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Diversity of aquatic plants in the Rote Dead Sea area, East Nusa Tenggara, Indonesia, based on *rbc*L marker

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Abstract. *Lonthor DW, Miftahudin, Kayat, Julzarika A, Subehi L, Iswandono E, Dima AOM, Dianto A, Setiawan F, Nugraha MFI. 2023. Diversity of aquatic plants in the Rote Dead Sea area, East Nusa Tenggara, Indonesia, based on* rbc*L marker. Biodiversitas 24: 810-818.* The Rote Dead Sea area in Rote Islands, Indonesia has two freshwater and 24 saltwater lakes. The saltwater lakes have salinity levels varied between 0-100 ppt. Every lake has specific aquatic plant species. Species identification through morphological analysis only has its limitations; therefore, DNA barcoding is a preference due to an effective, accurate, and faster method for species identification. In this study, we used the *rbc*L marker to identify and analyze the diversity of the aquatic plant species in lakes Ledulu, Oenduy, Oemasapoka, Oesotimori, and Landu Leko. Plants samples' DNA was amplified using *rbc*L primers, and the amplicons were sequenced. Species identification was based on morphological study and the *rbc*L sequence database. Diversity analyses were performed based on pairwise genetic distances, analysis of molecular variance, and genetic relationships based on the Kimura 2 parameter. The results showed that all 20 aquatic plants were correctly identified, and high genetic diversity was discovered. AMOVA analysis showed that genetic variations within the population were higher than among the population. All plant species were grouped into four groups based on the phylogenetic tree and fractional component analyses. The species belonging to the certain family were grouped in the same clade and the closely related plant families. The *rbc*L marker can be used to discriminate aquatic plant species and analyze their genetic diversity and relationships.

Keywords: Aquatic plant, Dead Sea area, *rbc*L marker, Rote Islands

INTRODUCTION

Rote Islands is located at Rote Ndao District, East Nusa Tenggara, the southernmost part of Indonesia. Geographically the Islands are located at 10°25'-11°00' S and 121°49'-123°26'E. The 2018 Oe, Rote team expedition revealed 82 lakes in Rote Islands (Julzarika et al. 2018), and most lakes are located in the Rote Dead Sea area (Julzarika et al. 2021). The Rote Dead Sea area has 24 saltwater and two freshwater lakes. For example, Lake Ledulu and Lake Oenduy are freshwater lakes. Lake Oemasapoka is a saltwater lake with a salinity level that varies between 45-50 ppt, while Lake Oesotimori has a salinity level >100 ppt. The Landu leko is a sea area that separates West and East Rote Island, forming lake saltwater's increasingly drying out, characterized by almost dry soil conditions during the dry season and wet soil conditions during the rainy season (Julzarika 2021). Lakes and wetlands have an important role as water sources to fulfill the needs of humans and other organisms. The presence of aquatic plant species affects the ecosystems in aquatic areas (Wood et al. 2021). Indonesia is blessed with 623 species of aquatic plants that belong to 105 families (Giesen 1991), which consist of more than 80% of aquatic plant species dwell on the water surface, 4% float freely on the surface, 12% are submerged, and 1% of them only show their leaves on the water surface (Molinari et al. 2021). Based on the type, aquatic plants are divided into four categories according to (Wetzel 2001), namely: (i). *Marginal* aquatic plants usually live in shallow water areas along rivers, lakes, swamps, or ponds; these plants provide food and protect other aquatic organisms. Examples: lotus,

rice, mangroves. (ii) *Floating aquatic* plant is a plant whose habitat is the same as marginal aquatic plants but different in the roots of floating leafy plants does not reach the ground; other parts, such as leaves and flowers, are above the water's surface. This plant has the function for absorbing sunlight so that plants can photosynthesize. For examples: *Salvinia natans* (Sampan Spikes), *Eichornia* sp., *Azolla pinnata*, and *Sargassum.* (iii) *Submerged aquatic* plants are plants that float above the surface of the water, most of the stems, leaves and other parts of the plant are submerged in water and only a small part appears above the surface. This plant has a function as an oxidizer in water. For example: Mangroves. (iv) *The deep aquatic* plant is a plant whose entire body is submerged in water and has roots attached to the bottom of the water, for examples: *Vallisneria*, *Chara*, *Hydrilla*, and *Cabomba.*

Barcoding DNA is chosen because fast identification process, accurate results, and insensitivity to environmental factors. The technology is a revolutionary breakthrough in identifying organisms (Gostel and Kress 2022). DNA barcoding has been utilized for species identification and discrimination, genetic variation and relationship analyses, ancestor genetic prevalence, and new species marking (Amandita et al. 2019). The *rbc*L (*ribulose-1.5-biphosphate carboxylase-oxygenase subunit large*) marker is a universal and ideal marker for plants' phylogenetic and taxonomic studies (Ismail et al. 2020). The *rbc*L as a marker has been previously utilized to identify endemic aquatic plants from Kalimantan, Indonesia (Nugraha et al. 2022). Identification of aquatic plant species from two different lake ecosystems and one wetland, i.e., the freshwater lake (Lake Ledulu and Oenduy), the saltwater lakes (Oemasapoka and Oesotimori), and the wetland (Landu Leko) using the *rbc*L marker. The main challenge is the capability of the barcode to analyze close relationships between species found in a vast geographical location. By taking advantage of the promising *rbc*L marker, we compared the results of the sequences we found with data from the gene bank database (www.ncbi.nlm.nih.gov/genbank/). The study's objective was to identify and analyze the diversity of aquatic plant species from four lakes and one wetland area (a total of five sample locations) at the rote dead sea area and rote islands using the *rbc*L marker.

MATERIALS AND METHODS

Sample collection

Samples of the native aquatic plants from the Rote Dead Sea area in Rote Islands, East Nusa Tenggara province, Indonesia, were collected during the expedition Oe Rote (Tropical Inland Water Project 2017-2018 and continued after Covid 19 at 2021-2022) from four important lakes and one wetland, namely Lake Ledulu (13 samples) and Lake Oenduy (2 samples), Lake Oemasapoka (3 samples), Lake Oesotimori (1 sample) and from Landu Leko wetland (1 sample) (Figure 1). Lake Oesotimori is adjacent to Lake Oemasapoka, while Landu Leko is 6 km from Ledulu Lake. All these areas are in the vicinity of the Rote Dead Sea area. The Rote Dead Sea area and other areas in Rote Islands have semi-arid climate conditions. The samples were collected, freeze-dried, and stored for DNA extractions.

DNA extraction, PCR amplification, and sequencing

Dried leaf samples of each aquatic plant were ground to obtain the fine powder using a pestle in liquid nitrogen and then subjected to a DNA isolation process. DNA isolation is carried out using the i-Genomic Plant DNA Extraction Kit (iNtRON Biotechnology, Korea) following the established protocol imprinted on the kit. First, the purity and quantity of the DNA were checked with a NanoDrop spectrophotometer (Thermo Fisher Scientific, U.S.A). Then, the *rbc*L gene fragment was amplified using a-F forward primer (ATG TCA CCA CAA ACA GAG ACT AAA GC) and a-R reverse primer (GTA AAA TCA AGT CCA CCR CG) (Wynns and Lange 2014; Alaklabi et al. 2014). A 50 µL PCR reaction consisted of 25 µL 2x master mix with 5x MyTaq™ Reaction Buffer Red (Bioline, U.S), 2.5 µL each 10 mM primer, 5 µL template DNA (50 ng/mL), 15 µL pure H_20 was used in the PCR amplification.

Cycling condition was as follows: 95°C for 5 min, followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1 min 30 sec, and extension at 72 °C for 1 min; and final extension at 10 min at 72°C, with the expected PCR amplicon was 510-560 bp. The PCR products (5 µL) were then electrophoresed on 1% agarose gel in 1x TBE buffer with a voltage of 120 V for 30 min and visualized using a GelDoc device to assess a single band and clear product. Finally, the remaining PCR products to 45 µL were sequenced using *rbc*L primer in First BASE Laboratories, Singapore.

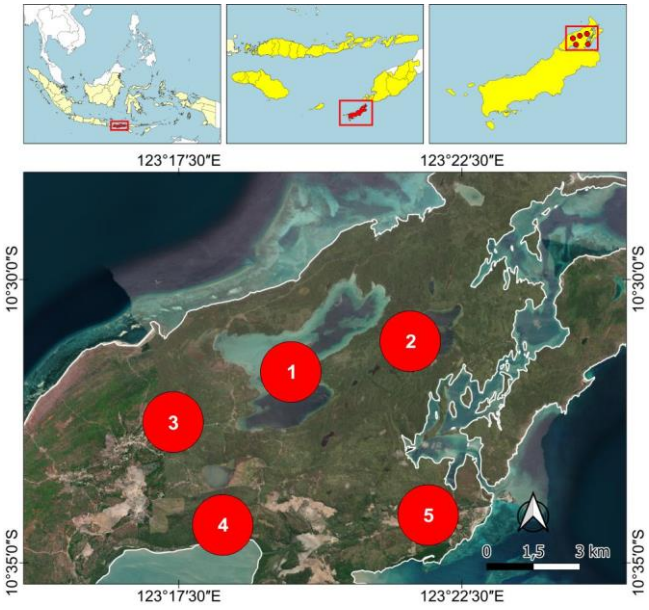

Figure 1. Aquatic plant sampling locations in the Rote Dead Sea area. 1. Lake Oemasapoka, 2. Lake Ledulu, 3. Oesotimori, 4. Lake Oenduy, and 5. Landu Leko

Data analysis

The *rbc*L sequence of each sample was used as a query sequence to search identical sequences at the gene bank National Center for Biotechnology Information (NCBI) at (https://www.ncbi.nlm.nih.gov), using the Basic Local Alignment Search Tool (BLAST) (Newell et al. 2013). The samples were identified based on morphological characteristics and the most identical sequence found in the NCBI GenBank database. The *rbc*L sequences of the 20 aquatic plants were aligned using the ClustalW algorithm (Korotkov and Kostenko 2022) provided in Bioedit ver. 6 (Kumar et al. 2018). Nucleotides variations among samples were analyzed using DnaSP v.6. The parameters of these variations were the number of sites, haplotype number, number of mutations, Parsimony informative site, singleton variable site, polymorphic site, haplotype diversity, and nucleotides diversity (Rozas et al. 2017). The genetic distances among the sample sequences were computed using The phylogenetic tree created using the Bayesian Information method and the appropriate model with (GTR+G) General Time Reversible + gamma 1000 cycle in Mr.Bayes version 3.1.2 (Laskar et al. 2017). Those results were then compared with the phylogenetic tree created using the Maximum Likelihood and the appropriate model with (K2+G) kimura 2 parameter + gamma 1000 cycle in IQ-TREE 2.2.2 (Minh et al. 2020). The resultant tree topology was made with a bootstrap percentage of up to 1,000 replications (Trujillo-Argueta et al. 2021). Analysis of molecular variance (AMOVA) was performed using Arlequin 3.5.1 (Excoffier et al. 2005). The FCA (Factorial Component Analysis) was performed with the PROXSCAL algorithm from the Multidimensional Scaling (MDS) (Campos et al. 2022) using Statistical Package for Social Sciences (SPSS) software version 26.

RESULTS AND DISCUSSION

Identification of aquatic plants based on *rbc*L sequences

Twenty samples of the aquatic plants collected from the research sites were identified based on morphological and molecular approaches. First, the samples were morphologically identified by comparing them with Herbarium specimen collections from Herbarium Bogoriense. Then, for molecular identification, each sequence was subjected to BLAST search at the NCBI database, and the result showed that all the accessions hit the identical sequences in the database with the query coverage ranging from 97 to 100%, the identity ranged from 99.20 to 99.82%, and all e-values were 0.00. Therefore, we named the accession with the name as the species name of the most identical sequence, and are presented in (Table 1). The plant species with 100% query cover are *Schoenoplectiella mucronata*, *Zostera marina*, *Cymodocea rotundata*, *Najas* sp, *Panicum repens*, *Cyperus alopecuroides*, *Pontederia korsakowii*, *Pontederia vaginalis*, and *Bacopa monnieri*. The other species showed 99% query cover except for *Ottelia alismoides* and *Ludwigia adscendens*.

Twenty aquatic plant DNA sequences were identified using BLAST in NCBI. According to Abbas et al. (2020), DNA sequences are identical when the percentage of identity is 97.70 to 100 %, with the query cover reaching 100% and the Expect value (E-value) 0.0. In this study, the Query cover and identity percentages for 20 aquatic plant species are 97 to 100% and 99.20 to 99.82%, respectively, with all E-values being 0.0. A high cover query percentage indicates all or most of the query sequence part is completely aligned with the sequence hit in the GenBank. The E-value is a parameter that describes the number of hits one can "expect" to see by chance when searching a database of a particular size. The lower the E-value, or the closer it is to zero, the more "significant" the match is. An E-value of 0.0 indicates the highest confidence level that the query sequence's alignment with a database sequence is true.

Twenty species from 13 families of aquatic plants were found in the Dead Sea Rote area. The largest population of aquatic plants at 13 species is found in Lake Ledulu; two live in Lake Oenduy, three from Lake Oemasapoka, one from Lake Oesotimori, and one from the Landu Leko. The Landu Leko is a water region that has seen a rapid uplift, characterized by almost dry soil conditions during the dry season and slightly wet soil conditions in the rainy season (Figure 2.A). Lake Ledulu and Oenduy are two freshwater lakes in the Rote Dead Sea area. Aquatic plants are more abundant in Lake Ledulu because this area is calm and nutrient-rich (Figure 2.B).

Nucleotide variation among the *rbc*L sequences

Analysis of nucleotide variation using DnaSP v.5 revealed 573 nucleotide sites among 20 haplotype sequences with haplotype and nucleotide diversity values of 1.000±0.0003 and 0.105±0.004, respectively (Table 2). The analysis also detected 130 polymorphic sites that can be parsimony informative.

Genetic distance among aquatic plant species

Based on the *rbc*L sequence variation, the paired species with the greatest genetic distance are *S. mucronata* and *Limnophila sessiliflora*, *L. adscendens* and *O. alismoides*, *Pseudoraphis spinescens* and *Lemna minor* with the genetic distance of 0.164, while the smallest genetic distance is found between *P. korsakowii* and *P. vaginalis* (0.004), *P. spinescens* and *P. repens* (0.006), followed by *L. sessiliflora* and *B. monnieri* (0.009). *Arthrocnemum macrostachyum* has the closest genetic distance to *Rhizophora apiculata* (0.094) and the greatest distance from *O. alismoides* (0.153). On the other hand, the species from the Oesotimori lake, *R. apiculata*, showed a small genetic distance to *B. monnieri* (0.075) and most distance to *P. spinescens* 0.154 (Table 3).

The species from the Oemasapoka lake, *Z. marina*, is most closely related to *P. vaginalis* (0.052) and most distantly related to *Hippuris vulgaris* (0.124). Meanwhile, *P. spinescens* is genetically closest to *P. repens* (0.006) and the furthest from *R. apiculata* (0.154). Furthermore, *C. rotundata* has the closest genetic distance to *Ottelia cordata* (0.056) and the furthest from *P. repens* (0.136).

Finally, a species from the Oenduy lake, *O. alismoides*, and *O. cordata* from Ledulu lake have a close genetic distance (0.054). Both species are also quite close to *Najas* sp. (Table 3).

Genetic distance within and among populations

The results of AMOVA using Arlequin 3 software showed that the percentage of variation within the population is higher (106.43) than between populations (-6.43) (Table 4). The high value of the variance component within the population (173.87) contributes significantly to the genetic variation within a population, causing very small or no genetic differentiation among the population, which is expressed by the negative value of F*st* (-0.0643).

Genetic relationship among aquatic plant species

Constructed using Bayesian Information (BI)

The phylogenetic tree was constructed using Mr. Bayes (Bayesian Information) (Figure 3) and IQ-TREE (Maximum Likelihood) (Figure 4). The phylogenetic tree was created using the Bayesian Information method and the appropriate model with (GTR+G) general time reversible + gamma 1000 cycle in Mr.Bayes version 3.1.2 (Laskar et al. 2017) into three main groups. Group I consisted of five species, i.e., *Najas* sp, *O. cordata, O. alismoides, C. rotundata* and *L. minor*. Group II consists of seven species: *Z. marina, P. korsakowii, P. vaginalis, P. spinescens, P. repens, S. mucronata,* and *C. alopecuroides*. Group III consists of eight species, i.e., *L. sessiliflora, B. monnieri, H. vulgaris, A. macrostachyum, L. adscendens, Rhizophora apiculata, Ipomoea aquatica, Nymphaea alba*.

Constructed using IQ-Tree (Maximum Likelihood)

Result analysis with IQ-TREE (Maximum Likelihood) the phylogenetic tree was created using the Maximum Likelihood and the appropriate model with (K2+G) kimura 2 parameter + gamma 1000 cycle in IQ-TREE 2.2.2 (Minh et al. 2020). The phylogenetic tree grouped the twenty aquatic plants into four main groups (Figure 4, 6). Group I consisted of eight species, i.e., *L. sessiliflora, B. monnieri, H. vulgaris, A. macrostachyum, L. adscendens, R. apiculata, Ipomoea aquatica, Nymphaea alba*. Group II consisted of five species, i.e., *Najas* sp, *O. cordata, O. alismoides, C. rotundata* and *L. minor*. Group III consisted of three species, i.e., *Z. marina, P. korsakowii*, and *P. vaginalis*. Group IV consisted of four species, i.e., *P. spinescens, P. repens, S. mucronata*, and *C. alopecuroides*.

All species of both analytical methods used occupy the same position. There are a difference between the 2 analytical methods was used, i.e in the IQ tree analysis species in groups 3 and group 4 are combined into the same group (Figure 4,6), but in the Bayesian Information (BI) analysis all species in that groups are combined into the same group (group 3) (Figure 3, 5). This grouping is also seen in the PCA analysis.

Table 1. Identification of *rbc*L sequences of 20 aquatic plant species based on BLAST analysis from NCBI nucleotide database

Species name based on morphological characters	Location lake	Species name based on BLAST	Query cover (%)	Percent identity (%)	e-value
Ottelia cordata (Wall.) Dandy	Ledulu	*Ottelia cordata*	99	99.63	0.00
Schoenoplectiella mucronata (L.) J.Jung & H.K.Choi.	Ledulu	*Schoenoplectiella mucronata*	100	99.28	0.00
Hippuris vulgaris L.	Ledulu	*Hippuris vulgaris*	99	98.88	0.00
Limnophila sessiliflora (Vahl) Blume	Ledulu	*Limnophila sessiliflora*	99	97.85	0.00
Panicum repens L.	Ledulu	*Panicum repens*	100	99.44	0.00
Ludwigia adscendens (L.) H. Hara.	Ledulu	*Ludwigia adscendens*	97	99.62	0.00
Cyperus alopecuroides Rottb	Ledulu	*Cyperus alopecuroides*	100	99.82	0.00
Ipomoea aquatica Forssk.	Ledulu	*Ipomoea aquatica*	99	99.82	0.00
Pontederia korsakowii (Regel & Maack) M.Pell. & C.N.Horn).	Ledulu	*Pontederia korsakowii*	100	99.27	0.00
Lemna minor L.	Ledulu	*Lemna minor*	99	96.54	0.00
Pontederia vaginalis Burm.f.	Ledulu	*Pontederia vaginalis*	100	99.82	0.00
Bacopa monnieri (L.) Wettst	Ledulu	*Bacopa monnieri*	100	99.82	0.00
Nymphaea alba L.	Ledulu	*Nymphaea alba L.*	99	99.74	0.00
Ottelia alismoides (L.) Pers	Oenduy	*Ottelia alismoides*	97	99.20	0.00
Najas sp.	Oenduy	*Najas* sp.	100	98.83	0.00
Zostera marina L.	Oemasapoka	*Zostera marina*	100	99.64	0.00
Pseudoraphis spinescens (R.Br) Vickery	Oemasapoka	*Pseudoraphis spinescens*	99	99.82	0.00
Cymodocea rotundata Asch. & Schweinf	Oemasapoka	*Cymodocea rotundata*	100	99.64	0.00
Rhizophora apiculata Blume	Oesotimori	*Rhizophora apiculata*.	99	99.82	0.00
Arthrocnemum macrostachyum (Moric.) K.Koch	Landu Leko	*Arthrocnemum macrostachyum*	99	99.26	0.00

A **B**

Figure 2. A. *Arthrocnemum macrostachyum* in the saline habitat of Landu Leko in the Rote Dead Sea area, Indonesia. B. Ecosystem of Ledulu Lake in the Rote Dead Sea area

Table 2. Nucleotide variations in aqu*a*tic plants in the Rote Dead Sea area using *rbc*L markers with DnaSP v.6

Samples	Nsi	Hn	Pi	Hd	Nd
Aquatic plant Rote Dead Sea area	573	20	130	1.000±0.0003	0.105±0.004

Note: Nsi: number of sites; Hn: haplotype number; Pi: polymorphic site; Hd: haplotype diversity; Nd: nucleotide diversity

Table 3. The genetic distances of the aquatic plants from the Rote Dead Sea region are based on the *rbc*L sequence variation

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	0																			
2	0.009	0																		
3	0.071	0.073	0																	
4	0.083	0.077	0.098	0																
5	0.075	0.085	0.102	0.089	0															
6	0.104	0.111	0.100	0.106	0.102	0														
7	0.105	0.105	0.107	0.105	0.094	0.102	0													
8	0.113	0.118	0.127	0.116	0.092	0.106	0.120	0												
9	0.133	0.140	0.135	0.129	0.122	0.137	0.138	0.111	0											
10	0.111	0.113	0.127	0.109	0.100	0.126	0.106	0.098	0.116	0										
11	0.122	0.124	0.129	0.113	0.111	0.133	0.119	0.107	0.104	0.086	0									
12	0.153	0.155	0.165	0.149	0.135	0.164	0.153	0.115	0.137	0.106	0.095	0								
13	0.109	0.111	0.120	0.105	0.093	0.113	0.108	0.090	0.100	0.056	0.038	0.054	0							
14	0.118	0.122	0.131	0.118	0.107	0.133	0.115	0.094	0.118	0.092	0.103	0.126	0.079	0						
15	0.113	0.118	0.127	0.114	0.103	0.133	0.111	0.094	0.113	0.088	0.103	0.121	0.079	0.004	0					
16	0.115	0.115	0.124	0.091	0.100	0.115	0.107	0.083	0.103	0.083	0.094	0.115	0.075	0.056	0.052	0				
17	0.139	0.148	0.149	0.148	0.150	0.145	0.145	0.142	0.159	0.136	0.136	0.151	0.126	0.116	0.111	0.098	0			
18	0.143	0.148	0.148	0.147	0.154	0.147	0.145	0.142	0.164	0.134	0.133	0.151	0.124	0.116	0.111	0.098	0.006	0		
19	0.159	0.164	0.140	0.134	0.142	0.137	0.142	0.131	0.135	0.125	0.118	0.142	0.109	0.096	0.096	0.094	0.116	0.116	0	
20	0.145	0.149	0.143	0.132	0.138	0.126	0.135	0.131	0.126	0.118	0.105	0.137	0.096	0.103	0.098	0.100	0.118	0.116	0.028	0

Note: 1. *Bacopa monnieri*; 2. *Limnophila sessiliflora*; 3. *Hippuris vulgaris*; 4. *Ipomoea aquatica*; 5. *Rhizophora apiculata*; 6. *Ludwigia adscendens*; 7. *Arthrocnemum macrostachyum*; 8. *Nymphaea alba*; 9. *Lemna minor*; 10. *Cymodocea rotundata*; 11. *Najas* sp; 12. *Ottelia alismoides*; 13. *Ottelia cordata*; 14. *Pontederia korsakowii*; 15. *Pontederia vaginalis*; 16. *Zostera marina*; 17. *Panicum repens*; 18. *Pseudoraphis spinescens*; 19. *Schoenoplectiella mucronata*; 20. *Cyperus alopecuroides*

Table 4. Analysis of Molecular Variance (AMOVA)

Source of variation	df	Sum of squares	Variance components	Percentage variation	F_{st}
Among populations	2	264.83	-10.51	-6.43	
Within populations	15	2608.00	173.87	106.43	
Total	17	2872.83	163.36		-0.0643

Note: df: degree of freedom; F_{st}: the degree of genetic differentiation among populations (Ledulu, Oenduy, and Oemasapoka populations). *All populations are calculated, except species from Landuleko and Oesotimori are not included in this calculation because only one species is in the population

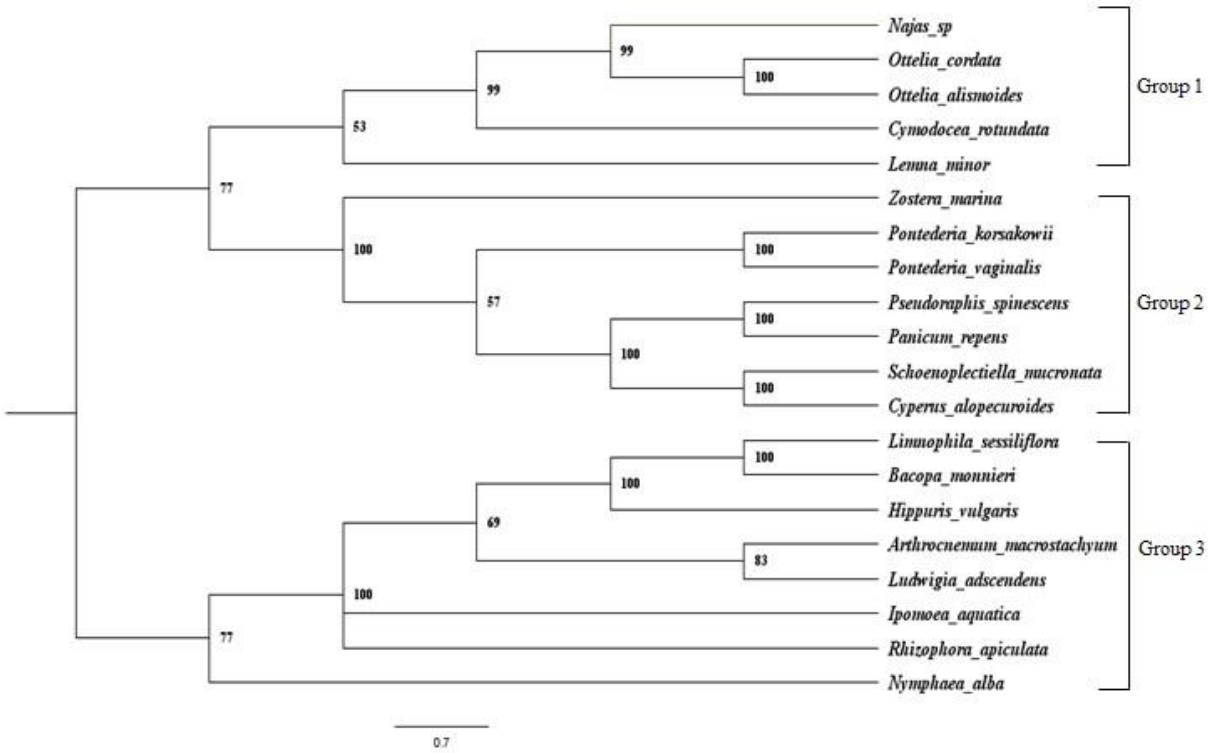

Figure 3. Phylogenetic tree of aquatic plants from the Rote Dead Sea area using Bayesian Information (BI) version 3.1.2

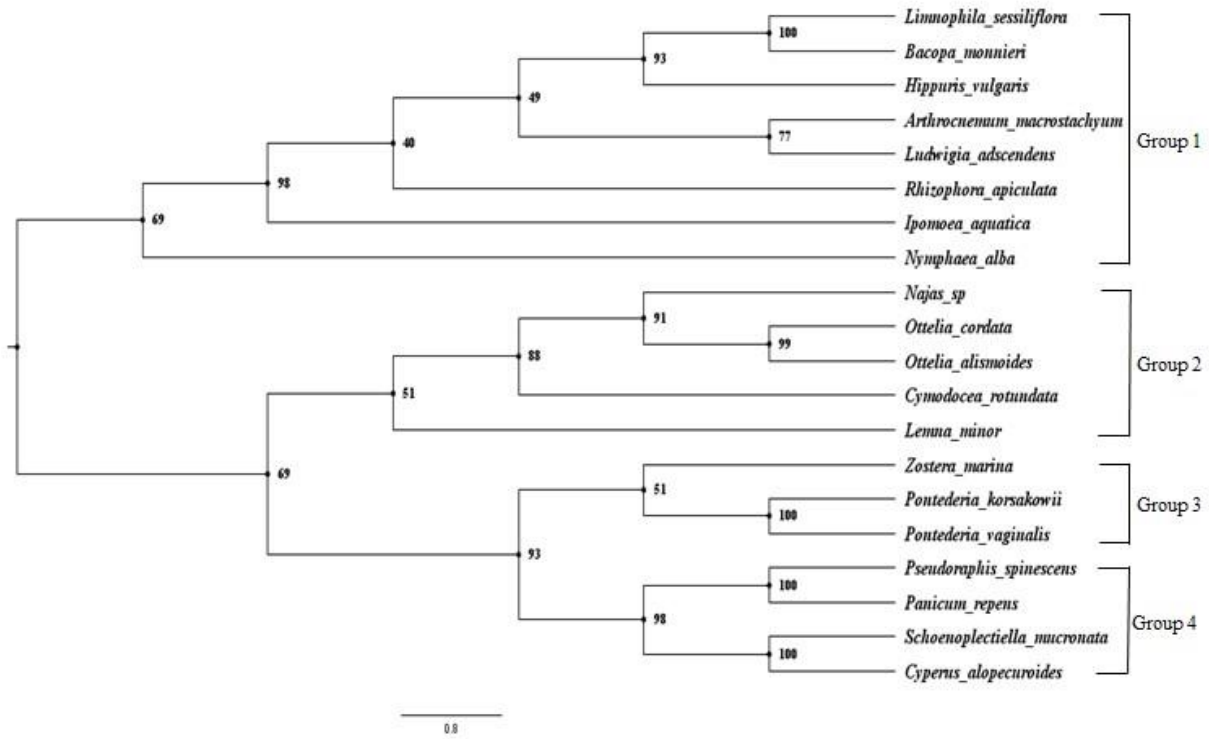

Figure 4. Phylogenetic tree of aquatic plants from the Rote Dead Sea area using IQ-TREE (Maximum Likelihood)

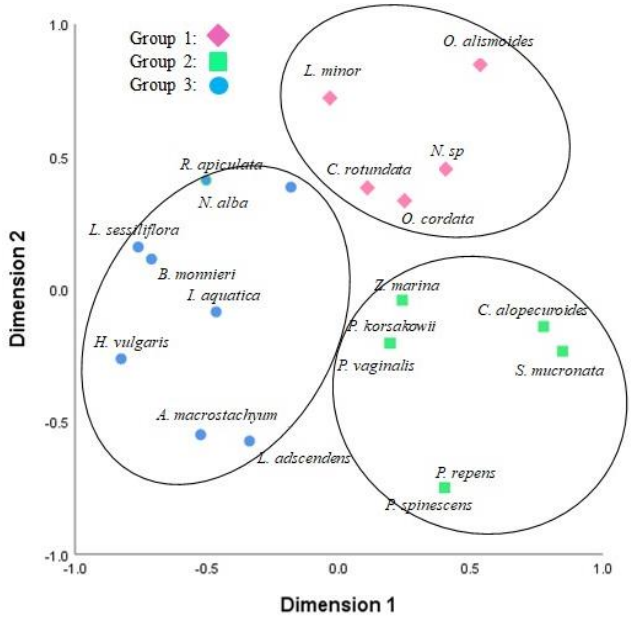

Figure 5. FCA (Fractional Component Analysis) with multidimensional scaling (MDS) of aquatic plants from the Rote Dead Sea area, from Bayesian Information (BI) results analysis

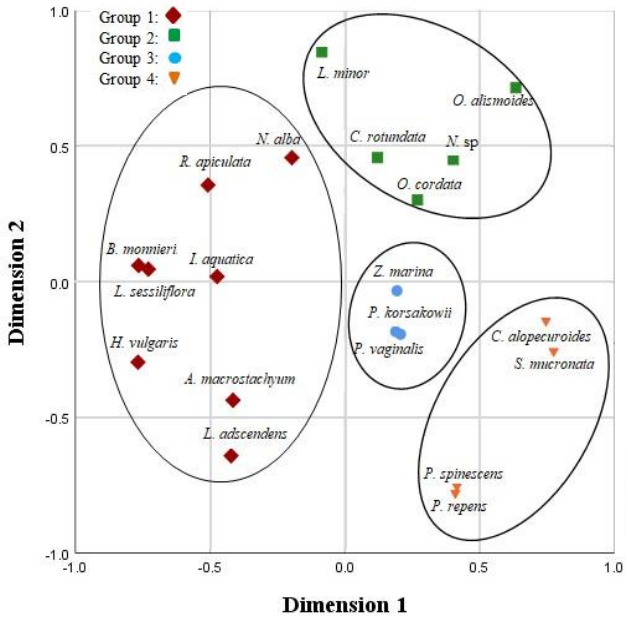

Figure 6. FCA (Fractional Component Analysis) with multi-dimensional scaling (MDS) of aquatic plants from the Rote Dead Sea area, from IQ-TREE (Maximum Likelihood) result analysis

Discussion

Rote Island waters is an island that has many lakes with unique characteristics, such as saltwater lakes which are very similar to marine ecosystems and freshwater lakes. Generally, in every marine or lake ecosystem, there are aquatic plants. Aquatic plants have important roles such as phytoremediation agents, trapping organic matter in eutrophic waters, and cleaning and controlling pollution of heavy metals, pesticides, and oil (Li et al. 2022). Identification of the diversity of aquatic plants needs to be done accurately because, from previous studies, the identification was only based on morphological information.

In this study, we carried out molecular identification using DNA barcoding. Identification using this method provides accurate results. Previously, research using DNA barcoding was carried out in Indonesia but was very limited. Several researchers carried out molecular identification on plants, including aquatic plants, such as in *Araceae lemnoideae* in West Java (Andriani et al. 2019) and soft coral, *Clavularia inflata* in 13 seawater sites in Indonesia (Anambas, Natuna, Tanjung Lesung, Kepulauan Seribu, Madura, Kangean, Bontang, Lombok, Manado, Wakatobi, Morotai, Kon, and Maluku Tenggara Barat) (Subhan et al. 2022). Limited information on the molecular identification of aquatic plants makes research on aquatic plants in Indonesia essential. This study observed molecular identification of aquatic plants using the *rbc*L marker, developed for a gene that encodes a photosynthetic enzyme Rubisco (Ribulose 1,5 bisphosphate carboxylase/oxygenase) (Tan et al. 2015). The *rbc*L can also be used to identify interspecific genetic variation and construct a phylogenetic tree. As a molecular marker, *rbc*L sequence data has been deposited in a tremendous amount in GenBank, making it easier to perform data analysis (Kim et al. 2014)

A high percentage of sequence identity in aquatic plants indicates a high degree of sequence similarity or homology between accession samples and sequences in the database. Nucleotide variations in the *rbc*L sequence comprised haplotype, nucleotide, and informative parsimony variations. The results showed that the 20 plant sequence data's haplotype diversity has a high value (1.0000 ± 0.003). Haplotype diversity is considered high if it has a value of 0,8-1,00 (Ausubel et al. 2011). The highest value of haplotype diversity indicates that the population of aquatic plants in the Rote Dead Sea area has a significant haplotypic variation. On the other hand, Nucleotide diversity (Nd), which measures the degree of polymorphism in a population, was 0.105 ± 0.004. Nucleotide diversity is declared to have a high value when Nucleotida diversity (Nd) >0,1 (Pardi et al. 2016). The result of the nucleotide diversity analysis of 20 rbcL sequences suggested that the aquatic plants from the Rote Dead Sea area have high nucleotide sequence variation.

Genetic distance indicates the differences between two or more species. In this study, the genetic distance value ranged from 0.004 to 0.164. *Pontederia korsakowii* and *P. vaginalis* have the closest genetic distance (0,004), while *P. spinescens* and *L. minor* showed the furthest distance (0,164). The kinship relationship between these species can influence genetic distance in species with a large or small value (Rinawati and Dinarti 2021). *Pontederia korsakowii* and *P. vaginalis* are related to each other. Both species belong to the same family Pontenderiaceae. In contrast, *P. spinescens* and *L. minor* are unrelated because they belong to different families, Poaceae and Araceae. Genetic distance has a range of values from 0 to 1. A value of 1 indicates the most significant genetic differences. Based on the pairwise distance results, the genetic distances among species are far less than 1. Similar results were obtained

from the study on seagrass using the *rbc*L marker reported by Stevanus and Pharmawati (2021). They obtained a genetic distance of 0.028 to 0.095 among seagrass species.

Phylogenetic tree reconstruction of aquatic plants from the Rote Dead Sea area using IQ-TREE (Maximum Likelihood) showed that all 20 aquatic plants from the Rote Dead Sea area were grouped into four groups. The branches formed on the phylogenetic tree are monophyletic. The bootstrap ranged from 33 to 100%. A bootstrap value of 33% was found in the *R. apiculata* branch. Many singleton characters and informative parsimony properties in the DNA sequence might cause a weak bootstrap value. A high bootstrap value indicates that the phylogenetic tree branching construction has a good level of accuracy and stability (Chen et al. 2012). The phylogenetic tree grouping pattern is similar to the FCA species grouping. The more related families, such as Poaceae and Cyperacea, are also grouped in one group, which indicates that the *rbc*L marker can be used for interspecific phylogenetic tree construction of aquatic plants.

In conclusion, the Rote Dead Sea area has a high diversity of aquatic plant species from freshwater to saltwater based on the *rbc*L sequences of the 20 types of aquatic plants that have been collected and identified. The values of nucleotide and haplotype diversity support the notion of the high diversity among the aquatic plant species in the area. Furthermore, the phylogenetic tree and Factorial Component Analysis grouped all aquatic plants into four groups. Therefore, the *rbc*L marker can accurately identify aquatic plant species and study genetic diversity and relationships among the aquatic plant species from the Rote Dead Sea area.

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