



Morphological Characterization and Phenetic Relationship Analysis of *Paphiopedilum* Orchids

I Made Wira Sumerjaya, Ida Ayu Putri Darmawati*, Rindang Dwiyani, I Putu Sudiarta,
Yuyun Fitriani, I Made Basma Redana

Agroecotechnology Study Program, Faculty of Agriculture, Udayana University,
Jln. PB. Sudirman Denpasar Bali 80232, **Indonesia**

*Corresponding author: darmawati@unud.ac.id

ABSTRACT

Paphiopedilum orchids are among the most distinctive and conservation-priority members of *Orchidaceae* because of their ornamental value, restricted distribution, and vulnerability to habitat loss and overexploitation. This study aimed to characterize the morphology of four *Paphiopedilum* species and to assess their morphology-based phenetic relationships as preliminary information for conservation and breeding programs. Plant materials were obtained from orchid collectors and nurseries associated with the Pancasari area, Buleleng Regency, Bali, from July to December 2024. Qualitative and quantitative characters of leaves, stems, flowers, and flower stalks were observed using standard orchid characterization descriptors, including the RHS Colour Chart for color identification. Morphological data were analyzed descriptively and subjected to UPGMA clustering using Euclidean distance in Minitab 22. Four species were characterized: *Paphiopedilum agusii*, *Paphiopedilum javanicum*, *Paphiopedilum lowii*, and *Paphiopedilum glaucophyllum*. Clear interspecific variation was observed in leaf shape, leaf margin, leaf apex, leaf surface texture, leaf symmetry, inflorescence type, flower aroma, sepal and petal morphology, flower color, and several quantitative characters. *Paphiopedilum lowii* showed the most prominent dimensions, particularly in leaf length, flower diameter, and flower stalk length, and appeared as the most divergent taxon in the cluster analysis. *Paphiopedilum agusii* and *Paphiopedilum javanicum* showed the closest phenetic relationship. These findings provide baseline morphological information for *Paphiopedilum* germplasm documentation, conservation planning, and preliminary parent selection in breeding programs. Molecular analysis and voucher-based taxonomic validation are recommended to strengthen the interpretation of evolutionary relationships.

Keywords: conservation, morphology, orchid, *Paphiopedilum*, phenetic relationship

INTRODUCTION

Orchidaceae is one of the largest families of flowering plants, with more than 32,000 species recognized worldwide (POWO, 2021). Indonesia is a megabiodiversity country and an important center of orchid diversity, with an estimated

4,000-5,000 orchid species (Setiawan et al., 2020). Orchids have high ornamental and economic value because of their diverse flower shapes, colors, rarity, and aesthetic appeal. These attributes make orchids important horticultural commodities at both national and international levels.

Natural orchids also have strategic value as genetic resources in breeding programs aimed at producing superior hybrids (Sarwono, 2002). The discovery of new species and endemic taxa in Indonesia emphasizes the importance of conserving orchid germplasm as a valuable biological resource (Djufri et al., 2015). However, natural orchid populations are increasingly threatened by overharvesting, habitat fragmentation, deforestation, forest fires, and land conversion, creating an urgent need for systematic conservation strategies (Wulanesa et al., 2017).

Paphiopedilum, commonly known as slipper orchids, is one of the rare orchid genera with high conservation value. The genus comprises approximately 80 species distributed from the Himalayas to Southeast Asia, including Indonesia (Chochai et al., 2012). *Paphiopedilum* species are generally terrestrial or lithophytic and commonly occur in humid tropical forest habitats at elevations from approximately 1 to 2,300 m above sea level. Their pouch-shaped labellum makes the genus morphologically distinctive, but their specific habitat requirements and high ornamental demand also make them vulnerable to population decline.

Morphological characterization is an important approach for supporting orchid conservation. It is conducted through systematic observation of plant organs such as leaves, stems, flowers, fruits, and roots (Susantidiana et al., 2009). Morphological data are useful for identification, species comparison, germplasm documentation, and analysis of relationships among taxa (Purwantoro et al., 2005). A systematic morphological study can also support germplasm conservation and the use of genetic resources in plant breeding programs (Pangestu et al., 2014).

This study was conducted to characterize the morphology of *Paphiopedilum* orchids and to analyze their

morphology-based phenetic relationships. The results are expected to provide baseline information for the conservation and sustainable use of *Paphiopedilum* genetic resources in Indonesia.

MATERIALS AND METHODS

Plant materials and study period

The *Paphiopedilum* plant materials used in this study were obtained from orchid collectors, including Limbata Orchid, and orchid nurseries associated with Desa Pancasari, Sukasada District, Buleleng Regency, Bali. The study was conducted from July to December 2024. The inventory was carried out using a direct exploration or survey approach by recording and identifying the available orchid materials.

Morphological characters observed

Morphological characters observed in this study included qualitative and quantitative traits of leaves, stems, flowers, and roots. Quantitative observations included plant height or stem length, stem diameter, leaf length, leaf width, sepal and petal dimensions, flower diameter, flower stalk diameter, flower stalk length, and root length where available. Qualitative observations followed the ornamental plant characterization guide for orchids and anthuriums (Kartikaningrum et al., 2004), including leaf shape, leaf margin, leaf apex, leaf surface texture, leaf symmetry, leaf color, growth habit, flower shape, inflorescence type, flowering position, flower stalk posture, flower aroma, sepal, petal, and labellum color, sepal and petal pattern, sepal and petal apex, and labellum notch position. Leaf and flower colors were determined using the Royal Horticultural Society (RHS) Colour Chart.

Paphiopedilum floral morphology reference

The main floral structures used as references for morphological observation are

shown in Figure 1, including the dorsal sepal, petal, synsepal, staminode, column, labellum, and whole-flower views.

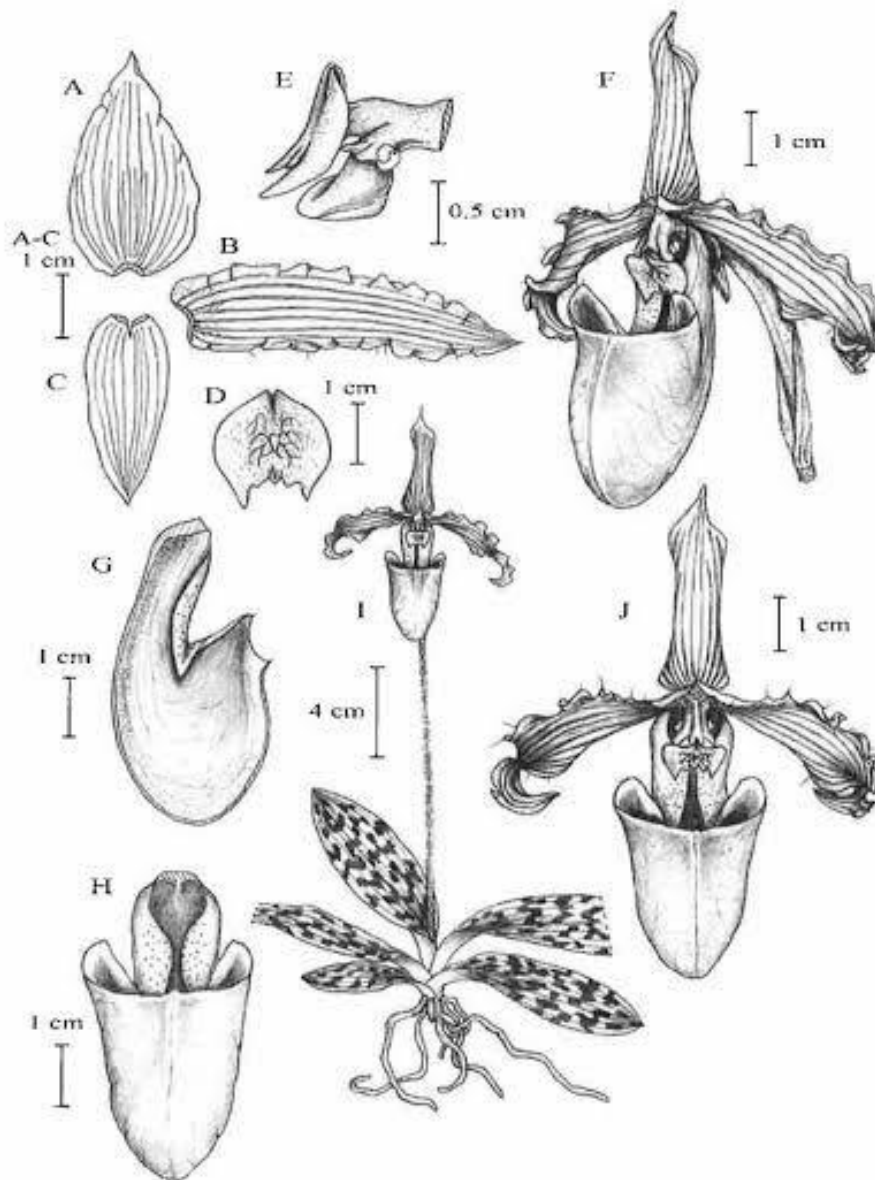


Figure 1. Floral and vegetative morphology of Paphiopedilum orchids. A = dorsal sepal; B = petal; C = synsepal; D = staminode; E = column; F = lateral flower view; G = elongated labellum; H = frontal labellum view; I = mature orchid plant; J = frontal flower view

Source: Destario Metusela (2017)

Data analysis

Qualitative and quantitative morphological data were tabulated and analyzed descriptively. Phenetic relationships among the four *Paphiopedilum* species were analyzed using cluster analysis with the Average Linkage/UPGMA approach and Euclidean distance in Minitab 22. Because the analysis was based on morphological characters only, the resulting clusters are interpreted as phenetic relationships rather than molecular phylogenetic relationships.

RESULTS AND DISCUSSION

Species inventory and morphological variation

The inventory conducted at Limbata Orchid and among orchid collectors in Pancasari identified four species of *Paphiopedilum*: *Paphiopedilum agusii*, *Paphiopedilum javanicum*, *Paphiopedilum lowii*, and *Paphiopedilum glaucophyllum*. Morphological characterization showed clear variation in both qualitative and quantitative characters. Qualitative variation was observed

in leaf shape, leaf margin, leaf apex, leaf surface texture, leaf symmetry, flower shape, inflorescence type, flowering position, and leaf and flower coloration. Quantitative variation was observed in stem length, stem diameter, leaf length, leaf width, flower diameter, flower stalk diameter, and flower stalk length.

Paphiopedilum lowii showed the most prominent overall morphology among the four species. It had the longest stem (8.0 cm), the longest leaves (39.2 cm), the largest flower diameter (14.5 cm), and the longest flower stalk (74.0 cm). These dimensions made *P. lowii* easily distinguishable from the other species. In contrast, *P. agusii* had the largest stem diameter (2.8 cm), which can be used as a diagnostic vegetative character. *Paphiopedilum glaucophyllum* had the widest leaves (5.3 cm), while *P. javanicum* generally showed smaller dimensions across most observed characters. The variation observed across these traits supports their use in taxonomic identification and germplasm grouping.

Table 1. Quantitative morphological characters of *Paphiopedilum* species

Character	<i>P. agusii</i>	<i>P. javanicum</i>	<i>P. lowii</i>	<i>P. glaucophyllum</i>
Stem length (cm)	3.7	2.6	8.0	2.9
Stem diameter (cm)	2.8	1.5	1.1	0.6
Leaf length (cm)	17.4	12.9	39.2	19.7
Leaf width (cm)	4.4	3.6	5.2	5.3
Flower diameter (cm)	7.5	7.5	14.5	9.5
Flower stalk diameter (cm)	0.3	0.2	0.4	0.3
Flower stalk length (cm)	21.3	20.0	74.0	18.0

Note: Values represent means from three samples.

Paphiopedilum agusii

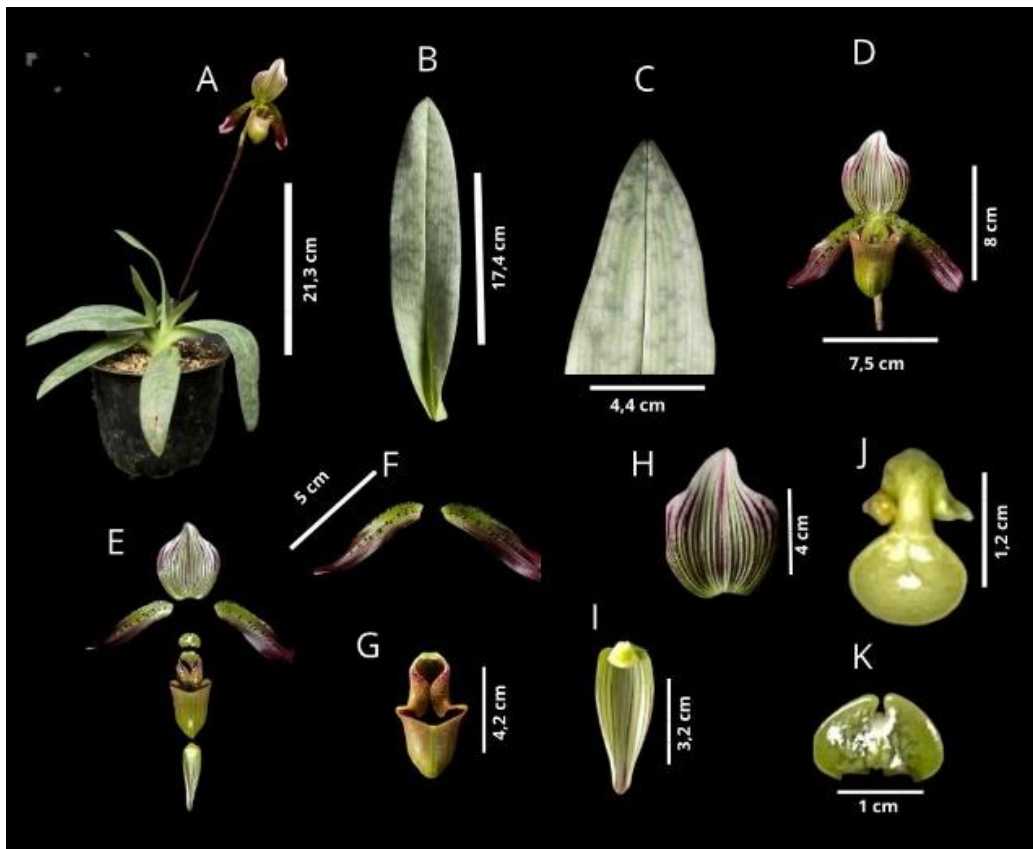


Figure 2. Morphological characters of *Paphiopedilum agusii*. A = habitus; B = leaf; C = leaf apex; D = flower; E = dissected flower; F = petal; G = labellum; H = dorsal sepal; I = synsepal; J = column; K = staminode.

Paphiopedilum agusii was originally reported from Java, particularly Mount Tugel, Central Java, at approximately 900 m above sea level. The species is described as having a narrow and specific habitat, occurring as a lithophytic or terrestrial orchid on moist mossy cliffs. In this study, *P. agusii* was

characterized by lanceolate leaves, serrated leaf margins, torn leaf apices, block-like leaf surface texture, symmetrical leaves, single-flowered inflorescence, aromatic flowers, ovate sepals, oblong petals with spotted patterning, and a strong yellowish-green flower base with purplish-red markings.

Paphiopedilum javanicum

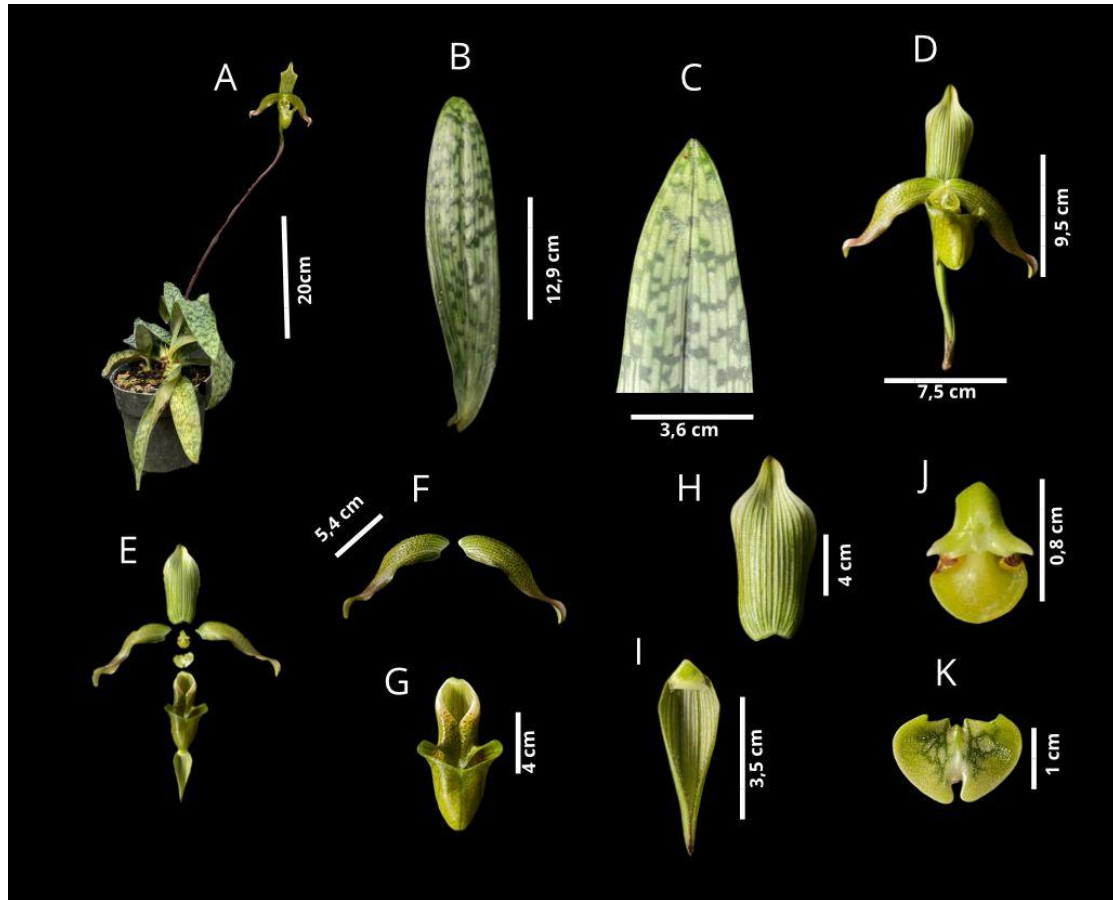


Figure 3. Morphological characters of *Paphiopedilum javanicum*. A = habitus; B = leaf; C = leaf apex; D = flower; E = dissected flower; F = petal; G = labellum; H = dorsal sepal; I = synsepal; J = column; K = staminode.

Paphiopedilum javanicum is a terrestrial slipper orchid that grows in humus-rich limestone substrates or rock crevices in humid and shaded forest environments. It is distributed across several Indonesian islands, although its habitat requirements are relatively specific. The species was

characterized by lanceolate leaves, serrated margins, three-toothed apices, irregular leaf surface texture, symmetrical leaves, single-flowered inflorescence, aromatic flowers, ovate sepals, oblong petals with spotted patterning, and pale yellowish-green flower coloration.

Paphiopedilum lowii

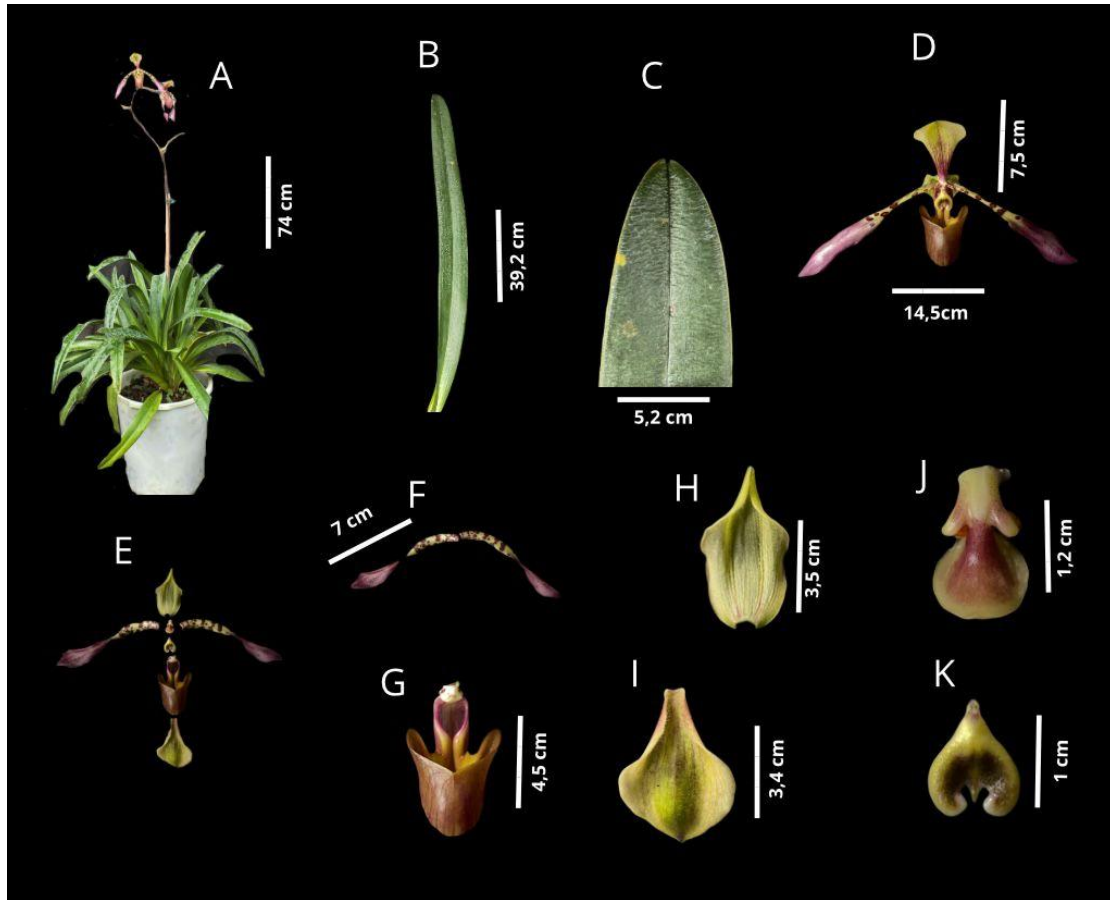


Figure 4. Morphological characters of *Paphiopedilum lowii*. A = habitus; B = leaf; C = leaf apex; D = flower; E = dissected flower; F = petal; G = labellum; H = dorsal sepal; I = synsepal; J = column; K = staminode.

Paphiopedilum lowii is one of the more widely distributed slipper orchids in Southeast Asia, occurring in Indonesia, Malaysia, and the Philippines. In Indonesia, it has been reported from major islands such as Sumatra, Kalimantan, Sulawesi, Bali, and Java. The species may grow as an epiphyte or lithophyte in humid forest habitats. In this study, *P. lowii* was distinguished by its large vegetative and

floral dimensions, including long leaves, a large flower diameter, and a very long flower stalk. Qualitatively, it had lanceolate leaves, serrated margins, three-toothed apices, block-like leaf surface texture, asymmetrical leaves, racemose inflorescence, non-aromatic flowers, spoon-shaped petals, and purplish-red floral markings.

Paphiopedilum glaucophyllum

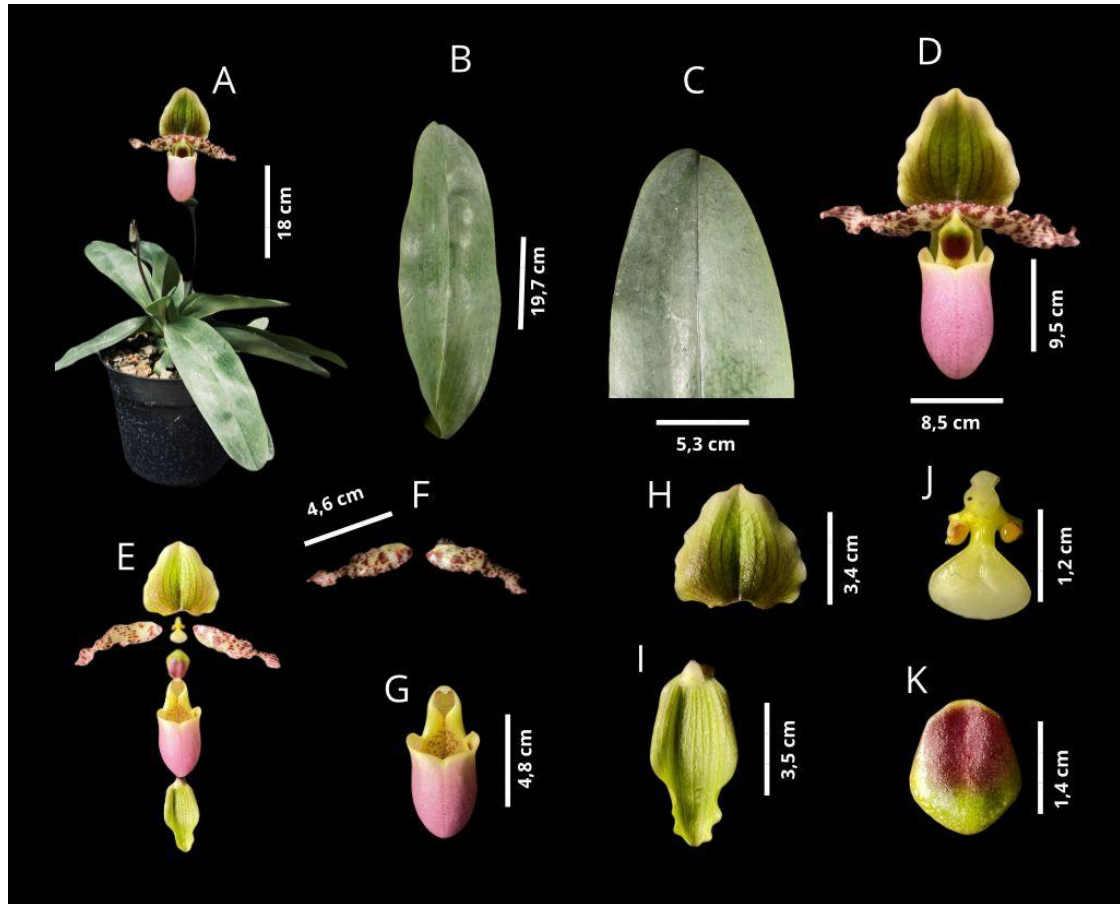


Figure 5. Morphological characters of *Paphiopedilum glaucophyllum*. A = habitus; B = leaf; C = leaf apex; D = flower; E = dissected flower; F = petal; G = labellum; H = dorsal sepal; I = synsepal; J = column; K = staminode.

Paphiopedilum glaucophyllum is an Indonesian endemic species naturally reported from East Java and Central Sumatra. It occurs at approximately 200-700 m above sea level, particularly on humid volcanic slopes and limestone cliffs with moss and humus. The species was characterized by ovate leaves, entire leaf margins, toothed leaf apices, glabrous leaf surface, asymmetrical leaves, racemose inflorescence, aromatic flowers, ovate sepals, oblong petals with spotted patterning, blunt sepal and petal

apices, and a brilliant yellowish-green flower base with strong purplish-red markings.

Qualitative morphological characters

The qualitative morphological data showed clear interspecific differences among the four *Paphiopedilum* species (Table 2). All observed species had monopodial growth and star-shaped flowers, but they differed in leaf form, leaf margin, leaf apex, leaf surface texture, symmetry, inflorescence type, aroma, petal form, floral coloration, and sepal-petal apex shape.

Table 2. Qualitative morphological characters of *Paphiopedilum* species

Character	<i>P. agusii</i>	<i>P. javanicum</i>	<i>P. lowii</i>	<i>P. glaucophyllum</i>
Specimen origin	Central Java	Bali	Bali	East Java
Growth habit	Monopodial	Monopodial	Monopodial	Monopodial
Leaf shape	Lanceolate	Lanceolate	Lanceolate	Ovate
Leaf margin	Serrated	Serrated	Serrated	Entire
Leaf apex	Torn	Three-toothed	Three-toothed	Toothed
Leaf surface texture	Block-like	Irregular surface	Block-like	Glabrous
Leaf symmetry	Symmetrical	Symmetrical	Asymmetrical	Asymmetrical
Basic leaf color	RHS 128C/163 Light Bluish Green	RHS 133C/163 Light Bluish Green	RHS 141C/131 Strong Yellowish Green	RHS 139C/120 Moderate Yellowish Green
Leaf pattern color	RHS 189B/149 Pale Green	RHS 131A/165 Bluish Green	-	-
Flower shape	Star-shaped	Star-shaped	Star-shaped	Star-shaped
Inflorescence type	Single	Single	Raceme	Raceme
Flowering position	Apex	Apex	Apex	Apex
Flower stalk posture	Erect	Erect	Erect	Erect
Flower aroma	Aromatic	Aromatic	Non-aromatic	Aromatic
Sepal shape	Ovate	Ovate	Ovate	Ovate
Petal shape	Oblong	Oblong	Spoon-shaped	Oblong
Petal color pattern	Spotted	Spotted	Spotted	Spotted
Sepal and petal apex	Pointed	Tapered with sharp sides	Tapered with sharp sides	Blunt
Basic flower color	RHS 144C/117 Strong Yellowish Green	RHS 149D/121 Pale Yellowish Green	RHS 11C/89 Pale Yellow	RHS 154B/116 Brilliant Yellowish Green
Flower pattern color	RHS 72A/255 Strong Purplish Red	RHS 142A/117 Strong Yellowish Green	RHS 186B/255 Moderate Purplish Red	RHS 64B/255 Strong Purplish Red

Phenetic relationship analysis

Cluster analysis of the four *Paphiopedilum* species was performed using the Average Linkage/UPGMA approach and Euclidean distance based on qualitative and quantitative morphological characters. At the 50% similarity level, the four species formed

separate clusters (Figure 6), indicating marked morphological differentiation. The Euclidean distance values ranged from 0.691 to 2.155. These values should be interpreted as morphological distances, not as direct percentage similarity or molecular genetic distance.

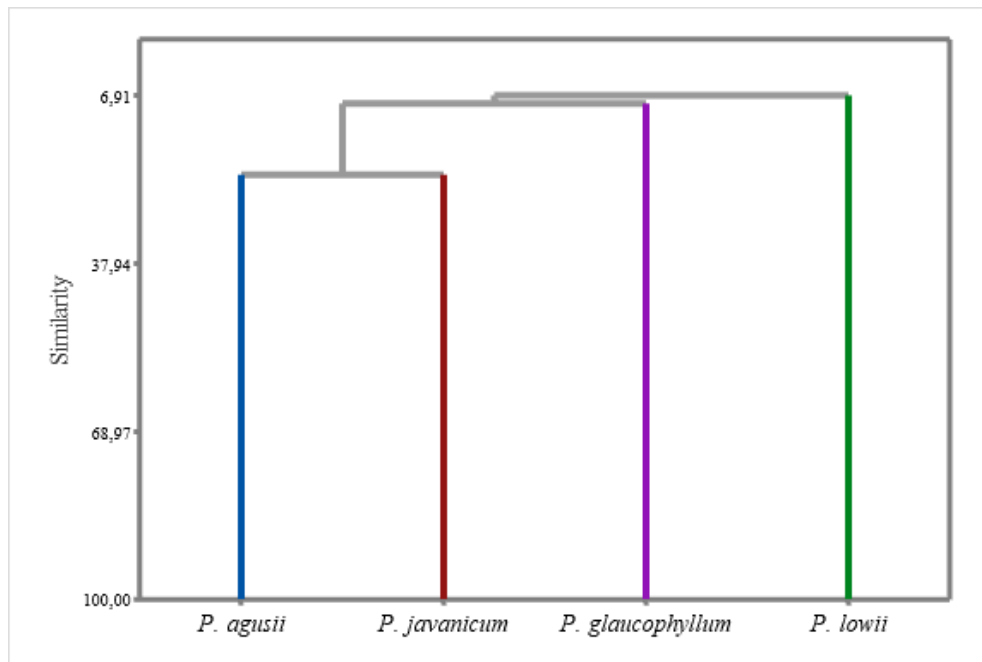


Figure 6. UPGMA dendrogram of four *Paphiopedilum* species based on qualitative and quantitative morphological characters.

The dendrogram showed that *P. lowii* was clearly separated from the other three species, supporting its status as the most morphologically divergent taxon in this dataset. Its divergence was mainly associated with the large leaf size, larger flower diameter, and considerably longer flower stalk. This morphology may be useful in breeding programs aimed at generating wider phenotypic variation; however, its use as a parent should be further evaluated through reproductive compatibility tests and molecular characterization.

In contrast, *P. agusii* and *P. javanicum* showed the closest phenetic relationship. Their similarity may be associated with shared vegetative and floral characters, including lanceolate leaves, serrated leaf margins, single-flowered inflorescences, aromatic flowers, ovate sepals, and oblong petals. *Paphiopedilum glaucophyllum* occupied an intermediate position, showing distinct characters such as ovate leaves, entire leaf margins, and blunt floral apices while still

sharing several floral traits with the other species.

Overall, the results indicate wide morphological variation among the four *Paphiopedilum* species. Such variation is valuable for taxonomy, germplasm documentation, conservation prioritization, and preliminary breeding consideration. Nevertheless, because morphology can be influenced by environmental conditions and phenotypic plasticity, molecular analysis is recommended to validate the inferred relationships and to provide a more comprehensive understanding of genetic diversity.

CONCLUSION

This study characterized four *Paphiopedilum* species, namely *Paphiopedilum agusii*, *Paphiopedilum javanicum*, *Paphiopedilum lowii*, and *Paphiopedilum glaucophyllum*. The species differed clearly in qualitative and quantitative morphological characters, including leaf

shape, leaf margin, leaf apex, leaf surface texture, symmetry, inflorescence type, aroma, flower color, flower diameter, and flower stalk length. UPGMA analysis based on morphological data separated the four species into distinct clusters. *Paphiopedilum lowii* was the most divergent taxon and may be useful as preliminary material for breeding programs, whereas *P. agusii* and *P. javanicum* showed the closest phenetic relationship. The findings provide baseline data for *Paphiopedilum* identification, germplasm documentation, breeding consideration, and conservation planning. Future studies should include molecular markers, voucher specimens, and broader population sampling to confirm taxonomic identity and evolutionary relationships.

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