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First Report on Molecular Identification of *Caulerpa* Green Algae from Mandangin Island Indonesia Using Partial 18SrRNA Genes

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Abstract

Caulerpa is one of the seaweed that grows naturally in Indonesian waters such as those in Mandangin Island. This study aimed to identify *Caulerpa* sp. based on molecular analysis using certain genetic markers. This research is expected to provide information on the identification of macroalgae from Indonesia waters, especially Mandangin Island, Madura with the use of molecular analysis based on 18SrRNA primers. The two green seaweed samples from the *Caulerpa* genus in this study were successfully analyzed using 18SrRNA primers. The BLAST results of samples 1and 2 are related to *Caulerpa taxifolia* 18SrRNA, but in the phylogenetic tree result, Sample 1 was more closely related to *Caulerpa sertularioides f. longipes*. 18SrRNA primers have been used for molecular identification of green seaweed from Mandangin for the first time and this shows that barcode markers can be used for molecular identification of seaweed, specifically *Caulerpa* in the waters of Mandangin Island, Indonesia.

INTRODUCTION

One of the marine resources that have high economic value is seaweed. Seaweed can be used as a source of natural products. One type of seaweed whose potential is still not widely utilized is the green seaweed of the genus *Caulerpa*. *Caulerpa* is one of the genera of green algae that can grow in tropical and subtropical regions and it is the object of this research because it has not been well studied up to date.

The potential development of *Caulerpa* is quite good because this seaweed contains nutrients needed by the body and has been known as a traditional food by coastal communities in Indonesia. Several types of bioactive substances found in *Caulerpa* such as alkaloids, flavonoids, terpenoids, tannins, and saponins can be used as food and pharmaceutical ingredients (Lantah *et al*., 2017). Geographically, Mandangin Island is located at coordinates 113°12'8.45" - 113°13'31.21" E and 7°18'22.38" - 7°18' 52.92" S. Mandangin Island is included in the administration of the Sampang

Regency and one of its villages; Pulau Mandangin. Sampang Village has sea borders throughout the island. Pulau Mandangin Village is divided into 3 kindreds which were called by Candin, Kramat, and Barat. Mandangin Island has the potential as a location for the development of seaweed cultivation, one of them is *Caulerpa*.

However, some reports mention that the invasive growth of *Caulerpa taxifolia* in nature can affect the diversity of coastal ecosystems. That is because the active compound called "Caulerpin" inhibits the multixenobiotic resistance mechanism of the sea sponge *Geodia cydonium* which can protect marine organisms from various water pollutants (Shanmugam *et al*., 2018). One identification method that can be used for seaweed is the determination of morphology, this refers to the research of Dawson (1954) but the weaknesses that occur in morphological identification is the difficulty in distinguishing intra species, species reference is still limited, and images of species referred to were still in the form of hand drawings hence they had a relatively low level of accuracy when compared with photographic results.

Research on molecular identification of *Caulerpa* is still rarely performed considering the abundant availability of this species in nature (Camacho *et al*., 2015) including coastal waters in Indonesia. The process of molecular identification of macroalgae also has constraints such as the difficulty of obtaining high purity in the DNA extraction process (Doyle and Doyle, 1990), high inhibitors in the extracted algae (Hoarau *et al*., 2007) and there are still few available DNA sequences of partial DNA genomes contained in Gen Bank.

Genetic markers have been widely used in molecular identification of macroalgae such as rbcL and 18SrRNA (Mahendran and Saravanan, 2017), DNA barcodes (Camacho *et al.,* 2015; Poong *et al*., 2014; Saunders and Moore, 2013; Kher *et al*., 2011; Le Gall and Saunders, 2010; Mattio and Payri, 2010; Lane *et al*., 2007), 18SrDNA (Soylu and Gönülol, 2012), microsatellite (Varela-Álvarez *et al*., 2006).

This study aims to identify *Caulerpa* sp. based on molecular analysis using 18SrRNA markers. This research is expected to be a breakthrough that will contribute to the identification of macroalgae from Indonesian waters, especially in the waters of Mandangin Island, Madura, East Java on a molecular basis based on the 18SrRNA markers.

METHODOLOGY

Place and Time

This research was conducted between September - December 2019. Samples were collected from Mandangin Island coastal waters and then treated in Cell and Molecular Biology Laboratory, Faculty of Science and Technology, Universitas Airlangga.

Research Material

The tools used in the study include plastic bags, cooler box, freezer (-20 °C), digital scale, centrifuge, microtube, incubator, NanoDrop 2000 (Thermo Scientific), electrophoresis equipment, thermocycler machine, pipette, and digital camera (Digitec, Japan).

The materials used to support this study were TRIzol, chloroform, ethanol, NaOH, EDTA, HEPES, universal primer 18SrRNA, agarose gel, and TBE buffer.

Research Design

The samples of *Caulerpa* were identified with DNA extraction, PCR, sequencing, and phylogenetic analysis. DNA extraction was carried out using the Trizol reagent method according to Chomczynski (1993). Sequencing was carried out by referring to the standard Sanger Sequencing method. Contiq sequence was aligned (multiple alignments) with the database using ClustalW and a phylogenetic tree was made with 1000 replications using the Neighbor-Joining (NJ) method with

MEGA 7 (Molecular Evolutionary Genetics Analysis) Software.

Work Procedures

Sampling Location

The two samples of *Caulerpa* sp. were obtained from the intertidal zone in the coastal waters of Mandangin Island, Madura, East Java, Indonesia (Figure 1). The obtained samples were then washed using fresh water and placed into plastic bag with a label and stored in a cooler box during transportation to the Cell and Molecular Biology Laboratory, Study Program of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya where the sample was stored in a freezer at -20 °C upon arrival at the laboratory.

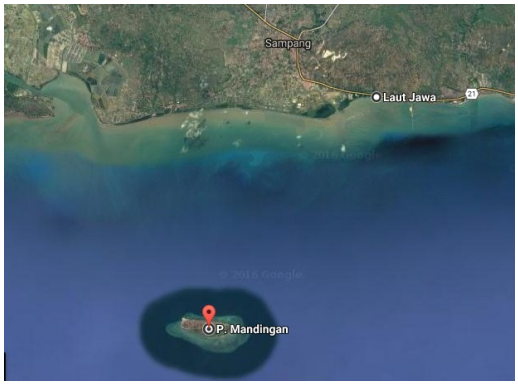

Figure 1. Map of the sampling location of *Caulerpa* sp. taken with Google Maps.

DNA Extraction

DNA extraction was carried out using the Trizol reagent method according to Chomczynski (1993). First, a sample weighing up to 250 g was added to 750 mL of Trizol then mixed by pipetting. Algae contain a lot of fat hence lysate was centrifuged at a speed of 12,000 × g, at 4 °C for 5 minutes. The resulting supernatant was then incubated for 5 minutes. After incubation, 200 µL of chloroform was added and incubated for 2-3 minutes, followed by sample centrifugation, 12,000 × g speeds at 4 °C for 15 minutes.

The results of the centrifuge formed three layers and DNA was in the interphase (middle layer) and 300 µL 100% ethanol (EtOH) was added and incubated for 2-3 minutes followed by centrifugation for 5 minutes with 2,000 × g speed at of 4 °C. The pellet was obtained by removing the supernatant. The result of the pellet in 1 mL 0.1 M Na citrate in 10% EtOH with a pH of 8.5 was incubated for 30 minutes while inverting slowly every 10 minutes for 30 minutes. After 30 minutes, it was centrifuged using 2,000 × g speed at 4 °C for 5 minutes. The supernatant was discarded and this process was repeated once again. The pellet was re-suspended in 1.5 mL 75% EtOH and incubated for 10-20 minutes.

The resulting product of incubation was centrifuged at 2,000 × g speed at 4 °C for 5 minutes. The supernatant was again discarded while the pellet was dried at room temperature for 5-10 minutes. The pellet was re-suspended in 0.3 - 0.6 mL of 8 Mm NaOH and centrifuged for 10 minutes using 12,000 × g speed at 4 °C. The supernatant was taken and put into a new microtube while the pH was adjusted to 7-8 with HEPES and 1 mM EDTA was added and stored at -20 °C. The obtained level of DNA purity was measured using NanoDrop followed by the electrophoresis process.

Polymerase Chain Reaction (PCR)

The PCR reaction was carried out on a Thermocycler machine in the following sequence in Table 1. The total cycle of this process was 35 cycles. The primer used in the PCR reaction is a universal primer

18SrRNATrackittm 1 Kb Plus DNA Ladder (Table. 1).

Table 1. Primers and PCR condition (Mahendran and Saravanan, 2017).

Primer Name	Sequence [5'-3']	PCR Condition
18SrRNA 1	CGATCTATTCAT-TCAATATTTC	Initial 2 min; denaturation at 95°C, 35 cycles of 93°C for 1 min, 54°C annealing for 45 sec, 72°C extension for 2 min, followed by 72°C final extension for 10 min.
18SrRNA 2	TCTAGCACACG-AAAGTCGAAGT	Initial 2 min; denaturation at 95°C, 35 cycles of 93°C for 1 min, 51°C annealing for 45 sec, 72°C extension for 2 min, followed by 72°C final extension for 5 min.

The amplification product was separated with 1% agarose gel (m/v) in 1X TBE buffer (addition of 0.75 µL Floro safe DNA strain) at 90 V for 15 minutes. 1000 bp DNA Ladders are used as long markers of product DNA bases. DNA bands were then documented using a digital camera (Digitec, Japan). The 18SrRNA DNA band appeared as a single band at a base length of 1000 bp.

Sequencing and Phylogenetic Analysis

Sequencing was carried out by the 1st BASE DNA Laboratory, Genetics Science, Singapore by referring to the standard Sanger Sequencing method to obtain contiq results and the results of primary and forward sequences. Contiq sequence results are aligned (multiple alignments) with the database stored in Gen bank referred to National Center for Biotechnology Information (NCBI) through the BLAST (Basic Local Alignment Searching Tool) program to obtain a percentage of similarity (sample similarity) by comparing the *Caulerpa* sample with other species in the database.

Contiq sequences of the 18SrRNA *Caulerpa* gene were aligned (multiple alignment) with the partial 18SrRNA genes selected in the database using ClustalW and a phylogenetic tree was made with 1000 replications using the Neighbor-Joining (NJ) method with MEGA 7 (Molecular Evolutionary Genetics Analysis) Software.

Data Analysis

The data obtained was numerically described since it needed no statistical package.

RESULTS AND DISCUSSION

Globally, the genus of green algae *Caulerpa* has a total of 85 species (Guiry *et al.*, 2014) which are broadly distributed in tropical to sub-tropical waters. One importance of the presence of *Caulerpa* species is that they can form one of the best coastal ecosystems that have been widely explained by many reports on the invasion of *C. taxifolia* (M.Vahl), *C. agardh* and *C. cylindracea* Sonder (formerly called *C. racemosa* var. Cylindraceae (Sonder) (Belton *et al.*, 2014). Different studies on the formation of population invasion of *C. taxifolia* and *C. cylindraceae* in the Mediterranean focused on the spread of these species and the impact of their invasion on the surrounding aquatic ecosystems (Meinesz *et al.*, 1993; Verlaque and Fritayre, 1994; Chisholm *et al.*, 1997; Jousson *et al.,* 1998; Piazzi *et al.,* 2001; Balata *et al.*, 2004; McKinnon *et al.*, 2009; Bulleri *et al.*, 2010; Vázquez-Luis *et al.*, 2010; Gennaro and Piazzi, 2011; Oakes *et al.*, 2011; Pacciardi *et al.*, 2011).

The spread of green algae also provides pharmaceutical benefits such as antioxidants, anticoagulants, anti-mutagenic, antibacterial and anticancer activities which must be taken into consideration (Cho *et al.*, 2011; Vinayak *et al.*, 2011; Farasat *et al.*, 2013). According to a previous study, among several green algae species, *Caulerpa* sp. had an inhibitory effect on telomerase in MOLT-4

cells while some species were reported to have antitumor activity (Kanegawa *et al*., 2000). *Caulerpa* sp. harvested from the Yucatan Peninsula, Mexico showed a higher phenolic content than other seaweeds around the environment (Zubia *et al*., 2007). "Caulerpin" which is an alkaloid that can be isolated from *Caulerpa* sp. has antitumor activity but there are very limited studies available on the compound (Liu *et al*., 2009). A study also mentioned that the antioxidant and phenolic content of *C. racemosa* was higher than the red algae species (Matanjun *et al*., 2008).

From our observations, part of the results of this study varies with those of other species, hence we hypnotized that some compounds in *Caulerpa* could be inhibitors and even impurities in the DNA extraction process as this is seen from the low quantity of DNA obtained from the extraction process after being read on NanoDrop 2000 (Thermo Scientific). This hypothesis is supported by the study of Setyawan (2019) who observed that compounds such as alginate in the *Sargassum* species are impurities, making it difficult to extract DNA from these algae. Thus, the process of depigmentation and removal of genomic DNA impurities in algae needs to be carried out in DNA extraction. The acidic alginate removal in the process of extracting algal DNA can give a positive effect in reducing impurities such that it can increase the number of peaks on the chromatogram when compared using standard CTAB. The feasibility of the DNA extraction process with Trizol can be further studied and compared with other extraction methods using higher DNA quantity in the future.

Morphological Identification of *Caulerpa* sp.

Morphological identification results showed that Sample 1 (Figure 2A) has similarities with *Caulerpa racemosa* (Forsskal) J. Agardh 1873. *C. racemosa* consists of several branches connected by stolon attached to the sandy substrate by rhizoid. The branches are several centimeters apart and grow to a height of 30 cm. The shoots are round hence the species is also called sea grapes and *C. lentilifera*.

Morphological identification results showed that Sample 2 (Figure 2B) had similarities with *Caulerpa sertularioides* (S.G Gmelin) with the description of branch-like feathers, flat and upright as high as 3-5 cm and 1-2 mm in diameter. Rhizoid is attached to the substrate. The opposite branchlets are attached to the midrib, slightly curved upward and tapered at the base and tip. The midrib is slightly flat and light green-yellow. This species bears little resemblance to *C. taxifolia*.

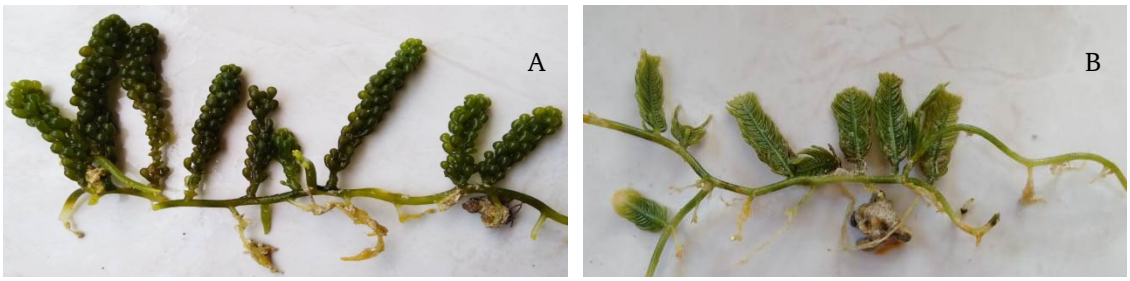

Figure 2. Morphology of *Caulerpa* sp. Which was collected from Mandangin Island, Madura, East Java. (A) Sample 1 and (B) Sample 2.

18SrRNA Amplification

The results of the amplification of the PCR reaction mixture, the temperature and time conditions as described in the method showed the 18SrRNA gene used as a primer for *Caulerpa* sp. samples gave rise to a thin single band at around 1000 bp (Figure 3).

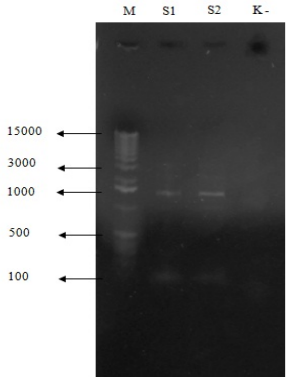

Figure 3. PCR results with 18SrRNA primers in *Caulerpa* sp. samples. The gene is amplified in the presence of a single band at a length of about 1000 bp. Description: M: Marker; S1: Sample 1; S2: Sample 2 and K-: Negative control.

The 18SrRNA primer used in this study was based on the research of Mahendran and Sarasvanan (2017) that studied out molecular identification of the green algae *Ulva lactuca* and *Caulerpa taxifolia* collected from rocky coasts around the coastal areas of the new port of Tuticorin, the Gulf of Mannar, Southeast Coast of India. In terms of yield, the rRNA yield of this study appears lower than the report on *Symbiodinium* sp. by Santiago-Vázquez *et al*. (2006) who obtained a high quantity of RNA using Trizol® reagent and found their result 3-4 times higher than their findings using RNeasy®. Such variations could be as a result of location, macroalgae adaptation, and evolution.

Sequencing Results

Sequencing results for *Caulerpa* sp. had the following nucleotide:

a. Nucleotide of Sample 1
CGTAGTGGACGGCTCGGTTCCCGTG
TCGATGAAGGACGTAGCGAAATACG
AAAGGTGTTGCGAATTGCAAGATTTC
GTGAGTCAATGAATATCCGAATGCAT
TGTCGTCCCTTCGCAGCTTCTGGGTT
GCAAGGGGCGCGTCGGCAACATCGG
CTAATTGTCGACCTTCATATGAGTCT
CTTTTGTTCACTCTCTTACTGAGCGA
GTGTGCTGGATTCTGTATGAAGCAGT
GGTTCAGATGAGCAAGCATTAACTTT
TCCATACCTGTATCTGTGGCTGAATG
CTTGTGATGACTCCCTCTCACAACTA
TCGACTACTGATTTTGAGATTAACGA
CAGTATATTTGTGACTTGCATCACTC
TATGTGCTCTTGTCCTATAGCAAGCT
CAATCGCATGAGATTCGAAGGTTGCC
TAGAAATCTGTGCTCAAAAATAGTAG
TCTGGTTCTGTCAGAACGTGTATCTG
TATGCCTTTGACGATGTTGTCGGCGA
GGAAACCCGCTGAACTTAAGCATATC
AATAAGCGGAGGAAAAGAAACCAAT
TTGGATGCCCCTAGTACTGGCGAGC
GAACAAGCGATGCGGTTGGCGTACG
TTCTCCAGAAGTTCGCTTCGTTATGA
GGAAGGCAACCAGACTGAGTTCCAA
ATTCTCGGTAAGCTTTATATTGAAGG
TTACAACGTGGATCTAGTGTTCGAAA
ATTTGGCGGACTTCCCTGGAGCGAA
GTACGACGCTAAGACCGTCAAGTTC
GTCCTGGAGTCTCTAACCACTGGTTT
CGTTGATTGCCGCTAACAGGTGGCG
ACGTCACTCTCGAGAGAACTTTGATA
CATCTTCGGCTATTGGCGTCGTAGCG
AGAGTGACAGAGAATGATCGACAAT
ACGGCGCTTGACATGAGCAGCACTC
GGGCGAGAACCATCAGGACATCAGA
AACA

b. Nucleotide of Sample 2
GCCCCGGTTTTCGGCCGGCAATTCCC
AGTAGGCGTCGGAAAAACAAACGTT
TGCCTTTACGGTAAAAAGGCTTTACT
TTCGGAATCCGGTTAAGGCGTTGGG
TAATTAACGTAGACCGGTGCCCGATG
GTTCCGGAAGCGAAAGGGTAACAGG
CGGCCGGTCCAAGCCTAGTTACGAG
CACGATGATGTATGGTTCGGTTTCCA
CTGGAGCCGGCTCCCTGTCGACCTTT
ACGCAAGAAGCGACGGTCAGATACC
GGCCTCGATCGCGGATGCGATAACA

GCCCGGGTTCAACACGCAACTTGAG
ACAAGTTCGTTGATGGTAAGAGTATG
GCATATCAACATTTTCGTAGGTGAAC
CTGCGAAAGGATCATTATCGGCAAAT
TCTATTTATGTATACACTACTGTGTG
TCTATATTCTTTGTGTAAAGACATGG
CTATGTTGTAATGAGATGTGTTGTTA
TTATTGTCTAAAGCATGTTCGTAGTT
GGACGGCATCGGTTCCCGTGTCGAT
GAAGGACGTAGCGAAAGTTTACGAA
AGGTGTTGCGTAATTGCAAGATTTCG
TGAGTCAATGAATAATCCGAATGCAT
TGTCGTCCCTTCGCAGCTTCGGTTGC
AAGGGGCGCGTCGGCAACATCGGCA
CTATAATTGTCGACCTTCATAGTCTC
TCTTGTATACTCTCTCTTACTGAGAG
CAAGGTTGCTCGACTCTATGAAGCAG
GTTTGGTTCAGTGAGCAAGCATTCCA
TACGCCGATGTGGCTATCGCTTGTCG
ACGACTCCCTCTCGGACTATCGACTA
CTGACTGAGATTAACGACAGTATTGT
GACTTGCATACTCTATGTGCTTGTCC
TATAGCAATCAATCGCTGAGATTGAA
TGCCTAGAAACTGTGCTCAGCAATAG
TAGTCTGGTGNTNTCAGAACGTGTA
TCTATATTTTGACGATGTTGTCGGCG
AGGAAACCCGCTGAACTTAAGCATAT
CACTAAGCGGAGGAAAAGAAACCAA
TTTGGATGC

BLAST Analysis

The results of the analysis with BLAST, which is an online search engine that matches a query sequence with database sequence showed that both *Caulerpa* sp. samples had similarities with *C. taxifolia* 18SrRNA gene with a percentage identity of sample 1 at 88.87% lower than that of sample 2 which was 95.20%. The BLAST results for each sample can be seen in the following Table 2 and Table 3:

Table 2. Result of BLAST analysis of sample 1.

No.	Description	Max Score	Total Score	Query Cover	E -Value	Per. Identity	Accession
1	*Caulerpa taxifolia* 18SrRNA gene	638	638	59%	2e-178	88.87%	AJ299788.1
2	*C. taxifolia* 18SrRNA gene	636	636	59%	6e-178	88.73%	AJ299789.1
3	*C. taxifolia* 18SrRNA gene	632	632	59%	8e-177	88.73%	AJ299784.1
4	*C. taxifolia* 18SrRNA gene	630	630	59%	3e-176	88.73%	AJ299775.1
5	*C. taxifolia* 18SrRNA gene	627	627	59%	4e-175	88.55%	AJ299790.1
6	*C. taxifolia* 18SrRNA gene	627	627	59%	4e-175	88.59%	AJ299776.1
7	*C. taxifolia* 18SrRNA gene	627	627	59%	4e-175	88.59%	AJ299772.1
8	*C. taxifolia* CTCB3-6	625	625	59%	1e-174	88.33%	AY034869.1
9	*C. taxifolia* isolate St-Cyprien France	625	625	59%	1e-174	88.41%	AJ228960.1
10	*C. taxifolia* 18SrRNA gene	623	623	59%	5e-174	88.50%	AJ299786.1

Table 3. Result of BLAST analysis of sample 2.

No.	Description	Max Score	Total Score	Query Cover	E - Value	Per. Identity	Accession
1	*C. taxifolia* 18SrRNA gene	1033	1033	65%	0.0	95.20%	AJ299767.1
2	CTCB3-6	1027	1027	66%	0.0	95.07%	AY034869.1
3	18SrRNA gene	1027	1027	65%	0.0	95.05%	AJ299753.1
4	18SrRNA gene	1022	1022	65%	0.0	94.89%	AJ299773.1
5	isolate Le Brusc France clone A	1020	1020	66%	0.0	94.77%	AJ228969.1
6	18SrRNA gene	1018	1018	66%	0.0	94.76%	AJ299769.1
7	18SrRNA gene	1018	1018	66%	0.0	94.76%	AJ299743.1
8	*C. taxifolia* 18SrRNA gene	1016	1016	66%	0.0	94.76%	AJ299776.1
9	*C. taxifolia* 18SrRNA gene	1016	1016	65%	0.0	94.74%	AJ299 775.1
10	*C. taxifolia* 18SrRNA gene	1014	1014	66%	0.0	94.76%	AJ299772.1

Phylogenetic Tree Analysis

Phylogenetic tree analysis results from the 18SrRNA gene were obtained from Neighbor-Joining and compared with 8 other sequences for Sample 1 and 13 sequences for Sample 2. The sequences used were the *Caulerpa* species sequences from NCBI. The results showed that the sample 1 species had a close kinship with *C. Sertularioides f. longipes*, whereas for sample 2 it had a close kinship with *C. taxifolia*. The results of the phylogenetic tree for both samples are presented in Figures 4 and 5 below.

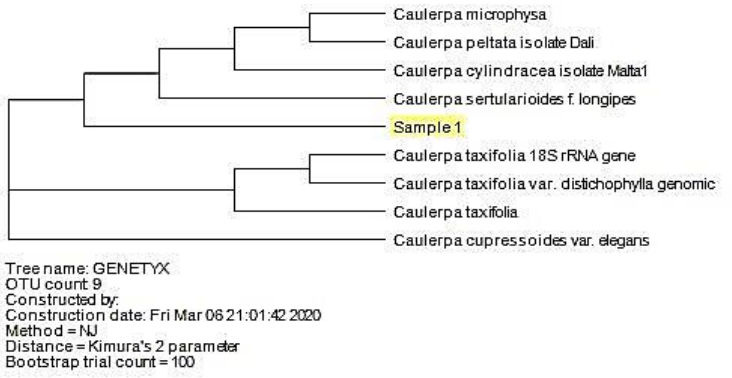

Figure 4. Phylogenetic tree of sample 1 showing a close kinship with *C. Sertularioides f. longipes*.

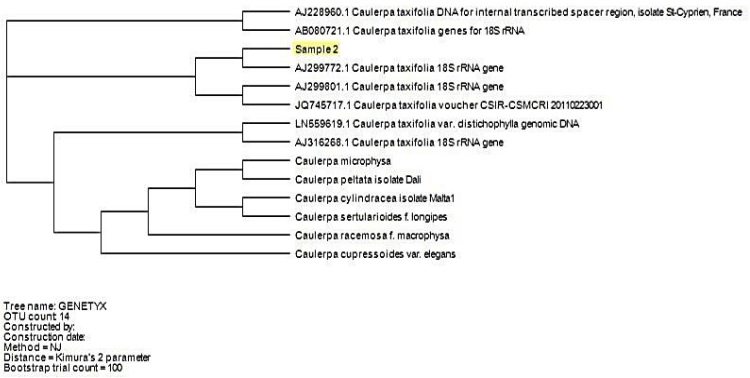

Figure 5. Phylogenetic tree of sample 2 showing close kinship with *C. taxifolia*.

The results of phylogenetic analysis with maximum consensus like-hood of sample 1 actually had similarities with *Caulerpa sertularioides f. longipes*. These results support the findings of previous studies such as Mahendran and Saravanan (2017) whose algae sample had a 96% similarity with *Caulerpa taxifolia* 18SrRNA gene, ascertaining that primer 18SrRNA can be used for molecular identification. However, the BLAST results were not supported by phylogenetic trees in sample 1 because synonymously *C. taxifolia* has similarities with *C. Mexicana* (Sonderex Kutzing, 1849) and *C. taxifolia* var. *falcifolia*. The similarity in our current study with previous studies (Wang *et al*., 2019; Mahendran and Saravanan, 2017) whom all found their studied *Caulerpa* species closely related to other *Caulerpa* species on their respective phylogenetic tree is one affirmation that our current study and those of the authors have been performed on related species of the same genus. The difference in the result of our sample 1 may be due to the small number of *Caulerpa* sequences used in phylogenetic tree analysis when compared to sample 2.

Various DNA Markers Used for Molecular Identification of Seaweed from Different Regions

Some research results from the use of DNA markers as a method of molecular identification of seaweed reported in different parts of the world are presented in Table 4 below.

Table 4. DNA markers were used for seaweed identification.

Species	DNA Marker	Region	Reference
Spirogyra ellipsospora	ISSR and rbcL	Thailand	Wongsawad and Peerapornpisal (2014)
Spirogyra maxima	rbcL	California	Stancheva *et al*. (2013)
- *Dictyota* sp.	ITS2	-Tomini Bay, Indonesia	Dharmayanti *et al*. (2018)
- *Halimeda* sp.		-Tomini Bay	
- *Padina* sp.		-Tomini Bay	
- *Polysiphonia* sp.		-Tomini Bay	
- *Sargassum* sp.		-Banten, Indonesia	
Kappachycus Doty and *Eucheuma* J. Agardh	*Cox*1, *Cox*2-3 spacer, *Cox*2 and rbcL	Southeast Asia	Tan *et al*. (2012)
Ulva lactuca and *Caulerpa taxifolia*	rbcL and 18SrRNA	Gulf of Mannar, South East coast India	Mahendran and Saravanan (2017)
Caulerpa taxifolia	18SrRNA	Mediterranean Sea	Jousson *et al*. (1998)
Sargassum sp.	COI	Krakal Beach, Yogyakarta, Indonesia	Setyawan (2019)
Brown Seaweed	rbcL and *partial Cox*1	Malaysia and Lombok, Indonesia	Poong *et al*. (2014)
*Caulerpa taxifolia*18SrRNA	18SrRNA	Mandangin Coastal, Indonesia	Current study

The use of DNA markers for molecular identification of seaweed in several regions was highlighted in Table 4. Moreover, studies have shown that molecular identification of *Spirogyra* can be determined through molecular biology approach using rbcL primer (Stancheva *et al.,* 2013; Wongsawad and Peerapornpisal, 2014). The results of research from Dharmayanti *et al.* (2018) submitted that the Internal Transcribed Spacers 2 (ITS2) DNA marker can be used for molecular identification of several types of algae collected from the Tomini Bay and Banten Bay in Indonesia.

This study has shown that molecular identification of *Caulerpa* species can use DNA barcoding as an 18SrRNA primer. In

addition to the use of this primer, several genetic markers such as ITS-2, Rubisco, MSTSP, Cox3 and rbcL can be used for molecular identification of algae. PCR results with 18SrRNA primers in this study showed a band at a length of about 1000 bp. The same results were reported by Yeh and Chen (2004) that rDNA sequences were around 872-1124 bp length when amplified using PCR in 11 *Caulerpa* individuals and compared with several other *Caulerpa* species. Some researchers also mentioned that the success of molecular identification with barcode markers can vary depending on the size, geographical range and availability of the species dataset identified (Mattio and Payri, 2010). The results of this study are the first step in molecular identification of *Caulerpa* green algae in Indonesia and further research is needed on the identification of green algae with the use of other markers to support its knowledge of molecular taxonomy.

CONCLUSION

Based on the research performed, 18S rRNA primers have been used for molecular identification of green seaweed from Mandangin for the first time and this shows that barcode markers can be used for molecular identification of seaweed, specifically *Caulerpa* in the waters of Mandangin Island, Indonesia. We suggest that further research be performed using other markers and different DNA extraction methods, order to facilitate the identification of other parts of the seaweed to obtain supporting results and develop *Caulerpa* green algae as medicinal ingredients and food for coastal people of Mandangin to improve their regional economy.

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