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Research Article

Diversity of Santigi (*Pemphis acidula* J.R.Forst. & G.Forst.), A Mangrove Association in Tomini Bay, Sulawesi, Indonesia

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ABSTRACT

Pemphis acidula is a wild plant in rocky or sandy coastal areas and mangrove ecosystems. Different geographic characteristics may affect plant adaptability and have an impact on the emergence of various genotypes. This study was performed to reveal the phenetic relationship and genetic variation of *P. acidula* in 3 different areas in Tomini Bay, Gorontalo Province, Indonesia. We took 3 samples from each location and analysed them using 14 morphological characters and molecular approaches based on ISSR markers and ITS gene. The results showed that *P. acidula* on Olele had bigger sizes in some morphological features compared to the plants in other study areas. The phenetic analysis showed that *P. acidula* at Biluhu and Dulanga were more closely related, although *P. acidula* at the 3 locations had 100% similarity. Genetic variation analysis showed the highest genetic similarity based on ISSR markers was found in Dulanga and Biluhu samples (76.8%). Phylogenetic based on ITS gene revealed that Olele samples were in the same clade with *P. acidula* accession from GenBank (genetic distance 0-0.19%), while Biluhu samples were a sister group (genetic distance 24.97-25.03%) even though their percentage identity corresponds to *P. acidula* (81.34%). Plant adaptation to different habitat conditions may affect the genetic diversity of *P. acidula*.

INTRODUCTION

Gorontalo, one region of Sulawesi Island, is located in the northern part of Sulawesi and is geographically bordered by Tomini Bay, the largest bay in Indonesia. Tomini Bay is included in the coral triangle. The coastal area of Gorontalo has varied geographical conditions, contains a high level of endemic biodiversity, and one of which is sourced from mangrove ecosystem (Baderan et al. 2022).

Pemphis acidula J.R.Forst. & G.Forst. is one of the mangrove association plants. It is known as the only accepted member of Genus *Pemphis* in Family Lythraceae (POWO 2022). It is considered as a shrub or small

tree growing in coastal areas which are rocky, sandy, or at the edge of mangrove forests (Giesen et al. 2006; Utina et al. 2019). This coastal tree is typically 4-10 meters in height. It has a wavy stem with irregular branches (Irwansah et al. 2017; Manek & Puay 2020). Many people admire *P. acidula* and utilise it as an expensive ornamental plant in the form of *bonsai* (dwarf potted plant) (Cunningham et al. 2017). *Pemphis acidula* is naturally distributed in East Africa, India, Southeast Asia, and Australia. In Indonesia, this plant is mostly found in all main islands including Java, Moluccas, Kalimantan, and Sulawesi (George 1990; Rao & Ellis 1995; de Wilde & Duyfjes 2016; POWO 2022). The locals are known this plant by the name "Santigi" (Baderan et al. 2018).

Recent study in Gorontalo revealed that *P. acidula* is specifically found in three coastal areas of Tomini Bay, namely Biluhu, Dulanga and Olele Beaches (Baderan et al. 2022). Biluhu, Dulanga and Olele Beaches display various environmental conditions and plant species. Biluhu Beach exposes a dominantly sandy ground structure with 117 plant species distributed around the area (Baderan & Utina 2021). Dulanga Beach has a rocky characteristic and 56 plant species were found in the steep rocky hills. Meanwhile, Olele Beach has a partially sandy-rocky soil structure with 82 plant species on the rock hill (Baderan & Angio 2019). The distribution, richness, and diversity of plant species may affected by developmental stages of plant, geographical condition and environmental factors (Ibrahim 2021).

At present, research about the variety of *P. acidula* has not been massively conducted and the available information is too general while there are need for specific ones. However, information on plant morphological and genetic diversity on the individual, species, or population levels is essential to be used as basic consideration for conservation, breeding, management, and sustainable use of the species (Abdelhamid et al. 2014; Raji & Siril 2021). Hence, this study aims to reveal the morphological and genetic diversity of *P. acidula* in three different areas in Tomini Bay, Gorontalo Province, Indonesia based on the phenetic and DNA molecular marker.

MATERIALS AND METHODS

Fieldwork and Sample collection

This study was carried out from June 2021 to July 2022. Based on our previous findings, we chose three study areas of *P. acidula* as follows: Site 1 Biluhu Beach, Site 2 Dulanga Beach, and Site 3 Olele Beach. The three locations are located in the coastal area of Gorontalo's South Beach with coral limestone as the bedrock. Sampling sites were shown in Figure 1.

We found that there are only 12 mature individuals of *P. acidula* (tree or generative phase) spread in three different locations. There are also several seedling of *P. acidula* in the location, but we were used it as exclusion criteria. We did purposive sampling with only *P. acidula* in the generative stage and reachable as inclusion criteria. Morphological observations were conducted by describing the habit and the characteristics of the leaves, roots, stems, fruits and seeds. Measurement of plant height, stem diameter, length, width and weight of leaves, fruit and seeds, as well as the height of the buttress root were also carried out. Each character was documented and detailed the information in terms of collector's name, collection number, date, location, and habit which was noted on the prepared observation sheet. Several abiotic factors including altitude, light intensity, substrate pH, air humidity, air temperature and habitat were also measured over several days with a single measurement every day.

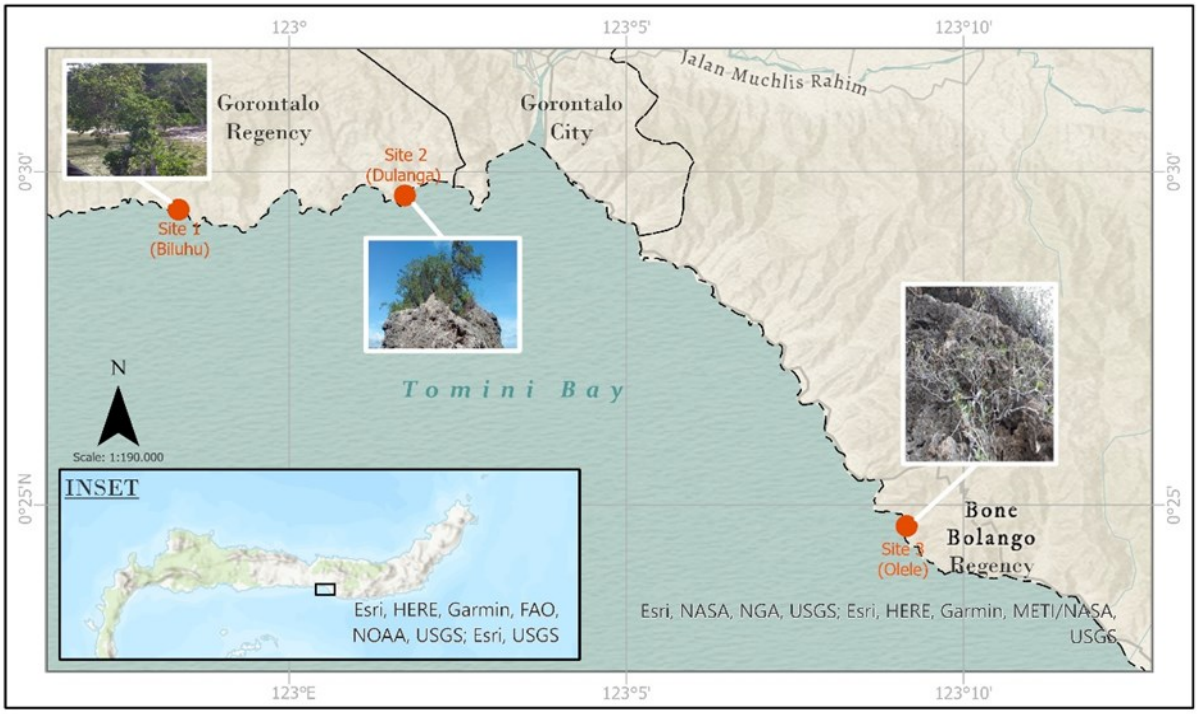

Figure 1. The study sites map of *P. acidula* at Tomini Bay of Gorontalo, Sulawesi: Site 1 Biluhu Beach, Site 2 Dulanga Beach, and Site 3 Olele Beach.

Leaf, root, stem, fruit and seed samples were collected from 3 *P. acidula* individuals in the generative stage from each provenance for further morphological analysis in laboratory. Leaves sample of *P. acidula* at each site were also taken for molecular analysis. Fresh young leaves sample from 3 individuals of *P. acidula* at each location were prepared by putting them into a separated plastic bags containing silica gel (Martida & Pharmawati 2019), then stored in cooler box for transportation. Leaf samples were sent to Integrated Research and Testing Laboratory of Universitas Gadjah Mada, Yogyakarta.

Species identification and Phenetic analysis

Identification of *P. acidula* was carried out by comparing the morphological features with the data in several references, namely Flora of Java Volume II (Backer & van den Brink 1965), Plant Identification Terminology (Harris & Harris 2001), Flora Malesiana Series 1 Moraceae: Ficeae (Berg & Corner 2005), as well as 4 herbarium specimens collection of Naturalis Biodiversity Center (Bijmoer et al. 2023). The speciment data were shown on Table 1.

Identification towards the validation of accepted names were conducted by using several sites i.e. https://www.gbif.org/ (GBIF Secretariat: GBIF Backbone Taxonomy), https://www.theplantlist.org/ (The Plant List 2022), and https://powo.science.kew.org/ (POWO 2022). Stipulated the conservation status of *P. acidula* were in reference to International Union of Conservation of Nature (IUCN) on Red List of Threatened Species (https://iucnredlist.org/) (IUCN 2022).

Phenetic analysis of *P. acidula* was carried out by comparing 14 qualitative morphological characters between samples from 3 locations with *Sonneratia alba* (Family Lythraceae) from Biluhu as outgroup considering *P. acidula* is the only member of Genus *Pemphis*. The qualitative morphological characters observed included habit; petiole, shape, tip, margin, surface, symmetry, colour, texture and section arrangement of leaves; petal colour; as well as texture, shape and colour of ripe fruit. The

morphological characters were converted into a binary matrix 0-1 based on the similarity and dissimilarity of each character with *S. alba* (Duncan & Baum 1981). Matrix data were analysed using MVSP 3.1 program and dendogram were constructed based on similarity index by using UPGMA with Jaccard's Coefficient.

Table 1. Herbarium specimen data of *Pemphis acidula* from Naturalis Biodiversity Center

Collection number	Collector	Location	Accessed URL
L.2487455	Turner, H.	Aru Islands, New Guinea	https://www.gbif.org/occurrence/2514336352
L.3923106	Afriastini, J.J.	Siberut Island, Sumatera	https://www.gbif.org/occurrence/2516885754
L.2487476	Snellius-II	Tiger Island, Sulawesi	https://www.gbif.org/occurrence/2514429373
L.2487469	Hidayat, A.	Pangkep Regency, Sulawesi	https://www.gbif.org/occurrence/2516308158

Molecular analysis

DNA Extraction and Amplification

Genomic DNA were extracted from 0.1g leaf sample according to Genomic DNA Mini Kit (Plant) (Geneaid) manufacturer's protocols, then were amplified using 10 ISSR primers (UBC-807, UBC-810, UBC-814, UBC-817, UBC-826, UBC-827, UBC-830, UBC-834, UBC-835, UBC-845) (Ibrahim 2021; Sevindik & Efe 2021). The PCR premix was contained 25 µL reaction volume consisting of 12.5 µL of 1x PCR-kit MyTaq™ HS Red Mix (Bioline), 20µM of each ISSR primer, 40 ng DNA template and nuclease free water. Thermal cycler was run at 35 cycles in condition of pre-denaturation at 94°C for 10 minutes, denaturation at 94°C for 1 minute, annealing at 45-55°C (according to the optimal annealing temperature of each ISSR primer) for 1 minute, extension at 72°C for 2 minutes, post extension at 72°C for 10 minutes, and hold at 12°C for infinity. The amplification products and 1 Kb DNA Ladder marker were electrophoresed using 2% agarose gel contained DNA staining in 1x TBE buffer at 100V for 1h (Seng et al. 2013), then were visualized on a UV transilluminator and documented using geldoc and optilab. All procedures were undertaken by Integrated Research and Testing Laboratory of Universitas Gadjah Mada, Yogyakarta (certificate number: 00875.01/IX/UN1/LPPT/2021).

Genetic variation analysis

Genetic variations of *P. acidula* were analysed by electrophoresis visualization of PCR products using ISSR primer. The DNA band patterns produced by ISSR markers were converted into a binary matrix 0-1 based on the absence or the presence of the DNA band (Ibrahim 2021). They were analysed using MVSP version 3.1 program with simple matching coefficient of UPGMA method to construct dendogram based on similarity index (Singh et al. 2014; Putri et al. 2023).

Phylogenetic analysis

Genetic relationship of *P. acidula* were analysed by Internal Transcribed Spacer (ITS) gene as the DNA barcode, using primer P674 (5′-CCTTATCATTTAGAGGAAGGAG-3′) and P675 (5′-TCCTCCGCTTATTGATATGC-3′) to amplify nuclear ITS region of 700-720 bp containing ITS1, 5.8S, and ITS2 (Nguyen et al. 2017). Ampli-

fication of the samples from Olele and Biluhu were undertaken by Integrated Research and Testing Laboratory of Universitas Gadjah Mada, Yogyakarta, according to a procedure by Nguyen et al. (2017) with some modifications. Sequencing of the amplification product were undertaken by Genetika Science Indonesia Ltd.

The ITS sequences data of samples Olele and Biluhu were BLAST with ITS gene sequences of *P. acidula* accession in GenBank (https://www.ncbi.nlm.nih.gov/genbank/) to determine the percentage of identity and query cover. The data of ITS gene sequences were also used to reconstruct a phylogenetic tree based on neighbor-joining (NJ) algorithm with Kimura 2 Parameter model using MEGA X software (Kumar et al. 2018), followed by the genetic distance analysis between samples and *P. acidula* accession in GenBank.

RESULTS AND DISCUSSION

Environmental condition

Pemphis acidula is a highly adaptive plant. Various geographical conditions, especially in the coastal area of Gorontalo may causing some morphological and genetic changes in the response of the plants toward the environmental condition. Based on our study, Biluhu and Dulanga Beaches (Site 1 and 2, respectively) have relatively similar physicochemical parameter values compared to Olele Beach (Site 3) which have slightly different altitude and habitat (Table 2). In Biluhu and Dulanga Beaches, *P. acidula* grows in coastal land with coral limestone at an altitude of 4 m asl. In contrast, *P. acidula* in Olele Beach grows in karst structural foothills at 11 m asl of altitude. *Pemphis acidula* in three locations were spread naturally and can only be found in certain points as it can only grow in an ideal location (Ellison et al. 2010).

Morphological description and Phenetic analysis

Comparison of morphological characters between samples from the three locations with a number of references and 4 herbarium specimens showed that all the samples studied were verified as *P. acidula* J.R.Forst. & G.Forst. The plant habit of *P. acidula* is perennial shrubs or small trees, with a height ranging from 1.78 to 3.8 meters (Figure 2 and Table 3). It is in contrast to *P. acidula* in East Africa, Australia and other Southeast Asian regions, which can reach 5-7 meters in height (George 1990; Rao & Ellis 1995; de Wilde & Duyfjes 2016). Even in the Andaman Islands, India, this plant can reach a height of 9.5 meters (Goutham-Bharathi et al. 2015). Plant growth can be affected by several factors, including developmental stage, age, nutrition and genetics. However, environmental condition also indirectly affects plant growth, thereby affecting plant height.

Differences in environmental conditions in Table 2, especially altitude and habitat, can affect the morphological characters at the three locations resulting in size variations (Table 3). *P. acidula* at Olele Beach

Table 2. The range measurements result of 6 physicochemical parameters in three research locations.

Physicochemical Parameter	Study Site		
	Site 1 Biluhu Beach	Site 2 Dulanga Beach	Site 3 Olele Beach
Altitude (m asl*)	4	4	11
Habitat	Coastal land	Coastal land	Structural foothills
Light intensity (mW/cm²)	0.428 – 0.485	0.493 – 0.552	0.516 – 0.523
pH	7.52 – 8.25	8.13 – 8.24	7.47 – 7.97
Humidity (%)	51 – 75	69 – 70	60 – 61
Temperature (°C)	23 – 28	24 – 25	26 – 26

*m asl = meters above sea level

Figure 2. Shrubs habit of *P. acidula* in three sites: (a) Biluhu Beach; (b) Dulanga Beach; and (c) Olele Beach.

performs a bigger size in plant height, diameter of stem, length and weight of leaf, length of petiole, fruit and peduncle, as well as the height of buttress roots than those observed in other study areas. At the Biluhu and Dulanga Beaches, these plants share relatively similar morphological character sizes, except that the seed weight of *P. acidula* at Dulanga Beach is twice as large as the other two locations. *P. acidula* which only grows on karst cliffs at an altitude of 11 m asl at Olele Beach has an advantage to their growth because it is not much affected by sea water. Meanwhile, *P. acidula* on Biluhu and Dulanga Beaches grows in rocky coastal areas at an altitude of 4 m asl which is still affected by tidal effects. Continuous exposure to salinity can be toxic and affect plant physiological processes, which in turn can suppress plant growth (Kodikara et al. 2018; Dittmann et al. 2022).

Pemphis acidula has such a knee root, where some parts of the root above the ground surface grow highly then bow and slip into the ground. It is bent, rounded, and slightly flat (Figure 3,4,5 A). The root surfaces are greyish-pale, rough, stiff enough, and are no spines. *Pemphis acidula* is growing in direction of perpendicular (*erectus*) with sympodial branching. It has a stiff and strong woody stem, grey-brownish, elongated round-like cylinder, rough surface and disclosed a cracked stem bark that looked like scales all over the stem surface (Figure 3,4,5 B). It has single leaves (*folium simplex*) which are arranged alternately, quite thick and stiff, succulent, ellipse (*elipticus*) to elongates (*oblongos*) in shape, and flat margin with no slices (*integer*) (Figure 3,4,5 D). The leaves are green with a hairy abaxial and adaxial surface and are not wrinkled. The petiole (*petiolus*) is cylindrical with a slightly flat upper side, green, thickened on the base

Table 3. The average measurement result of 15 morphological characters of *P. acidula* from 3 sites

Morphological Characters	Measurement result average at study area		
	Site 1 Biluhu Beach	Site 2 Dulanga Beach	Site 3 Olele Beach
Plant Height	1.787 m	2.157 m	3.831 m
Stem diameter	13 cm	14.8 cm	27 cm
Leaf length	19.11 mm	18.29 mm	27.34 mm
Leaf width	9.68 mm	9.03 mm	8.8 mm
Leaf weight	0.0963 g	0.073 g	0.917 g
Leaf thickness	0.68 mm	0.51 mm	0.44 mm
Petiole length	2.02 mm	2.05 mm	3.07 mm
Fruit length	7.09 mm	7.90 mm	9.1 mm
Fruit width	5.02 mm	5.55 mm	5.23 mm
Fruit weight	0.0958 g	0.1454 g	0.1336 g
Peduncle length	2.38 mm	4.83 mm	5.49 mm
Seed width	2.65 mm	3.32 mm	2.28 mm
Seed length	3.52 mm	4.09 mm	3.57 mm
Seed weight	0.0034 g	0.0072 g	0.0030 g
Buttress root height	13.5 cm	13.1 cm	50 cm

part, and a little hairy without a stipule. The leaves are pinnate (*penninervis*), attached laterally to the costa so that the leaves on both sides of the costa are not symmetrical. The vein does not always appear and stops before reaching the leaf margin. The vein has a smaller size, forms a net and do not stand out. The flowers are single or in pairs, axillary (*flos axillaris*) (Figure 3,4,5 C), with six white crumpled petals (Figure 3,4,5 F). The peduncle (*pedunculus*) is reddish-green, unbranched and a little hairy. The fruits are a true single, dry capsule, enclosed within hypanthium, containing about 2-10 tiny seeds with several pseudolayers and topped by style (Figure 3,4,5 E). The young fruit is green and turns brownish when it is ripe. These morphological features showed high similarities to *P. acidula* J.R.Forst. & G.Forst described elsewhere, such as in Andaman Island, India (Goutham-Bharathi et al. 2015) and in Somalia (POWO 2022).

Figure 3. *Pemphis acidula* J.R.Forst. & G.Forst. from Biluhu Beach: A - knee roots; B – Bark; C - Fruit in axilla; D – Leaves; E – Fruit with persistent style, side view; F – Flower with crumpled white petals.

A total of 14 qualitative morphological characters of *P. acidula* at three locations and *S. alba* from Biluhu were analysed in phenetics using the UPGMA method with Jaccard's Coefficient to determine the similarities between them. The similarity index may indicate their genetic relationship. If the similarity value is high, then the genetic relationship between them will also be close (Putri et al. 2023). The results shows that the analysis of phenetic relationship between *P. acidula* at the three locations and *S. alba* from Biluhu yielded in 3 clusters, namely A, B and C. Cluster A consist of *P. acidula* at Biluhu and Dulanga with similarity of 100%. Furthermore *P. acidula* at Biluhu and Dulanga fused with *P. acidu-*

Figure 4. *Pemphis acidula* J.R.Forst. & G.Forst. from Dulanga Beach: A - knee roots; B – Bark; C - Fruit in axilla; D – Leaves; E – Fruit with persistent style, side view; F – Flower with crumpled white petals.

Figure 5. *Pemphis acidula* J.R.Forst. & G.Forst. from Olele Beach: A - knee roots; B – Bark; C - Fruit in axilla; D – Leaves; E – Fruit with persistent style, side view; F – Flower with crumpled white petals.

la at Olele forming cluster B with similarity of 100%. Next, *S. alba* from Biluhu fused with *P. acidula* at 3 locations forming cluster C with similarity of 7.7% (Figure 6). It can be seen that *P. acidula* at 3 locations have a high level of similarity, so it can be said that the phenetic relationship between them is very close. However, *P. acidula* at Biluhu and Dulanga had the closest phenetic relationship.

Genetic diversity

The existence of associated mangroves particularly *P. acidula* species in three study areas with different habitats may show the possibility of plant to adapt towards the environmental condition which influenced by genet-

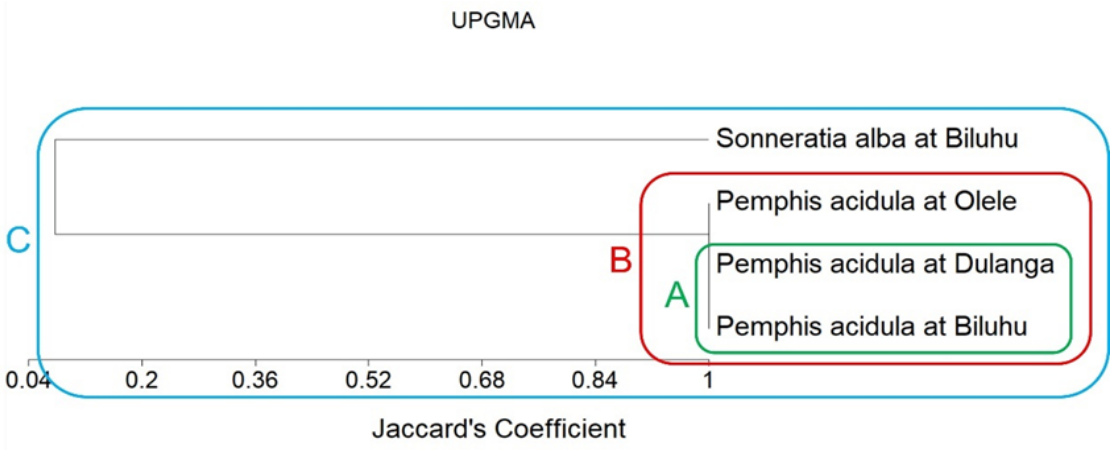

Figure 6. Dendogram showing the clustering of *P. acidula* at 3 locations and *S. alba* at Biluhu based on qualitative morphological characters using the UPGMA methods with Jaccard's Coefficient.

ic diversification of species level. Genetic diversity quantifies how big a genetic diversity is in or between populations. Genetic diversity can predict homogeneity or heterogeneity which makes the plants possibly adapt and survive in a dynamic environment (Ramzan et al. 2020).

Genetic variation analysis using ten primers ISSR showed that all primers are capable of detecting and amplifying the sequence of *P. acidula* and *S. alba* genome with 175 bp to 4400 bp in size (Figure 7). There were a total of 147 polymorphic bands from 156 DNA bands that appeared. This is in line with some previous studies which showed that ISSR primer had successfully detected the polymorphism between species (Wang et al. 2012; Hamza et al. 2013; Louati et al. 2019; Ramzan et al. 2020; Raji & Siril 2021; Sevindik & Efe 2021; Takele et al. 2021). The higher the level of polymorphism, the higher the genetic diversity in a species (Ezekiel et al. 2011). However, among the specimens, similarities were found mainly between *P. acidula* on the beaches of Biluhu and Dulanga compared to those in Biluhu and Olele or Dulanga and Olele.

Genetic variation similarities data based on ISSR marker (Figure 7) were supported by the analysis of similarities using UPGMA method with simple matching coefficient. A dendogram which revealed a genetic relationship resulted in a total of 3 clusters, namely I, II and III (Figure 8). Cluster I consist of *P. acidula* at Dulanga and Biluhu with similarity of 76.8%. Furthermore, *P. acidula* at Dulanga and Biluhu fused with *P. acidula* at Olele forming cluster II with similarity of 64.2% and 62.3%, respectively. Next, *S. alba* at Biluhu fused with *P. acidula* at Biluhu, Dulanga and Olele forming cluster III with similarity of 38.4%, 37.7% and 32.5%, respectively. This strengthens the results of the phenetic analysis (Figure 6) that *P. acidula* at Biluhu is more closely related genetically to *P. acidula* at Dulanga than to those in Olele.

Based on the results of genetic relationship analysis using ISSR marker (Figure 8) which showed high similarity between *P. acidula* at Biluhu and Dulanga (76.8%), we decided to only use *P. acidula* at Biluhu along with Olele sample for phylogenetic analysis based on ITS sequences. The ITS sequences of *P. acidula* at Biluhu and Olele were analysed using BLAST and obtained five *P. acidula* accession in GenBank (accession codes: MH768221.1, MH768222.1, MH768223.1, AY035762.1 and AF268394.1) with highest similar sequences to Biluhu and Olele sample, as well as *Sonneratia apetala*-MH244026.1, *Lafoensia pacari*-JN701292.1 and *Lafoensia acuminata*-AY905425.1 as outgroup. BLAST analysis of ITS sequences of *P. acidula* at Biluhu and Olele were shown in Table 4.

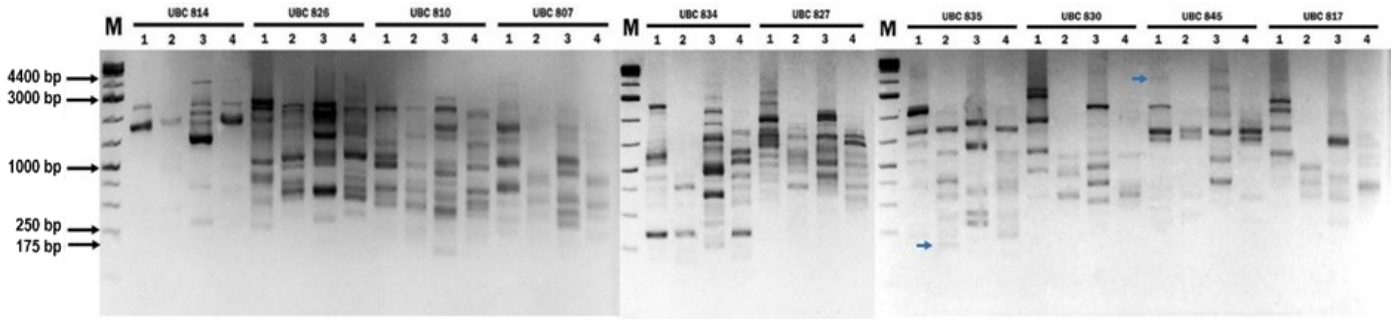

Figure 7. The pattern of ISSR bands of *P. acidula* and *S. alba* using 10 primers; M = 1 kb DNA Ladder Lane 1 = *P. acidula* from Olele; Lane 2 = *P. acidula* from Biluhu; Lane 3 = Sonneratia alba from Biluhu; Lane 4 = *P. acidula* from Dulanga; blue arrow = the longest and shortest band.

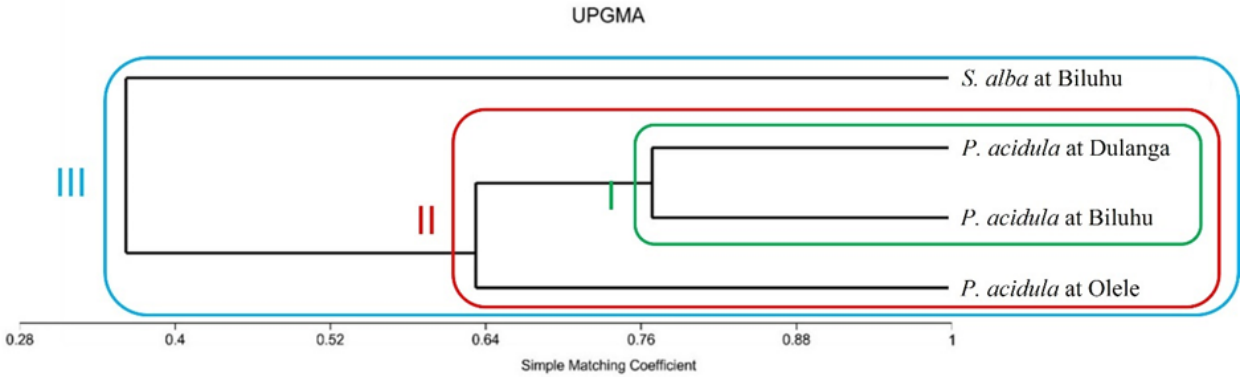

Figure 8. Dendogram showing the clustering of *P. acidula* at 3 locations and *S. alba* at Biluhu based on ISSR marker using the UPGMA methods with simple matching coefficient

Based on Table 4, the BLAST result of ITS gene sequences of Olele (Sample A1 R) was verified as *P. acidula* with the highest identity percentage of 100% and a query cover score of 98%. Meanwhile, ITS gene sequences of Biluhu (Sample B1 R) had acquired the highest identity percentage of 81.34% and a query cover score of 93%. It can be said that Biluhu sample has an ITS sequences with lower similarity to the ITS sequences of *P. acidula* accession in GenBank compared to the Olele sample.

Reconstruction of the phylogenetic tree with NJ method Kimura 2 Parameter model towards the samples from Olele and Biluhu, five accession samples of *P. acidula* from GenBank (accession codes: MH768221, MH768222, MH768223, AY035762 and AF268394), and 3 outgroup samples (*Sonneratia apetala*-MH244026, *Lafoensia pacari*-JN701292 and *Lafoensia acuminata*-AY905425) showed that *P. acidula* in Olele is located on the same clade with the accessions of *P. acidula* in GenBank with bootstrap score 97. Meanwhile, the sample located in Biluhu is considered as a sister group of *P. acidula* (Figure 9).

Cladogram and genetic distance data based on the ITS gene sequence among 5 accessions of *P. acidula* in GenBank with the Olele sample revealed an extremely low score (0 - 0.19%). Meanwhile, the samples located in Biluhu performed a high score (24.97 - 25.03%) (Table 5). This score implied a close phylogenetic relationship between Olele sample and five accessions of *P. acidula*, while the phylogenetic relationship between Biluhu sample and five *P. acidula* accessions is pretty far. According to Qin et al. (2017) and Ningrum et al. (2020), the genetic distance threshold for ITS2 in intraspecies of seed plants is 3.76%. Based on the value, it can be said that Olele sample and the fifth *P. acidula* accession were classified as intraspecies, while Biluhu sample was classified as different species. However, given the very high morphological similarity between the Biluhu and Olele samples, it is possible that the Biluhu sample is a species

complex of *P. acidula* in Olele.

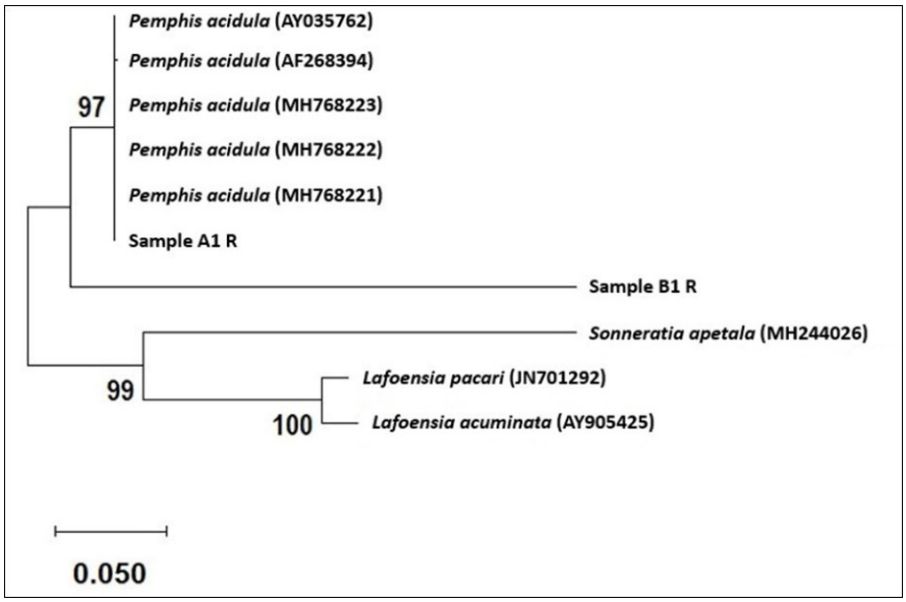

Figure 9. Reconstruction of phylogenetic tree using NJ method with Kimura 2 Parameter model (a bootstrap with 100 repetitions). Sample A1 R = Olele; Sample B1 R = Biluhu.

Implication for conservation

Our study underlined the influence of environmental variables on *P. acidula* diversities, with a focus on morphological and genetic variation. *Pemphis acidula* is the only member of Genus *Pemphis* in Family Lythraceae (Graham et al. 1987; POWO 2022). It is considered as the "least concern" plant by the International Union for Conservation of Nature (IUCN) since March 07, 2008 (Ellison et al. 2010). Due to its slow growth and limited availability in nature, *P. acidula* becomes one of the plants with extinction risks in wild nature.

Sulawesi Island, Indonesia, is known for its high level of endemicity of flora and fauna due to its isolation from the Asian and Australian continental shelves for a long period of time, thus allowing phenotypic and genotypic changes in individuals as an adaptation response to environ-

Table 4. BLAST analysis of ITS sequences of *P. acidula* at Olele and Biluhu showing the identity percentage and query cover.

Sample Code	Fragment Length (bp)	Accession number	Species	Identity (%)	Query Cover (%)
Sample A1 R (*P. Acidula* at Olele)	547	MH768222.1	*P. acidula*	100	98
		MH768221.1	*P. acidula*	100	98
		MH768223.1	*P. acidula*	100	97
		AY035762.1	*P. acidula*	99.43	95
		AF268394.1	*P. acidula*	99.23	94
		AY905425.1	*Lafoensia acuminata*	86.48	86
		JN701292.1	*Lafoensia pacari*	86.29	91
		MH244026.1	*Sonneratia apetala*	-	-
Sample B1 R (*P. Acidula* at Biluhu)	668	MH768221.1	*P. acidula*	81.34	93
		MH768223.1	*P. acidula*	81.06	92
		MH768222.1	*P. acidula*	81.18	90
		AY035762.1	*P. acidula*	79.93	85
		AF268394.1	*P. acidula*	79.62	84
		MH244026.1	*Sonneratia apetala*	78.01	27
		JN701292.1	*Lafoensia pacari*	-	-
		AY905425.1	*Lafoensia acuminata*	-	-

Table 5. Genetic distance between the studied samples with the accession of *P. acidula* in GenBank

	Olele R Sample	Biluhu R Sample	MH768221	MH768222	MH768223	AY035762	AF268394
Olele R Sample							
Biluhu R Sample	24.97						
MH768221*	0.00	24.97					
MH768222*	0.00	24.97	0.00				
MH768223*	0.00	24.97	0.00	0.00			
AY035762*	0.00	25.03	0.00	0.00	0.00		
AF268394*	0.19	25.30	0.19	0.19	0.19	0.19	

* *P. acidula* accession in GenBank

mental conditions. As one of the distribution areas of *P. acidula* in Indonesia, Gorontalo on Sulawesi Island provides a suitable habitat for the growth of this species, such as on Biluhu, Dulanga and Olele Beaches. The various environmental characteristics displayed by these three areas led to differences in the adaptive response of *P. acidula*. Our findings suggest that *P. acidula* at Biluhu and Dulanga Beaches developed distinct morphological and molecular characters, offering a rationale for conservation and management of *P. acidula* in Indonesia.

In addition, considering the low population of *P. acidula* in nature, especially in the three research locations, we hope that there will be an increase in public awareness to cultivate this plant as an effort to use it sustainably and prevent the destruction of coastal ecosystems. To date, *P. acidula* has been used as the ingredient of traditional medicine in various countries including Indonesia which perceives this plant as sacred. Local people also use it in traditional ceremonies and house construction (Baderan et al. 2022). This plant is also widely used as an expensive ornamental plant (one of the best bonsai species in the world) (Cunningham et al. 2017).

CONCLUSIONS

We demonstrated the importance of genetic analysis in biodiversity studies. We found that although samples from the three locations had the similarities in phenetics, they were genetically different. The differences in morphological and molecular characters between *P. acidula* at Biluhu and Dulanga Beaches and those at Olele Beach are thought to be the result of the adaptation of these plants over a long period of time to differences in environmental variables, especially the condition of limestone as a growth substrate. *Pemphis acidula* at Biluhu and Dulanga beaches evolved distinct morphological and molecular traits, providing a rationale for the conservation and management of *P. acidula* in Indonesia. Further studies are needed to study this species due to the possibility that the Biluhu and Dulanga samples are different species or even new species from the *Pemphis* genera.

AUTHORS CONTRIBUTION

D.W.K.B., S.R., Y.R. and R.U. designed the research; D.W.K.B., S.R., M.N.A., Y.R. and R.U. collected the data (field work); M.H.A. and D.W.K.B. analysed the morphological features; M.J. and M.N.A. analysed the genetic data; M.J. and D.W.K.B. wrote the manuscript, and all authors contributed to revisions. All the authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest regarding the research or the research funding.

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