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Research Article

Anatomical study of *Dendrobium* (Orchidaceae) of Nepal

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Abstract: *Dendrobium* Swartz is one of the largest and most problematic genus of subtribe Dendrobiinae, tribe Malaxideae of family Orchidaceae. Twenty-six species of *Dendrobium* investigated are characterized by the variations in anatomical characters of leaves, stems, stomata and stegmata. These anatomical characters are taxonomically significant and used as distinguishing characters for delimiting taxa at infrageneric level. An artificial dichotomous key based on anatomical characters of leaves and stems for the identification of Dendrobium species of Nepal is provided. The cluster analysis based on anatomical with micromorphological characters is carried out with the aim of investigating interrelationships among the species of *Dendrobium* present in Nepal.

Keywords: Dendrobium; Orchidaceae; Anatomy; Stomata; Stegmata; Cluster analysis

Introduction

Dendrobium is a dominant genus of Orchidaceae and was established by Olof Swartz in 1799. *Dendrobium moniliforme* (L.) Sw. is the type for the genus *Dendrobium* (Holttum, Brieger and Cribb 1979). Species of *Dendrobium* are mostly epiphytes with some are lithophytes. *Dendrobium* Swartz comes under subtribe Dendrobiinae and it consists of about 1450 species worldwide (Schuiteman, 2014). Rajbhandari *et al.,* (1999) reported that largest number of epiphytic orchids are found in the lower temperate regions of Central and Eastern Nepal, The Subtribe Dendrobiinae is represented by genera *Dendrobium, Epigeneium* and *Flickingeria* (Dressler, 1993). But according to update classification of Orchidaceae (Chase *et al.,* 2015) two genera *Epigeneium* and *Flickingeria* were merged into single genus *Dendrobium*. The distinguishing characters of the genus *Dendrobium* are presence of cane-like stem with pseudo-bulb covered with or without sheathing leaf bases, distichous or duplicate leaves, spiral flowers with basally jointed lips with prominent spurs formed by the column foot and naked pollinia.

Although morphological and floristic studies of Orchidaceae have been carried out extensively, anatomical investigations are yet inadequate on orchids from Nepal. Only a few anatomical characters have been explored by some of the scientists as a supplementary portion of the revision of respective genera (*Coelogyne, Dendrobium, Eria, Gastrochilus, Oberonia* and *Pholidota*) in Nepal. In subtribe Dendrobiinae, only certain species have been studied anatomically as a part of a broad investigation (Morris *et al.,* 1996). Kaushik (1983) explored the ecological and anatomical marvels of the Himalayan Orchids and discussed the impact of anatomy on the classification of the family and formulated the phylogenetic classification based on the morpho-anatomical characters. Morris *et al.,* (1996) studied the vegetative anatomy and systematic of subtribe Dendrobiinae. They found that the anatomy of plants reflects a high degree of morphological diversity and many of the anatomical characters appear to be homoplasous. They also recommended that a more detailed understanding of the phylogeny of subtribe Dendrobiinae will require the characters from morphology, micromorphology, anatomy and DNA studies. Carlsward *et al.,* (1997) studied the comparative leaf anatomy and systematic in *Dendrobium*, Sections *Aporum* and *Rhizobium* and concluded that two sections of *Dendrobium* are hypothesized to be sister taxa because of synapomorphies in their foliar anatomy. The cladistic analysis performed with various anatomical characters of the leaf demo-

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nstrates that both groups are monophyletic. Anatomical characterization helps in the identification of plant taxa when morphological characters are similar. It also helps in understanding the physiological processes and phylogenetic relationships among plant species (Liu and Zhu, 2011).

Micromorphological study on leaf epidermal surface had been widely used in taxonomic and phylogenetic studies. Singh and Singh (1974) studied the organization of stomatal complex in some Orchidaceae and concluded that the presence of tetracytic stomata was considered significant in evolutionary relationships of the family. Kaushik (1983) and Rasmussen (1987) studied the ecological significance of dermal characters. Khasim and Mohana Rao (1990) studied anatomy in relation to taxonomy in some members of Epidendrodoideae. Vij *et al.,* (1991) studied the epidermal features of Indian orchids for taxonomic and ecological implications. Yukawa *et al.,* (1992) reported the existence of two types of stomatal shape in the genus *Dendrobium* and its systematic significance. Arditti (1992) suggested that the shape of subsidiary cells had been used to distinguish the stomatal complex of Orchidaceae into several types. In concerning about Himalayan species, Shakya (1999) classified eight types of stomatal complex according to the arrangement of subsidiary cells among the twelve species of the genus *Oberonia.* Bajracharya (2003) observed seven types of stomata within the Himalayan genus *Eria.* Dangol (2006) studied the anatomical structures of subtribe Coelogyninae.

Stegmata or silica cell in orchids was reported and illustrated by Link (1849) for the first time but described them as projections from the sclerenchyma. The stegmata are restricted to the monocotyledon families Orchidaceae, Palmae, Maranthaceae, Musaceae, and with some doubt Juncaceae and not found in dicotyledons (Kohl, 1889). He distinguished two main kinds of stegmata in orchids one with conical bodies and other with spherical bodies. Stegmata with conical bodies are widespread within the family and those with spherical bodies are found in vandoid orchids and *Dendrobium*. Although the distribution of stegmata in orchids seems to be of systematic value and phylogenetic interest, little attention has been paid to them since then. Moller and Rasmussen (1984) studied the distribution of stegmata in 130 species representing 105 genera in Orchidales. They reported that the stegmata bearing silica bodies occur in longitudinal files lining fibre bundles and vascular bundle sheaths of rhizomes, stems and especially in the leaves and rarely in roots. Dressler (1993) reported that in most orchids the silica cells are roughly conical in shape but they are spherical and rather lumpy in the *Vandae*, *Eriinae*, *Podochilinae* and *Dendrobiinae*. Carlsward *et al.,* (1997) reported that stegmata containing rough-surfaced spherical bodies are found associated with phloic sclerenchyma of the larger bundles of *Dendrobiun leonis, D. anceps, D. brevimentum, D. aloifolium, D. distichum, D. indivisum, D. mannii, D. rosellum* and *D. nathaniele* and they are absent along vascular bundle sclerenchyma in *D. acinaciforme*. Bajracharya (2003) studied the stegmata on leaf surface of 28 species of *Eria*. He reported that the study of stegmata reveal valuable characters that had been used in delimiting the taxa *Eria* at species level.

Presently 29 species of *Dendrobium* are reported from Nepal Himalaya. Out of 29 species, 26 species of *Dendrobium* were studied for anatomical structures. Remaining 3 species (*D. darjeelingense* Pradhan, *D. chryseum* Rolfe and *D. plicatile* Lindley) were excluded from anatomical study because these species could not be collected from field visits. This may be due to extinction of orchids from their natural habitats because of their medicinal values, deforestation or landslides. In concerning about *D. darjeelingense* it was known that this species was destroyed due to forest firing. This study will provide the taxonomic significance, relationships and variations among different species of *Dendrobium* of Nepal.

Materials and Methods

Study sites and sample collection

In this study, the plant materials were collected from West, Central and East Nepal on the basis of those regions mentioned in the herbaria and also from the available literature. The collected specimens were preserved in FAA (Formalin

0.5 parts, glacial acetic acid 0.5 parts, 70% ethanol 9 parts) and stored in 70% ethanol for anatomical studies. These techniques involve killing and fixing the tissues in FAA and then storing in 70 % ethanol before sectioning.

Anatomical study

Anatomical studies of the different parts of the plant body such as leaves and stems or pseudobulbs were studied on the collected materials by two methods *viz.,* Free hand sections and Microtomy.

In Free hand sections method, the plant parts were hand sectioned into 25-30 µm with the help of sharp blades and dehydrated with different alcohol series. Double staining was done by Safraninfast green combination (Johansen, 1940) and the sections were mounted in DPX. The different anatomical characters were observed and measured using compound light microscopes (Olympus) fitted with ocular micrometer scale. Microscopic photography was carried out of the observed specimens. In Microtomy method, sections were prepared using standard micro-technical methods for anatomy by using a rotary microtome (Yorco Spencer type) as described by Cutler (1978). For microtome sections, leaf and stem parts of specimens were dehydrated in alcohol and xylene series, infiltrated and embedded in paraffin wax (melting point 60-62$^{0.}$c). The embedded materials in the block of wax were sectioned with a rotary microtome at a thickness of 15-30 µm. Staining, measurement and photography were same as in free hand sections method.

Micromorphological study

Stomata

The leaf material for epidermal peeling was prepared by immersing in Potassium hydroxide solution (5%) overnight, rinsed with deionized water and again immersed in fresh solution of potassium hydroxide solution for few hours, then washed with water and treated with glacial acetic acid for 2-5 minutes (Carpenter 2005; with some modification). The peeled epidermal layer was then treated with Sodium hypochlorite (4%) until the tissue got discolored. It was then washed with water for several times and stained in Safranin (1%) and mounted in glycerin. The leaf epidermis was observed under light microscope. The size of stomata and stomatal pore of each specimen were measured by using the standardized ocular micrometer scale. The microscopic photography was carried out. The stomatal distribution on the both surfaces were observed and noted. The terminologies used for stomatal complex types were followed those of Patel (1979). The data were stored in the tabulated form. Stomatal index and stomatal frequencies were calculated using the formula given by Salisbury (1928).

$$SI = S / E+S \times 100,$$

Where, SI = Stomata Index, E = Average number of epidermal cells per unit area or microscopic field, S = Average number of stomata in microscopic field.

$$SF=S/A \text{ per } mm^2$$

Where, SF= Stomata frequency, A= Area of microscopic field.

$$A= \pi r^2$$

Where, π = 3.14, r = radius of the microscopic field.

Stegmata

Stegmata were studied on the leaf surfaces of specimen by peeling the epidermal layer, stained in Safranin (1%) and mounted on glycerin. The leaf epidermis was observed under light microscope and microscopic photography was carried out.

Cluster analysis

In the present study, the character and character states based on collected live specimens and herbarium specimens were described to carry out the cluster analysis. Altogether 60 characters (anatomical with micromorphological) were considered to perform the cluster analysis.

Data matrix

Altogether 60 variable anatomical characters were scored as multistate characters. The multistate characters were coded as 0-4. The complete data matrix of characters and character states are presented in Table 1. The data matrix of different species is presented in Table 2.

Data analysis

The cluster analysis was performed on the anatomical with micromorphological data with pvclust (Suzuki and Shimodaira, 2011), an R package (R version 3.1.0, 2014). The pvclust is an implementation of bootstrap analysis on a statistical software R for hierarchical clustering with *p*-values. The pvclust calculates probability values (*p*-values) for each cluster during bootstrap resampling techniques. In pvclust, two types of *p*-values are available: Approximately Unbiased (AU) *p*-value and Boots-trap Probability (BP) *p*-value. Multiple bootstrap resampling is used for the calculation of AU *p*-value which has superiority in bias over BP value calculated by normal bootstrap resampling. R-pvclust analysis was performed on anatomical data with 10000 bootstrapping to get the stronger cluster supported by data. The average cluster analysis with correlation distance was used to analyze the data set.

Results

Anatomy with micromorphology

Leaf Surface

Epidermis: cells vary from square to rectangular to polygonal in shape. Rectangular to polygonal epidermal cells are present in species of all the species of *Dendrobium* except in *D. bicameratum, D. chrysanthum, D. crepidatum, D. denneanum, D. densiflorum, D. denudans, D. fuscescens, D. fugax* and *D. porphyrochilum* are foundsquare to polygonal epidermal cells are present.

Stomata: present on abaxial surface only in all species of *Dendrobium*. a, b and c tetra-monocyclic stomata are present in all species of *Dendrobium* except in *D. anceps* (eupara twi-monocyclic with hexa-monocyclic stomata) and *D. fugax* (eupara twi-monocyclic stomata).

Three types of stomata i.e. elliptical, circular and sub-orbicular are present in all species of *Dendrobium*. Largest stoma was found in *D. formosum* and smallest in *D. fuscescens*. Largest stomatal pore was found in *D. nobile* and smallest in *D. porphyrochilum*.

Stegmata: with roughsurfaced spherical silica bodies are present all the species of Dendrobiumand not present in *D. densiflorum, D. formosum, D. longicornu* and *D. polyanthum* (Table 3, Plates 1, 2, 3, 4, 5, 6, 7, 8).

Leaf (T.S.)

Cuticle: Thin cuticles are present in *D. aphyllum, D. crepidatum, D. denudans, D. eriiflorum, D. fimbriatum,D. formosum, D. gibsonii, D. heterocarpum, D. longicornu, D. monticola, D. moschatum, D. nobile, D. polyanthum, D. porphyrochilum* and *D. transparens* whereas thick cuticles are present in *D. amoenum, D. amplum, D. anceps, D. bicameratum, D. chrysanthum, D. denneanum, D. densiflorum, D. fuscescens, D. fugax, D. moniliforme* and *D. rotundatum*. Cuticle is intermittently domed in *D. anceps*.

Epidermis: cells vary from square to rectangular to hexagonal to polygonal. The adaxial cells are larger than abaxial cells.

Hypodermis: single layer of rounded small cells is present abaxially in *D. anceps* and adaxially in *D. densiflorum* and absent in other species.

Fibre bundles: present in one series both on adaxial and abaxial sides subtending the hypodermis in *D. anceps* and absent in other species.

Mesophyll: homogenous and heterogenous. Heterogenous mesophylls are present in *D. amplum* and *D. rotundatum* whereas homogenous mesophylls are present in remaining species. Cells are thin walled, columnar to oval to polygonal. Cell layers range from 6 in *D. monticola* to 18 in *D. anceps* in homogenous mesophylls. In heterogenous mesophylls, palisade layers 2 in *D. amplum* and *D. rotundatum* whereas spongy layers 7 in *D. amplum* and 8 in *D. rotundatum*.

Vascular bundle: conjoint, collateral and closed bundles are arranged in one row with a large bundle at midrib. Midrib vascular bundle is oval to oblong to flask shaped to circular to conical. U-shaped fibre caps are present at xylem poles and C-shaped fibre caps at phloem poles of midrib vascular bundles. In case of *D. moschatum,* and *D. densiflorum* dome-shaped fibre caps are present at xylem poles.

Table 1. Characters and character states used in Cluster analysis of *Dendrobium*

S.No.	Character	Character states
0	Stomatal shape	(0) elliptic; (1) circular; (2) sub-orbicular
1	Stomatal type	(0) a-tetra-monocyclic; (1) b-tetra-monocyclic; (2) c-tetra-monocyclic; (3)eupara-twi-monocyclic;(4) eupara-twi-monocyclic+hexa-monocyclic
2	Stomata length, µm	(0) < 33; (1) 33 - 37; (2) > 37
3	Stomata width, µm	(0) < 26; (1) 26 - 28; (2) > 28
4	Stomatal pore length, µm	(0) < 16; (1) 16 - 18; (2) > 18
5	Stomatal pore width, µm	(0) < 7; (1) 7 – 9.99; (2) > 9.99
6	Stomatal frequency, mm^2	(0) < 80; (1) 80-120; (2) > 120
7	Stomatal index, %	(0) < 7; (1) 7 - 10; (2) > 10 %
8	Leaf epidermal cell type	(0) parenchyma; (1) sclerenchyma
9	Stegmata	(0) absent; (1) present; (2) indistinct
10	Cuticle layer of leaf	(0) thick; (1) thin
11	Adaxial leaf epidermal cell width (largest), µm	(0) < 46; (1) 46 - 67; (2) > 67
12	Adaxial leaf epidermal cell length (largest), µm	(0) < 28; (1) 28 - 40; (2) > 40
13	Abaxial leaf epidermal cell width (largest), µm	(0) < 28; (1) 28 - 42; (2) > 42
14	Abaxial leaf epidermal cell length (largest), µm	(0) < 18; (1) 18 - 24; (2) > 24
15	Leaf hypodermis position	(0) absent; (1) adaxial; (2) abaxial
16	Leaf hypodermal layer	(0) absent; (1) 1
17	Mesophyll layer type	(0) homogenous; (1) heterogenous
18	Homogenous mesophyll layers	(0) absent; (1) ≤ 7; (3) 8 - 10
19	Heterogenous mesophyll spongy layers	(0) absent; (1) 7; (2) 8
20	Water storage cells in mesophyll	(0) absent; (1) present
21	Calcium oxalate crystals in mesophyll	(0) absent; (1) present
22	Midrib vascular bundle shape	(0) oval; (1) flask-shaped; (2) conical; (3) oblong; (4) circular
23	Midrib vascular bundle length, µm	(0) <140; (1) 140 - 200; (2) > 200
24	Midrib vascular bundle width, µm	(0) < 120; (1) 120 - 170; (2) > 170
25	Fibre bundle	(0) absent; (1) present
26	Major vascular bundle at leaf pole	(0) absent; (1) present
27	Fibre cap on midrib bundle	(0) absent; (1) present
28	Xylem fibre cap layers in midrib bundle	(0) absent; (1) < 4; (2) 4 - 6; (3) > 6
29	Phloem fibre cap layers in midrib bundle	(0) absent; (1) < 4; (2) 4 - 5; (3) > 5

30	Xylem fibre cap length, µm	(0) absent; (1) < 30; (2) 30 - 50; (3) > 50
31	Xylem fibre cap width, µm	(0) absent; (1) < 120; (2) 120 - 150; (3) > 150
32	Phloem fibre cap length, µm	(0) absent; (1) < 27; (2) 27 - 37; (3) > 37
33	Phloem fibre cap width, µm	(0) absent; (1) < 130 (2) 130 - 175; (3) > 175
34	Xylem length (largest), µm	(0 < 19; (1) 19 - 25; (2) > 25
35	Xylem width (largest), µm	(0) < 10; (1) 10 – 17; (2) > 17
36	Phloem length (largest), µm	(0) < 6; (1) 6 - 9; (2) > 9
37	Phloem width (largest), µm	(0) < 4.0; (1) 4.0 - 6.5; (2) > 6.5
38	Stem epidermal cell shape	(0) rectangular to polygonal; (1) rectangular to square; (2) rectangular to angular; (3) rectangular; (4) barrel-shaped
39	Stem epidermal cell width (largest), µm	(0) < 18; (1) 18 - 25; (2) > 25
40	Stem epidermal cell length (largest), µm	(0) < 12; (1) 12 – 16; (2) > 16
41	Stem hypodermal layer	(0) absent; (1) 1; (2) 2 - 3; (3) 4 - 5
42	Stem hypodermal cell shape	(0) absent; (1) oblong-Polygonal; (2) rectangular-polygonal; (3) polygonal; (4) oval-polygonal
43	Stem vascular bundle shape	(0) oval; (1) elongate
44	Stem vascular bundle no.	(0) 60; (1) 60 - 70; (2) > 70
45	Stem vascular bundle length (smallest), µm	(0) < 100; (1) 100 - 130; (2) > 130
46	Stem vascular bundle width (smallest), µm	(0) < 80; (1) 80 - 90; (2) > 90
47	Stem vascular bundle length (largest), µm	(0) < 190; (1) 190 - 230; (2) > 230
48	Stem vascular bundle width (largest), µm	(0) < 100; (1) 100 - 130; (2) > 130
49	Fibre cap in vascular bundle of stem	(0) absent; (1) present
50	Xylem fibre cap length (largest), µm	(0) absent; (1) ≤ 45; (2) > 45
51	Xylem fibre cap width (largest) µm,	(0) absent; (1) ≤ 100; (2) > 100
52	Phloem fibre cap length (largest), µm	(0) absent; (1) < 30; (2) 30 – 37; (3) > 37
53	Phloem fibre cap width (largest), µm	(0) absent; (1) < 80; (2) 80 - 100; (3) > 100
54	Xylem length (largest), µm	(0) < 18; (1) 18 - 27; (2) > 27
55	Xylem width (largest), µm	(0) < 15; (1) 15 - 20 ; (2) > 20
56	Phloem length (largest), µm	(0) < 7; (1) 7 - 10; (2) > 10
57	Phloem width (largest), µm	(0) < 5; (1) 5 - 8; (2) > 8
58	Stegmata in vascular bundle of stem	(0) absent; (1) present
59	Raphide bundles in stem	(0) absent; (1) present.

Table 2: Data matrix for cluster analysis of *Dendrobium*

S. N.	Taxa	Characters and character states with codes																													
	Dendrobium											**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	*D. amoenum*	0	1	0	0	0	0	0	0	0	1	1	2	1	2	1	0	0	0	1	0	0	1	0	1	1	0	0	1	2	1
2	*D. amplum*	2	1	1	2	1	0	1	0	1	1	0	1	1	2	1	0	0	1	0	1	0	0	1	1	1	0	0	1	2	3
3	*D. anceps*	0	4	0	0	0	0	0	0	0	2	0	0	0	0	0	2	1	0	3	0	1	1	4	0	1	1	1	1	1	1
4	*D. aphyllum*	0	0	1	0	1	1	0	1	0	1	1	2	2	2	2	0	0	0	2	0	1	0	0	0	0	0	0	1	1	1
5	*D. bicameratum*	0	0	1	2	2	1	0	1	0	2	0	1	1	1	0	0	0	0	2	0	0	0	0	0	0	0	0	1	1	1
6	*D. chrysanthum*	1	0	1	0	1	0	0	0	0	1	1	2	0	1	1	0	0	0	2	0	0	0	0	2	2	0	0	1	3	2
7	*D. crepidatum*	0	1	0	1	0	1	2	2	0	1	1	0	2	0	2	0	0	0	2	0	0	1	2	2	1	0	0	1	2	3
8	*D. denneanum*	1	0	0	1	1	1	2	1	1	1	0	0	0	0	0	0	0	0	2	0	0	1	2	2	2	0	0	1	2	2
9	*D. densiflorum*	0	0	1	1	2	2	0	0	1	0	0	0	0	0	1	1	1	0	3	0	0	1	2	2	2	0	0	1	3	3
10	*D. denudans*	0	0	2	1	2	1	1	2	0	2	1	0	0	1	0	0	0	0	1	0	0	1	3	0	0	0	0	1	1	1
11	*D. eriiflorum*	0	2	2	0	2	2	2	2	0	2	1	0	2	0	2	0	0	0	1	0	0	1	0	0	0	0	0	1	2	2
12	*D. fimbriatum*	1	0	1	2	1	1	2	2	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	1	2	2
13	*D. formosum*	1	2	2	2	2	0	0	2	1	0	1	2	2	2	2	0	0	0	2	0	0	1	0	2	2	0	0	1	3	2
14	*D. fugax*	1	3	0	2	0	2	2	2	1	2	0	0	0	0	0	0	0	0	3	0	0	1	1	2	1	0	0	1	3	3
15	*D. fuscescens*	1	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	0	1	0	1	0	0	1	2	1	0	0	1	2	3
16	*D. gibsonii*	0	0	1	0	2	0	0	1	0	1	1	1	1	1	0	0	0	0	2	0	0	1	0	2	2	0	0	1	2	2
17	*D. heterocarpum*	1	0	2	2	1	2	1	2	0	1	1	2	2	1	2	0	0	0	1	0	0	1	0	1	0	0	0	1	2	1
18	*D. longicornu*	0	0	0	0	0	0	2	2	0	0	1	1	2	2	2	0	0	0	2	0	0	1	4	1	1	0	0	1	2	2
19	*D. moniliforme*	1	0	2	2	1	0	2	2	0	2	0	1	2	0	1	0	0	0	2	0	0	1	0	0	1	0	0	1	1	2
20	*D. monticola*	0	0	2	1	2	1	2	2	0	2	1	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	1
21	*D. moschatum*	1	0	0	1	1	1	0	0	0	1	1	1	0	0	0	0	0	0	3	0	1	1	2	2	2	0	0	1	3	3
22	*D. nobile*	0	2	2	2	2	2	1	2	0	1	1	2	1	2	2	0	0	0	2	0	1	1	0	1	1	0	0	1	1	1
23	*D. polyanthum*	0	1	0	1	1	0	0	1	0	0	1	0	0	1	0	0	0	0	2	0	1	0	4	2	2	0	0	1	3	2
24	*D. porphyrochilum*	0	0	1	0	0	0	0	1	0	1	1	2	1	1	1	0	0	0	1	0	1	1	3	0	0	0	0	0	0	0
25	*D. rotundatum*	2	2	0	0	0	0	1	1	1	2	0	1	1	1	2	0	0	1	0	2	1	1	1	1	1	0	0	1	2	3
26	*D. transparens*	0	1	1	1	1	0	2	2	0	1	1	1	2	2	1	0	0	0	2	0	0	1	4	1	1	0	0	1	2	2

S. N.	**Taxa**	**Characters and character states with codes**																																							
	Dendrobium	**2**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**3**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**4**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	*D. amoenum*	3	2	2	2	2	A	1	1	0	1	0	2	3	0	2	2	2	0	1	1	0	0	2	2	2	2	2	2	1	0
2	*D. amplum*	1	2	3	2	1	2	1	1	1	1	1	2	3	1	0	2	0	2	2	1	2	2	3	3	2	1	1	1	1	0
3	*D. anceps*	1	1	1	2	0	0	0	0	4	0	0	2	3	0	1	1	0	1	0	1	1	1	3	2	0	0	0	1	0	0
4	*D. aphyllum*	1	1	1	1	1	1	1	1	4	0	2	1	3	0	2	2	2	2	1	1	0	0	2	2	1	1	2	1	1	1
5	*D. bicameratum*	1	1	1	1	0	0	1	0	4	0	0	2	3	1	2	0	1	1	0	1	0	0	2	1	2	2	0	1	1	0
6	*D. chrysanthum*	2	3	2	3	1	2	2	2	4	1	1	2	4	1	1	1	1	2	2	1	0	0	2	2	2	2	1	1	0	1
7	*D. crepidatum*	2	2	3	2	1	1	1	1	0	2	0	1	1	0	0	1	1	1	0	1	0	0	1	1	1	1	1	2	0	0
8	*D. denneanum*	2	3	2	3	1	1	1	1	4	1	1	3	2	1	2	2	2	2	2	1	0	0	3	3	2	2	2	2	0	0
9	*D. densiflorum*	3	3	3	3	2	1	2	2	3	0	2	3	2	0	2	1	1	1	2	1	0	0	2	3	0	1	0	0	1	1
10	*D. denudans*	0	1	1	1	1	0	1	1	3	2	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0
11	*D. eriiflorum*	2	1	1	1	1	1	1	1	3	2	1	0	0	0	1	1	1	1	1	1	0	0	1	1	0	0	0	0	0	1
12	*D. fimbriatum*	2	1	2	1	1	1	1	1	4	0	0	3	2	0	1	2	2	1	2	1	0	0	3	3	2	0	2	2	0	0
13	*D. formosum*	3	3	2	3	2	2	2	2	0	2	2	2	3	0	1	2	2	2	2	1	0	0	3	3	2	0	2	2	1	0
14	*D. fugax*	3	3	2	3	2	1	2	2	4	1	2	2	3	1	2	1	1	1	2	1	0	0	2	2	1	1	0	1	1	0
15	*D. fuscescens*	3	1	3	3	1	1	2	2	1	2	2	2	3	0	2	0	1	2	1	1	2	2	1	1	1	1	1	1	0	0
16	*D. gibsonii*	3	3	3	3	2	2	2	2	2	0	1	2	3	0	0	1	2	2	2	1	0	0	3	2	2	1	2	2	0	1
17	*D. heterocarpum*	1	1	1	1	1	1	1	1	2	1	1	2	3	0	2	1	2	0	1	1	0	0	3	2	0	0	1	1	0	1
18	*D. longicornu*	2	3	2	1	1	1	1	2	0	1	1	2	3	1	1	0	1	0	1	1	0	0	3	2	1	1	2	2	1	1
19	*D. moniliforme*	1	1	1	2	0	0	0	0	0	1	1	2	3	1	0	2	2	2	2	1	0	0	1	1	1	1	2	2	0	1
20	*D. monticola*	1	1	1	1	0	0	1	1	3	2	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0
21	*D. moschatum*	3	3	3	3	2	2	2	2	4	0	2	2	3	0	2	2	2	0	2	1	1	1	2	3	0	0	0	0	0	1
22	*D. nobile*	2	3	1	3	1	2	2	2	0	2	1	2	3	0	2	1	1	1	2	1	0	0	2	2	1	1	0	1	0	0
23	*D. polyanthum*	3	3	3	2	1	1	1	1	0	2	2	1	3	1	0	0	0	2	2	1	0	0	2	3	2	2	2	2	1	1
24	*D. porphyrochilum*	0	0	0	0	0	0	0	0	0	2	2	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
25	*D. rotundatum*	2	3	3	1	2	2	1	1	1	2	2	2	3	0	2	0	1	0	1	1	0	0	3	2	1	1	2	2	1	1
26	*D. transparens*	2	2	1	2	1	1	1	1	1	1	2	2	3	1	0	0	0	0	0	1	0	0	1	1	0	0	1	1	1	1

In *D. fimbriatum* xylem pole and phloem pole are completely surrounded by fibre cap. STEGMATA with rough surfaced spherical silica bodies are present along the outer margin of xylem and phloem caps in *D. amoenum, D. chrysanthum, D. fugax* and *D. moschatum*; along xylem cap in *D. bicameratum*; along phloem cap in *D. aphyllum, D. crepidatum, D. denudans, D. fuscescens, D. gibsonii, D. nobile*; outer margin of fibre bundle in *D. anceps*;indistinct in *D. amplum, D. denneanum, D. densiflorum, D. fimbriatum, D. formosum, D. heterocarpum, D.longicornu, D. polyanthum, D. porphyrochilum, D. transparens; D. rotundatum*and lacking in *D. eriiflorum, D. moniliforme* and *D. monticola* (Table 4, Plates 1,2, 3, 4, 5, 6, 7, 8).

Stem (T.S.)

Cuticle: thick and smooth in most species of *Dendrobium* but in *D. porphyrochilum* it is thin and smooth. Cuticle is intermittently in *D. anceps*.

Epidermis: cells vary from rectangular to square to polygonal to angular to barrel-shaped in shape. Cells are thin walled in *D. anceps, D. porphyrochilum* and thick walled in other species.

Hypodermis: one to two layers of polygonal parenchymatous cells are present in *D. anceps, D. aphyllum, D. crepidatum* and *D. polyanthum*. In remaining species, two to five layers of rectangular to oval to polygonal sclerenchymatous cells are present. Outer hypodermal cells are smaller than inner cells. Hypodermal cells are smaller than ground tissues.

Ground tissue: no differentiation of cortex, endodermis, pericycle and pith.A mass of thin walled parenchymatous cells with intercellular spaces are present in ground tissue. The cells are of two types: many, small, living, round to polygonal assimilatory parenchymatous cells and fewer, large, scattered, dead, water-storage cells with unpleated outlines in *D. anceps, D. crepidatum, D. densiflorum, D. fimbriatum,D. moschatum, D. nobile,D. polyanthum* and with pleated outlines in other species.

Starch grains are present in parenchymatous cells in all species except *D. amoenum, D. anceps, D. denneanum, D. denudans, D. monticola* and raphide bundles are present in *D. aphyllum, D. chrysanthum,D. densiflorum, D. eriiflorum, D. gibsonii, D. heterocarpum,D. longicornu, D. moniliforme, D. moschatum,D. polyanthum,D. rotundatum*and*D. transparens*. Calcium oxalate crystals are present in *D. porphyrochilum*.

Vascular bundle: conjoint, collateral and closed bundles are scattered in the ground tissue. Number of vascular bundle ranges from 16 (*D. polyanthum*) to 118 (*D. bicameratum*). Vascular bundles are oval to elongate in shape. The inner vascular bundles are larger than outer bundles. Sclerenchymatous bundle sheaths are present surrounding vascular bundles in *D. amoenum, D. anceps, D. chrysanthum, D. crepidatum, D. denudans, D. eriiflorum. D. fuscescens, D. heterocarpum,D. longicornu, D. porphyrochilum* and only xylem in *D. amplum, D. aphyllum, D. bicameratum, D. denneanum, D. densiflorum, D. fimbriatum,D. formosum, D. fugax, D. gibsonii, D. moniliforme, D. monticola, D. moschatumD. nobile, D. polyanthum, D. rotundatum*and *D. transparens*. Fibre caps are present above phloem in *D. amoenum, D. longicornu, D. monticola*; surrounding xylem and phloem in *D. anceps, D. fuscescens* and surrounding phloem in remaining species.

Stegmata with rough surfaced spherical silica bodies are present along the outer margin of phloem cap in *D. amoenum, D. amplum, D. aphyllum, D. bicameratum, D. densiflorum, D. formosum, D. fugax, D. longicornu, D. polyanthum, D. rotundatum, D. transparens*; absent in *D. eriiflorum, D. moniliforme, D. monticola* and indistinct in remaining species (Table 5, Plates 1, 2, 3, 4, 5, 6, 7, 8). From the present study, the stomatal complex, stegmata and anatomical characters of leaves and stems were found to be taxonomically important for delimiting the taxa. Based on characters such as stomatal complex, stegmata and anatomical characters of leaves and stems, an anatomical key has been prepared to delimit the taxa within genus *Dendrobium.*

Table 3: Stomatal and Stegmatal characters in species of *Dendrobium*

S.No.	Taxa	Stomatal size with guard cells (l x w) µm	Stomatal pore size (l x w) µm	Stomatal Frequency mm²	Stomatal Index %	Shape of Stomata	Type of Stomata	Shape of Epidermal cells	Type of Epidermal cells	Stegmata
1	*Dendrobium amoenum* Wallich ex Lindley	29.97 x 23.31	13.32 x 4.99	76.92	5.81	Elliptic	b-tetra-monocyclic	Rect. to poly.	Paren.	Spherical
2	*D. amplum* Lindley	33.30 x 29.97	16.65 x 6.66	107.69	6.03	Sub-orbicular	b-tetra-monocyclic	Rect. to poly.	Scleren.	Spherical
3	*D. anceps* Swartz	29.97 x 25.30	13.32 x 6.66	46.15	3.75	Elliptic	eupara twi + hexa-monocyclic	Rect. to poly.	Paren.	Indistinct
4	*D. aphyllum* (Roxburgh) C.E.C. Fischer	33.30 x 23.31	16.65 x 9.99	46.15	7.31	Elliptic	a-tetra-monocyclic	Rect. to poly.	Paren.	Spherical
5	*D. bicameratum* Lindley	33.30 x 29.97	23.31 x 9.99	76.92	7.04	Elliptic	a-tetra-monocyclic	Squa. to poly.	Paren.	Indistinct
6	*D. chrysanthum* Wallich ex Lindley	33.30 x 23.31	16.65 x 6.66	76.92	5.68	Circular	a-tetra-monocyclic	Squa. to poly.	Paren.	Spherical
7	*D. crepidatum* Lindley & Paxton	32.63 x 26.64	15.32 x 7.32	130.77	13.04	Elliptic	b-tetra-monocyclic	Squa. to poly.	Paren.	Spherical
8	*D. denneanum* Kerr	26.64 x 26.64	16.65 x 9.99	123.07	7.34	Circular	a-tetra-monocyclic	Squa. to poly.	Scleren.	Spherical
9	*D. densiflorum* Wallich	33.30 x 26.64	26.64 x 13.32	76.92	6.76	Elliptic	a-tetra-monocyclic	Squa. to poly.	Scleren.	N.P
10	*D. denudans* D. Don	39.96 x 26.64	26.64 x 9.99	92.3	10.71	Elliptic	a-tetra-monocyclic	Squa. to poly.	Paren.	Indistinct
11	*D. eriiflorum* Griffith	38.62 x 25.97	20.64 x 10.65	123.07	10.66	Elliptic	c-tetra-monocyclic	Rect. to poly.	Paren.	Indistinct
12	*D. fimbriatum Hooker*	33.30 x 34.60	16.65 x 9.99	123.07	10.38	Circular	a-tetra-monocyclic	Rect. to poly.	Scleren.	Spherical
13	*D. formosum* Roxburgh ex Lindley	45.96 x 41.96	24.64 x 6.66	76.92	12.5	Circular	c-tetra-monocyclic	Rect. to poly.	Scleren.	N.P
14	*D. fugax* Reichenbach	29.97 x 29.97	13.32 x 13.32	184.61	10.17	Circular	eupara twi-monocyclic	Squa. to poly.	Scleren.	Indistinct
15	*D. fuscescens* Griffith	23.31 x 23.31	13.32 x 6.66	76.92	5.37	Circular	a-tetra-monocyclic	Squa. to poly.	Scleren.	Spherical
16	*D. gibsonii* Paxton	33.30 x 24.98	19.98 x 6.66	76.92	8.33	Elliptic	a-tetra-monocyclic	Rect. to poly.	Paren.	Spherical
17	*D. heterocarpum* Wallich ex Lindley	38.30 x 33.30	16.65 x 10.00	92.3	13.04	Circular	a-tetra-monocyclic	Rect. to poly.	Paren.	Spherical
18	*D. longicornu* Lindley	29.97 x 23.31	13.32 x 4.99	123.07	10.53	Elliptic	a-tetra-monocyclic	Rect. to poly.	Paren.	N.P
19	*D. moniliforme* (L.) Swartz	38.30 x 34.00	16.65 x 6.66	138.5	12.5	Circular	a-tetra-monocyclic	Rect. to poly.	Paren.	Indistinct
20	*D. monticola* Hunt & Summerhayes	39.96 x 26.64	23.31 x 9.99	200	10.83	Elliptic	a-tetra-monocyclic	Rect. to poly.	Paren.	Indistinct
21	*D. moschatum* (Buchanan-Hamilton) Swartz	27.97 x 26.64	16.65 x 9.99	76.92	5	Circular	a-tetra-monocyclic	Rect. to poly.	Paren.	Spherical
22	*D. nobile* Lindley	44.95 x 39.96	27.30 x 17.98	92.31	15.79	Elliptic	c-tetra-monocyclic	Rect. to poly.	Paren.	Spherical
23	*D. polyanthum* Wallich ex Lindley	32.63 x 26.64	16.65 x 4.99	76.92	7.94	Elliptic	b-tetra-monocyclic	Rect. to poly.	Paren.	N.P
24	*D. porphyrochilum* Lindley	36.63 x 19.98	13.32 x 3.33	76.92	7.14	Elliptic	a-tetra-monocyclic	Squa. to poly.	Paren.	Spherical
25	*D. rotundatum* (Lindley) Hooker	26.64 x 23.31	13.32 x 6.66	92.3	8.33	Sub-orbicular	c-tetra-monocyclic	Rect. to poly.	Scleren.	Indistinct
26	*D. transparens* Wallich ex Lindley	33.30 x 27.31	17.98 x 6.66	138.46	10.71	Elliptic	b-tetra-monocyclic	Rect. to poly.	Paren.	Spherical

Abbreviations used: N.P = Not present; Rect. = Rectangular; poly. = Polygonal; Squa. = Square; Paren = Parenchyma; Scleren. = Sclerenchyma.

Table 4: Anatomical characters of leaf in species of *Dendrobium*

S.No.	Taxa	Cuticle layer	Epidermal cell Size (w x l) µm			Hypodermis		Fibre bundle	Mesophyll		Mid Vascular bundle		Fibre cap size		Xylem size (l x w) µm	Phloem size (l x w) µm
			Shape	Adaxial	Abaxial	Position	Layer		Shape	Layer	Shape	Size (l x w) µm	Xylem cap (l x w) µm	Phloem cap (l x w) µm		
1	*Dendrobium amoenum* Wallich ex Lindley	Thin	Rect. to Poly.	19.98–69.93 x 19.98–33.30	13.32–43.29 x 9.99–23.31	N.P	N.P	N.P	Colu. to Poly.	7	Oval	193.14 x 133.20	83.25 x 143.19	33.30 x 133.20	9.99 – 29.97 x 6.66 – 26.64	4.99–6.66 x 3.33–4.99
2	*D. amplum* Lindley	Thick	Rect. to Poly.	19.98–56.61 x 16.65–29.97	16.65–49.95 x 9.99–19.98	N.P	N.P	N.P	Pali. Poly. to Colu. Spon.Oval to Poly.	Pali. 2 Spon. 7	Flask-shaped	199.80 x 143.19	16.65 x 139.86	46.62 x 166.50	6.66–23.31 x 4.99–19.98	3.33–6.66 x 2.66–4.99
3	*D. anceps* Swartz	Thick	Poly.	16.65–36.63 x 6.66–13.32	16.65–23.31 x 6.66 – 13.32	Abaxial	1	Present	Oval to Poly.	18	Circular	139.06 x 136.53	23.31 x 119.88	26.64 x 139.86	6.66 – 9.99 x 4.99 - 9.99	3.33-4.99 x 2.66-3.33
4	*D. aphyllum* (Roxburgh) C.E.C. Fischer	Thin	Rect. to Hexa.	46.62–76.59 x 36.63–49.95	23.31–66.60 x 13.32–29.97	N.P	N.P	N.P	Oval to Poly.	9	Oval	126.54 x 106.56	19.98 x 86.58	19.98 x 89.91	9.99–19.98 x 6.66 – 16.65	4.99–6.66 x 3.33–4.99
5	*D. bicameratum* Lindley	Thick	Rect. to Poly.	13.32–49.95 x 19.98–29.97	13.32–36.63 x 9.99–13.32	N.P	N.P	N.P	Oval to Poly.	8	Oval	119.88 x 93.24	26.64 x 89.91	19.98 x 93.24	6.66 – 13.32 x 4.99 – 6.66	3.99–6.66 x 2.66–3.33
6	*D. chrysanthum* Wallich ex Lindley	Thin	Rect. to Poly.	19.98–73.26 x 16.65–26.64	9.99–36.63 x 9.99–19.98	N.P	N.P	N.P	Rect.	8	Oval	219.79 x 209.78	49.95 x 186.48	36.63 x 183.15	9.9–19.98 x 8.32–19.98	6.66–9.99 x 3.33–6.66
7	*D. crepidatum* Lindley & Paxton	Thin	Rect. to Poly.	19.98–43.29 x 13.32–43.29	8.32–26.64 x 13.32–26.64	N.P	N.P	N.P	Rect. to Poly.	10	Conical	233.10 x 166.50	43.29 x 126.54	53.28 x 173.16	13.32–19.98 x 11.65–13.32	4.99–8.32 x 3.33–4.99

8	*D. denneanum* Kerr	Thick	Rect. to Poly.	19.98–33.30 x 13.32–16.65	16.65–26.64 x 8.32–9.99	N.P	N.P	N.P	Rect. to Poly.	8	Conical	216.45 x 189.81	49.95 x 166.50	33.30 x 176.49	6.66–19.98 x 4.99–16.65	3.33–6.66 x 3.33–4.99
9	*D. densiflorum* Wallich	Thick	Rect. to Poly.	23.31-36.63 x 13.32–23.31	16.65–26.64 x 13.32–19.98	Adaxial	1	N.P	Oval to Poly.	13	Conical	586.52 x 386.57	213.28 x 319.92	146.63 x 413.23	16.65–29.97 x 6.66–16.65	4.99–9.99 x 3.33–6.66
10	*D. denudans* D. Don	Thin	Rect. to Poly.	23.31–43.29 x 9.99–19.98	23.31–29.97 x 9.99–16.65	N.P	N.P	N.P	Rect. to Poly.	7	Oblong	126.54 x 53.28	23.31 x 53.28	23.31 x 56.61	13.32–19.98 x 4.99–6.66	4.99–6.66 x 3.99–4.99
11	*D. eriiflorum* Griffith	Thin	Rect. to Poly.	19.98–43.29 x 26.64–43.29	13.32–23.31 x 19.98–29.97	N.P	N.P	N.P	Rect. to Poly.	7	Oval	116.55 x 69.93	43.39 x 59.94	23.31 x 76.59	6.66–19.98 x 4.99–13.32	3.33–6.66 x 2.66–4.99
12	*D. fimbriatum Hooker*	Thin	Rect. to Poly.	16.65–39.96 x 13.32–26.64	9.99–23.31 x 6.66–16.65	N.P	N.P	N.P	Rect. to Poly.	7	Oval	169.83 x 123.21	43.29 x 116.55	33.30 x 129.80	9.99–19.98 x 9.99–16.65	3.33–6.66 x 1.66–4.99
13	*D. formosum* Roxburgh ex Lindley	Thin	Rect. to Poly.	36.63–76.59 x 33.30–46.62	19.98–43.29 x 19.98–33.30	N.P	N.P	N.P	Oval to Poly.	9	Oval	216.45 x 193.14	73.26 x 183.15	33.30 x 206.46	13.32–29.97 x 6.66–19.98	3.33–13.32 x 3.33–9.99
14	*D. fugax* Reichenbach	Thick	Rect. to Squa.	19.98–33.30 x 9.99–16.65	13.32–23.31 x 9.99–16.65	N.P	N.P	N.P	Oval to Poly.	15	Flask-shaped	223.11 x 166.50	56.61 x 166.50	36.63 x 199.80	9.99–26.64 x 9.99–16.65	4.99–9.99 x 3.33–6.66
15	*D. fuscescens* Griffith	Thick	Rect. to Poly.	23.31–43.29 x 16.65–26.64	16.65–39.96 x 16.65–23.31	N.P	N.P	N.P	Pali. Poly. to Oval Spon.Oval to Poly.	Pali. 2 Spon. 7	Flask-shaped	209.79 x 156.51	56.61 x 113.22	49.95 x 179.82	9.99–23.31 x 6.66–16.65	4.99–9.99 x 4.99–6.66

16	*D. gibsonii* Paxton	Thin	Rect. to Poly.	23.31–66.60 x 13.32–33.30	16.65–33.30 x 13.32–16.65	N.P	N.P	N.P	Rect. to Poly.	8	Oval	289.71 x 233.10	83.25 x 199.80	49.95 x 233.10	13.32–29.97 x 6.66–23.31	4.99–9.99 x 3.33–8.32
17	*D. heterocarpum* Wallich ex Lindley	Thin	Rect. to Poly.	26.64–73.26 x 26.64–53.28	9.99–33.30 x 13.32–26.64	N.P	N.P	N.P	Rect. to Poly.	7	Oval	149.85 x 116.55	19.98 x 99.90	19.98 x 86.58	6.66–19.98 x 4.99–16.65	3.99–6.66 x 3.66–4.99
18	*D. longicornu* Lindley	Thin	Rect. to Hexa.	23.31–53.28 x 19.98–46.62	19.98–49.95 x 13.32–33.30	N.P	N.P	N.P	Oval to Poly.	8	Circular	166.50 x 166.50	33.30 x 156.51	33.30 x 126.54	9.99–23.31 x 6.66–16.65	4.99–6.66 x 3.33–6.66
19	*D. moniliforme* (L.) Swartz	Thick	Rect. to Poly.	23.31–56.61 x 36.63–46.62	9.99–26.64 x 13.32–19.98	N.P	N.P	N.P	Rect. to Poly.	8	Oval	119.88 x 133.20	26.64 x 106.56	23.31 x 133.20	9.99–10.65 x 4.99–6.66	3.99–5.99 x 2.66–3.33
20	*D. monticola* Hunt & Summerhayes	Thin	Rect. to Poly.	13.32–53.28 x 19.98–33.30	13.32–19.98 x 9.99–19.98	N.P	N.P	N.P	Rect. to Poly.	6	Oval	99.90 x 66.60	23.31 x 53.28	13.32 x 66.60	6.66–9.99 x 3.33–8.32	4.99–6.66 x 3.33–4.99
21	*D. moschatum* (Buchanan-Hamilton) Swartz	Thin	Rect. to Hexa.	26.64–66.60 x 16.65–26.64	16.65–23.31 x 9.99–16.65	N.P	N.P	N.P	Oval to Poly.	16	Conical	506.54 x 333.25	159.96 x 319.92	106.64 x 333.25	19.98–39.96 x 6.66–26.64	4.99–9.99 x 3.33–8.32
22	*D. nobile* Lindley	Thin	Rect. to Hexa.	36.63–83.25 x 73.26–99.90	16.65–53.28 x 23.31–46.62	N.P	N.P	N.P	Oval to Poly.	9	Oval	199.80 x 166.50	49.95 x 166.50	26.64 x 159.84	19.98–23.31 x 9.99–19.98	6.66–9.99 x 4.99–6.66
23	*D. polyanthum* Wallich ex Lindley	Thin	Rect. to Hexa.	16.65–33.30 x 9.99–26.64	13.32–29.97 x 9.99–13.32	N.P	N.P	N.P	Oval to Poly.	8	Circular	213.12 x 199.80	83.25 x 193.14	43.29 x 173.16	6.66–23.31 x 3.33–6.66	4.99–6.66 x 3.33–4.99

24	*D. porphyrochilum* Lindley	Thin	Rect. to Hexa.	49.95–73.26 x 19.98–39.96	23.31–39.96 x 16.65–23.31	N.P	N.P	N.P	Oval to Poly.	6	Oblong	99.90 x 93.24	N.P	N.P	4.99–13.32 x 3.99–9.99	3.33–4.99 x 2.66–3.33
25	*D. rotundatum* (Lindley) Hooker	Thick	Rect. to Poly.	23.31–49.95 x 16.65–33.30	9.99–39.96 x 16.65–26.64	N.P	N.P	N.P	Pali. Poly. to Colu. Spon.Oval to Poly.	Pali. 2 Spon. 8	Flask-shaped	183.15 x 133.20	39.96 x 193.14	53.28 x 126.54	13.32–26.64 x 6.66–19.98	4.99–6.66 x 3.33–4.99
26	*D. transparens* Wallich ex Lindley	Thin	Rect. to Hexa.	23.31–66.60 x 36.63–59.94	13.32–43.29 x 13.32–23.31	N.P	N.P	N.P	Oval to Poly.	8	Circular	149.85 x 146.52	33.30 x 149.85	26.64 x 146.52	13.32–19.98 x 9.99–16.65	3.33–6.66 x 3.33–4.99

Abbreviations used: N.P=Not Present; Rect.=Rectangular; Poly.=Polygonal; Squa.= Square; Hexa.=Hexagonal; Colu.=Columnar; Pali.=Palisade layer; Spon.=Spongy layer.

Table 5: Anatomical characters of stem in species of *Dendrobium*

S.N.	Taxa	Cuticle	Epidermal cell		Hypodermis		Vascular bundle			Fibre cap size		Xylem size (l x w) µm	Phloem size (l x w) µm
			Shape	Size (w x l) µm	Shape	Layer	Shape	No.	Size (l x w) µm	Xylem cap (l x w) µm	Phloem cap (l x w) µm		
1	*Dendrobium amoenum* Wallich ex Lindley	Thick	Rect. to Poly.	9.99–19.98 x 4.99-9.99	Poly.	2	Oval	76	143.19-183.15 x 93.24-113.22	N.P	26.64–33.30 x 83.25–96.57	9.99–29.97 x 6.66–23.31	6.66–16.65 x 4.99–9.99
2	*D. amplum* Lindley	Thick	Rect. to Squa.	9.99–19.98 x 9.99–13.32	Poly.	2	Elongate	50	133.20–333.00 x 66.60–149.85	43.29–56.61 x 106.56–133.20	33.30–99.90 x 83.25–116.55	11.65–26.64 x 9.99–16.65	3.33–9.99 x 3.33–6.66
3	*D. anceps* Swartz	Thick	Barr.	9.99–16.65 x 6.66-9.99	Poly.	2	Oval	64	116.55-199.80 x 73.26–99.90	16.65–26.64 x 79.92–86.58	19.98–49.95 x 73.26–99.90	13.32–16.65 x 4.99–13.32	4.99–6.66 x 3.33–6.66
4	*D. aphyllum* (Roxburgh) C.E.C. Fischer	Thick	Barr.	9.99-13.32 x 16.65-26.64	Poly.	1	Oval	82	133.20–236.43 x 103.23–106.56	N.P	29.97–33.30 x 99.90–93.24	13.32–26.64 x 9.99–19.98	6.66–13.32 x 4.99–6.66
5	*D. bicameratum* Lindley	Thick	Barr.	9.99–13.32 x 6.66-9.99	Poly.	2 to 3	Elongate	118	93.24–199.80 x 83.25–93.24	N.P	16.65–33.30 x 49.95–66.60	13.32–33.30 x 6.66–26.64	4.99–6.66 x 3.33–6.66

6	*D. chrysanthum* Wallich ex Lindley	Thick	Barr.	13.32-19.98 x 9.99-13.32	Oval to poly.	2	Elongate	70	103.23–266.40 x 83.25–133.20	N.P	16.65–33.30 x 83.25–96.57	9.99–33.30 x 9.99–29.97	4.99–9.99 x 3.33–6.66
7	*D. crepidatum* Lindley & Paxton	Thick	Rect. to Poly.	13.32–36.63 x 6.66-6.65	Oblo. to Poly.	1	Oval	60	123.21–209.79 x 83.25–99.90	N.P	19.98–26.64 x 49.95–66.60	13.32–26.64 x 6.66–19.98	4.99–9.99 x 3.33–9.32
8	*D. denneanum* Kerr	Thick	Barr.	9.99–19.98 x 6.66-13.32	Rect. to Poly.	4	Elongate	97	163.17–299.70 x 143.19–149.85	N.P	39.96–49.95 x 116.55–119.88	43.29–49.95 x 23.31–23.31	9.99- 13.32 x 6.66–9.99
9	*D. densiflorum* Wallich	Thick	Rect.	6.66–9.99 x 16.65–16.65	Rect. to Poly.	4 to 5	Oval	78	106.56–206.46 x 89.91–153.18	N.P	26.64–36.63 x 83.25–119.88	9.99–13.32 x 6.66–9.99	3.33–4.99 x 2.66–3.33
10	*D. denudans* D. Don	Thick	Rect.	13.32–26.64 x 6.66–13.32	N.P	N.P	Oval	50	66.60–119.88 x 56.61–76.59	N.P	16.65–16.65 x 49.95–56.6	9.99–13.32 x 6.66- 9.99	3.33–4.99 x 2.66–3.33
11	*D. eriiflorum* Griffith	Thick	Rect.	13.32–26.64 x 6.66–13.32	N.P	N.P	Oval	70	113.22–199.80 x 86.58- 126.54	N.P	19.98–29.97 x 49.95–66.60	13.32–16.65 x 9.99–13.32	3.33-6.66 x 3.33–4.99
12	*D. fimbriatum* Hooker	Thick	Barr.	13.32–16.65 x 9.99–11.65	Rect. to Poly.	4 to 5	Oval	62	133.20–199.80 x 109.89–133.20	N.P	39.96–43.29 x 106.56–123.21	26.64–49.95 x 9.99–13.32	6.66–16.65 x 4.99–9.99
13	*D. formosum* Roxburgh ex Lindley	Thick	Rect. to Poly.	16.65–39.96 x 9.99–16.65	Poly.	2	Oval	64	166.50–233.10 x 119.88–153.18	N.P	33.30–53.28 x 89.91–109.89	26.64–49.95 x 9.99–13.32	6.66–16.65 x 4.99–9.99
14	*D. fugax* Reichenbach	Thick	Barr.	13.32–23.31 x 9.99–16.65	Poly.	2	Elongate	71	116.55–216.45 x 86.58 – 166.50	N.P	26.64–33.30 x 79.92 – 99.90	13.32–23.31 x 9.99–16.65	4.99–6.66 x 3.33–6.66
15	*D. fuscescens* Griffith	Thick	Rect. to Squa.	9.99–26.64 x 6.66–19.98	Poly.	2	Oval	83	99.90–243.09 x 89.91-116.55	16.65–49.95 x 59.94–116.55	16.65–26.64 x 59.94–66.60	6.66–23.31 x 6.66–16.65	3.33–9.99 x 3.33–6.66
16	*D. gibsonii* Paxton	Thick	Rect. to Angu.	13.32–16.65 x 6.66–13.32	Poly.	2	Oval	30	123.31–316.35 x 93.24–183.15	N.P	16.65–69.93 x 76.59–93.24	16.65–29.97 x 9.99–19.98	4.99–13.32 x 3.33–9.99
17	*D. heterocarpum* Wallich ex Lindley	Thick	Rect. to Angu.	13.32–19.98 x 6.66-13.32	Poly.	2	Oval	80	116.55–179.82 x 99.90–109.89	N.P	16.65–43.29 x 73.26–83.25	9.99–16.65 x 4.99–13.32	6.66–9.99 x 4.99–6.66
18	*D. longicornu* Lindley	Thick	Rect. to Poly.	13.32–19.98 x 6.66–13.32	Poly.	2	Elongate	68	76.59–183.15 x 89.91–119.88	N.P	16.65–46.62 x 79.92–83.25	9.99–26.64 x 4.99–19.98	4.99–11.65 x 3.33–9.99
19	*D. moniliforme* (L.) Swartz	Thick	Rect. to Poly.	13.32-23.31 x 6.66-13.32	Poly.	2	Elongate	55	199.80–233.10 x 113.22–166.50	N.P	23.31–29.97 x 49.95–66.60	19.80–23.31 x 13.32–19.98	9.99–13.32 x 4.99–9.99
20	*D. monticola* Hunt & Summerhayes	Thick	Rect.	16.65–36.63 x 6.66–9.99	N.P	N.P	Elongate	20	66.60–89.91 x 49.95–53.28	N.P	13.32–16.65 x 43.29 – 49.95	6.66–13.32 x 4.99–9.99	4.99–6.66 x 3.33–4.99

21	*D. moschatum* (Buchanan-Hamilton) Swartz	Thick	Barr.	9.99–13.32 x 13.32–16.65	Poly.	3	Oval	78	133.20–166.50 x 113.22–133.20	26.64-43.29 x 66.60–99.90	33.30–36.63 x 113.22–133.20	13.32–16.65 x 9.99–13.32	3.33–6.66 x 3.33–4.99
22	*D. nobile* Lindley	Thick	Rect. to Poly.	13.32–26.64 x 6.66–13.32	Poly.	2	Oval	82	116.55–216.45 x 86.58–166.50	N.P	26.64–33.30 x 79.92 – 99.90	13.32–19.98 x 9.99–16.65	4.99–6.66 x 3.33–6.66
23	*D. polyanthum* Wallich ex Lindley	Thick	Rect. to Poly.	19.98–39.96 x 13.32–16.65	Poly.	1	Elongate	16	99.90–289.71 x 49.95–133.20	N.P	33.30–36.30 x 63.27–119.88	9.99–39.96 x 6.66–23.31	4.99–16.65 x 3.33–13.32
24	*D. porphyrochilum* Lindley	Thin	Rect. to Poly.	29.97–46.62 x 19.98–23.31	N.P	N.P	Elongate	40	89.91–166.50 x 66.60– 83.25	N.P	N.P	9.99–16.65 x 6.66–9.99	6.66–8.32 x 3.33–6.66
25	*D. rotundatum* (Lindley) Hooker	Thick	Rect. to Squa.	16.65–29.97 x 9.99–16.65	Poly.	2	Oval	82	99.90–166.50 x 83.25–116.55	N.P	13.32–39.96 x 33.30–99.90	16.65–23.31 x 13.32–19.98	3.33–13.32 x 3.33–9.99
26	*D. transparens* Wallich ex Lindley	Thick	Rect. to Squa.	13.32–19.98 x 9.99–19.98	Poly.	2	Elongate	50	96.57–133.32 x 63.27–79.92	N.P	23.31–26.64 x 59.94–76.59	9.99–16.65 x 6.66–9.99	6.66–8.32 x 3.33–6.66

Abbreviations used: N.P = Not present; Rect. = Rectangular; Poly. = Polygonal; Angu. = Angular; Squa. = Square; Barr. = Barrel-shaped.

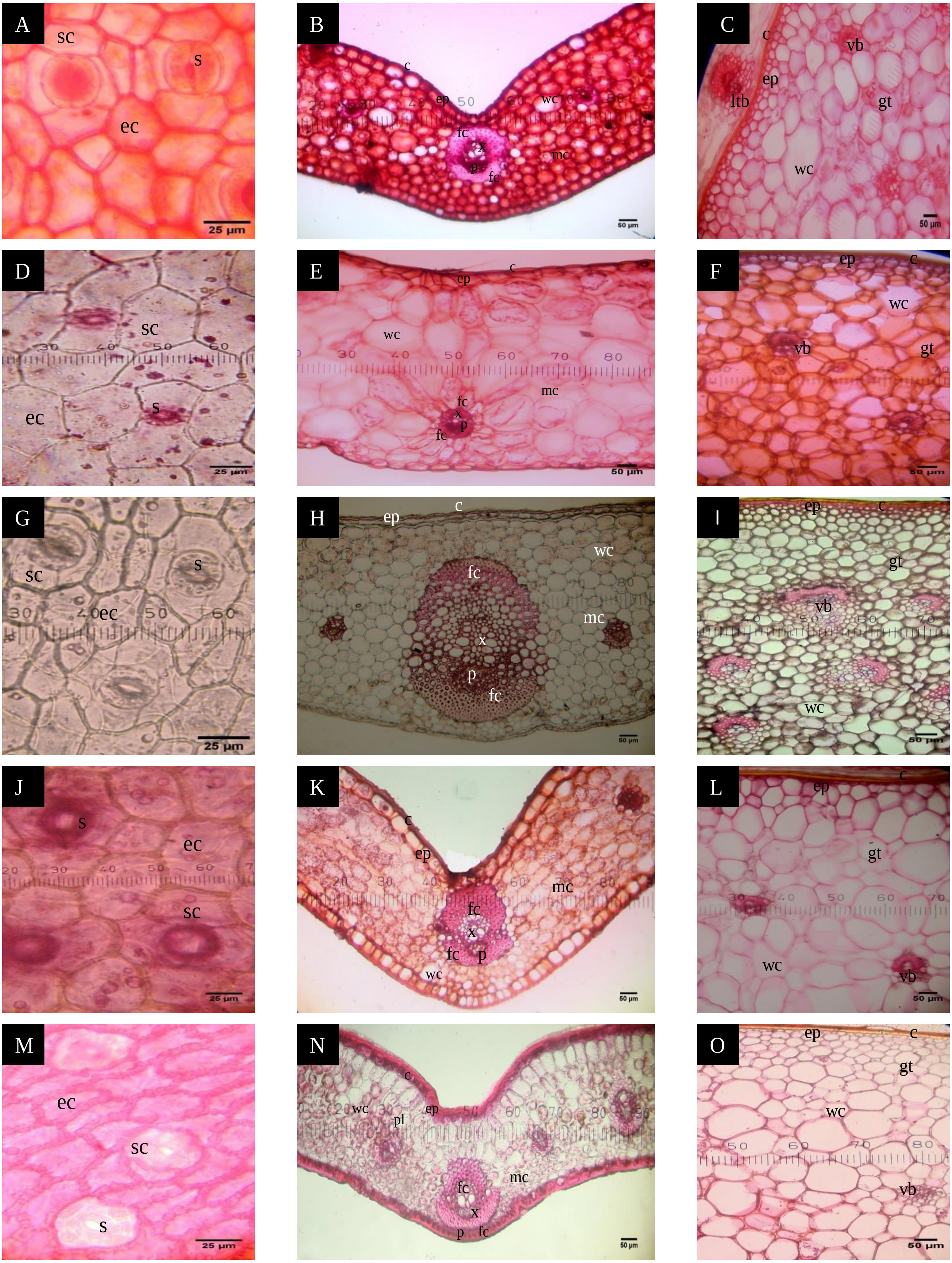

PLATE 1: ***D. longicornu*** A. Stomata , B. T.S. Leaf, C. T.S. Stem; ***D. aphyllum*** D., Stomata, E. T.S. Leaf, F. T.S. Stem; ***D. moschatum*** G. Stomata, H. T.S. Leaf, I. T.S. Stem; ***D. formosum*** J. Stomata , K. T.S. Leaf, L. T.S. Stem; ***D. rotundatum*** M. Stomata, N. T.S. Leaf, O. T.S. Stem. Stomata showing stoma (s), subsidiary cell (sc), epidermal cell (ec); T.S. Leaf showing cuticle (c), epidermis (ep), mesophyll cell (mc), xylem (x), phloem (p), fibre cap (fc), palisade layer (pl), water-storage cell (wc); T.S.Stem showing cuticle (c), epidermis (ep), leaf trace bundle (ltb), ground tissue (gt), vascular bundle (vb), water-storage cell (wc).

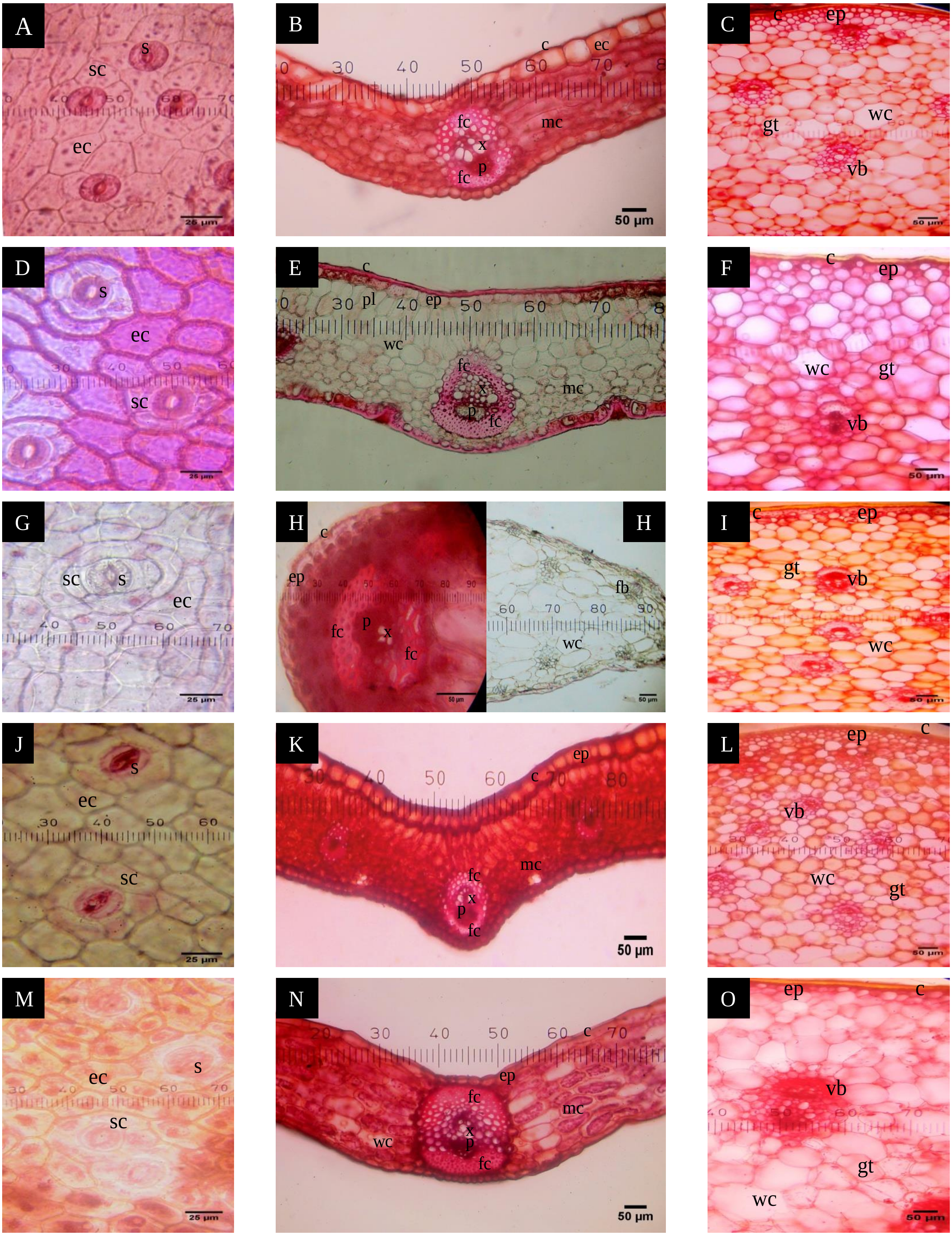

PLATE 2: ***D. amoenum*** A. Stomata , B. T.S. Leaf, C. T.S. Stem; ***D. amplum*** D. Stomata , E. T.S. Leaf, F. T.S. Stem; ***D. anceps*** G. Stomata, H. T.S. Leaf, I. T.S. Stem; ***D. bicameratum*** J. Stomata , K. T.S. Leaf, L. T.S. Stem; ***D. chrysanthum*** M. Stomata, N. T.S. Leaf, O. T.S. Stem. Stomata showing stoma (s), subsidiary cell (sc), epidermal cell (ec); T.S. Leaf showing cuticle (c), epidermis (ep), mesophyll cell (mc), xylem (x), phloem (p), fibre cap (fc), palisade layer (pl), water-storage cell (wc); T.S.Stem showing cuticle (c), epidermis (ep), ground tissue (gt), vascular bundle (vb), water-storage cell (wc).

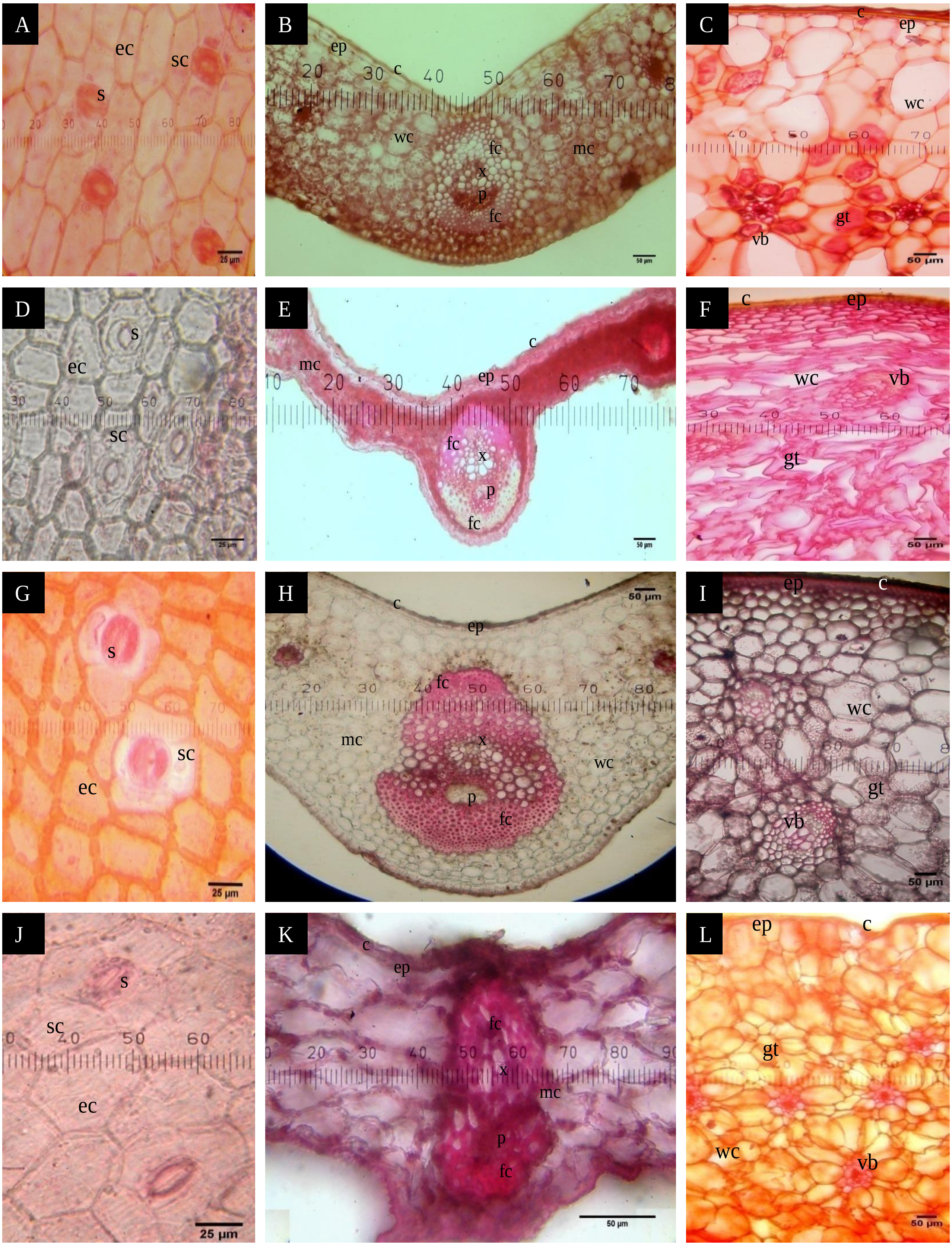

PLATE 3: ***D. crepidatum*** A. Stomata , B. T.S. Leaf, C. T.S. Stem; ***D. denneanum*** D. Stomata , E. T.S. Leaf, F. T.S. Stem; ***D. densiflorum*** G. Stomata, H. T.S. Leaf, I. T.S. Stem; ***D. denudans*** J. Stomata, K. T.S. Leaf, L. T.S. Stem. Stomata showing stoma (s), subsidiary cell (sc), epidermal cell (ec); T.S. Leaf showing cuticle (c), epidermis (ep), mesophyll cell (mc), xylem (x), phloem (p), fibre cap (fc), water-storage cell (wc); T.S. Stem showing cuticle (c), epidermis (ep), ground tissue (gt), vascular bundle (vb), water-storage cell (wc).

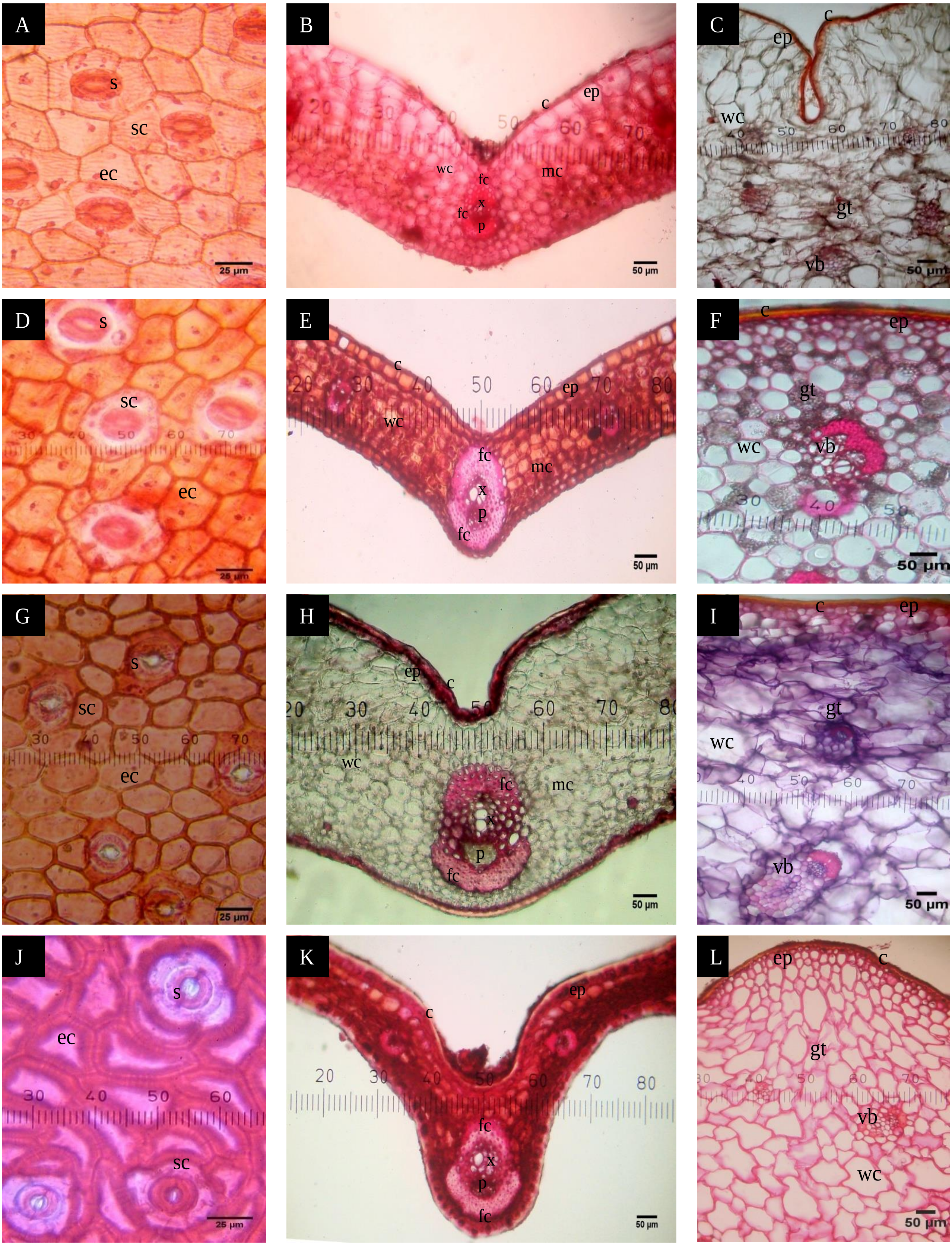

PLATE 4: ***D. eriiflorum*** A. Stomata , B. T.S. Leaf, C. T.S. Stem; ***D. fimbriatum*** D. Stomata , E. T.S. Leaf, F. T.S. Stem; ***D. fugax*** G. Stomata, H. T.S. Leaf, I. T.S. Stem; ***D. fuscescens*** J. Stomata , K. T.S. Leaf, L. T.S. Stem. Stomata showing stoma (s), subsidiary cell (sc), epidermal cell (ec); T.S. Leaf showing cuticle (c), epidermis (ep), mesophyll cell (mc), xylem (x), phloem (p), fibre cap (fc), water-storage cell (wc); T.S. Stem showing cuticle (c), epidermis (ep), ground tissue (gt), vascular bundle (vb), water-storage cell (wc).

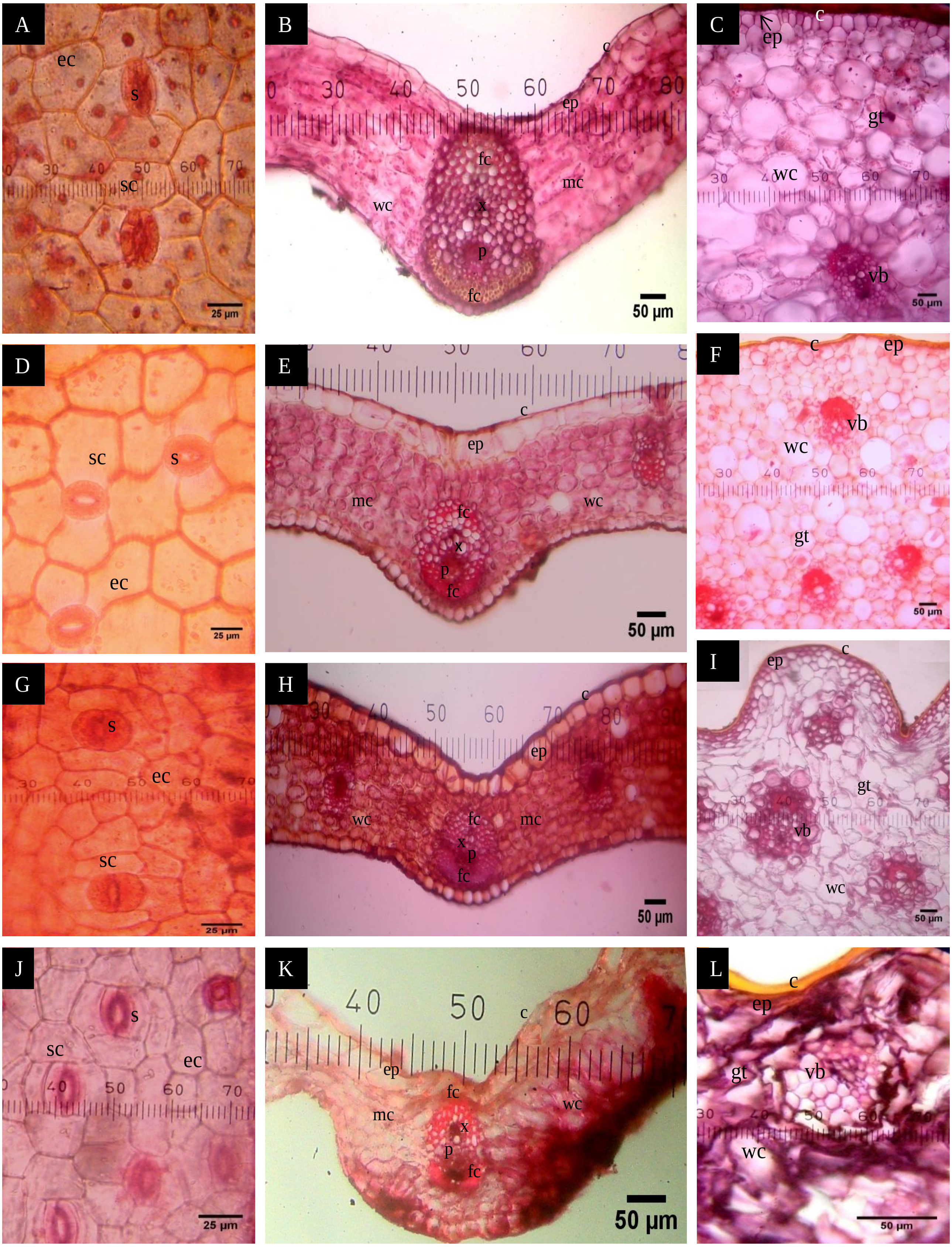

PLATE 5: ***D. gibsonii*** A. Stomata , B. T.S. Leaf, C. T.S. Stem; ***D. heterocarpum*** D. Stomata , E. T.S. Leaf, F. T.S. Stem; ***D. moniliforme*** G. Stomata, H. T.S. Leaf, I. T.S. Stem; ***D. monticola*** J Stomata , K. T.S. Leaf, L. T.S. Stem. Stomata showing stoma (s), subsidiary cell (sc), epidermal cell (ec); T.S. Leaf showing cuticle (c), epidermis (ep), mesophyll cell (mc), xylem (x), phloem (p), fibre cap (fc), water-storage cell (wc); T.S. Stem showing cuticle (c), epidermis (ep), ground tissue (gt), vascular bundle (vb), water-storage cell (wc).

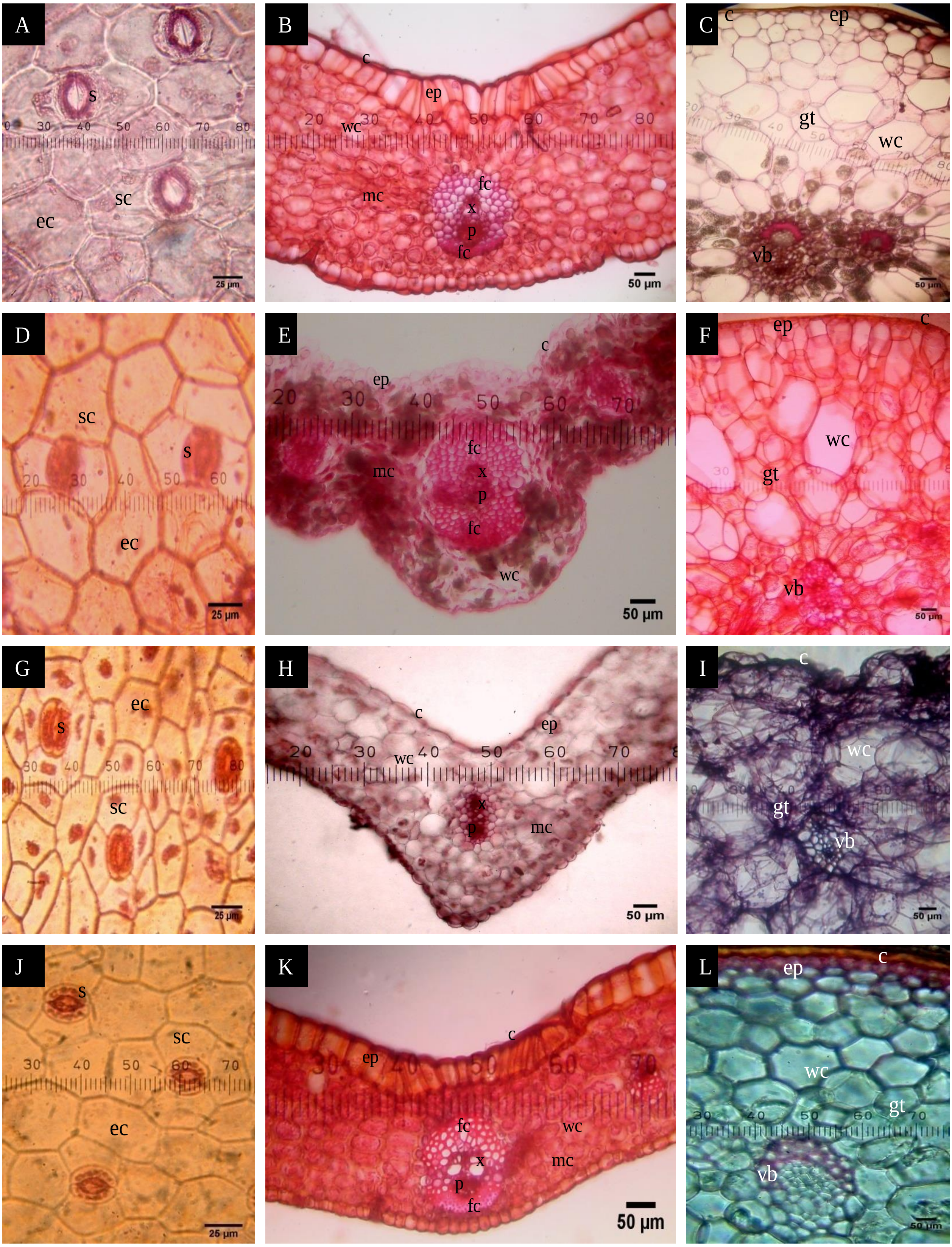

PLATE 6: ***D. nobile*** A. Stomata, B. T.S. Leaf, C. T.S. Stem; ***D. polyanthum*** D. Stomata, E. T.S. Leaf, F. T.S. Stem; ***D. porphyrochilum*** G. Stomata, H. T.S. Leaf, I. T.S. Stem; ***D. transparens*** J. Stomata, K. T.S. Leaf, L. T.S. Stem. Stomata showing stoma (s), subsidiary cell (sc), epidermal cell (ec); T.S. Leaf showing cuticle (c), epidermis (ep), mesophyll cell (mc), xylem (x), phloem (p), fibre cap (fc), water-storage cell (wc); T.S. Stem showing cuticle (c), epidermis (ep), ground tissue (gt), vascular bundle (vb), water-storage cell (wc).

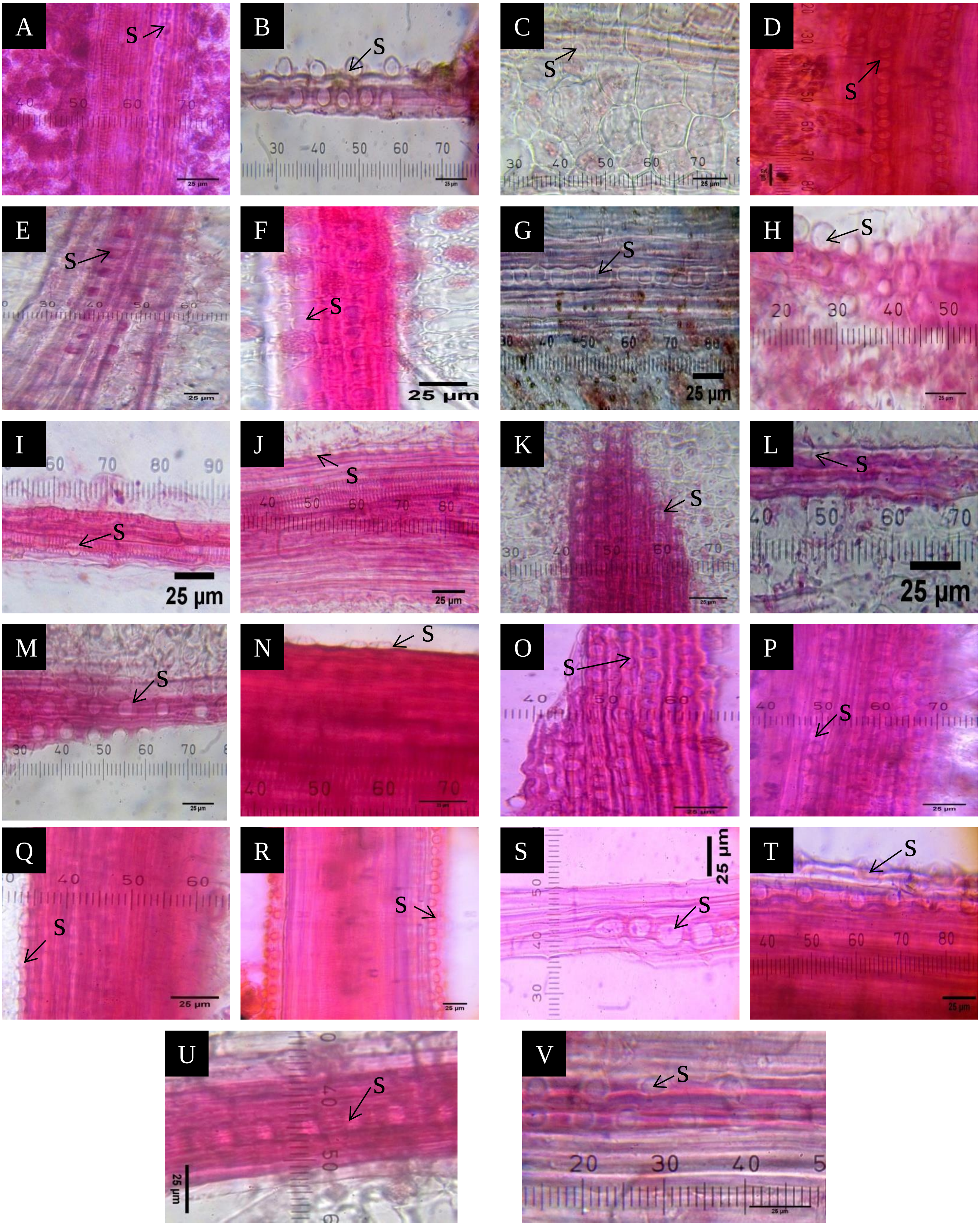

PLATE 7: Stegmata in Leaf surface of *Dendrobium* species (A-V). A. ***D. amoenum***; B. ***D. amplum***; C. ***D. anceps***; D. ***D. aphyllum***; E. ***D. bicameratum***; **F. *D. chrysanthum***; G. ***D. crepidatum***; H. ***D. denneanum***; I. ***D. denudans***; J. ***D. eriiflorum***; K. ***D. fimbriatum***; L. ***D. fugax***; M. ***D. fuscescens***; N. ***D. gibsonii***; O. ***D. heterocarpum***; P. ***D. moniliforme***; Q. ***D. monticola***; R. ***D. moschatum***; S. ***D. nobile***; T. ***D. porphyrochilum***; U. ***D. rotundatum***; V. ***D. transparens***. Leaf surface showing stegmata (s).

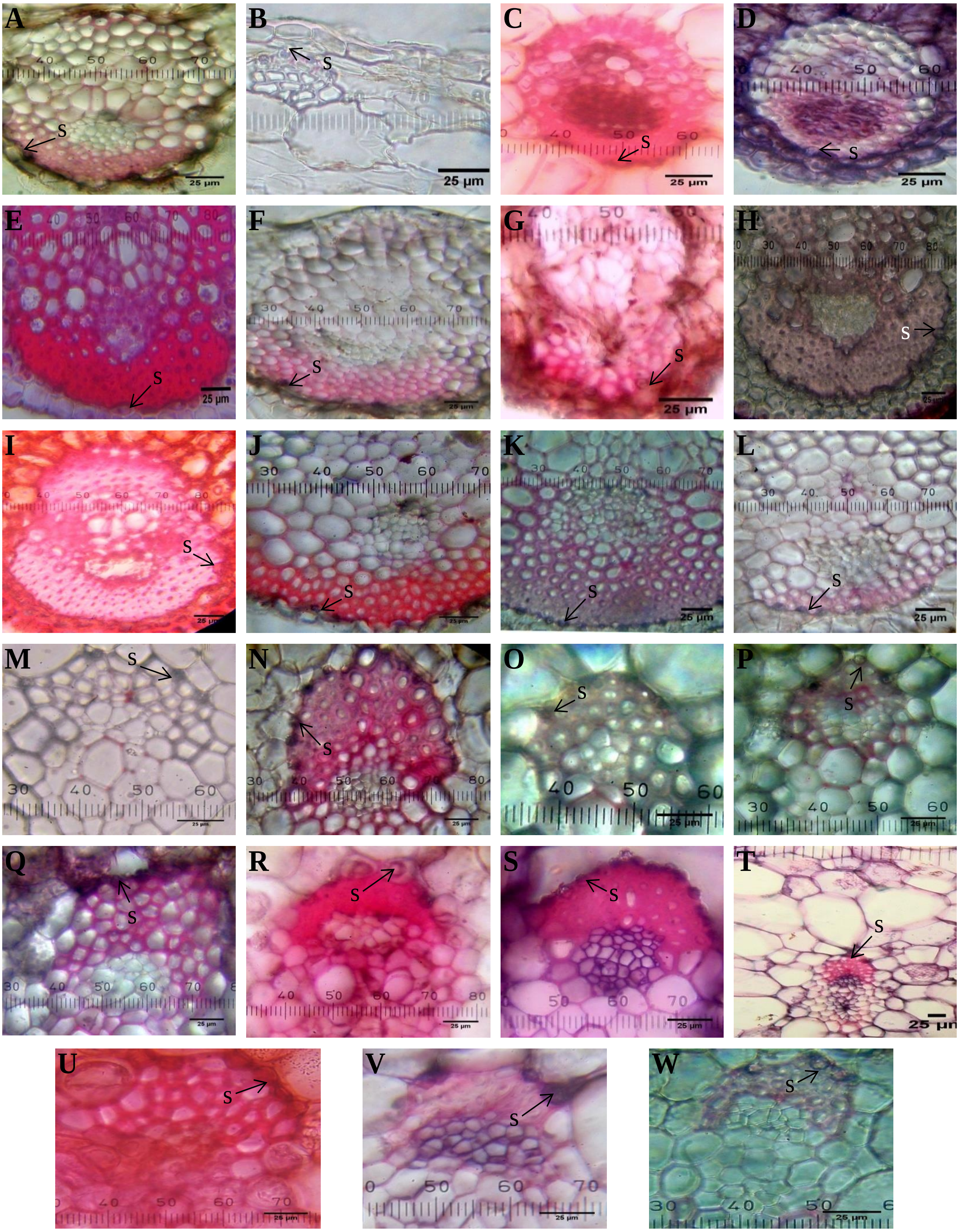

PLATE 8: Stegmata in Dendrobium species. T.S. Leaf (A-L) A. ***D. amoenum***, B. ***D. anceps***, C. ***D. aphyllum***, D. ***D. bicameratum***, E. ***D. chrysanthum***, F. ***D. crepidatum***, G. ***D. denudans***, H. ***D. fugax***, I. ***D. fuscescens***, J. ***D. gibsonii***, K. ***D. moschatum***, L. ***D. nobile;*** T.S. Stem (M-W) M. ***D. amoenum***, N. ***D. amplum***, O. ***D. aphyllum***, P. ***D. bicameratum***, Q. ***D. densiflorum***, R. ***D. formosum***, S. ***D. fugax***, T. ***D. longicornu***, U. ***D. polyanthum***, V. ***D. rotundatum***, W. ***D. transparens***. T.S. Leaf showing stegmata (s) and T.S. Stem showing stegmata (s).

Anatomical Key to the species of *Dendrobium*

1a. Leaf with thick cuticle ---------- 2

1b. Leaf with thin cuticle ---------- 10

2a. Thick cuticle with homogenous mesophyll in leaf ---------- 3

2b. Thick cuticle with heterogenous mesophyll in leaf ---------- 8

3a. Homogenous mesophyll with major vascular bundle at one leaf pole ---------- ***D. anceps***

3b. Homogenous mesophyll without major vascular bundle at one leaf pole ---------- 4

4a. Elongate shaped vascular bundle in stem ---------- 5

4b. Oval shaped vascular bundle in stem ---------- ***D. densiflorum***

5a. Stem hypodermal layers 4 ---------- ***D. denneanum***

5b. Stem hypodermal layers < 4 ---------- 6

6a. Oval shaped vascular bundle in leaf ---------- 7

6b. Flask-shaped vascular bundle in leaf ---------- ***D. fugax***

7a. Barrel shaped epidermal cells in stem ---------- ***D. bicameratum***

7b. Rectangular to polygonal shaped epidermal cells in stem ---------- ***D. moniliforme***

8a. Fibre cap surrounding xylem and phloem in stem ---------- 9

8b. Fibre cap surrounding phloem in stem ---------- ***D. rotundatum***

9a. Stem vascular bundle 333.0 µm long ---------- ***D. amplum***

9b. Stem vascular bundle 243.09 µm long ---------- ***D. fuscescens***

10a. Thin leaf cuticle without hypodermis in stem ---------- 11

10b. Thin leaf cuticle with hypodermis in stem ---------- 14

11a. Fibre caps surrounding xylem and phloem present in leaf ---------- 12

11b. Fibre caps surrounding xylem and phloem absent in leaf ---------- ***D. porphyrochilum***

12a. Number of vascular bundles 20 in stem ---------- ***D. monticola***

12b. Number of vascular bundles > 20 in stem ---------- 13

13a. Stem vascular bundle 119.88 µm long ---------- ***D. denudans***

13b. Stem vascular bundle 199.80 µm long ---------- ***D. eriiflorum***

14a. Midrib vascular bundle 506.54 µm long in leaf ---------- ***D. moschatum***

14b. Midrib vascular bundle < 506.54 µm long in leaf ---------- 15

15a. Mesophyll layers 10 in leaf ---------- ***D. crepidatum***

15b. Mesophyll layers < 10 in leaf ---------- 16

16a. Phloem fibre cap 233.10 µm wide in leaf ---------- ***D. gibsonii***

16b. Phloem fibre cap < 233.10 µm wide in leaf ---------- 17

17a. Xylem 16.65 µm long in stem ---------- ***D. transparens***

17b. Xylem > 16.65 µm long in stem ---------- 18

18a. Xylem fibre cap 86.58 µm wide in leaf ---------- ***D. aphyllum***

18b. Xylem fibre cap > 86.58 µm wide in leaf ---------- 19

19a. Stem vascular bundle 289.71 µm long ---------- ***D. polyanthum***

19b. Stem vascular bundle < 289.71 µm long ---------- 20

20a. Hypodermal layers 2 in stem ---------- 21

20b. Hypodermal layers 5 in stem ---------- ***D. fimbriatum***

21a. Oval shaped midrib vascular bundle in leaf ---------- 22

21b. Circular shaped midrib vascular bundle in leaf ---------- ***D. longicornu***

22a. Phloem fibre cap 206.46 µm wide in leaf ---------- ***D. formosum***

22b. Phloem fibre cap < 206.46 µm wide in leaf ---------- 23

23a. Oval shaped vascular bundle in stem ---------- 24

23b. Elongate shaped vascular bundle in stem ---------- ***D. chrysanthum***

24a. Mesophyll layer 7 in leaf ---------- 25

24b. Mesophyll layer 9 in leaf ---------- ***D. nobile***

25a. Mesophyll cell columnar to polygonal in leaf ---------- ***D. amoenum***

25b. Mesophyll cell rectangular to polygonal in leaf ---------- ***D. heterocarpum***

Cluster analysis

The anatomical data were analyzed to find the interrelationship among the species of genus *Dendrobium*. The analysis results in one dendrogram with AU *p*-value (printed in red colour by default) and BP value (printed in green colour by default). In the dendrogram, AU *p*-value above 95% was the significant clusters.

The dendrogram of cluster analysis of species of *Dendrobium* is presented in Fig. 1. The topology showed that the species of *Dendrobium* were divided into two major clusters: cluster A with AU *p*-value of 45% and BP *p*-value of 19% and cluster B with AU *p*-value of 49% and BP *p*-value of 18% with significant values.

Cluster A was further divided into two clusters: cluster C with a single species *D. anceps* (section Aporum) and cluster D with *D. longicornu, D. polyanthum, D. porphyrochilum, D. denudans* and *D. transparens.* Cluster D was again divided into two clusters: cluster D1 with *D.longicornu* and *D. polyanthum* with AU *p*-value of 60% and BP *p*-value of 24% and cluster D2 with *D. porphyrochilum, D. denudans and D. transparens* with AU *p*-value of 68% and BP *p*-value of 24%. Cluster D2 was again divided into two clusters: cluster D2a with single species *D. porphyrochilum* (section Stachyobium) and cluster D2b with *D. denudans* and *D. transparens*.

Cluster B was further divided into two clusters: cluster E with AU *p*-value of 66% and BP *p*-value of 3% and cluster F with AU *p*-value of 69% and BP *p*-value of 1%. Cluster E was again divided into two clusters: cluster E1 with *D. moniliforme, D. eriiflorum* and *D. monticola* and cluster E2 with *D. bicameratum, D. amoenum, D. aphyllum and D. heterocarpum.* Cluster E1 was again divided into two clusters: cluster E1a with a single species *D. moniliforme* (section Dendrobium) and cluster E1b with *D. eriiflorum* and *D. monticola.* Cluster E2 was again divided into two clusters: cluster E2a with a single species *D. bicameratum* (section Dendrobium) and cluster E2b with *D. amoenum, D. aphyllum* into two clusters: cluster E2bi with *D. amoenum* (section Dendrobium) and cluster E2bii with *D. aphyllum* and *D. heterocarpum.* Cluster F was divided into two clusters: cluster F1 with *D. rotundatum,D. amplum* and *D. fuscescens* (section Sarcopodium)with AU *p*-value of 98% and BP *p*-value of 64% with significant values and cluster F2 with *D. fugax, D. formosum, D. nobile, D. densiflorum, D. moschatum, D. chrysanthum, D. gibsonii, D. crepidatum*. *D. denneanum* and *D. fimbriatum* with AU *p*-value of 87% and BP *p*-value of 1%. Cluster F1 again divided into two clusters: cluster F1a with *D. rotundatum* and cluster F1b with *D. amplum* and *D. fuscescens.* Cluster F2 further divided into two clusters: cluster F2a with *D. fugax, D. formosum* and *D. nobile* with AU p-value of 94% and BP p-value of 35% with significant values and cluster F2b with *D. densiflorum, D. moschatum, D. chrysanthum, D. gibsonii, D. crepidatum, D. denneanum* and *D. fimbriatum* with AU *p*-value of 96% and BP *p*-value of 0% with significant values. Cluster F2a again divided into two clusters: cluster F2ai with *D. fugax* (section Crinifera) and cluster F2aii with *D. formosum* and *D. nobile* with AU *p*-value of 92% and BP *p*-value of 54% with significant values. Cluster F2b again divided into two clusters: cluster F2bi with *D. densiflorum* and *D. moschatum* with AU *p*-value of 98% and BP *p*-value of 85% with significantValues and cluster F2bii with *D. chrysanthum, D. gibsonii, D. crepidatum, D. denneanum* and *D. fimbriatum* with AU *p*-value of 81% and BP *p*-value of 0% with significant values. Cluster F2bii again divided into two clusters: cluster F2biii with *D. chrysanthum* and *D. gibsonii* with AU *p*-value of 94% and BP *p*-value of 77% with significant values and cluster F2biv with *D. crepidatum, D. denneanum* and *D. fimbriatum* with AU *p*-value of 83% and BP *p*-value of 8% with significant values. Cluster F2biv again divided into two clusters: cluster F2bv with *D. crepidatum* and cluster F2bvi with *D. denneanum* and *D. fimbriatum.*

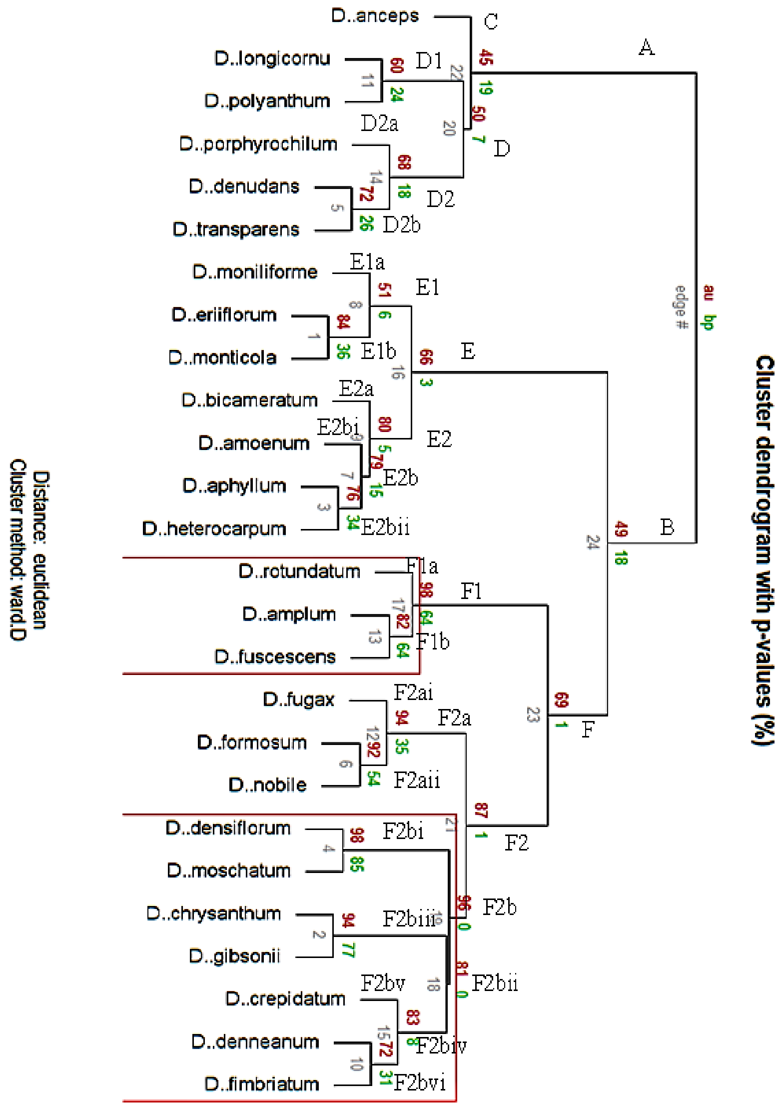

Figure 1. Cluster dendrogram constructed according to pvclust cluster analysis with AU/BP *p*-values in % and the bootstrapping of 10000. The AU/BP *p*-values are indicated below the branches. The AU *p*-vlues above 95% indicates the significant clusters.

Discussion

The present study revealed that the stomatal complex, stegmata, anatomy of leaves and stems of different species of *Dendrobium* showed variations in stomatal, stegmatal and anatomical characters of stem and leaves.

The stomatal complex of *Dendrobium* showed variation in types of epidermal cells, shape, size and types of stomata, stomatal frequency and stomatal index. All species of *Dendrobium* showed hypostomatic leaf surface. The leaves are found hypostamic condition in most oforchids (Avadhani *et al.,* 1982). Stomata were found only on the abaxial surface (hypostomatic). This is because these plants are adapted to aerial habitats to minimize water loss through stomata (Stebbins and Khush, 1961). The epiderm-al cells on the abaxial surface varied from rectangular, squarish, pentagonal, hexagonal to polygonal, irregular with thick or thin walled parenchymatous cells and some species with sclerenchymatous cells. The shape of stomata varied from elliptical, circular to suborbicular. In *Dendrobium* most species had elliptical stomata and few species with circular stomata whereas in *D. amplum* and *D. rotundatum* suborbicular stomata were present. Various modifications of stomatal shape such as elliptical, circular, transversely elliptical and angular are known to exist within Orchidaceae (Rasmussen 1987). Two types of stomatal shape (Stoma I and Stoma II) were reported in *Dendrobium* (Yukawa *et al.,* 1992). In the present study, the presence of two types of stomatal shape i.e. elliptical and circular is supported by the result of Yukawa *et al.,* (1992). In addition, suborbicular stomata were found in *D. amplum* and *D. rotundatum* which are the accepted names of *Epigeneium amplum* and *Epigeneium rotundatum* according to update classification (Chase *et al.,* 2015).

Tetracytic type of stomata had been reported in many monocotyledons (Metcalfe, 1961). Mostly three types of stomata paracytic, tetracytic and anomocytric were reported in mostly in monocots (Cheadle, 1953; Stebbins and Khush, 1961). Patel (1979) distinguished five types of stomata i.e. tetracytic (a-tetra-monocyclic, b-tetra-monocyclic and c-tetra-monocyclic), twicytic, perihaplocytic, anisocytic and hexacytic and each type with four to six subsidiary cells. In the present study tetracytic type (a-tetra-monocyclic, b-tetra-monocyclic and c-tetra-monocyclic) was found in most species of *Dendrobium* whereas eupara twi-monocyclic with hexa-monocyclic types was found in one species (*D. anceps*). In the stomatal type the monocyclic means the guard cells of the stomata are surrounded by a single cycle of subsdiary cells (Patel, 1979). Anisocytic stomata were observed in *D. amplum* according to Rasmussen (1987) but it was not observed in our study.

The size of stomata showed a wide variation from 620.97 µm^2 to 1928.48 µm^2. The largest stoma was found in *D. formosum* and the smallest in *D. fugax.* The stomatal frequency ranged from 46.15 mm^2 to 123.07 mm^2. The highest stomatal frequency was found in *D. longicornu* and lowest in *D. anceps* and *D. aphyllum*. The reduced stomatal frequency was distinctly related with the extent of leaf succulence and more the succulence lesser the frequency of stomata (Goh *et al.,* 1977). The stomatal index ranged from 3.75 to 12.50. The highest stomatal index was found in *D. formosum* and lowest in *D. anceps.* Similar variation in stomatal index was reported in orchid species (Saadu*et al.,* 2009).

Stegmata was found in all species of *Dendrobium* except *D. densiflorum, D. formosum, D. longicornu* and *D. polyanthum*. Presence of stegmata in Dendrobiinae was supported by the result of Dressler (1993). Stegmata containing rough surfaced spherical bodies present in *D. anceps* was found similar to the result of Carlsward *et al.,* (1997).Transverse section of leaf showed some variations in cuticle, shape of epidermal cells, hypodermis, mesophyll, vascular bundle and fibre caps in different species of *Dendrobium*.Intermittently domed cuticle was present in *D. anceps*. The result was found similar to the result of Morris *et al.,* (1996). In epidermis of *D. fugax*, cell lumina were found occluded with Saffranin staining in adaxial and abaxial sides. A single layer of hypodermis was present in abaxial side in *D. anceps* and adaxial side in *D. densiflorum* and absent in other species. This result was found similar to the result of Morris *et al* (1996). Fibre bundles were present subtending the hypodermis only in *D. anceps*. Presence of fibre bundle in *D. anceps* is supported by the result of Morris *et al.,* (1996). Mesophyll cells were found homogenous and heterogenous. Heterogenous mesophyll was found in *D. amplum* and *D. rotundatum* and homogenous in remai-ning species. Palisade cells were columnar in *D. amplum* which is supported by the result of Morris *et al.,* (1996). Vascular bundles were arranged in a single row with large midrib bundle in most of species of *Dendrobium* except in *D. anceps* in which the vascular bundles were arranged in two rows on either side of midline with a major vascular bundle at one pole of leaf section. This result is supported by the result of Carlward *et al.,* (1997). Dome-shaped fibre cap was present at xylem pole of *D. moschatum.* Transverse section of stem showed variations in cuticle, shape of epidermal cells, shape and no. of hypodermal layers, ground tissue layers, shape and no. of vascular bundles and fibre caps in different species of *Dendrobium.*

Cuticle is thin in *D. porphyrochilum* and thick in remaining species. Cuticle is intermittently undulating in *D. anceps*. This result was found similar to the result of Morris *et al.,* (1996). The epidermal cells vary from rectangular to square to polygonal to barrel shaped in different species of *Dendrobium*. In ground tissue two kinds of water-storage cells *i.e.* pleated or unpleated were present in all *Dendroboium* species. Water-storage cells have been reported in Orchidaceae (Stern and Morris, 1992). Water-storage cells with pleated or unpleated walls lack the secondary thickenings. This result is similar to the result of Morris *et al.,* (1996). Vascular bundle shape and number varies from species to species of *Dendrobium*. Stegmata with rough-surfaced silica bodies were found in stems and leaves of *Dendroboium* species. Presence of stregmata with spherical bodies in this study is supported by the result of Kohl (1889). This result supports the hypothesis of Moller and Rasmussen (1984) in possessing stegmata with spherical silica bodies in all Dendrobiinae.

From the present study, the stomatal complex, stegmata, anatomical characters of leaves and stems were found to be taxonomically significant for delimiting the taxa within *Dendrobium* of Nepal.

Cluster analysis

Cluster analysis was carried out to find out the interrelationships among the species of *Dendrobium*. The cluster analysis based on anatomical with micrpmorphological characters revealed two major significant clusters: cluster A comprising 6 species with AU *p*-value of 45% and BP *p*-value of 19% belonging to sections Aporum, Formosae, Dendrobium and Stachyo-bium and cluster B comprising 20 species with AU *p*-value of 49% and BP *p*-value of 18% belonging to sections Stachyobium, Dendrobium, Crinifera, Formosae and Sarcopodium. The species under cluster A were distinguished by elliptic shaped a-tetra-monocyclic, b-tetra-monocyclic to eupara-twi-monocyclic+hexa-monocyclic stomata, parenchymatous leaf epidermal cells, abaxial leaf hypodermis, homogenous mesophyll, oblong to circular midrib vascular bundle, presence or absence of fibre bundle, presence or absence of fibre cap on midrib vascular bundle of leaf, major vascular bundle at leaf pole, one to three stem hypodermal layer. In cluster B, the species were distinguished by circular to suborbicular shape with a-tetra-monocyclic, b-tetra-monocyclic, c-tetra-monocyclic to eupara-twi-monocyclic typed stomata, parenchymatous or sclerenchymatous leaf epidermal cells, adaxial leaf hypodermis, presence of homogenous and heterogenous mesophyll, oval, flask-shaped to conical midrib vascular bundle, absence of fibre bundle and major vascular bundle at leaf pole, presence fibre cap on midrib vascular bundle of leaf, one to five stem hypodermal layer.

Cluster A was divided into two clusters: cluster C and cluster D. In cluster A, a separate cluster C was formed by *D. anceps* (section Aporum) as it is differed from cluster D by eupara-twi-monocyclic+hexa-monocyclic stomata, thick cuticle, one layered abaxial leaf hypodermis, major vascular bundle at leaf pole and fibre bundle. In cluster D1 of cluster D, *D. longicornu* (section Formosae) was found close to *D. polyanthum* (section Dendrobium)as they had elliptic stomata, parenchymatous leaf epidermal cell, thick cuticle, circular midrib vascular bundle and elongate stem vascular bundle. In cluster D2a of cluster D, *D. porphyrochilum* (section Stachyobium) was found separated from cluster D2b with *D. denudans* (section Stachyobium) and *D. transparens* (section Dendrobium) as it had a-tetra-monocyclic stomata, water storage cells in mesophyll, no fibre cap on midrib bundle of leaf and stem vascular bundle. In cluster D2b, *D. denudans* was found close to *D. transparens* as both species had elliptic stomata, thin cuticle, no leaf hypodermis, homogenous mesophyll layers, stem vascular bundle number up to 60.

Cluster B was divided into two clusters: cluster E and cluster F. In cluster E1 of cluster E, *D. moniliforme* (section Dendrobium) of cluster E1a was found separated from cluster E1b in having circular shaped a-tetra-monocyclic stomata, thick cuticle and two to three polygonal stem hypodermal layers. In cluster E1b, *D. eriiflorum* (section Stachyobium) was found close to *D. monticola* (section Stachyobium) because both species had elliptic stomata, thin cuticle, no leaf hypodermis, rectangular stem epidermal cell and no stem hypodermis. In cluster E2a, *D. bicameratum* (section Dendrobium)differed from cluster E2b in having stomatal pore more than 28 μm, thick cuticle of leaf and elongate stem vascular bundle. In cluster E2bi, *D. amoenum* (section Dendrobium) differed from cluster E2bii in having b-tetra-monocyclic stomata with length up to 33 µm, rectangular to polygonal stem epidermal cell and larger xylem in stem vascular bundle. In E2bii, *D. aphyllum* (section Dendrobium) was found close to *D. heterocarpum* (section Dendrobium) as both species had a-tetra-monocyclic stomata, thin cuticle, parenchymatous leaf epidermal cell, stegmata, no leaf hypodermis, oval midrib vascular bundle, oval stem vascular bundle and raphide bundles in stem.

In cluster F, cluster F1 with *D. rotundatum, D. amplum* and *D. fuscescens* (section Sarcopodium) form a significant cluster with AU *p*-value of 98% and BP *p*-value of 64% with significant values. *D. rotundatum* of cluster F1a was found separated from cluster F1b in having suborbicular c-tetra-monocyclic stomata, water storage cell and calcium oxalate crystals in mesophyll and no xylem fibre cap in stem vascular bundle. *D. amplum* and *D. fuscescens* of cluster F1bwere placed closely as both species had thick cuticle, sclerenchymatous leaf epidermal cell, stegmata, no leaf hypodermis, heterogenous mesophyll layers, flask-shaped midrib vascular bundle and two to three layers of polygonal stem hypodermis. As *D. rotundatum, D. amplum* and *D. fuscescens* were more closely related to each other, these three species may be merged into single species with further additional research. In cluster F2, *D. fugax* (section Crinifera) of cluster F2ai differed from cluster F2aii in having eupara-twi-monocyclic stomata, thick cuticle, more than ten homogenous mesophyll layers, flask-shaped midrib vascular bundle, barrel-shaped stem epidermal cell and elongate stem vascular bundle. In cluster F2aii, *D. formosum* (section Formosae) was found close to *D. nobile* (section Dendrobium)with AU *p*-value of 94% and BP *p*-value of 35% as both species had c-tetra-monocyclic stomata, eight to ten homogenous mesophyll layers, calcium oxalate crystals in mesophyll, oval midrib vascular bundle and two to three layers of polygonal stem hypodermis, oval stem vascular bundle. In cluster F2b, cluster F2bi formed a significant cluster with *D. densiflorum* and *D. moschatum* (section Dendrobium) with AU *p*-value of 98% and BP *p*-value of 85% with significant values. *D. densiflorum* was found close to *D. moschatum* as both species had a-tetra-monocyclic stomata, more than ten homo-genous mesophyll layers, conical midrib vascu-lar bundle, oval stem vascular bundle, stem vascular bundle no. more than 70 and raphide bundles in stem. *D. densiflorum* and *D. moscha-tum* were closely interrelated, with further research, these two species may be merged into a single species.

In cluster F2biii, *D. chrysanthum* (section Dendrobium) was found close to *D. gibsonii* (section Dendrobium) as both species had a-tetra-monocyclic stomata, thin cuticle, no leaf hypodermis, eight to ten homogenous mesophyll layers, oval midrib vascular bundle, two to three stem hypodermal layers, no xylem fibre cap in stem vascular bundle. Cluster F2biv again divided into two clusters: cluster F2bv and cluster F2bvi. Cluster F2bv with *D. crepidatum* (section Dendrobium) differed from cluster F2bvi with *D. denneanum* and *D. fimbriatum* in having elliptic shaped b-tetra-monocyclic stomata, parenchymatous leaf epidermal cell, phloem fibre cap layers more than 5 in midrib bundle, one stem hypodermal layer, stem vascular bundle number less than 61. In cluster F2bvi, *D. denneanum* was found close to *D. fimbriatum* as both species had circular shaped a-tetra-monocyclic stomata, sclerenchymatous leaf epidermal cell, no leaf hypodermis, barrel-shaped stem epidermal cell, four to five layers of rectangular to polygonal stem hypodermis and no xylem fibre cap in stem vascular bundle.

D. amplum, *D. fuscescens* and *D. rotundatum* belong to section Sarcopodium, came under genus *Epigeneium* previously but now it was merged into genus *Dendrobium* s.l. on the basis of molecular data (Schuiteman and Adams, 2014). The merging of genus *Epigeneium* into genus *Dendrobium* s.l. was seemed to be good as they formed a distinct significant cluster within genus *Dendrobium* s.l. This indicated that the present result of cluster analysis based on anatomical characters correlated with molecular data. *D. fugax* of section Crinifera came under genus *Flickingeria* in previous classification, but in update classification, the genus

Flickingeria was merged into genus *Dendrobium* s.l. based on the molecular data (Schuiteman and Adams, 2014). The inclusion of genus *Flickingeria* into genus *Dendrobium* s.l. was seemed correct as *D. fugax* showed close relationship with other species of *Dendrobium*. This cluster analysis result revealed that the analysis result based on anatomical characters with micromorphological characters correlate with molecular data.

In the present study, according to result of cluster analysis based on anatomical characters with micro-morphological characters, *Dendrobium* species of Nepal can be divided into new 6 sections with closely interrelated species. Sections are as follows:

Section I. includes species with eupara-twi-monocyclic+hexa-monocyclic stomata, one layered abaxial leaf hypodermis, major vascular bundle at leaf pole and fibre bundle. -- *D. anceps*

Section II. includes species with elliptic stomata and small stem vascular bundle less than 100 µm ----- *D. longicornu, D. polyanthum, D.porphyrochilum, D.denudans* and *D. transparens*

Section III. includes species with oval midrib vascular bundle and no xylem fibre cap in stem vascular bundle -------------------*D. moniliforme, D. eriiflorum, D. monticola, D. bicameratum, D. amoenum, D. aphyllum* and *D. heterocarpum*

Section IV. includes species with circular or suborbicular stomata, sclerenchymatous leaf epidermal cell, heterogenous mesophyll layer and rectangular to square stem epidermal cell -- *D. rotundatum, D. amplum* and *D. fuscescens*

Section V. includes species with stomatal index more than 10%, xylem fibre cap in midrib vascular bundle more than 150 µm in width and polygonal stem hypodermal cell -------------------------------*D. fugax, D. formosum* and *D. nobile*

Section VI. includes species with a-tetra-monocyclic stomata ----------------*D. densiflorum, D. moschatum, D. chrysanthum, D. gibsonii,D. crepidatum, D. denneanum* and *D. fimbriatum* but b-tetramonocyclic stomata - *D. crepidatum,* no leaf hypodermis but with one layered adaxial leaf hypodermis - *D. densiflorum,* large stem vascular bundle width more than 130 µm but less than 100 µm - *D. crepidatum* and no stegmata but present - *D. densiflorum.*

Conclusion

The present study was based on anatomical characters of leaves and stems of *Dendrobium* species of Nepal Himalaya. Based on characters such as stomatal complex, stegmata and anatomical characters of leaves and stems, an anatomical key was prepared to delimit the taxa within genus *Dendrobium.*

Based on anatomical with micromorphological characters, the cluster analysis was carried out in species of *Dendrobium* to find out the interrelationships among the species of *Dendrobium*. With the result of cluster analysis, *Dendrobium* of Nepal was divided into 6 sections: section I with *D. anceps,* section II with *D. longicornu, D. polyanthum, D. porphyrochilum, D. denudans, D. transparens*, section III with *D. moniliforme*, *D. eriiflorum, D. monticola, D. bicameratum, D. amoenum, D. aphyllum, D. heterocarpum*, section IV with *D. rotundatum, D. amplum,D. fuscescens*, section V with *D. fugax, D. formosum, D. nobile* and section VI with *D. densiflorum*, *D. moschatum, D. chrysanthum, D. gibsonii, D. crepidatum, D. denneanum* and *D. fimbriatum.*

The present study represents the first attempt to bring the cluster analysis of *Dendrobium* of Nepal based on anatomical with micromorphological characters. The proposed classification based on the cluster analysis is slightly similar to classification of Schuiteman and Adams (2014).

Due to unavailability of the modern equipment like Scanning Electron Microscope (SEM), Transmission Electron Microscope (TEM) and DNA sequences, a detailed study of the species could not be carried out. However, the result obtained from the present study will provide the working hypothesis for further study. Therefore, it is recommended that additional researches should be needed to fully resolve the relationships between several species of *Dendrobium.*

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