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MORPHO-ANATOMICAL ADAPTATIONS OF *COELOGYNE OVALIS* AND THEIR TAXONOMIC IMPLICATIONS IN RELATION TO ECOLOGICAL HABITAT

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Abstract

The present study was aimed to describe the morphological and anatomical characteristics of the leaf, stem/pseudobulb, and root in *Coelogyne ovalis* Lindl., (classified under section *Fuliginosae*) with a view to understand adaptations related to water regulation and their taxonomic implications. The vegetative tissues were fixed and preserved in 70% alcohol, and sections were prepared using a rotary microtome. Anatomical observations revealed that the key features namely the leaf exhibits stomata, a thick cuticle, and a vascular bundle in the mid-rib; the stem contains specialized water storage cells and distinct tracheids; and the root possesses a multilayered velamen and endodermal lignification. The inflorescence of *Coelogyne ovalis* is characterized as a stachyoid (or stachyoid-type) structure, which is defined as a closed, determinate bracteous spike.

Introduction

THE FAMILY Orchidaceae is one of the largest and overwhelmingly diverse families of angiosperms that encompass about 70% of the epiphytic vascular species with 29,481 species recognized across 693 genera (POWO, 2025; WFO, 2023) with 1,256 species reported from India (Singh *et al*., 2019). Orchids are captivating plants admired for their stunning flowers, long-lasting blooms, ornamental appeal, medicinal value, and significance in evolutionary studies. The family is segregated into five subfamilies namely *Apostasioideae, Cypripedioideae, Epidendroideae, Orchidoideae,* and *Vanilloideae,* with *Epidendroideae* consisting of highest number of species *i.e.* 21,160; there are 516 genera and 16 tribes within the *Epidendroideae* (Chase *et al*., 2015). The genus *Coelogyne* with nearly 601 species, distributed throughout SouthEast Asia and extended into Pacific Islands (POWO, 2025). Chase *et al*. (2015) and Pridgeon *et al*. (2014) subdivided the genus into fourteen sections, amongst which section Fuliginosae is characterized by a scape with scales inserted on its base, small and shiny 2-leaved pseudobulbs, inflorescence with a relatively low number of small flowers open in succession, and a lip with fimbriate margin. Pelser *et al.* (2000) studied flowering period, flower colour, habitat, elevation and geographical distribution of the species with an aim to tracing possible correlations with clusters found in the phenetic analysis. Hence, the present studies were made to study the qualitative and quantitative traits and analyze the data on absorbing trichomes, cuticle thickness, stomatal width, midrib vascular bundle size, laminar vascular bundle size, water storage cell and phloem cap layers in leaf; length of tracheid, length of xylem fiber in stem; and number of velamen layers, exodermal cell lignification, passage cell size and endodermal cell lignification in roots.

Material and Methods

The plant material *Coelogyne ovali*s was collected from Karuman Code, Kerala at an elevation of 980 amsl which was growing as an epiphyte on *Azadirachta indica* with a view to evaluate the leaf, stem/pseudobulb and root anatomy. The vegetative parts of 1.5 cm were fixed in 50% FAA and preserved in 50% alcohol (Johansen, 1940). Transverse sections were subjected to double staining with 0.05% Safrablau and mounted in glycerin (cf. Bukatsh, 1972; Khasim *et al*, 2025). Sections were cut using a rotary microtome at a thickness of 15-18µm. For starch identification, Lugol solution was used (Bücherl, 1962). The most important aspects were recorded with a digital Camera attached to an Olympus microscope. Macerations of leaf, stem and root were carried out in a solution of 20% nitric acid (HNO_3) and chromium trioxide by heating on the spirit lamp, for lignin staining with hydrochloric phloroglucin (Jansen, 1962). To study the stomatal index, the epidermis was peeled carefully without disturbing the stomata, the peeled

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abaxial layer stained with safranin was mounted in glycerine on the slide. The observation were taken under the microscope. The stomatal index is the number of stomata per unit area of the leaf surface and is expressed as a percentage. The number of stomata and its epidermal cells per unit area were counted and the stomatal index was calculated as using the formula as

$$\text{Stomatal index} = \frac{\text{stomatal density}}{\text{stomatal denisity+epidermal cell density}} \times 100$$

The results of the anatomical studies are mentioned in Tables 1-4.

Results and Discussion

The morphological and anatomical characteristics of the leaf, stem/pseudobulb, and root in *Coelogyne ovalis* were studied with a view to understand adaptations related to water regulation and their taxonomic implications.

Leaf (Fig. 1A-F; Table 1,2)

Thick leaves help reduce the transpiring surface under stress conditions. In the present study, adaxial epidermal cells were larger than abaxial epidermal cells, and stomata were confined to the abaxial surface. The stomata were surrounded by four to five subsidiary cells (Fig. 1A). Smooth cell walls, a characteristic feature of advanced epiphytic orchids as mentioned by Solereder and Meyer (1976), were also presently observed. A hypodermis with prominent bulliform cells was evident in surface view of the leaf. Anomocytic and tetracytic stomatal types have earlier been reported in *Orchis* (Sevgi *et al*., 2012). Leaf anatomical features of epiphytic orchids have also been documented by Arévalo *et al*. (2011) and Riverón-Giró *et al*. (2017).

Mesophyll

Presently, the mesophyll was found to be homogeneous and it comprised various tracheoidal elements, including specialized water-storage cells with multispiral cellulosic thickenings, as well as simple water-storage cells containing chloroplasts. These observations were also made earlier by a few authors (Pridgeon, 2014; Singh *et al*., 2021). The mesophyll exhibited uniseriate vascular bundles, consisting of a larger central midrib and smaller laminar vascular bundles (Fig. 1B). In addition, thick walled water storage cells forming a hypodermal layer beneath the adaxial epidermis were evident (Fig. 1C). Prominent absorbing trichomes were also observed in the presently investigated species (Fig. 1D), reflecting thereby the ecological adaptations to its habitat.

Vascular Bundles

The vascular bundles were arranged in a single series, with both xylem and phloem surrounded by a sclerenchymatous sheath, as mentioned in earlier reports (Kurzweil *et al*., 1995; Ramudu and Khasim, 2018). The midrib vascular bundle measured 0.078 µm, while the laminar vascular bundles measured 0.056 µm (Table 1). A prominent commissural vascular bundle extending between adjacent vascular bundles was also observed (Fig. 1E), indicating thereby ecological adaptations in the presently investigated species. Tracheids with helical thickenings and vessel-like tracheids were abundant in the species examined (Fig.

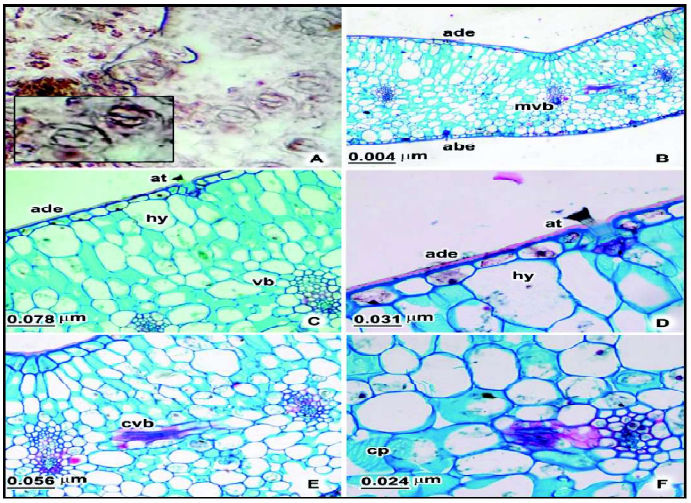

Fig. 1: Anatomy of *C. ovalis* Root: A, Cross section of root in cross section showing multilayer velamen, exodermis and vascular cylinder; B, Root cross section showing exodermal thickening; C, Root cross section showing exodermis with passage cells; D, Root cross section showing vascular cylinder with alternating xylem and phloem patches; E, Root cross section showing endodermis and passage cell; F, Vessel like tracheid from root maceration (v, Velamen; end, endodermis; exo, exodermis; pe, passage cell; ph, phloem; xy, xylem).

Table 1. Leaf anatomical features in *C.ovalis* (in µm).

Absorbing trichome	Cuticle thickness	Stomatal width (two guard cells including pore)	Stomatal length (only guard cell)	Midrib vb. size	Laminar vb. size	Water storage cell	Substomatal chamber size	Number of ph. Cap layers	Number of xy. Cap layers
+	0.004	0.024	0.011	0.078	0.056	0.028	0.031	6	7

Vb, vascular bundle; xy, xylem; ph, phloem.

Table 2. Diagnostic (leaf and root) anatomical features (quantitatively) in *C.ovalis*.

Size of Adaxial epidermal cells in leaf (µm)	Number of subsidiary cells in stoma	Number of phloem cap layers in leaf	Number of velamen layers in root	Number of proto Xylem poles in root
0.025	4-6	6	5-6	6

1F). The number of phloem and xylem cap layers was found to be six and seven, respectively (Table 1).

Stem/Pseudobulb (Fig. 2A-F; Table 3)

In outline, the stem was found to be oval in shape.

Epidermis

The epidermal cells were elongated with thick cuticle (0.006 µm).

Cortex and Ground Tissue

The cortex comprised irregularly shaped, large parenchymatous cells forming the ground tissue, within which numerous vascular bundles were observed to be scattered (Fig. 2A).

Vascular Bundles

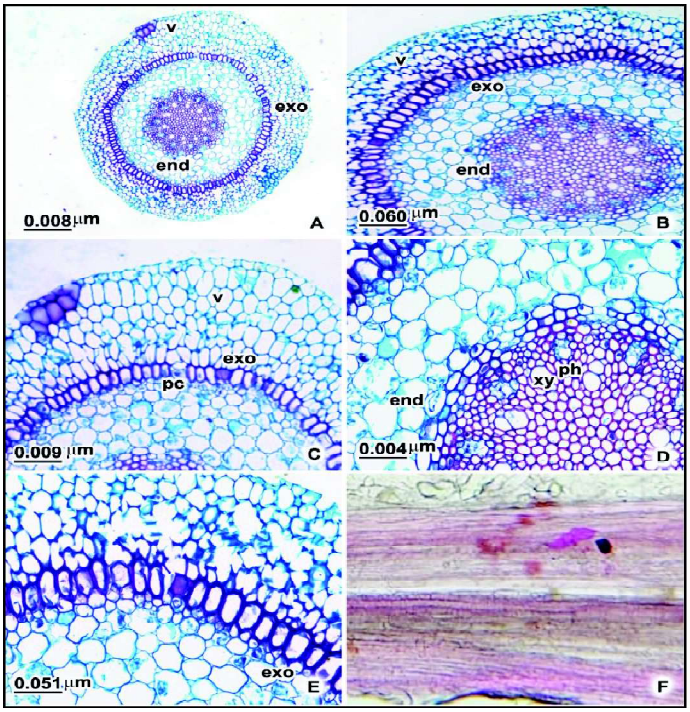

Fig. 2. Anatomy of *C. ovalis* Stem/Pseudobulb: A,Pseudobulb cross section showing scattered vascular bundles in the ground tissue; B,Pseudobulb cross section showing smaller cells towards outer cortex and larger cells towards inner cortex; C, Pseudobulb cross section showing larger vascular bundles in the ground tissues; D, Part of cross section of Pseudobulb showing starch cells; E, Parts of cross section of Pseudobulb showing vascular bundle with phloem cap and xylem cap and simple water storage cells; F, Water storage cell with multi spiral cellulosic thickening from maceration (epi, epidermis; g, ground tissue; vb, vascular bundle; chl, chloroplast; phc, phloem cap cell).

Numerous small and large vascular bundles were found as scattered throughout the tissue, with 2-3 layers of smaller bundles near the epidermis and larger bundles towards the center (Fig. 2B), measuring 0.040 µm in size (Table 3; Fig. 2C). The ground tissue region contained abundant mucilage and chloroplasts as reported earlier by Kluge *et al.* (2012) and Ramudu and Khasim (2018). The chloroplasts contained starch grains (Fig. 2D). The vascular bundles exhibited six layers of phloem and seven layers of xylem caps (Fig. 2E). The xylem consisted of tracheids with helical thickenings, vessel-like tracheids, and fibers, as well as water-storage cells with multispiral cellulosic thickenings. These features, particularly the water-storage cells (Fig. 2F) observed in pseudobulb maceration, are indicative of ecological adaptation as mentioned earlier by Ramesh *et al.* (2020) The vegetative structures of *Oeceoclades maculata* are notable for their role in ecological succession (cf. Ramudu *et al.*, 2025).

Root (Fig. 3A-F; Table 4)

The outline of the root was found to be circular in cross section, with velamen consisting of 6-7 layers (Fig. 3A). The exodermis was well-developed, featuring long, thick-walled cells (Fig. 3B), along with shorter, thin-walled passage cells. These passage cells were not arranged alternately, and their number varied (Fig. 3C). The root and leaf anatomy of a few terrestrial species from the Cranichideae subfamily (Orchidaceae) has earlier been studied by Ramudu and Khasim (2020) and Sajo and Chase (1999).

Cortex

The cortex consisted of 5-7 layers, with the outermost layer made up of smaller cells and the inner layers consisting of larger, oval-shaped cells with intercellular spaces. These cells were filled with abundant mucilage (Fig. 3D), reflecting the ecological adaptation of the studied species.

Endodermis and Vascular Cylinder

The endodermis comprised of a single layer of prominent, thick-walled cells, with interruptions at the protoxylem poles where thin-walled passage cells were present (Fig. 3E). Such observations were also made by Rai *et al.* (2024) Xylem and phloem strands alternated within the vascular cylinder, which had a diameter of

Table 3. Stem/Pseudobulb anatomical features in *C.ovalis* (in mm).

Cuticle thickness	Water storage cells	Size of vb	Number of ph. cap layers	Number of xy. cap layers	Length of tracheid / vessel member	Length of xy. Fibre	Length of ph. Fibre
0.006	0.039	0.040	6	7	0.025	0.041	0.057

0.060 µm (Table 4). The number of protoxylem poles ranged from 6 to 8. The xylem consisted of tracheids with helical thickenings, vessel-like tracheids, and fibers, all of which were clearly visible in root maceration. These features are indicative of ecological adaptations and are distinctive characteristics of *Coelogyne ovalis*, which may serve as key diagnostic anatomical traits in the species (Table 4).

Table 4. Root anatomical features in *C.ovalis* (in mm).

Number of velamen layers	Fibrous mats/ tilosomes	Exodermis cell lignification	Passage cell size	Endoermis cell lignifiction	Vascular cylinder diamter	Number of protoxylem poles
6-7	+	0.051	0.009	0.004	0.060	6

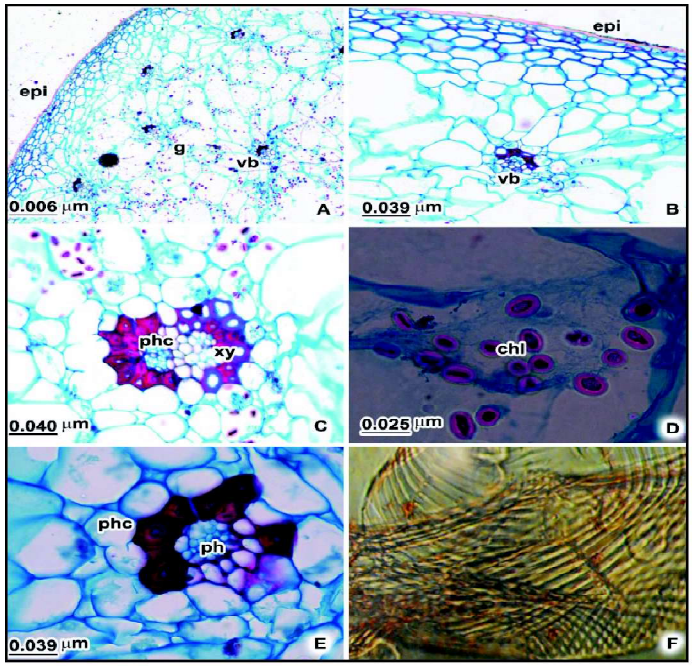

Fig. 3.Anatomy of *C. ovalis* Leaf: A, Leaf epidermis in surface view with stomata; B, Leaf cross section showing single layered midrib vascular bundle and smaller laminar vascular bundles; C, Leaf cross section showing hypodermis towards adaxial epidermis; D, Leaf cross section showing absorbing trichome towards adaxial epidermis; E, Leaf cross section showing commissural vascular bundle between the two vascular bundles; F, Leaf cross section showing pitted cell wall thickenings and commissural vascular bundles (ade, adaxial; abe, abaxial; mvb, midrib vascular bundle; cp, pitted thickening; hy, hypodermis; at, absorbing trichome).

The present data on leaf, stem/pseudobulb and root anatomical features may prove valuable for species discrimination in *C. ovalis*, particularly within the context of the Fuliginosae section of orchids. Similar observations were made by Adit and Kumar (2024) and Johnson and Linder (1995). Bhukhari and Gangaprasad (2023) also studied Root anatomical features in ten epiphytic orchids from Western Ghats, Goa and indicated their taxonomic importance. Further Behera, (2024), in an ecological study to conserve orchids studied ecological adaptation and phorophytic relationship of orchid flora in Similipal biosphere reserve, Odisha.

Conclusion

The present data on leaf, stem/pseudobulb and root anatomical features may prove valuable for species discrimination in *C. ovalis*, particularly within the context of the Fuliginosae section of orchids. The anatomical studies are meager and hence need to be undertaken in other related taxa as well so as to understand ecological adaptations and their significance in taxonomy.

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