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ROOT ANATOMICAL STUDIES IN SOME SPECIES OF COELOGYNEAE (ORCHIDACEAE) WITH REFERENCE TO ECOLOGICAL ADAPTATIONS

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Abstract

Presence of multiseriate velamen, parenchymatous cortex and defined endodermis was observed in roots of all the presently studied species of tribe Coelogyneae (*Coelogyne breviscapa, C. corymbosa, C. flaccida, C. nervosa, C. nitida, C. ovalis, C. prolifera, C. stricta,* and *Pholidota pallida*). The geographical conditions and type of habitat, besides the host-tree on which orchid grows has a vital role in survivability of epiphytic orchids. The orchids that are getting poor supply of nutrients from the host plant undergo adaptations so as to survive under extreme environmental conditions.

Introduction

THE ORCHIDACEAE is one of the largest families of flowering plants. It comprises about 779 genera and 22,500 species (Mabberley, 2008). In India, with 1141 species spreading over 166 genera (Kumar and Manilal, 1994), it represents one of the largest flowering plant family and contributes about 10% of Indian flora. In South India, there are about 250 orchid species spreading over 70 genera (Abraham and Vatsala, 1981). The vegetative anatomy of this important family has neither been completely taken up nor received much attention. During the last two decades, a few important monographs on orchid biology and systamatics have appeared (Dressler, 1993; Pridgeon *et al.,* 1999, 2001, 2003, 2005; Vermeulen, 1993); these authors have studied the anatomy in relation to only systamatics and not taken into account the ecological adaptation of orchids. From the ecological point of view, Sanford (1974) did some work on African orchids and Kaushik (1983) has made anatomical studies in Himalayan orchids and has correlated these with ecological adaptations. Perusal of literature (Chattopadhyay *et al*., 2014; Kaushik, 1983; Mohana Rao and Khasim, 1986, 1987a,b; Ramudu *et al*., 2012; Vij *et al*., 1991) in some Indian orchid species also advocates the ecological significance of leaf dermal features. Hence presently, an attempt was made to study root anatomical features in some species of Coelogyneae with reference to ecological adaptations.

Materials and Methods

The roots of total of nine orchid species (eight species of *Coelogyne* and one species of *Pholidota*; Table 1) were collected from different parts of Southern India. These were fixed in FAA (0.5: 0.5: 9 of formalin, acetic acid, and 70% ethanol), later these were preserved in 70% ethanol, before processing. Roots were dehydrated in alcohol and xylene series, infiltrated and embedded in paraffin wax (melting point 60-62^{o}C), and sectioned with a rotary microtome at a thickness of 15-20 mm. Double staining was done by safranin-fast green combination and sections were mounted in DPX mount (Khasim, 2002; Vijayaraghavan and Shukla, 1990). Optical photomicroscope (Motic 2.0, 5 megapixels) was used to take anatomy photographs.

Results

The roots of all the presently studied taxa were observed as circular in outline, in transverse sections (Fig. 1A). The velamen was formed by dead tissue and cells were polygonal to oval shaped (Fig. 1A). The number of velamen layers were highest (7) in *Coelogyne stricta* (Table 2). In *C. flaccida*, epivelamen was distinct (Fig. 1B). However, in *C. corymbosa*, velamen was bi-stratified with characteristic wall thickenings (Fig. 1C). The outer layer of cortex had long thick walled, passage cells which did not necessarily alternate with each other (Fig. 1C). The exodermal size was equal in all the studied taxa. However, the presence of secondary thickenings in these cell walls seemed to play an important role in water storage function and mechanical support. Exodermal cell lignifications was found highest in *Pholidota pallida* as compared to other studied *Coelogyne* species (Fig. 2A, B). The highest number of cortical layers was found in *C. breviscapa* (Fig. 2C, D), *C. flaccida,* and *C. nervosa* (Fig. 2E). Cortex comprised of small and large oval shaped cells with inter-cellular spaces. Some of the cortical cells were with pitted thickenings (Fig. 1E). In *C. stricta*, some

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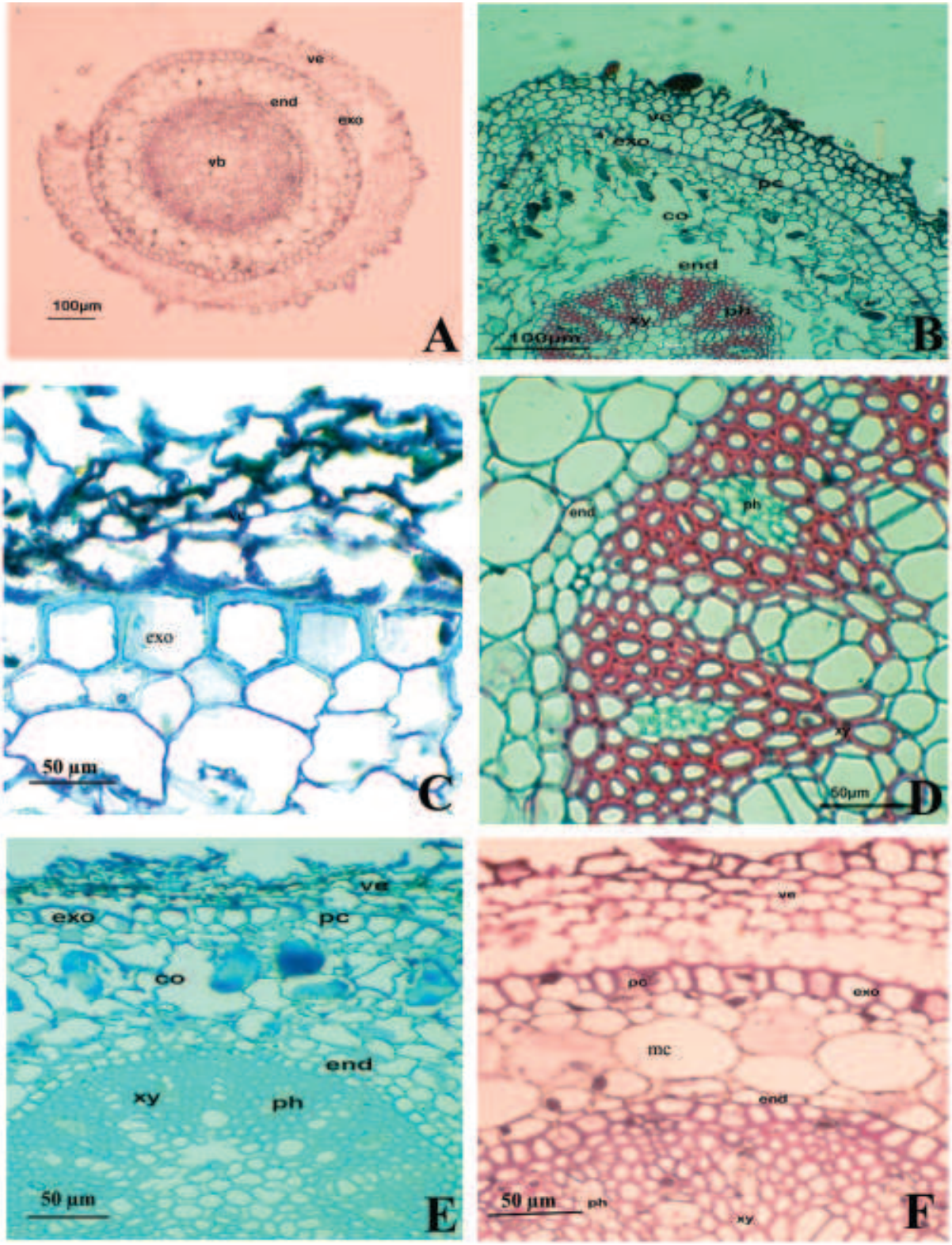

Fig. 1. A-F. Anatomical studies in *Coelogyne nervosa, C. flaccida, C. corymbosa, C. stricta,* and *C. ovalis* roots, by transverse cross sections: A, Root showing gross structure with cortex and vascular cylinder in *C. nervosa*; B, Root showing velamen, exodermis, cortex and endodermis in *C. flaccida*; C, Root showing epivelamen and exodermis and cortex in *C. corymbosa*; D, Root showing endodermis in *C. stricta*; E, Root showing cortex in *C. stricta*; F, Root showing velamen, cortex and endodermis in *C. ovalis.* (co, cortex; end, endodermis; exo, exodermis; mc, mucilage cavity; pc, passage cell; ph, phloem; vb, vascular bundle; ve, velamen; xy, xylem).

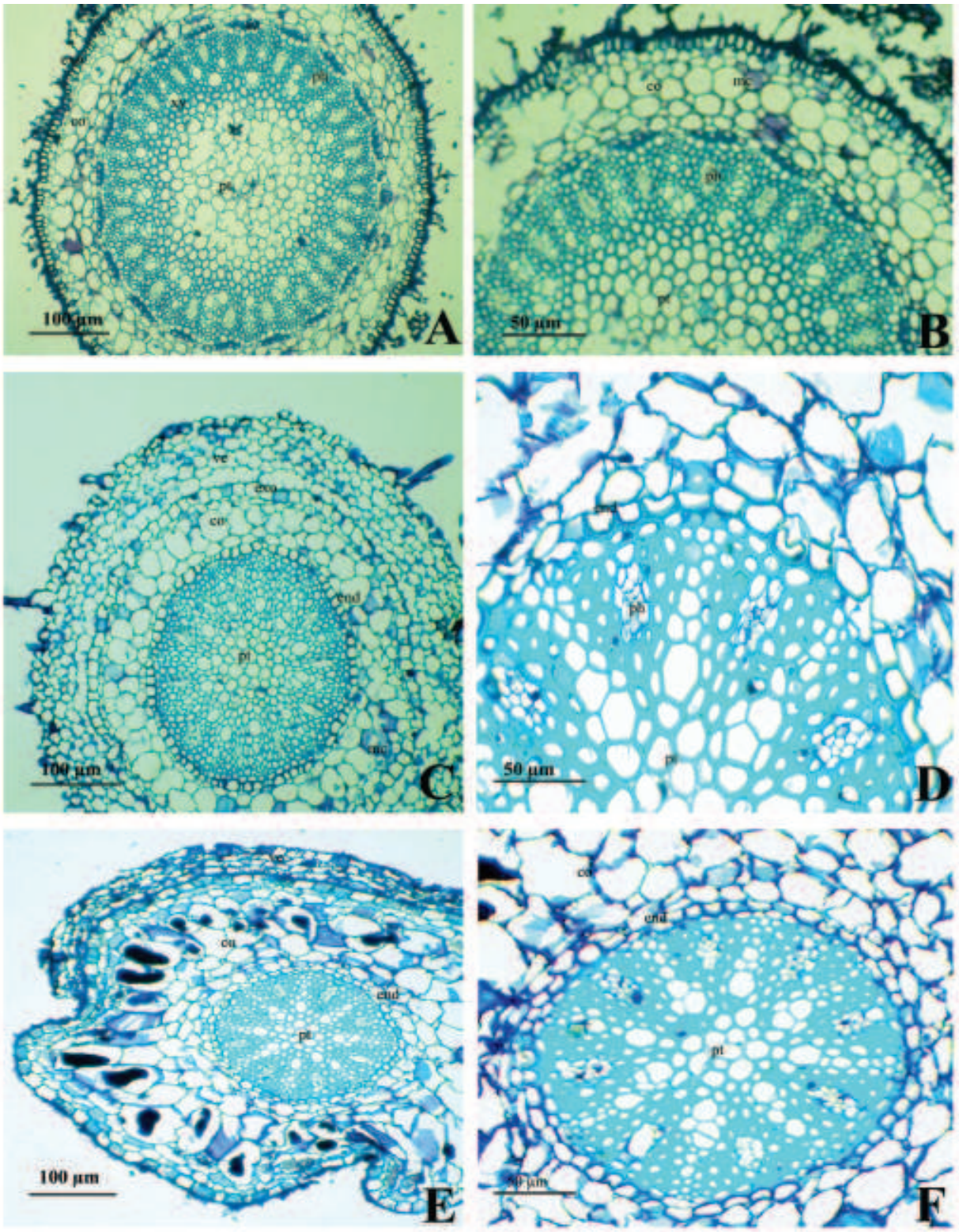

Fig. 2. A-F. Anatomical studies of *Pholidota pallida, Coelogyne breviscapa, C. nitida,* and *C. flaccida* roots, by transverse cross sections: A, Root showing gross structure with cortex and vascular cylinder in *Pholidota pallida*; B, Root showing exodermis, cortex and endodermis in *P. pallida*; C, Root showing epivelamen, exodermis, and cortex in *C. breviscapa*; D, Root showing cortex and endodermis in *C. breviscapa*; E, Root cross section showing cortex in *C. nitida*; F, Root showing velamen, cortex, and endodermis in *C. flaccida.* (co, cortex; end, endodermis; exo, exodermis; mc, mucilage cavity; pc, passage cell; ph, phloem; pt, pith; ve, velamen; xy, xylem).

layers of cortical cells were observed as hyaline and mucilaginous probably their role is related to perform water storage function (Fig. 1E). The endodermis cell walls were thickened; however, in *C. stricta*, the highest endodermal cell lignifications were observed (Fig. 1D). In vascular cylinder, phloem and xylem strands alternate with each other. In *P. pallida*, xylem had tracheids with helical thickenings (Fig. 2A, B). Vessel members and vessel like tracheids were abundantly observed in root macerations of *C. ovalis*. Pith was parenchymatous (Fig. 1F, Fig. 2F).

Discussion

All the presently investigated orchid species possessed velamen roots; the epidermis of mature root was multiseriate with velamen tissue. According to Benzing (1986, 1989a, b) and Dycus and Knudson (1957), the epiphytic roots may function in two ways i) If the velamen is modified by mechanical injury or after the aerial root becomes attached to a solid substratum, then the root may serve as an absorbing organ for water and nutrients; and ii) aerial roots that are totally exposed to air and provide mechanical strength to plant body. The velamen is formed of dead cells, which could be polygonal, elliptic or rectangular. According to Dahlgren and Clifford (1982), the velamen is also found in other monocots such as Araceae, Liliaceae, Dioscoreacee and Amarillidaceae. Epi-velamen, which differs from the inner velamen layers was also reported earlier by Khasim (1986) in *Cymbidium grandiflorum, C. mastersii,* and *Oberonia wightiana*, but it gets peeled off at maturity.

Fibrous mats (tilosomes) were completely absent in the roots of the presently studied taxa except their presence in the *Pholidota pallida.* Perusal of literature reports the presence of such mats in roots in the tribes Polystacheae, Dendrobieae and other subtribes of Epidendroideae (Khasim, 1986; Mohana Rao and Khasim, 1987b; Pridgeon *et al.*, 1983; Ramesh, 2014).

According to Oliveira and Sajo (1999) and Shushan (1959), the outermost layer of cortex, close to the velamen is differentiated as an exodermis. Exodermis comprises of two types of cells, i) larger cells, along with root axis, with thickened walls, without protoplast, and isodiametric; and ii) shorter cells with thin walls, known as passage cells with a dense cytoplasm and prominent nucleus. The longer lignified cells of exodermis protect the root cortex against dehydration while shorter cells, with thin walls, drive nutrients from velamen to root cortex (Dycus and Knudson, 1957; Oliveira and Sajo, 1999). In almost all presently investigated taxa, root exodermis comprised of 'O' shaped thickenings.

In the present species, cortex comprised of thin-walled oval to circular shaped chlorenchymatous cells of various sizes; the cortical layers close to the exodermis and endodermis were smaller than those of the central region. Occurrence of endotrophic mycorrhiza in the velamen and cortex is a regular feature in the family Orchidaceae (Leitgeb, 1864).

Endodermis was uniseriate in the roots of all the investigated taxa. It was made up of thick-walled protective cells and interrupted at protoxylem poles by thin-walled passage cells; the endodermal cells possessed 'O' shaped thickenings. Vascular cylinder comprised of pericycle, phloem, xylem and pith. Phloem strands alternate with xylem strands. Vessel members were reported in almost all taxa except *Pholidota pallida*. Carlquist and Schneider (2006) and Dahlgren and Clifford (1982) reported vessel members in several species of the Orchidaceae. According to

Table 1. List of orchid species studied for root anatomical studies.

Species	Place of collection and altitude	Habitat and host tree	Voucher Number
Coelogyne breviscapa Lindl.	Shevroy Hills, Yercaud (TN), 2000 m	Epiphyte and *Alnus nepalensis*	ANUH 1001
C. corymbosa Lindl.	Dodabetta, Ooty (TN), 2200 m	Epiphyte and *Mangifera indica*	ANUH 1002
C. flaccida Lindl.	Dodabetta, Ooty (TN), 2200 m	Epiphyte and *Castanopsis indica*	ANUH 1003
C. nervosa A. Rich.	Dodabetta, Ooty (TN), 1800 m	Epiphyte and *Schima wallichii*	ANUH 1004
C. nitida (Wall. ex D. Don) Lindl.	National Orchidarium,Yercaud (TN)	Epiphyte	ANUH 1005
C. ovalis Lindl.	Dodabetta, Ooty (TN), 2200 m	Epiphyte and *Terminalia bellirica*	ANUH 1006
C. prolifera Lindl.	Dodabetta, Ooty (TN), 2100 m	Epiphyte and *Terminalia alata*	ANUH 1007
C. stricta (D. Don) Schltr.	National Orchidarium,Yercaud (TN)	Epiphyte	ANUH 1008
Pholidota pallida Lindl.	Shevroy Hills, Yercaud (TN), 1800 m	Epiphyte and *Mangifera indica*	ANUH 1009

TN, Tamil Nadu.

Dahlgren and Rasmussen (1983), vessel types and their occurrence constitutes an important aspect for estimating evolutionary sequence and degree of advancement in monocots. According to Cheadle and Kosakai (1980), the presence of vessels in roots is considered to be more advanced than the rhizome, stem and leaf. Since all the investigated taxa were epiphytes, vessels were rarely observed, but very long tracheids and vessel-like tracheids were found to be present abundantly, in their vegetative parts.

Ecological Adaptations

Velamen found in the orchid roots is a dead tissue filled with air in the dry condition, giving the roots, the characteristic grey colour; this tissue is reported to act as a sponge, absorbing the moisture from atmosphere. In fact, the velamen stores water which is utilized by plant during dry conditions. The diversification of velamen character was also exemplified by the type of habitat and host tree on which both *Coelogyne* and *Pholidota* species were growing continuously. However, velamen was well developed in the species collected from Yercaud (Tamil Nadu) at an elevation of 1500 m than those collected from Dodabetta (Ooty, 2200 m). The present investigation further indicates that the Yercaud plant collections *i.e. C. breviscapa, P. pallida* growing in Eastern Ghats show more xeric elements than those of Ooty and Dodabetta collections, Western Ghats where luxuriant growth of orchids was found.

Root exodermis had 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 tissue. Benzing *et al*. (1983) reported that just like velamen, the exodermal thickenings in the roots causes reduction of water lost by transpiration. Thickenings of exodermal cell walls were highly developed in *P. pallida*, collected from Yercaud (Eastern Ghats, Tamil Nadu). Cortical cells with pitted thickenings were found in *C. ovalis*. These were earlier reported in *C. cristata* (Mohana Rao and Khasim, 1987a) and *Eria bicolor* (Isaiah *et al*., 1990). Bur and Barthlott (1991), however, described these cells as pseudovelamen cells. These cells provide mechanical strength to the plant body. Moreira *et al*. (2013) opined that the well developed velamen, distinct exodermis, and specialized thick-walled cortical cells are the characteristic features of epiphytic orchids.

Endodermis was interrupted by thin-walled passage cells at protoxylem poles. In all investigated taxa, endodermal cells were uniformly lignified ('O' shaped

Table 2. Root anatomical features in presently studied orchid species.

Species / Anatomical Feature	*Coelogyne breviscapa*	*C. corymbosa*	*C. flaccida*	*C. nervosa*	*C. nitida*	*C. ovalis*	*C. prolifera*	*C. stricta*	*Pholidota pallida*
Number of velamen layers	5	4	5	6	5	4	4	7	6
Exodermis cell size	32.12	33.32	34.01	30.08	34.04	31.87	29.85	31.90	33.46
Exodermis cell lignifications (in µm)	2.1	2.0	2.1	2.2	1.9	1.8	2.1	2.0	2.3
Cortex (number of layers)	7	6	7	7	5	6	6	6	6
Endodermis cell Thickness (in µm)	18.81	23.41	21.59	18.48	16.61	21.21	14.53	21.21	14.53
Endodermal cell lignifications (in µm)	12.11	13.22	12.91	11.11	13.00	15.99	9.08	16.44	9.20
Vascular cylinder (diameter; in µm)	316.02	499.9	452.05	5808	486.6	730.16	328.32	395.5	664.17
Number of protoxylem poles	10	9	8	9	10	19	8	9	14
Presence of Vessel members	+	+	+	+	+	+	+	+	-

(+), present; (-), absent.

thickenings). Species such as *Coelogyne nervosa*, *C. prolifera* and *Pholidota pallida* collected from Ooty and Dodabetta (Western Ghats) and Yercaud (Eastern Ghats), showed high lignification in exodermal cells, indicating thereby that not only habitat conditions (including altitude) but also the host tree supplying nutrients play a vital role for the survival of epiphytic taxa. Ramesh (2014) had also made similar observations in *Dendrobium anceps,* collected from Darjeeling and Sikkim Himalaya. However, detailed studies need to be made in inter-population diversity in relation to ecology, in many such orchid species so as to draw better conclusions.

In vascular cylinder of roots, the maximum number of protoxylem poles (protoxylem points) was observed in *C. ovalis* followed by *P. pallida*. Based on 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. Vascular cylinder was found to be polyarch in orchid roots. Xylem strands alternate with phloem. In most of the investigated species, fibre sheath was present around xylem and phloem. Sclerenchymatous pith in some of the species was merging with vascular sclerenchyma. Vessels members were present in all investigated *Coelogyne* species whereas these were absent in *Pholidota*. Carlquist and Schneider (2006) also reported vessels in other members of Epidendroideae. Cheadle (1942) reported vessels with both simple and sclariform perforation plates. However, in most of the cases, vessels were not reported but vessel-like tracheids were abundant. In this context, Kaushik (1983) opined that vessels must have eliminated due to development of other water storage mechanisms in the plant body.

It may be concluded that there is no generalized pattern in epiphytic orchids; not only the geographical conditions and type of habitat, but also the host-tree on which orchid grows, plays vital role in survivability of epiphytic orchids. Those orchids that are getting poor supply of nutrients by host plant undergo adaptations so as to survive under extreme environmental conditions (Kaushik 1983, Khasim and Ramesh, 2010; Ramudu *et al*., 2012).

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