DNA barcoding of *Eucheuma* sp. collected from Ekas Bay, East Lombok, West Nusa Tenggara, Indonesia using the *Cytochrome Oxidase Subunit I* (COI) gene

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Abstract. Seaweed is a fundamental component of the marine ecosystem. As a primary producer seaweed has the ability to providing food resources, habitat, supporting coastal and marine biodiversity. Molecular identification is needed to determine the genetic diversity of a seaweed species, so that differences between types can be seen clearly and provide opportunities to find new characters of a species. This study aims to determine the species of *Eucheuma* sp. cultivated in Ekas Bay, East Lombok, NTB. Sample identification was carried out by morphological observation and molecular identification through DNA barcoding techniques. The morphological features of *Eucheuma* sp. included a cylindrical thallus, a disc-shaped holdfast, pointed or blunt branches, and the presence of nodules. The results of the molecular study identified the species *Eucheuma* sp. identical 100% having a level of similarity with *Eucheuma denticulatum* from the Philippines using *the Cytochrome Oxidase Subunit I* (COI) Gene. The results of the most similar kinship were marked by a Query Coverage value approaching 92%, an E-value approaching 0, and Per. Ident approaching 100% in each database. This research enhances the understanding of *Eucheuma* sp. genetic diversity, which can be utilized for the sustainable management of marine biological resources.

1. Introduction

Seaweed is a plant that contains chlorophyll and is classified as a lower plant that does not have true roots, stems, or leaves, but only resembles a stem called a thallus [1]. As a multicellular alga, seaweed can live by attaching to two types of substrates: soft and hard, such as sand, mud, a mixture of sand, gravel, rocks, dead coral, shells, live coral, and other hard objects. Based on their pigments and morphological characteristics, seaweed is divided into three main categories: brown algae (Phaeophyta), red algae (Rhodophyta), and green algae (Chlorophyta) [1]. This shows that seaweed can adapt to all environmental changes. In Indonesia, West Nusa Tenggara is the main center of seaweed production, especially in the East Lombok region and specifically in Ekas Bay, Jerowaru District.

The main role of seaweed in aquatic environments is as a supporter of substrate base and a provider of carbonate to maintain the balance and sustainability of coral reefs. Furthermore, seaweed acts as a primary producer in the aquatic food chain and is considered a natural food source for various marine organisms [2], while also providing protection, supporting coastal and marine biodiversity, maintaining ecosystem balance, and contributing to aquatic productivity. As a country with abundant seaweed diversity, Indonesia has massive economic potential if this resource is properly utilized, including for pharmaceuticals, food, and carious other industries.

Eucheuma sp. is a seaweed belonging to the Rhodophyta group, characterized by a cylindrical thallus, blunt or pointed branch tips, and the presence of nodules or thorns [3]. In Indonesia, seaweed identification has traditionally relied on morphological observations. However, misidentification may occur because morphological characteristics can vary depending on environmental conditions, leading to errors in biodiversity assessments. Therefore, molecular identification based on DNA sequences offers a more accurate approach for species determination and provides more precise information regarding the genetic aspects of species and biodiversity [4]. Genetic uniqueness based on mitochondrial DNA sequences and plastid genes or spacers of this variety has been questioned [5]. Red algae are difficult to identify at the

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species level because of their high phenotypic plasticity under varying environmental conditions and the difficulty of finding reliable diagnostic morphological characters. Molecular methods have proven to be reliable for species identification and determining genetic variation within species. DNA barcoding has been widely applied to identify species in red algae, evaluate genetic variation, reveal cryptic species, and map the level and distribution of genetic variation within habitats. The mitochondrial *Cytochrome Oxidase Subunit I* gen (COI-5P) is a molecular marker commonly used in species identification [6]. This marker has a length of approximately 600 base pairs, longer than other molecular markers previously used. In taxonomy and phylogenetic studies, COI has been widely utilized to identify *Kappaphycus* species. In red algae, the COI marker shows high reliability and greater genetic variation compared to the COX2-3 spacer marker, particularly in *eucheumatoid* groups [7, 8].

DNA barcoding is a technology used for species identification based on the detection of target genes. This technique uses universal primers and specific mitochondrial DNA to identify difficult-to-identify species [9] . Therefore, this study aims to identify and confirm the species identity of cultivated *Eucheuma* sp. from Ekas Bay, East Lombok, using DNA barcoding based on the *Cytochrome Oxidase Subunit I* (COI) gene. Sustainable utilization of biological natural resources without considering environmental conditions may threaten national biodiversity, thus sustainable regeneration efforts are required to improve both quantity and quality [4].

2. Materials and Methods

Sample Collections. This study was conducted from July 2024 to January 2025. Seaweed samples were collected from a cultivation site in Ekas Bay, East Lombok, West Nusa Tenggara (NTB), Indonesia. Healthy and undamaged thalli with typical morphological characteristics were selected for analysis. After harvesting, the samples were cleaned to remove dirt and epiphytic organisms attached to the thalli [10]. Morphological observations were then carried out based on several characteristics, including colour, holdfast structure, branching pattern, and thallus shape [11]. Subsequently, thallus fragments were collected and placed in sterile zip-lock bags. The samples were stored in an ice box containing ice packs to maintain sample quality and minimize DNA degradation during transportation to the laboratory before DNA extraction and molecular analyses.

Molecular identification. Molecular identification is carried out through several stages, namely isolation by taking 0,3 g of sample, placing it into a mortar and pestle, and adding liquid nitrogen to grind it into a fine powder. This is followed by DNA extraction using KOD^{TM} One PCR Master Mix. PCR amplification was carried out using the primer pair GazF1 5'-TCA ACA AAT AAA GAT ATT GG -3' dan GazR1 5'- ACT TCT GGA TGT CCA AAA AAY CA -3' [12]. DNA amplification is performed in a volume of 50 µL with the following composition: KOD^{TM} One PCR Master Mix 25 µL, primers GazF1 and GazR1 each 1,5 µL, DNA template 1,25 µL, and PCR water 20,75 µL.

PCR was performed an IKA HB Eco (China) using the amplification conditions as follows: 98°C 10 min (pre-denaturation), 98°C 10 s (denaturation), 49°C 5 s (annealing), 68°C 10 s (elongation), and 68°C 10 min (final elongation). The final stage is electrophoresis to examine DNA amplification results, using 1% agarose gel that has been supplemented with 2 µL of florosafe in an electrophoresis apparatus under an electric field to visualize DNA bands.

3. Result and Discussion

The sampling of this seaweed *Eucheuma* sp. (ESL1) showed results where the seaweed was studied morphologically and compared with molecular identification results. Morphologically, the specimen exhibited a cylindrical thallus measuring approximately 5–30 cm in length, with a transparent reddish-brown to yellowish-red coloration (**Figure 1).**

Figure 1. Morphology of the red seaweed *Eucheuma* sp. (ESL1)

The holdfast was disk-shaped, used to firmly attach to rocky and coral substrates. The branching pattern is irregular with pointed or blunt tips. The thallus surface was covered with irregular spine-like protuberances, some of which developed into branch-like structures. Dense branching gave the thallus an upright and clumped appearance. These characteristics are generally consistent with those reported for Eucheuma species. Based on the report in [4], this seaweed has morphological characteristics including a cylindrical thallus, branching with pointed or blunt ends, and the

presence of nodules. The classification of *Eucheuma* sp. is follows.

Classification of *Eucheuma* sp.
Division: Rhodophyta
Class: Rhodophyceae
Order: Gigartinales
Family: Solieriaceae
Genus: *Eucheuma*
Species: *Eucheuma spinosum* [13]

Seaweed morphology may change in response to environmental factors, even within the same species. These characteristics are influenced by environmental factors such as temperature, salinity, pH, currents, and light intensity. These differences include thallus length, number of branches, and leaf size, all of which are affected by local environmental conditions such as wave exposure and nutrient availability [14]. Seaweed morphology is not only influenced by its environmental but also by interactions between genetic and environmental factors. This indicates that morphological adaptation of seaweed is a complex and inseparable combination of genetic and environmental. DNA barcoding was used to verify the taxonomic identity of ESL1 and to examine its phylogenetic relationship with other Eucheuma species and related taxa included in this study.

The PCR visualization results through UV transilluminator irradiation, marked by sample code ESL1 using *Cytochrome Oxidase Subunit I* (COI) primers showed a DNA band size of approximately 700 bp. Based on COI gene sequencing results analysed using *Basic Local Alignment Search Tool* (BLAST) showed alignment results consistent with morphological identification(**Table 1).** *Eucheuma* sp. shows similarity with *Eucheuma denticulatum*, where the NCBI database records a similarity value (Per. Ident) of 100% from the Philippines and an E-value of 0.0. This finding is consistent with previous [5], who stated that the most similar results are indicated by Query Coverage close to 100%, E-value close to 0, and Per. Ident close to 100% in each database. According to this reference, *Eucheuma* sp. can be considered 100% identical to *Eucheuma denticulatum* from the Philippines, which is also illustrated in the phylogenetic tree in **Figure 2**. *Eucheuma* sp. (ESL1) has a closer and shorter evolutionary distance to *Eucheuma denticulatum* from the Philippines.

Phylogenetic analysis results show that *Eucheuma denticulatum* isolate from the Philippines (EU0334419.1), as indicated by a bootstrap value of 100 in BLAST analysis. Bootstrap values between 70 and 100 indicate high confidence in evolutionary relationships within small clades, although some changes may still occur. Meanwhile, bootstrap values below 70 indicate that larger clade structures are unstable and may change. If two species have high bootstrap values and are located on adjacent or parallel branches, this indicates a close evolutionary relationship [15]. As shown in **Figure 2**, the bootstrap value for the relationship between *Eucheuma* sp. and *Eucheuma denticulatum* is 100 in a major clade.

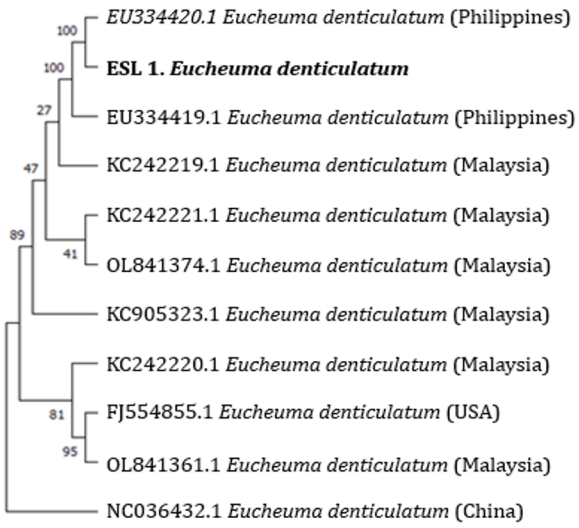

Figure 2. Phylogenetic tree of *Eucheuma* sp. (ESL1) based on the COI (*Cytochrome c Oxidase Subunit I*) gene.

Table 1. BLAST results of the COI gene sequence from *Eucheuma* sp

Sample code	Morfology identification	Moleculer identification				
		GenBank Strain	*Query Cover*	*E Value*	*Per. Ident*	*Accession Number*
ESL 1	*Eucheuma* sp.	*Eucheuma denticulatum*	92%	0.0	100%	EU334419.1 (Philippines)
ESL 1	*Eucheuma* sp.	*Eucheuma denticulatum*	100%	0.0	99.85%	NC036432.1 (China)

[a] Sequence obtained from GenBank

4. Conclusion

The COI gene sequence analysis successfully identified the cultivated seaweed specimen from Ekas Bay, East Lombok, West Nusa Tenggara (NTB), as *Eucheuma denticulatum*, showing 100% sequence similarity with reference sequences in public databases. This molecular identification was supported by morphological characteristics consistent with those of *Eucheuma* sp.*Eucheuma* sp. (ESL1) showed a DA band size of ~700 bp and similarity level of 100% with *Eucheuma denticulatum* from the Philippines. Therefore, molecular approaches based on the COI gene can be used as a reliable method for validating seaweed species, including *Eucheuma* genera, particularly to support proper aquaculture management and sustainable development.

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