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Research Article

Morphological and Molecular Identification Using the *Cox1* Gene in Wild Populations of *Gracilaria* sp. from Ekas Hamlet

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Abstract: Ekas, located in East Lombok Regency, is the center of seaweed cultivation. One of the seaweeds found in Ekas waters is the genus *Gracilaria*. Seaweed is an organism with high phenotypic plasticity so that its morphological characters change easily. In addition to morphological identification, molecular identification is also carried out as a more accurate alternative. Cox1 is one of the genes in mitochondrial DNA used in determining the genetics of organisms, especially seaweed genetics. This study aims to determine the results of the identification of *Gracilaria* sp. morphologically and molecularly. Morphological identification refers to identification books, AlgaeBase, and related journals. Data analysis used ChromasPro and BioEdit programs. The fasta data results were blasted on the NCBI web to determine the species. Fasta results are also used in phylogeny analysis to determine kinship information of research samples with data samples in NCBI. In this study, 1 research sample was obtained at 1 sampling point. The results of morphological identification were identified as *Gracilaria edulis* and the results of molecular identification with a series of bioinformatics analysis were also confirmed as *Gracilaria edulis*. *Gracilaria* EKS_035 has the closest kinship with *G. edulis* KY995636.1 Philippines, *H. edulis* JQ026083.1 Malaysia and *G. edulis* KY995635.1 Philippines.

Keywords: Morphological Identification, Molecular Identification, Cox1 Gene, *Gracilaria* sp., Ekas

INTRODUCTION

One of the provinces in Indonesia, West Nusa Tenggara (NTB), has the potential to develop seaweed cultivation, especially on Lombok Island. Seaweed is widely spread in tidal areas, one of which is in the waters of Ekas. Ekas is a well-known area as a center for seaweed cultivation. Environmental conditions in Ekas are very good so that they can support the growth of seaweed. Various kinds of seaweed can be found in Ekas waters, one of which is the genus *Gracilaria* seaweed [1].

Seaweed from the genus *Gracilaria* is a very potential resource, especially used as a source of phycocolloids [2][3]. Phycocolloids is a polysaccharide found in seaweed cell walls [4]. The seaweed genus *Gracilaria* is the largest producer of extracted phycocolloids, namely agar. Global agar production was recorded in 2016 at 14.500 tons [5].

Seaweed is an organism with high phenotypic plasticity [6]. Unstable environmental conditions can cause morphological changes in seaweed [7]. The identification process is generally based on the morphological characteristics of the

organism. The limitations that exist in the morphological identification process cause misidentification of species [6].

Molecular identification based on genetic information using DNA sequences is more accurate. Cox1 (cytochrome c oxidase subunit 1) is one of the genes in mitochondrial DNA used to determine the genetics of an organism. The Cox1 gene can be used to determine the genetics of seaweed species, especially in red seaweed (Rhodophyta) [8]. Therefore, morphological and especially molecular identification is needed to provide more accurate information about the genetics of an organism, especially in the genus *Gracilaria* seaweed.

MATERIALS AND METHODS

Study Area

Gracilaria sp. seaweed samples were taken in the waters of Ekas Hamlet (8°52.133'S 116°26.222'E), East Lombok, West Nusa Tenggara (Figure 1). Seaweed was taken at low tide using Purposive Random Sampling technique by taking seaweed at the location on the substrate of muddy sand, dead coral, and seagrass. Samples obtained were then documented and isolated for identification at the PUI Laboratory, Bioscience and Biotechnology, Faculty of Mathematics and Natural Sciences, Mataram University. The samples obtained were then cleaned, labeled with a code, and dried at room temperature for analysis at a later stage.

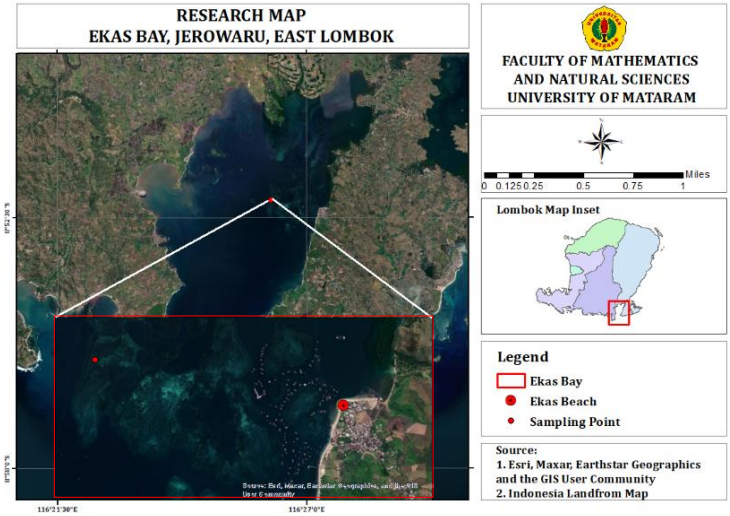

Figure 1. Research Map of *Gracilaria* sp. Sampling (ArcGis)

Morphologycal Identification

Identification was carried out using qualitative analysis by paying attention to the morphological characteristics of seaweed samples such as color, talus, durability, branching patterns, and so on. Species identification refers to reference identification books, AlgaeBase, and related journals.

Molecular Identification

Powder samples were extracted using the Plant DNeasy KIT (Qiagen). PCR process using Cox1 gene primers COXI43F and COXI1549R, mitochondrial Cox1 sequences were aligned and then used in designing specific primers for amplification of the *Gracilaria* sp. gene region: COXI43F-5' TCA ACA AAT CAT AAA GAT ATT GGW ACT 3' and reverse: COXI1549R-5' AGG CAT TTC TTC TTC AAA NGT ATG ATA 3'). PCR was programmed for 35 cycles consisting of pre-denaturation at 94°C for 4 minutes, denaturation at 94°C for 30 seconds, annealing at 55°C for 1 minute, and extension at 72°C for 1 minute. DNA ladder and DNA

products from PCR were injected into the wells of agarose gel, electrophoresis was performed at 250 volts within 45 minutes, then the results were documented through UV Transluminator. PCR products were sequenced using the Sanger Sequencing method. BLAST analysis was performed on the NCBI web with the aim of observing similarities in GenBank. Sample sequences that were a combination of F' and R' were entered into the BLAST process. Phylogenetic tree created using bootstrap analysis (2.000 replicates).

RESULTS AND DISCUSSION

Individual samples of *Gracilaria* sp. taken at 1 point in the waters of Ekas Hamlet, Jerowaru District, East Lombok Regency were labeled with sample code EKS_035 (Figure 2). Morphological characters observed were color, talus, attachment, branching pattern, and also habitat (Table 1).

Figure 2. *Gracilaria* sp. Research Sample

Table 1. Morphological Characteristics of *Gracilaria* sp. Research Sample

Morphological Characteristics	*Gracilaria* sp.
Color	Reddish Brown with Yellowish Tips
Thallus	Cylindrical, Length= 22cm Height= 20cm Diameter= 1mm
Holdfast	Disc
Branching Pattern	Dichotomous
Habitats	Muddy sand, Dead corals and Seagrasses

After identification of the research sample *Gracilaria* sp. morphologically referring to identification books, Algaebase and related journals, the research sample was confirmed as *Gracilaria edulis*. Furthermore, molecular identification is based on the results of sequencing analysis. Molecular identification, especially in red seaweed (Rhodophyta) has been successfully applied, including *Gracilaria* sp. [8]. Molecular identification starts from sample preparation, sample DNA isolation and measurement of DNA concentration and purity, gel electrophoresis and UV transilluminator, sequencing analysis, sequencing result data analysis, blast analysis, and finally phylogeny analysis.

The result of DNA concentration was 10.8 ng/µL and DNA purity at 260/280 ratio was 2.00. The DNA concentration obtained is sufficient for use in molecular analysis. The purity value is measured to determine the presence or absence of contaminants, if the purity value is below 1.8 and above 2.0 then the DNA is

contaminated with RNA and Protein. DNA from PCR is inserted into gel wells which are then electrophoresed then in UV transilluminator and produce DNA bands. The results of sequencing analysis in the form of sequence data were then analyzed by blast by looking for database sequence similarities on the NCBI page which was identified as *Gracilaria edulis* with the highest similarity of 99.79%. Phylogeny analysis using bootstrap 2000 replicates. The bootstrap analysis used shows replication in phylogeny tree visualization. The scale used in tree construction is 0.1 which shows the number of different bases in each sequence. The value of the analysis results from the research sample is 89. The value obtained is quite good, the higher the value obtained indicates good sequence quality, close kinship, and a high level of confidence in the research sample and database sample. The research sample *Gracilaria* (*Gracilaria* EKS_035) is most closely related to *G. edulis* KY995636.1 Philippines, *H. edulis* JQ026083.1 Malaysia, and *G. edulis* KY995635.1 Philippines.

Table 2. The BLAST results obtained from the partial sequence of the Cox1 gene for samples from the waters of Ekas Hamlet were compared with the results of morphological identification.

Sampling Location	Waters of Ekas Hamlet
Coordinate Point	8°52.133'S 116°26.222'E
Code	EKS_035
Morphological Identification Results	*Gracilaria edulis*
Molecular Identification Results	*Gracilaria edulis*
Accession Number	MZ336086.1
Percent Similarity	99,79%

> Fasta Sequence EKS_035 Cox1
TATTGGTACTTTATATTTAATTTTTGGTGCCTTTTCAGGAGTTCTAGGAGGAT
GTATGTCAATATTAATTCGTATGGAGTTAGCACAACCAGGAAATCAATTATTA
TTGGGAAATCATCAGATTTATAATGTTTTAATAACTGCACATGCATTTTTAATG
ATTTTTTTTATGGTTATGCCTGTAATGATAGGAGGCTTTGGTAATTGGTTAGT
ACCTATTATGATAGGAAGTCCTGATATGGCTTTTCCTCGCTTAAATAATATAT
CTTTTTGATTGTTGCCACCTTCTCTTTGTTTACTTATAGCTTCTGCAATTGTG
GAAGTAGGTGTGGGAACAGGATGAACTGTATATCCCCCATTAAGTTCAATTC
AAAGTCATTCTGGAGGAGCTGTAGATTTGGCAATATTTAGTTTACATATATCA
GGAGCATCTTCAATTTTAGGAGCAATAAATTTTATTTCAACAATTTTAAATATG
CGTAACCCTGGTCAAAGCATGTATCGTATGCCTTTATTTGTTTGATCAATTTT
TATAACCGCGTTTTTATTATTATTAGCCGTACCTGTTTTAGCTGGGGCAATTA
CCATGCTTTTAACAGACCGTAATTTTAATACAGCCTTTTTTGATCCAGCCGGA
GGAGGAGATCCTGTATTATATCAACACCTTTTTTGATTTTTTGGACATCCAGA
GGTTTATATTTTAATACTTCCGGGTTTTGGTATGGTTAGTCATATAGTAGCAA
CTTTTTCCCGAAAACCTGTTTTTGGTTACATTGGAATGGTTTATGCTATGGTT
TCAATTGGAGTGTTAGGTTTTATAGTTTGGGCACATCATATGTATACTGTAGG
TTTAGATGTAGATACAAGAGCTTATTTTACTGCAGCTACAATGATTATAGCTG
TACCTACAGGAATTAAAATTTTTAGTTGAATAGCAACAATGTGAGAAGGTTC
GATACATTTTAAAACACCTATGTTATTTGCAACTGGATTTATTTTTTTATTTAC
TATAGGAGGCTTAACAGGTATTGTTTTAGCAAATTCTGGTTTGGATATTAGTT
TGCATGATACTTATTATGTAGTCGCACATTTTCATTATGTATTATCAATGGGT
GCTGTATTTGCAATATTTGCTGGATTTTATTATTGATTTGGTAAAATTACTGG
AGTGCAATATCCTGAATTATTAGGGAAAATACATTTTTGATCTACTTTTATTG
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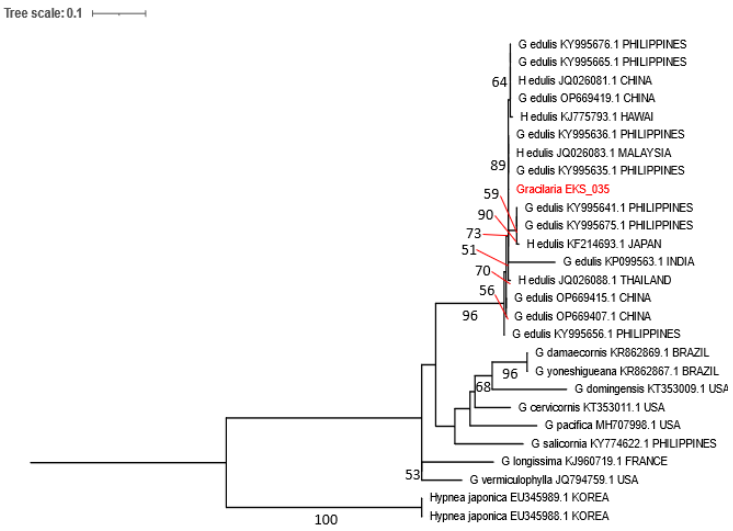

Figure 3. Phylogeny Tree Analysis Results

CONCLUSION

Based on the research that has been done, it can be concluded that the research sample of *Gracilaria* sp. obtained in the waters of Ekas Hamlet, Jerowaru District, East Lombok Regency is morphologically confirmed as *Gracilaria edulis* and molecularly identified as *Gracilaria edulis*.

REFERENCE

[1] Cokrowati, N., Diniarti, N., Setyowati, D. N. A., Waspodo, S., & Marzuki, M. 2019. Ekplorasi dan Penangkaran Bibit Rumput Laut (*Eucheuma cottonii*) di Perairan Ekas Lombok Timur. *Jurnal Biologi Tropis*. 19(1), 51-53.

[2] Arulkumar, A., Rosemary, T., Paramasivam, S., Babu Rajendran, R. 2018. Phytochemical Composition, In Vitro Antioxidant, Antibacterial Potential and GC-MS Analysis of Red Seaweeds (*Gracilaria corticata* And *Gracilaria* sp.) From Palk Bay, India. *Biocatal. Agric. Biotechnol*. 15, 63–71.

[3] Toy, T. S., Lampus, B. S., dan Hutagalung, B. S. 2015. Uji Daya Hambat Ekstrak Rumput Laut *Gracilaria* sp. terhadap Pertumbuhan Bakteri Staphylococcus Aureus. *E-Gigi*. 3(1), 153.

[4] Yang, Y., Chai, Z., Wang, Q., Chen, W., He, Z., and Jiang, S. 2015. Cultivation of Seaweed *Gracilaria* in Chinese Coastal Waters and Its Contribution to Environmental Improvements. *Algal Res*. 9, 236–244.

[5] Porse, H., and Rudolph, B. 2017. The Seaweed Hydrocolloid Industry: 2016 Updates, Requirements, dan Outlook. *J. Appl. Phycol*. 29, 2187–2200.

[6] Hassan, R., Othman, M. N. A., Harith, M. N., and Md Sah, A. S. R. 2019. Morphological Diversity of *Gracilaria blodgettii* Harvey 1853 (*Gracilaria*ceae, Rhodophyta) from Sarawak, Malaysian Borneo. *Scientifica (Cairo)*. 2019: 3430968. DOI: 10.1155/2019/3430968.

[7] Kongkittayapun, N., and Chirapart, A. 2011. Morphometric dan Molecular Analysis of *Gracilaria salicornia* dan Its Adelphoparasite in Thailand. *Scienceasia*. 37: 6-16. DOI: 10.2306/Scienceasia1513-1874.2011.37.006.

[8] Meinita, M. D. N., Akromah, N., Danriyani, N., Setijanto, S., Harwanto, D., and Liu, T. 2021. Molecular Identification of *Gracilaria* Species (*Gracilaria*les, Rhodophyta) Obtained from The South Coast of Java Island, Indonesia, Biodiversitas. *Journal of Biological Diversity*. 2 (7), 3048.