SPECIES DIVERSITY AND PHYLOGENETIC RELATIONSHIP OF SEAGRASS FROM INTERTIDAL ZONE OF EKAS, LOMBOK

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Abstract: Lombok Island is one of the areas which are rich in seagrass. Most of research in seagrass were focusing on their ecology, whereas the study of morphological and molecular in taxonomy context is still limited. The purpose of this research is to observe diversity and phylogenetic relationship of seagrass in intertidal zone of Ekas. In this research, samplesware collected byexplorating in intertidal zones of Ekaswaters, Lombok, West NusaTenggara. The data was analyzed to observe species diversity of seagrass andtheir phylogenetic relationship using rbcL gene sequence. Phylogenetic analysiswas carried rut using Neighbor-Joining method by MEGA 6 with 1000 bootstrap. Diversity seagrassin Ekasconsisted 7 species:*Enhalusacoroides, Halophilaspinulosa, Halophilaovalis, Cymodoceaserrulata, Cymodocearotundata, Syringodiumisoetifolium*and*Halodulepinifolia*. Phylogenetic analysis showed that 2 clades were formed at by more than 91% of bootstrap. Clade I consisted of *Enhalusacoroides,* and*Halophilaovalis*, while Clade II consist of *Cymodoceaserrulata, Cymodocearotundata, Syringodiumisoetifolium*and*Halodulepinifolia.* In conclusion, rbcL gene can be a marker to differentiate cluster seagrassbased ontheir family and genus.

Keywords: Diversity, Phylogenetic Relationship, Seagrass, Rbcl

I. INTRODUCTION

Seagrass is a flowering plant (Angiospermae) which have unique characteristics with the entire process of life were in the sea waters (McKenzie & Yoshida, 2009). The reproduction could be by vegetative and sexual organs, which occurs with the help of water (Philips &Milchakova, 2003). Seagrassfunction as a protection area, the spawning and nurturing fishlarva,preventing erosion at the surface sediments (Kuriandewa, 1998) and carbon sinks in the Sea (Kawaroe, 2009).

Seagrass bed in the area of East Lombok covers 1.631, 66 Ha (Dermawan*et al.*, 2014). Results of the research of Syukur (2015) about diversity of seagrassin Lombok Island retrieved the 9 species of seagrass, covers*C. rotundata, C. serrulata, E. acoroides, H. pinifolia, H. ovalis, H. minor, H. spinulosa, S. isoetifolium*and*T. hemprichii*.

rbcLgene was one locus for the molecular identification recommended by the Consortium for the Barcode of Life (CBOL, 2009). The rbcL gene is encoding gene of sub-unit of large protein Ribulose-1.5-bifosphate Carboxylase (RuBisCo), located on the genomes of chloroplasts.

This is very abundant in chloroplast and the reaction can catalyze all carbon fixation (Palumbi, 1991). rbcL sequences have an important role in the phylogenetic analysis due to its conserve nature (Albert *et al.*, 1994).
Seagrass distribution in the intertidal zone of the waters in Ekas is quite wide. The extents of seagrass distribution in these waters is not yet supported by the data on seagrassphylogenetic relationship. The research on seagrass were frequently conducted in terms of ecology, whereas in the field of taxonomy, especially the use of DNA markers, is still limited. The purpose of this research is to observe the diversity and the phylogenetic relationship of seagrass species from Ekas, Lombok.

II. DETAILS EXPERIMENTAL

Materials and Procedures
This researchwas conducted on seagrass populations in intertidal areas along the coastal waters of Ekas. The data were collected in the form of various types of seagrass for morphological identification using den Hartog, (1979); Philips &Menez (1988) in Azkab (1999). The DNA extraction used CTAB. DNA extraction using CTAB.

The condition of the PCR using Primer P609 5 '-GTAAAATCAAGTCCACCRCG-3 ' and P610 (5 '-ATGTCACCACAAACAGAGACTAAAGC-3') (Lucas *et al.*, 2012). PCR component consist of 12.5 µl of Bioline, 1 µltemplate DNA concentrations 30-80 ng/µl, 9.5 µl ddH_2O, primer P609 and P610 1 µl of each in a total volume of 25 µl.

Conditionsof the PCR: initial denaturation (3 min at 95^{o}C), followed by 35 cycles with denaturationof 95^{o}C for 30 s, annealing of 51^{o}C for 30 s, extension of 71^{o}Cfor 1 min 30 s, and final extension of 72^{o}C for 10 min. Sequencing in 1st Base did in Malaysia.

Methods phylogenetictree reconstruction using Neighbour-Joiningwith the model of substitution the Kimura's two-parameterby MEGA 6 with 1000 bootstrap.

III. RESULTS AND DISCUSSION

3.1 Results

Species diversity of Seagrass

Result of sample identification based on morphological characters using identification key from den Hartog, (1979); Philips &Menez (1988) in Azkab (1999). Show that there are 7 species of seagrass in the intertidal zone of Ekas. There are: *EnhalusacoroidesHalophilaovalis, HalophilaspinulosaSyringodiumisoetifolium, Cymodoceaserrulata, Cymodocearotundata*and*Halodulepinifolia* (Fig. 1).



Fig. 1. Spesies of seagrass: (1)*Enhalusacoroides*(2)*Halophilaovalis*(3)*Halophilaspinulosa,* (4)*Syringodiumisoetifolium,* (5) *Cymodocearotundata*(6)*Cymodoceaserrulata*(7)*Halodulepinifolia*

Phylogenetic Relationship

Phylogenetic tree construction based onrbcLgene on the seagrass, molecular character indicated 2 main clades. Clade I consists of *E. acoroides,* and *H. ovalis*. Clade II consists of *C. serrulata, C. rotundata, S. isoetifolium*and *H. pinifolia.* Clade I belongs to the familyHydrocharitaceae and clade II belongs to Cymodoceaceae.

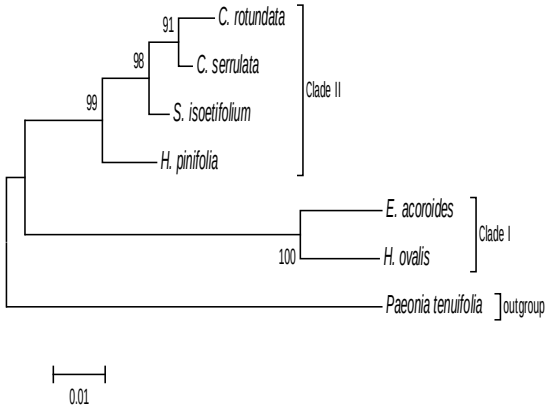


Fig. 2. The reconstruction result of phylogenetic tree of rbcL gene

The number of family Hydrochariteceaethis studyshowed the degree of confidence of 91-99%, whereas for family Cymodoceaceae is 100%. The value of the genetic distance of a clade within one family showed smaller the value compared to a clade their familial. The range of genetic distance at family Hydrocharitaceae is 0,031 while the family Cymodoceaceae is 0,020.

3.2. Discussion

Species diversity of Seagrass

Species of seagrassfound in this study are among from 13 species of seagrass that existed in Indonesia. Intertidal zone is characterized by the pioneerplant dominated by *Halophilaovalis Cymodocearotundata*and *Halodulepinifolia* (Hutomo, 1997). Syukur(2015) reported that the diversity of species of seagrass in the coastal waters of the Lombok Island is9 species of 12 species of seagrass in the coastal waters of Indonesia, or 75% of the seagrass species that exist in the coastal waters of Indonesia can be found in the coastal waters of the Lombok Island.

Phylogenetic Relationship

Genetic distancefrom species clade I is 0,031 and clade II is 0,020. Clade originating from the same family will have genetic distance smaller than from different family. On the family Hydrocharitaceaethe closest genetic distance is between *Halophilaovalis* and *Enhalusacoroides.* In the phylogenetic clade with *Halophila* separation was assumed because it has a different ancestor (*paraphyletic*).Family Cymodoceaceae have the nearest genetic distances between *C. rotundata*and*C. serrulata* when compared to *S. isoetifolium* and *Halodulepinifolia*. The species *C. rotundata*and*C. serrulata*still are in one genus (*Cymodeacea*).

Research on the seagrassphylogenetichas been done by Nguyen *et al.* (2013) usingrbcL genes in a sample of the genus *Halophila* from Vietnam. Analysis of rbcL sequences produce a pattern of low resolution of most species. *Halophila* complexare grouped into two main groups, namely *H. beccari* to two sub-sections below and besides *Halophiladecipiens* complex.

Molecular markers of rbcLgenehas not been able to detect the species *Halophilaspinulosa*from the genus *Halophila.* rbcLgene has limitations for the identification of species. This can be resolveby using additional molecular markers to settle relationship on seagrass.

CONCLUSIONS

Species diversity of seagrassin intertidal zone of Ekas, Lombok is consisted7 species of seagrass, i.e: *Enhalusacoroides Halophilaovalis, Halophilaspinulosa, Syringodiumisoetifolium, Cymodoceaserrulata, Cymodocearotundata*and *Halodulepinifolia.*

Phylogenetic relationship using rbcl genes forming 2 main clades. Clade I consists of *E. acoroides,*and *H. ovalis*. Clade II consists of *C. serrulata, C. rotundata S. isoetifolium,* and*H. pinifolia.* The seagrass is

classified into two families,Hydrocharitaceae and Cymodoceaceae.

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REFERENCES

[1] Azkab, M. H. PedomanInventarisasiLamun. *Oseana* XXIV (1): 1-16.1999.

[2] Albert, V.A., Backlund, A., Bremer, K., Chase, W.K, Manhart, J.R, Mishler, B.D & Nixon K.C. Functional constraints and *rbcL* evidence for land plant phylogeny. *Annals of Missouri Botanical Garden* 81(3): 534-567.1994.

[3] Consortium Barcode of Life (CBOL). A DNA Barcode for Land Plants. *PNAS*, 106 (31).2009.

[4] Dermawan, A.S., Lubis, B. & Suraji. *Status Pengolahan Efektif Kawasan Konservasi Perairan, Pesisir dan Pulau-Pulau Kecil di Indonesia: Profil 113 Kawasan Konservasi Perairan, Pesisir dan Pulau-Pulau Kecil.* DKJI, KP3K, Kementrian Kelautan dan Perikanan RI. Jakarta.2014.

[5] Hutomo, H. Padang lamun Indonesia: salahsatuekosistemlautdangkal yang belumbanyakdikenal. Jurnalpuslitbangoseanologi LIPI-JAKARTA. pp35.1997.

[6] Kuriandewa, T. E. Lamun di Teluk Ambon dan Permasalahannya. *Seminar Pengenalan Lingkungan Pesisir Pulau Ambon*. Ambon. LIPI.1998.

[7] Kawaroe, M. 2009. *Perspektif lamun sebagai blue carbon sink di laut.* Lokakarya Lamun. Departemen Ilmu dan Teknologi Kelautan Fakultas Perikanan dan Ilmu Kelautan. IPB. Bogor.

[8] Lucas, C., Thangaradjou, T., Papenbrock, J. Development of DNA Barcoding System for Seagrass: Successful but Not Simple. *PLoS ONE* 7(1): e29987. doi: 10.1371/journal.pone.0029987. 2012.

[9] McKenzie, L.J. &Yoshida. Seagrass Watch: Proceedings of a Workshop for Monitoring Seagrass Habitats in Indonesia. *The Nature Conservancy, Coral Triangle Center, Sanur Bali, 9 th May 2009*, (p. 56). 2009.

[10] Nguyen, X.V., Bujang, J.S. & Papenbrock, J. Variability of leaf morphology and marker genes of members of the *Halophila* complex collected in VietNam. *AquaticBotany*, 110: 6– 15.2013.

[11] Palumbi, S.R. Nucleid Acids II: The Polymerase Chain Reaction. In hillis, M.H, Craig, M & Mable, B.K. 1996. *Molecular Systematics*, 2nd ed. Sinaeur Associates, Inc. Massachusetts, USA.1991.

[12] Phillips, R.C. & Milchakova N.A. Seagrass Ecosystems. *Морський Екологічний Журнал*: 29-39.2003.

[13] Syukur, A. Distribusi, Keragaman Jenis Lamun (Seagrass) dan Status Konservasinya di Pulau Lombok. *Jurnal Biologi Tropis*, 15 (2): 163-176.2015.

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