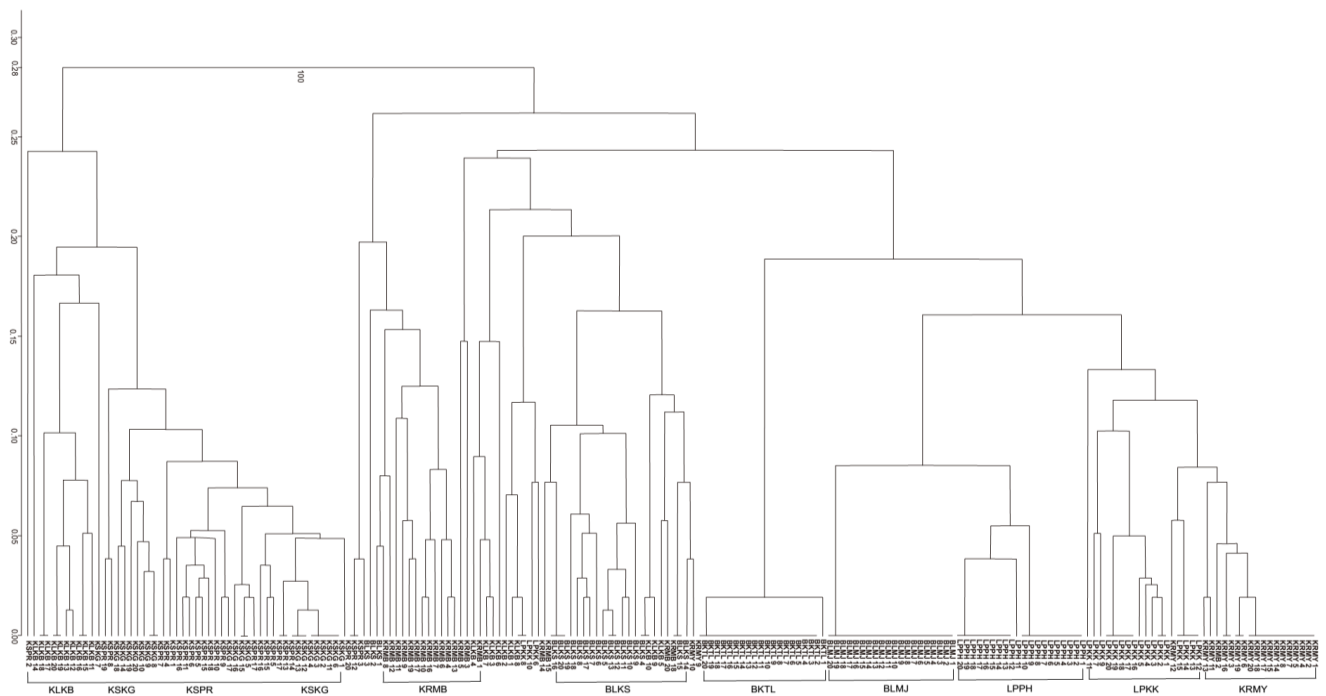


Figure 3. UPGMA dendrogram of *Sargassum polycystum* populations based on Gower's Similarity Index, showing distinct clustering by habitat typology.

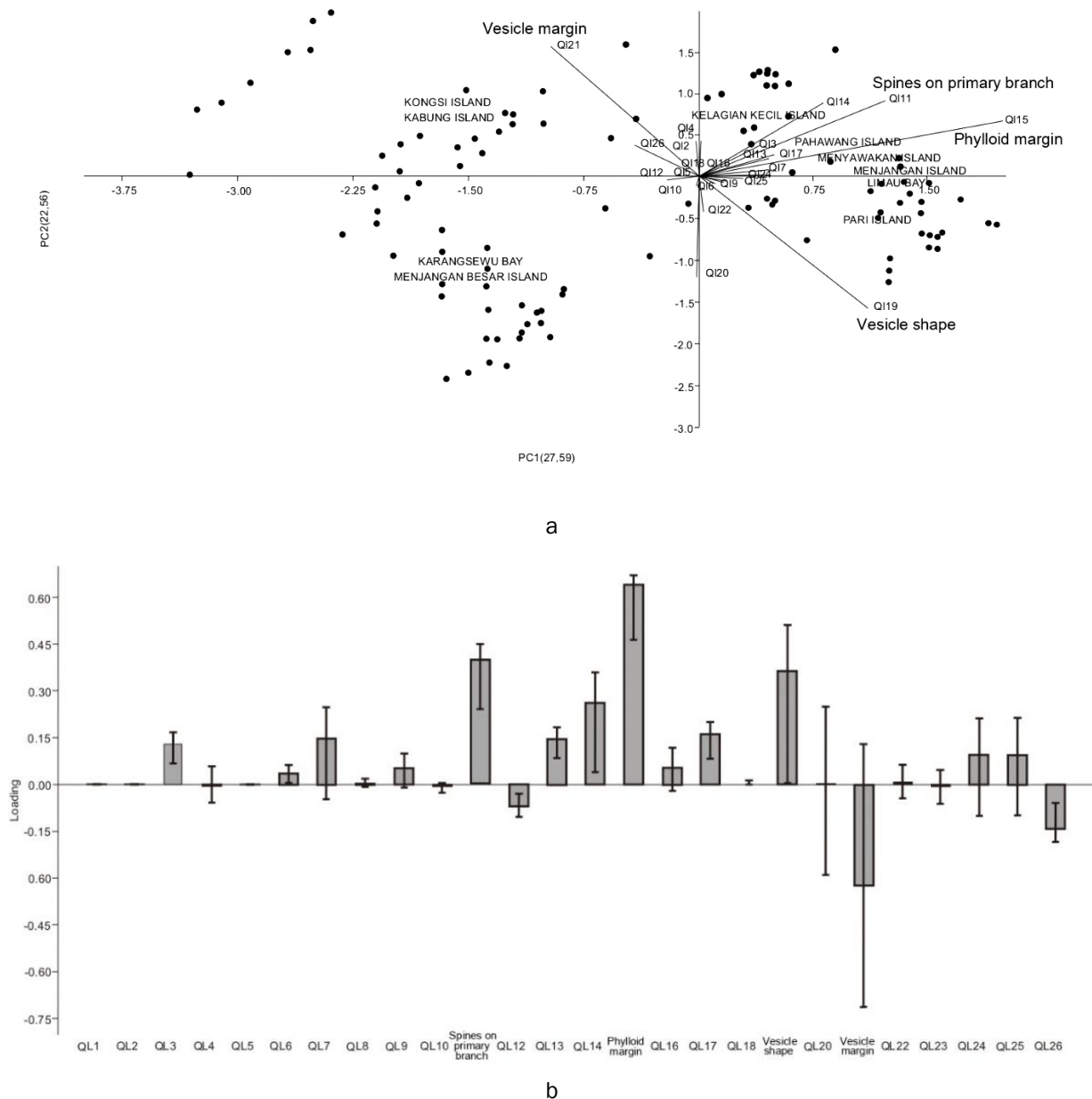


Figure 4. Scatter Plot (A) and PCA Loading Plot (B) of Qualitative Morphological Characters in *S. polycystum* from the Sundaland Region.

PCA clarified this ecological differentiation by reducing variation to two principal axes, explaining 50.15% of the total variation. The loading plots showed that phylloid margin shape was the most dominant character (loading value 0.7; eigenvalue 27.59), followed by the presence of spines on primary branches (loading value 0.4; eigenvalue 22.56) and vesicle margin widening (loading value 0.35; eigenvalue 11.74). The full eigenvalues are presented in Table S2 of the Supplementary Material. Visualization of the scatterplots and loading plots (Figures 4A and 4B) showed separation along the PC1 axis, with intertidal morphotypes characterized by

serrated phylloids and densely spined primary branches, whereas subtidal morphotypes exhibited winged vesicle margins and additional variations in vesicle shape (Figure 5). These distinct diagnostic traits represent adaptive responses to physical stress in each microhabitat.

In the intertidal zone, the dominance of serrated phylloids and dense spiny axes serves to modulate hydrodynamics by increasing micro-turbulence for nutrient exchange while reducing drag during strong currents (Stewart, 2006). These rugged structures also serve as defense against benthic

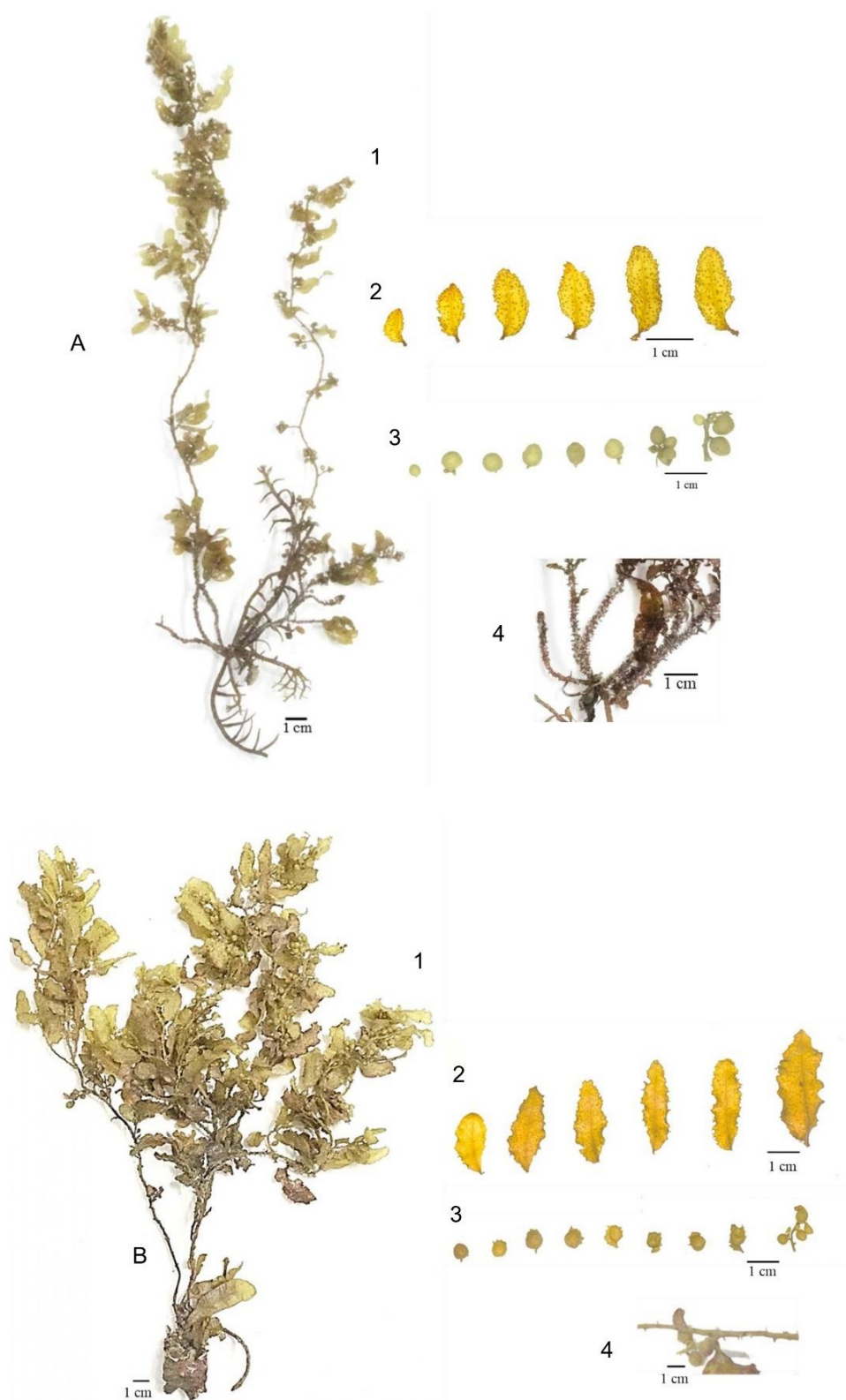


Figure 5. Morphological variation of *Sargassum polycystum* from intertidal and subtidal habitats. Intertidal morphotype (A). Characterized by: Whole thallus habit (1); Phylloids with distinctly serrated margins (2); Vesicles predominantly small, spherical, and wingless (3); Main axis and basal branches densely covered with abundant spines (heavily muriccate) (4). Subtidal morphotype (B). Characterized by: Whole thallus habit (1); Phylloids with entire to undulate margins (2); Vesicles exhibiting fusiform shapes or winged appendages (3); Main axis with sparse spines (4). All scale bars = 1 cm.

Table 1. Ecological Parameters of Sampling Locations in the Sundaland Region

Sampling Location		Zonation	Substrate Type	pH value *)	Salinity (%)*)	Total Nitrogen (%)*)	Total Phosphate (ppm)*)
Location Name	Coordinate points						
Pari Island, Seribu Islands	S 5° 51' 37.68"	Subtidal	Fine to medium sand	8.16± 0.005	3.067± 0.001	0.18± 0.001	0.012± 0.001
Kongsi Island, Seribu Islands	E 106° 37' 10.8" S 5° 51' 25.254"	Subtidal	and a mixed substrate of dead coral fragments and shell fragments	8.24± 0.009	3.093± 0.001	0.12± 0.001	0.028± 0.001
Menyawakan Island, Karimunjawa Islands	E 106° 36' 2.358" S 5° 47' 56.4"	Intertidal	Mud, sand, and coral fragments	7.63± 0.009	2.977± 0.001	0.12± 0.001	0.347 ±0.003
Menjangan Besar Island, Karimunjawa Islands	E 110° 20' 37.56"	Subtidal		8.18± 0.001	3.078± 0.001	0.12± 0.001	0.018± 0.003
Kelagian Kecil Island, Lampung	S 5° 52' 56.820" E 110° 25' 39.360"	Intertidal	Fine sand and coral/dead coral	8.21± 0.005	2.906± 0.001	0.12± 0.007	0.064± 0.001
Pahawang Island, Lampung	S 5° 39' 54.30" E 105° 14' 15.420"	Intertidal		8.29± 0.005	2.908± 0.001	0.12± 0.001	0.046± 0.001
Menjangan Island, Bali	S 8° 8' 15.480" E 114° 31' 5.680"	Intertidal	A stretch of dead coral mixed with sand and mud	8.42± 0.001	3.038± 0.003	0.12± 0.001	0.147 0.002
Karang Sewu Bay, Bali	S 8° 10' 3.180" E 114° 27' 28.440"	Subtidal	Mud, sand, and coral fragments	8.27± 0.005	3.039± 0.003	0.12± 0.004	0.017 0.001
Kabung Island, Kalimantan	N 0° 49' 18.300" E 108° 46' 4.640"	Subtidal	Fine sand, mud, rocks, and dead coral fragments	8.36± 0.019	2.814± 0.001	0.12± 0.001	0.178± 0.008
Limau Bay, Bangka	S1° 33' 21.78" E105° 36' 22.20"	Intertidal	A stretch of fine sand, mixed with stone fragments	8.49± 0.004	2.68± 0.003	0.18± 0.001	0.123± 0.001

Note: *) There were no significant differences in all environmental parameters between sampling locations.

herbivores (Kilar *et al.*, 1992). Conversely, in the subtidal zone, where light availability is reduced, an adaptive strategy involves enhancing buoyancy by modifying vesicles into a winged (fusiform) shape. This adaptation increases vesicle surface area without adding excessive biomass, thereby maintaining thallus uprightness and optimizing photosynthetic efficiency (Norton, 1991; Yip *et al.*, 2018).

The adaptation patterns of *Sargassum polycystum* in Sundaland exhibit unique

characteristics compared to subtropical populations, which are more influenced by temperature gradients or rocky substrates (Norton, 1991; Yip *et al.*, 2018). In clear but deep tropical water habitats, selection pressures favor adaptations that enhance light acquisition through buoyancy mechanisms. Notably, there is functional convergence with populations in the Pacific and the Philippines, where compact thallus formation and rough texture serve as universal defense mechanisms of *S. polycystum* across the Indo-Pacific region (Mattio *et al.*, 2013; Trono, 1998).

In general, the findings reinforce the decoupling potential of *S. polycystum* in Sundaland. Regional nutrient homogeneity maintains growth stability, while plastic modification of vegetative organs is actively used as a strategy to optimize survival in specific physical microhabitats. As a practical outcome, a diagnostic identification key was developed based on these key functional traits (Table S3, Supplementary Material). By prioritizing phylloid margin morphology, the presence of spines on primary branches, and vesicle modifications, this diagnostic identification key offers a more practical approach. A method for identifying ecological status in the field, rather than complex morphometric analyses. A description of other supporting characters is provided in Supplementary Material Table S1.

Conclusion

In conclusion, this study showed that intraspecific variation in *S. polycystum* in Sundaland is controlled by phenotypic plasticity as an adaptive response to the physical environment. Stability in chemical parameters (e.g., nutrient concentrations) across the study sites maintains uniformity in quantitative traits through bottom-up control mechanisms. In contrast, variations in physical factors such as depth and hydrodynamics drive differences in qualitative traits, resulting in the formation of two distinct ecotypes at a Gower distance of 0.28. The intertidal population is characterized by serrated phylloids and tightly packed spiny axes to reduce drag, while the subtidal population modifies vesicles into winged shapes to maximize buoyancy. These findings showed a decoupling strategy between growth metrics and thallus shape as a vital adaptation mechanism for tropical macroalgae in the face of dynamic environmental constraints.

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Supplementary Table S1. List of observed morphological characters (quantitative and qualitative) of *Sargassum polycystum* with their respective measurement methods and scoring criteria

Code	Character	Data Type	Measurement Method / Scoring States
A. Quantitative Characters			
QN1	Holdfast thickness	Ratio	Thickness from the base to the junction with the main axis (mm)
QN2	Holdfast diameter	Ratio	Maximum diameter of the holdfast (mm)
QN3	Main axis length	Ratio	Length from the holdfast base to the thallus apex (cm)
QN4	Secondary holdfast length	Ratio	Length from attachment on main axis to the tip (mm)
QN5	Phylloid length	Ratio	Length from base to apex of the leaf-like structure (mm)
QN6	Phylloid width	Ratio	Maximum width of the phylloid (mm)
QN7	Vesicle length	Ratio	Length from base to apex of the vesicle (mm)
QN8	Vesicle diameter	Ratio	Maximum diameter of the vesicle (mm)
QN9	Vesicle stalk length	Ratio	Length from vesicle base to attachment point on axis (mm)
QN10	Vesicle stalk diameter	Ratio	Diameter at the middle of the stalk (mm)
QN11	Number of vesicles	Count	Total count of vesicles per cluster/branchlet
B. QUALITATIVE CHARACTERS			
QL1	Holdfast surface	Nominal	1: Smooth, 2: Rough
QL2	Secondary holdfast presence	Binary	0: Absent, 1: Present
QL3	Secondary holdfast surface	Nominal	1: Smooth, 2: Rough
QL4	Secondary holdfast color	Nominal	1: Greenish-brown, 2: Yellowish-brown, 3: Brown
QL5	Sec. holdfast cross-section	Nominal	1: Circular, 2: Triangular, 3: Compressed/Flat
QL6	Main axis surface texture	Nominal	1: Smooth, 2: Muricate (covered with small bumps/spines)
QL7	Branching order	Ordinal	1: Primary only, 2: Up to secondary, 3: > Secondary
QL8	Primary branch shape	Nominal	1: Compressed & smooth, 2: Flattened, 3: Twisted
QL9	Secondary branch shape	Nominal	1: Slightly compressed, 2: Compressed
QL10	Primary branch surface	Nominal	1: Smooth, 2: Spiny
QL11	Spines on primary branch	Ordinal	1: Sparse, 2: Moderate, 3: Abundant/Dense
QL12	Dwarf branch presence	Binary	0: Absent, 1: Present
QL13	Dwarf branch surface	Nominal	1: Smooth, 2: Rough
QL14	Phylloid shape	Nominal	1: Lanceolate, 2: Elliptical-lanceolate, 3: Oblanceolate
QL15	Phylloid margin	Nominal	1: Undulate-serrulate, 2: Doubly serrate, 3: Serrate (Saw-toothed)
QL16	Phylloid apex	Nominal	1: Rounded, 2: Obtuse, 3: Acute
QL17	Petiole surface	Nominal	1: Smooth, 2: Spiny, 3: Verrucose (warty)
QL18	Vesicle distribution	Nominal	1: Solitary, 2: Clustered
QL19	Vesicle shape	Nominal	1: Spherical, 2: Elliptical, 3: Obovate
QL20	Vesicle apex modification	Ordinal	1: None, 2: Blunt mucronate, 3: Sharp mucronate, 4: Leafy/Winged
QL21	Vesicle margin/wing	Ordinal	1: None, 2: Blunt mucronate, 3: Sharp mucronate, 4: Winged/Auriculate
QL22	Cryptostomata on vesicle	Binary	0: Absent, 1: Present
QL23	Cryptostomata location	Nominal	1: Scattered, 2: Upper part, 3: Middle part, 4: Lower part
QL24	Vesicle stalk type	Nominal	1: Smooth, 2: Spiny/Muricate
QL25	Vesicle stalk surface	Nominal	1: Smooth, 2: Soft spines, 3: Warty
QL26	Cryptostomata on stalk	Binary	0: Absent, 1: Present

Supplementary Table S2. Statistical summary of eigenvalues and variance contribution from the Principal Component Analysis (PCA)

PC	Eigenvalue	% Variance	Cumulative %
1	1.93	27,59	27,59
2	1.58	22,56	50,15
3	0.82	11,74	61,89
4	0.61	8,73	70,62
5	0.36	5,21	75,83
6	0.33	4,73	80,56
7	0.33	4,69	85,25
8	0.26	3,74	88,99
9	0.16	2,32	91,31
10	0.14	1,98	93,29
11	0.12	1,68	94,97
12	0.09	1,39	96,36

Supplementary Table S3. Diagnostic key for identifying *Sargassum polycystum* ecotypes in Sundaland based on principal morphological traits

No	Diagnostic Characteristics	Ecotype Identification	Representative Populations
1a	The phylloid margin is serrated; the spines on the main branches are dense (abundant spines).	To number 2	
1b	Phylloid margins are even or gently wavy; Spines on the main branches are rare.	To number 3	
2	Vesicles are generally small, round, wingless; Habitat in the intertidal zone exposed at low tide.	Morfotipe Intertidal (<i>Intertidal Ecotype</i>)	KRMY, LPKK, LPPH, BLMJ,BKTL
3	Vesicles have winged or fusiform edge expansions; Habitat in the subtidal zone	Morfotipe Subtidal (<i>Subtidal Ecotype</i>)	KSPR, KSKG, KLKB KRMB, BLKS