

Extended distribution of three *Bulbophyllum* species (Orchidaceae) in India and some observations about their foliar anatomy^a

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Abstract

Bulbophyllum dissitiflorum Seidenfaden is reported for the first time for India and *Bulbophyllum appendiculatum* (Rolfe) J.J.Smith and *Bulbophyllum gamblei* (J.D.Hooker) J.D.Hooker are reported for the first time for the Indian State of Manipur. The three taxa are described in detail. Comments on their foliar anatomy, phylogeny and ecology are included and each species is illustrated by a photograph.

Résumé

Extension en Inde de la distribution de trois espèces de *Bulbophyllum* (Orchidaceae) et observations sur leur anatomie foliaire – *Bulbophyllum dissitiflorum* Seidenfaden est enregistré pour la première fois en Inde et *Bulbophyllum appendiculatum* (Rolfe) J.J.Smith ainsi que *Bulbophyllum gamblei* (J.D.Hooker) J.D.Hooker sont enregistrés, également pour la première fois, dans l'état indien de Manipur. Les trois espèces sont décrites en détail et illustrées de photographies. L'article propose aussi des commentaires sur leur anatomie foliaire, leur phylogénie et leur écologie.

Introduction

Bulbophyllum Thouars is one of the largest genera of the family Orchidaceae and comprises about 3,048 species (Govaerts *et al.*, 2014) which are

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distributed mainly throughout the tropical regions of both old and new worlds. A review of the literature (Hooker, 1890; Mukerjee, 1953; Pradhan, 1979; Kataki, 1986; Hyniewata *et al.*, 2000; Rao, 2007; Lucksom, 2007) reveals that the genus is represented by c. 100 species in India, of which about 75 species and 3 varieties are known from north-eastern India (Misra, 2007). About 29 species are found in the State of Manipur (Kumar & Kumar, 2005; Chowlu *et al.*, 2013; Chowlu *et al.*, 2014; Chowlu & Nageswara Rao, 2015).

Manipur, one of the biodiversity rich states of North East India, is located between 23° 68' N - 25° 68' N and 93° 03' E - 94° 78' E and covers a total area of about 22,327 km². The state is enclosed by lofty mountains, the highest being Mount Tenupi of Iso which is 2,999 m high.

During field explorations in various districts of Manipur, the authors could collect some interesting orchids including the three *Bulbophyllum* species discussed here.

Material and Methods

Specimens of the three taxa were collected and deposited as herbarium material in the centre for Orchid Gene Conservation of Eastern Himalayan Region, Hengbung, India for future study.

The identification of these taxa was based on a critical study of the relevant literature (Hooker, 1890; Mukerjee, 1953; Pradhan, 1979; Ghatak & Devi, 1986; Kataki, 1986; Deb, 1961; Shukla & Baishya, 1979; Seidenfaden, 1979; Seidenfaden, 1995; Hynniewata *et al.*, 2000; Chauhan, 2001; Pearce & Cribb, 2002; Lucksom, 2007; Misra, 2007; Chen *et al.*, 2009) and consultation of herbaria (ARUN, ASSAM & CAL).

Anatomical observations. Thin cross sections from the middle portion of matured leaves of seven plants of each *Bulbophyllum* species were obtained using a sharp razor blade and stained with safranine (Guan *et al.*, 2010). The sections were examined under a light microscope (Olympus CX31) using different magnifications. The adaxial and abaxial epidermises of matured leaves were peeled from fresh leaves for the observation of stomatal type, density, wax secreting cells etc. The area of stomatal apparatus was calculated by using the formula $\frac{1}{4} \times \pi \times l \times w$ (Guan *et al.*, 2010), where l=length and w=width and the stomatal density was calculated by following the formula: Number of stomata/Number of

grids × area of 1 grid square. All sections were photographed under the light microscope and digital images were manually analyzed with Adobe Photoshop.

Results

The taxa were identified as *Bulbophyllum appendiculatum* (Rolfe) J.J.Smith, *Bulbophyllum dissitiflorum* Seidenfaden and *Bulbophyllum gamblei* (J.D.Hooker) J.D.Hooker. Among them, *Bulbophyllum dissitiflorum* has hitherto been reported only from Thailand. *Bulbophyllum appendiculatum* and *B. gamblei* were hitherto known from India but not from Manipur. Hence the present report of occurrence of these species from Manipur forms an extended distribution.

The present communication provides their detailed description, foliar anatomy, comments about their phenology as well as photographic illustrations for future identification.

Taxonomic description

Bulbophyllum appendiculatum (Rolfe) J.J.Smith in *Bulletin du Jardin Botanique de Buitenzorg*, sér. 2 (8): 22 (1912)

Cirrhopetalum appendiculatum Rolfe in *Bulletin of Miscellaneous Information Kew* 148 (1901)

Mastigion appendiculatum (Rolfe) Garay, Hamer & Siegerist in *Nordic Journal of Botany* 14: 637 (1994)

Type: India, Sikkim, *Pantling* 197 (*holo* CAL!, *iso* BM K)

C. ornatissimum sensu J.D.Hooker in *Flora of British India* 5(2): 773 (1890) non Reichenbach f.

Roots generated from the base of the pseudobulbs; pseudobulb 2.0-2.2 × 0.8-1.1 cm, ribbed, rhizome 2 cm long; leaf solitary, 3.7-5.2 × 1.7-2.0 cm, oblong, obtuse, minutely notched at apex, thick; inflorescence single flowered, 6-7 cm long, from the base of the pseudobulb, peduncle sheathed at base; floral bract 1.2-1.5 × 0.5-0.6 cm, transparent, pedicellate ovary *c.* 1.5 cm long, creamy; inflorescence solitary; flowers 1.2-1.5 cm across, creamy with dark purple linings; dorsal sepal 1.2-1.5 × 0.7-0.9 cm, creamy, elliptic, acute-apiculate, with palea at the tip, 5 veined; lateral sepals 9.5-11 × 0.7-0.9 cm, oblong, acuminate, 5 veined; petals 1.2-1.5 × 0.5 mm, oblong-lanceolate, acuminate, 1 veined, with palea at the tip; lip from the tip

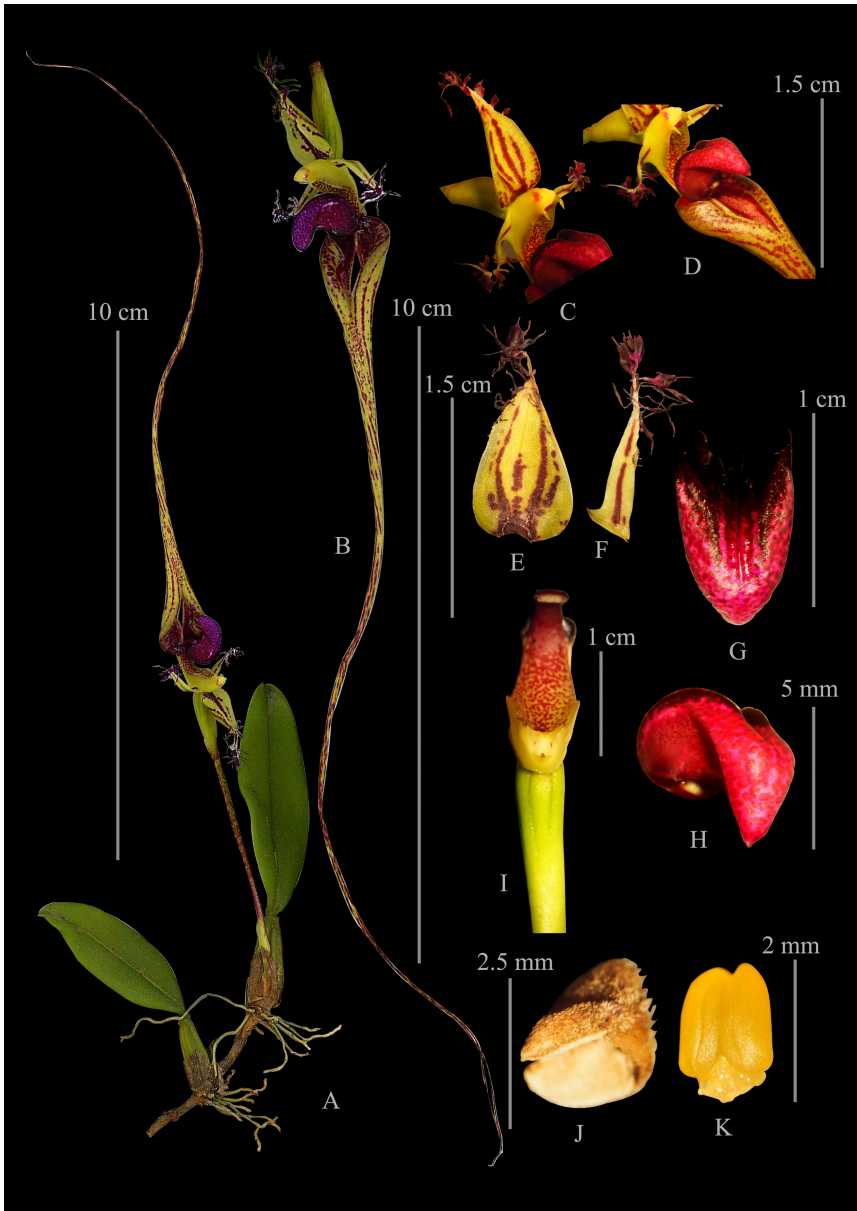


Plate 1. *Bulbophyllum appendiculatum*

A. Habit; B. Flower; C. Microscopic view of dorsal sepal, column and lip; D. Microscopic view of column and lip; E. Dorsal sepal; F. Petal; G. Lip (dorsal view); H. Lip (side view); I. Column with pedicel ovary; J. Anther cap; K. Pollinia. [Photographs by K. Chowlu]

of the column foot, 9-10 × 5-6 mm, dark purple, disc grooved and with 3 lamellae; column 3-4 mm long, creamy, foot 13-15 mm long, curved; anther cap *c.* 2.5 × 1.8 mm, brown, hairy; pollinia 4, *c.* 2 mm long, oblong, yellow (Plate 1).

Flowering: November-December

Ecology: Epiphyte in tropical mixed deciduous forest.

Specimen examined: India, Manipur, Senapati district, Ngatan, *Kipgen* 00569.

Distribution: India (Arunachal Pradesh, Manipur, Meghalaya, Sikkim) Malaya, Myanmar, Sumatra, Philippines.

Notes: This species is sometimes confused with *Bulbophyllum putidum* J.J.Smith and *Bulbophyllum fascinator* Rolfe but it can be easily identified by having a pinkish purple structure at the apex of the dorsal sepal which is called palea.

Bulbophyllum dissitiflorum Seidenfaden, *Dansk Botanisk Arkiv* 33 (3): 116 (1979)
Type: Thailand, Doi Suthep, GT 2785 (*holo.* C).

Plant epiphytic; rhizome 3-4 mm thick and 4-7 cm long, creeping; roots arising from nodes; pseudobulbs 3-5 × 1.5-2.5 cm, oblong, longitudinally 4-5 grooved or angled; leaf solitary, 4-12 × 1.5-3.0 cm, oblong-lanceolate, obtuse to shortly bilobed at apex, thick, tapered at base, petiole 0.8-1.5 cm long; inflorescence 5-13 cm long, racemose, lax or dense, arising from the base of the pseudobulbs, stout, peduncle 1.5-5 cm long, terete, thick, dark maroon, covered with sheaths all along or only towards the base, rachis drooping, laxly or densely many flowered, sheaths 0.8-1.5 cm long, lanceolate, acute, overlapping; floral bracts 4.5-5.5 × 1.2-1.5 mm, lanceolate, acute, membranous, brown, longer than the pedicellate ovary, pedicellate ovary 2.2-2.6 mm long, slender; flower 2-3 mm across, yellow with purple linings or spots; sepals sub equal, yellow spotted with purple, 3 nerved, dorsal sepal 4-5 × 1.5-1.6 mm, ovate-lanceolate, acute at apex, lateral sepals 6-7 × 1.5 mm, ovate, acute-acuminate, sub-falcate, shortly keeled at base, connate or free; petals *c.* 1.5 × 0.7 mm, triangular to narrowly elliptic, acuminate, 1 nerved, pale yellow with purple markings; lip 2.4-2.8 × 1.1-1.4 mm, simple, slightly recurved, slightly papillose on the surface, especially along edges, dorsal side with a furrow at the basal two thirds, ventral side somewhat

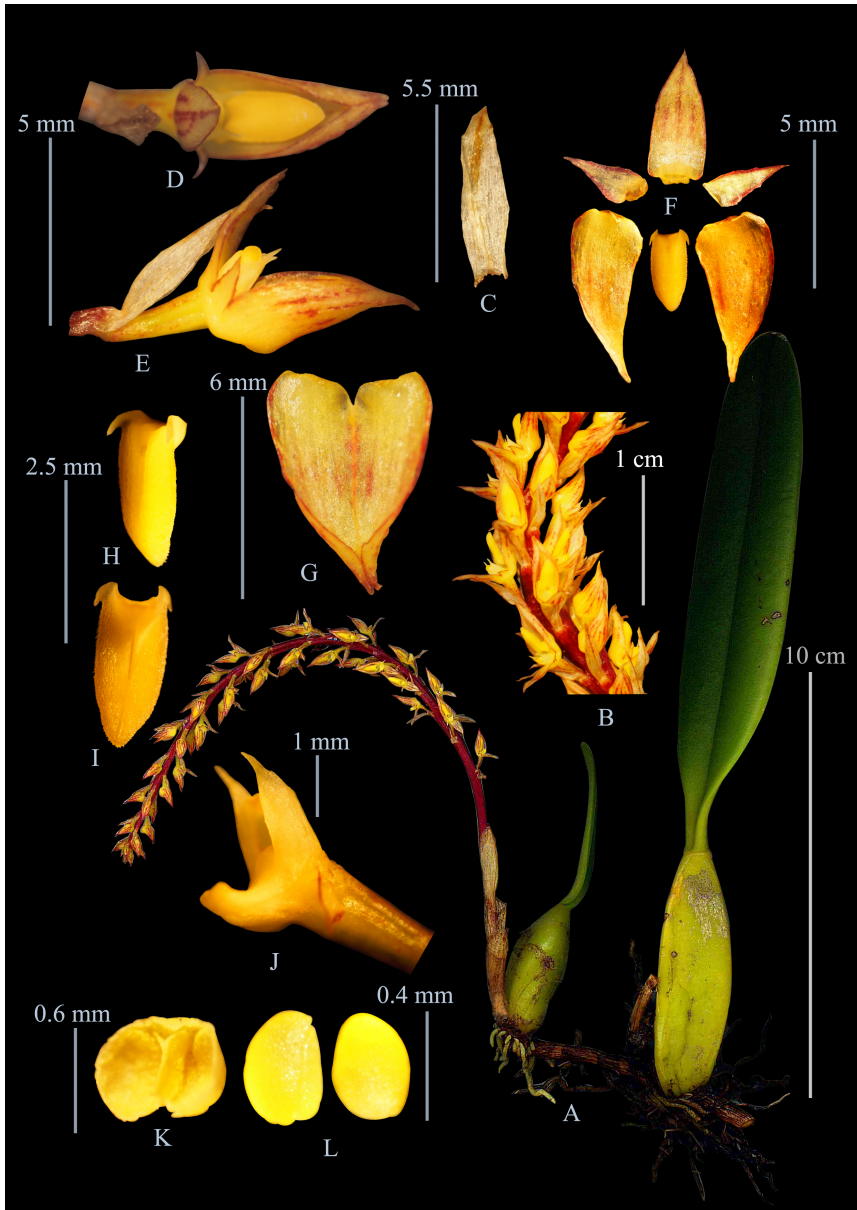


Plate 2. *Bulbophyllum dissitiflorum*

A. Habit; B. Inflorescence; C. Bract; D. Flower; E. Flower (side view); F. Dissected parts; G. Lateral sepals; H. Lip (side view); I. Lip (dorsal view); J. Column; K. Anther cap; L. Pollinia.
[Photographs by K. Chowlu]

deeper, bright yellow, 3 lobed, lateral lobes very small, c. 1 mm long, lanceolate, acuminate, pointing forwards, not very erose edges, mid lobe oblong, grooved above, margins entire, papillose; column 1.1-1.3 mm long, yellow, foot 1.5-1.6 mm long, curved, stylids triangular, acuminate; anther cap 0.6-0.7 × 0.8-0.9 mm, 2-chambered, yellow, pollinia 4, in two pairs, c. 0.4 mm long, oblong-ovate, orange (Plate 2).

Flowering: December-January.

Ecology: Found growing on oak tree branches along with other orchids and fern species.

Specimen examined: INDIA, Manipur, Chandel District, Lokchao, *Chowlu* 00568, 00571.

Distribution: India (Manipur), Thailand.

Note: Range of variation observed in populations: (1) dense or lax inflorescence, (2) peduncle with sheaths covered all the length or only at base and (3) lateral sepals connate or free (Plate 3).

Bulbophyllum gamblei (J.D.Hooker) J.D.Hooker, *Flora of British India* 6 (1): 188 (1890)

Bulbophyllum leptanthum var. *gamblei* J.D.Hooker, *Flora of British India* 5 (2): 759 (1890)

Phyllorchis gamblei (J.D.Hooker) Kuntze, *Revisio Generum Plantarum* 2: 677 (1891)

Type: India, Darjeeling, Goompahar (Ghumpahar), *Gamble* 783 (*holo.* K!).

Plant epiphytic or lithophytic; rhizome 2-3 mm thick, filiform, sheathless, branched; roots arising from the nodes; pseudobulbs 1-1.3 × 0.3-0.4 cm, subcylindric, inserted at an interval of 1.5-4 cm along rhizome; leaf solitary, 2-3 × 0.8-0.9 cm, oblong to oblong-elliptic, obtuse at apex, minutely retuse, thick, petiole 2-3 mm long; inflorescence 2-3 cm long, erect, umbellate, from the base of the pseudobulbs, 3-6 flowered, peduncle with 3 bracts, filiform, sheathed, basal sheaths bladeless, 1-2, tubular, lanceolate; floral bracts 2-3 × 1-1.5 mm, narrowly ovate, acute, pedicelate ovary 2-4 mm long; flowers 1-1.2 cm across, yellowish-green; sepals sub equal, 3 nerved, dorsal sepal 5-6 × 1.3-1.6 mm, lanceolate, acuminate, lateral sepals 5.3-7 × 1.5-2 mm, elliptic-lanceolate, acute-acuminate, joined above the base and widen at base; petals 2-2.5 × 1-1.5 mm, lanceolate-elliptic, acute, 1 nerved; lip 2-2.5 × 1.2-1.5 mm, simple, oblong-lanceolate, acute, base cordate, grooved centrally, minutely recurved; column broad, 0.4-1 mm, very short, yellow;

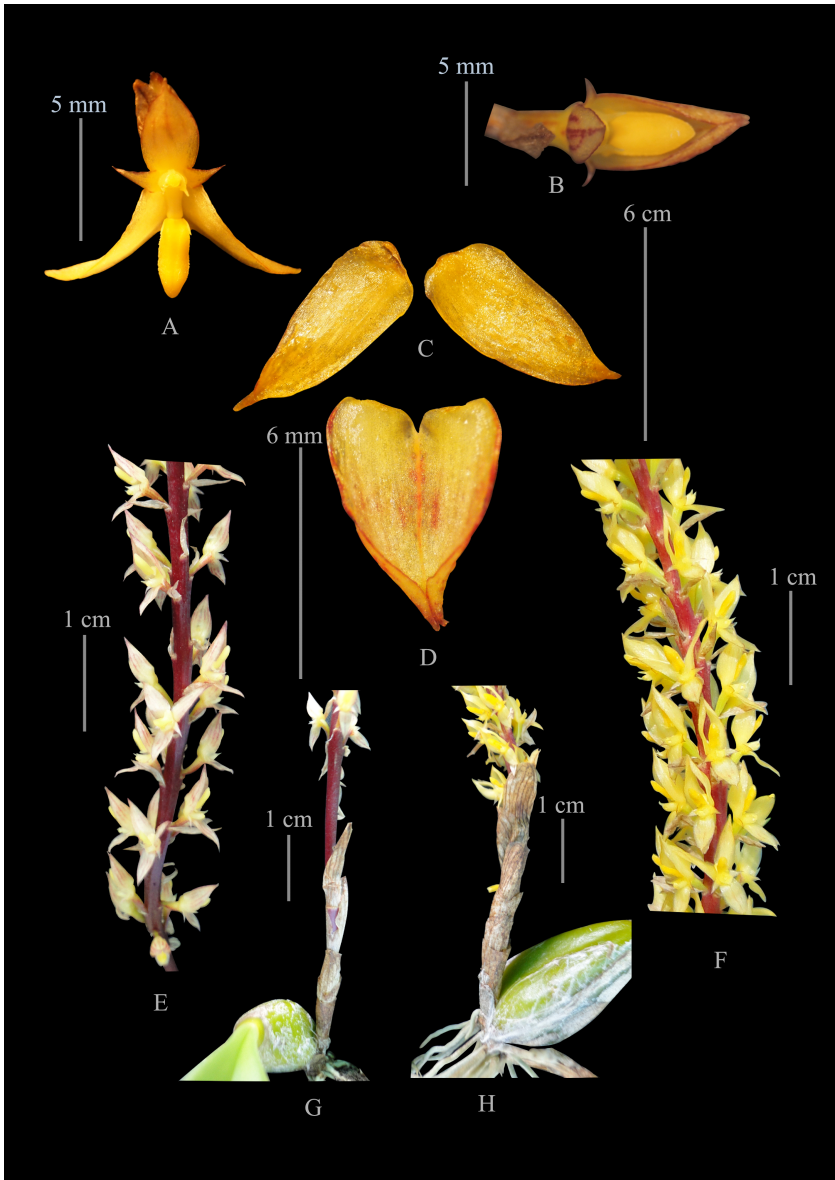


Plate 3. variations in *Bulbophyllum dissitiflorum*

A-B. Flowers showing the variations; C-D. Lateral sepals showing the connet and free sepals; E-F. Inflorescence showing lax and dense type; G-H. Peduncle sowing half and full covered. [Photographs by K. Chowlu]

column foot 1.5-2 mm long; anther cap $0.3-0.4 \times 0.4-0.4$ mm, light brown, pollinia 4, c. 0.3 mm long, minutely triangular-oblong (Plate 4).

Flowering: September-October.

Ecology: Found growing on the rock surface in temperate forest.

Specimen examined: INDIA, Manipur, Senapati District, Hengbung Kipgen 00547, 00548, 00549.

Distribution: India (Arunachal Pradesh, Manipur, Meghalaya, Nagaland, Sikkim), Nepal and Bangladesh.

Anatomical observations (see Table 1)

Leaves of *Bulbophyllum dissitiflorum* are thicker than *Bulbophyllum appendiculatum* and *Bulbophyllum gamblei*. Cuticle and epidermis are also slightly thicker in *Bulbophyllum dissitiflorum*. All the three species have polygonal epidermal cells. Mesophyll cells are differentiated into palisade and spongy tissues in *Bulbophyllum appendiculatum* and *Bulbophyllum dissitiflorum*, whereas it is not differentiated in *Bulbophyllum gamblei*. Mesophyll cells are also thicker in *Bulbophyllum dissitiflorum*. Middle vascular bundles are ovate in *Bulbophyllum dissitiflorum*, almost globular in *Bulbophyllum gamblei* and elongate in *Bulbophyllum appendiculatum*. The stomata are observed only on lower surface (hypostomatic) in all three species. *Bulbophyllum dissitiflorum* has floating stomata, whereas anisocytic type of stomata is observed in *Bulbophyllum gamblei* and *Bulbophyllum appendiculatum*. The shapes of the guard cells are sub-orbicular to orbicular in all the species. Stomatal apparatus area is larger in *Bulbophyllum dissitiflorum* but this species shows lower stomatal density. Stomatal density is observed to be maximum in *Bulbophyllum appendiculatum*. Multicellular glandular hairs and wax secreting cells on both adaxial and abaxial surfaces are observed in three species. Density of wax secreting cells is observed to be maximum in *Bulbophyllum appendiculatum* (1.25 mm^{-2}).

Discussion

The foliar anatomical study of three *Bulbophyllum* species may reflect adaptations to their habitat. Thickness of cuticle plays an important role for preventing water loss from the leaf interior (Bargel *et al.*, 2004; Mill & Stark Schilling, 2009). The presence of thick cuticles on the leaf surface is an indicator of aridity (Haworth & McElwain, 2008). The thick cuticles layers

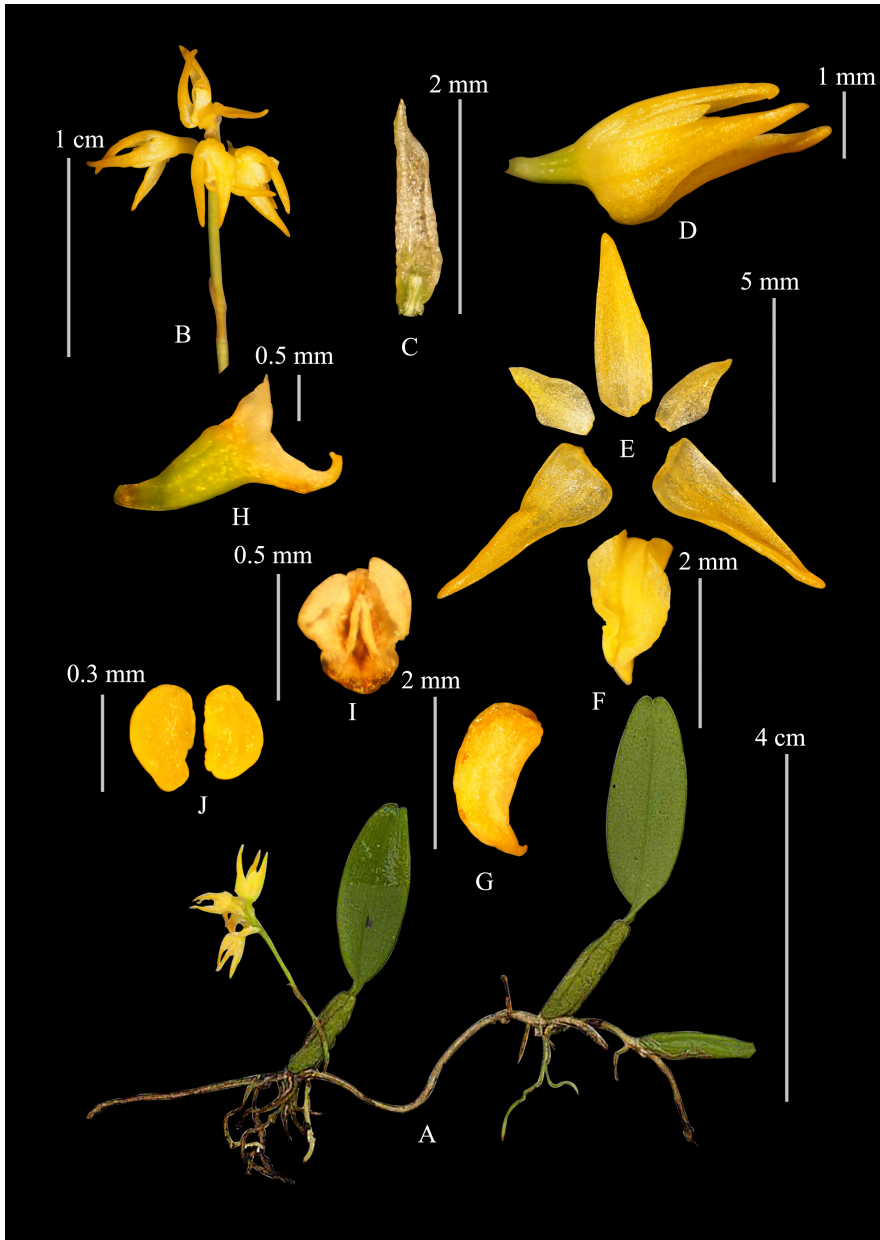


Plate 4. *Bulbophyllum gamblei*

A. Habit; B. Inflorescence; C. Bract; D. Flower; E. Dissected parts; F. Lip (dorsal view); G. Lip (side view); H. Column with pedicel ovary; I. Anther cap; J. Pollinia. [Photographs by K. Chowlu]

Species	LT	CT _{ad}	CT _{ab}	ET _{ad}	ET _{ab}
<i>Bulbophyllum</i>	(μm)	(μm)	(μm)	(μm)	(μm)
<i>appendiculatum</i>	1312 ± 15.5	14.5 ± 0.5	13.0 ± 0.5	36.5 ± 1.0	34.6 ± 0.8
<i>dissitiflorum</i>	1705.0 ± 18.6	15.8 ± 0.7	13.4 ± 0.5	38.6 ± 1.7	36.6 ± 1.5
<i>gamblei</i>	385.0 ± 5.7	13.5 ± 0.4	10.5 ± 0.3	35.0 ± 0.8	32.0 ± 0.6

Species	MT (μm)	VBML	VBMW	<i>d</i>	A _s
<i>Bulbophyllum</i>		(μm)	(μm)	(mm^{-2})	(μm^2)
		(middle)	(middle)		
<i>appendiculatum</i>	1225 ± 14.9	204 ± 15.5	112.5 ± 11.5	13.04 ± 0.7	769.38 ± 48.6
<i>dissitiflorum</i>	1606.0 \pm 14.9	337.9 ± 22.6	212.5 ± 18.6	05.06 ± 0.82	1777.08 ± 58.8
<i>gamblei</i>	286.8 ± 6.7	45.5 ± 1.8	42.5 ± 1.4	09.02 ± 0.4	602.8 ± 52.8

Table 1. Leaf anatomical traits
of *Bulbophyllum appendiculatum*, *B. dissitiflorum* and *B. gamblei*

LT: leaf thickness, CT_{ad}: adaxial cuticle thickness, CT_{ab}: abaxial cuticle thickness, ET_{ad}: adaxial epidermis thickness, ET_{ab}: abaxial epidermis thickness, MT: mesophyll thickness, VBML: length of middle vascular bundle, VBMW: width of middle vascular bundle, *d*: stomatal density, A_s: stomatal apparatus area. (Mean \pm SEM)

on *Bulbophyllum dissitiflorum* leaves can reduce rate of transpiration and increase water use efficiency during scarcity of water. Mesophyll cells also absorb sunlight to perform photosynthesis. The differentiated large mesophyll cells in *Bulbophyllum dissitiflorum* leaves can store more water than other species, thereby assisting this species in maintaining normal physiological metabolism at times of limited water availability (Guan *et al.*, 2010). Generally, the stomatal density of orchid leaves is low compared to the entire plant kingdom (Karasawa & Saito, 1982; Willmer & Fricker, 1996). Stomatal distribution, size, density and morphology are closely associated with plant transpiration (Willmer & Fricker, 1996). Plants with lower stomatal density are usually able to tolerate a more arid environment than plants with higher stomatal density (Kebede *et al.*, 1994). In the present study *Bulbophyllum dissitiflorum* with its least stomatal density appears to be more tolerant to the arid environment. Wax secreting cells act

not only as a form of sunscreen, but also aid in shedding rain so the leaf cells do not become over saturated with water and burst (Ferry, 2008). In this study *Bulbophyllum dissitiflorum* has maximum density of wax secreting cells which may protect the species from high intensity of sunlight and regulating the inner water content during heavy rain fall. This study of the foliar anatomical traits will help to understand their adaptive significance and importance in devising cultural conditions for successful conservation and also to find out some important anatomical characters for identification of taxa at species level besides usual morphological features (Plate 5).

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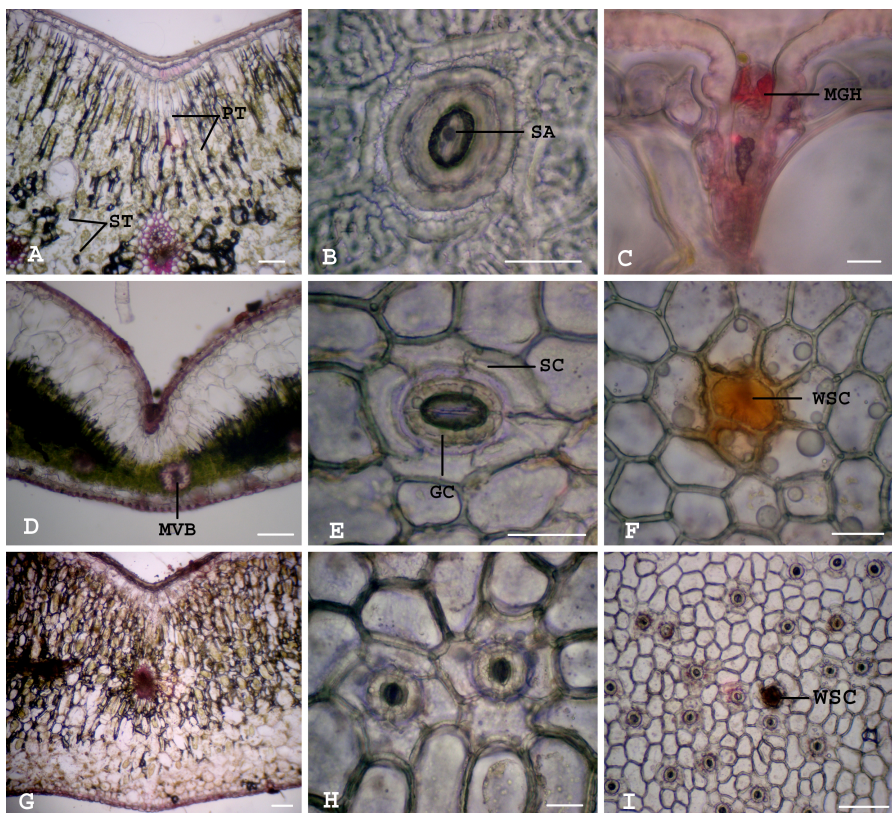


Plate 5. Leaf anatomical traits of
Bulbophyllum appendiculatum, *B. dissitiflorum* and *B. gamblei*
 under light microscope

A. Cross section of leaf of *Bulbophyllum dissitiflorum* showing distinctly differentiated mesophyll cells into palisade and spongy tissues, B. Floating type of stomata of *Bulbophyllum dissitiflorum*, C. Multicellular glandular hair of *Bulbophyllum dissitiflorum*, D. Cross section of leaf of *Bulbophyllum gamblei* showing undifferentiated mesophyll cells, E. Anisocytic type of stomata of *Bulbophyllum gamblei*, F. Wax secreting cells of *Bulbophyllum gamblei*, G. Cross section of leaf of *Bulbophyllum appendiculatum* showing slightly differentiated mesophyll cells, H. Anisocytic twin stomata of *Bulbophyllum appendiculatum* and I. Stomatal density observed to be maximum in *Bulbophyllum appendiculatum*.

GC guard cell, MGH multicellular glandular hair, MVB median vascular bundle, PT palisade tissue, SA stomatal aperture, SC subsidiary cell, ST spongy tissue, WSC wax secreting cell. Scale bars, A, D, G, I – 100 µm, B, C, E, F, H- 25 µm. [Photographs by Bishwajit].

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