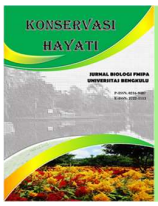

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Morphological and Physiological Adaptations of Venus Slipper (*Paphiopedilum*)

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ABSTRACT

Paphiopedilum, commonly known as venus slipper or lady slipper, is a genus of orchids that boasts over 70 species, most of which are critically endangered to endangered. These orchids are characterized by the presence of a sac on the flower that serves a unique function closely related to pollination. The pollination of Paphiopedilum is facilitated by bees, which are lured to the flower by producing pheromones such as €-β-farnese and β-pinene. Pouched orchids also produce compounds from the terpenoid group to synthesize a scent that attracts pollinators. Paphiopedilum orchids have evolved a fascinating mechanism to ensure successful pollination. They trap pollinators by making the pollination area slippery so that when the pollinator enters the area, it is likely to slip and try to get out along with the attached pollen. This unique mechanism ensures that the pollen is transferred effectively, increasing the chances of successful fertilization.

Keywords: *Cypripedium, Paphiopedilum, Pouched orchid*

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INTRODUCTION

Paphiopedilum Pfitzer belongs to the Orchidaceae family or orchid group (Zhang *et al*., 2018). The genus *Paphiopedilum* was discovered by Ernst Hugo Heinrich Pftzer in 1886, the name is taken from the Latin '*Paphos*' the name of the city of Cyprus which is the sacred place of Aphrodite, and the Greek *pedilon* which is 'slipper'. Sometimes *Paphiopedilum* is mixed up with its holo-arctic relative *Cypripediums* L. which is native to the Mediterranean region (Diengdoh *et. al*, 2022). *Cypripedium* can live in diverse climatic zones including temperate forests in Northeast Asia, North America, and Europe in contrast to *Paphiopedilum* which is distributed in tropical and subtropical forests in Southeast Asia (Pemberton, 2013).

More than 70 species of *Paphiopedilum* are known for their enormous, colorful, and distinctive blossoms, as well as their extended flower life. This plant often flowers in the spring or the fall once a year (Feng *et al*., 2021). Because *Paphiopedilum* has a distinctive sac on its foliage, it is also known as the lady slipper or venus slipper (Kemper, 2020). Two

extremely different petals, three dorsal sepals—one at the apex of the flower—and two joined lateral sepals make up the sac on *Paphiopedilum* flowers (Arwatchananukul *et al*., 2020). Here is one example of the morphology of the *Paphiopedilum* species that existed in the wild habitat of Papua, namely *P. papuanum* (Figure 1 and Figure 2).

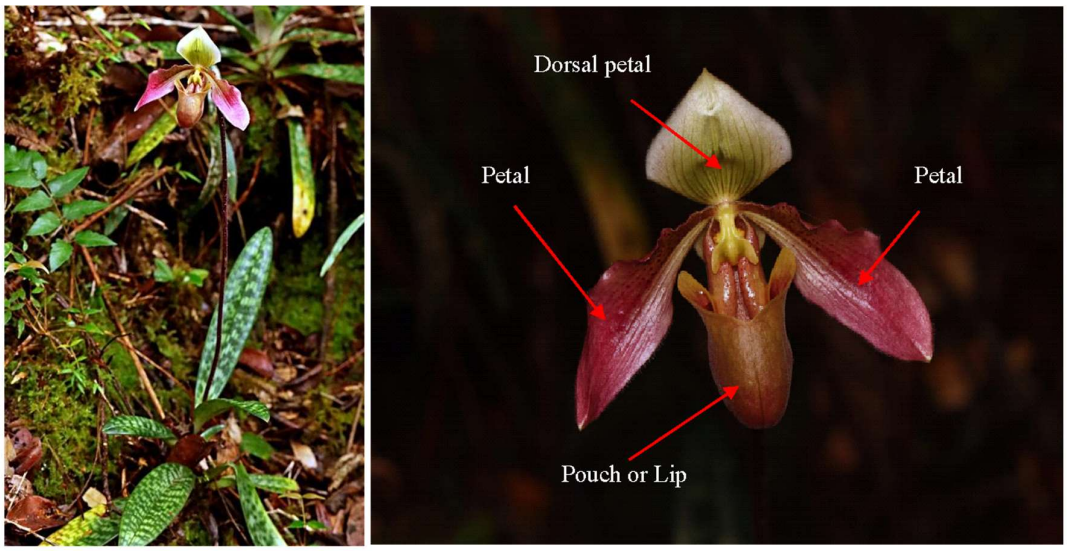

Figure 1. Morphology of *P. papuanum* (Ridl.) L.O. Williams in the wild habitat and its unique flower appearance (close up). Photographs by Reza Saputra (Gruss *et al.*, 2022)

Previous research in 2022 reported species of *Paphiopedilum, P. sandyanum* Cavestro, and G. Benk from Southeast Maluku Regency, Moluccas, Indonesia (Cavestro and Benk 2022) has morphology similarity with *P.papuanum* therefore it has been concluded that *P. sandyanum* is conspecific with *P. papuanum* (Ridl.) L.O.Williams (Gruss et al., 2022). Other studies discuss the conservation of *Paphiopedilum* both *in situ* and *ex-situ*. A review of IUCN red list categories data reveals that the majority of *Paphiopedilum* species are Critically Endangered (47.5%) and Endangered (43.4%). Furthermore, the majority of these species are overexploited. In addition, *P. rothschildianum* (Rchb.f) Stein in Kinabalu National Park, Malaysia (van der Ent *et al*., 2015), *P. fairrieanum* (Lindl.) in Bhutan (Samdrup *et al*., 2020) and *P. javanicum* in Gunung Lawu, Java, Indonesia (Romadlon *et al*., 2021) has been reported to exist, and need to be concern for their conservation. This study aims to explore the morphological, anatomical, and physiological adaptations of *Paphiopedilum*. This article might serve as a reference for further research in the fields of botany and conservation, as well as provide a scientific basis for studying the adaptations of the Venus slipper and its relevance to biodiversity conservation.

METHODS

The method employed in this study is the systematic scientific review method, which begins with topic determination, literature search, journal selection, interpretation of the results, and impact of the conclusions in the field (Ferrari, 2015). The method of scientific review. The writing was carried out by examining various authentic sources related to the morphological, anatomical, and physiological adaptation of *Paphiopedilum* and especially the pouch. Keywords of this review are "anggrek berkantung, slipper orchids, *Paphiopedilum,* and *Cypripedium*". To confirm the results of the literature review, we also made direct observations of *P. papuanum* (Ridl.) L.O. Williams flowers in their natural

habitat. The results of these observations are presented in the form of illustrations of the flower morphology.

RESULT AND DISCUSSION

3.1 Species List and Morphology of the *Paphiopedilum*

Paphiopedilum is known to have more than 70 species (Feng *et al.*, 2021). Synonyms of species names are based on the Royal Botanic Gardens Kew | Plants of the World Online website are mentioned in Table 1.

Table 1. Characteristics of *Paphiopedilum* and its pollinators

No	Species Name	Characteristic of the Pouch	Pollinators	References
1	*P. bellatulum* **Synonym**: *Cordula bellatula* (Rchb.f.) Rolfe; *Cypripedium bellatulum* Rchb.f.	The Pouch is white with scattered violet spots	Hoverflies: Syrphidae	Arwatchananukul (2020); Bänziger (2002)
2	*P. callosum* **Synonym:** *Cordula callosa* (Rchb.f.) Rolfe; *Cypripedium callosum* Rchb.f.	Red-purple pouch	Hoverflies: Syrphidae	Bänziger (2002); Arwatchananukul (2020)
3	*P. charlesworthii* **Synonym**: *Cordula charlesworthii* (Rolfe) Rolfe; *Cypripedium charlesworthii* Rolfe	Greenish-pale yellow-purple pouch	Hoverflies: Syrphidae	Bänziger (2002); Arwatchananukul (2020);
4	*P. godefroyae* **Synonym**: *Cordula godefroyae* (God.-Leb.) ; *Cypripedium concolor* var. godefroyae (God.-Leb.) ; *Cypripedium godefroyae* God.-Leb	The color of the pouch is white	Hoverflies: *Milesiine hoverflies* viz., *Eumerus nicobarensis* and E. *figurans*	Banziger *et al*. (2012); Arwatchananukul (2020)
5	*P. hirsutissimum* **Synonym:** *Cordula hirsutissima* (Lindl. ex Hook.) Rolfe; *Cypripedium hirsutissimum* Lindl. ex Hook.	Pouch with shades of green and brown	Hoverflies: *Allobaccha* sp.; *Episyrphus* sp.	Shi *et al*. (2009); Arwatchananukul (2020)
6	*P. parishii* **Synonym:** *Cordula parishii* (Rchb.f.) Rolfe; *Cypripedium parishii* Rchb.f.; *Selenipedium parishii* (Rchb.f.) Jolibois	Light pink or yellow-brown pouch	Hoverfly: *Allographa* sp.	Bänziger (2002); Arwatchananukul (2020)
7	*P. purpuratum* **Synonym:** *Cordula purpurata* (Lindl.); *Cypripedium purpuratum* Lindl.	Pale green, mottled, and veined purple pouch	Hoverfly: *Ischiodon* sp.	Liu *et al*. (2004); Arwatchananukul (2020)
8	*P. spicerianum* **Synonym:** -	Yellow-brown pouch	Hoverflies: *Allobaccha nubilipennis* and *Episyrphus balteatus* (main)	Arwatchananukul (2020); Liu *et al*. (2020)
9	*P. villosum* **Synonym:** -	Yellow-brown pouch	Hoverflies: *Betasyrphus* sp.; *Episyrphus* sp.; *Syrphus* sp.	Bänziger (1996); Arwatchananukul (2020)

Based on the observation, *P. papuanum* has pink petals, brownish sac, and greenish white dorsal petals. This species is terrestrial, similar to other *Paphiopedilum* members.

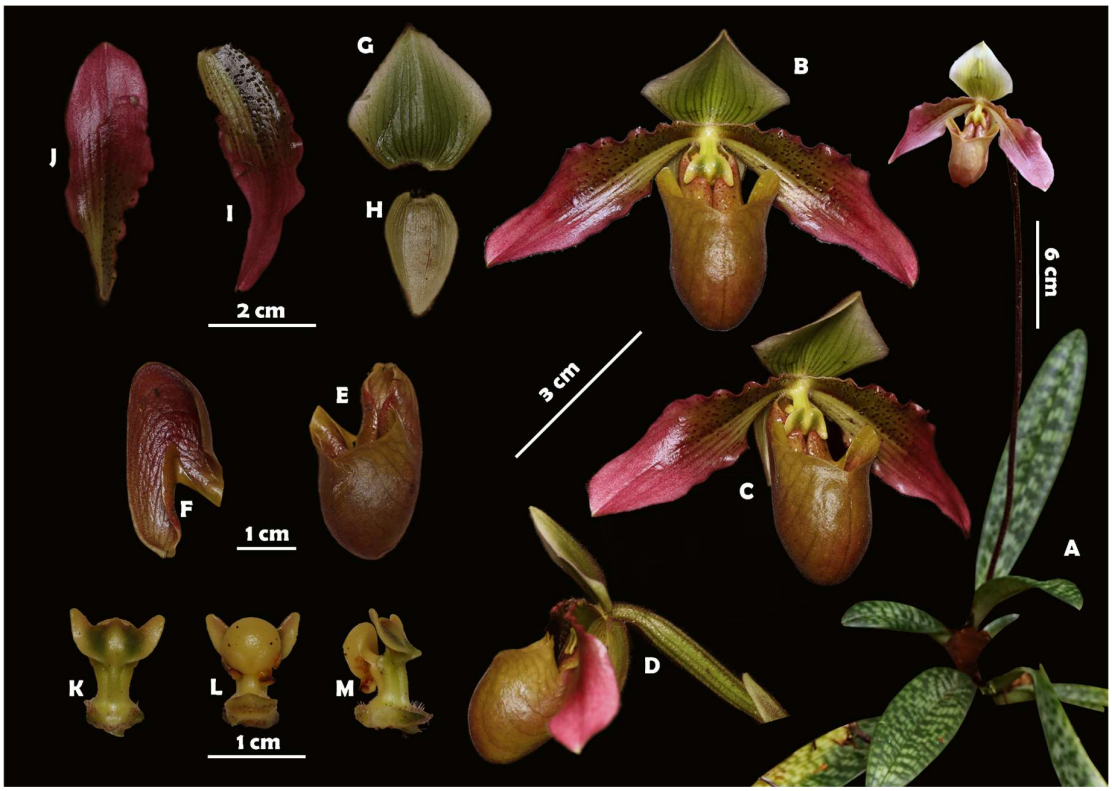

Figure 2. *Paphiopedilum papuanum* (Ridl.) L.O.Williams. A. Plant habit with inflorescence; B-D. Flower (front, oblique, side view); E-F. Lip (outside and inside view); G. Dorsal sepal; H. Synsepal; I-J. Petal (adaxial, abaxial); K-M. Column with staminode (front, dorsal, side view) (Photographs and compiled by Reza Saputra)

3.2 Mechanisms of Venus Slipper Trap Pollinator

The pollination processes of the venus slippers are aided by insects. It has been observed that the rate of pollination increases with the number of visits made by pollinating insects (Syari *et al.,* 2023). Venus slippers adapted to trap pollinators by mimicking their kind. One of the families Orchidaceae, *Cypripedium subtropicum* has a labellum that is concealed behind hairy, tufted structures and may produce pheromones to attract hoverflies. The pheromones are primarily €-β-farnesene and smaller amounts of β-pinene (Jiang *et al.,* 2020). Metabolite compounds related to flower scent that can attract pollinators are terpenoid groups. Generally, genes involved in aroma formation in orchid flowers include farnesyl diphosphate synthase (*FDPS),* acetyl-CoA C-acetyltransferase *(AACT),* hydroxymethylglutaryl-CoA reductase *(HMGR), linalool synthase (LIS),* 3-hydroxy-3-methylglutaryl-CoA synthase *(HMGS),* and jasmonic acid carboxyl methyltransferase (*JMT)* in methyl jasmonate (Zhang *et al*., 2022). Volatile compounds such as terpenoids are synthesized by orchids in general through two types of pathways, namely the MEP pathway and the MVA pathway. Phosphoenolpyruvate (PEP) and erythrose 4-phosphate (E4P) will be converted into pyruvate and glyceraldehyde-3-phosphate (G3P) to enter the MEP pathway. Dimethylallyl pyrophosphate (DMAPP) is reversible with isopentenyl pyrophosphate (IPP). Pyruvate and G3P are converted to geranylgeranyl pyrophosphate (GGPP) or geranyl pyrophosphate (GPP) and terpenoids such as monoterpenes and

diterpenes are obtained. Whereas in the MVA pathway, Acetyl-CoA will be converted into IPP, IPP is reversible into DMAPP then both are converted into farnesyl pyrophosphate synthase (FPPS), then into farnesyl pyrophosphate (FPP) which will become sesquiterpenes (Ramya *et al*., 2020). The following is an illustration of the biosynthesis of terpenoid compounds.

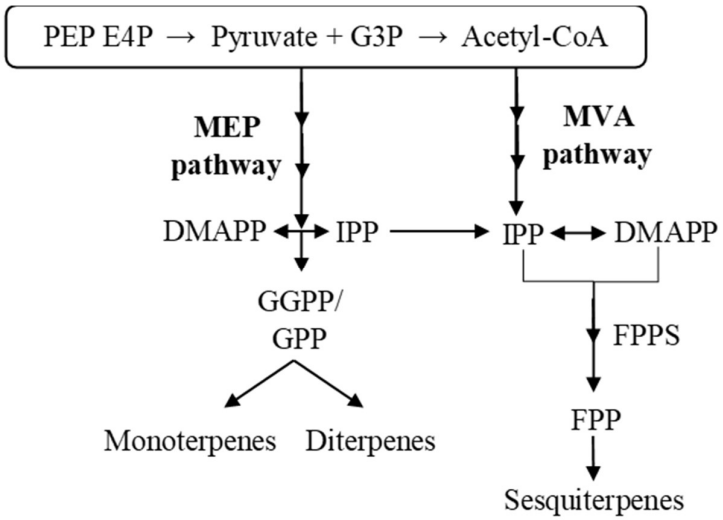

Figure 3. Biosynthesis terpenoids compound pathway. Abbreviations: MEP: methyl erythritol phosphate; MVA: mevalonic acid; PEP: phosphoenolpyruvate; G3P: glyceraldehyde-3-phosphate; E4P: erythrose 4-phosphate; DMAPP: dimethylallyl pyrophosphate; FPPS: farnesyl pyrophosphate synthase; FPP: farnesyl pyrophosphate; GGPP: geranylgeranyl pyrophosphate; GPP: geranyl pyrophosphate; IPP: isopentenyl pyrophosphate (Ramya *et. al*, 2020)

Venus or lady slipper would lure pollinators by food deception. Long-distance attraction is most likely by odor (Bänziger, 1996; Jiang *et al*., 2020), while close rage attraction other than mimicking pollinators is a slippery wart, lady slipper would make pollinators loosen the grip and tumble to the pouch and it would exit with stuck pollen on its body (Bänziger, 1996). *Cypripedium parviflorum* also imprisons pollinators temporarily, the pollinators will press the stigma to escape, making pollination easier and widening the escape hole (Case & Bradford, 2009). Micromorphology of floral such as glandular trichomes, papillae, and floral stomata has essential roles in fragrance and metabolite that are released to ensure pollinators revisit (Besi *et al*., 2021). For instance, *Paphiopedilum spicerianum* in China is listed as a Critically Endangered species that has been confirmed to have two distinct pollinators: *Allobaccha nubilipennis* (Austen) and *Episyrphus balteatus* (De Geer). The primary pollinator of *P. spicerianum* is *E. balteatus*, which exhibits higher visitation rates. Over time, *P. spicerianum* becomes the primary pollinator, resulting in the sharing of co-flowering with *Polygonum pubescens* (Blume), another nectar-rewarding species (Liu *et al*., 2020). Further research is needed on the synthesis of compounds that can attract pollinators, especially in *Paphiopedilum,* and more observations of the pollinators.

CONCLUSION

The morphological variations, particularly in the flowers encompassing structures, floral colors, floral scent, and lip or pouch shape are closely associated with the pollination method in venus slipper. The pouch of the Venus slipper is modified from labellum. The

color of the pouch varies between species. The existence of a pouch is proposed to attract pollinators by mimicking the pollinator's appearance and it releases pheromones such as β-farnese and β-pinene. The compounds that can be synthesized by orchids come from the terpenoid group which produce aromas that can attract pollinators. Trapping temporary pollinators is also an orchid's adaptation and the pollinators may escape after facilitating pollination or attaching with pollen.

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