International Journal of Agricultural Technology 2026 Vol. 22(1):435-450
DOI: https://doi.org/10.63369/ijat.2026.22.1.435-450
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Characterization of morphological variation in wild orchids of Kepahiang Regency, Bengkulu Province

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Romeida, A., Jenawie, B., Marlin, Herawati, R., Ganefianti, D. W., Rustika Wati, Masdar and Supanjani (2026). Characterization of morphological variation in wild orchids of Kepa Hiang Regency, Bengkulu Province. International Journal of Agricultural Technology 22(1):435-450.

Abstract A dendrogram based on an analysis of 66 morphological characters was found, and the coefficient of similarity between natural orchid species ranged from 0.29 to 0.71. Orchid species of the same genus were clustered in a specific form. The correlation matrix of the morphological marker reached a goodness of fit (r) of 0.88. This value is proved that the grouping based on morphological characterization of species of orchids can be categorized accordingly and can be used as a reference for determining the parent cross.

Keywords: Accession, Characterization, Genetic resources, Orchid, Phylogenetic

Introduction

Kepahiang Regency, Bengkulu Province, Indonesia, is a natural habitat for various species of orchids. In recent years, their natural diversity has faced threats as a consequence of human activities. Conversion of habitat areas of natural orchids for plantation and mining eliminates a great number of wild orchid species. Besides that, natural disasters, like forest fires, floods, and landslides, can destroy the natural habitats of wild orchids. Characterization of orchid plants can be done through the appearance of roots, stems, leaves, flowers, and fruits, because they generally have different characteristics from those of other plants (Ornamental Plant Research Institute, 2007; Hartati *et al.,* 2021). Wild orchids can be used as a source of germplasm for the development of commercial orchids with unique characteristics (Prayoga *et al.,* 2022; Sophia *et al.,* 2024).

Ganefianti *et al.* (2025) identified 35 wild orchids from the forests of Bengkulu and constructed a phylogenetic tree based on morphological data of natural orchid accessions in Bengkulu Province. Accurate identification and classification of wild orchid species are very important for the conservation and sustainability of orchids in the future in their natural habitat. Most natural orchids

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grow as epiphytes, and some species are known to grow terrestrially (Ganefianti *et al.,* 2023).

Morphological variability has been conducted and observed in *Phalaenopsis* spp. orchids and their hybrids, which can be used as material for morphological characterization. The research results show there are 70 characters separated morphologically into 490 subcharacters, consisting of 484 subcharacters polymorphic (98.78%) and 6 subcharacter monomorphic (1.22%) (Aziz *et al.,* 2015). The change of monomorphic character is not expected to change with the cross between accessions (Mujaffar *et al*., 2013).

Dendrogram results based on a similarity matrix resulted in two groups: the five species in the similarity of 1.32 produced two groups, the first group consisted of *P. amboinensis, P. indigoferus,* and *P. montanus*. The second group consists of *P. callosus,* and *P. tankervillae*. Dendrogram results based on coefficient similarities in *Phalaenopsis* spp. orchids resulted in two orchid groups of five Phalaenopsis orchid species with a similarity level of 1.32. The first group consists of *Phalaenopsis amboinensis, P. indigoferus, and P. montanus;* whereas the second group consists of *P. callosus* and *P. tankervillae* (Hartati *et al.,* 2021).

Morphological characterization of orchid species on Bangka Island resulted in moderate diversity, a high evenness index, and a low species richness index. The morphological similarity of orchid species on Bangka Island is 60%, divided into nine clusters. Prevention of orchid population decline on Bangka Island can be achieved through *in situ* and *ex situ* conservation methods. Furthermore, future orchid development will prioritize crossbreeding between orchids with unique characteristics and distant morphological relationships, which can also be used to produce commercial orchid varieties (Prayoga *et al.,* 2022). Variation in morphological characteristics also implies varying patterns in fragment molecular (DNA) (Khasim and Ramesh, 2010; Wahba *et al*., 2014; Ferdiani *et al*., 2015).

The natural orchids of Kepahiang Regency, Bengkulu Province, are highly diverse. It is crucial to understand their species, potential, and diversity status. Therefore, a morphological characterization is necessary. The study objective was to obtain complete morphological characters and a phylogenetic tree to determine the phenotypic relationships of 30 natural orchid accessions from Kepahiang Regency, Bengkulu Province.

Materials and methods

The natural habitat of the orchids used in this study is located in the Bukit Daun Protected Forest in Kepahiang Regency, Bengkulu Province. Exploration

and identification were conducted through direct surveys of the natural habitat. This study used the Tracking Line Transect method for exploration (Figure 1).

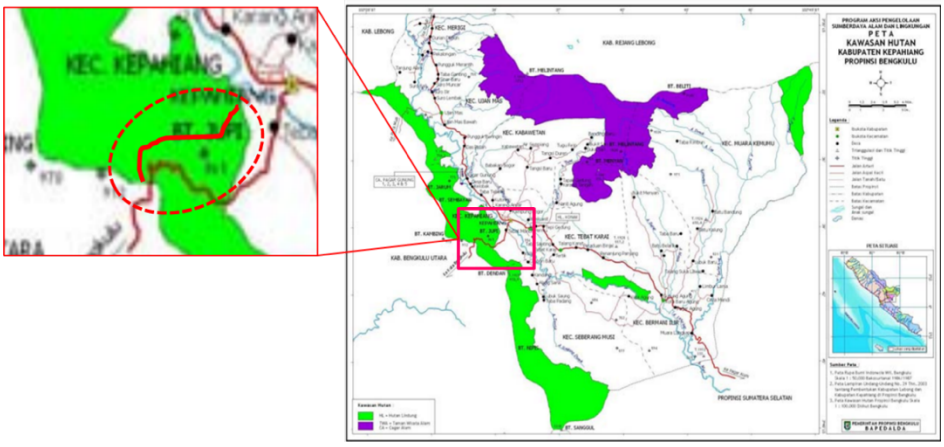


Figure 1. Map of Kepa Hiang Regency, Bengkulu Province: Insert: Research Location and Line transect route in the Bukit Daun Protected Forest Area, Kepahiang Regency, Bengkulu Province, Indonesia

The coordinates and habitat of each orchid species found were recorded. Each wild orchid species found in bloom was immediately and thoroughly characterized in its natural habitat. Meanwhile, orchids that had not yet flowered were domesticated and induced to flower ex situ at the Orchid Breeding House of the Faculty of Agriculture, University of Bengkulu, at an altitude of approximately 50 meters above sea level.

The 30 natural orchid accessions that were characterized can be characterized morphologically, provided that they have flowered both in their natural habitat and after being maintained in captivity. The natural orchids characterized in this study consist of 15 genera, including: Acriopsis, Aerides, Arachnis, Arundina, Bulbophylum, Cymbidium, Coelogyne, Dendrobium, Epidendrum, Gramathophylum, Phalaenopsis, Pomatocalpa, Phaius, Spathoglottis, and Vanda.

The morphological characters included roots, stems, bulbs, leaves, flowers, and fruits, rated from two fully grown plants (Ornamental Plant Research Institute, 2007). The qualitative data served to identify types of morphism for the observed features. The quantitative data's conversion to binary codes received a score of one (1) for observable and zero (0) for unobservable traits. The data sets' arrangement in a matrix carries out further analysis. Using the NTSYS-PC version 2.02i (Rohlf, 2000) ran the SIMQUAL routine to generate the Dice

similarity coefficient and the SAHN routine to construct the unweighted pair group method with arithmetic mean (UPGMA) clustering and the dendrogram. Cophenetic correlation (r) application measures the 'goodness of fit' of the similarity between cluster outputs, where $r > 0.9$ is scored as a very good fit, $0.7 < r < 0.9$ = a good fit, and $r < 0.7$ is a less fit (Ganefianti *et al.*, 2025).

Results

Morphological marker analysis

The natural orchid collection of Kepahiang Regency, Bengkulu Province, which is successfully domesticated in the orchid breeding facility, obtained 56 accessions. From these, a total of 26 accessions are not successfully induced to flower, so morphological characterization cannot be carried out. A total of 30 species are fully characterized because they flowered both in their natural habitat (12 accessions) and in the Breeding House (18 accessions); hence, they were used in this research. The natural orchid species of Kepahiang Regency, Bengkulu Province, which is fully characterized morphologically, are 30 species as presented in Table 1.

Table 1. Wild orchid species and their natural habitat in Kepahiang Regency, Bengkulu Province, Indonesia

No	Species	Habitat	No	Species	Habitat
1	*Phalaenopsis violacea*	Epiphytic	16	*Spathoglottis alba*	Terrestrial
2	*Phalaenopsis tetraspis*	Epiphytic	17	*Spatoghlottis plicata*	Terrestrial
3	*Phalaenopsis amboinensis*	Epiphytic	18	*Arundina graminifolia*	Terrestrial
4	*Phalaenopsis modesta*	Epiphytic	19	*Vanda Helvola*	Epiphytic
5	*Phalaenopsis cornucervi*	Epiphytic	20	*Dendrobium secundum*	Epiphytic
6	*Phalaenopsis bellina*	Epiphytic	21	*Dendrobium antenatum*	Epiphytic
7	*Phalaenopsis javanicum*	Epiphytic	22	*Dendrobium tetradon*	Epiphytic
8	*Bulbophylum claptonense*	Epiphytic	23	*Acriopsis lifolia*	Epiphytic
9	*Bulbophylum dearei*	Epiphytic	24	*Pomatocalpa latifolia*	Epiphytic
10	*Bulbophylum metachillum*	Epiphytic	25	*Epidendrum paniculatum*	Epiphytic
11	*Bulbophylum ovalifolium*	Epiphytic	26	*Grammatophilum stapeliaeforum*	Epiphytic
12	*Coelogyne asperata*	Epiphytic	27	*Cymbidium chlorantum*	Epiphytic
13	*Coelogyne rochussenii*	Epiphytic	28	*Cymbidium bicolor*	Epiphytic
14	*Coelogyne pandurate*	Epiphytic	29	*Aerides odorata*	Epiphytic
15	*Phaius tancarvilleae*	Terrestrial	30	*Arachnis Flosaeris*	Epiphytic

Morphological diversity of 30 wild orchid species from Kepahiang Regency, Bengkulu Province has been successfully characterized. The number of morphological characters used to identify morphological differences in orchid plants is as many as 66 characters, which include general characters, such as plant growth type and plant size, as well as organic characters, including the type and size of roots, stems, leaves, and flowers. In accordance with the characterization guide published by the Ornamental Plant Research Institute (2007), it is identified 506 subcharacters. These consisted of 504 polymorphic sub-characters (99.60%) and 2 monomorphic sub-characters (0.40%). The two monomorphic characters are the main characteristics of wild orchid species that grow in Kepahiang Regency, Bengkulu Province. Main characteristics the always found in every type of wild orchid found in Kepahiang Regency, Bengkulu Province, even though different species still have the same shape of the round cross-root section and of the edge of the leaves. The results of morphological characterization, which includes roots, stems, leaves, flowers, fruit, and seeds, are presented in Table 2.

Table 2. Morphological Characteristics of Natural Orchids in Kepahiang Regency, Bengkulu Province

No	Morphological Characters	Number of sub characters	Number of polymorphic sub characters	Number of monomorphic sub characters
1.	Stem growth type	2	2	0
2.	Plant size	3	3	0
3.	Plant height	3	3	0
4.	Stem size	3	3	0
5.	Peduncle position on the stem	3	3	0
6.	Stem erectness	3	3	0
7.	Stem color	16	16	0
8.	Leaves arrangement	7	7	0
9.	Stem shape	9	9	0
10.	Stem diameter	3	3	0
11.	Bulb number	5	5	0
12.	Root tip color	19	19	0
13.	Root base color	18	18	0
14.	Root hair	2	2	0
15.	Root type	2	2	0
16.	Root cross-section shape	1	0	1
17.	Leaf cross-section	2	2	0

No	Morphological Characters	Number of sub characters	Number of polymorphic sub characters	Number of monomorphic sub characters
18.	Leaf tip symmetry	2	2	0
19.	Leaf shape	4	4	0
20.	Leaf tip shape	7	7	0
21.	Leaf edge shape	1	0	1
22.	Leaf surface texture	3	3	0
23.	Mature leaf color	9	9	0
24.	Young leaf color	9	9	0
25.	Leaf size	4	4	0
26.	Leaf length	4	4	0
27.	Leaf width	5	5	0
28.	Inflorescence type	3	3	0
29.	Flower resupination	2	2	0
30.	Flower shape	4	4	0
31.	Flower arrangement	4	4	0
32.	Dorsal sepal shape	7	7	0
33.	Lateral sepal shape	8	8	0
34.	Sepal tip shape	5	5	0
35.	Petal Shape	8	8	0
36.	Petal tip shape	5	5	0
37.	Callus	3	3	0
38.	Spur	2	2	0
39.	Polinia Number	3	3	0
40.	Spike color	22	22	0
41.	Dorsal sepal color	21	21	0
42.	Dorsal sepal pattern	21	21	0
43.	Lateral sepal color	16	16	0
44.	Lateral sepal pattern	19	19	0
45.	Flower base color	22	22	0
46.	Lip color	23	23	0
47.	Lip tip color	25	25	0
48.	Lip sidelobe color	2	2	0
49.	Lip mid lobe color	19	19	0
50.	Lip sidelobe	20	20	0
51.	Number of flower colors	4	4	0
52.	Petal twist	4	4	0
53.	Petal color	22	22	0
54.	Petal pattern	13	13	0
55.	Flower size	4	4	0

No	Morphological Characters	Number of sub characters	Number of polymorphic sub characters	Number of monomorphic sub characters
56.	Flower length	4	4	0
57.	Flower width	4	4	0
58.	flower arrangement length	5	5	0
59.	Peduncle length	5	5	0
60.	Peduncle diameter	4	4	0
61.	Pedicel number per peduncle	3	3	0
62.	Flower number per pedicel	4	4	0
63.	Flower freshness	4	4	0
64.	Mature fruit color	6	6	0
65.	Fruit length	4	4	0
66.	Fruit diameter	3	3	0
Total		506	504	2
Percentage (%)		100	99.60	0.40

Note: Characterization based on Ornamental Plant Research Institute book of Characterization, 2007

Polymorphic sub-characters in morphological markers showed a very large variation, reaching 99.60%. This highly morphological variability is not only influenced by genetic factors, but also greatly influenced by environmental factors at every developmental stage of the plants. Every type of polymorphic character of an accession can become a differentiator and become a characteristic typical of every accession plant orchid observed in nature.

Grouping of accessions based on morphological characters

Based on the dendrogram analysis of 66 morphological characters from 30 wild orchid accessions in Kepahiang Regency, it was found that the similarity coefficient between natural orchid species in Kepahiang Regency, Bengkulu Province ranged from 0.29 to 0.71 with a correlation value (r) of 0.8815 (Figure 2). The similarity coefficient approaching 1 means the accession has a higher level of similarity The flower appearance of each accession and its grouping are presented in Figure 3.

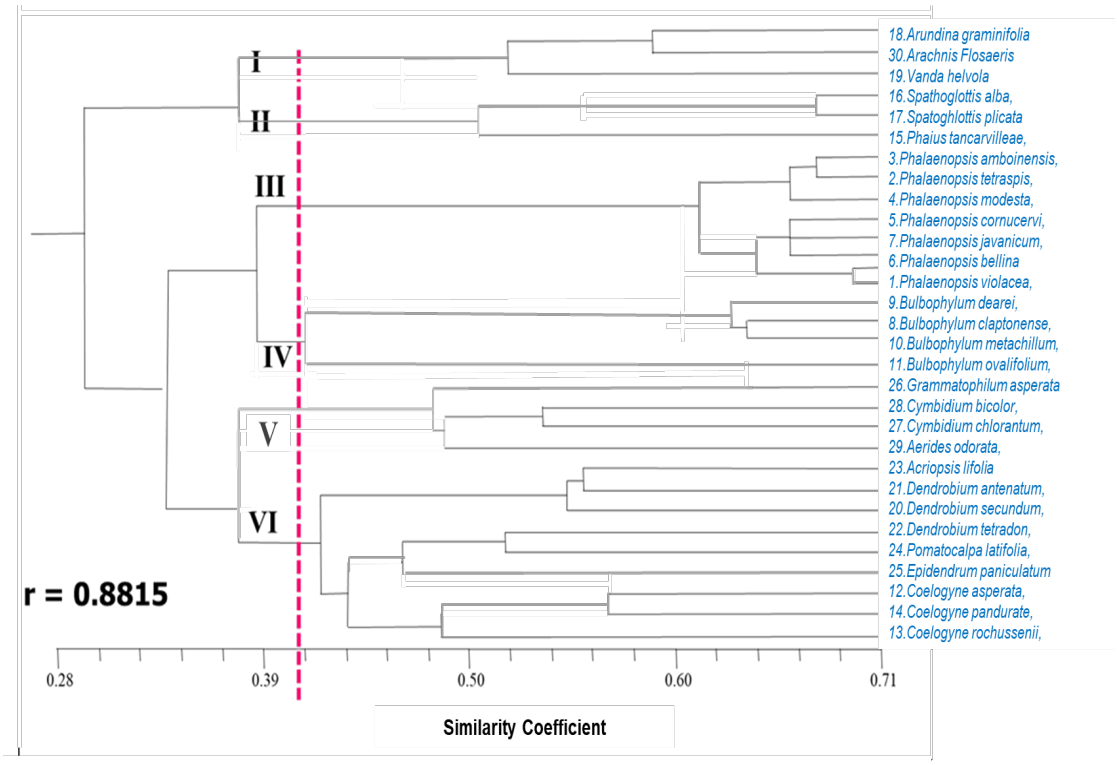
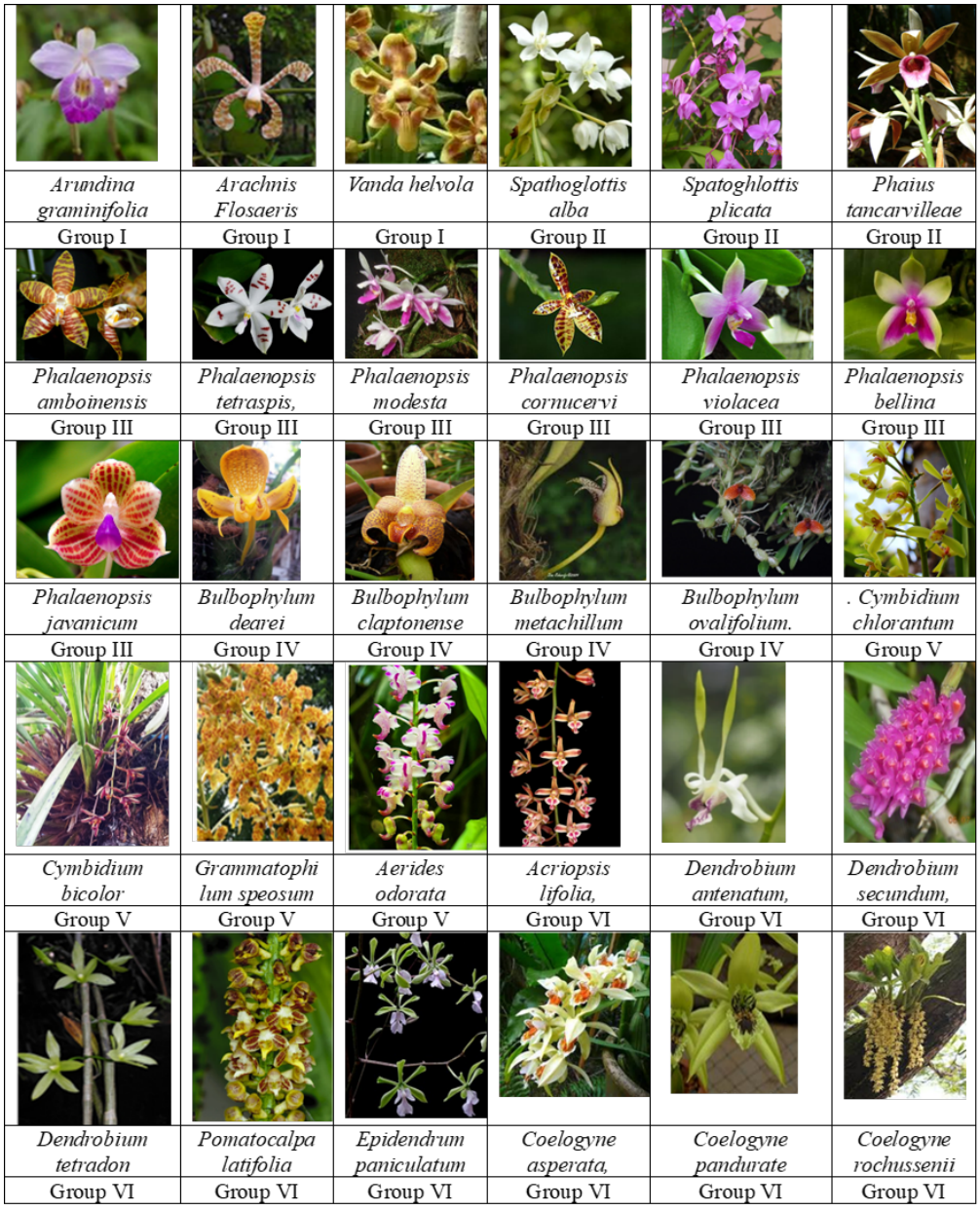


Figure 2. Dendrogram of cluster analysis of 30 wild orchid accessions in Kepahiang Regency, Bengkulu Province based on morphological characters



Arundina graminifolia	*Arachnis Flosaeris*	*Vanda helvola*	*Spathoglottis alba*	*Spatoghlottis plicata*	*Phaius tancarvilleae*
Group I	Group I	Group I	Group II	Group II	Group II
Phalaenopsis amboinensis	*Phalaenopsis tetraspis,*	*Phalaenopsis modesta*	*Phalaenopsis cornucervi*	*Phalaenopsis violacea*	*Phalaenopsis bellina*
Group III	Group III	Group III	Group III	Group III	Group III
Phalaenopsis javanicum	*Bulbophylum dearei*	*Bulbophylum claptonense*	*Bulbophylum metachillum*	*Bulbophylum ovalifolium.*	*. Cymbidium chlorantum*
Group III	Group IV	Group IV	Group IV	Group IV	Group V
Cymbidium bicolor	*Grammatophi lum speosum*	*Aerides odorata*	*Acriopsis lifolia,*	*Dendrobium antenatum,*	*Dendrobium secundum,*
Group V	Group V	Group V	Group VI	Group VI	Group VI
Dendrobium tetradon	*Pomatocalpa latifolia*	*Epidendrum paniculatum*	*Coelogyne asperata,*	*Coelogyne pandurate*	*Coelogyne rochussenii*
Group VI	Group VI	Group VI	Group VI	Group VI	Group VI

Figure 3. Flower Appearance of 30 Wild Orchid Accessions from Kepahiang Regency, Bengkulu Province, Indonesia. Grouping Based on Dendrogram Clustering of Morphological Characters

Discussion

Based on morphological characters, the dendrogram of cluster analysis of 30 natural orchid accessions of Kepahiang Regency, Bengkulu Province can be clustered into 6 groups at a similarity coefficient of 40%. The goodness of fit value of the correlation matrix (r) of morphological markers reaches r = 0.8815, which can be categorized as appropriate criteria. This is in line with the results of a research reported by Wang *et al.,* (2009), which states that the level of grouping harmony is determined by the goodness of fit value, namely the suitability between the similarity coefficient value (r) with the criteria of very appropriate (r> 0.9), of appropriate (0.8 <r <0.9), of inappropriate (0.7 <r <0.8) and of very inappropriate (r <0.7).

Group 1 consists of 3 accessions, namely *Arundina graminifolia, Arachnis flosaeris,* and *Vanda helvola.* These accessions are all in different genus, but based on their growing habitat, Arundina is a terrestrial orchid, whereas Arachnis and Vanda are Epiphytic orchids, which require a medium to attach their roots to grow. All three accessions have a similarity of about 52%. Based on taxonomy, the similarity coefficient is <0.6, so the differences are categorized at the genus-level differences. The similarities of these three types of orchids are located in the morphology of the roots, stems, and large leaves. All three types of orchids have lanceolate-shaped leaves, alternate leaf positions on the stem, and shaped bunches-like flower stalks. stated that the orchid species *Arachnis flosaeris,* and *Vanda helvola* are in one cluster, namely the Vanda tribe, which has the same type of monopodial plant growth, flower stalks growing in the leaf axils (Li *et al.,* 2016; Risdiana *et al.,* 2023). Arundina graminifolia has a sympodial growth type, the flower stalk being at the terminal end of the stem.

Group 2 consists of three accessions belonging to three orchid species: *Spathoglottis alba, Spatoghlottis plicata,* and *Phaius tancarvilleae.* Based on their growing habitat, these three species are classified as terrestrial orchids, requiring full sunlight for growth and flowering. Based on the UPGMA Cluster, the similarity is approximately 52%, meaning that *Spathoglottis* and *Phaius* are in different genera. *Spathoglottis alba* and *Spathoglottis plicata* are in the same genus. The similarities between these three orchids include their growth location, sympodial growth, alternating leaf growth, leaf surfaces having folds/plicates, upright flower stalks, emerging from the base of the stem, and large flowers in the form of resemose (Kartikaningrum and Effendi, 2005; Aziz *et al.*, 2016; Romeida, 2008; Romeida *et al.,* 2012). This morphological characterization is

very important for orchid conservation, whether *in-situ, ex-situ*, and *in-vitro* (Ganefianti *et al.*, 2023).

Group 3 consists of 7 natural orchid species, namely *Phalaenopsis amboinensis, Phalaenopsis tetraspis, Phalaenopsis modesta, Phalaenopsis cornucervi, Phalaenopsis javanicum, Phalaenopsis bellina, and Phalaenopsis violacea.* The seven accessions are included in the genus Phalaenopsis, which has a similarity between accessions of 62%-70%. The seven orchid accessions are different species. The similarities of these orchids are found in the growth type, leaf shape, and rosette stem. Flower stalks and roots grow at the stem node, flower stalks grow upright. The differences are found in the shape, size, and color of the flowers. The seven Phalaenopsis orchids have great potential to be parents for crossbreeding so that they can produce new hybrid orchids. All Phalaenopsis hybrids grouped together at a similarity coefficient of 0.729, while one replicate of the yellow-flowered hybrid had a similarity coefficient of 0.435 with the main differences being leaf shape and flowering type. *Phalaenopsis amabilis* ecotype 'Cidaun' has a similarity coefficient of 0.528 with hybrid Phalaenopsis, the main differences are in flower shape and flowering type (Pangestu *et al*., 2015; Handini *et al*., 2016).

Group 4 consists of *Bulbophylum dearei, Bulbophylum claptonense, Bulbophylum metachillum,* and *Bulbophylum ovalifolium.* The four accessions have approximately 65% similarity, meaning they are in the same genus, and differing only by species. The four accessions have similarities in their sympodial growth pattern, with bulbs connected by connectors, relatively rounded to oval bulb shapes, each bulb having only one leaf, a single flower stalk growing at the base of the bulb, and a very short blooming period of approximately 2-4 days. The differences lie in the shape, size, and color of the flowers. *Bulbophyllum ovalifolium* has a single flower, the smallest of which is red. The shape of the flower resembles a fly . The uniqueness of *Bulbophyllum* lies in its lips, which can move when blown by the wind. Based on the high diversity of *Bulbophyllum* orchids in Bengkulu Province, it has great potential to become a new type of orchid for the development of commercial varieties.

Group 5 consists of accession orchids classified in species *Grammatophilum speosum, Cymbidium bicolor, Cymbidium chlorantum,* and *Aerides odorata*. This consists of three genuses that have several similarities, including habitat, type orchid Epiphyte, type sympodial growth, a ribbon-like leaf, and having fluorescent flower stalks. The difference lies in the position of the flower stalk. Accession *Grammathophillum speosum* and *Cymbidium*

chlorantum poses flower stalk with erect position, whereas *Cymbidium bicolor* and *Aerides odorata* have hanging flower stalks. Accessions Grammatophillum and Cymbidium and Aerides own coefficient similarity of 47%-49%. Based on taxonomy, if the coefficient similarity is < 0.6, then the differences are included in Genus level. *Grammatophilum asperata* is the largest orchid compared to other types. The flower stalk reaches a height of 2.5-3 m. The number of flower buds reaches > 100 buds per flower stalk. Based on RBCL nucleotide sequence data, Cameron *et al.* (2009) found that the genera Cymbidium and Grammatophyllum have the same grouping in the subtribe Cyrtopodiinae cluster (Cymbidieae tribe).

Group 6 consists of *Acriopsis lifolia, Dendrobium antennatum, Dendrobium secundum, Dendrobium tetradon, Pomatocalpa latifolia, Epidendrum paniculatum, Coelogyne asperata, Coelogyne pandurate,* and *Coelogyne rochussenii.* The similarity coefficient of the accessions in group 6 ranges from 42%-57%. There are 4 genera consisting of 7 species. The similarities are found in the sympodial growth type, and having an oval-shaped bulb covered with leaves. The difference lies in the growth of leaves on the stem. Dendrobium orchids have the highest diversity (55%) in the shape, size, and color of their flowers. The general characteristics of Dendrobium are having one leaf on each stem node, and flowers growing upright in the leaf axils. This genus can grow more than a flower stalk per bulb. Flowers were in bunches and non-resupinate, oval dorsal and lateral sepals, sepals with sharp tips, lip side lobes, side pieces, and no flower spurs. Zed *et al*. (2020) also reported that species *D. sanguinolentum* was predominant in the protected forest of Gunung Raya, Temajuk Village, Sambas Regency, Kalimantan, Indonesia. De (2020) reported that, in *Dendrobium aphyllum,* the leaf morphology was lanceolate, and the leaf surface texture was bald. Epidendrum accessions are very popular in kinship with Dendrobium accessions. Similarities covering roots, leaves, and flowers. The difference lies in the flower stalks which are shaped like bunches that have lots of branching. The accession *Acriopsis lifolia* also has similarities with Dendrobium, especially in the shape of the roots and flower shape. The difference is in the bulb which is more round with leaves found in the section end terminal. The cross section transverse leaf is round, flower stalks grow in the axils leaf with hanging position, small flower size and larger big lips as compared to *A. lifolia* with petals and sepals. Accession Coelogyne has similarity in bulb shape, with 2 leaf strands at the terminal end of the bulb. Flower stalks grow from bulb base. The flower stalk grows semi-erect in *Coelogyne pandurate* and

Coelogyne asperata, and hangs in *Coelogyne rochussenii.* Knowledge and information about parental relationships is the main point for developing ornamental plant breeding programs. Selection of native orchids as potential parents is imperative where selected accessions will be used as a source of genetic diversity. The results of this study are expected to provide scientific information and phenotypic selection for the development of hybrid orchids (Indraloka *et al.,* 2019).

Orchid species from the same genus are grouped to form a special cluster, with a similarity coefficient ranging from 0.29 to 0.71. Complete morphological characterization of 30 natural orchid species in Kepahiang Regency, Bengkulu Province were grouped into 6 groups at a similarity coefficient of 40%, with a correlation value of morphological markers reaching r = 0.8815. A total of 66 morphological characters produced 506 sub-characters, consisting of 504 (99.60%) polymorphic sub-characters and 2 sub-characters (0.40%) monomorphic, which can be used to group orchid accessions accurately.

Acknowledgements

Thank you to Bengkulu University, Indonesia, for providing funds through the 2023 Faculty of Agriculture Superior Research.

Conflicts of interest

The authors declare no conflict of interest.

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(Received: 29 September 2025, Revised: 30 December 2025, Accepted: 7 January 2026)