Jurnal Ilmu Kelautan **P-ISSN: 2460-0156**
SPERMONDE (2024) 10(2): 53-60 **E-ISSN: 2614-5049**

IDENTIFICATION OF GREEN ALGAE (CHLOROPHYTA) GENUS *HALIMEDA* IN THE WATERS OF MABA DISTRICT, EAST HALMAHERA

Wilya Ananda[1], Khairul Amri[2], Supriadi[2], Inayah Yasir[2]*

Submitted: June 6, 2024 Accepted: July 22, 2024

[1]Student of Marine Science Study Program, Faculty of Marine and Fishery Sciences, Hasanuddin University
[2]Marine Science Study Program, Faculty of Marine and Fishery Sciences, Hasanuddin University
Corresponding Author:
*Inayah Yasir
E-mail: inakhae@gmail.com

ABSTRACT

Halimeda is a genus of calcified green algae that inhabits tropical aquatic environments that function as buffers to neutralize pH.. The presences of *Halimeda* is essential for waters around mining sites, such as the waters of Maba District, wich is the center of mining in East Halmahera. Although the presence of *Halimeda* in waters is not rare, studies on *Halimeda* taxonomy in Indonesia are very limited. Identification of *Halimeda* species can be done by looking at morphological and anatomical structures as an alternative based on the key to determining *Halimeda* species in the Indo-Pacific by Hillis & Collinvaux (1980), in addition to looking at DNA sequences. This study was aimed at identifying *Halimeda* species distributed in the waters of Maba District, East Halmahera based on morphological and anatomical characteristic and to determine the distribution and similarity of species between sampling locations. Sampling was carried out using purposive sampling method. *Halimeda* sample collection was prepared before identification. Eight species of *Halimeda* were found from four sampling sites, namely *Halimeda cylindracea, H. distorta, H. macroloba, H. opuntia, H. simulans, H. discoidea, H. melanesica,* and *H. tuna.* In additional to these eight species, seven unidentified *Halimeda* 'species' were also found based on morphological and anatomical characteristc. Most similarity percentage of *Halimeda* species was found between Tanjung Buli-Monoropo at 75% and the lowest between Gee Island-Pakal Island (0%). The distribution of *Halimeda* species in waters in influenced by many factors, including substrate type, nutrients, light intensity, salinity, pH, temperature, depth, wave and current action.

Keywords: *Halimeda*; Identification; Morphological; Anatomical

INTRODUCTION

Besides being dubbed as one of the largest and most complexes of the green algae, *Halimeda* is also known as calcareous green algae, because all types of *Halimeda* store calcium carbonate ($CaCO_3$) in the form of aragonite which plays an important role in the growth of coral reefs (Hillis & Colinvaux, 1980). The content of $CaCO_3$ in *Halimeda* also acts as a buffer to maintain pH in the waters (Rukminasari et al., 2014) so it is very susceptible to the phenomenon of ocean acidification (Campbell et al., 2016). Morphologically, *Halimeda* is described as a plant with calcified green flattened segments and has a holdfast that is used to attach to the substrate. While anatomically, *Halimeda* is described as a segment consisting of one giant tubular cell with siphons that branch to form the medulla and utricle in the cortex. The structure of the cortex in one type of *Halimeda* is different from that of another species. Therefore, the structure of the cortex can be used as a reference in determining the type. In addition, the structure of the cortex can affect the high and low potential of $CaCO_3$ deposition, especially in the form of solid aragonite crystals in the *Halimeda* segment (Hillis & Colinvaux, 1980). To date, there are 44 known *Halimeda* species worldwide and 30 of them are found in the South China Sea, the waters of Malaysia, Thailand, Vietnam, Singapore, Philippines, and Indonesia (Arina et al., 2019).

The morphological structure of *Halimeda* consists of 2 parts, namely holdfast and thallus or commonly known as segments. Holdfast on *Halimeda* is described as irregular branches resembling tangled threads formed by rhizoids (Hillis & Colinvaux, 1980). According to Pongparadon (2009), there are 3 holdfasts at *Halimeda*, namely bulbous, rock-grower and sprawling. While the *Halimeda* segment is formed by organized filaments to form a segment (Hillis & Colinvaux, 1980). The shape of the segments on *Halimeda* varies greatly from shape to size (Pongparadon, 2009). While the anatomical structure of *Halimeda* consists of the medulla, cortex, and interfilament space. A medulla is several central filaments that run along with segments, generally trichotomous branches that produce branches called the cortex. The structure of the cortex consists of 2-5 layers of the utricle which differ between types. This cortical structure was also used as a reference in the anatomical identification of *Halimeda* by Hillis & Colinvaux (1980). While the interfilament space is the space formed by the union of the primary utriculus to form the space under the attachment of the primary utricle. This interfilament space is where calcium

carbonate is deposited in the form of aragonite (Hillis & Colinvaux, 1980).

In Indonesia, studies on the taxonomy of *Halimeda* are still very limited, although *Halimeda* is spread in almost all waters. Generally, the identification of *Halimeda* is based solely on morphological characteristics. Meanwhile, according to Perdosa et al. (2004), one of the problems in identifying *Halimeda* species is the morphology which varies between the same species but grows in different habitats. This is because the variety of *Halimeda* species is influenced by many factors, such as depth, substrate, light intensity, as well as wave and current action (Pongparadon et al., 2015). Therefore, morphological characteristics cannot be used as the main reference in determining the species (Perdosa et al., 2004). Thus, anatomical characteristics become an alternative in determining the type other than looking at the DNA sequence (El-Manawy & Shafik, 2008). Therefore, this study was conducted to identify macroalgae of the *Halimeda* genus in four different locations, namely in Tg. Buli, Pakal Island, Moronopo and Gee Island located in Maba District, East Halmahera Regency which is a mining center in North Maluku.

MATERIALS AND METHODS

This research was conducted from December 2021 to June 2022. Sampling was conducted in four locations with 17 stations (figure 1). Identification and analysis carried out at the Multitropic Research Group Laboratory, Institute for Research and Community Service (LP2M), Hasanuddin University.

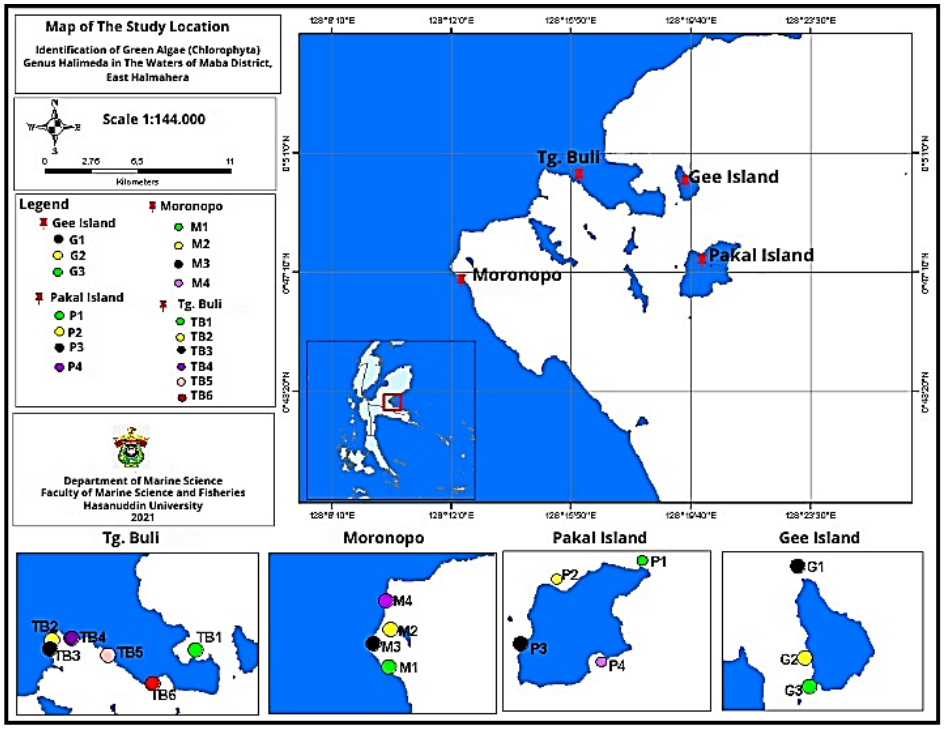


Figure 1 Map of the study location

Research Procedure

Sampling was carried out using purposive sampling method by only targeting *Halimeda* at a distance of 100 meters along the coast and 20-50 towards the sea. Sample preparation in the field is done by removing epiphytes attached to the thallus of the sample using fresh water, then the samples is immersed in 70% alcohol for 10-15 seconds and make sure that all segments are submerged in alcohol. The sample that has been given alcohol is then place on newsprint and left at room temperature for 5-12 hours or until the sample is completely dry, then the dried sample is put into a plastic sample that has been labeled. Given alcohol to the sample last longer during the trip to the laboratory.

Morphological analysis was carried out by identifying the type of holdfast, the shape of the segment and measuring the length and width of the *Halimeda* segment. *Halimeda* segment is measured using a caliper by applying the conventional method. The segment will be devided into 4 landmarks, one is at the center of the segment attachment, one is at the highest part of the segment, and the other landmarks are at the left and right ends

of the segment, repectively (Pongparadon, 2009). In one individual there are 10-20 segments that are measured depending on the number of segments in that individual. The measured segments are taken randomly from the base to the apex (the basal segment is not included) so that later data on the range of segment sizes in one type will be obtained.

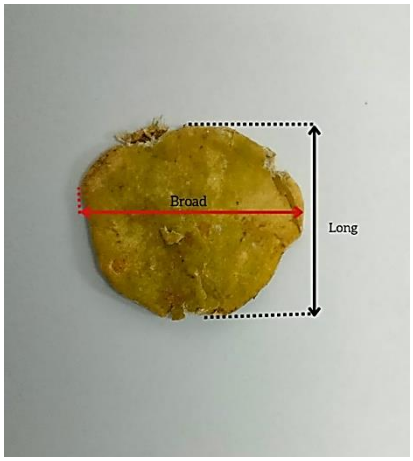


Figure 2. *Halimeda* segment measurement using conventional method

After the morphological analysis, it was continued with the decalcification process to see the anatomical structure of the sample. The decalcified segment should take the unmeasured segment to avoid damage to the edge of the segment due to contact with the caliper. The decalcification process aims to remove the content of $CaCO_3$ in the *Halimeda* segment to be observed. In one individual, 2-3 segments are taken for decalcification. It aims to get a picture of the structure of cortex and the diameter range of the peripheral utricle. The sample to be observed is first sliced crosswise using a razor blade. Then, the slices will be decalcified using a 10% HCl as much as 3-10 drops (depending on the width of the dacalcified segment) for 20-30 seconds. The cessation of the decalcification process was indicated by the absence of a bubble reaction in the decalcified segment. After the decalcification process is complete, the HCl solution in the decalcified segment is then absorbed using a tissue, then rinsed using distilled water before being observed under a microscope.

The anatomical structures observed were the structure and shape of the cortex, as well as the shape and size of the peripheral utricle. The structure and shape of the cortex will be observed under a microscope with a magnification of x4-x40. Meanwhile, the shape and size of the peripheral utricle will be observed with a magnification of x40. Then the diameter of theperipheral utricle will be measured using the Image View software. The results of the peripheral utricle diameter measurement will then be exported to Microsoft Excel to get the range of utricle peripheral diameter values from the measured segment.

Data Analysis

The results obtained will be analyzed descriptively by describing the morphological and anatomical characteristics of each type of *Halimeda* obtained in the form of tables and figures. Moreover, the Sorensen Similarity Index (Mueller-Dombois & Ellenberg, 1976)was also used to see the similarity of species between locations.

$$\text{Iss} = \frac{2C}{(A + B)} \text{ X } 100$$

Where:
A: Number of species in community a
B: Number of species in community b
C: Number of species in common between both communities.

RESULTS AND DISCUSSION

Out of 17 sampling stations, *Halimeda* was only found in 15 stations. Based on the result of morphological and anatomical analysis, eight *Halimeda* species and seven unidentified *Halimeda* 'species' were found, distributed as follow (Tabel 1).

P-ISSN: 2460-0156
E-ISSN: 2614-5049

Table 1. *Halimeda* species found at 17 sampling stations

No.	*Halimeda* Species	Location																
		Gee Island			Tg. Buli						Pakal Island				Moronopo			
		G1	G2	G3	TB1	TB2	TB3	TB4	TB5	TB6	P1	P2	P3	P4	M1	M2	M3	M4
1	*H. cylindracea*	✓																
2	*H. distorta*			✓														
3	*H. macroloba*					✓	✓	✓			✓			✓		✓		
4	*H. opuntia*				✓	✓	✓	✓	✓	✓	✓	✓		✓	✓	✓		✓
5	*H. simulans*				✓			✓										
6	*H. discoidea* (Morphotype1)			✓														
7	*H. discoidea* (Morphotype 2)						✓											
8	*H. discoidea* (Morphotype 3)																	✓
9	*H. melanesica*										✓							
10	*H. tuna*														✓			
11	Unknown 1	✓																
12	Unknown 2	✓																
13	Unknown 3	✓																
14	Unknown 4		✓															
15	Unknown 5										✓							
16	Unknown 6				✓													
17	Unknown 7							✓										

Figure 3. Morphological characteristics of *Halimeda* species found at sampling sites; (a) *H. cylindracea*; (b) *H. distorta*; (c) *H. macroloba*; (d) *H. opuntia*; (e) *H. simulans*; (f) *H. discoidea* Morphotype 1; (g) *H. discoidea* Morphotype 2; (h) *H. discoidea* Morphotype 3; (i) *H.melanesica;* (j) *H. tuna*; (k) Unknown 1; (l) Unknown 2; (m) Unknown 3; (n) Unknown 4; (o) Unknown 5; (p) Unknown 6; (q) Unknown 7

Table 2. Anatomical characteristics of Halimeda found t sampling sites.

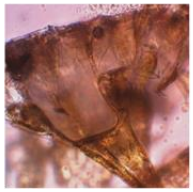
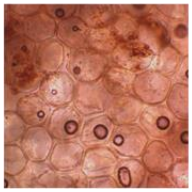
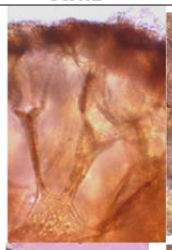
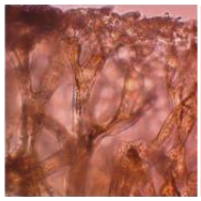
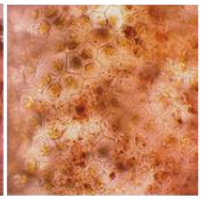
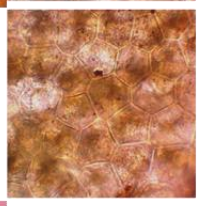
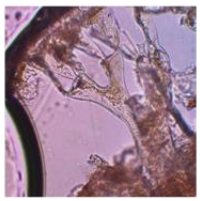
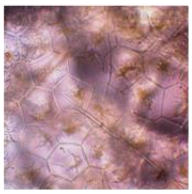
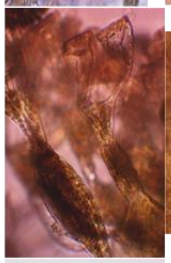
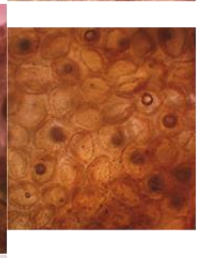
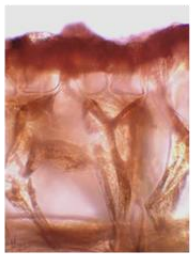
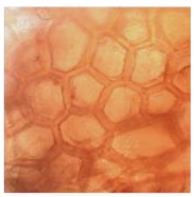
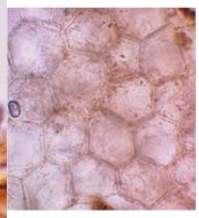
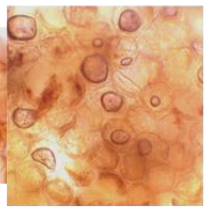
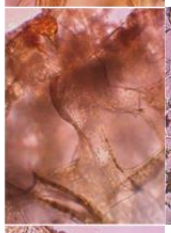
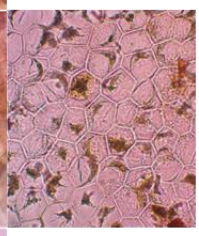
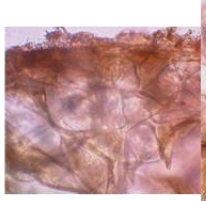
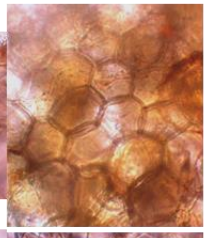
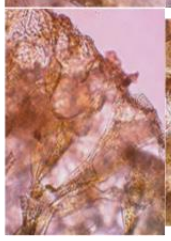
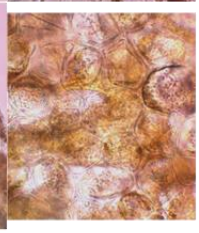
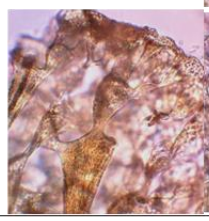
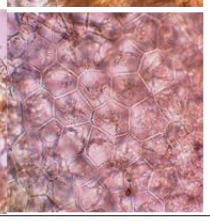
Species	Cortex	Peripheral Utricle	Species	Cortex	Peripheral Utricle
H. macroloba			*H. melanesica*		
H. opuntia			*H. tuna*		
H. simulans			Unknown 1		
H. discoidea Morphotype 1			Unknown 2		
Unknown 3			Unknown 6		
Unknown 4			Unknown 7		
Unknown 5					

Table 3. Similarity index calculation results (%)

Pair Location	Similarity (%)
Gee Island-Tg. Buli	29
Tg. Buli-Pakal Island	57
Pakal Island-Moronopo	57
Gee Island-Pakal Island	0
Gee Island-Moronopo	29
Tg. Buli-Gee Island	29
Tg. Buli-Moronopo	75

Out of the eight species of *Halimeda* identified, *H. opuntia* was the species that was distributed in almost all sampling sites, followed by *H. macroloba.* Both of them were only absent from one location, Gee Island. These species have good tolerance to various environmental conditions. The ability of a *Halimeda* species to tolerate various environmental conditions is based on the morphology, reproduction and physiological traits (Verbruggen et al., 2009). Furthermore, both species are also included in the cosmopolitan *Halimeda* species or *Halimeda* species that are widely distributed in waters (Arina et al., 2019). This is different from the species of *H. cylindracea, H. distorta, H. melanesica* and *H. tuna* which were only found in one station. This is shows that *Halimeda* found is location indicates the suitability of environmental conditions for *Halimeda* species in building populations (Xu et al., 2015).

Among the eight species of *Halimeda* found, there is one species that has three different morphologies, namely *H. discoidea.* This is because these three morphotypes grow in different locations and habitats. *H. discoidea* Morphotype 1 was found in Gee Island, Morphotype 2 was found in Tg. Buli and Morphotype 3 was found in Moronopo. The existence of morphological variations in the same species is one of the obstacles in identifying *Halimeda* if only based on morphological characteristics (Perdosa et al., 2004). This morphological variation can be caused by enviromental conditions and as a result the plastic properties of macroalgae (Pongparadon et al., 2015). This means that morphological characteristics cannot be used as a reference in determining species.

In addition to the morphological differences found in *H. discoidea*, there were seven 'species' of *Halimeda* that were not identified based on morphological and anatomical characteristics. Some samples showed morphological and anatomical characteristics of two different species of *Halimeda* in one individual, while others showed variations in morphological and anatomical characteritics that were not describes in references. Sample Unknown 1 has a cortex structure and peripheral utricle shape that resembles *H. macroloba* (Hillis & Colinvaux, 1980), but the peripheral utricle diameter of sample Unknown 1 is smaller than *H. macroloba*. Differently from the Unknown 1 sample, the Unknown 2 sample has variations in the shape and number of utricle layers on cortex. Although the number of utricle layer on *Halimeda* can be affected by environmental conditions (Pongparadon, 2009), there is no references that explains that the difference in the number of layers on the utricle is also followed by a change in shape. A similar case applies to the Unknown 3 sample, which shows variations in the shape and number of utricle layers. One of the cortex structures found in the sample, ponting to *H. bikinensis* (Hillis & Colinvaux, 1980) is supported by the shape and diameter of the peripheral utricle.

Variations in the number of layers and the shape of the utricle are also present in the Unknown 4 sample. One of the cortex variations in this sample refers to the cortex structure of *H. discoidea* (Hillis & Colinvaux, 1980) and is very similar to the cortex structure of *H. discoidea* Morphotype 3. In addition to variations in the number of layers and shape of the utricle, the peripheral utricle in this sample also shows a stacked hexagonal shape that is not shared by others species, making the anatomical characteristics of this sample very distinctive. Different from the previous samples, Unknown 5 morphologically refers to *H. micronesica* found by stone (1967) in Merizo Bay, Mariana Island. However, the anatomonical structure shows different result. *H. micronesica* has 3-4 utricle layers with a rounded utricle periphery (Hillis & Colinvaux, 1980). This is very different from the results observed during the research.

Sample Unknown 7, morphologically (segment shape) resembles *H. distorta* (Hillis & Colinvaux, 1980). However, the segments of sample Unkown 7 are poorly calcified when compared to the heavily calcified segments of *H. distorta.* The peripheral utricle shape of the Unkwon 7 samples refers to *H. opuntia* (Hillis & Colinvaux, 1980) with a predominantly hexagonal and small polygonal peripheral utricle shape. When referring to the shape of the peripheral utricle, it could be concluded that sample Unknown 7 is a type of *H. opuntia* that grows at depth, as stated by El-Manawy & Shafik (2008) that *H. opuntia* that grows at depth has the mophological characteristic of forming loose tufts with branching that tends to be unclear. However, despite this, the utricle characteristic of the sample consisted of only 3 layers and this overlapped with the anatomical characteristics described earlier. Similar to the previous cases, variations in the

number of layers and shapeof the utricle were also found in Unknown 6 sample. The cortex characteristics refer to *H. simulans* (Hillis & Colinvaux, 1980),but the shape of the peripheral utricle of the sample is different from the shape of the peripheral utricle in the determination key. One of the reasons for not identifying these samples is that there is no reference to include them in a particular species, but to declare them a new species is not possible due to the lack of a species identification key.

The results of the calculation of the Sorensen Similarity Index (Mueller-Dombois & Ellenberg, 1976) show that the highest species similarity is in Moronopo-Tg. Buli with a similarity percentage of 75%. The highest percentage similarity in these two locations in most likely due to the type of substrate that dominates the two locations is a muddy sand substrate and the type of *Halimeda* that grows in both locations is a cosmopolitan *Halimeda* species.

The lowest level of similarity is on Gee Island-Pakal Island, which does not have the same species (0%). Gee Island is dominated by varied substartes, such as fine sand, mud and coarse sand, while Pakal Island is only dominated by fine and coarse sand. *Halimeda* species that grow on Gee Island is rare and not found in other locations, such as *H. cylindracea* and *H. distorta.* This is very inversely proportional to the species that growing on Pakal Island, the dominating species are cosmopolitan *Halimeda* species, such as *H. macroloba* and *H. opuntia.* The difference species grown in the two locations can be caused by very different environmental conditions. This is because the distribution of macroalgae species in the waters is influenced by many factors, such as type of substrate, nutriens, light intensity, salinity, pH, temperature, depth, and wave and current action (Arina et al., 2019; Pongparadon et al., 2015).

CONCLUSION

Among the 17 sampling stations, eight *Halimeda* species were identified based on morphological and anatomical characteristics, namely *H. cylindracea, H. distorta, H. macroloba, H. opuntia, H. simulans, H. discoidea, H. melanesica,* and *H. tuna.* Meanwhile, the highest percentage of similarity is in Tg. Buli-Moronopo waters, which is 75% dominated by cosmopolitan *Halimeda* species.

ACKNOWLEDGMENT

We would like to thank Dr. Inayah Yasir, M. Sc. who have funded this research and provided suggestions and input so that this research can reach this point. Thanks also to Dr. Supriadi, ST., M. Si. who helped a lot during field sampling and thanks to Dr. Khairul Amri, ST., M. Sc. Stud. who assisted in processing the results of the data analysis.

REFERENCES

Arina, N., Rozaimi, M., & Zainee, N. F. A. (2019). High localised diversity of Halimeda (Chlorophyta: Bryopsidales) in a tropical marine park from Pahang, Malaysia. Regional Studies in Marine Science, 31, 100773. https://doi.org/10.1016/j.rsma.2019.100773

Campbell, J. E., Fisch, J., Langdon, C., & Paul, V. J. (2016). Increased temperature mitigates the effects of ocean acidification in calcified green algae (Halimeda spp.). Coral Reefs, 35(1), 357–368. https://doi.org/10.1007/s00338-015-1377-9

El-Manawy, I. M., & Shafik, M. A. (2008). Morphological Characterization of Halimeda (Lamouroux) from Different Biotopes on The Red Sea Coral Reefs of Egypt. American-Eurasian Journal of Agricultural and Environmental Science, 3(4), 532–538.

Hillis, L., & Colinvaux. (1980). Ecology and Taxonomy of Halimeda : Primary Producer of Corals Reefs. Adv. Mar. Biol., 17, 1–327.

Mueller-Dombois, D., & Ellenberg, H. (1976). Aims and Methods of Vegetation Ecology. Geographical Review, 66(1), 114. https://doi.org/10.2307/213332

Perdosa, M. E. B., Pereira, S. M. B., & Oliveira, E. C. (2004). Taxonomy and Distribution of The Green Algal Genus Halimeda (Bryopsidales, Chlorophyta) in Brazil. Revista Brasileira de Botânica, 27(2). https://doi.org/10.1590/s0100-84042004000200015

Pongparadon, S. (2009). Diversity, Distribution and Variations within Species of Genus Halimeda J. V. Lamour. (Chlorophyta) in Peninsular Thailand. In Journal of Biogeography (Vol. 60, Issue 1). Prince Songkla University.

Pongparadon, S., Zuccarello, G. C., & Phang, S. M. (2015). Diversity of Halimeda (Chlorophyta) from The Thai-Malay Peninsula. Phycologia, 54(4), 349–366. https://doi.org/10.2216/14-108.1

Rukminasari, N., Nadiarti, & Awaluddin, K. (2014). Pengaruh Derajat Keasaman (pH) Air Laut Terhadap Konsentrasi Kalsium dan Laju Pertumbuhan Halimeda sp. Jurnal Ilmu Kelautan Dan Perikanan, 24(1), 28–34. https://www.scribd.com/document/363166182/ph-derajat-air-laut-pdf

Verbruggen, H., Tyberghein, L., Pauly, K., Vlaeminck, C., Nieuwenhuyze, K. Van, Kooistra, W. H. C. F., Leliaert, F., & de Clerck, O. (2009). Macroecology Meets Macroevolution: Evolutionary Niche Dynamics in The Seaweed Halimeda. Global Ecology and Biogeography, 18(4), 393–405. https://doi.org/10.1111/j.1466-8238.2009.00463.x

Xu, H., Zhao, X., Eberli, G. P., Liu, X., Zhu, Y., Cai, Y., Luo, W., Yan, G., Zhang, B., Wei, K., & Shi, J. (2015). Biogenic Carbonate Formation and Sedimentation in The Xisha Islands: Evidences from Living Halimeda. Acta Oceanologica Sinica, 34(4), 62–73. https://doi.org/10.1007/s13131-015-0584-0