

Anatomical study of *Paphiopedilum fairieanum* (Lindley) Stein - an endangered orchid of Arunachal Pradesh, North-East India

Akhil Baruah¹ and Jayanta Sonowal²

Phytodiversity Research Cell, Department of Botany, Darrang College, Tezpur – 784001, Assam, India

²Present address: Rubber Board Regional Office, Giri Bhawn 3rd floor, NS-Avenue, Rangirkhari,
Silchar – 788005, Assam, India

¹Corresponding Author: E-mail: drakhilbaruah@yahoo.co.in

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Abstract

The anatomy of leaf, rhizome, root and peduncle of *Paphiopedilum fairieanum* (Lindley) Stein, an endangered orchid of Arunachal Pradesh, has been investigated. Leaves hypostomatic. The stoma on leaf and peduncle are anomocytic. Vascular bundles numerous, arranged in a single series in leaf-lamina, while scattered in rhizome, root and peduncle. Based on anatomical characters of *P. fairieanum* with that of two other species of *Paphiopedilum* Pfitzer viz. *P. insigne* (Wallich ex Lindley) Pfitzer and *P. venustum* (Sims) Pfitzer, a comparative account has been provided.

Key words: *Paphiopedilum fairieanum*, Anatomy, Leaf, Rhizome, Root, Peduncle, Arunachal Pradesh

INTRODUCTION

Paphiopedilum Pfitzer (Orchidaceae), commonly known as ‘Asian Lady’s Slipper Orchid’ consists of about 65 species distributed in India, Burma, S.E. Asia, China, Indonesia, New Guinea and Philippines (Chowdhery 1998). They are commonly terrestrial, but sometimes also grow as epiphyte. In India, the genus is represented by nine species, out of which eight are growing in Northeastern region. However, only three species viz. *P. fairieanum* (Lindley) Stein, *P. venustum* (Sims) Pfitzer and *P. wardii* Summerhayes are so far known from Arunachal Pradesh (Chowdhery 1998).

P. fairieanum is one of the most popular terrestrial orchids known for its exquisite colour, longevity of bloom and curious shape of flowers. It is popularly called the ‘Lost orchid’ as its habitat could not be traced for nearly half a century after its first discovery. It was however, rediscovered in West Bhutan in 1905. In Arunachal Pradesh, it is found to occur in a few isolated patches in West Kameng District (Sharma *et al.* 2006). The plant is generally found growing in calcareous soils on steep hills at an elevation between 1500-1800 m MSL in association with grasses in blue and chir pine habitat. This orchid is listed as ‘endangered’ because of its restricted habitat and rarity of occurrence (Hedge 1984).

Except the studies on *P. insigne* (Wallich ex Lindley) Pfitzer and *P. venustum* (Sims) Pfitzer (Kaushik 1983), no other work with regard to the anatomy of Indian species of *Paphiopedilum* have so far been done. However, anatomical characters can be used to distinguish species in vegetative stage or when the flowers are not available

(Baruah 1998; Baruah & Saikia 2002; Baruah & Sonowal 2010; Kaushik 1983; Khasim & Ram 1989; Stern & Judd 1999). The present paper reports the result of anatomical investigation of *P. fairieanum* and its comparison with the reported *P. insigne* and *P. venustum* (Kaushik 1983).

MATERIALS AND METHODS

Materials (leaf, rhizome, root and peduncle) for the present investigation were collected from a location (altitude: \pm 1600 m AMSL; N-27°12.8372 & E-92°28.0152) of Tenga valley of West Kameng district of Arunachal Pradesh during April' 2009. The free hand transections of the leaf, rhizome, root and peduncle were made with the help of a sharp razor. Epidermal peels were taken from 3 x 5 mm leaf fragments after treating those with 5 % aqueous solution of Potassium hydroxide for 30 minutes at 40°C as macerating agent for the mesophyll. Epidermal peels were taken from 5 mm long segments using similar treatment. The peels were stained with aqueous safranin, mounted in 50 % glycerin and sealed with Dibutylphthalate xylol. Camera lucida sketches were prepared under an optical microscope. The quantitative data were calculated from the average of 10 readings of each character. The stomatal index (I) was also calculated by the standard formula *i.e.* $I = S/E+S \times 100$, where S and E refer to the number of stomata and epidermal cell, respectively per unit area. The voucher specimens (Jayanta S, Biplob C & A. Baruah 014 dated 07-04-2009) of *P. fairieanum* have been deposited in the Herbarium of the Department of Botany, Darrang College (DCH), Tezpur, Assam, India.

RESULT

EAF: Morphologically, it is dorsiventral and flat, V-shaped adaxially in the mid-vein region, tapering towards the edges and slightly turning downwards (Fig. 1C).

Epidermis in surface view: Both, upper and lower leaf epidermises are single layered and consist of thin walled cells. Upper epidermal cells are pentagonal to polygonal, while the lower epidermal cells are tetragonal to polygonal. The upper epidermal cells are significantly larger than the lower epidermal cells. These larger epidermal cells function as 'water-storage cell' (Fig. 1C). The number of epidermal cell/mm² on the upper surface 167, while on the lower epidermis, it accounts 503. The size of epidermal cell on upper surface is 153.80 x 146.68 μ m and on lower surface, it is 70.49 x 60.52 μ m. Stomata are present only in lower epidermis *i.e.* hypostomatic. Stomata are anomocytic with 4 – 5 subsidiary cells which are alike with those of adjacent epidermal cells. The number of stomata/mm² is 118 and their size 43.47 x 36.31 μ m. The stomatal index is 19.00.

Transection: Both the upper and lower leaf epidermis uniseriate and thickly cuticle. The upper and lower cuticles measure 40.94 and 21.95 μ m, respectively (Fig. 2A). Mesophyll is of spongy parenchyma only, 11-13 layered and rich in chloroplast. Mesophyll cells of one or two upper and lowermost layers are comparatively smaller than those of middle layers and are tetragonal to polygonal with small intercellular spaces; a few cells contain acicular raphid bundles and are devoid of starch grains.

Vascular bundles 13 – 16, arranged in single series in the mesophyll, comparatively smaller (135.28 x 109.65 μ m) towards the edges than the middle ones (250.62 x 155.21 μ m); shape ranged from oval to elliptic-oblong (Fig. 2B). In each vascular bundle, xylem is lying towards the adaxial surface and the phloem towards the abaxial surface of the leaf.

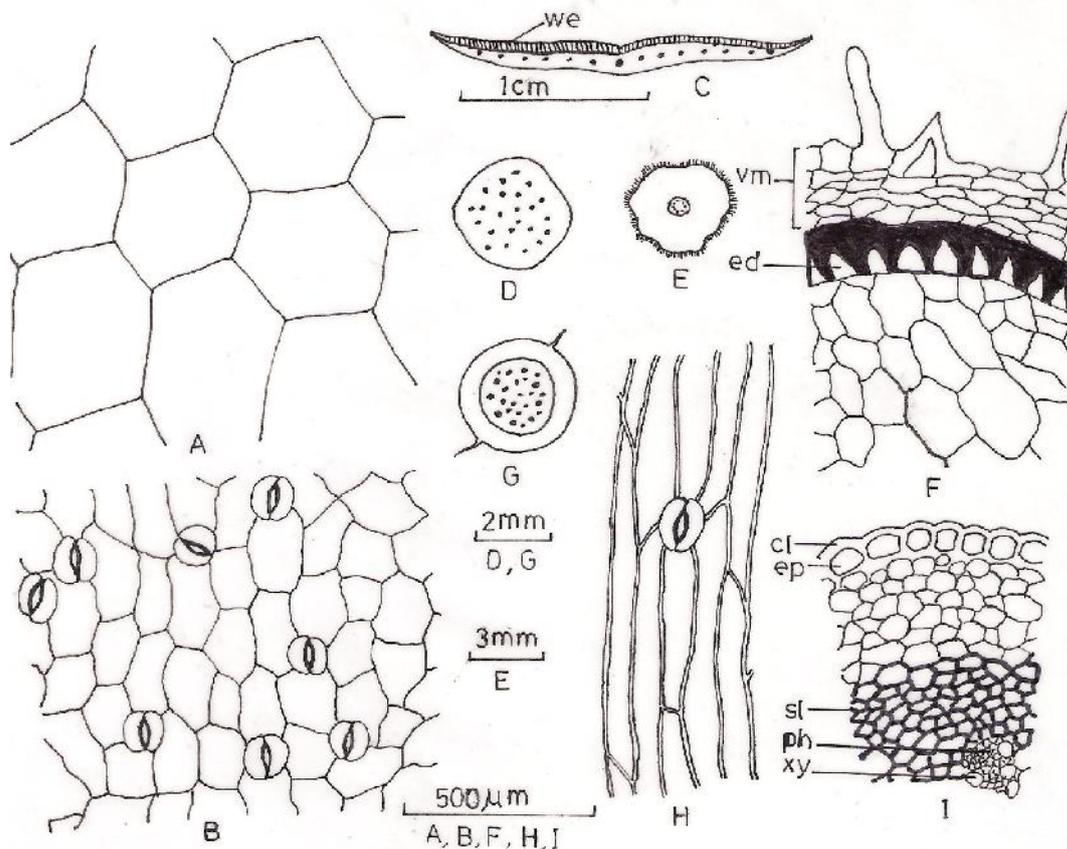


Fig. 1A-I – Anatomical structures of *Paphiopedilum fairieanum* (we, water storage epidermal cell; vm, velamen; ed, endodermis; cl, cuticle; ep, epidermal cell; sl, sclerenchyma; ph, phloem; xy, xylem). **A.** Upper epidermis of leaf. **B.** Lower epidermis of leaf. **C.** Outline drawing of transection of leaf showing water storage epidermal cell. **D.** Outline drawing of transection of rhizome. **E.** Outline drawing of transection of root. **F.** T.S. through root showing velamen and exodermis. **G.** Outline drawing of transection of peduncle. **H.** Peduncle epidermis in surface view. **I.** T.S. through peduncle showing cuticle, epidermis, sclerenchyma, phloem and xylem.

RHIZOME: It is almost circular in outline with broad U to V shape ridges. Cortical cells tetragonal to polygonal. Peripheral cortical cells are comparatively smaller than middle one. Vascular bundle numerous, lie scattered in the ground tissue, oval to elliptic-globose in shape, size ranged from 111.07 – 143.82 x 82.59 – 115.34 μm . (Fig. 2C)

ROOT: wavy in outline with long, filiform surface hairs (Fig. 1E). Transection revealed that velamen consists of 4 – 6 layered, elongated, rectangular to polygonal, thinly cutinized cells (Fig. 1F). Epivelamen generally gets ruptured in matured roots and bears long and filiform hairs. Exodermis 1- layered, polygonal to rectangular, elongated cells with U to V-shaped thickenings. Cortex 10-13 layered, comprising rectangular to polygonal, thin walled, parenchymatous cells whose outer one or two layers are comparatively smaller and slightly lignified but the innermost layers are unlignified (Fig. 1F). Cortical cells are packed with starch grains. Air cavities are not so prominent. Vascular bundles 9, radially arranged, 159.48 – 192.24 x 135.28 – 163.76 μm . Medullary cells rounded to polygonal, parenchymatous with small intercellular spaces.

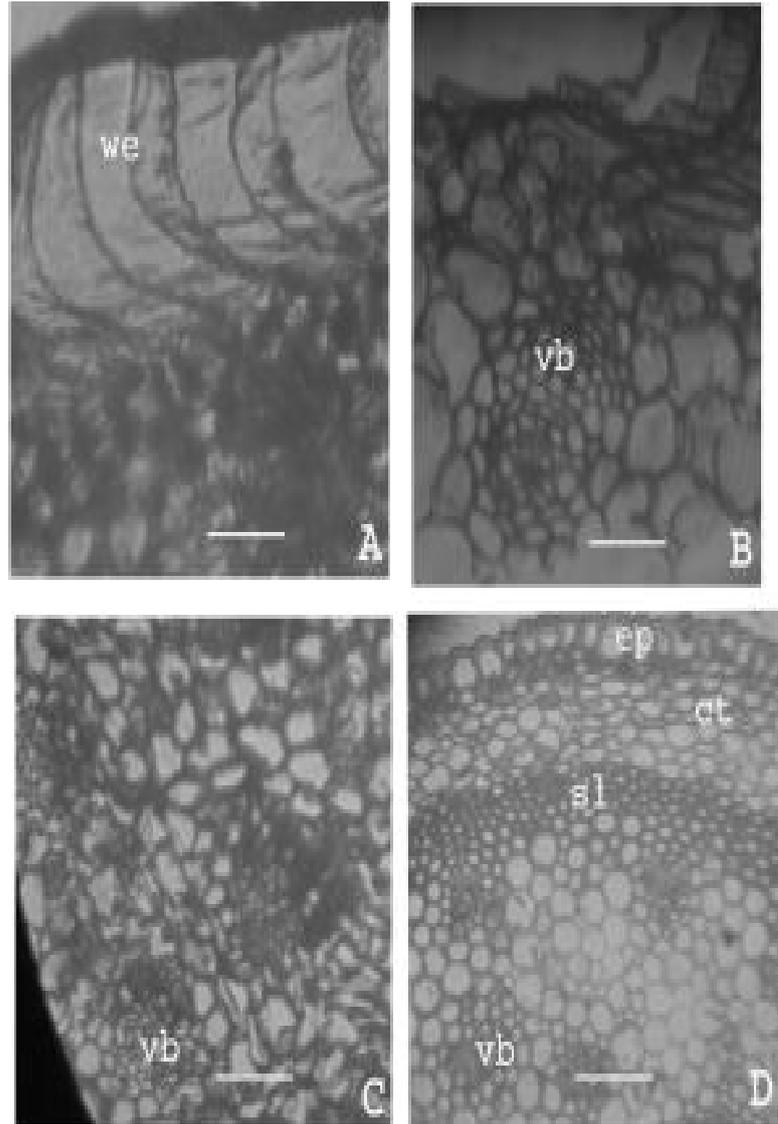


Fig. 2A-D. Anatomical structures of *Paphiopedilum fairieanum* (we, water storage epidermal cell; vb, vascular bundle; ep, epidermal cell; ct, cortex; sl, sclerenchyma): **A.** T.S. of leaf on abaxial side showing water storage epidermal cell. **B.** T.S. of leaf showing vascular bundle. **C.** T.S. of rhizome showing vascular bundle. **D.** T.S. of peduncle showing epidermis, cortex, sclerenchyma and vascular bundle. (A-D x 800), (Scale = 200 μ m).

PEDUNCLE: It is circular in outline (Fig. 1G).

Epidermis in surface view: cells thick walled, elongated and polygonal. Stomata anomocytic, 62.30 x 45.43 μ m. Stomata and epidermal cell/mm² accounts 7 and 234, respectively. epidermal cells 359.56 x 27.63 μ m. The stomatal index is 2.73.

Transection: Epidermis is uniseriate (Fig. 1I & 2C). Cuticle 24.20 μ m thick. The cortical zone is 5-7 layered and composed of circular to polygonal, thin walled, parenchymatous cells. It is followed by a 4-7 layered, polygonal, thick walled sclerenchymatous cells.

Vascular bundles 24-26, scattered in the ground tissue, size 82.59 – 113.92 x 55.45 – 86.87 μm , and shape oval to globular (Fig. 2D).

DISCUSSION

Vegetative anatomical characters are reported to be useful in distinguishing orchid species (Baruah 1998; Baruah & Saikia 2002; Baruah & Sonowal 2010; Kaushik 1983; Khasim & Ram 1989; Stern & Judd 1999). Kaushik (1983) studied the anatomical characters of leaf, rhizome and root of *P. insigne* and *P. venustum*. A comparative account on the anatomical characters of *P. fairieanum* evaluated in the present investigation with that of reported (Kaushik 1983) *Paphiopedilum* species viz. *P. insigne* and *P. venustum* are presented in Table 1.

Table 1. Comparative anatomical characters of *P. fairieanum*, *P. insigne* and *P. venustum*

<i>P. fairieanum</i> (Present observation)	<i>P. insigne</i> (Kaushik 1983)	<i>P. venustum</i> (Kaushik 1983)
Leaf: Hypostomatic; stomata anomocytic, 118 per mm^2 , 43.47 x 36.31 μm in size; -subsidiary cells 4-5, similar to epidermal cells; upper epidermal cells much larger than the lower ones; upper-epidermal cell 153.80 x 146.68 μm	Leaf: Hypostomatic, stomata anomocytic, 62 per mm^2 , 64 x 49 μm in size; subsidiary cells 4-6, similar to epidermal cells; upper epidermal cells much larger than the lower ones, upper epidermal cell 256-320 x 96-128 μm .	Leaf: Hypostomatic, stomata anomocytic, 25 per mm^2 , 70 x 60 μm in size; subsidiary cells 4-6, similar to epidermal cells; upper epidermal cells much larger than the lower ones, upper epidermal cell 288-320 x 224-256 μm .
Rhizome: Cortical cells tetragonal to polygonal. Vascular bundle numerous, scattered in the ground tissue, oval to elliptic-globular in shape.	Rhizome: Cortical cells globular to polygonal. Vascular bundle numerous, scattered in the ground tissue, oval-globular in shape.	Rhizome: Cortical cells globular to polygonal. Vascular bundle numerous, scattered in the ground tissue, oval-globular in shape.
Root: Velamen of 4-6 layered cells. Cortex 10-13 layered. Vascular bundles nine.	Root: Velamen of 9-10 layered cells. Cortex 13-16 layered. Vascular bundles 8-9.	Root: Velamen of 9-10 layered cells. Cortex 13-16 layered. Vascular bundles 8-9.

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