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FOLIAR ANATOMY IN SOME SPECIES OF *BULBOPHYLLUM* THOU.

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Abstract

The present paper deals with foliar anatomy of ten species of *Bulbophyllum i.e., Bulbophyllum affine, B. careyanum, B. polyrhizum, B. reptans, B. retusiusculum, B. rufinum, B. scabratum, B. secundum, B. trichocephalum,* and *B. xylophyllum* from NorthEast India. The aim has been to understand the inter-specific leaf anatomical variations within the genus *Bulbophyllum* and their divergent adaptations. The leaves varied in thickness from 332.5 *µ*m (*B. reptans*) to 4180 *µ*m (*B. xylophyllum*) Cuticle was most well developed in *B. xylophyllum* (31.25 *µ*m) and least so in *B. scabratum* (7.26 *µ*m). Epidermis thickness was maximum in *B. polyrhizum* (121.25 *µ*m) and minimum in *B. secundum* (17.66 *µ*m). Water storage cells were present in all the species. The leaves were invariably hypostomatic with polygonal epidermal cells. The most common stomatal type observed was floating type. Guard cells were cuticularised in *B. retusiusculum*, *B. secundum* and *B. trichocephalum.* The stomatal apparatus area was largest in *B. affine* (3996.63 *µ*m²) and smallest in *B. xylophyllum* (145.23 *µ*m²). Stomatal density was highest in *B. secundum* (51.15 mm^{-2}) and lowest in *B.affine* (6 mm^{-2}). These data suggest the drought tolerant nature of all the presently studied species. An attempt has also been made for the formation of identification key, based on anatomical features, with a view to helping in their identification under vegetative state.

Introduction

BULBOPHYLLUM IS an epiphytic genus of orchids with nearly 1900 species distributed all over the world. In India, it is represented by 80 species (Misra, 2007), majority of which are NorthEast Indian in distribution. The genus exhibits wide distribution amplitude extending from tropical to temperate countries. It stands characterised by having creeping rhizomes with pseudobulbs that carry one or two leaves, rarely three. Dunbar-Co *et al*. (2009) observed that the effect of environment on plants and their adaptation to the environment is exhibited by leaf traits. The presence of xeromorphic features in plants is an indication of aridity (Haworth and McElwain, 2008).

Owing to the great variation and heterogeneity of shapes (morphology), variations in the anatomy are also expected. In the present paper, leaf and dermal anatomy of ten species of the genus *Bulbophyllum* Thou. *i.e.*, *Bulbophyllum affine* Lindl., *B. careyanum* (Hook.) Spreng., *B. polyrhizum* Lindl., *B. reptans* (Lindl.) Lindl., *B. retusiusculum* Reichb. f., *B. rufinum* Reichb. f., *B. scabratum* Reichb. f., *B. secundum* Hook. f., *B. trichocephalum* (Schltr.) Tang & Wang and *B. xylophyllum* Par. & Reichb. f. were studied with a view to understanding their significance in devising cultural tactics under green house conditions. An identification key based on anatomical features for easy identification under vegetative condition was also prepared.

Materials and Methods

Plant Material

The plants for the present study were collected from various districts of Manipur (Table 1) and grown in the Orchidarium of the Centre for Orchid Gene Conservation of Eastern Himalayan Region at Hengbung. Approximately after a year, the flowering plants were critically studied for taxonomical and anatomical characters.

Preparation of Leaf Sections

For histological observations, mature leaves were collected and free hand transverse sections were made with a sharp razor blade. The thin sections were stained with safranin and mounted on glass slides. The sections were examined and photographed under × 100 and × 400 of a light microscope (Model-CX31, Olympus Corp. Japan). Selected parameters like thickness of cuticle, epidermis, mesophyll and leaf were measured at the midpoint of each transverse section with a standardised ocular micrometer scale.

The adaxial and abaxial epidermis of middle leaf parts of mature leaves were peeled from fresh leaves and photographed under × 100 and × 400 of a light

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microscope. Digital images were manually analysed with Adobe Photoshop 7.0. The length and width of stomata were recorded. Stomatal apparatus area (A_s) and stomatal density (d) were also recorded. Stomatal apparatus area (A_s) was calculated using,

A_s = ¼ x ð x l x w (Shelley and David, 2001)
Stomatal density (d) was also calculated using the standard formula,

d = Number of stomata in one grid/Number of grids × Area of one grid square.

For leaf histological observations, 10 leaves from 10 different individuals were examined for each species.

Statistical Analysis

Statistical analysis was carried out using One way Analysis of Variance (ANOVA) followed by Duncan's Post Hoc test. Results were presented as a value ± standard deviation (SD). Significant levels were defined at $p < 0.05$ as analyzed by ANOVA.

Results

Foliar Anatomy (Fig.1A-E)

The leaf section in all the species studied were V-shaped in outline. Cuticle was present on both the adaxial and abaxial surfaces and it was smooth in all the species except that of in *B. polyrhizum* and *B. reptans* where, it was slightly ridged. Adaxial cuticle

Table 1. Ecological traits in presently studied species of *Bulbophyllum*.

	Source			
Species	Locality	Altitude (m)	Flowering	Distribution in India
Bulbophyllum affine	Tamenglong (Dailong, Longku)	1029 –1179	June	Eastern and Western Himalayas
	Senapati (Willong Khunou, Sadim Pukhri)	800–1485		
	Ukhrul (Kamjong)	1598		
B. careyanum	Tamenglong (Longku, Longchum, Dailong Rangan)	403–1422	Feb-March	Arunachal Pradesh, Meghalaya, Sikkim,
	Senapati (Sadim Pukhri)	1485		Manipur, Uttar Pradesh and West Bengal
	Chandel (Kwatha)	470		
B. polyrhizum	Chandel (Kwatha) Ukhrul (Kamjong)	402 1480	April	Sikkim, Darjeeling, Manipur and Western Himalaya
B. reptans	Tamenglong (Longku)	1303	Oct-Dec	Arunachal Pradesh, Manipur, Meghalaya,
	Senapati (Mao)	2390		Sikkim and Western Himalaya
B. retusiusculum	Senapati (Willong, Khunou, Mao)	1028-2390	August	Nagaland and Manipur
B. rufinum	Senapati (Hengbung)	1298	Sept-Oct	Manipur
B. scabratum	Tamenglong (Longku)	1303	April	Arunachal Pradesh, Manipur, Meghalaya, Darjeeling and Garhwal Himalaya
B. secundum	Senapati (Sadim)	1512	June-Aug	Nagaland, Manipur and Sikkim
B. trichocephalum	Ukhrul (Kamjong)	1460	August	Sikkim, Manipur and Meghalaya
B. xylophyllum	Chandel (Kwatha)	490	January	Manipur and Meghalaya

was thicker than the abaxial one in all the species (Table 2), with the exception of *B. affine* (CT_{ad} 22.5 μm and CT_{ab} 26.25 μm) *and B. trichocephalum* (CT_{ad} 14.38 μm and CT_{ab} 15.63 μm). Cuticular ledges were visible in *B. rufinum, B. scabratum, B. secundum,* and *B. xylophyllum*. Epidermis was single-layered in all the species and it was slightly modified above the mid-rib in *B. affine*, *B. careyanum, B. rufinum, B. scabratum, B. secundum, and B. xylophyllum*. Epidermis was followed by hypodermis in all the species except in *B. polyrhizum, B. reptans,* and *B. rufinum*. Hypodermis was composed of thin-walled water-storage cells with pitted or banded thickenings. Vascular bundles in all the species were collateral and arranged in one row with the median bundle being the largest and lateral bundles smaller with the exception of *B. secundum* where, the lateral bundles were larger than the median bundle. Both xylem and phloem in all the species were bounded by fibrous caps except in case of *B. polyrhizum*. Phloem cap was more prominent in most of the species. Mesophyll was modified into spongy and palisade cells except in *B. polyrhizum*. Multicellular glandular hairs within epidermal crypt were observed on both the adaxial and abaxial surfaces in all the species. Mesophyll tissue was 17-20 layers in *B. affine*, 8-12 in *B. careyanum*, 4-7 in *B. polyrhizum*, 6-12 in *B. reptans* and *B. retusiusculum*, 11-15 in *B. rufinum*, 7-10 in *B. scabratum*, 6-9 in *B. secundum*, 10-14 in *B. Trichocephalum,* and 14-20 in *B. xylophyllum*.

Dermal Anatomy (Fig.2A-E)

Stomata were observed only on the abaxial surface in all the species. Size of stomata and stomatal apparatus area was largest in *B. affine* (71.75 × 70.5 μm & 3996.63 μm^2) and smallest in *B. xylophyllum* (15.25 × 12.25 μm & 145.23 μm^2)*;* see Table 2. Epidermal cells were polygonal. Stomata were very small and sunken in *B. polyrhizum* and *B. xylophyllum* and slightly sunken in *B. affine.* Wax-secreting cells were found on both the adaxial and abaxial surfaces. Guard cells were with chloroplasts in all the species and cuticularised in *B. retusiusculum, B. Secundum,* and *B. trichocephalum*. Stomatal clustering was observed in all the species. Three stomatal types were observed: Foating type was the most common and observed in seven of the species studied (*B. affine, B. careyanum, B. polyrhizum, B. reptans, B. rufinum, B. scabratum* and *B. xylophyllum*), Cyclocytic type was found in *B. retusiusculum* and *B. Trichocephalum*, and Tetracytic type was found in *B. secundum*.

One way Analysis of Variance (ANOVA) followed by Duncan's Post Hoc test values were statistically significant at $p < 0.05$ ($n = 10$).

Based on the various anatomical characters, an attempt has been made to form a key for identification of the species studied under vegetative state which is as follows:

Identification Key

1a. Cuticular ledges present	2
1b. Cuticular ledges absent	5
2a. Sunken stomata present	*B. xylophyllum*
2b. Sunken stomata absent	3
3a. Guard cells cuticularised	*B. secundum*
3b. Guard cells not cuticularised	4
4a. Hypodermis present	*B. scabratum*
4b. Hypodermis absent	*B. rufinum*
5a. Abaxial cuticle thicker than adaxial	6
5b. Abaxial cuticle thinner than adaxial	7
6a. Guard cells cuticularised	*B. trichocephalum*
6b. Guard cells not cuticularised	*B. affine*
7a. Phloem cap absent	*B. polyrhizum*
7b. Phloem cap present	8
8a. Hypodermis absent	*B. reptans*
8b. Hypodermis present	9
9a. Guard cells cuticularised	*B. retusiusculum*
9b. Guard cells not cuticularised	*B. careyanum*

Discussion

Foliar Anatomy

Amongst the ten species studied, it was observed that leaf thickness was maximum in *B. xylophyllum* (4180 μm) and minimum in *B. reptans* (332.5 μm). Cuticle was found on both sides of the lamina in all the species. Thickest adaxial cuticle was observed in *B. xylophyllum* (31.25 μm) while abaxial cuticle thickness was maximum in *B. polyrhizum* (26.88 μm). *B. scabratum* showed minimum cuticle thickness; both adaxial and abaxial (Table 2). Cuticle helps in reducing water loss from the leaf interior (Mill and Schilling, 2009). Thick cuticle is usually found in plants of dry habitats (Haworth and McElwain, 2008). Adaxial epidermal thickness was maximum in *B. polyrhizum* (121.25 μm) and minimum in *B. secundum* (17.66 μm) while abaxial epidermal thickness was maximum in *B. affine* (68.75 μm) and minimum in *B. reptans* (15.84 μm). Large

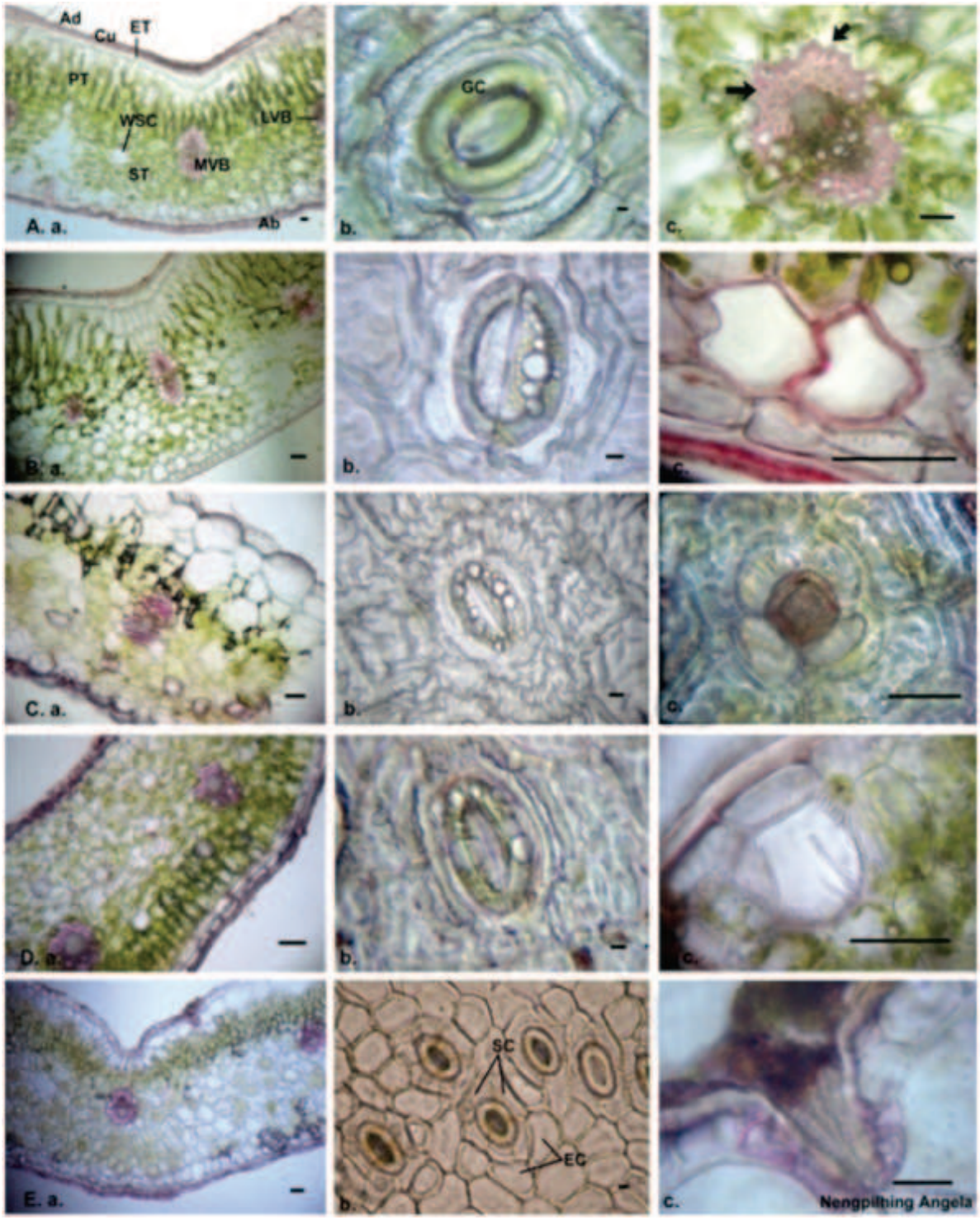

Fig. 1A-E. A. *Bulbophyllum affine:* a, T.S. of leaf (×100); b, Floating stomata (×400); c, Vascular bundles with fibrous caps (arrows) (×100); B. *B. careyanum:* a, T. S. of leaf (X100); b, Floating stomata (×400); c, Water-storage cells with pitted walls (×400); C. *B. polyrhizum:* a, T. S. of leaf (×100); b, Floating stomata (×400); c, *B. careyanum*. Wax-secreting cell on upper epidermis (×400); D. *B. reptans:* a, T. S. of leaf (×100); b, Floating stomata (×400); c, Water-storage cells with banded thickenings (×400); E: *B. retusiusculum.* a, T. S. of leaf (×100); b, Cyclocytic stomata (×400); c, Multicellular glandular hair within epidermal crypt (×400); Ad, adaxial epidermis; Ab, abaxial epidermis; Cu, cuticle; ET, epidermal tissue; PT, palisade tissue; ST, spongy tissue; MVB, median vascular bundle; LVB, lateral vascular bundle; WSC, water storage cells; GC, guard cell; EC, epidermal cell; SC, subsidiary cell. Scale bars, Column 1,100 μm; Column 2,10 μm; Column 3,50 μm.

Table 2. Foliar anatomy in presently studied species of *Bulbophyllum*.

Species	LT (μm)	CT_{ad} (μm)	CT_{ab} (μm)	ET_{ad} (μm)	ET_{ab} (μm)	MT (μm)
Bulbophyllum affine	1897.50 ± 37.64^{g}	22.50 ± 3.23^{c}	26.25 25 ± 2.64^{f}	49.38 ± 8.04^{b}	68.75 ± 8.84^{g}	1820 ± 63.25^{f}
B. careyanum	977.50 ± 68.41^{e}	14.38 ± 3.02^{b}	13.13 ± 1.98^{cd}	47.5 ± 14.49^{b}	38.75 ± 2.64^{d}	803.75 ± 6.79^{d}
B. polyrhizum	463.75 ± 10.94^{c}	28.75 ± 6.04^{d}	26.88 ± 7.82^{f}	121.25 ± 14.49^{e}	63.13 ± 15.72^{g}	288.75 ± 24.62^{a}
B. reptans	332.50 ± 69.27^{a}	16.50 ± 0.03^{b}	11.22 ± 1.70^{bc}	19.47 ± 2.89^{a}	15.84 ± 2.60^{a}	295 ± 64.60^{a}
B. retusiusculum	758.75 ± 61.53^{d}	14.19 ± 2.72^{b}	9.90 ± 3.11^{abc}	23.76 ± 4.87^{a}	21.45 ± 1.74^{ab}	671.25 ± 62.37^{c}
B.rufinum	1313.77 ± 300.37^{f}	25.25 ± 4.48^{c}	24.25 ± 4.72^{e}	74.50 ± 32.36^{c}	47 ± 14.76^{e}	1159.34 ± 248.7^{e}
B.scabratum	441.25 ± 37.75^{bc}	7.26 ± 1.39^{a}	6.80 ± 1.22^{a}	22.44 ± 3.03^{a}	21.45 ± 1.74^{ab}	397.50 ± 40.74^{b}
B. secundum	346.25 ± 64.5^{ab}	10.40 ± 3.02^{a}	9.24 ± 2.36^{ab}	17.66 ± 3.12^{a}	16.01 ± 2.34^{a}	289.38 ± 49.31^{a}
B. trichocephalum	852.50 ± 53.94^{d}	14.38 ± 3.02^{b}	15.63 ± 3.29^{d}	42.50 ± 5.74^{b}	27.5 ± 4.37^{bc}	680 ± 22.97^{c}
B. xylophyllum	4180 ± 27.13^{h}	31.25 ± 5.89^{d}	25.63 ± 1.98^{f}	101.25 ± 30.45^{d}	56.25 ± 5.1^{f}	3968.75 ± 8.83^{g}

Mean ± SD (n = 10). Different letters in the same column indicate statistical difference $p < 0.05$ (ANOVA).

LT , leaf thickness; CT_{ad}, adaxial cuticle thickness; CT_{ab}, abaxial cuticle thickness; ET_{ad}, adaxial epidermis thickness; $ET_{ad,}$ abaxial epidermis thickness; MT, mesophyll thickness.

Table 3. Dermal anatomy in presently studied species of *Bulbophyllum*.

Species	L_s (μm)	W_s (μm)	A_s (μm²)	S_a (μm)	d (mmÉ²)
Bulbophyllum affine	71.75 ± 8.5^{g}	70.50 ± 8.96^{g}	3996.63 ± 853.12^{i}	40 ± 4.71^{g}	6.00 ± 0.69^{a}
B. careyanum	64.02 ± 1.7^{f}	48.18 ± 1.7^{f}	2442.38 ± 106.32^{h}	44.55 ± 1.74^{h}	15.83 ± 2.29^{b}
B. polyrhizum	57.50 ± 4.86^{e}	38.75 ± 3.78^{e}	1753 ± 257.44^{g}	39.00 ± 4.28^{g}	15.40 ± 2.41^{b}
B. reptans	54.12 ± 3.55^{e}	39.93 ± 4.52^{e}	1611.42 ± 202.99^{fg}	34.98 ± 1.7^{f}	26.4 ± 2.66^{d}
B. retusiusculum	46.53 ± 3.95^{d}	34.32 ± 1.7^{cd}	1252 ± 102.76^{de}	25.74 ± 3.41^{d}	35.83 ± 3.46^{e}
B.rufinum	48.18 ± 3.55^{d}	37.45 ± 2.47^{de}	1416.51 ± 142.77^{ef}	29.37 ± 2.56^{e}	20.15 ± 0.66^{c}
B.scabratum	40.59 ± 4.41^{c}	25.08 ± 3.55^{b}	801.01 ± 154.98^{bc}	23.43 ± 2.31^{cd}	18.75 ± 3.76^{bc}
B. secundum	32.50 ± 3.02^{b}	27.39 ± 1.77^{b}	699.28 ± 82.15^{b}	16.33 ± 1.82^{b}	51.15 ± 5.07^{h}
B. trichocephalum	37.95 ± 2.46^{c}	27.23 ± 2.83^{b}	812.55 ± 113.31^{bc}	22.77 ± 1.87^{c}	39.25 ± 3.75^{g}
B. xylophyllum	15.25 ± 2.49^{a}	12.25 ± 2.49^{a}	145.23 ± 30.61^{a}	10.25 ± 1.42^{a}	36.55 ± 4.71^{fg}

Mean ± SD (n = 10). Different letters in the same column indicate statistical difference $p < 0.05$ (ANOVA).

$L_{s,}$ stomatal length; W_s, stomatal width; A_s, stomatal apparatus area; $S_{a,}$ stomatal aperture; d, stomatal density.

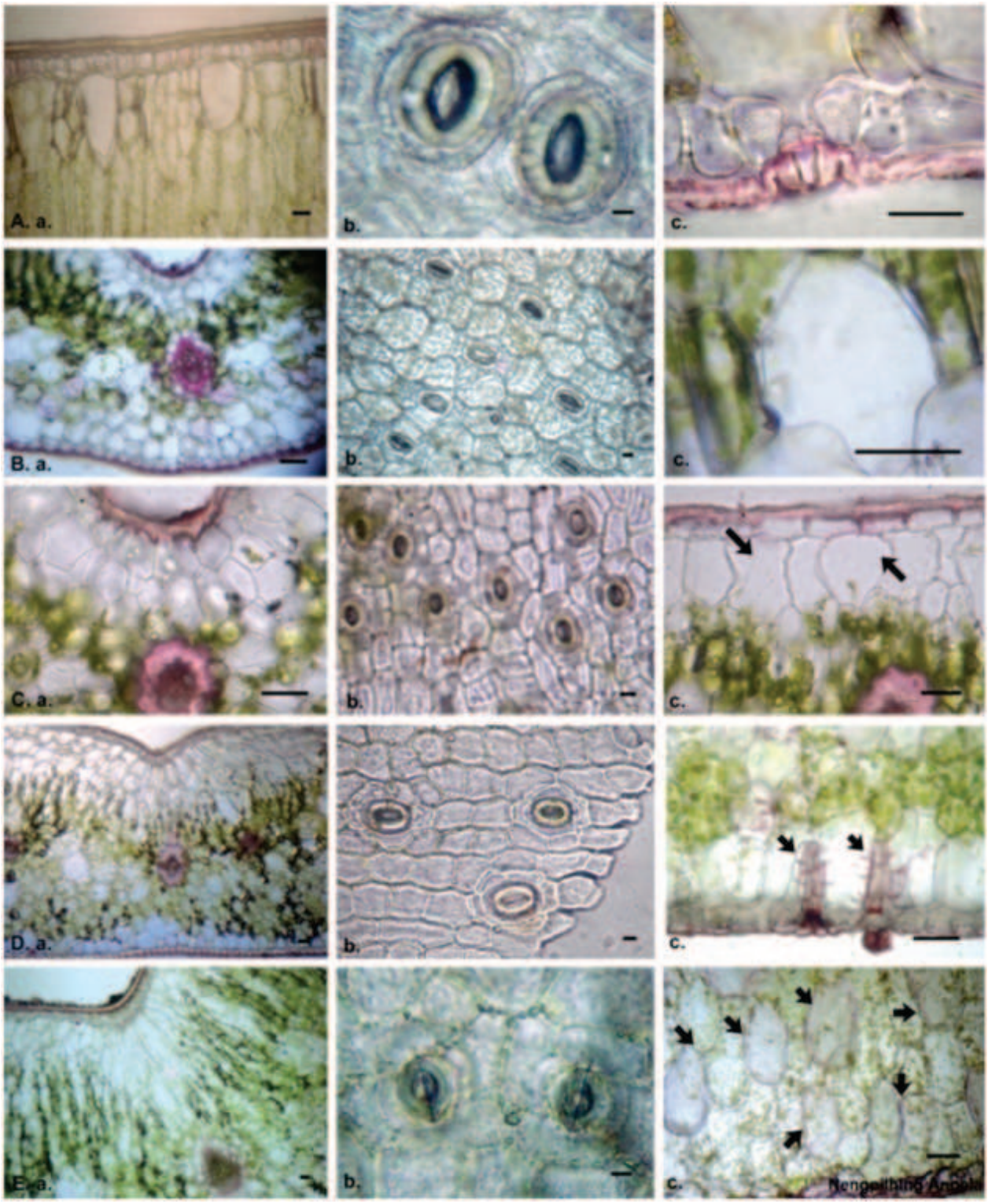

Fig.2A-E. A. *Bulbophyllum rufinum*: a, T.S. of leaf (×100); b, Floating stomata (×400); c, Cuticular ledges (×400); B. *B. scabratum:* a, T. S. of leaf (×100); b, Floating stomata (×100); c, Water-storage cells with pitted walls (×400); C. *B. secundum:* a, T. S. of leaf (×100); b, Tetracytic stomata (×400); c, Hypodermis made up of water-storage cells with pitted walls (arrows) (×100); D. *B. trichocephalum:* a, T. S. of leaf (×100); b, Cyclocytic stomata (×400); c, Multicellular glandular hairs within epidermal crypt (arrows) (×100); E. *B. xylophyllum*: a, T. S. of leaf (×100); b, Floating stomata (×400); c, Water-storage cells with banded thickenings (arrows) (×100). Scale bars, Column 1,100 μm; Column 2,10 μm; Column 3, 50 μm.

epidermal cells in many orchid species serve as water-storage cells. In some species of orchids, the water stored in epidermal cells can account for up to 80% of the entire leaf volume (Pridgeon and Stern, 1982). Maximum mesophyll thickness (3968.75 μm) was observed in *B. xylophyllum* and minimum in *B. polyrhizum* (288.75 μm). In all the species studied, the median vascular bundle was larger than the lateral ones except in *B. secundum*. The presence of multicellular glandular hairs within epidermal crypt in all the species is an indication of the plant's adaptability to reduce the rate of transpiration. It also protects the plants from outer injurious agencies (Pandey, 2001).

Dermal Anatomy

Stomatal size and stomatal apparatus area was largest in *B. affine* and smallest in *B. xylophyllum* (Table3). The exchange of gases takes place through the stomata (Buckley, 2005). The distribution, size, density, morphology and behaviour of stomata are closely associated with plant transpiration (Willmer and Fricker, 1996). Under severe water scarcity, smaller stomata are more efficient than larger stomata (Aasamaa *et al.,* 2001). Maximum stomatal density was observed in *B. secundum* and minimum in *B. affine* (Table 3). Plants with lower stomatal density are usually able to tolerate a more arid environment than plants with higher stomatal density (Kebede *et al*., 1994). All the species studied were hypostomatic with polygonal epidermal cells. A distinct predominance of hypostomatic over amphistomatic leaves was shown by Lavarack (1971), Williams (1979), and Avadhani *et al*., (1982). Guard cell chloroplast was present in all the species studied. Guard cell chloroplasts can contribute to stomatal opening (Zeiger *et al.,* 2002). However, guard cell cuticularisation was observed only in *B. retusiusculum, B. secundum* and *B. trichocephalum*. The presence of wax-secreting cells in all the species help in reducing the rate of transpiration thereby, aiding in water conservation. These cells also protect the leaves from shedding rain so the leaf cells don't become overly saturated with water and burst. Hoover (1986) observed that stomatal clusters may help in conserving water in plants. The most common stomatal type observed was floating type. However, cyclocytic and tetracytic types were also observed in some of the species. Floating condition is suggested to arise when anticlinal walls between subsidiary cells in a tetracytic configuration dissolve (Singh and Singh, 1974). These data suggest that all the ten species studied are xeromorphic in nature and can tolerate long periods of drought. *B. Xylophyllum*, however, showed maximum xeromorphic features. The leaves of this orchid are very thick and has the thickest adaxial cuticle with extremely small and sunken stomata. Thus, it can be concluded that all the species studied are able to tolerate long periods of drought and efficient in water-use and these traits can be useful for conservation of these species under green house conditions.

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