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Biodiversity and phylogenetic analyses using DNA barcoding *rbc*L 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 *rbc*L 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 rbc*L 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 *rbc*L 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 *rbc*L 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, *rbc*L 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 (Göltenboth et al. 2012). Seagrass has also function as a habitat for highly-economic species, such as sea cucumber (Holothuroids), 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).

Studies on seagrass genetic diversity and genetic population have been carried out in several places, such as India (Thangaradjou and Bhatt 2018), South China Sea (Hernawan 2018), Isles of Scilly, an archipelago in England (Alotaibi et al. 2019), and Western Australia (Sinclair et al. 2020). The studies covered tropical and temperate seagrass. Southeast Asia has the highest diversity of seagrass with 12-15 species (Fortes et al. 2018). 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. sulawesii*, *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 (*rbc*L) as one of the high potential candidate loci for plant barcode. This is because the *rbc*L gene has been well characterized, therefore primer design can be easily improved. Besides that, *rbc*L has high universality and high discriminating power. The *rbc*L 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 at 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. 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 *rbc*L 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 *rbc*L 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'-GTAAAATCAAGTCCACCRCG-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.

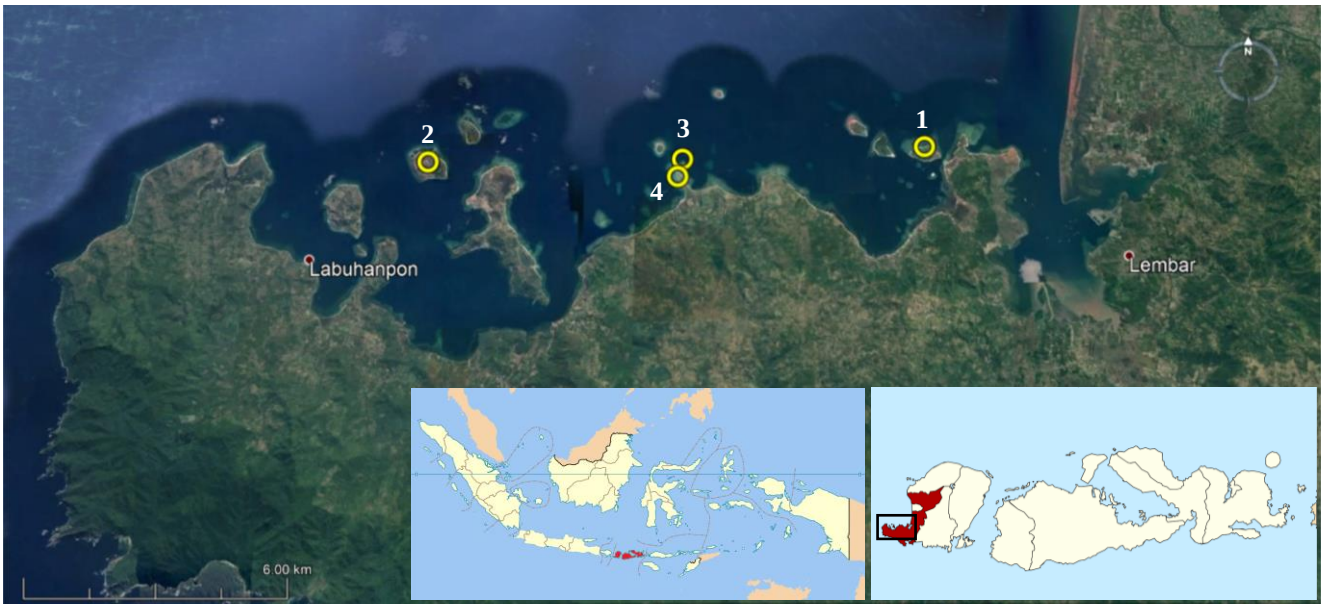

Figure 1. Seagrass sampling locations in Sekotong, West Lombok, Indonesia. 1. Gili Sudak, 2. Gili Layar, 3. Gili Genting, and 4. Ela-Ela

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

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	-

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 hydrophily (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 *rbc*L 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 *rbc*L 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 *rbc*L 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 *rbc*L 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 *rbc*L 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. hemrichii* 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 polymorphic 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
Enhalus 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
Halophyla minor	Leaves are small, oval, 0.5-0.9 cm long, 0.4-0.6 cm wide, 6-12 pairs of leaf veins, smooth leaf edges
Halophila ovalis	Oval shape of leaves, leaf length 0.9-2 cm, leaf width 0.4-0.9 cm, number of leaf veins are 8-15 pairs, smooth leaf edges
Syringodium isoetifolium	Cylindrical leaf shape, contain air cavities
Thalassia hemprichii	Ribbon-like leaves and slightly curved with 5–10 cm long, segmented rhizomes
Cymodocea rotundata	Ribbon-like leaves, the tips of the leaves are rounded with smooth edges, non-segmented rhizomes
Halodule pinifolia	Small and delicate ribbon-like leaves, rounded leaf tip, pale rhizome

Table 3. The BLAST results obtained from the partial sequences of the *rbc*L 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 identified[a]	Second similarity rank (Q/I)	Species identified[b]
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	*Halophyla minor*	Gili Genting	100/100	*Halophila decipiens* JN225340	100/100	*Halophila 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	*Halophila ovalis*	Gili Sudak	100/100	*Halophila decipiens* JN225340	100/100	*Halophila 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	100/100	*C. rotundata* JN225334	92/100	*C. rotundata* JQ031763
073161	*C. rotundata*	Gili Layar	100/100	*C. rotundata* JN225334	92/100	*C. rotundata* JQ031763
073162	*C. rotundata*	Gili Layar	100/99	*C. rotundata* KF488490	100/99	*C. rotundata* KF488489
073163	*Halodule pinifolia*	Gili Sudak	98/100	*Halodule uninervis* KF488495	97/100	*Halodule pinifolia* U80690
073164	*H. pinifolia*	Gili Sudak	98/100	*H. uninervis* KF488495	97/100	*H. pinifolia* U80690
073165	*H. pinifolia*	Gili Sudak	100/99	*H. uninervis* JN225344	100/99	*H. pinifolia* JN225345
073166	*H. pinifolia*	Gili Sudak	100/99	*H. uninervis* JN225344	100/99	*H. pinifolia* JN225345
073167	*H. pinifolia*	Gili Sudak	100/99	*H. uninervis* 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

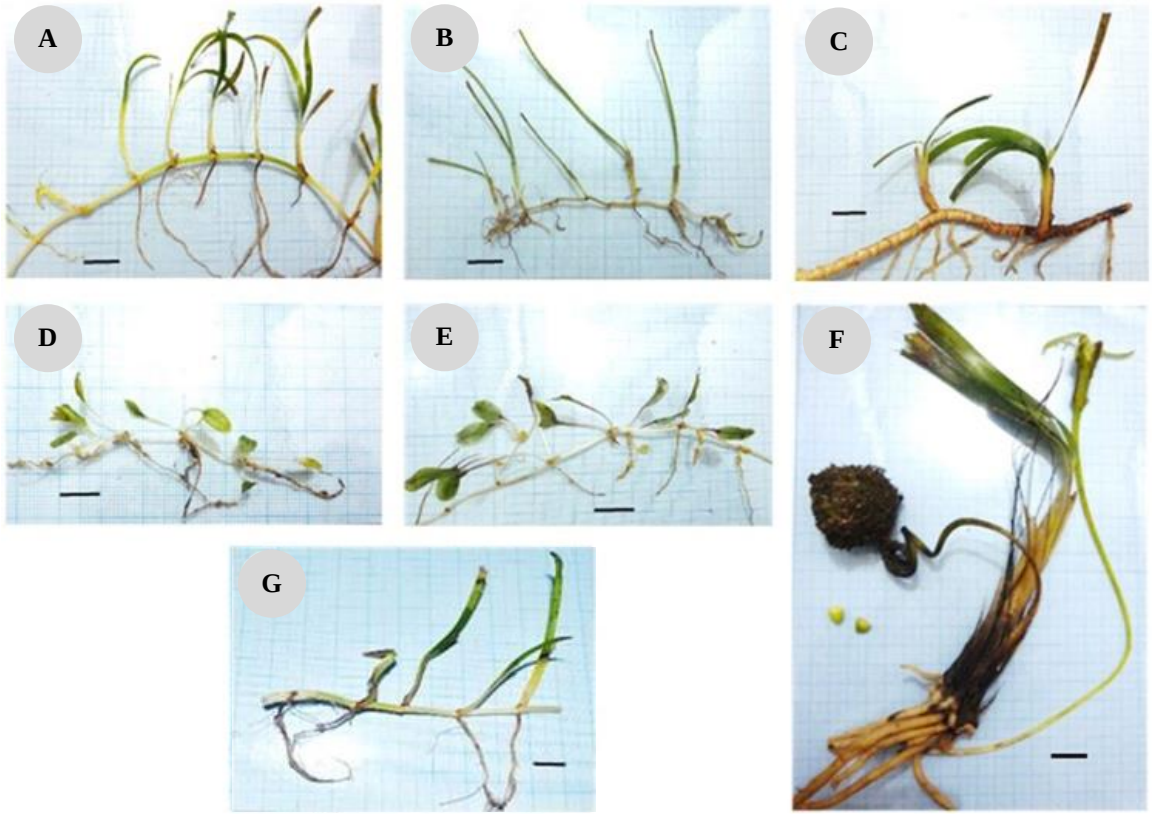

Figure 2. Seagrass species were found in Sekotong, West Lombok, Indonesia. A. *C. rotundata*, B. *S. isoetifolium*, C. *T. hemprichii*, D. *H. minor*, E. *H. ovalis*, F. *E. acoroides*, G. *H. pinifolia*. Bar = 2 cm

Table 4. The p-distance value between clades in the phylogenetic tree of seagrass was based on the partial *rbc*L 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 using18S 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).

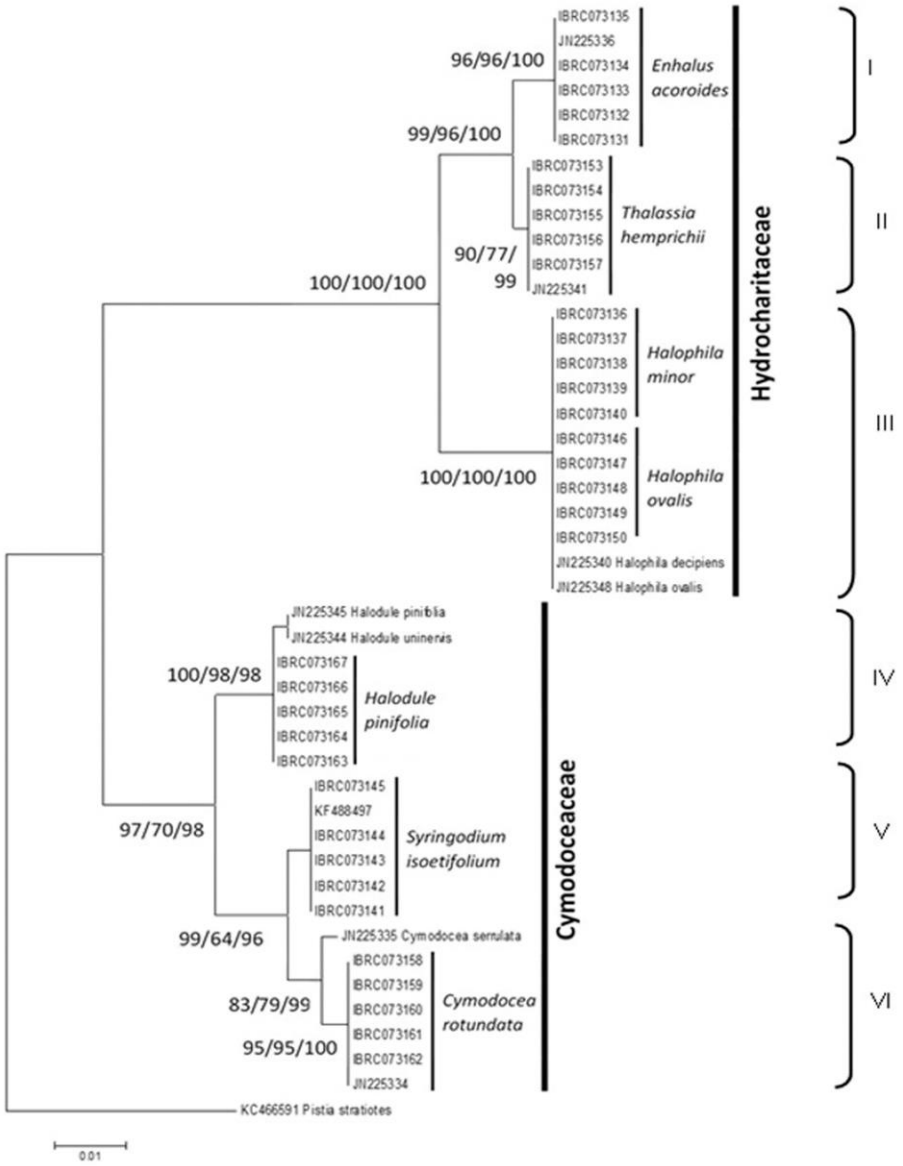

Figure 3. Phylogenetic tree of *rbc*L sequences of seagrass from Sekotong, West Lombok, Indonesia. The bootstrap values for each clade are sequential based on Neighbor-Joining, Maximum Likelihood, and Bayesian.

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 *Cymodocea* 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 *Cymodocea* and *Syringodium* was also

demonstrated by Lucas et al. (2012) using combined *mat*K and *rbc*L loci. Based on *rbc*L data, *Cymodocea* and *Syringodium* were found to be closely related and grouped in one clade (Les and Tippery 2013). Besides, analysis using *mat*K, *rbc*L 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 *rbc*L gene sequence has not been able to separate the *Halophila* species. Lucas et al (2012) stated that *rbc*L/*mat*K 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* subsp. *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. hawaiiana*, 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 *mat*K sequences of seagrass from Bali showed that *H. pinifolia* and *H. uninervis* formed polytomy lineage (Pharmawati et al. 2016). The *rbc*L 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. univervis* 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 *rbc*L 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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