

Chapter I

INTRODUCTION

1.1. Background

Nepal is a Himalayan country, representing one of the world's richest spots in floral diversity with wide altitudinal variation and diverse climatic condition within a small geographical area. It is estimated that Nepalese territory comprises a large number of flowering plants with extreme diversity of floristic patterns ranging from low altitude of tropics to the highest altitude of alpine zone. With regard to the phytogeography of the world, Nepal lies on the cross-road of various floristic regions of the world, thus has an influence of differing flora of various provinces of those regions, varying from drier atmosphere in the west to the more humid in the east and from flat plains in the south to high Himalayas in the north.

Orchids are considered as the most beautiful, diverse and highly evolved flowering plants comprising 25,000 species throughout the world (Atwood, 1986). Nepal harbors about 377 species of native orchids belonging to 100 genera (Rajbhandari & Dahal, 2004) out of which 11 species are endemic. They are *Bulbophyllum ambrosia* subsp. *nepalensis*, *Eria baniaae*, *E. nepalensis*, *E. pokherensis*, *Liparis olivacea*, *Listera nepalensis*, *Malaxis monophyllos* var. *obtusae*, *M. tamurensis*, *Oberonia nepalensis*, *Oreorchis porphyranthus* and *Pleione coronaria* (Rajbhandari & Dahal, 2004).

1.2. Characteristics of Orchidaceae

Orchids have two basic habits of growth. Sympodial orchids, such as *Cattleya*, form a horizontal stem called a rhizome that produces new shoots from buds. Monopodial orchids, such as *Vanda* and *Vanilla*, have no rhizomes or pseudobulbs, but produce an erect or pendent stem with leaves growing continuously from the same growing point. Orchids are also classed as epiphytes, those that grow naturally on trees, etc., terrestrial, those that grow in the soil, subterranean, those that grow beneath the surface of the growing medium, or lithophytes, those that grow on the surface of rocks. The leaves of epiphytes usually persist on the plant from year to year, but leaves of

terrestrials often die at the end of the growing season. Leaves may be plicate, i.e., folded many times longitudinally or conduplicate, folded once in the middle. Plicate leaves are always thin, but conduplicate leaves may be thick, fleshy, and leathery. Some leaves are terete, pencil-like and round in cross-section.

This family contains the most varied plants and beautiful flowers. The beautiful flowers consist of a corolla of three petals, one of which, the lip, also called as labellum, may be large or small, long and thin, or shaped like a bucket. One interesting feature that distinguishes the orchid from other flower group is the union of the male parts and female parts of the flower. Orchids have three pistils and three stamens and they are fused into a column. Most orchids have only one fertile stamen. The pollen grains of the fertile stamens are clustered into packets or more sticky units called pollinia. Orchid groups are ordinarily defined by the number of pollinia present.

1.3. Taxonomic Account of Subtribe Coelogyninae

Orchids exhibit extraordinary characteristics with a great modification in their floral as well as vegetative structures. The classification of family Orchidaceae is somewhat technical and centers mostly on the morphology of the column and pollinia. The family is usually considered to have three subfamilies, six tribes, and about 750 to 1000 genera (Dressler 1993).

Subtribe Coelogyninae was placed under the tribe Epidendreae by Bentham and Hooker (1883) in Flora of British India. King and Pantling (1898) also included the member of this subtribe under the tribe Epidendreae in Orchids of Sikkim Himalaya. Banerjee and Pradhan (1982) followed the classification of Schechter (1926) in which subtribe Coelogyneae has been placed under subfamily Monandreae, division Acrotonae, tribe Kerosphaeroideae and series Acranthae. Deva and Naithani (1986) included this subtribe in subfamily Epidendroideae and tribe Epidendreae. Rasmussen (1990) gave the classification of orchids and placed the subtribe under subfamily Epidendroideae and tribe Coelogyneae. Dressler (1981, 1993) also classified family Orchidaceae and kept the subtribe Coelogyninae Bentham in tribe Coelogyneae Pfitzer and subfamily

Epidendroideae. Pearce and Cribb (2002) included this subtribe under subfamily Epidendroidae and tribe Coelogyneae in Flora of Bhutan.

Hooker (1889) gave the characteristics of subtribe Coelogyneae as: "Inflorescence terminal, pollinia 8 or 4, subequal, connected by viscus or an appendage (in *Calanthe*), and in others the pollinia are appendages, but the appendage is an undeveloped portion of the pollen itself, and is not derived from the rostellum."

The members of this subtribe are mostly epiphytic plants with characteristic features of sympodial growth, pseudobulbs having single internode, terminal inflorescence, winged column and massive caudicles (Dressler 1981). The velamen is *Coelogyne* type except in *Pleione* which is *Calanthe* type. Leaves are plicate or conduplicate, or articulate. Inflorescences are simple with few to many flowers. Flowers small to large with short or elongated winged column hooded over the anther. Number of pollinia may be two or four.

Taxonomically, this subtribe has been divided and subdivided by numerous taxonomists with different approaches. Bentham (1881) divided the subtribe Coelogyneae into 14 genera including *Coelogyne*, *Otochilus* and *Pholidota*. Pfitzer and Kraenzlin (1907) divided it into 15 genera including *Coelogyne*, *Otochilus*, *Pholidota*, *Panisea*, *Pleione* and *Neogyne*. Butzin (1992) divided it into 15 genera and Pedersen *et al.* (1997) divided it into 16 genera including the six above mentioned genera. Gravendeel (2000) divided it into 12 genera including *Coelogyne*, *Otochilus*, *Panisea* and *Pleione*, in which *Neogyne* and *Pholidota* are placed under *Coelogyne*.

Dressler (1993) attributed 20 genera and 285 species to the subtribe Coelogyneae. Dressler characterized this subtribe as an unusually clear group with the column resembling that of some *Bletinae* and considered this as the primitive feature retained in this group. Out of the total 20 genera as described by Dressler, Nepal harbors only six genera, *Coelogyne*, *Neogyne*, *Otochilus*, *Panisea*, *Pholidota*, and *Pleione* (Bajracharya and Shakya 2002).

1.4. Objectives

1. To study the anatomical features of leaves and roots of the species belonging to the subtribe Coelogyninae of Nepal.
2. To investigate intrageneric anatomical variation of leaves and roots of subtribe Coelogyninae of Nepal.
3. To determine the taxonomic and phylogenetic significance of the anatomy of the species belonging to the subtribe Coelogyninae of Nepal.
4. To establish probable hypothetical classification of subtribe Coelogyninae of Nepal on the basis of leaf and root anatomy.

1.5. Limitation of the Study

1. Anatomical study of only leaves and roots could be done due to the unavailability of plant parts of many of the species.
2. Since the study of some of the plants was done from dry herbarium specimens, the clear differentiation of cells and tissues of some species could not be made.
3. Anatomical studies were done in a traditional manner by freehand section cutting and compound microscopic study. Modern methods could not be applied.

Chapter II

LITERATURE REVIEW

2.1. Literatures related to Morphology and Floristic

Study and enumeration of orchid flora of Nepal was done by many scientists. Many morphotaxonomical studies have also been conducted in Nepal. Among the orchid species studied, almost all studies comprise the members of subtribe Coelogyninae. D. Don (1825) enlisted two species of *Pleione* in *Prodromus Florae Nepalensis*. Tuyama (1966) did the study of Eastern Himalaya which was updated in 1971 and later in 1978 by Hara *et al.* (1978) and published *An Enumeration of Flowering plants of Nepal (EFPN)* in which he has presented 12 species of *Coelogyne*, four species of *Otochilus*, two species of *Panisea*, five species of *Pholidota* and five species of *Pleione*. Press *et al.* (2000) again prepared a checklist in which one more species of *Coelogyne* was added. Many other floristic studies have been done locally throughout the country and all of those studies represent the members of this subtribe. Banerji (1978) did an extensive study on orchids of Nepal representing the five genera namely *Coelogyne* six species, *Otochilus* three species, *Panisea* one species, *Pholidota* four species and *Pleione* three species. Banerji and Pradhan (1984) studied these species from herbarium specimen deposited at KATH. Shakya *et al.* (1994) reported six species of *Coelogyne*, one species of *Otochilus*, two species of *Panisea*, three species of *Pholidota*, and three species of *Pleione* from Kathmandu Valley. Bajracharya (1999) reported six species of *Coelogyne*, one species of *Panisea* and two species of *Pholidota* from lower Gorkha of Manaslu region. White and Sharma (2000) recorded seven species of *Coelogyne*, three species of *Otochilus*, two species of *Panisea*, five species of *Pholidota* and two species of *Pleione* from Tribhuvan Rajpath and Chitwan Jungle. Recent compilation of Nepalese orchids done by Rajbhandari and Bhattarai (2001) includes eight species of *Coelogyne*, three species of *Otochilus*, two species of *Panisea*, one species of *Pholidota* and two species of *Pleione*. Subedi (2002) recorded eight species of *Coelogyne*, three species of *Otochilus*, two species of *Panisea*, four species of *Pholidota* and three species of *Pleione* from around Pokhara Valley. Ghimiri (2002) reported three species of *Coelogyne* and two species of *Pholidota*; Bajracharya *et al.* (2003) reported six species of *Coelogyne*, one species of *Otochilus*, two species of *Pholidota* and one species of *Pleione* from Raja Rani (Morang

District) and adjoining areas. Subedi (2003) enlisted five species of *Coelogyne*, three species of *Otochilus*, two species of *Panisea*, four species of *Pholidota*, three species of *Pleione* from Seti and Marsyangdi river Valley of Central Nepal. Amatya (2005) reported six species of *Coelogyne* and four species of *Pholidota* from Doti District.

2.2. Literatures related to General Anatomical Studies of Orchidaceae

Although the morphological and floristic studies of orchids have done extensively, there has not been any study performed so far on the anatomical aspect of orchids in Nepal. Only few anatomical characters have been explored by some of the workers as a supplementary portion on the revision of respective genera. Limited work has been done by scientist from India and abroad on the anatomy of orchids.

Mehra and Vij (1974) made observations on the ecological adaptations and distribution pattern of the East Himalayan orchids and highlighted features of morphological adaptations of the orchids in relation to their habitats. From the variations found in the anatomical structures of orchids, they were regarded as the most highly ecologically adapted flowering plants.

Singh and Singh (1974) studied the organization of stomatal complex in some Orchidaceae including some species of *Coelogyne* and *Pholidota* in which the presence of tetracytic stomata were considered significant in evolutionary relationships of the family.

Kaushik (1983) explored the ecological and anatomical marvels of the Himalayan orchids based on the anatomical study of 53 species belonging of 28 genera. He discussed the impact of anatomy on the classification of the family and formulated the phylogenetic classification based on morphoanatomical characters. He divided the genus *Coelogyne* into two genera, *Coelogyne s.s.* with hyaline water storage cells and *Hydroplecellulosa* with special type water storage cells.

The adaptive significance of anatomical features has been stressed by Rasmussen (1986). Zankowski *et al.* (1987) studied the developmental anatomy of velamen and exodermis in the aerial roots of *Epidendrum ibagnense*.

Rao and Khasim (1987) analyzed the evolutionary trends in Orchidaceae on the basis of growth habits and vegetative anatomy of 43 species. It was concluded that the epiphytic habit has evolved from the terrestrial one and the monopodial growth in the epiphytes is derived from sympodial habit. The evolution of stomatal types has progressed from anomocytic to diacytic to cyclocytic to paracytic. The absorbing trichomes, absent in the terrestrials, are abundant in the epiphytes. The water conducting system in the relatively advanced epiphytes comprise well developed fibrous cap, vessel like trachieds and different types of water storage cells.

Chase and Peacord (1987) observed calcium oxalate crystals in perianth of *Stelis*. Porembski and Barthlott (1988) classified the combination of root characters of orchids into 12 types. The distribution of the root characters showed a high conformity with existing classification of the family.

Rao *et al.* (1988) studied the vegetative anatomy of *Panisea uniflora* and observed the differences in several characters from the species of *Coelogyne*. Trichomes and special water storage cells were found absent in *Panisea* whereas present in *Coelogyne*. Stegmata are present in *Panisea* roots and absent in *Coelogyne* roots. Pith is parenchymatous in *Panisea* and fibrous in *Coelogyne*. These features showed the distinctiveness of genus *Panisea* form the genus *Coelogyne*.

Vij *et al.* (1991) studied the leaf epidermal features in 43 species of ground growing and epiphytic orchids which revealed a direct correlation of these parameters with habit and habitat of the plants and their leaf orientation. The study was considered to be useful in identifying various taxonomic categories..

Rao *et al.* (1991) studied the vegetative anatomy of some species of subfamily Epidendroideae and found the differences among the species with respect to stomatal features and presence or absence of trichomes, cellulose thickened mesophyll cells and fibrous bodies. The comparative analysis of the anatomical features indicated the close relationship between *Bulbophyllum fischeri* and *Coelogyne odoratissima*.

Stern (1997) studied the vegetative anatomy of subtribe Orchidinae and subtribe Habenarinae. Stern and Judd (2000) studied comparative anatomy and systematics of the orchid tribe Vanilleae excluding *Vanilla*. The study has showed the usefulness of vegetative anatomical characters in phylogenetic analysis.

Shakya (1998) did a review of genus *Oberonia* of the Himalaya and adjoining area. On his study of micromorphological characters of the leaf surface of *Oberonia*, he observed the variation in size of stomata, arrangement of subsidiary cells and surface sculpturing of epidermal cells. Out of 12 species studied, he traced out eight different types of stomatal complex according to the arrangement of subsidiary cells. Among the taxa studied, a tetra-monocytic arrangement was common type.

Bajracharya (2003) studied the leaf epidermis of 28 species of Himalayan genus *Eria*. On his study, he observed two types of stoma within the genus, i.e., elliptical and round shape. In his results he had classified the stomatal complex on the basis of variation in size of stomata, arrangement of subsidiary cells and surface sculpturing of epidermal cells and nature of stoma. Seven distinct stomatal complex had been recorded. Among the taxa studied, a tetracytic arrangement was common type. Besides that silica bodies are also recorded on 28 species of *Eria* and on the basis of silica bodies it has been classified into four groups.

2.3. Literatures related to the Anatomy of subtribe Coelogyinae

Singh and Singh (1974) described the significance of tetracytic stomata in *Coelogyne* and *Pholidota*. Kaushik (1983) described the stomata type as cyclocytic in *Coelogyne* and *Pholidota* and as tetracytic in *Otochilus*. Rao and Khasim (1987) described the type of stomata in *Pholidota imbricata* as paracytic and in *Pleione humilis* and *P. praecox* as both cyclocytic and paracytic type. Rao *et al* (1988) described the stomata type in *Panisea uniflora* as hypostomatic and cyclocytic. Kaushik (1983) found distinct strand and interstrand regions in all species of *Coelogyne*, *Otochilus* and *Pholidota* except in *C. flaccida* on the surface view of leaf epidermis. He reported that the anticlinal walls of the subsidiary cells are partly or completely dissolved in *Coelogyne* and *Otochilus*. Kaushik (1983) reported that trichomes are absent on both the surfaces of

leaves in *Coelogyne*, *Otochilus* and *Pholidota* and also in *Panisea uniflora* (Rao *et al.*, 1988), but Rao (1991) found the trichomes on both the surfaces of leaves in *C. odoratissima*. Rao and Khasim (1987) described the presence of absorbing trichomes on both the abaxial and adaxial surfaces of leaves in the species of *Coelogyne*, *Otochilus* and *Pholidota*, which Kaushik (1983) described as handle cells.

On the transverse section of leaves, hypodermis is absent in *Otochilus*, *Pholidota* and *Panisea* and mesophyll is not differentiated into palisade and spongy parenchyma in *Coelogyne*, *Otochilus*, *Panisea* and *Pholidota*. Vascular bundles are arranged in a single series except in *C. viscosa*. U or V shaped fiber caps are present at the phloem and xylem end. Rao and Khasim (1987) found the fibre cap girdling all around the large vascular bundles and found only at phloem end in smaller bundles which he described as suggestive of direct correlation of fiber cap development with the plant habit, i.e., fiber caps are well-developed in advanced epiphytic orchids.

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On the root, velamen is present in *Coelogyne*, *Otochilus*, *Panisea* and *Pholidota* (Kaushik 1983, Rao *et al.* 1988, Rao *et al.* 1991) but *Pleione* is nonvelametous (Rao and Khasim, 1987). They also observed lignified excrescences from the walls of cells of the innermost velamen layer adjacent to thin-walled passage cells of exodermis in epiphytic orchids including *Otochilus albus* which they called as fibrous mats. U-shaped thickening on the endodermal cells of the root are present in *Coelogyne*, *Otochilus*, *Panisea* and *Pholidota*. (Kaushik 1983, Rao *et al.* 1988, Rao *et al.* 1991). Rao *et al.* (1988) illustrated parenchymatous pith in *Panisea* and fibrous in *Coelogyne*. Kaushik (1983) also described few species of *Coelogyne* with fibrous pith and also presence of lignified and pitted cells in pith of *Otochilus* and *Pholidota*.

Chapter III

MATERIALS AND METHODS

The plant materials were collected from the habitat growing in nature or under cultivation in the garden of Department of Botany, Amrit Campus, Lainchour and from National Herbarium, Godavari (KATH). Few species were collected from the different locality of Kathmandu Valley including local nurseries. Some dry plant materials of the specimens were collected from the herbarium from TUCH and KATH. The collected plants were identified with the help of standard literatures and then confirmed by tallying them with the standard herbarium specimens in KATH and TUCH. Small pieces of the materials were preserved in FAA and then stored in 70% alcohol for anatomical studies.

For the study of epidermis of leaves, the leaf surfaces were taken out by scratching or by simply peeling. For the dry specimen, the leaf pieces were first boiled with glacial acetic acid and hydrogen peroxide and then the leaf surfaces were taken out (Bajracharya and Shrestha, 2004). The leaf surfaces were then cleaned with soft brush and the pieces were stained with safranin and mounted with glycerin so as to prepare the temporary slides. Prepared temporary slides were sealed with nail polish for later observations. The leaf epidermis was studied under the light microscope. Measurements of stomata of each specimen were taken with the help of standardized ocular micrometer scale. The nature of stomata, orientation of subsidiary cells around the stomata and the shape of the stomata were also studied under the microscope. Many readings were taken invariably to find out the mean value of size and number of stomata per unit area.

For the transverse section of leaves and roots, the materials were taken and by free hand section cutting with the help of blade, the anatomical characteristics were studied. For the dry specimens, first of all the materials were boiled with 1:1 glycerin and water. Then the permanent slides of the thin sections of leaves and roots were prepared. For that, double staining was done by the safranin and fastgreen combination and sections were mounted in DPX mountant. Anatomical characteristics of leaf and root were studied by observing through the light microscope (LM). The series of preparation of slides were given in the chart below.

Drawings of the plants were made from fresh or dried specimens and the overall morphological study in general was done. Anatomical drawings were made with the help of camera lucida or simply observing through the Light Microscope (LM). Photographs of anatomical characteristics were also taken through the microscope.

Chart 1: Preparation of Temporary Slide

Plant materials

Free hand section cutting or peeling or scratching

Wash the section with water

Stain with safranin – 30 minutes

Wash with water – 5 times

Acid water – in case of excessive staining

Mount in glycerin

Chart 2: Preparation of Permanent Slide

Plant materials

Free hand section cutting

Wash the section with water

Cover the section with 30% alcohol – 5 minutes

50% alcohol – 5 minutes

Stain with safranin (1% solution in water) – 30 minutes

70% alcohol – 5 minutes

Stain with fast green – 30 seconds

90% alcohol – 5 minutes

Absolute alcohol – 10 minutes

Xylol – 15 minutes

Mount in DPX mountant on a clear slide

Chapter IV

OBSERVATIONS

4.1. Genus *Coelogyne* Lindl.

The genus is epiphytic having pseudobulb usually with two leaves. Leaves coriaceous or membranous. Flowers in raceme or in short scape at pseudobulb apex, often at apex of young pseudobulb with young leaves; usually several flowered, rarely more than 20-flowered or reduced to a solitary flower. Flowers small to large or medium-sized, often white, or greenish yellow, labellum with or without stripes. Sepals usually concave, mentum or spur absent, sometimes abaxially with keeled protrusions. Petals usually narrower than the sepals, rarely as wide as sepals. Labellum three-lobed, lateral lobes broader, erect on both sides of the column, labellum disc with two to five longitudinal lamella or ridges. Column long, winged or hooded round the tip. Anthers overhanging forward by short filaments, tip resting on large rostellum which is divaricate. Pollinia 4.

The genus was established in 1821 by John Lindley in his *Collectanea Botanica*. The genus comprises over 100 species distributed in India, China, SE Asia, the Malay Archipelago and the Pacific (Pearce and Cribb, 2002). The name is derived from the Greek words “coelo” meaning hollow and “gynus” meaning female indicating the deeply excavated stigma in the genus.

1. *Coelogyne corymbosa* Lindl., *Fol. Orchid.*, *Coelogyne*: 7 (1854). Hook. f., *Fl. Brit. Ind.* 5: 831 (1890); op. cit. 6: 194 (1890). King & Pantl. in *Ann. Roy. Bot. Gard. Calcutta* 8: 134, t. 185 (1898). Banerji & Thapa in *J. Bombay Nat. Hist. Soc.* 66: 578, t. 1 (1969). Hara *et al.*, *EFPN* 1: 36 (1978). Press *et al.*, *ACFPN*: 211 (2000).

Epiphytic herb. Pseudobulbs short clustered, dense, oblong-ovate or nearly rhombic-oblong, ridged, sheathed at base. Leaves two, arising from the apex of the pseudobulb, shortly petioled, oblong lanceolate to obovate lanceolate. Inflorescence raceme from the base of the pseudobulb, two to four flowered. Sheaths broad, ovate, acute. Floral bracts caducous. Flowers white, fragrant. Sepals and petals subequal, lanceolate. Lateral sepals slightly narrower than dorsal sepal. Lip white, with orange-red

bordered yellow blotches, oblong or subovate with three lobes, lateral lobes erect, suborbicular, serrulate at apex, midlobe ovate. Disc with three ridges elongated from the base to midlobe. Column slightly bent forward, broadly winged. (Fig 1A)

Flowering time: March-May.

Distribution: This species is found in India, Nepal, Bhutan and China. It occurs as an epiphytic herb in the subtropical and temperate zones of central and east Nepal at 1500-2900 m altitude.

Collection: Godavari, 1600m, 22.4.2000, D.M. Bajracharya, 316 (ASCOL), Daman, 2400m, 15.5.2003, D.M. Bajracharya, 487 (ASCOL)

Specimens examined: Jiri, 2500m, 2054.2.28 (10.6.1997), Tek Bdr. Gurung, 53 (TUCH); Shivapuri, 2600m, 2051.2.26 (9.6.1994), Baniya, N., 04/51 (TUCH); Phulchoki, 2018m, 2053.11.25, Ritesh Shrivastava, 67 (TUCH); Melanchi gaun, Sindhuplachok, 2250m, 23.5.1993, N.P. Manandhar, 89-93 (KATH); Yarsa Chisapani, 1860-2260m, 23.4.1978, I. Sharma, H.K. Sainju, A. Karki, 78/212 (KATH).

Anatomy

Epidermis in Surface View (Fig 1B)

Stomata are present on adaxial surface only. Strand and interstrand regions are slightly differentiated. Epidermal cells are rectangular in shape. Trichomes are absent. Stomata are tetracytic. Anticlinal walls of subsidiary cells are dissolved. Stomatal size is 28 x 24 μm with the frequency of 124 per sq. mm.

Transverse Section of Leaf (Fig 1C)

Epidermis – It consists of single layer of small rectangular cells surrounded by thick cuticle on both adaxial and abaxial surfaces. Adaxial cells are smaller than abaxial cells.

Hypodermis – It is represented by two layers of large and vertically elongated thin-walled parenchymatous cells towards the abaxial surface above the vascular bundle. It is distinguishable only in midrib region and the midrib vascular bundle is pushed adaxially by the large hypodermal cells.

Mesophyll – Not differentiated into palisade and spongy parenchyma. However, abaxial cells are larger than adaxial cells. It is four to five layers thick. Mesophyll cells are oval to spherical in shape.

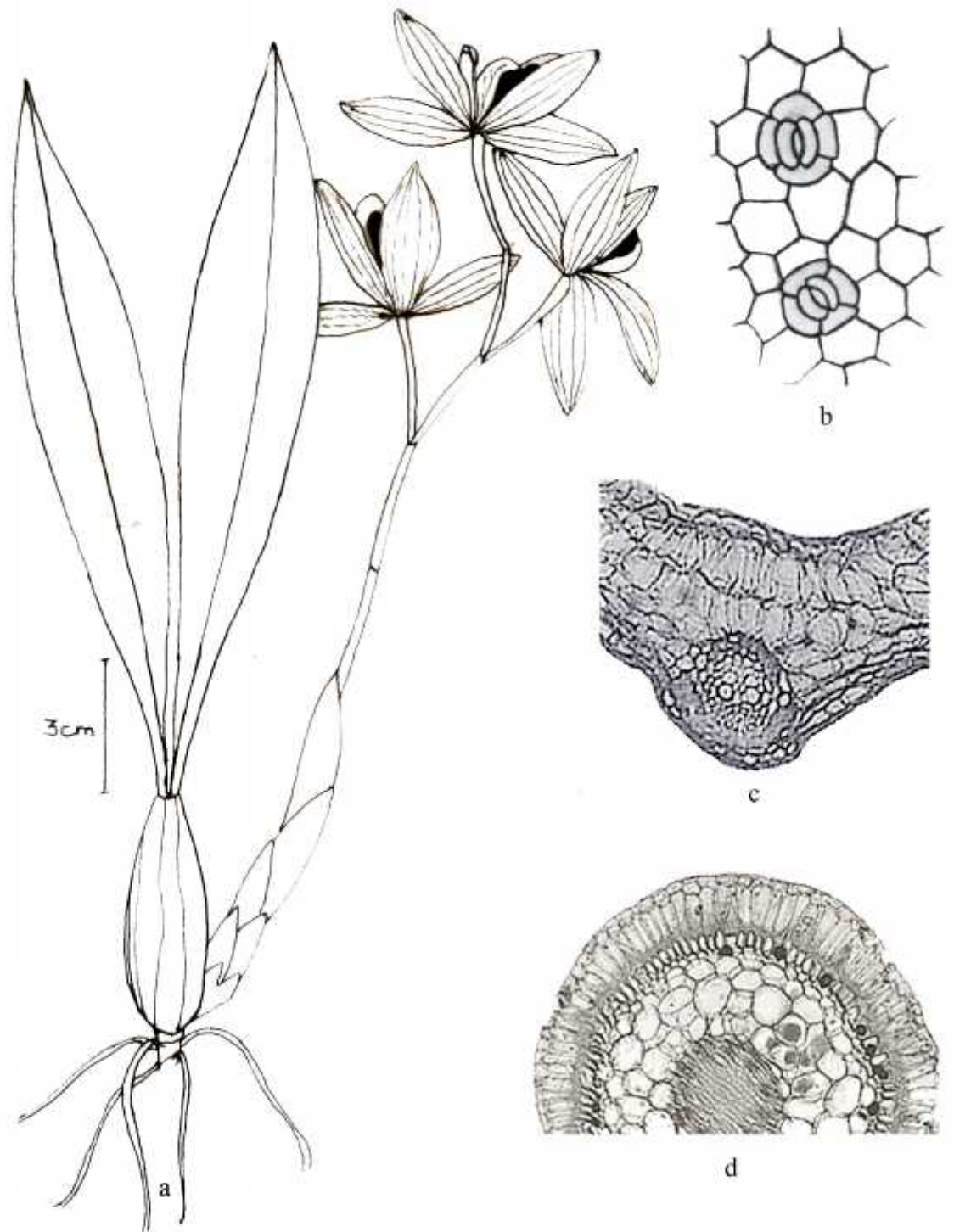


Fig 1: *Coelogyne corymbosa*; a. Habit sketch, b. Stomata, c. T. S. leaf, d. T. S. root

Vascular bundle – Large midrib vascular bundle is in the center and other smaller vascular bundles are present throughout the lamina in a single series. Along the lamina, small and large bundles are found alternating with each other. Midrib vascular bundle is slightly conical in shape with U- or V- shaped fibre caps towards xylem and phloem end. Fibre cap is narrow towards the xylem end and broader towards the phloem. Phloem is present on the adaxial side and xylem on the abaxial side. Only one phloem patch is present just above the adaxial fibre cap in the centre.

Transverse Section of Root (Fig 1D)

Velamen – Two layers of velamen cells are present. Outer layer is small-celled and inner layer cells are more than three times elongated than the outer cells. Inner walls of inner cells are highly cutinized.

Exodermis – Below the velamen, lies a single layered exodermis with anticlinally arranged long thick-walled cells. Few cells are alternating with short rectangular to squarish passage cell. Long cells are highly lignified.

Cortex – It is composed of three to four layers of thin walled round to oval cells. The outermost layer is small celled with thick outer wall. Middle one or two layers consist of large cells and the innermost cells are more or less smaller than the middle layer

Endodermis - Not clearly seen.

Vascular cylinder –Xylem strands alternating with phloem strand. These are 11 each in number.

Pith – It is formed of small oval to round cells.

2. Coelogyne cristata Lindl., *Collect. Bot.*: sub t. 33 (1824). Hook. f., *Fl. Brit. Ind.* 5: 829 (1890); op. cit. 6: 193 (1890). King & Pantl. in *Ann. Roy. Bot. Gard. Calcutta* 8: 133, t. 184 (1898). Banerji & Thapa in *J. Bombay Nat. Hist. Soc.* 66: 578, t. 2 (1969). Hara *et al.*, *EFPN* 1: 36 (1978). Press *et al.*, *ACFPN*: 211 (2000).

Cymbidium speciosissimum D. Don, *Prodr. F. Nepal.*: 35 (1825).

Pendulous epiphytic herbs. Pseudobulbs arising from a stout rhizome, oblong or ovoid-cylindric, sheathed at the base. Leaves two arising from the apex of the pseudobulb, almost sessile, linear to linear-lanceolate. Inflorescence racemose arising

from the base of the pseudobulb, two to eight flowered. Scape pendulous sheathed by numerous small bracts. Floral bracts persistent, oblong, acute. Flowers white, fragrant. Sepals narrowly elliptic-oblong or oblong-lanceolate, undulate margins, subacute apex. Petals slightly shorter than the sepals, ovate-lanceolate, undulate. Lip white, tinged with yellow, oblong, large, three-lobed, with long rounded lateral lobes, erect, large and separated from the mid lobe by an isthmus, mid lobe broad, short, orbicular, with two broad, rectangular, yellow lamellae on its upper surface; disc between the lateral lobes with four yellow fimbriate lamellae. Column white straight, apex hooded due to wings. (Fig 2A)

Flowering time: February-April.

Distribution: This species is found in India, Nepal and Bhutan. In Nepal, it occurs as an epiphytic herb in the subtropical and temperate zones at 1400-2600 m altitude.

Collection: Rajarani, Dhankuta, 500m, 4.6.2003, D.M. Bajracharya, 540 (ASCOL)

Specimens examined: Phulchoki, 4700ft, D.H. Shai (TUCH); Tadapani, 1900m, 26.3.1997, Ritu Gurali, 13 (TUCH); Phulchoki, 1980m, 15.4.1995, Sameeta Malla, 159 (TUCH); Sanguri dannda, 4500ft, 22.5.1969, T.B. Shrestha, 15112 (KATH); Danda gaun, Myagdi, 1760m, 28.5.1992, N.P. Manandhar, 477-92 (KATH).

Anatomy

Epidermis in surface view (Fig 2B)

Stomata are present on adaxial surface only. Strand and interstrand regions are not clearly differentiated. Epidermal cells are rectangular to polygonal in shape. Trichomes are absent but root hairs are present. Stomata are tetracytic. Anticlinal walls of subsidiary cells are dissolved in mature stomata. Stomatal size is 42 x 33 μm and stomatal frequency is 113 per sq. mm.

Transverse Section of Leaf (Fig 2C)

Epidermis – It consists of single layer of squarish and domed cells surrounded by thick cuticle on both abaxial and adaxial surfaces. Adaxial cells are smaller than abaxial cells.

Hypodermis – It is clearly distinguishable in this species. A single layer of large, thin walled hyaline cells are present towards the abaxial surface. The shape of the cell varies from squarish to elongated cylindrical. On the adaxial side, a single layer

of small and thin walled cells are present. Hypodermal cells on the abaxial surface are more than three times larger than the cells on adaxial surface.

Mesophyll – It is not differentiated into palisade and spongy parenchyma. It consists of six to eight layers of cells with chlorophyll. Cell shape varies from spherical to oval and size varies from small peripherally to large centrally. Intercellular spaces are not present.

Vascular bundle – Vascular bundles are arranged in a single series with a large midrib vascular bundle in the centre and other smaller and larger bundles are found alternating with each other. Midrib vascular bundle is oval in shape with U-shaped fibre caps towards both xylem and phloem ends. Only one phloem patch is present towards the adaxial fibre cap in the centre. Xylem is present above the phloem bundle.

Transverse Section of Root (Fig 2D)

Velamen – Unicellular root hairs are present radiating from the epidermal cells which are small in size and not differentiated with other velamen cells. Velamen is composed of four to five layers of small polygonal cells. The innermost layer is elongated and thin walled in comparison to other cells. Inner wall of the innermost layer is highly cutinized.

Exodermis – It is single layered below the velamen. It consists of thick walled elongated to rectangular cells having squarish passages cells alternating with few exodermal cells.

Cortex – It is composed of four to five layers of thin walled oval cells. Inner one layer and outer one or two layers are composed of very small cells but the middle two to three layers are composed of large cells which are more than five times larger than other cells.

Endodermis – A single layer of endodermal cells are present. Endodermal cells consist of U-shaped thickening on its inner tangential and radial walls which are interrupted by one or two thin walled cells at protoxylem poles after every five to six lignified cells.

Vascular cylinder – Xylem strands alternate with phloem strands. 16 bundles of xylem and phloem each are present.

Pith – It is formed of small oval to round thin walled parenchymatous cells.

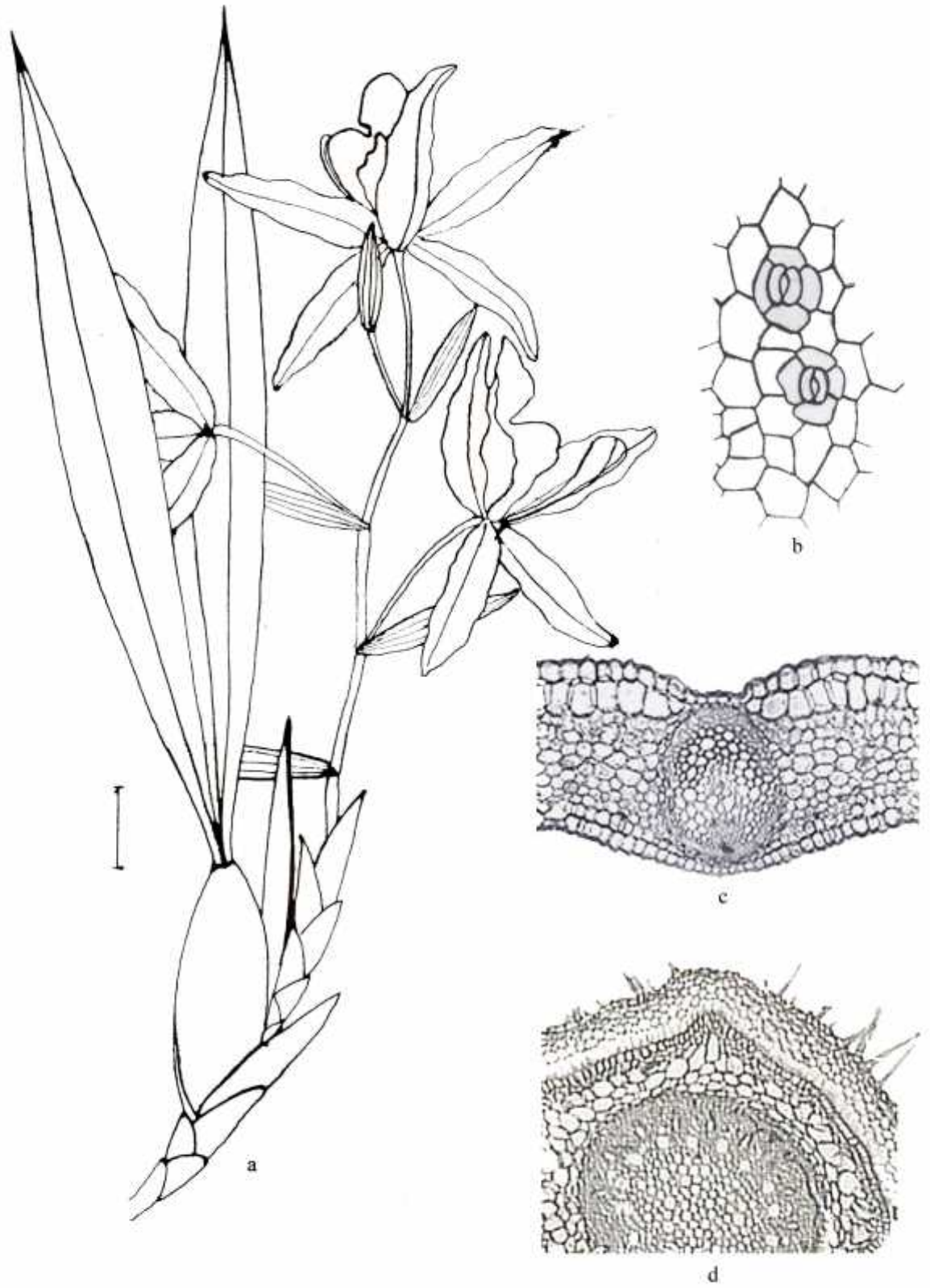


Fig 2: *Coelogyne cristata*; a. Habit sketch, b. Stomata, c. T. S. leaf, d. T.S. root

3. *Coelogyne flaccida* Lindl. [ex Wall., Number. List: 54, n. 1961 (1829), nom.nud.], *Gen. Sp. Orchid. Pl.*: 39 (1830). Hook. f., *Fl. Brit. Ind.* 5: 829 (1890). King & Pantl. in *Ann. Roy. Bot. Gard. Calcutta* 8: 133, t. 183 (1898). Banerji & Thapa in *J. Bombay Nat. Hist. Soc.* 66: 578, t. 1 (1969). Hara *et al.*, *EFPN* 1: 36 (1978). Press *et al.*, *ACFPN*: 211 (2000).

Coelogyne lacteal Rehb. F. in *Gard. Chrono.* n.s. 23: 692 (1885). Type: Myanmar (Burma), cult, Day (holo. W Herb. No. 42299).

Epiphytic herbs. Pseudobulbs clustered conical or ovoid-cylindric, ridged, sheathed at the base. Leaves two, at the apex of the pseudobulb, linear-lanceolate or narrowly oblong, acuminate, with long channeled petiole. Inflorescence racemose, arising from the base of the pseudobulb, pendulous about nine-flowered. Floral bracts obovate-cymbiform, acute, caducous. Flowers creamy white, fragrant. Sepals lanceolate or oblong-lanceolate, acute, longer than the petals. Petals linear or oblanceolate, acute, reflexed. Lip oblong, three-lobed, mid lobe oblong or ovate-lanceolate with yellow in the middle and spotted red at base of mid-lobe, lateral lobes long, with striped red, disc with three yellow flexuose ridges between the lateral lobes. Column white, broadly winged and erosely hooded at the apex. (Fig 3A)

Flowering time: April-June.

Distribution: This species is distributed in India, Nepal, Myanmar, West China and Laos. It occurs as an epiphytic herb in the subtropical zone of central Nepal at 900-1400 m altitude.

Collection: Bhedetar, 1600m, 1.6.2003, D.M. Bajracharya, 503 (ASCOL)

Anatomy

Epidermis in surface view (Fig 3B)

Stomata are present on adaxial surface only. Strand and interstrand regions are not clearly differentiated. Epidermal cells are rectangular to polygonal in shape. Trichomes are absent but root hairs are present. Stomata are tetracytic. Anticlinal walls of subsidiary cells are dissolved in mature stomata. Stomatal size is 59 x 38 μm and stomatal frequency is 78 per sq. mm.

Transverse Section of Leaf (Fig 3C)

Epidermis – Both adaxial and abaxial single layered epidermal cells are of same size and barrel-shaped. Cells are small in the midrib region. Thick cuticle is present on both the surfaces.

Hypodermis – Below the epidermis lies a one-cell layer thick hypodermis with distinct large hyaline cells on both surfaces. Hypodermal cells are four to six times larger than the mesophyll cells. The cells contain annular cellulose thickenings. This character of the species differentiates it from other species.

Mesophyll – Mesophyll cells are oval to rectangular in shape and are three to four layers. Mesophyll cells are very small in comparison to hypodermal cells but not differentiated into palisade and spongy cells.

Vascular bundle – Midrib vascular bundle is large and oval in shape whereas the laminar bundles are comparatively very small present in a single series embedded on the mesophyll tissue. Since hypodermis is absent on the midrib, the midrib bundle is large. U-shaped fibre cap on the abaxial side and only a small sheath at the top on adaxial side are present on the midrib vascular bundle. Three phloem patches are present, one at the center and two on the periphery towards the adaxial side.

Transverse Section of Root (Fig 3D)

Velamen – Root hairs present. Velamen consists of 4 layers of polygonal cells. Outer three layers are small celled and almost equal in size whereas the innermost layer is elongated with cutinized inner wall.

Exodermis – Single layer of thick walled cells are present below the velamen. Elongated small walled cells are frequently interrupted by a thin walled squarish cell which is known as passage cell.

Cortex – Two outermost cortical layers are composed of small celled polygonal to oval cells, middle layer with large parenchymatous cells. Innermost cortical layer is single layered consisting of small cells.

Endodermis – As in other species, the endodermal cells do not possess U-shaped thickening but the inner walls are thick and every three to four lignified cells are interrupted by two to four thin walled cells at protoxylem poles.

Vascular cylinder – Below the endodermis lies pericycle layer which is similar to endodermis surrounding the vascular cells. 19 bundles of phloem and xylem are alternating with each other. Pith is formed of rounded or oval cells at the center.

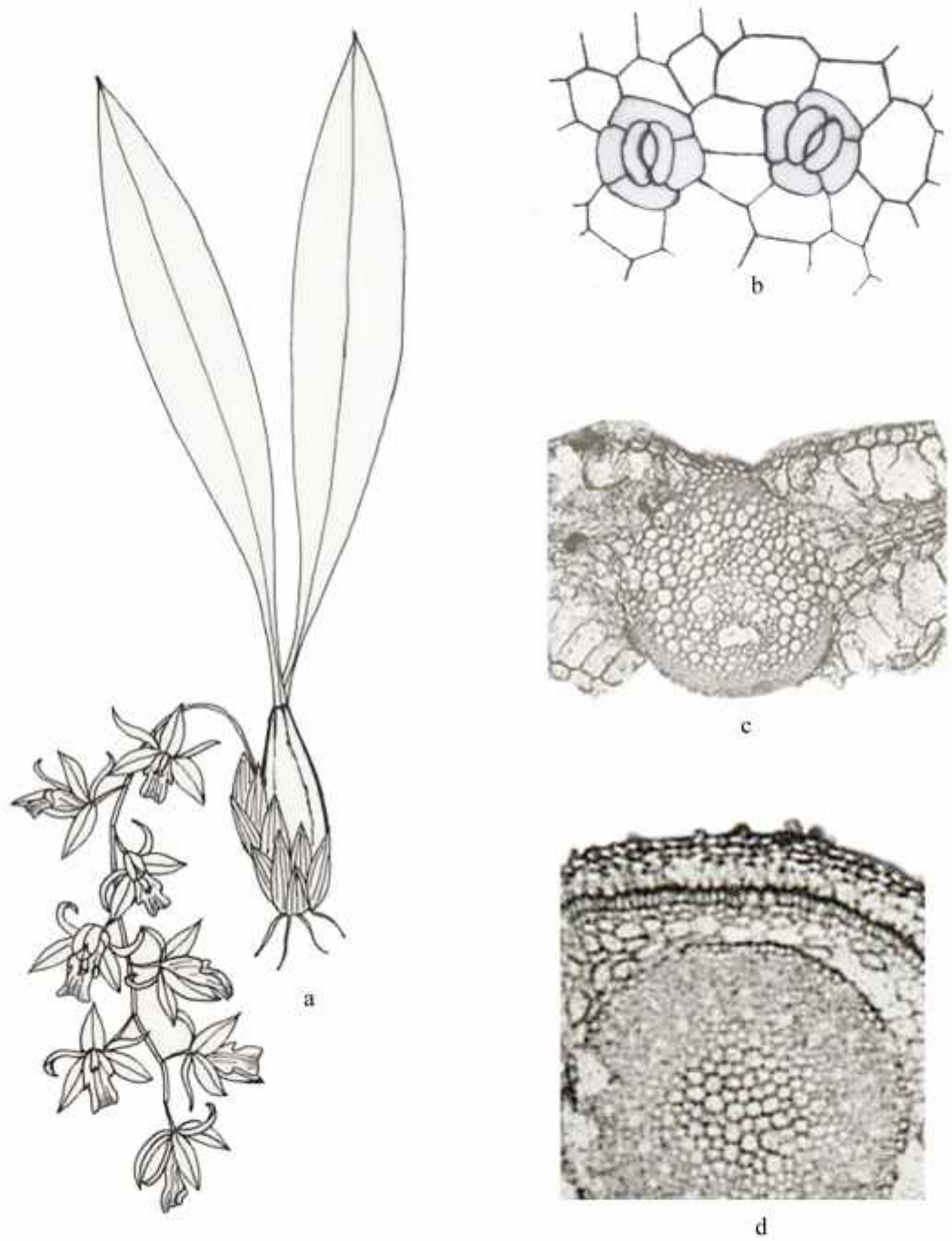


Fig 3: *Coelogyne flaccida*; a. Habit Sketch, b. Stomata, c. T. S. leaf, d. T. S root

4. *Coelogyne flavida* Hook. f. ex Lindl., *Fol. Orchid.*, *Coelogyne*: 10 (1854). Hook. f., *Fl. Brit. Ind.* 5: 839 (1890). King & Pantl. in *Ann. Roy. Bot. Gard. Calcutta* 8: 138, t. 191 (1898). Banerji in *Candollea* 19: 218 (1964). Tuyama in Hara, *Fl. E. Himal.*: 429 (1966). Banerji & Thapa in *J. Bombay Nat. Hist. Soc.* 66: 580, t. 5 (1969). Tuyama in Hara, *Fl. E. Himal.* 2: 181 (1971). Hara *et al.*, *EFPN* 1: 36 (1978). Press *et al.*, *ACFPN*: 211 (2000).

Creeping epiphytic herbs. Pseudobulbs distant, on stout creeping scaly rhizome, conical or ovoid, sheathed at the base. Leaves two, at the apex of the pseudobulb, narrowly elliptic oblong, acute apex, petioled. Inflorescence raceme, proliferating, arising from between the leaves at the apex of the pseudobulb, erect, five to eleven flowered. Scape terminal, zigzag with many small imbricating scaly sheaths just below the flowers. Floral bracts large, scarious. Flowers pale yellow or greenish yellow. Sepals subequal, spreading, ovate, acute. Petals linear, spreading as long as the sepals, obtuse. Lip oblong, three-lobed, the basal half concave, the side lobes small, oblong, obtuse, the mid lobe shortly recurved, obcordate, separated from the side lobes by two broad lamellae, disc faintly two- ridged. Column long, straight, narrowly winged neat the apex. (Fig 4A)

Flowering time: April-June.

Distribution: This species is distributed in Nepal, India (East Himalaya), Bhutan, Myanmar and Thailand. It occurs as an epiphytic herb in the subtropical and temperate zones of central and east Nepal at 1000-2300 m altitude.

Collection: Rajarani, Morang, 500m, 14.1.2001, D.M. Najracharya, 398 (ASCOL)

Specimens examined: Chitlang-Chandragiti-Pharping-Suryabinayak, 11.4.1981, G. Poudyal (TUCH); Shivapuri-Gokarna, 20.3.1981, G. Amatya (TUCH); Lamauwa Basinda, Sankhuwasabha, 1300m, 8.6.1994, P.R. Shakya, K.K. Dangol, 100/9 (KATH); Ramche 1800m-Garam 1900m, 6.6.1969, H. Kanai, 11884 (KATH). Sheopuri, S. Slope, 1800m, 17.11.1966, D. H. Nicolson, 2759 (KATH).

Anatomy

Epidermis in Surface View (Fig 4B)

Stomata are present only on the adaxial surface of the leaf. Strand and interstrand regions are differentiated. Epidermal cells are polygonal in shape. Stomata are tetracytic. Subsidiary cells are similar to other epidermal cells but they are smaller in size. Anticlinal

walls of the subsidiary cells are not dissolved. Stomatal size is 54 x 51 μm and stomatal frequency is 85 per sq. mm.

Transverse Section of Leaf (Fig 4C)

Epidermis – Epidermal cells are smaller in size. Cells are squarish to rectangular on both surfaces. Cells on the adaxial surface are slightly larger than on the abaxial surface. Epidermal cells on the midrib region are comparatively smaller.

Hypodermis – Single layer of larger rectangular cells are present on the abaxial surface. However it is absent on adaxial surface.

Mesophyll – Mesophyll cells are composed of nine to ten layers of cells which are slightly differentiated into palisade type on adaxial side and spongy type on abaxial side at laminar regions. Adaxial cells are smaller and rounded whereas abaxial cells are slightly larger and slightly elongated in shape.

Vascular bundle – Pear-shaped large vascular bundle is present in the midrib surrounded by a single layer of very small and thin walled bundle sheath cells. Adaxial U-shaped and abaxial V-shaped fibre caps are present. There are six phloem patches on the adaxial side out of which one patch is large and centered and five are small located peripherally.

Transverse Section of Root (Fig 4D)

Velamen – Four layers of velamen cells are present out of which outer three layers are composed of rounded cells and one innermost layer is composed of elongated cells with cutinized inner wall.

Exodermis – Single layer of thick walled cells as in other species are present inner to the velamen.

Cortex – One outermost layer of cortical cells are small, a single middle layer with large rounded cells, two innermost layers again are small celled.

Endodermis – Single layer as in other species. U-shaped thickening is present on the inner wall of endodermal cells. Four to five thick walled cells are alternating with one to three thin walled passage cells. Pericycle similar to endodermis

Vascular cylinder – 11 strands each of xylem and phloem are present. Separate xylem and phloem patches alternate with each other.

Pith – Central pith is present with rounded cells.

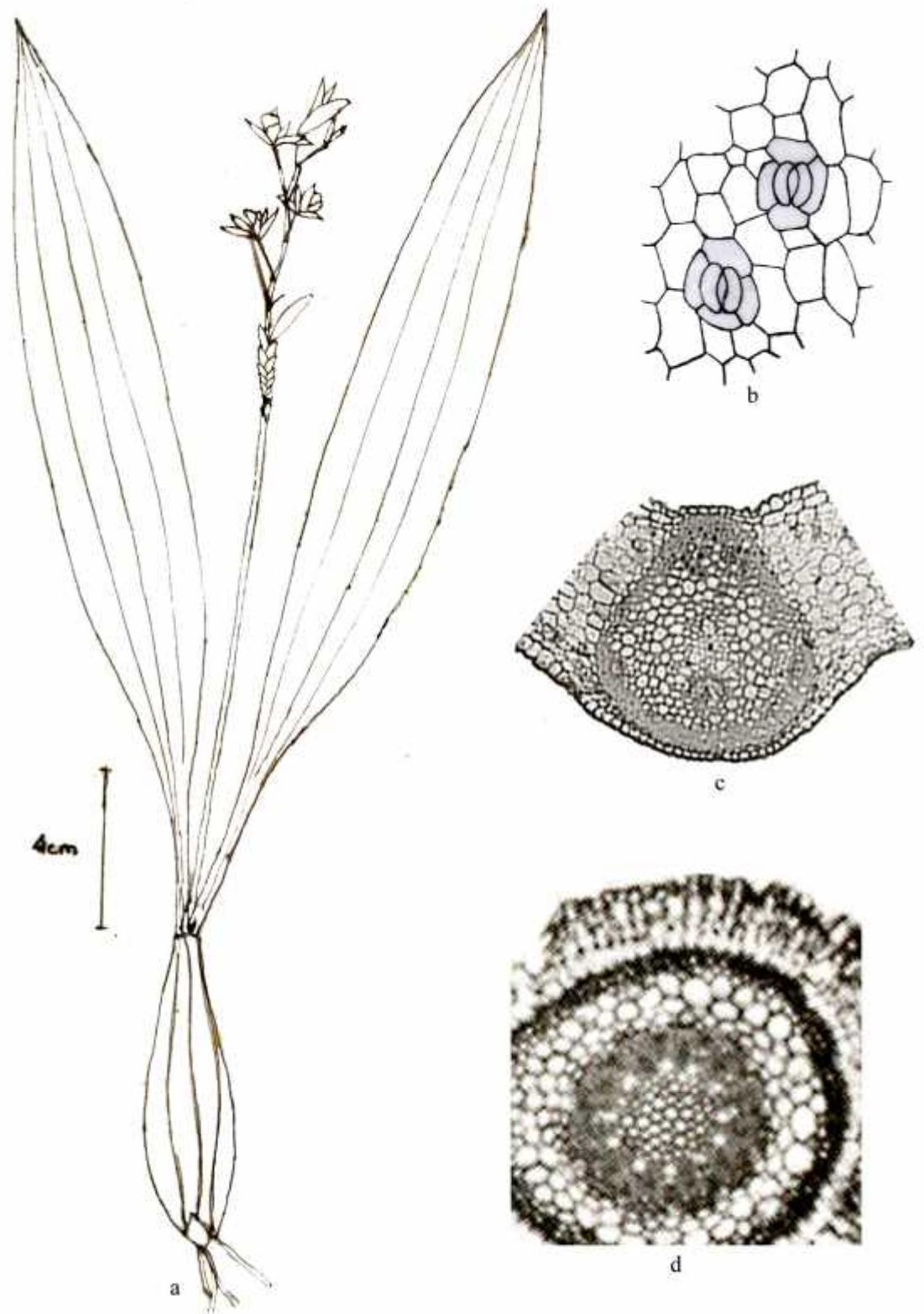


Fig 4: *Coelogyne flavida*; a. Habit sketch, b. Stomata, c. T. S. leaf, d. T. S. root

5. *Coelogyne fuscescens* Lindl. [ex Wall., Numer. List.: 54, n. 1962 (1829), nom. nud.], Gen. Sp. Orchid. Pl.: 41 (1830). Hook. f., Fl. Brit. Ind. 5: 833 (1890). King & Pantl. in Ann. Roy. Bot. Gard. Calcutta 8: 132, t. 181 (1898). Banerji & Thapa in J. Bombay Nat. Hist. Soc. 66: 580, t. 6 (1969). Hara *et al.*, EFPN 1: 36 (1978). Press *et al.*, ACFPN: 211 (2000).

Lithophytic herbs. Pseudobulbs on stout rhizome, cylindrical or fusiform, sheathed at base. Leaves two, at the apex of the pseudobulb, oblanceolate to oblong-elliptic, acute apex, shortly petioled. Inflorescence racemose, drooping from the base of the pseudobulb, four to ten flowered. The peduncle stout, arising from the rhizome, enveloped for more than half its length in imbricate sheaths. Floral bracts broadly ovate, caducous. Flowers pale yellow or yellowish brown. Sepals subequal, oblong lanceolate, acute. Petals linear, acute, reflexed. Lip whitish with a pale yellow-green central stripe, oblong or elliptic-oblong, three lobed, lateral lobes short rounded, recurved with marginal bands, mid lobe broadly ovate, elliptic, wider than side lobes. Disc between the side lobes with three smooth elongated lamellae or ridges. Column long, curved, broadly winged at the apex. (Fig 5A)

Flowering time: October-December.

Distribution: This species is found in Nepal, India (Sikkim), Bhutan and Myanmar. It occurs as an epiphytic herb in the subtropical and temperate zones of central and east Nepal at 1200-2100 m altitude.

Collection: Bhedetar, 1600m, 1.6.2003, D.M. Bajracharya, 502 (ASCOL)

Specimens examined: Dhulikhel, 1650m, 15.10.1982, Rhidaya Shrestha (TUCH); Chandrakot, Kaski, 1300m, 12.9.1979, R. Niraula, N. Pradhan, 10/1979 (KATH); Dandakateci, Dundarijal, 5800 ft, 13.9.1977, P. Pradhan, N. Shrestha, 389 (KATH).

Anatomy

Epidermis in Surface View (Fig 5B)

Stomata are present on adaxial surface only. Strand and interstrand regions are differentiated. Epidermal cells are squarish to rectangular in shape. Subsidiary cells are slightly differentiated from other epidermal cells. Trichomes are absent. Stomata are tetracytic. Anticlinal walls of the subsidiary cells are not dissolved. Stomatal size is 33 x 29 μm with the stomatal frequency of 141 per sq. mm.

Transverse Section of Leaf (Fig 5C)

Epidermis – Epidermal layer is formed by single layer of square to rectangle cells. Thick cuticle on both surfaces are present. Abaxial cells are larger than adaxial cells.

Hypodermis – It is absent on abaxial surface. Two layers of small celled hypodermis is present towards the adaxial side at the midrib region below the vascular bundle.

Mesophyll – There is no distinct spongy and palisade cells in the mesophyll. The cells are oval to round in shape. This portion is made up of five to seven layers of parenchymatous cells.

Vascular bundle – Oval shaped vascular bundle is present at the center in the midrib region. The smaller bundles are in a series throughout the lamina. U-shaped abaxial and adaxial fibre caps are present. Abaxial fibre caps are more curved. Single phloem bundle is present towards the adaxial surface in the middle.

Transverse Section of Root (Fig 5D)

Velamen – Three to four layers of more or less polygonal velamen cells are present.

Exodermis – This layer is represented by single layer of slightly elongated cells on which the radial walls are thick and the outer walls are cutinized.

Cortex – It is composed of outer one to two layers of small cells, middle one layer of large cells and inner two layers of small cells. The cells are oval to spherical in shape. The cells are thin walled and parenchymatous.

Endodermis – It is composed of U-shaped highly thickened cells in a single layer, 10-15 cells interrupted by a single thin walled cell towards the protoxylem pole only. Pericycle is not distinguishable.

Vascular cylinder – There are 14 bundles of xylem and phloem alternating with each other. Phloem bundles are ruptured. Connective tissue is sclerenchymatous.

Pith -- Pith is present at the center with small rounded cells.

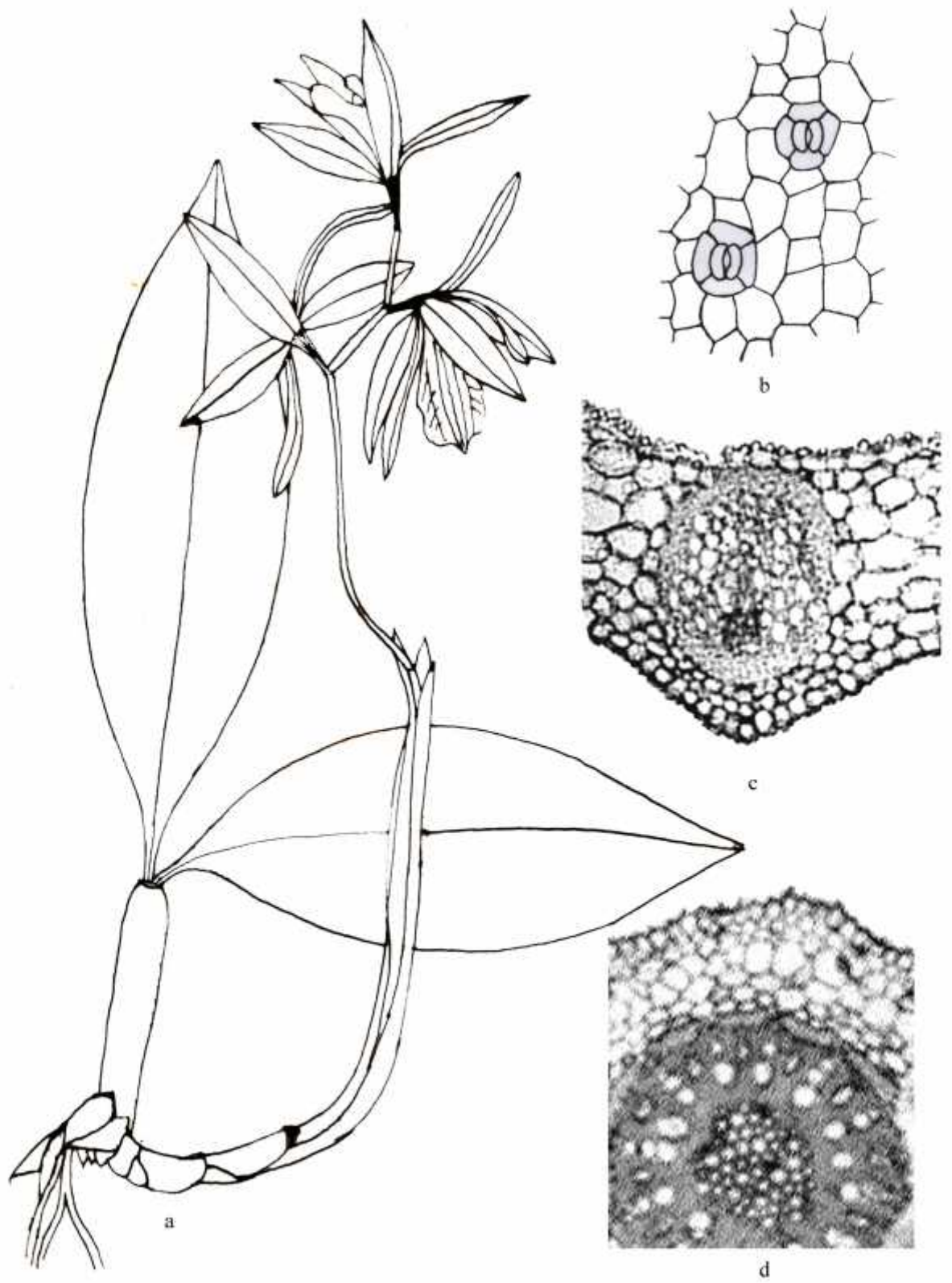


Fig 5: *Coelogyne fuscescence*; a. Habit sketch, b. Stomata, c. T. S. leaf, d. T. S. root

6. *Coelogyne nitida* Lindl. [ex. Wall., Numer. List; 53, n. 1954 (1829), nom. nud.] P. F. Hunt in Kew Bull. 20:54, in nota (1966).

Cymbidium nitidum Wall. ex. D. Don, Prodr. Fl. Nepal.: 35 (1825); non Roxb. (1832).

Coelogyne ochracea Lindl. in Edw., Bot. Regist. 32: t, 69 (1846). Hook. in Curtis, Bot. Mag. 78: t. 4661 (1852). Hook. f., Fl. Brit. Ind. 5: 831 (1890). King & Pantl. in Ann. Roy. Bot. Gard. Calcutta 8: 132, t. 182 (1898). Banerji & Thapa in J. Bombay Nat. Hist. Soc. 66: 580, t. 7 (1969). Hara *et al.*, EFPN 1: 36 (1978). Press *et al.*, ACFPN: 212 (2000).

Epiphytic herbs. Pseudobulb on stout rhizomes, cylindrical, ovoid to conical, furrowed. Leaves two arising from the apex of the pseudobulb, narrowly oblong, elliptic-lanceolate, acute, with channeled petiole. Inflorescence racemose, from the base of the pseudobulb, three to six flowered, erect or drooping. Flower bracts deciduous. Flowers white, fragrant. Sepals subequal, narrowly oblong, subacute to obtuse. Petals narrower, oblong-lanceolate, subacute. Lip oblong or almost ovate, upper surface of lip white with two elongated blotches of yellow bordered with red, lateral lobes large, oblong to rounded, incurved, the edges serrulate, mid lobe ovate, blunt, entire or very slightly notched at the apex. The disc with two smooth ridges and two yellow spots at the base of the mid lobe. Column slightly curved, broadly winged. (Fig 6A)

Flowering time: April-June.

Distribution: This species is distributed in India (Central and East Himalaya), Nepal, Bhutan, Bangladesh, Myanmar, China (Yunnan), Thailand, and Laos. It occurs as an epiphytic herb in the subtropical and temperate zones at 1300-2400 m altitude.

Collection: Okhare, Dhankuta, 1600m, 3.6.2001, D.M. Bajracharya, 518 (ASCOL)

Specimens examined: 1035, Pot plant from department of botany, T.C. College (TUCH); Chhap Bhanjyang, Shivapuri, 1700m, 052.2.13, Chitra Bdr. Baniya, 196cbb (TUCH); Thankot, 4500ft, 026.2.3. K.H. Shrestha (TUCH); Hanspokhari, Illam, 1330, 12.7.1974, P. Pradhan, M. Amatya, Rajani, 259/74 (KATH); West of Phewa, 5500 ft, 18.5.1966, T.B. Shrestha, 2005 (KATH).

Anatomy

Epidermis in Surface View (Fig 6B)

Stomata are present on adaxial surface only. Strand and interstrand regions are not differentiated. Epidermal cells are rectangular in shape. Subsidiary cells are differentiated

from other epidermal cells. Stomata are tetracytic and anticlinal walls of the subsidiary cells are not dissolved. Stomatal size is 52 x 45 with the stomatal frequency of 141 per sq. mm.

Transverse Section of Leaf (Fig 6C)

Epidermis – A single layer of rectangular and cuticularized cells present. Abaxial cells are larger than adaxial cells. Epidermal cells are smaller at the midrib region.

Hypodermis – One to two layers of large hyaline rectangular cells are present on abaxial surface only.

Mesophyll – It is not differentiated into spongy and palisade cells. It is composed of four to six layers of small rectangular or barrel-shaped chlorophyllous cells.

Vascular bundle – A single series of small vascular bundles are present below the hypodermis throughout the lamina and a large spherical bundle is present at the midrib. The bundle is surrounded with fibre sheath and in the center lies three phloem patches with central large and two small peripheral patches. Xylem is present towards the abaxial side.

Transverse Section of Root

Velamen – Three to four layers of round to oval shaped cells are present.

Epidermis – Single layer of small barrel shaped cells are present below the velamen.

Cortex – Below the epidermis lies three to four layers of thin walled, slightly larger cells with innermost layer with smaller rounded cells.

Endodermis – There is presence of U-shaped thickening on the endodermal cells interrupted with few thin walled cells towards the protoxylem end.

Vascular cylinder – 10 strands each of xylem and phloem are present alternating with each other. Connective tissues are thick walled.

Pith – Pith consists of small rounded cells in the center.

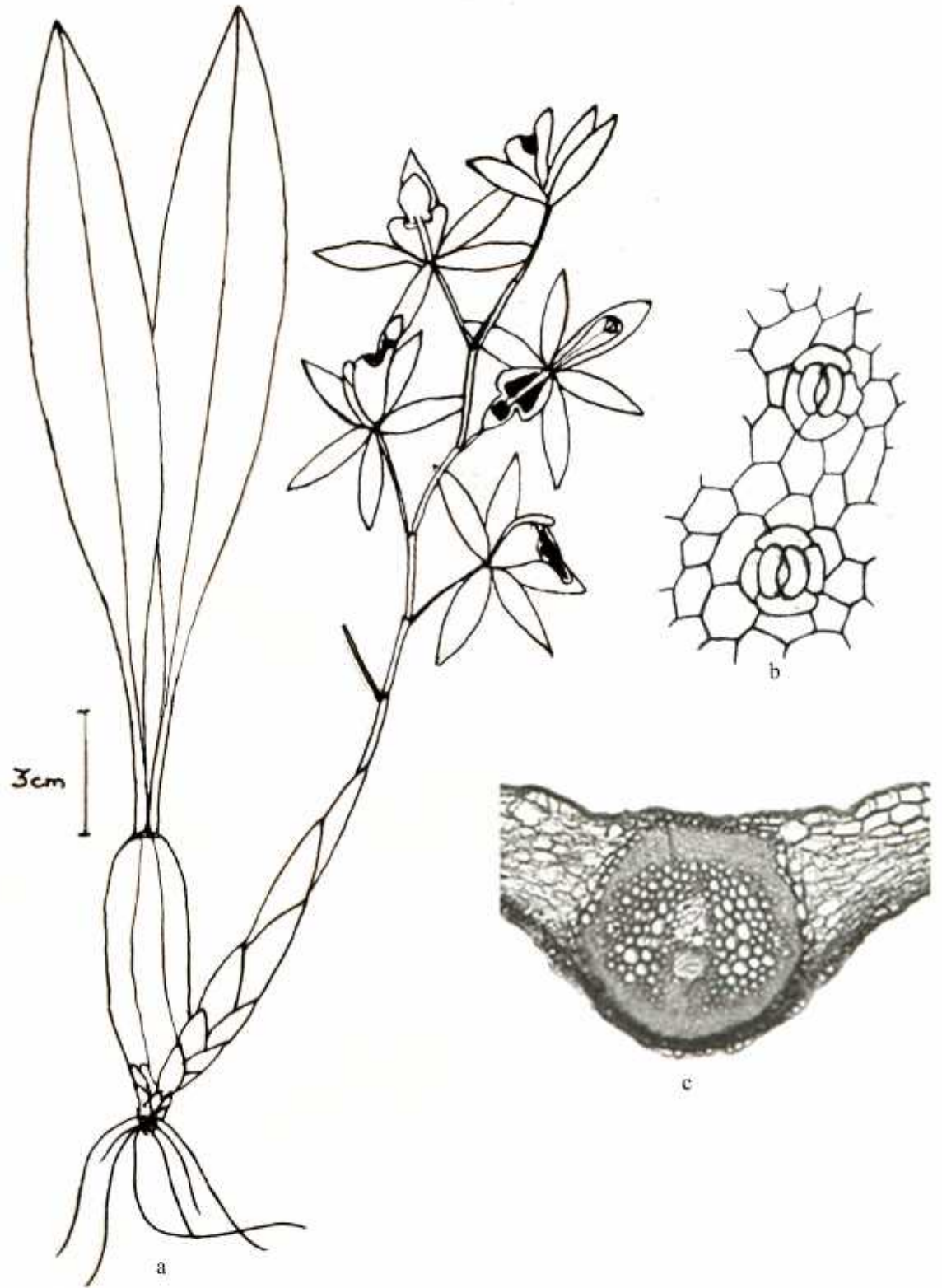


Fig 6: *Coelogyne nitida*, a. Habit sketch, b. Stomata, c. T. S. leaf

7. *Coelogyne ovalis* Lindl. in Edw., Bot. Regist. 24, Misc.: 91 (1838). Hook. f., Fl. Brit. Ind. 5: 836 (1890). King & Pantl. in Ann. Roy. Bot. Gard. Calcutta 8: 135, t. 187 (1898). Banerji & Thapa in J. Bombay Nat. Hist. Soc. 66: 580, t. 8 (1969). Hara *et al.*, EFPN 1: 36 (1978). Press *et al.*, ACFPN: 212 (2000).

Coelogyne fimbriata auct. Non Lindl. (1825): Wall., Numer. List: 54, n. 1957 (1829). Lindl., Gen. Sp. Orchid. Pl.: 41 (1830), p.p.

Creeping epiphytic herbs. Pseudobulbs arising from sheathed rhizome, long, cylindrical to fusiform, sheathed at the base. Leaves two arising from the apex of the pseudobulb, narrowly elliptic or elliptic-oblong, coriaceous. Shortly petioled. Inflorescence racemose, arising from between the pair of leaves on the apex of pseudobulb, one to three flowered, erect, sheathed at the base. Floral bracts coriaceous, ovate-lanceolate, deciduous. Flowers lemon yellow to yellowish brown. Sepals subequal, ovate-lanceolate, dorsal sepal slightly broader. Petals linear, reflexed. Lip light yellow tinged with brown on the mid lobe, oblong, three-lobed, the side lobes narrow, recurved, the mid lobe broadly ovate, fimbriate with ciliate upper surface and edges. The disc with two crisped lamellae from base to apex. Column curved, broadly winged in its upper half, yellow in colour or light coloured than the rest of the flower. (Fig 7A)

Flowering time: September-December.

Distribution: This species is found in India, Nepal, Bhutan and China (Southeast Tibet). In Nepal it occurs as an epiphytic herb in the subtropical and temperate zones at 1300-2100 m altitude.

Collection: Rajarani, Morang, 500, 14.1.2001, D.M. Bajracharya, 380 (ASCOL)

Specimens examined: Hanspokhari, 5200 ft, 11.4.037, R.B. Tamang, 127 (KATH); Sundarijal, 5300 ft, 13.9.1977, P. Pradhan, N. Shrestha, 102 (KATH).

Anatomy

Epidermis in Surface View (Fig 7B)

Stomata present only on adaxial surface. Strand and interstrand regions are not differentiated. Stomata are tetracytic. Shape of subsidiary cells are somewhat similar to other epidermal cells but smaller. Epidermal cells are rectangular to polygonal in shape. Anticlinal walls are not dissolved. Size of stomata is 60 x 42 μm with the stomatal frequency of 99 per sq. mm.

Transverse Section of Leaf (Fig 7C)

Epidermis – Single layer of rectangular cells on the abaxial side and rectangular to squarish cells on the adaxial surface. Adaxial cells are comparatively smaller than abaxial cells.

Hypodermis – One to two layers of large oval hyaline cells below the epidermis only towards the abaxial surface is present.

Mesophyll – Four to five layers of small, oval shaped cells are not differentiated into palisade and spongy cells below the hypodermis.

Vascular bundle – Pear-shaped or flask-shaped large bundle is present at the midrib. Midrib bundle consists of three phloem patches, one large centrally and two small peripherally. V-shaped fibre cap on the adaxial and U-shaped on the abaxial side are seen distinctly. The bundle is surrounded with a circle of small bundle sheath cells.

Transverse Section of Root (Fig 7D)

Velamen – Root hairs present. Four layers of velamen are present. The velamen cells are rounded except the innermost layer which is composed of elongated cells.

Exodermis – Single layer of highly cutinized, thick walled cells are present inner to the velamen. The thick walled cells are interrupted with thin-walled squarish passage cells.

Cortex – Two layers of small rounded to polygonal cells are on the outer side. Middle one to two layers are with large cells and one innermost layer is also made of small oval cells.

Endodermis – Below the cortex lies a single endodermal layer. Three to four thick walled barrel shaped cells to squarish endodermal cells are alternating with two to three thin-walled cells towards the protoxylem poles. There is no U-shaped thickening on the endodermal cells. Pericycle is similar to endodermis but with small sized cells.

Vascular cylinder – 19 bundles are present. Other vascular tissues are similar as in other species.

Pith – It is composed of rounded cells in the center.

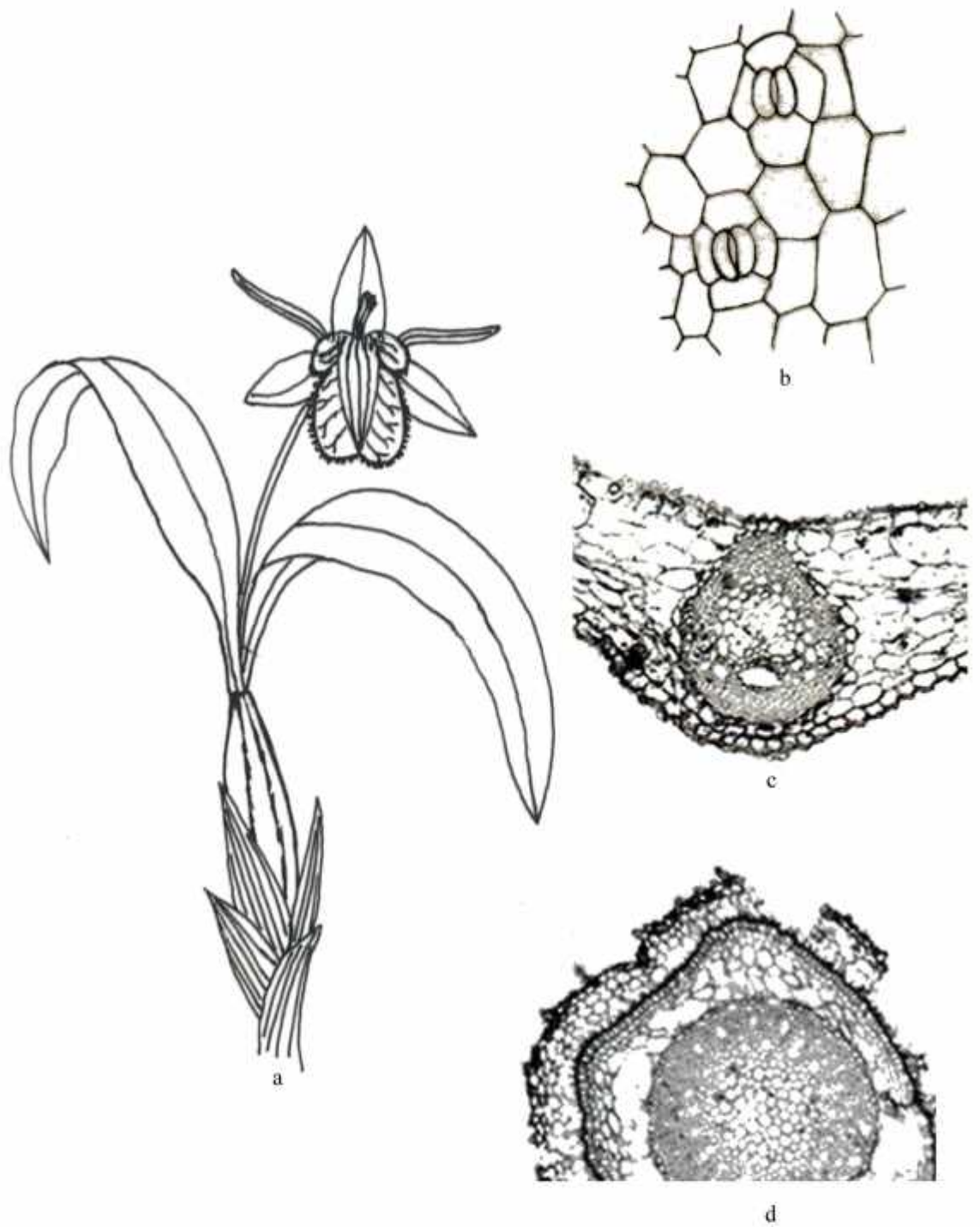


Fig 7: *Coelogyne ovalis*; a. Habit sketch, b. Stomata. c. T. S. leaf, d. T. S. root

8. *Coelogyne stricta* (D. Don) Schlechter in Fedde, Repert. Beih. 4: 184, 300 (1919).

Cymbidium strictum D. Don prodr. Fl. Nepal. 35:1825.

Coelogyne elata Lindl. [ex Wall., Numer. List: 54, n. 1959 (1829), nom. Nud.], Gen. Sp. Orchid. Pl.:40 (1830). Wall., Pl. Asoat. Rarior. 3:12, t. 218 (1831). Hook. F., Fl. Brit. Ind. 5: 838 (1890); op. cit. 6:194 (1890). King & Pantl. in Ann. Roy. Bot. Gard. Calcutta 8; 136, t. 188 (1898). Tuyama in Hara, Fl. E. Himal.: 429 (1966). Banerji & thapa in J. Bombay Nat. Hist. Soc. 66: 578, t. 3 (1969). Herklots in Orchide Rev. 78:188 (1970). Tuyama in Hara, Fl. E. Himal. 2:181 (1971). Hara *et al.*, EFPN 1: 37 (1978). Press *et al.*, ACFPN: 212 (2000).

Epiphytic herb. Pseudobulbs on the stout sheathed rhizome, oblong-cylindric, sheathed at the base. Leaves in pairs, coriaceous, narrowly elliptic-oblong, acute, narrowed to a long petiole. Inflorescence racemose, arising from between the pair of leaves on the apex of pseudobulb, peduncle naked below, having many short imbricate sheaths, many-flowered. Floral bract sub-rhomboid or ovate-elliptic, acute, caducous. Flowers ochraceous white. Sepals subequal, oblong lanceolate, spreading. Petals as long as the sepals but much narrower, linear lanceolate. Lip shorter than the sepal, elongate or oblong, three lobed, side lobes narrow, entire, erect, with yellow tinge at the apex, mid lobe ovate, rounded or subrhomboid, erose, tinged with golden yellow. Disc with two orange coloured lamellae extending from the base to near the apex. Column white, slightly curved, broadly winged in its upper half. (Fig 8A)

Flowering time: April-June.

Distribution: This species is distributed in India, Nepal, Bhutan and North Myanmar. It occurs as an epiphytic herb in the subtropical and temperate zones of central and east Nepal at 1400-2100 m altitude.

Collection: Godavari, 1600m, 2.5.2000, D.N. Bajracharya, s.n. (ASCOL)

Specimens examined: Godawari-Pharping, 19.4.1981, G. Paudyal (TUCH); Tel-kot Ban, Manichur, 2037.12.22, G. Amatya (TUCH); Godavari, 6000 ft, 19.4.1978, P. Pradhan, 404 (KATH); Tinpani Bhanjyang, 7000 ft, 2.5.1978, no collector, 424 (KATH).

Anatomy

Epidermis in Surface View (Fig 8B)

Stomata are present only on adaxial surface. Epidermal cells are large and polygonal to rectangular in shape. Strands and interstrand regions are differentiated.

Stomata are tetracytic. Subsidiary cells are clearly distinguished from the other epidermal cells. Anticlinal walls of subsidiary cells are not dissolved. Size of stomata is 62 x 45 μm and the stomatal frequency is 75 per sq. mm.

Transverse Section of Leaf (Fig 8C)

Epidermis – Both adaxial and abaxial cells are squarish in shape but abaxial cells are larger than the adaxial cells.

Hypodermis – It is present on both sides below the epidermis. Single layer of large hyaline cells on both the sides are present. Abaxial hypodermal cells are larger than adaxial cells. They are oval in shape.

Mesophyll – 10-12 layers of undifferentiated small chlorophyllous cells are present in the middle. Mesophyll cells are comparatively much smaller than the hypodermal cells. They are oval to round in shape.

Vascular bundle – Shape of the large midrib vascular bundle is elongated surrounded by fibre sheath all around. Thick fibre cap is present on the abaxial surface and U-shaped fibre cap is on the adaxial side. Three phloem patches are present, one in the center and other two on the periphery as in other species.

Transverse Section of Root (Fig 8D)

Velamen – Five to six layers of velamen cells are present which are small in size. The innermost layer is composed of elongated cells with inner wall cutinized.

Exodermis – Single layer of exodermal cells are thick and cutinized and few cells are interrupted by a single parenchymatous thin walled squarish cells.

Cortex – It is composed of inner two and outer two layers of small cells and middle four to six layers of comparatively larger cells.

Endodermis – A single layer of endodermal cells with U-shaped thickening is present. Three to six such cells are alternating with two to four thin walled small cells.

Vascular cylinder – 20 patches each of xylem and phloem are found alternating with each other.

Pith – Pith is made of smaller rounded cells in the centre. It occupies a large space at the center. It is ruptured with hole at the center forming a hollow root.

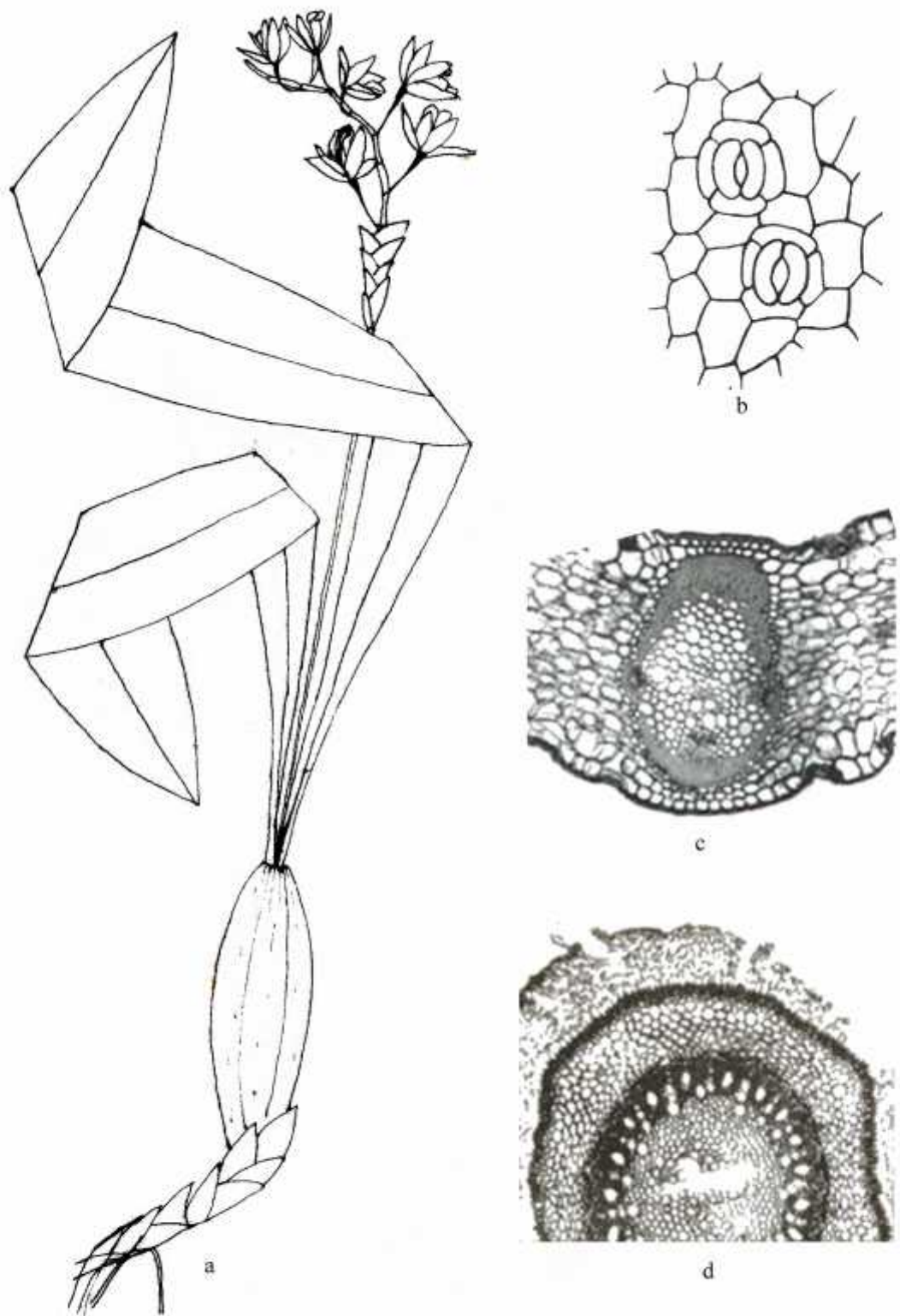


Fig 8: *Coelogyne stricta*; a. Habit sketch, b. Stomata, c. T. S. leaf, d. T. S. root

4.2. Genus *Otochilus* Lindl.

The genus is epiphytic with articulate branched stems formed by elongated, superimposed pseudobulbs. Leaves in pairs on the uppermost pseudobulb, elliptic or lanceolate. Inflorescence raceme, many small flowered. This species produce chains of white or brownish white flowers. Flowers small with scarious floral bracts with the sides rolled inwards. Sepals and petals subequal, narrow, free and spreading. Lip short and sessile on base of the column, lateral lobes short, erect, midlobe entire. Column long and slender, foot absent.

The genus *Otochilus* was established in 1830 by John Lindley in his *Genera and Species of Orchidaceous Plants*. The genus comprises five species distributed in Himalayas, China, Myanmar, Thailand and Vietnam. (Pearce and Cribb, 2002) The name is derived from the Greek words “otos” meaning ear and “cheilos” meaning lip. The name signifies the auriculate lateral lobes of the lip which enclasp the base of the column.

1. *Otochilus albus* Lindl. [ex Wall., Numer. List; 54, n. 1967 (1829), nom. Nud.], Gen. Sp. Orchid. Pl.: 35 (1830). Hook. f., Fl. Brit. Ind. 5: 843 (1890); op. cit. 6:195 (1890). King & Pantl. in Ann. Roy. Bot. Gard. Calcutta 8:143, t. 200 (1898). Banerji & Thapa in J. Bombay Nat. Hist. Soc. 68:662 (1972). Hara *et al.*, EFPN 1: 51 (1978). Press *et al.*, ACFPN: 222 (2000).

Coelogyne alba (Lindl.) Reichenb. f. in Walp., Ann. Bot. Syst. 6:236 (1861).

Epiphytic, pendulous herbs. Internodes of the stem pseudobulb, subcylindric, grooved, enclosed in a tubular sheath. Leaves two at the terminal nodes, elliptic lanceolate, tapering at the base into a petiole. Inflorescence raceme with young leaves, shorter and enveloped in large imbricating sheaths. Floral bracts large, ovate-lanceolate, caducous. Flowers white. Sepals oblong lanceolate, acute, dorsal sepal smaller. Petals narrower than sepals, linear-lanceolate. Lip having three lobes, equaling the sepals, side lobes broad and acute, mid lobe oblong, apiculate, deflexed from the base. Column long and slender, winged with hooded like extension, bent at the base. (Fig 9a)

Flowering time: June.

Distribution: This species is found in Nepal, India (East Himalaya) and Thailand. It occurs as an epiphytic herb in the subtropical and temperate zones of central and east Nepal at 1500-2400 m altitude.

Collection: Pathevara, Dhankuta, 1600m, 1.6.2003, D. M. Bajracharya, 511 (ASCOL)

Specimens examined: Kande to Deurali Village, 1800m, 8.7.2002, Subedi 970, (TUCH). Hanspokhari, 8000ft, 7.6.1978, P. Pradhan and R. Niraula 469, (KATH). Ramche, 2.6.1969, H. Kanai 11885 (KATH).

Anatomy

Epidermis in Surface View (Fig 9B)

Stomata are present on adaxial surface only. Epidermal cells are polygonal in shape. Strands and interstrand regions are differentiated. Stomata are tetracytic. Subsidiary cells are not clearly distinguished from the other epidermal cells but smaller. Anticlinal walls of subsidiary cells are dissolved in mature stomata. Stomatal size is 29 x 24 μm and the frequency of stomata is 75 per sq. mm.

Transverse Section of Leaf (Fig 9C)

Epidermis – Single layer of squarish to rectangular cells are present which are larger in size.

Hypodermis – Not distinguishable

Mesophyll – Five to seven layers of cells are present below the epidermis. The cells are not differentiated into spongy and palisade cells

Vascular bundle – Shape of vascular cylinder is oval or elongated surrounded by U shaped fibre sheath on both phloem and xylem ends. One large phloem patch is present almost at the center.

Transverse Section of Root (Fig 9D)

Velamen – Two to three layers of barrel shaped cells are present of which outer layer is composed of small cells and innermost layer with large cells which are squarish in shape.

Exodermis – Single layer of thick walled cells are present below the velamen cells. Number of thick walled cells are alternating with single thin walled barrel-shaped cell called as passage cell.

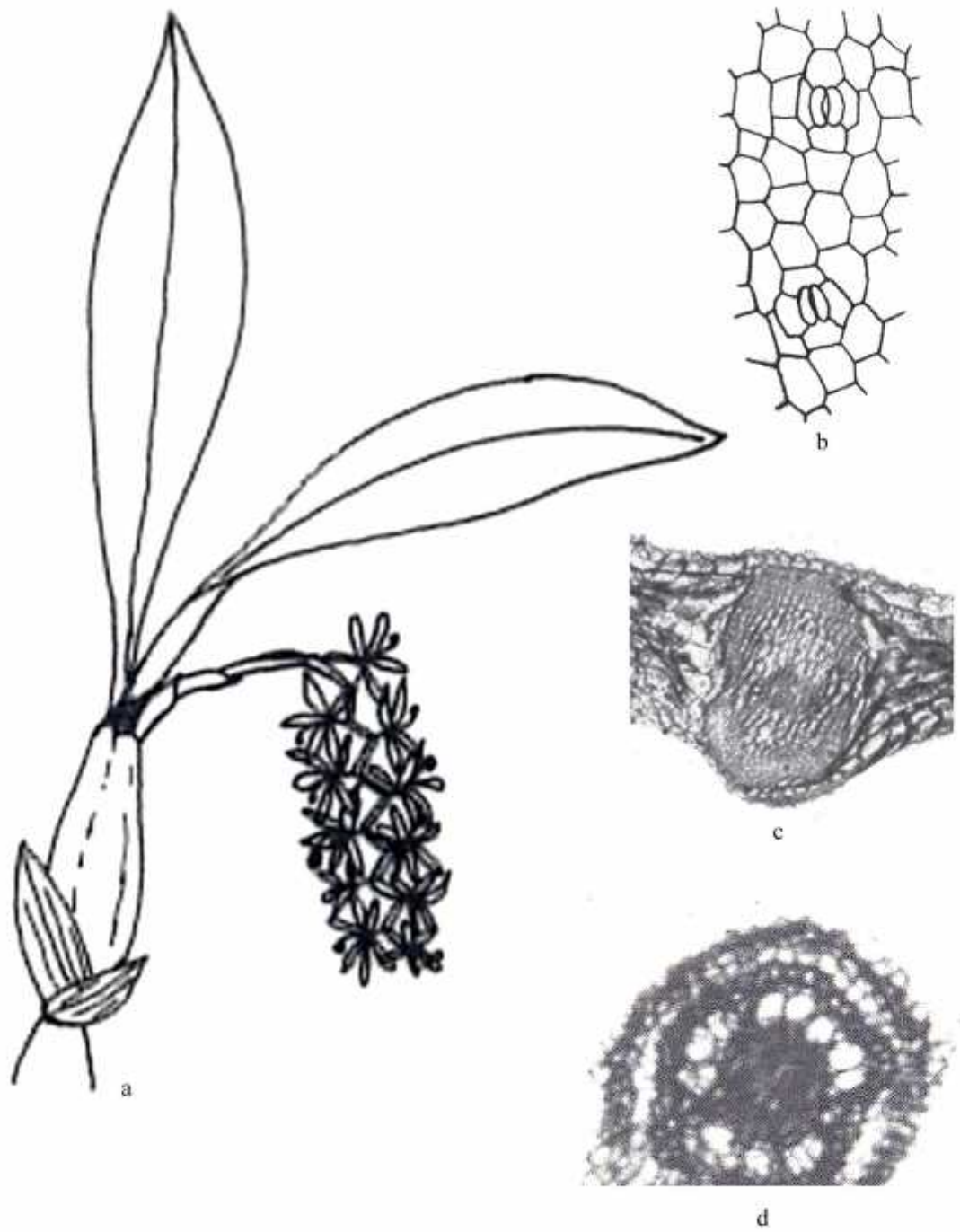


Fig 9: *Otochilus albus*; a. Habit sketch, b. Stomata, c. T. S. leaf, d. T. S. root.

Cortex – Two outer layers and one inner layer made of thin walled small cells. A single middle layer is made of large cells. Shape of cells are round to oval.

Endodermis – U-shaped thickening is present on single layer of endodermal cells. Three to four such thick walled cells are alternating with one to two thin walled cells.

Vascular cylinder – 7 strands of xylem alternating with phloem patches are present in the stellar region with thick walled connective tissue.

Pith – Pith in the center is made of small rounded cells.

2. *Otochilus fuscus* Lindl. [ex Wall., Numer. List: 54, n. 1969 (1829); nom. nud.], Gen. Sp. Orchid. Pl.: 35 (1830). Hook. f., Fl. Brit. Ind. 5: 844 (1890). King & Pantl. in Ann. Roy. Bot. Gard. Calcutta 8: 143, t. 199 (1898). Banerji & Thapa in J. Bombay Nat. Hist. Soc. 68: 662 (1972). Hara *et al.*, EFPN 1: 51 (1978). Press *et al.*, ACFPN: 222 (2000).

Coelogyne fusca (Lindl.) Reichenb. f. in Walp.m Ann. Bot. Syst. 6:236 (1861).

Epiphytic pendulous herb. Internodes pseudobulb like, cylindrical or fusiform, pendulous covered with fibrous sheaths. Leaves two, from the apex of the terminal node, linear lanceolate, slightly narrowed at the base to form a short petiole. Inflorescence raceme, peduncles enclosed in imbricated sheaths, semi erect, equaling leaves. Floral bracts narrow, oblong, scarious. Flowers white. Sepals subequal, linear-oblong. Petals narrower than sepals, linear-oblong. Lip with mid lobe oblong, shorter than sepals, lateral lobes small teeth like. Column winged at the apex. (Fig 10A)

Flowering time: December-January.

Distribution: This species is distributed in Nepal, Northeast India, Bhutan and North Myanmar. It occurs as an epiphytic herb in the subtropical and temperate zones of central and east Nepal at 1100-2100 m altitude.

Collection: Bhadaure to Deurali Village, 1800m, 15.1.2001, Subedi 863, (TUCH)

Specimen examined: Bhadaure to Deurali Village, 1800m, 15.1.2001, Subedi 863, (TUCH). Hanspokhari, 1550m, 13.4.1979, P. Pradhan, R. Niraula, M. Gorkhali 692, (KATH). Makawanpur, 1600m, 24.2.1992, K. J. White 14, (KATH). Sankhuwasabha, 1660m, 22.12.1990, P. R. Shakya 9545, (KATH).

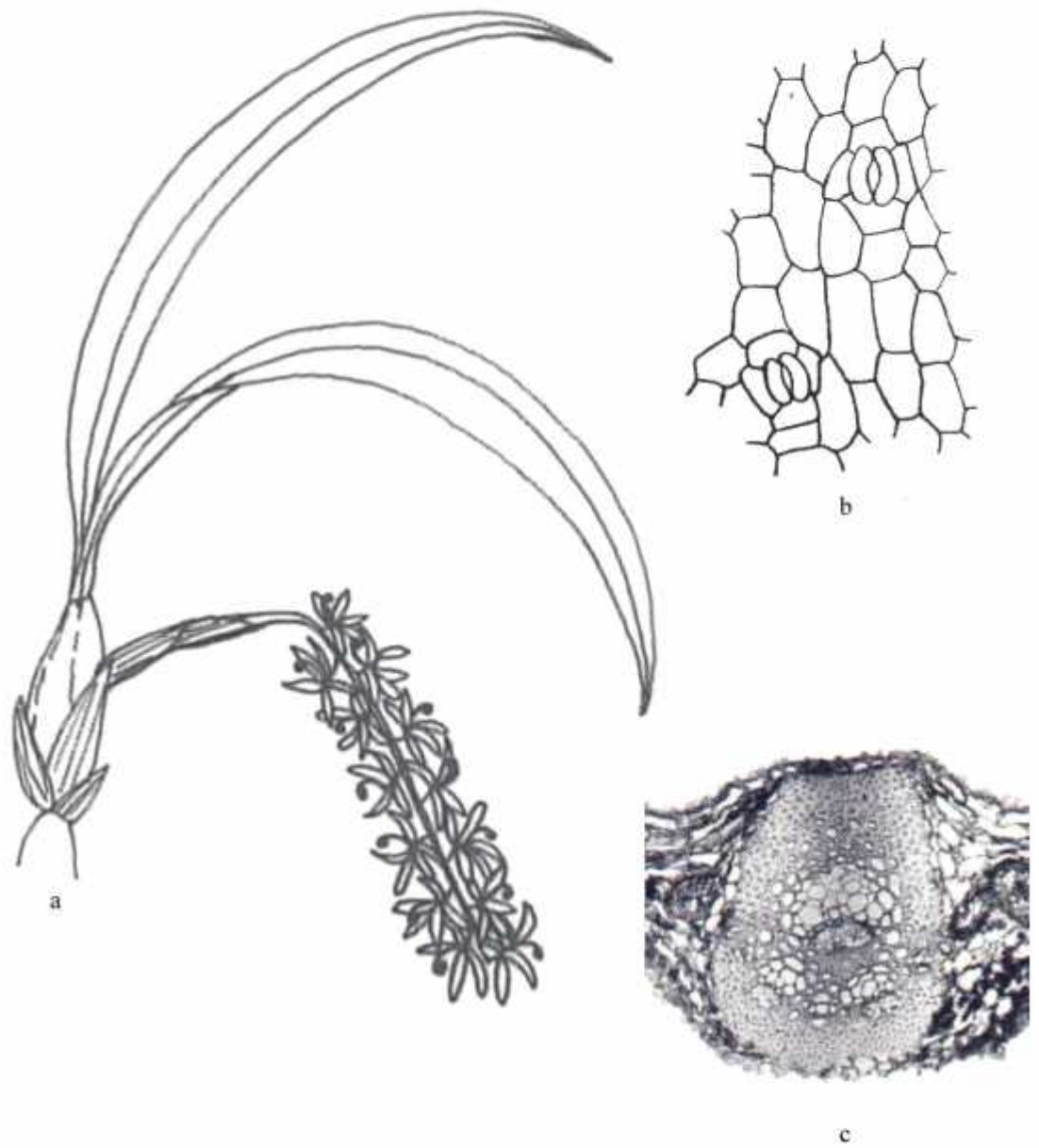


Fig 10: *Otochilus fuscus*; a. Habit sketch, b. Stomata, c. T. S. leaf through midrib

Anatomy

Epidermis in Surface View (Fig 10B)

Stomata are present on adaxial surface only. Epidermal cells are rectangular in shape. Strands and interstrand regions are only slightly differentiated. Stomata are tetracytic. Subsidiary cells are polygonal in shape and much smaller than other epidermal cells. Stomatal size is 30 x 26 μm and the stomatal frequency is 74 per sq. mm.

Transverse Section of Leaf (Fig 10C)

Epidermis – Single layer of large rectangular cells are present.

Hypodermis – Not distinguishable.

Mesophyll – Below the epidermis, five to seven layers of thin walled cells are present which are not differentiated into spongy and palisade cells

Vascular bundle – Vascular bundles are elongated with thick fibre caps surrounded the xylem and phloem all around. Altogether three patches of phloem are present out of which one is large in the center whereas other two are smaller on the periphery towards the abaxial surface.

3. Othochilus porrectus Lindl., Gen. Sp. Orchid. 36. 1830; Hook. f., F. Brit. Ind. 5:844. (1890) [ex Wall., Numer. List: 54, n. 1968 (1829), nom. nud.], Gen. Sp. Orchid. Pl.:36 (1830). Hook. f., Fl. Brit. Ind. 5:844 (1890). King & Pantl. in Ann. Roy. Bot. Gard. Calcutta 8:142, t. 198 (1898). Hara *et al.*, EFPN 1: 51 (1978). Press *et al.*, ACFPN: 222 (2000).

Tetrapeltis fragrans Wall. ex Lindl. In Edw., Bot. Regist. 18: sub t. 1522 (1832).

Epiphytic herbs with subcylindric, elongated, fusiform pseudobulbs arising from the apex of older one, sheathed. Leaves two, narrowly elliptic, narrowed at the base to the petiole. Inflorescence raceme, shorter than the leaves, pendulous. Floral bracts acute, caducous. Flowers white. Sepals subequal, oblong lanceolate. Petals equaling the sepals, linear, acute. Lip three lobed, linear-lanceolate, acute, lateral lobes overlapping each other above the column, shorter than the mid lobe, mid lobe linear-ovate, acute, pendulous. Column slender and winged. (Fig 11A)

Flowering time: October -January

Distribution: Found in Nepal, Sikkim, Darjeeling, Naga, Khasia Hills and extending further to Burma at elevation of 1300-2000 m.

Collection: Lele Bhanjyang, 7000ft, 27.10.1978, P. Pradhan 661 (KATH)

Specimens examined: Panchase Lekh, 2350m, 13.12.1973, D. P. Joshi and M. M. Amatya 73/1171, (KATH). Tinpani Bhanjyang, 7000ft. 18.11.1974, P. Pradhan 382, (KATH). Bhimbunk Lekh, Dhanding, 2000m, 2.12.1988, N. P. Manandhar 12751, (KATH). Mangsang Danda, 6000 ft. 4.10.1981, P. R. Shakya 6894, (KATH).

Anatomy

Epidermis in Surface View (Fig 11B)

Stomata are present on adaxial surface only. Epidermal cells are rectangular to polygonal in shape. Strands and interstrand regions are differentiated. Stomata are tetracytic. Subsidiary cells are distinguished from other epidermal cells being smaller in size. Size of stomata is 30 x 23.33 μm and the stomatal frequency is 108 per sq. mm.

Transverse Section of Leaf (Fig 11C)

Epidermis – Single layer of rectangular shaped cells. Abaxial cells are larger than adaxial cells. Cells are smaller in midrib region.

Hypodermis – Not distinguishable.

Mesophyll – Not differentiated into palisade and spongy cells. Six to seven layers of thin walled cells are present below the epidermis.

Vascular bundle – Shape of vascular bundle is oval. U-shaped fibre caps on the adaxial side and V-shaped on the abaxial side are present surrounding the conducting tissues. A single phloem patch is present towards the adaxial side which is ruptured in mature plant.

Transverse Section of Root (Fig 11D)

Velamen – It is composed of four layers of small cells.

Exodermis – Thick walled single layer of cells which are elongated to squarish in shape are present below the velamen layers. The cells are highly cutinized interrupted by rectangular passage cells.

Cortex – One inner and one outer layer of cortical cells are small celled, middle layer with large cells as in other species.

Endodermis – U-shaped thickening is present in the endodermal cells. Four to five lignified cells are alternating with one to two thin-walled cells at protoxylem poles.

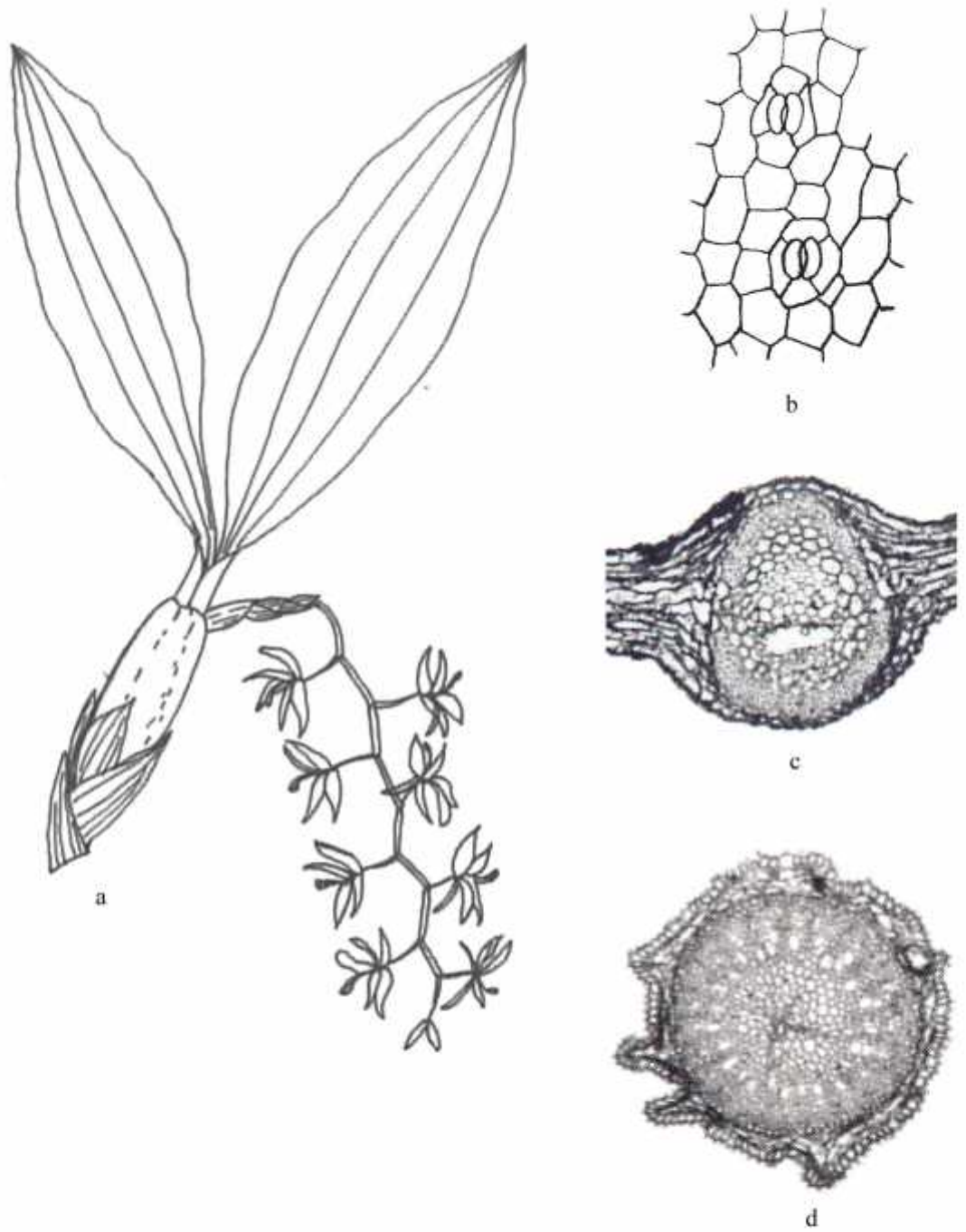


Fig 11: *Otochilus porrectus*; a. Habit sketch, b. Stomata, c. T. S. leaf through midrib, d. T. S. root

Vascular cylinder – 20 bundles of xylem and phloem archs are present alternating with each other embedded on the connective tissue.

Pith – Pith is of greater diameter in comparison to that in other species. Cells are small and round.

4.3. Genus *Panisea* (Lindl.) Steudel

The genus is epiphytic with small and tufted plants having small pseudobulbs. Leaves usually two, membranous and narrow elliptic. Scape is slender and few flowered, one in *P. uniflora*. Sepals are keeled with oblique base, free. Petals are subequal, free and also with oblique base, lanceolate. Lip narrow and as long as the sepals, clawed. Column slender, erect and slightly two winged above. The genus is very much similar to the *Coelogyne* but differ in having the clawed lip.

The genus *Panisea* was first proposed in 1830 as a section within *Coelogyne* by John Lindley in his genera and species of Orchidaceous Plants. In 1841, E.G. Steudal raised the section rank in his Nomenclator Botamincus. The genus comprises 7 species distributed in N. India, Nepal, Bhutan, and S.E. Asia. (Pearce and Cribb, 2002) The name is derived from Greek words “pan” meaning all and “iso” meaning equal referring to the similarity between the sepals and petals of the flower. Only two species are found in Nepal which are studied here.

1. *Panisea demissa* (D.Don.) Pfitz. in Engler, Pfl.-reich IV-50, IIB-7, Ht. 32:141, t. 49 (1907). Hara *et al.*, EFPN 1: 51 (1978). Press *et al.*, ACFPN: 222 (2000).

Dendrobium demissum D. Don, Prodr. Fl. Nepal.: 34 (1825).

Erect, epiphytic herbs with long creeping hairy roots. Pseudobulbs crowded, narrowly ovoid, fusiform, reticulately veined, translucent, partly enclosed in membranous basal sheaths. Leaves two, narrowly linear-oblong to narrowly elliptic-lanceolate, acute, membranous, tapering to the base into a short indistinct petiole, subsessile. Inflorescence pendulous raceme from the base of the pseudobulb, slightly decurved, five to six flowered, the base with membranous sheaths. Floral bract membranous, lanceolate, deciduous. Flowers white with brown streaks. Sepals keeled, unequal, the dorsal linear-

oblong, the laterals lanceolate, acute. Petals shorter, oblong, blunt. Lip narrowly lanceolate, subacute, three-nerved. Column brown. (Fig 12A)

Flowering time: October-February.

Distribution: This species is found in the Eastern Himalayas. In Nepal, it occurs in the subtropical and temperate zones of central and eastern Nepal at an altitude ranging from 1500-2400 m.

Collection: Panchase Danda, 2300m, 11.2.2002, Subedi, Chaudhari and Shakya, 1012, (TUCH).

Specimens examined: Chisapani, 7800ft, 21.7.1978, P. Pradhan 587, (KATH). Mansang Danda, 6800ft, 3.10.1981, P. Pradhan 6862, (KATH). Chitlang Phedi, 2300m, 10.1.1975, D.P. Joshi, Rajbhandari, Ghimire 75/217 (KATH). Doko Bhanjyang, 2400m, 16.3.1975, D.P. Joshi, K. R. Rajbhandari 75/646, (KATH).

Anatomy

Epidermis in Surface View (Fig 12B)

Stomata are present on adaxial surface only. Epidermal cells are polygonal in shape. Strands and interstrand regions are not differentiated. Stomata are anomocytic as the surrounding cells cannot be clearly distinguished as subsidiary cells from other epidermal cells although they are slightly smaller in size. Size of stomata is 28 x 22 μm and the frequency of stomata is 151 per sq. mm.

Transverse Section of Root (Fig 12C)

Velamen – Velamen consists of four layers of polygonal cells. The cells in all layers are almost of equal size.

Exodermis – Single layer of exodermal cells are somewhat thick walled and cutinized.

The cells are elongated and slightly larger than velamen cells. Passage cells are small and squarish in shape which are found alternating with few exodermal cells.

Cortex – Cortex is composed of one inner layer of small cells, one middle layer of large cells and one outer layer of small cells. The cells are oval to round in shape. Cells in the middle layer are larger than double the size of the cells of inner and outer layers.

Endodermis – U-shaped thickening is present on the single layer of endodermal cells.

Vascular cylinder – Eight bundles of xylem and phloem each are present alternating with each other. Below the phloem bundles, there are air spaces present.

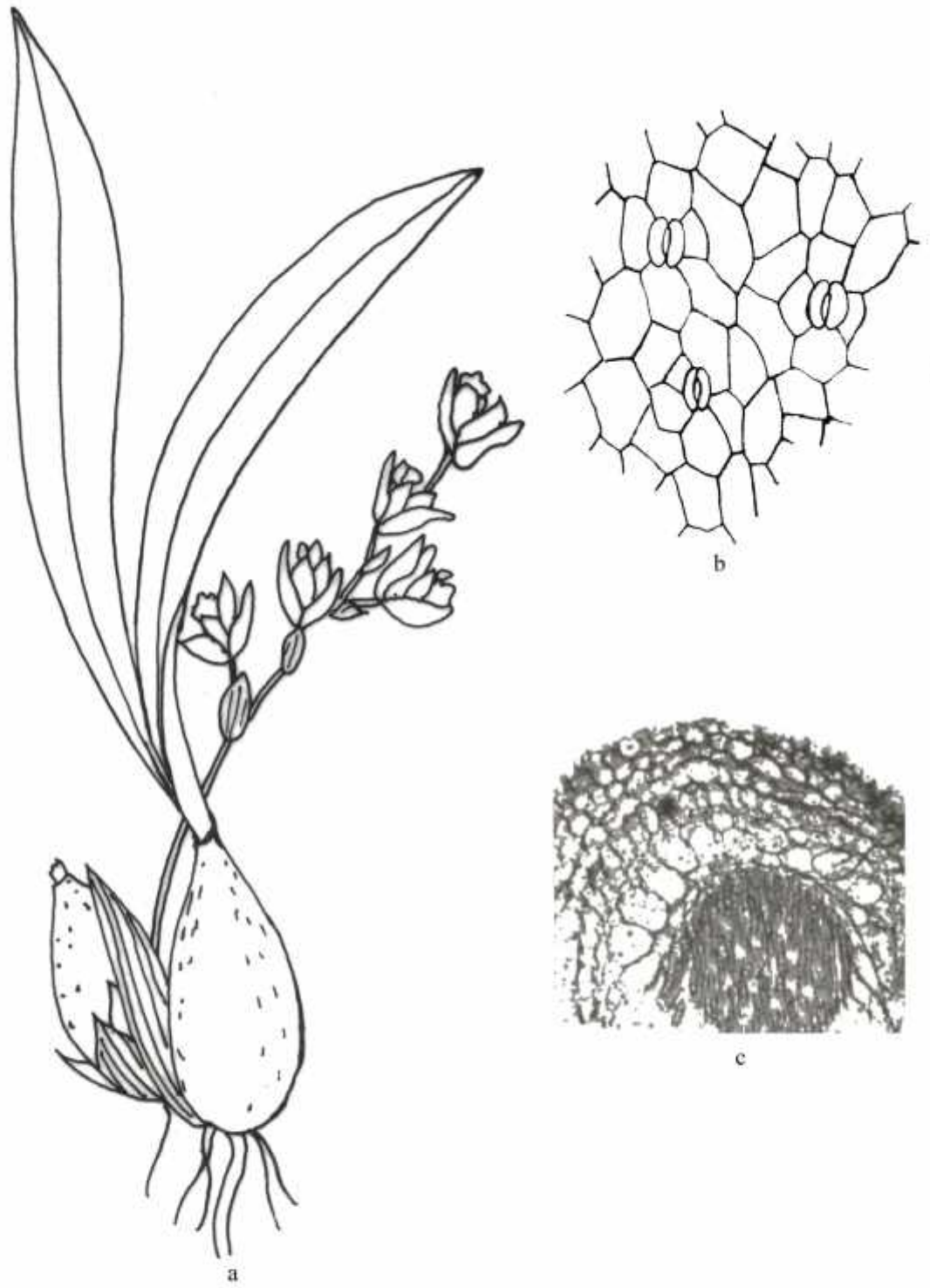


Fig 12: *Panisea demissa*; a. Habit sketch, b. Stomata, c. T. S. root

2. *Panisea uniflora* (Lindl.) Lindl., Fol. Orchid. 1, Panisea: 2 (1854). Seidenf. in Bot. Tidsskr. 70: 77, 12 (1975). Hara *et al.*, EFPN 1: 51 (1978). Press *et al.*, ACFPN: 222 (2000).

Coelogyne uniflora Lindl. [ex Wall., Numer. List; 54, n. 1966 (1829), nom. nud.]. Gen. Sp. Orchid. Pl.: 42 (1830). Hook. f., Fl. Brit. Ind. 5: 842 (1890). King & Pantl. in Ann. Roy. Bot. Gard. Calcutta 8: 138, t. 192 (1898). Banerji & Thapa in J. Bombay Nat. Hist. Soc. 66: 580 (1969).

Epiphytic herbs. Pseudobulbs densely crowded, broadly ovoid, with some loose fibrous sheaths at the base. Leaves two arising from the pseudobulb, linear to linear lanceolate, slightly narrowed at the base, sessile. Flower solitary on a short peduncle from the base of the pseudobulb, scape usually shorter than the pseudobulbs, erect. Floral bracts lanceolate, imbricating. Flower pale ochraceous. Sepals oblong-lanceolate, subacute, spreading. Petals broadly lanceolate, slightly shorter than the sepals. Lip oblong, three lobed, lateral lobes erect, narrow, acute, mid lobe ovate, blunt, with a very short claw at the base, pale brown with four elongated spots of dark brown. The disc between the lamellae with two slight ridges. Column winged, white, with two broad marginal lobes. (Fig 13A)

Flowering time: April-June.

Distribution: This species is distributed in Nepal, India, Bhutan and Myanmar. It occurs in the subtropical and temperate zones of central and east Nepal at an altitude of 1000-2300m.

Collection: Phulchoki, 7500 ft, 19.4.1978, P. Pradhan 405 (KATH).

Specimens examined: Godak, Ilam, 3500 ft, 9.6.1978, P. Pradhan 491, (KATH). Yaru-Jagat, Gorkha, 1300m, 25.5.1983, P. R. Shakya, M. K. Adhikari, M. N. Subedi 7621, (KATH).

Anatomy

Epidermis in Surface View (Fig 13B)

Stomata are present on adaxial surface only. Epidermal cells are rectangular to polygonal in shape. Strands and interstrand regions are not differentiated. Stomata are anomocytic to tetracytic. Subsidiary cells in few stomata are clearly distinguishable being small in size and also different in shape whereas in other, the surrounding cells are similar

to other epidermal cells. Stomata is 25 x 22 μm in size and its frequency is 197 per sq. mm.

Transverse Section of Leaf (Fig 13C)

Epidermis – Single layer of cells, squarish in shape are present. Abaxial cells are comparatively larger than adaxial cells. Trichomes are absent on both the surfaces.

Hypodermis – Absent.

Mesophyll – Not differentiated into palisade and spongy cells. The cells are oval to spherical in shape.

Vascular bundle – Large midrib vascular bundle is oval in shape. Fibre cap is very much distinguished and dense towards the adaxial side in comparison to the abaxial side. A single phloem patch is present towards the adaxial side and xylem lies somewhat at the center embedded on the connective tissue.

Transverse Section of Root (Fig 13D)

Velamen – It is four to five layers thick consisting of almost equal sized cells.

Exodermis – It is composed of single layer of thick walled small and large cells.

Cortex – One middle layer of large cells with one outer layer and one inner layer of small thin walled cells are present in the cortical region.

Endodermis – U-shaped thickening is present on the inner and radial walls of endodermal cells. Four to five lignified cells are interrupted by thin-walled cells at protoxylem poles.

Vascular cylinder – 10 strands of xylem and phloem are present alternating with each other embedded on the connective tissue.

Pith – Pith is parenchymatous with small and rounded thin walled cells.

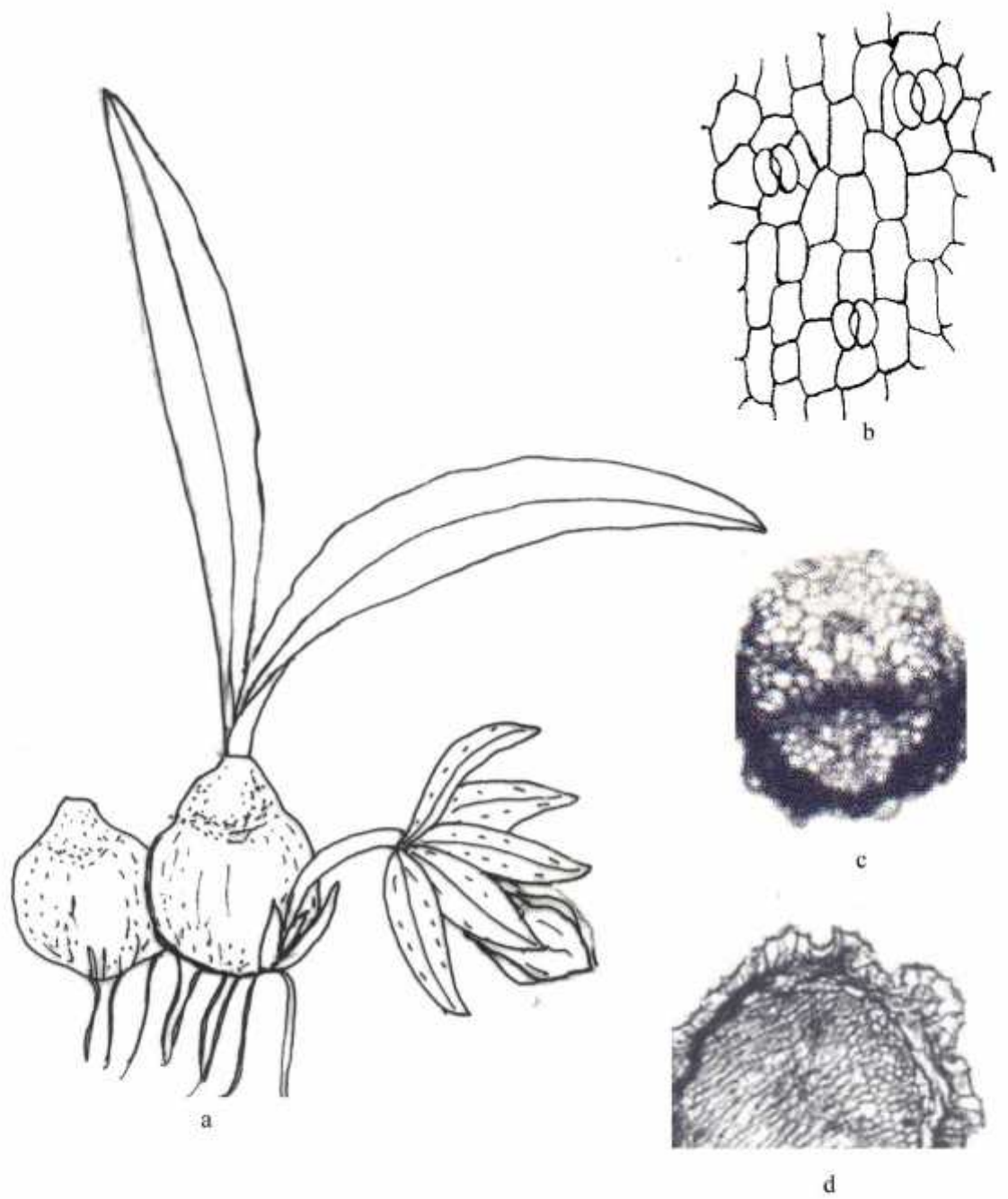


Fig 13: *Panisea uniflora*; a. Stomata, b. Midrib vascular bundle, c. T. S. root

4.4. Genus *Pholidota* Lindl. ex Hook.

The genus is epiphytic with one or two leaves and flowers in long pendulous racemes, the rachis of the racemes is flexuous. The flowers are small and bracteate. Bracts are stiff and distichous. Column is very short and with wide wings.

The genus *Pholidota* was established in 1825 by W. J. Hooker in his Exotic Flora. The genus comprises 29 species distributed in tropical SE Asia, the Malay, Archipelago, Australia, and Pacific Islands. (Pearce and Cribb, 2002) The name was derived from the Greek word “pholidotos” meaning scaly covering referring to the large scales or sheaths surrounding the pseudobulbs and also the base of the inflorescence is covered by bracts. Only four species are studied here.

1. *Pholidota articulata* Lindl. [ex Wall., Numer. List: 55, n. 1992 (1829), nom. nud.], Gen. Sp. Orchid. Pl.: 38 (1830). Hook. f., Fl. Brit. Ind. 5:844 (1890). King & Pantl. in Ann. Roy. Bot. Gard. Calcutta 8: 146, t. 205 (1898). Banerji & Thapa in J. Bombay Nat. Hist. Soc. 68: 664, t. 1 (1972). Hara *et al.*, EFPN 1: 53 (1978). Press *et al.*, ACFPN: 223 (2000).

Epiphytic herbs with jointed branched stem. Internodes furrowed with few scarious sheaths at the nodes. Leaves in pairs, elliptic-lanceolate, thickly membranous. Inflorescence drooping raceme, as long as the leaves, densely flowered, originating from the apex of young pseudobulbs from between the leaves. Floral bracts pale brown, rhomboid-ovate, deciduous when the flowers expand. Flowers off-white. Sepals subequal, dorsal sepal sub-orbicular, lateral sepals ovate, keeled. Petals ovate-orbicular, blunt. Lip as long as the sepals, broadly cymbiform, with broad oblong bilobulate middle lobe, disc with five bold lamellae. Column slightly winged at the apex, rostellum large. (Fig 14A)

Flowering time: April-July.

Distribution: This species is found in India (Himalaya) and Nepal. In Nepal, it occurs as an epiphytic herb in the subtropical and temperate zones at 500-2300 m altitude.

Collection: Letang, Morang, 500m, 8.6.2003, D.M. Bajracharya, 598 (ASCOL)

Specimens examined: Khorlak (gorkha district), 24.5.1983, Shakya, Adhikari and Subedi, 7612 (KATH); Hanspokhari, Ilam 12.6.1974, Pradhan, Amatya and Shrestha 74/260 (KATH); Chitray 11.5.1978, P. Pradhan, 435 (KATH).

Anatomy

Epidermis in Surface View (Fig 14B)

Stomata are present on adaxial surface only. Epidermal cells are polygonal in shape. Strands and interstrand regions are differentiated. Stomata are tetracytic. Four subsidiary cells of the stomata are clearly distinguishable from other epidermal cells. Stomata is 39 x 26 μm in size and the frequency of stomata is 92 per sq. mm.

Transverse Section of Leaf (Fig 14C)

Epidermis – Single layer of cells are squarish or rectangular to barrel shaped. Abaxial cells are larger than adaxial cells. Trichomes are absent on both the surfaces. Epidermal cells on the midrib region are smaller than those on the laminar region.

Hypodermis – Hypodermis is absent.

Mesophyll – Not differentiated into palisade and spongy cells. The cells are oval to spherical in shape. Lower cells are smaller than the upper cells. The cells are chlorophyllous.

Vascular bundle – Arranged in a single series with large midrib vascular bundle which is oval in shape. Sclerenchymatous fibre sheaths are present all around the bundle. Fibre sheath is dense towards the phloem end than xylem end.

Transverse Section of Root (Fig 14D)

Velamen – Velamen is five to seven layers in thickness consisting of spherical to oval shaped small sized cells.

Exodermis – It is composed of single layer of thick-walled anticlinally arranged small cells. Few slightly large square-shaped to rectangular cells interrupt the layer.

Cortex – Three to four layers of cortical cells are present below the exodermis. The outermost single layer is composed of very small cells below which lies larger thin walled rounded cells. These cells are more than three times larger than the outer cells. The innermost one or two layers of cortical cells are larger than the outer cells but smaller than the middle layer.

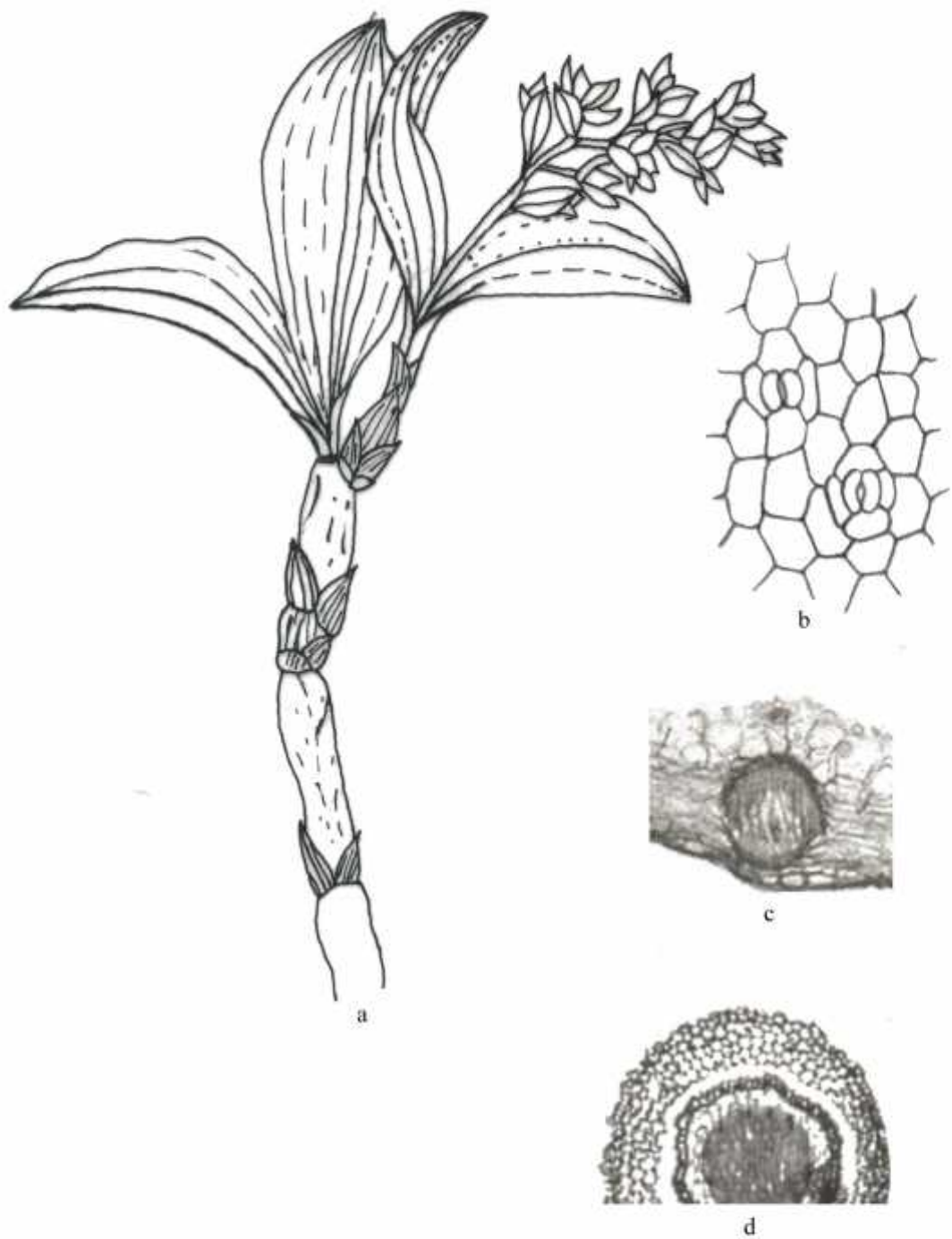


Fig 14: *Pholidota articulata*; a. Habit sketch, b. Stomata, c. T. S. leaf, d. T. S root

Endodermis – There is U-shaped thickening on the inner and radial walls of endodermal cells. Four to five lignified cells are interrupted by unlignified cells towards the protoxylem poles.

Vascular cylinder – Xylem and phloem strands are present alternating with each other. 14 strands of xylem and phloem each are present embedded on the ground tissue.

Pith – Pith is parenchymatous with small and rounded thin walled cells. Central cells are larger than other ground cells.

2. Pholidota imbricata Hook., Exot. Fl. 2: t. 138 (Jan. 1825)/ Hook. f., Fl. Brit. Ind. 5: 845 (1890). King & Pantl. in Ann. Roy. Bot. Gard. Calcutta 8: 144, t. 201 (1898). Banerji & Thapa in J. Bombay Nat. Hist. Soc. 68:664, t. 2 (1972). Hara *et al.*, EFPN 1: 53 (1978). Press *et al.*, ACFPN: 223 (2000).

Cymbidium imbricatum Roxb. [Hort. Bengal.: 63 (1814), nom. nud.], Fl. Ind. Ed. 2,3: 460 (1832).

Epiphytic herbs with aggregated, ovate cylindrical, furrowed pseudobulbs, partly enclosed in conspicuous fibrous sheaths. Stem creeping, rhizomatous. Leaves solitary, elliptic-lanceolate, petiole stout. Inflorescence, from the base of the pseudobulb covered by few imbricating sheaths, densely flowered, drooping racemes. Flowers enveloped in a large floral bracts, pale brown with dark brown dots, persistent, many nerved. Flowers pale white, distichous. Dorsal sepals orbicular, lateral sepals boat shaped, joined at the base with keel. Petals linear oblong. Lip three lobed, side lobes large rounded, terminal lobe divided at the apex into two small lobules. Column more or less circular, broad towards the apex and winged. (Fig 15A)

Flowering time: May-August.

Distribution: This species is found in India and Nepal and distributed eastwards to South East Asia, South China to Australia.

Collection: Bhedetar, 1600m, 1.6.2003, D. M. Bajracharya, 507 (ASCOL)

Specimens examined: Sera, 900m, 16.7.2002, Subedi, Chaudhari and Shakya 965, (TUCH). Telok, 5500 ft, 28.6.1969, T. B. Shrestha 15958, (KATH). Ghandruk, Kaski 2100m, 29.6.1986, N. P. Manandhar, L. P. Katel 10997 (KATH). Ghasa, Mustang, 1950m, 16.7.1983, K. R. Rajbhandari 7825 (KATH). Ranital Danda, Sankhuwasabha, 1740m, 19.6.1994, P. R. Shakya, K. K. Dangol 10201, (KATH).

Anatomy

Epidermis in Surface View (Fig 15B)

Stomata are present on adaxial surface only. Epidermal cells are polygonal to elongated in shape. Strands and interstrand regions are differentiated with narrowly elongated cells in the interstrand region. Stomata are tetracytic. Subsidiary cells are clearly distinguishable from other epidermal cells being small and narrow. Stomatal size is $48 \times 30 \mu\text{m}$ and the stomatal frequency is 71 per sq. mm.

Transverse Section of Leaf (Fig 15C)

Epidermis – It is composed of single layer of small cells. The cells are squarish to rectangular in shape. The cells on the abaxial surface are slightly larger than the cells of the adaxial cells. Epidermal cells on the midrib region are even smaller. Trichomes are also absent on both the surfaces.

Hypodermis – Hypodermis is absent.

Mesophyll – Mesophyll is not differentiated into palisade and spongy cells but the size of the cells are different in different regions. Cells on the upper surface are more than three times larger than the cells on the lower surface of the leaf. The cells are four to six layers and are round to oval in shape.

Vascular bundle – A single series of vascular bundle is present throughout the leaf lamina with large bundle at the midrib. The shape of the midrib vascular bundle is flask shaped or slightly elongated. Fibre sheaths are present on both the upper and lower surfaces. Fibre sheath is dense towards the xylem end than the phloem end. One large phloem patch is present on the lower surface of the leaf.

Transverse Section of Root (Fig 15D)

Velamen – Four to five layers of round to oval shaped cells are present on the velamen.

Exodermis – A single layer of thick walled cutinized square shaped cells are present on the outer layer. Thin walled passage cells interrupt the exodermis in frequent intervals.

Cortex – There is altogether four to five layerd of cells in the cortex. The outermost single layer of cortical cells are very small is size. Two layers of central cells are large and oval whereas the inner one to two layers of cells are again small in size.

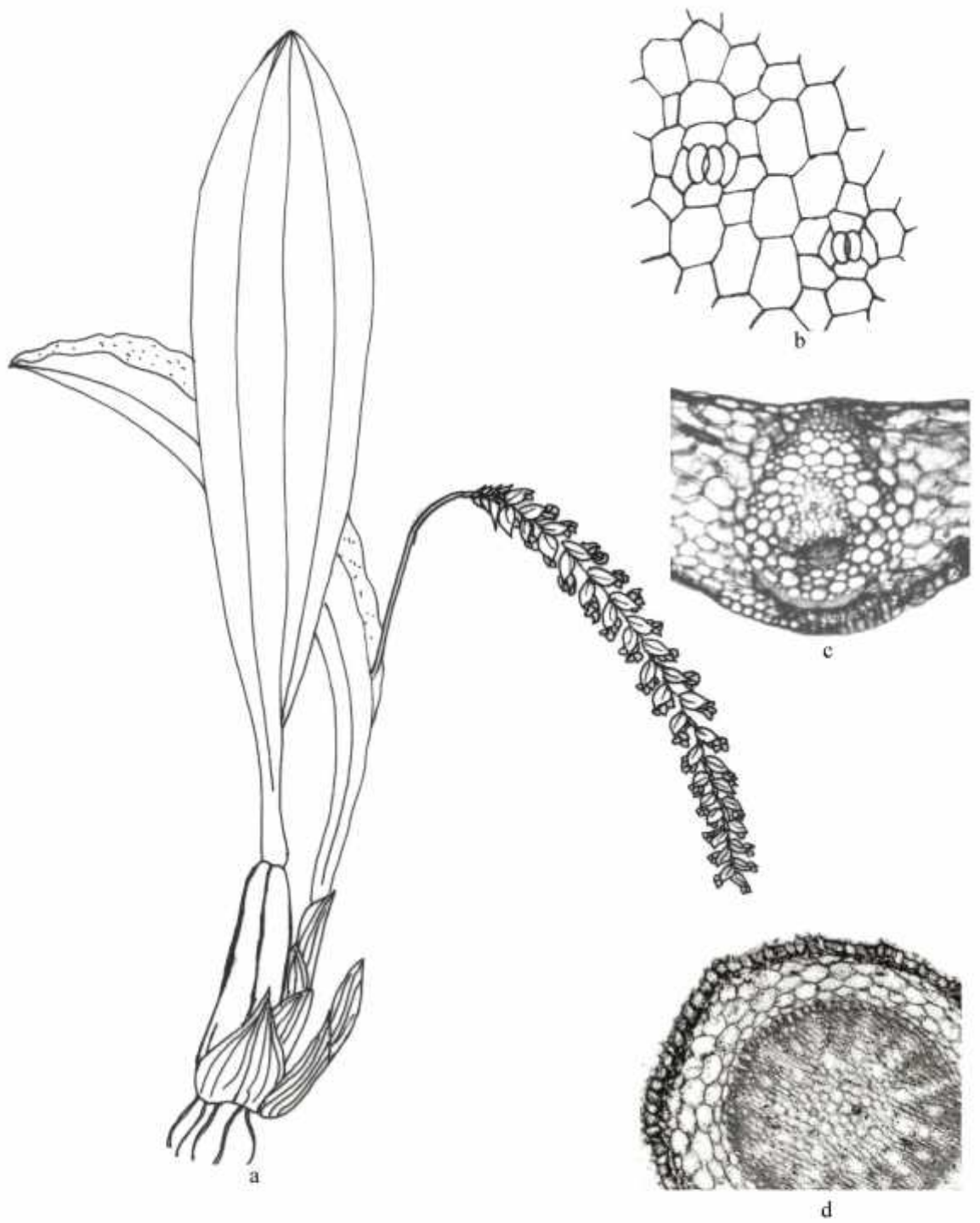


Fig 15: *Pholidota imbricata*; a. Habit sketch, b. Stomata, c. T. S. leaf through midrib, d. T. S. root

Endodermis – Endodermal cells are also almost squarish in shape. These cells have U-shaped thickening being lignified on the inner and radial walls. Few lignified cells are interrupted by one to two unligified cells towards the protoxylem poles.

Vascular cylinder – There are 11 strands of xylem and phloem each. Xylem and phloem strands are present alternating with each other as in other species.

Pith – Pith cells are small, round and parenchymatous at the center occupying a large area at the center.

3. Pholidota protracta Hook. f. in Hook., Icon. Pl. 19: t. 1877 (1889); Fl. Brit. Ind. 5: 845 (1890). King & Pantl. in Ann. Roy. Bot. Gard. Calcutta 8: 146, t. 203 (1898). Banerji & Thapa in J. Bombay Nat. Hist. Soc. 68: 665 (1972). Hara *et al.*, EFPN 1: 53 (1978). Press *et al.*, ACFPN: 223 (2000).

Epiphytic herbs. Stems flexuous slender with scarious sheaths. Pseudobulbs distant, elongated, fusiform, bracteate at the base. Leaves two at the apex, elliptic lanceolate, membranous, shortly petioled. Inflorescence several, racemes, arising from the joints of rhizome, much shorter than leaves, few-flowered. Floral bracts ovate. Flowers greenish or white. Sepals subequal, broadly ovate. Petals broadly elliptic, shorter than the sepals. Lip three lobed, lateral lobes narrow, mid lobe broadly oblong, slightly notched at the apex. Column broadly winged. (Fig 16A)

Flowering time: October-December

Distribution: 1500-2500 m.

Collection: Panchase forest, Kaski, 2300m, 15.8.1999, Subedi, A. 251 (TUCH).

Specimens examined: Thulokhorka, 2100m, 15.6.1975, Joshi and Bhatta, 2697 (KATH). Phulchowki, 2120m, 5.10.1967, P. Pradhan, 7461 (KATH). Pothana, KAski, 2000m, 8.8.1983, RAjbhandari, 7349 (KATH); Panchase forest, Kaski, 2300m, 15.8.1999, Subedi, A., 251 (TUCH).

Anatomy

Epidermis in Surface View (Fig 16B)

Stomata are present on adaxial surface only. Epidermal cells on surface view are small squarish to large rectangular and polygonal in shape and size. Strands and interstrand regions are differentiated. Stomata are tetracytic. Subsidiary cells are clearly

distinguishable being small in size and also different in shape. Stomatal size is 38 x 33 μm and stomatal frequency is 59 per sq. mm.

Transverse Section of Leaf (Fig 16C)

Epidermis – Squarish to rectangular in shape with abaxial cells are larger than adaxial cells.

Hypodermis – Absent.

Mesophyll – Not differentiated into palisade and spongy cells. Cells are round to oval in shape.

Vascular bundle – A single series of vascular bundles are present throughout the lamina. Two to three small bundles are alternating with a large vascular bundle but midrib vascular bundle is the largest of all. Shape of the midrib vascular bundle is elongated to flask shaped as in *P. imbricata*. Fibre caps are present in both the phloem and xylem end but dense in phloem end. Only one phloem patch is present on the adaxial surface.

Transverse Section of Root (Fig 16D)

Velamen – Root hairs are present on this species. Velamen is three to four layers thick consisting of small cells. The innermost layer is comparatively larger.

Exodermis – It is composed of single layer of thick walled small hexagonal to squarish cells.

Cortex – There are only three layers of cortical cells. One outer layer is small celled. Middle layer is large celled and the innermost layer is also small celled. The large cells in the middle layer are round in shape and the smaller cells on the outer and inner layers are round to oval in shape.

Endodermis – There is presence of U-shaped thickening in small endodermal cells.

Vascular cylinder – Ten strands of xylem and phloem are present alternating with each other. Vascular strands are embedded on the ground tissue.

Pith – Pith occupies a small area at the center. It is made of small parenchymatous cells.

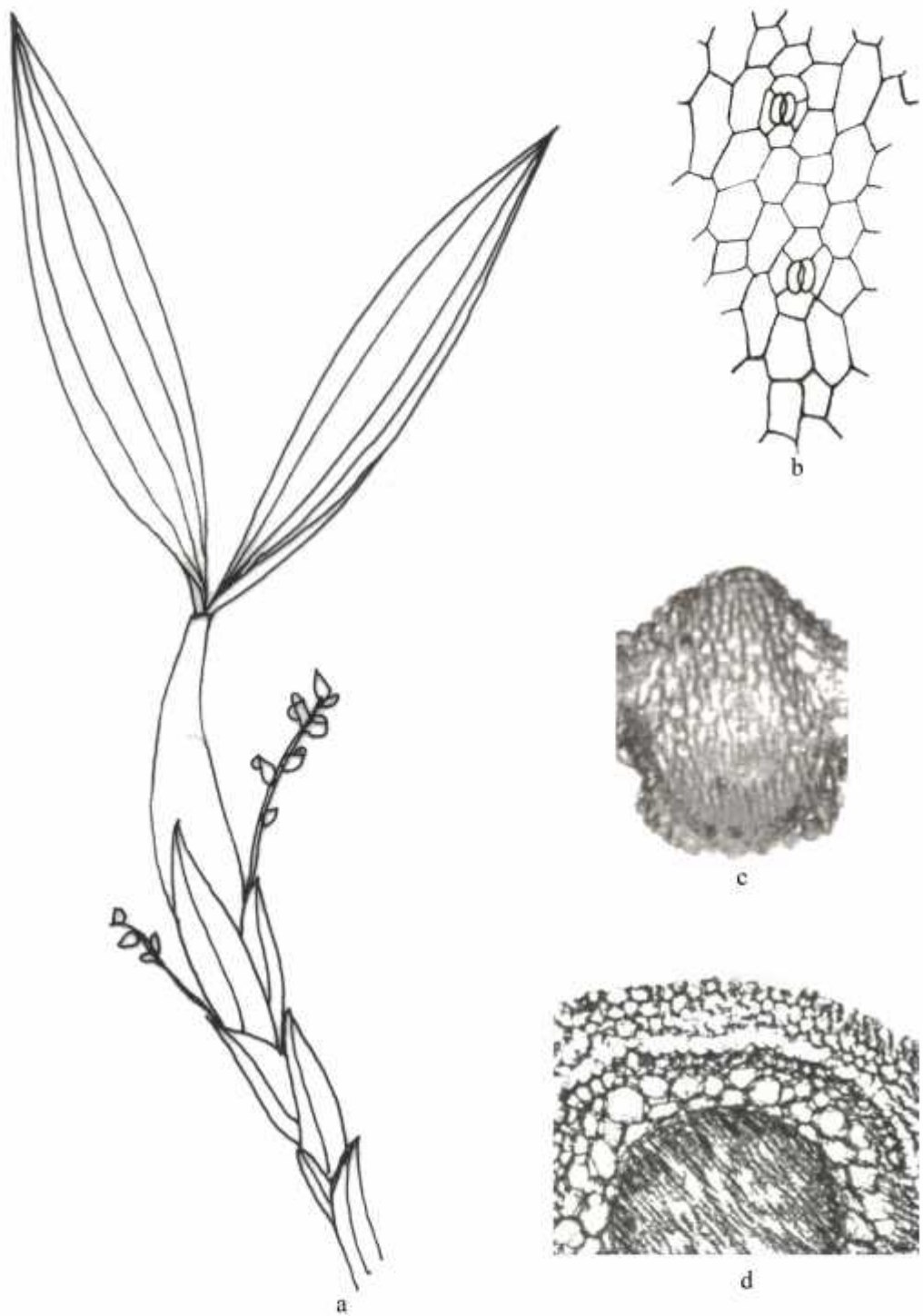


Fig 16: *Pholidota protracta*; a. Habit sketch, b. Stomata, c. Midrib Vascular bundle, d. T. S. root

4. *Pholidota recurva* Lindl., Gen. Sp. Orchid. Pl.: 37 (1830). Hook. f. in Hook., Icon. Pl. 19: t. 1878 (1889); Fl. Brit. India 5: 844 (1890). King & Pantl. in Ann. Roy. Bot. Gard. Calcutta 8: 147, t. 206 (1898). Hara *et al.*, EFPN 1: 53 (1978). Press *et al.*, ACFPN: 223 (2000).

Coelogyne recurva (Lindl.) Reichenb. f. in Walp., Ann. Bot. Syst. 6: 237 (1861).

Epiphytic herbs with internodes pseudobulb like. Nodes enclosed with few scarious sheaths. Leaves two, membranous, lanceolate-elliptic, petioled. Inflorescence raceme, as long as the leaves arising from between the leaves. Floral bracts fan shaped, imbricated, persistent. Flowers pale brown. Dorsal sepal orbicular, concave, lateral sepals broadly ovate. Petals ovate-oblong, spreading. Lip three lobed, terminal lobe two-auricled, five short ridges at the bottom of the lip on the upper surface. (Fig 17A)

Flowering time: August-September

Distribution: Nepal, Sikkim, Darjeeling, Bhutan at 700-1300 m.

Collection: Makawanpur, 1600m, 24.2.1992, K. J. White, 22 (KATH)

Anatomy

Epidermis in Surface View (Fig 17B)

Stomata are present on adaxial surface only. Epidermal cells are rectangular to polygonal in shape. Strands and interstrand regions are differentiated. Stomata are tetracytic but the subsidiary cells are not very much different from other epidermal cells. The subsidiary cells are larger, more or less equal to other epidermal cells. Size of stomata is 30 x 31 μm with the frequency of 74 per sq. mm.

Transverse Section of Leaf (Fig 17C)

Epidermis – Single layer of square to rectangular cells are present. Abaxial cells are larger than adaxial cells.

Hypodermis – Absent.

Mesophyll – Not differentiated into palisade and spongy cells. The cells are oval to spherical in shape.

Vascular bundle – Large midrib vascular bundle is slightly elongated cylindrical in shape. Fibre sheath is dense towards phloem end. Single patch of phloem is present on the adaxial side. Xylem lies at the abaxial side embedded on the connective tissue. Fibre cap is not dense towards the xylem end.

Transverse Section of Root (Fig 17D)

Velamen – Three to four layers of round or oval shaped velamen cells are present.

Exodermis – It is composed of single layer of thick walled small cells. The exodermal cells are thick on the radial wall and inner wall.

Cortex – Three to four layers of cortical cells are present below the exodermis. The cells are round to oval in shape. Inner one layer is composed of small cells.

Endodermis – Endodermal cells are highly lignified. U-shaped thickening is present on the inner and radial walls of endodermal cells. Four to six lignified cells are interrupted by one to two thin-walled cells at protoxylem poles.

Vascular cylinder – 15 strands of xylem and phloem each are present alternating with each other and embedded on the connective tissue.

Pith – Pith is parenchymatous with small and rounded thin walled cells. Pith occupies large space at the center.

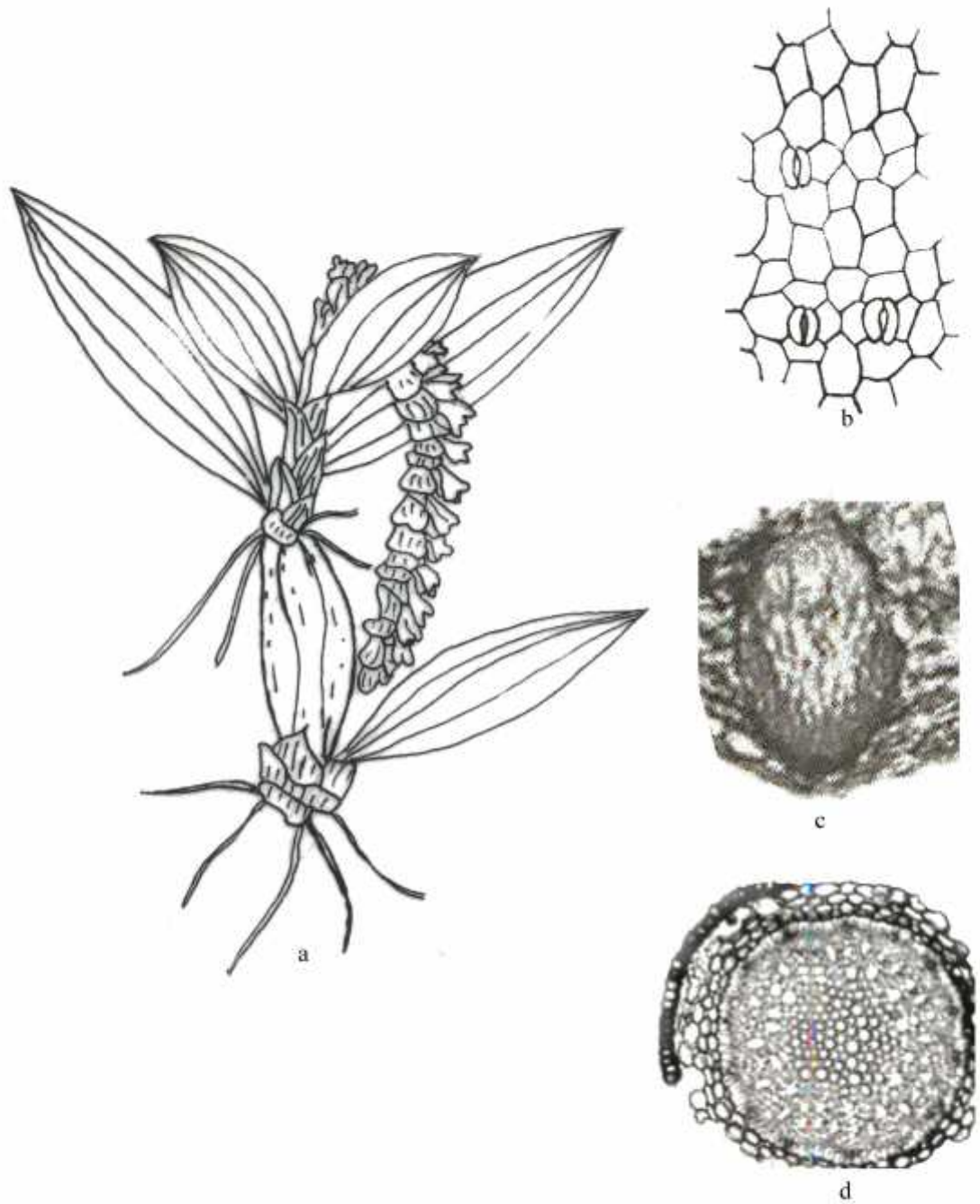


Fig 17: *Pholidota recurva*; a. Habit sketch, b. Stomata, c. T. S. leaf through midrib, d. T. S. root

4.5 Genus *Pleione* D. Don

The genus is closely related to *Coelogyne* but is unique in habit being cluster-forming mostly rather dwarf plants found growing in the ground or on rocks of trees with peculiar flask-shaped irregular pseudobulbs, deciduous folded leaves and solitary, often proportionately very large flowers which arise concurrently with the new growth. Flowers arise from the base of the pseudobulbs. Sepals valvate. Lip with obscure lateral lobes or they are absent, margin serrate or denticulate, apically lobed.

The genus *Pleione* was established in 1925 by David Don in his *Prpdromus Flora Nepalensis*. The genus comprises about 16 species distributed from Central Nepal eastward to Northeast India, Myanmar, China, Taiwan, Thailand, and Laos. (Pearce and Cribb, 2002) The genus was named after “Pleione” the mother of Pleiades (the 7 daughters of Atlas) in Greek mythology who transformed into a cluster of stars by Zeus. Only 3 species are studied here.

1. *Pleione hookeriana* (Lindl.) J. Moore in Williams, *Orchid-Grow. Man. Ed. 6: 548* (1885). Banerji & Thapa in *J. Bombay Nat. Hist. Soc. 66:582, t. 9* (1969). Hara *et al.*, *EFPN 1: 54* (1978). Press *et al.*, *ACFPN: 224* (2000).

Coelogyne hookeriana Lindl., *Fol. Orchid., Coelogyne: 14* (1854). Hook. f. in *Curtis, Bot. Mag. 104: t. 6388* (1878); *Fl. Brit. Ind. 5: 842* (1890). King & Pantl. in *Ann. Roy. Bot. Gard. Calcutta 8: 139, t. 193* (1898).

Epiphytic or lithophytic herbs with sheathed smooth conical pseudobulbs. Leaf solitary, persistent arising from the base of the adult pseudobulb, elliptic, acute, membranous, tapering to the petiole, enclosed in tubular imbricate sheaths. Inflorescence sheathed. Floral bracts broadly obovate-elliptic, obtuse, persistent. Flowers solitary, violet white. Dorsal sepals elliptic oblong, spreading, blunt, the lateral sepals oblong, acute, petals spreading, as long as the sepals, oblanceolate, narrower. Lip three-lobed, reniform, with ciliated margin, lateral lobes rounded, mid lobe emarginated with six to seven ciliate keels arising from the base to apex. Column long curved, broadly winged in its upper half. (Fig 18A)

Flowering time: May-June.

Distribution: This species is distributed in Nepal, India (Sikkim, Assam), Bhutan and China (Southeast Tibet) at 2300-3700 m altitude.

Collection: Dharaban, 1000m, 6.6.2003, D. M. Bajracharya, 557 (ASCOL)

Specimens examined: Maily, Okhaldhunga, 1900m, 28.5.1979, N. P. Manandhar and M. K. Adhikari 1842, (KATH). Helambu, 1987m, 5.6.1972, John nad Naomi Bishop WF 9, (KATH). Phulchoki, 2000m, 10.6.1969, H. Kanai 673241, (KATH). Tarapati, Sindhupalchok, 3300m, 24.5.1994, N. P. Manandhar 134-93, (KATH).

Anatomy

Epidermis in Surface View (Fig 18B)

Stomata are present on adaxial surface only. Epidermal cells vary in their shape and size. The cells are small to large in size. Cells are polygonal to elongated in shape. Stomata are anomocytic without distinct surrounding cells. The subsidiary cells are not differentiated from other epidermal cells. Size of stomata is 31 x 29 μm and the stomatal frequency is 71 per sq. mm.

Transverse Section of Root (Fig 18C)

Velamen – Velamen is composed of two to three layers of small rectangular cells.

Exodermis – It is a single layer of large square to rectangular cells. Exodermal cells are not thick walled in this species.

Cortex – There are five to six layers of cortical cells out of which the outmost layer is composed of small cells. The other cells are larger in size. There is no differentiation of innermost layer in cortex. Cells are round to oval in shape and thin walled.

Endodermis – A single layer of barrel shaped endodermal cells are present below the cortex. The cells are thick walled as in other species but there is absence of U-shaped thickening.

Vascular cylinder – Eight strands of xylem and eight strands of phloem are present alternating with each other.

Pith – The central part of root is pith composed of small parenchymatous cells. The cells are polygonal or round without intercellular spaces.

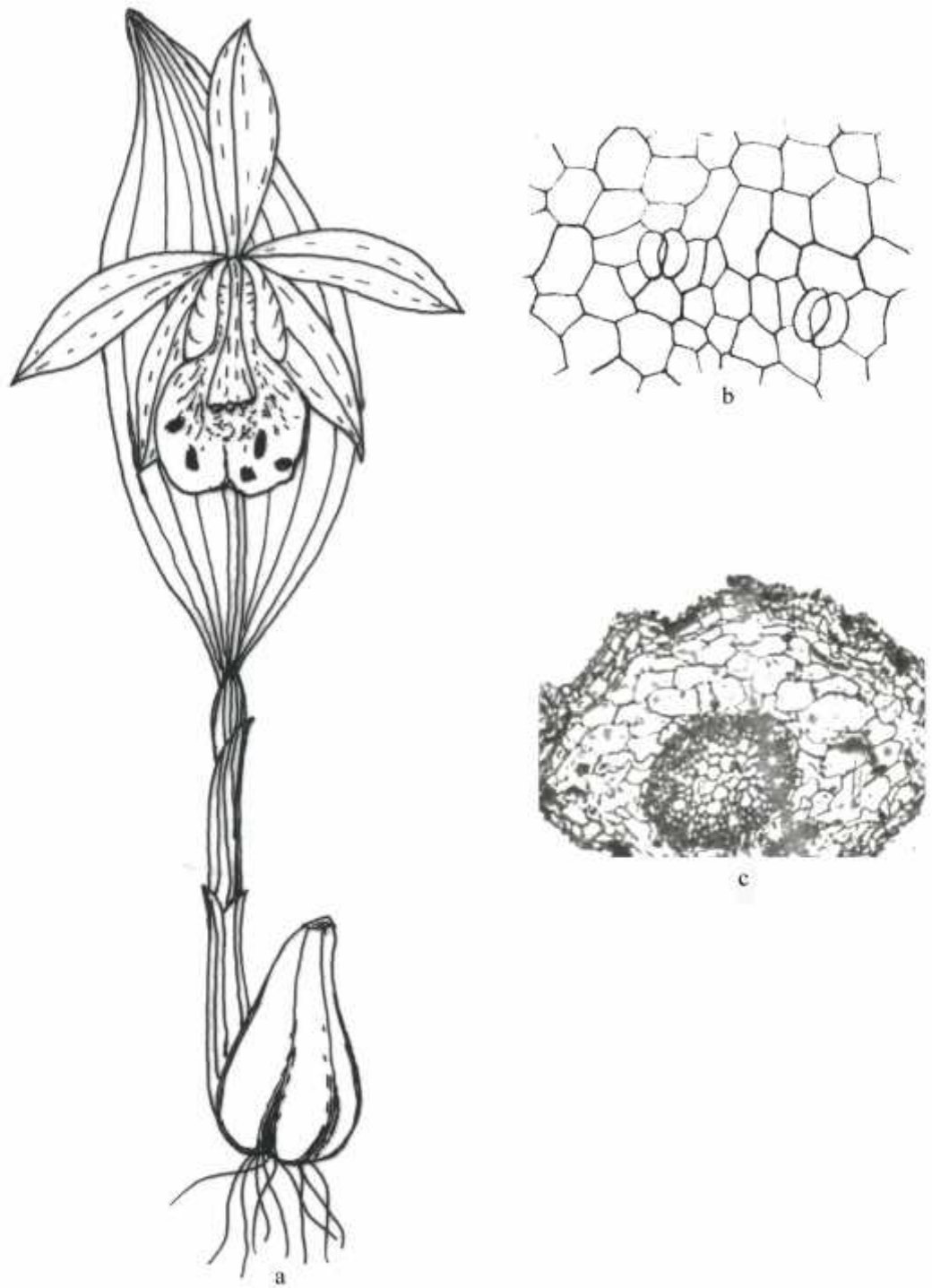


Fig 18: *Pleione hookeriana*; a. Habit sketch, b. Stomata, c. T. S. root

2. *Pleione humilis* (Smith) D. Don, Prodr. Fl. Nepal.: 37 (1825). anerji & Thapa in J. Bombay Nat. Hist. Soc. 66: 582, t. 10 (1969). Hara *et al.*, EFPN 1: 55 (1978). Press *et al.*, ACFPN: 224 (2000).

Epidendrum humile Smith, Exot. Bot. 2:75, t. 98 (1806).

Coelogyne humile (Smith) Lindl., Collect. Bot.: sub t. 37 (1825). Hook. f. in Curtis. Bot. MAg. 93: t. 5674 (1867); Fl. Brit. Ind. 5:840 (1890); op. cit. 6:194 (1890). King & Pantl. in Ann. Roy. Bot. Gard. Calcutta 8: 139, t. 194 (1898).

Epiphytic or lithophytic herbs with ovoid conical pseudobulbs, partly enclosed in fibrous sheaths. Leaves solitary, narrowly elliptic, membranous, tapering to the sessile base, present only when plant is not flowering. Inflorescence short appearing before the leaves, one flowered. Flowers white, on a sheathed scape, erect or nodding. Sepals subequal, narrowly oblong, acute. Petals narrowly oblanceolate, obtuse. Lip elliptical without lobes, concave basally but expanding into a wide apical portion, dentate and fimbriate, apex bilobulate. Disc with many fimbriate lamellae. Column long winged. (Fig 19A)

Flowering time: February-March.

Distribution: This species is distributed in Nepal, India (Sikkim, Darjeeling), Khasia hills, upper Burma at 2300-2800 m.

Collection: Daman, 2400m, 15.5.2003, D. M. Bajracharya, 486 (ASCOL)

Specimens examined: Simbhanjyang, Daman, 2400m, 668 (TUCH), M. Shrestha, 182c (TUCH). PAnchase, Danda, 2500m, 4.2.2002, Subedi, 668 (TUCH). Chitray, Dhankuta, 2350m, 14.11.1978, P PRadhan, N. P. Manandhar, N. Amatya 684, (KATH). Sankhuwaasbha, 2400m, 28.3.1991, P. R. Shakya 9644, (KATH). Doko Bhanjyang, 2400m, 16.3.1975, D. P. Joshi and K. R. Rajbhandari 75/640, (KATH).

Anatomy

Epidermis in Surface View (Fig 19B)

Stomata are present on adaxial surface only. Epidermal cells are elongated polygonal in shape. Strands and interstrand regions are differentiated. Stomata are anomocytic. Subsidiary cells are slightly smaller than the other epidermal cells but not distinguishable. Stomatal size is 30 x 28 μm and the stomatal frequency is 66 per sq. mm.

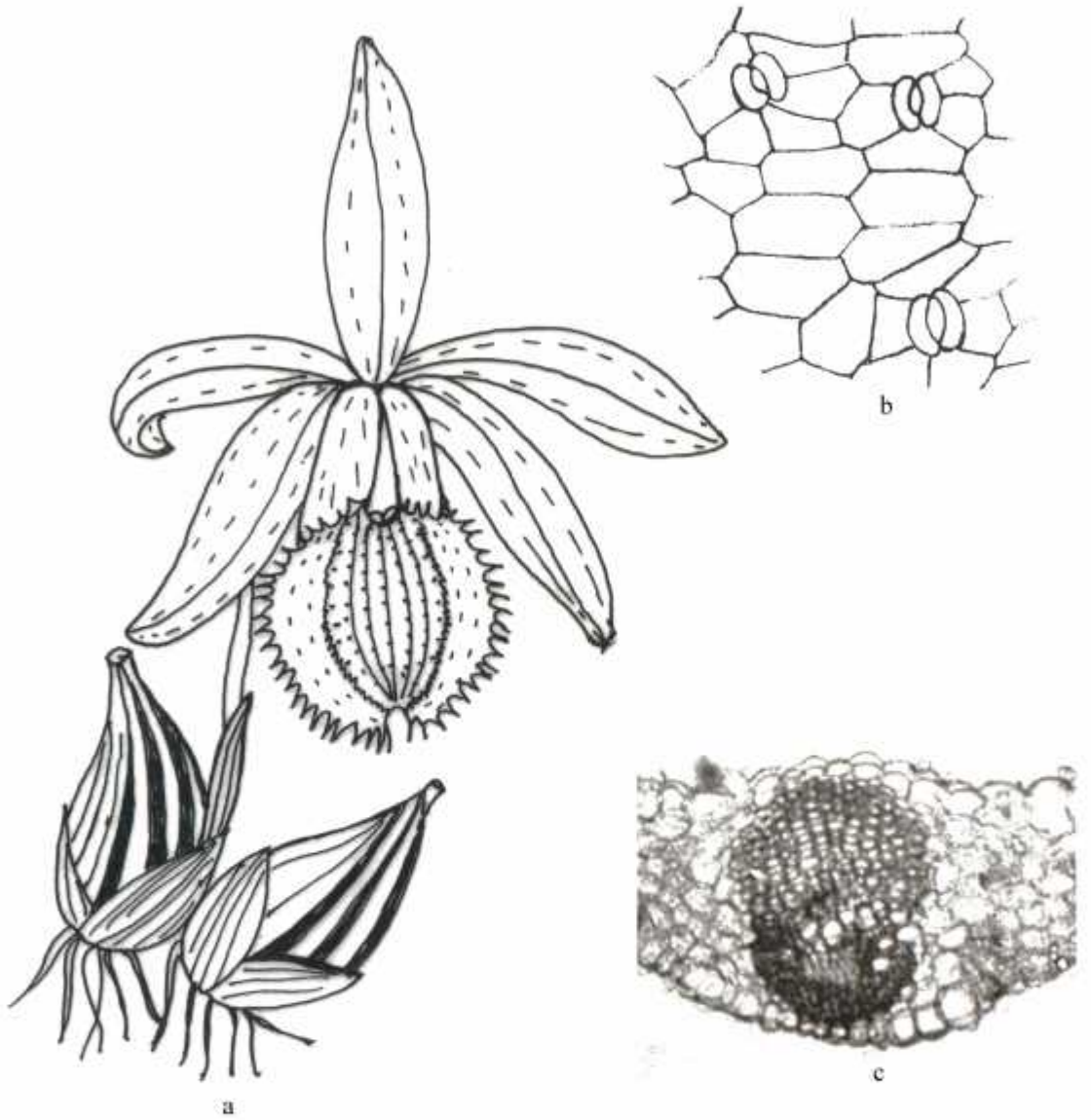


Fig 19: *Pleione humilis*; a . Habit sketch, b. Stomata, c. T. S. leaf through midrib

Transverse Section of Leaf (Fig 19C)

Epidermis – Epidermis is squarish in shape. Abaxial epidermis consists of larger cells than adaxial epidermis.

Hypodermis – Absent.

Mesophyll – Not differentiated into palisade and spongy cells. The cells are spherical and oval to hexagonal in shape.

Vascular bundle – Midrib vascular bundle is oval in shape with single phloem patch towards the adaxial surface. Fibre cap is dense and U-shaped towards the phloem end. Xylem patch is present at the center lying embedded in sclerenchymatous cells of vascular bundle.

3. Pleione praecox (Smith) D. Don, Prodr. Fl. Nepal.: 37 (1825). Nakao, Living Himal, Flow.: t. 98 (1964). Banerji & Thapa in J. Bombay Nat. Hist. Soc. 66: 582, t. 11 (1969). Hara *et al.*, EFPN 1: 55 (1978). Press *et al.*, ACFPN: 224 (2000).

Epidendrum praecox Smith, Exot. Bot. 2:73, t. 97 (1806).

Coelogyne praecox (Smith) Lindl., Collect. Bot. : sub t. 37 (1825). Hook. f., Fl. Brit. Ind. 5: 840 (1890); op cit. 6: 194 (1890). King & Pantl. in Ann. Roy. Bot. Gard. Calcutta 8: 141, t. 196 (1898).

Epiphytic or lithophytic herbs with shortly cylindrical pseudobulbs, barrel shaped, maroon in colour covered with green wart or net like loose brown fibres. Leaves two, elliptic, lanceolate, not present while flowering. Inflorescence one to two flowered arising from the base of the pseudobulb. Flowers rose violet. Floral bracts obovoid. Sepals subequal, oblong lanceolate. Petals equally long, narrowly lanceolate. Lip white with purple stripes at base, purple blotches at the apical margins, orbicular ovate, three lobed. Lateral lobes rounded, mid lobe with irregular lobulate dentate edges, apex bilobulate, disc with five papillate keels. Column narrowly winged with wide hood at the apex. (Fig 20A)

Flowering time: September-November.

Distribution: This species is found in India (Himalaya), Nepal, Bhutan, Myanmar, West China and Thailand. It occurs as an epiphytic herb in the subtropical and temperate zones of central and east Nepal at 1500-2500 m altitude.

Collection: Daman, 2400m, 15.5.2003, D. M. Bajracharya, 490 (ASCOL)

Specimens examined: Panchase Danda, Kaski, 2300m, 2.11.2002, Subedi, Chaudhari and Shakya, 996 (TUCH). Hile, Ilam, 7900ft, 6.10.1977, P. Pradhan, K. R. Rajbhandari and R. Niraula 268, (KATH). Okhle, BAglung, 2300m, 8.9.1991, N. P. Manandhar, S. K. Acharya 984-91, (KATH). Sheopuri, 2000m, 23.11.1966, D. H. Nicolson 2769, (KATH).

Anatomy

Epidermis in Surface View (Fig 20B)

Stomata are present on adaxial surface only. Epidermal cells are polygonal in shape. Strands and interstrand regions are differentiated. Stomata are tetracytic and anomocytic. Some of the stomata are surrounded by four subsidiary cells but the subsidiary cells are not very much different from other epidermal cells. Surrounding cells of most of the stomata are not distinguishable from other epidermal cells. Size of stomata is 32 x 25 μm and the frequency of stomata is 95 per sq. mm.

Transverse Section of Leaf (Fig 20C)

Epidermis – Single layer of squarish epidermal cells are present.

Hypodermis – Absent.

Mesophyll – Not differentiated into palisade and spongy cells. The cells are oval in shape.

Vascular bundle – Midrib vascular bundle is large and oval in shape. Fibre sheath is present only towards phloem end. Single patch of phloem is present on the adaxial side. Xylem is present at the center of the vascular bundle lying embedded at the sclerenchymatous tissue.

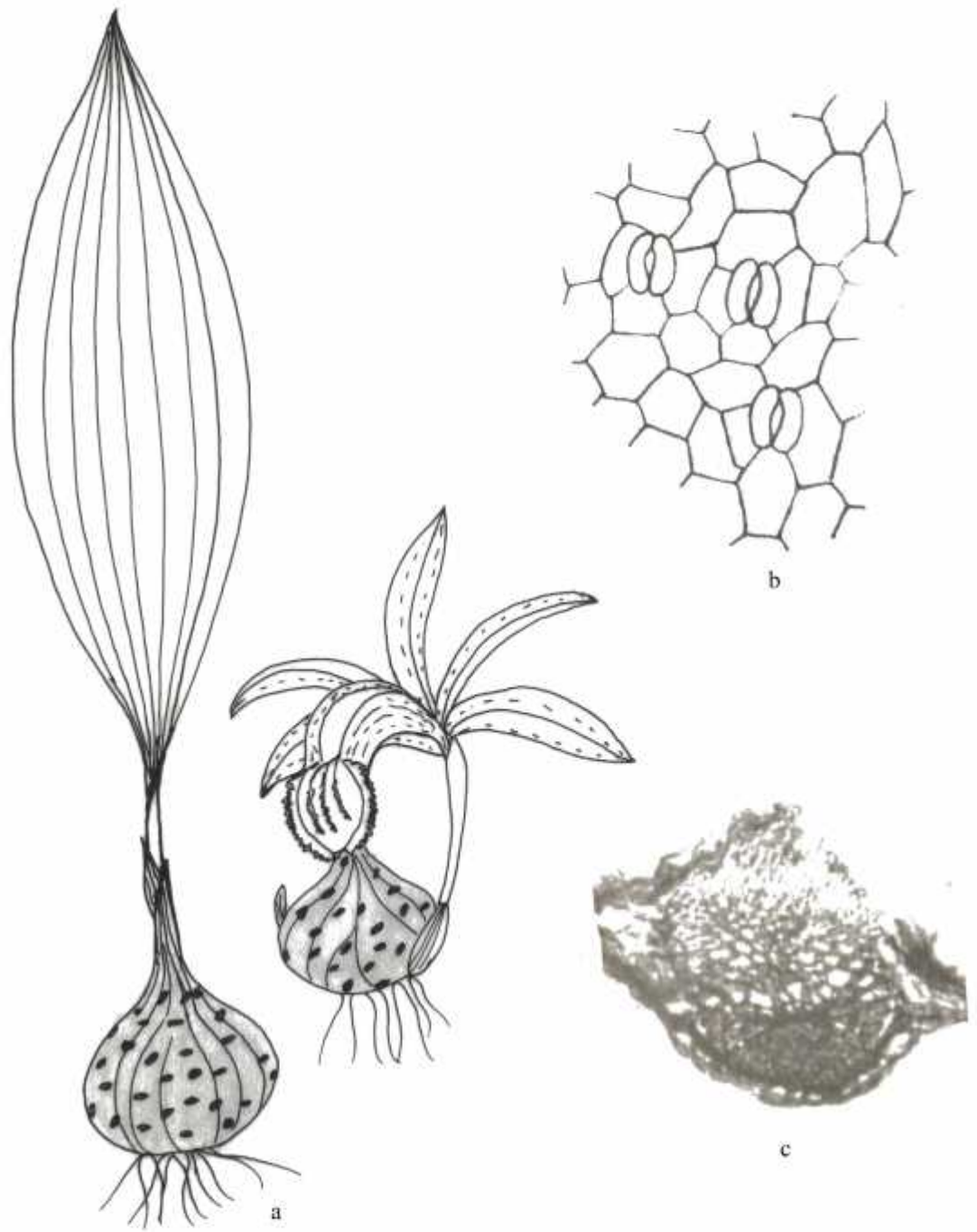


Fig 20: *Pleione praecox*; a. Habit sketch, b. Stomata, c. Midrib vascular bundle

Chapter V

CLADISTIC ANALYSIS

5.1. Introduction

Cladistics is a method of hypothesizing relationships among organisms. Cladistics has now become the most commonly used method to classify organisms. It is the best method available for phylogenetic analysis, as it provides a precise and assessable hypothesis of relationships among organisms. Concept of cladistic is based on the fact that members of a group share a common evolutionary history, and are closely related more to members of the same group than to other organisms. These shared derived characters, which were not present in distant ancestors, are called synapomorphies. One of the most important assumptions in cladistics is that the characteristics of organisms change over time. When such characteristic changes, different lineages or groups become recognizable. Hence the original state of characteristic is called as plesiomorphic and the changed state is apomorphic. These two terms are sometimes replaced by primitive and derived.

Application of cladistic principle to the study of generic and species relationship is relatively a recent phenomenon. The goal of phylogenetic analysis is to produce classifications that correspond to monophyletic groups and thus convey the maximum amount of information. (Thapa, 1995)

Willi Hennig (1950) published a short book in which he proposed the basic ideas that revolutionized systematics and launched the new science of cladistics. Cladistics takes its name from Hennig's concept of a "clade", which he defined as a group of organisms related by common descent.

Cladistics is a hypothetical relationship among taxa and considered as an alternative method of classification. Classifications based on relative positions of the divisions (branching) lines of descent, which is determined by appropriate study of characters ignoring their similarity or dissimilarity. It requires knowledge or assumptions as to which are ancestral and which are derived states of characters. Hence it is also called

as phylogenetic system by several proponents (Hennig 1966, Threkmorton 1986, Michener 1970).

5.2. Review of Literatures

Neyland, *et al.* (1994) did a phylogenetic analysis of subtribe Pleurothallidinae (Orchadaceae) based on 45 anatomical/morphological characters of 24 genera including the large genus *Pleurothallis*. The cladistic analysis suggested that *Pleurothallis* is not a natural genus and may be divided into several discrete genera

Repetur *et al.* (1997) analyzed phylogeny of the genus *Bromheadia* using 27 characters. It shows that *Bromheadia* is monophyletic genus with one very distinctive apomorphic character, the shape of the stipe of the pollinia.

Cozzoline *et al.* (1998) analyzed phylogenetic relationship in *Orchis* and some related genera by using chloroplast DNA. The cladistic analysis showed that *Orchis* as a paraphyletic group, and the genus is divided into two clades. These results, which agree to a great extent with literature evidence on chromosome and isozymes, have been compared with various traditional systematic hypotheses for the genus.

Cameron, *et al.* (1999) did the cladistic parsimony analyses of *rbcL* nucleotide sequence data from 171 taxa representing nearly all tribes and subtribes of Orchidaceae. These analyses divide the family into five primary monophyletic clades: apostasioid, cypripedoid, vanilloid, orchidoid, and epidendroid orchids

Freudenstein & Rasmussen (1999) performed the cladistic analysis of Orchidaceae undertaking 98 genera and using 71 morphological apomorphic characters based on a reconsideration of previous character analyses and newly discovered variation. The equally weighted analysis found 60,000 most parsimonious trees with low consistency (CI=0.29), but high retention (RI=0.83). The strict consensus reveals a significant amount of structure, and most traditionally recognized subfamilies are supported as monophyletic, including Apostasioideae, Cypripeioideae, Spiranthoideae and

Epidendroideae. Subfamily Orchidoideae in the broad sense are paraphyletic, giving rise to spieranthoids.

Stern and Judd (2000) conducted a study on anatomy and systematics of the orchid tribe Vanilleae excluding *Vanilla*. In the cladistic analysis, two equally parsimonious trees were obtained both constituting a paraphyletic complex giving rise to a clade containing the monophyletic Lecanorchidinae and Galeolinae.

Gravendeel (2000) performed a phylogenetic analysis of subtribe Coelogyninae (Epidendroideae, Orchidaceae) on the basis of 41 macromorphological and 4 anatomical characters scored from 43 taxa in Coelogyninae (27 *Coelogyne* species and 13 representatives of other genera) and three outgroups from Blettiinae and Thuniinae. This analysis confirmed the monophyly of the Coelogyninae but *Coelogyne* appeared to be polyphyletic with the species falling in at least two different clades.

Bajracharya (2003) performed a cladistic analysis of the genus Himalayan *Eria* on the basis of 35 morphological, three anatomical and two cytological characters scored from 40 species of Himalayan *Eria* and two outgroups from *Arundina* and *Dendrobium*. One hundred maximally parsimonious trees were obtained having length = 272; CI= 0.26. The analysis also confirmed that Himalayan *Eria* species fall in at least two different clades. The key characters for the delimitation are presence of stigmata, inflorescence types, pseudobulb type and number of flower/inflorescence, type of bracts and length of labellum appeared to be good characters for defining major clade in the Himalayan *Eria*. Morphological characters also play an important role in the cladistic analysis for reconstruction of the groups within the genus.

Until now there has been no morphological or anatomical cladistic analysis of Coelogyninae performed in the context of Nepal. This study is not broad enough to propose any phylogenetic hypotheses but it will serve to fill in the gap on the anatomy of the genera and species where the information is seriously lacking. Once the data are combined with other information on the comparative anatomy of Coelogyninae, they will add to a comprehensive analysis of this species-rich group of plants. This study represents a partial effort toward the goal. The study was designated to investigate the anatomy and

potential phylogenetic applications of microscopic structure to determine the usefulness of these characters in setting forthline of relationship among the taxa of subtribe Coelogyninae. Furthermore, this group is unknown anatomically and this study will provide a basis for further investigation of the assemblage of the genera within the subtribe.

Present study attempts an anatomical cladistic analysis of subtribe Coelogyninae. With the variation in the anatomical characters of roots and leaves, this analysis presents the interrelationship among the closely related taxa within the species and the genera consisted in subtribe Coelogyninae.

5.3. Materials and Methods

The cladistic analysis was performed with the help of closely related taxa *Phaius* as an outgroup and other species within the subtribe. The included species and their acronyms were shown in Table 1. The data coding was carried out selecting the gross anatomical and morphological characters on the basis of their variation. Each character is divided into different character states for character coding and multistate character coding was done. Those characters and corresponding states were mentioned in Table 2.

Data matrix for cladistic analysis was generated in computer using Winclada version 1.00.08 developed by K. Nixon (1999-2002). The parsimonious tree was obtained by analyzing the data matrix given in Table 3 through the “heuristics” command of the “Analysis” menu. For the heuristics analysis, the value for maximum trees to keep hold was given 100, number of replication 10 and starting trees per replication was given 10. The search strategy was given multiple TBR +TBR. Unconstrained search mode was applied for the analysis. The analysis gave 83 trees with CI = 35 and RI = 56. Only the best tree was kept deleting all the suboptimal trees.

Table 1: Species included and their acronyms for cladistic analysis of subtribe Coelogyntinae

<i>Phaius</i>	<i>phaiu</i>
<i>Coelogyne coymbosa</i>	<i>corym</i>
<i>C. cristata</i>	<i>cris</i>
<i>C. flaccida</i>	<i>flacc</i>
<i>C. flavida</i>	<i>flavi</i>
<i>C. fuscescens</i>	<i>fusce</i>
<i>C. nitida</i>	<i>nitid</i>
<i>C. ovalis</i>	<i>ovali</i>
<i>C. stricta</i>	<i>stric</i>
<i>Otochilus albus</i>	<i>albus</i>
<i>O. fuscus</i>	<i>fuscu</i>
<i>O. porrectus</i>	<i>porre</i>
<i>Panisea demissa</i>	<i>demis</i>
<i>P. uniflora</i>	<i>unifl</i>
<i>Pholidota articulata</i>	<i>artic</i>
<i>P. imbricate</i>	<i>imbri</i>
<i>P. protracta</i>	<i>protr</i>
<i>P. recurva</i>	<i>recur</i>
<i>P. hookeriana</i>	<i>hooke</i>
<i>P. humilis</i>	<i>humil</i>
<i>P. praecox</i>	<i>praec</i>

Table 2: Characters and character states used in cladistic analysis

Characters	Character States
0. Leaf texture	(0) coriaceous, (1) membranous
1. Stomatal type	(0) tetracytic, (1) anomocytic (2) both
2. Number of stomata/sq. mm	(0) 40-80, (1) 80-120, (2) >120
3. Stomatal index	(0) 5-10, (1) 10-15, (2) >15
4. Strand and interstrand	(0) not differentiated, (1) slightly differentiated, (2) differentiated
5. Leaf epidermal cell shape	(0) rectangular, (1) polygonal (2) both
6. Leaf epidermal cell arrangement	(0) isodiametric, (1) periclinal, (2) anticlinal
7. Leaf epidermal cell size	(0) adaxial=abaxial, (1) adaxial>abaxial, (2) adaxial<abaxial
8. Hypodermis in leaf	(0) absent, (1) present
9. No. of mesophyll layers in leaf	(0) <5, (1) 5-10, (2) >10
10. Mesophyll cell shape	(0) round to oval, (1) oval
11. Shape of midrib bundle	(0) round, (1) oval, (2) conical
12. Fibre cap on midrib bundle	(0) U or V shaped (1) surrounded
13. No. of phloem patches in midrib	(0) 1, (1) 1-3, (2) >3
14. Root hairs	(0) absent, (1) present
15. No. of velamen layers	(0) <=3, (1) >3
16. Velamen cell type	(0) round to oval, (1) polygonal
17. Cortical cell layers	(0) <=5, (1) >5
18. U-shaped endodermal thickening	(0) absent, (1) present
19. No. of vascular strand	(0) <=10, (1) >10
20. Habitat	(0) epiphytic, (1) epiphytic + lithophytic, (2) terrestrial
21. Stem	(0) jointed, (1) pseudobulbs clustered, (2) pseudobulbs apart
22. Shape of pseudobulb	(0) ovoid, (1) cylindrical
23. Number of leaves in pseudobulb	(0) 1, (1) 2
24. Leaf petiole	(0) sessile, (1) subsessile, (2) petioled
25. Inflorescence origin	(0) apex of pseudobulb, (1) base of pseudobulb, (2) top of immature leaves
26. Inflorescence type	(0) heteranthous, (1) proteranthous, (2) synanthous
27. Inflorescence position	(0) erect, (1) semierect, (2) pendulous
28. No. of flowers in an inflorescence	(0) <=5, (1) >5
29. Floral bracts	(0) persistent (1) deciduous

Table 3: Data matrix for the cladistic analysis of subtribe Coelogyninae

Taxa	Characters																														
	0	1	2	3	4	5	6	7	8	9	1	1	1	1	1	1	1	1	1	1	2	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	
<i>phaiu</i>	?	0	0	0	2	2	2	?	0	1	0	1	1	1	1	0	?	1	0	1	2	?	?	2	0	1	?	2	1	?	
<i>corym</i>	0	0	1	1	1	1	1	1	0	0	0	2	0	0	0	1	?	0	0	1	0	1	0	1	2	2	1	2	0	1	
<i>cris</i>	0	0	1	0	0	2	0	1	1	1	0	1	0	0	1	1	1	0	1	1	0	2	0	1	1	2	1	2	1	0	
<i>flacc</i>	0	0	0	1	1	2	0	0	1	0	1	2	0	1	0	1	1	0	0	1	0	1	0	1	2	1	1	2	1	1	
<i>flavi</i>	0	0	1	0	2	1	0	2	1	1	0	2	0	2	0	1	0	0	1	1	0	2	0	1	2	0	3	0	1	1	
<i>fusce</i>	0	0	1	1	1	2	0	1	0	1	0	1	0	0	0	1	1	0	1	1	0	1	1	1	1	2	1	2	0	1	
<i>nitid</i>	0	0	2	1	1	1	1	1	1	0	0	0	1	1	0	1	0	0	0	1	0	1	1	1	2	2	1	2	0	1	
<i>ovali</i>	0	0	1	1	0	2	0	1	0	0	1	2	0	1	0	1	0	1	0	1	0	2	0	1	1	0	3	1	0	1	
<i>stric</i>	0	0	0	2	2	2	0	1	1	2	0	1	0	1	0	1	0	1	1	1	0	2	1	1	1	0	3	0	1	1	
<i>albus</i>	0	0	0	0	2	0	1	1	0	1	1	1	1	0	0	0	0	0	1	0	0	0	1	1	2	2	2	1	1	1	
<i>fuscu</i>	0	0	0	0	1	1	1	1	0	1	1	1	1	1	0	1	0	0	1	0	0	0	1	1	1	2	1	1	1	1	
<i>porre</i>	0	0	1	0	2	2	1	1	0	1	1	1	0	0	0	1	0	0	1	1	0	0	1	1	1	2	1	2	1	0	
<i>demis</i>	1	1	2	2	0	1	1	1	0	1	1	0	0	0	0	1	1	0	1	0	0	1	0	1	0	1	0	1	1	1	
<i>unifl</i>	1	1	2	1	0	2	1	1	0	1	0	0	0	0	0	1	1	0	1	0	0	1	0	1	0	1	0	0	0	1	
<i>artic</i>	0	0	1	1	2	2	1	1	0	1	0	1	0	0	0	1	0	0	1	1	0	0	1	1	1	2	2	2	1	1	
<i>imbri</i>	0	0	0	1	2	1	1	1	0	1	0	1	0	0	0	1	1	1	1	1	0	1	1	0	2	2	2	2	1	0	
<i>protr</i>	1	0	0	1	2	2	1	1	0	1	0	1	0	0	0	1	1	0	1	1	0	0	1	1	1	2	2	1	1	0	
<i>recur</i>	0	0	1	1	2	2	1	1	0	1	0	1	0	0	0	1	0	0	1	1	0	0	1	1	1	2	2	2	1	0	
<i>hooke</i>	1	1	1	0	2	1	0	1	0	1	0	1	0	0	0	0	1	1	0	0	1	1	0	0	1	2	2	0	0	0	
<i>humil</i>	1	1	0	0	2	1	0	1	0	1	0	1	0	0	0	0	1	1	0	0	1	1	0	0	1	1	1	0	0	0	
<i>praec</i>	1	1	1	0	2	1	0	1	0	1	1	1	0	0	0	0	1	1	0	0	1	1	0	1	2	1	1	0	0	0	

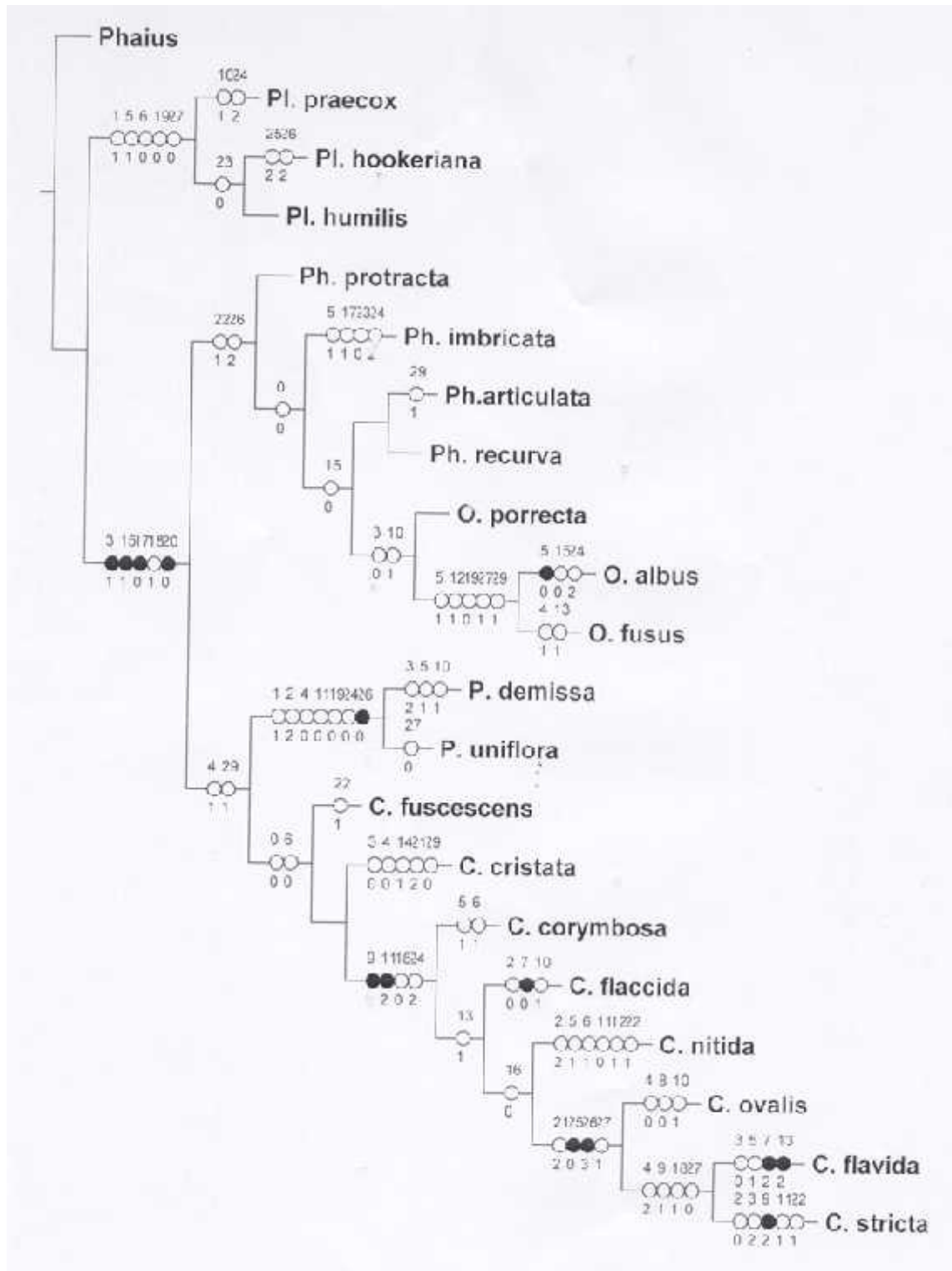


Fig 21: Cladogram of subtribe Coelogyinae

The dots represent synapomorphies. Numbers above the bar correspond characters and below the bars correspond the character states.

5.4. Results and Discussion

The cladistic analysis of subtribe Coelogyinae on the basis of anatomical and morphological characters gave a distinct picture of genus differentiation. The first major clade differentiated the genus *Pleione* from other genera confirming it a monophyletic group. Within the genus *Pleione*, *P. hookeriana* and *P. humilis* again form another group excluding *P. praecox*. This is consistent with the classification of *Pleione* into different sections.

Similarly, the second major clade differentiated the remaining four genera into two clades, the first clade comprising *Pholidota* and *Otochilus* and the second clade comprising *Panisea* and *Coelogyne*. *Panisea* and *Coelogyne* are again divided into two groups confirming each of them as monophyletic.

There is no clear differentiation into *Pholidota* and *Otochilus* as separate groups. However, *Pholidota articulate* and *P. recurva* has been separated from other two species of *Pholidota*. Within the genus *Otochilus*, *O. porrectus* has been separated from other two species, *O. albus* and *O. fuscus*.

Chapter VI

RESULT AND DISCUSSION

There is considerable uniformity of morphology and anatomy among the species of subtribe Coelogyntinae. However, the anatomical features show little difference from genus to genus and among the species. The anatomical features of the subtribe Coelogyntinae have been summarized with the characteristics of each genus.

6.1. Leaf Morphology

Leaves are simple, lanceolate to elliptic-lanceolate, linear, single (*Pholidota imbricata* and *Pleione sp.*) or in pairs arising from the top of the pseudobulb, rarely from the base of the pseudobulb. Texture of the leaves are coriaceous in most of the species of *Coelogyne* and some species of *Pholidota*. Other species of the subtribe Coelogyntinae have membranous leaves excepting few slightly thick-leaved species. Leaves are comparatively large in *Coelogyne* and *Pholidota* whereas small in *Otochilus* and *Panisea*. In the species of *Pleione*, leaves are usually not present when flowering.

6.2. Epidermal and Stomatal Features

Shape of the epidermal cells varies from rectangular to polygonal with four to many sides. The epidermis does not possess any trichomes. Stomata are present only on the adaxial surface of leaf in all the genera studied. Tetracytic type of stoma is almost universal in species of *Coelogyne*, *Pholidota* and *Otochilus*. Four subsidiary cells are distinct from other epidermal cells in these genera. *Panisea* and *Pleione* differ from other genera in having anomocytic stomata. In these genera, surrounding cells are not distinct from the other epidermal cells. Size of the stomata varies from the smallest of 25 x 22 μm in *Panisea uniflora* to the largest of 62 x 45 μm in *Coelogyne stricta*. The frequency of stomata also varies from the least of 59 per sq. mm. in *Pholidota protracta* to the highest of 197 in *Panisea uniflora*.

Shape of epidermal cells are rectangular to polygonal in all the species of *Coelogyne*. Stomata are surrounded by four subsidiary cells, i.e., tetracytic, which are

alike in all the species of *Coelogyne* under investigation. The stomatal characters of the species of *Coelogyne* show variation in shape, size and distribution of stomata, however, there is no significant variation in the arrangement of subsidiary cells. Anticlinal walls of the subsidiary cells are partly dissolved in mature stomata of *Coelogyne*. Among the eight species studied, *C. stricta* has been found to comprise the largest stomata and smallest stomata has been recorded in *C. corymbosa*.

In *Otochilus*, epidermal cells are almost square to rectangular. There is not much variation in the shape and size of epidermal cells in the three species investigated. Stomata are tetracytic. Anticlinal walls of the subsidiary cells are slightly dissolved in mature stomata.

Epidermal cells in *Panisea demissa* are large and polygonal whereas small and rectangular in *P. uniflora*. No distinct subsidiary cells are present in *P. demissa*. *P. uniflora* exhibits alongside both anomocytic and tetracytic arrangement of stomata. Some stomata have a tendency to be paracytic having two distinct subsidiary cells parallel to the guard cells.

Species of *Pholidota* show the same pattern of epidermal and stomatal complexes as in *Coelogyne*. Epidermal cells and stomata of *Pholidota imbricate* are largest among the four species studied and those of *P. recurva* are the smallest.

Stomatal arrangement in *Pleione* is anomocytic with the surrounding cells not distinct from other epidermal cells but comparatively smaller than other epidermal cells. Existence of paracytic stomata in *P. humilis* as reported by Rao and Khasim (1987) was not found. Epidermal cells are smallest in *P. hookeriana* and largest in *P. humilis* among the three species studied. In *P. humilis*, surrounding cells are arranged such that they are elongated perpendicularly to the guard cells while in other species of *Pleione* surrounding epidermal cells are elongated parallel to the long axis of guard cells.

Table 4: Stomatal features in species of subtribe Coelogyninae

S.N.	Taxa	Leaf Texture	Stomata Type	Stomata size (µm) (L x B)	Frequency per sq mm	Stomata Index
1	<i>Coelogyne coymbosa</i> Lindl.	C	T	28.33 x 24.17	124.13	11.46
2	<i>C. cristata</i> Lindl.	C	T	41.93 x 33.43	112.84	8.65
3	<i>C. flaccida</i> Lindl.	C	T	58.93 x 37.97	77.58	11
4	<i>C. flavida</i> Wall. ex Lindl.	C	T	53.83 x 51	84.63	7.84
5	<i>C. fuscescens</i> Lindl.	M	T	32.87 x 29.47	141.05	10.96
6	<i>C. nitida</i> Wall. ex D. Don) Lindl.	C	T	51.57 x 45.33	141.05	10.1
7	<i>C. ovalis</i> Lindl.	C	T	59.5 x 41.93	98.74	12.96
8	<i>C. stricta</i> (D. Don) Schltr.	C	T	61.77 x 45.33	75.23	15.38
9	<i>Otochilus albus</i> Lindl.	Th	T	29.17 x 24.17	75.23	5.63
10	<i>O. fuscus</i> Lindl.	Th	T	30 x 25.5	73.9	9.6
11	<i>O. porrectus</i> Lindl.	Th	T	30 x 23.33	108.14	8.68
12	<i>Panisea demissa</i> (D. Don) Pfitz.	M	A	28.33 x 21.67	150.46	22.86
13	<i>P. uniflora</i> (Lindl.) Lindl.	M	A+T	25 x 21.88	197.47	14.89
14	<i>Pholidota articulata</i> Lindl.	Th	T	39 x 25.5	91.6	11.04
15	<i>P. imbricata</i> (Roxb.) Lindl. in Hook.	C	T	47.5 x 30	71	11.2
16	<i>P. protracta</i> Hook. f.	M	T	37.5 x 32.7	59.1	12.1
17	<i>P. recurva</i> Lindl.	M	T	30 x 30.75	73.9	13.6
18	<i>P. hookeriana</i> (Lindl.) William.	M	A	30.75 x 29.47	71	8.67
19	<i>P. humilis</i> (Sm.) D. Don.	M	A	30 x 28.33	65.82	9.72
20	<i>P. praecox</i> (Sm.) D. Don.	M	A	31.67 x 25	84.63	9.74

C= Coriaceous, Th=Thick, M=Membranous T=Tetracytic, A=Anomocytic,

6.3. Transverse Section of Leaf

Transverse section of leaf demonstrates some variation in the structure of epidermis. The epidermal cells are covered with cuticle and vary in shape from square to rectangle. The adaxial epidermal cells are usually smaller than abaxial cells. Single layer of hypodermis is present in the species of *Coelogyne* and absent in others. Mesophyll cells are not differentiated into palisade and spongy tissue. The cells are round to oval in shape with varied size. Vascular bundles are present in a single series along the lamina with a large midrib bundle. They are surrounded by thick walled fiber caps. In the midrib vascular bundles, phloem patch is present at the center with other smaller patches in the periphery in some of the species.

Abaxial epidermal cells are usually periclinal in species of *Coelogyne* except in few such as *Coelogyne cristata* in which they are squarish. Adaxial cells are usually square and smaller than adaxial cells. Hypodermis consists of single layer of squarish to periclinal hyaline cells both abaxially and adaxially in *Coelogyne cristata*, *C. flaccida* and *C. stricta*. Hypodermis is present only adaxially in other species and absent in *Coelogyne fuscescens*. In *C. flaccida*, hypodermal cells are large hyaline and barrel-shaped with annular cellulosic thickenings. These cells are five to six times larger than mesophyll cells. Mesophyll cells are homogeneous, not differentiated into palisade and spongy parenchyma, thickness ranging from four to five cells wide in *C. ovalis* to 10-12 cells wide in *C. stricta*. Cells are thin walled, variously shaped, circular to elliptical to polygonal. Vascular bundles are conjoint and collateral, arranged in a single series with large bundle in the midrib. Midrib vascular bundle is globular to ovate or flask shaped. U or V shaped thick walled fibre caps are present at phloem and xylem ends. In the midrib vascular bundle, there are five phloem patches in *Coelogyne flavida*, three in *C. nitida*, *C. stricta* and *C. ovalis*. In these species, one large phloem patch is in the center and other patches are small in the periphery. In other species a single patch of phloem is present at the center.

In *Otochilus* species, the epidermal cells are barrel shaped on both adaxial and abaxial surfaces, adaxial cells being smaller than abaxial cells. Hypodermis is absent in this species. Mesophyll cells are not differentiated into spongy and palisade tissue. They

are homogeneous with five to seven layers in *O. albus* and *O. fuscus* and six to eight layers in *O. porrectus*. The cells are small to large oval shaped. Single series of vascular bundles are present. Midrib vascular bundles are oval in shape and are surrounded by fiber caps in *O. albus* and *O. fuscus* but U or V shaped fibre caps are present in *O. porrectus*. Single phloem patch is present in the center with additional two small peripheral patches in *O. fuscus*.

In *Panisea*, the epidermis is single layer, squarish in shape and abaxial cells are larger than adaxial cells. Hypodermal cells are absent. Mesophyll cells oval to round in shape, four to six layers and are not differentiated. U or V shaped fibre caps are present both towards the xylem and phloem ends in the midrib vascular bundle. Single phloem patch is present in the center.

T. S. of *Pholidota* leaves demonstrate single layer of periclinally arranged epidermal cells covered with thick cuticle. Hypodermis is absent in species of *Pholidota*. Mesophyll cells are not differentiated but are homogeneous with round to oval shaped cells. Number of layers varies from four to eight. Midrib vascular bundle is large and round in *P. articulata* and is oval in shape in other species of *Pholidota*. Fibre caps are U or V shaped towards the xylem and phloem ends. Single phloem patch is present at the center in all the species of *Pholidota*.

In the species of *Pleione*, the epidermal cells are squarish in shape with adaxial cells smaller than abaxial cells. Hypodermal cells are absent. Mesophyll cells are round to oval in shape, four to six layers and homogenous with no differentiation of spongy and palisade cells. Large midrib vascular bundles are oval in shape. In *Pleione*, U-shaped fibre caps are dense towards the phloem end than the xylem end.

Table 5: Anatomical features of leaf in species of subtribe Coelogyinae

S.N.	Taxa	Hypodermis	Mesophyll layers	Mesophyll cell shape	VB shape	Fibre cap	Phloem patches
1	<i>Coelogyne coymbosa</i> Lindl.	absent	4 to 5	round to oval	conical	U or V shape	1
2	<i>C. cristata</i> Lindl.	present	6 to 8	round to oval	oval	U or V shape	1
3	<i>C. flaccida</i> Lindl.	present	3 to 4	round to oval	conical	U or V shape	1+2
4	<i>C. flavida</i> Wall. ex Lindl.	present	9 to 10	round to oval	conical	U or V shape	1+5
5	<i>C. fuscescens</i> Lindl.	absent	5 to 7	round to oval	oval	U or V shape	1
6	<i>C. nitida</i> Wall. ex D. Don) Lindl.	present	4 to 6	rectangular	round	surrounding	1+2
7	<i>C. ovalis</i> Lindl.	absent	4 to 5	oval	conical	U or V shape	1+2
8	<i>C. stricta</i> (D. Don) Schltr.	present	10 to 12	round to oval	elongated	U or V shape	1+2
9	<i>Otochilus albus</i> Lindl.	absent	5 to 7	oval	oval	surrounding	1
10	<i>O. fuscus</i> Lindl.	absent	5 to 7	oval	oval	surrounding	1+2
11	<i>O. porrectus</i> Lindl.	absent	6 to 7	oval	oval	U or V shape	1
12	<i>Panisea. uniflora</i> (Lindl.) Lindl.	absent	4 to 6	round to oval	round	U or V shape	1
13	<i>Pholidota articulata</i> Lindl.	absent	5 to 7	round to oval	round	U or V shape	1
14	<i>P. imbricata</i> (Roxb.) Lindl. in Hook.	absent	4 to 6	round to oval	oval	U or V shape	1
15	<i>P. protracta</i> Hook. f.	absent	4 to 6	round to oval	oval	U or V shape	1
16	<i>P. recurva</i> Lindl.	absent	5 to 7	round to oval	oval	U or V shape	1
17	<i>Pleione. humilis</i> (Sm.) D. Don.	absent	5 to 6	round to oval	oval	U shaped	1
18	<i>P. praecox</i> (Sm.) D. Don.	absent	4 to 6	oval	oval	U shaped	1

VB=Vascular bundle

6.4. Transverse Section of Root

Subtribe *Coelogyinae* comprises epiphytic species thus velamen is present in all the species. Velamen is composed of few layers of small cells with slight variation in their structures. Single layer of exodermal cells with thickened outer and radial walls are interrupted by thin walled passage cells at frequent intervals. Cortex consists of few layers of cells, middle layer having large cells and outer and inner layers having smaller cells. Endodermal cells are highly lignified with U-shaped thickening except in *Pleione* and some species of *Coelogyne*. Thick walled cells are interrupted by thin-walled passage cells adjacent to the xylem strands which are present in radial rows alternating with circular to elliptical to elongated clusters of phloem cells. Vascular tissues are embedded on polygonal sclerenchyma cells. Pith cells are small circular without intercellular spaces.

T.S. of root in *Coelogyne cristata* shows distinct root hairs which is not evident in other species. Velamen cells are small in size and polygonal in shape. Velamen in *C. corymbosa* is different from others with a large elongated anticlinally arranged inner layer of cells. In other species velamen cells are almost of equal size with slightly elongated innermost cells. Number of velamen layers varies from two in *C. corymbosa* to seven in *C. stricta* and *C. cristata*. Exodermal cells are thick walled and are anticlinally arranged with thin walled passage cells at frequent intervals. Cortical cells consist of outer and inner small cells with large middle layer. Intercellular spaces are present in cortical cells. This type of arrangement is present in all species. Endodermal cells having U-shaped thickening opposite to phloem strands are present in all species of *Coelogyne* except in *C. flaccida* and *C. ovalis*. Number of xylem and phloem strands ranges from 10 in *C. nitida* to 20 in *C. stricta*.

In the *Otochilus* species, root hairs are absent. Two to four layers round to oval shaped velamen cells are present. Exodermis and cortical cells are similar to that in the species of *Coelogyne*. Distinct U-shaped thickenings are present in the species of *Otochilus*. Vascular cylinder consists of alternating xylem and phloem strands, number of strands ranging from seven in *O. albus* to 20 in *O. porrectus*.

Root hairs are absent in *Panisea* also. Velamen is four to five layers thick and the cells are polygonal in shape. Exodermis and cortex are similar to other genera. Endodermis cell wall thickening is very distinct in the species of *Panisea*. Number of xylem or phloem strands is eight in *P. demissa* and ten in *P. uniflora*.

Species of *Pholidota* does not show much variation in the anatomical structures of root. There is absence of root hairs. Velamen cells are round to polygonal in shape. Exodermal cells are anticlinally arranged and thick walled. Cortical cells are thin walled with small outer and inner cells and large middle cells in all species of *Pholidota*. Endodermis is highly lignified in *P. recurva*. In other species also, the endodermal cells consists of U-shaped thickening lying opposite to phloem. Number of xylem or phloem strands varies from 10 in *P. protracta* to 15 in *P. recurva*.

Two to three layers of polygonal velamen cells are present in *Pleione*. Exodermal cells are thick walled and almost squarish in shape. Cortical cells are thick walled and five to six layers thick, two to three middle layers are of large cells. No distinct U-shaped thickening is present in *Pleione*. There are eight to ten xylem and phloem strands present alternating with each other. Pith consists of large cells without intercellular spaces.

Table 6: Anatomical features of root in species of subtribe Coelogyinae

S.N.	Taxa	Velamen layers	Velamen cell type	Cortical cell layers	Endodermal cell thickening	Vascular bundles
1	<i>Coelogyne coymbosa</i> Lindl.	2	elongated	3-4	-	11
2	<i>C. cristata</i> Lindl.	5-7	polygonal	4-5	present	16
3	<i>C. flaccida</i> Lindl.	3-4	polygonal	4	absent	19
4	<i>C. flavida</i> Wall. ex Lindl.	4	round to oval	4	present	11
5	<i>C. fuscescens</i> Lindl.	3-4	round to oval	4-5	present	14
6	<i>C. nitida</i> Wall. ex D. Don) Lindl.	3-4	round to oval	3-4	-	10
7	<i>C. ovalis</i> Lindl.	4-5	round to oval	4-5	absent	19
8	<i>C. stricta</i> (D. Don) Schltr.	5-7	round to oval	8-10	present	20
9	<i>Otochilus albus</i> Lindl.	2-3	round to oval	4	present	7
10	<i>O. porrectus</i> Lindl.	4	round to oval	3	present	20
11	<i>Panisea demissa</i> (D. Don) Pfitz.	4	polygonal	3	present	8
12	<i>P. uniflora</i> (Lindl.) Lindl.	4-5	polygonal	3	present	10
13	<i>Pholidota articulata</i> Lindl.	5-7	round to oval	3-4	present	14
14	<i>P. imbricata</i> (Roxb.) Lindl. in Hook.	4-5	round to oval	4-5	present	11
15	<i>P. protracta</i> Hook. f.	3-4	polygonal	3	present	10
16	<i>P. recurva</i> Lindl.	3-4	round to oval	3-4	present	15
17	<i>P. hookeriana</i> (Lindl.) William.	2-3	rectangular	5-6	absent	8

6.5. Classification on the Basis of Anatomical Characters

6.5.1. Tentative Anatomical Key to the Genera

- 1a. Tetracytic stomata with four distinct subsidiary cells..... 2
- 1b. Anomocytic stomata without distinct subsidiary cells..... 3
- 2a. Presence of hypodermis in transverse section of leaf..... *Coelogyne*
- 2b. Absence of hypodermis in transverse section of leaf..... 4
- 3a. Endodermis with U-shaped thickenings in transverse section of root..... *Panisea*
- 3b. Absence of U-shaped thickening in transverse section of root..... *Pleione*
- 4a. Stomatal Index <10..... *Otochilus*
- 4b. Stomatal Index >10..... *Pholidota*

6.5.2. Tentative Anatomical Key to the species *Coelogyne*

- 1a. Anticlinal walls of subsidiary cells of stomata dissolved..... 2
- 1b. Anticlinal walls of subsidiary cells of stomata not dissolved..... 3
- 2a. Hypodermal cells of lamina made up of cells with cellulosic thickening...*C. flaccida*
- 2b. Hypodermal cells without cellulosic thickening.....4
- 3a. Only one phloem patch present in vascular bundle of leaf.....*C. fuscescens*
- 3b. More than one phloem patches in vascular midrib vascular bundle of leaf..... 5
- 4a. Distinct root hairs present.....*C. cristata*
- 4b. Root hairs absent.....6
- 5a. U-shaped thickening absent in endodermal cells of root.....*C. ovalis*
- 5b. Presence of U-shaped thickening in endodermal cells of root.....7
- 6a. Velamen only two layers, inner cells more than three times the outer cells.....*C. corymbosa*
- 6b. Velamen layers more than five cell layers.....*C. stricta*
- 7a. Flask shaped midrib vascular bundle in leaf.....*C. flavida*
- 7b. Rounded midrib vascular bundle in leaf.....*C. nitida*

6.5.3. Tentative Anatomical Key to the species *Otochilus*

- 1a. Number of phloem patches in midrib vascular bundle one.....2
- 1b. Number of phloem patches in midrib vascular bundle three.....*O. fuscus*
- 2a. Frequency of stomata per sq. mm <100.....*O. albus*
- 2b. Frequency of stomata per sq. mm >100.....*O. porrectus*

6.5.4. Tentative Anatomical Key to the species *Panisea*

- 1a. Stomata are anomocytic. Sotmatal index >20. Number of xylem and phloem strands in the root eight each.....*P. demissa*
- 1b. Both anomocytic and tetracytic stomata are present. Sotmatal index < 20. Number of xylem and phloem strands in the root 10 each.....*P. uniflora*

6.5.5. Tentative Anatomical Key to the species *Pholidota*

- 1a. Velamen layers more than five cells. Midrib vascular bundle round.....*P. articulata*
- 1b. Velamen cells less than five cells. Midrib vascular bundle oval or conical.....2
- 2a. Xylem and phloem strand 15 each.....*P. recurva*
- 2b. Xylem and phloem strand less than 12..... 3
- 3a. Frequency of stomata per sq. mm >60.....*P. imbricata*
- 3b. Frequency of stomata per sq. mm <60.....*P. protracta*

6.5.6. Tentative Anatomical Key to the species *Pleione*

- 1a. Surrounding cells of the stomata are perpendicular to the guard cells.....*P. humilis*
- 1b. Surrounding cells of the stomata are parallel to the long axis of guard cells.....2
- 2a. Frequency of stomata, less than 80 per sq. mm.....*P. hookeriana*
- 2b. Frequency of stomata, more than 80 per sq. mm.....*P. praecox*

Chapter VII

CONCLUSION

The comparative analysis of anatomical features in the present species indicate the relationship among the five genera within the subtribe *Coelogyne* the genera shares common characteristics among themselves; however, they differ with each other in various characters. The anatomical characteristics give some significance in understanding the affinity and evolutionary relationships among the species of subtribe *Coelogyne* of Nepal. From the observations and findings of the study, following conclusions have been drawn.

-) From the stomatal point of view, *Panisea* and *Pleione* differ from other genera in having anomocytic stomata. In *Coelogyne*, *Otochilus* and *Pholidota*, stomata are similar in feature with four subsidiary cells distinct from others.
-) Presence of hyaline cells in hypodermis is a distinct feature in *Coelogyne*, especially in *C. flaccida*. Hypodermis is not distinguishable in other genera.
-) Number of phloem patches in the midrib vascular bundles are inconsistent in the species studied. *Pholidota*, *Panisea* and *Pleione* possess only one phloem patch whereas few species of *Coelogyne* and *Otochilus* have more than one phloem patches.
-) Distinct root hairs in *Coelogyne cristata* exclude the species from other species and genera.
-) U-shaped thickening in the endodermal cells of roots are the characteristic feature of the species studied. They are absent in *Pleione* and some species of *Coelogyne*.
-) Undifferentiated homogeneous mesophyll cells, well-developed fibre caps in the vascular system of leaf, well-developed velamen, thick-walled exodermal cells, vascular tissues lying on the sclerenchyma cells in root are the common characters in subtribe *Coelogyne*.
-) From the cladistic standpoint, the genus *Pleione* has been separated from other genera among the five species studied. Similarly, *Pholidota* and *Otochilus* have been separated from *Panisea* and *Coelogyne*.

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-) Undifferentiated homogeneous mesophyll cells, well-developed fibre caps in the vascular system of leaf, well-developed velamen, thick-walled exodermal cells, vascular tissues lying on the sclerenchyma cells in root are the common characters in subtribe *Coelogyne*.
-) From the cladistic standpoint, the genus *Pleione* has been separated from other genera among the five species studied. Similarly, *Pholidota* and *Otochilus* have been separated from *Panisea* and *Coelogyne*.

Chapter VIII

SUMMARY

The family Orchidaceae consists of three subfamilies, six tribes, and about 750 to 1000 genera according to Dressler 1993 and attributed 20 genera and 285 species to the subtribe Coelogyninae. Taxonomically, this subtribe has been divided and subdivided by numerous taxonomists with different approach. Nepal harbors only six genera under subtribe Coelogyninae, they are *Coelogyne*, *Neogyne*, *Otochilus*, *Panisea*, *Pholidota*, and *Pleione*. Present anatomical study has been carried out on the vegetative parts, leaves and roots of five genera of subtribe Coelogyninae and their variation and taxonomic significances are identified. Only few anatomical studies have been done in Nepal and limited work has been done by scientist from India and abroad on the anatomy of orchids. Previous anatomical studies on different subtribes of family Orchidaceae have shown the usefulness of vegetative anatomical characters in phylogenetic analysis of the group.

From the present study, some variations among the genera and the species are elicited. Species of *Coelogyne*, *Pholidota* and *Otochilus* consist of tetracytic stomata whereas *Panisea demissa* and *Pleione spp.* have anomocytic stomata and *Panisea uniflora* comprises both anomocytic and tetracytic stomata. In leaves, hypodermal cells are present only on *Coelogyne* and absent in other genera. Number of phloem patches varies from one to five among the species of *Coelogyne* and *Otochilus* in the midrib vascular bundles but only one phloem patch is present in other genera. In root, velamen cells show similar type of arrangements except in *C. corymbosa*. U-shaped thickening of endodermal cells are present on species of *Coelogyne*, *Otochilus*, *Panisea* and *Pholidota* whereas absent in *Pleione*. The thickening of endodermal cells are uniform in *Pleione*. Number of xylem and phloem strands varies among species.

In an attempt to delineate the relationship among the species of subtribe Coelogyninae with cladistic approach, the present study has shown the demarcation of the genera as different clade in a cladogram. The genus *Pleione* has been separated from other four genera whereas *Pholidota* and *Otochilus* formed second group in a tree and *Coelogyne* and *Panisea* formed the other group. Hence, from the present study, it is evident that the anatomical characters are taxonomically and phylogenetically important.

