

# Diversity in aerial root anatomy of *Bulbophyllum* (Orchidaceae) and its significance as source for subsidiary characters in species identification

Madhavi Singh<sup>1</sup>, Vimala Y.<sup>2</sup>, Lavania S.<sup>1\*</sup> & D. Verma<sup>3</sup>

<sup>1</sup>Department of Botany, University of Lucknow, Lucknow, Uttar Pradesh – 226 007, India

<sup>2</sup>Department of Botany, C.C.S. University, Meerut, Uttar Pradesh – 250 004, India

<sup>3</sup>Botanical Survey of India, Dehradun, Uttarakhand – 248 195, India

\*E-mail: lavaniaseshu@yahoo.co.in

**Abstract:** The morphology and anatomy of aerial roots of twelve epiphytic species of *Bulbophyllum* Thouars were investigated to analyse the interspecific variation and their potential for species differentiation and identification. Anatomical features of various structures such as velamen tissue, exodermis, cortex, endodermis, stele, thickening pattern, the occurrence of raphides, crystals, druses in the cortical region and types of tracheoidal (water storage) cells were examined. Tilosomes were lamellate in half of the species studied, while in *B. umbellatum* Lindl. they were spongy. The exodermal cells showed uniform cell wall thickening all around cells in all the species analysed except in *B. affine* Wall. ex. Lindl. and *B. striatum* (Griff.) Rchb.f. where they were inverted U-shaped. Raphides, druses and a few geometrically shaped calcium oxalate crystals were found in the cortical cells, while they were absent in *B. affine* and *B. umbellatum*. A positive correlation was found between stele diameter with root area and cortex thickness, number of xylem strands with root diameter and velamen thickness, velamen thickness with root diameter and stele diameter as in root cross sections. The variation in exodermis thickening, ideoblast cells, crystal forms, presence or absence of chloroplast cells in cortex, tilosome type as observed in the present study may be useful as potential distinguishing features and additional characters for taxonomic identification of *Bulbophyllum* species.

**Keywords:** Druse, Epiphytic, Exodermis, Tilosomes, Tracheoidal cells, Velamen.

## Introduction

The family Orchidaceae Juss. is a highly evolved plant family represented by 736 recognised genera (Chase *et al.*, 2015) and 25,000 species distributed throughout the world (except Antarctica) and showing a wide range of variation in its morphology and anatomy (Atwood, 1986; Dressler, 1993). Orchids are also known for their morphological and anatomical adaptations, which produce an array of unique characters for identification and classification (Pridgeon & Stern, 1982; Aybeke *et al.*, 2010; Fan *et al.*, 2014).

Among various genera, the genus *Bulbophyllum* Thouars has a pantropical distribution, and includes about 2,200 species inhabiting diverse climates across the world (Pridgeon *et al.*, 2014). Amongst them nearly 100 species are reported from India. In this genus, growth and survival of a plant is supported by a number of morphological and anatomical adaptations in the floral and vegetative parts including the roots (Micco & Aronne, 2012). The aerial root anatomy of *Bulbophyllum careyanum* (Hook.) Spreng. was first investigated by Chatin (1856). Subsequently, Oudemans (1861) described the epidermal layer and its position in aerial roots of *Bulbophyllum*. Afterward, Leitgeb (1864) reported single-layered velamen in *B. muscicola* Rchb.f., and Meinecke (1894) in *Bulbophyllinae* Schltr. Dressler (1981) pointed out the presence of a white coloured sheath that enclosed the root in most orchids and called it 'velamen radicum'. Later

Pridgeon (1987) defined velamen radicum as the outermost, spongy, single to multi-layered, thick walled, dead (at maturity) structure of aerial roots. Porembski and Barthlott (1988) also reported single-layered velamen in *Bulbophyllum*. Pridgeon *et al.* (1983) described the morphological types of tilosomes, protrusions or wall ingrowths in the form of branched lignified structures on the inner periclinal wall of the velamen (Stern, 2014).

Aybeke (2012) studied the taxonomic importance of anatomical characters of leaf, rhizome and root of various species of orchids for the identification of some taxa of the subfamilies Orchidoideae and Epidendroideae. Epiphytic plants grow on substrata like rocks, tree bark (at different heights on tall trees of different species), and their roots emerge from rhizomes that are exposed to light having intermittent access to water and minerals. Therefore, the aerial root morphology is entirely different from that of underground roots of orchids (Thangavelu & Kowsalya, 2017). As such, aerial roots of orchids have been shown to provide valuable anatomical characters for species diagnosis

in taxonomy (Singh, 1986; Piazza *et al.*, 2015). The present study was conducted to elucidate anatomical features of the aerial roots to identify the supplementary diagnostic characters that could complement the application of leaf epidermal characters in the context of species identification (Singh *et al.*, 2020).

## Materials and Methods

### Sample collection

Living plants of twelve epiphytic taxa of the genus *Bulbophyllum*, namely, *B. affine* Wall. ex. Lindl., *B. ambrosia* (Hance) Schltr., *B. bisetum* Lindl., *B. cauliflorum* Hook.f., *B. cherrapunjeense* Barbhuiya & D.Verma, *B. gymnopus* Hook.f., *B. leopardinum* (Wall.) Lindl. ex. Wall., *B. pteroglossum* Schltr., *B. reptans* (Lindl.) Lindl. ex. Wall., *B. striatum* (Griff.) Rchb.f., *B. sunipia* J.J.Verm., Schuit. & de Vogel, and *B. umbellatum* Lindl. were collected in the field from the north-eastern region of India representing a broad range of habitats located at varying heights on tree bark and rocky surfaces (Table 1). The plants

**Table 1.** Details of collection sites and altitude for the twelve species of *Bulbophyllum* used in the present study

Sl. No.	Species	Collection site	Altitude range (m)
1.	<i>B. affine</i> Wall. ex. Lindl.	Mukhaialong Community Reserve, Jaintia Hills, Meghalaya	100–1800
2.	<i>B. ambrosia</i> (Hance) Schltr.	Planted at Botanical Survey of India, Eastern Regional Centre Garden, Shillong (collected from Mizoram)	300–1300
3.	<i>B. bisetum</i> Lindl.	Cherapunjee, Khasi Hills, Meghalaya	1500–2000
4.	<i>B. cauliflorum</i> Hook.f.	Chyrmang Community Reserve, Jaintia Hills, Meghalaya	600–2000
5.	<i>B. cherrapunjeense</i> Barbhuiya & D.Verma	Cherapunjee, Khasi Hills, Meghalaya	1460
6.	<i>B. gymnopus</i> Hook.f.	Chyrmang Community Reserve, Jaintia Hills, Meghalaya	600–2000
7.	<i>B. leopardinum</i> (Wall.) Lindl. ex. Wall.	Mukhaialong Community Reserve, Jaintia Hills, Meghalaya	1300–3300
8.	<i>B. pteroglossum</i> Schltr.	Upper Shillong, Khasi Hills, Meghalaya	1000–2500
9.	<i>B. reptans</i> (Lindl.) Lindl. ex. Wall.	Tuber Community Reserve, Jaintia Hills, Meghalaya	300–1600
10.	<i>B. striatum</i> (Griff.) Rchb.f.	Tuber Community Reserve, Jaintia Hills, Meghalaya	1500–2330
11.	<i>B. sunipia</i> J.J.Verm., Schuit. & de Vogel	Chyrmang Community Reserve, Jaintia Hills, Meghalaya	900–2300
12.	<i>B. umbellatum</i> Lindl.	Upper Shillong, Khasi Hills, Meghalaya	1000–2200

were maintained at the Botanical Survey of India (BSI), Shillong (Meghalaya), for the purpose of further anatomical and morphological investigations. The collected taxa were also matched with the online herbaria on the World Checklist of the Royal Botanic Gardens, Kew (<https://wcsp.science.kew.org/qsearch.do>).

### Microscopic observations and data acquisition

Fresh aerial roots from each species were fixed in FAA, *i.e.*, formalin : acetic acid : ethanol 50% v/v (10 : 5 : 50), and then preserved in 70% ethanol according to Johansen (1940). Hand-cut transverse sections of fresh as well as FAA preserved roots were taken 1–2 cm from the base of the root. The sections were stained with 1% safranin in 70% alcohol and observed under a compound microscope. The number of cell layers in the velamen and the cortex and their thickness, thickening pattern of the exodermis and the endodermis and their thickness, morphology of tilosomes, number of xylem strands in vascular tissue, root and stele diameter, type of tissue in the stele, the occurrence of crystals and their types were determined. The thickness of the layers in root transverse sections was measured with a calibrated stage micrometer. From one plant per species, three mature roots were sectioned, except for *B. affine*, *B. ambrosia*, *B. cauliflorum* and *B. striatum*, where only one root was taken due to the paucity of root material available. For each root ten measurements were taken from random sections. Photomicrographs were taken on a H600L microscope (Nikon, Tokyo, Japan) fitted with a colour high-definition DS-Fi2 camera.

### Data analysis

Mean values and standard errors of the mean (SE) were calculated for the quantitative characters, and correlation coefficients (*r*) between selected anatomical character pairs, *i.e.* velamen thickness and number of xylem strands in the vascular cylinder, velamen thickness and root diameter, root diameter and number of xylem strands in the

vascular cylinder, stele diameter and root area, stele diameter and cortex thickness, were calculated using the Microsoft Excel ToolPak.

## Results

The results of qualitative observations and quantitative assessments on the morphology and anatomy of aerial roots of *Bulbophyllum* are summarized in Tables 2 and 3, and illustrated with photomicrograph evidence in figures 1 and 2, with some data also represented graphically using a bar chart (Fig. 3).

**External morphology of the roots:** The aerial roots were all grey, greenish-grey, or white in colour with a green apex. Root hairs were absent.

**Histological examination of the roots:** Transverse sections of the roots were mostly circular in outline and were composed of velamen tissue, exodermis, cortex and endodermis (Fig. 1a). The endodermis was single layered, separating the cortex from the vascular tissue. The diameter of the root and its cross-section area ranged from  $0.57 \pm 0.040$  mm and  $0.25 \pm 0.009$  mm<sup>2</sup> respectively in *B. striatum* to  $1.32 \pm 0.051$  mm and  $1.37 \pm 0.008$  mm<sup>2</sup> in *B. umbellatum*. The roots of half of the examined species *viz.*, *B. bisetum*, *B. cherrapunjeense*, *B. gymnopus*, *B. leopardinum*, *B. sunipia* and *B. umbellatum* were photosynthetic since the cortical cells had chloroplasts (Fig. 1c, e–g, k, l).

The thickness of the single outermost layer, the velamen, ranged from  $40.70 \pm 1.09$  µm to  $151.70 \pm 3.44$  µm (Table 3). This layer represented the smallest proportion of the root diameter in *B. cherrapunjeense* (13.1%) while largest in *B. gymnopus* (29.9%) (Fig. 3; Table 3). The cells of the velamen were commonly columnar, thick walled, stained with safranin (*i.e.* lignified) and devoid of nucleus and cytoplasmic inclusions, indicating that they were dead (Fig. 2a–h, k, l).

In all the species studied, an exodermis beneath the velamen layer was single layered with the thickness ranging between  $20.72 \pm 0.84$  and  $38.48 \pm 0.84$  µm. The thickest exodermis was recorded in *B. sunipia*

**Table 2.** Root morphological and anatomical features in twelve *Bulbophyllum* species from Northeast India

Sl. No.	Species	Tilosome morphology	Exodermal wall thickening (shape)	Number of cortical layers	Tracheoidal element thickening	Endodermal wall thickening (shape)	Number of xylem strands in stele	Stelar tissue	Crystal type
1	<i>B. affine</i> Wall. ex. Lindl.	Baculate	∩- shaped	7-8	Reticulate	O- shaped	8	Sclerenchymatous	Absent
2	<i>B. ambrosia</i> (Hance) Schltr.	Lamellate	O- shaped	3-4	Spiral	O- shaped	7	Sclerotic	Druses & Geometric crystals
3	<i>B. bisetum</i> Lindl.	Lamellate	O- shaped	4-5	Spiral	O- shaped	7	Sclerenchymatous	Raphides
4	<i>B. cauliflorum</i> Hook.f.	Lamellate	O- shaped	3-5	Spiral	O- shaped	10	Sclerotic	Raphides
5	<i>B. cherrapunjeense</i> Barbhuiya & D. Verma	Baculate	O- shaped	4-5	Absent	O- shaped	8	Sclerenchymatous (central)	Raphides
6	<i>B. gymnopus</i> Hook.f.	Baculate	O- shaped	6-7	Annular & Reticulate	O- shaped	12	Sclerenchymatous	Raphides
7	<i>B. leopardianum</i> (Wall.) Lindl. ex. Wall.	Baculate	O- shaped	6-7	Spiral (few)	O- shaped	15	Sclerenchymatous (highly thick)	Raphides, Druses
8	<i>B. pteroglossum</i> Schltr.	Baculate	O- shaped	3-4	Spiral	O- shaped	8	Sclerenchymatous	Raphides
9	<i>B. reptans</i> (Lindl.) Lindl. ex. Wall.	Lamellate	O- shaped	4-5	Annular	O- shaped	7	Sclerenchymatous	Raphides
10	<i>B. striatum</i> (Griff.) Rchb.f.	Lamellate	∩- shaped	5-6	Spiral	O- shaped	5	Sclerenchymatous	Geometric crystals
11	<i>B. sunipia</i> J.J.Verm., Schuit. & de Vogel	Lamellate	O- shaped	6-7	Annular & Reticulate	O- shaped	9	Sclerenchymatous	Raphides
12	<i>B. umbellatum</i> Lindl.	Spongy	O- shaped	7-8	Absent	O- shaped	14	Thick walled	Absent

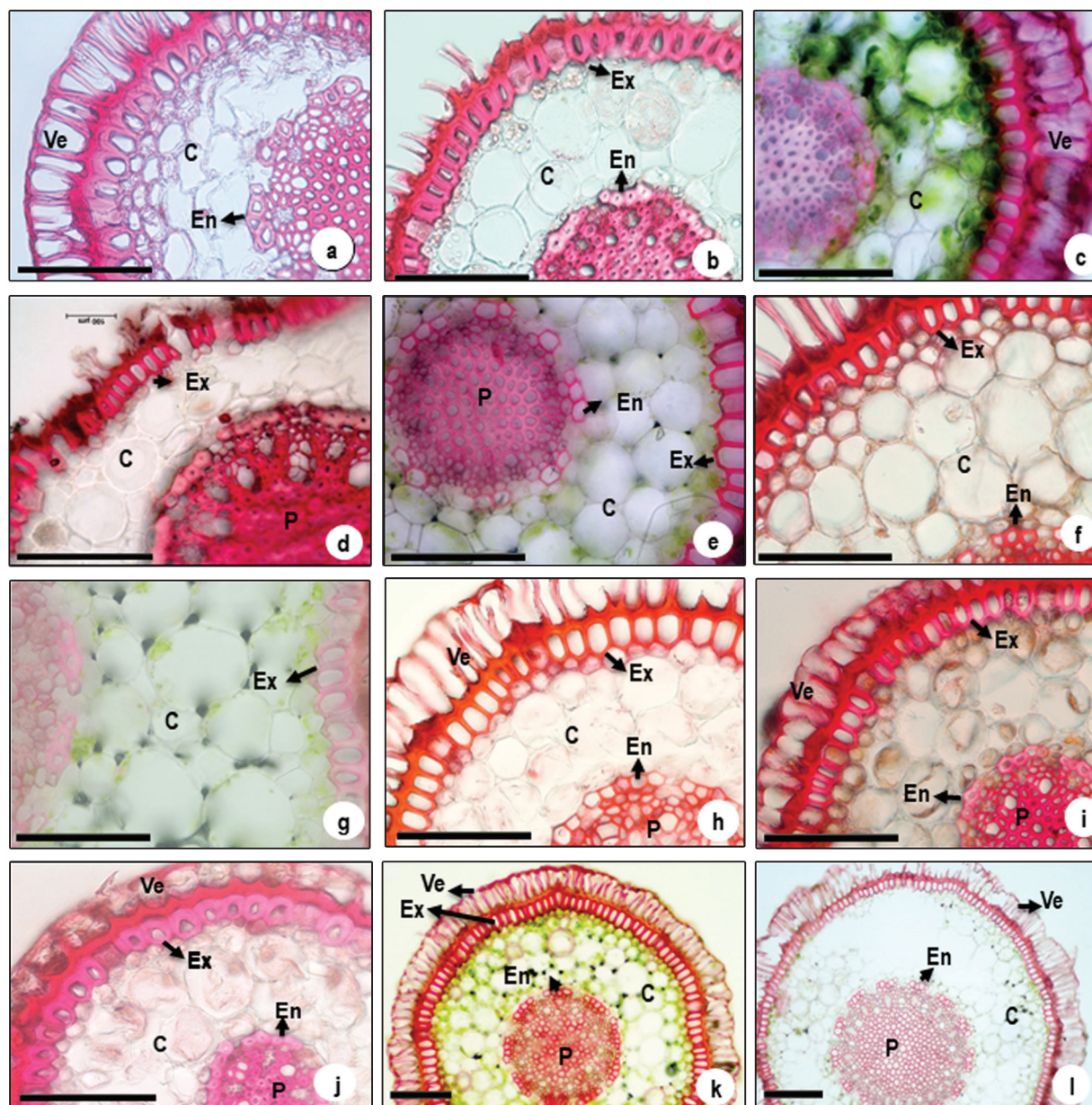
**Table 3.** Root characters and variation in their quantitative values represented as means, ± standard error of the mean (SE), in twelve *Bulbophyllum* species from Northeast India

S. No.	Species	Root diameter (mm)	Root cross section area (mm <sup>2</sup> )	Stele diameter (mm)	Cortex thickness (mm)	Velamen % of ring root diameter	Thickness as part of radius (µm)		
							Velamen	Exodermis	Endodermis
1	<i>B. affine</i> Wall. ex. Lindl.	0.76 ±0.010	0.45 ±0.012	0.20 ±0.003	0.16 ±0.005	17.9	68.08 ±1.75	34.78 ±0.84	17.02 ±0.84
2	<i>B. ambrosia</i> (Hance) Schltr.	0.77 ±0.040	0.46 ±0.023	0.16 ±0.001	0.18 ±0.002	18.1	69.66 ±3.34	25.16 ±1.68	26.64 ±0.68
3	<i>B. bisetum</i> Lindl.	0.58 ±0.031	0.27 ±0.001	0.16 ±0.002	0.11 ±0.005	17.8	51.80 ±1.54	29.60 ±1.54	16.28 ±0.64
4	<i>B. cauliflorum</i> Hook.f.	0.77 ±0.010	0.47 ±0.031	0.26 ±0.002	0.13 ±0.003	20.1	77.70 ±1.54	35.52 ±0.84	16.28 ±0.84
5	<i>B. cherrapunjeense</i> Barbhuiya & D.Verma	0.69 ±0.010	0.37 ±0.051	0.18 ±0.002	0.16 ±0.005	13.1	45.14 ±1.28	36.26 ±1.28	13.32 ±0.84
6	<i>B. gymnopus</i> Hook.f.	1.02 ±0.053	0.80 ±0.019	0.26 ±0.006	0.18 ±0.009	29.9	151.70 ±3.44	31.08 ±0.84	18.13 ±1.00
7	<i>B. leopardinum</i> (Wall.) Lindl. ex. Wall.	1.14 ±0.054	1.01 ±0.014	0.41 ±0.003	0.21 ±0.008	16.2	91.76 ±1.28	38.48 ±0.84	19.24 ±1.28
8	<i>B. pteroglossum</i> Schltr.	0.71 ±0.022	0.39 ±0.004	0.21 ±0.001	0.11 ±0.003	25.1	89.54 ±2.01	34.04 ±0.68	17.02 ±0.84
9	<i>B. reptans</i> (Lindl.) Lindl. ex. Wall.	0.59 ±0.025	0.27 ±0.006	0.13 ±0.002	0.14 ±0.003	16.0	47.36 ±1.28	28.86 ±0.68	13.38 ±1.42
10	<i>B. striatum</i> (Griff.) Rchb.f.	0.57 ±0.040	0.25 ±0.009	0.13 ±0.006	0.14 ±0.003	14.3	40.70 ±1.09	20.72 ±0.84	14.06 ±0.68
11	<i>B. sunipia</i> J.J.Verm., Schuit. & de Vogel	0.95 ±0.061	0.70 ±0.012	0.29 ±0.002	0.20 ±0.005	15.4	73.26 ±2.28	38.48 ±0.84	16.28 ±0.84
12	<i>B. umbellatum</i> Lindl.	1.32 ±0.051	1.37 ±0.008	0.49 ±0.003	0.23 ±0.003	18.7	123.58 ±1.75	35.52 ±1.75	26.64 ±1.68



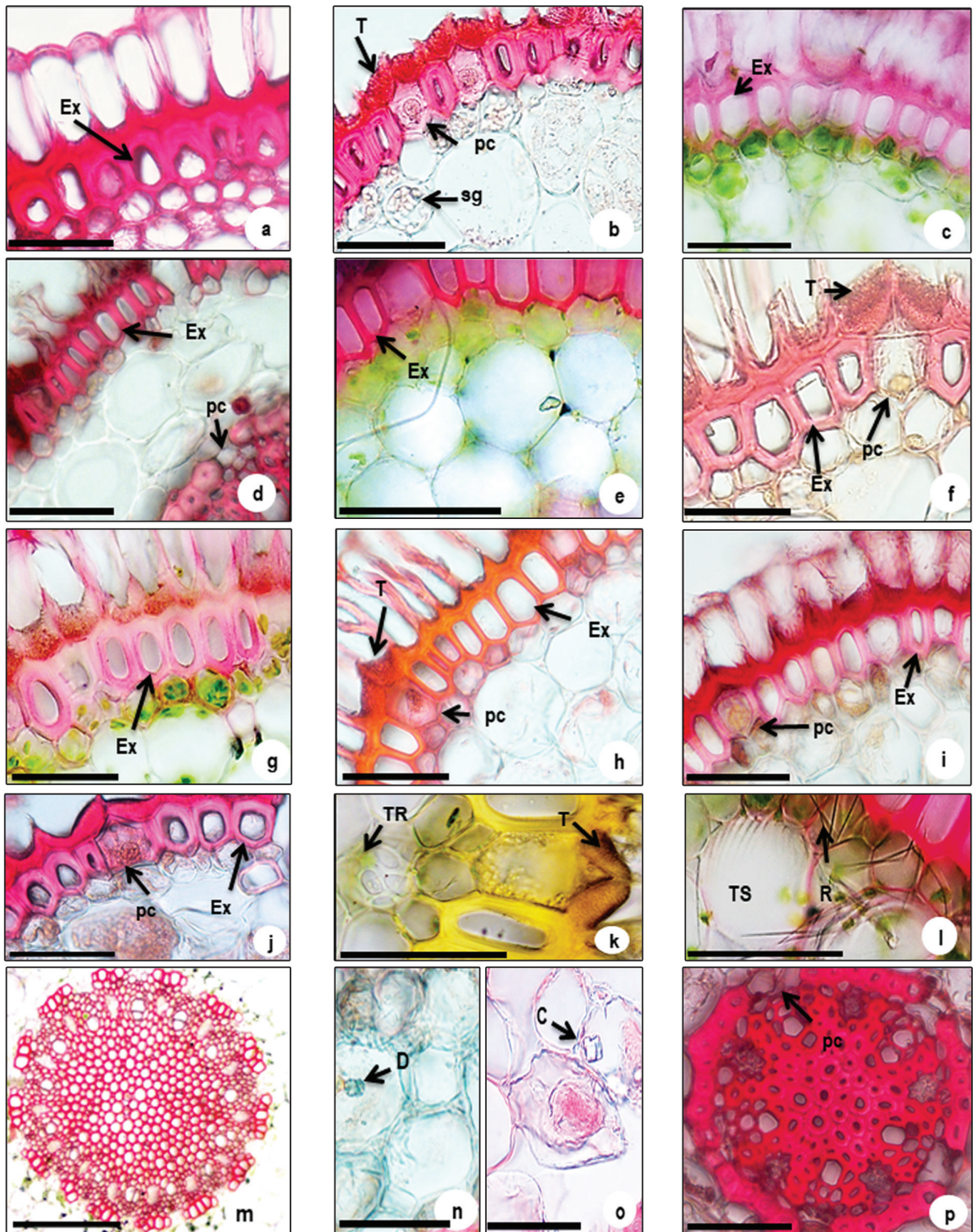
and *B. leopardinum* while it was thinnest in *B. striatum* (Table 3). The shape of the exodermal cells was polygonal (Fig. 1b–e, g–i, k, l), or radially elongated (Fig. 1a, f, j). Their cell wall thickening was uniform all around (O-shaped) in the roots of all the species (Fig. 2b–i, k; Table 3), except in *B. affine* and *B. striatum*. In those two species, wall thickening of the outer and radial wall of the

exodermal cells was greater compared to the inner walls, thus giving an appearance of an inverted U (*i.e.*,  $\cap$ -shaped) (Fig. 2a, j). There were a few cells occurring solitary or in pairs in both the exodermal and endodermal layers. Unlike the other cells, they were smaller, thin walled and had a large prominent densely stained nucleus, referred to by Haberlandt (1914) as passage cells (Fig. 2b, f, h, i, j). In the



**Fig 1.** Photomicrographs of root transverse sections of the species of *Bulbophyllum* Thouars showing layers, viz., velamen, exodermis, endodermis and pith: **a.** *B. affine* Wall. ex. Lindl.; **b.** *B. ambrosia* (Hance) Schltr.; **c.** *B. bisetum* Lindl., chlorenchyma in cortex; **d.** *B. cauliflorum* Hook.f.; **e.** *B. cherrapunjeense* Barbhuiya & D.Verma; **f.** *B. gymnopus* Hook.f.; **g.** *B. leopardinum* (Wall.) Lindl. ex. Wall., chlorenchyma in cortex; **h.** *B. pteroglossum* Schltr.; **i.** *B. reptans* (Lindl.) Lindl. ex. Wall.; **j.** *B. striatum* (Griff.) Rchb.f.; **k.** *B. sunipia* J.J.Verm., Schuit. & de Vogel, chlorenchyma in cortex; **l.** *B. umbellatum* Lindl., chlorenchyma in the cortex. Abbreviations: C = cortex; En = endodermis; Ex = exodermis; P = pith; Ve = velamen. Scale bars a – j = 100  $\mu$ m, k & l = 200  $\mu$ m.





**Fig. 2.** Photomicrographs of root transverse sections of the species of *Bulbophyllum* Thouars showing variations in morphological features: **a.** *B. affine* Wall. ex Lindl., outer and radial walls of exodermal cells thickened ( $\gamma$ -shaped); **b.** *B. ambrosia* (Hance) Schltr., uniformly thickened walls of exodermal cells, passage cell, starch grains, tilosomes; **c.** *B. bisetum* Lindl., exodermal cell wall thickened all over uniformly; **d.** *B. cauliformum* Hook.f., all over uniformly thickened exodermal cell; chlorenchymatous, circular cells in cortex; **e.** *B. cherrapunjeense* Barbhuiya & D.Verma, all over uniformly thickened exodermal cell; **f.** *B. gymnopus* Hook.f., all over uniformly thickened exodermal cell, passage cell, tilosomes; **g.** *B. leopardinum* (Wall.) Lindl. ex. Wall., all over uniformly thickened exodermal cell, passage cells; **h.** *B. pteroglossum* Schltr., all over uniformly thickened exodermal cell, passage cell, tilosomes; **i.** *B. reptans* (Lindl.) Lindl. ex. Wall., all over uniformly thickened exodermal cell, passage cells; **j.** *B. striatum* (Griff.) Rchb.f., outer and radial wall thickened ( $\gamma$ -shaped) exodermal cell, passage cell; **k.** *B. sunipia* J.J.Verm., Schuit. & de Vogel, all over uniformly thickened (O-shaped) exodermal cell, tilosome, tracheoidal idioblast with reticulate thickening; **l.** *B. sunipia* J.J.Verm., Schuit. & de Vogel, tracheoidal idioblasts spirally thickening, raphides; **m.** *B. umbellatum* Lindl., passage cell, sclerenchymatous stele; **n.** *B. reptans* (Lindl.) Lindl. ex. Wall. Druse in cortex of root transverse section; **o.** *B. striatum* (Griff.) Rchb. f., crystal; **p.** *B. striatum* (Griff.) Rchb.f. **Abbreviations:** C = crystal; D = druse; Ex = exodermis; pc = passage cell; R = raphide; sg = starch grain; T = tilosome; TR = tracheoidal idioblast with reticulate thickening; TS = tracheoidal idioblast with spiral thickening; ) - inverted 'U'. Scale bars a–l, n–p = 25  $\mu$ m, m = 500  $\mu$ m.

endodermis, the passage cells occurred precisely opposite the xylem conduits. Fibrous tilosomes were present on tangential walls of the passage cells in the exodermis facing the velamen. In the present investigation, three morphological types of tilosomes, viz., baculate, lamellate and spongy, were observed. The baculate type was observed in *B. affine*, *B. cherrapunjeense*, *B. gymnopus*, *B. leopardinum* and *B. pteroglossum* (Fig. 2f-h) while lamellate type was most common in half of the species such as *B. ambrosia*, *B. bisetum*, *B. cauliflorum*, *B. reptans*, *B. striatum*, and *B. sunipia* (Fig. 2 b-d, f-h, j, k). In contrast, the third type, i.e., spongy type, tilosomes were seen only in *B. umbellatum* (Table 2).

Across the examined species, the cortex was radially 3–8-celled thick, parenchymatous, in which the cells beneath the exodermis were small polygonal and sclerenchymatous (Fig. 1a, b, f, h, i, k), while in the lower cortex, they were circular to oval and / or polygonal (Fig. 2b, d, e, h). Specialized water storage cells, i.e., tracheoidal idioblasts, were also

observed in the cortex region. In all the species, they were circular, polygonal or sac like, with spiral or reticulate thickening, except in *B. cherrapunjeense*, where they were absent. In the cortex of *B. sunipia* the water storage cells had both reticulate as well as spiral thickening (Fig. 2k, l). A preliminary histochemical analysis also showed the presence of calcium oxalate crystals, raphides, and druses (Fig. 2l, n, o) in cortex cells except in *B. affine* and *B. umbellatum*, in which they were absent (Table 2).

The innermost part of root transverse section i.e., stele was circular and sclerotic. The stele of *B. leopardinum* and *B. umbellatum* proportionally occupied the largest part of the root diameter (36–37%) while it was smallest in *B. ambrosia* (21%). The cells of the pith were thick walled, lignified and sclerotic in *B. cauliflorum* and *B. ambrosia*. The pith region was irregularly sclerenchymatous in *B. cherrapunjeense* while it was uniformly sclerenchymatous in the rest of the species analysed here. *Bulbophyllum umbellatum* had slightly thick-

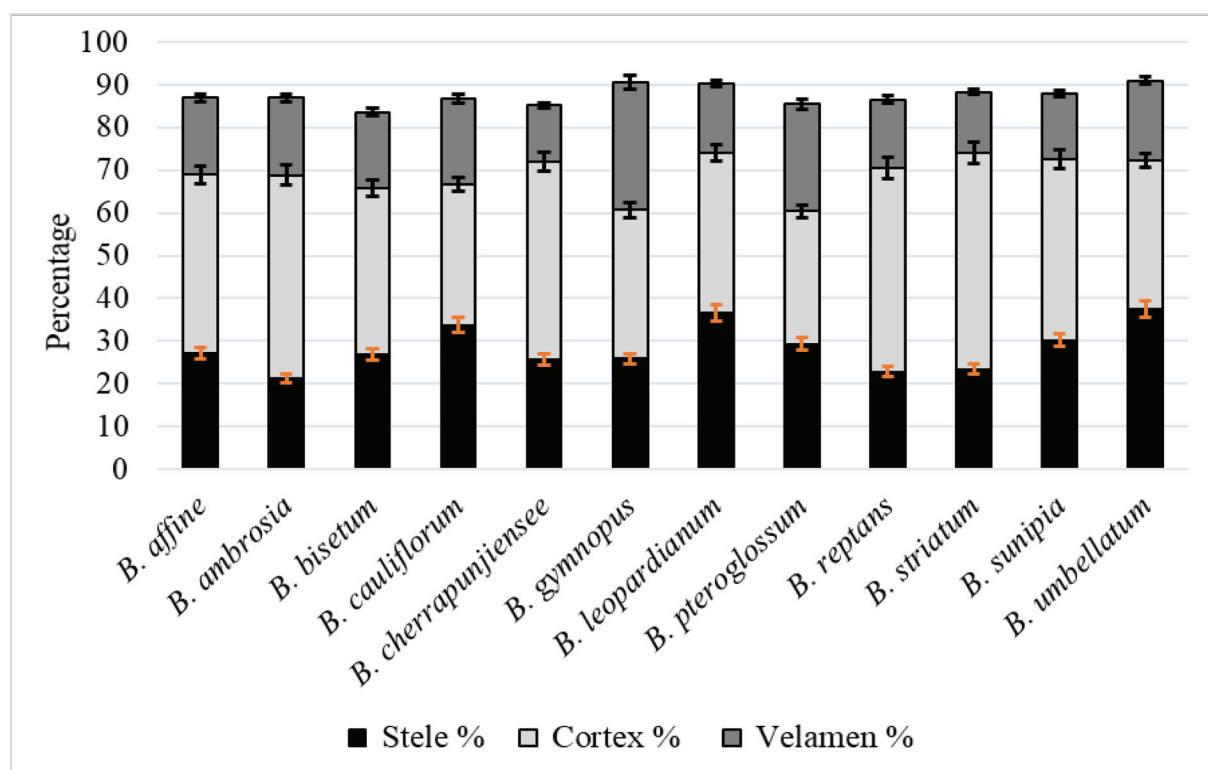


Fig. 3. Percentage of root thickness (diameter) occupied by velamen, cortex and stele in twelve species of *Bulbophyllum* Thouars from Northeast India.



walled sclerenchyma (Table 2). The endodermal cells were polygonal, barrel shaped with uniformly thickened walls. They were moderately to highly thick-walled in *B. bisetum*, *B. cherrapunjeense*, *B. gymnopus*, *B. pteroglossum*, *B. sunipia* and *B. umbellatum* or sclerenchymatous in the remaining species (Fig. 1a-l). The endodermis was uniseriate, thick walled, lignified and separate the cortex from the stele in all the species. These cells were thin walled opposite to all xylem sectors of the vascular cylinder. The stele was polyarch, with xylem and phloem arranged at different radii. The number of xylem strands ranged between 5 and 15.

Statistically, a positive correlation was found between velamen thickness and number of xylem strands in the vascular cylinder ( $r = 0.7145$ ,  $p = 0.020243$ ), velamen thickness and root diameter ( $r = 0.7513$ ,  $p = 0.03164$ , root diameter and number of xylem strands in the vascular cylinder ( $r = 0.9198$ ,  $p = 0.000164$ ), stele diameter and root area ( $r = 0.9625$ ,  $p = 0.00001$ ) and stele diameter and cortex thickness ( $r = 0.7511$ ,  $p = 0.012296$ ).

## Discussion

The root of all species of *Bulbophyllum* possessed single cell-layered velamen. This outermost layer of dead cells is a specialized epidermis in Orchidaceae (Pridgeon, 1987) and was first described by Oudemans (1861) in *Bulbophyllum*. This layer was also reported in several other monocot families such as Amaryllidaceae, Araceae, Commelinaceae, Dioscoreaceae and Taccaceae (Dahlgren & Clifford, 1982). According to an earlier study (Went, 1940), it was believed that the velamen principally absorbs rainwater laden with nutrients that flows down the surface of trees. In contrast, Dycus and Knudson (1957) described that the dead cells of velamen were impermeable to water and provide mechanical support. However, in several later studies it was indicated that the velamen facilitated water absorption (Moreira & Isaias, 2008; Muthukumar & Shenbagam, 2018). There are reports that thick-walled exodermal cells are impermeable to water and are said to be the

characteristic of orchids growing in drier habitats (Dycus & Knudson, 1957; Sanford & Adanlawo, 1973). In several studies the velamen was described as a structure that prevents water loss through the roots (Pridgeon, 1987; Benzing, 1996; Lüttge, 2008).

The cell wall thickening of the exodermis was uniform all around the cell wall (giving an appearance O-shape) in all the species examined here but *B. affine* and *B. striatum* showed that the outer and radial walls were thickened (∩-shaped). Stern (2014) also reported similar results and mentioned the predominance of uniform thickening in the genus *Bulbophyllum*. The exodermal thickness was found to be moderate to highly thickened in the species examined in present study, as also observed by Porembski and Barthlott (1988). The species like *B. affine*, *B. cauliflorum*, *B. ambrosia*, *B. striatum*, *B. leopardinum* and *B. pteroglossum* could be assumed to be better adapted to dry conditions by preventing water loss from interior root tissues as the thick walled exodermal cells are impermeable to water.

According to Haberlandt (1914) the 1–2 distinguishable cells in the exodermis of the aerial roots of orchids are referred to as passage cells. These passage cells may facilitate uptake of water and minerals flowing down along rocks or bark of trees during and after rainfall. Similar passage cells were also recorded in the present study. Presumably such cells may be playing important role in the supply of nutrients to each layer of root. The fibrous tilosomes reported in the present study on the tangential walls of the passage cells (facing the velamen) could possibly enhance the carrier activity of the passage cells.

Three morpho-typic forms of tilosomes were detected in *Bulbophyllum* species viz., baculate, lamellate and spongy, which were among the seven morphological types of tilosomes identified in orchids as reported by Pridgeon *et al.* (1983) who also reported their importance as taxonomic marker (Pridgeon, 1987). Scanning electron microscopy details are warranted, which could help to

substantiate this feature in the investigated species. The available literature indicates that tilosomes act as a water holding structures that help in minimizing the water loss through passage cells (Haberlandt, 1914). It has been shown that tilosomes assist in the condensation of water vapour and other gases such as oxygen and carbon dioxide (Pridgeon, 1987). Figueroa *et al.* (2008) proposed that the tilosome morphotypes could be indicators of environmental conditions wherein various orchids may be growing and adapted for successful completion of their life cycle. The observations recorded here show two distinct cortex types; either thin with just 3–5 cell layered with various types of thickening pattern and inclusions as a potential storage resource or possessing chloroplast, demonstrating photosynthetic activity. Therefore, histomorphological organization of the cortex could be of some help in species identification. The specialized water storage cells referred to as ‘tracheoidal idioblasts’ (Foster, 1956) are characteristic to the epiphytic orchids that play an important role in providing mechanical support and protect them from desiccation (Olatunji & Nengim, 1980; Kaushik, 1983; Porembski & Barthlott, 1988). Porembski and Barthlott (1988) listed two types of idioblastic cells in the cortex: (i) sac-like cells have different wall thickening patterns and pores, (ii) cells containing raphide-bundles or druses. Tracheoidal idioblasts are absent in *B. cherrapunjeense*, whereas *B. sunipia* showed the presence of reticulate and spiral thickening. Prychid and Rudall (1999) concluded that druses and raphides are the most common types of calcium oxalate crystals in monocotyledons and their presence is also reported by earlier researchers in Orchidaceae (Smith, 1923; Sandoval, 1993; Pridgeon, 1994; Stern, 1997). Druses have been reported in *Dendrobium* Sw. (Carlsward *et al.*, 1997). Crystals in the roots of *Bulbophyllum* are of great importance in their occurrence, distribution and type, *i.e.* druse, raphides and other geometric crystals in the cortex of a particular species (Piazza *et al.*, 2015). The accumulation of such ergastic substances is considered to be genetically controlled (Franceschi

& Nakata, 2005). Therefore, they may be considered consistent features from a taxonomic point of view.

The endodermis was sclerenchymatous with a considerable variation in the wall thickness. A sclerenchymatous pericycle with presence of passage cells opposite to the xylem strands was considered to facilitate water movement (Pridgeon *et al.*, 2014). The stele was sclerenchymatous in all the *Bulbophyllum* species studied here.

## Conclusion

The root characters of twelve *Bulbophyllum* species from Northeast India exhibited several identification features such as the presence of uneven thickening of exodermis cells in *B. affine* and *B. striatum* and absence or presence of particular type of crystals in the cortical cells in *B. affine* and *B. umbellatum*. The occurrence of raphides in the cortex was found to be a very common feature, whereas geometric crystals were rare and occurred only in *B. ambrosia* and *B. striatum*, which could be used as a diagnostic feature. The root anatomical characters *viz.*, tilosome type, crystal type, cell wall thickening patterns described in the present study may be used as species distinguishing features. In the future, scanning electron microscopic (SEM) details of tilosomes might help to explore their taxonomic significance. The interspecific variation in the root morphological characters could assist in the identification of species such as *B. gymnopus*, *B. leopardinum*, and *B. umbellatum*. However, the sample of 12 species is at present too small to draw more far-reaching conclusions for the genus until more species are investigated. The application of histochemical techniques will help to develop anatomical tools for the characterization of species.

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