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Vegetative anatomy and mycorrhizal morphology of *Schoenorchis nivea* (Lindl.) Schltr., (Orchidaceae) and their adaptive significance

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ABSTRACT   The anatomical description of the vegetative parts (leaf, leaf sheath, stem and root) and mycorrhizal morphology of *Schoenorchis nivea* (Lindl.) Schltr., belonging to the subfamily Epidendroideae of Orchidaceae was investigated. Leaves were amphistomatic covered by 10-12 μm thick cuticle, stomata paracytic with small and irregular substomatal chambers. Mesophyll homogenous, composed of thin-walled chlorenchymatous cells. Banded water-storage cells abundant in the mesophyll and the largest vascular bundle occurred at the centre of the leaf. The leaf sheath has both adaxial and abaxial epidermis covered with cuticle, homogenous mesophyll, water-storage cells, raphides and vascular bundles. The stem is surrounded by a uniseriate epidermis, cortex consisting of thick-walled fibers and collateral vascular bundles scattered in the ground tissue. Cortical proliferation was evident in *S. nivea* stem. Root hairs present in root regions were in contact with the substratum. Root hairs frequently branched at their tips. Root possess 2-3 layered velamen, ∩-thickened exodermal cells, O-thickened uniseriate endodermis, and cortex of thin-walled parenchymatous cells containing raphides and water-storage cells. Cover cells present. Xylem arches are 9-11, with vascular tissues embedded in sclerenchymatous cells. Pith composed of thick-walled sclerenchymatous cells with intercellular space. The stomatal characteristics in leaf, the size of water-storage cells and vascular bundles exhibited significant variation in different plant parts. Intact and degenerating pelotons of orchid mycorrhizal fungi were observed in the root cortical cells. The observations of the present study clearly indicate that *S. nivea* possesses several anatomical adaptations to thrive in epiphytic habitats.
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Introduction

The plant family Orchidaceae with approximately 25000 species distributed in 780 genera is one among the largest plant families in angiosperms (Pridgeon et al. 2009). Orchids occur in a wide range of habitats with different life forms like terrestrial, epiphytes or lithophytes (Dearnaley 2012). Generally, epiphytic habitats are stressful due to the lack of substrate that can sufficiently hold moisture and provide nutrient essential for plant growth (Adhikari et al. 2012). Nevertheless, plant species belonging to different families have adopted several strategies to tolerate and thrive in the harsh epiphytic habitats. One such adaptation of the orchids to the epiphytic conditions is the development of multi-layered dead tissue covering the roots called velamen that helps in the acquisition and storage of water under limiting conditions (Benzing 2000).

Another unique characteristic of orchids is the mutualistic association with mycorrhizal fungi. Orchids, unlike other graminaceous members, are obligately mycorrhizal either throughout or part of their lifecycle. The dependence of orchids on mycorrhizal symbiosis arises from the fact that orchids produce minute seeds that have minimal or lack seed reserves (Arditti and Ghani 2000). Mycorrhizal fungi provide essential nutrients to the orchids from the soil or substrates needed for their growth (Smith and Read 2008, Nurfadilah et al. 2013). Orchid mycorrhiza is unique by the production of highly coiled fungal hyphal structures in the root cortical cells called pelotons. These pelotons enlarge the contact surface area between fungus and the cells, but are short-lived, senesce and collapse within the host cells (Smith and Read 2008). It is presumed that green orchids during their adult stage are less mycorrhizal dependent than during their earlier stages. However, this claim was refuted in many recent

studies where several mature green orchids were shown to adopt a mixotrophic mode of nutrition (Bertolini et al. 2014; Selosse and Martos 2014; Lallemand et al. 2019). Mycorrhizal morphology in roots of epiphytic orchids is not well studied than those of terrestrial forms despite more than 70% of orchids have an epiphytic life-form (Atwood 1986; Sathiyadash et al. 2012).

Commonly known as flea orchids, *Schoenorchis* belongs to the subtribe Aeridinae of tribe Vandeae, subfamily Epidendroideae of the plant family Orchidaceae. At present, there are 25 species of this genus distributed specifically in the subtropical and tropical Asia and the Western Pacific. In India, *Schoenorchis* is represented by five species of which *Schoenorchis nivea* (Lindl.) Schltr., is a small (up to 15 cm tall) epiphytic monopodial orchid distributed in peninsular India and Sri Lanka (Kumar and Sequiera 2000; Mathew and George 2015). Although *S. nivea* has no recorded economic importance, it is listed in the CITES Appendix II where trade on this species is stringently regulated to prevent utilization irreconcilable with its survival (Mathew and George 2015; ENVIS Centre on Floral Diversity 2019).

Anatomical investigations of orchids assist in resolving the problems related to the identification of plants in their vegetative stage (Kowsalya et al. 2017; Balachandar et al. 2019). Anatomy also helps to overcome the systematic problems and is a useful tool in the precise identification of plants in same genera sharing similar morphological characteristics (Kowsalya et al. 2017). Though detailed observation of vegetative anatomy of Aeridineae taxa like *Acampe, Amesiella, Chiloschista, Microtatorchis, Neofinetia, Phalaenopsis, Taeniophyllum, Trichoglottis,* and *Vanda* are available (Carlsward et al. 2006; Carlsward 2014; Kowsalya et al. 2017) the vegetative anatomy of members belonging to *Schoenorchis* are unknown except for a couple of studies involving foliar and nectary anatomical investigations (Stpiczyńska et al. 2011; Angela et al. 2015). The leaf anatomy of *Schoenorchis gemmata* (Lindl.) J. J. Sm., along with five other terete-leaved orchids was examined by Angela et al. (2015) to see if these orchids exhibited any xeromorphic characters. The leaves of *S. gemmata* were amphistomatic and the stomata were of a paracytic type. Moreover, the leaves were broadly V-shaped, cuticle ridged, adaxial cuticle thicker than the abaxial, presence of cuticular papillae, epidermal cells spherical to polygonal, presence of specialized water-storage cells with banded thickenings, small and large vascular bundles distributed together, and sclerenchymatous tissue covering both xylem and phloem. From these results, it was concluded that *S. gemmata* like all other terete-leaved orchids examined exhibited xeromorphic characters that were directed towards efficient use of water for survival in water stressed environments (Angela et al. 2015). A study

on the anatomy of the nectary spurs in the entomophilous flowers of *S. gemmata* pollinated by hymenoptera indicated the absence of secretary trichomes, and a very short and saccate spurs with glabrous to a minutely papillose inner surface (Stpiczyńska et al. 2011).

As there is paucity of information on the anatomy of *Schoenorchis* in general we examined the anatomy of the vegetative structures of *S. nivea* growing in the Western Ghats region of southern India. Moreover, we also determined the various adaptive characters exhibited by *S. nivea* and recorded the extent of variation in similar structures occurring in different parts of the plant. In addition, the mycorrhizal morphology and the extent of mycorrhizal association were also studied in *S. nivea*.

Materials and method

Plant samples of *S. nivea* were collected during October 2018 from Indian Cardamom Research Institute campus (09°05′N and 77°09′E) located at the Myladumpara of Idukki district under Kerala state, peninsular India. The campus located at an altitude of 1050–1060 m a.s.l., spreads over an area of 64.60 ha with undulating terrain consisting of hills and valleys covered by lush evergreen forests (Balan and Harikrishnan 2017). This region is characterized by a cool and humid climate with an average maximum and minimum temperature of 25.70 °C and 17.05 °C, respectively. The hottest days occur during April-May and the coolest days occur between December-January. The relative humidity ranges from 90% to 96% and the average annual precipitation is 2153 mm. The rainfall data for 2007–2016 indicate that the amount of rainfall received consistently decline in the alternate years (Balan and Harikrishnan 2017). The floristic diversity of the site indicates the presence of 392 angiospermic taxa in 303 genera under 94 families consisting of both indigenous and naturalized flora. This angiospermic diversity also includes 15 orchids belonging to *Aerides, Bulbophyllum, Cottonia, Dendrobium, Disperis, Eria, Liparis, Luisia, Oberonia, Papilionanthe, Polystachya, Schoenorchis, Sirhookera, Thelasis* and *Trias* (Balan and Harikrishnan 2017).

Four mature plants of *S. nivea* were collected of which one plant was in late flowering phase. The specimen in flowering was used for authentication. The specimens were authenticated by the Botanical Survey of India, Southern Circle, Coimbatore, India and a voucher was deposited in the Bharati Herbarium, Department of Botany, Bharathiar University, Coimbatore, India (accession number: 007740). The plant samples were placed in an icebox and transported to the laboratory. In the lab, plants were washed thoroughly free of dirt and attached debris prior to sectioning. For uniformity, fully opened fourth
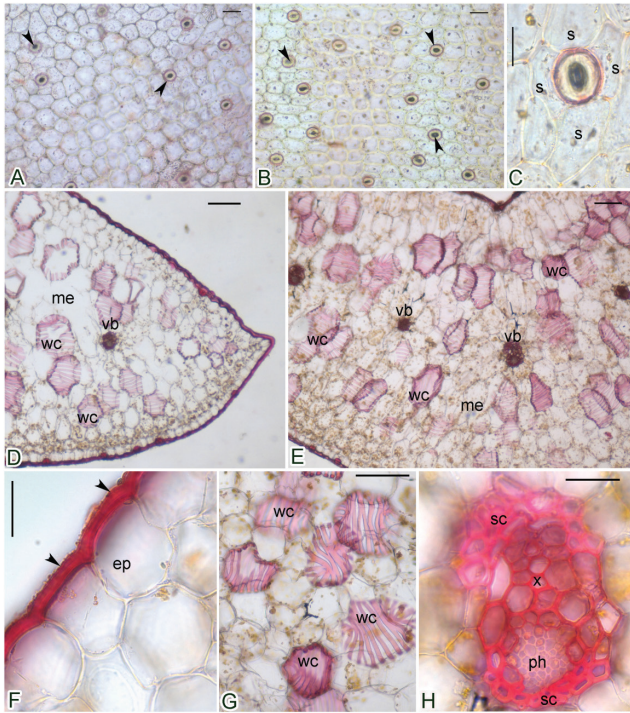
Figure 1. Leaf anatomy of *Schoenorchis nivea* (A,B) Epidermal peeling of leaf showing stomata (black arrow heads) on the adaxial (A) and abaxial (B) surface (C) stomata with guard cells and subsidiary cells; (D–E) cross section of leaf showing epidermis, mesophyll , vascular bundle and water- storage cells; (F) epidermis with cuticle (black arrow heads); (G) banded water-storage cells; (H) vascular bundle with phloem and xylem capped by sclerenchymatous cells.
ep = epidermis, me = mesophyll, ph = phloem, s = subsidiary cells, sc = sclerenchymatous cells, vb = vascular bundle, wc = water-storage cells, x = xylem. Scale bars = 50 μm (A, B, D, E, G) and 30 μm (C, F, H).

leaf from the tip, 1-cm stem or root from 3 cm below the shoot or root tip was used in the study. The transverse sections for histological observations were prepared by freehand sectioning (Lux et al. 2005). The sections of desired plant parts were made using smooth strokes with a sharp razor blade and transferred onto a drop of water on a microscopic slide. The water was later removed using a filter paper and various types of stains such as safranin, Iodine-Potassium iodide, Phloroglucinol-HCl, and Tolu-dine Blue O were used for identifying cell inclusions and other compounds such as cutin, lignin, starch grains, and polysaccharides (Johanson 1940). For the observation of epidermal surface, 1 cm square leaf pieces were placed in Jeffrey's maceration solution for 72 hours at 35 °C (Kigkr 1971). The specimens were later washed, stained with safranin and examined under an Olympus BX51 light microscope (Olympus, Tokyo, Japan). Cellular details like the cuticle thickness, length and width of different types of cells, and vascular bundles were measured us-

ing a calibrated ocular micrometer. The stomatal index (SI %) was calculated by using the formula $(S/S+E) \times 100$ according to Salisbury (1927) where S and E denote the number of stomata and epidermal cells. The stomatal type was determined according to Van Cotthem (1970).

For estimation of mycorrhizal colonization, the roots were cut into 1 cm long bits, cleared by boiling in 2.5% KOH at 100 °C for 60 minutes. The cleared roots were washed and treated with 5 N HCl for 30 minutes. The acidified roots were stained by placing them in trypan blue (0.05%, w/v) solution overnight (Koske and Gemma 1989). Squashes of stained root bits were prepared and observed for the presence orchid mycorrhizal fungal structures. McGonigle et al. (1990) magnified intersec-tion method was used to calculate the percentage of total root length colonization and root length containing intact and degenerating pelotons. The pelotons were considered intact when the fungal hyphae were undamaged and degenerating when the hyphae were damaged or were a crumbling mass.

To determine the extent of variation in structures occurring in different plant parts we performed statis-tical analysis [t-test or Analysis of Variance (ANOVA)] using SPSS version 9.0 for windows (SPSS Inc., Chicago, USA). The data were examined for homogeneity prior to statistical analysis (Levene's test) and Post Hoc analysis (Duncan's Multiple Range Test) was performed when the Fishers values were found significant. Box plot analysis was performed for assessing the extent of mycorrhizal colonization. The values presented in tables as mean.

Results

Leaf

Leaves are fleshy and succulent. Stomata are of paracytic type observed on both the leaf surfaces with two subsid-iary cells parallel to the guard cells (Fig. 1 A-C) The abaxial leaf surface had significantly more stomata (19-21 mm^{-2}) than the abaxial surface (42-47 mm^{-2}). The stomatal index (SI %) on the abaxial surface (8.62%) was significantly higher than the adaxial surface (5.51%). Similarly, the length and width of guard cells (0.87% and 18.92%) and stomatal pores (47.05% and 43.05%) were significantly greater on the adaxial surface than abaxial leaf surface (Table 1). Cuticle ridged, 10-12 μm thick, present on both adaxial and abaxial surfaces (Fig. 1D). The thickness of the cuticle was 11.59% higher on the adaxial than the abaxial surface. Cells of adaxial and abaxial epidermis were compactly arranged. Cell wall on the outer tangential surface much thicker than the other walls. The epider-mal cells on the adaxial surface were 25.03% longer and 27.77% wider respectively when compared to the abaxial

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surface of the leaf (Table 1). Substomatal chambers small and irregular. Hypodermis and fiber bundles absent. Mesophyll cells: homogenous, chlorenchymatous, and composed of thin-walled, elongated to oval-shaped cells (Fig. 1D-E). Banded water-storage cells present in the mesophyll (Fig. 1G). Bundle sheath indistinct. Vascular bundles oriented towards the upper epidermis. Largest vascular bundle present in the centre of the leaf. Xylem and phloem are covered by sclerenchymatous cells (Fig. 1H). The walls of sclerenchymatous cells enclosing the phloem tissues are more thickened than those enclosing the xylem. Stegmata absent in the sclerenchyma cells of the xylem and phloem poles.

Table 1. Cell dimensions in transverse section of *Schoenorchis nivea* leaf and leaf sheath.

| Variables	Leaf (L)	Leaf sheath (LS)
Stomata number (mm^2)		
Adaxial	21.76 ± 1.52	--
Abaxial	47.06 ± 1.37	--
Guard cell (adaxial) (µm)		
Length	37.67 ± 0.17	--
Width	9.67 ± 0.33	--
Guard cell (abaxial) (µm)		
Length	38.00 ± 0.50	--
Width	11.50 ± 0.41	--
Stomatal pore length (µm)		
Adaxial	17.00 ± 0.27	--
Abaxial	13.17 ± 0.30	--
Stomatal pore width (µm)		
Adaxial	9.00 ± 0.48	--
Abaxial	7.50 ± 0.42	--
Cuticle thickness (µm)		
Adaxial	12.25 ±0.51	8.58 ± 0.23
Abaxial	10.83 ± 0.64	19.17 ± 0.78
Epidermis (adaxial) (µm)		
Cell Length	53.25 ± 1.19	16.67 ± 0.77
Cell Width	51.92 ± 1.56	33.50 ± 1.25
Epidermis (abaxial) (µm)		
Cell length	39.92 ± 0.62	47.33 ± 1.09
Cell width	37.50 ± 1.35	46.00 ± 0.88
Mesophyll (µm)		
Cell length	135.42 ± 5.60	42.83 ± 1.02
Cell width	76.58 ± 2.27	59.92 ± 1.46
Water-storage cell (µm)		
Length	168.25 ± 6.60	39.42 ± 0.84
Width	127.00 ± 3.77	99.33 ± 4.24
Vascular bundle (µm)		
Length	99.50 ± 5.22	98.67 ± 2.93
Width	69.00 ± 3.09	87.08 ± 3.67
Vascular bundle (No. per L/LS)	9.13 ± 0.83	11.40 ±0.21

Leaf sheath
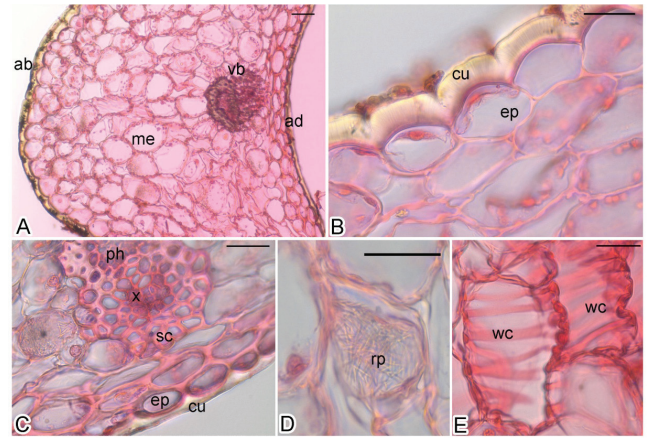
Figure 2. Leaf sheath anatomy of *Schoenorchis nivea* (A) cross section of leaf sheath showing adaxial and abaxial epidermis, mesophyll and vascular bundle; (B) epidermis with thick cuticle on the abaxial side; (C) epidermis and cuticle on the adaxial side and vascular bundle with phloem and xylem capped by sclerenchymatous cells; (D) raphides present in the mesophyll cell; (E) water-storage cell with banded thickening.
ab = abaxial, ad = adaxial, cu =cuticle, ep = epidermis, ep = epidermis, me = mesophyll, ph = phloem, rp = raphides, sc = sclerenchymatous cells, vb = vascular bundle, wc = water-storage cell, x = xylem. Scale bars = 50 µm (A) and 30 µm (B–E).

Stomata absent. Cuticle present on both adaxial and abaxial surfaces. Cuticle heavily ridged along the contours and is twice more thickened on the abaxial side when compared to the adaxial surface (Table 1). Adaxial epidermis uniseriate, compactly arranged with thick-walled rectangular cells. Abaxial epidermis single layered, composed of thin-walled round to oval-shaped cells (Fig. 2A, B). Both radial and tangential walls of the adaxial epidermal cells are much thicker than those on the abaxial side of the leaf sheath. The dimension including length (64.53%) and width (27.17%) of the abaxial epidermal cell is significantly larger than the adaxial epidermis (Table 1). Hypodermis absent. Mesophyll undifferentiated into palisade and spongy parenchyma, chlorenchymatous. Mesophyll cells are periclinally oriented, smaller and compactly arranged towards the adaxial surface. Idioblasts containing raphides and banded water-storage cells are present in the mesophyll (Fig. 2D, E). Vascular bundles oriented towards the adaxial surface. Xylem and phloem tissues are surrounded by sclerenchymatous cells. The phloem tissue is covered by thickened sclerenchymatous cells (Fig. 2C).

Stem

Stem consists of cuticle, epidermis, cortex, ground tissue containing vascular bundles (Fig. 3A). Stem circular in shape and covered by cuticle of 10-13 µm thickness (Table
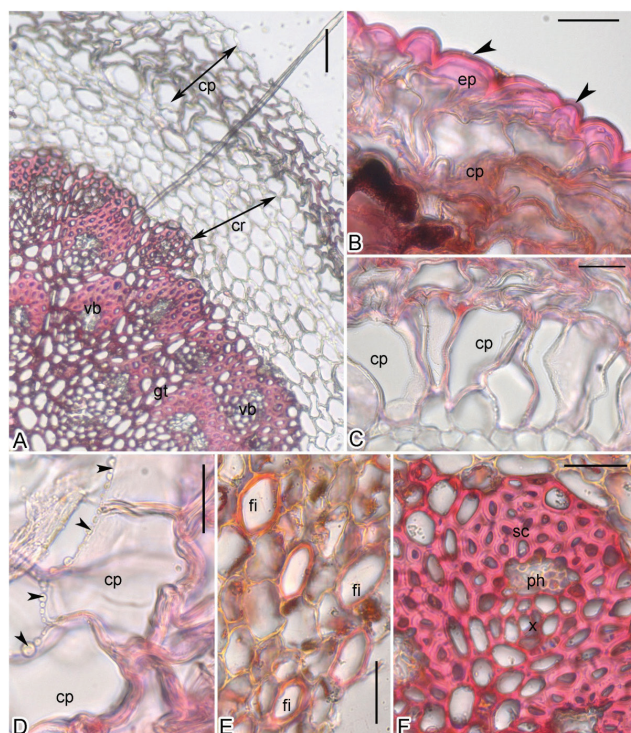
Figure 3. Stem anatomy of *Schoenorchis nivea* (A) transverse section of stem showing cortical proliferation, cortex and vascular bundle embedded in ground tissue; (B) epidermis covered by cuticle (black arrow heads) and cortical proliferation; (C) elongated cortical cells during cortical proliferation; (D) vesicles (black arrow heads) arranged close to the expanding cortical cell walls in cortical proliferation; (E) fibers in the cortical region; (F) vascular bundle with phloem and xylem capped by sclerenchymatous tissue .
cp = cortical proliferation, cr =cortex, ep = epidermis, fi = fibers, gt = ground tissue, ph = phloem; sc = sclerenchymatous cell, vb = vascular bundle, x = xylem. Scale bars = 50 μm (A), 30 μm (B-F).

Table 2. Cell dimensions in transverse section of *Schoenorchis nivea* stem.

Variables	Measurements (μm)
Cuticle thickening	13.17 ± 0.27
Epidermis	
Cell length	19.83 ± 0.87
Cell width	17.33 ± 0.75
Hypodermis	
Cell length	31.08 ± 1.05
Cell width	32.58 ± 0.99
Ground tissue	
Cell length	26.42 ± 0.99
Cell width	25.67 ± 1.31
Vascular bundle	
length	95.75 ± 2.37
width	80.17 ± 2.02

2). Epidermis single layered, consisting of compactly arranged parenchymatous cells (Fig. 3B). Cortex subtending the epidermis is 6-9 layered and composed of thin-walled parenchymatous cells. Thick-walled fibers are present in the cortex (Fig. 3E). This region is characterized by the absence of vascular bundles. Ground tissue: comprising of circular to angular, thick-walled sclerenchymatous cells enclosing triangular intercellular air spaces. Cortical proliferation is evident during the process of stem thickening (Fig. 3B-D). The outer most cells of the cortical region expand radially which is later on followed by tangential expansion. During the cortical proliferation, small vesicle-like structures are formed which could help in lignifications (Fig. 3D). The cell walls of expanding cells get isolated from the primary cortical cells and buckles up providing thickness to the stem. Vascular bundles are collateral and scattered in the ground tissue. Larger vascular bundles are present at the central portion and smaller ones are oriented towards the peripheral region of the ground tissue. Xylem and phloem tissues are enclosed by thick-walled sclerenchymatous tissues (Fig. 3F).

Root

Roots are mostly attached to the substratum. Circular in outline when aerial and the region attached to the substratum is not much flattened. Root hairs present in the portions of the roots that were in contact with the substratum. Root hairs are frequently branched at their tips (Fig. 4A). Velamen: 2-3 layered, differentiated into exovelamen and endovelamen. The cells of both exovelamen and endovelamen possess striations and both the radial as well as the tangential walls are thicker (Fig. 4B). The cells of endovelamen are longer when compared to exovelamen whereas; the exovelamen cells were wider than those of endovelamen (Table 3). Exodermis: Uniseriate, dimorphic and ∩-thickened and composed of both long and short cells. Long-cells of exodermis are thick-walled. Thin-walled short passage cells are present in-between the long exodermal cells (Fig. 4C). Tilosomes absent. Cover cells present above the passage cells. Cortex is 6-8 layered and composed of thin-walled parenchymatous cells (Fig. 4B). Raphides and water-storage cells are abundant in the cortex (Fig. 4D, E). Endodermis is O-thickened and present opposite to the phloem tissue and interrupted by thin-walled passage cells oriented opposite to the xylem. Pericycle located opposite to the xylem is thin-walled and thick-walled pericycle is oriented opposite to the phloem. Xylem and phloem elements are embedded in sclerenchymatous tissues. Pith consists of thick-walled cells with intercellular space of varied shapes (Fig. 4F).

Table 3. Cell dimensions in transverse section of *Schoenorchis nivea* root.

Variables	Measurements (µm)
Root hair	
Length	146.17 ± 5.34
Width	47.92 ± 1.41
Exovelamen	
Cell length	29.25 ± 1.09
Cell width	30.33 ± 1.03
Endovelamen	
Cell length	50.25 ± 1.68
Cell width	23.67 ± 0.65
Exodermis	
Cell length	54.67 ± 1.97
Cell width	33.42 ± 1.19
Cortex	
Cell length	53.50 ± 2.68
Cell width	45.50 ± 1.29
Water cell	
Length	97.42 ± 2.84
Width	68.50 ± 2.46
Endodermis	
Cell length	26.00 ± 0.68
Cell width	20.00 ± 0.74
Passage cell	
Length	15.75 ± 0.71
Width	10.08 ± 0.46
Meta xylem	
Cell length	13.50 ± 0.23
Cell width	12.50 ± 0.24
Phloem patch	
Length	24.08 ± 0.53
Width	13.08 ± 0.57
Pith	
Cell length	15.08 ± 0.58
Cell width	14.50 ± 0.57

Figure 4. Root anatomy of *Schoenorchis nivea* (A) branched root hair; (B) transverse section of root showing velamen, exodermis and cortex; (C) exodermis long cell, cover cell and passage cell; (D) raphides in cortical cell; (E) banded water-storage cell; (F) stele showing O- thickened endodermis, thin-walled passage cell, pericycle, phloem, xylem, and thick-walled pith.
cc = cover cell, co = cortex, en = endodermis, ex = exodermis, lc = long cell, pc = passage cell; pe = pericycle, ph = phloem, pi = pith, rp = raphides, vel = velamen, wc = water-storage cell, x = xylem. Scale bars = 50 µm (B, F) and 30 µm (A, C-E).

Mycorrhizal morphology and extent of colonization

Mycorrhizal colonization was observed in the roots that were attached to the substrate. The fungal hyphae entered the roots through the root hairs and transacted through the velamen and the passage cells of the exodermis into the cortex (Fig. 5A, B). Regularly septate hyphae, hyphal coils, microsclerotia and moniliform cells were observed in the velamen tissue (Fig. 5C, F). Both intact and degenerating pelotons were present in the root cortical cells (Fig. 5D, E). The proportion of root length with degeneration pelotons was several folds higher than the root length with intact pelotons (Fig. 6). Root infection by nematodes was occasionally seen (Fig. 5G). Nevertheless, no distortion or damage was visible in roots containing nematodes.
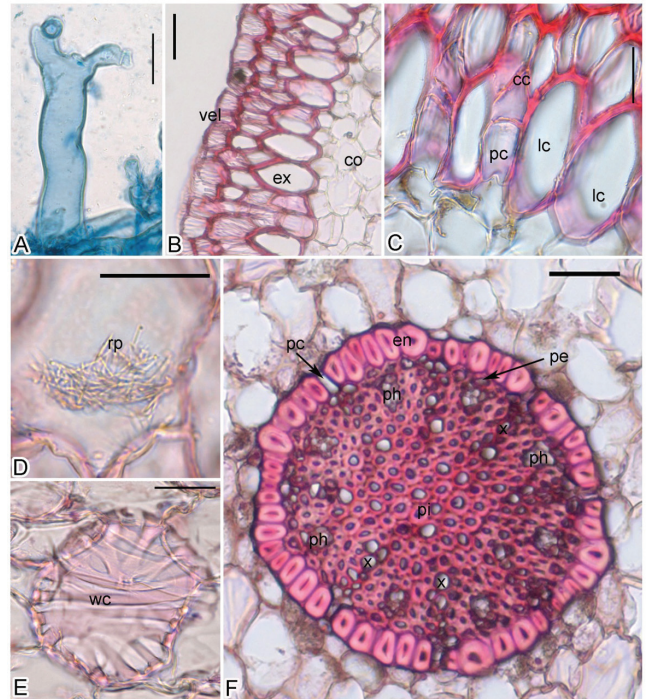
The orchid mycorrhizal fungal structures including intact pelotons and degenerating pelotons varied significantly ($F_{2,8}$ = 72.756; P < 0.001) in *S. nivea*. The percentage of root length colonization with degenerating pelotons was higher than the percentage of root length colonization with intact pelotons. The percentage of total root length colonization was 50.15% (Fig. 6).

Comparison of common structures in plant parts

The stomatal characteristics including the width of the guard cell, size of the stomatal pore and the number of stomata were significantly different on both adaxial and abaxial surface of the leaf except for guard cell length. The cell dimension of epidermal cells, cuticle thickening, number and width of vascular bundles on adaxial and abaxial surfaces had significant variation between leaf and leaf sheath except for the length of vascular bundles (Table 4). Similarly, the size of the water-storage cells and width of the vascular bundles also exhibited significant variation except for the length of the vascular bundles
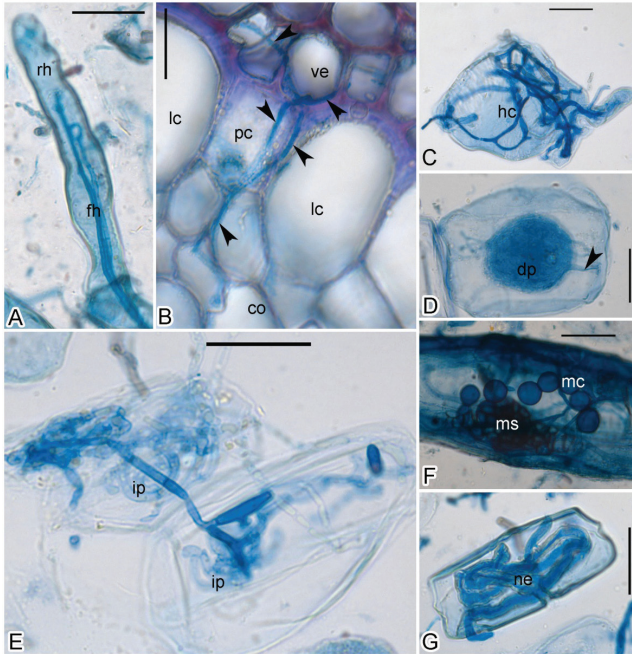
Figure 5. Mycorrhizal morphology of *Schoenorchis nivea* root. (A) fungal hyphae within root hair; (B) fungal hyphae (black arrow heads) traversing the velamen into the cortex through the passage cell of the exodermis; (C) hyphal coil in velamen cell; (D) degenerating peloton with hyphal remnants (black arrow head); (E) intact pelotons in cortical cells; (F) moniliform cells and microsclerotia; (G) nematode in cortical cell. co = cortex, dp = degenerating peloton, fh = fungal hyphae, hc = hyphal coil, ip = intact pelotons, lc = long cell, mc = moniliform cells, ms = microsclerotia, ne = nematode, pc = passage cell, rh = root hair, ve = velamen. Scale bars = 50 μm (B) and 30 μm (A, C–F).

Table 4. Comparison of stomata, epidermis and cuticle characteristics using student's t-test.

Variables	t-value	df	Significance (2-tailed)
Stomata (adaxial and abaxial)			
Guard cell			
Length	-0.695	14	0.499
Width	-3.556	14	0.003
Stomata pore			
Length	11.500	14	0.001
Width	2.806	14	0.014
Stomata number	-21.767	14	0.001
Stomatal index (%)	9.953	14	0.001
Leaf and Leaf sheath			
Upper epidermis			
Cell length	-7.891	29	0.001
Cell width	28.039	29	0.001
Lower epidermis			
Cell length	-5.877	29	0.001
Cell width	9.062	29	0.001
Cuticle thickening			
Lower side	-8.118	29	0.001
Upper side	6.279	29	0.001
Vascular bundle			
Length	0.141	29	0.889
Width	-3.935	29	0.001
Number	-2.639	14	0.019

among plant parts (Table 5). A three-way ANOVA involving cuticle thickness, length and breadth of cells (cell dimension) of adaxial and abaxial surfaces (sides) of leaf and leaf sheath (plant parts) indicated a significant variation among these variables (Table 6). In addition, the two-way and three-way interactions among these factors were also highly significant.

Discussion

Schoenorchis nivea exhibited several adaptive characteristics to thrive in the harsh epiphytic habitats. Cuticle, the key barrier between the comparable dry atmosphere and the hydrated aerial parts of plants covered all the airy parts of *S. nivea*. Cuticular thickness covering the leaves can substantially vary among plant species in spite of environmental influence on this anatomical trait (Zhong et al. 2018). This is clearly evident in the present study where the cuticle covering the adaxial and abaxial surfaces of the leaves in *S. nivea* were respectively 9.26% and 8.96% thinner those of *S. gemmata* examined from Tamenglong, Manipur, India (Angela et al. 2015). The altitudes and temperature ranges of Myladumpara, Kerala and Tamenglong, Mani-
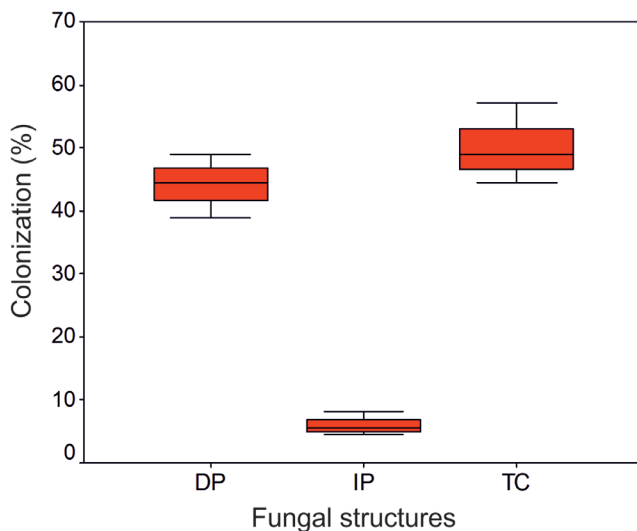

Figure 6. Box plots showing the distribution of total mycorrhizal colonization (TC) and root length with degenerating (DP) and intact (IP) pelotons in *Schoenorchis nivea* root.

Table 5. Characteristics of water-storage cells and vascular bundle in different parts of *Schoenorchis nivea*.

Plant organs	Water-storage cell (µm)		Vascular bundle (µm)	
	Length	Width	Length	Width
Leaf	168.25 ± 6.59a	127.00 ± 3.77a	99.50 ± 5.22a	69.0 0 ± 3.09a
Leaf sheath	39.42 ± 0.83c	99.33 ± 4.23b	98.67 ± 2.92a	87.08 ± 3.67b
Stem	-	-	95.75 ± 2.37a	80.17 ± 2.02b
Root	97.42 ± 2.84b	68.50 ±2.45c	-	-
df- 29	238.793***	67.226***	0.281ns	9.214***

Means ± S.E., in a row followed by a same letter(s) are not significantly (P > 0.05) different according to Duncan's Multiple Range Test.
*** significant at 0.01%, ns- not significant

pur are almost similar except for Tamenglong receiving twice the average rainfall of Myladumpara. Moreover, the cuticle in *Schoenorchis* is several times thicker than those reported for other epiphytic orchids like *Ascochilus, Bulbophyllum, Epidendrum, Dendrobium, Thrixspermum* and *Vanda* (Yang et al. 2016; Kowsalya et al. 2017; Muthukumar and Shenbagam 2017, 2018; Rindyastuti et al. 2018). The cuticle is a hydrophobic covering made up of cutin and solvent soluble cuticular waxes. This covering plays an important role in effectively controlling the loss of water from the cell interior, protects against radiation and biotic stresses (Mill and Stark Schilling 2009; Hen-Avivi et al. 2014). In addition, cuticle development has been shown to be closely related to cell patterning and organ development (Shi et al. 2013). According to Haworth and McElwain (2008), the occurrence of cuticle on the leaf surface is an indication of aridity. The thick cuticle on *S. nivea* aerial parts prevents water loss and improves the efficiency of water when it is scarce (Guan et al. 2011). Yang et al. (2016) also suggested that the presence of thick cuticle is one of the strategies adopted by epiphytic orchids to maintain the water balance. In the present study, the cuticle was

Table 6. F-values from three-way ANOVA for epidermis cell length, width and cuticle thickening in both sides of *Schoenorchis nivea* leaf and leaf sheath. Numerator and denomenator difference is presented as subscripts in paranthesis.

Source	F-value
Plant parts$_{(1,348)}$	102.990***
Surface$_{(1,348)}$	597.779***
Cell dimension$_{(1,348)}$	1104.3***
Plant parts × Surface$_{(1,348)}$	52.546***
Plant parts × Cell dimension$_{(1,348)}$	75.120***
Surface × Cell dimension$_{(1,348)}$	66.878***
Plant parts × Surface × Cell dimension$_{(1,348)}$	24.350***

Plant parts- Leaf and Leaf sheath, Sides- adaxial and abaxial Cell dimension-Length, Width, cuticle thickening. *** significant at P< 0.001

thickest on the abaxial surface of the leaf sheath which was 56.49% and 77.01% thicker than the cuticle covering the adaxial and abaxial surface of the leaves respectively and 45.56% thicker than those covering the stem. Some of the leaf anatomical characters *S. nivea* shares with *S. gemmata* include paracytic stomata, water-storage cells with banded thickenings, and fibrous caps bordering xylem and phloem. However, cuticle papillae, conjunct vascular bundles, and cuticularized guard cells reported in leaves of *S. gemmata* was absent in *S. nivea* (Angela et al. 2015). Similarly, the absence of hypodermis and fiber bundles, homogenous mesophyll and sclerenchymatous caps bordering xylem and phloem resembles the leaves of Vandeae members. However, *S. nivea* differs from other Vandean taxa in amphistomatous condition and absence of glandular hairs, distinct bundle sheath, and stegmata associated with the vascular tissue (Carlsward et al. 2006).

Despite the widespread occurrence of hypostomatic in many orchids, *S. nivea* had an amphistomatal leaves. Amphistomatal condition is also reported in other epiphytic orchids like *Acampe, Aerides, Bulbophyllum, Rhynchostylis,* and *Vanda* (Muthukumar and Shenbagam 2017; Mulgaonkar 2005a,b; Sonowal and Baruah 2010). Richardson et al. (2017) suggested that the existence of amphistomatic condition has a strong relationship with the environment based on their commonality in high light and/or dry environments. In addition, amphistomatic leaves have an advantage of increased CO_2 conductance for photosynthesis and efficient supply water to both the surfaces of the leaves (Buckley et al. 2015). The stomata in *S. nivea* were smaller and less dense than some of the epiphytic orchids. For example, the stomatal aperture dimensions in *S. nivea* were several folds smaller than those reported for some Vandoideae members. The stomatal index of 7.06% in *S. nivea* is higher than *S. gemmata* (5%) reported by Angela et al. (2015). Zhang et al. (2016) indicated that plants with small and dense stomata respond more rapidly to changes in the environment or to the reduction in leaf water potential than those with larger stomata.

Moreover, the results of that study also indicated that evergreen epiphytic orchids were better adapted to cope with water limitations during the growing season than the deciduous conspecifics (Zhang et al. 2016). This condition is generally advantageous for evergreen orchids like *S. nivea* occurring in monsoonal dry environments.

Limited studies have examined the stem anatomy of orchids than root and leaves (Kowsalya et al. 2017; Balachandar et al. 2019). Stems of *S. nivea* possess a thick cuticle which is in accordance with the observation of Kowsalya et al. (2017) in Vandas growing in south India. The hardy stem of *S. nivea* was completely covered by the leaf sheath as in *Luisia*, where, the leaf sheath covered almost the entire internode (Balachandar et al. 2019). Further, Muthukumar and Shenbagam (2017) suggested that leaf sheath aids in adding stiffness to the stem based on the difference in the thickness of the cuticle on the stems that were covered and uncovered by leaf sheath. The hard woody nature of the stem in *S. nivea* arises through progressive cortical proliferation and lignification as reported in *Luisia* (Balachandar et al. 2019). The uniseriate epidermis in *S. nivea* also provides mechanical support in addition to the lignified cortical proliferation. The stem cortex composed of parenchymatous cells was devoid of vascular bundles as observed in *Luisia* species (Balachandar et al. 2019). The sclerenchymatous ground tissue comprising of vascular bundles in *S. nivea* were similar to the stems of *Vanda spathulata* Spr. and *Vanda wightii* Rchb.f. reported by Kowsalya et al. (2017). These sclerenchymatous ground tissues provide additional mechanical support to the stems (Balachandar et al. 2019). Unlike the stems of some Vandae members, the ground tissue in *S. nivea* was characterized by the absence of idioblasts containing raphides, water-storage cells and aeration units (Carlsward et al. 2006; Kowsalya et al. 2017; Balachandar et al. 2019). The vascular bundles were scattered in the ground tissue and embedded in sclerenchymatous cells. The xylem and phloem were capped by sclerenchyma cells and the size of vascular bundles tended to be larger in the centre as reported in several species of *Luisia*, *Vanda* and *Epidendrum radicans* Pav. ex Lindl. (Muthukumar and Shenbagam 2017; Kowsalya et al. 2017; Balachandar et al. 2019).

Roots of *S. nivea* are mostly attached to the substratum. These roots were cylindrical and not much flattened in regions that were attached to the substratum. The substrate roots bear root hairs that were often branched. This is in line with the observations of Balachandar et al. (2019) where the roots of epiphytic orchid *Luisia tenuifolia* Blume were branched. The branched root hairs could be the result of stress experienced by the root hairs (Medeiros 2006) or mutation in genes involved in the root hair formation (Schiefelbein 2000).

The size of the velamen tissue and the number of cell layers could suggest the condition in which the orchid occurs. The 2-3 layered velamen of *S. nivea* resembles those of *V. spathulata* and *Luisia tristis* Hook.f. of the tribe Aeridinae (Kowsalya et al. 2017; Balachandar et al. 2019). The few layered velamen tissue may be the result of humid conditions in which *S. nivea* occurs. All the cells of the exovelamen and endovelamen in *S. nivea* possessed striations and wall thickenings. The striations were of type IIA according to Sanford and Adanlawo (1973) classification of velamen striations. The thickened walls render mechanical support to the velamen cells by preventing cell collapse during dehydration (Oliveira and Sajo 1999).

The uniseriate exodermis in *S. nivea* was dimorphic comprising of long and short cells. Similarly, Muthukumar and Kowsalya (2017) also observed long and short exodermal cells in substrate roots of *Acampe praemorsa* (Rox.) Blatt. & McCann. Although the importance of long exodermal cells is unknown, Pridgeon (1982) reported that long cells give rise to secondary wall thickenings during maturity and die. Further, the long cells of exodermis in plants could be helpful to tolerate drought (Chimungu et al. 2014). The exodermal cells were larger than the velamen cells in *S. nivea* which resembles with the *Vanda*-type of velamen according to Porembski and Barthlott (1988) classification of velamen radicum. The exodermal proliferations unlike reported in some of members of Aeridinae subtribe, (Carlsward et al. 2006, Muthukumar and Kowsalya 2017) was not observed in any of the *S. nivea* roots examined. The exodermal cell thickenings in *S. nivea* may prevent the loss of water through transpiration (Benzing et al.1982). The exodermis is interrupted by thin-walled passage cells in *S. nivea* as observed in the roots of *Luisia* species and other members of Vandeae tribe (Balachandar et al. 2019). The nutrients and water transferred into the cortical cells through the thin-walled exodermal passage cells (Benzing et al. 1982, Bercu et al. 2011). Moreover, Chomichi et al. (2014) suggested that the distribution and frequency of passage cells in the exodermis might be a strategy adopted by orchids to localize and control fungal invasion. Tilosomes are absent in the roots of *S. nivea* as reported for some of the orchid species in Aeridinae (Carlsward et al. 2006). However, Leitgeb (1864) observed tilosomes in some members of Vandaceous taxa. The cover cells are present above the exodermal layer which is similar to the observations of Carlsward et al. (2006) and Kowsalya et al. (2017) in roots of some *Vanda* species. These cover cells are associated with the condensation of gases and water (Pridgeon 1987).

The cortex in *S. nivea* is parenchymatous (Kowsalya et al. 2017; Balachandar et al. 2019). The water-storage cells with banded thickening in *S. nivea* are similar to those observed by Carlsward et al. (2006) and Balachandar et

al. (2019) in the roots of Vandeae members. The water-storage cells with uniformly thickened walls can enhance the apoplastic movement of substance in the vascular tissues (Kowsalya et al. 2017). In epiphytic habitats, the rooting zones are limited to small patches of organic matter accumulating on the branches. These patches get soaked with water during rain and then quickly dry off. So epiphytic plants like orchids that are able to maintain constant water content in their tissues independent of fluctuating water content of the rooted medium have a selective advantage (Tulyananda and Nilsen 2017). The cortical cells are thin-walled in *S. nivea*. Nevertheless, wall thickenings in the root cortical cells were recorded in species of Aeridinae subtribe (Carlsward et al. 2006, Kowsalya et al. 2017) which contradicts with the results of the present study. Idioblasts comprising of raphides are present in the root cortex of *S. nivea*. The function of raphides in roots is yet to be ascertained (Balachandar et al. 2019). Nevertheless, their function in leaves and stem are well documented (Paiva and Machado 2005; Moreira et al. 2013).

The endodermis was uniseriate and O-thickened in *S. nivea* as observed in the endodermal cells of roots in other species of Vandeae (Carlsward et al. 2006; Kowsalya et al. 2017; Balachandar et al. 2019). The endodermal cell walls were heavily thickened in *S. nivea*. This is in accordance with Balachandar et al. (2019), who also revealed the presence of thick-walled endodermal cells in *Luisia pulniana Vatsala* roots. Nevertheless, most of the Vandeae taxa exhibit thin to slightly thick endodermal cell walls, and endodermis interrupted by thin-walled passage cells oriented towards the xylem. Similarly, cells of the pericycle are thick-walled opposite to the phloem tissue. The arrangement and orientation of cells in endodermis and pericycle in *S. nivea* are similar to other epiphytic orchid species of Vandeae taxa (Kowsalya et al. 2017; Balachandar et al. 2019). The thickenings in the endodermal cell walls act as an apoplastic barrier for water and nutrient transport and protect vascular bundles from pathogens (Moreira and Isaias 2008; Muthukumar and Kowsalya 2017).

The number of xylem arches (9-11) in *S. nivea* is in line with the observations of Carlsward et al. (2006) who also reported 9-12 xylem arches in *Neofinetia* of Aeridinae. Like in other Vandeae members, the vascular tissues in *S. nivea* were embedded in the sclerenchymatous cells. The sclerenchymatous cells enclosing the phloem patches were heavily thickened than those cells around the xylem (Kowsalya et al. 2017; Balachandar et al. 2019). In most of the epiphytic orchids, the occurrence of vascular elements in the sclerenchyma cells is considered as an anatomical feature that is related with drought tolerance (Nawaz et al. 2013; Muthukumar and Shenbagam 2017). Pith in *S.*

nivea was of sclerenchymatous enclosing intercellular spaces as observed in the Vandeae taxa (Carlsward et al. 2006). However, parenchymatous pith was reported in *L. tenuifolia* (Balachandar et al. 2019). Cell inclusions such as silica bodies and starch grains are absent in pith of *S. nivea*. In contrast, many members of Vandeae are characterized by the presence of such cell inclusions (Carlsward et al 2006; Balachandar et al. 2019).

Mycorrhizal colonization in *S. nivea* was evident in the root portions that were attached to the substrate which is in line with the observations of Sathiyadash et al. (2012) and Muthukumar and Kowsalya (2017) in various epiphytic and terrestrial roots. The fungal hyphae entered *S. nivea* roots through root hairs and traversed the velamen tissue and passage cells of exodermis and then moved into cortical cells where the fungal hyphae formed highly coiled structures called pelotons. Both degenerating and intact pelotons was observed in the root cortex. This is in accordance with the observations reported for epiphytic orchids (Sathiyadash et al. 2012; Kowsalya et al. 2017). It is well known that intact pelotons act as a reservoir for the exchange of nutrient between the orchid and fungus (Dearnalay et al. 2012). The total root length colonization was 50% in *S. nivea* with a high proportion of degenerating pelotons when compared to the intact pelotons. This suggests that *S. nivea* could have adopted mixotrophic mode of nutrition as it occurs in shady regions as observed for *L. tenuifolia* and *L. tristis*. This could have been the reason for the higher percentage of degenerating pelotons in *S. nivea* as suggested by Balachandar et al. (2019).

Conclusion

Schoenorchis nivea exhibited anatomical traits in all the vegetative parts that enable it to survive in the epiphytic habitats. These include thick-walled cuticle covering the entire surface of the leaves, leaf sheath, and the stem, abundant water-storage cells in all the vegetative parts except stem, well developed velamen tissue, presence of cover cells and thick-walled exodermis and endodermis in the roots. All the anatomical characteristics directed towards the acquisition and prevention of water loss could be attributed to the adaptation of *S. nivea* to the xeromorphic conditions of the epiphytic habitat. Moreover, the results of the study clearly revealed that the dimensions of the same structures occurring in different plant parts could vary significantly. In addition, the presence of mycorrhizal association along with the large proportion of degenerating pelotons in the roots suggests that *S. nivea* could adopt a mixotrophic mode of nutrition. Further anatomical and mycorrhizal investigations of other spe-

cies of the *Schoenorchis* could help in the understanding of the precise adaptation of these taxa to epiphytic habitats and resource acquiring strategies.

Acknowledgments

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