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Phenetic Relationships of Red Seaweeds from Gunungkidul Coastal Area Using Morphological, Anatomical, and Biochemical Characters

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ABSTRACT

The coastal region of Gunungkidul is characterized by coral reefs and sandy substrates, creating an ideal habitat for the abundant growth of red seaweeds (Rhodophyceae). This favorable environment supports a high diversity of life forms, necessitating precise taxonomic approaches such as numerical taxonomy. This study aimed to evaluate species diversity and phenetic relationships among red seaweeds based on morphological, anatomical, and biochemical (pigment content) characteristics, and identify the traits that most significantly influence clustering patterns through principal component analysis (PCA). Morphological characterization focused on the thallus structure, encompassing both vegetative and generative organs, while anatomical analysis involved observations of thallus cell arrangement. Pigment content was assessed using thin-layer chromatography. Cluster and PCA analyses were conducted using MVSP version 3.1. Cluster analysis delineated three distinct groups at the 25% phenon line, with PCA indicating that branching type, blade shape, and blade margin were the most influential traits in defining these groupings. The results suggest that morphological and anatomical features play a more critical role in red seaweed clustering than biochemical characteristics.

INTRODUCTION

Rhodophyceae, commonly known as red seaweed, offers various benefits and has high economic potential. These include supporting the lives of other marine organisms by serving as sources of feed, energy, and nutrients, as well as providing a substrate for settlement. Currently, red seaweeds have been commercialized for use in functional foods, pharmaceuticals, and nutraceuticals **(Hafting *et al*., 2012)**. Their growth responds to environmental factors such as light, nutrients, and water temperature **(Awasthi & Kumar, 2015)**. Generally, red seaweeds are found abundantly at depths of up to 200 meters below sea level **(Hoek *et al.*, 1995)**.

Red seaweeds are characterized by phycobilins (phycocyanin, phycoerythrin) in their chloroplasts **(Dubinsky & Stambler, 2011)**. Their reserve carbohydrates include floridean starch and the galactoside floridoside. The cell wall of red seaweeds comprises an inner layer of cellulose and an outer layer of agar or carrageenan **(Wher & Sheath, 2003)**, along with polysulfated esters. The outer thallus structure consists of cortical cells, while the inner thallus comprises medullary cells, which are generally larger than the cortical cells. Thallus morphology varies among species and can be simple, filamentous, foliose, or cylindrical. Some species also possess blades (leaf-like structures) and stipes (stem-like structures).

Red seaweeds are widely distributed along the Gunungkidul coastal area. The region's geographical and geological conditions, including its equatorial tropical climate, support the growth of red seaweeds. Numerous studies have investigated the diversity of red seaweeds in this region, such as that by **Anisa ad Chasani (2022)**. However, most of these studies focus only on species counts or diversity indices in one or two specific locations. Therefore, a broader study is needed to examine species diversity and phenetic relationships across more of the Gunungkidul coastline.

In their natural habitat, red seaweeds exhibit high levels of morphological variation across different life phases and habitat conditions. This variability gives rise to "cryptic species"—distinct species that are taxonomically grouped as one due to minimal morphological differences **(Yang & Kim, 2023)**. Such variation presents taxonomic challenges, making accurate species identification and classification difficult. Traditional taxonomy may be insufficient to address these complexities; thus, more advanced tools, knowledge, and technologies are required.

One such approach is numerical phenetic taxonomy, which has been successfully used to reveal diversity in various marine organisms, including seaweeds **(Susanti *et al.*, 2022)**, *Vibrio* in shellfish **(Ihsan & Retnaningrum, 2020)**, clams **(Chen *et al.*, 2021)**, and corals **(Ghafari *et al.*, 2022)**.

Numerical phenetic taxonomy, or taxometric taxonomy, aims to determine phenetic relationships among organisms or taxa based on similarities across all observable characters. These characters may be morphological, anatomical, embryological, chemical, ecological, or physiological **(Hasanuddin & Fitriana, 2014)**. This method is particularly useful for identifying and classifying organisms based on phenetic relationships.

Therefore, this study aimed to investigate the species diversity and phenetic relationships of red seaweeds (Rhodophyceae) based on morphological, anatomical, and biochemical characters and to determine which characters most influence clustering patterns using Principal Component Analysis (PCA).

MATERIALS AND METHODS

1. Materials

This research was conducted from September 2019 to February 2020. Red seaweed sampling was carried out monthly on the 15th, following the lunar calendar, along six coastal locations in Gunungkidul, Special Region of Yogyakarta: Porok, Sepanjang, Drini, Wediombo, Sarangan, and Krakal (Fig. 1 & Table 1). Identification and observation of morphological and anatomical characteristics—including pigment detection—were conducted at the Plant Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada.

The tools and materials used in this study included ziplock plastic bags, an Olympus microscope, an Optilab Advance V1 camera (5 MP resolution), an oven, a TLC chamber, a 10 mL Pyrex measuring cylinder, a 500 mL Pyrex beaker, 2 mL and 1 mL Pyrex volumetric pipettes, a UV lamp, 1 mm capillary tubes, silica gel plates, Whatman No. 1 filter papers (9 cm diameter), formalin, aquadest, hexane, ethyl acetate, methanol, and chloroform.

Fig. 1. Six sampling sites used in this study (Google Earth 2020, 9km in height)

Table 1. Coordinates of sampling location

No.	Sampling location	Coordinate site
1	Porok Beach	8°08'02.9"S, 110°33'28.3"E
2	Sepanjang Beach	8°08'10.0"S, 110°33'46.1"E
3	Drini Beach	8°08'18.8"S, 110°34'39.3"E
4	Wediombo Beach	8°11'10.5"S, 110°42'35.8"E
5	Sarangan Beach	8°08'50.5"S, 110°35'48.0"E
6	Krakal Beach	8°08'42.0"S, 110°36'00.4"E

2. Sample preparation

Macroalgae samples were preserved as wet collections by rinsing them with fresh water to remove any attached dirt and substrate. The preservation solution consisted of 10% formaldehyde and seawater in a 1:9 ratio. This mixed solution was placed in transparent jars, into which the cleaned macroalgae samples were inserted and then sealed tightly.

3. Identification and morphological observation

Species identification was conducted using references from **Readdie *et al.* (2006)** and **Kasanah *et al*. (2018)**. Moreover, the dichotomous key of **Abbot and Hollenberg (1976)** as used. Morphological, anatomical, and biochemical observations were carried out based on both vegetative and reproductive characteristics, incorporating quantitative and qualitative traits (Table 2).

Several previous studies have examined the presence of red seaweeds in similar habitats along the Gunungkidul coastal area, including work by **Hadisusanto *et al*. (2013)**, **Suryandari (2017)** and **Susanti *et al.* (2022)**.

Table 2. Morpho-anatomical and biochemical characters used in this study

No.	Character	Score
1.	Appearance	1=cylindrical, 2=hollow tube, 3=foliose, 4=filamentous, 5=feathery
2.	Branching	1=sympodial, 2=simple, 3=dichotomous, 4=opposite, 5=monopodial, 6=irregular, 7=alternate, 8=sub-dichotomous
3.	Holdfast	1=irregular, 2=rhizoid, 3=discoid, 4=haptera
4.	Chlorophyll a	1=present, 2=absent
5.	Chlorophyll b	1=present, 2=absent
6.	Chlorophyll c	1=present, 2=absent
7.	β-carotene	1=present, 2=absent
8.	Fucoxanthin	1=present, 2=absent
9.	Lutein	1=present, 2=absent
10.	Violaxanthin	1=present, 2=absent
11.	Cortex cell shape	1=elongated, 2=rounded, 3=polygonal, 4=ovoid
12.	Medulla cell Shape	1=angular, 2=rounded, 3=irregular, 4=elongated
13.	Type of thallus substance	1=cylindrical cartilaginous, 2=flat cartilaginous, 3=filamentous, 4=subcartilaginous
14.	Surface of thallus	1=spiny, 2=pappilous, 3=smooth, 4=hairy
15.	Spine	1=present, 2=absent
16.	Spine position	1=branch, 2=absent
17.	Spine arranged	1=radially, 2=absent
18.	Hairy position	1=entire talus, 2=absent
19.	Tip of thallus	1=pyramidal, 2=blunt, 3=roundish, 4=incurved, 5=pointed
20.	Segmentation	1=present, 2=absent
21.	Color	1=red, 2=brown, 3=brownish green

No.	Character	Score
22.	Growth thallus	1=apical growth, 2=difused
23.	Habit	1=erect, 2= decumbent
24.	Midrib	1=present, 2=absent
25.	Blade	1=present, 2=absent
26.	Blade shape	1=irregularly, 2=pyramidal, 3=absent
27.	Blade margin	1=ruffled, 2=smooth, 3=absent
28.	Cystocarp position	1=branchlet, 2=thallus surface, 3=branch, 4=main axis
29.	Cystocarp shape	1=globose, 2=urn-shaped, 3=spatulata, 4=ovoid
30.	Cystocarp prominent or not	1=bulging, 2=embedded
31.	Texture of thallus	1=tough, 2=soft
32.	Stipe	1=present, 2=absent
33.	Carposporangia shape	1=globose, 2=ovoid
34.	Branchlet	1=hook-like, 2=oval, 3=pipih, 4=filamen, 5=konikal, 6=spinose, 7=wart-like, 8=cylindical, 9=absent
35.	Stolon	1=present, 2=absent
36.	Stolon shape	1=cylindrical, 2=absent
37.	Rhizome	1=present, 2=absent
38.	Branches	1=cylindrical, 2=flattened, 3=filamentous, 4=decumbent, 5=lanset
39.	Central axis	1=smooth, 2=with spines, 3=with papilla
40.	Node	1=present, 2=absent
41.	Node type	1=no spinae, 2=absent
42.	Central axis shape	1=rounded, 2=flattened, 3=filamentous
43.	Branches thin/thick	1=thin, 2=thick
44.	Transparent hair	1=present, 2=absent
45.	Internodes	1=present, 2=absent
46.	Thallus size	1= <5cm, 2= 5-10 cm, 3= >10cm
47.	Substrat	1=rockreef, 2=sandy
48.	Habitat	1=intertidal, 2=mid-littoral, 3=upper-littoral
49.	Tetrasporangia cleavage type	1=cruciate, 2=zonnate, 3=tetrahedral
50.	Tetrasporangia shape	1=globose, 2=elips, 3=ovoid
51.	Life form	1=clumped, 2=solitary
52.	Vesicle	1=present, 2=absent
53.	Vesicle position	1=tip of thallus, 2=absent
54.	Epiphyte/non-epiphyte	1=epiphyte, 2=non-epiphyte
55.	Branchlet arranged	1=spirally, 2=irregular, 3=pinnate, 4=absent, 5=simple
56.	Branchlet position	1=short axis, 2=distal region, 3=apical branchlet, 4=surface thalus, 5=absent

4. Anatomical observation

Anatomical characters were examined through transverse and paradermal sections using the free-hand sectioning method. The sections were observed under a microscope at 10× and 40× magnification. For visualization and image capture, the microscope was connected to an Optilab Advance V1 camera (5 MP resolution).

5. Thin layer chromatography (TLC)

Pigment analysis of red seaweed was conducted using Thin Layer Chromatography (TLC), following the method described by **Ludanes *et al.* (2009)**. Powdered red seaweed samples were mixed with 2.5mL of chloroform and 2.5mL of methanol as solvents. The mixture was then filtered using filter paper. The stationary phase consisted of a silica gel plate, while the mobile phase was a mixture of 7mL hexane and 3mL ethyl acetate.

The red seaweed extract was applied to the silica plate using a capillary tube, with the application point set 0.5cm from the tip. Once the sample spot became visible, the silica plate was placed in the TLC chamber. The mobile phase was allowed to move through the stationary phase until it reached approximately 10cm from the origin. The plate was then removed and left to dry in open air. Pigment spots were detected visually under a UV lamp, and the Rf (retention factor) value for each spot was calculated.

All visible spots were recorded and compared to the standard Rf values for seaweed pigments as reported by **Alam *et al.* (2019)** (Table 3).

Table 3. Estimated Rf pigment values

Pigment	Rf
Chlorophyl a	0.68
Chlorophyl b	0.54
B-carotene	0.94
Fucoxanthin	0.51
Lutein	0.43

6. Data analysis

Phenetic relationships between OTUs were analyzed using Multi-Variate Statistical Package (MVSP) version 3.1. Two types of characters were used: two-state characters and multi-state characters. Data standardization was done before doing any further analysis. The Jaccard coefficient was used to measure the similarities between OTUs, and the OTUs were grouped by UPGMA (Unweighted Pair Group Method with Arithmetic Mean) **(Stuessy, 2009)**. The results of the phenetic relationship analysis were portrayed as a dendrogram. In addition to morphological, anatomical, and biochemical analyses, the UPGMA approach is often used in other phenetic clustering analyses, such as the DNA fingerprint study conducted by **Sangvirotjanapa *et al*. (2019)** using RAPD markers. Principal Component Analysis (PCA) was performed in order to reveal which characters had important roles in the clustering **(Tabachnick & Fidell, 2001)**. The PCA approach can be used to represent the relationship between samples and variables, and provide information on how variables differ from each other **(Munawar & Hasanuddin, 2020)**.

RESULTS

Morpho-anatomical and Biochemical observation

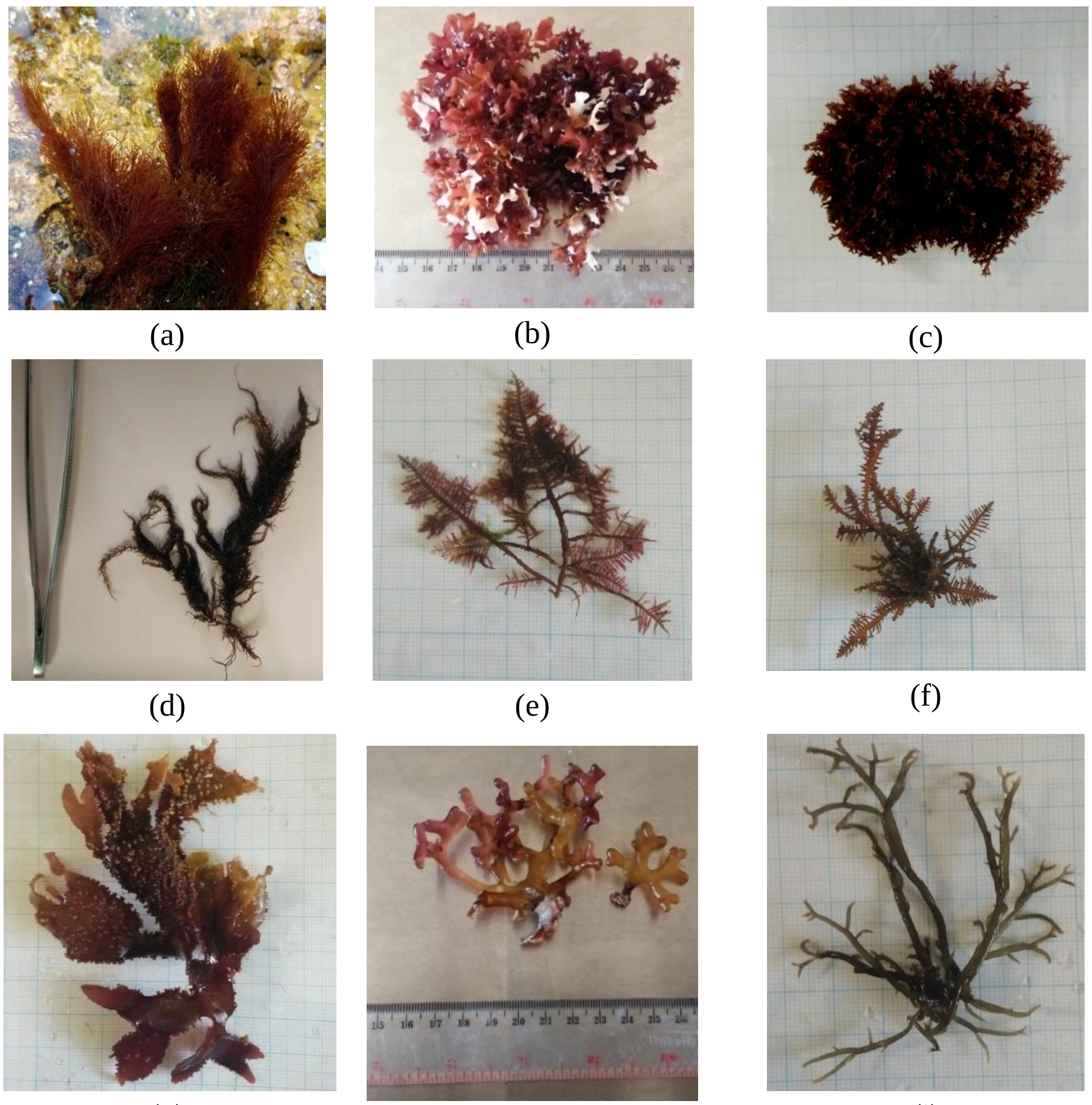
Our results showed that 15 species of red seaweeds were found on the southern coast of Gunungkidul, which were included in 4 orders and 8 families. The species diversity and morphological images of red seaweeds are presented simultaneously in Table (4) and Fig. (2). The highest number of red seaweed species was found in Porok. From these coastal areas there were 13 collected species, i.e., *C. virgatum*, *C. crispata*, *H. pannosa*, *H. esperi*, *G. corneum*, *G. acerosa*, *G. polycarpa*, *G. canaliculata*, *G. salicornia*, *G. verrucosa*, *A. spicifera*, *A. nana*, and *P. concreta*. The lowest number of species was in Wediombo, with only two species obtained (*C. virgatum* and *G. polycarpa*). The difference in the number of species obtained was probably due to differences in the tidal zoning of the sampling locations. For example, Porok has a wider expanse of coral reef at maximum low tide than Wedi Ombo. A total of 56 characters were used in this study, comprising 40 morphological characters, 9 anatomical characters, and 7 biochemical characters. The result of biochemical analysis using the TLC method is shown in Table (5).

Table 4. Red seaweeds from six sampling sites in the Gunungkidul coastal area

Order	Family	Species	Location					
			P	Sp	D	W	Sr	K
Ceramiales	Ceramiaceae	*Ceramium virgatum* Roth	√			√		
	Rhodomelaceae	*Acanthophora spicifera* (M.Vahl) Børgesen	√	√	√		√	√
		Acrocystis nana Zanardini	√		√			
		Osmundea pinnatifida (Hudson) Stackhouse			√			
		Palisada concreta (A.B.Cribb) K.W.Nam	√	√	√			
Gigartinales	Kallymeniaceae	*Callophyllis crispata* Okamura	√	√	√		√	
	Cystocloniaceae	*Hypnea pannosa* J.Agardh	√	√	√		√	√
		Hypnea esperi Bory de Saint-Vincent	√		√		√	√
	Gigartinaceae	*Gigartina polycarpa* (Kützing) Setchell & N.L.Gardner	√	√	√	√		
Gelidiales	Gelidiaceae	*Gelidium corneum* (Hudson) J.V.Lamouroux	√	√	√		√	√
	Gelidiellaceae	*Gelidiella acerosa* (Forsskål) Feldmann & Hamel	√	√	√		√	√
Gracilariles	Gracilariaceae	*Gracilaria canaliculata* Sonder	√					

	Gracilaria edulis (S.G.Gmelin) P.C.Silva		√	√			
	Gracilaria salicornia (C.Agardh) E.Y.Dawson	√	√	√		√	
	Gracilaria verrucosa (Hudson) Papenfuss	√				√	

Descriptions: **P**=Porok, **Sp**= Sepanjang, **D**=Drini, **W**=Wediombo, **Sr**=Sarangan, **K**=Krakal



(a) (b) (c) (d) (e) (f) (g) (h) (i)

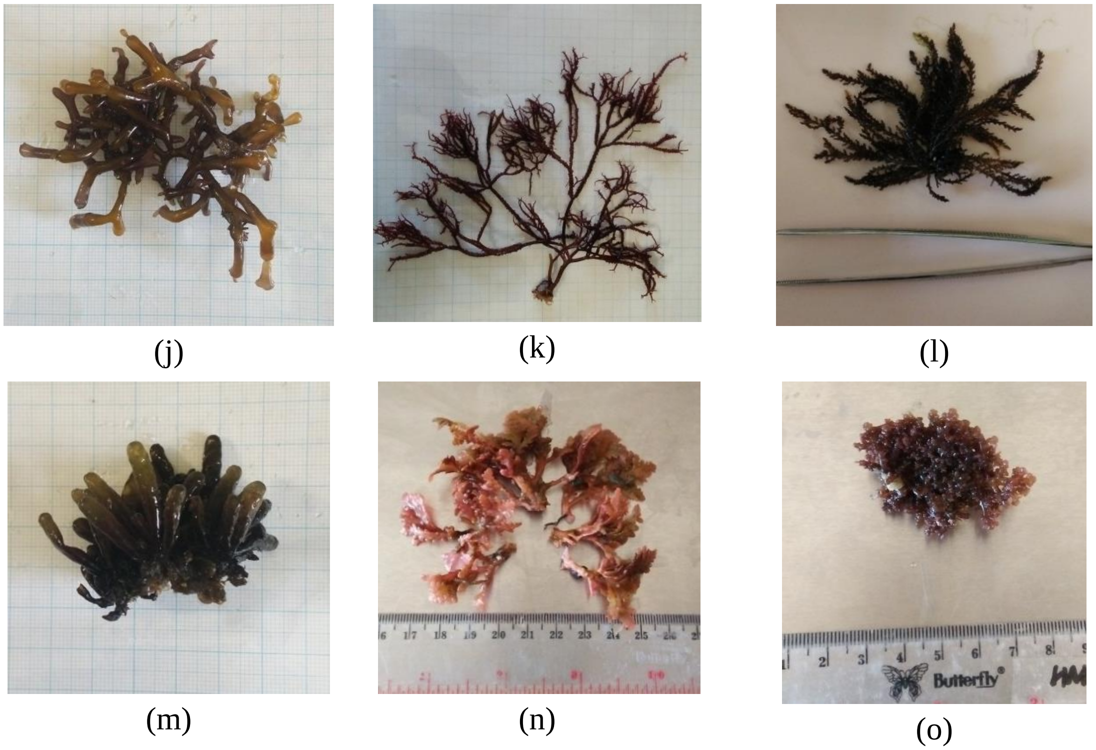


Fig. 2. Red seaweed members of Rhodophyceae in the South Coast Region of Gunungkidul, Yogyakarta. (a) *C. virgatum*, (b) *C. crispata*, (c) *H. pannosa*, (d) *H. esperi*, (e) *G. corneum*, (f) *G. acerosa*, (g) *G. polycarpa*, (h) *G. canaliculata*, (i) *G. edulis*, (j) *G. salicornia*, (k) *G. verrucosa*, (l) *A. spicifera*, (m) *A. nana*, (n) *O. pinnatifida*, (o) *P.concreta*

Fig. (3) displays the anatomical structures of the red macroalgae cells under observation. The observation results showed that species of *C. virgatum* had a thallus with filaments composed of angular periaxial cells. Each thallus branch comprises approximately 15 segments. Whereas *A. spicifera* is composed of angular medulla cells and elongated cortex tissue layers. A sac-like thallus, also known as "sea sac," in *A. nana* consists of *pseudo-parenchymatous* medulla cells that contain a mucus-like fluid and cortex cells that are smaller than the medulla cells. Observations of *O. pinnatifida* showed a thallus composed of medulla cells and cortex cells, both of which were round in shape. *P. Concreta* has a thallus composed of irregular medulla cells and elongated cortex cells. In *C. crisspata*, the medulla cell layer has a large size. A similar anatomical appearance of the thallus is shown by *H. pannosa* and *H. esperi*, which are composed of irregular parenchymatous medulla cells and round-shaped cortex cells.

G. polycarpa thallus consists of angular medulla cells with cystocarp that contains carpospores. Thallus in *G. corneum* is formed of elongated medulla cells and smaller ovoid cortex cells. *G. acerosa* has a thallus that is composed of angular-shaped cells and polygonal cortex cells. In both *G. cannaliculata* and *G. edulis*, the thallus consists of irregularly shaped cortex cells without intercellular spaces and polygonal-shaped cortex cells, respectively. Irregularly shaped and polygonal cortex cells were also found in the thallus of *G. salicornia*. In addition, inner medulla cells, outer medulla cells, and cortex cells in the outer layer were also observed in *G. verrucosa*.

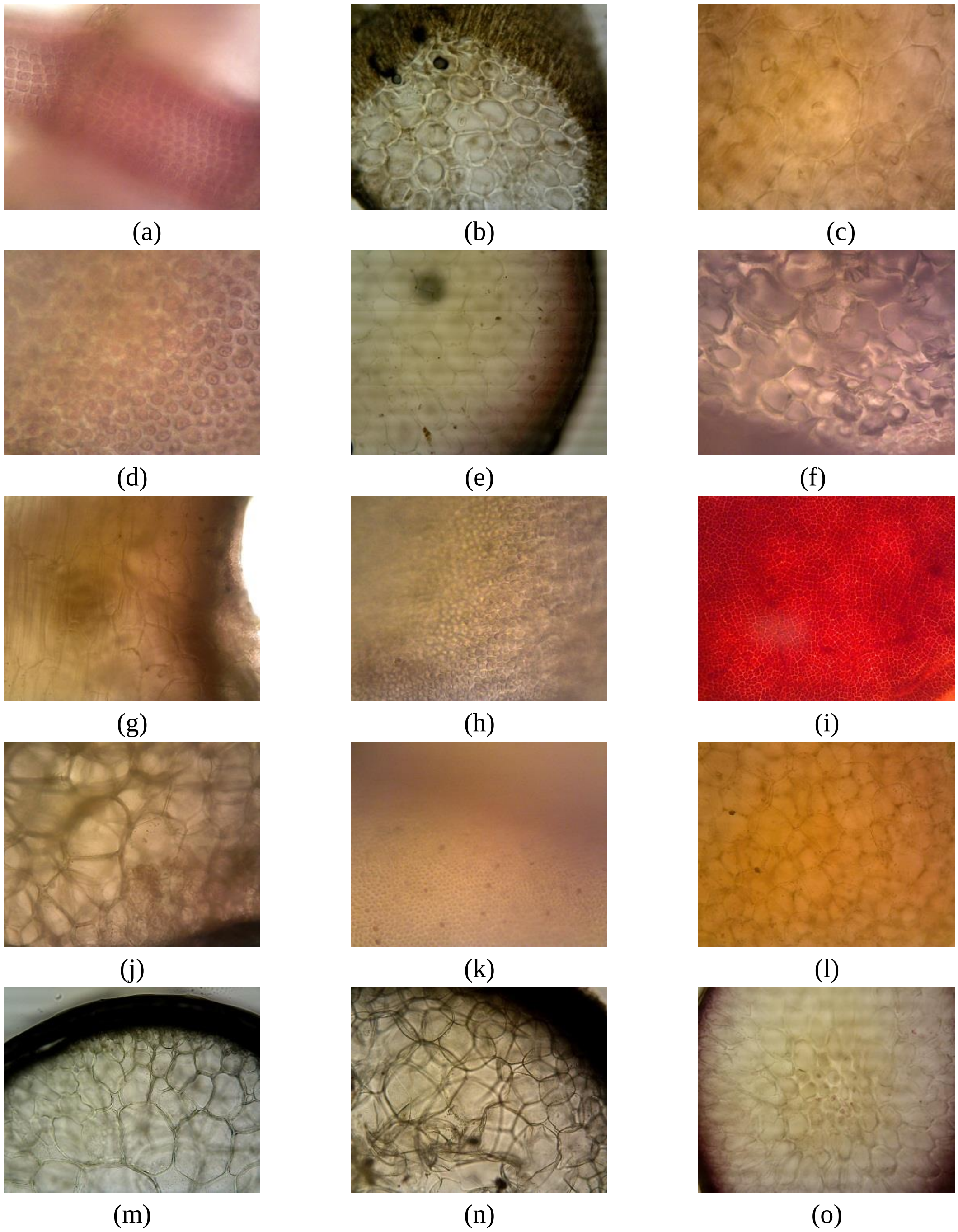


Fig. 3. Anatomical variation in Rhodophyceae cell structure from the South Coast of Gunungkidul, Yogyakarta. (a) *C. virgatum,* (b) *A. spicifera,* (c) *A. nana, (d) O. pinnatifida, (e) P. concreta, (f) C.crispata, (g) H. pannosa, (h) H. esperi, (i) G. polycarpa, (j) G. corneum, (k) G. acerosa, (l) G. cannaliculata, (m) G. edulis, (n)G. salicornia, (o) G. verrucosa*

Table 5. Pigment content of red seaweed from Gunungkidul coastal area

Species	Chlorophyll a	Chlorophyll b	β-carotene	Fucoxanthin	Lutein
A. spicifera	-	-	√	-	√
A. nana	-	-	-	-	√
C. crispata	-	-	-	-	√
C. virgatum	-	-	√	-	-
G. acerosa	-	-	√	-	-
G. corneum	√	-	-	-	-
G. polycarpa	√	-	-	-	-
G. cannaliculata	√	-	-	-	-
G. edulis	-	-	-	-	-
G. salicornia	-	√	-	-	-
G. verrucosa	-	√	-	-	-
H. esperi	-	-	√	-	√
H. pannosa	-	√	-	√	-
O. pinnatifida	-	√	-	-	√
P. concreta	-	√	√	-	√

Phenetic relationship of red seaweed

Based on the UPGMA dendrogram in Fig. (4), red seaweeds from the Gunungkidul coastal area are grouped into three main clusters, as determined by the phenon line at the 25% similarity level. The similarity index represents the degree of resemblance between species—the higher the value, the greater the similarity in their characters.

Cluster I, with a similarity index of 33.5%, consists of *Hypnea pannosa*, *Hypnea esperi*, and *Centroceras virgatum*. These species share common features such as a cylindrical thallus, discoid holdfast, presence of chlorophyll *b*, and a subcartilaginous thallus texture.

Cluster II is further divided into two sub-clusters:

- Sub-cluster IIa, with a 51.7% similarity index, includes *Gracilaria verrucosa*, *Gracilaria edulis*, *Gracilaria salicornia*, and *Gracilaria canaliculata*. These species exhibit cylindrical thalli, cartilaginous textures, smooth thallus surfaces, clumped growth forms, thick central axes, acute thallus tips, and predominantly inhabit rocky reef substrates.
- Sub-cluster IIb, with a 35.3% similarity index, comprises *Padina concreta*, *Osmundea pinnatifida*, *Gracilaria acerosa*, *Gracilaria polycarpa*, *Gracilaria corneum*, and *Callophyllis crispata*. Common traits include flat thalli, soft apical growth, and red to brownish-red pigmentation.

Cluster III, showing the highest similarity index at 60%, consists of *Acanthophora nana* and *Acanthophora spicifera*. These species share oval-shaped cortical cells, cylindrical thalli, cystocarps located on branchlets, cystocarp bulges on the thallus surface, intertidal habitats, and a clumped thallus growth form.

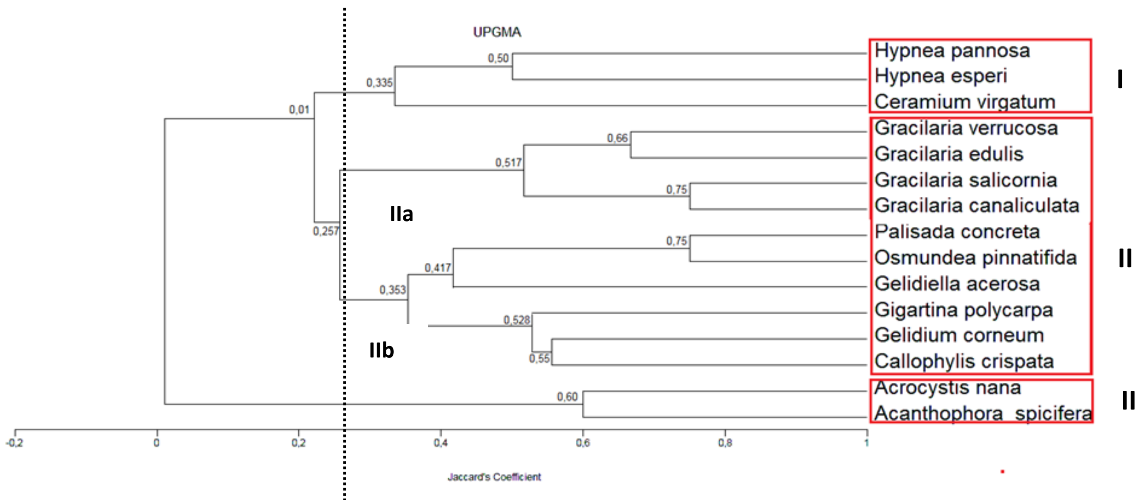


Fig. 4. UPGMA dendrogram of red seaweeds from Gunungkidul coastal area

Based on the scatter plot in Fig. (5), the influence of individual characters on the grouping pattern is illustrated by the vector lines. *Thallus size* shows the shortest vector line from the origin, indicating it has the least effect on species separation.

In the first quadrant, *Centroceras virgatum*, *Hypnea pannosa*, and *Hypnea esperi* cluster together, sharing similarities in characters such as thallus tip shape, thallus substance type, surface texture, branch shape, cystocarp position, branchlet arrangement, central axis structure, and habitat preference.

In the second quadrant, *Gracilaria acerosa* and *Callophyllis crispata* are grouped based on similarities in branchlet position and shape, overall appearance, and medullary cell structure.

In the third quadrant, *Gracilaria edulis*, *Padina concreta*, *Osmundea pinnatifida*, *Gracilaria polycarpa*, and *Gracilaria corneum* cluster together due to shared traits including larger thallus size, cortex cell shape, cystocarp morphology, type of holdfast, and branching pattern.

In the fourth quadrant, *Acanthophora spicifera* and *Acanthophora nana* are grouped by similarities in blade type, thallus growth form, and type of tetrasporangial cleavage.

Meanwhile, *Gracilaria salicornia*, *Gracilaria canaliculata*, and *Gracilaria verrucosa* are located near the center of the plot, indicating that these species exhibit relatively uniform characteristics across all measured traits.

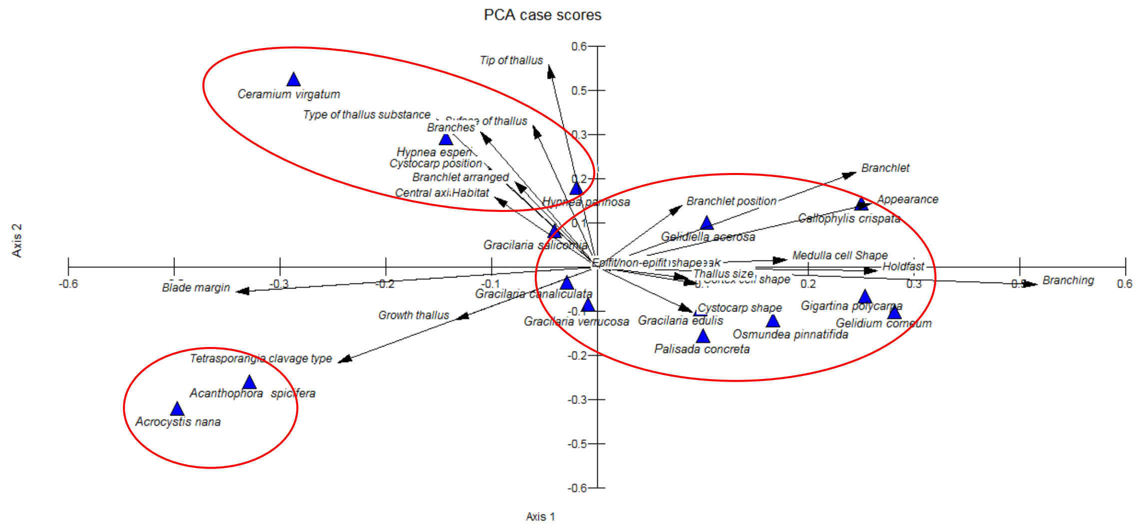


Fig. 5. Scattered plot of PCA (Principal Component Analysis)

Each character has a different main component value of the phenetic character (Table 6). Based on the 56 characters analyzed by PCA, it was found that 16 characters had component values less than zero (>0). These characters are the principal components that define the clustering pattern of red seaweeds from Gunungkidul, i.e., branching type, blade shape, and blade margin. Moreover, the first main component (1st axis) contributed to clustering at 25.076%, while the second main component (2nd axis) contributed to clustering at 22.712%, with a cumulative diversity reaching 47.788%. The higher the main component value, the higher the effect of these characters on the clustering pattern.

Table 6. Principal component value

	Axis 1	**Axis 2**
Branching type	**0,454**	-0,043
Holdfast	0,289	-0,009
Appearance	0,283	0,158
Branchlet	0,267	0,235
Medulla cell shape	0,196	0,018
Cortex cell shape	0,104	-0,041
Cystocarp shape	0,098	-0,112
Thallus size	0,093	-0,026
Branchlet position	0,087	0,153
Chlorophyll a	0	0
Chlorophyll b	0	0
Chlorophyll c	0	0
β-carotene	0	0
Fucoxanthin	0	0
Lutein	0	0
Violaxanthin	0	0

	Axis 1	**Axis 2**
Spines	0	0
Spine's position	0	0
Spines arranged	0	0
Hairy position	0	0
Segmentation	0	0
Habitus	0	0
Midrib	0	0
Blade	0	0
Cystocarp position	0	0
Thallus texture	0	0
Stipe	0	0
Carposporangia shape	0	0
Stolon	0	0
Stolon shape	0	0
Rhizome	0	0
Central axis	0	0
Nodus	0	0
Nodus type	0	0
Branches thickness	0	0
Transparent hairs	0	0
Internodus	0	0
Substrate	0	0
Tetrasporangia shape	0	0
Life form	0	0
Vesicles	0	0
Vesicles shape	0	0
Ephyphytic	0	0
Tip of thallus	-0,05	**0,499**
Thallus surface	-0,066	0,351
Branchlet arranged	-0,085	0,212
Central axis shape	-0,106	0,173
Habitat	-0,106	0,173
Cystocarp position	-0,113	0,246
Branches shape	-0,12	**0,334**
Thallus color	-0,146	-0,13
Growth thallus	-0,146	-0,13
Type of thallus substance	-0,166	**0,365**
Tetrasporangia cleavage type	-0,267	-0,235
Blade shape	**-0,373**	-0,062
Blade margin	**-0,373**	-0,062
Eigenvalues	**0,075**	**0,068**
Percentage	**25,076**	**22,712**
Cum. Percentage	**25,076**	**47,788**

DISCUSSION

The red macroalgae specimens obtained in this study exhibited variations in several thallus characters, including shape, size, color, texture, and surface type. Surface variations observed among the red macroalgae included smooth (slippery), papillose, spinous, and hairy types. Holdfast shapes also showed diversity, such as irregular, discoid, rhizoidal, and hapterous forms. Branching patterns observed included sympodial, simple, dichotomous, opposite, monopodial, irregular, and alternate. This morphological variability in macroalgae provides selective advantages, enhancing adaptation to environmental conditions. These patterns also reflect the influence of the habitat on the morphological expression of macroalgae **(Kim *et al.*, 2022)**.

Anatomical observations revealed thalli with both soft and cartilaginous textures. The thallus structure typically consisted of an outer layer of cortex cells and an inner layer of medulla cells. In some species, such as *Gracilaria verrucosa*, the medulla was further differentiated into inner and outer regions. Medulla cells play important roles in starch storage, transporting photosynthetic products to actively growing areas, and supporting cellulose production **(Goldberg, 2013)**.

The presence of chlorophyll *a* was confirmed in *Gracilaria corneum*, *Gracilaria polycarpa*, and *Gracilaria canaliculata*, while chlorophyll *b* was detected in *Gracilaria salicornia*, *Gracilaria verrucosa*, *Hypnea pannosa*, *Osmundea pinnatifida*, and *Palisada concreta*. Similar findings were reported in studies by **Lumbessy *et al.* (2021)** and **Yalçın *et al.* (2021)**, which also identified chlorophyll *b* in red seaweed species. These results contrast with earlier claims that red seaweeds contain only one type of chlorophyll—specifically chlorophyll *a* **(Freitas *et al.*, 2021)**.

Additionally, fucoxanthin was detected in several red macroalgae in this study. Previous research using HPLC methods also found fucoxanthin in red seaweed species such as *Actinotrichia* sp., *Hypnea musciformis*, *Jania rubens*, and *Polysiphonia scopulorum* **(Yalçın *et al.*, 2020; Noviendri *et al.*, 2023)**. However, this finding contrasts with **Cunningham *et al.* (2023)**, who reported that fucoxanthin is exclusive to brown algae (Phaeophyceae), where it functions as a light-harvesting pigment and antioxidant. Fucoxanthin is an accessory pigment located in the chloroplast that contributes to the characteristic color of brown algae **(Lourenço-Lopes *et al.*, 2021)**.

Lutein, identified as a major carotenoid in red macroalgae **(Mikami & Hosokawa, 2013; Balasubramaniam *et al.*, 2020)**, was observed in species such as *Acanthophora spicifera*, *Acanthophora nana*, *Callophyllis crispata*, *Hypnea esperi*, *Osmundea pinnatifida*, and *Palisada concreta*. The variability in biochemical pigment composition between samples and compared to the literature may be due to factors such as temperature and drying duration, which can lead to pigment degradation and evaporation.

Phenetic characters are essential for understanding the relationships among species. Morphological data are based on visible traits at the time of observation and are supported by evolutionary and adaptive hypotheses **(Knapp, 2001)**. In this study, morpho-anatomical and biochemical characterizations were used to construct an artificial key for validating species identity and classification.

In general, red seaweed species from the same genus clustered together in the UPGMA dendrogram. According to **Singh (1999)**, a minimum similarity index of 65% is typical for grouping species within the same genus. An exception was noted with *Palisada concreta* and *Osmundea pinnatifida*, which clustered with a similarity index of 75%, despite belonging to different genera. This is likely because both species belong to the family Rhodomelaceae, characterized by thalli composed of an axial filament surrounded by pericentral cells and sympodial branching patterns **(Womersley, 2003)**.

PCA (Principal Component Analysis) further supported these groupings, indicating that the type or pattern of branching may have significantly influenced the similarity between *P. concreta* and *O. pinnatifida*. PCA allows classification of samples based on character correlations, highlighting the main traits that drive clustering **(Hetharie *et al.*, 2018)**. **Wongsa *et al.* (2018)** noted that PCA is a multivariate method used to analyze metabolite similarities and differences, with loading scores indicating the strength and direction of variable contributions to the principal components.

In this study, red seaweed species were grouped according to the similarity of character vectors. Traits located in the same quadrant were found to significantly influence clustering patterns. Conversely, species found in different quadrants at approximately 90° angles are considered distantly related **(Setiawati *et al.*, 2013)**.

Our results indicate that morphological and anatomical characters had the highest principal component values, meaning these traits had a greater influence on red seaweed clustering patterns than biochemical characters. Phenetic variation in macroalgae can be shaped by various physicochemical factors. For example, **Karsten (2020)** reported that varying salinity conditions can elicit different tolerances even within the same macroalgae species. Other influential factors include temperature, substrate type, nutrient availability, wave exposure, and sedimentation, all of which can drive macroalgal distribution **(Voerman *et al.*, 2019)**. Furthermore, understanding the correlations among characters in macroalgae is critical for selecting and developing genotypes with desirable traits **(Mofokeng *et al.*, 2017)**.

CONCLUSION

Based on our study, it can be concluded that there are 15 species of red seaweeds in the Gunungkidul coastal area, belonging to 8 families and 4 orders. Analysis of phenetic relationships resulted in three clusters of red seaweeds at a 25% phenon line. The principal components defining the clustering patterns are morphological and anatomical characteristics, such as branching type, blade shape, and blade margin. These results indicate that morphological and anatomical traits have a greater influence than biochemical characteristics on the clustering of red seaweeds.

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