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 1 and 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 GenBank.

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).

![Figure 1. Map of the sampling location of *Caulerpa* sp. taken with Google Maps.](image)

**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 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.
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 contig results and the results of primary and forward sequences. Contig 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.

Contig 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 subtropical 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*.  

**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
CGTATGAGGGGACGCTCGGTTCGGTCCG
TCGATGAAAGACGTGAGAGCTAGCA
AAGGTGTGGAGGAACTCGTCTGTGAG
GTGTGACGCTCTCTGCTAATATGGATC
AGGACGGGCGGCTGGAAATACATCG
CTAATCTCGAGCTTTCTATGACTGT
CTTTGCTACATCTCTACTGAGCAG
GTGTGGCTGAATCCGAGGAGCTG
GGTAGCATGAGAAGACGCTATTAC
TCCATAAGCTTCTGTGGTCGTAAG
CTTGTGATGACTCCCTCACAACACT
TCGAATCTATGGATTAGATAC

b. Nucleotide of Sample 2
GCCGGGTGTTTGGCGCGGCAATTCCC
AGTAGGCGTCGCAAAAAAACAGTT
GGTAGTGCAGGATACACTTGCTTTAC
TTCGGAATCGCTGATAGGCTGGG
TAATTACGTAGACCGGCTGGCCAGTA
GTCGGGAGGCAAGGAGTCGAAC
CGCGCGTGCCAAATCTAGTTAC
AGAGGCGTCCGAGAAGGACGCTTT
CTGGAGAGGCTGACGATAGGCG
AACA

CAGTATATTGTGACTTGCATCACTC
TATGTGGCTTTGCTCTATAGCAGCT
CAATCGCATGAGATTTGAGGTGTTCCG
TAGAAATGTGTCTCAGAAAAATAGTAG
TCTGGTTCTGTACAGGTGATCTG
TATGCCCTTGGACGATGTTGTCCGGCAG
GGAAACCCTGGAATCTTAAAGATCT
AATAAGCGGAGAAAGAAAACAAAT
TTGGAATGCCCTAGTACTGGCGGAC
GAACAGGATGCGTGGCGTAGC
TTCTCCGACAGGTCTTTCTATGTA
GAAGAACAAACAGCTAGTCTCCCAA
ATTCCTCGTAAAGCTTATATTTGAAG
TTACAACGTGGATCTAGTGTCTGGAAA
ATTTGGCGGACTTCCCTGGAGCGA
GTACGACGCTAAAGCGTCAAGTG
GTCCTCGGATTCTACTACACTGGTT
CGTGGATTGCCGCTAACAGGGTTGCG
ACGTCACTCTCGAGAGACTTTGATA
CATCTGGCTATTGGCTCGTAGC
AGAGTCAAGGAAATGATCGACAAT
ACGGCGTCTACGATAGCAGCACTC
GGGCAGAAACATCGACAGATCAA
AACA
GCCCGGGTTCACAGCAAATTTGAG
ACAGTTTCTGTTGTAAGATAG
CAGATAAAGTTCGTTGATGGTAAGTATG
CTGCGAATCATATTTACGTAGGTGAC
CTGCGAAAGGATCATTATCGGCAAAT
TCTATTTATGTATACACTACTGTGTG
TCTATATTCTTTGTGTAAAGACATGG
CTATGTTGTAATGAGATGTGTTGTTA
TTATTTGTCTAAAGCAGATGTTGTTTA
TTATTTGTCTAAAGCAGATGTTGTTTA
TTATTTGTCTAAAGCAGATGTTGTTTA
TTATTTGTCTAAAGCAGATGTTGTTTA
TTATTTGTCTAAAGCAGATGTTGTTTA
TTATTTGTCTAAAGCAGATGTTGTTTA
TTATTTGTCTAAAGCAGATGTTGTTTA
TTATTTGTCTAAAGCAGATGTTGTTTA
TTATTTGTCTAAAGCAGATGTTGTTTA
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TTATTTGTCTAAAGCAGATGTTGTTTA
TTATTTGTCTAAAGCAGATGTTGTTTA
TTATTTGTCTAAAGCAGATGTTGTTTA
TTATTTGTCTAAAGCAGATGTTGTTTA
TATAGCAATCAATCGGTCAGATTGAA
TGCCCTGAAACTGTGCTACGCAATAG
TAGTCTGAGCTNGAAGACGTGA
TGATATTTGAGCTGTTGTCGCG
AGGAAACCCCGCTGAACCTAAAGCATAT
CACTAAGCGAGAAAAGAACCAC
TTTGAGATGC

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%        | AJ299776.1  |
| 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               | 1022      | 1022        | 65%         | 0.0     | 95.05%        | AJ299753.1      |
| 4   | isolate Le Brusc France clone A | 1020   | 1020        | 66%         | 0.0     | 94.89%        | AJ299773.1      |
| 5   | *C. taxifolia* 18SrRNA gene | 1018      | 1018        | 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%        | AJ299775.1      |
| 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*.](image1)

![Figure 5. Phylogenetic tree of sample 2 showing close kinship with *C. taxifolia*.](image2)
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.

| 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 | Cox1, Cox2-3 spacer, 279 spacer, Cox2 and rbcL | Southeast Asia | Tan et al. (2012) |
| Ulva lactuca and Caulerpa taxifolia | 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    | Malaysia and Lombok, Indonesia | Poong et al. (2014)                |
| Caulerpa taxifolia      | 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, in 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.

ACKNOWLEDGEMENT

We thank all parties who have aided in the completion of this research.

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