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3.3.2 Number of books and chapters in edited volumes/books published and papers published in national/ international conference proceedings per teacher during last five years

Links to Source website - 2017-22

Sl. No.	Name of the teacher	Title of the book/chapters published	Title of the paper	Year of publication	ISBN/ISSN number of the proceeding	Affiliating Institute at the time of publication	Name of the publisher	Link to Website
1	Dr. G. Ramesh	Orchid Biology: Recent Trends and Challenges	*Structural Adaptations of Bulbophyllum and Dendrobium (Orchidaceae) to the Epiphytic Habitat and their Phylogenetic Implications*	2020	https://doi.org/10.1007/978-981-32-9456-1_15	Hindu College, Guntur	Springer	https://link.springer.com/chapter/10.1007/978-981-32-9456-1_15
2	Dr. Krishna Naragani	Medicinal Plants : Biodiversity, Sustainable Utilization and Conservation	*Rare Actinobacteria Nocardiopsis lucentensis VLK-104 Isolated from Mangrove Ecosystem of Krishna District, Andhra Pradesh*	2020	ISBN 978-981-15-1635-1 ISBN 978-981-15-1636-8 (eBook)	Hindu College, Guntur	Springer	https://doi.org/10.1007/978-981-15-1636-8
9	Dr. S.V.S. Girija	*Angular Statistics,*	*Angular Statistics,*	2019	ISBN 9780367030001	Hindu College, Guntur	CRC Press	https://www.routledge.com/Angular-Statistics/Rao-Girija/p/book/9780367030001

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Orchid Biology: Recent Trends & Challenges

Springer

15 Structural Adaptations of *Bulbophyllum* and *Dendrobium* (Orchidaceae) to the Epiphytic Habitat and Their Phylogenetic Implications

G. Ramesh, J. Ramudu, S. M. Khasim, and K. Thammasiri

Abstract

The morpho-anatomical studies in *Bulbophyllum* and *Dendrobium* (Orchidaceae) with special reference to ecological adaptation and phylogenetic implications have been carried out. The vegetative parts were collected from different parts of North-East Himalaya and Western and Eastern Ghats of India. All were epiphytes belonging to tribe Dendrobieae. These plant parts were fixed in FAA (Formaline-Acetic acid-Alcohol) and usual methods of microtomy had followed. Stomata were confined to abaxial surface in all the investigated taxa. The presence of stomatal ledges and substomatal chambers is helpful in reducing leaf transpiration and evaporation of water. Absorbing trichomes were recorded only in Sikkim collections of *D. anceps* whereas they were absent in Darjeeling collections. In case of *D. herbaceum* and *D. moschatum*, these were present only in Kerala collections and absent in Karnataka collections. Single- or multi-layered velamen has been reported in both genera. It was observed that tilosomes were always associated with single layered velamen roots whereas completely absent in multilayered velamen taxa. Based on anatomical data, sectional delineation and phylogenetic interrelationships have been discussed.

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Keywords
Bulbophyllum · *Dendrobium* · Anatomical adaptations · Habitat tolerance · Phylogenetic implications

15.1 Introduction

The Orchidaceae constitutes one of the largest families of flowering plants comprising about 28,484 species (Govaerts et al. 2017). It contributes about 40% of the monocotyledons (Rasmussen 1985). In India, it represents the second largest flowering plant family with 1350 species (Jalal and Jayathi 2012) and contributes about 10% of Indian flora (Jain 1980; Kumar and Manilal 1994). A majority of orchid habitats in India are dwindling in state due to many anthropogenic activities. The present paper deals with some of the insights in anatomy related to ecological adaptability and phylogenetic interrelationship of genera *Bulbophyllum* and *Dendrobium*.

Both *Bulbophyllum* and *Dendrobium* belong to the tribe Dendrobieae Endl. and sub tribes Bulbophyllinae Schltr. and Dendrobiinae Lindl. respectively (Dressler 1993). In India, the tribe Dendrobieae is represented by about 189 species, distributed in Western and Eastern Ghats and, Eastern and Western Himalayas. Most of the taxa are primarily epiphytic, although some are lithophytic or terrestrial. In general, the great diversity of orchids and their different habitats have been made possible by structural, ecological and physiological adaptations (Mehra and Vij 1974; Khasim and Mohana Rao 1986; Mohana Rao and Khasim 1987a, b; Pridgeon 1986; Arditti 1992; Stern and Morris 1992). Vegetative structures such as roots, stems and leaves are specialized in water and nutrient absorption (Benzing et al. 1983; Moreira and Isaias 2008). Physiologically, the Crassulacean Acid Metabolism (CAM) helps in water economy by closure of stomata during the day (Luittge 2004) and, photosynthesis in roots is equally important in the maintenance of oxygen supply (Dycus and Knudson 1957; Moreira et al. 2009).

However, the vegetative anatomy of this highly evolutionary important family is completely neglected or has received little attention. From the ecological point of view Sanford (1974) did some work on African orchids, Kaushik (1983) on some Himalayan orchids and Metusala et al. (2017) on *Dendrobium* of Indonesia. During the last two decades few important monographs on orchid biology and systematics have appeared (Dressler 1993; Vermeulen 1993; Pridgeon et al. 1999, 2001, 2003, 2005; Ramesh et al. 2017). By critical reading of the available literature, it is evident that the authors had studied the anatomy with respect to systematics; but they did not explain the ecological adaptation of orchids. From the ecological point of view Sanford (1974) did some work on African orchids and Kaushik (1983) on some Himalayan orchids. As such, there has been no single paper on anatomy of orchids in relation to ecological adaptability for the last 20 years. In view of this the present anatomical investigation has been undertaken in the *Bulbophyllum* and *Dendrobium* species, the largest genera in the family Orchidaceae, so as to throw light on their

ecological adaptability and also ascertain the tribal, subtribal and sectional delineation, and phylogenetic relationships.

Plant materials were collected from Arunachal Pradesh, Darjeeling, Sikkim, Himalayas, Karnataka and Kerala at various altitudes over a period of 3 years (Table 15.1, Fig. 15.1). Plants were identified with the help of standard floras (Hooker 1894, 1895; King and Pantling 1898; Brühl 1926; Bose and Bhattacharjee 1980; Abraham and Vatsala 1981; Hegde 1984; Dressler 1993; Manilal and Kumar 2004; Mabberley 2008); these were confirmed by comparing them with the authentic herbarium specimens stocked at the Botanical Survey of India, Coimbatore,

Table 15.1 [a]Details of collections of orchid plant materials

S.No.	Species	Place, altitude and date of collection	Host tree	Accession No.
	Family: **ORCHIDACEAE**			
1	Subfamily: **EPIDENDROIDEAE**			
	Tribe: **DENDROBIEAE LINDL.**			
	Subtribe: **BULBOPHYLLINAE**			
	BULBOPHYLLUM Thouars			
	Bulbophyllum affine Lindl.	(i) Tipi (Arunachal Pradesh), 1500 m; May, 2011	*(i) Castonopsis indica*	(i) **RO1** (Arunachal Pradesh)
		(ii) Araria (Darjeeling), 1650 m; April, 2011	*(ii) Azadirachta indica*	(ii) **RO2** (Darjeeling)
2	*B. bisetum* Lindl.	(i) Jalalgarh (Darjeeling), 2250 m; February, 2011	(i) *Azadirachta indica*	***(i)*** *RO3*
3	*B. careyanum* W.J. (Hook.) Spreng.	(i) Packyong (Sikkim), 2500 m; February , 2011	(i) *Saurauia nepalensis*	(i) **RO4**
4	*B. cauliflorum* Hk. f.	(i) Jalalgarh (Darjeeling), 2250 m; February, 2011	(i) *Mangifera indica*	(i) **RO5**
5	*B. cornutum* (Lindl.) Rchb.f.	(i) Araria (Darjeeling), 1650m; February, 2011	(i) *Alnus nepalensis*	(i) **RO6**
6	*B. crassipes* J.D. Hook. f.	(i) Qasba (Darjeeling), 1250 m; February, 2011	(i) *Schima wallichii*	(i) **R07**
7	*B. fischerii* Seidenf.	(i) Jalalgarh (Darjeeling), 2250 m;	(i) *Mangifera indica*	(i) **R08**
		(ii) Lingtam (Sikkim), 2680 m; February, 2011	(ii) *Meliosma dillenifolia*	(ii) **R09**
8	*B. khasyanum* Griff.	(i) Taplejorg (Darjeeling), 1650 m; February, 2011	(i) *Schima wallichii*	(i) **R10**

(continued)

Table 15.1 (continued)

S.No.	Species	Place, altitude and date of collection	Host tree	Accession No.
9	*B. protractum* Hook. f.	(i) Ramda (Arunachal Pradesh), 1650 m, May 2011.	(i) *Elaeocarpus floribundus*	(i) **R11**
10	*B. scabratum* Rchb. f.	(i) Saddlepoint (Arunachal Pradesh, 2000 m; May 2011	(i) *Bischofia jaramica*	(i) **R12**
11	*B. stenobulbon* Par et Rchb. f.	(i) Qasba (Darjeeling), 1250 m; February, 2011	(i) *Alnus nepalensis*	(i) **R13**
12	*B. tremulum* Wight.	(i) Lingtam (Sikkim), 2680 m; February, 2011	(i) *Castanopsis indica*	(i) **R14**
13	*B. umbellatum* Lindl.	(i) Packyong (Sikkim), 2500 m; February, 2011	(i) *Alnus nepalensis*	(i) **R15** (Sikkim)
		(ii) Araria (Darjeeling), 1650 m; February, 2011	(ii) *Alnus nepalensis*	(ii) **R16** (Darjeeling)
	SUBTRIBE*: DENDROBIINAE*			
1	***DENDROBIUM* Swartz**	(i) Araria (Darjeeling), 1850 m; February, 2011	(i) *Alnus nepalensis*	(i) **R17 (**Darjeeling)
	Dendrobium anceps Sw.	(ii) Packyong (Sikkim), 2500 m; February, 2011	(ii) *Persea oderatissima*	(ii) **R18** (Sikkim)
2	*D. bicameratum* Lindl.	(i) Phidim (Darjeeling), 2000 m; February, 2011	(i) *Mangifera indica*	(i) **R19**
3	*D. densiflorum* Lindl.	(i) Phidim (Darjeeli ng), 2000 m; February, 2011	(i) *Schima wallichii*	(i) **R20**
4	*D. haemoglossum* Thw.	(i) Qasba (Darjeeling), 1785 m; February, 2011	(i) *Schma wallichii*	(i) **R21**
5	*D. herbaceum* Lindl.	(i) Karuman code (Kerala), 985 m; January, 2011	(i) *Mangifera indica*	(i) **R22** (Kerala)
		(ii) Khanapur (Karnataka), 850 m; June, 2011	(ii) *Terminalia elliptica*	(ii) **R23** (Karnataka)
6	*D. heyneanum* Lindl.	(i) Karuman code (Kerala), 985 m; June, 2011	(i) *Phoenix sylvestris*	(i) **R24**
7	*D. jenkinsii* Wall. ex. Lindl.	(i) Jalalgarh (Darjeeling), 1750 m; February, 2011	(i) *Azadirachta indica*	(i) **R25**

(continued)

Table 15.1 (continued)

S.No.	Species	Place, altitude and date of collection	Host tree	Accession No.
8	*D. microbulbon* A. Rich.	(i) Palavara (Kerala), 950 m; January, 2011	(i) *Terminalia bellirica*	(i) **R26** (Kerala)
		(ii) Halsi (Karnataka), 850 m; June, 2011	(ii) *Syzygium cumini*	(ii) **R27** (Karnataka)
9	*D. moschatum* (Buch.-Ham.) Sw.	(i) Karuman code (Kerala), 925 m; January, 2011	(i) *Mangifera india*	(i) **R28** (Kerala)
		(ii) Hanbur (Karnataka), 875 m; June, 2011	(ii) *Phoenix sylvestris*	(ii) **R29** (Karnataka)
10	*D. nobile* Lindl.	(i) Araria (Darjeeling), 2210 m; February, 2011	(i) *Alnus nepalensis*	(i) **R30**
11	*D. nutantiflorum* Hawkes & Heller	(i) Peringammala (Kerala), 950 m; June, 2011	(i) *Madhuca latifolia*	(i) **R31**
12	*D. pendulum* Roxb.	(i) Rongli (Sikkim), 1950 m; February, 2011	(i) *Albizia gamblei*	(i) **R32**

[a]Arranged according to Dressler (1993)

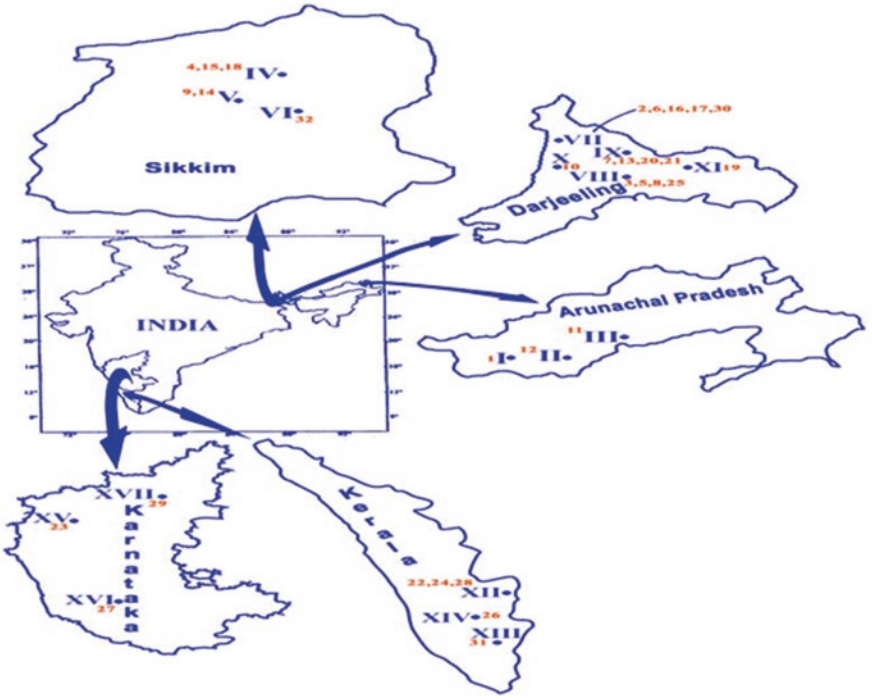

Fig. 15.1 India map and places of collection of orchid plant material

India. Voucher specimens were deposited in the Department of Botany and Microbiology, Acharya Nagarjuna University, India.

Vegetative organs such as leaves, stems, pseudobulbs and roots were fixed in FAA (5 cc formalin + 5 cc acetic acid + 90 cc 70% ethanol) for 24 h and then they were transferred to 70% alcohol and stored in it for laboratory studies. Free-hand cross sections of all vegetative organs were made at standardized levels (Metcalfe 1963; Cutter 1978). Cross section of mature leaves was done in a region equidistant from the base and apex of lamina. Stems were sectioned at median internodes, and mature roots at half way between the apex and junction with the rhizome. Sections were stained with safranin and fast green. For leaf epidermal peelings, small bits of leaves were put in 10% potassium hydroxide solution and then boiled until the epidermis was loosened from the mesophyll and veins. These peelings were mounted in 50% glycerine.

15.2 General Anatomy of *Bulbophyllum* and *Dendrobium*

The genera *Bulbophyllum* and *Dendrobium* are sympodial orchids, in which growth of the stem is arrested at certain stage and shoots are produced laterally from the base.

15.2.1 Leaf

Leaf anatomical features of *Bulbophyllum* and *Dendrobium* were given in the Tables 15.2 and 15.3. In a majority of the taxa studied here, the leaf is thick and fleshy. A fully developed leaf consists of a tubular leaf sheath and a lamina, often separated by an abscission layer, which involves in shedding and consequently helps in reducing the transpiring surfaces under stress conditions (Goh and Kluge 1989). In cross section, the leaf is generally V-shaped at the midrib and flattened at the laminar region.

Epidermal cells possess smooth and thin walls in almost all investigated taxa belonging to tribe Dendrobieae. According to Solereder and Meyer (1930) smooth cell walls are present in advanced epiphytic orchids whereas sinuous walls in primitive terrestrial ones.

In most of the presently studied taxa, the size of the adaxial epidermal cells is comparatively larger than abaxial ones. In some cases, these cells are two or three times larger in their size than the abaxial epidermal cells (Fig. 15.2a–d). Khasim (1996) reported adaxial epidermal cells that are three times larger than abaxial ones in *Paphiopedilum fairrieanum*. Mohana Rao and Khasim (1987b) reported bulliform cells on adaxial surface in *Anthogonium gracile* of Thuniinae. Bulliform cells are also reported in presently investigated taxon viz., *D. moschatum*.

Stomata The stomata are hypostomatic in distribution, restricted to abaxial surface of leaf. Similarly hypostomatic distribution is found in other groups of Orchidaceae (Möbius 1887; Singh 1981; Williams 1979; Avadhani et al. 1982). Interestingly Vij et al. (1991) observed the hypostomatic leaves in mesophytic orchids. Rasmussen

Table 15.2 **Leaf:** anatomical features in *Bulbophyllum* (in μm)

Anat. feat.	Access. no.															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Absorbing trichome	–	–	+	–	+	+	+	–	–	–	–	+	+	–	–	+
2. Cuticle thickness	0.008	0.015	0.011	0.009	0.007	0.004	0.012	0.009	0.008	0.005	0.005	0.019	0.008	0.008	0.007	0.005
3. Stomatal width (two gua rd cells including pore)	0.023	0.018	0.021	0.019	0.016	0.024	0.028	0.024	0.020	0.011	0.021	0.022	0.015	0.018	0.017	0.014
4. Stomatal length (only guard cell)	0.017	0.012	0.019	0.015	0.019	0.011	0.021	0.011	0.015	0.008	0.012	0.011	0.018	0.019	0.015	0.012
5. Midrib vb. Size	0.089	0.075	0.062	0.084	0.058	0078	0071	0.075	0.069	0.08	0.086	0.083	0.077	0.054	0.095	0.038
6. Laminar vb. size	0.041	0.047	0.052	0.051	0.044	0.049	0.057	0.064	0.058	0.061	0.047	0.058	0.062	0.042	0.050	0.032
7. Water storage cell	0.064	0.051	0.059	0.062	0.054	0.064	0.057	0.060	0.069	0.068	0.066	0.054	0.056	0.058	0.061	0.051
8. Substomatal chamber size	0.023	0.021	0.021	0.021	0.020	0.019	0.025	0.018	0.021	0.026	0.020	0.029	0.027	0.022	0.024	0.024
9. No.of ph. cap layers	3	4	3	3	2	3	2	3	2	3	5	3	5	4	2	2
10. No.of xy. cap layers	2	4	1	2	A	A	1	2	1	2	2	1	4	2	2	1

[1. *Bulbophyllum affine* (Arunachal Pradesh), 2. *B. affine* (Darjeeling), 3. *B. bisetum,* 4. *B. careyanum,* 5. *B. cauliflorum,* 6. *B. cornutum,* 7. *B. crassipes,* 8. *B. fischerii* (Darjeeling), 9. *B. fischerii* (Sikkim), 10. *B. khasyanum,* 11. *B. protractum,* 12. B. scabratum, 13. B. stenobulbon, 14. B. tremulum, 15. B. umbellatum (Sikkim).16. B. umbellatum (Darjeeling)]

ph. = phloem, xy. = xylem, vb. = vascular bundles; + = present, – = absent

Table 15.3 **Leaf:** anatomical features in *Dendrobium* (in μm)

Anat. feat.	Access. No.															
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1. Absorbing trichome	–	+	–	–	–	+	–	+	+	–	+	+	–	–	–	+
2. Cuticle thickness	0.010	0.007	0.009	0.006	0.004	0.008	0.006	0.011	0.006	0.009	0.004	0.004	0.008	0.007	0.005	0.012
3. Stomatal width (two guard cells including pore)	0.020	0.025	0.028	0.019	0.029	0.015	0.014	0.022	0.019	0.021	0.015	0.022	0.017	0.025	0.028	0.011
4. Stomatal length (only guard cell)	0.015	0.018	0.010	0.012	0.012	0.014	0.017	0.012	0.019	0.022	0.015	0.012	0.018	0.011	0.015	0.025
5. Mid vb. Size	0.082	0.070	0.078	0.071	0.057	0.068	0.083	0.079	0.088	0.081	0.078	0.076	0.081	0.085	0.073	0.084
6. Laminar vb. size	0.051	0.055	0.047	0.060	0.041	0.048	0.055	0.063	0.059	0.044	0.057	0.048	0.041	0.062	0.054	0.059
7. Water storage cell	0.065	0.062	0.067	0.064	0.068	0.060	0.067	0.057	0.066	0.026	0.059	0.068	0.061	0.069	0.053	0.057
8. Substomatal chamber size	0.026	0.027	0.021	0.029	0.025	0.023	0.029	0.028	0.026	0.021	0.025	0.020	0028	0.022	0.022	0.024
9. No. of ph. Cap layers	2	2	3	3	3	3	2	3	2	2	2	2	3	2	3	2
10. No. of xy. Cap layers	1	–	2	2	1	2	1	2	2	–	1	1	-	+	1	1

[17. *Dendrobium anceps* (Darjeeling), 18. *D. anceps* (Skkim), 19. *D. bicameratum*, 20. *D. densiflorum*, 21. *D. haemoglossum*, 22. *D. herbaceum* (Kerala), 23. *D. herbaceum* (Karnataka), 24. *D. heyneanum*, 25. *D. jenkinsii*, 26. *D. microbulbon* (Kerala), 27. *D. microbulbon* (Karnataka), 28. *D. moschatum* (Kerala), 29. *D. moschatum* (Karnataka), 30. *D. nobile*, 31. *D. nutantiflorum*, 32. D. pendulum

ph. = phloem, xy. = xylem, vb. = vascular bundles; + = present, – = absent

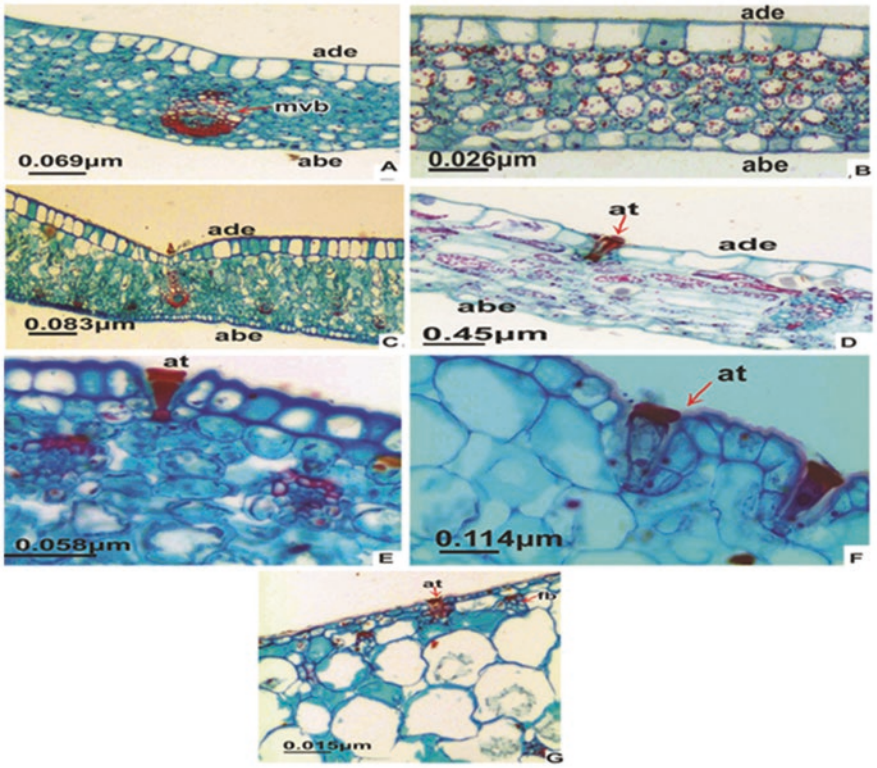

Fig. 15.2 **a**–**g**. Leaf
(**a**) *Bulbophyllum fischerii.* Leaf cross section showing larger adaxial epidermal cells and midrib vascular bundle
(**b**) *B. khasyanum.* Leaf cross section showing adaxial and abaxial epidermis
(**c**) *B. herbaceum.* Leaf cross section showing larger adaxial epidermal cells and midrib vascular bundle
(**d**) *B. umbullatum* (Sikkim collection). Leaf cross section showing absorbing trichome towards adaxial epidermis
(**e**) *B. herbacetum*. Leaf cross section indicating elongated 3-celled absorbing trichome on adaxial epidermis
(**f**) *B. umbellatum* (Darjeeling collection). Leaf cross section showing absorbing trichomes towards adaxial epidermis
(**g**) *Dendrobium anceps.* Leaf cross section indicating the absorbing trichome and fibre bundles towards adaxial epidermis

(1987) opined that hypostomaty is more frequent in mesophytic orchids whereas amphistomaty dominates in those of dry and humid habitats. Parkhurst (1978) observed that thick leaves tend to be amphistomatous, thus producing a secondary dependence of stomatal distribution on the environment. The thick leaves, generally associated with crassulacean acid metabolism have been considered an additional feature promoting amphistomaty in orchids (Rasmussen 1987). During the unfavourable period leathery leaves get folded and, in *R. retusa* two sides of lamina come so close to each other that there is no chance of transpiration from the adaxial

side of leaf (Kaushik 1983). With few exceptions, cyclocytic stomata with 5–6 subsidiary cells have been observed in presently investigated taxa. Epiphytes generally have smaller stomata than terrestrials. In the presently investigated taxa, the width of guard cells (including pore) varies among *Bulbophyllum* species (minimum of 0.011 μm to maximum 0.028 μm) and also *Dendrobium* (from 0.011 to 0.029 μm). Guard cells with prominent cuticular ledges (stomatal ledges) were observed on the leaf surface view of presently investigated taxon *B. affine* and *B. careyanum*. In *D. nobile* also, cuticular projections were observed around the stomatal apparatus; this type of projections has not been reported so far in any other orchid.

Absorbing trichomes The trichomes known to be absorbing in function, are 2 or 3-celled structures with dome-shaped apical cell and basal stalk cell (Fig. 15.2e–g). Kaushik (1983) preferred to call them as 'Handle cells'. The presence of absorbing trichomes is a regular feature in the members of Epidendroideae except tribe Vandeae (Khasim 1986). However, in the present investigation, these were observed in some species such as *Bulbophyllum bisetum, B. scabratum, B. stenobulbon*, *B. umbellatum* and also in *Dendrobium anceps, D. densiflorum, D. herbaceum, D. heyneanum* and *D. jenkinsii*. Pridgeon (1981) also studied the absorbing trichomes in Pleurothallidinae. He stated that the movement of water-soluble stain in these trichomes indicates an absorbing function similar to that of absorbing process of some bromeliad trichomes (Schimper 1888, quoted in Tomlinson 1969; Benzing et al. 1976).

Hypodermis In the presently investigated taxa, hypodermis is almost absent. However, fibre bundles at hypodermal position have appeared in *D. anceps* (Fig. 15.2g). Isaiah (1993) also reported fibre bundles in *Agrostophyllum khasianum, B. bhotanense* and *Epidendrum xanthum*. Mohana Rao and Khasim (1987b) observed these fibre bundles in *Agrostophyllum callosum, Cymbidium grandiflorum, C. lowianum, C. marstersii, C. traceyanum* and *Epidendrum radicans*. They also stated that fibre bundles provide mechanical strength to the plant body.

Mohana Rao and Khasim (1987b, c) reported multispiral thickenings in hypodermal cells in *B. dyerianum, Phaius maculatus, Pholidota imbricata* and *Otochilus alba.* Isaiah (1993) also observed hypodermal cells with multispiral thickenings in *B. bhotanense, B. gymnopus* and *D. jenkinsii*.

Mesophyll In all the investigated taxa, mesophyll is homogeneous, not differentiated into palisade and spongy parenchyma. Mesophyll tissue is tightly packed in some cases, which favours the fixation of carbon through C_4 pathway. Various tracheoidal elements including water storage cells with cellulosic thickenings and without thickenings were observed in the presently studied taxa. Olatunji and Nengim (1980), who coined the term 'tracheoidal elements', opined that certain specialized elements which possess annular, spiral or pitted secondary wall thicken-

ings, resemble the tracheids of vascular system. Pridgeon (1986) referred to these tracheoidal elements as 'spirally thickened idioblasts'.

In general, vascular bundles are arranged in a single series in all the presently investigated taxa. In all vascular bundles of leaf, phloem is situated towards abaxial side, and xylem towards adaxial side. The phloem and xylem ends possess some amount of sclerenchyma (sclerotic sheath). Tracheids with helical thickenings and vessel-like tracheids are abundant in leaves and also other parts of the plant body. Vessel-like tracheids were also reported by Ayensu and Williams (1972) in *Palumbina* and *Odontoglossum,* and also by Kaushik (1983) in several Himalayan orchids.

15.2.2 Pseudobulb/Stem

Anatomical features of Pseudobulb/stem were given in Tables 15.4 and 15.5. The stem shows morphological variation. In some species of *Dendrobium* and other orchids, the upper portion of the stem is fleshy whereas lower portion is thick and hard. Pseudobulbs are present in epiphytic orchids. Both fleshy stem and pseudobulb are concerned with storage of water. Pseudobulbs are consistent with sympodial growth, that leads to the shortening of shoots and thus to a compact habit reducing the transpiring surface; at the same time, sympodial habit promotes water storage and accumulation of starch materials (Benzing 1989a, b, c; Goh and Kluge 1989).

Fleshy stem and pseudobulb show anatomical similarities such as cuticle on the epidermis, and barrel-shaped or squarish and turgid epidermal cells; cortex and ground tissue with large polygonal to oval-shaped cells, function in storage of water (Fig. 15.3a, b). However, pseudobulb differs from stem in certain features. In pseudobulb, distinct cortex is absent; directly ground tissue in which numerous vascular bundles are scattered, appeared immediately below the epidermis (Fig. 15.3a, b). In case of stem, in the presently investigated taxa viz., *B. bisetum, B. cauliflorum* and *D. nobile*, a distinct cortex is present; this cortex is demarcated from the ground tissue by a ring of 3–4 layered sclerenchyma. Such type of demarcation was also reported by Morris et al. (1996) in some members of the subtribe Dendrobiinae.

Some of the cortical cells in the ground tissue region are showing pitted wall thickenings in most of the dendrobiums, such as *D. anceos, D. microbulbon, d. densiflorum* and *D. haemoglossum* (Fig. 15.3c–f). In some cases, cortical cells with multispiral cellulosic thickenings are involved in water storage (Fig. 15.3g). Vascular bundles showed well developed phloem cap made up of sclerenchymatous tissue. Large and small, numerous, collateral vascular bundles are scattered in the ground tissue region. In general, small vascular bundles are scattered at the peripheral region and large vascular bundles located in the centre.

Table 15.4 Stem/pseudobulb: anatomical features in *Bulbophyllum* (in μm)

	Access. no.															
Anat. feat.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. Cuticle thickness	0.006	0.004	0.005	0.006	0002	0.003	0.004	0.005	0.004	0.003	0.003	0.003	0.007	0.004	0.002	0.003
2. Water storage cells	0.021	0.024	0.020	0.021	0.026	0.021	0.024	0.018	0.021	0.019	0.017	0.025	0.038	0.022	0.024	0.025
3. Size of vb.	0.041	0.046	0.049	0.095	0.051	0.048	0.032	0.038	0.065	0.041	0.051	0.040	0.049	0.041	0.065	0.059
4. No. of ph. cap layers	3	3	3	2	1	3	2	2	3	3	3	3	2	2	2	2
5. No. of xy cap layers	2	2	1	–	–	–	–	1	2	–	1	1	–	–	1	–
6. Length of tracheid/vessel member	0.029	0.031	0.041	0.021	0.019	0.025	0.019	0.029	0.028	0.022	0.029	0.041	0.019	0.021	0.018	0.029
7. Length of xy. Fibre	0.035	0.045	0.019	0.025	0.042	0.037	0.029	0.039	0.027	0.035	0.039	0.019	0.032	0.022	0.041	0.035
8. Length of ph. Fibre	0.044	0.031	0.038	0.041	0.032	0.038	0.015	0.037	0.028	0.039	0.037	0.038	0.041	0.045	0.033	0.040

[1. *Bulbophyllum affine* (Arunachal Pradesh), 2. *B. affine* (Darjeeling), 3. *B. bisetum,* 4. *B. careyanum,* 5. *B. cauliflorum,* 6. *B. cornutum,* 7. *B. crassipes,* 8. *B. fischerii* (Darjeeling), 9. *B. fischerii* (Sikkim), 10. *B. khasyanum,* 11. *B. protractum,* 12. *B. scabratum,* 13. *B. stenobulbon,* 14. *B. tremulum,* 15. *B. umbellatum* (Sikkim), 16. *B. umbellatum* (Darjeeling)]

ph. = phloem, xy = xylem, vb. = vascular bundles; + = present, – = absent

Table 15.5 Stem/pseudobulb: anatomical features in *Dendrobium* (in μm)

	Access. No.															
Anat. feat.	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1. Cuticle thickness	0.006	0.005	0.005	0.003	0.006	0.003	0.004	0.003	0.005	0.006	0.003	0.004	0.006	0.004	0.005	0.006
2. Water storage cells	0.021	0.026	0.030	0.021	0.025	0.019	0.020	0.024	0.027	0.016	0.014	0.021	0.024	–	0.021	0.028
3. Size of vb.	0.041	0.047	0.040	0.031	0.044	0.051	0.031	0.042	0.044	0.049	0.040	0.045	0.039	0.047	0.047	0.042
4. No. of ph. cap layers	2	3	4	3	4	3	3	3	3	3	4	4	3	1	2	2
5. No. of xy. cap layers	–	1	2	–	1	2	3	–	1	1	2	2	1	–	1	–
6. Length of tracheid/vessel member	0.021	0.025	0.031	0.025	0.018	0.021	0.018	0.021	0.018	0.022	0.027	0.019	0.024	0.026	0.025	0.020
7. Length of xylem fibre	0.031	0.042	0.030	0.034	0.031	0.039	0.031	0.041	0.036	0.029	0.038	0.01	0.034	0.038	0.032	0.029
8. Length of phloem fibre	0.034	0.031	0.040	0.031	0.044	0.032	0.039	0.024	0.041	0.039	0.031	0.042	0.041	0.037	0.033	0.039

[17. *Dendrobium anceps* (Darjeeling), 18. *D. anceps* (Skkim), 19. *D. bicameratum*, 20. *D. densiflorum*, 21. *D. haemoglossum*, 22. *D. herbaceum* (Kerala), 23. *D. herbaceum* (Karnataka), 24. *D. heyneanum*, 25. *D. jenkinsii*, 26. *D. microbulbon* (Kerala), 27. *D. microbulbon* (Karnataka), 28. *D. moschatum* (Kerala), 29. *D. moschatum* (Karnataka), 30. *D. nobile*, 31. *D. nutantiflorum*, 32. *D. pendulum*]
ph. = phloem, xy. = xylem, vb. = vascular bundles; + = present, – = absent]

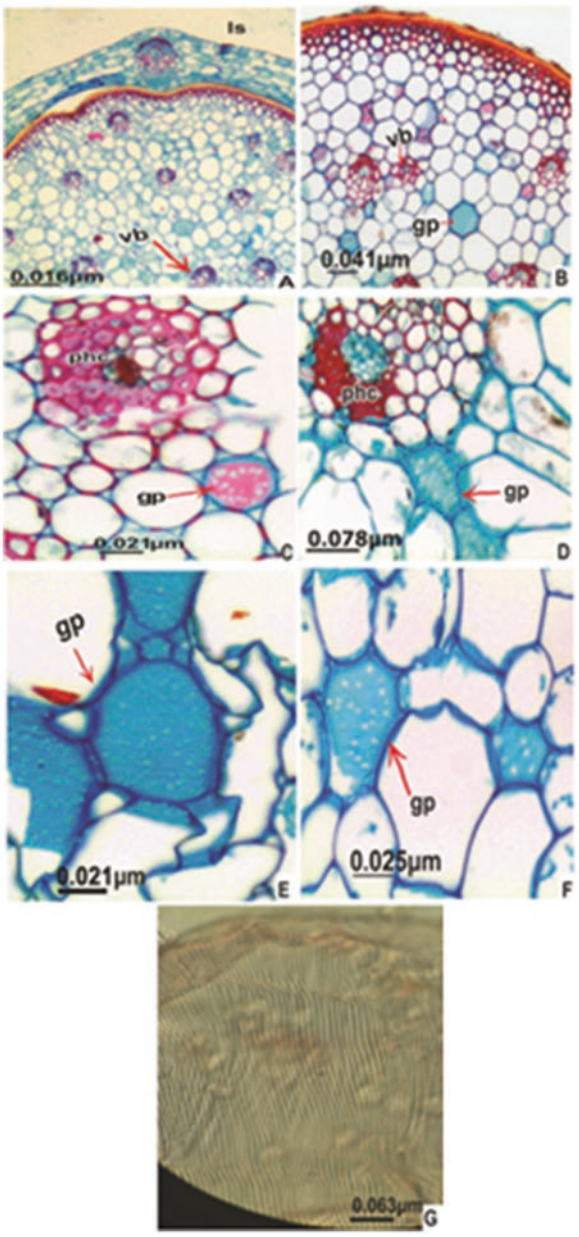

Fig. 15.3 (**a**–**g**). Pseudobulb/stem (**a**) *B. khasyanum*. Pseudobulb cross section indicating leaf sheath and scattered vascular bundles in the ground tissue (**b**) *B. tremulum.* Pseudobulb cross section showing ground tissue and vascular bundles (**c**) *D. anceps*. Fleshy stem cross section indicating the phloem cap and pitted wall thickenings in the ground tissue cell (**d**) *D. microbulbon* Pseudobulb cross section indicating vascular bundle with well-developed phloem cap and cells with pitted thickenings in ground tissue region (**e**) *D. densiflorum* Part of cross section of fleshy stem indicating pitted cell wall thickenings in ground tissue region (**f**) *D. haemoglossum* Stem cross section indicating pitted thickenings in cells of ground tissue (**g**) *D. bicameratum* Water storage cell with multispiral thickenings from stem maceration

15.2.3 Root

In general velamen roots are present in all epiphytic taxa of *Bulbophyllum* and *Dendrobium* (Tables 15.6 and 15.7) and occasionally in terrestrials. The epidermis

Table 15.6 Root: anatomical features in *Bulbophyllum* (in μm)

	Access. No.															
Anat. feat.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. No. of velamen layers	1	2–3	1	1	1	1	6	7	–	5	8	5–7	5–7	1	6–8	4–6
2. Fibrous mats/tilosomes	+	–	+	–	+	+	–	–	–	–	–	+	–	+	–	–
3.Exodermis cell lignification	0.021	0.028	0.031	0.019	0.015	0.021	0025	0.024	0.023	0.020	0.022	0.031	0.026	0.021	0.025	0.030
4.Passage cell size	0.004	0.007	0.003	0.004	0.006	0.003	0.002	0.003	0.005	0.004	0.006	0.003	0.008	0.007	0.004	0.005
5.Endoermis cell lignifiction	0.008	0006	0.010	0.009	0.004	0.007	0.011	0.008	0.005	0.015	0009	0.010	0.006	0.008	0.011	0.013
6.Vascular cylinder diameter	0.051	0.051	0.047	0.053	0.072	0.061	0.052	0.054	0.050	0049	0.044	0.047	0.051	0.049	0.057	0.041
7. No. of protoxylem poles	8	10	13	9	9	8	16	10	12	12	8	13	8	6	10	9

[1. *Bulbophyllum affine* (Arunachal Pradesh), 2. *B. affine* (Darjeeling), 3. *B. bisetum,* 4. *B. careyanum,* 5. *B. cauliflorum,* 6. *B. cornutum,* 7. *B. crassipes,* 8. *B. fischerii* (Darjeeling), 9. *B. fischerii* (Sikkim), 10. *B. khasyanum,* 11. *B. protractum,* 12. *B. scabratum,* 13. *B. stenobulbon,* 14. *B. tremulum,* 15. *B. umbellatum* (Sikkim), 16. *B. umbellatum* (Darjeeling)]

Table 15.7 Root: anatomical features in *Dendrobium* (in μm)

Anat. feat.	Access. No.															
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1. No. of velamen layers	9	3–4	6	5–7	1	1	1	1	1	1	2–5	3–6	5–8	1	6	3–5
2. Fibrous mats/tilosomes	–	–	–	9	+	+	+	+	+	+	–	–	–	+	–	–
3. Exodermis cell lignification	0.029	0.028	0.027	0.019	0.030	0.026	0.027	0.021	0.027	0.021	0.028	0023	0.029	0.026	0.029	0.015
4. Passage cell size	0.004	0.006	0.004	0.004	0.005	0.003	0.005	0.004	0.006	0.007	0.004	0.003	0.007	0.005	0.002	0.003
5. Endoermis cell lignifiction	0.012	0.005	0.007	0.010	0.011	0008	0.012	0.011	0.007	0.013	0.009	0.014	0.016	0.010	0.009	0.009
6. Vasclar cylinder diameter	0.0 39	0.047	0.054	0.050	0.055	0.048	0.047	0.045	0.037	0.051	0.058	0.053	0.043	0.046	0.060	0.049
7. No. of protoxylem poles	8	10	12	10	8	10	11	10	11	8	10	10	8	9	10	9

[17. *Dendrobium anceps* (Darjeeling), 18. *D. anceps* (Skkim), 19. *D. bicameratum*, 20. *D. densiflorum*, 21. *D. haemoglossum*, 22. *D. herbaceum* (Kerala), 23. *D. herbaceum* (Karnataka), 24. *D. heyneanum*, 25. *D. jenkinsii*, 26. *D. microbulbon* (Kerala), 27. *D. microbulbon* (Karnataka), 28. *D. moschatum* (Kerala), 29. *D. moschatum* (Karnataka), 30. *D. nobile*, 31. *D. nutantiflorum*, 32. *D. pendulum* + = present, – = absent

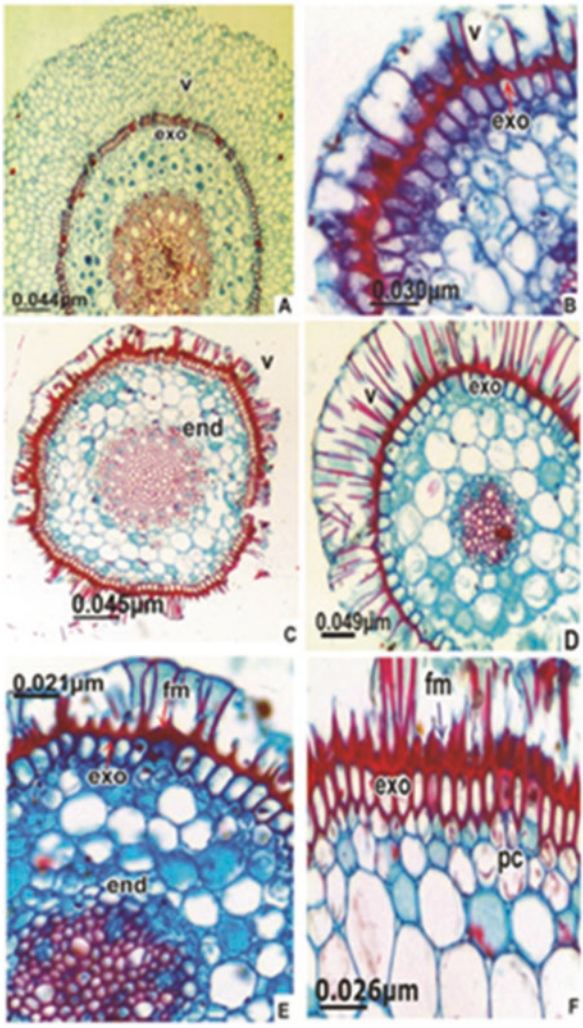

Fig. 15.4 (**a**–**f**) Root (**a**) *B. protractum*. Gross structure of root in cross-section showing multilayered velamen, exodermis and vascular cylinder (**b**) *D. haemoglossum* Root cross section showing single-layered velamen and exodermis with highly thickened inner tangential walls (**c**) *D. heyneanum* Gross structure of root in cross section showing single-layered velamen and vascular cylinder (**d**) *B. tremulum.* Water storage cell from root maceration (**e**) *B. cornutum.* Root transection showing velamen-exodermis complex with fibrous mats (tilosome) and endodermis (**f**) *D. nobile.* Root cross section indicating, fibrous mats, exodermis and also cortical cells possessing pitted thickenings

of mature root is multiseriate with velamen tissue (Fig. 15.4a). In epiphytic taxa, an extensive root system is developed to collect humus from the surrounding area. These roots are classified into two types: (1) substrata roots which penetrate the soil and absorb water and nutrients, and (2) aerial roots that are totally exposed to air and invariably they have multilayered velamen for water absorption, conservation and to provide mechanical strength to the plant body (Dycus and Knudson 1957; Morriset 1964; Benzing 1986, 1989a, b). Roots with single layered velamen (Fig. 15.4b–e) were recorded in almost all the presently studied taxa of *Bulbophyllum* and *Dendrobium*. In young roots, the cells in the outermost velamen layer are smaller than the inner ones; this layer is known as ‘epivelamen’ which is ruptured in mature

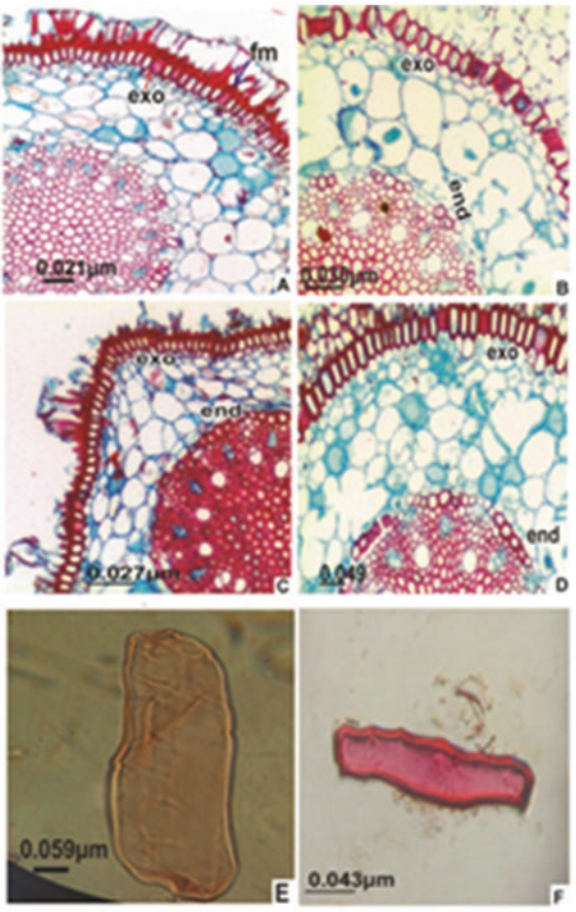

Fig. 15.5 (**a**–**f**) Root (**a**) *D. heyneanum.* Part of cross section of root showing velamen with thickened inner tangential walls, cortex and interrupted endodermis (**b**) *B. umbellatum.* Root cross section indicating exodermis and endodermis (**c**) *D. jenkinsii.* Root cross section showing peeled-off velamen with thickened inner tangential walls, exodermis, cortex, endodermis and vascular cylinder (**d**) *D. pendulum* Part of root cross section showing slightly elongated thick-walled exodermis, cortex and interrupted endodermis (**e**) *B. tremulum*. Water storage cell from root maceration (**f**) *D. moschatum.* Vessel-like tracheid from root maceration

roots. Wide bands of thickenings are found in velamen roots of some presently studied taxa.

Fibrous mats also known as tilosomes (Pridgeon et al. 1983) or fibrous bodies/fibrous mats (Benzing et al. 1983) in more specialized form are observed in some taxa of present investigation, such as *B. affine, B. bisetum, B. careyanum, B. cauliflorum, B. cornutum, B. tremulum, D. haemoglossum, D. herbaceum, D. heyneanum, D. jenkinsii, D. microbulbon and D. nobile* (Fig. 15.4f). Tilosomes also appeared in other Indian species such as *B. leopardianum, D. rotundatum* and *Otochilus alba* (Khasim 1986; Mohana Rao and Khasim 1987a). Tilosomes were observed only in epiphytes whereas absent in terrestrial taxa (Pridgeon et al. 1983; Khasim 1986; present work).

The exodermis, a single layer of cells, is situated in-between the velamen and cortex (Fig. 15.5a–d); in fact, it is the outermost layer of the cortex (Janczewski 1885; Leitgeb 1864; Engard 1944; Shushan 1959). It differs from the velamen by its greater degree of vacuolation and its elongation parallel to the long axis of the root.

At maturity, most exodermal cells do not contain protoplast and they are thick-walled, although a few remain living, thin-walled and these cells are known as "passage cells" (Fig. 15.5b). It is believed that water and solutes pass into the cortex through these cells (Dycus and Knudson 1957).

Cortex is situated in between exodermis and endodermis. It comprises thin-walled cells with cellulosic nature (Fig. 15.5e) and some may be chlorenchymatous. Some cortical cells may give an illusory appearance of vessel-like elements but the thickenings are cellulosic in nature (Fig. 15.5f). Occurrence of endotrophic mycorrhiza in the velamen and cortex is a regular feature in the family Orchidaceae.

Endodermis is uniseriate in all the investigated taxa. It is made up of thick-walled protective cells and interrupted at protoxylem poles by thin-walled passage cells. However, multiseriate endodermis was also reported in *Paphiopedilum venustum, Phragmipedium caudatum* and *P. achroederos* (Rosso 1966). Endodermal cells possess 'O' shaped thickenings (uniform lignification) in all the presently studied taxa.

Vascular cylinder comprises pericycle, phloem, xylem and pith. Phloem strands alternate with xylem strands. Vessel elements are not found in all the presently investigated taxa. However, these were reported in the roots of *Dendrobium peirardii* (Singh 1986). Vessel types and their occurrence constitute an important aspect for estimating evolutionary sequence and degree of advancement in monocotyledons (Dahlgren and Rasmussen 1983). Dahlgren and Clifford (1982) reported vessels in some orchid roots. The presence of vessels in roots is considered to be more advanced than the rhizome, stem and leaf (Cheadle and Kosakai 1980). Since all the investigated taxa are epiphytes, vessels are absent but recorded very long tracheids and vessel-like tracheids in their vegetative parts.

15.3 Anatomy in Relation to Ecological Adaptability

Some of the anatomical features of ecological interest are given below in the Table 15.8. In *Bulbophyllum* leaves are fleshy and differ in their size and form; in some cases leaf is deciduous at flowering. Leaves in *Dendrobium* are commonly distichous, conduplicate and articulate, but they range from terete and coriaceous, to laterally flattened and fleshy (Morris et al. 1996). Leaves may be persistent or deciduous. Persistent leaves are succulent ones and they commonly store water (Holttum 1960), whereas deciduous leaves remained present during wetter season of the year.

Stomatal ledges are prominent on the guard cells in *B. affine, B. careyanum, D. jenkinsii, D. moschatum* and *D. nobile*. These stomatal ledges are helpful in reducing the rate of transpiration from leaf surface and increases resistance to water loss (Yukawa et al. 1991, 1992; Ramesh et al. 2017). The presence of substomatal chambers in all taxa is an added advantage for epiphytic orchids in reducing leaf transpiration and evaporation of water.

In general, adaxial epidermal cells are larger in their size than abaxial epidermal cells. In some cases, e.g. *B. fischerii, B. khasyanum, B. pendulum, B. protractum, B. scabratum, B. stenobulbon, B. umbellatum, D. haemoglossum, D. herbaceum, D.*

Table 15.8 Morphological and anatomical features of ecological interest

Taxa	Habitat	External features	Ade cells size; stomata, distribution; ssc	Absorbing trichomes	Water storage cells and other trachaeoidal elements; fb, vlt	No. of velamen layers	Fibrous mats/ tilosomes
Bulbophyllum affine	E	Thick leaves, fleshy pseudobulbs	**ade** cells comparatively larger; with 2 or 4 subsidiary cells, prominent stomatal ledges, **h**; well-developed **ssc**	–	Simple water storage cells with abundant mucilage; **fb** absent; **vlt** abundant	Single layered	+
B. bisetum	E	Thick leaves, fleshy pseudobulb	**ade** cells comparatively larger; with 4 subsidiary cells, **h**; **ssc** present	+	Simple water storage cells with mucilages; **fb** absent	Single-layered	+
B. careyanum	E	Fleshy pseudobulb, leathery leaves	**ade** cells comp aratively larger; with 2 subsidiary cells (paracytic), stomatal ledges present **h**; well-developed **ssc**	–	Special type of water storage cells with cellulosic thickenings, other cells rich with mucilage; **fb** absent. **vlt** numerous	Single-layered	+
B. cauliflorium	E	Long-sheathed rhizome, fleshy pseudobulbs	**ade** cells comparatively larger; with 2 or 4 subsidiary cells, **h**; small **ssc**	+	Simple water storage cells rich with mucilage; **fb** absent; **vlt** numerous	Single-layered	+
B. cornutum	E	Thick leaves, fleshy pseudobulb	**ade** cells are comparatively larger; with 4–6 subsidiary cells (mostly cyclocytic, **h**; **ssc** present	–	Simple water storage cells rich with mucilage; **fb** absent; **vlt** numerous	Single-layered	+
B. crassipes	E	Leathery leaves, fleshy pseudobulb	**ade** cells are comparatively larger; with 4 subsidiary cells (tetracytic), **h**; **ssc** present	–	Simple water storage cells rich with mucilage; *fb* absent; **vlt** numerous.	6-layered	–

B. fischerii	E	Fleshy pseudobulb	**ade** *c*ells two times larger; with 4–5 subsidiary cells, **h; ssc** very small	–	Special type of water storage cells with multi- spiral cellulosic thickenings, simple water storage cells rich with mucilage; **fb** absent; **vlt** present	7–8 layered	–
B. khasyanum	E	Leaves coriaceous, fleshy pseudobulbs	**ade** cells two times larger; with 5 subsidiary cells, **h**; **ssc** present	–	Simple water storage cells rich with mucilage; **fb** absent	5- layered	–
B. protractrum	E	Fleshy pseudobulb	**ade** cells two times larger; with 5 subsidiary cells, **h**; **ssc** present	–	Simple water storage cells rich with mucilage; **fb** absent; **vlt** present	8- layered	–
B. scabratum	E	Fleshy pseudobulb	**ade** cells two times larger; with 4 subsidiary cells, **h; ssc** present.	+	Simple water storage cells with mucilage	Single-layered	+
B. stenobulbon	E	Fleshy leaves, cylindrical pseudobulbs	**ade** *c*ells 2–3 times larger; with 2–3 subsidiary cells, **h; ssc** present	+	Simple water storage cells rich with mucilage; cells with pitted thickenings **fb** absent; **vlt** present	5- layered	–
B. tremulum	E	Fleshy pseudobulb	**ade** cells comparatively larger; with 4–5 subsidiary cells, **h; ssc** absent	–	Simple water storage cells rich with mucilage, cells with pitted thickenings; **fb** absent; vessel-like tracheids	Single-layered	+

(continued)

Table 15.8 (continued)

Taxa	Habitat	External features	Ade cells size; stomata, distribution; ssc	Absorbing trichomes	Water storage cells and other trachaeoidal elements; fb, vlt	No. of velamen layers	Fibrous mats/ tilosomes
B. umbellatum	E	Ovoid pseudobulb, fleshy	**ade** cells 2–3 times larger, with 4–5 subsidiary cells or anomocytic in some cases. **h; ssc** very small	+	Special type of water storage cells with multispiral cellulosic thickenings, simple water storage cells with mucilage; **vlt** present	4–6 layered	–
Dendrobium anceps	E	Stem stout, leaves leathery	**ade** cells comparatively larger; with 4–5 subsidiary cells, **h**; no. **ssc**	+	Special water storage cells with cellulosic thickenings, cells with pitted thickenings; **fb** present, **vlt** numerous	5–9 layered	–
D. bicameratum	E	Stem fleshy	**ade** cells comparatively larger; with 4–5 subsidiary cell, **h; ssc** present	–	Special type of water storage cells with multispiral cellulosic thickenings, **fb** absent; **vlt** present.	6- layered	–
D. densiflorum	E	Thick leaves; fleshy stems	**ade** cells comparatively larger; with 45 subsidiary cells, **h**; very small, **ssc** present	–	Simple water storage cells rich with mucilage, some cells with pitted thickenings; **fb** absent; **vlt** present.	5–6 layered	–
D. haemoglossum	E	Fleshy stem	**ade** cells two times larger; with 5 subsidiary cells, **h**; smaller size, **ssc** present	–	Simple water storage cells rich with mucilage and also abundant starch **fb** absent; **vlt** numerous	Single layered	+
D. herbaceum	E	Fleshy stem	**ade** cells, 2–3 times larger; with 4–5 subsidiary cells, **h**; small **ssc**	+	Simple water storage cells with abundant mucilage; **fb** absent; **vlt** numerous	Single layered	+

D. heyneanum	E	Leathery leaves, fleshy stems	**ade** cells two times larger; with 4–6 subsidiary cells, **h**;	+	Simple water storage cells rich with mucilage, cells with pitted thickenings; **fb** absent.	Single layered	+
D. jenkinsii	E	Leathery leaves, fleshy pseudobulb	**ade** cells comparatively larger; with 4 subsidiary cells, prominent stomatal ledges, **h; ssc** present	+	Simple water storage cells with abundant mucilage; **fb** absent; **vlt** numerous.	Single layered	+
D. microbulbon	E	Thick leaves, fleshy pseudobulb	**ade** cells 2–3 times larger; with 4 subsidiary cells, **h; ssc** present	+, some times absent	Simple water storage cells with abundant mucilage, cells with pitted thickenings; **vlt** present	1–4 layered	+ (inconspicuous)
D. moschatum	E	Leaves leathery, fleshy stems	**ade** cells 2 or 3 times larger; with 5 subsidiary cells, prominent stomatal ledges, **h; ssc** not seen	+	Simple water storage cells with abundant mucilages, cells with pitted thickenings**;** **fb** absent; **vlt** numerous	6–8 layered	–
D. nobile	E	Fleshy pseudobulb like stem	**ade** cells 2 times larger; with 4 subsidiary cells, **h; ssc** present	–	Simple water storage cells with abundant mucilage, **vlt** present	Single-layered	+
D. nutantiflorum	E	Thick stems	**ade** cells 2–3 times larger; with 5 subsidiary cells, **h**; **ssc** present	–	Simple water storage cells rich with mucilage; **fb** absent, **vlt** numerous	6- layered	–
D. pendulum	E	Thick leaves, fleshy pseu- dobulbs	**ade** cells comparatively larger; with 4–5 subsidiary cells, **h; ssc** present	+	Simple water storage cells rich with mucilage, presence of bulliform ells, **fb** absent; **vlt** present	3–5 layered	–

ade = adaxial**, e** = epiphyte, **h** = hypostomatic, **fb** = fibre bundle, **ssc** = substomatal chamber, **vlt** = vessel-like tracheid; **+** = present, **–** = absent

heyniyanum, D. microbulbon, D. moschatum, D. nutantiflorum, D. nobile and *D. pendulum*, adaxial epidermal cells are 2–3 times larger in their size than abaxial ones. Such type of larger adaxial epidermal cells were also reported in *D. cumulatum, D. falconeri, D. gibsonii and D. parishii* (Isaiah 1993). All these large epidermal cells are thin-walled, mucilagenous, hyaline and function in storage of water.

Absorbing trichomes are present in both *Bulbophyllum* and *Dendrobium* species. These may be 3-celled in *B. bisetum, B. umbellatum, D. anceps*, *D. herbaceum, D. jenkinsii* and *D. pendulum;* whereas 2-celled in *B. cauliflorum, B. stenobulbon, B. heyneanum, D. microbulbon* and *D. moschatum*. Systematic occurrence of absorbing trichomes were recorded in Pleurothallidinae (Pridgeon 1981). These are sunken and glandular with a basal cell. Apical cells of trichomes on adaxial leaf surface generally rupture and a brown opaque residue covers the exposed portion of the basal cell (stalk cell). Pridgeon (1981) observed that amylacetate-alcohol dissolves the residue, rendering the stalk cell's lateral walls clearly visible. Inward movement of eosin or safranin stain from the apical cell through the stalk cell and into hypodermal cells indicates an absorptive function.

As such distinct hypodermis is absent; however, fibre bundles are observed in *D. anceps* at hypodermal position. Fibre bundles provide mechanical strength to the plant body along with special type of water storage cells with multispiral cellulosic thickenings.

Pseudobulbs are uninodal or polynodal organs. They show a great range of variation in their size and shape. Pseudobulbs are conical-ovoid in *B. bisetum* and *B. fischerii*, ellipsoidal in *B. crassipes*, cylindrical in *B. protractum, B. umbellatum* and *B. stenobulbon* and sub-globose in *B. tremulum*. In the case of *D. jenkinsii*, pseudobulbs are bottle-shaped; mostly in presently studied taxa of *Dendrobium*, stems are fleshy and erect or pendulous without bulbous nature. Thick cuticle and sinuous walls of epidermal cells are helpful in reducing the transpiration and also harden the tissue (Yukawa and Uehara 1996).

In some cases, these larger cells are modified into special type of water storage cells with multispiral cellulosic thickenings observed in *B. careyanum, B. fischerii, B. umbellatum, D. anceps* and *D. bicameratum*. All these larger parenchymatous cells with or without thickenings serve as water storage cells and comprise the succulent tissue of the organ (Wilder 1985; Koller and Rost 1988a, b; Stern and Morris 1992).

As it was opined by Moreira et al. (2013) that the well-developed velamen roots, distinct exodermis and endodermis, and specialized thick-walled cortical cells are the characteristic features of epiphytic orchids. This tissue is supposed to act as a sponge, absorbing the moisture from the atmosphere. In fact, the velamen stores water which is utilized by plant during dry conditions. Pridgeon (1987) reviewed the functional aspects of velamen. Tangential walls of cells in the innermost velamen layer are much thickened and form fibrous mats, also known as fibrous bodies or tilosomes (Benzing et al. 1982; Pridgeon et al. 1983). It was observed in the presently investigated taxa that the well-developed fibrous mats (tilosomes) are always associated with single-layered velamen (Table 15.8). This type of situation was also reported from *D. rotundatum* (Khasim and Mohana Rao 1984). Dycus and Knudson

(1957) called these fibrous bodies as "layers of even mats" situated on velamen cell walls immediately above the passage cells in several epiphytic taxa. The fibrous bodies in *Sobralia macrantha* (Benzing et al. 1982) and *D. rotundatum* (Khasim and Mohana Rao 1984) have been described as hygroscopic device designated to facilitate the condensation of atmospheric moisture prior to its absorption through underlying passage cells, and as a possible aid to the acquisition of atmospheric ammonia (Haberlandt 1914). The fibrous mats/tilosomes promote water economy in orchids. The labyrinthine structure of tilosome lengthens the pathway, the water vapour must traverse to breach the exodermis-velamen barrier during transpiration (Khasim and Mohana Rao 1984). This notion parallels to the plug hypothesis of (Leitgeb 1856). If the fibrillar components of tilosome alternately shrink and swell upon desiccation and hydration, its mass would function as a one way valve and not as a plug (Benzing et al. 1982).

Velamen may be single-layered or multi-layered. In single-layered velamen roots, exodermis is well developed with long thick-walled and short thin-walled passage cells. This single-layered velamen is peeled-off in mature roots. As a result, the entire interior part of the root is exposed out and there is a possibility of losing water from the root very easily. So as to prevent the water loss, tilosomes were conspicuously seen just above the exodermis in these single-layered velamen roots. Tilosomes are completely absent from the multi-layered velamen roots of *Bulbophyllum* and *Dendrobium* species. From this discussion, it can be presumed that the single-layered velamen roots have undergone anatomical adaptations so as to protect the root from desiccation and transpiration of water from interior parts of root.

The diversification of velamen characters is also exemplified by the type of habitat and host tree on which *Bulbophyllum* and *Dendrobium* species are growing continuously (Ramesh et al. 2017). When compared to Darjeeling collections, plants collected from Arunachal Pradesh habitat show some xeric characters; with respect to velamen, it is well-developed in Arunachal collections of *B. umbellatum* at altitude 1500 m when compared to Darjeeling at 1650 m elevation. However, velamen is well developed in *D. anceps* plants collected from Darjeeling (9-layered) than that of Sikkim collections at 2500 m elevation (3–4 layered velamen). Besides, tilosomes are observed in *B. affine* of Arunachal collections whereas absent in Darjeeling accessions. Isaiah (1993) reported 1–2 layered velamen with tilosomes in *B. protractum*, whereas in the presently studied collection at 1650 m elevation from Arunachal Pradesh velamen is multilayered without tilosomes. This report further indicates that Arunachal Pradesh habitat shows more xeric elements than that of Darjeeling where luxuriant growth of orchids is found. Similarly Karnataka region at 850 m altitude shows some xeric condition when compared to Kerala at 950 m elevation, both come under Western Ghats of India. This is evident from the well developed velamen roots of *D. microbulbon* and *D. moschatum* collected from Karnataka region (Tables 15.6 and 15.7). Moreover, more number of water storage cells with abundant mucilage is observed in *D. microbulbon* collected from Karnataka than that of Kerala collections. From the above discussion, it is evident

that those plants growing in lower elevation are showing more xeric conditions than those plants of higher elevation.

Exodermis in root possesses long, thick-walled and broad thin-walled passage cells. The thick-walled cells prevent water escaping from the conducting tissues in the interior of roots; thin-walled passage cells allow the water-soluble nutrients to pass through from outside into the conducting tissues. Water-soluble nutrients are checked by tilosomes and these pass through the passage cells into the interior of roots. Just like velamen, the exodermal thickenings aid in the reduction of water loss by root transpiration (Benzing et al. 1983), turning into an important apoplastic barrier (Hose et al. 2001; Ma and Peterson 2003).

Endodermis is interrupted by thin-walled passage cells at protoxylem poles. In all the investigated taxa, endodermal cells are uniformly lignified ('O' shaped thickenings). In both samples of *D. anceps* collected from Darjeeling and Sikkim have showed poor lignification of endodermal cells. But Isaiah (1993) reported high lignification in endodermis of some species, also collected from Sikkim. This can be attributed that not only the habitat conditions but also the supply of nutrients by host plant plays a vital role for the survival of epiphytic orchids.

Vascular cylinder in the root consists of pericycle, phloem, xylem and pith. In *Bulbophyllum* the number of protoxylem poles is 6–16 whereas in *Dendrobium*, it is 8–12 (Tables 15.6 and 15.7). On the basis of the number of protoxylem poles Rosso (1966) classified orchids belonging to Cypripedioideae into two groups: (I) protoxylem points 8 or less and (ii) protoxylem points 9 or more.

In orchid root, vascular cylinder is polyarch in nature. In most of the investigated taxa, fibre sheath was observed around xylem and phloem. Vessels were not found in majority of taxa, instead vessel-like tracheids were abundant in almost all the taxa. However, vessels were reported from the roots of *Dendrobium pierardii* (Singh 1986), *D. amplum* and *D. thyrsiflorum* (Isaiah 1993). Carlquist and Schneider (2006) reported vessels in other members of Epidendroideae. Cheadle (1942) found vessels with scalariform perforation plates and also with simple perforation plates in some orchid taxa; he opined that the vessels do not occur in the shoot system of typically bulbose or cormose plants, but occur most commonly in roots, less commonly in leaves, and an intermediate way in aerial roots. This can be interpreted as an adaptation for rapid uptake of water during brief periods of water availability (Carlquist and Schneider 2006). Kaushik (1983) also opined that vessels must have been eliminated due to development of other water storage mechanisms in the plant body; in fact, epiphytes, which are cut-off from the ground perhaps, have no need of possessing vessels.

Mycorrhizal association is found in the roots of presently investigated taxa. It was also observed in rhizomes of *Zeuxine gracilis* (Muthu Kumar et al. 2011). In fact orchid seed germinates only after being infected by fungal mycelium. No other members of angiospermous family, except Orchidaceae, have maximum exploitation of endotrophic fungus for their nutritional requirements. Withner (1974) postulated that orchid-fungus association in various types of soils as indicative of deficient soil nutrient supply rather than that of a particular host-fungus specificity. Rayer (1927) opined that the possession of mycorrhiza is infrequently beneficial to

vascular plants. Phytoalexins undoubtedly play an important role in this respect (Arditti 1979).

Having no direct root contact to the soil, epiphytes lack access to the most important nutrient source of ground-rooted plants. Sources for epiphytic orchids are atmospheric inputs (rain, dust and intercepted mist), nutrients released from ground-rooted host plants through leaching or decomposition and to a lesser extent, remains of animals as well as mineral and organic matter (Benzing 1990). Awasti et al. (1995) reported that stemflow leachates are the main source of ammonium-N and nitrate-N for uptake by orchids of Sikkim Himalaya. Nutrient scavenging in epiphytes is assisted by unusual morphological structures such as velamen roots with tilosomes, extensive development of roots, absorbing trichomes etc. However, some ecologists pointed out that though nutrients are scarce, this may not be of much importance, but the prime limiting factor is water (Zotz and Heitz 2001). So as to store and conserve the water, orchid has undergone various morphological adaptations such as presence of pseudobulbs, succulent/leathery leaves, presence of water storage cells with multispiral cellulosic thickenings.

From the entire discussion of this chapter, it is evident that there is no generalized pattern of growing of epiphytic orchids; not only the geographical conditions and type of habitat, but also the host-tree on which orchid grows, is playing vital role in survivability of epiphytic orchids (Ramesh et al. 2017) However, this needs further study to confirm. Those orchids that are getting poor supply of nutrients from host plant, undergo anatomical adaptations so as to survive under extreme environmental conditions (Khasim and Ramesh 2010; Ramudu et al. 2012). Sikkim-Himalaya is known to be congenial for orchid growth as it has sufficient rainfall and, warm and humid conditions prevailing throughout the year. However, leaves and roots of *B. fischerii* collected from Sikkim itself showed much larger size of leaf adaxial epidermal cells and well-developed, 12-layered velamen roots. This could be attributed to the host-tree, *Meliosma dillenifolia*, on which it is growing (Table 15.1); leaves and roots have undergone structural adaptations so as to conserve the nutrient supply appropriately (Khasim and Ramesh 2010). As Zotz and Heitz (2001) pointed out, a more integrative approach to study the epiphytic biology is needed including physiological investigations, substrate instability, dispersal limitation and competition (intra and inter specific level).

15.3.1 Tribal and Subtribal Delineation in Dendrobieae of Subfamily Epidendroideae

Lindley (1830–1840), Bentham and Hooker (1883), Rolfe (1909), Mansfeld (1937a, b), Hatch (1954), Dressler and Dodson (1960), and Melchior (1964) treated the Epidendroideae as one of the major tribes in family Orchidaceae; whereas Vermeulen (1966), Garay (1972), Thorne (1976), Dressler (1981), Rasmussen (1985) and Dressler (1986, 1993) regarded the Epidendroideae as a subfamily of Orchidaceae.

The subfamily Epidendroideae resembles the other members of Orchidaceae in possessing both terrestrial and epiphytic habits, larger adaxial epidermal cells and

homogeneous mesophyll. However, Epidendroideae deviate markedly in having hypodermis in leaf; stomata of cyclocytic, diacytic and in some cases paracytic type, heterogeneous mesophyll; well-developed sclerenchymatous sheath around vascular bundles; and velamen roots with uniformly thickened or U-shaped thickenings in exodermal and endodermal cells. With respect to embryology, they show similarities with members of Orchidaceae in possessing zygomorphic flowers, column, rostellum, unilocular ovary, parietal placentation, capsular fruit and numerous tiny transparent non-endospermic seeds. But Epidendroideae deviate from other members of Orchidaceae in possessing well-developed suspensor and thick cell-walled seed coat.

With respect to chemistry, Epidendroideae show affinity with members of Orchidoideae in having flavone C-glycosides, anthocyanins, phenanthrenes and coumarins, but they deviate significantly in other chemical constituents like alkaloids, 9, 10-dihydrophenanthropyrans and pyrones, steroides, triterpenoids and bibenzyls (Veerraju 1990). Due to these significant differences in anatomy, embryology and chemical constituents, Epidendroideae deserves the status of subfamily.

The members of tribe Dendrobieae are characterized by the presence of naked pollinia without caudicles or any other appendages, a prominent column foot and *Dendrobium* seed type. *Bulbophyllum* belonging to subtribe Bulbophyllinae is quite distinct from subtribe Dendrobiinae in its habit (pseudobulbs of a single internode and basal inflorescence), absence of silica bodies, presence of leaf hypodermis and more number of protoxylem poles upto 16 in roots (Table 15.9). However, both genera *Bulbophyllum* and *Dendrobium* share several common anatomical features such as presence of prominent stomatal ledges, substomatal chambers, well-developed phloem cap, water storage cells with multispiral cellulosic thickenings and single-layered velamen roots (Table 15.10). These resemblances strongly

Table 15.9 Dissimilar anatomical features (quantitatively) of *Bulbophyllum* and *Dendrobium*

Anatomical features	*Bulbophyllum*	*Dendrobium*	References
Leaf Cuticle thickness in leaf	0.005–0.019	0.004–0.012 µm	Present study
Length of guard cells	0.008–0.021	0.010–0.025 µm	Present study
Hypodermis	Present; however, absent in most of the taxa of present study	Absent	Isaiah (1993); present study
Laminar vascular bundles	0.032–0.062 µm	0.041–0.063 µm	Present study
Root Exodermis lignification	0.015–0.31 µm	0.015–0.029 µm	Present study
Endodermis lignification	0.003–0.013 µm	0.005–0.016 µm	Present study
Vascular cylinder diameter	0.041–0.061 µm	0.037–0.060 µm	Present study
No. of protoxylem poles	6–16	8–12	Kaushik 1983; present study

Table 15.10 Common anatomical features shared by *Bulbophyllum* and *Dendrobium*

Anatomical features	*Bulbophyllum*	*Dendrobium*	References
Stomata	With 2–6 subsidiary cells (cyclocytic)	With 4–6 subsidiary cells (cyclocytic)	Mohana Rao and Khasim (1987c), present study
Stomatal ledges	Prominent	Prominent	Mohana Rao and Khasim (1987c), present study
Substomatal chambers	Present	Present	Mohana Rao and Khasim (1987c), Isaiah 1993 present study
Absorbing trichomes	Present	Present	Mohana Rao and Khasim (1987c), present study
Fibre bundles in leaf	Present in few cases	Present in few cases	Mohana Rao and Khasim (1987c), present study
Mesophyll	Homogeneous, in few cases differentiated	Homogeneous	Mohana Rao and Khasim (1987c), present study
Phloem cap in leaf and stem	Well developed	Well developed	Mohana Rao and Khasim (1987c), present study
Special water storage cells	Present, columnar or oval shaped	Present, club or oval shaped	Mohana Rao and Khasim (1987c), present study
Single-layered velamen	Present in some cases with fibrous mats (tilosomes)	Present, in some cases with fibrous mats (tilosomes)	Isaiah (1993), present study

support the view of Dressler (1993) that both subtribes Bulbophyllinae and Dendrobiinae are sister groups of tribe Dendrobieae.

15.4 Interrelationships and Phylogenetic Implications

Morris et al. (1996) stated that the comparative anatomy and systematics are common strategies to understand the relationships among *Dendrobium* and also *Bulbophyllum* spp. Nor Hazlina et al. (2013) opined that morphological characters are very important in distinguishing various species and also interspecific hybrid progenies. They also stated that the data on relationships among the species and hybrids are useful to select the parents for hybrid production.

In *Bulbophyllum* sections (Table 15.11), viz., *Desmosanthus* is characterized by larger adaxial epidermal cells (two to three times larger in their size); similarly in the section *Cirrhopetalum* species such as *B. scabratum* and *B. umbellatum* larger adaxial epidermal cells are found; further phloem cap is well developed in the section *Cirrhopetalum*. Both single- and multi-layered velamen is observed in the

Table 15.11 [a]Sectional delineation in *Bulbophyllum* Thou. and *Dendrobium* Sw

Section	Species
BULBOPHYLLUM Thouars	
Sestochilos (Breda) Benth. Hk.f.	(i) *Bulbophyllum affine* Lindl.
Desmosanthes (Bl.) J.J.Sm.	(i) *B. cauliflorum* Hk. f.
	(ii) *B. protractum* Hk.f.
	(iii) *B. stenobulbon* Par. et Rchb. f.
Racemosae Benth. ex Hk. f.	(i) *B. bisetum* Lindl.
	(ii) *B. careyanum* (Hook.) Sprngl.
	(iii) *B. crassipes* Hk.f.
Cirrhopetalum Lindl.	(i) *B. cornutum* (Lindl.) Rchb.f. (*Cirrhopetalum cornutum* Lindl.)
	(ii) *B. fischerii* Seidenf. (*Cirrhopetalum gamblei* Hk.f. *C. thomsonii* Hk.f.)
	(iii) *B. scarbratum* Rchb. f. (*C. caespitosum* Lindl.)
	(iv) *B. umbellatum* Lindl. [=*B.maculosum* (Lindl.) Rchb. f.]
Globiceps Schltr.	(i) *B. khasyanum* Griff.
Pleiophyllus J.J. Sm.	(i) *B. tremulum* Wt.
DENDROBIUM Swartz	
Aporum Bl.	(i) *D. anceps* Sw.
Breviflores Hk.f.	(i) *D. bicameratum* Lindl.
Dendrobium Sw.	(i) *D. nobile* Lindl.
	(ii) *D. pendulum* Roxb. (*D. crassinode* Benson & Rchb.f.)
Densiflora Finet	(i) *D. densiflorum* Lindl.
	(ii) *D. jenkinsii* Wall. ex Lindl.
Grastidium Bl.	(i) *D. haemoglossum* Thw.
Formosae (Benth. et. Hk.f.) Hk.f.	(i) *D. nutantiflorum* Hawk. et Helr. (D. *jerdonianum* Wt.)
Herbacea Krzl.	(i) *D. herbaceum* Lindl.
Holochrysa Lindl.	(i) *D. moschatum* (Buch.-Ham.) Sw.
Stachyobium Lindl.	(i) *D. heyneanum* Lindl.
	(ii) *D. microbulbon* A.Rich.

[a]According to Garay et al. (1994), Wood (2006)

section *Cirrhopetalum.* Single-layered velamen is also found in other studied taxa belonging to *Sestochilus* and *Pleiophyllus*. Mohana Rao and Khasim (1987c) reported the same type of velamen roots in *B. andersonii*. Further well-developed multi-layered velamen is also recorded in sections *Desmosanthes, Racemosae* and *Globiceps*. This anatomical data clearly indicates that the section *Cirrhopetalum* is a unique one, from which other groups have originated. This strengthens the Schlechter's (1912) opinion that *Cirrhopetalum* species are true bulbophyllums. The assemblage of some vegetative characters in the section *Cirrhopetalum* and appearance of these characters in other sections of *Bulbophyllum*, justify that *Cirrhopetalum* must have existed prior to the origin of other *bulbophyllums*. Molecular data also support this assumption (Ramesh et al. 2017). However, some more studies are needed to ascertain this statement.

In case of genus *Dendrobium*, both single and multi-layered velamen was recorded in the sections *Dendrobium* and *Densiflora*. The section *Stachyobium* in the present study had sheerly showed single-layered with well-developed tilosomes and also with larger adaxial epidermal cells in leaf; whereas in the sections of *Aporum, Breviflores, Formosae* and *Holochrysa*, well-developed multi-layered velamen was recorded.

The presently investigated taxon *D. anceps* of *Dendrobium* section *Aporum*, is characterized by the presence of 3-celled absorbing trichomes, suberized epidermal cells and fibre bundles at subepidermal region in leaf. Similar anatomical features were recorded in *D. aloifolium* also belongs to the section *Aporum* by Solereder and Meyer (1930) and Morris et al. (1996).

Leaf anatomy of *D. anceps*, only species representing the section *Aporum* in the present investigation, is similar to that of species of the section *Rhizobium* in possessing three-celled absorbing trichomes, suberized epidermal cells and fibre bundles (Carlsward et al. 1997). This anatomical data supports the view of Stern et al. (1994) that the section *Aporum* is a sister group of the section *Rhizobium*. Based on cladistic analysis with leaf anatomical features, Carlsward et al. (1997) demonstrated that both these groups are monophyletic.

The morphological characters (quantitative data; Tables 15.12 and 15.13) from various species of *Bulbophyllum* and *Dendrobium* are taken and subjected to Hierarchical cluster analysis using Euclidean distance to determine the distance among various species (Tables 15.14 and 15.15).

Bulbophyllum In *Bulbophyllum*, a range of 1.00–11.87 Euclidean distance values are observed (Table 15.14). *B. crassipes* has highest (11.87) and *B. careyanum* lowest (1.00) values. The dendrogram based on anatomical features of *Bulbophyllum* (Fig. 15.6) revealed 3 clusters as follows:

Cluster I –	*B. umbellatum, B. scabratum, B. fischerii, B. khasyanum, B. stenobulbon, B. protractum.*
Cluster-II –	*B. cauliflorum, B. careyanum, B. cornutum, B. affine, B. tremulum, B. bisetum.*
Cluster-III –	*B. crassipes*

From the dendrogram (Fig. 15.6), it is evident that *B. umbellatum* is closely related to *B. scabratum*; in the same way *B. stenobulbon* has close affinity with *B. protractum*; similarly *B. cauliflorum* with *B. careyanum*.

It is also noted from the dendrogram (Fig. 15.6) that the section *Cirrhopetalum* species, such as *B. cornutum, B. fischerii, B. scabratum* and *B. umbellatum*, are scattered among two clusters. This indicates that all species of the section *Cirrhopetalum* are in one way or other related to other sections of *Bulbophyllum*. In other words, other *Bulbophyllum* species show some affinity with this section. This supports the view that all other *Bulbophyllum* species might have derived from the section

Table 15.12 Diagnostic anatomical features (quantitatively) in *Bulbophyllum* used for dendrogram construction

Taxa	Size of adaxial epidermal cells in leaf (μm)	No. of subsidi- ary cells in stoma	No. of phloem cap layers in leaf	No. of velamen layers in root	No. of proto- xylem poles in root
Bulbophyllum affine	0.025	2–4	2–4	1	8–10
B. bisetum	0.023	4	3	1	13
B. careyanum	0.026	4–5	3	1	9
B. cauliforum	0.028	4–5	2	1	9
B. cornutum	0.025	4–6	3	1	8
B. crassipes	0.029	4	2	5–7	16
B. fischerii	0.029	4–5	2–3	7–8	10–12
B. khasyanum	0.005	5	2	5	12
B. protractum	0.024	5	5	8	8
B. scabratum	0.037	4	3	5–7	10
B. stenobulbon	0.025	4–5	6	5–7	8
B. tremulum	0.024	4–5	4	1	6
B. umbellatum	0.031	4	2	6–8	10

Table 15.13 Diagnostic anatomical features (quantitatively) in *Dendrobium* used for dendrogram construction

Taxa	Size of adaxial epidermal cells in leaf (μm)	No. of subsidi- ary cells in stoma	No. of phloem cap layers in leaf	No. of velamen layers in root	No. of proto- xylem arches in root
Dendrobium anceps	0.019	4–5	2	7–9	8–10
D. bicameratum	0.021	2	3	6	12
D. densiflorum	0.025	4	3	5–7	8
D. haemoglosum	0.031	5	3	1	8
D. herbaceum	0.027	4–5	2–3	1	10–11
D. heyneanum	0.032	4–6	3	1	10
D. jenkinsii	0.002	4–5	2	1	11
D. microbulbon	0.024	5	2	2–5	8–10
D. moschatum	0.026	4	2–3	5–8	8–10
D. nobile	0.029	4	2	1	9
D. nutantiflorum	0.003	4	3	6	7
D. pendulum	0.028	4–5	2	3–5	9

Table 15.14 Distance Matrix (Euclidean Distance) based on anatomical features in *Bulbophyllum*

Taxa	*B. affine*	*B. bisetum*	*B. careyanum*	*B. cauliflorum*	*B. cornutum*	*B. cressipes*	*B. fischeri*	*B. Khasyanum*	*B. protractum*	*B. scabratum*	*B. stenobulbon*	*B. tremulum*	*B. umbellatum*
Bulbophyllum affine	–												
B. bisetum	3.162	–											
B. careyanum	1.732	4.123	–										
B. cauliflorum	2.449	4.243	1.000	–									
B. cornutum	3.000	5.385	1.414	1.732	–								
B. crassipes	8.718	6.782	9.327	9.274	10.247	–							
B. fischerii	7.416	7.141	7.616	7.681	8.124	4.359	–						
B. khasyanum	5.000	4.359	5.099	5.000	5.831	4.583	3.162	–					
B. protractum	7.416	8.888	7.348	7.681	7.348	8.660	4.472	5.831	–				
B. scabratum	6.083	6.708	6.164	6.245	6.633	6.083	2.450	3.162	3.162	–			
B. stenobulbon	6.708	8.426	6.782	7.280	6.782	9.000	5.099	6.000	1.414	3.742	–		
B. tremulum	4.123	7.141	3.162	3.606	2.449	11.874	9.274	7.483	7.348	7.348	6.633	–	
B. umbellatum	7.280	7.681	7.211	7.141	7.616	6.083	2.449	3.742	3.742	1.414	4.690	8.367	–

Table 15.15 Distance Matrix (Euclidean Distance) based on anatomical features in *Dendrobium*

Taxa	*D. anceps*	*D. bicameratum*	*D. densiflorum*	*D. haemoglosum*	*D. herbaceum*	*D. heyneanum*	*D. jenkinsii*	*D. microbulbon*	*D. moschatum*	*D. nobile*	*D. nutantifium*	*D. pendulum*
Dendrobium anceps	–											
D. bicameratum	4.796	–										
D. densiflorum	3.162	4.583	–									
D. haemoglosum	8.307	7.071	6.083	–								
D. herbaceum	8.124	5.916	6.782	3.000	–							
D. heyneanum	8.124	6.708	6.633	2.236	1.414	–						
D. jenkinsii	8.063	6.000	6.856	3.162	1.000	1.732	–					
D. microbulbon	4.000	3.873	3.162	4.583	4.243	4.243	4.123	–				
D. moschatum	1.732	3.464	2.236	7.348	7.141	7.280	7.211	3.317	–			
D. nobile	8.124	6.245	6.164	1.732	2.449	2.449	2.236	4.243	7.141	–		
D. nutantiflorum	4.472	5.385	1.414	5.196	6.481	6.164	6.557	3.464	3.606	5.477	–	
D. pendulum	4.123	4.472	2.646	4.243	4.583	4.359	4.472	1.000	3.464	4.123	2.646	–

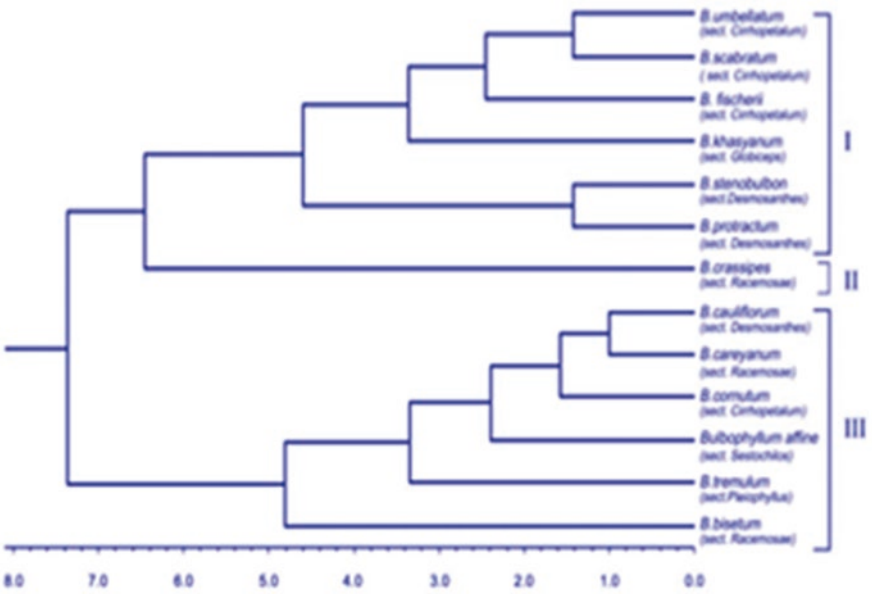

Fig. 15.6 Dendrogram showing dissimilarity among *Bulbophyllum* based on anatomical features

Cirrhopetalum which is considered to be the ancestral one to others. However, this needs further study. *B. crassipes* showing 6.5 dissimilarity value, does not form cluster with any other group of species.

Dendrobium

In *Dendrobium*, a range of 1.00–8.30 Euclidean Distance values are observed (Table 15.15); *D. haemoglossum* has the highest (8.30) whereas *D. jenkinsii* lowest (1.00) Euclidean distance values. The dendrogram (Fig. 15.7) reveals the following clusters.

Cluster-I – *D. nutantiflorum, D. densiflorum, D. pendulum, D. microbulbon, D. moschatum, D. anceps, D. bicameratum.*

Cluster-II – *D. nobile, D. haemoglossum, D. jenkinsii, D. herbaceum, D. heyneanum.*

The *Dendrobium* section *Formosae*, to which *D. nutantiflorum* (=*D. jerdonianum* Wt.) belongs (Table 15.11), was thoroughly analysed by Sathapattayanom (2008); according to him, the two morphologically aberrant species, such as *D. nutantiflorum* and *D. trigonopus*, remain unplaced. But from this study, preliminarily dendrogram shows that *D. nutantiflorum* has close affinity with *D. densiflorum* (section *Densiflora*) based on quantitative anatomical features.

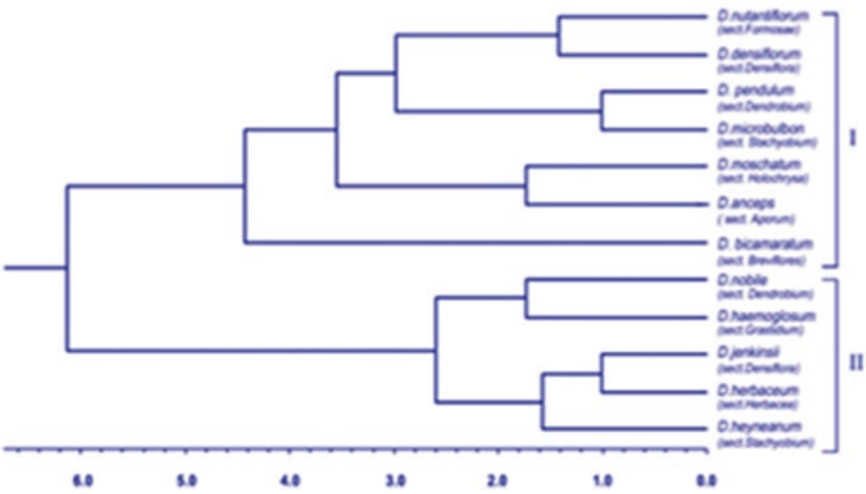

Fig. 15.7 Dendrogram showing dissimilarity among *Dendrobium* based on anatomical features

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Rare Actinobacteria *Nocardiopsis lucentensis* VLK-104 Isolated from Mangrove Ecosystem of Krishna District, Andhra Pradesh

33

Krishna Naragani

Abstract

The aim of the study was to isolate, identify, and analyze the phylogenetic relationship of the secondary metabolite-producing actinobacterial strains isolated from the mangrove ecosystem of Krishna district, Andhra Pradesh. The potent bioactive metabolite-producing strain was isolated and designated as VLK-104. The identification of the strain was carried out by employing the micromorphological, cultural, physiological, and biochemical methods. The antimicrobial efficacy of the strain was evaluated by using four solvents such as chloroform, ethyl acetate, methanol, and acetone. Among the solvents used, ethyl acetate extract exhibited maximum antimicrobial activity, whereas the other solvent extracts showed moderate to minimum activity against the Gram-positive and Gram-negative bacteria and fungi. Phylogenetic analysis of 16S rRNA gene sequence showed that the strain VLK-104 forms a distinct clade within the *Nocardiopsis* 16S rRNA gene tree and is closely related to *Nocardiopsis lucentensis.*

Keywords

Mangrove ecosystem · *Nocardiopsis lucentensis* · Phylogenetic analysis · Antimicrobial activity

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33.1 Introduction

Mangrove ecosystem has diverse groups of microorganisms. Among the microorganisms, Actinobacteria are the microbial poulation with a broad variety of secondary metabolites with various biological activities. It has rich biological diversity due to tremendous conditions like high moisture, salinity, pH, and temperature (Amrita et al. 2012). It is essential that new groups of microbes from unexplored habitats are pursued as sources of novel antibiotics and other bioactive compounds (Goodfellow and Fiedler 2010). The importance of cultivation of these microorganisms is necessary for a viable opportunity to bio-discovery (Joint et al. 2010). The phylum Actinobacteria stands for the most prominent group of microorganisms for the production of bioactive compounds, especially antibiotics and antitumor agents (Stach et al. 2003). Close to 40% of all microbial bioactive compounds derived from Actinobacteria, where approximately 80% of them are produced by the genus *Streptomyces* (Bérdy 2012). In fact, two of the four new classes of antibiotics discovered in recent years have been derived from actinobacterial strains (Hardesty and Juang 2011). In view of that, the mangrove environment is a good source for actinobacterial diversity and secondary metabolites. Hence, I have switched over to extreme environments to identify rare actinobacteria with promising bioactive compounds from mangrove sediments of Krishna district, Andhra Pradesh, India.

The soil samples were collected from the coastal region of Krishna district of Andhra Pradesh. The collected soil samples were air-dried at room temperature and transported to the laboratory in sterile bags. The air-dried mangrove soil samples were pretreated with calcium carbonate to selectively isolate rare actinobacteria. The identification of the strain was carried out by employing the micromorphological, cultural, physiological, and biochemical methods. The antimicrobial efficacy of the strain was evaluated by using four solvents such as chloroform, ethyl acetate, methanol, and acetone. After incubation for a week at 30 °C, distinct strains were selected for subculturing to maintain pure culture on agar slants.

33.2 Polyphasic Characterization of the Actinobacterial Strains

The potent actinobacterial strain was identified by using cultural, morphological, biochemical, and physiological characteristics together with genomic (16S rRNA gene sequencing) analysis. Morphological characteristics of the strain were observed by employing slide culture as well as scanning electron microscopy (SEM) as per the procedure described by Bozzola and Russell. Biochemical and physiological characters of the strain such as starch hydrolysis, urea hydrolysis, gelatin hydrolysis, acid production, temperature tolerance test, sodium chloride tolerance test, citrate utilization test, indole test, methyl red and Voges-Proskauer test, hydrogen sulfide test, and melanin tests were tested according to various standard methods (Holding and Collee 1971). Cultural characteristics were studied by culturing the strains on different International *Streptomyces* Project (ISP) media including ISP-1

(tryptone-yeast extract agar), ISP-2 (yeast extract-malt extract-dextrose agar), ISP-3 (oatmeal agar), ISP-4 (inorganic salts-starch agar), ISP-5 (glycerol-asparagine salts agar), ISP-6 (starch-casein agar), and ISP-7 (tyrosine agar) as well as on maltose-tryptone agar, Czapek-Dox agar, and nutrient agar (Shirling and Gottlieb 1966). In addition, the sensitivity of the strain to different antibiotics was determined by paper disc method (Williams 1989). Molecular identification of the strain based on 16S rRNA sequencing analysis was carried out.

33.3 Screening of Potent Actinobacterial Strains for Bioactive Metabolites

Pure culture of the actinobacterial strain was tested for secondary metabolites by the method of agar well diffusion (Cappuccino and Sherman 2004). The pure culture of the strain was transferred aseptically into the seed medium. Fermentation was carried out at 30 °C for 1 week under agitation at 120 rpm. At every 24 h interval, the flasks were harvested. Solvent extracts were evaporated to dry in water bath, and residues obtained were used to determine antimicrobial assay by employing seeded plate techniques. The inoculated plates were examined for zones of inhibition after incubation period. Diameter of the inhibition zone against the test microorganisms was taken as criteria for determining the antimicrobial potential of the actinobacterial strains.

33.4 Test Microorganisms

The test bacteria and fungi used for tetsing antimicrobial activity such as *Bacillus megaterium* (ATCC-10231), *B. subtilis* (ATCC 6633), *Escherichia coli* (ATCC-15597), and *Staphylococcus aureus* (ATCC-6538) and fungi *Candida albicans* (NCIM 2187). The inoculated plates were examined after 24–48 h of incubation at 37 °C for bacteria and 48–72 h at 28 °C for fungi.

Sample was fixed in 5.5% glutaraldehyde in 8.1 M phosphate buffer (pH 7.2) for 24 h at 40 °C and postfixed in 2% aqueous osmium tetraoxide for 4h, dehydrated in series of graded alcohols, and dried to critical point drying with CPD unit. The processed samples were mounted over the stubs with double-sided carbon conductivity tape, and a thin layer of gold coat over the sample was done by using an automated sputter coater (Model—JOEL JFC-5600) for 3 min and scanned under scanning electron microscope (SEM) (Model— JOEL-JSM 1600) at required magnifications as per the standard procedures (Bozzola and Russell 1999) at RUSKA Lab's College of Veterinary Science, SVVU, Rajendranagar, Hyderabad, India.

33.5 Molecular Identification

The molecular identification of the potential strain was carried out by using 18S rRNA gene sequencing analysis. The gene sequence of the strain was aligned using MEGA against the gene library available for potential actinobacteria strains in the NCBI. The phylogenetic tree was constructed using the maximum parsimony method. The closely related homologous strains were identified, retrieved, and compared to the sequence of the isolated strain using BLAST available with the MEGA 6.0 version (Tamura et al. 2013).

The 16S rRNA gene sequence of the strain has been deposited in the National Center for Biotechnology Information (NCBI).

33.6 *Nocardiopsis lucentensis* VLK-104 from Mangrove Ecosystem

33.6.1 Sample Collection

The mangrove sediment samples were collected at bimonthly intervals from mangrove ecosystems of the different places of the coastal region of Krishna district located along the east coast of Andhra Pradesh, India. Samples were collected from 6–10 cm depth and transported to the laboratory in sterile bags and air-dried at room temperature.

Table 33.1 Cultural characteristics of the strain VLK-104

S. no.	Medium	Growth	Aerial mycelium	Substrate mycelium	Pigmentation
1	Tryptone yeast-extract agar	Moderate	White to grayish	Nil	Nil
2	Yeast extract-malt extract-dextrose agar	Good	White to grayish	Brown	Nil
3	Oatmeal agar	Poor	Nil	Brown	Nil
4	Inorganic salts-starch agar	Good	White to grayish	Brown	Nil
5	Glycerol asparagine agar	Moderate	White to grayish	Brown	Nil
6	Starch-casein agar	Good	White to grayish	Brown	Nil
7	Tyrosine agar	Good	White to grayish	Nil	Nil
8	Maltose-tryptone agar	Moderate	White to grayish	Brown	Nil
9	Nutrient agar	Moderate	White to grayish	Nil	Nil
10	Czapek-Dox agar	Moderate	White to grayish	Brown	Nil

33.6.2 Isolation of Actinobacteria

During our search for potent actinobacterial strains from the coastal region of Krishna district, Andhra Pradesh, a total of 520 strains were isolated by using different pretreatment techniques and the selective media. Among them, one of the potent strains was designated as VLK-108, and it was identified by cultural, morphological, physiological, and biochemical characteristics.

33.6.2.1 Cultural Morphological, Physiological, and Biochemical Characteristics

The cultural characteristics of the strain VLK-108 grown on ten culture media are presented in Table 33.1. The strain exhibited good growth on ISP-2, ISP-4, and ISP-6 media out of ten culture media tested. The growth was moderate on ISP-1, ISP-5, ISP-7, nutrient agar, maltose-tryptone agar, and Czapek-Dox agar media, while it was poor on ISP-9. The color of aerial mycelium was white to ash when cultured on different media, while the substrate mycelium was brown. However, melanin pigmentation was not found on ISP-7.

Morphological Characteristics of the Strain VLK-104

The strain VLK-108 exhibited typical morphological characteristics of the genus *Nocardiopsis* spp. Micromorphology of the strain was examined by cover slip method and SEM analysis (Fig. 33.1). The strain showed zigzag hyphae and spore with a smooth surface. The culture was grown on ISP-2 medium supplemented with 3% NaCl for 6 days.

Physiological and Biochemical Characteristics of the Strain VLK-104

Physiological and biochemical characteristics of the strain VLK-104 are recorded in Table 33.2. Kampfer et al. (1991) suggested the physiological tests as indispensable tools for classification and identification of actinobacteria. The temperature range for growth was 25–40 °C with the optimum at 30 °C. NaCl tolerance is also serves as an important characteristic for species identification. VLK-104 was sensitive to

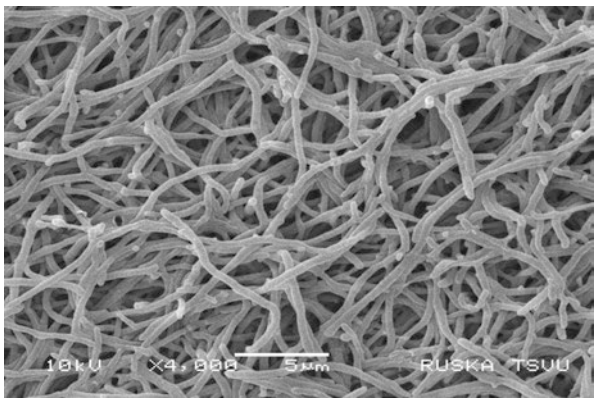

Fig. 33.1 Scanning electron micrograph of strain VLK-104

Table 33.2 Morphological, physiological, and biochemical characteristics of strain VLK-104

Characteristic	Response
Morphological characteristics	
Color of aerial mycelium	White to grayish
Color of substrate mycelium	Brown
Physiological characteristics	
Gram's reaction	+
Production of melanin pigment	–
Optimum temperature for growth	40 °C
NaCl tolerance	9%
Biochemical characteristics	
Catalase production	+
Urease production	+
Hydrogen sulfide production	–
Nitrate reduction	–
Starch hydrolysis	+
Gelatin liquefaction	–
Methyl red test	–
Voges-Proskauer test	–
Indole production	–
Citrate utilization	+

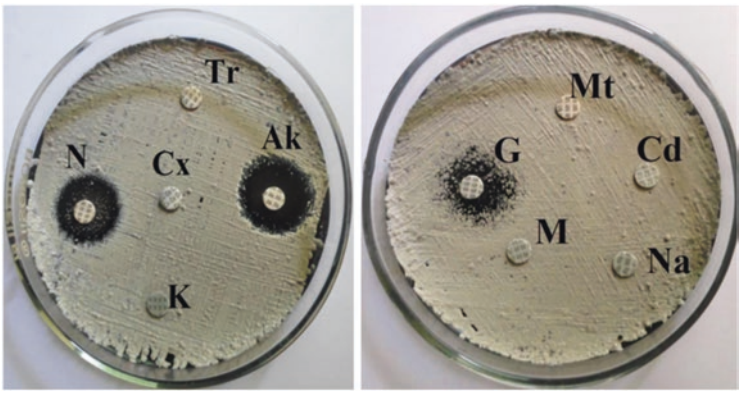

Fig. 33.2 Antibiotic susceptibility of the strain VLK-108. *N* neomycin, *Tr* trimethoprim, *Cx* cloxacillin, *Ak* amikacin, *K* kanamycin, *G* gentamycin, *Mt* metronidazole, *Cd* clindamycin, *M* methicillin, *Na* nalidixic acid

amikacin, gentamicin, and neomycin but resistant to kanamycin, trimethoprim, metronidazole, methicillin, nalidixic acid, clindamycin, and cloxacillin (Fig. 33.2; Table 33.3).

The strain VLK-104 has the ability to hydrolyze starch and exhibited positive response to citrate utilization and catalase production but negative for indole, methyl red, Voges-Proskauer, and nitrate reduction tests and also hydrogen sulfide

Table 33.3 Antibiotic susceptibility testing of VLK-104

S. no.	Name of the antibiotic (μg/disc)	Response
1	Gentamycin (10)	S
2	Kanamycin (30)	R
3	Trimethoprim (5)	R
4	Metronidazole (5)	R
5	Neomycin (30)	S
6	Amikacin (30)	S
7	Methicillin (5)	R
8	Nalidixic acid (30)	R
9	Cloxacillin (1)	R
10	Clindamycin (2)	R

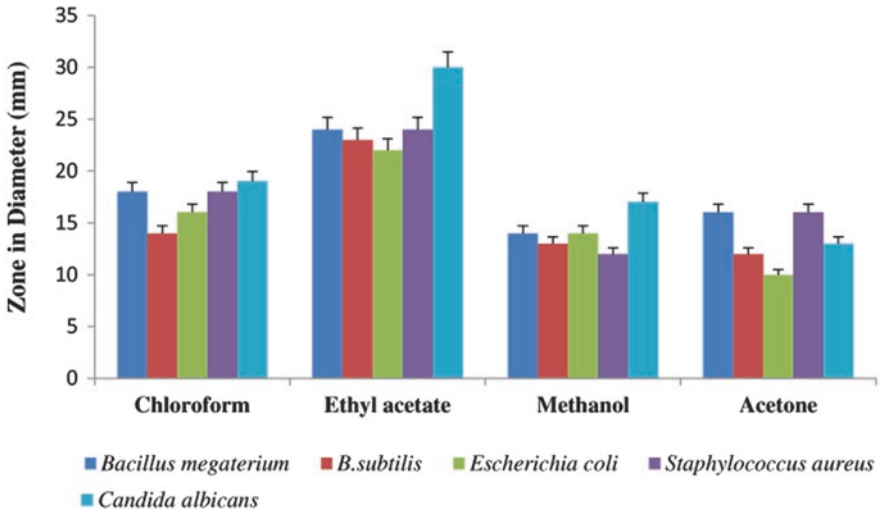

Fig. 33.3 Antimicrobial activity of the strain 104 by using different solvent extracts

production. The utilization of starch revealed the ability of the strain to produce extracellular amylase and protease respectively. Positive reaction with catalase revealed its potential to withstand the stress generated by reactive oxygen species.

33.6.3 Antimicrobial Activity

The antimicrobial efficacy of the strain was evaluated by using four solvents such as chloroform, ethyl acetate, methanol, and acetone (Fig. 33.3). Among the solvents used, ethyl acetate extract exhibited maximum antimicrobial activity, whereas the other solvent extracts showed moderate to minimum activity against the test microorganisms (Figs. 33.4 and 33.5). The ethyl acetate extract is highly effective against *Candida albicans*, *Staphylococcus aureus*, *Bacillus megaterium, Bacillus subtilis*, and *Escherichia coli*.

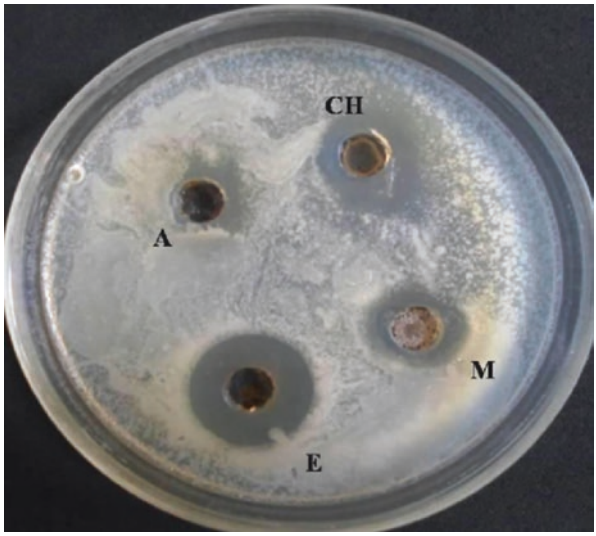

Fig. 33.4 Antibacterial activity of the crude extract of the strain 104 with different solvent extracts against *E. coli*. *CH* chloroform, *E* ethyl acetate, *M* methanol, *A* acetone

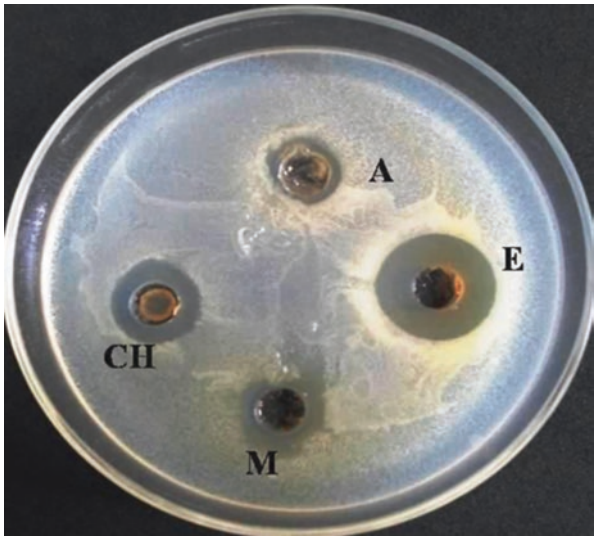

Fig. 33.5 Antifungal activity of the crude extract of the strain 104 with different solvent extracts against C*andida albicans*. *CH* chloroform, *E* ethyl acetate, *M* methanol, *A* acetone

33.6.4 Analysis of the 16S rRNA Gene Sequence of the Strain VLK-104

The 16S rRNA sequence data supported the assignment of this strain VLK-104 to the genus *Nocardiopsis* and species *lucentensis* (Fig. 33.6). The partial 16S rRNA sequence of the strain VLK-104 was submitted to the GenBank database under an accession number **KF317772**. The partial sequence was aligned and compared with all the 16S rRNA gene sequence available in the GenBank database by using the multi-sequence advanced BLAST comparison tool.

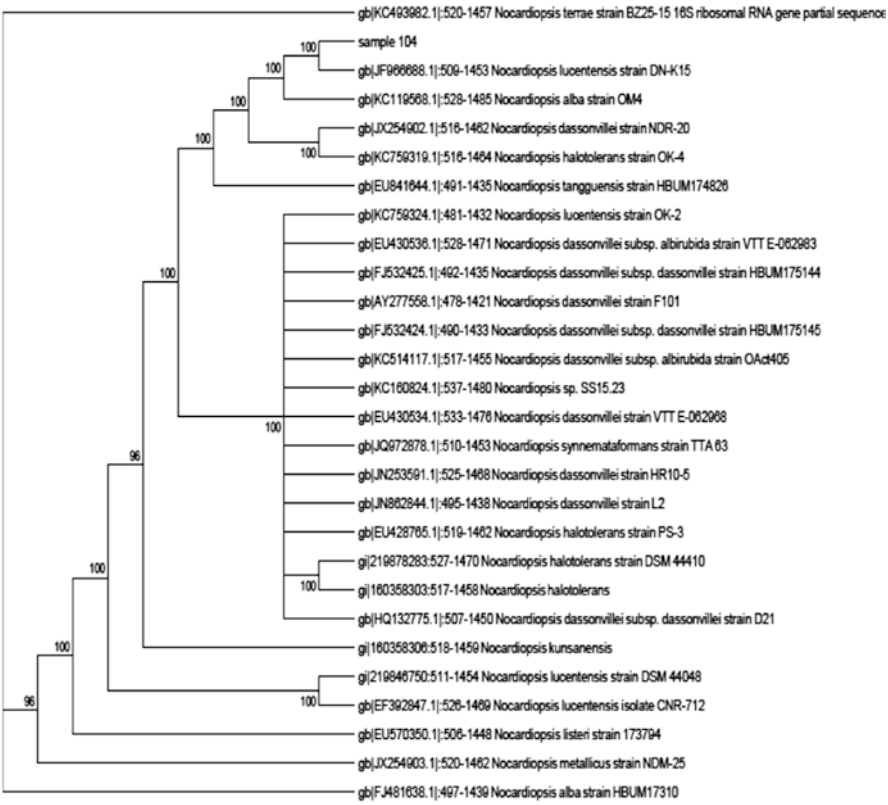

Fig. 33.6 Maximum parsimony tree based on partial 16S rRNA gene sequence showing relationship between isolate (Sample 108) VLK-104 and related members of the genus *Nocardiopsis.* The numbers at the nodes indicate the level of bootstrap support based on maximum parsimony analysis of 1000 resampled datasets; only values above 50% are given

33.7 Conclusion

The present study was aimed at the isolation of novel actinobacterium *Nocardiopsis lucentensis* VLK-104 having potent antimicrobial properties from mangrove ecosystem of Krishna district, Andhra Pradesh, and its identification based on cultural, physiological, and biochemical characteristics. Further study on optimization, purification, and chemical characterization of bioactive compounds of the strain is in progress.

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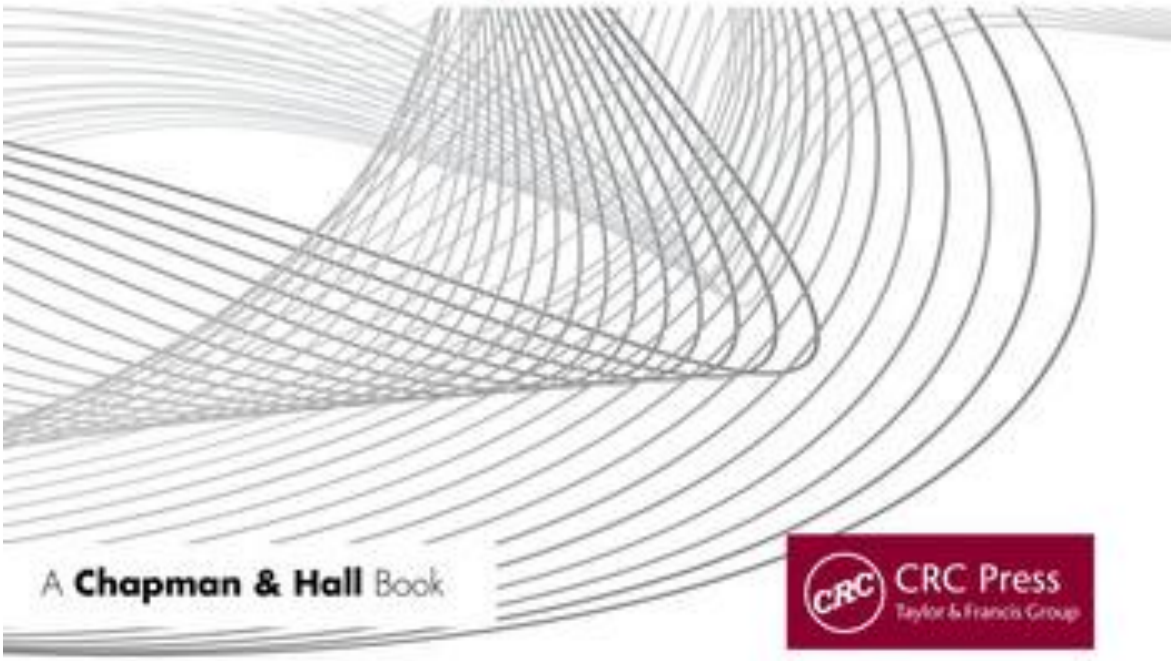
ANGULAR STATISTICS

A V Dattatreya Rao and S V S Girija



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PROLOGUE

Directional data arise in many scientific fields including Biology, Geology, Geography, Meteorology, Physics, Political Science, Image Analysis etc. Examples of such applications of directional data are given in the much acclaimed book titled Directional Statistics by Mardia and Jupp. A set of observations on directions is referred to as Directional Data. In particular, directional data of two dimensions is called circular data. Directional data arise in the form of circular / semicircular / axial, symmetric / asymmetric, uni / bimodal data, in practical situations of varied fields listed above. For the purpose of modeling such kind of data sets, the data scientists found that existing models as inadequate. As there is paucity of angular models, and to fill the gap, this book is designed at constructing new angular models with the existing techniques and to develop new tools of constructing angular models with an application to control charts in angular models. This book will be useful for data scientists / researchers / research students of Statistics and allied fields.

The celebrated book on **Topics in Circular Statistics** by Jammalamadaka and Sengupta is the source of inspiration for us to start working in this new emerging area somewhere in 2003. Around that period, a few wrapped models were available for modeling circular data, though different methods of constructing circular models were cited in the above book and we found that not much was done basing on other construction procedures. At the same time it came to our attention that there is paucity of suitable models to work on the above types of data sets. That is the motivating point to take up the present work.

With this backdrop, and with the encouragement of CRC press group of publishers, we have planned this book on ANGULAR STATISTICS, consisting of nine chapters. Chapters 2, 3 and 4 are devoted to present methodologies of wrapping, inverse stereographic projection and

offsetting respectively. Our endeavor is to present various circular models derived using these procedures. In addition to the above, we could develop new techniques of construction of angular models using Rising Sun, Positive Definite sequences and differential approach, and the models derived using these new techniques are included in Chapter 5. Besides, discretization of continuous circular models and its application to derive discrete circular models are presented.

When the semicircular data analysis is to be analyzed, the existing practice is to double the angles to carry out the analysis based on Circular Statistics and back - transforming the results for the interpretation of semicircularity. Further, Jammalamadaka and Sengupta have defined axial distributions restricting a circular model to an arc of an arbitrary length for the data analysis of axial data. It was observed that when inverse stereographic projection is applied on a linear model spanning from o to ∞ , it turned out to be a semicircular model automatically/ extemporaneously and when offsetting is adopted on Bivariaate Beta model, the resultant model happened to be axial model automatically/ extemporaneously. Therefore Chapter 6 is devoted to constructing such extemporaneous angular models.

Some of circular and semicircular models are extended to l – axial (arc) models. Also the procedure of Marshall – Olkin transformation is extended to angular models to construct asymmetric l – axial models which are included in Chapter 7.

Having discussed various construction procedures of angular models and deriving them, the next logical step would be to use them for modeling by applying one of the inferential techniques called fitting of distributions. In case several models are found to be good fits then discriminating among good fits based on their relative performance for the choice of the best fit in the class is the essence of the penultimate chapter.

In the last chapter, on the lines of Laha and Gupta (2011), the idea of control charts for circular and semicircular models are presented as one of the applications of these angular models.

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- AUTHORS

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