Biodiversity and phylogenetic analyses using DNA barcoding rbcL gene of seagrass from Sekotong, West Lombok, Indonesia

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Abstract. Stevanus, Pharmawati M. 2021. Biodiversity and phylogenetic analyses using DNA barcoding rbcL gene of seagrass from Sekotong, West Lombok, Indonesia. Biodiversitas 22: 50-57. West Lombok, Indonesia is one of the locations that is thought to have a quite high diversity of seagrass. Information on the diversity of seagrass species is important due to the important value of seagrass in the marine ecosystem. This research aimed to analyze biodiversity and phylogenetic of seagrass from Sekotong, West Lombok, Indonesia using DNA barcoding of rbcL gene. As many as 35 samples from seven morphologically identified species (Enhalus acoroides, Thalassia hemprichii, Cymodocea rotundata, Syringodium isoetifolium, Halodule pinifolia, Halophila ovalis, and H. minor) were taken from four Gilis (small island) in Sekotong. The DNA was amplified for the rbcL gene and sequence analyses using BLAST were conducted to determine the species. Phylogenetic analyses were carried out using three evolutionary algorithms using Neighbor-Joining, Maximum Likelihood and Bayesian analysis with 1000 bootstrap. The rbcL gene was successfully amplified from all samples with a maximum length of 552 bp. The phylogenetic analysis showed that clades were split by family and genera where six clades were formed (Enhalus acoroides, T. hemprichii, Halophila complex, H. pinifolia, S. isoetifolium, and C. rotundata) with more than 95% of bootstrap values for Neighbor-Joining and Bayesian. The p-distance values between species were 0.008-0.097 and the polymorphic site was not found within species. The rbcL sequences only confirmed five seagrass species out of seven morphologically identified species and the sequences generated from this study cannot discriminate Halophila ovalis and H. minor.

Keywords: Biodiversity, phylogenetic, rbcL gene, seagrass, West Lombok

INTRODUCTION

One of the most important components in coastal biomes is the seagrass ecosystem. It plays an important role in supporting the coral reef ecosystem through filtration and sedimentation of pollutant substances coming from land (Göttenboth et al. 2012). Seagrass also has function as a habitat for highly-economic species, such as sea cucumber (Holothuroidea), crabs and shrimps (Crustaceans), rabbitfishes (Siganus spp.), sea horses (Hippocampus spp.), and the rare and protected dugong (Dugong dugon) (McKenzie et al. 2003; Short et al. 2007; Christanty et al. 2008). Studies on tropical seagrass are generally on ecological studies including the distribution and density of seagrass species (Soe-Htun et al. 2017; Jahnke et al. 2019; Lamit and Tanaka 2019; Clarito et al. 2020). Molecular studies such as evaluation of genetic diversity, genetic population, and seagrass phylogenetic are important to understand seagrass distribution, evolution, and conservation (von der Heyden et al. 2014). However, only a few studies are available on genetic diversity and phylogenetic of seagrass in this region.

Indonesia has 13 seagrass species, i.e. Cymodocea rotundata, C. serrulata, Enhalus acoroides, Halodule pinifolia, H. uninervis, Halophila decipiens, H. minor, H. ovalis, H. spinulosa, H. sulawesi, S. isoetifolium, Thalassodendron ciliatum, and Thalassia hemprichii (Hutomo and Moosa 2005; Kuo 2007). Studies on seagrass genetic diversity in Indonesia are still limited to certain species. For example, studies on the population structure of E. acoroides (Putra et al. 2018), S. isoetifolium, and T. hemprichii (Wainwright et al. 2018) C. rotundata (Ramili et al. 2020).

DNA barcoding is one of the fast identification techniques of the species using a short DNA fragment containing 400-800 bp (Selvaraj et al. 2013). The Consortium for Barcode of Life (CBOL) (2009) recommended ribulose biphosphate carboxylase/oxygenase (rbcL) as one of the high potential candidate loci for plant barcode. This is because the rbcL gene has been well characterized, therefore primer design can be easily improved. Besides that, rbcL has high universality and high discriminating power. The rbcL region has been used to differentiate four seagrass species from Thailand (Osathanunkul et al. 2015), identify Halophila spp. from Sri Lanka (Liu et al. 2020), and identify other flowering plants such as Sonchus arvensis (Wahyuni et al. 2019) and genus Piper (Naim and Mahboob 2020).
Lombok Island, Nusa Tenggara Barat Province, Indonesia has recently become Indonesia's top tourism destination besides Bali and Raja Ampat. There are high numbers of tourism activities in Lombok, especially in Sekotong, West Lombok that could lead to ecosystem damage including seagrass (Kurniawan et al. 2016; Syukur et al. 2020). Information on the genetic variation of seagrass in West Lombok is still limited. West Lombok is one of the locations that is thought to have quite high seagrass diversity. Besides as a tourism destination, Sekotong area is also a waste disposal area for traditional gold mining (Budiyanto 2016). It was reported that the waste from mining activities in Sekotong contain heavy metals which are harmful to marine biota (Budiyanto 2016). Therefore, it is important to examine the biodiversity, both morphologically and genetically, to support policy making for the conservation of coastal areas.

This study aimed to analyze biodiversity and phylogenetic relationships of seagrass from Sekotong, West Lombok, using DNA barcoding rbcL gene.

**MATERIALS AND METHODS**

**Study areas**

Samples of seagrass were taken from four sites in Sekotong, West Lombok District, West Nusa Tenggara Province, Indonesia. The four sample sites were Gili Layar, Gili Sudak, Gili Genting, and Ela-ela (Figure 1, Table 1).

**Procedure**

The purposive sampling method was used on seagrass sampling by taking five random individuals from each morphologically-distinct species and one additional individual of each species to be preserved as herbarium and deposited at Herbarium Biologi Udayana. The samples were washed to remove any attached epiphytes. Leaf was cut approximately 3-5 cm long and 8-10 cuts were then kept in a labeled zip-lock plastic bag with silica gels to absorb the moisture content and kept the leaf samples dry. Morphological identification was conducted according to Kuo and den Hartog (2001) and Waycott et al. (2004).

The DNA extractions were done using DNeasy® Plant Mini Kit (Qiagen) according to the manufacturer's protocols. Amplifications of rbcL gene were done in 25 µL reaction mixture containing 10 µL ddH2O, 1.25 µL each of forward and reverse primer (P610 5'-ATGTCACCACAAACAGAGACTAAAGC-3' and P609 5'-GTAAAATCAAGTCCACRCG-3') (Lucas et al. 2012), 12.5 µL KAPA2GTM Fast Ready Mix and 2 µL DNA template. The PCR conditions used in this study followed the protocol of Lucas et al. (2012) with 1 cycle of pre-denaturation at 95°C for 5 seconds, followed by 30 cycles of denaturation at 94°C for 35 seconds, annealing at 57°C for 3 minutes, extension at 70°C, and 1 cycle of post-extension at 72°C for 8 minutes, then store at 4°C. Amplification results were verified by 1% agarose gel electrophoresis running in Sodium Borat (SB) buffer at 100 volts for 30 minutes with EtBr staining. The PCR products were then sequenced at UC Berkeley Sequencing Facility, USA.

![Figure 1](image1.png)  
**Figure 1.** Seagrass sampling locations in Sekotong, West Lombok, Indonesia. 1. Gili Sudak, 2. Gili Layar, 3. Gili Genting, and 4. Ela-Ela

| Locality  | Coordinate                  | Substrate                          | Water temp. (°C) | Main tourism activity       |
|----------|-----------------------------|------------------------------------|------------------|-----------------------------|
| Gili Sudak | 8°43'40.55"S, 115°54'36.65"E | Sand and rubble                    | 24-26            | Snorkeling and diving       |
| Gili Layar | 8°44'55.51"S, 115°53'33.34"E | Sand and rubble                    | 26-27            | Snorkeling and diving       |
| Gili Genting | 8°44'17.94"S, 115°57'51.70"E | Sand, rubble, and dead coral       | 26-27            | -                           |
| Ela-ela    | 8°44'7.27"S, 115°58'0.26"E   | Silt, sand, and rubble             | 26-27            | -                           |

Table 1. Sampling locations and environmental condition in Sekotong, West Lombok, Indonesia
Data analysis

Reconstruction of the phylogenetic tree was conducted using three evolutionary algorithms, which were Neighbor-Joining (NJ), Maximum Likelihood (ML) with 1000 bootstrap, and Bayesian. *Pistia stratiotes* was used as an outgroup, due to the more distant relationship of this species to seagrass species studied. *Pistia stratiotes* is an aquatic plant, commonly known as water lettuce. This species has been used as outgroup species in the phylogenetic study of seagrass and hydrophyll (Les et al. 1997). The DNA sequences were then analyzed using MEGA5 (Tamura et al. 2011), GeneiousR7 (Kearse et al. 2012), and MrBayes 3.2 (Ronquist et al. 2012). To decide the evolutionary model, the j Model Test was used (Guindon and Gascuel 2003; Posada 2008) and a comparison between Akaike Information Criterion (AIC) (Akaike 1974), and Bayesian Information Criterion (BIC) (Drummond and Rambaut 2007) was conducted. Bayesian analysis was performed with 10,000,000 generations with sampling for carried out every 1,000 generations and a flat prior approach was applied to the analyses. The phylogenetic tree from Bayesian analyses was visualized by FigTree v1.3.1 (Rambaut 2009). Species identification of each sequence was determined by Basic Local Alignment Search Tool (BLAST) (Altschul et al. 1990) with 99-100% similarity cut-off. The BLAST values were gained by comparing sequence samples to GenBank databases. The category observed included sequence identity and query cover. Species determination was also conducted by comparing genetic distance value with p-distance of each clade by MEGA5.

RESULTS AND DISCUSSION

Seagrass diversity

Based on morphological identification, seven species of seagrass were found that form the seagrass ecosystem in Sekotong, West Lombok. The seven species were *Enhalus acoroides* (L.f.) Royle, *Thalassia hemprichii* (Ehrenb. ex Solms) Asch., *Cymodocea rotundata* Asch. & Schweinf. *Syringodium isoetifolium* (Asch.) Dandy, *Halodule pinifolia* (Miki) Hartog, *Halophila ovalis* (R.Br.) Hook. F. and *Halophila minor* (Zoll.) Hartog (Figure 2). This study covered wider locations than Shalihah et al. (2012) which reported four species in Gili Genting which were *E. acoroides*, *S. isoetifolium*, *H. ovalis*, and *C. rotundata*. The main morphological characteristics of each species are presented in Table 2.

Seagrass species diversity in Sekotong was lower than seagrass diversity in Sanur coastal water, Bali, Indonesia that were nine seagrass species (Pharmawati et al 2016). Recent studies found seven seagrass species in Pramuka Island, Seribu Archipelago, Jakarta, Indonesia (Haviarini et al. 2019), while in coastal water of Kei Besar Utara Timur Sub-district, South-East Maluku District, there were only four seagrass species reported (Beruat et al. 2016). Annual monitoring and biodiversity assessment need to be put into further consideration to produce an up to date data.

Molecular identification

The *rbcL* sequences of a total of 35 samples were successfully amplified and the size varied from 524-552 bp. The sizes were slightly less than the sizes obtained by Lucas et al. (2012) which was up to 599 bp. The averages of nucleotide compositions (calculated by MEGA5) were Thymine 28.7%, Cytosine 20.6%, Adenine 28.3%, and Guanine 22.3%.

The BLAST results showed that of the seven species identified morphologically, the similarity percentage obtained by BLAST only confirmed five species (*E. acoroides*, *T. hemprichii*, *S. isoetifolium*, *C. rotundata*, and *H. pinifolia*) based on the *rbcL* gene sequence with similarity values in the range of 99-100% in the top two similarities in GenBank. The value of the cover query obtained was in the range of 98-100% in the first similarity and 92-100% in the second similarity (Table 3).

The BLAST results showed that the *rbcL* gene sequences of the *H. ovalis* and *H. minor* samples had similarities to *H. decipiens* in the first order, even though there were differences in the morphological characteristics of *H. decipiens* and *H. ovalis*. The leaf blade of *H. decipiens* is elliptical and the margin is serrated, whereas in *H. ovalis* the leaf shape is oval with smooth margin. Also, leaves of *H. decipiens* have minute hair-like structures on both sides of the leaf blade (Kuo and den Hartog 2001; Waycott et al. 2004). Analysis of genetic distance demonstrated that the *rbcL* gene sequences of *H. ovalis* and *H. minor* had 0 genetic distance which indicated that the sequences are 100% identical (Table 2). It means that the *rbcL* primer used has not been able to discriminate *H. ovalis* and *H. Minor*. Based on morphological characters, the leaves of *H. ovalis* and *H. minor* are oval-shaped. The leaf size of *H. ovalis* is generally larger (1-2.2 cm in length, 0.4-1 cm in width) than that of *H. minor* (0.6-1.2 cm in length, 0.35-0.6 cm in width). The vein number of *H. ovalis* is 8-15, while *H. minor* has <7-12 vein number (Waycott et al. 2004). This range of sizes overlaps between *H. ovalis* and *H. minor*. Besides, several species of *Halophila* are not clearly distinguished morphologically. *Halophila* spp. has small and oval to oblong leaf shapes as their general characters. Waycott et al. (2004) grouped them into the *H. ovalis* complex, which included *H. minor*, *H. ovata*, *H. ovalis* ssp. *bullosa*, *H. ovalis* ssp. *Linearis*, and *H. hawaiiana*.

Genetic distance and phylogenetic analyses

Genetic distance (p-distance) showed that the farthest genetic distance was between *Halophila* and *C. rotundata* with p-distance value 0.097; while the closest genetic distance was between *E. acoroides* and *T. hemprichii* clade with p-distance value 0.008 respectively (Table 4).

The analyses of genetic distance for all five sequences for each species within the clade showed that the genetic distance value is 0.000 (data not shown). It means that the sequences that are in one clade are considered to be 100% identical to each other. Analysis of 552 bp of each sequence in each species showed no variation of nucleotides or polymeric sites in one clade, which means that each clade has only one haplotype.
Table 2. Diversity of seagrass species in Sekotong, West Lombok, Indonesia

| Species                        | Morphological characteristics                                                                 |
|--------------------------------|------------------------------------------------------------------------------------------------|
| Halodule acoroides             | The leaves are ribbon-shaped, have a rigid structure at the edges of the leaves, and the leaves are 30-50 cm long, thick rhizome with black bristles |
| Cymodocea rotundata            |                                                                                                  |
| Thalassia hemprichii           |                                                                                                  |
| Syringodium isoetifolium       | Cylindrical leaf shape, contain air cavities                                                    |
| Cylindrical leaf shape, contain air cavities |

Table 3. The BLAST results obtained from the partial sequences of the rbcL gene for each sample from Sekotong, West Lombok, Indonesia were compared with the results of morphological identification. The BLAST similarity value was selected from the highest value of the query cover (Q) and identity percentage (I).

| ID IBRC | Morphological identification | Location | First similarity rank (Q/I) | Species identifieda | Second similarity rank (Q/I) | Species identifiedb |
|---------|-------------------------------|----------|----------------------------|--------------------|-----------------------------|---------------------|
| 073131  | Enhalus acoroides             | Gili Sudak | 100/100 | E. acoroides JN225336  | 97/100 | E. acoroides AB004889 |
| 073132  | E. acoroides                  | Gili Sudak | 100/100 | E. acoroides JN225336  | 97/100 | E. acoroides AB004889 |
| 073133  | E. acoroides                  | Gili Sudak | 100/100 | E. acoroides JN225336  | 97/100 | E. acoroides AB004889 |
| 073134  | E. acoroides                  | Gili Sudak | 100/100 | E. acoroides JN225336  | 97/100 | E. acoroides AB004889 |
| 073135  | E. acoroides                  | Gili Sudak | 100/100 | E. acoroides JN225336  | 97/100 | E. acoroides AB004889 |
| 073136  | Halophyta minor               | Gili Genting | 100/100 | Halophyta decipiens JN225340 | 100/100 | Halophyta ovalis JN225348 |
| 073137  | H. minor                      | Gili Genting | 100/100 | H. decipiens JN225340  | 100/100 | H. ovalis JN225348 |
| 073138  | H. minor                      | Gili Genting | 100/100 | H. decipiens JN225340  | 100/100 | H. ovalis JN225348 |
| 073139  | H. minor                      | Gili Genting | 100/100 | H. decipiens JN225340  | 100/100 | H. ovalis JN225348 |
| 073140  | H. minor                      | Gili Genting | 100/100 | H. decipiens JN225340  | 100/100 | H. ovalis JN225348 |
| 073141  | Syringodium isoetifolium      | Ela-Ela   | 98/99  | S. isoetifolium KF488497 | 97/99  | S. isoetifolium U80691 |
| 073142  | S. isoetifolium               | Ela-Ela   | 98/99  | S. isoetifolium KF488497 | 97/99  | S. isoetifolium U80691 |
| 073143  | S. isoetifolium               | Ela-Ela   | 98/99  | S. isoetifolium KF488497 | 97/99  | S. isoetifolium U80691 |
| 073144  | S. isoetifolium               | Ela-Ela   | 98/99  | S. isoetifolium KF488497 | 97/99  | S. isoetifolium U80691 |
| 073145  | S. isoetifolium               | Ela-Ela   | 98/99  | S. isoetifolium KF488497 | 97/99  | S. isoetifolium U80691 |
| 073146  | Halophyta ovalis              | Gili Sudak | 100/100 | Halophyta decipiens JN225340 | 100/100 | Halophyta ovalis JN225348 |
| 073147  | H. ovalis                     | Gili Sudak | 100/100 | H. decipiens JN225340  | 100/100 | H. ovalis JN225348 |
| 073148  | H. ovalis                     | Gili Sudak | 100/100 | H. decipiens JN225340  | 100/100 | H. ovalis JN225348 |
| 073149  | H. ovalis                     | Gili Sudak | 100/100 | H. decipiens JN225340  | 100/100 | H. ovalis JN225348 |
| 073150  | H. ovalis                     | Gili Sudak | 100/100 | H. decipiens JN225340  | 100/100 | H. ovalis JN225348 |
| 073153  | Thalassia hemprichii          | Gili Sudak | 100/100 | T. hemprichii JN225341 | 97/100 | T. hemprichii AB004897 |
| 073154  | T. hemprichii                 | Gili Sudak | 100/100 | T. hemprichii JN225341 | 97/100 | T. hemprichii AB004897 |
| 073155  | T. hemprichii                 | Gili Sudak | 100/100 | T. hemprichii JN225341 | 97/100 | T. hemprichii AB004897 |
| 073156  | T. hemprichii                 | Gili Sudak | 100/100 | T. hemprichii JN225341 | 97/100 | T. hemprichii AB004897 |
| 073157  | T. hemprichii                 | Gili Sudak | 100/100 | T. hemprichii JN225341 | 97/100 | T. hemprichii AB004897 |
| 073158  | Cymodocea rotundata           | Gili Layar | 100/99 | C. rotundata JN225334  | 92/99  | C. rotundata JQ031763 |
| 073159  | C. rotundata                  | Gili Layar | 100/99 | C. rotundata JN225334  | 92/99  | C. rotundata JQ031763 |
| 073160  | C. rotundata                  | Gili Layar | 100/99 | C. rotundata JN225334  | 92/99  | C. rotundata JQ031763 |
| 073161  | C. rotundata                  | Gili Layar | 100/99 | C. rotundata JN225334  | 92/99  | C. rotundata JQ031763 |
| 073162  | C. rotundata                  | Gili Layar | 100/99 | C. rotundata JN225344  | 100/99 | C. rotundata JF488489 |
| 073163  | Halodule pinifolia            | Gili Sudak | 98/100 | Halodule unineris KF488495 | 97/100 | Halodule pinifolia U80690 |
| 073164  | H. pinifolia                  | Gili Sudak | 98/100 | H. unineris KF488495   | 97/100 | H. pinifolia U80690 |
| 073165  | H. pinifolia                  | Gili Sudak | 100/99 | H. unineris JN225344   | 100/99 | H. pinifolia JN225345 |
| 073166  | H. pinifolia                  | Gili Sudak | 100/99 | H. unineris JN225344   | 100/99 | H. pinifolia JN225345 |
| 073167  | H. pinifolia                  | Gili Sudak | 100/99 | H. unineris JN225344   | 100/99 | H. pinifolia JN225345 |

Note: first similarity rank was based on the first highest score of query cover (Q) and identity percentage (I), second similarity rank was based on the second highest of Q and I. The codes behind the species name are the accession number of sequences from NCBI. a=species identified based on first highest Q and I, b=species identified based on second highest Q and I.
Figure 2. Seagrass species were found in Sekotong, West Lombok, Indonesia. A. C. rotundata, B. S. isoetifolium, C. T. hemprichii, D. H. minor, E. H. ovalis, F. E. acoroides, G. H. pinifolia. Bar = 2 cm

Table 4. The p-distance value between clades in the phylogenetic tree of seagrass was based on the partial rbcL gene sequence

|   | 1   | 2          | 3          | 4          | 5          | 6          | 7          |
|---|-----|-----------|------------|------------|------------|------------|------------|
| 1 | 0   |           |            |            |            |            |            |
| 2 | 0.032 | 0         |            |            |            |            |            |
| 3 | 0.032 | 0         | 0          |            |            |            |            |
| 4 | 0.093 | 0.091     | 0.091      | 0          |            |            |            |
| 5 | 0.008* | 0.028     | 0.028      | 0.093      | 0          |            |            |
| 6 | 0.095 | 0.097*    | 0.097*     | 0.014      | 0.095      | 0          |            |
| 7 | 0.087 | 0.087     | 0.087      | 0.020      | 0.083      | 0.028      | 0          |

Note: * indicates the value of the farthest genetic distance and + indicates the value of the closest genetic distance. 1. E. acoroides, 2. H. minor, 3. H. ovalis, 4. S. isoetifolium, 5. T. hemprichii, 6. C. rotundata, 7. H. pinifolia

The value of the genetic distance from clades within one family is smaller than the value of the genetic distance between clades in different families. The genetic distance that occurred in Hydrocharitaceae was 0.008-0.028, while Cymodoceaceae was in the range of 0.014-0.028. The genetic distance that arose between Hydrocharitaceae and Cymodoceaceae was 0.083-0.097. These values indicate that the greater the genetic distance, the more distant the relationship between taxa.

In Hydrocharitaceae, the genetic distance between clade E. acoroides and clade T. hemprichii was much closer than that of the Halophila complex. This is also consistent with the morphology where E. acoroides and T. hemprichii both have ribbon-shaped leaves, in contrast to the Halophila complex which has oval leaf morphology. Enhalus acoroides and T. hemprichii also have thick rhizomes (Waycot et al. 2004). Based on phylogenetic analysis, the clades of E. acoroides and T. hemprichii are monophyletic, which means they have the same ancestry and are paraphyletic when compared to the Halophila complex (Figure 3).

In Cymodoceaceae, the genetic distance between Cymodocea was closer to S. isoetifolium than H. pinifolia. Phylogenetically, it appears that the Cymodocea clade is monophyletic against the Syringodium clade but is paraphyletic to the Halodule clade (Figure 3). This finding is supported by analysis using 18S rDNA sequences which showed that Cymodocea spp., Syringodium spp. and Halodule spp. were clustered together. Cymodocea spp. were sister to Syringodium spp. and formed a sub-group, while Halodule spp. were in another sub-group (Dilipan et al. 2016). The leaf morphology of Cymodocea and Halodule is more similar because the leaves are ribbon-shaped and arise from the vertical stem, while S. isoetifolium leaves have tubular shape, contain air cavities and pointed leaf tip (Kuo and den Hartog 2001; McKenzie 2008).
Reconstruction of the phylogenetic tree by Neighbor-Joining (NJ) with p-distance and Maximum Likelihood (ML) using General Time Reversible + Gamma (GTR+G) and Bayesian analysis showed that the samples tested formed six distinct clades. The samples were grouped based on genus and the larger clade grouping occurred based on family, namely Hydrocharitaceae which consisted of *E. acoroides* clade, *T. hemprichii* and *Halophila* complex and Cymodoceaceae which include the *S. isoetifolium* clade, *C. rotundata* and *H. pinifolia* (Figure 3).

The groupings that occurred between samples were following the existing classification system and supported by the polytomic branching in each clade as well as the high bootstrap values of the three evolutionary algorithms used. The bootstrap values ranged from 64% to 100%. The higher the bootstrap value that appears at each branch indicates that the level of confidence in the branching formed on the phylogeny tree is higher. In the Neighbor-Joining algorithm, the lowest bootstrap value was 83% and was in the branching separation of *C. rotundata* clade and *C. serrulata*. Meanwhile, for the Maximum Likelihood and Bayesian algorithms, the lowest bootstrap was 64% and 96% respectively, and was in the same place, namely at the branching between the *Cymodoce* clade and *S. isoetifolium*. It means that Maximum Likelihood and Bayesian are less sure about the branching formed. However, even though the Maximum Likelihood bootstrap value at the branching was the smallest, the level of confidence in the formation of the branch was still relatively high because it was supported by the bootstrap values of Neighbor-Joining and Bayesian. The branching between *Cymodoce* and *Syringodium* was also
demonstrated by Lucas et al. (2012) using combined matK and rbcL loci. Based on rbcL data, Cymodocea and Syringodium were found to be closely related and grouped in one clade (Les and Tippery 2013). Besides, analysis using matK, rbcL and mitochondrial genes placed Cymodocea and Syringodium in one clade (Petersen et al. 2014).

Two other clades, namely the Halophila complex and Halodule, become more interesting to study. This is because in the Halophila clade there were three sequences of morphologically different species, namely H. ovalis, H. minor, and H. decipiens. The cladding that occurs in the Halophila sequence shows that the rbcL gene sequence has not been able to separate the Halophila species. Lucas et al. (2012) stated that rbcLmatK sequences were not fully discriminate members of Halophila. Another study reported that between H. ovalis from Vietnam and H. ovalis sequences downloaded from Gene Bank, H. ovata, and H. ovalis subspp. ramamurthiana in India there were no nucleotide differences found (Nguyen et al. 2013). A recent study on the genus Halophila in Sri Lanka using ITS sequences showed a clade of Halophila ovalis complex which consisted of H. ovata, H. minor, H. ovalis, H. hawaiiiana, and H. johnsonii (Liu et al. 2020).

In the Halodule clade, the BLAST results showed that the H. pinifolia sequence from Sekotong had similarities to H. uninervis and H. pinifolia with 100% of the query cover and identity. A previous study using matK sequences of seagrass from Bali showed that H. pinifolia and H. uninervis formed polymer line (Pharmawati et al. 2016). The rbcL sequences of H. pinifolia (JN225345) and H. uninervis (JN225344) from Lucas et al. (2012) were then reconstructed along with the H. pinifolia sequence from Sekotong. The two sequences (JN225345 and JN225344) formed a small branch and grouped as one clade with bifurcate branches with five H. pinifolia sequences from Sekotong. This indicates that both H. pinifolia and H. uninervis sequences from GenBank are slightly different from H. pinifolia from Sekotong which are shown by short branches on the phylogeny tree.

Morphologically, both Halodule pinifolia and H. uninervis have ribbon-like leaf with a pointed tip and white-colored rhizome. They differ in their leaf tips, where H. pinifolia has a blunter-toothed leaf tip, whereas H. uninervis had sharper leaf tips with two to three-toothed leaf tip (Kuo and den Hartog 2001; Waycott et al. 2004). Nevertheless, at a glance, the leaf width of H. uninervis and H. pinifolia was also different. Halodule. uninervis was slightly wider than H. pinifolia, which makes it closely resembles the leaves of Cymodocea spp.

Low genetic diversity was identified for seven seagrass species from Sekotong, West Lombok, Indonesia, since no intraspecific variation was identified. Based on rbcL gene as a single barcode, the phylogeny analysis of seagrass plants in Sekotong had not shown clade separation up to the species level at several genera.

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