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Morphological and Genetic Diversity of Mangrove Species *Ceriops tagal* (Perr.) C.B. Rob. Around Java Island

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Abstract

Ceriops tagal (Perr.) C.B. Rob can be found in Baluran National Park, Kepulauan Seribu National Park, Karimunjawa National Park, and Segara Anakan Cilacap in Java and its surrounding areas. These locations have different demographics and environmental conditions. Plant species found in different geographical areas have varied genetic structures and morphological traits as a result of their adaptability to their environment. The goal of this study is to evaluate the morphological and genetic differences between the four populations of C. tagal in Java and its surroundings. Internode length, leaf length, leaf width, petiole length, and leaf area were the morphological characters used. The Internal Transcribed Spacer (ITS) region is used as a molecular marker. The UPGMA dendogram was used to analyze the morphological similarity, and multidimensional unfolding analysis was used to determine which character most determined the grouping. Mega X software was used to analyze genetic variation using the neighbor-joining approach, while Arlequin 3.5 software was used to estimate genetic variation within and among populations. The results of this study showed that the population of Baluran National Park has features or is distinct from the other three populations based on morphological and genetic characters; this could be because of variations in geographic conditions, and that population diversification was aided by a combination of genetic and physical divergence, restricted gene flow, and local adaptation.

Keywords: *Ceriops tagal, genetic, ITS, morphological*

Introduction

Ceriops tagal (Perr.) C.B. Rob is one of the mangrove species in the Rhizophoraceae family, which has an important role both in terms of ecology and economy. Like mangroves in general, *C. tagal* also has an ecological role as a shoreline protector from abrasion, sedimentation, and breakwaters (Zulkarnaini *et al.*, 2017). In addition, *C. tagal* also acts as a habitat for mangrove biota, especially molluscs (Partiwi *et al.*, 2023) and crustaceans (Ginantra *et al.*, 2021). *C. tagal* is also associated with epifauna (Setiawan *et al.*, 2021; Candri *et al.*, 2022) and infauna macrobenthos (Schrijvers *et al.*, 1998). For the economic use of *C. tagal*, for example, tannins from the bark are used as batik dyes and preservatives for toddy fermentation in the Philippines, and the wood is commonly used for fuel in Madagascar (Chan *et al.*, 2015). In addition, owing to the chemical content of diterpenoids and triterpenoids, *C. tagal* has the potential to be used as a raw material for medicine, because it has anti-hyperglycemic, cytotoxic properties (Chan *et al.*, 2015; Ahad *et al.*, 2021), antioxidant, anticoagulant, sedative effect, anthelmintic (Ahad *et al.*, 2021), antibacterial, and inhibitory properties of glucosidase (Chan *et al.*, 2015). *C. tagal* also acts as an anticorrosive (Shamsuzzaman *et al.*, 2021), and plays a role in mitigating disasters due to climate change because it is able to store carbon (C) from CO_2 compounds that have the potential to cause global warming (Ashuri and Patria, 2020).

C. tagal grows to form dense thickets on the edge of the land, from tidal to intertidal areas. *C. tagal*

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is also found along ponds, can grow on clay substrates, and associates with *C. decandra* (Giesen *et al.*, 2006). *C. tagal* can also grow in downstream mangrove forests, which are least affected by fresh water and can be found together with other species that are more tolerant to salt, such as *Sonneratia alba*, *Bruguiera sexangula*, *Lumnitzera racemosa*, and *Rhizophora stylosa* (Wang *et al.*, 2019).

Geographically, the distribution of *C. tagal* ranges from Mozambique and East Africa to the West Pacific and is spread across northern Australia and Southeast Asia, as well as Taiwan, South China (although it is very rare), and New Caledonia. In Southeast Asia, *C. tagal* is found in Myanmar, Cambodia, Thailand, Vietnam, the Philippines, Malaysia, Brunei, Singapore, Indonesia, and Papua New Guinea (Giesen *et al.*, 2006). *C. tagal* grows on the island of Java and its surroundings, for example in Baluran National Park (Hariyanto *et al.*, 2019), Kepulauan Seribu National Park (Wigati and Aminah, 2011), Karimunjawa National Park (Malik, 2022), and Segara Anakan Cilacap (Hilmi *et al.*, 2021; Jennerjahn *et al.*, 2022).

The four populations in this study have different geographical conditions; the Kepulauan Seribu National Park is a coral archipelago located in the Java Sea (Cahyadi *et al.*, 2019). Mangroves in the Kepulauan Seribu National Park are located in a core zone far from anthropogenic activity (Suraji *et al.*, 2015). Karimunjawa National Park has similarities with Kepulauan Seribu National Park because it is a conservation area located in the Java Sea (Nehren and Wicaksono, 2018). The existence of *C. tagal* in the eastern part of Java Island is in the Baluran National Park area, which is located at the tip of the island and is affected by the currents of the Madura Strait in the north, east of the Bali Strait, south of the Bajulmati River, and west of the Klokoran River (Fahmi *et al.*, 2017). The mangroves in Segara Anakan Cilacap are the only ones that are not in a conservation area and are found in almost the entire area. The western part of the area was converted into residential areas, while the eastern part of the area was used as an industrial area and a small area for aquaculture (Ardli *et al.*, 2022).

It has been proven from previous studies that plant species spread over different geographical areas also differ in their genetic structure and morphological characteristics. Morphological characteristics as such as Involucre color, trunk surface, twig surface, twig color, leaf shape, leaf color, leaf pubescence, number of primary leaf vein, primary leaf vein divergence, fruit and pedicles shape, fruit skin thickness, flesh texture, seed shape, cotyledon surface (Abbaszadeh *et al.*, 2020; Juma *et al.*, 2021). This can happen because of the adaptation of plants to their environment, both morphologically and genetically (Hu *et al.*, 2019; Gul *et al.*, 2021). Because of this, alongside morphological characters with emphasizing on the leaf (Permata and Susandarini, 2022), molecular markers have been extensively used in population genetics studies (Forough *et al.*, 2018; Susanto *et al.*, 2018; Marpaung and Susandarini, 2022). The physiological and biochemical processes of plants are directly impacted by the morphological variety of leaves, which also represents the plants' adaptive tactics for acquiring resources (Li and Wang, 2021). As the most plasticity organs of the plant the variation of leaf morphology commonly used as basis identification to determine plant species (Mollick *et al.*, 2021).

One of the markers that can be used in universal population genetic studies is the Internal Transcribed Spacer (ITS) (Forough *et al.*, 2018; Surya and Hari, 2018). ITS is a region on ribosomal DNA (rDNA), which is present in every eukaryotic organism. There are two ITS regions in eukaryotic organisms. ITS-1 is located between the 18S gene and the 5.8S gene, while ITS-2 is located between the 5.8S gene and the 28S gene (Baldwin, 1993). According to Baldwin *et al.* (1995), there are three things on the basis of which this ITS area is suggested for use in phylogenetic analysis of angiosperms. First, compared to other components in nrDNA (nuclear ribosomal DNA), the ITS region has relatively many copies, even thousands, which supports the detection, amplification, cloning, and sequencing of nrDNA. Second, the rate of evolution is relatively fast, so it can be used to study inter- or intraspecific genetic variation for evolution or phylogeny. Third, the ITS region has a relatively short base length (<700 bp in angiosperms) and is supported by its position, which is flanked by three conservative genes, making the ITS region easy to amplify. As far as is known, there have not been many studies on the genetic variability of *C. tagal* in Indonesia, especially in Java and its surroundings, and none have used ITS markers, so that this research can provide genetic information about *C. tagal* as a basis for further conservation and utilization of *C. tagal*.

Materials and Methods

A total of 16 samples of *C. tagal* were taken from four populations, namely Baluran National Park (Kalitopo 7°50'26.1" LS 114°27'46.4" E and Popongan 7°51'59.24" S 114°27'26" E, Figure 1 and 2.a), Kepulauan Seribu National Park (Penjaliran Timur Island 5°27'53.26" S 106°33'10.13" E, Figure 1 and 2.b), Karimunjawa National Park (Kemujan Island 5°49'39.8" S 110°28'36.0" E, Figure 1 and 2.c), and Segara Anakan Cilacap (eastern part 7°43'38.5" S, 108°59'2.6" BT, Figure 1 and 2.d). Four individuals from each population were taken. The organs needed are the youngest leaves and up to

2-3 older branches. Each sample is labeled according to the location and individual number, with the following description: B = Baluran National Park; KS = Kepulauan Seribu National Park; KJ = Karimunjawa National Park; C = Segara Anakan Cilacap; 1-2-3-4 = Sample number

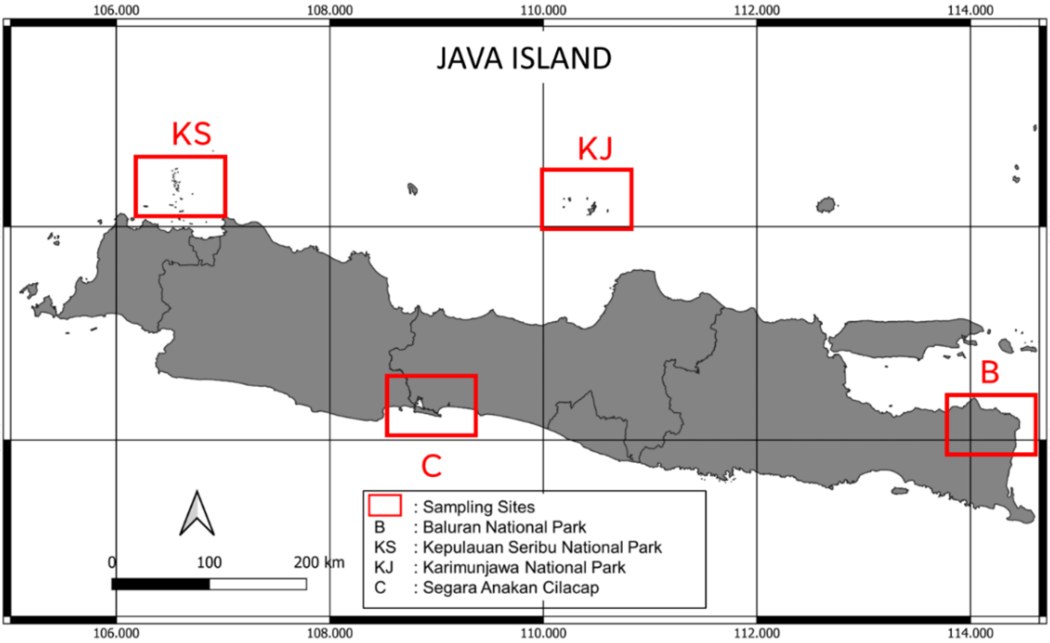


Figure 1. Sampling map around Java Island

a. Baluran National Park

b. Kepulauan Seribu National Park

c. Karimunjawa National Park

d. Segara Anakan Cilacap

Figure 2. Sampling habitats around Java Island

Morphological characteristics evaluation of the leaves

As the most plastic organs of the plant and closely related to adaptation to environmental change, we use the internode length, leaf length, leaf width, petiole length, and leaf area as characters for the determination of *C. tagal* species from four sampling locations (Figure 3.). Morphological character measurements were performed with ImageJ software. Morphological similarity analysis with the UPGMA dendogram was performed with NTsys software. Multidimensional unfolding analysis was performed in SPSS software to determine the characters that most determined the grouping.

Genetic variation analysis

DNA extraction was performed using the GENEAID Genomic DNA Mini Kit following the Kit's protocol. DNA amplification (PCR) was performed with primer pairs ITS 5P (5' GGA AGG AGA AGT CGT AAC AAGG 3') and 8P (5' CAC GCT TCT CCA GAC TACA 3'), using the Promega GoTaq Flexi DNA Polymerase Kit, with a total reaction mixture of 25μL (5 μL buffer 5X; 2.5 μL MgCl2 25 mM; 0.5 μL dNTPs 10mM; 0.5 μL forward primer 10 mM; 0.5 μL reverse primer 10 mM; 0.14 μL Taq Polymerase; 13.86 μL nuclease-free water; and 2 μL DNA template), and was carried out at initial denaturation temperature conditions 94ºC- 3 minutes, 35 cycles (denaturation 94ºC- 1 min, annealing 55ºC- 1 min, elongation 72ºC- 1 mins), final elongation 72°C- 5 mins, and maintained at 12°C. PCR amplification results were then visualized using 1% agarose gel electrophoresis in 1X TBE and BIOTIUM GelRed Nucleic Acid Gel Stain for 30 minutes at 100 volts. Furthermore, sequencing was carried out at 1st Base, Singapore. Alignment was performed using BioEdit software (Hall, 1999). Analysis of genetic variation from the ITS area was carried out using the neighbor-joining method with a 1000-replication bootstrap test, and genetic distances were calculated using the p-distance method in the MEGA X software (Kumar *et al.*, 2018). To determine the genetic variation within and between populations, analysis of molecular variance (AMOVA) was used in Arlequin 3.5 software (Excoffier and Lischer, 2010).

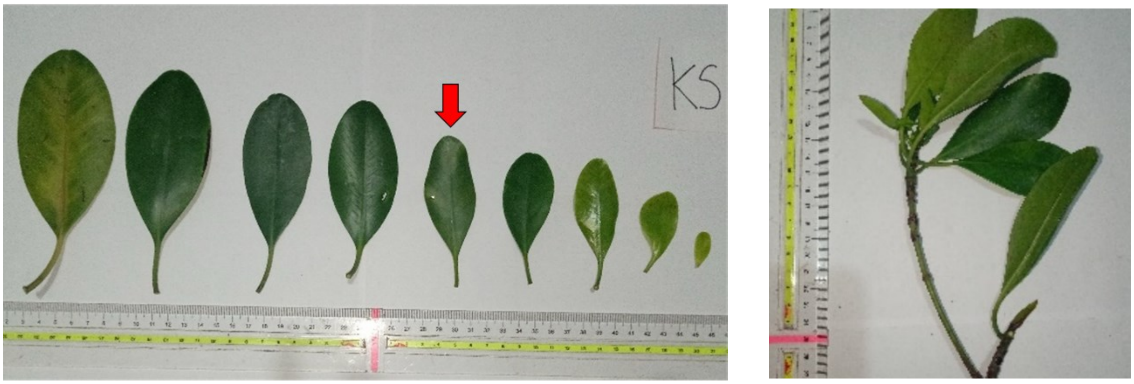
Result and Discussion

Morphological characters of Ceriops tagal (Perr.) C.B. Rob

Based on the results of measurements of internode length, leaf length, leaf width, petiole length, and leaf area presented in Figures 4 to 7 and Table 1, the number of leaves of *C. tagal* that curved backwards in Baluran National Park was greater than that of Segara Anakan Cilacap, Seribu Islands National Park, and Karimunjawa National Park, which is one of the effects of drought stress (Cal *et al.*, 2019). In addition, samples from Baluran National Park were found to have longer internodes compared to other populations, while leaf length, leaf width, and leaf area were relatively smaller than in other populations. On the other hand, the petiole was not significantly different in the four populations. The morphology of smaller leaves like those in Baluran National Park is also described in a study by (Zahidi *et al.*, 2013), which found that dry conditions can be the main factor in leaf width and that leaf area tends to be narrower when compared to humid conditions. Given that the habitat of *C. tagal* in Baluran National Park is on drier land when compared to other populations, due to the topography of the land, *C. tagal* is inundated only during the highest daily tide (in Kalitopo) and even during the highest tide during one period of the full moon (in Popongan). On the other hand, *C. tagal* in the Kepulauan Seribu National Park was inundated during medium and low tides; in Karimunjawa National Park it was inundated during medium and high tides; and in Segara Anakan Cilacap it was inundated during medium or even the lowest tides.

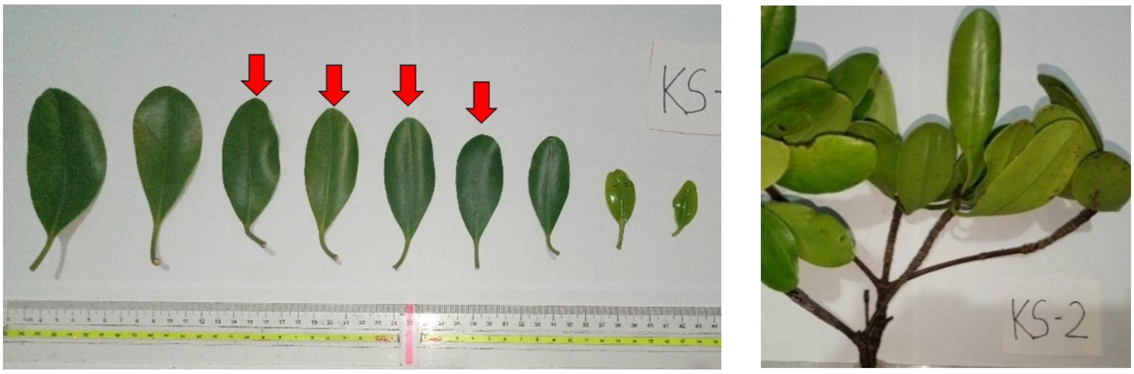
a b

Figure 3. Measurement of morphological characters: (a) internode length (b) leaf length, leaf width, petiole, and leaf area



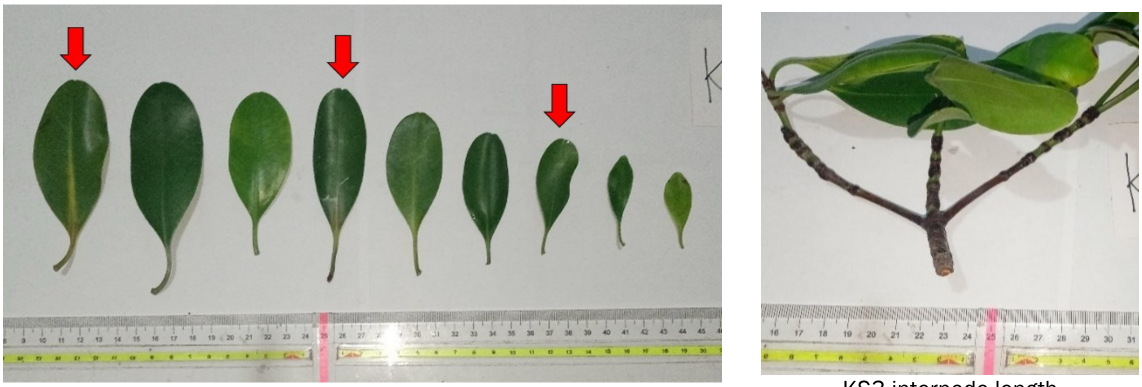
KS1 leaves

KS1 internode length



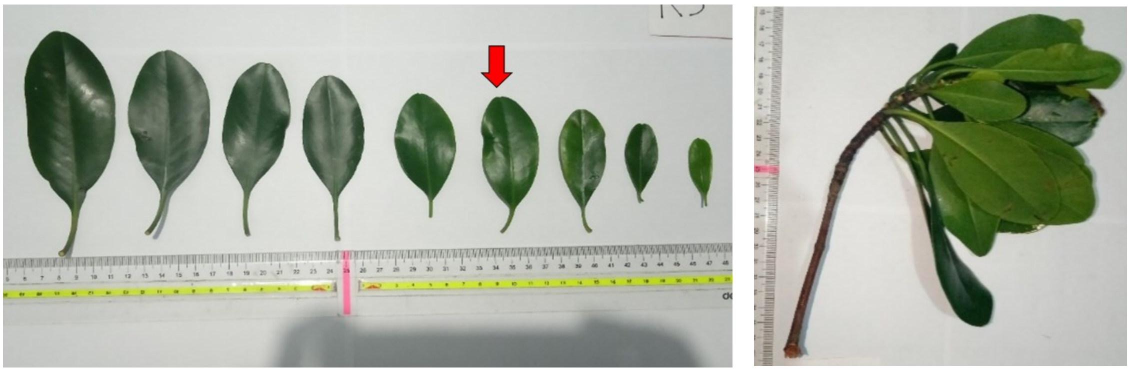
KS2 leaves

KS2 internode length



KS3 leaves

KS3 internode length



KS4 leaves

KS4 internode length

Note: ↓: leaves curved backwards

Figure 4. Morphological characters of leaves and internode length of *Ceriops tagal* (Perr.) C.B. Rob samples from Kepulauan Seribu National Park

KJ1 leaves

KJ1 internode length

KJ2 leaves

KJ2 internode length

KJ3 leaves

KJ3 internode length

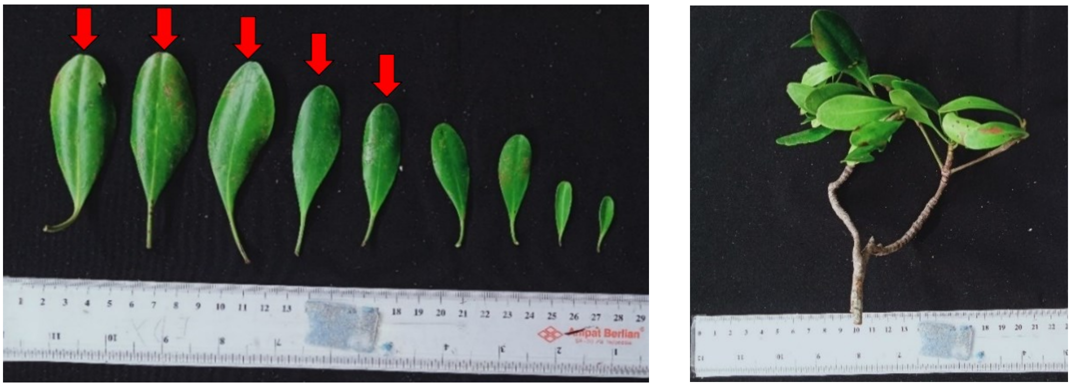
KJ4 leaves

KJ4 internode length

Note: ↓: leaves curved backwards

Figure 5. Morphological characters of leaves and internode length of *Ceriops tagal* (Perr.) C.B. Rob samples from Karimunjawa National Park

B1 leaves B1 internode length



B2 leaves B2 internode length

B3 leaves B3 internode length

B4 leaves B4 internode length

Note: ↓: leaves curved backwards

Figure 6. Morphological characters of leaves and internode length of *Ceriops tagal* (Perr.) C.B. Rob samples from Baluran National Park

C1 leaves

C1 internode length

C2 leaves

C2 internode length

C3 leaves

C3 internode length

C4 leaves

C4 internode length

Note: ↓: leaves curved backwards

Figure 7. Morphological characters of leaves and internode length of *Ceriops tagal* (Perr.) C.B. Rob samples from Segara Anakan Cilacap

Table 1. Results of measurements of the morphological characters of *Ceriops tagal* (Perr.) C.B. Rob using ImageJ

Sample	Internode length ± SD (cm)	Leaf length ± SD (cm)	Leaf width ± SD (cm)	Petiole length ± SD (cm)	Leaf area ± SD (cm^2)
B1	1.23±0.84	5.74±1.78	2.36±0.79	1.93±0.57	10.95±5.94
B2	1.33±0.75	5.02±1.85	1.96±0.89	1.52±0.54	7.95±5.19
B3	1.17±0.80	4.92±1.91	1.99±0.99	1.36±0.60	8.15±6.24
B4	1.46±0.55	5.38±1.58	2.16±0.66	1.440.58	9.05±5.11
KS1	0.76±0.59	7.11±3.40	3.38±1.66	2.02±1.22	21.22±16.74
KS2	0.66±0.41	6.52±2.45	3.01±1.09	1.82±0.69	16.65±10.53
KS3	1.03±0.87	6.20±2.32	2.75±1.12	1.88±0.75	14.45±9.88
KS4	0.76±0.74	7.71±2.56	3.72±1.25	2.09±0.91	24.44±15.02
KJ1	0.75±0.51	7.00±2.28	3.21±0.96	1.85±0.45	17.33±8.90
KJ2	0.80±0.71	6.65±2.57	3.18±1.30	1.81±0.86	18.72±10.98
KJ3	1.08±0.66	8.76±2.53	4.24±1.36	2.59±0.82	32.00±17.44
KJ4	0.86±0.37	6.72±3.09	3.23±1.50	2.05±0.92	17.77±11.97
C1	0.66±0.23	6.33±2.96	3.17±1.62	1.52±0.84	17.48±14.15
C2	0.91±0.85	5.89±2.29	2.92±1.30	1.54±0.41	14.37±9.43
C3	0.97±0.67	6.51±1.95	3.24±1.17	1.54±0.62	16.71±9.18
C4	0.91±0.45	6.29±1.81	3.11±0.83	1.30±0.45	15.00±7.39

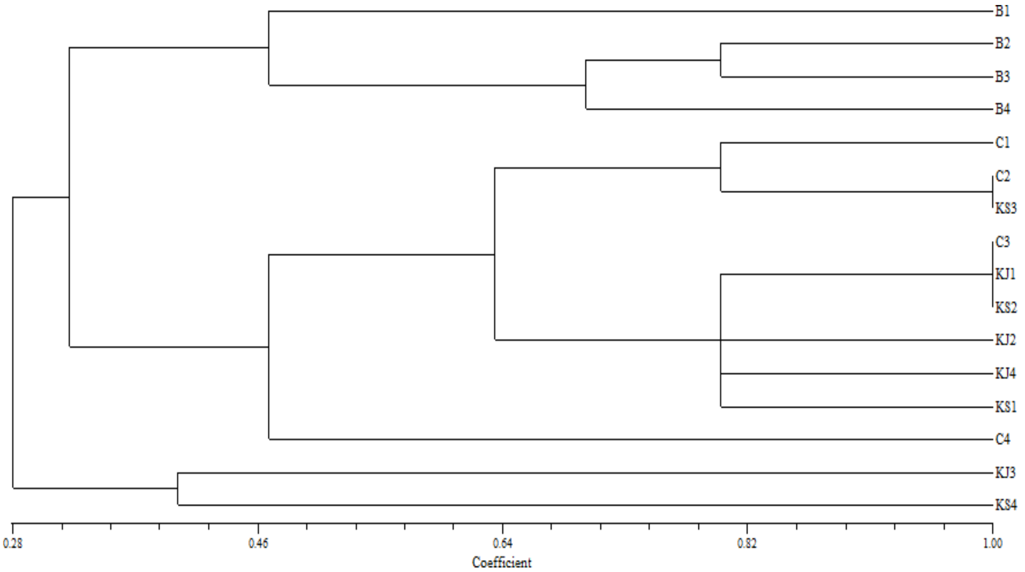


Figure 8. Dendogram of *Ceriops tagal* (Perr.) C.B. Rob morphology using UPGMA

To see the grouping of samples from the four locations, a dendrogram was compiled based on the similarity of the morphological characters of each sample (Figure 8). The UPGMA dendrogram results show that the sample from Baluran National Park is separated from other populations. Furthermore, samples from Kepulauan Seribu National Park, Karimunjawa National Park, and Segara Anakan Cilacap did not appear to be clustered with samples from the same population; in fact, they were closer to samples from other populations, or it could be said that the three populations had morphological similarities.

Multidimensional unfolding analysis (Figure 9.) was carried out to see the morphological characters that became the basis for grouping each sample. The morphological characters that form the strongest basis for separating Baluran National Park population groups are internodes length, supported by the characters of leaf area, leaf width, and leaf length, which support the grouping of samples from Kepulauan Seribu National Park, Karimunjawa National Park, and Segara Anakan Cilacap, except for C1. Meanwhile, the petiole character is located at a coordinate point far from the others, indicating that it is not a determining character for population grouping.

It is well known that the main function of leaves is to act as a link between plants and the environment, for example through gas exchange, exposure to light, and thermoregulation. This is also the cause of the diversification in leaf shape and size.

Leaf with high of Leaf Water Content (LWC), clustering of picophyll, fleshy and succulent leaf mostly found in dried environment, whereas those traits in plant as the mechanism of adaptation of physiology of plan to survive in drought condition (Goldsmith *et al.*, 2017). In addition, rainfall and local temperature are also strong factors affecting leaf morphology (Souza *et al.*, 2018; Li *et al.*, 2020). In the face of mild drought stress, plants are responding by regulating signalling pathway by specific hormones to mediate development of meristem cell, restricting shoot growth to conserve water and energy resources that they invest in growing root crops along with increasing water consumption and reproduction (Kim *et al.,* 2022). Meanwhile, the humid environment will have a detrimental effect on development of plants by facilitating plant growth with photosynthesis and excessing water such with flooding (Seleiman *et al.*, 2021).

The morphological variations can also occur due to the influence of geography (Zahidi *et al.*, 2013; Alcántara-ayala *et al.*, 2020), seasonal variations, genotypes, and genotype x environment interactions (Zahidi *et al.*, 2013). In this study, the habitat of *C. tagal* in Baluran National Park was on dry land, which was inundated only during the daily high tide, and was also adjacent to salt flats, which were inundated only during the highest tide in one full moon period. Furthermore, it is known that Kepulauan Seribu National Park has a similarity with Karimunjawa National Park, namely that it is a small island in the open waters, north of Java Island, and is affected by the waves of the Java Sea. This geographical condition may be related to the similar morphology of *C. tagal* in the two populations. On the other hand, from a habitat perspective, Segara Anakan Cilacap is similar to Karimunjawa National Park because *C. tagal* grows in mangrove forests that are not directly exposed to waves but are inundated at low or moderate tides. In addition to habitat factors and geographical conditions, the seasonal change at the time of sampling were also take role in the variation of the leaf morphology. On Woody plant, for example *Croton blanchetianus's* demonstrates seasonal flexibility on the leaf shape, possibly to maximize water usage effectiveness in seasons of water abundance and scarcity (Mendes *et al.*, 2022).

DNA visualization and phylogenetic tree

PCR results using ITS markers were visualized using agarose gel electrophoresis. It shows that the length of the amplified base is between 750 and 1000 bp (Figure 10.). Following molecular analysis, there were a total of 783 bp in the final dataset. Alignment was done using Clustal W on the BioEdit software. Blasting results at NCBI for *C. tagal* samples from the four locations showed 99.35% to 100% homology with the ITS sequence regions of *C. tagal* in the database with accession numbers EF119022.1, EF119032.1, and EF119040.1. This means that the sequences obtained in this study are from the ITS regions of *C. tagal*.

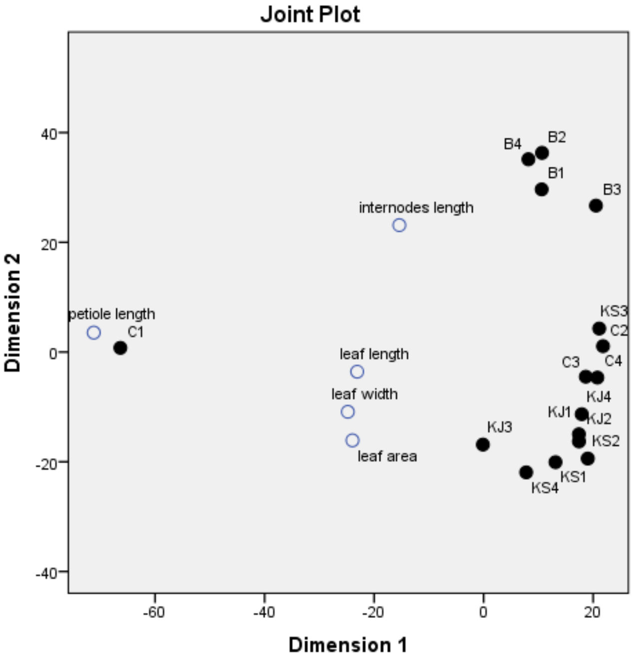


Figure 9. Clustering results based on the morphological characters of *Ceriops tagal* (Perr.) C.B. Rob using Multidimensional Unfolding

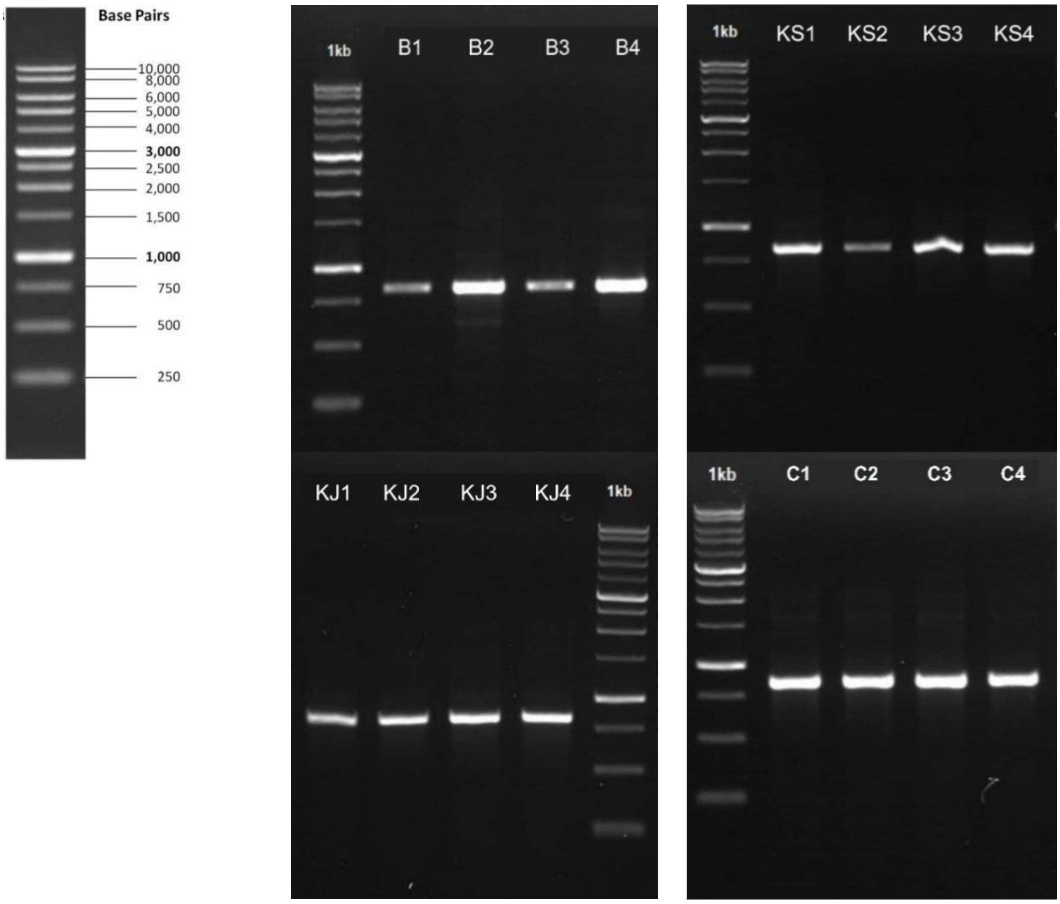


Figure 10. Visualization results of *Ceriops tagal* (Perr.) C.B. Rob PCR products using ITS 5P and ITS 8P primers

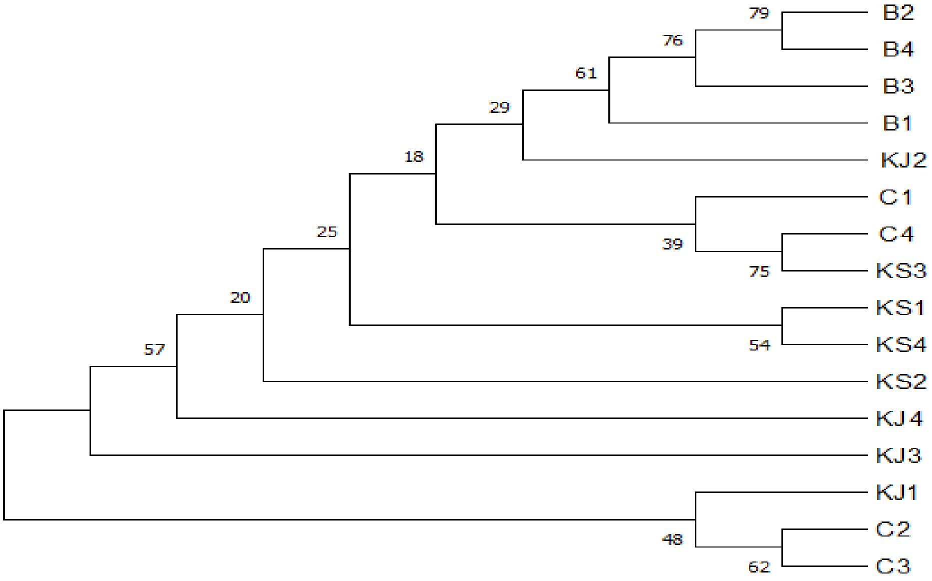


Figure 11. *Ceriops tagal* (Perr.) C.B. Rob phylogenetic tree using neighbor-joining p-distance method

The most optimal phylogenetic tree is selected from the construction results using the neighbor-joining (NJ) method with distance calculations using p-distance, as shown in Figure 11. The percentage of bootstrap test results is displayed for each branch. The results of the phylogenetic tree analysis from the ITS area show the similarity of patterns with morphological groupings. The sample from Baluran National Park is close to the same population sample. Meanwhile, samples from Kepulauan Seribu National Park, Karimunjawa National Park, and Segara Anakan Cilacap appear to have genetic similarity to samples from other populations.

A close relationship between populations can occur due to gene flow in these populations (Susanto *et al.*, 2018; Abbaszadeh *et al.*, 2020). Kepulauan Seribu National Park and Karimunjawa National Park are in the same sea zone due to the influence of the moonson that occurs in the waters of the Karimata Strait (Simanjorang *et al.*, 2018; Purba *et al.*, 2021). The similarity of marine zones certainly affects the distribution of *C. tagal* in the Kepulauan Seribu National Park and Karimunjawa National Park populations, which also allows for gene flow between the two populations. Different sea zones apply in the waters of Baluran National Park due to the influence of the moons coming from the waters of the Makassar Strait (Purba *et al.*, 2021). Meanwhile, what affects the waters in Segara Anakan Cilacap are the currents that pass through Nusa Tenggara, south of Java Island, to the west of Sumatra Island (Simanjorang *et al.*, 2018). According to the simulation of dispersal of the mangrove propagules by Van der Stocken *et al.,* (2019) showed that Segara Anakan Cilacap is part of Java Transtitional region that potentially received mangrove propagules from Sunda Shelf region, Northeastern Australia and Northwestern Australia. The current flows in the Java Transitional region were output flow from Indonesian throughflow (ITF) current that confirmed flowing through Java Sea then mix with the Indian South Equatorial Current by the Injection of the Leeuwin Current (Van der Stocken *et al.*, 2019; Gruenburg *et al.*, 2023). However, apart from the sea current factor, one of the possibilities for gene flow is the distribution of seedlings from Segara Anakan Cilacap to other mangrove forests in Indonesia, especially in Central Java and its surroundings by the mangrove restoration program from the conservationist and regional government (Darmawan, 2020; Gatra, 2020).

Genetic distance and genetic variation

The degree of gene difference (genome differences) between species or populations as measured by various numerical methods is referred to as genetic distance (Doğan and Doğan, 2016). Regarding to the criteria for determining genetic distance in Table 2.2, according to Nei (1972), if the genetic distance is 0.010–0.099, it means low; if it is 0.1–0.99, it means medium; and if the value is 1.00–2.00 it means high. According to Tallei *et al.* (2016), the lower the value of genetic distance, meaning that the more they have similarities, the closer the relationship; conversely, the greater the genetic distance, the greater the dissimilarity, or the greater the relationship. Based on the results of the analysis using the MEGA X software in Table 2, the genetic distance is in the range of 0.000 to 0.022. It can be said that the samples from the four locations are genetically similar.

The AMOVA in Table 3 shows that the percentage of genetic variation within the population (61.42%) is higher than the genetic variation between populations (38.58%). The results of this AMOVA analysis strengthen the previous analysis (Table 2.), which shows a low genetic distance value between the four populations, which means that the four populations have a lot of genetic similarities or low genetic variation between populations.

High genetic variation in populations may be related to cross-breeding that occurs within a species (Abbaszadeh *et al.*, 2020). In addition, it can also be caused by the introduction of new genes from outside the population or gene flow between different geographical populations (Jin *et al.*, 2020; Smith *et al.*, 2020). If gene flow in a population is limited, high genetic variation within the population can occur due to isolation by distance or local adaptation, interactions between genotype and

Table 3. AMOVA *Ceriops tagal* (Perr.) C.B. Rob from four populations

Source of variation	*d.f.*	*Sum of squares*	*Variance components*	*Percentage of variation*
Among populations	3	25,688	1,53125	38,58
Within populations	12	29,250	2,43750	61,42
Total	15	54,938	3,96875	
Fixation Index (FST) = 0,38583 p = 0,00000				

Table 2. Genetic distance of *Ceriops tagal* (Perr.) C.B. Rob using p-distance method

Sampel	B1	B2	B3	B4	KS1	KS2	KS3	KS4	KJ1	KJ2	KJ3	KJ4	C1	C2	C3	C4
B1	0															
B2	0,005	0														
B3	0,003	0,003	0													
B4	0,006	0,004	0,004	0												
KS1	0,004	0,008	0,005	0,009	0											
KS2	0,006	0,01	0,008	0,012	0,001	0										
KS3	0,006	0,005	0,003	0,006	0,003	0,004	0									
KS4	0,004	0,008	0,005	0,009	0	0,003	0,003	0								
KJ1	0,008	0,012	0,009	0,013	0,004	0,004	0,006	0,004	0							
KJ2	0,004	0,006	0,004	0,008	0,003	0,005	0,003	0,003	0,006	0						
KJ3	0,006	0,01	0,008	0,012	0,003	0,003	0,005	0,003	0,001	0,005	0					
KJ4	0,003	0,008	0,005	0,009	0,001	0,001	0,004	0,001	0,003	0,003	0,001	0				
C1	0,008	0,006	0,004	0,008	0,004	0,003	0,001	0,004	0,005	0,004	0,004	0,003	0			
C2	0,01	0,012	0,009	0,013	0,006	0,006	0,006	0,006	0,005	0,006	0,006	0,005	0,005	0		
C3	0,017	0,021	0,018	0,022	0,013	0,013	0,015	0,013	0,009	0,015	0,01	0,012	0,014	0,009	0	
C4	0,006	0,005	0,003	0,006	0,003	0,004	0	0,003	0,006	0,003	0,005	0,004	0,001	0,006	0,015	0

environment (Chen *et al.*, 2023), and even genetic drift (genetic drift) until the population has a specific gene structure (Abbaszadeh *et al.*, 2020). Genetic variations due to local adaptations or specific gene structures can also be formed due to genetic mutations (Mba, 2013). Genetic variation is important for plant survival and adaptation to environmental conditions. High genetic variation in the population helps to cope with environmental changes (Razgour *et al.*, 2019; Zhao *et al.*, 2021).

Conclusion

Regarding to the morphological and genetic of *C. tagal* leaf on this study, the population of Baluran National Park has can be clearly distinguished from the other three populations; this could be due the local environmental condition of this population has strongly drive to the plasticity of morphology of *C. tagal* leaf. Meanwhile the population of Segara Anakan Cilacap which geographically different with Kepulauan Seribu National Park and Karimunjawa National Park, has high genetic variation within population and a close genetic distance to other populations due to the genetic flow. Hence, the genetic and environmental factor not always work together in all sample population to form the morphology of *C. tagal* leaf. Population diversification of *C. tagal* on this study are indicated by morphological divergence of leaf as a mechanism of adaptation and limited gene flow between *C. tagal* population. Regarding to those matters, the further consideration of the conservation of *C. tagal* in general and particularly on Java Island and its surroundings could be implied from this study.

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References

Abbaszadeh, M., Sheidai, M., Azizi, N. & Koohdar, F. 2020. Genetic and Morphological Variability in Medicinal Plant *Helichrysum oocephalum* Boiss. (Asteraceae) in Iran. *Hacquetia*, *19*(2): 317–324. https://doi.org/10.2478/hacq-2020-000 2

Ahad, F., Zilani, N.H., Akter, A. & Sadat, A. 2021. Comparative Pharmacological Potential of *Ceriops decandra* (Griff) and *Ceriops tagal* Linn : Medicinal Plants of the Sundarbans. *J. Med. Plants Stud.*, *9*(4): 14–23.

Alcántara-ayala, O., Oyama, K., Ríos-muñoz, C. A., Rivas, G., Ramirez-Barahona, S. & Luna-Vega, I. 2020. Morphological Variation of Leaf Traits in the *Ternstroemia lineata* Species Complex (Ericales : Penthaphylacaceae) in Response to Geographic and Climatic Variation. *PeerJ*, 8: 1–26. https://doi.org/10.7717/peerj.8307

Ardli, E.R., Yuwono, E. & Purwanto, A.D. 2022. Land Cover Changes and Impacts of Massive Siltation on the Mangrove Segara Anakan Lagoon System , Cilacap Indonesia. *J. Ecol. Eng.*, *23*(7): 29–41.

Ashuri, N.M. & Patria, M.P. 2020. Vegetation and Carbon Stock Analysis of Mangrove Ecosystem in Pancer Cengkrong , Trenggalek , East Java , Indonesia. *IOP Conf. Ser. Earth Environ. Sci.*, 481(1): p.012029 https://doi.org/10.1088/1755-1315/481/1/012029

Baldwin, B.G. 1993. Molecular Phylogenetics of Calycadenia (Compositae) Based on ITS Sequences of Nuclear Ribosomal DNA: Chromosomal and Morphological Evolution Reexamined. *Am. J. Bot., 80*(2)" 222–238. https://doi.org/10.2307/2445043

Baldwin, B.G., Sanderson, M.J., Porter, J.M., Wojciechowski, M.F., Campbell, C.S. & Donoghue, M.J. 1995. The its Region of Nuclear Ribosomal DNA: A Valuable Source of Evidence on Angiosperm Phylogeny. *Ann. Missouri Bot. Gard.,* 82(2): 247. https://doi.org/10.2307/2399880

Cahyadi, A., Ramadhoan, F. & Sasongko, M.H.D. 2019. Water Resources in Pari Cay , Kepulauan Seribu , Jakarta , Indonesia Water R. *IOP Conf. Ser. Earth Environ. Sci.*, 256(1): p. 012012. https://doi.org/10.1088/1755-1315/256/1/012012

Candri, D.A., Rahmani, M.S., Ahyadi, H. & Zamroni, Y. 2022. Diversity and Distribution of Gastropoda and Bivalvia in Mangrove Ecosystem of Pelangan, Sekotong, West Lombok. *Jurnal Biologi Tropis*, 22(3): 1092–1100. https://doi.org/10.29303/jbt.v22i3.1413

Chan, E.W.C., Tangah, J., Kezuka, M., Hoan, H.D. & Binh, C.H. 2015. Botany, Uses, Chemistry and Bioactivities of Mangrove Plants II: *Ceriops tagal*. *ISME/GLOMIS Electronic Journal*, 13(6): 39–43.

Chen, S., Huang, C., Cheng, Y., Wang, C., Li, C., Lai, I. & Hung, K. 2023. Heliyon Effect of Geographic Isolation on Genetic Variation and Population Structure of *Euphrasia nankotaizanensis*, A Threatened Endemic Alpine Herb in Taiwan. *Heliyon*, *9*(3): e14228. https://doi.org/10.1016/j.heliyon.2023.e14228

Darmawan, L. 2020. *Konservasi Mangrove, Ternyata Mampu Datangkan Rp650 Juta per Tahun*. Mongabay.

Doğan, İ. & Doğan, N. 2016. Genetic Distance Measures: Review. *Turk. Klin. J. Biyoistatistik*, 8(1): 87–93. https://doi.org/10.5336/biostatic.2015-49517

Fahmi, M.Y., Muttaqin, A.D., Nurjanah, I., Bama, P., Massive, C., Coral, A., Enceusting, A.C., Branching, A.C., Follios, C., Submassive, C., Coral, D. & Karang, T. 2017. Monitoring Ekosistem Laut dan Pesisir di Taman Nasional Baluran, Situbondo. *Prosiding Seminar Nasional Kelautan Dan Perikanan III 2017*, *September*, 40–54.

Forough, J.F., Shivaji, T.A. & Mallikarjun, D.R. 2018. Assessment of Genetic Diversity Among Different Sugarcane Genotypes Using Internal Transcribed Spacer (ITS) Region of the Ribosomal DNA (rDNA). *GSC Biological and Pharmaceutical Sciences*, 5(2): 017–025. https://doi.org/10.30574/gscbps.2018.5.2.0108

Fritz, M.A., Rosa, S. & Sicard, A. 2018. Mechanisms Underlying the Environmentally Induced Plasticity of Leaf Morphology. *Front. Genet.*, 9: 1–25. https://doi.org/10.3389/fgene.2018.00478

Gatra. 2020. Arobretum Cilacap Suplai 600 Bibit Mangrove Per Tahun. Gatra.Com. https://www.gatra.com/detail/news/470858/gaya-hidup/arobretum-cilacap-suplai-600-bibit-mangrove-per-tahun

Giesen, W., Wulffraat, S., Zieren, M., Sch, L. & Olten. 2006. Mangrove Guidebook For Southeast Asia. FAO and Wetlands International

Ginantra, I.K., Muksin, I.K. & Joni, M. 2021. Crab Diversity as Support for Ecotourism Activities in Pejarakan Mangrove Forest, Buleleng, Bali, Indonesia. *Biodiversitas*, *22*(10): 4139–4145. https://doi.org/10.13057/biodiv/d221003

Goldsmith, G.R., Bentley, L.P., Shenkin, A., Salinas, N., Blonder, B., Martin, R.E., Castro-Ccossco, R., Chambi-Porroa, P., Diaz, S., Enquist, B. J., Asner, G.P. & Malhi, Y. 2017. Variation in Leaf Wettability Traits Along A Tropical Montane Elevation Gradient. *New Phytologist*, 214(3): 989–1001. https://doi.org/10.1111/nph.14121

Gruenburg, L.K., Gordon, A.L. & Thurnherr, A.M. 2023. Indonesian Throughflow Partitioning Between Leeuwin and South Equatorial Currents. *J. Phys. Oceanogr.,* 53(9): 2159-2170 https://doi.org/https://doi.org/10.1175/JPO-D-22-0205.1

Gul, J., Saeed, S., Ahmed, A., Laghari, S. ullah khan, Basit, A., Rehman, A. & Zahid, M. 2021. Genetic Diversity and Morphological Variation of Pinus Gerardiana Along the Environmental Gradient From Zhob, Balochistan, Pakistan. *Nusantara Bioscience*, 13(1): 121–128. https://doi.org/10.13057/nusbiosci/n130116

Hariyanto, S., Fahmi, A. K., Soedarti, T. & Suwarni, E. E. 2019. Vegetation and Community Structure of Mangrove in Bama Resort Baluran National Park Situbondo East Java. *Biosaintifika*, 11(1): 132–138. https://doi.org/10.15294/biosaintifika.v11i1.19111

Hilmi, E., Sari, L.K., Cahyo, T.N., Muslih, Mahdiana, A. & Samudra, S.R. 2021. The Affinity of Mangrove Species Using Association and Cluster Index in North Coast Of Jakarta and Segara Anakan of Cilacap, Indonesia. *Biodiversitas*, *22*(7): 2907–2918. https://doi.org/10.13057/biodiv/d220743

Hu, C.C., Wu, Y.Q., Ma, L., Chen, Y.J. & Ji, X. 2019. Genetic and Morphological Divergence Among Three Closely Related Phrynocephalus Species (Agamidae). *BMC Evolutionary Biology*, 19(1): 1–15. https://doi.org/10.1186/s12862-019-1443-y

Jennerjahn, T.C., Ardli, E.R., Boy, J., Heyde, J., Lukas, M.C., Nordhaus, I., Sastranegara, M.H., Máñez, K.S. & Yuwono, E. 2022. 7 - Mangrove Ecosystems Under Threat in Indonesia: the Segara Anakan Lagoon, Java, and Other Examples. *Science for the Protection of Indonesian Coastal Ecosystems (SPICE)* (pp. 251–284). https://doi.org/https://doi.org/10.1016/B978-0-12-815050-4.00004-3

Jin, C., Huixia, K. & Shubin, D. 2020. Population Genetic Structure and Gene Flow of Rare and Endangered *Tetraena mongolica* Maxim . Revealed by Reduced Representation Sequencing. *BMC Plant Biology*, 20(391): 1–13.

Juma, I., Geleta, M., Hovmalm, H.P., Nyomora, A., Saripella, G.V., Carlsson, A.S., Fatih, M. & Ortiz, R. 2021. Comparison of Morphological and Genetic Characteristics of Avocados Grown in Tanzania. *Genes*, 12(1): 1–21. https://doi.org/10.3390/genes12010063

Kim, G., Ryu, H. & Sung, J. 2022. Hormonal Crosstalk and Root Suberization for Drought Stress Tolerance in Plants. *Biomolecules*, 12(6): 1–16. https://doi.org/10.3390/biom12060811

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis Across Computing Platforms. *Mol. Biol. Evol.,* 35(6): 1547–1549. https://doi.org/10.1093/molbev/msy096

Li, Y. Q. & Wang, Z.H. 2021. Leaf Morphological Traits: Ecological Function, Geographic Distribution and Drivers. *Chin. J. Plant Ecol.*, 45(6): 1154–1172. https://doi.org/10.17521/cjpe.2020.0405

Li, Y., Zou, D., Shrestha, N., Xu, X., Wang, Q., Jia, W. & Wang, Z. 2020. Spatiotemporal Variation in Leaf Size and Shape in Response to Climate. *J Plant Ecol.,* 13(1): 87–96. https://doi.org/10.1093/JPE/RTZ053

Malik, A. 2022. Endemic Plants on Karimunjawa Island as an Environmental Conservation Effort. *Al-Hayat: Journal of Biology and Applied Biology*, 5(2): 157–168.

Marpaung, A.A. & Susandarini, R. 2022. Genetic diversity of Dicranopteris and Sticherus from Rokan Hulu, Riau based on ISSR marker. *J. Tropical Biodiversity and Biotechnology*, 7(1): p.66552. https://doi.org/10.22146/JTBB.66552

Mba, C. 2013. Induced Mutations Unleash the Potentials of Plant Genetic Resources for Food and Agriculture. *Agronomy*, 3(1): 200–231. https://doi.org/10.3390/agronomy3010200

Mendes, K.R., Batista-Silva, W., Dias-Pereira, J., Pereira, M.P S., Souza, E.V., Serrão, J.E., Granja, J.A.A., Pereira, E.C., Gallacher, D.J., Mutti, P. R., da Silva, D.T.C., de Souza Júnior, R.S., Costa, G. B., Bezerra, B.G., Silva, C.M.S.E. & Pompelli, M.F. 2022. Leaf Plasticity Across Wet and Dry Seasons in *Croton blanchetianus* (Euphorbiaceae) at a Tropical Dry Forest," *Sci. Rep.*, 12(1): 1–12. https://doi.org/10.1038/s41598-022-04958-w.

Mollick, A.S., Sultana, R., Azad, M.S. & Khan, M.N.I. 2021. Leaf Morphological Plasticity in Three Dominant Tree Species in The Sundarbans Mangrove Forest of Bangladesh in Different Salinity Zones. *Wetl. Ecol. Manag.,* 29(2): 265–279. https://doi.org/10.1007/s11273-020-09782-5.

Nehren, U. & Wicaksono, P. 2018. Mapping Soil Carbon Stocks in An Oceanic Mangrove Ecosystem in Karimunjawa Islands, Indonesia. *Estuar. Coast. Shelf Sci.,* 214: 185-193. https://doi.org/10.1016/j.ecss.2018.09.022

Partiwi, S., Idrus, A. Al & Umami, R. 2023. Mangrove Ecosystem Services in Mitigation for Mollusca Communities in Lembar Sheet District, West Lombok Regency. *Jurnal Biologi Tropis*, 23(1): 164–173. https://doi.org/10.29303/jbt.v23i1.4367

Permata, D.A. & Susandarini, R. 2022. Morphological Diversity and Phenetic Relationship of Wild and Cultivated Begonia Based On Morphology and Leaf Venation. *Biodiversitas*, 23(2): 928–937. https://doi.org/10.13057/biodiv/d230235

Purba, N. P., Pranowo, W. S., Ndah, A. B. & Nanlohy, P. 2021. Seasonal Variability of Temperature, Salinity, and Surface Currents at 0° Latitude Section of Indonesia Seas. *Regional Studies in Marine Science*, 44: p.101772. https://doi.org/10.1016/j.rsma.2021.101772

Razgour, O., Forester, B., Taggart, J. B., Bekaert, M., Juste, J. & Ibáñez, C. 2019. Considering Adaptive Genetic Variation in Climate Change Vulnerability Assessment Reduces Species Range Loss Projections. *Proc. Natl. Acad. Sci. U.S.A.,* 116(21): 10418–10423. https://doi.org/10.1073/pnas.1820663116

Schrijvers, J., Camargo, M.G., Pratiwi, R. & Vincx, M. 1998. The Infaunal Macrobenthos Under East African *Ceriops tagal* Mangroves Impacted by Epibenthos. *J. Exp. Mar. Biol. Ecol.*, 222(1–2): 175–193. https://doi.org/10.1016/S0022-0981(97)00144-5

Seleiman, M.F., Al-Suhaibani, N., Ali, N., Akmal, M., Alotaibi, M., Refay, Y., Dindaroglu, T., Abdul-Wajid, H.H. & Battaglia, M.L. 2021. Drought Stress Impacts on Plants and Different Approaches to Alleviate Its Adverse Effects. *Plants*, 10(2): 1–25. https://doi.org/10.3390/plants10020259

Setiawan, R., Siddiq, A.M., Wimbaningrum, R., Sulistiyowati, H. & Aditiya, M.Y. 2021. Diversity of Gastropod Types in Mangrove Forest Jatipapak Resort Kucur Alas Purwo National Park. *Bioeduscience*, 5(3): 257–263. https://doi.org/10.22236/j.bes/536235

Shamsuzzaman, M., Kalaiselvi, K. & Prabakaran, M. 2021. Evaluation of Antioxidant and Anticorrosive Activities of *Ceriops tagal* Plant Extract. *Applied Sciences*, 11(21): p.10150. https://doi.org/10.3390/app112110150

Simanjorang, J.E., Pranowo, W.S., Sari, L.P., Purba, N. P. & Syamsuddin, M.L. 2018. Building Up the Database of the Level-2 Java Sea Ecoregion

Based on Physical Oceanographic Parameters. *IOP Conf. Ser.: Earth Environ. Sci.*, 176(1): 1–13. https://doi.org/10.1088/1755-1315/176/1/012009

Smith, A.L., Hodkinson, T.R., Villellas, J., Catford, J.A., Csergő, A.M., Blomberg, S.P., Crone, E.E., Ehrlén, J., Garcia, M.B., Laine, A.L., Roach, D.A., Salguero-Gómez, R., Wardle, G.M., Childs, D. Z., Elderd, B.D., Finn, A., Munné-Bosch, S., Baudraz, M.E.A., Bódis & J., Buckley, Y.M. 2020. Global Gene Flow Releases Invasive Plants from Environmental Constraints on Genetic Diversity. *Proc. Natl. Acad. Sci. U.S.A.*, 117(8): 4218–4227. https://doi.org/10.1073/pnas.1915848117

Souza, M.L., Duarte, A.A., Lovato, M.B., Fagundes, M., Valladares, F. & Lemos-Filho, J.P. 2018. Climatic Factors Shaping Intraspecific Leaf Trait Variation of A Neotropical Tree Along A Rainfall Gradient. *PLoS ONE*, 13(12): 1–20. https://doi.org/10.1371/journal.pone.0208512

Suraji, Rasyid, N., Kenyo, A.S., Jannah, A.R., Wulandari, D.R., Saefudin, M., Ashari, M., Widiastutik, R., Kuhaja, T., Juliyanto, E., Afandi, Y.A., Wiyono, B., Syafrie, H. & Handayani, S. N. 2015. Profil Kawasan Konservasi DKI Jakarta. Direktorat Konservasi Kawasan dan Jenis Ikan Direktorat Jenderal Kelautan, Pesisir dan Pulau-Pulau Kecil Kementerian Kelautan dan Perikanan.

Surya, S. & Hari, N. 2018. DNA Barcoding of Mangroves Using Ribosomal ITS Marker in Rhizophoraceae. *Int. J. Sci. Res. Sci. Technol,*, *3*(7): 066–609.

Susanto, A. H., Nuryanto, A. & Daryono, B. S. 2018. High Connectivity Among *Synedrella nodiflora* Populations in Java Island Based on Intergenic Spacer atpB-rbcL. *Biosaintifika*, 10(1): 41–47. https://doi.org/10.15294/biosaintifika.v10i1.12038

Tallei, T. E., Rembet, R. E., Pelealu, J. J. & Kolondam, B. J. 2016. Sequence Variation and Phylogenetic Analysis of *Sansevieria trifasciata* (asparagaceae). *Bioscience Research*, 13(1): 1–7.

Van der Stocken, T., Wee, A.K.S., De Ryck, D.J. R., Vanschoenwinkel, B., Friess, D. A., Dahdouh-Guebas, F., Simard, M., Koedam, N. & Webb, E. L. 2019. A General Framework for Propagule Dispersal In Mangroves. *Biological Reviews*, 94(4): 1547–1575. https://doi.org/10.1111/brv.12514

Wang, W., Li, X. & Wang, M. 2019. Propagule Dispersal Determines Mangrove Zonation at Intertidal and Estuarine Scales. *Forests*, 10(245): 1–19. https://doi.org/10.3390/f10030245

Wigati, R. & Aminah, N. 2011. Potential Plants in The Coast and Mangrove Forest Area of Seribu Archipelago. *Bul. Penelit. Kesehat.*, 39(1), 42–51.

Zahidi, A., Mousadik, A.El & Bani-Aameur, F. 2013. Morphological Diversity of Leaf Characters in Three Natural Populations of *Argania spinosa* L. Skeels. *J. Exp. Biol. Agric.*, 1(4): 297–316.

Zhao, W., Wang, X., Li, L., Li, J., Yin, H., Zhao, Y. & Chen, X. 2021. Evaluation of Environmental Factors Affecting the Genetic Diversity, Genetic Structure, and The Potential Distribution of *Rhododendron aureum* Georgi Under Changing Climate. *Ecol. Evol.*, 11(18): 12294–12306. https://doi.org/10.1002/ece3.7803

Zulkarnaini, Saam, Z., Amrivo, V. & Miswadi, D. 2017. Community Structure and Economic Evaluation Mangrove Village In Bengkalis District. *Int. J. Oceans Oceanogr.*, 11(1): 63–74.