Advances in Biological Sciences Research, volume 22
7th International Conference on Biological Science (ICBS 2021)

Numerical Taxonomy of Marine Macroalgae Gracilariaceae from Southern Coast of Gunungkidul Based on Morpho-Anatomical and Phytochemical Characters

Adinda Nur Anisa[1] Abdul Razaq Chasani[1,*]

[1]*Faculty of Biology, Universitas Gadjah Mada, Jl. Teknika Selatan, Sekip Utara, Yogyakarta 55281, Indonesia*
[*]*Corresponding author. Email: ar.chasani@ugm.ac.id*

ABSTRACT
Gracilariaceae is a macroalgal family of Rhodophyta which can be found abundantly in tropical waters, including in Southern Coasts of Gunungkidul D.I.Yogyakarta, Indonesia. Coral and sand dominate the substrate of Southern Coasts of Gunungkidul, so it is an ideal habitat for Gracilariaceae. Along with the rapid development of coastal tourism areas, the existence of Gracilariaceae in this area needs to be studied intensively. Hence, this study aimed to determine species diversity and phenetic relationships of Gracilariaceae from Southern Coasts of Gunungkidul, including defining principal characters that influence clustering patterns. Field sampling was done by purposive method, followed by identification, characterization of morpho-anatomical, and phytochemical characters in Plant Systematic Laboratory Faculty of Biology UGM. Numerical Taxonomy for phenetic relationships analysis was computed using Clustering and Principal Component Analysis of Ordination methods by MVSP 3.1. The results of the Clustering analysis were presented as a phenogram while the PCA ordinance as a scattered plot. This study succeeded in finding five species of Gracilariaceae, i.e *Gracilaria fergusonii, Gracilaria edulis, Gracilaria salicornia, Glacilaria canaliculata,* and *Glacilaria verrucosa*. Phenetic relationships of Gracilariaceae were grouped into two main clusters and two sub-clusters. Clustering patterns were determined by the shape of holdfast, tip of thallus, surface texture of thallus, presence of cortex cells, fucoxanthin, neoxanthin, carotenoid, beta-carotenoid, cystocarp, pericarp, color, location, size, and characters of cystocarp, texture of thallus, and substrate characters.

Keywords: *Gracilariaceae, Numerical Taxonomy, Phenetic, PCA, Species diversity.*

1. INTRODUCTION

Macroalgae are large algae, mostly living in marine waters, growing attached to substrates such as mud, sand, rock, hard objects, inanimate objects, or attached to other plants. The plant body of algae called thallus, is a simple vegetative body containing chlorophyll pigment for photosynthesis [1]. Most macroalgae structures can be recognized as holdfast (roots-like part), stipe (stem-like part), and blades (leave-like part) [2]. Gracilariaceae is macroalgal family belong to Rhodophyta (red algae). There are more than 100 species belonging to the Gracilariaceae, generally living in tropical waters ranging from intertidal to subtidal areas [3]. In Indonesia, especially on Southern Coasts, Gunungkidul D.I.Yogyakarta, there are at least four species of macroalgae belonging to the Gracilariaceae, i.e., *Gracilaria canaliculata* Sonder, *G. edulis* (S.H.Gmelin) P.C.Silva, *G. salicornia* (C. Agardh) E.Y.Dawson, and *G. verrucosa* (Hudson) Papenfuss [4]. Gracilariaceae has a varied thallus color from red to purplish green and olive-yellow, varies shaped from cylindrical to flat, branching with irregular subdichotomous, dichotomous, alternate, pinnate, and other branching forms. The surface of the thallus is smooth or nodule, a tip of the thallus is tapered, the length of the thallus varies (3.4-8

cm), in some species, it reaches >60 cm and has a disc-shaped and hard holdfast [5]. Gracilariaceae has simple anatomy, thallus arranged by epidermal cells, cortical cells, and medulla cells. Similar to other Rhodophyta, Gracilariaceae contain photosynthetic pigments such as chlorophyll *a*, chlorophyll *b*, and phycobilins (phycocyanins, phycoerythrins, carotenoids) [2].

The southern coast of Gunungkidul is the most visited destination for tourists in Yogyakarta that has beautiful scenery and abundant natural resources, like the diversity of macroalgae species. Southern Coast of Gunungkidul has a substrate dominated by dead coral and sand that is an ideal habitat for macroalgae, including Gracilariaceae. As reported by [5], there are four species of macroalgae belong to Gracilariaceae, *G. canaliculata* Sonder, *G. edulis* (S.G.Gmelin) P.C.Silva, *G.salicornia* (C.Agardh) E.Y.Dawson, and *G. verrucosa* (Hudson) Papenfuss. Even if it provides economic benefits, tourism can cause damage to macroalgae habitats because visitors are littering and do not know the benefits of macroalgae. They provide benefits for ecosystem as primary producers, food sources for other biota, habitats, shelters, and carbon sinks to reduce global warming. Also, they are beneficial for humans as research materials, learning materials, industrial raw materials, and food ingredients [6]. Therefore, it is necessary to support all environmental conservation, such as updating data regularly and studying macroalgae diversity using various approaches, including numerical taxonomy. Currently, research on species diversity of macroalgae Gracilariaceae already exists, but the study of macroalgae diversity using the numerical taxonomy method has not been widely carried out. Hence, this study aimed to determine species diversity and phenetic relationships of Gracilariaceae from the Southern Coast of Gunungkidul, including defining principal characters that influence clustering patterns using the numerical taxonomy method.

2. METHODOLOGY

2.1 Sampling Macroalgae

Sample of Gracilariaceae was collected from three beaches, i.e., Sepanjang, Kukup, and Porok in Southern Coast of Gunungkidul D.I.Yogyakarta, Indonesia. The three research sites were chosen because they have sand and coral substrate and a dry surface at maximum low tide. Sampling procedures were done according to Chasani, A.R, and E.A. Suyono [7].

Figure 1. Sampling sites in Southern Coasts of Gunungkidul, D.I.Yogyakarta, Indonesia: a. Kukup, b. Porok, and c. Sepanjang.

2.2 Sampling Collection and Identification

Samples were cleaned using fresh water and preserved as wet herbarium for morphological observations by formulation of seawater and alcohol 70% (6:4). Identification was made by standard references [2, 8, 9].

2.3 Morphological and Anatomical Observation

Morphological characters of macroalgae were observed and documented with a digital camera equipped with a scale. Anatomical characters observations were done by the freehand section. The thallus sliced longitudinally and transversely, then placed on a glass slide, dripped water, and covered with cover glass. The anatomical observation was implemented using a light microscope connected to optilab and displayed to a laptop.

2.4 Pigment Analysis

Pigment analysis using TLC (Thin Layer Chromatography) method. Macroalgae samples were

dried in the oven and mashed with blender or mortar and pestle. Powdered samples then weighed as much 2 g. Extraction by maceration method using acetone and methanol 70% (7:3). The TLC method was done according to Ningrum, A.M and Chasani, A.R [10]. After forming a pigment separation pattern, samples were visualized using UV light with wavelengths (λ) 254 and 366 nm, and the distance of patterns was measured. The Retention factor (Rf) value was calculated using Equation (1) [11].

$$Rf = \frac{\text{Distance moved by the compound}}{\text{Distance moved by the solvent}} \quad (1)$$

2.5 Data Analysis

The phenetic relationships of macroalgae Gracilariaceae were analyzed using the numerical taxonomy method based on overall similarity characters, consisting of two-state and multistate characters. The numerical taxonomy method was implemented in 7 procedures, i.e. characterization, scoring including standardization and re-scoring, calculation the similarity values based on Gower (General Similarity Coefficient), UPGMA clustering analysis and ordinance analysis by Principal Component Analysis. The clustering and PCA analysis were computed by MVSP 3.1.

3. RESULTS AND DISCUSSION

This study found five species of Gracilariaceae, i.e. *Gracilaria fergusonii*, *G. edulis*, *G. canaliculata*, *G. salicornia*, *G. verrucosa*. These species belong to Class Florideophyceae, Ordo Gracilariales. Family Gracilariaceae, and Genus *Gracilaria*. All macroalgae Gracilariaceae were found attached to coral and sand because the substrate of the Southern Coast of Gunungkidul is dominated by coral and sand, an ideal habitat for Gracilariaceae.

Numerical taxonomy is an analysis of various types of taxonomic data using mathematical or computerized methods based on characters similarity [12]. The morphological, physiological, phytochemical, anatomical, embryological, palinological, and ultrastructure [13]. The first step is characterization and scoring. The characterization obtained 50 characters including, morphological, anatomical, and phytochemical characters (Table1). The results show that the highest similarity values are *G. fergusonii* and *G. verrucosa* 0.643 (Table 2) it means two species are closely related.

Clustering analysis using the UPGMA among five species of Gracilariaceae obtained two main clusters (Figure 2). Cluster I consisted of *G. canaliculata* (OTU4), *G. edulis* (OTU2), *G. verrucosa* (OTU5), and *G. fergusonii* (OTU1), while cluster II consisted of *G. salicornia* (OTU3). Cluster I divided into two sub-clusters i.e., Ia (*G. fergusonii* and *G. verrucosa*) and Ib (*G. edulis* and *G. canaliculata*).

PCA aims to determine the most influential characters in grouping species [14]. Ordinance analysis with PCA using Euclideon biplot produces a scatter plot. The PCA scatter plot (Figure 4) displayed the most

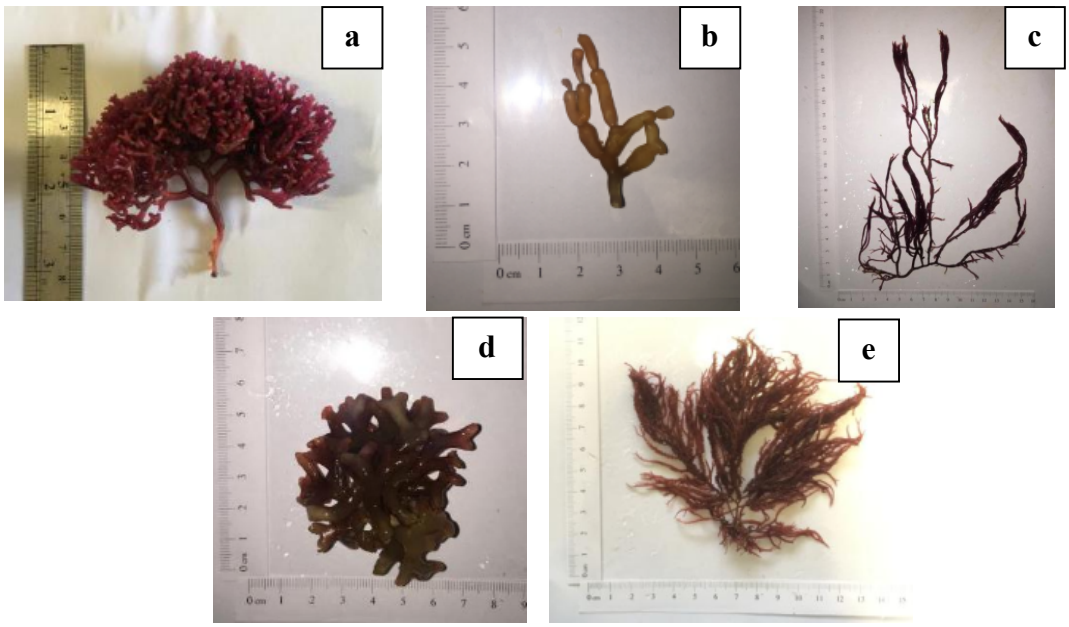

Figure 2. Species of Gracilariaceae from Southern Coast of Gunungkidul : *G. fergusonii* (a); *G. salicornia* (b); *G. edulis* (c); *G. canaliculata* (d); *G. verrucosa* (e).

Table 1. Taxonomic characters of macroalgae Gracilariaceae

No.	Characters	Character Types
1	Appearance	Categorical
2	Thallus branching types	Continuous
3	Growth direction	Continuous
4	Thallus types	Categorical
5	Thallus growth	Continuous
6	Colors of thallus	Categorical
7	Surface texture of thallus	Categorical
8	*Spine* (duri)	Binary
9	*Spine position*	Categorical
10	*Spine arranged*	Categorical
11	Tip of thallus	Categorical
12	Constriction at tip of the talus	Binary
13	Segmentation	Binary
14	*Node*	Binary
15	*Internodes*	Binary
16	Central axis	Binary
17	Branches	Binary
18	Branchlet	Binary
19	Branchlet arranged	Categorical
20	Substrate	Categorical
21	Life cylce	Categorical
22	Habitat	Categorical
23	Shape of holdfast	Categorical
24	Cystocarp	Binary
25	Colors of cystcarp	Categorical
26	Position of cystocarp	Categorical
27	Character of cystocarp	Categorical
28	Height of thallus	Continuous
29	Diameter of thallus	Continuous
30	Size of cystocarp	Continuous
31	Epidermis cells	Binary
32	Medulla cells	Binary
33	Shaped of medulla cells	Categorical
34	Cortex cells	Binary
35	Shape of cortex cells	Categorical
36	Pericarp	Binary
37	Epidermis cells in cystocarp	Binary
38	Medulla cells in cystocarp	Binary
39	Shaped of medulla cells in cystocarp	Categorical
40	Shape of cystocarp	Categorical
41	Carpospore	Binary
42	Shape of carpospore	Categorical
43	*nutritive filament/Gonimoblast*	Binary
44	Chlorophyll *a*	Binary
45	Carotenoid	Binary
46	beta-carotenoid	Binary
47	Lutein	Binary
48	Fucoxanthin	Binary
49	Neoxanthin	Binary
50	Violaxanthin	Binary

Table 2. Results of similarity values between OTUs

	OTU1	OTU2	OTU3	OTU4	OTU5
OTU1	1				
OTU2	0.563	1			
OTU3	0.527	0.383	1		
OTU4	0.573	0.597	0.56	1	
OTU5	0.643	0.5	0.363	0.417	1

Table 3. The value of each main component on the characters of marine macroalgae Gracilariaceae

Characters	Axis 1	Axis 2
Surface texture of thallus	0.12	**0.221**
Central axis	**0.216**	-0.032
Branches	**0.214**	-0.034
Substrate	0.13	**-0.208**
Cystocarp	0.047	**0.238**
Colors of cystcarp	0.046	**0.238**
Position of cystocarp	0.025	**0.249**
Character of cystocarp	0.119	**0.219**
Cortex cells	**0.215**	-0.032
Pericarp	0.049	**0.238**
Fucoxanthin	**-0.22**	0.033
Size of cystocarp	-0.062	**0.231**

Note : The value of ≥0.2 or ≥-0.2 indicate that the characters has most influential in clustering.

influential characters for grouping OTUs, while the value of the most influential characters showed in Table 3. The value of ≥0.2 or ≥-0.2 indicates that the characters are the most influential in clustering. Percentage axis 1 (35.72%) is higher than axis 2 (31.24%), showed that characters on axis 1 have more influence than axis 2.

Based on Figure 3, cluster I divided into two sub-cluster. Sub-cluster Ia and Ib have a similarity value of 0,513. The similarity value of Sub-cluster Ia (*G. verrucosa* and *G. fergusonii*) was higher than other clusters, which means that the two species have more similar characters. Both *G. verrucosa* and *G. fergusonii* have red to purplish and grow erect with a size more than 10 cm thallus, holdfast attached to coral and sand, and cystocarp. As reported by [2], *G. fergusonii* has cylindrical thallus, dichotomous, and the holdfast

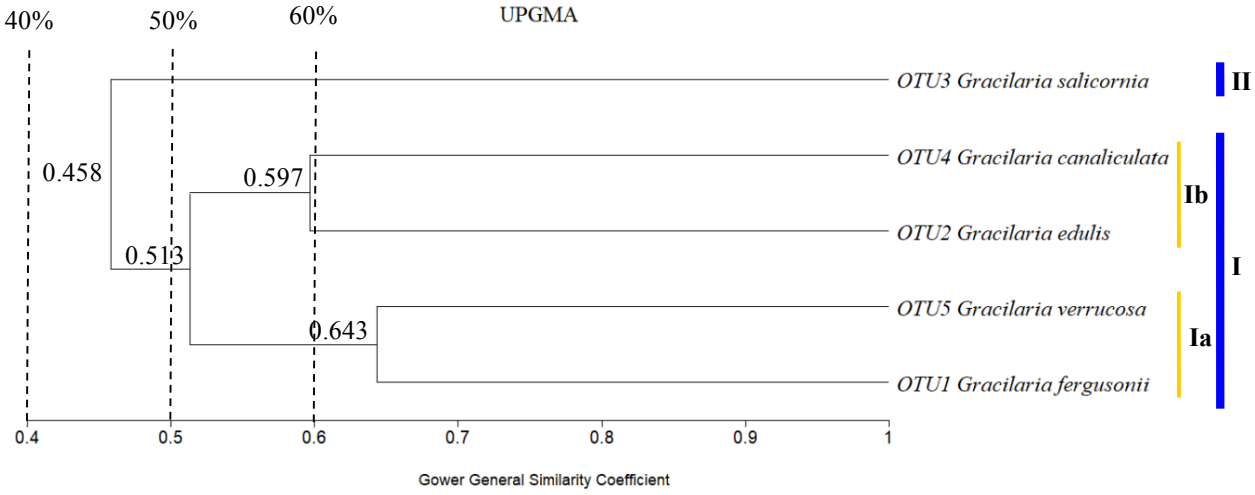

Figure 3. The UPGMA phenogram of marine macroalgae Gracilariaceae. Note: - - - = *phenon line*

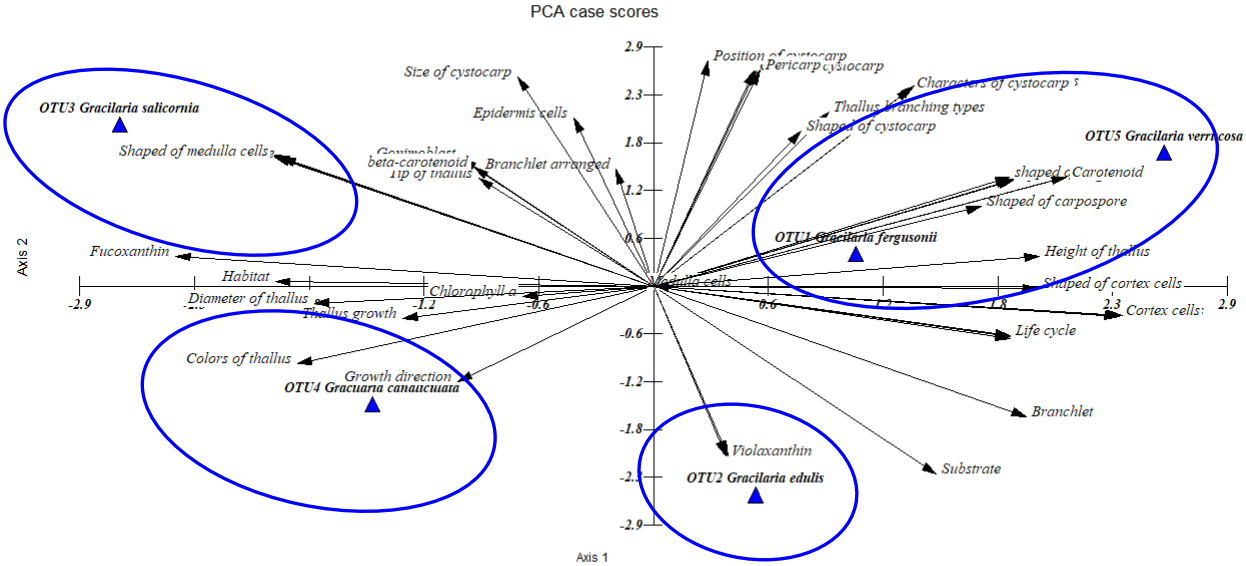

Figure 4. Scatter plot of the PCA of Gracilariaceae based on morphology, anatomy, and phytochemical characters.

attached to rocks or coral. *G. verrucosa* has thallus color red to greenish, thallus growth erect, presence cystocarp and spine, branching types alternate to irregular, and size of thallus can reach 50 cm [15,16]. Subcluster Ib (*G. canaliculata* and *G. edulis*) have smooth, cylindrical, cartilaginous, dichotomous thallus, and discoid-shaped holdfast. According to [9], *G. canaliculata* has a cylindrical to flat thallus, dichotomous, cartilaginous, and discoid-shaped holdfast. Cluster II consist of only *Gracilaria salicornia.* This species has a cylindrical thallus and is cartilaginous like others, but has segmented thallus and polystichous branching type [2].

Based on Figure 4, the characters that most influenced the clustering patterns of Cluster I were shaped of holdfast, presence, and size of cystocarp, and presence of carotenoid. While the tip of the thallus, the presence of nutritive filament, the shape of medulla cells in the cystocarp, and the presence of beta-carotenoid were the most influenced the clustering patterns Cluster II.

This study succeeded in finding five species of Gracilariaceae. Phenetic relationships of marine macroalgae Gracilariaceae based on numerical taxonomic analysis resulted in two main clusters, i.e., Cluster I consisting of *G. fergusonii*, *G. edulis*, *G. canaliculata*, and *G. verrucosa,* and Cluster II consisted of *G. salicornia*. Characters that most determines the clustering patterns were shape of holdfast, tip of thallus, surface texture of thallus, presence of cortex cells, fucoxanthin, neoxanthin, carotenoid, beta-carotenoid, cystocarp, pericarp, color, location, size, and characters of cystocarp, texture of thallus, and substrate characters.

AUTHORS' CONTRIBUTIONS

A.N.A collected samples, analyzed the data and wrote the manuscript. A.R.C gave research idea, revised the manuscript, and supervised all research process.

ACKNOWLEDGMENTS

This study was supported by KDM Grant 1016/UN1/FBI/KSA/PT.01.03/2021. The gratitude are expressed towards the Plant Systematic Laboratory, Faculty of Biology, Universitas Gadjah Mada, Indonesia.

REFERENCES

[1] R.C. Kepel, D.M.H. Mantiri, Naspiranto, Macroalgae Biodiversity in Tongkaina Coasts, Kota Manado, Jurnal Ilmiah Platax, vol. 6(1), 2018, pp.160-173.

[2] N. Kasanah, Setyadi, Triyanto, T. Ismi, Indonesian Seaweed Series 1: Seaweed Diversity in Gunung Kidul, Yogyakarta, Gadjah Mada University Press, Yogyakarta, 2018, pp.1-4, 76-84.

[3] M.N.A. Othman, R. Hassan, M.N. Harith, A. Shah, R.M. Sah, Morphological Characteristics and Habitats of Red Seaweed Gracilaria spp. (Gracilariaceae, Rhodophyta) in Santubong and Asajaya, Sarawak, Malaysia, Tropical Life Sciences Research, vol. 29(1), 2018, pp.87-101.

[4] D. Sartika, A.R. Chasani, A.M. Ningrum, S.L. Nafiah, S.W. Cahyani, Diversity and Composition

of Marine Macroalgae Species in Different Coastal Typologies in Gunungkidul D.I. Yogyakarta, Berita Biologi, vol. 20(1), 2021, pp.13-21.

[5] R. Iyer, O.D. Clerck, J.J. Bolton, V.E. Coyne, Morphological and taxonomic studies of Gracilaria and Gracilariopsis species (*Gracilariales, Rhodophyta*) from South Africa, South African Journal of Botany. 70(4), 2004, pp.521-539.

[6] T. Handayani, The Role of Microalgae Ecology for Marine Ecosystems, Oseana, vol. 44(1), 2019, pp.1-14.

[7] A.R. Chasani, E.A. Suyono, Comparison of Structure and Composition of Seaweeds Population in Porok and Greweng Coasts, Gunungkidul, Indonesia, AIP Conference Proceedings, vol. 2260(1), 2020, pp.020011.

[8] K.E. Carpenter, V.H. Niem, FAO Spesies Identification Guide For Fishery Purpose: The Living Marine Resources of The Western Central Pacific Volume 1 (Seaweeds, corals, bivalves, and gastropods), Food And Agriculture Organization of The United Nations, Rome, 1998, pp.84-89.

[9] A.S. Buriyo, E.C. Oliveira, M.S. Mtolera, A.K. Kivaisi, Taxonomic Challengers and Distribution of Gracilarioid Algae (Gracilariales, Rhodophyta) in Tanzania, Western Indian Ocean J.Mar Sci, vol. 3(2), 2004, pp.135-141.

[10] A.M. Ningrum, A.R. Chasani, Numerical phenetic and phylogenetic relationships in silico among brown seaweeds (Phaeophyceae) from Gunungkidul, Yogyakarta, Indonesia, Biodiversitas, vol. 22(6), 2021, pp.3057-3064.

[11] A.A. Bale, A. Khale, An Overview on Thin Layer Chromatography, International Journal Pharmaceutical Sciences and Research, vol. 2(2), 2011, pp.256-267

[12] O.P. Sharma, Plant Taxonomy 2nd Edition, Tata Mc Graw Hill Educatin Pivate Limited, New Delhi, 2009, pp.115-116.

[13] G. Singh, Plant Systematics: An Integrated Approach, Science Publisher, New Hampshire, 2004, pp.181.

[14] M.S.N. Delsen, A.Z. watiimena, S.D. Saputri, Application of Principal Component Analysis Method to Reduce Inflation Factors in Kota Ambon, Jurnal Ilmu Matematika dan Terapan, vol. 11(2), 2017, pp.109-118.

[15] A.A. Abbott, G.J. Hollenberg, Marine Algae of California, Stanford University Press, California, 1976, pp.500.

[16] M. Farchan, M. Mulyono, Fisheries Cultivation Basics, STP Press, Jakarta, 2011, pp.53