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*Research Article*

# The Genetic Diversity of Macroalgae on The Ekas Coast of East Lombok Based on Chromatogram Data from PCR Ampification

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## Abstract

Macroalgae are multicellular photosynthetic organisms that live in water. Some species of macroalgae are very difficult to identify based on their morphology alone because they have great similarities. Therefore, identification of macroalgae diversity based on its genotype is very important to do in order to enrich the genetic information of macroalgae found on one of the beaches on the island of Lombok. This study aimed to analyze the genetic diversity of macroalgae from Pantai Ekas Beach, Jeowaru District, East Lombok, NTB using 18S rRNA gene markers. The research was carried out in 4 stages, namely sampling, morphological analysis of samples, isolation of total macroalgae DNA, isolation of genes by PCR amplification, agarose gel electrophoresis. Sampling was carried out at certain coordinates at east longitude and south latitude using the Ekas Beach quadratic transect method. The morphological analysis of the samples was guided by an identification key. Total DNA, macroalgae were isolated based on the spin column I DNA kit easy method. Genes were isolated by means of PCR amplification. Total DNA isolates and gene amplicons were confirmed by agarose gel electrophoresis. The results obtained were based on their morphology, all samples obtained were grouped into 9 macroalgae samples which could be divided into brown macroalgae (3 samples), green macroalgae (2 samples) and red macroalgae (4 samples). All samples have successfully isolated total DNA and its 18S rRNA gene which was confirmed by agarose gel electrophoresis chromatogram.

**Keywords:** *Genetic diversity, 18S rRNA gene, PCR amplification, macroalgae, Ekas beach*.

## 1. Introduction

Macroalgae is one of the natural resources with species richness that has a very important role in the world of health. This is because macroalgae contain several types of compounds that are reported to have anti-cancer activity, for example kahalalide F from Bryopsis algae, -carotene from the algae Rhodymenia pseudopalmata, Eucheuma serra agglutinin (ESA), protein fraction from red algae, Euchema spinosum, and crude extracts. fucoidan isolated from the brown algae Sargassum polycystum (Smith, 2014).

Karnan et al (2016) stated that ecologically, the complexity formed by this macroalgae has the same function as coral reefs, seagrass beds, and mangroves, namely as a nursery ground, a feeding ground, and a spawning ground. ground) and lay eggs for various kinds of marine life such as fish and squid. The existence of this macroalgae community can also reduce the current velocity so that it plays an important role in saving the coast and can play a role in increasing the value of biodiversity.

Indonesia has great potential in macroalgae production, both cultivated macroalgae and wild macroalgae. This great potential is supported by Indonesia's long coastline. This is evidenced by Indonesia's aquaculture production which has increased from 2000 to 2015, where macroalgae is one of the main aquatic plants for aquaculture production. During the last decade, Indonesia's macroalgae production has increased sharply where in 2015 the production of Indonesian aquatic plants, especially macroalgae, reached 10 million tons (FAO, 2017).

On the other hand, Indonesia's macroalgae biodiversity has generally only been classified based on its morphology, not genetically. Some macroalgae species are very difficult to distinguish based on morphology, such as red macroalgae (Gracilaria spp.) (Sze-Looi et al., 2015). Diversity starts at the gene level (genetic diversity) to the ecosystem level (population diversity). Genetic diversity occurs due to differences in the sequence of nucleotide bases in the same species (Ellegren, 2016). Differences in the base sequence in a gene allow changes in the morphology of an organism that can be monitored with the naked eye without the help of special tools (Suryanto, 2013).

The 18S rRNA gene is one of the genetic markers used to identify species. The 18S rRNA gene has a conserved region and a variable region. Variable areas are areas that can be used as genetic markers. Variable region V4 is the variable region of the 18S rRNA gene which is highly recommended in analyzing and identifying genetically species (Hadziavic, 2014).

Indonesia's macroalgae habitats are scattered in coastal areas that have coral substrates, coral fragments and muddy sand, such as the Riau Islands, Bangka Belitung, Thousand Islands, Karimunjawa, Sunda Strait, South Java Sea Coast, West Nusa Tenggara, East Nusa Tenggara, islands in Sulawesi and Maluku (Kadi, 2016). Ekas Beach, Jerowaru District, East Lombok Regency, West Nusa Tenggara Province is one of the beaches on the island of Lombok, West Nusa Tenggara which has coral substrate, coral fragments and sand.

The West Nusa Tenggara region is an area that has enormous potential in the development of macroalgae, especially seaweed. This is evidenced by the establishment of the cattle, corn and seaweed development program (PIJAR) as a regional flagship program (NTB Provincial Government, 2013). Based on the description above, it is very necessary to carry out research on Identification of Macroalgae Genetic Diversity in Ekas Beach, Jerowaru District, East Lombok Regency, NTB Province using 18S rRNA Gene Markers.

## 2. Materials and Methods

### 2.1 Materials

The tools used in the study were clear plastic, cooler box, micro pipettes measuring 1–10 L, 10–100 L, and 100–1000 L (Biologix), mortar and pestle, tweezers,

100 mL measuring cup (Iwaki), Erlenmeyer 100 mL (Iwaki). The instruments used consisted of a DSC-H300 digital camera (Sony), MAC-601 autoclave (EYELA), micro centrifuge (Prism TM R), TM water bath (MyBath), vortex mixer (Labnet), refrigerator (Toshiba), analytical balance (Ohaus Pioneer), PCR machine (BioRad), agarose gel electrophoresis apparatus (Biorad), GPS (Global Positioning System) (Garmin), thermometer, multiparameter HI 9828 (Hanna Instruments), light microscope (Nikon) and UV wave transilluminator 254 nm (Vilber Lourmat).

The materials used in the research were aquadest, ice cubes, liquid nitrogen, PVP (polyvinyl pyrrolidone), CTAB (cetyl trimethyl ammonium bromide) (SigmaAldrich), EDTA (ethylenediamine tetra acetic acid), SDS (sodium dodecyl sulfate), mercaptoethanol, tris base, HCl, chloroform, isoamyl alcohol, NaCl, ethanol, isopropanol (Merck), DNeasy Plant Mini Kit (Qiagen), 1kb DNA marker (Promega), lambda DNA (Promega), nucleus-free water (Thermo scientific), gene primer marker (18S rRNA region V4 and partial rbcL) (Macrogen), intact 18S rRNA marker gene primer (1st BASE), white tip, yellow tip, blue tip (Biologix), agarose (Promega), ethidium bromide (Sigma-Aldrich), RNase A (Thermo scientific), PCR buffer solution 10X (Thermo scientific), MgCl2 (Thermo scientific), dNTP (Thermo scientific), Taq DNA polymerase (Thermo scientific) and loading dye (Thermo scientific).

## 2.2 Methods

*Macroalgae Sampling*

Sampling of macroalgae was carried out in tidal beach conditions with sunny weather. Seawater conditions at the time of sampling were analyzed with several parameters, including: pH, temperature (°C), conductivity (µS/cm), total dissolved solids (ppt) and salinity (PSU). Seawater analysis was carried out on each square that was submerged in seawater during sampling.

*Morphological Analysis of Macroalgae Samples*

Morphological analysis was carried out based on visual observations and documented using digital camera photos. Parameters observed included color, shape and branching of the thallus. The morphology of macroalgae samples was evaluated and compared with the morphology of macroalgae found in the literature (Guiry and Guiry, 2018).

*Isolation of Macroalgae Total DNA Based on Spin Column Metode Method*

The procedure used is based on the spin column method of the DNeasy Plant Mini Kit (Qiagen) with some modifications.

*Agarose Gel Electrophoresis*

The results of total DNA isolation and gene isolation were analyzed and visualized by electrophoresis on 1% (w/v) agarose gel in 1X TAE buffer solution (1 L

TAE 50X). Electrophoresis was carried out with a voltage of 70 volts for 40 minutes. Visualization of DNA bands after electrophoresis was carried out by irradiating UV light at 254 nm.

*Gene Isolation by PCR Amplification*

Isolation of the 18S rRNA gene in the V4 region was carried out by PCR amplification using primer pairs F-5'- CAG CAG CCG CGG TAA TTC C - 3' (advanced primer) and R-5'- CCC GTG TTG AGT CAA ATT AAG C - 3 ' (backward primary) (Hadziavdic et al., 2014). In general, PCR is carried out using the Taq DNA polymerase enzyme from Thermo scientific. The composition of the reaction mixture with a total volume of 25 L consisted of: 1 L DNA isolate, 1µM forward and reverse primers, 200 M dNTP mixture, 2.5µL 10X PCR buffer solution, 2.5 mM MgCl2, 1 unit Taq DNA polymerase , and nucleus-free water up to a volume of 25 L.

## 3. Result and Discussion

### 3.1 Result

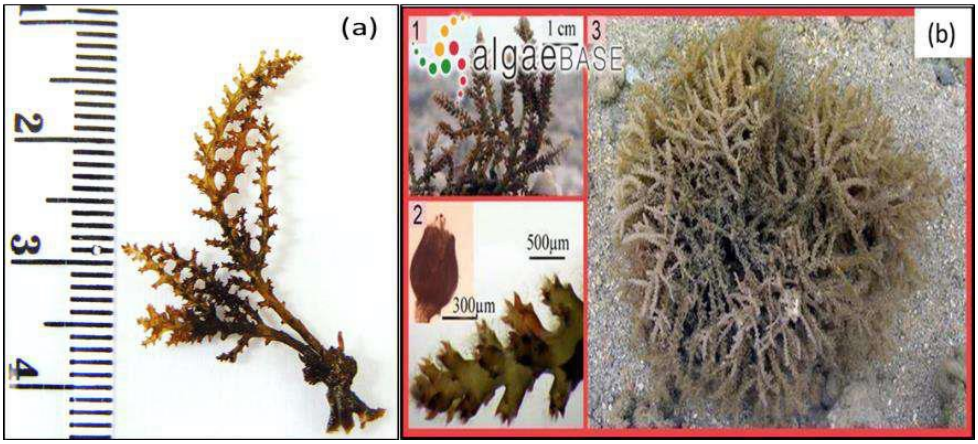


**Figure 1**. The morphology of the red macroalga Acanthophora spicifera. (a) the morphology of the AS1 sample, and (b) the morphology of Acanthophora spicifera from the literature (Eduard and Titlyanova in Guiry and Guiry, 2018).

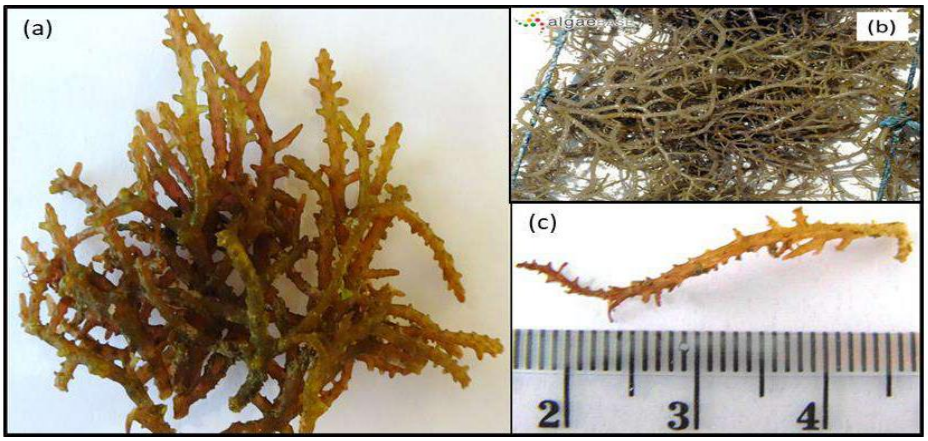


**Figure 2.** Morphology of red macroalgae Eucheuma sp. (a and c) morphology of Eu1 samples, and (b) morphology of Eucheuma denticulatum (Guiry, 2015 in Guiry and Guiry, 2018).

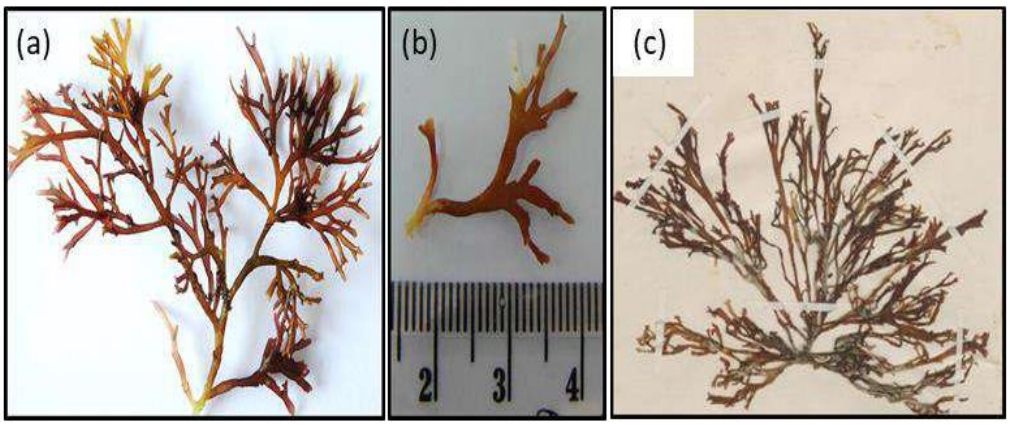


**Figure 3**. The morphology of the red macroalgae Gracilaria foliifera. (a-b) the morphology of the S14 sample, and (c) the morphology of the Gracilaria foliifera specimen from the literature (Natural History Museum, 2014).

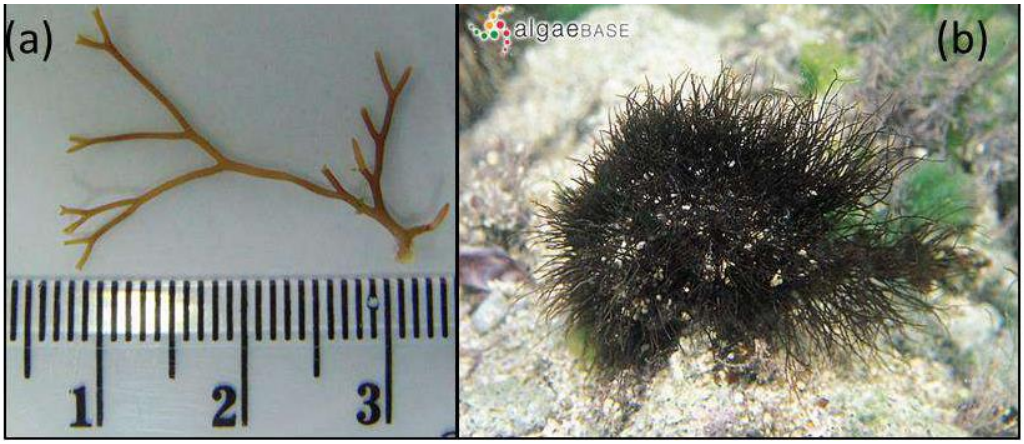


**Figure 4**. The morphology of the red macroalga Wurdemannia miniata. (a) morphology of GC1 samples, and (b) morphology of Wurdemannia miniata from the literature (Oesterlund in Guiry and Guiry, 2018)


**Figure 5**. Morphology of brown macroalgae Hormophysa cuneiformis. (a) morphology of Dsp samples, (b) morphology of Hormophysa cuneiformis from the literature (Suzuki, 2015 in www.natural-history.main.jp).


**Figure 6**. The morphology of the brown macroalgae Padina australis. (a) morphology of PA samples, and(b) morphology of Padina australis from the literature (Palau International Coral Reef Center in Guiry and Guiry, 2018). The morphology of the brown macroalgae Padina australis. (a) morphology of PA samples, and (b) morphology of Padina australis from the literature (Palau International Coral Reef Center in Guiry and Guiry, 2018).


**Figure 7**. Morphology of brown macroalga Turbinaria ornata. (a) morphology of TO samples, and (b) morphology of Turbinaria ornata from literature (Huisman in Guiry and Guiry, 2018).


**Figure 8**. Green macroalgae morphology Ulva intestinalis. (a) morphology of EI samples, and (b) morphology of Ulva intestinalis from the literature (Sotogawa-cho in Guiry and Guiry, 2018).


**Figure 9**. Ulva reticulata green macroalgae morphology. (a) morphology of UR samples, and (b) morphology of Ulva reticulata from the literature (ABC Taxa in Guiry and Guiry, 2018).

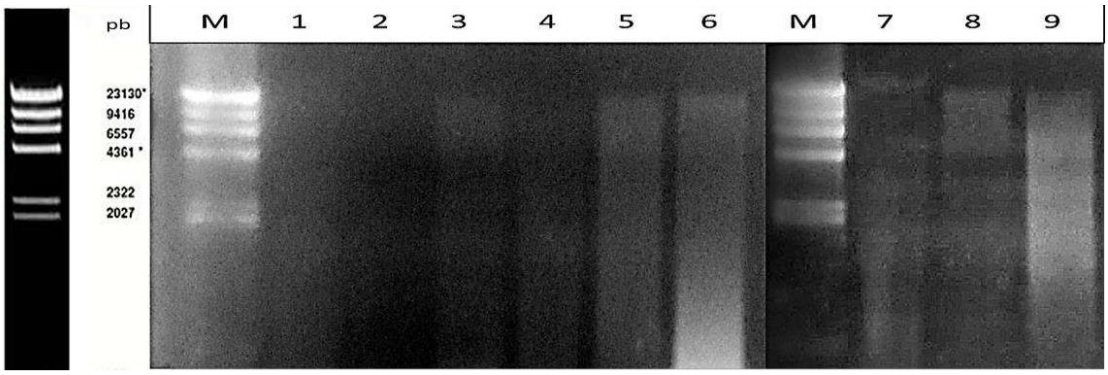


**Figure 10**. Electrophorogram of the total DNA of red macroalgae samples using the spin column-based isolation method from the DNeasy Plant Mini Kit. M = Lambda DNA/HindIII markers, 1 = UR, 2 = EI, 3 = TO, 4 = PA, 5 = Dsp, 6 = GC1, 7 = S14, 8 = Eu1 and 9 = AS1

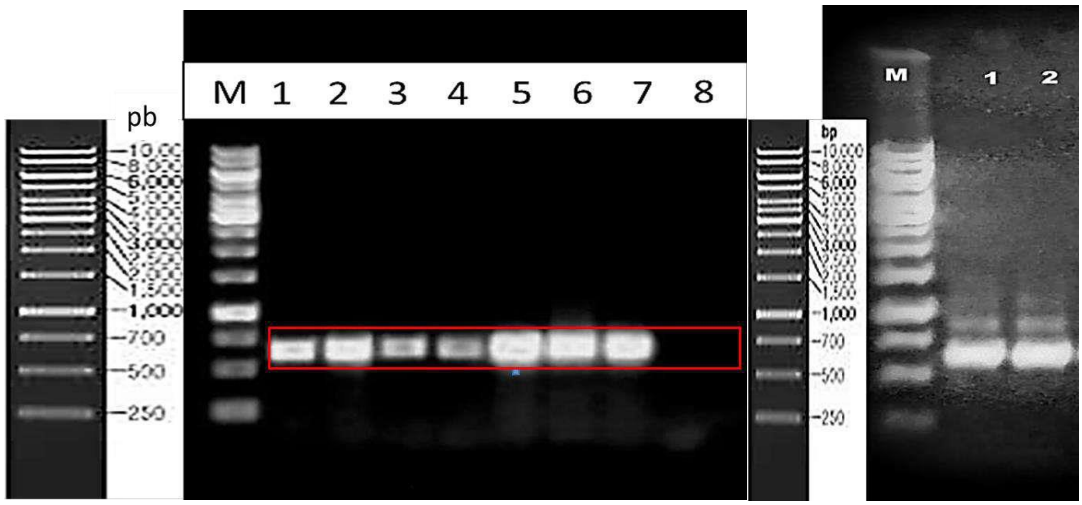


**Figure 11**. Electrophorogram of the 18S rRNA gene amplicon region V4 from several macroalgae samples. (A) M = 1kb DNA ladder marker, 1 = UR, 2 = EI, 3 = TO, 4 = PA, 5 = Dsp, 6 = GC1, 7 = S14 and 8 = ddH20 / control (-), (B) M = marker 1kb DNA ladder, 1 = Eu1 and 2 = AS1 (all samples used 10x dilution of total DNA isolate as PCR template).

### 3.2 Discussion

Morphological analysis of macroalgae was carried out at the Integrated Laboratory of the Biology Unit of UIN Mataram. The results of morphological analysis showed that 9 samples of macroalgae could be distinguished into brown macroalgae (3 samples), green macroalgae (2 samples) and red macroalgae (4 samples). The identities of the 9 samples based on their morphology in the literature are as follows: Acanthophora spicifera, Eucheuma sp., Gracilaria foliifera, Wurdemannia miniata (red macroalgae), Hormophysa cuneiformis, Padina australis, Turbinaria ornata (brown macroalgae), Ulva intestinalis and Ulva reticulata (green macroalgae).

Total DNA isolation has been successfully carried out for 9 samples of macroalgae, it can be seen from the chromatogram of the electrophoresis result. All red, brown and green macroalgae samples were isolated by spin column based method from the DNeasy Plant Mini Kit (Qiagen). The electrophorogram of the five total DNA isolates is shown in Figure 10. The electrophorogram shows that DNA isolation has been successful in the presence of bright bands of DNA or polish. Bright bands indicate large (intact) fragments of total DNA. Polishing indicated that DNA was degraded during the isolation process.

The isolation of the 18S rRNA gene in the V4 region has also been successfully carried out using the PCR amplification method with a primer that has been previously designed. The results can be seen in the chromatogram image of the electrophoresis results. Total DNA from red, brown and green macroalgae samples is believed to have been isolated based on the positive results of amplification of the V4 region of the 18S rRNA gene in each macroalgae sample. The 18S rRNA gene in the V4 region from 9 samples of macroalgae has been successfully amplified by PCR (Polymerase Chain Reaction). The electrophorogram of the 18S rRNA gene region V4 from samples UR, EI, TO, PA, Dsp, GC1, S14, Eu1 and AS1 is shown in Figure 11. The 18S rRNA gene region V4 is ~600 bp in size as indicated by the position of the amplicon band between bands 2 ( 500 bp) and 3 (700 bp) of 1kb DNA ladder markers. The Dsp sample (morphologically brown macroalgae) was re-PCR (re-PCR) on the amplicons from the first PCR (used as a PCR template for the second). re-PCR of Dsp samples was carried out because the results of the first PCR had a small quantity/concentration of DNA. The first PCR electrophorogram and re-PCR of the 18S rRNA gene amplicon in the V4 region of the Dsp sample can be seen in Figure 11.

### Conclusion

The results obtained were based on their morphology, all samples obtained were grouped into 9 macroalgae samples which could be divided into brown macroalgae (3 samples), green macroalgae (2 samples) and red macroalgae (4 samples). All samples have successfully isolated total DNA and its 18S rRNA gene which was confirmed by agarose gel electrophoresis chromatogram.

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