

Phenetic analysis and habitat preferences of wild orchids in Gunung Gajah, Purworejo, Indonesia

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Abstract. Purba THP, Chasani AR. 2021. Phenetic analysis and habitat preferences of wild orchids in Gunung Gajah Purworejo. *Biodiversitas* 22: 1371-1377. Orchid (Orchidaceae) is one of the largest flowering plant families comprising more than 25.000 species. Knowledge of wild orchid diversity and their habitat preferences is useful for its conservation efforts. Research of wild orchid diversity in Indonesia has been done before, however, there is still few information about phenetic analysis and habitat preferences of wild orchids in Indonesia particularly in Gunung Gajah, Purworejo, Indonesia. Therefore, our research aimed to study species diversity and phenetic relationship of wild orchids and their habitat preferences in Gunung Gajah, Purworejo. Exploration was done using purposive sampling method. Phenetic analysis was carried out using MVSP 3.1, implemented by clustering method to understand phenetic relationships and ordination by PCA method to reveal morpho-anatomical characters which determine the clustering pattern. Thirteen orchid species of subfamily Vanilloideae, Orchidoideae, and Epidendroideae were found. Two clusters were formed on dendrogram and each of them was separated into two sub-clusters. PCA analysis based on stem, leaf, and flower characters showed different clusters compared to dendrogram. Habitat preferences of wild orchids in Gunung Gajah are alkaline soil, low soil moisture, air temperature 24-29°C, and low to high light intensity. Epiphytic orchids preferences on their host tree are dominated by median and upper part of main stem.

Keywords: Gunung Gajah, habitat, phenetic, wild orchids

INTRODUCTION

Orchids (Orchidaceae) are the second-largest families of flowering plant group after Asteraceae with more than 25.000 species estimated and among them 4.000 species can be found in Indonesia (Kusmana 2015; Teoh 2005). Some species are endemic, including *Dendrobium capra*, *Paphiopedilum glaucophyllum*, and *Vanda foetida* (Yulia and Ruseani 2008; Metusala and Rindyastuti 2016). The high diversity and endemicity of wild orchid species encourage researchers to explore the diversity of wild orchids in Indonesia.

A number of research on wild-orchid diversity in Indonesia has been done before. According to Fardhani et al. (2015), fifty orchid species were found in Mount Sanggara, Bandung, West Java with *Bulbophyllum flavescens* and *Tainia speciosa* were the most abundant species. Twenty-three orchid species were found in Hutan Lindung Gunung Talang, West Sumatra including an endemic species, *Eria merapiensis* (Musa et al. 2013). High orchid species diversity in Wawonii Island, Southeast Sulawesi was reported by Sulistiarini (2008) who stated that ninety-one species of orchid were found and the most abundant species was *Grammatophyllum pictum*. Furthermore, orchid species diversity has been recorded at several areas of Pegunungan Menoreh. Setiaji et al. (2018) reported thirteen orchid species that were found at Pegunungan Menoreh. These include *Acriopsis liliifolia*, *Appendicula* sp., *Appendicula ramosa*, *Bryobium retusum*, *Coelogyne*

speciosa, *Dendrobium crumenatum*, *Dendrobium mutabile*, *Dendrobium plicatile*, *Dienia ophrydis*, *Liparis parviflora*, *Spathoglottis plicata*, *Cymbidium bicolor*, and *Vanilla planifolia*. As well, an exploration by Nugroho et al. (2010) in Bukit Cokro, Krengseng, Ngasinan, and Watublencong of Pegunungan Menoreh resulted from data that were similar species to Setiaji et al. (2018), except *Liparis condylobulbon*.

Numerous research of orchid diversity in Pegunungan Menoreh has been done excluding Gunung Gajah. Gunung Gajah, located in Purworejo, Central Java, is a part of Pegunungan Menoreh where orchid diversity has never been explored before and also, there is no research about phenetic relationship and habitat preferences of wild orchids in this area. Phenetic analysis or also called taximetry, is the grouping of organisms into taxa groups based on the character they have (Singh 2010). Phenetic analysis is useful in helping taxonomists choosing characters and grouping organisms appropriately (Stuessy 2009). A habitat preference is one of the limiting factors of plant diversity and distribution. Specific habitat conditions will allow plants including wild orchids to grow, survive and reproduce (Lambers et al. 2008). Wild orchid's habitat is widely varied, from wet, dry, calcareous and even saline environments (Pridgeon 2014). Therefore, orchid survivability highly depends on environmental factors, including soil pH, altitude, temperature and humidity (Djordjević et al. 2016). Understanding orchid habitat preferences is important for orchid conservation efforts

because orchids have a wide range of habitats and environmental factors (Irawati 2012). Hence, our research is not only investigating the species diversity, but also examined the phenetic relationships and habitat preferences of wild orchids in Gunung Gajah, Purworejo, Jawa Tengah.

MATERIALS AND METHODS

Our research was conducted from March to June 2019 both in field and laboratory. Wild orchid samples were taken from two different sites in Gunung Gajah, Pandanrejo Village, Kaligesing Sub-district, Purworejo District, Central Java Province, Indonesia. The two sites were pine forests and forests with calcareous soil. Morphological characters were recorded in the field while anatomical characters were observed in Laboratory of Plant Structure and Development, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Wild orchid samples were collected using purposive sampling method. Morphological characters of all the wild orchids under investigation were recorded. For investigation of anatomical characters, the leaves were sliced and soaked in chloralhydrate solution for about a week to make an epidermal slide. The slides then observed using microscope. Soil pH, soil moisture, air temperature, and light intensity of wild orchids habitat were recorded. Every epiphytic wild orchid's host tree species were also noted and host tree stem's condition was described.

Morphological characters were used to determine and classify wild orchids species while morphological and anatomical characters were used for phenetic analysis. Using clustering method in Multi-Variate Statistical Package (MVSP) version 3.1, phenetic relationships between

OTUs were analyzed. Two types of characters were used, two-state character and multi-state character. Data standardization was conducted before doing any further analysis. Jaccard coefficient was preferred to measure the similarities between OTUs and finally, the OTUs were grouped by UPGMA (Unweighted Pair Group Method with Arithmetic Mean) approach. The result of phenetic relationship analysis was portrayed as a dendrogram. Principal Component Analysis (PCA) in MVSP 3.1 was performed in order to reveal which character(s) has important roles in the clustering patterns.

RESULTS AND DISCUSSION

Based on our study, there were 13 wild orchid species in Gunung Gajah, Purworejo, Central Java. In our study, only terrestrial and epiphytic orchids were found in Gunung Gajah. This is slightly different from Setiaji et al. (2018) that stated there is a holomycotropic orchid *Epipigeum roseum* in Pegunungan Menoreh. Further, there are seven species that are commonly found in Pegunungan Menoreh but were not found in our study, i.e. *Appendicula ramosa*, *Coelogyne speciosa*, *Dienia ophrydis*, *Dendrobium mutabile*, *Cymbidium bicolor*, *Liparis parviflora* and *Spathoglottis plicata*. However, our study found six species that were not reported by Setiaji et al. (2018), that was *Oberonia similis*, *Arundina graminifolia*, *Nervilia concolor*, *Crepidium koordersii*, *Peristylus goodyeroides*, and *Habenaria reflexa*. For phenetic analysis, all individual samples from thirteen wild orchids were treated as OTUs. There were 41 samples that were determined as OTUs (Table 1).

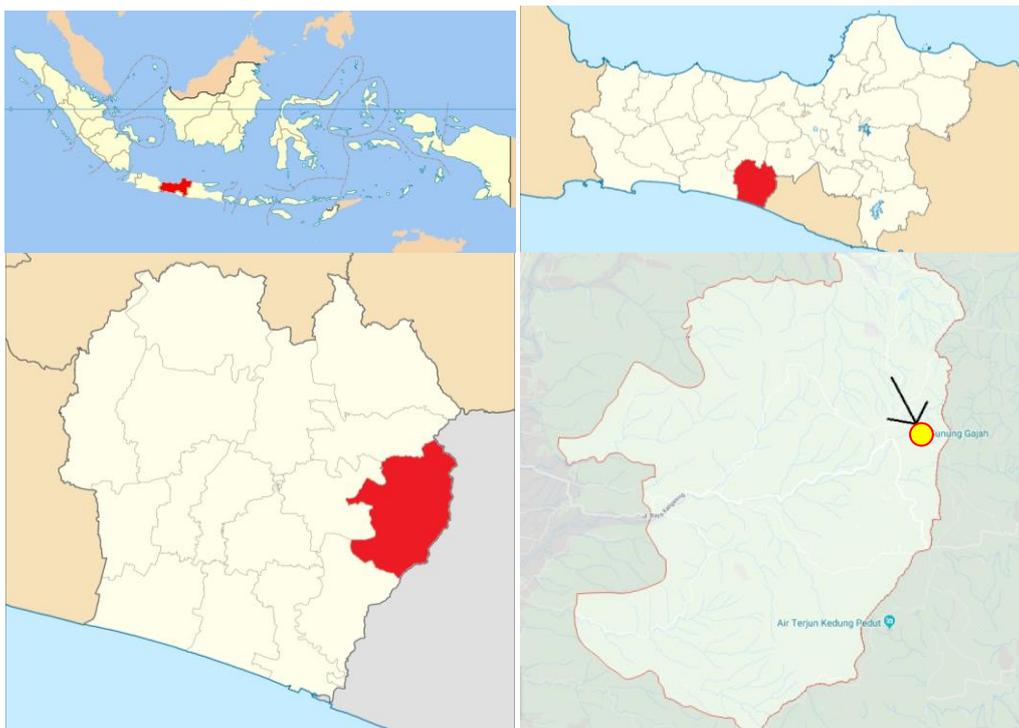


Figure 1. Sampling site in Gunung Gajah (arrow) of Pandanrejo Village, Kaligesing Subdistrict, Purworejo District, Central Java Province, Indonesia ($7^{\circ}43'29.98''S$ $110^{\circ}07'38,31''E$)

Table 1. Wild orchids in Gunung Gajah, Purworejo, Indonesia

OTUs	Subfamily	Species	Habitat
14-15 16 17 18	Vanilloideae	<i>Vanilla planifolia</i> Jacks. ex Andrews	Stem of <i>Macaranga</i> sp. Stem of <i>Syzygium</i> sp. Stem of <i>Theobroma cacao</i> Stem of <i>Albizia chinensis</i>
24-25	Orchidoideae	<i>Peristylus goodyeroides</i> (D.Don) Lindl.	Pine forest, on moist soil with leaf litters, covered by a surrounding plant canopy
36-41		<i>Habenaria reflexa</i> Blume	Calcareous soil forest, on moist soil with leaf litters, near <i>C. koordersii</i>
1-4 5-9 10-13	Epidendroideae	<i>Acriopsis liliifolia</i> (J.Koenig) Seidenf. <i>Bryobium retusum</i> (Blume) Y.P.Ng & P.J.Cribb <i>Oberonia similis</i> Lindl.	Stem of <i>Cocos nucifera</i> Stem of <i>Pinus merkusii</i> Stem of <i>Macaranga</i> sp.
20 21-23 19		<i>Dendrobium crumenatum</i> Sw.	Stem of <i>Syzygium</i> sp. Stem of Species A Stem of <i>Macaranga</i> sp.
27-31 32-33 26		<i>Dendrobium plicatile</i> Lindl. <i>Nervilia concolor</i> (Blume) Schltr. <i>Arundina graminifolia</i> (D.Don) Hochr.	Rocks on limestone cliffs, not covered by a canopy. Dry soil near limestone cliffs and covered by a canopy Cliff with a height of ± 3 m above the ground, not covered by a canopy.
35		<i>Crepidium koordersii</i> (J.J.Sm.) Szlach.	Calcareous soil forest, on moist soil with leaf litters, covered by a canopy
42		<i>Crepidium</i> sp.	Limestone with a little soil on the rock surface, not covered by a canopy
34		<i>Arachnis flos-aeris</i> (L.) Rchb.f.	Dry soil near limestone cliffs and covered by a canopy

A total of 59 morpho-anatomical characters were used in this study. Morphological characters of root, stem, leaf, and flower organs were used. Anatomical characters of leaf were obtained from epidermal slide observation, comprising of stomatal type, stomatal occurrence location and presence of raphide crystal on epidermal cells. The result of morpho-anatomical characterization was used to construct this following artificial determination key.

Based on the similarity matrix, the phenetic relationship of wild orchids in Gunung Gajah can be observed in the dendrogram in Figure 2.

Key to species

1. a. Monopodial 2
b. Sympodial 3
2. a. Climbing vines; terrestrial roots only present during seedling; leaf apex acuminate and symmetry
..... *Vanilla planifolia*
b. Not a vine; terrestrial roots persistent; leaf apex obtuse and asymmetry *Arachnis flos-aeris*
3. a. Terrestrial 4
b. Epiphyte 9
4. a. Stem more than 1 m long; grass-like leaves, linear-shaped *Arundina graminifolia*
b. Stemless than 1 m 5
5. a. Tuberiferous stem; single leaf, cordate, dark green with purple spots *Nervilia concolor*
b. Stem not appear as tuber; multiple leaves 6
6. a. Tuberiferous root; no petiole; leaves succulent, dark green7
b. No tuber; short petiole; leaves thin, light green8
7. a. Leaves oblong, margin entire
..... *Peristylus goodyeroides*
b. Leaves lanceolate, margin wavy
..... *Habenaria reflexa*
8. a. Leaves oblong *Crepidium* sp.
b. Leaves ovate *Crepidium koordersii*
9. a. Stem flat and short, covered by equitant leaf arrangement *Oberonia similis*
b. Pseudobulbous stem 10
10. a. Pseudobulb ovoid to rounded 11
b. Pseudobulb fusiform 12
11. a. Pseudobulb dark green, furrowed; leaves thin, linear, apex symmetry, dark green; flower white with purple spots *Acriopsis liliifolia*
b. Pseudobulb light green, not furrowed; leaves succulent, lanceolate, apex asymmetry, light green; flower light green-yellow *Bryobium retusum*
12. a. Pseudobulb flat; single leaf, oval, light green; single flower, pale white with red dots
..... *Dendrobium plicatile*
b. Pseudobulb rounded; multiple leaves, oblong, dark green; raceme inflorescence; flower white, labellum yellow *Dendrobium crumenatum*

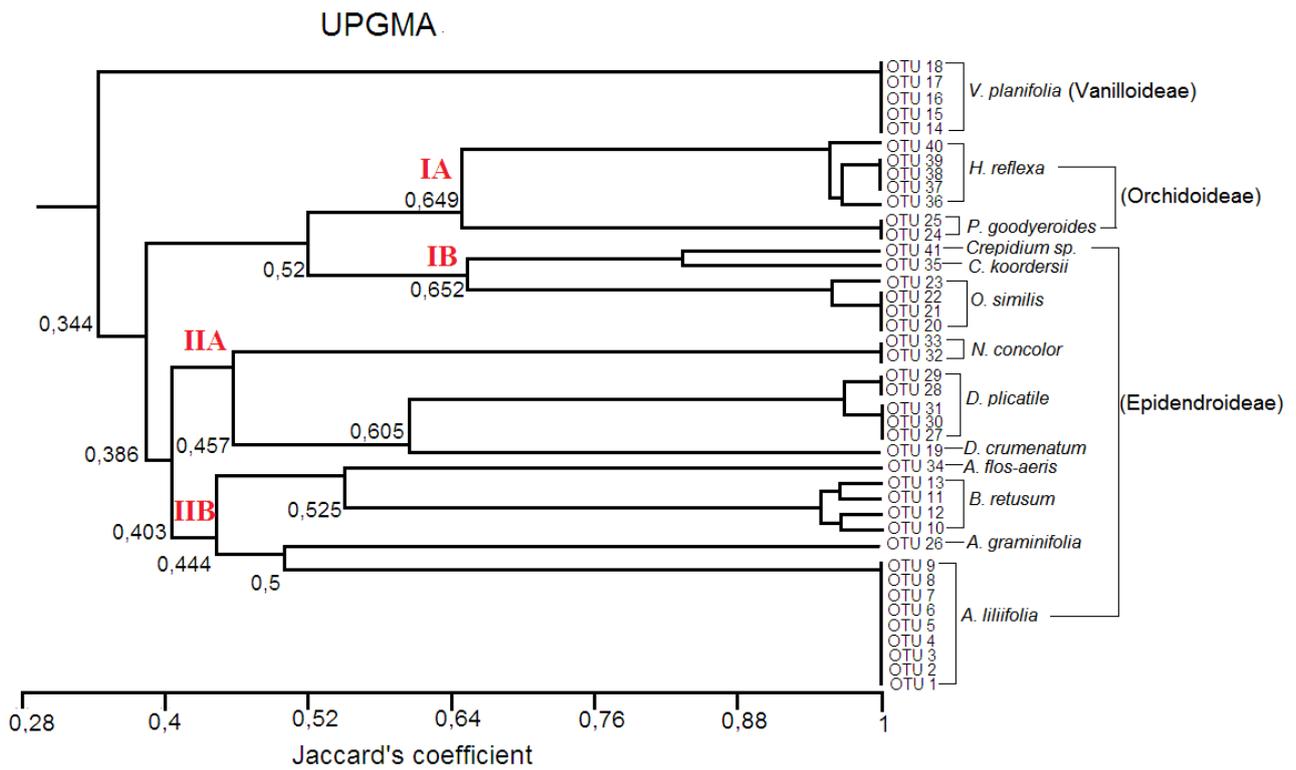


Figure 2. Phenetic relationship of wild orchids in Gunung Gajah, Purworejo, Indonesia

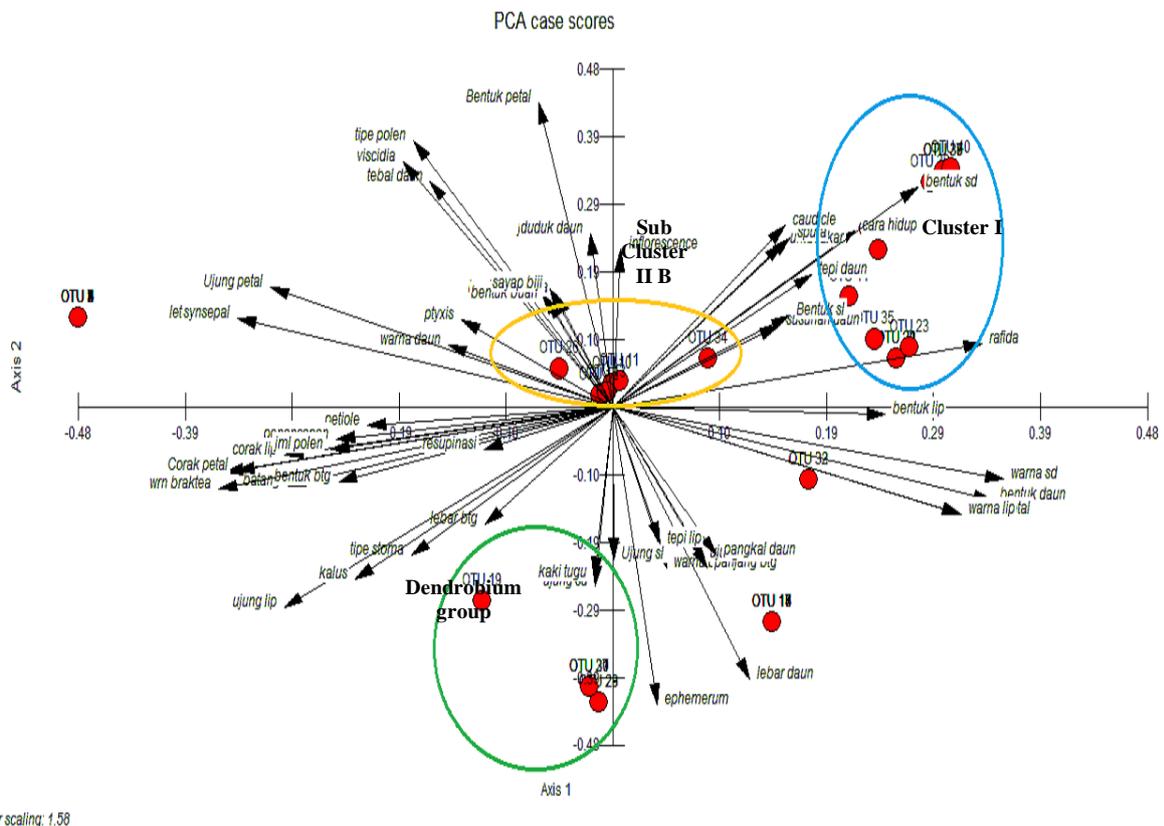


Figure 3. PCA diagram of wild orchids in Gunung Gajah, Purworejo, Indonesia

Table 2. Environmental parameters per wild orchid habitat

Habitat of	Air temp.	Soil hum.	Soil	Light intens.
	(°C)	(%)	pH	(lux)
<i>Acriopsis liliifolia</i>	24	-	-	1000
<i>Bryobium retusum</i>	24	-	-	500
<i>Vanilla planifolia</i>	24-25	-	-	300-800
<i>Dendrobium crumenatum</i>	25	-	-	800
<i>Oberonia similis</i>	24-26	-	-	800-1000
<i>Peristylus goodyeroides</i>	24	5.5	8	300
<i>Crepidium sp.</i>	29	-	-	1500
<i>Arundina graminifolia</i>	27	-	-	1000
<i>Dendrobium plicatile</i>	27	-	-	1000
<i>Nervilia concolor</i>	27	2.5	8	700
<i>Crepidium koordersii</i>	26	3.5	8	400
<i>Habenaria reflexa</i>	26	3	8	300
<i>Arachnis flos-aeris</i>	25	1.5	8	1000

Table 3. Host-tree attachment zone of Gunung Gajah, Purworejo, Indonesia epiphytic orchids

Host tree	Orchid species	Zone
<i>Cocos nucifera</i>	<i>A. liliifolia</i>	I
<i>Pinus merkusii</i>	<i>A. liliifolia</i>	I
<i>Macaranga sp.</i>	<i>B. retusum</i>	I, III
	<i>D. crumenatum</i>	I
	<i>V. planifolia</i>	I
	<i>V. planifolia</i>	I
<i>Theobroma cacao</i>	<i>V. planifolia</i>	I
<i>Albizia sinensis</i>	<i>V. planifolia</i>	I
<i>Syzygium sp.</i>	<i>O. similis</i>	I, III
	<i>V. planifolia</i>	I
	<i>D. crumenatum</i>	I, III
	<i>B. retusum</i>	I, III
Species A	<i>O. similis</i>	I, III
	<i>B. retusum</i>	I

Based on the dendrogram in Fig. 2, it was found that *V. planifolia* (OTU 14 - OTU 18) is separate from other groups. *V. planifolia* is the only member of subfamily Vanilloideae and morphologically differs from other species by the length of the stem (more than 2 meters) and its growth form as a vine/liana. Cluster I was composed of species from two different subfamilies. Subcluster IA consists of *P. goodyeroides* and *H. reflexa* (Orchidoideae) while subcluster IB includes *Crepidium sp.*, *C. koordersii* and *O. similis* (Epidendroideae). Cluster II entirely consists of species from subfamily Epidendroideae. Subcluster IIA includes *D. crumenatum*, *D. plicatile* and *N. concolor*. Subcluster IIB includes *A. flos-aeris*, *B. retusum*, *A. graminifolia*, and *A. liliifolia*.

Further, PCA method was done to determine the significant and relevant characters that play a role in clustering patterns. Specifically, PCA is aimed to identify character that determines the grouping of taxon groups tested (Quicke 1993). The result showed that the grouping of wild orchids using PCA analysis (Figure 3) was slightly different by cluster analysis (Fig 2). For instance, *A. liliifolia* which belonged to sub-cluster IIB (see Fig. 2) is separated from other species in PCA analysis due to the

presence of synsepal and amphistomatic stomata. These characters are unique to *A. liliifolia*. Other species of sub-cluster II B (shown by yellow circle in Fig. 3) grouping together due to the similarity of their leaves color, presence of seed wings, fruit shape, inflorescence type, stem growth and shape of bract. Based on Fig. 3, the species of sub-cluster IIA were separated into the *Dendrobium* group (green circle) in Quadrant III and *Nervilia* in Quadrant IV. The unique character of *Nervilia* is the cordate leaf shape while *Dendrobium* group has ephemeral flowers and presence of mentum. The species of cluster I (blue circle) was grouped in Quadrant I based on their shapes of dorsal sepals, growth habit, presence of caudicles, root tubers, leaf margin, lateral sepals shape, and presence of spura.

According to Purwanto et al. (2005), *Phalaenopsis* species form a cluster based on the similarity of the characters of stems, leaves, and flowers while the species of *Dendrobium* forms four different clusters due to variations in flower characters. Hidayati et al. (2016) conducted a phenetic analysis of 20 *Dendrobium* species using three different characters such as vegetative and flower characters (inflorescence, sepal and petal characters), which show similar grouping, while dendrogram based only on labellum character shows lower similarity coefficient result. In our study, it was not only the character of the flower and the labellum that play an important role in determining the grouping of the wild orchid species, but also the stem and leaf characters. It is likely due to the wild orchid species in this study were belonging to several genera, thus the vegetative characters are useful to distinguish between species. In other studies, the flower characters played an important role because the phenetic analysis was carried out on the same genus which having similarities in their vegetative characters. It can be seen through the artificial determination key that the thirteen orchid species from our study can be identified using vegetative characters such as habitus, stem characters, and leaf characters.

There were only epiphytic wild orchid species found in the pine forest area of Gunung Gajah, *A. liliifolia*. This is likely due to pine trees (*Pinus merkusii*) secrete resins from root, stem, and leaf organs as an allelopathy mechanism. The rare presence of wild orchids in pine forest areas is similar to the results of the research by Acharya et al. (2011) which stated that the number of epiphytic orchid species is reduced in forest areas that dominated by pine.

In our study, various environmental parameters were measured, i.e. air temperature, soil moisture, soil pH, and light intensity (Table 3). Environmental factors affect the diversity and distribution of wild orchid species. This is in accordance with Sadili (2013), who reported that some factors including light intensity, humidity, altitude, and associated vegetation will form a microclimate that will determine the survival of orchids. Here, the range of air temperature of orchid habitats was not too high since it was located at the same elevation. Besides air temperature, altitude is also related to atmospheric humidity. So it can be assumed that the atmospheric humidity of wild orchid habitats in Gunung Gajah was likely to be similar.

The soil moisture of terrestrial orchid habitats at study sites tends to be low. *A. flos-aeris* was recorded as the only species that grow in this calcareous soil. Research by Djordjević et al. (2016) showed that orchids were more likely to grow in alkaline or slightly acidic soils. Some orchid species prefer calcium-rich soils, or alkaline soils with high pH (Harrap and Harrap 2009). Calcareous soil is also known to be favored by fungi that form a symbiosis with orchids (Tremblay et al. 1998). This is due to orchid exalbuminous seed germination is facilitated with fungal symbiosis.

Light intensity in orchid habitat was quite varied. The wild orchids that grow in sites with the lowest light intensity were terrestrial wild orchids *P. goodyeroides*, *C. koordersii* and *H. reflexa*. Mostly, terrestrial orchids grow in low light intensity and under shade, except for a few species such as *Spathoglottis plicata* or *Arundina graminifolia* that require full light exposure (Sadili 2013). According to Yulia and Budiharta (2011), epiphytic orchids tend to grow on open canopy trees. Based on Table 2, it can understand that epiphytic orchid *A. liliifolia*, *O. similis*, *D. plicatile* and *D. crumenatum* grow in high light intensity.

Unlike the study by Setiaji et al. (2018), there was no holomycotrophic orchid in our study. Research by Muhaimin et al. (2017) showed that the preferred habitat for holomycotrophic orchids is moist and canopy-covered areas, for example in wet primary forests within mountainous areas and bamboo forests. From Table 2 we know that soil humidity in Gunung Gajah was quite low. In addition, the tree canopy especially in the forest area was not dense and the leaf litter was not thick, thus Gunung Gajah habitat is not suitable for holomycotrophic orchids.

Our result showed that five epiphytic orchid species grow on host trees. Johansson (1974) divided the epiphytic plant attachment zones on the host tree into 5 zones. Zone I is part of the stem with a height of 0-3 meters above ground level. Zone II is the trunk above 3 meters from ground level up to the first ramification. Zone III is the base of a large branch which covers 1/3 of the total length of the branch. Zone IV is the center of a large branch, covering 1/3 of the total length of the branch. Zone V is the tip of a large branch and covers 1/3 of the total length of the branch. Adhikari et al. (2016) stated there are some orchid species that can grow on various tree species and others only grow on certain trees because of their specific microclimate requirements. Based on Table 4, it can be known that those five epiphytic orchid species do not have any specific host tree species. The host tree *Syzygium* sp. and *Macaranga* sp. were the most preferred species because of their stem characters. Its stem with rough and cracked bark and are covered with a lot of moss will support the growth of orchids. The epiphytic orchid zonation on host tree was dominated by Zone I. Zone I tend to be stable and strong, not easily broken by wind. Rasmussen and Rasmussen (2018) stated that epiphytic orchids that grow in outer branches are short-lived because the outer branches are vulnerable to being separated from the main stem. According to Yulia and Budiharta (2011), Zone III is considered as the most suitable part for the growth of epiphytic orchids because orchid seeds are easily trapped

and the orchid will receive adequate water, nutrient, and light intake.

Based on our results and discussion, we conclude that the 13 wild orchid species in Gunung Gajah comprised of *Acriopsis liliifolia*, *Bryobium retusum*, *Vanilla planifolia*, *Dendrobium crumenatum*, *Oberonia similis*, *Peristylus goodyeroides*, *Dendrobium plicatile*, *Crepidium* sp., *Nervilia concolor*, *Arachnis flos-aeris*, *Habenaria reflexa*, *Crepidium koordersii* and *Arundina graminifolia*. Phenetic relationships of wild orchids in Gunung Gajah clustering into two groups, each divided into two sub-groups, except *V. planifolia* which separated from the other groups. PCA diagram shows that vegetative characters are more significant to determine the species into groups. The wild orchids in Gunung Gajah grow in alkaline soil conditions and warm environment. The terrestrial orchids in Gunung Gajah grow in humid and littered soil and low light intensity, whereas epiphytic orchids grow on host trees with rough and cracked bark.

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