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Root anatomical diversity in epiphytic Orchids: Taxonomic implications and adaptive significance

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ABSTRACT

Root anatomy is fundamental to both ecological adaptations and taxonomic understanding in Orchids, yet it has received far less attention than floral morphology. To address this gap, the present study investigates the root anatomical features of 20 epiphytic Orchid species representing 12 genera. Transverse sections were analysed both qualitatively and quantitatively, focusing on velamen, cortex, exodermis, endodermis, vascular tissue, pith composition, root hairs, mycorrhizal associations and crystalline idioblasts. Our results showed a significant variation among the studied species. The velamen ranges from a single layer to as many as nine layers, while the cortex ranges between three and thirty-one layers. Similarly, the vascular cylinders differed considerably, with the xylem pole varying from five to thirty-six. Furthermore, distinct differences in exodermal and endodermal thickening types, the presence of passage cells and the structure of pith highlight the underlying taxonomic diversity. Mycorrhizal pelotons and calcium oxalate crystals (raphides and druses) were recorded in several species, highlighting their functional roles in nutrient acquisition and defence. The multivariate analysis which includes Cluster Analysis and Principal Component Analysis showed strong associations between root anatomical traits. Species like *Aerides multiflora* and *Vanilla planifolia* are distinct due to their unique root anatomy. Overall, this study demonstrates that root anatomical traits not only underpin adaptive strategies to the epiphytic niche but also provide valuable diagnostic features for taxonomy and conservation of Orchids.

Key words: Epiphytic orchids, Root anatomy, Systematics, Multivariate analysis

1. INTRODUCTION

The Orchidaceae Juss. represents one of the largest and most diverse families among flowering plants, with more than 22,500 species under 779 genera distributed worldwide (Pineiro et al., 2019; Kuo et al., 2022). Within India, the family is particularly well represented comprising about 1,331 species, out of which 856 are recorded from the Himalayan region while 320 are from southern India (De and Medhi, 2014; Khasim et al., 2020). *Epidendroideae* is the largest subfamily within Orchidaceae, comprising over 21,000 species across 516 genera, 16 tribes and 28 subtribes. The majority of its members are epiphytic (Chase et al., 2003, 2015; Versiane et al., 2021).

The extraordinary floral diversity, ornamental appeal and commercial importance

of orchids are globally valued (Zhang et al., 2018). They exhibit remarkable variation in size, colour, scent and texture leading their cultivation into a highly profitable industry. Morphologically, orchids are typically perennial herbs with racemose inflorescences, sympodial stems and simple leaves (Sarachai et al., 2022).

Orchids can be epiphytic, terrestrial or mycoheterotrophic depending on their life form. Epiphytic types are most common, representing about 80% of orchid species (Chase et al., 2015; Ngugi et al., 2020). Epiphytic orchids are most abundant in tropical forests. They grow on host trees non-parasitically and play vital roles in ecosystem functioning (Diaz et al., 2010; De and Medhi, 2014; Djordjevic et al., 2022; Shah et al., 2022). They obtain nutrients from atmospheric sources like rain, mist, dust and organic debris since they do not have direct contact with soil (Zotz and Hietz, 2001; Favre-Godal et al., 2020). Epiphytic orchids exhibit specialised adaptations to survive under challenging environmental conditions. Among these are the presence of succulent leaves, thick cuticles, modified stomata, Crassulacean Acid Metabolism (CAM) photosynthesis and specialised aerial roots with velamen radicum (Zhang et al., 2018; Gamisch et al., 2021). Velamen is a multi-layered epidermis composed of dead cells. It is essential for water and nutrient absorption. They also provide mechanical support and reduce desiccation (Benzing et al., 1982; Hauber et al., 2020). Additionally, the cortex contains parenchymatous cells rich in chloroplasts and idioblasts further contributing to root function (Thangavelu and Ayyasamy, 2017; Joca et al., 2017; Bona et al., 2020).

Although the anatomy of orchid roots has been studied in particular species (Kaushik, 1983; Singh, 1986; Oliveira and Sajo, 1999; Moreira and Isaias, 2008; Pedroso-de-Moraes et al., 2012; Silva et al., 2015), interspecific variation among Himalayan epiphytic orchids remains poorly understood. Anatomical studies serve an vital role in orchid systematics. Despite species identification, they provide knowledge on evolutionary relationships and adaptive mechanisms of taxa (Thangavelu and Ayyasamy, 2017; Senel et al., 2019; Ravi et al., 2021). However, detailed anatomical studies of epiphytic orchids from the Himalayan region are still scanty. Thus, the present study investigates the root anatomy of 20 epiphytic orchid species belonging to 12 genera. Specifically, our study aims to: (i) document the interspecific root anatomical variations, (ii) identify traits with potential taxonomic importance and (iii) assess how these characters may contribute to adaptation in epiphytic habitats.

2. MATERIAL AND METHODS

Sample collection

The root samples from 20 epiphytic orchid species under 12 genera were collected from different forests of Darjeeling eastern Himalaya during September 2024 to March 2025. However, two species *Vanda cristata* Wall. *ex* Lindl., *Vanill*a *planifolia* Andrews. were collected from A.K. Sharma Botanic Garden, Ballygunge Science College, University of Calcutta, West Bengal. The collected specimens include *Aerides multiflora* Roxb., *Bambuseria bambusifolia* (Lindl.) Schuit., Y.P.Ng and H.A.Pedersen, *Bulbophyllum cirrhatum* (Lindl.) Hook.*f*., *Bulbophyllum hirtum* (Sm.) Lindl. *ex* Wall., *Bulbophyllum leopardinum* (Wall.) Lindl. *ex* Wall., *Coelogyne articulata* (Lindl.) Rchb.*f*., *Coelogyne barbata* Lindl. *ex* Griff., *Coelogyne corymbosa* Lindl., *Coelogyne cristata* Lindl., *Coelogyne flaccida* Lindl., *Coelogyne fuscescens* Lindl., *Coelogyne lancilabia* (Seidenf.) R.Rice, *Cymbidium aloifolium* (L.) Sw., *Cymbilabia undulata* (Lindl.) D.K.Liu and Ming H.Li, *Eria coronaria* (Lindl.) Rchb.*f*., *Oberonia caulescens* Lindl., *Pinalia spicata* (D.Don) S.C.Chen and J.J.Wood, *Thunia alba* (Lindl.) Rchb.f.., *Vanda cristata* Wall. *ex* Lindl. and *Vanill*a *planifolia* Andrews.

We referred to relevant literature to ensure the correct identification of the species (Hara, 1966, 1971; Ohashi, 1972; Hara et al., 1978; Pearce and Cribb, 2002) and maintained proper nomenclature following Plants of the World Online (POWO, 2025). Additionally, we sought assistance from the Lloyd Botanical Garden Herbarium and the Calcutta University Herbarium (CUH) for confirmation of the taxa. The herbarium specimens have been deposited at the Calcutta University Herbarium (CUH) for future study.

We collected aerial root samples and fixed them in FAA (formalin: acetic acid: ethanol 50% v/v in the ratio 10:5:50) following the methodology by Johansen (1940). Samples were preserved in 50% alcohol until further analysis. The preserved root samples were hand-sectioned (transverse sections) using a fine razor. Each samples were stained with different histology reagents to make it easier to identify thickenings and accurately recognise the cells. The different stains used were 1% safranin (Gurav et al., 2013) and Alcian blue 8GX (Tolivia and Tolivia, 1987). Sections were immersed in 1ml of the stain or reagents for 2–5 minutes, depending on the specimen. They were then washed with distilled water and passed through different alcohol concentrations (95–100%). Finally, the samples were mounted in 30% glycerine and examined them under a compound microscope for qualitative and quantitative observations.

Light Microscopy

To analyse both qualitative and quantitative traits, thin transverse sections of roots were examined under a compound microscope. Structural features recorded included the number of cell layers in the velamen and cortex, tissue areas, thickening patterns of the exodermis and endodermis, the number of xylem strands in the vascular bundle, root and stele diameter, stele tissue type, presence of mycorrhizal associations and occurrence of root hairs. We used a stereo microscope (Wild M3 Heerbrugg) and a binocular microscope (Leitz Laborlux D) to examine the specimen. Cellular dimensions were measured at suitable magnifications with ImageJ software and permanent slides were prepared using Canada balsam as the mounting medium.

Data Analysis

To understand the structural characteristics of roots, we took five repeated measurements for each trait. We then calculated the mean, standard deviation and standard error to analyse the data. In total, 25 quantitative and nine qualitative features were analysed separately. Quantitative data were subjected to hierarchical clustering to generate a dendrogram, while Principal Component Analysis (PCA) using Pearson's correlation was applied to assess relationships among taxa based on seven key traits. All statistical analyses were performed in R (R Core Team, 2013) and a dichotomous artificial key to the genera was developed in bracketed or parallel format.

3. RESULTS

Cross-sectional analysis revealed that the roots of all studied specimens had a circular outline. Root diameter varied widely with *Aerides multiflora* showing the largest and *Oberonia caulescens* the smallest. The highest number of velamen layers occurred in *Cymbidium*. Species of *Bulbophyllum*, *Aerides*, *Vanilla* and *Pinalia* exhibited a single-layered velamen while *Coelogyne* species displayed a broad range from one to nine layers (Table 1). Multilayered velamen (two to nine layers) was recorded across several taxa: bi-layered velamen in *Eria* and *Oberonia*; five layers consistently in *Cymbilabia* and *Vanilla*; and multiple layers in some *Bambuseria* and *Thunia* species (Fig. 1; Fig. 4).

Table 1. Root anatomical characteristics in the studied taxa

Species	Root hairs	Number of velamen layers	Exodermal wall thickening (shape)	Number of cortical layers (cells)	Endodermal wall thickening (shape)	Passage cells	Mycorrhizal fungi colonization	Number of xylem strands in stele	Stelar tissue
Aerides multiflora	–	1	U-type	27–31	O-type	endodermis	–	36	parenchymatous
Bambuseria bambusifolia	–	7–8	O-type	6–7	O-type	present in both	–	14	sclerenchymatous
Bulbophyllum cirrhatum	–	1	O-type	4–5	O-type	present in both	–	11	sclerenchymatous
Bulbophyllum hirtum	–	1	O-type	5–6	O-type	present in both	+	9	parenchymatous
Bulbophyllum leopardinum	+	1	O-type	5–6	O-type	Endodermis	+	16	sclerenchymatous
Coelogyne articulata	+	4–5	O-type	4–5	O-type	endodermis	–	9	sclerenchymatous
Coelogyne barbata	+	4–5	O-type	3–5	O-type	present in both	–	10	sclerenchymatous
Coelogyne corymbosa	+	1	O-type	3–4	O-type	present in both	–	12	sclerenchymatous
Coelogyne cristata	+	4	O-type	4–5	O-type	exodermis	+	9	sclerenchymatous
Coelogyne flaccida	+	1	O-type	4–5	O-type	present in both	–	13	sclerenchymatous
Coelogyne fuscescens	–	7–8	O-type	4–5	O-type	present in both	–	13	sclerenchymatous
Coelogyne lancilabia	–	5	O-type	6–7	O-type	endodermis	–	23	sclerenchymatous
Cymbidium aloifolium	–	9–10	O-type	13–15	O-type	present in both	+	16	parenchymatous
Cymbilabia undulata	–	3–4	U-type	17–20	O-type	present in both	–	13	sclerenchymatous
Eria coronaria	–	2	Ot-type	11–13	O-type	endodermis	+	11	parenchymatous
Oberonia caulescens	–	2	U-type	5–6	O-type	exodermis	+	5	sclerenchymatous
Pinalia spicata	+	1	U-type	4–5	O-type	present in both	+	11	parenchymatous
Thunia alba	–	8–9	O-type	4–5	O-type	present in both	+	17	parenchymatous
Vanda cristata	+	4–5	Ot-type	20–25	O-type	present in both	+	15	parenchymatous
Vanilla planifolia	+	1	U-type	15–20	O-type	endodermis	+	15	parenchymatous

Cortical cell layers also varied considerably among taxa ranging from 3 to 31. The maximum (27–31) was recorded in *Aerides multiflora*, while the minimum (3–4) occurred in *Coelogyne corymbosa* and *C. barbata*. Species of *Bulbophyllum*, *Pinalia* and *Thunia* consistently showed 4–6 layers. *Coelogyne* species also showed variation, some having 4–5 layers (*C. articulata*, *C. cristata*, *C. flaccida* and *C. fuscescens*) and others having 6–7 layers (*C. lancilabia*). *Bambuseria bambusifolia* exhibited 6–7 layers, *Oberonia* 5–6, *Eria* and *Cymbidium* 11–15 and *Cymbilabia undulata*, *Vanda cristata* and *Vanilla planifolia* 15–25 layers (Fig. 1).

All studied orchids exhibited a single-layered exodermis but the distribution of passage cells varied. In most species, passage cells occurred in both the exodermis and endodermis while in *Coelogyne cristata* and *Oberonia caulescens* they were restricted to the exodermis. Conversely, in *Aerides multiflora*, *Bulbophyllum leopardinum*, *Coelogyne articulata*, *C. lancilabia*, *Eria coronaria* and *Vanilla planifolia*, they appeared only in the endodermis. Cell wall thickening also showed diversity: O-type in *Bambuseria*, *Bulbophyllum*, *Coelogyne*, *Cymbidium* and *Thunia*; U-type in *Aerides*, *Cymbilabia*, *Oberonia* and *Vanilla*; and Ot-type in *Vanda* and *Eria*. Notably, *Pinalia spicata* showed sclerenchymatous thickening around the endodermis (Table 1).

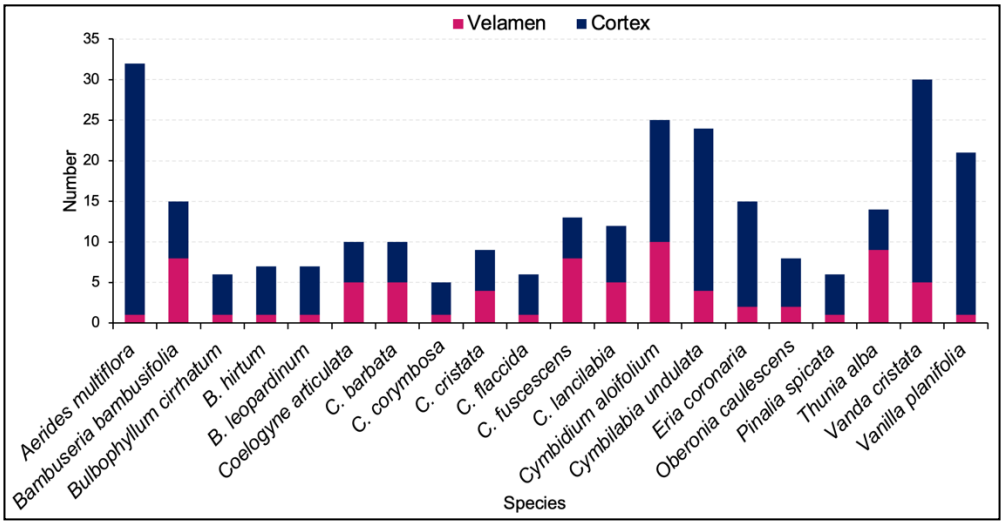

Figure 1. Graph showing the variation in the number of velamen and cortex cell layers

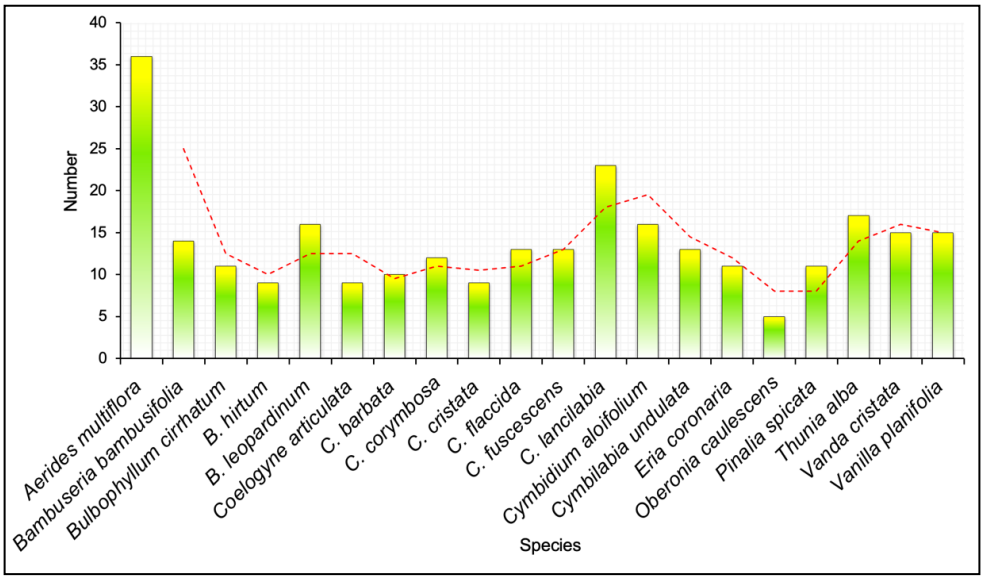

Figure 2. Graph showing the distribution of xylem strands among species

The vascular cylinder features varied among the species studied. The result showed the maximum number of xylem poles in *Aerides multiflora* (36 in number) followed by *Coelogyne lancilabia* (23) and *Thunia alba* (17). In contrast, *Oberonia caulescens* exhibited the lowest count with only five strands. *Bulbophyllum* (9–16) and *Coelogyne* (9–23) showed intermediate ranges while specific count observed in

other species: *Cymbidium aloifolium* (16), *Vanda cristata* and *Vanilla planifolia* (15 each) and *Eria coronaria* and *Pinalia spicata* (11 each) (Fig. 2).

Stele composition was also variable: parenchymatous pith occurred in *Aerides*, *Cymbidium*, *Eria*, *Pinalia*, *Thunia*, *Vanda* and *Vanilla* while sclerenchymatous pith was consistent in all *Coelogyne* species as well as *Bambuseria bambusifolia*, *Cymbilabia undulata* and *Oberonia caulescens*. *Bulbophyllum cirrhatum* and *Bulbophyllum leopardinum* have exsclerenchymatous tissue whereas parenchymatous tissue is found in *Bulbophyllum hirtum* (Fig. 5-6). Root hairs were present in particular species. All *Coelogyne* species possessed root hairs except *C. fuscescens* and *C. lancilabia*. Root hairs were also present in *Bulbophyllum leopardinum*, *Pinalia spicata*, *Vanda cristata* and *Vanilla planifolia* (Fig. 3).

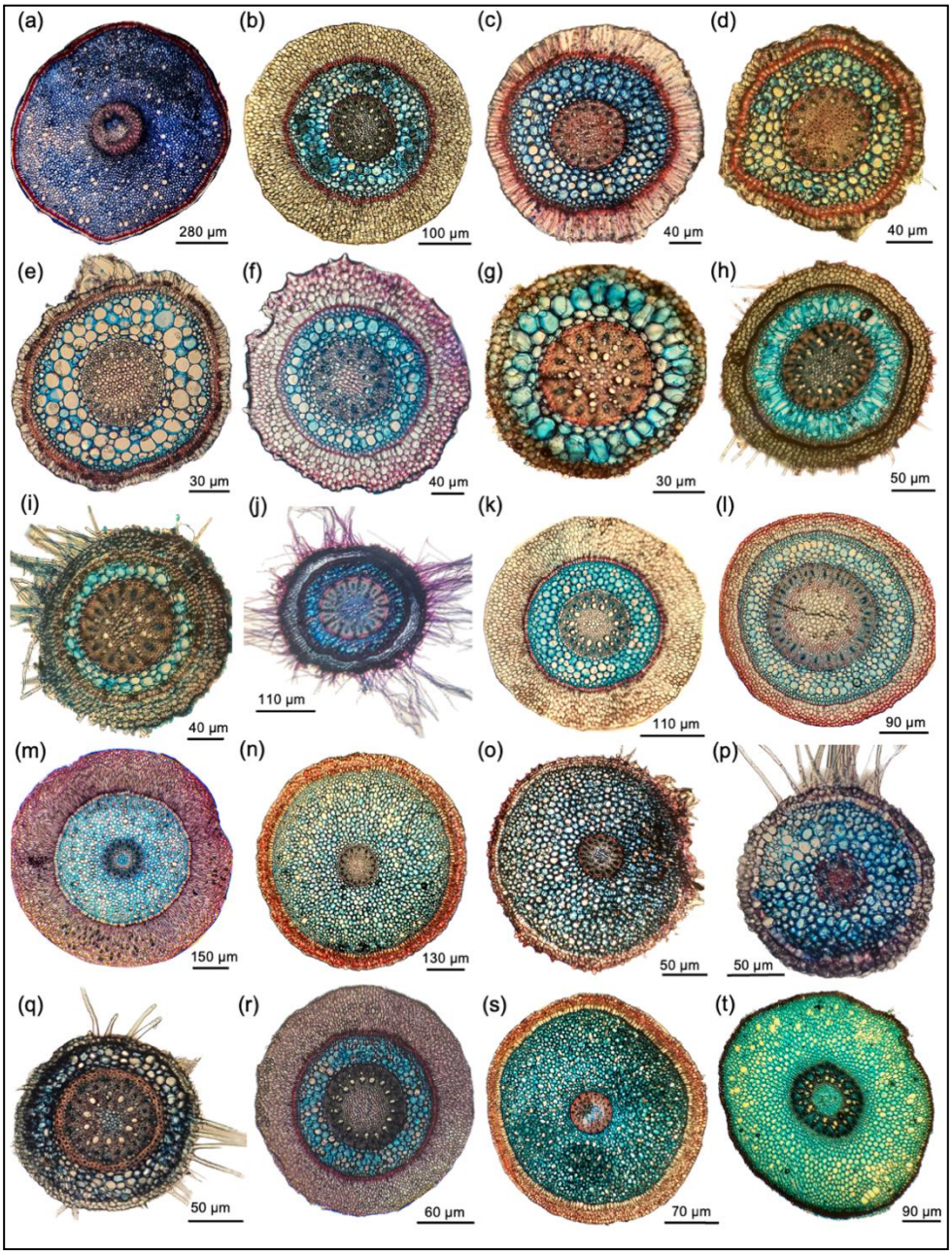

Figure 3. Cross-section of root; (a) *Aerides multiflora*; (b) *Bambuseria bambusifolia*; (c) *Bulbophyllum cirrhatum*; (d) *B. hirtum*; (e) *B. leopardinum*; (f) *Coelogyne articulata*; (g) *C. corymbosa*; (h) *C. cristata*; (i) *C. barbata*; (i) *C. flaccida*; (k) *C. fuscescens*; (l) *C. lancilabia*; (m) *Cymbidium aloifolium*; (n) *Cymbilabia undulata*; (o) *Eria coronaria*; (p) *Oberonia caulescens*; (q) *Pinalia spicata*; (r) *Thunia alba*; (s) *Vanda cristata*; (t) *Vanilla planifolia*

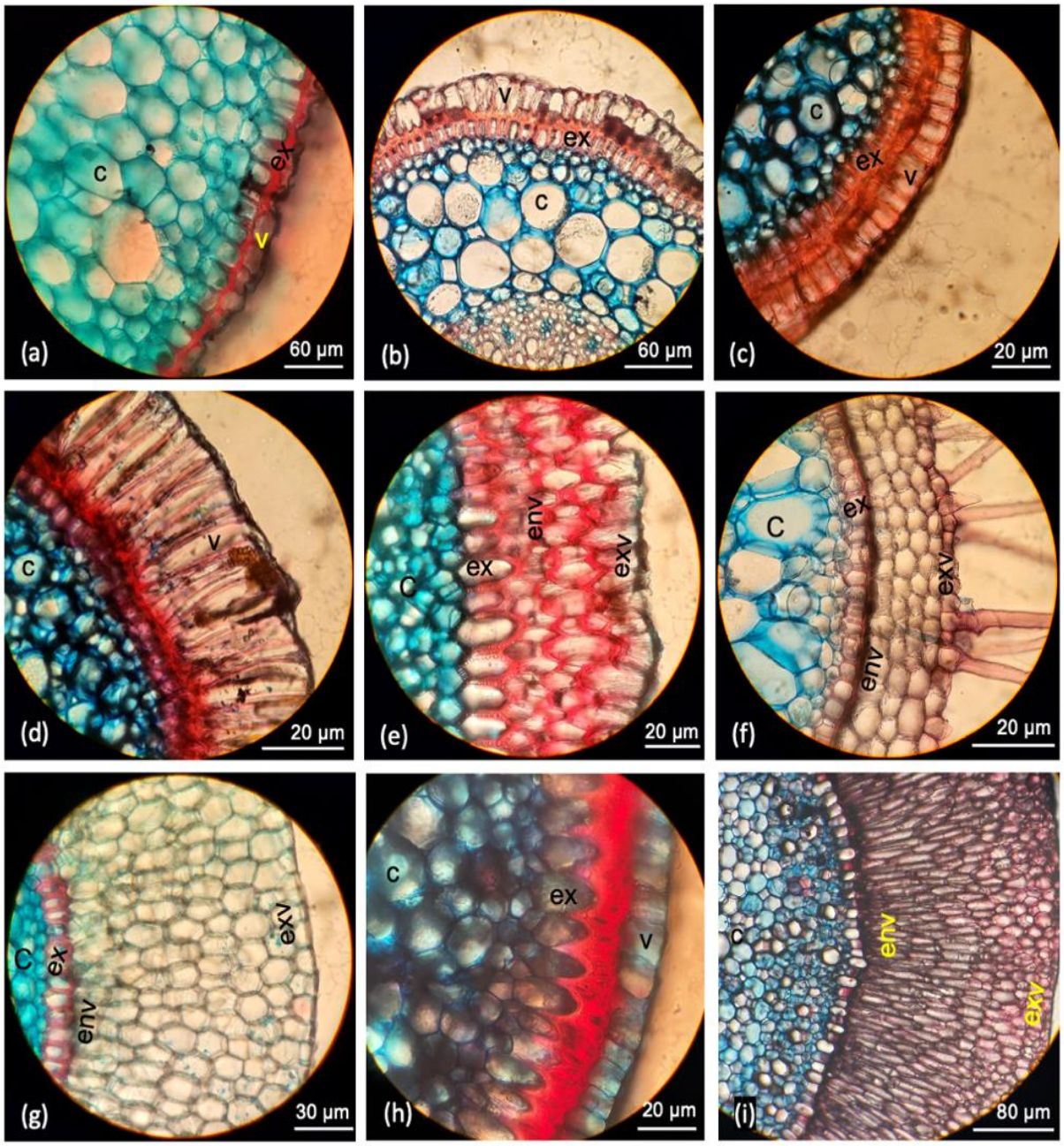

Figure 4. Variation in number of velamen cells; (a) *Vanilla planifolia*; (b) *Bulbophyllum leopardinum*; (c) *Coelogyne corymbosa*; (d) *Bulbophyllum cirrhatum*; (e) *Cymbilabia undulata*; (f) *C. cristata*; (g) *C. fuscescens*; (h) *Aerides multiflora*; (i) *Cymbidium aloifolium*

We observed mycorrhizal fungal pelotons in the cortical cells of several taxa including *Bulbophyllum hirtum, B. leopardinum, Coelogyne cristata, Cymbidium aloifolium, Eria coronaria, Pinalia spicata, Thunia alba, Vanda cristata* and *Vanilla planifolia* (Fig. 7). Additionally, *Vanilla planifolia* possessed crystals, raphides in *Coelogyne cristata* and druses in the velamen and cortex of *Eria coronaria* (Fig. 7).

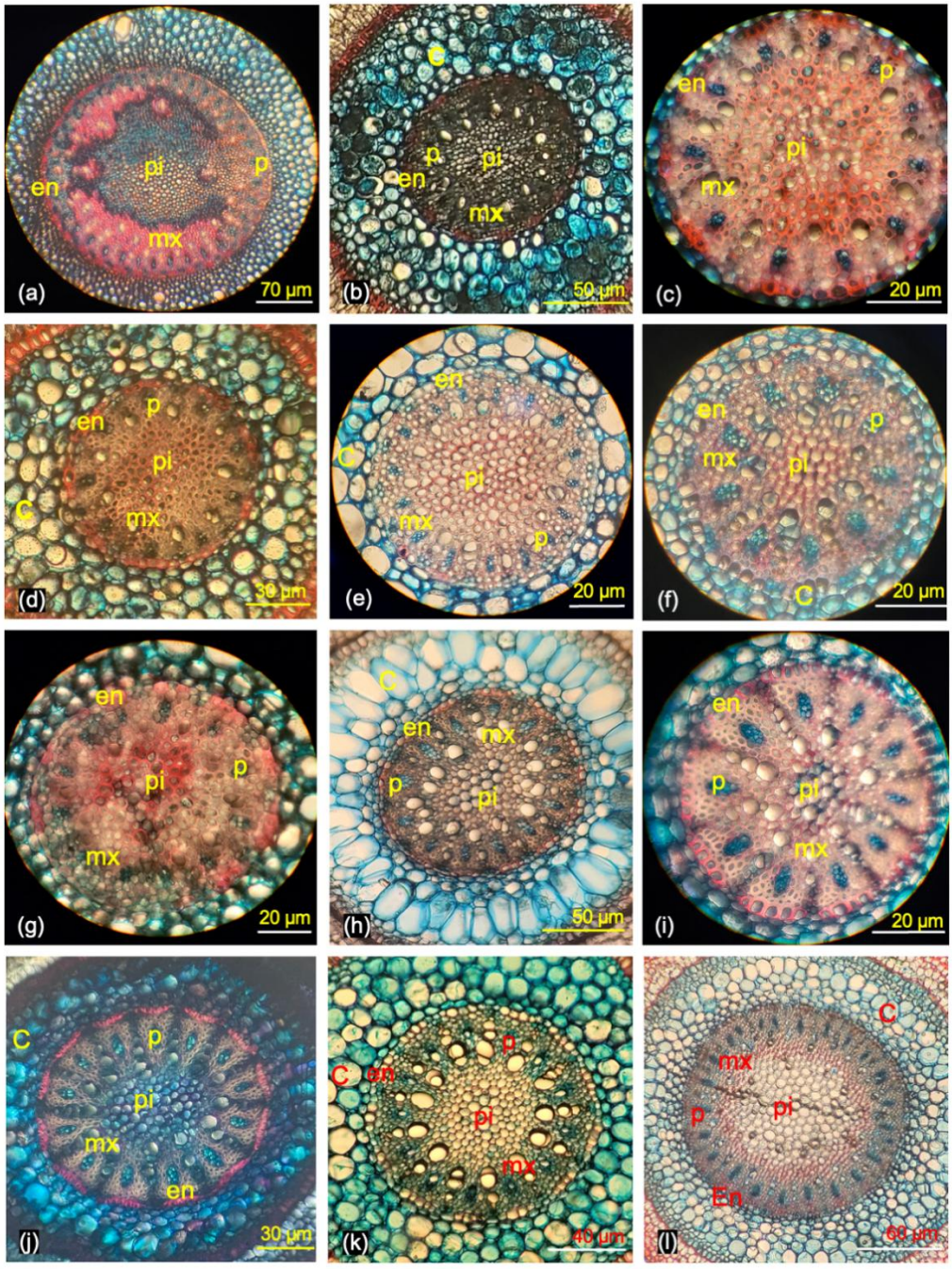

Figure 5. Stelar region; (a) *Aerides multiflora*; (b) *Bambuseria bambusifolia*; (c) *Bulbophyllum cirrhatum*; (d) *B. hirtum*; (e) *B. leopardinum*; (f) *Coelogyne articulata*; (g) *C. corymbosa*; (h) *C. cristata*; (i) *C. barbata*; (j) *C. flaccida*; (k) *C. fuscescens*; (l) *C. lancilabia*

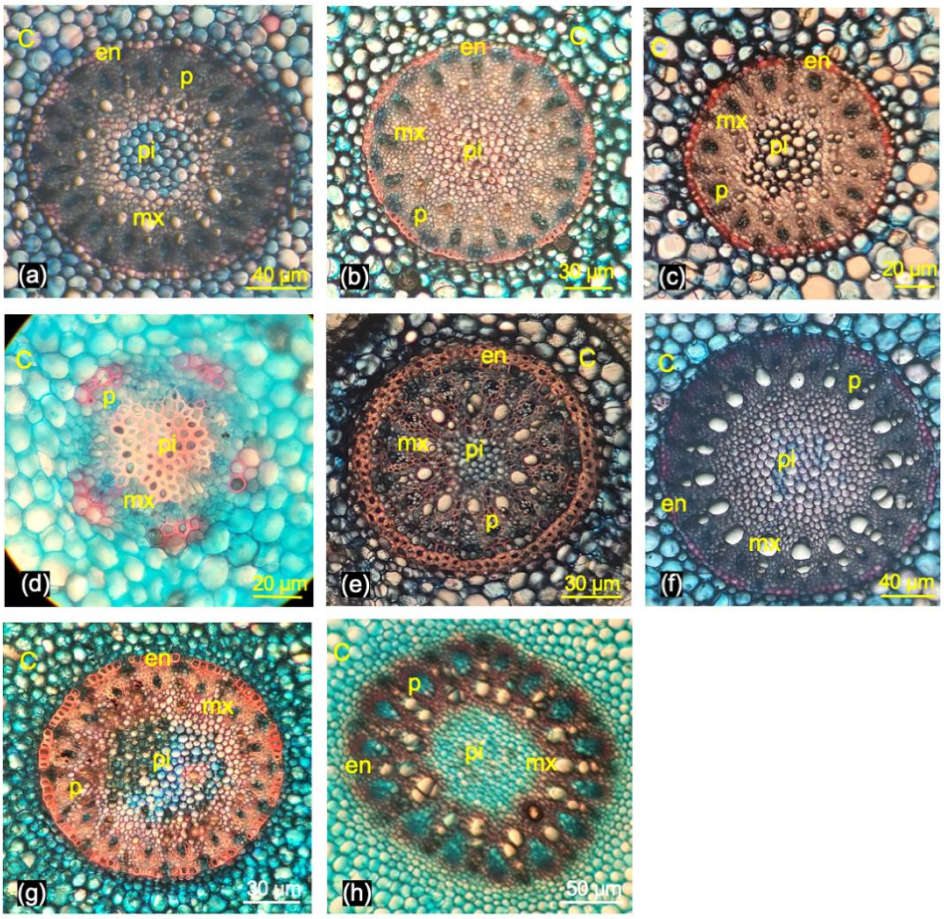

Figure 6. Stelar region; (a) *Cymbidium aloifolium*; (b) *Cymbilabia undulata*; (c) *Eria coronaria*; (d) *Oberonia caulescens*; (e) *Pinalia spicata*; (f) *Thunia alba*; (g) *Vanda cristata*; (g) *Vanilla planifolia*

Cluster analysis

Cluster analysis based on quantitative anatomical traits including root diameter, velamen layers, cortex, exodermis, endodermis, stele and pith dimensions and vascular bundle revealed two major clusters (Fig. 8). The first cluster separated into two sub-groups: *Aerides multiflora* formed an independent branch while *Cymbidium aloifolium*, *Cymbilabia undulata*, *Vanda cristata*, *Eria coronaria* and *Vanilla planifolia* grouped closely reflecting substantial similarity in root diameter and cortex area. These species were distinct from the second cluster which also split into two sub-groups. The first included *Thunia alba* clustered with *Coelogyne lancilabia*, *C. fuscescens* and *Bambuseria bambusifolia* sharing common anatomical traits. The second sub-group comprised intermixed taxa placed in proximity likely due to their comparatively minor root, stele and pith diameters.

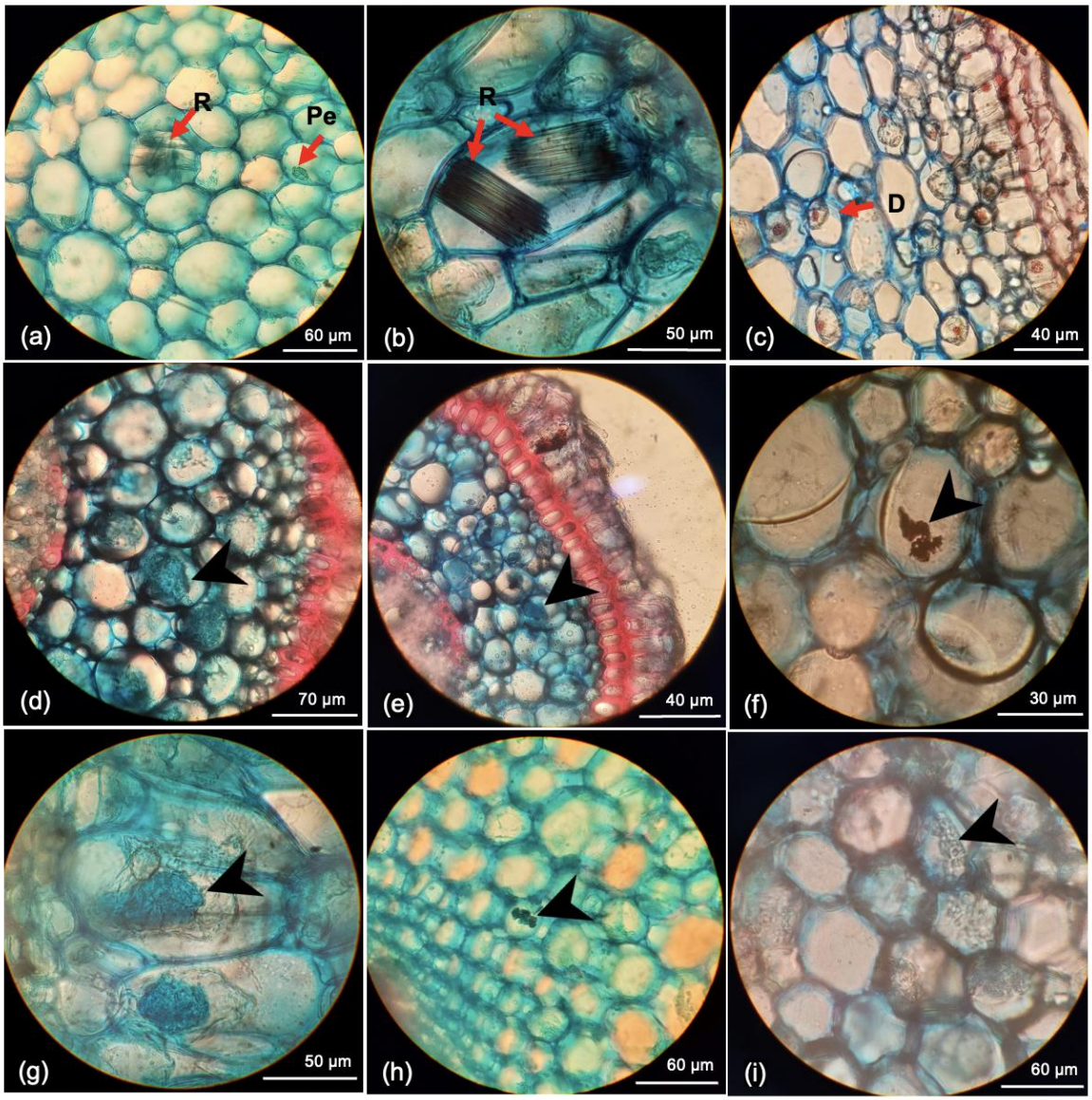

Figure 7. (a) Peloton and Crystals in *Vanilla planifolia*; (b) Raphide and peloton in *Coelogyne cristata*; (c) Druses in *Eria coronaria*; Peloton in (d) *Bambuseria bambusifolia*; (e) *Bulbophyllum hirtum*; (f) *Coelogyne corymbosa*; (g) *C. cristata*; (h) *Vanilla planifolia*; (i) *Oberonia caulescens*

Principal Component Analysis

We performed Principal Component Analysis (PCA) on seven key root anatomical traits to examine patterns of variation among the studied species (Fig. 9). The first two components accounted for the majority of variance with PC1 explaining 63% and PC2 16.4% both with eigenvalues >1. The Principal Component Analysis (PCA) highlighted strong trait associations. Root diameter, cortex area, exodermis length and breadth, stele area and pith diameter all showed high loadings on the first principal component (PC1). However, the velamen area was primarily aligned with the second principal component (PC2) (Table 2).

The biplot further illustrated these relationships with velamen area grouped alongside pith and stele diameter while cortex dimensions were grouped more closely with exodermal traits. In contrast, root diameter and velamen area were uncorrelated underscoring that velamen thickness does not scale with root size. Notably, *Aerides multiflora* and *Vanilla planifolia* separated distinctly from the other taxa reflecting their unique anatomical profiles.

Figure 8. Circular hierarchical cluster analysis (HCA) dendrogram grouping different species

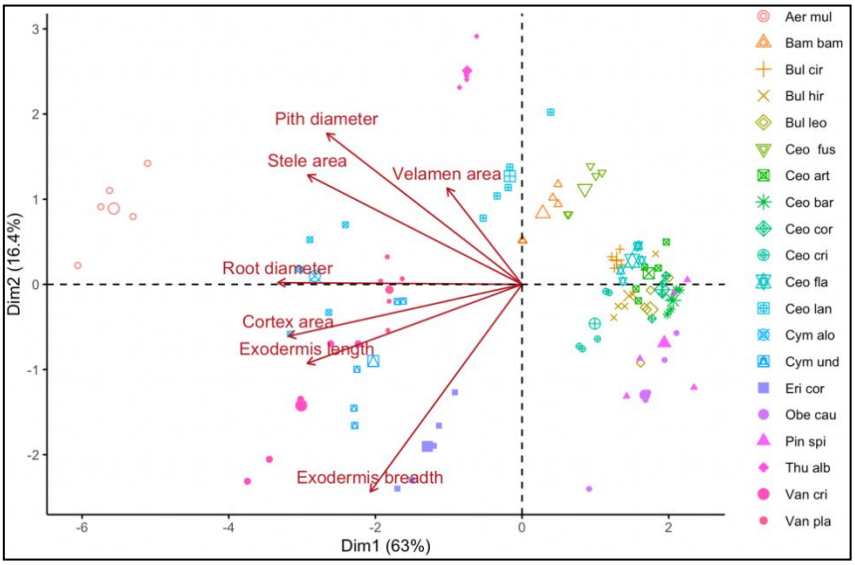

Figure 9. Principal Component Analysis biplot for PC1 and PC2 among the variables

Table 2. PCA loadings of root anatomical variables on the first four principal components

Variable	Principal Component (PC)			
	PC 1	PC 2	PC 3	PC 4
Root diameter	0.93	0.19	-0.10	0.08
Velamen area	0.04	0.73	-0.19	-0.24
Cortex area	0.32	-0.65	-0.18	-0.18

Stele area	0.15	0.00	0.77	-0.56
Pith diameter	0.08	0.06	0.55	0.70
Exodermis length	0.05	-0.03	-0.10	0.30
Exodermis breadth	0.02	-0.06	-0.12	-0.09
Standard deviation	2.10	1.07	0.98	0.50
Proportion of Variance	0.63	0.16	0.14	0.04
Cumulative Proportion	0.63	0.79	0.93	0.97

Key to the species based on the observed features

1a. Velamen single layered …….. ……..…..……..……..2

b. Velamen multi-layered ….…...…….……...……...8

2a. Root hair absent ……...…..…….…….3

b. Root hair present …….....…….…….4

3a. Stele with parenchymatous pith ……..……..…… *Bulbophyllum hirtum*

b. Stele with sclerenchymatous pith ……..…….…… *B. cirrhatum*

4a. Exodermal thickening O-type…….……..…5

b. Exodermal thickening U-type …….……..…..7

5a. Mycorrhizal colonisation present ……..…….. *B. leopardinum*

b. Mycorrhizal colonisation absent ……..…… 6

6a. Metaxylem length and breadth 30 µm and 16 µm …...……..…. *Coelogyne corymbosa*

b. Metaxylem length and breadth less than 30 µm and 16 µm …...……..…..*C. flaccida*

7a. Five cell layers in cortex ……..……...…… *Pinalia spicata*

b. More than five cell layers in cortex……..…..…… *Vanilla planifolia*

8 a. Root hair present ……..……...…….. 9

b. Root hair absent ……..…….…. 12

9a. Exodermal thickening O-type ……..……..……..10

b. Exodermal thickening Ot-type ……..…… *Vanda cristata*

10a. Number of xylem strands 9 ……..……..……11

b. Number of xylem strands more than 9 ……..……..... *Coelogyne barbata*

11a. Exodermis area 14 µm ……..……..…….....……..…… *C. articulata*

b. Exodermis area more than 14 µm ……..…… *C. cristata*

12a. Exodermal thickening O/U-type ……..…….. 13

b. Exodermal thickening Ot-type ……..…..…….. *Eria coronaria*

13a. Exodermal thickening U type; cortex cell 30 cell layered;

stele with parenchymatous pith ……...……..…… *Aerides multiflora*

b. Exodermal thickening O type; cortex cell less than 30 layered;

stele with sclerenchymatous pith ……..……..……... 14

14a. Cortical region 6 cell layered ……..……. *Oberonia caulescens*

b. Cortical region more than 6 cell layered ……..……..……. *Cymbilabia undulata*

15a. Velamen with more than 5 cell layers ……..…….. 16

b. Velamen up to 5 cell layers ……. *Coelogyne lancilabia*

16a. Number of xylem strands less than 15; sclerenchymatous pith ……..… 17

b. Number of xylem strands more than 15; parenchymatous pith ……..…… 18

17a. 5 cell layered cortex ……..……..……. *C. fuscescens*

b. 7 cell layered cortex ……..……..…… *Bambuseria bambusifolia*

18a. Cortical region with 5 cell layers ……..……. *Thunia alba*

b. Cortical region with 15 cell layers ……..……. *Cymbidium aloifolium*

4. DISCUSSION

The present study revealed striking anatomical variation in orchid roots particularly in the velamen which ranged from single to multi-layered forms. Multi-layered velamen with complex thickening is widely recognised as an epiphytic adaptation enhancing water storage and resilience to desiccation (Porembski and Barthlott, 1988; Benzing, 2008; Kowsalya et al., 2017). We recorded 4–6 layered velamen in all *Coelogyne* species, except *C. corymbosa* and *C. flaccida*. Although this result contrasts with earlier observations, our findings for *Bulbophyllum* and *Vanda* species are consistent with previous reports (Porembski and Barthlott, 1988). The presence of a single-layered velamen in *Bulbophyllum* is also consistent with Singh et al. (2021). These findings underscore the taxon-specific diversity of velamen structure reflecting both phylogenetic constraints and ecological pressures.

The velamen layer provides several ecological functions. For instance, it reduces moisture loss, mechanical stress and infrared radiation while immobilising nutrients from precipitation (Moreira and Isaias, 2008; Chomicki et al., 2015). The varied velamen features give an idea of habitat conditions. The single-layered velamen is most common in humid habitats while multi-layered velamen predominates in drier and more exposed areas (Sanford and Adanlawo, 1973; Sharma and Bhattacharyya, 2022). Thus, velamen not only functions as a protective and absorptive layer but also serves as an ecological indicator of a species' microhabitat.

Velamen wall striations are categorised into three types that vary among taxa (Sanford and Adanlawo, 1973). Type I is a parallel and unbranched type that was observed in *Thunia alba* and *Vanda cristata*. *Bambuseria bambusifolia*, *Coelogyne cristata*, *C. fuscescens* and *Cymbilabia undulata* showed Type II (branched and occasionally crossing). Similarly, type III thin, thread-like and crossing was found in *Cymbidium aloifolium*. The functional role of endovelamen thickenings is not clear. However, previous reports suggest that they are associated with water loss reduction (Benzing et al., 1982; Pridgeon et al., 1983).

The presence of spiral cortical thickenings in *Cymbidium aloifolium* was previously reported (Yukawa and Stern, 2002). However, no such feature was observed in the present study. As per Leroux et al. (2011), these thickenings may have developed from nutrient and moisture deficiency. Furthermore, passage cells were consistently present in both exodermis and endodermis facilitating the nutrient transport from velamen to cortex (Dycus and Knudson, 1957; Oliveira and Sajo, 1999). They also serve as entry points for mycorrhizal fungi (Senthilkumar et al., 2000; Nurfadilah et al., 2016).

In addition, O-type exodermal thickenings were uniform among most taxa except in *Vanilla planifolia* which exhibited U-type thickenings. Both exodermis and endodermis contribute to apoplastic barriers regulating water and solute movement (Schreiber and Franke, 2011). Our study is consistent with earlier studies where endodermal cells in *Bulbophyllum* and *Coelogyne* are O-thickened, lignified and interrupted only by passage cells (Singh et al., 2019; Ramudu and Khasim, 2020). The observed suberin thickenings provide a mechanical strength and water storage (Olatunji and Nengim, 1980). These anatomical modifications collectively strengthen orchid roots against bot hydraulic stress and mechanical strain, ensuring survival in variable environments.

The vascular cylinder was predominantly polyarch. As per the classification proposed by Rosso (1966), only *Oberonia caulescens* is classified into the ≤8 protoxylem poles category. The remaining other species had 9–23 protoxylem poles exceeding the previous report for *Coelogyne* (Ramudu and Khasim, 2020). A positive correlation between root diameter and protoxylem pole number was observed in *Aerides multiflora* with the widest roots and the most significant number of xylem arches (Rutter and Stern, 1994). However, root diameters of *Coelogyne barbata* are relatively narrow with a high number of protoxylem poles. Similarly, there is a variation in the pith composition among species. For example, *Vanda cristata* showed a parenchymatous pith which is consistent with the previous finding

(Kowsalya et al., 2017). *Vanilla planifolia* also showed parenchymatous pith (Thangavelu and Ayyasamy, 2017). However, *Bulbophyllum* has both parenchymatous and sclerenchymatous pith (Sharma and Bhattacharyya, 2022).

Species like *Bambuseria bambusifolia*, *Bulbophyllum hirtum*, *Coelogyne corymbosa*, *C. cristata*, *Oberonia caulescens* and *Vanilla planifolia* appeared with mycorrhizal associations. Such symbioses are central to orchid ecology, supporting seed germination and nutrient acquisition (Zhang et al., 2018). While many epiphytes retain mycorrhizal dependence, some tropical orchids gradually lose this reliance at maturity (Smith and Read, 2008). Our observations suggest the importance of the mycorrhizal relationship in shaping life history and ecological success.

In addition, idioblastic cells containing raphides or druses were recorded in *Vanilla planifolia*, *Coelogyne cristata* and *Eria coronaria*. These calcium oxalate crystals which are common across Orchidaceae function as a structural defence against herbivory (Konno et al., 2014; Mani et al., 2021). The presence of such features shows how anatomical adaptations can mediate the biotic interactions in natural environments. Vessels were absent in all the species examined. Interestingly, their presence was previously reported in orchid roots (Dahlgren and Clifford, 1982). The absence of vessels suggests a conservative hydraulic strategy of epiphytic orchids. It also highlights that orchids adapt to their environment through special structural features.

5. CONCLUSION

This study highlights the remarkable anatomical diversity in the roots of epiphytic Orchids. The observed variations in velamen stratification, cortical patterning, vascular cylinder and pith structure reflect the adaptive strategies to epiphytic conditions. These also serve as informative taxonomic characters. The presence of passage cells, mycorrhizal associations and calcium oxalate crystals emphasises the multifunctional nature of orchid roots in water regulation, nutrient uptake and survival under environmental stress. Moreover, these features illustrate how structural innovation contributes to the ecological success and evolutionary persistence of orchids. This also provides valuable insights for taxonomy and conservation on this diverse plant family.

Authors contributions

PR: Methodology, Writing – original draft; KB: Investigation. SM: Conceptualisation, Supervision, Writing – review and editing.

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Conflict of Interest

The authors declare that they have no conflicts of interests, competing financial interests or personal relationships that could have influenced the work reported in this paper.

Informed consent

Not applicable.

Ethical approval & declaration

In this article, as per the plant regulations followed in the Department of Botany, University of Calcutta, 35, B.C. Road, Kolkata-700019, West Bengal, India; the authors observed the Root anatomical diversity in epiphytic Orchids were collected from A.K. Sharma Botanic Garden, Ballygunge Science College, University of Calcutta, West Bengal. The ethical guidelines for plants & plant materials are followed in the study for species observation, identification & experimentation.

Data and materials availability

All data associated with this study will be available based on the reasonable request to corresponding author.

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