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Phenetic Diversity and Relationships of Sea Lettuce (*Ulva* spp.) on the Southern Coast of Gunungkidul Yogyakarta Indonesia

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Abstract— The development of tourism areas in the Gunungkidul coastal zone was a potential threat that can affect the marine macroalgae diversity. In order to prevent the decline of macroalgae diversity from these anthropological threats, a comprehensive study is needed to record the population dynamics that occur. The objective of this study is revealing the species diversity and phenetic relationship of the sea lettuce (*Ulva spp.*). Phenetic analysis of sea lettuce was conducted by observing morphological, anatomical, and biochemical characters. Clustering analysis was done by the UPGMA method, whereas ordination analysis was conducted using the PCA (Principal Component Analysis) method; both of them were calculated using the MVSP 3.1 program. The result of the study discovered four species of *Ulva*, i.e., *Ulva lactuca*, *Ulva rigida*, *Ulva compressa*, and *Ulva linza*. The reconstruction of phenetic relationships revealed two main clusters of sea lettuce, exhibiting a percentage difference of 75.2. The PCA analysis exhibited eight characters that significantly influence clustering patterns, including thallus color, blade width, blade thickness, blade hole, stiff edge cells, cell surface diameter, elongated cell shape, and the presence of violaxanthin and neoxanthin.

Keywords-Gunungkidul, PCA, phenetic relationships, Ulva diversity, UPGMA

I. INTRODUCTION

Commonly referred to as seaweed, macroalgae

are non-vascular plant-like organisms (Thallophyta) made up of unclearly differentiated root, stem, and leaf structures [1]. Ecologically, macroalgae contribute significant biomass and play a major role in primary productivity within marine ecosystems [2]. The taxonomic classification of macroalgae is based on their dominant pigments: Chlorophyta (green algae), Phaeophyta (brown algae), and Rhodophyta (red algae) [3]. *Ulva* Linnaeus is a large genus within the family Ulvaceae, which includes approximately 100 species worldwide. This genus inhabits diverse aquatic ecosystems, encompassing both freshwater and marine environments [4].

Gunungkidul Regency has a diverse range of beaches, with a coastline extending approximately 65 kilometres [5]. Essentially, the southern coast of Yogyakarta is predominantly characterized by rocky formations, which serve as natural habitats for various species of macroalgae [6]. Currently, the marine macroalgae on the southern coast of Gunungkidul are vulnerable to disturbances due to the increasing beach tourism development programs, potentially leading to a decline in macroalgae diversity.

Numerous studies have been documented regarding macroalgae diversity in the Gunungkidul coastal zone,

specifically the studies performed by Chasani & Suyono [7], Sartika et al. [8], Faradilla et al. [9], and Aisyah and Widodo, which involved the inventory and identification of macroalgae species diversity in 2023. According to these studies, marine macroalgae belonging to the genus *Ulva* were predominantly represented by one species. Therefore, it is essential to conduct further research on the diversity of *Ulva* spp. on the southern coast of Gunungkidul, encompassing a broader range of coastal regions.

The macroalgae inventory is crucial for maintaining the diversity of macroalgae, especially for sea lettuce. The inventory includes analysing diversity data and among sea lettuce relationships species. The relationships among macroalgae can be interpreted using phenetic taxonomy methods. Phenetic taxonomy can be determined by comparing the differences and similarities of phenetic characters such as morphological, anatomical, and biochemical characters [10]. This has evolved with advancements taxonomy in multivariate analysis, one of which is the phenetic analysis approach using taxometrics or computerized numerical taxonomy [11].

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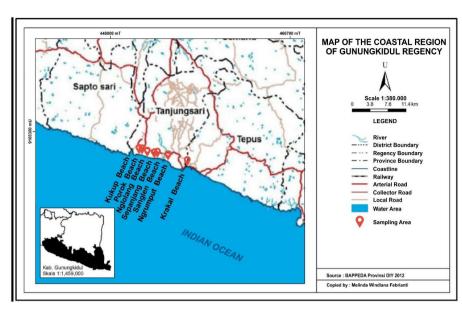


Figure 1. Sampling locations at Gunungkidul coastal region

Although information on the diversity of macroalgae from Gunungkidul Beach has been extensively studied, research examining the correlation between diversity and phenetic relationships in sea lettuce (Ulva) has been limited. The objective of this study is to reveal the species diversity and phenetic relationship of the sea lettuce (Ulva spp.) based on their morpho-anatomical and biochemical characters. This novel research on the diversity of Ulva spp. macroalgae in Gunungkidul coastal regions is essential, with a broader research scope than previous studies. Evidence of evolution in macroalgae, based on species distribution and abundance data, can provide insights into environmental changes, potential harvesting pressures, habitat loss, and the increase of non-native species [12]. Additionally, to enhance objectivity and reliability, it is important to study the characteristics of Ulva spp., which will complement the taxonomic evidence of the genus Ulva, particularly from Gunungkidul coastal area.

II. METHOD

A. Sampling location

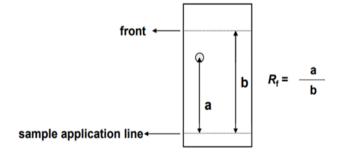
Macroalgae samples were collected from several beaches in Gunungkidul, Yogyakarta, namely Kukup Beach, Porok Beach, Ngelolang Beach, Sepanjang Beach, Krakal Beach, Sanglen Beach, and Ngrumput Beach. Location map can see in Figure 1.

B. Sample collection and morpho-anatomical observation

Macroalgae samples were collected using purposive sampling. The intertidal zone was thoroughly explored during low tide. *Ulva* spp. were collected using tweezers and then placed into ziplock bags. Each macroalgal thallus was spread out on a millimeter block for documentation. Morphological and anatomical characteristics were observed by comparing the color, size, texture, and distinguishing shapes among species. Anatomical characteristics were examined using freehand sectioning, observed with a light microscope, and visualized with Optilab 2.2.

C. Biochemical observation

Pigment detection was conducted using the Thin Layer Chromatography (TLC) method with dried samples. Two grams of each sample were dissolved in a chloroform and methanol mixture at a 1:1 ratio as the solvent. The solution was left to stand for 24 hours. Subsequently, it was filtered using Whatman No. 1 paper. Silica plates were prepared by cutting them into pieces measuring 10 cm in length and 2 cm in width, with a bottom margin of 0.5 cm and a top margin of 1 cm from the edges of the plate. The chamber was prepared by filling it with a mobile phase consisting of n-hexane and ethyl acetate in a 7:3 ratio. Extracts from each collected species were then spotted onto the silica plate (stationary phase) using a capillary pipette, precisely at the bottom margin. The plates were then placed in the chamber and allowed to develop until spots appeared on the silica plate. The Retention Factor (Rf) value was calculated using the following [13]. formula Furthermore, the Rf value was compared with the standard Rf value of seaweed by Bhatia et al. [14] (Table 1).



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TABLE 1. ESTIMATED Rf PIGMENT VALUES		
Pigment	Rf	
Chlorophyl a	0.68	
Chlorophyl b	0.54	
Chlorophyl c	0.03	
B-carotene	0.94	
Fukoxanthin	0.51	
Lutein	0.43	
Violaxanthin	0.22	
Neosanthin	0.08	

D. Data analysis

Analysis of the data begins with scoring all identified characteristics, distinguished into two types:

- 1) Two state characters: These are characteristics that can be distinguished into two states (binary). Scores assigned are typically 0 or 1.
- 2) Multi state characters: These are characteristics that can be distinguished into more than two states. Scores assigned can range from 0, 1, 2, 3, to 4.

Standardization is necessary for Multi state characters using the formula (1) [15]:

$$X'tj = \frac{(Xtj - Xt)}{SD}$$
(1)

Explanation:

- X'tj = standardized value of trait t for species j that has been standardized
- Xtj = trait t of species j that has not been standardized

Xt = mean of trait t

SD = standard deviation

The clustering process is performed using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) algorithm with Gower's coefficient of similarity (Gower general similarity). Subsequently, further analysis is conducted using Principal Component Analysis (PCA). Both stages are carried out using the MVSP 3.1 software program.

III. RESULTS AND DISCUSSION

A. Diversity of Ulva spp.

Diversity of marine macroalgae Ulva spp. in the study area includes four species: *Ulva lactuca* L., *Ulva rigida* C. Agardh., *Ulva compressa* L., and *Ulva linza* L. The distribution of these species across each beach can be seen in Table 2, whereas their morphological appearance is illustrated in Figure 2.

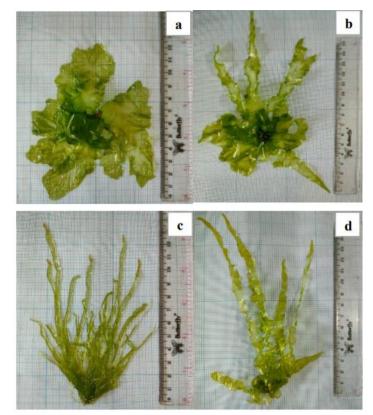


Figure 2. Collection of macroalgae species: (a) Ulva lactuca, (b) Ulva rigida, (c) Ulva compressa and (d) Ulva linza

Location	Species			
	U. lactuca	U. rigida	U. compressa	U. linza
Sepanjang beach	\checkmark	\checkmark	\checkmark	-
Krakal beach	\checkmark	\checkmark	\checkmark	-
Nglolang beach	\checkmark	\checkmark	\checkmark	\checkmark
Porok beach	\checkmark	\checkmark	\checkmark	-
Ngrumput beach	\checkmark	-	-	-
Sanglen beach	\checkmark	\checkmark	\checkmark	\checkmark
Kukup beach	\checkmark	\checkmark	\checkmark	\checkmark

 TABLE 2.

 DISTRIBUTION OF Ulva spp. MACROALGAE IN THE SOUTHERN BEACHES OF GUNUNGKIDUL

Based on the exploration result, the highest diversity of macroalgae was exhibited by Ngelolang Beach, Sanglen Beach, and Kukup Beach, where species found include *Ulva lactuca*, *Ulva rigida*, *Ulva compressa*, and *Ulva linza*. The beach with the lowest macroalgae diversity is Ngrumput Beach, where only *Ulva lactuca* was found. This could be attributed to Ngrumput Beach being a frequent tourist destination. Additionally, algae farming activities also influence species diversity in each beach area. This is in line with Handayani et al. [16], who stated that anthropogenic activity is one of the factors that caused low diversity in macroalgae. According to Sartika et al. [8], differences in the diversity and distribution of macroalgae species in a region can be influenced by both environmental

characteristics and the biological traits of the macroalgae themselves. The compatibility between these factors determines the growth potential of macroalgae. In addition, the number of species itself, the higher the number of species, and higher diversity are factors that greatly affect the high and low diversity of macroalgae [16].

B. Phenetic Relationships of Ulva spp.

Morphological, anatomical, and biochemical characteristics that have been tested are used as references to analyze the phenetic relationships among the macroalgae species found. The results of this characterization are presented in Table 3.

TABLE 3. TAXONOMIC CHARACTERISTICS OF *Ulva* spp. IN GUNUNGKIDUL BEACH

	Character	Character type	Character	Character type
Living form		Binary	Basal form	Binary

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Thallus color	Categorical	Edge fading (white)	Binary
Appearance of the thallus	Binary	Serrated edge	Categorical
Thallus shape	Categorical	Blade holes	Binary
Thallus surface texture	Binary	The blade grows narrower upwards	Binary
Growth type	Binary	Axis length	Binary
Growth direction	Binary	Axis shape	Binary
Thallus branching	Categorical	Holdfast form	Binary
Talus transparency level	Binary	Presence of rhizoids	Binary
Water content	Binary	Presence of stolons	Binary
Mucus on the surface of the thallus	Binary	Stolon form	Binary
Compressed/terete form	Binary	Cell layer	Binary
Slender shape	Binary	Cell arrangement	Categorical
Blade length	Categorical	Rigid edge cells	Binary
Blade width	Categorical	Cell surface diameter	Categorical
Blade shape	Binary	Chloroplast form	Binary
Blade edge	Categorical	Pyrenoid	Binary
Edge thickness	Binary	Cell wall	Binary
Overall blade thickness	Binary	Elongated cell shape	Binary
Segment blades	Binary	Tapered cell shape	Binary
Surface appearance	Binary	Interspace	Binary
Blade turgid	Binary	Violaksantin	Binary
Blade tip shape	Binary	Neosantin	Binary

TABLE 4. MILARITY VALUES BETWEEN OT

	U. lactuca	U. rigida	U. compressa	U. linza
U. lactuca	1.000			
U. rigida	0.784	1.000		
U. compressa	0.275	0.216	1.000	
<u>U. linza</u>	0.667	0.569	0.255	1.000

Dendrogram of phenetic relationships of *Ulva* spp. in southern coast of Gunungkidul based on UPGMA method using the maximum similarity from multiple characters of each species as presented in Table 4 and Figure 3. The characters tested are given equal or equivalent values (unweighted). The association coefficient used is Gower's similarity coefficient, which measures the agreement between characteristics among compared species. This function is calculated for each pair of species within the group.

In general, *Ulva* spp. macroalgae are divided into two main cluster groups. Cluster I consists of *Ulva lactuca*, *Ulva rigida*, and *Ulva linza*. Cluster II consists of *Ulva compressa*. *Ulva lactuca* and *Ulva rigida* exhibit

phenetic similarity with a similarity index of 78.4%. Next, Ulva linza joins with a similarity index of 61.8%, and Ulva compressa follows with a similarity index of 24.8%. According to Singh [17], hierarchical classification can be determined based on the level of their similarity threshold. The similarity threshold for species was established at 85%, for genera at 65%, and for families at 45%. According to these thresholds, three species belonging to cluster II were categorized under the same genus. Whereas U. compressa was shown in a separated cluster with a similarity index that is beneath the genera threshold. This could indicate that U. compressa is a distinct genus based on their morphoanatomical and biochemical characters.



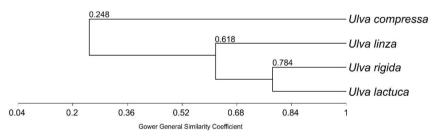


Figure 3. Dendrogram of phylogenetic relationships of Ulva spp. in Gunungkidul Beach

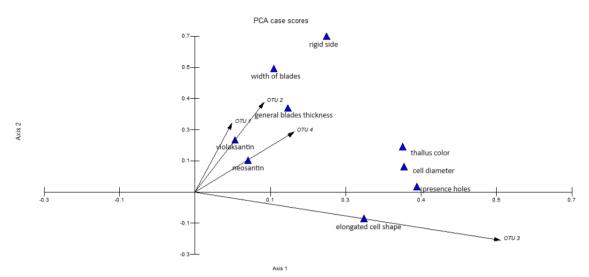


Figure 4. PCA scatter plot of Ulva spp. in Gunungkidul Beach

Species with the same taxonomic rank generally exhibit many similar characteristics, thus they are grouped into the same cluster. Based on the threshold percentage of similarity, Ulva compressa has the lowest similarity and does not cluster with other species classified under the same taxonomic rank (genus). This may be due to the subjective nature of the phenetic character testing and observations conducted in this study, which could be a contributing factor to the discrepancy between the research findings and existing theories. As follows from a study by Steinhagen et al. [18] U. compressa has two extremely different morphological characters and have been misinterpreted as distinct species. According to Leksono & Hakim [19], a weakness of phenetic approaches is their tendency to overlook the phylogenetic relationships derived from evolutionary history reconstruction. Therefore, numerical taxonomy classifies based on the number of characters without attempting to determine whether the similarities arise from common ancestry or evolutionary outcomes.

U. compressa is a species that frequently undergoes taxonomic revision due to morphological variation. This species was originally described as a highly branched

species and named *Tremella marina tenuissima*, *compressa* by Dillenius [20], and Linnaeus [21] integrated this species in his binomial system and renamed it as *Ulva compressa*. Morphological flexibility of *U. compressa* was genetically studied by Liu and Melton [22], showing seven novel gene orders of cpDNA (chloroplast genome) that were different from other *Ulva* cpDNA genes. However, based on maximum likelihood phylogenetic analysis by Xia et al. [23], complete cpDNA of *U. compressa* showed the closest sister species of *U. linza*.

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PCA analysis with biplot visualization in Figure 4 is used to map clustered species based on specific characteristics that define each group, showing the relationship between OTUs (cases) and variables (characteristics). Rifqi and Chasani [24] state that one can use PCA to identify the sources of variation and the contribution of observed characters to the overall variability. Each characteristic has different values in the clustering process, known as principal component scores. The influential principal component scores of the four macroalgae species are presented in Table 5.

TABLE 5.	

THE DE U				
STRONGLY INFLUENTIAL PRINCIPAL COMPONENT SCORES				
Character	Axis 1	Axis 2		
Thallus color	0.357	0.194		
Blade width	0.131	0.518		
Overall blade thickness	0.154	0.352		
Holes blade	0.381	0.028		
Rigid edge cells	0.219	0.653		
Cell surface diameter	0.359	0.110		
Elongated cell shape	0.293	-0.107		
Violaksantin	0.067	0.217		
Neosantin	0.088	0.135		

PCA analysis with biplot visualization yields similar results to the clustering analysis previously conducted using the UPGMA method, which also separates the four species into two main clusters or groups. Cluster I consists of *Ulva lactuca*, *Ulva rigida*, and *Ulva linza*. Cluster II consists of *Ulva compressa*. Characteristics with principal component scores ≥ 0.1 or ≥ -0.1 are considered influential in clustering. Among all characteristics, nine are identified as strongly influential in species grouping: thallus color, blade width, overall blade thickness, holes in blade, rigid edge cells, surface cell diameter, elongated cell shape, violaxanthin, and neoxanthin.

The distribution of characteristics on the scatter plot has specific values; the farther a characteristic is from the center point, the greater its influence can be indicated. Based on the visualization in Figure 4, the characteristic that has the greatest influence is rigid edge cells, which aligns with the principal component scores

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where rigid edge cells have values influencing axis 1: 0.219 and axis 2: 0.653. Rigid edge cells play a strong role in clustering Cluster I because *Ulva lactuca*, *Ulva rigida*, and *Ulva linza* possess this characteristic, while *Ulva compressa* does not.

The genus *Ulva* is a complex species with morphological varieties and shapes that are still poorly understood. In most cases, the high morphological variation and different morphotypes of *Ulva spp*. within a single species remain unknown and are frequently regarded as distinctive taxa [18]. This highlights the utmost importance of applying methods for identification that are conserved and not affected by environmental factors such as molecular DNA. This was proved by Ng and Danwei [25] that the combination of molecular analysis and morphological examination was useful for identifying and characterizing *Ulva* diversity.

IV. CONCLUSION

The diversity of marine macroalgae *Ulva* spp. found in Gunungkidul coastal area consists of four species: *Ulva lactuca*, *Ulva rigida*, *Ulva compressa*, and *Ulva linza*. Phenetic relationships analyzed showed the establishment of two main clusters. Cluster I includes *Ulva lactuca*, *Ulva rigida*, and *Ulva linza*, while Cluster II consists of *Ulva compressa*. PCA analysis exhibits characters that influencing clustering patterns are thallus color, blade width, overall blade thickness, holes in blade, rigid edge cells, surface cell diameter, elongated cell shape, violaxanthin, and neoxanthin.

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