



## VARIATION IN *RHODODENDRON ARBOREUM* SM. COMPLEX (ERICACEAE): INSIGHTS FROM EXOMORPHOLOGY, LEAF ANATOMY AND POLLEN MORPHOLOGY

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**Abstract.** *Rhododendron arboreum* Sm., placed under the genus *Rhododendron* L. in the family Ericaceae Juss. consists of c. 1000 species, of these c. 102 species occur in India. *R. arboreum* Sm. is restricted to a few South Eastern Asian countries. In India, the species is distributed in the Himalayas, North Eastern India and hill tops of South Western Ghats. Detailed investigations of the genus were studied by several workers but nobody studied variation in *R. arboreum* complex. A few workers described pollen morphology of Ericaceae including *Rhododendron*, but they did not study different subspecies of *R. arboreum*. No detailed investigation on leaf anatomy was also reported. The purpose of the present study is to evaluate the contribution of leaf anatomy and pollen morphology along with herbarium and field based exomorphological data to delimit infraspecific variations in *R. arboreum* complex.

**Key words:** *Rhododendron arboreum*, Ericaceae, variation, exomorphology, leaf anatomy, pollen morphology

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### Introduction

*Rhododendron arboreum* Sm., commonly called as Lali Guras is placed under the subsection *Arborea* Sleumer, section *Ponticum* G. Don, subgenus *Hymenanthes* (Bl.) K. Koch and the genus *Rhododendron* L. in the family Ericaceae Juss. The species was first described and named by SMITH (1805: 9). The genus *Rhododendron* L. consists of c. 1000 species (MINGYUAN *et al.* 2005; MABBERLEY 2008). However, CRAVEN *et al.* (2008: 435-442) reported a range between 600-1000 species in the world as they mentioned “this number range depending upon the breadth of specific variation accepted by individual workers”, of these c. 102 species occur in India by BHATTACHARYYA & SANJAPPA (2014: 9).

*R. arboreum* Sm. is restricted to a few South Eastern Asian countries viz India, Nepal, Bhutan, Sri Lanka, South Western China, Northern Myanmar, Northern Thailand and Northern Vietnam. In India, the species is distributed in the Himalayas, North Eastern

India and hill tops of South Western Ghats (Tamil Nadu & Kerala).

Detailed investigations of the genus were studied by several workers like CLARKE (1882: 493-498), CHAMBERLAIN (1982: 328-332), PRADHAN & LACHUNGPA (1990: 65), LONG (1991:372), CHAMBERLAIN *et al.* (1996:1-184), KRON *et al.* (2002: 335-423); MINGYUAN *et al.* (2005: 260-455) and BHATTACHARYYA (2007: 131-138).

NAIR & KOTHARI (1985: 1-7) as well as PARIA & PAL (1990: 95-104) described pollen morphology (LM & SEM) of some Indian Ericaceae including a few species of *Rhododendron*, but they did not study different populations of *R. arboreum*, rather they studied based on herbarium material from a single collection. Similarly, VASANTHY & POCKOCK (1987: 213-245) studied pollen tetrads of a few South Indian Ericaceae including *R. nilagiricum*, but they did not study other subspecies of *R. arboreum*. Meanwhile, ZHANG *et al.* (2009: 123-138) studied pollen morphology (LM & SEM) of 80 taxa of *Rhododendron* subgen.

*Tsutsusi*, but they did not include *R. arboreum* Sm. Similarly, SARWAR & TAKAHASHI (2013: 185-199) and PARK & SONG (2010: 663-672) studied pollen grains of 40 taxa of *Rhododendron* and its closely related genera and pollen morphology of 11 species of *Rhododendron* in Korea respectively, but they did not include *R. arboreum* like ZHANG *et al.* (2009). No detailed investigation on leaf anatomy (leaf-stomata, leaf areole patterns, vein endings) were reported. Researchers like NIEDENZU (1890: 134-263), COX (1948: 493-498) and STEVENS (1971: 1-53) contributed a little works on leaf-stomata, vein islets and vein endings.

The purpose of the present study is to evaluate the contribution of leaf anatomy (LM) and pollen morphology (both LM & ESEM) along with herbarium and field based exomorphological data to delimit infraspecific variations in *R. arboreum* complex.

### Material and methods

The present work is the outcome of detailed light microscopic (LM; Olympus, Tokyo) as well as environmental scanning electronic microscopic (ESEM; FEI Quanta-200 MK2, Leiden) studies of leaf stomata, leaf areolar pattern (vein islets and vein endings) and pollen morphology of different populations of *R. arboreum* complex based on Indian (live as well as herbarium materials in CAL, BSIS, ASSAM & Barasat Govt College (BGC) herbaria), Nepal, Bhutan, China, Sri Lanka and Myanmar materials (duplicate herbarium materials in CAL & BSIS). This work was carried out partly in the Taxonomy and Biosystematics Laboratory, Barasat Government College and partly in the Angiosperm Taxonomy & Ecology Laboratory, Darjeeling Government College. All measurements are given in metric system. The dimensions "D", "(d)" and "2f" corresponding to the tetrad diameter, diameter of individual pollen grains and colpi lengths respectively were measured according to OLDFIELD (1959: 37). These pollen measurements are based on at least 10 grains from each specimen.

**Methodology for stomatal study.** Mature leaves were obtained from live specimens

collected during field tour in Arunachal Pradesh and Meghalaya as well as from the herbarium specimens (CAL, ASSAM, BSIS and BGC herbaria). Small cubical pieces (c. 1 cm<sup>2</sup>) were excised from the base, middle and apical regions of the blade. Several existing methods viz 10% HNO<sub>3</sub>-boiling for 10 minutes, 5% KOH overnight (12-24 hours) treatment without boiling and with boiling were done. Pieces were ringed in sterilized water until clear. After clearing, pieces were dehydrated in an ethanol series followed by staining with 10% safranin and mounted onto microscope slide in DPX (pieces of basal, middle and apical regions in one slide). The slide was examined under Olympus (Tokyo, Japan) light microscope using ×10, ×40 and ×100 objectives and drawings were made with the help of camera lucida. The descriptive terminology follows METCALFE & CHALK (1950:1-806), DILCHER (1974: 1-53), STACE (1965: 3-78; 1989: 78-80), FAHN (1997: 168) and CARPENTER (2005: 1595-1615).

**Methodology of leaf clearing for venation study.** Entire mature leaves were immersed in 2.5% NaOH solution until clear (closed condition). In the present study, most of the leaves were cleared after 15 days of NaOH treatment. After 7-12 days, these NaOH-treated leaf samples were again immersed in 2.5% NaOH solution for 2-3 days followed by 1 drop chloral hydrate treatment overnight. Leaf samples were then washed in distilled water. After clearing, pieces were dehydrated in an ethanol series followed by staining with 1% safranin and mounted onto microscope slide in DPX (pieces of basal, middle and apical regions in one slide or entire leaf when small size). The descriptive terminology follows HICKEY (1973: 17-33) and DILCHER (1974: 1-53).

**Preparation of pollen slides.** The method used in this study was by ERDTMAN (1952: 1-539; 1969: 486; 1986: 553). The descriptive terminology follows ERDTMAN (1952, 1969, 1986) and SARWAR *et al.* (2006: 15-34).

**Slide preparation for ESEM.** Acetolysed pollen grains (following ERDTMAN 1952) were prepared for ESEM observation. Pollen grains at least from 10 flowers of each species were acetolysed and studied. Observations were

made with FEI Quanta-200 MK2 (Leiden, Netherlands) in the high vacuum mode at an applied voltage of 10 KV. For ESEM, above samples were mounted on the metallic stub using double stick tape.

## Results

***Rhododendron arboreum*** Sm., Exot. Bot. **1**: 9, t. 6, 1805; Hook. f., *J. Hort. Soc. London* **7**: 78, 92, 1852; C.B. Clarke in Hook. f., *Fl. Brit. India* **3**: 465. 1882; Tagg in J.B. Stev., *Sp. Rhodod.*: 14. 1930; Kanjilal in Kanjilal et al., *Fl. Assam* **3**: 152. 1939; Hara in Hara et al., *Enum. Fl. Pl. Nepal* **3**: 58. 1982; D.F. Chamb., *Notes Roy. Bot. Gard. Edinburgh* **39** (2): 328. 1982; Ghosh & Samaddar, *J. Econ. Taxon. Bot.* **13** (1): 206. 1989; Pradhan & Lachungpa, *Sikkim-Himalayan Rhododendr.*: 75. 1990; Long in Grierson & Long, *Fl. Bhutan* **2**: 372. 1991; Mingyuan et al., in Ruizheng & Chamberlain (eds.), *Fl. China* **14**: 368. 2005; Bhattacharyya, *Rev. Gen. Rhododendr. India* (Ph.D. thesis): 131. 2007; Bhattacharyya & Sanjappa in Sanjappa & Sastry, *Fasc. Fl. India* no. **25** (Ericaceae): 87-93. 2014.

The species is variable in respect with indumentums present or absent, lamina surface, margin, apex, flower colour, stamens length, style indumentum.

5 subspecies recognized (CHAMBERLAIN 1982).

### KEY TO THE SUBSPECIES

(based on exomorphology, leaf anatomy and pollen morphology)

1. Abaxial leaf with a unistrate compacted indumentum, if rarely bistrate, then adaxial surface rufous floccose; stomata variable paracytic, amphiparacytic, brachyparacytic to desmocyctic types; stomatal dimensions 16-21 × 12.5-21.5 µm; 300 µm long; tetrads 30.4-45.6 µm in diameter; exine surface reticulate with viscin threads ..... 2
- 1\*. Abaxial leaf with a fawn to light brown spongy tomentum, always unistrate; stomata variable paracytic, amphiparacytic, brachyparacytic to desmocyctic types; stomatal dimensions 15.5-17.5 × 15.5-17.5

µm; leaf areoles up to 176 µm long; tetrads 38.7-46.6 µm in diameter; surface reticulate with viscin threads ..... 3

2. Abaxial leaf surface with white to silvery compacted indumentum; stomata brachypara to desmocyctic besides para and amphiparacytic; stomatal dimension 16 × 12.3 µm; tetrad 30.4-45.6 µm in diameter ..... 1. subsp. *arboreum*
- 2\*. Abaxial leaf surface with fawn indumentums; stomata only amphiparacytic; stomatal dimension 21.5 × 21.5 µm; tetrad 38.5-42.6 µm in diameter ..... 2. subsp. *cinnamomeum*
3. Leaves strongly concave with bullate upper surface ..... 5. subsp. *zeylanicum*
- 3\*. Leaves with a ± plane, reticulate or rugose upper surface ..... 4
4. Leaf apex rounded, margins revolute, rugulose to rugose; stomata para and amphiparacytic; stomatal dimension 15.5 × 15.5 µm; tetrad 38.7-40.6 µm in diameter ..... 4. subsp. *nilagiricum*
- 4\*. Leaf apex acute, margins not recurved, not rugulose; stomata only paracytic; stomatal dimension 17.5×17.5 µm; tetrad 42.4-46.6 µm in diameter ..... 3. subsp. *delavayi*

### 1. subsp. *arboreum*

Figs 1-2.

Description based on duplicate herbarium specimens in CAL, BSIS & ASSAM which include all probable natural habitats of its distribution (Himalayas, North-Eastern India, Nepal, Bhutan, China & Myanmar) as well as live collections from Sikkim, Arunachal Pradesh and Nagaland.

**Type.** A plate accompanying the protologue, drawn from the plant seen near Srinagar (Kashmir) by Capt. Hardwicke in 1796 (Icono, CAL!). *R. puniceum* Roxb., *Hort. Beng.*: 33. 1814 & *Fl. India* **2**: 409. 1832. Type: North India, mountains north of Nohilkhund, *Hardwicke s.n. (n.v.)*. *R. windsorii* Nutt., *Hooker's J. Bot. Kew.*





**Fig. 1.** Habit photographs of *Rhododendron arboreum* subsp. *arboreum*: **A** – Salari forest, Arunachal Pradesh (S.Panda 111, BGC); **B**–Jabrang, Arunachal Pradesh (G. Panigrahi 61878, CAL); **C**–Lachung, N Sikkim (S.Panda 16, BGC).





**Fig. 1.** Continued. **D** – Lachung (*J.D. Hooker s.n.*, CAL); **E** – Tonglu, Darjeeling (*Anderson s.n.*, CAL); **F** – Shillong peak, Meghalaya (*S. Panda 177*, BGC).





**Fig. 2.** Habit photographs of *Rhododendron arboreum* subsp. *arboreum*: **A** – Uttarakhand (Strachey & Winterbottom 169, CAL); **B** – Himachal Pradesh (Lace 1307, CAL); **C** – Nepal (Scully s.n., CAL); **D** – Bhutan (Griffith 3487/2, CAL).



Fig. 2. Continued. E – China, Yunnan, (Henry 10983, CAL); F – China, Chumbi valley (Seawright s.n., CAL).

*Gard. Misc.* 5: 357. 1853. Type: Nepal, on the ridges and slopes of Ropprye, 7000-9000 ft, *Nuttall s.n.* (K, photo!).

**Vernacular names.** Pullasa (Sanskrit), Brons (Almora), Etok (Bhutia), Zalatni (Burmese), Cheu (Chamba), Burans (Hindi), Bras, Burans (Kumaon), Al-etok-Koong (Lepcha), Guras, Lal-guras, Laliguras, Bhorans, Dotial, Taggu (Nepali), Ardawal, Aru, Broa, Chacheon, Mandal (Punjabi), Baras (Bengali), Chhan, Chiu (Kashmiri), Tin-saw, Dieng-tin-thuin (Khasi).

**Description.** Lamina (6)8-15 × (2)3-5 cm, usually oblong-lanceolate, apex acute to rarely acuminate (*J. Scully* 44, Nepal, CAL!), adaxial surface reticulate, abaxial surface compacted, usually white to silvery indumentums; petioles 5-14 mm long. Flowers mostly 33 mm long and 32 mm across, occasionally 40 mm long (*K. Biswas* 9277, Sikkim, CAL!); pedicels 4-7 mm long, sparsely to densely pubescent. Corolla bright red to carmine, occasionally pink to white. Stamens 10, longer one 16-28 mm long (anther lobes c. 2 mm long in all cases). Ovary 4-7 mm long, style 18-24 mm long, glabrous.

**Distribution.** India: Himalayas (Jammu & Kashmir, Himachal Pradesh, Uttarakhand, Sikkim, West Bengal (Darjeeling) and Arunachal Pradesh), North-Eastern States (Meghalaya, Nagaland, Manipur, Mizoram); Nepal; Bhutan; South-Western China.

**Habitat.** Grows in dry as well as moist rocky slope in dense or open forests at altitudes ranging from (800)1500-2800(3400) m in association with *Gaultheria fragrantissima*, *G. seshagiriana*, *Leucothoe griffithiana* and *Rhododendron vaccinioides* at altitudes ranging in 2200-3200 m.

**Flowering.** March – May.

**Fruiting.** July – September.

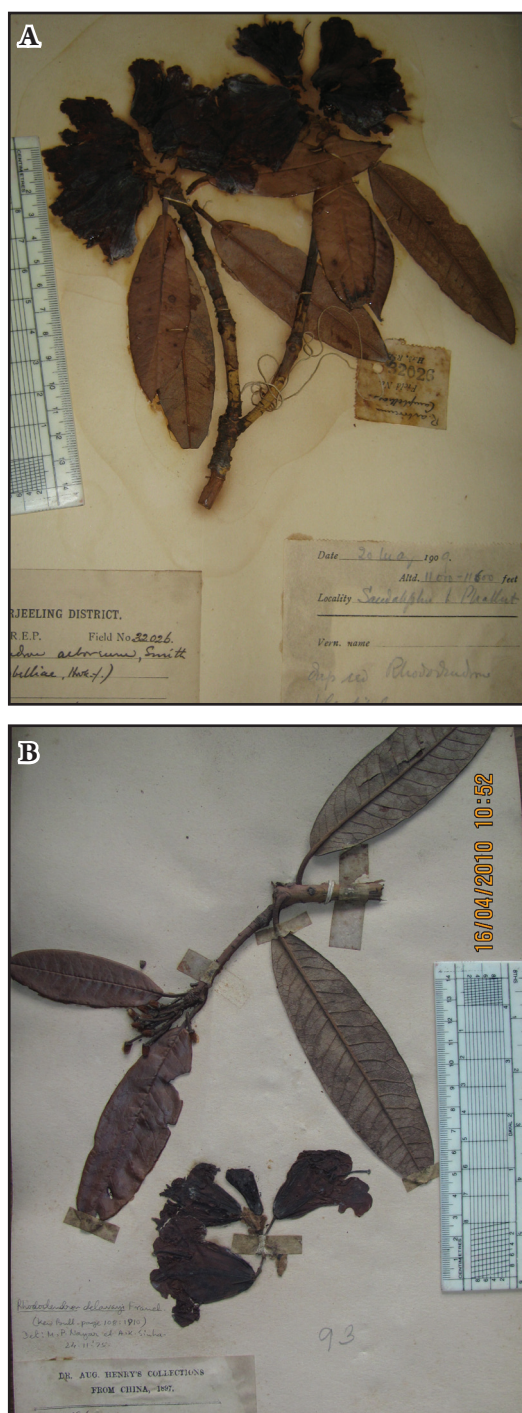
**Specimens examined in CAL otherwise mentioned.** **INDIA: EASTERN HIMALAYA: Arunachal Pradesh:** West Kameng district: New road from Bomdi-La to Rupa, 2484 m, 14.04.1957, *G. Panigrahi* 6893; Senge Dzong, ½ mile from Rest house, Kameng F.D., 3231 m, 21.05.1957, *R.S. Rao* 7656; Duphla hills, 7000 ft, 1874, *J.L. Lister s.n.*, Acc. No. 268259; Salari village, West Kameng district, 24.05.2010, 2200 m, *S. Panda* 197 (Barasat Govt. College Herbarium). **West Bengal (Darjeeling):** Senchel, 2250 m, 24/05/1909, *I.H. Burkill* 32143; Darjeeling, 2700 m, 08.05.1977, *J.L. Lister s.n.*, Acc. no. 268304; Sandakphoo, 3800 m,



18.06.1961, *Lepcha Jagat* 135; 24/11/1996; Sangachelling, 2300 m, 24.05.1909, *I.H. Burkill* 32143 (BSIS). **Sikkim:** Phadonchen, 3100 m, 19.05.1950, *Dr. K. Biswas* 9175; Lachung, 2750 m, 19.09.1892, *G.A. Gammie* 10. **NORTH-EASTERN STATES:** **Meghalaya:** Shillong peak, 1850 m, 05.04.1959, *H. Deka* 18301; Khasia hills, 05.04.1894, *G.A. Gammie* 374; Elephant falls, 1700 m, 23.12.1958, *G.K. Deka* 14082. **Nagaland:** Kohima, 5000-5500 ft, 23/04/1886, *Dr. D. Prain s.n.*, acc no. 268263. **Manipur:** Mao, 2100 m, Feb. 1882, *George Watt* 6126; Ching Sow, 2100 m, May 1882, *G. Watt* 5178. **WESTERN HIMALAYA:** **Uttaranchal:** Near Mussourie, May 1870, *G. King s.n.*, acc. no. 268345; Kedarnath north side, 3800 m, June 1893, *J.S. Gamble* 24429. **Himachal Pradesh:** Simla, 2300 m, Oct. 1907, *A. Meebold* 8676. **NEPAL:** Manichur, 2300 m, 18.03.1961, *Dr. P.N. Juwal & Party* 133; Chandragiri pass, 2350 m, 03.12.1907, *I.H. Burkill* 29808; On the way from Chitlong to Sisagan, 03/11/1950, *K.S. Srinivasan s.n.*, acc. no. 44626 (BSIS). **BHUTAN:** Thachu, 2500 m, 24.08.1963, *N.P. Balakrishnan* 1304. **CHINA:** Near Rima, 2450 m, 26.03.1950, *F. Kingdonward* 19245; Chumbi, 2700 m, April 1909, *G.L. Searight* 3. **MYANMAR:** Haka, 2500 m, 05.04.1939, *F.G. Dickason* 7386; Shan hills, upper Burma, Feb. 1892, *Abdul Huk* 135.

**Field notes.** The species is a variable from population to population in lamina size and shape, petiole length, flower size, corolla colour varying from blood red, crimson, pink, pinkish-white to white, pedicel indumentums and length, stamens length, pistil length and capsule diameter.

**Leaf stomata** (Fig. 4). The study of LM ( $\times 40$ ,  $\times 100$ ) stomatal architecture includes number, form and arrangement of specialized epidermal cells associated with the stomatal guard cells. **Distribution and orientation:** 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. **Type:** The investigated species shows different forms of paracytic stomata, mostly euparacytic to amphiparacytic (Sikkim population, *Panigrahi* 6385), occasionally brachiparacytic (Himachal population, *Burkill* 28678) to desmocyctic (Arunachal population, acc. no. 268259). **Dimensions of stomata:** The average dimension is  $16 \times 12.3 \mu\text{m}$  in apex, middle and base. The length varies from  $12.6 \mu\text{m}$  to  $19.4 \mu\text{m}$  and breadth – from  $10.5 \mu\text{m}$  to  $14.2 \mu\text{m}$  ( $19 \mu\text{m}$  in Sikkim population, *Panigrahi* 6385). **Size of guard cells:** The average dimension is  $10.6 \times 4.4 \mu\text{m}$ . **Size of epidermal cells:** The epidermal cells are usually pentagonal, isodiametric to rarely irregular,

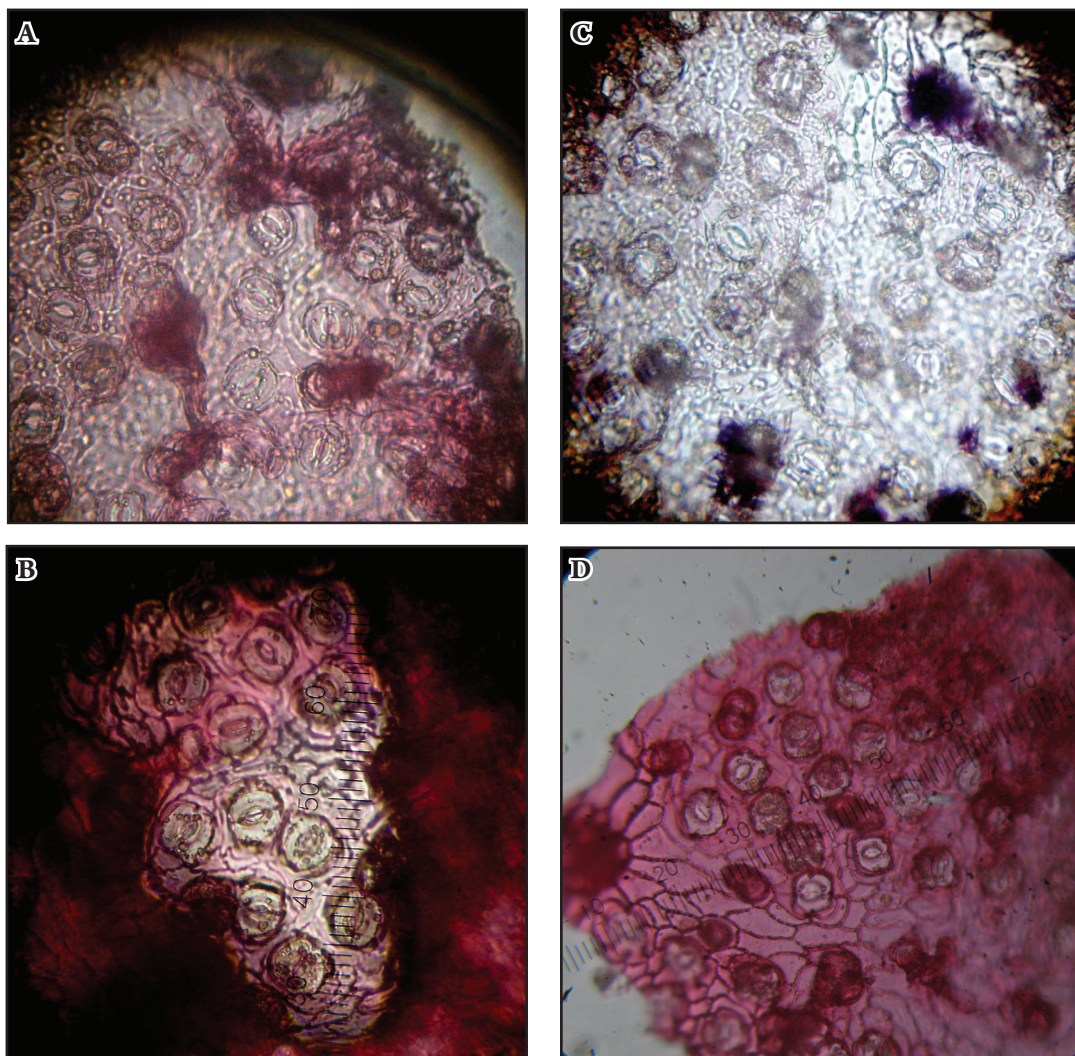


**Fig. 3.** Habit photographs of *Rhododendron arboreum*: **A** – var. *cinnamomeum* (Burkill 32026); **B** – subsp. *delavayi* (China, Henry 10983, CAL).



**Fig. 3.** Continued. **C-F** – subsp. *nilagiricum* (in CAL: **C** – Kerala, Pandurangan 62539; **D** – Tamil Nadu, 2071; **E** – Tamil Nadu, C.B. Clarke; **F** – Tamil Nadu, Subramanyam 5540).





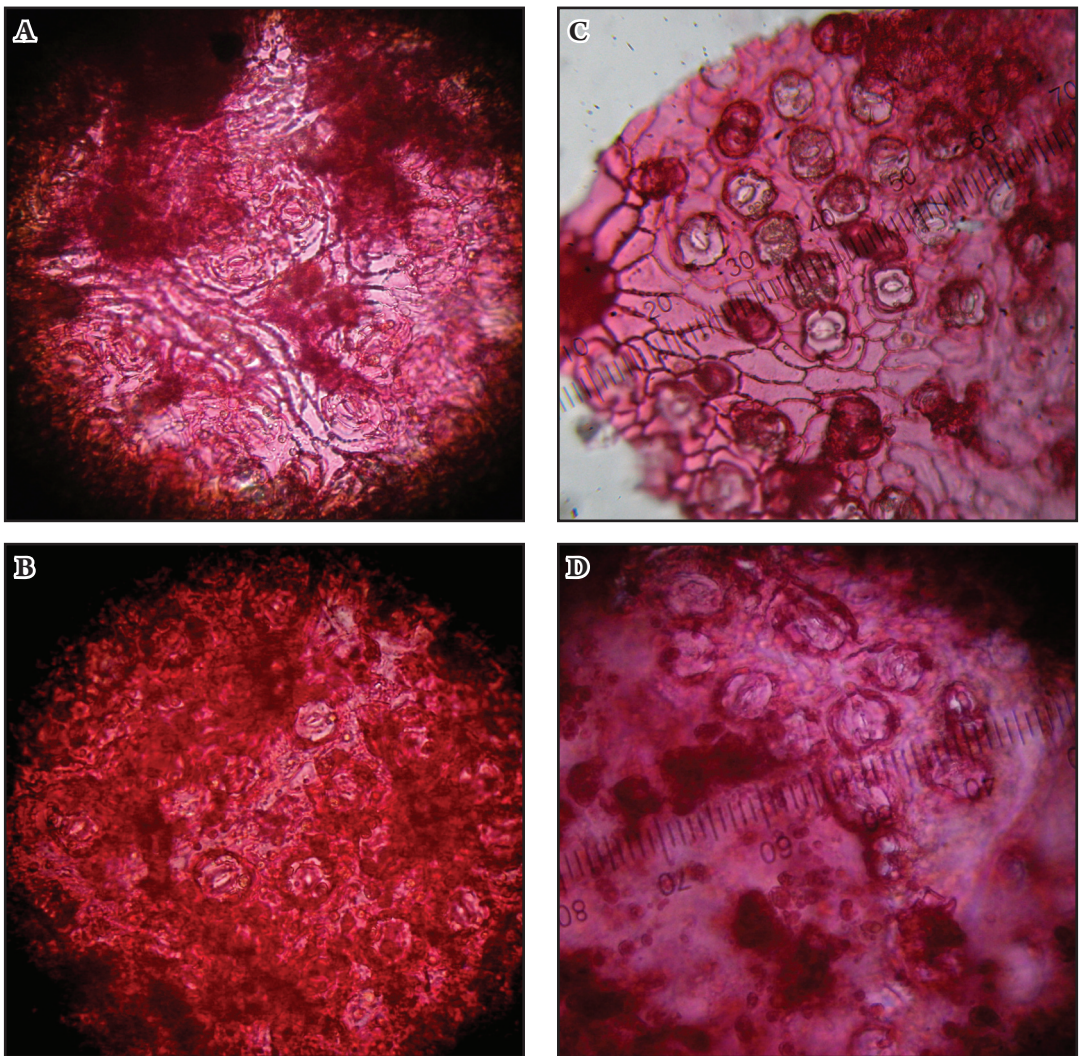
**Fig. 4.** Stomatal complex of *Rhododendron arboreum* subsp. *arboreum*: **A** – stomata (LM  $\times 40$  at apex, *Panigrahi* 15422, Arunachal Pradesh); **B** – stomata (LM  $\times 40$  at middle, *Townaud* 766, Sikkim); **C** – stomata (LM  $\times 40$  at base, *Burkill* 29808, Sikkim); **D** – stomata (LM  $\times 40$  at base, *Balakrishnan* 1304, Bhutan).

some are quadrangular, elongated to deltoid. There is no definite pattern of arrangement of epidermal cells. The epidermal walls in surface view are mostly straight to slightly wavy to sinuous (Sikkim and Meghalaya populations). The epidermal walls in the adaxial surface are also straight. *Dimensions of epidermal cells*: The maximum length is  $44.4\ \mu\text{m}$  and breadth is  $19.3\ \mu\text{m}$ . The minimum length is  $7.5\ \mu\text{m}$  and breadth is  $4.3\ \mu\text{m}$ .

**Leaf areolar (vein islets) pattern** (Figs 6-7). *Shape of areole*: Most populations

show deltoid shape, occasionally quadrangular, pentangular, rhomboid (Sikkim population, *G. Panigrahi* 15747) to very rarely irregular in shape. *Larger areole*:  $282 \times 70\ \mu\text{m}$ . *Smaller areole*:  $58 \times 35\ \mu\text{m}$ . *Vein islets (areoles)*: 73 (average) per  $1\ \text{mm}^2$ . (116 per  $\text{mm}^2$  in Sikkim population, *K. Biswas* 9277). *Vein endings*: 34 (average) per  $1\ \text{mm}^2$ ; veinlets usually simple unbranched to occasionally obscurely branched (once). Branched and unbranched veinlets occur in the same areole. *Vein ends*: Bulbous, bulbous-acute, bulbous-truncate (Sikkim population,





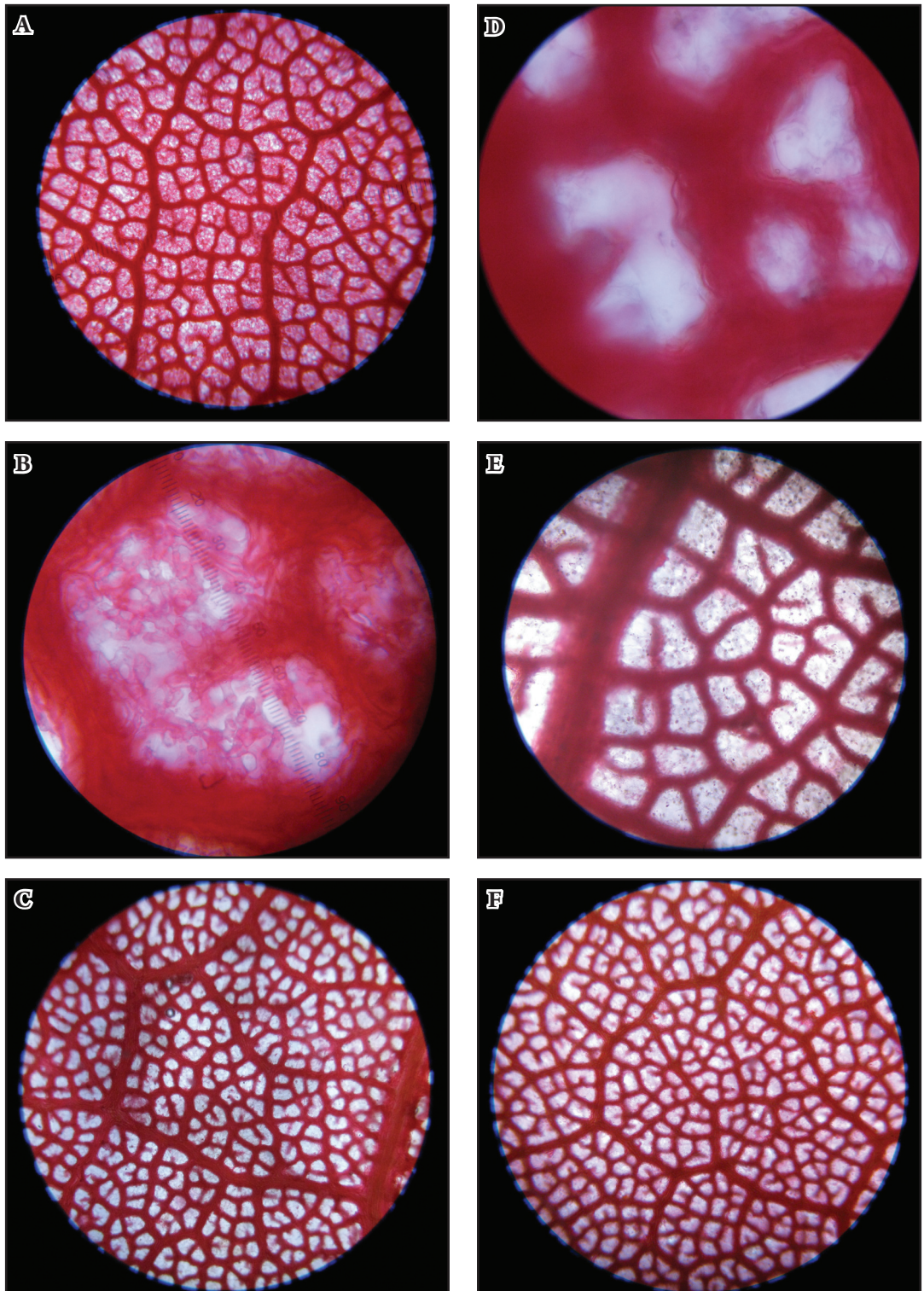
**Fig. 5.** Stomatal complex of *Rhododendron arboreum*: **A** – subsp. *cinnamomeum* var. *cinnamomeum* (LM  $\times 40$  at base, King's Collector s.n., Sikkim); **B** – subsp. *delavayi* (LM  $\times 40$  at apex, Mc Laren 38AA, China); **C** – subsp. *nilagiricum* (LM  $\times 40$  at apex, Ramamurthy 66376, Kerala); **D** – subsp. *nilagiricum* (LM  $\times 40$  at apex, Fischer 2530, Tamil Nadu).

*G. Panigrahi* 15747) to rarely bulbous-rounded (Sikkim population).

**Pollen morphology** (Figs 9-11). Pollen grains are variable in size. Grains occur mostly in tetrahedral tetrads, occasionally decussate tetrads (Nepal population 23 & *Burkill* 28678 from Himachal Pradesh), 3-zonocolporate. *Tetrad size (D)*: 30.4-45.6  $\mu\text{m}$  in diameter (30.4  $\mu\text{m}$  in Manipur, 33  $\mu\text{m}$  in Western Himalaya, 34.2  $\mu\text{m}$  in Darjeeling in West Bengal and China, 35.5  $\mu\text{m}$  in Bhutan and Myanmar, 38  $\mu\text{m}$  in Nepal, 43.1  $\mu\text{m}$  in Meghalaya and

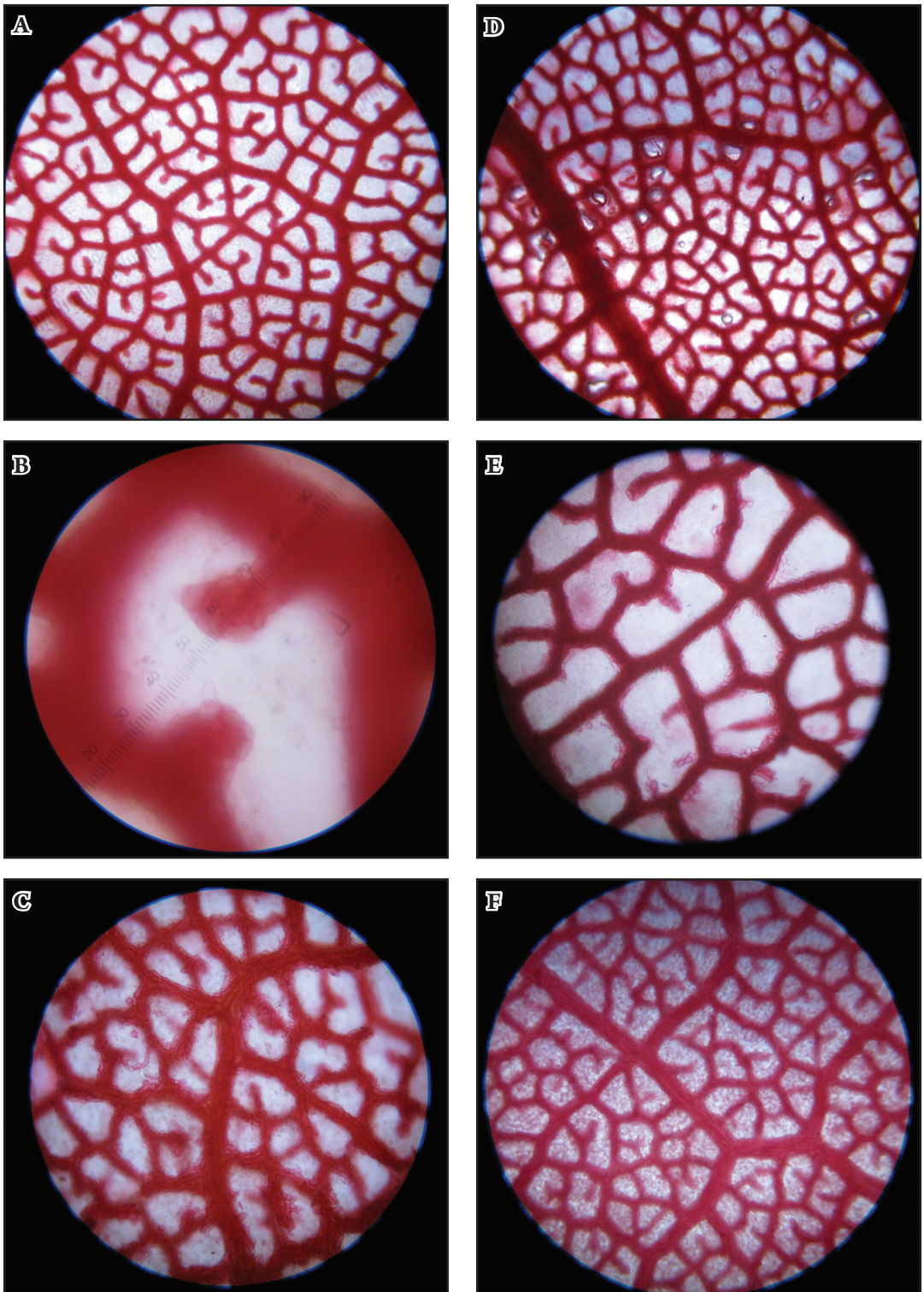
Nagaland, 45.6  $\mu\text{m}$  in Sikkim populations), subspheroidal. *Individual grain size (d)* also variable, 15-31.7  $\mu\text{m}$  in diameter, mostly 19.3  $\mu\text{m}$ . *Exine* tectate, 2.8-3.9  $\mu\text{m}$  thick, *surface reticulate with viscin threads under ESEM*, aperture margin granulated and compact. *Colpi* distinct, 10.8-14.4  $\mu\text{m}$  long, width 2.4-2.8  $\mu\text{m}$ . *2f/D* (ratio of colpus length, 2f to tetrad diameter, D): 0.26-0.33  $\mu\text{m}$ , colpus margin distinct, acute to tapering towards ends. *Septum thickness* 1.7-3.2  $\mu\text{m}$ .





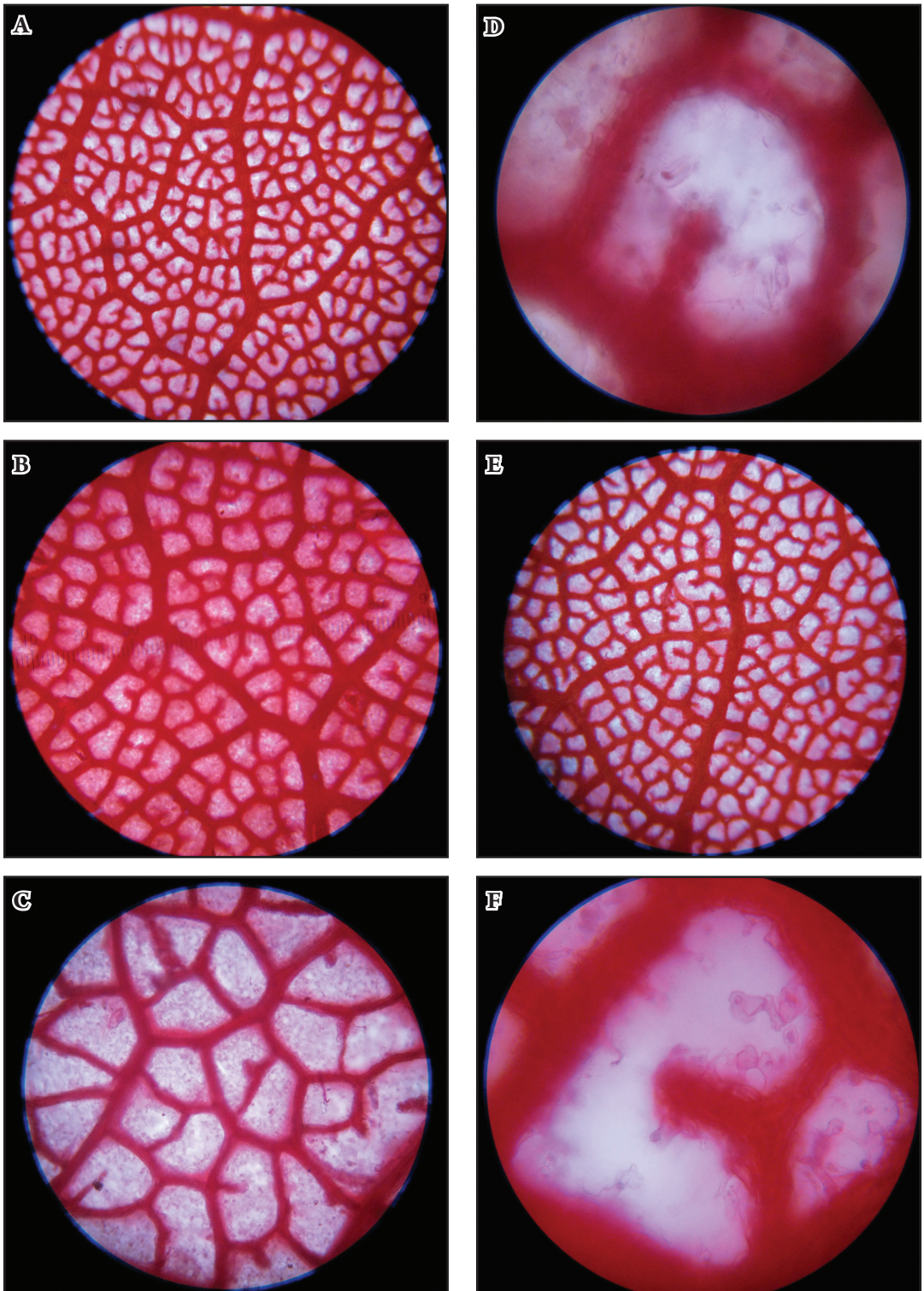
**Fig. 6.** Leaf areolar (vein islets) pattern of *Rhododendron arboreum* subsp. *arboreum*: **A-B** – Arunachal Pradesh (LM  $\times 100$ ,  $\times 400$ ); **C-E** – Sikkim (LM  $\times 50$ ,  $\times 400$ , and  $\times 100$ ); **F** – Darjeeling (LM  $\times 50$ ).





**Fig. 7.** Leaf areolar (vein islets) pattern of *Rhododendron arboreum* subsp. *arboreum*: **A-B** – Nagaland (LM  $\times 100$ ,  $\times 400$ ); **C** – Manipur (LM  $\times 100$ ); **D, E** – Nepal (LM  $\times 50$ ,  $\times 100$ ); **F** – Bhutan (LM  $\times 50$ ).





**Fig. 8.** Leaf areolar (vein islets) pattern of: **A** – *Rhododendron arboreum* subsp. *cinnamomeum* var. *cinnamomeum* from Himachal Pradesh (LM  $\times 50$ ); **B** – *R. arboreum* subsp. *delavayi* from China (LM  $\times 100$ ); **C-F** – *R. arboreum* subsp. *nilagiricum* from Kerala (**C, D** – LM  $\times 100$ ,  $\times 400$ ) and Tamil Nadu (**E, F** –  $\times 50$ ,  $\times 400$ ).

**2. subsp. *cinnamomeum*** (Lindley) Tagg in J.B. Stev., Sp. Rhodod.: 17. 1930; D.F. Chamb., *Notes Roy. Bot. Gard. Edinburgh* **39** (2): 330. 1982; Bhattacharyya, Rev. Gen. Rhododendr. India (Ph.D. thesis): 135. 2007. Bhattacharyya & Sanjappa in Sanjappa & Sastry, Fasc. Fl. India no. 25 (Ericaceae): 91. 2014.

3 varieties recognized (BHATTACHARYYA & SANJAPPA 2014).

#### KEY TO THE VARIETIES

1. Abaxial leaf unistrate, compacted, fawn or whitish ..... 2
- 1\*. Abaxial leaf bistrate, upper layer loose, floccose, rufous, lower layer compacted, whitish to fawn ..... 2a. var. *cinnamomeum*
2. Corolla pink to carmine ..... 2b. var. *roseum*
- 2\*. Corolla white ..... 2c. var. *album*

**2a. var. *cinnamomeum***. *R. campbelliae* Hook. f., Rhododendr. Sikkim-Himalaya: t. 6. 1849.

Fig. 3 A.

**Type.** India, Sikkim Himalaya, 9000-10000 ft, J.D. Hooker s.n. (CAL!). *R. arboreum* Sm. subsp. *campbelliae* (Hook. f.) Tagg in J. B. Stev., Sp. Rhodod.: 15. 1930; Bhattacharyya, Rev. Gen. Rhododendr. India (Ph.D. thesis): 135. 2007.

**Vernacular names.** Etok (Bhutia); Guras, Lal Guras (Nepali).

**Description.** Lamina 6-13 × (1.5) 2.5-3.3 cm, usually elliptic-lanceolate to rarely oblong-lanceolate, apex acute, adaxial surface reticulate, abaxial surface with a bistrate indumentum, the upper layer loose and floccose, rufous, lower surface whitish to fawn and compacted; petioles 5-8 mm long. Flowers c. 32 mm long and 32 mm across; pedicels 4-8 mm long, sparsely to densely pubescent. Corolla pink to carmine.

**Distribution.** India: Eastern Himalaya [Sikkim, Darjeeling in West Bengal]; Eastern Nepal; Bhutan; South-Western China.

**Habitat.** Grows in dry as well as moist rocky slope in dense or open forests at altitudes ranging in 2500-3400 m.

**Flowering.** March – June.

**Fruiting.** September – November.

**Specimens examined in CAL otherwise mentioned.**

**INDIA: EASTERN HIMALAYA: Sikkim:** J.E. Lister s.n., acc. no. 268304; J.D. Hooker s.n., acc. no. 268303; Dr. King s.n., acc. no. 268326. No specimens available from Nepal, Bhutan and China in CAL.

**Field notes.** The species is a variable from population to population in lamina size and shape, petiole length, flower size, corolla colour.

**Leaf stomata** (Fig. 5 A, B). The investigated species shows only amphiparacytic type. *Dimensions of stomata:* The average dimension is 21.05 × 21.05 μm in apex, middle and base. The length varies from 19.3 μm to 22.8 μm and breadth – from 19.3 μm to 22.8 μm. *Size of guard cells:* The average dimension is 11.5 × 5.8 μm. *Size of epidermal cells:* The epidermal cells are usually penta- to polygonal, isodiametric to rarely irregular, some are quadrangular. There is no definite pattern of arrangement of epidermal cells. The epidermal walls in surface view are straight. The epidermal walls in the adaxial surface are also straight. *Dimensions of epidermal cells:* The maximum length is 25.3 μm and breadth is 6.3 μm. The minimum length is 8.5 μm and breadth is 6.3 μm.

**Leaf areolar (vein islets) pattern** (Fig. 8 A). *Shape of areole:* Most populations show deltoid shape, occasionally quadrangular and pentangular in shape. *Larger areole:* 164 × 70 μm. *Smaller areole:* 58 × 47 μm. *Vein islets (areoles):* 119 (average) per 1 mm<sup>2</sup>. *Vein endings:* 46 (average) per 1 mm<sup>2</sup>; veinlets simple unbranched occurring inside areole. *Vein ends:* Bulbous-rounded (Sikkim population).

**Pollen morphology** (Fig. 12 A, B). Pollen grains occur in tetrahedral tetrads, 3-zonocolporate. *Tetrad size (D):* 38.5-42.6 μm in diameter, subspheroidal. *Individual grain size (d):* 25-27.7 μm in diameter. *Exine* tectate, 3.6-4 μm thick, *surface reticulate with viscid threads under ESEM*, aperture margin granulated and compact. *Colpi* distinct, 8.5-15.4 μm long, width 2.4-2.8 μm. *2f/D:* 0.24-0.27 μm, colpus margin distinct, acute to tapering towards ends. *Septum thickness* 1.7-1.9 μm.

Duplicate specimens of var. *roseum* and var. *album* were not available for study either

in herbaria or in field. Therefore, these two varieties are not included in this work.

**3. subsp. *delavayi*** (Franchet) D.F. Chamberlain, *Notes Roy. Bot. Gard. Edinburgh* **37**: 328. 1979; D.F. Chamb., *Notes Roy. Bot. Gard. Edinburgh* **39** (2): 331. 1982; Bhattacharyya, *Rev. Gen. Rhododendr. India* (Ph.D. thesis): 136. 2007. *R. delavayi* Franchet, *Bull. Soc. Bot. France* **33**: 231. 1886.

Fig. 3 C, D.

**Type.** China, Yunnan, in monte Calcareo Houangli-pin, 2500 m, *Delavoy* 242 (iso. K, photo!).

**Vernacular name.** Lali gurans (Nepalese of Tenga, Arunachal Pradesh). Bhattacharyya & Sanjappa in Sanjappa & Sastry, *Fasc. Fl. India* no. 25 (Ericaceae): 92. 2014.

**Description.** Lamina 8-15 × 2-4 cm, usually elliptic-lanceolate, apex acute, adaxial surface reticulate, abaxial surface with a unistrate spongy, whitish to fawn indumentums; petioles 5-14 mm long. Flowers 32-55 mm (Yunnan population, *Henry s.n.*) long and 22-40 mm across; pedicels 4-6 (-10 in Yunnan population) mm long, sparsely to densely pubescent. Corolla deep crimson to carmine.

**Distribution.** India: Eastern Himalaya (Arunachal Pradesh), North-Eastern India (Meghalaya, Nagaland, Manipur, Mizoram); China (Yunnan, Guizhou); Northern Myanmar; Northern Thailand.

**Habitat.** Grows in dry as well as moist rocky slope in dense or open forests at altitudes ranging in 1500-2800 m.

**Flowering.** Late March – May.

**Fruiting.** September – November.

**Specimens examined in CAL otherwise mentioned.** INDIA: EASTERN HIMALAYA: Arunachal Pradesh: West Kameng district, 16 km from Bomdi-La toward Tenga valley, road side slope, 2150 m, 27.11.2011, *S. Panda* 212 (BGC). CHINA: Pai-Ching Summit, Mc laren 48AA; Yunnan, 1897, *Dr. A. Henry* 10983 & 11330; Soshuich mt. Mc Laren 60A; hills west of Lung-fang, 9000-10000 ft, April, 1925, acc. no. 26475.

**Field notes.** The species is a variable from population to population in lamina size and shape, petiole length, flower size, corolla colour.

**Leaf stomata** (Fig. 5 C, D). The investigated species shows only paracytic type. *Dimensions of stomata:* The average dimension is 17.5 ×

17.5 µm in apex, middle and base. The length varies from 15 µm to 20.3 µm and breadth – from 15 µm to 20.3 µm. *Size of guard cells:* The average dimension is 14.5 × 6.1 µm. *Size of epidermal cells:* The epidermal cells are usually polygonal to rarely irregular. There is no definite pattern of arrangement of epidermal cells. The epidermal walls in surface view are straight. The epidermal walls in the adaxial surface are also straight. *Dimensions of epidermal cells:* The maximum length is 21.5 µm and breadth is 6.3 µm. The minimum length is 15 µm and breadth is 6.3 µm.

**Leaf areolar (vein islets) pattern** (Fig. 8 C, D). *Shape of areole:* Most populations show quadrangular, pentangular to deltoid in shape. *Larger areole:* 176 × 141 µm. *Smaller areole:* 94 × 58 µm. *Vein islets (areoles):* 67 (average) per 1 mm<sup>2</sup>. *Vein endings:* 22 (average) per 1 mm<sup>2</sup>; veinlets simple unbranched, occurring inside areole. *Vein ends:* Bulbous-rounded (Sikkim population).

**Pollen morphology** (Fig. 12 C, D). Pollen grains occur mostly in tetrahedral tetrads, occasionally decussate tetrads (Yunnan population), 3-zonocolporate. *Tetrad size (D):* 42.4-46.6 µm in diameter, spheroidal. *Individual grain size (d):* 30-32.7 µm in diameter. Exine tectate, 2-2.8 µm thick, *surface reticulate with viscin threads under ESEM*, aperture margin granulated and compact. *Colpi* distinct, 12.8-14 µm long, width 2.4-2.8 µm. *2f/D:* 0.26-0.33 µm, colpus margin distinct, acute to tapering towards ends. *Septum thickness:* 1.7-3.2 µm.

**4. subsp. *nilagiricum*** (Zenker) Tagg in J. B. Stev., *Sp. Rhodod.*: 15. 1930; D.F. Chamb., *Notes Roy. Bot. Gard. Edinburgh* **39** (2): 331. 1982; Bhattacharyya, *Rev. Gen. Rhododendr. India* (Ph.D. thesis): 137. 2007. Bhattacharyya & Sanjappa in Sanjappa & Sastry, *Fasc. Fl. India* no. 25 (Ericaceae): 93. 2014. *R. nilagiricum* Zenker, *Amer. Sci. Nat. Ser. 2* **6**: 150. 1836.

Fig. 3 E, F.

**Type.** S. India, Saladia, Nilgiris Hills, Utacamund, *Schmidt s.n.* (iso. E n.v.).

**Vernacular names.** Alingi, Bili (Tamil), Badaga, Bili (Kanarese), Kaattu-poovarasu



(Malayalam), Pumaram (local inhabitants of Nilgiris).

**Description.** Lamina 8-12 × 2-3.6 cm, usually oblong, oblong-lanceolate, apex rounded-acute with a short mucro, adaxial surface rugose, abaxial surface with a spongy, yellowish-brown indumentum; petioles 5-10 mm long. Flowers c. 32 mm long and c. 32 mm across; pedicels 4-6 mm long, sparsely to densely pubescent. Corolla carmine. Stamens 10, c. 16 mm long.

**Distribution.** India: hill tops of Western Ghats (Tamil Nadu, Kerala). Endemic.

**Habitat.** Grows in open rocky slopes, mostly in shola forests at altitudes ranging from 2000-2500 m.

**Flowering.** February – May.

**Fruiting.** September – November.

**Specimens examined in CAL otherwise mentioned.**

**INDIA: WESTERN GHATS: Tamil Nadu:** Bison Swamp, Kuyndak, Nilgiri hill, 2250 m, 3.2.1916, C.E.C. Fischer 2530; Naduvattam, Nilgiri hill, 2060 m, 18.01.1961, B.V. Shetty 11920; Edapalli, Nilgiri Dt., 2067 m, 19.10.1956, K.M. Sebastine 991; Pillar rock, Kodaikanal, Madurai dt., 2333 m, 9.3.1958, K. Subramanyam 55; Perumalmalai, Madurai Dt., 12/02/1978, M. Chandrabose 53390. **Kerala:** Deviculum, 19.02.1957, G.S. Puri 15470; Kodanad-Kotagiri road, 2000 m, 5.1.1957, K. Subramanyam 1925; Coonoor, upper Tiger Shola, 1400 m, 19.01.1957, K.M. Sebastine 2071. Munnar to Bodi road 1800 m, 25.03.1980, K. Ramamurthy 66376.

**Field notes.** The species is a variable from population to population in lamina size and shape, petiole length, flower size, corolla colour.

**Leaf stomata** (Fig. 5 E, F). The investigated species shows both paracytic and amphiparacytic types. *Dimensions of stomata:* The average dimension is 15.5 × 15.5 µm in apex, middle and base. The length varies from 11.4 to 25.3 µm and breadth – from 11.4 to 25.3 µm. *Size of guard cells:* The average dimension is 14.5 × 6.4 µm. *Size of epidermal cells:* The epidermal cells are polygonal to irregular. There is no definite pattern of arrangement of epidermal cells. The epidermal walls in surface view are straight. The epidermal walls in the adaxial surface are also straight. *Dimensions of epidermal cells:* The maximum length is 19.4 µm and breadth is 6.3 µm. The minimum length is 12.6 µm and breadth is 6.3 µm.

**Leaf areolar (vein islets) pattern** (Fig. 8 E, F). *Shape of areole:* Most populations show quadrangular, pentangular to deltoid in shape (Kerala population (Sebastine S695, CAL) does not show deltoid shape). *Larger areole:* from 141 µm (Tamil Nadu population, Fischer 2530) to 235 µm (Kerala population, Sebastine S695) × 117 (× 235) µm. *Smaller areole:* 94 × 84 µm. *Vein islets (areoles):* 46 (average) (Kerala, Sebastine S695, CAL) to 78 (Tamil Nadu population, Fischer 2530) per 1 mm<sup>2</sup>. *Vein endings:* 24 in Tamil Nadu and 30 in Kerala (average) per 1 mm<sup>2</sup>; veinlets simple unbranched occurring inside areole. *Vein ends:* Bulbous-rounded to bulbous acute.

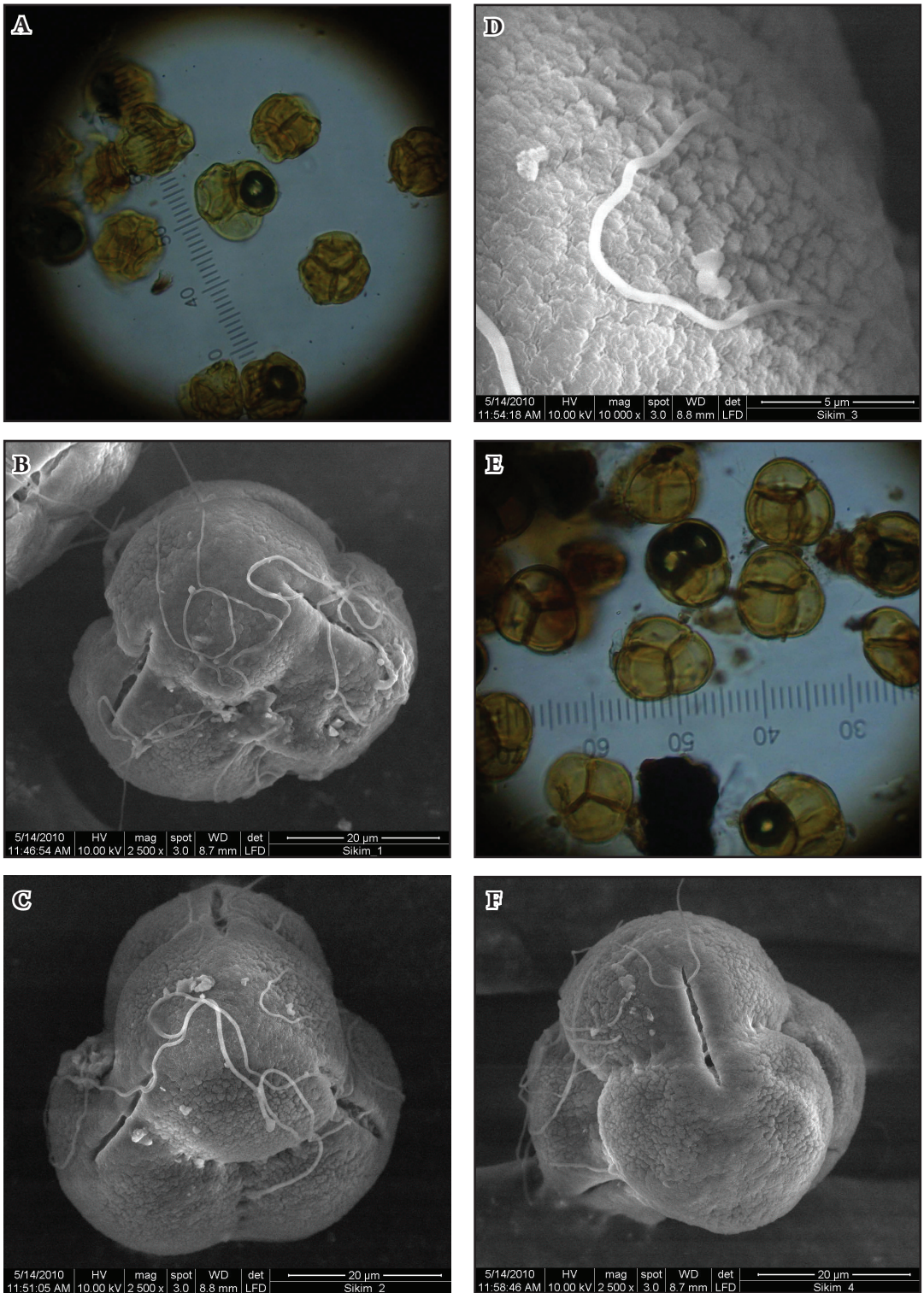
**Pollen morphology** (Fig. 12 E, F). Pollen grains are slightly variable in size. Grains occur in tetrahedral tetrads, 3-zonocolporate. *Tetrad size (D):* 38.7-40.6 µm in diameter (Tamil Nadu & Kerala populations), spheroidal. *Individual grain size (d)* also variable, 21.9-30.4 µm in diameter. Exine tectate, 3-3.9 µm thick, *surface reticulate with viscin threads under ESEM*, aperture margin granulated and compact. *Colpi* distinct, 12.8-14.4 µm long, width 2.4-2.8 µm. *2f/D:* 0.26-0.33 µm, colpus margin distinct, acute to tapering towards ends. Septum thickness: 5-5.07 µm.

**5. subsp. zeylanicum** (Booth) Tagg in J.B. Stev., Sp. Rhodod.: 16. 1930; D.F. Chamb., *Notes Roy. Bot. Gard. Edinburgh* **39** (2): 332. 1982. *R. zeylanicum* Booth, *Gard. Chron.*: 150. 1850.

**Type.** Described from a plant cultivated in Sir Charles Lemon's Garden in Cornwall.

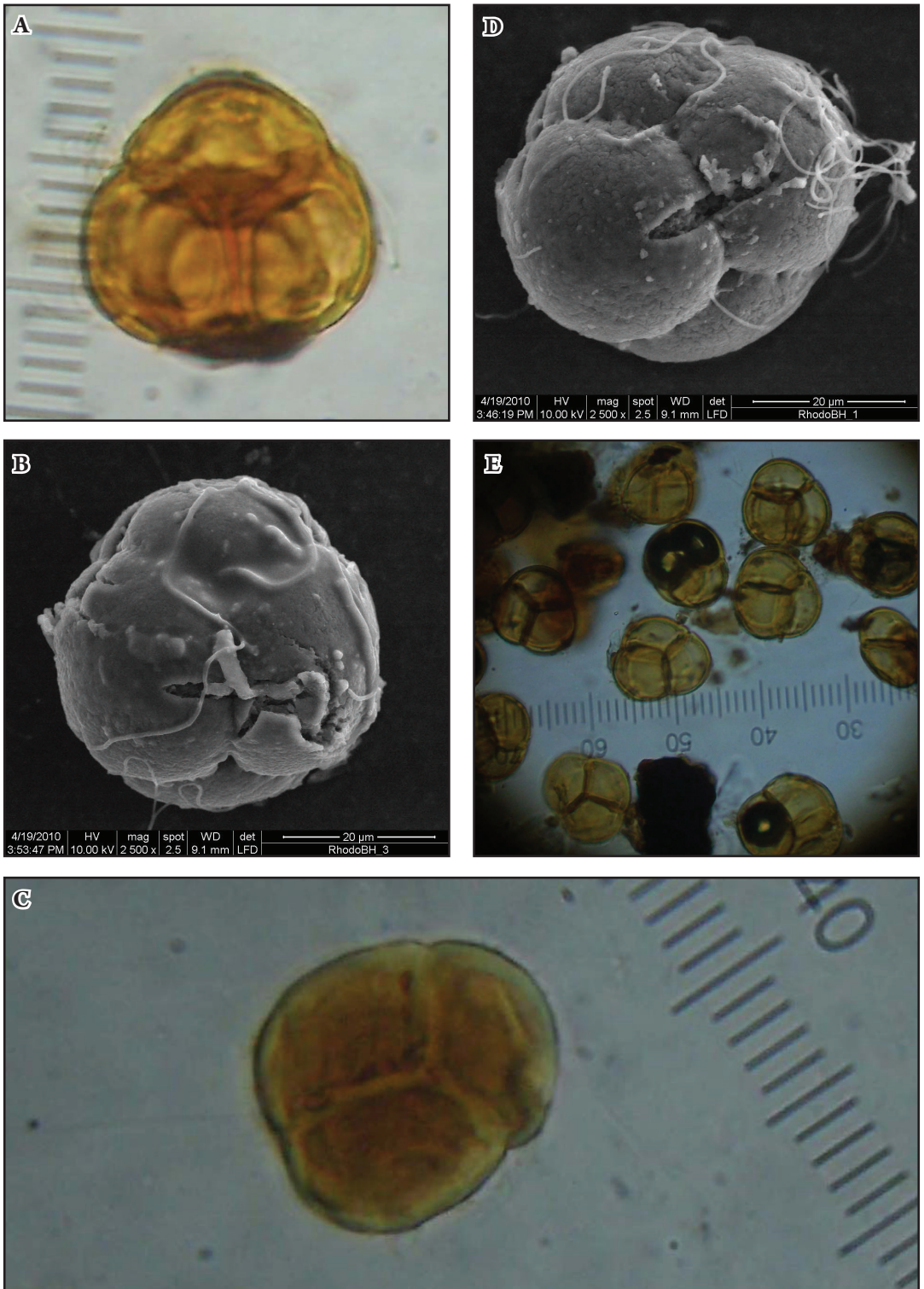
**Vernacular names.** Ma-ratmal, Asoka (Sinhalese of Sri Lanka – JAYAWEERA & SENARATNA 2006); Alingi, Bili (Tamilese of Sri Lanka – JAYAWEERA & SENARATNA 2006).

**Description.** Lamina 8-11 × 3-4 cm, usually oblong, oblong-lanceolate, apex blunt-acute, adaxial surface with a strongly impressed veins, bullate, margins strongly recurved, abaxial surface with a spongy, brownish indumentum; petioles 8-10 mm long. Flowers c. 32 mm long and c. 32 mm across; pedicels 4-6 mm long, sparsely to densely pubescent. Corolla carmine.

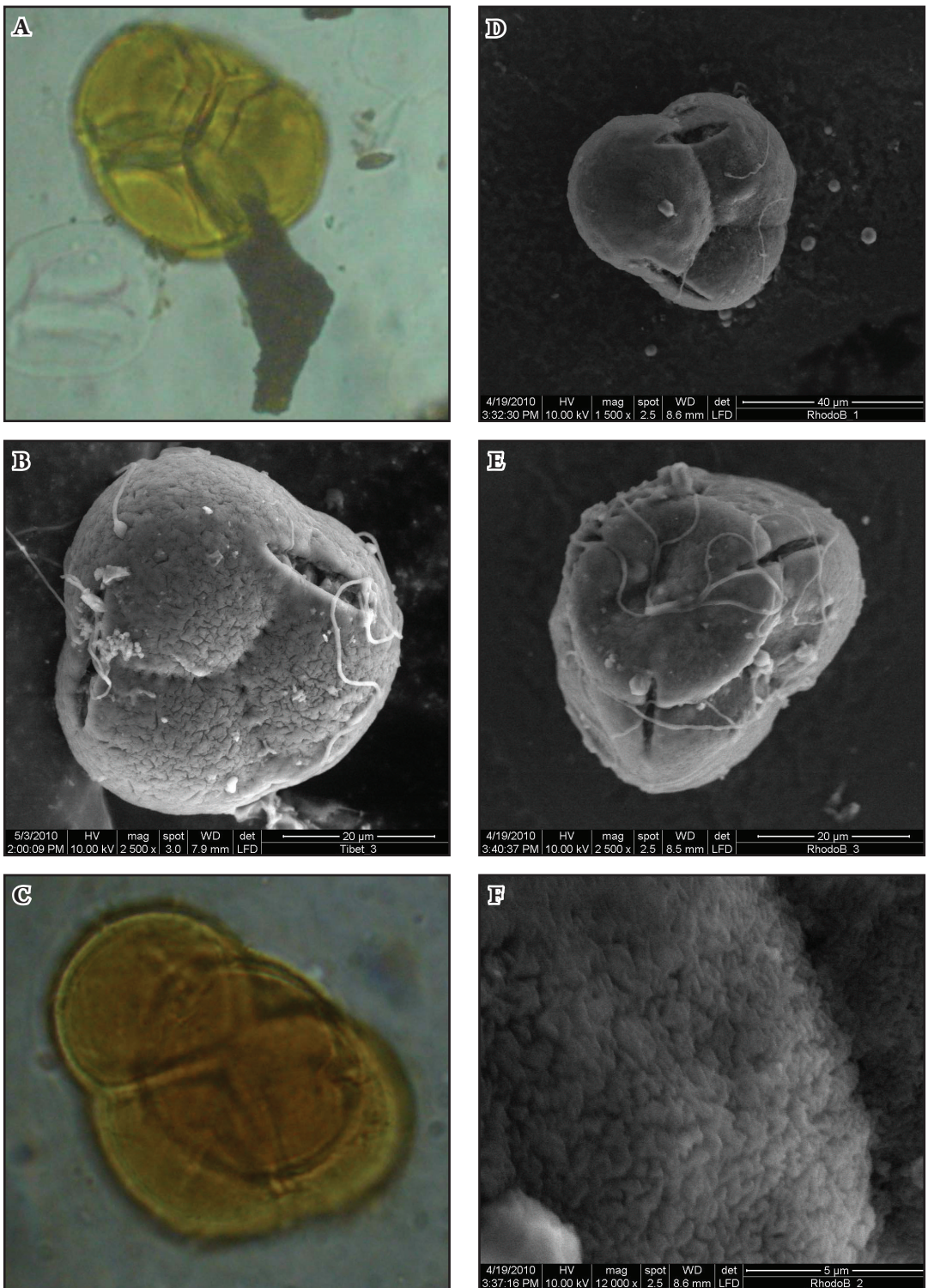


**Fig. 9.** Pollen morphology of *Rhododendron arboreum* subsp. *arboreum*: **A-D** – tetrads of Sikkim population (**A** – LM  $\times 400$ ; **B, C** – ESEM; **D** – part mag. ESEM); **E, F** – tetrads of Darjeeling population (**E** – LM  $\times 400$ ; **F** – ESEM).



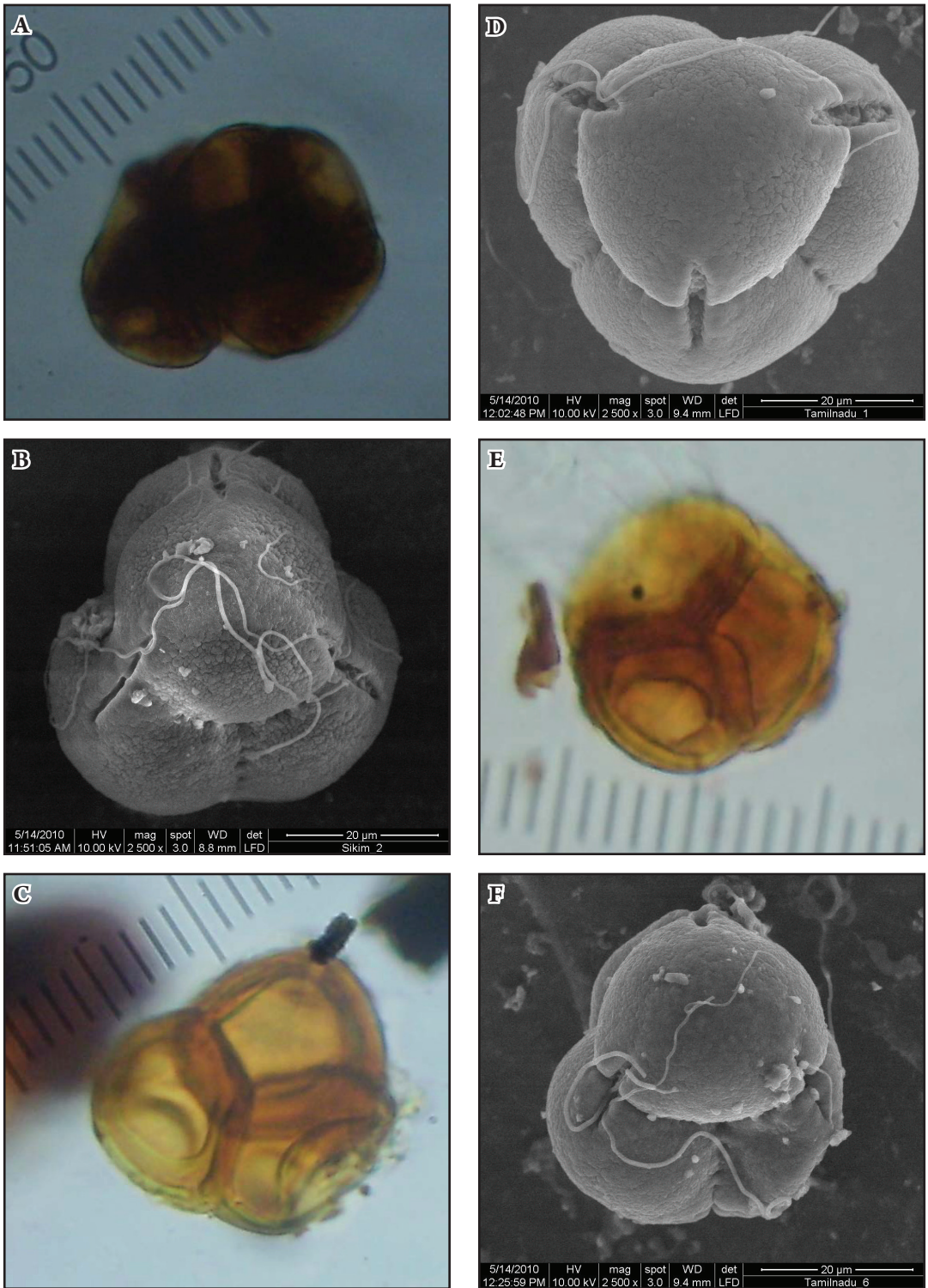


**Fig. 10.** Pollen morphology of *Rhododendron arboreum* subsp. *arboreum*: **A, B** – tetrads of Nepal population (**A** – LM ×400; **B** – ESEM); **C-E** – tetrads of Bhutan population (**C** – LM ×400; **D** – ESEM; **E** – ESEM part mag).



**Fig. 11.** Pollen morphology of *Rhododendron arboreum* subsp. *arboreum*: **A, B** – tetrads of Yunnan population, China (**A** – LM  $\times 400$ ; **B** – ESEM); **C-F** – tetrads of Myanmar population (**C** – LM  $\times 400$ ; **D, E** – ESEM of tetrads; **F** – part of tetrad mag.).





**Fig. 12.** Pollen morphology of *Rhododendron arboreum*: **A-B** – tetrads of *R. arboreum* subsp. *cinnamomeum* from Sikkim population (**A** – LM  $\times 400$ ; **B** – ESEM); **C-F** – tetrads of *R. arboreum* subsp. *nilagiricum* from Tamil Nadu (**C** – LM  $\times 1000$ ; **D** – ESEM), and Kerala (**E** – LM  $\times 400$ ; **F** – ESEM) populations.

**Distribution.** Sri Lanka (Upland regions).

**Habitat.** Grows in open rocky slopes at an altitude of *c.* 2300 m.

**Flowering.** April – May.

**Fruiting.** September – November.

**Specimens examined.** SRI LANKA: Ceylon: Upland region, *Kurz s.n.*, acc. no. 268375, veg. (CAL).

**Field notes.** The species is a variable from population to population in lamina size and shape, petiole length, flower size, corolla colour.

Duplicate materials were not available for leaf anatomical and pollen morphological studies.

### Discussion and conclusions

Live and herbarium specimens-based analysis on different populations of *R. arboreum* complex including its 5 existing subspecies (subsp. *arboreum*, subsp. *cinnamomeum*, subsp. *delavayi*, subsp. *nilagiricum* and subsp. *zeylanicum*) and 2 varieties (var. *arboreum* and var. *cinnamomeum*) shows that exomorphological variations are qualitative among different infraspecific taxa which are justified as valid taxa in leaf anatomical and pollen morphological data (LM & ESEM).

Subsp. *arboreum* is more variable in respect to exomorphology, leaf anatomy and pollen morphological data comparing to other subspecies in respect to leaf texture, lamina shape & size, corolla colour, the variable range of stomatal types (from euparacytic, amphiparacytic, brachiparacytic to desmocyctic), variable epidermal walls on surface view (straight, slightly wavy to sinuous), longer epidermal cells (up to 44 µm long), largest vein islets (up to 282 µm long), variable vein endings (bulbous-acute, bulbous-truncate to bulbous-rounded) and variable size of pollen tetrads (30.4-45.6 µm in diameter). Among the existing subspecies, subsp. *arboreum* is more close to subsp. *cinnamomeum* in respect to leaf texture, amphiparacytic stomata, variable epidermal cells, deltoid vein islets and subspheroidal tetrads in both, and more isolated from subsp. *nilagiricum* in respect to leaf texture, leaf apex, corolla size, epidermal cells, vein islets shape, vein endings, diameter of pollen tetrads as well as its shape and septum thickness.

Subsp. *nilagiricum* is more close to subsp. *zeylanicum* (in respect to exomorphology) and subsp. *dilavayi* (in respect to leaf anatomy and pollen morphology), but more isolated from subsp. *arboreum* (in respect to exomorphology, leaf anatomy and pollen already mentioned). Subsp. *cinnamomeum* and subsp. *delavayi* are comparatively more closely related in respect to exomorphology, leaf anatomy and pollen morphological data. Thus, the present work shows that exomorphological variations are qualitative among different infraspecific taxa which are justified as valid taxa in leaf anatomical and pollen morphological data.

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