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# MORPHOLOGICAL VARIATION IN ENKIANTHUS DEFLEUS ( GRIFF. ) C. K. SCHNEID : LEAF ANATOMY AND POLLEN MORPHOLOGY

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## ABSTRACT

*Enkianthus deflexus* (Griff.) C. K. Schneid. is represented by three varieties, of which var. *deflexus* and var. *acuminatus* S. Panda & Sanjappa occur in India. Microscopic studies of leaf anatomy and pollen morphology of these two varieties have been summarized and compared in present paper.

**Key words**: *Enkianthus deflexus*, var. *deflexus*, var. *acuminatus*, Ericaceae, India.

## Introduction

The genus *Enkianthus* Lour. consists 17 species (Anderberg, 1994; Mabberley, 2008), among which *Enkianthus deflexus* (Griff.) C. K. Schneid. is the only species reported from India (Eastern Himalaya, Darjeeling, Sikkim and Arunachal Pradesh). *E. deflexus* is represented by three varieties viz., var. *deflexus*, var. *glabrescens* R.C. Fang and var. *acuminatus* S. Panda & Sanjappa

Detail investigations on the genus *Enkianthus Lour. have been* studied by Anderberg (1994), Ruizheng and Stevens (2005), Panda (2008) and Panda and Sanjappa (2006, 2011, 2014). Present investigation represents results obtained on microscopic studies of leaf anatomy and pollen morphology of the two varieties of *E. deflexus* (var. *deflexus* and var. *acuminatus*) to establish exo-morphological varietal delimitation between them.

## Material and Methods

The plant material was collected in the month of May, 2016 from Sikkim and Arunachal Pradesh. Small cubical pieces (*ca*. 1 sq.cm) were excised from the base, middle and apical regions of leaf blade, treated with 5% KOH for 1224 hours. The pieces were dehydrated in ethanol, followed by staining with 57 % safranin, and mounted on microscope slide in DPX. The slide was examined under light microscope and Camera Lucida drawings were made.

For the studies on venation, mature leaves were immersed in 2 % NaOH solution until clear leaf lamina was obtained. After clearing, the pieces were dehydrated in ethanol, stained with 1% safranin and mounted on microscope slide in DPX.

The pollen slides were prepared as described by Erdtman (1952, 1969, 1986). Leaf samples and acetolysed pollen grains were subjected to Scanning Electron Microscopic observations. Observations were made with Hitachi S530 (SEM, Tokyo, Japan) in the high vacuum mode at an applied voltage of 15 KV.

## Results and Discussion

### Description:

***Enkianthus deflexus*** (Griff.) C. K. Schneid., Ill. Handb. Laubh. 2: 521. 1911; Rehder & E. H. Wilson in Sarg. Pl. Wilson. 1: 550. 1913; Hara in Hara *et al*., Enum. Fl. Pl. Nepal 3: 55. 1982; Rae in A. J. C. Grierson & D. G. Long, Fl. Bhutan 2 (1): 394. 1991; Anderb., *Nord. J. Bot*. 14 (4): 390. 1994. F. Ruizheng & P. F. Stevens in Wu et al. Fl. China 14: 244. 2005; S. Panda & Sanjappa, J. Jap. Bot. 86: 41. 2011; Panda & Sanjappa in Sanjappa & Sastry, Fasc. Fl. India 25: 160. 2014.

Stout and erect, 1.54 (6) m tall. Stem and branches glabrous; branches reddish or sometimes greenish when young, when older developing a smooth brownish-grey minutely longitudinally wrinkled bark. Leaves alternate or in pseudowhorls at the apices of the branches, 47 in each pseudowhorl, papery to chartaceo-coriaceous, lamina elliptic to ovate-elliptic, 27.5×12.5 cm, serrulate to serrulate-ciliate at margin, cuneate at base, acuminate at apex, pale greenish-pink, sparsely pubescent above, green with pinkish stripes, densely pubescent beneath; petioles spatulate, pink to pale green, 510 mm long. Inflorescence usually axillary to rarely terminal corymbs, often appearing as pseudoumbel, perulate, pendulous, 6-16-flowered; rachis greyish-white to light green, up to 10 cm long, glabrous or pubescent. Flowers pendulous, 45 cm long; pedicels light green, 34 cm long, deflexed, pubescent; bract 1, basal to subbasal, pale green with pinkish stripes, ovate-triangular, linear to linear-lanceolate, 19×1 mm. Calyx deeply 5-lobed, lobes equal, connate basally, light green, 35×11.5 mm. Corolla narrowly to broadly campanulate to rarely urceolate-campanulate, blood red, pinkish-red or pale green with pinkish veins, 1015 mm long, 415 mm diameter, glabrous, lobes broadly ovate, *c*. 2 mm long, 58 mm diam., rounded or obtuse at apex. Stamens 10, usually in 2 whorls (5+5), outer longer *c*. 3.5 mm long, inner shorter *c*. 2.5 mm long or rarely in a single whorl, *c*. 4.5 mm long, loosely epipetalous; filaments light green, longer *c*. 2.5 mm long, shorter *c*. 1.5 mm long or if in single whorl *c*. 3 mm long, slender, dilated at base, papillose, pilose; anther lobes light green to blackish-brown, oblong to obovate, 11.5 mm long, granular, each lobe with single slightly recurved warty awn of 11.5 mm long. Pistil *c*. 1 cm long; ovary ovoid, *c*. 4 mm long, *c*. 3 (base)1.5 (apex) mm diam., densely puberulous, 5-locular; style light green, *c*. 5 mm long, slender, pilose; stigma capitate. Fruit loculicidally 5-valved capsule, globose to oblong, 57×4 mm including *c*. 5 mm long persistent style with 20 35 mm long, pilose and curved pedicel, glabrous or pubescent. Seeds numerous, ellipsoid, *c*. 2 × 1.5 mm, scariose, winged.

Key to the varieties of *Enkianthus deflexus* **(Griff.) C. K. Schneid.**

1a. Calyx lobes acute, *c*. 1 mm long; bracts ovate-triangular, 1-2 mm long; stomata paracytic to brachyparacytic, epidermal walls in surface view sinuous; pollen 3-colporoid, exine surface finely rugulate with minute granules to rarely rugulate-psilate; colpi 4.110.8 μm long

**1a.** var. ***deflexus***

1b. Calyx lobes long acuminate, *c*. 5 mm long; bracts linear to linear-lanceolate, *c*. 9 mm long; stomata desmocytic, epidermal walls in surface view straight; pollen 2-colporoid, exine surface psilate to faintly rugulate; colpi 14.4 18 μm long**……………………………1b.**var. ***acuminatus***

**1a.** var. ***deflexus***

*Rhodoraceae deflexa* Griff., Itin. pl. Khasyah mts. (Posthumous Papers 2): 148. 1848. *Rhodora deflexa* Griff., Itin. pl. Khasyah mts. (Posthumous Papers 2): 187. 1848. *Meisteria deflexa* (Griff.) Nakai, *Bot. Mag.* Tokyo 38: 39. 1924. Type: Bhutan, between Jaisa and Tongsa, 90009500 ft, *Griffith* 717 and 969 (K, cibachrome image!). *Enkianthus himalaicus* Hook. f. & Thomson in Hook., *J. Bot. Kew Gard. Misc.* 7: 125, t. 3, 1855. Type: India, Sikkim, Lachen, 8000 10000 ft, May, 28, *J. D. Hooker s.n.* (K, Cibachrome image!). *E. indicus* M. R. Debta & H. J. Chowdhery, *J. Non-Timber For. Prod.* 16 (2): 173. 2009. Type: India, West Bengal, Darjeeling, Tonglu to Gairibas, 2900 m, 29 May 2006, *M. R. Debta* 40542 (holo. CAL!). (Figs. 1AC; 2A, C-D; 3A-D; 4A-B, D-F; 5A-B, D) **(Fig. 1)**

**Distribution**: INDIA: Eastern Himalaya (Sikkim, Darjeeling in West Bengal and Arunachal Pradesh); Eastern Nepal, Bhutan, South western China; Northern Myanmar.

**Habitat**: This species grows gregariously in loose rocky soils, rarely in rock crevices at altitudes ranging from 2100 3450 m in association with *Gaultheria stapfiana*, *Lyonia villosa* and *Pieris formosa*.

**Flowering***:* AprilMay. ***Fruiting****:* Late MayJuly.

**Leaf-stomata**

Stomata are distributed more or less evenly over the entire abaxial leaf surface in between the veins, but generally not over the finer veins and main veins. Epidermal walls in surface view is usually sinuous. The investigated variety in three different populations shows stomatal diversity from paracytic to brachyparasitic. Stomatal dimension ranges from 20.6 µm×32 µm to 27.4 µm×20.6 µm. (Figs. 2A, C-D; 3A-D)

Leaf Areoles (Vein-Islets) varies from pentagonal, hexagonal, polygonal to irregular in shape. Vein-islets size ranges from 312 µm×192 µm to 1380 µm×512 µm. (Table 2, Figs. 4, A-B, D-F)

**Pollen Morphology**

Pollen grains are in monads, 3-zonocolporate, prolate-spheroidal, oblate-spheroidal to sub-oblate in shape, 3-colporoid, 20 µm**×**19 µm to 22.6 µm**×**21.5 µm. Exine surface varies from finely regulate to faintly rugulate-psilate. (Table 3, Figs. 5A, B, D).

**1b.** var. ***acuminatus*** S. Panda & Sanjappa, *Indian J*. *For*. 29 (3): 277279. 2006; S. Panda & Sanjappa in Sanjappa & Sastry, Fasc. Fl. India 25: 162. 2014. (Figs. 1D, 2B, 4C, 5C). Closely allied to var. *deflexus* from which it differs in having 56 mm long calyx lobes always with acuminate apex, 79 mm long linear or linear-lanceolate basal bract and 89 cm long densely puberulous rachis.

***Distribution***: India (Eastern Himalaya: Sikkim), endemic.

***Habitat***: This variety grows in rocky slopes along the river bank in association with *Lyonia ovalifolia, Pieris formosa* and *Rhododendron triflorum* at an altitude of *c*. 2050 m.

***Flowering***: April. ***Fruiting***: JuneJuly.

**Leaf stomata:**

The study of Light Microscopic (45X, 100X) as well as Scanning Electron Microscopic stomatal architecture includes number, form and arrangement of specialized epidermal cells associated with the stomatal guard cells. The stomata are uniformly distributed in abaxial surface only, they are widely separated from each other by epidermal cells. The investigated variety shows only one type, *desmocytic*. The average stomatal dimension is 27.7 µm×19.9 µm in apex, middle and base. The length slightly varies from 25.8 µm29.7 µm and breadth 19.8 µm20.1 µm. Epidermal cells are usually isodiametric-polygonal to irregular. The epidermal walls in surface view are straight. (Fig. 2B) Leaf Areoles (Vein-Islets) varies from Pentagonal, hexagonal to polygonal in shape. Vein-islets ranges from 231 µm×153µm to 692 µm×346 µm (Fig. 4C).

**Pollen Morphology**

Pollen grains are in monads, 3-zonocolporate, prolate spheroidal in shape, 2-colporoid, 20 µm x 19 µm (20 24.4 x 23.4 25.9). Exine surface psilate to faintly regulate. (Fig. 5C).

*Enkianthus deflexus* (Griff.) C. K. Schneid. showed variations in leaf, stomata and pollen morphology. The leaf anatomy and pollen morphological data support validity of var. *acuminatus viz*., desmocytic stomata, larger stomatal dimension, strictly straight epidermal walls in surface view, larger P/E value of pollen and larger exine with psilate to faintly rugulate surface (SEM). On the other hand, var. *deflexus* from three distinct populations of Eastern Himalaya (Lachen, Lachung-Yumthang and Talle Valley populations) studied in this work shows minor variations leading these populations under the same variety.

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**Table 1. Source of leaf anatomy and pollen morphological materials of *Enkianthus deflexus* (Griff.) C.K. Schneid. var. *deflexus* and var. *acuminatus* S. Panda & Sanjappa**

| Name of species | Collector and Number | Voucher information (Herbarium acronym) |
|---|---|---|
| *Enkianthus deflexus* (Griff.) C.K. Schneid.<br>var. *deflexus*<br>1. Sikkim populations (Lachen) | *S. Panda* 29904; 29907; *R. S. Rao* 423; *P. Chakraborty* 2291; *S. Panda* 137 .<br>(**5 samples**) | India: North Sikkim, Lachen, 2950 m, 07.05.2001, *S. Panda* 29904 (CAL); Lachen to Chhaten, 2700 m, 08.05.2001, *S. Panda* 29907 (CAL); Lachen to Chhaten, *R. S. Rao* 423 (BSHC); Lachen, 25.08.1982, *P. Chakraborty* 2291 (BSHC); Near Lachen Monastery, 3020 m, 17.05.2016, *S. Panda* 137 (MAC). |
| 2. Sikkim population (Lachung-Yumthang) | *S. Panda* 29994; 29993; *315*.<br>**(3 samples**) | India: North Sikkim, Yakche to Phuney, Lachung-Yumthang Road, 3100 m, 01.06.2002, *S. Panda* 29994; Yumthang to Lachung, 3400 m, 01.06.2002, *S. Panda* 29993; Lachung to yumthang, 18.05.2016, *S. Panda* 315 (MAC). |
| 3. Arunachal population (Talle Valley) | *D. Banik* 27977; *Haridasan* 1111; *Haridasan* & *C. Barooa* 6904.<br>(**3 samples**) | India: Arunachal Pradesh, Lower Subansiri district, Talle valley, 2500 m, May, 2002, *D. Banik* 27977 (CAL); Lower Subans iri district, Talle Valley, 10.06.1984, *Haridasan* 1111 (APFH); Talle Valley, 17.06.1994, *Haridasan* & *C. Barooa* 6904 (APFH). |
| var. *acuminatus* Panda & Sanjappa<br>4. Sikkim populations (Bitchu-Lachung) | *S. Panda* 30080; *S. Panda* 327.<br>(2 **samples**)<br>***<u>Only natural habitat</u>*** | India: North Sikkim, Lachung to Chungthang, near Bitchu bridge, *c*. 2050 m, 03.05.2001, *S. Panda* 30080; on the way to Chungthang from Lachung, near Bitchu bridge, *c*. 2050 m, 18.05.2016, *S. Panda* 327(MAC). |

**Table 2. Comparative account of leaf anatomy (leaf-stomata and leaf vein-islets patterns) between three different populations of *Enkianthus deflexus* var. *deflexus* and one population of var. *acuminatus* .**

| Criteria | var. *deflexus* | | | var. *acuminatus* |
|---|---|---|---|---|
| | Lachen population (Sikkim Himalaya) | Lachung-Yumthang population (Sikkim Himalaya) | Talle valle population (Arunachal himalaya) | Lachung population (Sikkim) |
| **Leaf-stomata** | Paracytic. | Paracytic. | Brachyparacytic. | Desmocytic. |
| Stomatal Dimension | 21.4 µm×16.5 µm. | 27.4 µm×20.6 µm. | 20.6 µm×32 µm | 27.7 µm×19.9 µm |
| Epidermal cells | polygonal to irregular, walls sinuous in surface view. | polygonal to irregular, walls sinuous in surface view. | irregular, walls sinuous in surface view. | polygonal to irregular, walls straight in surface view. |
| Dimension of epidermal cells | 19.6 µm×9.6 µm | 25.6 µm×13.4 µm | 29.7 µm×16.9 µm | 24.4 µm×12.4 µm |
| **Leaf areole** (vein-islets) Shape: | polygonal to irregular in shape. | polygonal to irregular in shape. | penta- to polygonal in shape. | penta-, hexa - to polygonal. |
| Size: | Larger areole : 612 µm × 322 µm. Smaller areole: 312 µm × 192 µm. | Larger areole: 692 µm × 276 µm. Smaller areole: 379 µm × 192 µm. | Larger areole: 1380 µm × 512 µm. Smaller areole: 692 µm × 347 µm. | Larger areole: 692 µm × 346 µm. Smaller areole: 231 µm × 153µm. |
| Vein-islets: | 8 (average) per sq. mm. | 7 (average) per sq. mm. | 2 (average) per sq. mm. | 6 (average) per sq. mm. |
| Vein endings: | 62 (average) per sq. mm. | 54 (average) per sq. mm. | 15 (average) per sq. mm. | 37 (average) per sq. mm. |
| Vein ends: | Spoon-like bulbous | Spoon-like bulbous | bulbous-acute. | Spoon like bulbous to bulbous-acute. |

**Table 3. Comparative account of Pollen morphology between three different populations of *Enkianthus deflexus* var. *deflexus* and one population of var. *acuminatus***

| Criteria | var. *deflexus* | | | var. *acuminatus* |
|---|---|---|---|---|
| | Lachen population (Sikkim Himalaya) | Lachung-Yumthang population (Sikkim Himalaya) | Talle valle population (Arunachal himalaya) | Lachung population (Sikkim) |
| Pollen grains<br><br>Shape:<br><br>Dimension: | Monads<br>3-zonocolporate,<br>prolate-spheroidal<br>3-colporoid<br>20 µmx19 µm (18 –<br>20x19 – 2). | Monads<br>3-zonocolporate,<br>oblate spheroidal<br>3-colporoid<br>22.6 µmx21.5 µm (21.6–23.7x20.2–22.8). | Monads<br>3-zonocolporate,<br>sub-oblate<br>3-colporoid<br>20 µmx19 µm (20.8–21.7x17– 19) | Monads<br>3-zonocolporate,<br>prolate spheroidal<br>2-colporoid<br>20µmx19µm (20 – 24.4x23.4– 25.9). |
| P/E ratio | 1.05 | 0.95 | 0.84 | 1.10 |
| Exine | Tectate<br>1.24 – 1.28 µm thick<br>surface finely rugulate with minute granules | Tectate<br>1.20 – 1.24 µm thick, surface finely rugulate with minute granules. | Tectate<br>1.4 – 1.6 µm thick, surface faintly rugulate-psilate. | Tectate<br>2.4 – 2.8 µm thick, surface psilate to faintly rugulate. |
| Colpi | distinct, 3.<br>4.5 µm – 8.1 µm long (L)<br>0.9 µm – 2.7 µm width (P).<br>L/P = 0.31, colpus margin slightly wavy, acute to tapering toward s ends. | distinct, 3.<br><br>4.1 µm – 7.1 µm long (L)<br>0.9 µm – 2.1 µm width (P).<br>L/P = 0.26, colpus margin slightly wavy, acute to tapering towards ends. | distinct, 3.<br><br>9 µm – 10.8 µm long (L)<br>0.7 µm – 1.7 µm width (P).<br>L/P = 0.55, colpus margin smooth, acute to tapering towards ends. | distinct, 3.<br><br>14.4 µm – 18 µm long (L)<br>0.7 µm – 1.7 µm width (P).<br>L/P = 0.65, colpus margin smooth, acute to tapering towards ends. |

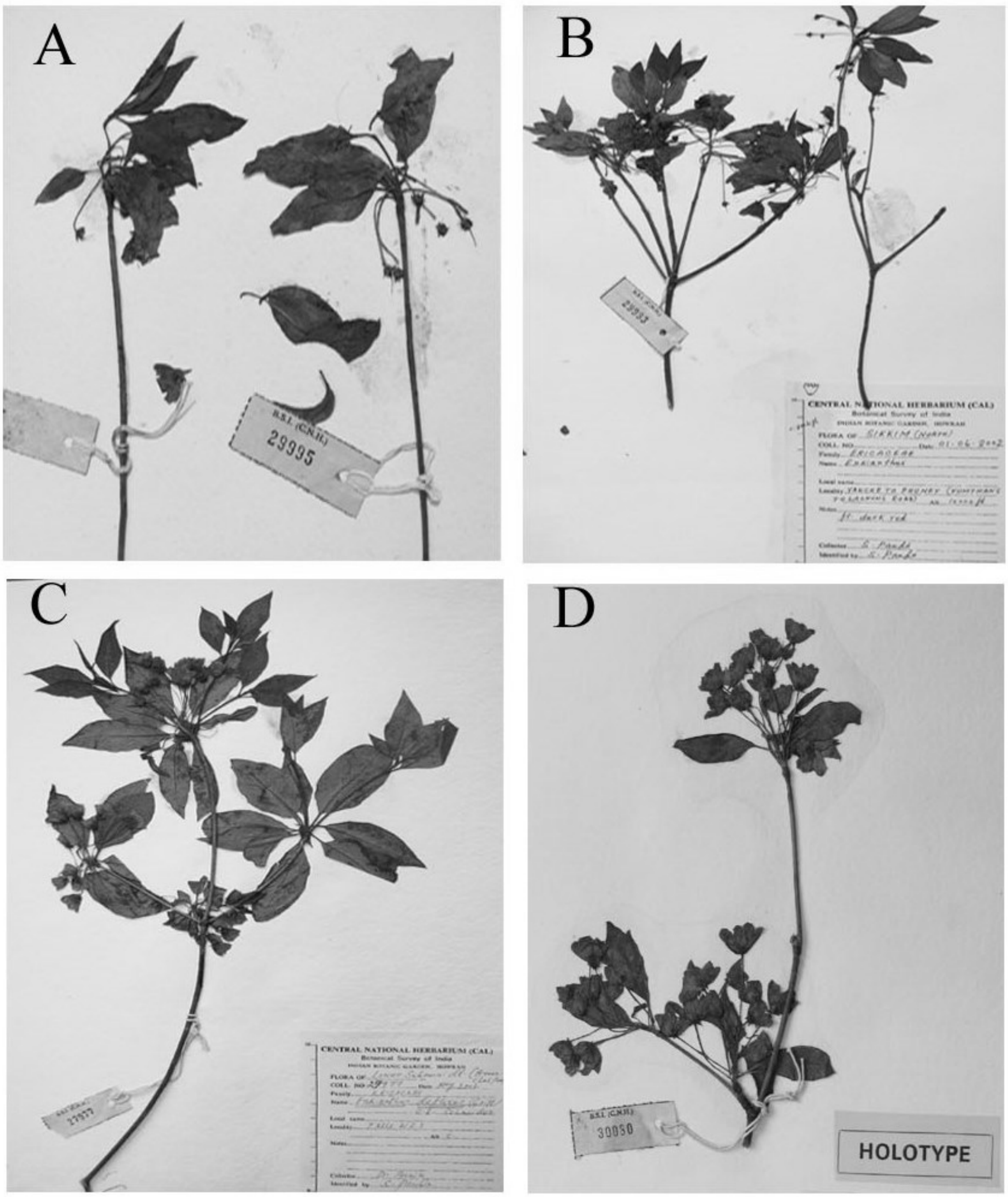


**Fig. 1.** A - C. populations of *Enkianthus deflexus* (Griff.) C.K.Schneid. var. *deflexus* (A. Lachen in Sikkim Himalaya, B. Lachung-Yumthang in Sikkim Himalaya, C. Arunachal Himalaya); D. var. a*cuminatus* Panda & Sanjapppa (Lachung in Sikkim).

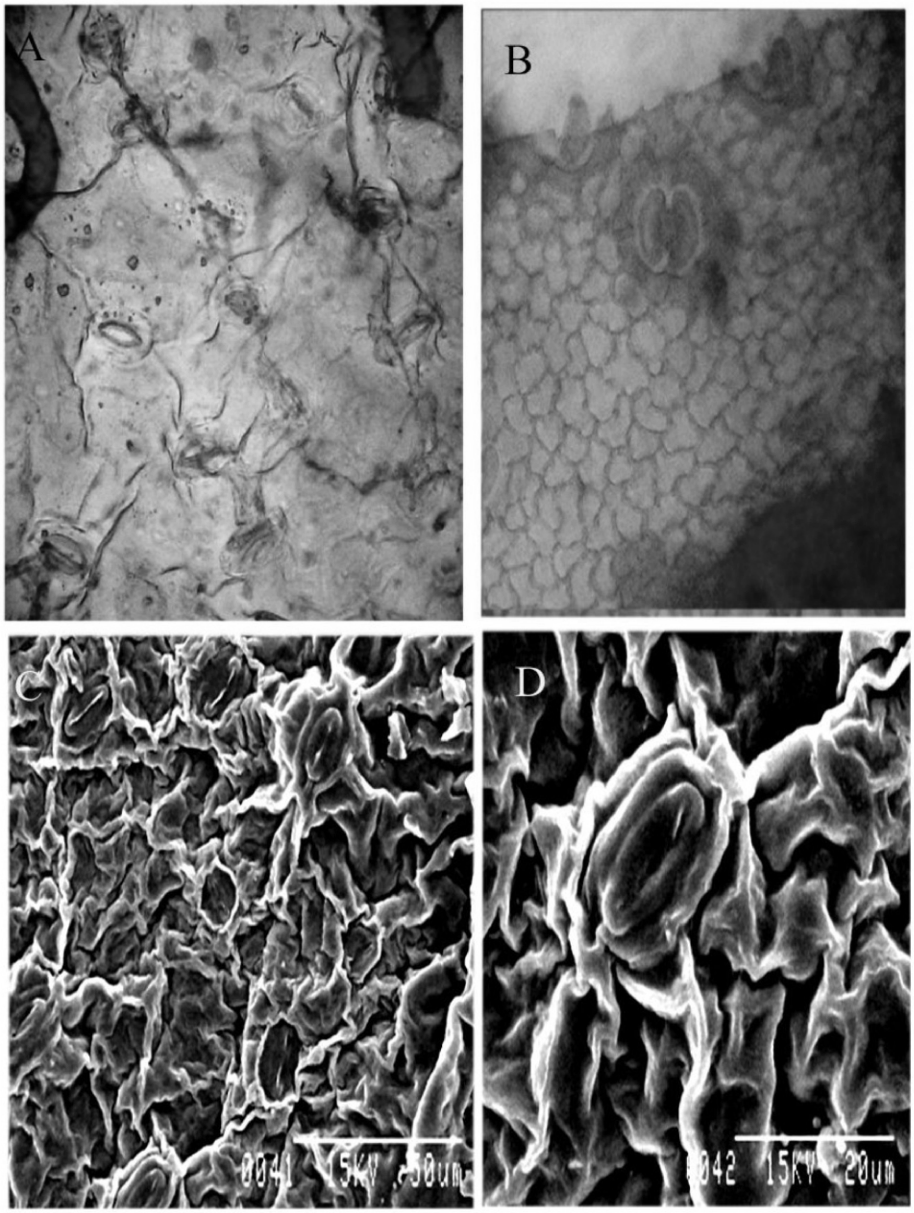


Fig. 2. Stomatal complex of *Enkianthus deflexus* (Griff.) C. K. Schneid. complex: A. stomatal complex of var. *deflexus* (LM, 40X, Lachen in Sikkim); B. stomatal complex of var. *acuminatus* (LM, 40X, Bitchu of Lachung in Sikkim); C. stomatal complex of var. *deflexus* (SEM, 1000X, Lachung); D. Stoma magnified (SEM, 4000X, Lachung).

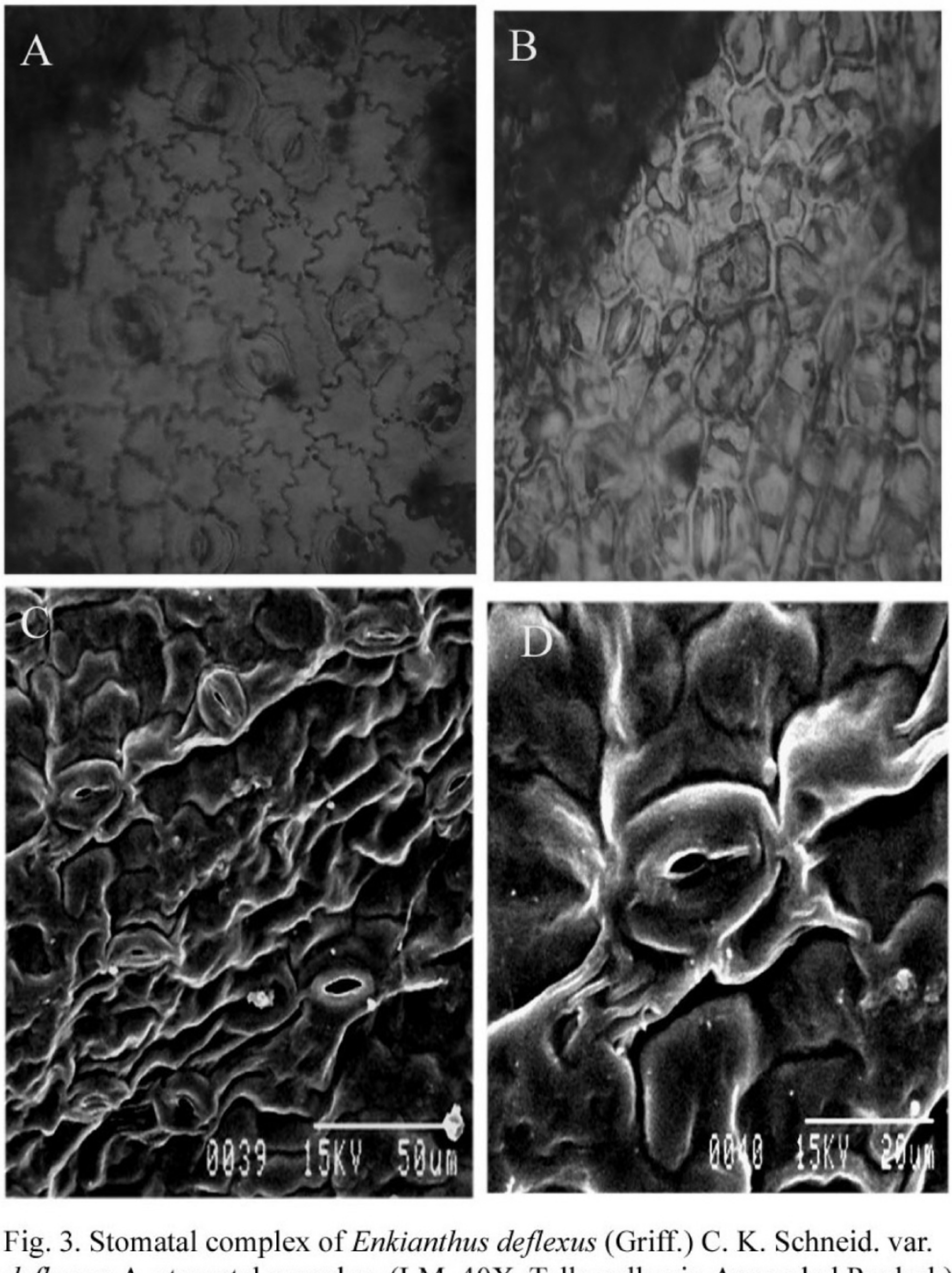


Fig. 3. Stomatal complex of *Enkianthus deflexus* (Griff.) C. K. Schneid. var. *deflexus*: A. stomatal complex (LM, 40X, Talle valley in Arunachal Pradesh); B. stomatal complex (LM, 40X, Yakche of Lachung in Sikkim); C. stomatal complex (SEM, 1000X, Arunachal Pradesh); D. Stoma magnified (SEM, 4000X, Lachen).

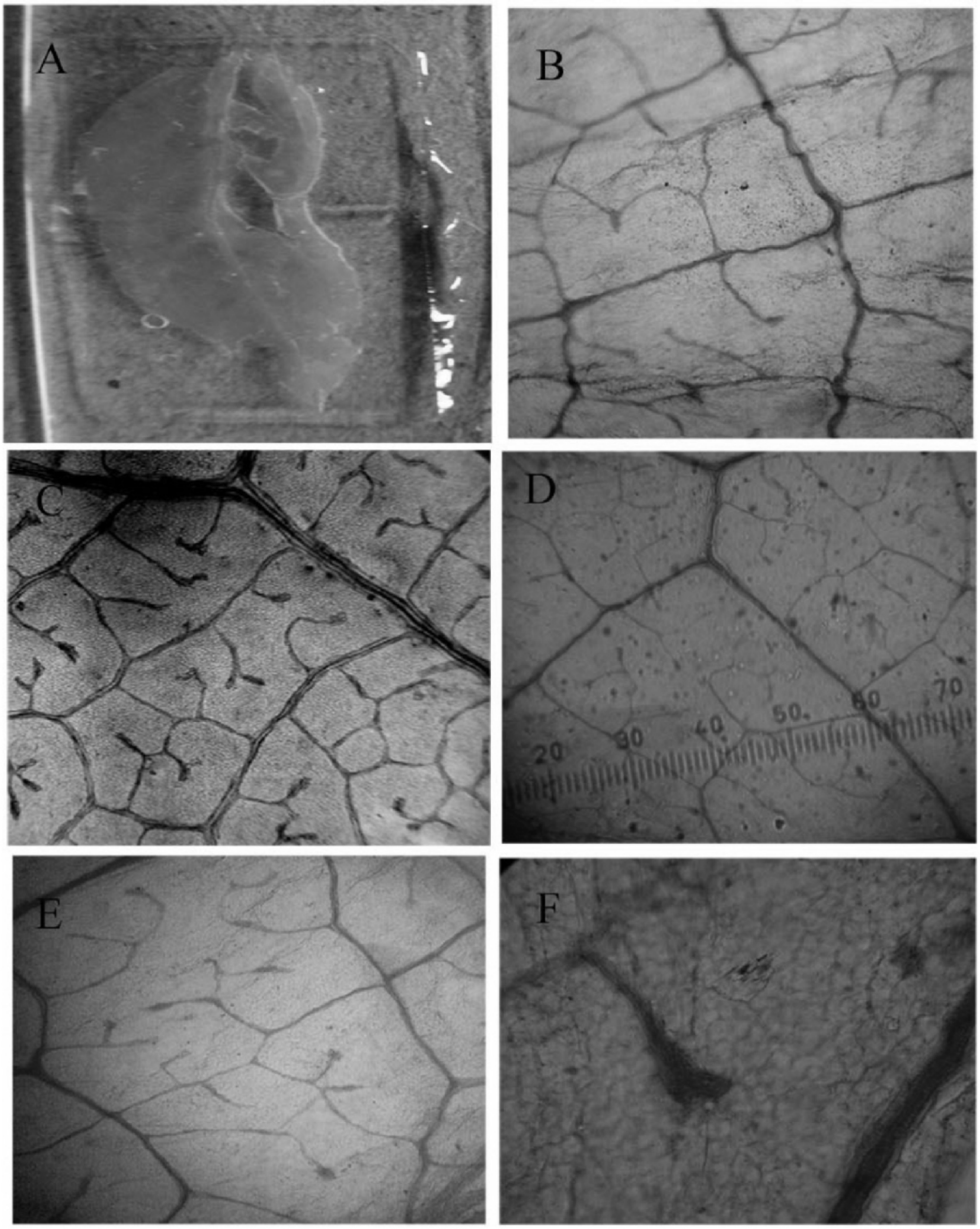


**Fig. 4.** Leaf areolar patterns of *Enkianthus deflexus* (Griff.) C. K. Schneid. complex: A. entire leaf venation of var. *deflexus* (Lachen in Sikkim); B. vein islets of var. *deflexus* (LM, 5X-5X, Lachen in Sikkim); C. vein islets of var. *acuminatus* (LM, 5X-10X, Bitchu of Lachung in Sikkim); D. vein islets of var. *deflexus* (LM, 5X-10X, Yakche, Lachung in Sikkim); E - F. vein islets of var. *deflexus* (E: LM, 5X-10X; F: 10X-40X, Talle Valley, Arunachal Pradesh).

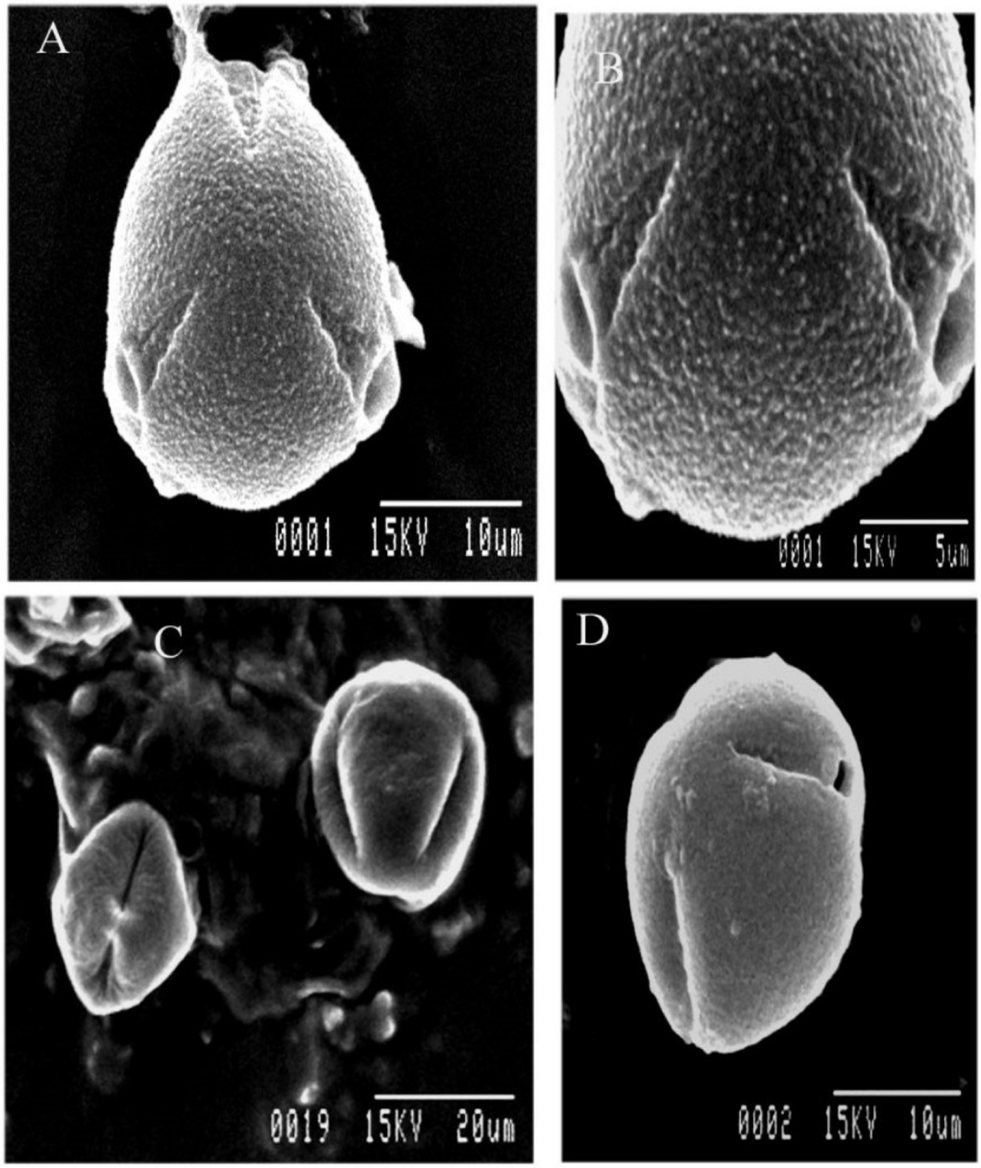


Fig. 5. Pollen morphology of *Enkianthus deflexus* (Griff.) C. K. Schneid. complex: A. monad of var. *deflexus* (SEM, 1000X, Lachen in Sikkim); B. part magnified of A (SEM 4000X); C. monad of var. *acuminatus* (SEM, 1000X, Bitchu of Lachung in Sikkim); D. monad of var. *deflexus* (SEM, 1000X, Talle WLS in Arunachal Pradesh).