Journal of Tropical Biodiversity and Biotechnology
Volume 09, Issue 02 (2024): jtbb86597
DOI: 10.22146/jtbb.86597

Research Article

# Morphological Variation of *Diospyros* spp. Native to Sulawesi Based on Vegetative Organ Characters

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**Keywords:**
Cluster
Ebenaceae
Leaves
Morphological similarity
Phenotypic
**Submitted:**
06 July 2023
**Accepted:**
08 December 2023
**Published:**
03 May 2024
**Editor:**
Furzani Binti Pa'ee

## ABSTRACT
*Diospyros* spp. is a tree or shrub species belonging to the family Ebenaceae. Researchers have not extensively conducted studies on the morphological characteristics of *Diospyros* spp. to examine their diversity or enhance information for taxonomic hierarchy purposes. *Diospyros* spp. in Indonesia has great potential for in-depth research due to its unique characteristics, particularly in Sulawesi. This research aims to fill the knowledge gap regarding investigating phenotypic variations in *Diospyros* spp., specifically in the Sulawesi region. Nine species of *Diospyros* spp. from the Bogor Botanical Gardens collection were observed for their morphological characteristics and described according to their character traits. The results indicate 25 variations in morphological characteristics out of the 50 characters used. These 14 characteristics are key characteristics that influence the grouping of *Diospyros* spp. from Sulawesi. Phenetic analysis generates three clades of 9 accessions *Diospyros* spp. analyzed.



## INTRODUCTION
*Diospyros* spp. is a flowering plant species classified in the family Ebenaceae. This species generally has a tree or shrub habit (Singh 2005). The number of accepted species of *Diospyros* spp. has reached 780 individuals. Initially, *Diospyros* spp. was only found in Asia, Australia, Africa, and the Americas, making it a native species. However, it has now spread to Europe, particularly in Italy, Switzerland, France, Greece, and Yugoslavia. The presence of *Diospyros* spp. is commonly found in Indonesia, on all of its islands, including Sulawesi and Sumatra which are part of the Malesian group (POWO 2023). Malesia is known for its tropical rainforest region, which supports the growth of this plant species (Kinho 2014). In Indonesia, the available number of *Diospyros* spp. is 32 species (Ariati et al. 2019).

*Diospyros* spp. has morphological characteristics such as being a medium- to large-sized tree with an upright stem reaching a height of 40 meters (Gunawan et al. 2019). The stem of *Diospyros* is black, although some species have a green color. The branch exhibits grooves, lacks latex, and features a swollen tip. The leaves are single and have an entire edge, an elliptical or elongated shape, a pointed-blunt tip, and an alter-

nate arrangement. The lower surface of the leaves frequently bears fine hairs. When young, the leaves are greenish-yellow and will change to green or dark green as they develop. *Diospyros* flowers are *dioecious*, with more male flowers than female flowers. The flowers are located in the leaf axils and belong to the cauliflorous type, where the flowers and fruits grow on the main stem. The fruit is fleshy with a fibrous pericarp and contains about 1–16 seeds per fruit (Wallnöfer 2001; Singh 2005).

Researchers have not extensively conducted morphological studies of *Diospyros* spp. based on vegetative and generative characteristics. This study is crucial to examine as it can ensure the accuracy of each taxonomic hierarchy of *Diospyros* spp., which often changes nomenclature due to limited morphological information (Rideng 1989; Puglisi et al. 2022). Another challenge frequently encountered in the morphological study of *Diospyros* spp. is that its generative organs have a short period, with flowering occurring only once a year (Yuniastuti et al. 2021). However, flower organs will not undergo phenotypic changes due to external factors. Therefore, an alternative solution is to utilize vegetative organs such as leaves, stems, or roots, as they can serve as alternative sources of information for *Diospyros* spp. plants instead of relying solely on flower organs. The science of morphology is crucial in taxonomy as it enables a comprehensive understanding of various plant organs, including their genetic variations and environmental influences (Stuessy 2009). Gnonlonfin's research (2022), which focused on phenotypic variations in *Diospyros mespiliformis*, demonstrates that leaf and flower organs continue to adapt due to environmental and geographical influences.

Several morphological studies have been conducted, such as the research by Putri and Chikmawati (2015), which revealed variations among the eight *Diospyros* spp. species based on their vegetative characteristics, precisely leaf flushing. Meanwhile, Wanda et al. (2022) successfully uncovered morphological variations within the species, albeit restricted to various locations by their morphometric analysis of the *Diospyros discolor* leaf organ. Morphological variation studies have also revealed relationships based on phenetic analysis (Rindyastuti et al. 2021) and identified new *Diospyros* species that have not been previously discovered (Puglisi et al. 2022).

*Diospyros* species that are native to Indonesia, specifically in the Sulawesi region, have a distinctive feature, which is their unique wood pattern. This species is *Diospyros celebica*, or ebony (Mustari 2021). Efforts to rescue ebony from extinction have been carried out by various methods, including forestry with soil repairs and various shading treatments to boost its growth optimally (Kurniawan 2013; Rauf et al. 2016). The study of data morphological variations is also one of the approaches used in plant conservation (Santos et al. 2011). Previous studies on the morphological variation of *D. celebica* have shown differences in leaf and stem characteristics across several regions in Sulawesi (Wahyuningsih et al. 2014). However, researchers have not studied the morphological variations of other distinctive *Diospyros* species from Sulawesi. Therefore, this research aims to fill the knowledge gap regarding the study of phenotypic variations in *Diospyros* spp., specifically in the Sulawesi region.

## MATERIALS AND METHODS

### Materials

The research utilized *Diospyros* spp. sourced from the Bogor Botanical Gardens (BBG) collection, which resulted from explorations in various regions of Sulawesi (Table 1). The age of the trees ranged from 10 to 28 years.

**Table 1.** The *Diospyros* spp from the Sulawesi region that used in the research.

| No. | Bed number in BBG | Registration number | Species name | Origin |
|---|---|---|---|---|
| 1. | X.G.130b | IRF 706 | *D. buxifolia* (Blume) Hiern | Central Sulawesi |
| 2. | XIX.B.41 | IRF 719 | *D. ridleyi* Bakh. | North Sulawesi |
| 3. | XIX.B.37 | IRF 721 | *D. sumatrana* Miq. | Gorontalo |
| 4. | IV.D.190a | IRF 722 | *D. celebica* Bakh. | South Sulawesi |
| 5. | XIII.F.16 | IRF 727 | *D. andamanica* (Kurz) Bakh. | Central Sulawesi |
| 6. | IV.C.II4 | IRF 729 | *D. malabarica* (Desr.) Kostel. | SE. Sulawesi |
| 7. | XXIV.A.202-202a | IRF 740 | *D. maritima* Blume | North Sulawesi |
| 8. | XXIV.A.270 | IRF 714 | *Diospyros sp.* 1 | SE. Sulawesi |
| 9. | IV.D.205 | IRF 738 | *Diospyros sp.* 2 | South Sulawesi |

## Methods

The determination of morphological characters refers to the books and journals authored by Indriyanto (2012), Tjitrosoepomo (2009), Ellis et al. (2009), and Ghazalli et al. (2017). Fifty morphological characters, quantitative and qualitative, were used (Table 2). Leaf sampling took ten leaf blades (Alcántara-Ayala et al. 2020) from the third order of mature leaves per species (Semagn 2014). We took stem bark samples using a knife with a thickness of approximately 0.5 cm. The pieces were placed in plastic clips and labeled with species identification (British Columbia Ministry of Forest 1996). We directly observed each morphological character using a microscope 1.3 MP Dino-Lite edge plus 3.0. We utilized the Royal Horticultural Society (RHS) color chart to keep the color characters (Asih et al. 2022), while quantitative characters were measured using a ruler with a precision of 1 mm (Handayani 2013).

**Table 2**. Morphological characteristics observed.

| Plant parts | Morphological character |
|---|---|
| Stem | Habit, canopy shape, stem development, stem base, stem surface texture, shape of stem, outer bark color, inner bark color, stem color, types of stem fiber, branch pattern, branch growth direction |
| Leaf | Leaf stalk shape, leaf stalk position, leaf stalk length (cm), leaf stalk type, leaf shape, leaf apex shape, leaf base shape, leaf margin type, type, leaf shape, leaf apex shape, leaf base shape, leaf margin type, leaf symmetry, leaf length (cm), leaf width (cm), laminar ratio, apex angle, base angle, young leaf color of an upper surface, young leaf color of a lower surface, mature leaf color (upper surface), mature leaf color (lower surface), leaf texture, upper leaf surface, lower leaf surface, leaf bones, variation of major secondary angle to midvein, number of secondary leaf vein, leaf venation, areolation, marginal ultimate (venation), areolar venation, lateral venation pattern, anastomosing of lateral venation, leaf glands, placement of leaf glands, number of glands per leaf, trichomes on leaf stalk |

### Data Analysis

The observational data on *Diospyros* spp. morphology consists of documentation of each character's traits. We analyzed it descriptively, quantitatively, and qualitatively to determine the morphological differences among *Diospyros* spp. The morphological character data were arranged in an n × t using Microsoft Excel software (Prasgi et al. 2022). Each descriptive morphological character was transformed into quantitive data by assigning score based on the observed characterictics. The binary data

from the quantitive were then scored, standardized, rescored and were further processed using MVSP 3.22 software to construct a phenogram using the UPGMA algorithm (Sneath & Sokal 1973). The similarity index was calculated based on the Simple Matching Coefficient (Sokal & Meichener 1958). Additionally, Principal Component Analysist was performed to clarify the pattern of character grouping that influenced the variations (Haekal et al. 2020).

## RESULTS AND DISCUSSION

The results of observations on 50 morphological characters within the nine *Diospyros* species in the Sulawesi region indicate that only 25 characters exhibit variations representing 50% polymorphism, while the remaining characters show similarities. The morphological similarities of *Diospyros* spp. include having a tree habit, a pyramid-shaped canopy, and a monopodial type of central stem development. The stem is cylindrical, and the stem fibers are fine. The branching pattern of *Diospyros* spp. is continuous, with horizontal branch growth. The leaves of *Diospyros* spp. are simple, with an *alternate* leaf arrangement and a *semi-terete* leaf stalk positioned at the leaf margin. The leaves are symmetrical and have an *acute* leaf apex. The color of *Diospyros* spp. leaves is generally greenish-yellow, and the type of leaf bones is *penninervis*. Leaf venation is *pinnate*, areolar venation shows simple and uni-veinlets, and the lateral venation pattern is ascending, with anastomosis positioned near the leaf edge. The leaves of *Diospyros* spp. also exhibit glandular characteristics located on the leaf surface in small dots.

### Leaves

Variations in leaf morphology are evident in the leaflet character, with variations in shape including *elliptical*, *ovate*, *oblongata*, and *lanceolate*. The *oblongata* shape dominates in species such as *D. malabarica*, *D. celebica*, *D. ridleyi*, *D. sumatrana*, and *Diospyros* sp.1. The *elliptical* shape is found in *D. buxifolia* and *Diospyros* sp.2. *Diospyros maritima* exhibits a unique shape, unlike other species, with an *ovate* form, while *Diospyros andamanica* has a *lanceolate* shape (Figure 1. I).

The leaflets of *Diospyros* spp. exhibit variations in leaf flesh, either *papyraceous* or *perkamenteus*, with a thin and stiffly fine texture. The upper and lower surfaces of the leaves differ but are predominantly *glabrous* (without hairs or scales), except for *D. buxifolia* and *Diospyros* sp.2. The upper surface of the leaves of *D. buxifolia* and *Diospyros* sp.2 has a smooth texture (*laevis*). The lower surface of *D. buxifolia* is *pilosus* (covered with short and fine hairs) (Putri & Chikmawati 2015), while *Diospyros* sp.2 has a smooth texture (*laevis*). The lower surface of *D. celebica* leaves is *pilosus*, or covered with fine hairs (Wahyuningsih et al. 2014) (Figure 1. II).

Leaf margin characteristics show two variations: wavy and entire. *D. andamanica* is the only species with wavy leaf margins (Figure 1. III), but Rindyastuti et al. (2021) found that the leaf margins of *D. andamanica* are entire. Regarding the leaf apex, there are two variations: *acutus* and *acuminatus*. Species like *D. buxifolia*, *D. maritima*, and *D. malabarica* have an *acutus* leaf apex. In contrast, *D. celebica*, *D. ridleyi*, *D. sumatrana*, *D. andamanica*, *Diospyros* sp.1, and *Diospyros* sp.2 have an *acuminatus* leaf apexes (Figure 1. IV). The shape of the leaf base in *Diospyros* spp. shows three variations: *acutus*, *obtusus*, and *rotundatus*. *Acutus* shapes are found in species such as *D. buxifolia*, *D. malabarica*, *D. celebica*, *D. andamanica*, and *Diospyros* sp.2. In contrast, *D. ridleyi*, *D. sumatrana*, and *Diospyros* sp.1 exhibit *obtusus* leaf bases. *D. maritima* is the only species with a *rotundatus* leaf base. Silalahi & Mustaqim (2020) also described *D. maritima* as having

*rotundatus* leaf bases (Figure 1. V). Variations in qualitative leaf characteristics indicate that each species undergoes adaptation to its environment, leading to the conditioning of organ shapes (Anatov & Mallaliev 2022). Similarly, trichomes on leaf stalk show variations in their abundance, ranging from few to many, in response to environmental conditions (Figure 2. I).

*Diospyros* spp. generally have leaf glands located on the upper or lower surface of the leaf. The number of leaf glands found in *Diospyros* spp. in the Sulawesi region ranges from approximately 1-14, depending on the leaf size for each species. Leaf bones play a crucial role as a character in identifying species (Setiaji et al. 2016). The secondary leaf veins of *Diospyros* spp. show two variations: uniform angle and inconsistent angle, with the number of veins ranging from 5 to 16 in each species.

The characters of leaf length, leaf width, leaf ratio, leaf base angle, and leaf stalk length exhibit quantitative variations. The size and width of leaves in *Diospyros* spp. vary greatly, ranging from approximately 2-32.3 cm in length and 0.7 to 7.3 cm in width. *D. celebica* has the largest leaf size, while *D. buxifolia* has the smallest. Based on the leaf length and width measurements, the resulting leaf ratios are 1:2.5, 1:3.5, and 1:4. Regarding the leaf base angle, *D. maritima*, which has a *rotundatus* leaf base, produces *obtuse* leaf base angles. Other species have *acute* leaf base angles. *Diospyros* spp. also vary in size, ranging from 0.1-1.1 cm. *D. buxifolia* has the shortest leaf stalk, measuring 0.1 cm. The most extended leaf stalk, measuring 1.1 cm, is found in *D. celebica* and *D. ridleyi*.

Variations in leaf color can often be difficult to identify accurately due to subjective interpretation. Therefore, the use of an RHS *color chart* helps determine leaf colors. The variations in color on the upper surface of mature leaves include green and greenish-yellow. The leaves have the same color but differ in contrast levels (Figure 2. II). The leaf stalk and leaf buds also exhibit color variations. The leaf stalk of *Diospyros* spp. shows various color variations, including grayish-brown, green, and greenish-yellow. *D. celebica* is the only species that has a different color variation compared to the other species, which is grayish-brown (Figure 2. III). Leaf buds are essential in leaf organs as they represent the initial stages of leaf formation. Color variations in leaf buds include greenish-yellow, brown, grayish-brown, and grayish-yellow. *D. buxifolia* and *Diospyros* sp.2 have different colors for their leaf buds compared to other species, with greenish-yellow and grayish-yellow colors, respectively. *D. maritima*, *D. malabarica*, *D. ridleyi*, *D. sumatrana*, and *D. andamanica* exhibit brown-colored buds. In contrast, grayish-brown buds are found in *D. celebica* and *Diospyros* sp.1 (Figure 2. IV).

## Stem

The characteristics of outer bark color, inner bark color, stem color, stem surface texture, and stem base indicate the variations in morphology found in the stem organ. Generally, the outer bark color of *Diospyros* spp. is black (Singh 2005), but this research shows color variations such as brown, grayish-brown, and black (Figure 2 V). The color of the inner bark is predominantly grayish-orange, while the stem color is dominated by yellow. The stem base of *Diospyros* spp. usually does not have buttresses, but occasionally buttresses are found, as in the case of *D. celebica* and *D. maritima* species (Figure 2 VI). Some stems of *Diospyros* spp. have a smooth stem texture, while others have soft and rough grooves. *D. celebica* is the only species with a rough, grooved texture, unlike other species (Figure 2 VII). Gunawan et al. (2019) stated that *D. celebica* has a fluted stem. Rindyastuti et al. (2021) also revealed that the stem surface

**Table 3**. Variations in leaf morphology of *Diospyros* spp.

| No. | Morphological Character | *D. buxifolia* | *D. maritima* | *D. malabarica* | *D. celebica* | *D. ridleyi* | *D. sumatrana* | *D. andamanica* | *Diospyros* sp.1 | *Diospyros* sp.2 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Leaf stalk length (cm) | 0-0.1 | 0.6-0.9 | 0.5-0.8 | 0.8-1.1 | 0.7-1.1 | 0.5-0.9 | 0.2-0.4 | 0.6-1 | 0.2-0.4 |
| 2. | Leaf stalk color | Greenish-yellow | Greenish-yellow | Greenish-yellow | Grayish-brown | Green | Greenish-yellow | Greenish-yellow | Greenish-yellow | Greenish-yellow |
| 3. | Leaf bud color | Greenish-yellow | Brown | Brown | Grayish-brown | Brown | Brown | Brown | Grayish-brown | Grayish-yellow |
| 4. | Leaf flesh type | *Papyraceus* | *Perkamenteus* | *Perkamenteus* | *Papyraceus* | *Perkamenteus* | *Perkamenteus* | *Papyraceus* | *Perkamenteus* | *Papyraceus* |
| 5. | Laef shape | *Elliptical* | *Ovate* | *Oblongata* | *Oblongata* | *Oblongata* | *Oblongata* | *Lanceolate* | *Oblongata* | *Elliptical* |
| 6. | Leaf apex shape | *Acutus* | *Acutus* | *Acutus* | *Acuminatus* | *Acuminatus* | *Acuminatus* | *Acuminatus* | *Acuminatus* | *Acuminatus* |
| 7. | Leaf base shape | *Acutus* | *Rotundatus* | *Acutus* | *Acutus* | *Obtusus* | *Obtusus* | *Acutus* | *Obtusus* | *Acutus* |
| 8. | Leaf margin type | Entire | Entire | Entire | Entire | Entire | Entire | Wavy | Entire | Entire |
| 9. | Leaf length (cm) | 2-3.8 | 12.5-18.2 | 12.1-18.5 | 25.5-32.3 | 16.2-22.8 | 15.5-22 | 7.4-10.9 | 15.5 – 23 | 4.6-7.3 |
| 10. | Leaf width (cm) | 0.7-1.5 | 5.1-7.2 | 3.2-5 | 6-7.3 | 3.5-6.2 | 3.1-5.2 | 2-2.6 | 3.5 – 5.5 | 1.4-2.1 |
| 11. | Laminar ratio | 1:2.5 | 1:2.5 | 1:3.5 | 1:4 | 1:4 | 1:4 | 1:4 | 1:4 | 1:3.5 |
| 12. | Base angle | *Acute* | *Obtuse* | *Acute* | *Acute* | *Acute* | *Acute* | *Acute* | *Acute* | *Acute* |
| 13. | Mature leaf color (upper surface) | Greenish-yellow | Greenish-yellow | Greenish-yellow | Green | Green | Greenish-yellow | Greenish-yellow | Green | Greenish-yellow |
| 14. | Leaf texture | Thin-fine | Stiff-fine | Stiff-fine | Stiff-fine | Stiff-fine | Stiff-fine | Thin-fine | Stiff-fine | Thin-fine |
| 15. | Upper leaf surface | *Laevis* | *Glaber* | *Glaber* | *Glaber* | *Glaber* | *Glaber* | *Glaber* | *Glaber* | *Laevis* |
| 16. | Lower leaf surface | *Pilosus* | *Glaber* | *Glaber* | *Pilosus* | *Glaber* | *Glaber* | *Glaber* | *Glaber* | *Laevis* |
| 17. | Variation of major secondary angle | Secondary angle uniform | Secondary angle uniform | Secondary angle uniform | Secondary angle inconsistent | Secondary angle uniform | Secondary angle uniform | Secondary angle uniform | Secondary angle uniform | Secondary angle uniform |
| 18. | Number of secondary leaf vein | 5-8 | 7-8 | 5-10 | 9-16 | 5-8 | 8-10 | 5-8 | 7-10 | 7-10 |
| 19. | Number of glands per leaf | 5-8 | 5-10 | 5-12 | 5-8 | 5-9 | 5-9 | 1-4 | 8-14 | 1-2 |
| 20. | Trichomes on leaf stalk | Many | Few | Few | Many | Few | Few | Few | Few | Many |

**Table 4.** Contd.

| No. | Morphological Character | *D. buxifolia* | *D. maritima* | *D. malabarica* | *D. celebica* | *D. ridleyi* | *D. sumatrana* | *D. andamanica* | *Diospyros* sp.1 | *Diospyros* sp.2 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Stem base | Non-buttress | Buttress | Non-buttress | Buttress | Non-buttress | Non-buttress | Non-buttress | Non-buttress | Non-buttress |
| 2. | Stem surface texture | Smooth | Smooth | Smooth grooved | Rough grooved | Smooth grooved | Smooth grooved | Smooth | Smooth grooved | Smooth |
| 3. | Outer bark color | Grayish-brown | Brown | Black | Black | Brown | Brown | Black | Brown | Black |
| 4. | Inner bark color | Grayish-orange | Grayish-orange | Orange-white | Orange-white | Grayish-orange | Grayish-orange | Grayish-orange | Grayish-orange | Yellow-orange |
| 5. | Stem color | Yellow | Yellow-white | Orange-white | Yellow-white | Orange-white | Yellow-white | Yellow-white | Yellow-white | Yellow-white |

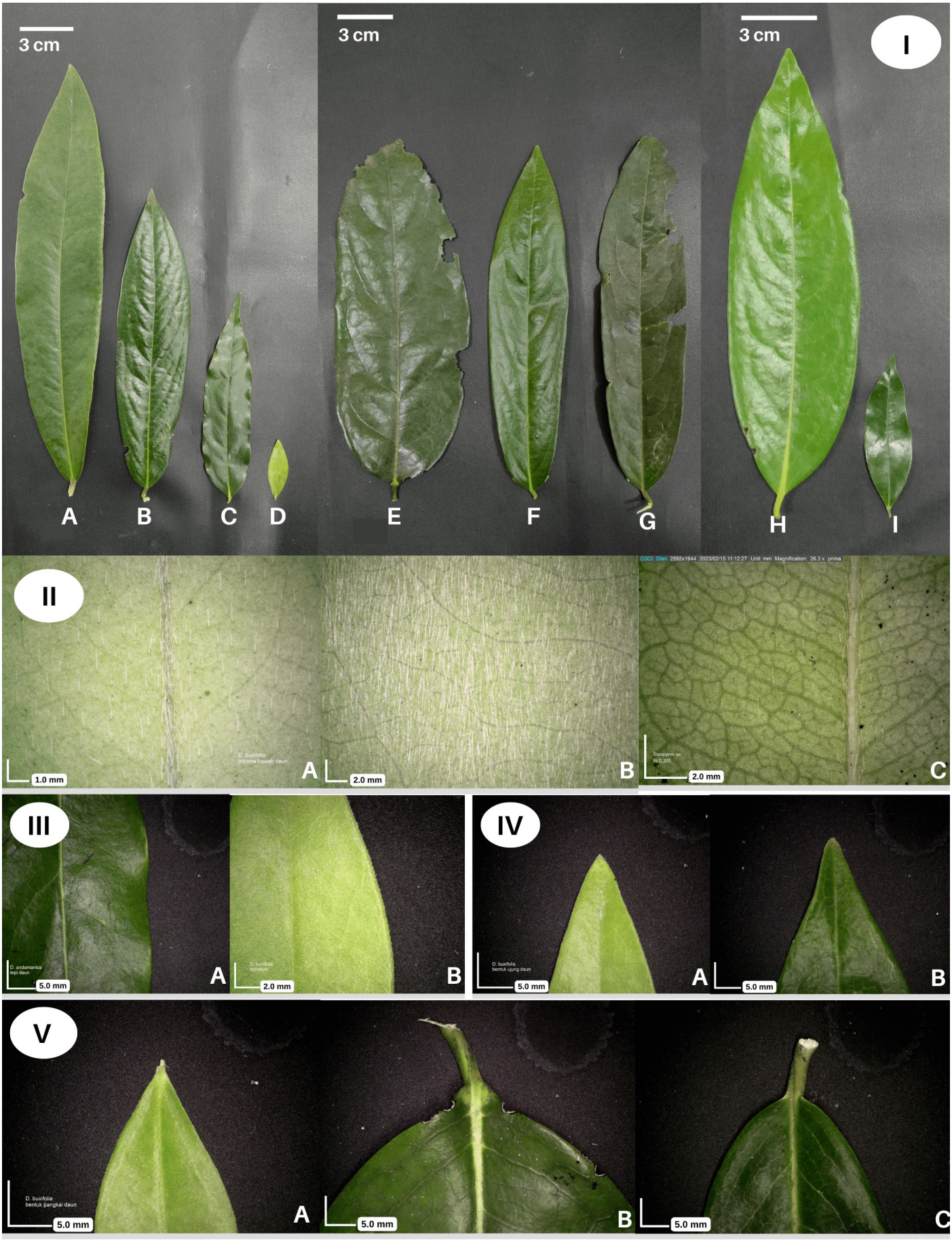


**Figure 1.** I) Leaf variations of *Diospyros* spp. in Sulawesi region. Description: (A) *D. celebica*, (B) *D. malabarica*, (C) *D. andamanica*, (D) *D. buxifolia*, (E) *D. maritima*, (F) *D. sumatrana*, (G) *D. ridleyi*, (H) *Diospyros* sp.1, (I) *Diospyros* sp.2.; II.) Lower leaf surface. Description (A). *D. buxifolia* (B). *D. celebica* (*pilosus*), (C). *Diospyros* sp.2 (*laevis*); III.) Types of leaf margins. (A). wavy, (B). entire.; IV.) Shape of leaf apex. (A). *acutus*, (B). *acuminatus*; V.) Shape of leaf base. (A). *acutus*, (B). *rotundatus*, (C). *obtusus*

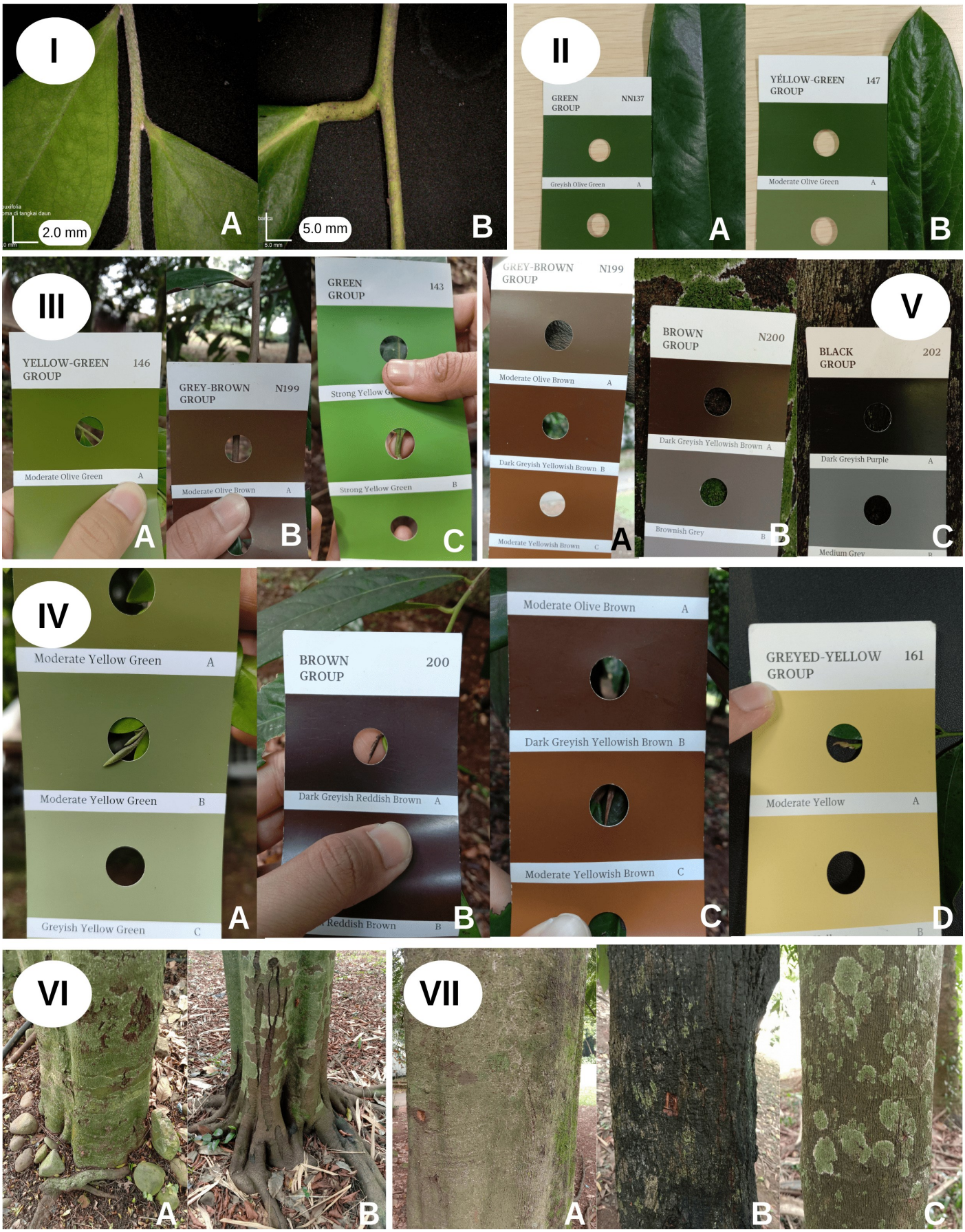


**Figure 2**. I) Number of trichomes on the leaf . (A). Many, (B). Few; II.) Color of mature leaves on the upper surface. (A). Greenish-yellow, (B). Green; III.) Leaf stalk color. (A). Greenish-yellow, (B). Grayish-brown, (C). Green; IV.) Leaf buds color. (A). Greenish-yellow, (B). Brown, (C). Grayish-brown, (D). Grayish-yellow; V.) Outer bark color. (A). Grayish-brown, (B). Brown, (C). Black; VI.) Types of stem base. (A). Non-buttress, (B). Buttress; VII.) Surface texture of stem. (A). Smooth, (B). Rough grooved, (C). Smooth grooved

texture of *D. celebica* is rough and grooved, while *D. andamanica* and *D. malabarica* have smooth stem surfaces.

Identification key for *Diospyros* spp. native to Sulawesi

1) a. The variation of the major secondary angle is inconsistent, number of secondary leaf veins ranges ≥10 ..... *D. celebica* Bakh.
   b. The variation of the major secondary angle is uniform, number of secondary leaf veins ranges ≤10 ..... 2
2) a. The stem base is a buttress, with an *obtuse* leaf base angle ..... *D. maritima* Blume
   b. The stem base is non-buttress, with an *acute* leaf base angle ..... 3
3) a. Smooth stem surface texture, leaf stalk length is <5 cm, leaf texture is thin-fine ..... 4
   b. Smooth-grooved stem surface texture, leaf stalk length is ≥5 cm, leaf texture is stiff-fine ..... 6
4) a. The shape of the leaf apex is *acutus*, outer bark color is grayish-brown ..... *D. buxifolia* (Blume) Hiern
   b. The shape of the leaf apex is *acuminatus*, outer bark color is black ..... 5
5) a. Wavy leaf margin, *glaber* upper leaf surface, *glaber* lower leaf surface ..... *D. andamanica* (Kurz) Bakh.
   b. Entire leaf margin, *laevis* upper leaf surface, *laevis* lower leaf surface ..... *Diospyros* sp.2
6) a. The shape of leaf base is *acutus*, inner bark color is orange-white ..... *D. malabarica* (Desr.) Kostel.
   b. The shape of leaf base is *obtusus*, inner bark color is grayish-orange ..... 7
7) a. The stem color is orange-white ..... *D. ridleyi* Bakh.
   b. The stem color is yellow-white ..... 8
8) a. Brown leaf bud color, greenish-yellow upper mature leaf color ..... *D. sumatrana* Miq.
   b. Grayish-brown leaf bud color, green upper mature leaf color ..... *Diospyros* sp.1

Based on the morphological similarities data of *Diospyros* that has been shown the key identification, the next step is phenetic analysis to reveal more definitely the relationship. Phenetic techniques systematically classify species based on morphological similarities and establish phylogenetic correlations. The phenetic method encompasses two analytical techniques, namely cluster and PCA analysis (Kovach 2007; Singh 2010). The phenogram analysis yielded two primary groups, Cluster I and Cluster II which had a similarity index of 0.83 as depicted in Figure 1. The morphological disparities seen between *D. celebica* and other *Diospyros* species result in their classification into two primary groupings. *Diospyros celebica* is classified as a separate species (Cluster I) based on its distinct morphological characteristics, including variances in the texture of the stem surface, the color of the leaf stalk, variation in the angle between the major secondary vein and the midvein, and the number of secondary leaf veins. Cluster II can be subdivided into two distinct subclusters, B1 and B2, with a similarity index value of 0.907. The primary distinguishing features differentiating subclusters B1 and B2 from the center cluster are the leaf shape variations and the upper leaf surface characteristics.

Within subcluster B2 are *Diospyros* sp.1 and *D. sumatrana*, which have a similarity index 1.00. Due to the considerable physical resemblances between these two species, they can be deemed comparable and closely affiliated. The homogeneity of physical characteristics can be in-

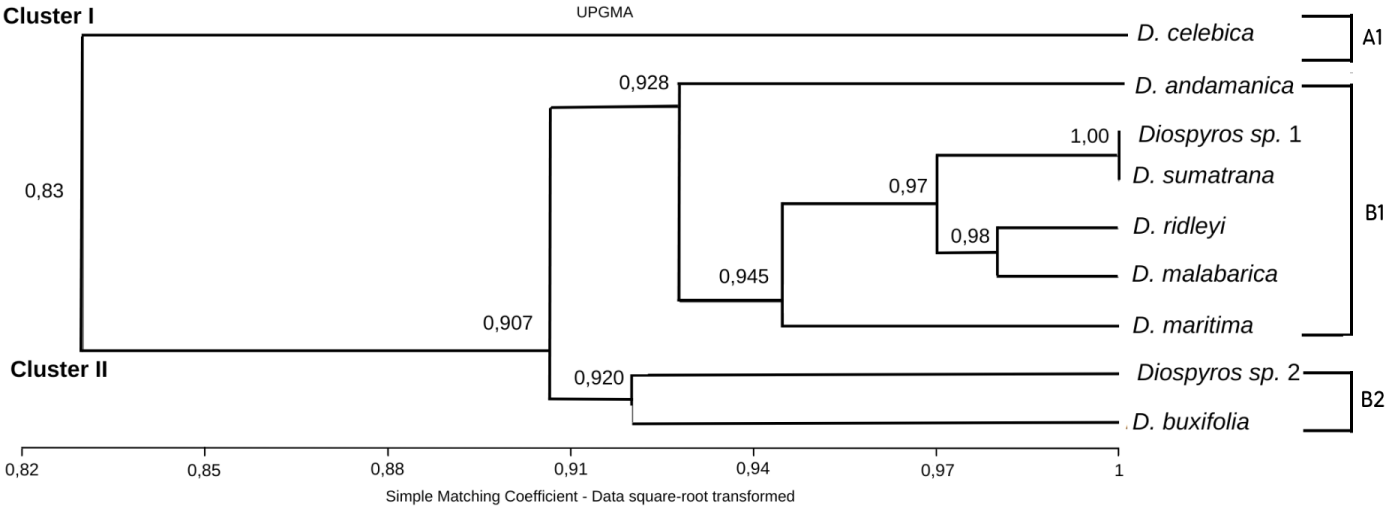


**Figure 3**. *Diospyros* phenogram of Sulawesi region based on 50 morphological characters.

fluenced by both genetic and environmental variables (Bramasto et al. 2015). Nevertheless, subtle variations in coloration exist between the physical attributes of leaf buds and fully developed upper leaves.

The analysis of similarity index values reveals that *Diospyros* sp.1 and *D. sumatrana* exhibit the highest resemblance, as indicated by a similarity index of 1.00. On the other hand, *D. celebica* and *D. ridleyi* show the most divergent relationship, as noted in a similarity value of 0.80 (Table 4). According to Singh (2010), when the similarity values between operational taxonomic units (OTUs) surpass 85%, they are still considered to belong to the same species. The utilization of vegetative traits in the biosystematic investigation of *Diospyros* is of utmost importance due to its role in species identification and the elucidation of relationships (Rindyastuti et al. 2021).

The grouping of *Diospyros* spp. is influenced by various morphological characteristics, including stem surface texture, the color of the outer bark of the stem, stem color, leaf bud color, leaf stalk color, leaf flesh type, leaf shape, leaf base shape, leaf width, leaf ratio, lower leaf surface, number of secondary leaf veins, and the number of trichomes on the leaf stalk (refer to Table 5 and Figure 2). The grouping is highly influenced by Principal Component 1 (PC1) due to its greatest eigenvalue of 42.287%, surpassing that of other principal components. According to Ningrum and Chasani (2021), there is a positive relationship between the value of the major component and its impact on the grouping of species.

The scatter plot analysis of OTU grouping using cluster analysis demonstrates consistent findings, revealing the existence of two distinct clusters. The first cluster, labeled A1 and corresponding to *D. celebica*, remains unchanged. The second cluster, denoted as B, can be further divided into two subclusters: B1, consisting of *D. andamanica*, *D. ridleyi*, *D. sumatrana*, *Diospyros* sp.1, *D. malabarica*, and *D. maritima*; and B2, comprising *D. buxifolia* and *Diospyros* sp.2. According to Setiawati et al. (2013), the proximity of clusters B1 and B2 within the same quadrant suggests a high degree of relatedness among the species belonging to these groupings.

## CONCLUSIONS

This research reveals the presence of morphological variations in *Diospyros* spp. based on the characteristics of their vegetative organs. The observations of 50 morphological characters indicate the presence of 25 var-

**Table 4**. Similarity index between *Diospyros* species based on morphological characters.

| No. | Species Name | A | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|---|---|
| A | *D. buxifolia* | 1,00 | | | | | | | | |
| B | *D. maritima* | 0,90 | 1,00 | | | | | | | |
| C | *D. malabarica* | 0,90 | 0,92 | 1,00 | | | | | | |
| D | *D. celebica* | 0,88 | 0,82 | 0,82 | 1,00 | | | | | |
| E | *D. ridleyi* | 0,92 | 0,94 | 0,98 | 0,80 | 1,00 | | | | |
| F | *D. sumatrana* | 0,94 | 0,96 | 0,96 | 0,82 | 0,98 | 1,00 | | | |
| G | *D. andamanica* | 0,92 | 0,90 | 0,94 | 0,84 | 0,92 | 0,94 | 1,00 | | |
| H | *Diospyros* sp.1 | 0,94 | 0,96 | 0,96 | 0,82 | 0,98 | 1,00 | 0,94 | 1,00 | |
| I | *Diospyros* sp.2 | 0,92 | 0,86 | 0,90 | 0,84 | 0,88 | 0,90 | 0,92 | 0,90 | 1,00 |

**Table 5.** Morphological characters that affect PC1, PC2, PC3, and PC4.

| No. | Morphological character | PC1 | PC2 | PC3 | PC4 |
|---|---|---|---|---|---|
| 1 | Stem surface texture | 0,280 | -0,219 | 0,162 | -0,019 |
| 2 | Outer bark color | 0,295 | 0,337 | 0,629 | 0,242 |
| 3 | Inner bark color | 0,084 | 0,352 | -0,116 | 0,190 |
| 4 | Stem color | -0,189 | -0,021 | 0,484 | -0,406 |
| 5 | Leaf stalk color | 0,280 | -0,219 | 0,162 | -0,019 |
| 6 | Leaf bud color | 0,084 | 0,352 | -0,116 | 0,190 |
| 7 | Leaf flesh | 0,440 | 0,338 | -0,119 | 0,003 |
| 8 | Leaf shape | 0,007 | 0,170 | 0,208 | 0,207 |
| 9 | Leaf base shape | -0,072 | -0,219 | -0,174 | 0,491 |
| 10 | Leaf width (cm) | 0,280 | -0,219 | 0,162 | -0,019 |
| 11 | Leaf ratio | 0,208 | -0,438 | -0,012 | 0,473 |
| 12 | Lower leaf surface | 0,349 | -0,185 | -0,211 | -0,393 |
| 13 | Number of secondary leaf vein | 0,280 | -0,219 | 0,162 | -0,019 |
| 14 | Trichomes on leaf stalk | 0,433 | 0,168 | -0,327 | -0,203 |

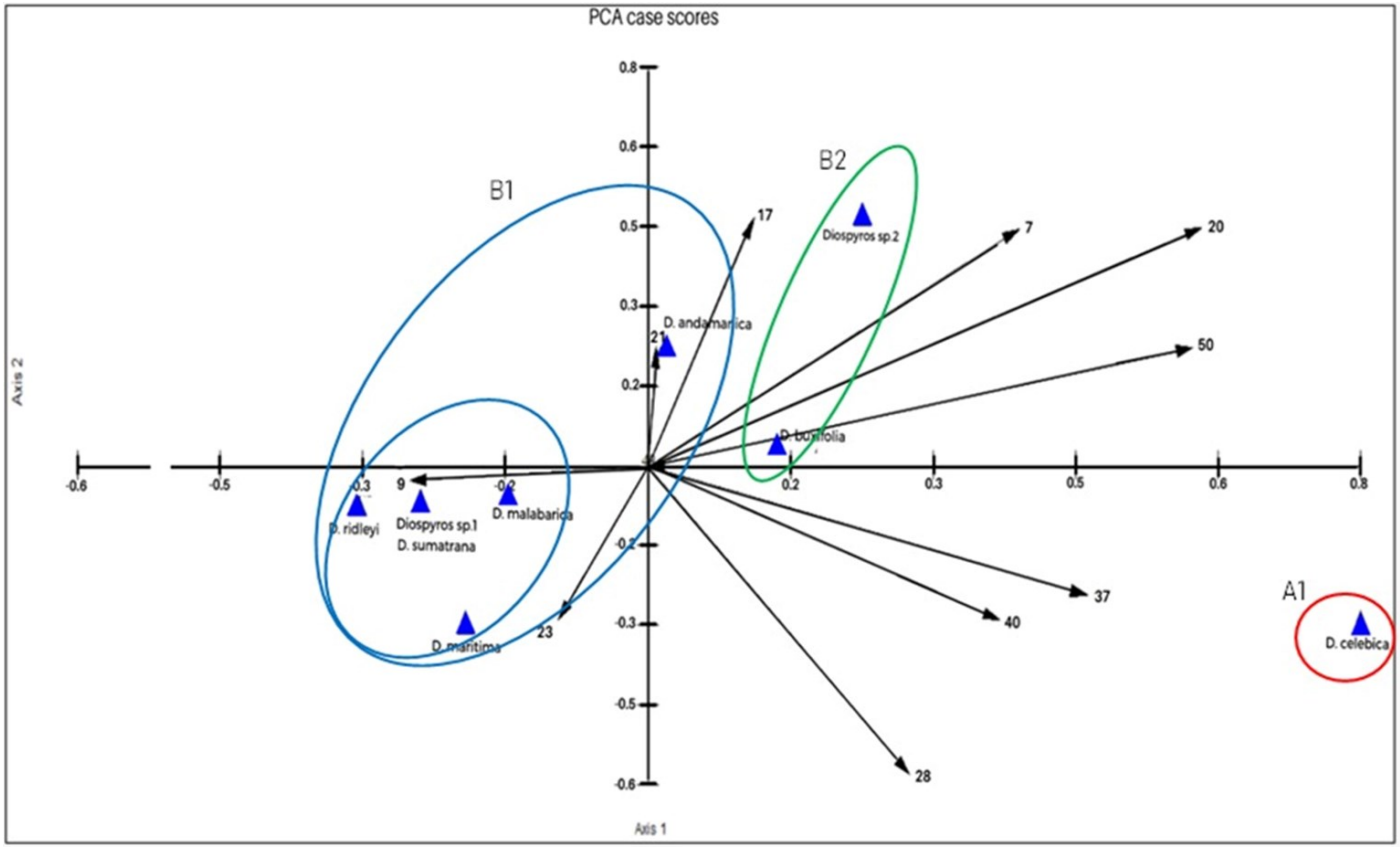


**Figure 4.** Scatter plot of *Diospyros* Sulawesi region.

iations in both qualitative and quantitative traits. These 14 characteristics are key characters that influence the grouping of *Diospyros* spp. from Sulawesi. The vegetative organs depict the diversity of morphological variations within *Diospyros* spp. While the vegetative organs can represent the diversity of morphological characters, the generative organs are still necessary to show other variations in the morphological traits of *Diospyros* spp.

## AUTHOR CONTRIBUTION

The author's contribution: AAW designed the research, collected and examined the data, and wrote the manuscript. LK designed the research, collected, analyzed the data, and improved the manuscript. IFW developed the study, collected, analyzed the data, and improved the manuscript.

## ACKNOWLEDGMENTS

The study was supported by The Research Organization for Life Sciences and Environment, National Research and Innovation Agency (BRIN) under grant Rumah Program OR HL. The author would like to thank the Directorate of Science and Technology Resource Management, National Research and Innovation Agency (BRIN), particularly the MBKM program, for facilitating this research. The author would also like to thank the Center for Biosystematics and Evolutionary Research, the Center for Plant Conservation Research, Botanic Gardens and Forestry, and the Molecular Laboratory of the Scientific Conservation Area, Bogor Botanic Gardens (BRIN) for their assistance in conducting this research. We want to extend our special thanks to the Department of Biology, Faculty of Science and Mathematics, Diponegoro University, for their continuous support and assistance throughout the study.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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