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The Genetic and Morphology Diversity of the Wild Seaweeds Found in Parigi Moutong Coast, Center Sulawesi-Indonesia

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Abstract. Parigi Moutong district is one of the potential area where the economic seaweeds are produced, but the wild seaweed is less noticed. This research was aim to investigate the potential and diversity of wild seaweed at Parigi Moutong coast Center Sulawesi-Indonesia. Samples were collected from the coastal area by diving and using a boat. The fresh seaweeds were morphologically identified while the dried samples were used for DNA amplification and testing. The water quality testing was carried out to determine the dominance of wild seaweed that occupies the coast of Parigi Moutong. The classification and DNA testing were carried out on the collected seaweeds which were distributed as *Agarum clatharum*, *Polysiphonia akkeshiensis*, *Palmaria lucida*, and *Ectocarpus* sp. This present work was conducted to study the diversity of the wild seaweeds found in Parigi Moutong coast and open the opportunities to established specific infrastructure to utilize the variety of seaweed at Parigi Mouting coast. Other than that, such findings represent an important scientific support concerning the Parigi Moutong wild seaweed, which provides an assist in algal studies and its application.

Keywords: DNA amplification, Parigi Moutong coast, wild seaweed

INTRODUCTION

Seaweed is one of the primary producers in marine aquatic ecosystems whose presence in Parigi Moutong Center Sulawesi-Indonesia is quite abundant. Seaweed is an famous alga and becomes one of the marine plant that is commonly interesting to study for its diversity [1]. Seaweeds also shown to produce a variety of compounds whose some of them have been reported for diverse biological activities [2]. This marine algae has been used in food or as a new development for food source [3] and other medicine [4, 5]. Many studies have been conducted to characterize seaweed including studies of a wide range of biological such as antiinflammatory, anticoagulant, anti-obesity, antibacterial, antiviral, antifungal, antioxidant, antitumoral and antiproliferative [6].

In term to support the advantages knowledge of algae or seaweeds diversity, marine algae need to be studied to discover another potency in developing algae utilization. Parigi Moutong coast in this case and especially that of its seaweeds have never been investigated. The first step was started by searching the other potential algae around the coast line of Parigi Moutong coast. The next step was collecting the algae and characterizing their morphological parts and their DNA structures. The adaptation of molecular techniques enabled a more reliable and systematic way of inferring evolutionary among different strains or species

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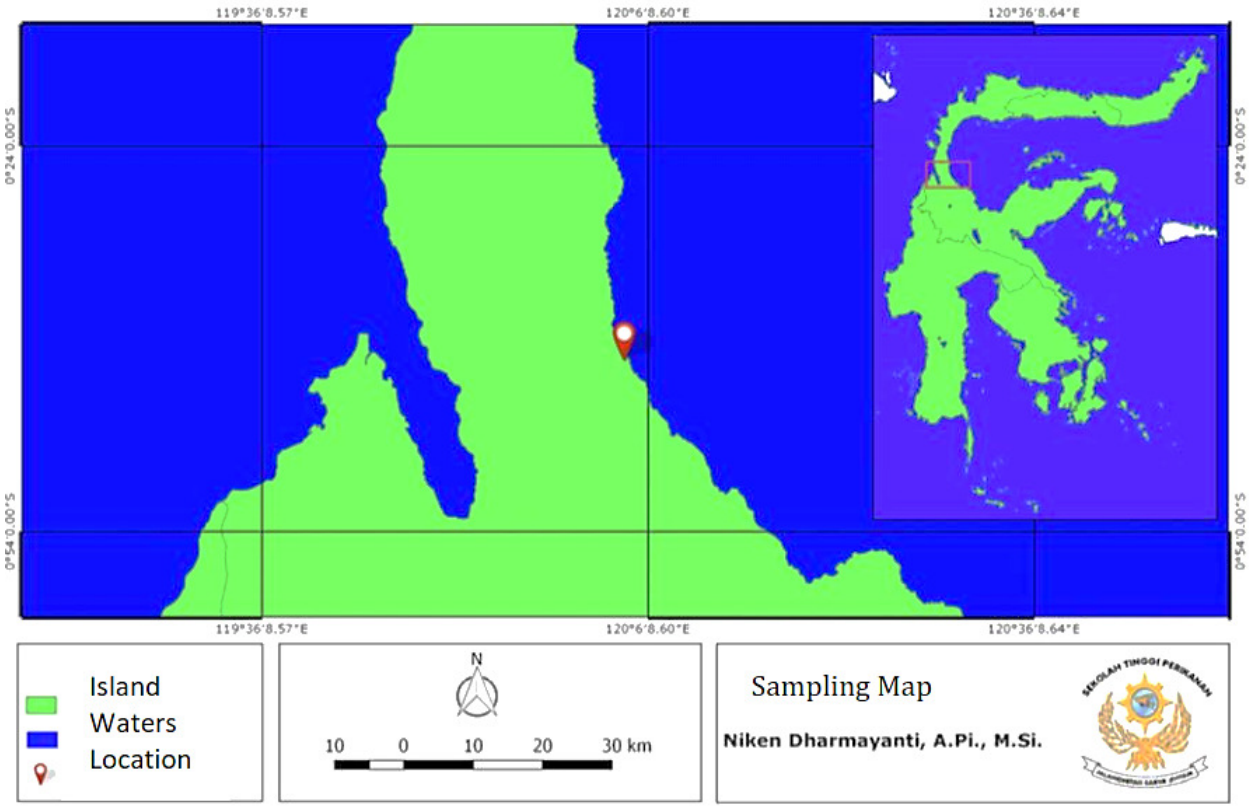

FIGURE 1. Location for sampling of seaweed in Parigi Moutong, Center Sulawesi.

MATERIALS AND METHOD

Samples were freshly collected from the costal area of Parigi Mountong on April 2018. The distance of the seaweed growth from the coastline was about 20 m. Samples were selected randomly using square method. Seaweed that appears in the quadran then collected for biomass calculation and herbarium of the simplicia. The seaweeds were picked up carefully included their base or holdfast structures, since it could help the observation of a fundamental character on morphology testing.

DNA extraction using the modified TIANGEN (DP121221) protocol in Genetica Lab UI. About 200 µg seaweed samples from tip of thalus shoots were put in the cryotube added with the addition of 1.4 ml of buffer, stored in ice and extracted at room temperature for 1 minute. Cryotube is heated at 70 °C in a water bath for 5 seconds. Cryotube was extracted at room temperature for 15 seconds and continued with centrifugation at 12,000 rpm at room temperature for 1 minute. The supernatant was transferred to a sterile sample tube and supplemented with 1 tablet inhibit (1x volume of Chloroform: Isopropanol (24: 1)). The solution was homogenized with vortex for 1 minute (rotator or shaker for 15 minutes), then centrifuged at a speed of 12,000 rpm at room temperature (24 °C for 10 minutes). The stage is repeated once, then the top layer formed is transferred to a sterile sample tube. The solution was added as much as 2 per 3 x volume of isopropanol and incubated at -20 °C for overnight. Total DNA was precipitated by centrifugation at a rate of 13,000 rpm at 24 °C for 10 minutes. Supernatant is removed, the total DNA produced is dried and resuspended with ddH2O.

DNA extraction results were quantified using a nanodrop spectrophotometer. DNA extract was diluted to obtain DNA concentrations of 10-50 ng/µL for DNA amplification (PCR) purposes. DNA extraction results were visualized by 1 % agarose gel electrophoresis to see the quality of the DNA produced.

DNA amplification was carried out using ITS2 markers. Primary and primary base sequences for the markers can be seen in Table 1. Amplification of DNA using Go Taq polymerase (Promega) PCR kit, PCR mixture is a

modification of the PCR mixture recommended by the Canadian Center for DNA Barcoding (CCDB). The types of markers and primary sequence of DNA barcodes are presented in Table 1. The composition of the PCR reaction is presented in Table 2. The results of amplification were visualized using electrophoresis with TAE buffer.

DNA Sequencing

DNA sequencing will be carried out by the sequencing service company, 1st Base DNA Sequencing Division, in Singapore. Samples are sent in the form of amplification results (PCR products).

Data Analysis

Analysis in the form of DNA sequence contig were done automatically using the Chromaspro program. The results of the contig were then made BLAST to look for the similarity of DNA sequences that had been deposited on NCBI GenBank. DNA alignment (DNA alignment) was done by adding MEGA7 programs with multiple alignment. Phylogeny analysis was performed using Maximum Parsimony using PAUP * 4.0b10 (Swofford 1998) through the Heuristic search method.

The species from the genus Rhodophyceae and Chlorophyceae will be used as outgroups in the construction of phylogenetic trees. Gaps were treated as missing data. All characters were treated as unorder data and have the same weight. Branch swapping algorithm was ran using tree-bisection-reconnection (TBR). Evaluation of phylogeny trees was carried out with Felsentein's bootstrap test (FB) with 1000x bootstrap. Data on raw sequences were edited and trimmed manually using the new version of Molecular Evolutionary Genetics Analysis v. X (MEGA X). The number of sequences determined by using GenBank database which is hosted at NCBI (National Center for Biotechnology Information, USA) [Link: http://www.ncbi.nlm.nih.gov/]. NCBI hosts a number of biological databases for example whole-genome databases for human, mouse, chimp, seaweed or algae and another specific genome of organisms.

RESULTS AND DISCUSSION

Based on this study, there were four wild seaweeds species which recorded in the Parigi Moutong coast. Those were *Polysiphonia* sp. (Rhodophytes), *Dictyota* sp. and *Padina* sp. (Phaeophytes) and *Halimeda* sp. (Chlorophytes) as shown in Fig. 1.

TABLE 1. Types of markers and the primary sequence of DNA barcodes [7].

No	Marker	AT	Primary	Primary and the Sequence of DNA Nucleotides
1	ITS2	55	5.8S-BF	5'-ATGAAGAACGCAGCGAAATGCGAT-3'
	Nuclear		25 BR-2	5'-TCCTCCGCTAGTATATGCTTAA-3'

TABLE 2. The composition of the PCR reaction used in amplification [7].

No	Reagent	Concentration	Volume (µL)	
1	Buffer reagent		1.25	2.5
2	$MgCl_2$	1.50 (mM)	1.25	2.5
3	DNTPs	0.20 (mM)	0.25	0.5
4	Primer f	0.10 (µM)	0.25	0.5
5	Primer r	0.10 (µM)	0.25	0.5
6	Taq DNA polymerase (Sigma St. Louis, MI, USA)	1.25 (unit)	0.07	0.14
7	ddH_2O		8.18	16.36
8	Mold DNA		1.00	2.0
	Total		12.5	25.0

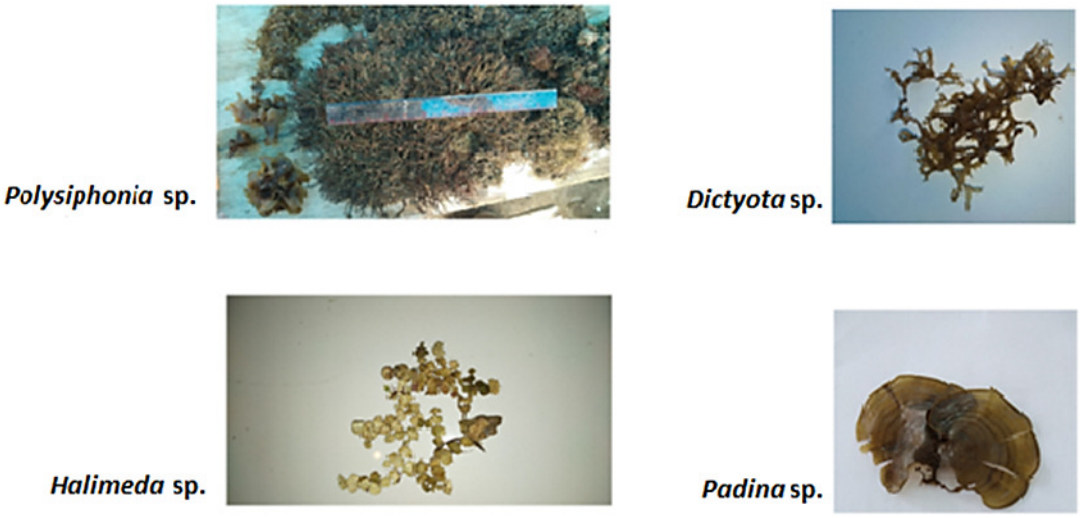

FIGURE 2. Result sampling of wild seaweed found from Parigi Moutong Center Sulawesi-Indonesia [8].

TABLE 3. Water quality and ambient characteristic.

Reagent	Concentration
Ambient temprature	34 °C
Water temprature	30 °C
Depth	30 M
Salinity	30 $^{o}/_{oo}$
Ph	7
Disolved oxygen	0.93
NO_2	-
NO_3	-
NH_3	-
Phosphate	4

Water Quality

The seawater physicochemical characteristics of the sites from which the seaweeds were collected are summarized in Table 3.

Seaweed Genetic Identification

The sample PCR (Fig. 3) products were visualized using a gel documentation system measuring 600 bp and the negative control did not contain the following DNA bands. Data analysis of sequences after trimmed by using MEGA X, resulting a vary sequences that equals for their pairs. Here the sequences based on their correct pairs.

1. *Polysiphonia* sp. #3217322_1N_ITS2-5_8S-BF
   GGTCACAAGTGACGCTGCTTGCCGCCGAGGCGGCCCGCGTGGCGGTCGAGCCAGACAACTGAC
   TGACTGACTGACCATCGTGGGTCGGACGCGCAAAGCCTAATAGCGGGGCCTGGACATCACCCG
   CACACCTCGCCCCGGTGGGGACAAGGCGGGGCGCCGAGTCTCGATCGCCCGTCGCGTACCCCA
   CGCCCCCCCCCCTGCCAAGAGAAATGGCGGGAGAGAGGAATGGGGACGGGCAAGAAACATTG

GCCCTCCACTAGTCGCAATGCCGCAGTCATACTTCTTAGGCCGGCTCACGAGACGAAGCTCGC
GGCCGGCTCATAGTCCGCCGCCCCCGAGCGCGCGCGAGTCACCATTTCTGGCGTCTCAAGCGG
GCCACGCACGGGGGGGCGAGTGGGGTGGACAGACACTCCGACAAGCATGCTCCCAGGAGTAT
CCCGGAAGCGCAAGATGCGTTCAAAGGTTTGATGATTCAC

2. *Polysiphonia* sp. #3217324_2N_600bp_ITS2-5_8S-BF
GAGATCCCGTAGGAAATAAGGACTGCTGCTTTTTTTTCTAGGAAATAAAAAAACAGCATTGAC
AGCCTAAAATACCCACATTTCTGTAGATATACTGTCGATCTGAGCGTTGTAATGTAAAAATCGA
AAGCCATGATCACTCATTTATTTTGAACCTTTGATATCTCTCTCCACCTCAACAAAGCAAGAAC
AAGTAAACTCTGAATGACAAGTTCAATTCATGCCTGCCGGCTCAAAAGAATCAATTCATTTTTG
TAAACAAAAACAAAGCGGCGCAAAAAACACTGGAAATACACTTTCTACAACTAGAATTTCAGG
TGACCCCGCTTGTAGGTTATTCAAGCCTGGTAGCAAGGACGCACCCATCAGCTGCGATTTCGGT
GCGGTCAGACCGCTAAAATCGCAAAGTTATGTTGTGAAATTTTACCTACACATGGGACACTCA
CACAGGTATGCTCGCAGATTAAACCGCGAGCGCCATTTGCGTTCAAAAATTCGATGATTCA

3. *Halimeda* sp. #3217326_2N_1400bp_ITS2-5_8S-BF
CCAAAAGCAGGACCGCTACCAACGCCACACCTCGCCTCCACAAACACACACTCAACACTCCGA
CTCAGACGGACCACTGCAAATGCCAAGCCCTAACAAACCCGCAAGCACGCACACACACACAC
ACGCACACACACCCACCAGTGCAGGACTCAACTTCAGCACTCACACCCGCCCGCTTTCGCGAA
CAAGCACAAACACATCCATGAATTCATCTACACCAACAAGCGCGCGCACGCACGCACACACAT
ACACACTCACACGCAAAGCTCAAACACAGAACTTCGACATACACAGCACCGCCACCAGTTCAC
ACTCCGCACACCAACTGCTCCGATCAAAGATTGCTCTCCTTACCTTCACAGCCAGTGAATTGTA
GTAACCGATCCCCAACAACGAAGCCATGCGCACAGGTCGCCCTGCACACCTGCCTCTATTCCA
CATCCCGTCGCCTCACTAAGTTCACCAGAACTAAAGAATGCCTCAACTCGCTTCCACAAAGCA
GGACTCAGCTTTGGCTTGCGCCTCCACGAA

4. *Padina* sp. #3217328_3N_ITS2-5_8S-BF
CCACTATCCCACAGCAGTCACTGGATCGTAGTTACTTCCCAGAGACTGAGCAAAAAAAAGGGG
GAAGGGATGGGATAACGTTTTCCAACTCTGTCGACCGAAACAACAGAAGTTTCTCTTCGGAAA
ACTCTCATCACCCCCCTCCCCTTCTCTTTCTTGCCAGGACCCAGAACCGCAAAAGACAACCAAG
CTAATATAGTACAATACACACACACAAATCCATTTCTCCCTTCTTGTATAATATTCCTTTCCACA
GTTCACTGAAAAAGTGTAACCGGTACTAGGAACCAAAAAGTGAGAAAGTGGGCGCGGCTTTG
GGTTCTCAAGTATTCCATAAGCCAGATGTTTGTATATTTTAGCAAAACACACAACTCTTGCGGG
ATGCGGTGTCGACACGCTGTACCAACTGGCCGTGTCCAGTTGATACTATTAAAAGGCAAAGAG
AATGTCACCTTTCTCCTGGTTTCGCACACTCACGAAAAGTCAAAACGCTTGGGGAAAAACAGT
AACAACACTGGTTTCT

5. *Dictyota* sp. #3217330_4N_ITS2-5_8S-BF
CGGAGGTCCGTAAAGACGACGCGCTGCCCGATAGCAAGCAAGCAAGCAAGCAAGCTAACTAA
CGGGCAGTCACTTACCTGACCGCGGTCGGAGGGGTCTCGGCGGGCCGGACGGTCGGGTGAAAG
GGCAGCACCGCAACAGCAGAAATCGATCAATCACTCGATCAATGGATCAAACAATCAACACA
ACCTCGCCGCCACGGGCCACACGGCCACCCAACTTCCTACTCTCGCTCTCTGCTACTCGGCGCG
CAACCCGACTTGCGCCGGACTAACGCTGCCGCGTATACGATCCGAGACCCTTCCTCCGGGTTGG
ACAGTTTTTGCTACTCTTCGCGGGGCTCGGCGATGGTCGTTCGCACGTCGGTAGAGGAGACGA
AGAGACAGCTAGGATAACAACAACAACAACAACAACAACAACAACAACAACAACAACAGCACTAC
ACCATCATCGTCATCATCATCGTCATCATCATCATCACCATCATCATCATCAGCATCCCTACTAC
CACCGGGAGAACGGCCACCACCGGCGCTCGCATAGTCCGCCCAAGTACTCACCCTTTCCCCGC
CGCGGCCAAGTCATCTTGGTACCGCGAGAAGCGGTGCCGCGATGACGAGGCTAGGCGAAGAC
CGAGTGTGCACAAGGACGAGTGGGGTGAACGAAGACTCCGACAAGCATGCTCCCAGGGATAT
CCCGGAAGCGCAAGATGCGTTCAAAGTTTTGATGATTCGC

Blast of the rbcL Parigi Moutong Coast sampels against the GenBank database did not support precise genetic identification for all samples. One sample showed similarity in their phylum, those were *Padina* sp. and *Dityota* sp., although come from different branch (clade). In the other branch, the samples showed same species that is was *Polysiphonia*. *Halimeda* sp. from Parigi Moutong coast was located within a very well supported branch (clade)

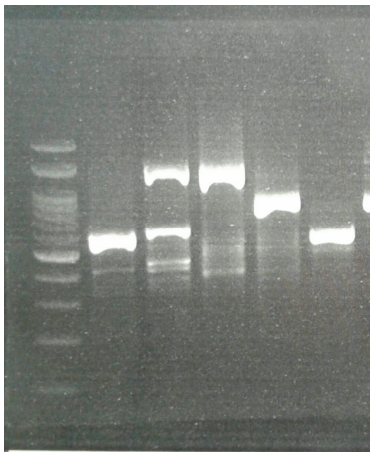

FIGURE 3. Result of genetic identification PCR.

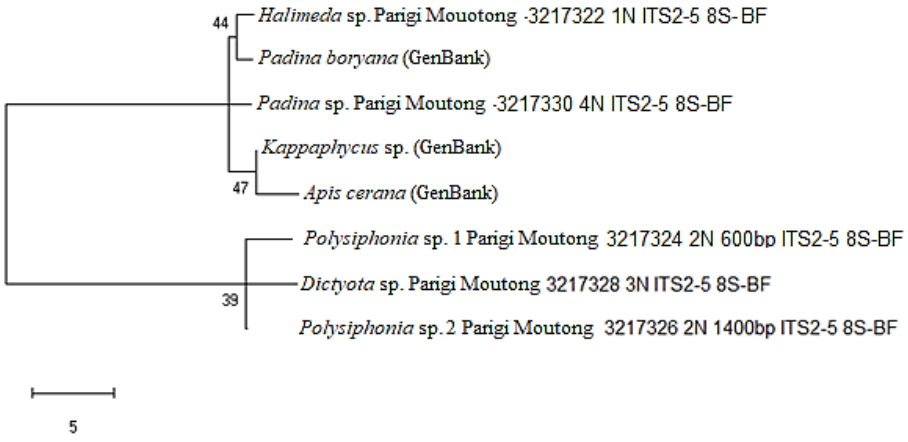

FIGURE 4. Neighbor joining consensus trees using partial sequences of GenBank-NCBI.

with *Padina boryana*, and the other seaweed species from Genbank which were *Kappaphycus* sp. and *Apis cerana* sp. (Fig. 4).

In all phyla the largest frequency of the species at the sites was *Dictyota* sp. Geographical distributions are more sensitive to environmental changes, especially water temperature because physiological activities of marine organisms depend on water temperature, Especially seaweeds [8, 9]. Other studies showed that climatic factors such as temperature, surrounding waters as rivers, etc. could influence the distribution of marine algae [10, 11]. Photoperiod, along with temperature, regulates seaweed reproduction [12] Shipping, pollution and many other factors may play key roles in marine algae distribution, a change in their metabolism and then in their chemical composition and their biological activities.

CONCLUSION

The classification and DNA testing were carried out on the collected seaweeds. Our results shows that the collected seaweeds were distributed as Dictyota sp., Halimeda sp., Padina sp., and Polysiphonia sp.

REFERENCES

1. M. B. Araújo and A. Guisan, Journal of Biogeography **33**, 1677-1688 (2006).
2. H. Admassu, W. Zhao, R. Yang, M. A.A. Gasmalla and E. Alsir, International Journal of Scientific and Technology Research **4**, 108-115 (2015).
3. T. Shrestha-Bhattarai and V. M. Rangnekar, Oncogene **29**, 3873-3880 (2010).
4. P. Laurienzo, Mar Drugs **8**, 2435-2465 (2010).
5. T. Komatsu, A. Meinesz and D. Buckles, Ma. Ecol. Prog. Ser. **146**, 145-153 (1997).
6. A. Mikami, T. Komatsu, M. Aoki and Y. Yokohama, La Mer **44**, 109-118 (2006).
7. L. Mattio and C. E. Payri, Bot. Rev. **77**, 31-70 (2011).
8. W. S. Atmadja, *Pengenalan Jenis-Jenis Rumput Laut Indonesia* (Puslitbang Oseanologi, LIPI, Jakarta, Indonesia, 1996).
9. A. M. Breeman, *Expected Effects of Changing Seawater Temperatures on the Geographic Distribution of Seaweed Species*, Expected Effects of Climatic Change on Marine Coastal Ecosystems, edited by J. J. Baukema (Springer, Dordrecth, 1990) pp. 69-76.
10. B. Santelices, Oceanogr. Mar. Biol. **28**, 177-276 (1990).
11. A. Jueterbock et al., Ecol Evol **3**, 1356-1373 (2013).
12. S. H. Brawley and L. E. Johnson, British Phycology Journal **27**, 233-252 (1992).