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Exomorphological Variation in *Gaultheria fragrantissima* Wall. (Ericaceae: Vaccinioideae) in India: A Micromorphological Solution from Leaf Stomata and Pollen Morphology

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Abstract

Gaultheria fragrantissima Wall., a variable species in the family Ericaceae, is restricted to some south-eastern Asian countries *viz.*, India, Nepal, Bhutan, China, Myanmar, Sri Lanka, Malaysia and Vietnam. This work includes field and herbarium-based exomorphology and Light as well as Scanning Electron Microscopic studies of leaf stomata and pollen morphology of six distinct populations of *G. fragrantissima* Wall. collected from three phytogeographical regions in India including tables of comparative studies of different populations to stabilize the species delimitation based on leaf stomata and pollen morphological data.

Gaultheria fragrantissima Wall., a variable species in the family Ericaceae Juss., was first described and named by Nathalien Wallich[28] based on the plant collected from Nepal Himalaya. The etymology of the specific epithet, "*fragrantissima*" was due to its wintergreen fragrance. The species is restricted to some south-eastern Asian countries *viz.*, India, Nepal, Bhutan, China, Myanmar, Sri Lanka, Malaysia and Vietnam. In India, the species is distributed in Eastern Himalaya, North eastern India and hill tops of South western Ghats.

Detailed investigations of the genus *Gaultheria* L. were studied by workers like Clarke[4], Airy Shaw[1], Middleton[15], Kron *et al.*,[12], Ruizheng & Stevens[23], Apte *et al.*,[2], Panda[19,20], S. Panda *et al.*,[21] and Panda & Sanjappa[22]. In India, the genus is studied in detailed by S. Panda & Sanjappa[22] who revised the genus and described 23 species including *G. fragrantissima* Wall.

Apte *et al.*,[2] studied genetic diversity analysis in *Gaultheria fragrantissima* Wall. based on Western Ghats and Meghalayan populations using ISSR markers. They observed high gene flow within Western Ghat population, which may be enough to prevent

genetic drift, compare to very low gene flow within Meghalayan populations, while Panda[19] revised the genus *Gaultheria* L. including *G. fragrantisima* Wall. based on herbarium-based exomorphology.

The present work embodies field and herbarium-based exomorphology and Light Microscopic studies of leaf-stomata (LM) as well as Light and Scanning Electron Microscopic studies of pollen morphology (LM & SEM) of six distinct populations of *G. fragrantissima* (in case of Pollen morphology, five populations were studied due to unavailability of flower buds in one) collected from three phytogeographical regions (Eastern Himalaya, North eastern India and Western Ghats) in India to stabilize delimitation of *G. fragrantissima* Wall. based on leaf-stomata and pollen morphological data to show that exomorphological variations among these six populations are not soundly observed in micromorphological data, which may justify that all variable populations are within the circumscription of the same species, *G. fragrantissima* Wall. This work also includes tables of comparative studies of six different populations under three phytogeographical regions in India. Nair and Kothari[16] described pollen morphology of this species based on herbarium material collected only from South India (DD, no 26147). Workers like Niedenzu[17], Cox[5], Hagerup[11], Watson[29,30], Lems[13] and Stevens[27] contributed a little or no works on leaf-stomata.

Present work is the result of detailed Light (Olympus, Tokyo) as well as Scanning Electronic Microscopic (Hitachi-S530, Japan at Burdwan University Centre) studies of leaf stomata (LM) and pollen morphology (LM & SEM) of six distinct populations of *Gaultheria fragrantissima* Wall. in India. Leaf-stomatal and polliniferous materials used in this investigation were taken from total 26 dried duplicate herbarium specimens deposited in Barasat Govt. College Herbarium (BGC) as well as from live materials collected from Arunachal Pradesh, Sikkim and Meghalaya (deposited in BGC). This work was carried out partly in the Taxonomy and Biosystematics Laboratory, Post Graduate Department of Botany, Barasat Government College (first author supervised second author under PG Dissertation Project) and partly in Botany Department, Maulana Azad College, Kolkata. 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[18]. These pollen measurements are based on at least 10 grains from each specimen.

Methodology for stomatal study:

Mature leaves were obtained from duplicate herbarium specimens deposited in CAL and BGC Herbarium as well as from live collections from Arunachal Pradesh. Small cubical pieces (*c.* 1 sq.cm) were excised from the base, middle and apical regions of the blade. Several existing methods *viz*., 10% HNO_3-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 40X and 100X objectives and Camera Lucida drawings were made with the help of drawing prism. The descriptive terminology follows Metcalfe and Chalk[14], Dilcher[6], Stace[25,26], Fahn[10] and Carpenter[3].

Preparation of Pollen slides:

The method used in this study was by Erdtman[7-9]. Dry polliniferous materials (mature flower buds) were taken from duplicate herbarium specimens deposited in CAL and BGC Herbarium as well as from fresh flower buds collected from Arunachal Pradesh. The descriptive terminology follows Erdtman[9] and Sarwar *et al*.,[24].

Slide preparation for SEM :

Leaf samples for stomata and acetolysed pollen grains (following Erdtman's method[8]) were prepared for Scanning Electron Microscope observation. Pollen grains at least from 10 flowers of each species were acetolysed and studied. Observations were made with Hitachi S530 (SEM, Tokyo, Japan at Burdwan University Instrument Centre) in the high vacuum mode at an applied voltage of 15 KV. For SEM, above samples were mounted on the metallic stub using double stick tape.

Taxonomy and Exomorphological observation :

Gaultheria fragrantissima Wall., Asiat. Res. 13: 397. 1820 & Numer. List: no. 765. 1829; G. Don, Gen. Syst. 3: 840. 1834; C. B. Clarke[4] in Hook. f., Fl. Brit. India 3: 457. 1882; 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): 388. 1991; Ruizheng & P. F. Stevens[23] in Wu *et al*., Fl. China 14: 472. 2005; S. Panda, W. Kameng Ericaceae: 155. 2013; Panda & Sanjappa in Sanjappa & Sastry, Fasc. Fl. India 25: 196. 2014. Panda *et al*.,[21] Div. Gen. *Gaultheria* India: 105 – 113. 2014. *G. fragrans* D. Don, Prodr. fl. nepal.: 151. 1825. Type: Nepal, 1818, *Wallich s.n.* (K, Cibachrome image!). *Arbutus laurifolia* Buch.Ham. ex D. Don, Prodr. fl. nepal.: 151. 1825. *Gaultheria ovalifolia* Wall., Numer. List: no. 1523. 1829. *Gaultheria leschenaultii* DC., Prodr. 7 (2): 593. 1839. *Leucothoe? katagherensis* DC., Prodr. 7 (2): 606. 1839. *Andromeda katagherensis* Hook., Icon. pl. 3: t. 246. 1839. *Gaultheria forrestii* Diels, Notes Roy. Bot. Gard. Edinburgh 5 (25): 210. 1912. (Figs. 1-7). *Vernacular names*: Chanchhewaa, Dhasingare, Goenhli (Nepalese of Sikkim); Shep-Sheng (Monpas of Bomdila); Jirhapkynthai, Jirhap, Soh-lyngthrait (Khasis of Meghalaya); Kolakkaai, Moolai (Tamil of Tamil Nadu).

Stout, erect shrub, 0.3–2 (–3.5) m high, or drooping down from rock crevices. Stems always glabrous, greyish-brown to light brown, profusely branched; branchlets blood red to deep pink, glabrous, glaucous, winged or triangular. Leaves coriaceous, lamina ovate-elliptic, oblong-elliptic, ovate-lanceolate to rarely ovate or obovate, 3–12×2–4 (–6) cm, serrate to serrulate at margin, broadly cuneate at base, always mucronate at apex, deep green, glabrous above, light green, punctate beneath;

Fig. 1. Plant of ***Gaultheria fragrantissima*** Wall. : A. Bomdila, Arunachal Pradesh; B. Dodhray, Darjeeling Himalaya; C. Manipur; D. Nilgiri Hills, South Western Ghat.

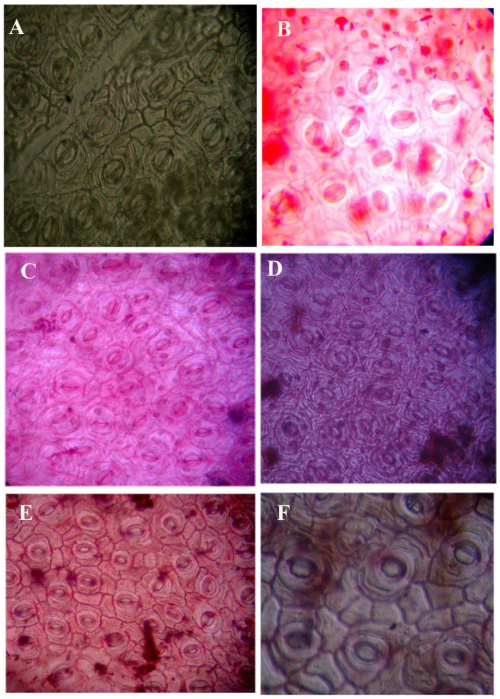

Fig. 2. Stomatal complex of ***Gaultheria fragrantissima*** Wall. (Eastern himalayan populations): A - B (40x)- Arunachal populations (Bomdi-La & Walong), C - D (40X)-Sikkim populations (Yuksum-Bakhim), E (40X) - F (100X)-Darjeeling populations.

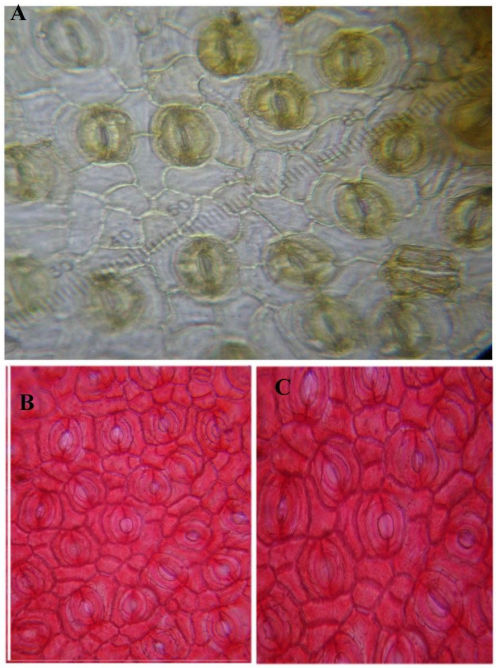

Fig. 3. Stomatal complex of ***Gaultheria fragrantissima*** Wall. (North eastern Indian populations): A (40x)- Meghalaya populations (Shillong peak), B (40X) - C (100X)- Nagaland populations (Japhu hill).

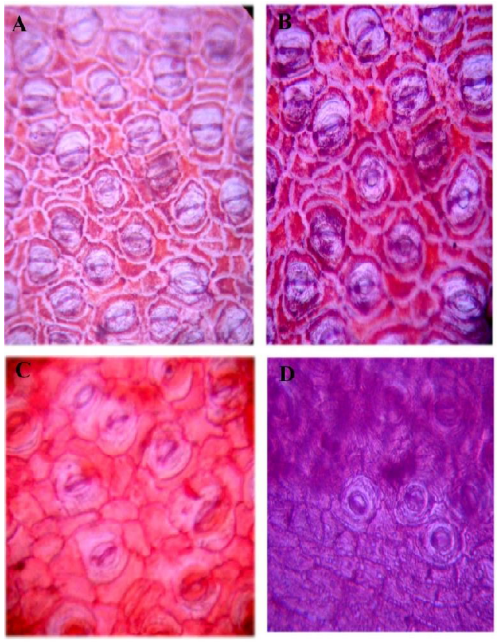

Fig. 4. Stomatal complex of ***Gaultheria fragrantissima*** Wall. (Western Ghat populations): A - C (40X)- Kerala populations (Palghat), D (40X)-Tamil Nadu populations (Nilgiris hill).

petioles stout, 5–11 mm long, glabrous or punctate beneath. Racemes axillary; rachis 2–6 (–10) cm long, 10–26-flowered, puberulous. Flowers always 5-merous, 8–14 mm long; pedicels greyish-white, 2–8 mm long, puberulous; bract 1, basal, light green, broadly ovate, 2–4×1.5–2.5 mm long, ciliate at margin, acute or subacute at apex, glabrous; bracteoles 2, opposite, mostly apical, rarely subapical to median on the pedicel, pink with greenish stripes, broadly ovate, 1.5–2.5×1–2 mm, ciliolate at margin, acute at apex, puberulous. Calyx lobes light pink, ovate-triangular, 2–2.5×1–1.5 mm, ciliolate at margin, acute at apex, glabrous or puberulous inside. Corolla ovoid-urceolate, greyish-white to light greenish-white, *c*. 5 mm long, 3–4 mm diameter, glabrous outside, pilose inside. Stamens 10, *c*. 3 mm long, loosely epipetalous; filaments greyish-white, *c*. 1 mm long, basally dilated; anther lobes blackish-brown, oblong, *c*. 1 mm long, each lobe with 2 apical awns of *c*. 1 mm long or minute. Pistil 2–3.5 mm long; ovary globose to subglobose, light green, 1–1.5×1.5–2 mm, tomentose; disc minutely 10-dentate; style light green, 1–2 mm long, slender, glabrous; stigma truncate. Fruit loculicidally 5-valved capsule, enclosed in a fleshy accrescent calyx, light green (immature) to deep blue or sky blue (mature), globose to subglobose, 5 – 8 × 3 – 6 mm. Seeds numerous, minute, obconical, scariose.

Distribution:

India (Eastern Himalaya: Sikkim, West Bengal and Arunachal Pradesh; NE India: Meghalaya, Nagaland and Manipur and Hill-tops of SW Ghats: Tamil Nadu, Kerala and Karnataka); Nepal; Bhutan; China; Myanmar and Sri Lanka.

Flowering: March–May; December–January. *Fruiting*: May – October.

Habitat : grown in moist rocky soil, often hanging down from rock crevices, rarely in loose humus-covered boulders or in landslide areas at altitudes ranging from 1600–2300 m.

Results (Micromorphological observation)

Leaf Stomata of *Gaultheria fragrantissima* Wall.

The study of Light Microscopic (40X, 100X) stomatal architecture includes number, form and arrangement of specialized epidermal cells associated with the stomatal guard cells. 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.

Eastern Himalayan Population

1. Arunachal population (Walong and Bomdi-La)

Specimens studied: 7 duplicate specimens were studied (Table-1). The investigated species shows two types, amphiparacytic and brachiparacytic. *Dimensions of stomata:* The average dimension is 35.6 µm×30.8 µm in apex, middle and base. *Size of guard cells:* The average dimension is 25 µm×4.9 µm. *Size of epidermal cells:* the epidermal cells are usually isodiametric

Table-1. Source of leaf anatomy and pollen materials of *G. fragrantissima* Wall. in India

Name of species	Collector and Number	Voucher information (Herbarium acronym)
Gaultheria fragrantissima Wall. Eastern Himalayan populations 1. Arunachal Pradesh	*S. Panda* 30890; S. Panda 30762, & 30763; *T. Majumder & S. Panda* 46; *S. Panda 30826; D. K. Singh & Party* 9302 (BSD); S. Panda 30847. (=07 samples)	India: Arunachal Pradesh, Lohit district, Namti to Kibithu, 1350 m, 30.04.2003, *S. Panda* 30890 (CAL); Seru to Malinja, 2100 m, 04.05.2003, *S. Panda* 30762; Malinja Base Camp, 2100 m, 04.05.2003, *S. Panda* 30763; West Kameng district, Bomdi-La, *T. Majumder & S. Panda* 46 (Barasat Govt. College Herbarium); 3 km from Nechephu toward Jamiri, 5500 ft, 25.12.2002, *S. Panda* 30826; Dibang Valley district, Eastern bank of Mehao Lake, 26.11.2000, *D. K. Singh & Party* 9302 (BSD); Lower Subansiri district: Pange to Talle Valley, 2500 – 2800 m, 31.12.2002, *S. Panda* 30847.
2. Sikkim	*S. Panda* 29928; 30022; *R. C. Srivastava* 13162; *D. Maity* 21558. (=04 samples)	India: West Sikkim, Yuksum to Bakhim, West district, 6000 ft, 12.05.2002, *S. Panda* 29928 (CAL); Hilley to Versay, 2650 m, 27.04.2001, *S. Panda* 30022 (CAL); Phamtam, South district, 10.05.1991, *R. C. Srivastava* 13162 (BSHC); Yuksum to Bakhim, West district, 2400 m, 23.03.1999, *D. Maity* 21558 (BSHC).
North eastern Indian populations 3. Meghalaya	*S. Panda* 30708; 30701; 30703; 30712; 30822. (05 samples)	India: Meghalaya, East Khasi Hill district, Upper Shillong, 5000 ft, 17.03.2002, *S. Panda* 30708 (CAL); Jongsha Village, 4600 ft, 16.03.2002, *S. Panda* 30701; Shillong Peak, 5000 ft, 17.03.2002, *S. Panda* 30703; Mawkdak, 4500 ft, 22.03.2002, *S. Panda* 30712; Sohrareim Forest, 5000 ft, 27.11.2002, *S. Panda* 30822.
4. Nagaland	*Watt* 6873. *Dr. D. Prain s.n.*; *Dr. A. A. Mao* 106486.(03 samples)	India: Nagaland, Pegwima to Japhu Hill, 6000 ft, 19.05.1882, *G. Watt* 6873 (CAL); Pulinalodza, 7800 ft, April, 1886, *Dr. D. Prain s.n.* (CAL); Banrew Forest, 06.02.1999, *Dr. A. A. Mao* 106486 (ASSAM).
Western Ghat populations 5. Kerala	*N. C. Nair* 77213; *A. Meebold* 13337; *Ramamurthy* 66385. (03 samples)	India: Kerala, Sispara to Palghat, Palakkad district, 2000 m, 28.03.1983, *N. C. Nair* 77213 (CAL); Devicolam, Idduki district, 6000 ft, December, 1909, *A. Meebold* 13337 (CAL); Munnar to Kumili Road, Idduki district, 2000 m, 25.03.1980, *Ramamurthy* 66385 (CAL).
6. Tamil Nadu	*K. M. Sebastine* 2619; *K. Subramanyam* 2009; *K. Subramanyam* 1882; *A. Meebold* 11915. (=04 samples)	India: Tamil Nadu, Gymkhana Hills, 1766 m, 24.03.1957, *K. M. Sebastine* 2619 (CAL); Dodabetta Road, 2000 m, 07.01.1957, *K. Subramanyam* 2009 (CAL); Kotagiri, 2000 m, 04.01.1957, *K. Subramanyam* 1882 (CAL); Udagamund hills, 6000 ft, October, 1909, *A. Meebold* 11915 (CAL).

Table-2. Comparative account of field and herbarium-based exomorphological data of *Gaultheria fragrantissima* Wall. among EH, NE and WG populations

Characters	Eastern Himalayan population	North eastern Indian population	Western Ghat population
Habit	bushy erect shrub to treelet up to 3.5 m tall (usually 0.5–2 m tall).	bushy erect shrub up to 1.5 m tall (usually 0.5–1 m tall).	bushy erect shrub up to 1 m tall (rarely 1.5 m) (usually 0.4–0.7 m tall).
Branches and twigs	blood-red to greenish; trigonous to terete	blood-red; trigonous	blood-red; trigonous to terete
Leaf shape	elliptic, ovate-elliptic to ovate-lanceolate	Ovate-elliptic to elliptic	Oblong-elliptic, obovate-elliptic to obovate
Leaf size	up to14 cm long and 8 cm wide	up to 7 cm long and 5 cm wide	up to 6 cm long and 4 cm wide
Leaf apex	mucronate to shortly acuminate.	always mucronate.	mucronate to mucronulate.
Petiole	up to 12 mm long.	up to 7 mm long.	up to 5 mm long.
Inflorescence length & number of flowers per rachis	rachis up to 11 cm long; 18-30-flowered (rachis usually 4–7 cm long)	rachis up to 5 cm ong; 10-16-flowered (rachis usually 3–4 cm long)	rachis up to 4 cm long; 8-14-flowered (rachis usually 2.5–3.5 cm long)
Pedicellar bracteoles position	usually apical	apical to subapical	apical to subapical
Pedicel length	4–8 mm long	2–4 mm long	2–4 mm long
Corolla shape	ovoid-urceolate, urceolate to narrowly urceolate.	ovoid-urceolate.	ovoid-urceolate.
Ovary size	1.5 × 2 mm.	1 × 1.5 mm.	1 × 1.5 mm.
Style length	2–3 mm long.	1–2 mm long.	1–1.5 mm long.
Capsule size (maximum)	8 × 6 mm.	6 × 4 mm.	6 × 4 mm.

EH = Eastern Himalaya; NE = North eastern India; WG = Western Ghat

hexa- to polygonal. The epidermal walls in surface view are straight. *Dimensions of epidermal cells:* The average dimension is 26.9 µm ×9.6 µm (Fig. 2A–B).

2. Sikkim population (Yuksum-Bakhim)

Specimens studied: 4 duplicate specimens were studied (Table-1). The investigated species shows only one type, brachyparacytic. *Dimensions of stomata:* The average dimension is 28 µm×31.3 µm in apex, middle and base. *Size of guard cells:* The average dimension is 21.4 µm×4.9 µm. *Size of epidermal cells:* the epidermal cells are variable quadrangular, pentagonal, hexagonal to polygonal and isodiametric. The epidermal walls in surface view are straight. *Dimensions of epidermal cells:* The average dimension is 23.9 µm×13.2 µm (Fig. 2C–D).

North Eastern Indian Population :

3. Meghalaya population (Shillong peak)

Specimens studied: 5 duplicate specimens were studied (Table-1). The investigated species shows only one type, paracytic. *Dimensions of stomata:* The average dimension is 30.05 µm×26.8 µm. *Size of guard cells:* The average dimension is 20.3 µm×5.2 µm. *Size of epidermal cells:* epidermal cells are isodiametric-hexagonal to polygonal. Epidermal walls in surface view are straight. *Dimensions of epidermal cells:* The average dimension is 27.4 µm×11.3 µm (Fig. 3A).

4. Nagaland population (Japhu hill)

Specimens studied: 3 duplicate specimens were studied. The investigated species shows two types, mostly paracytic to rarely brachyparacytic. *Dimensions of stomata:* The average dimension is 24.2 µm×33.1 µm in apex, middle and base. *Size of guard cells:* The average dimension is 20.3 µm×5.7 µm. *Size of epidermal cells :* the epidermal cells are usually polygonal to occasionally quadrangular and pentagonal in shape, isodiametric. The epidermal walls in surface view are straight. *Dimensions of epidermal cells:* The average dimension is 27.2 µm×13.2 µm (Fig. 3B-C).

Western Ghat Population

5. Kerala population (Palghat)

Specimens studied: 2 duplicate specimens were studied. The investigated species shows two types, mostly amphiparacytic to rarely brachyparacytic. *Dimensions of stomata:* The average dimension is 29.4 µm×26.1 µm in apex, middle and base. *Size of guard cells:* The average dimension is 23.6 µm×4.9 µm. *Size of epidermal cells:* the epidermal cells are usually polygonal to occasionally pentagonal in shape, isodiametric. The epidermal walls in surface view are straight. *Dimensions of epidermal cells:* The average dimension is 22.3 µm×14 µm (Fig. 4A-C).

6. Tamil Nadu population (Nilgiris)

Specimens studied: 4 duplicate specimens were studied. The investigated species shows only paracytic type. *Dimensions of stomata:* The average dimension is 24.2 µm×23.1 µm in apex, middle and base. *Size of guard cells:* The average dimension is 14.9 µm×4.9 µm. *Size of epidermal cells:* the epidermal cells are usually quadrangular, penta to polygonal in shape, isodiametric. The epidermal walls in surface view are straight. *Dimensions of epidermal cells:* The average dimension is 23.9 µm×14.8 µm (Fig. 4D).

Pollen Morphology **(**for each specimen, total 10 flower buds were taken for study).

Eastern Himalayan Population

1. Arunachal population (Walong)

Pollen grains occur in tetrahedral to rhomboidal tetrads, 3-zonocolporate. *Tetrad size (D):* 23.4 µm (average) diameter. *Individual grain size (d)* variable, 11 µm–13 µm diameter. Individual grain possesses no distinct furrows. *D/d = 1.9. Exine* tectate, 3.9–5.2 µm thick, surface rugulate-psilate to faintly rugulate, the rugulae faintly striate. *Colpi* distinct, 7 µm–7.8 µm long, width 1.6 µm–1.8 µm. Ratio of colpus length (2f) to tetrad diameter (2f/D), 0.2 µm–0.3 µm, colpus margin distinct, significantly wider at middle, bluntly acute towards ends. *Septum thickness* 1.2 µm– 2.8 µm (Fig. 5A-C).

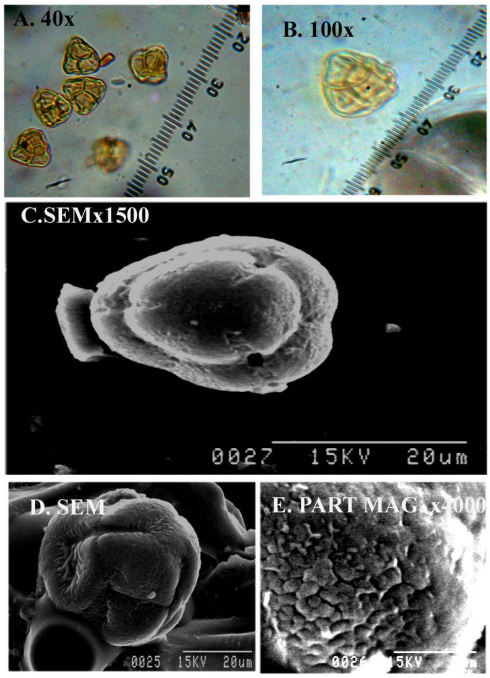

Fig. 5. Pollen morphology of ***Gaultheria fragrantissima*** Wall. (Eastern himalayan populations): A - C (40X, 100X, SEM).-Arunachal populations (Walong). D - E (SEM)-Sikkim populations (Yuksum-Bakhim).

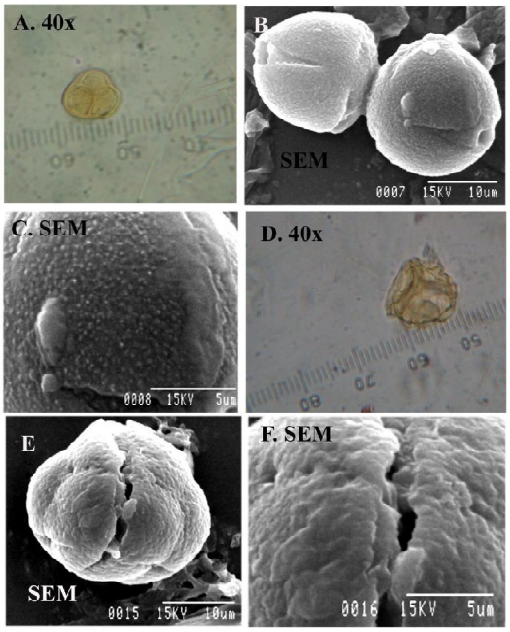

Fig. 6. Pollen morphology of ***Gaultheria fragrantissima*** Wall. (North eastern Indian populations): A - C (LM, SEM)-Meghalaya populations (Shillong Peak), D - F (LM, SEM) -Nagaland populations (Japhu hill).

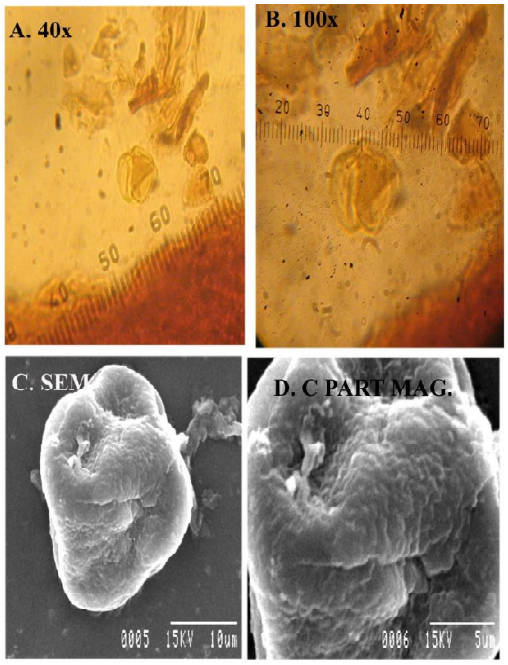

Fig. 7. Pollen morphology of ***Gaultheria fragrantissima*** Wall. (Western Ghat populations): A - D (40X,100X, SEM)-Kerala populations (Palghat). A - B. LM photographs, C . SEM photograph, D.SEM part magnified .

2. Sikkim population (Yuksum-Bakhim)

Pollen grains occur in tetrahedral tetrads, 3-zonocolporate. *Tetrad size (D):* 23.4 µm–24.1 µm diameter. *Individual grain size (d)* variable, 11.7 µm–12.2 µm diameter. Individual grain possesses no distinct furrows. *Exine* tectate, 3.9–4.2 µm thick, surface coarsely rugulate, the rugulae finely striate. *D/d = 1.99. Colpi* distinct, 10.8 µm–12.6 µm long, width 1.3 µm–1.6 µm. Ratio of colpus length (2f) to tetrad diameter (2f/D), 0.4 µm–0.5 µm, colpus margin distinct, significantly wider at middle, bluntly acute towards ends. *Septum thickness* 1.9 µm–2.8 µm (Fig. 5D-E).

North Eastern Indian Population

3. Meghalaya population (Shillong peak)

Pollen grains occur in tetrahedral to rhomboidal tetrads, 3-zonocolporate. *Tetrad size* (D): 20.7 µm–21.6 µm diameter. *Individual grain size (d)* variable, 7.2 µm–10.8 µm diameter. Individual grain possesses no distinct furrows. *D/d = 2.31. Exine* tectate, 2.2–2.8 µm thick, surface finely granular. *Colpi* distinct, 10.8 µm–11.7 µm long, width 0.9 µm (apex)–3.6 µm (middle). Ratio of colpus length (2f) to tetrad diameter (2f/D), 0.52 µm–0.54 µm, colpus margin distinct, significantly wider at middle, bluntly acute towards ends. *Septum thickness* 1.6 µm–1.8 µm (Fig. 6A-C).

4. Nagaland population (Japhu hill)

Pollen grains occur in tetrahedral tetrads, 3-zonocolporate. *Tetrad size (D):* 22.5–25.2 µm diameter. *Individual grain size (d)* slightly variable, 10.8–12.6 µm diameter. Individual grain possesses no distinct furrows. *D/d = 2.03. Exine* tectate, 3–3.6 µm thick, surface uneven and rugged, primary exine sculpture finely rugulate, the rugulae faintly striate. *Colpi* distinct, 10.8 µm–12.2 µm long, width 1.4 µm–1.6 µm. Ratio of colpus length (2f) to tetrad diameter (2f/D), 0.4 µm–0.5 µm, colpus margin distinct, significantly wider at middle, luntly acute towards ends. *Septum thickness* 1.2 µm–2.8 µm (Fig. 6D-F).

Western Ghat Population (sufficient and suitable flower buds not available for study for Tamil Nadu population)

5. Kerala population (Palghat)

Pollen grains occur in tetrahedral to decussate tetrads, 3-zonocolporate. *Tetrad size (D):* 21.9 µm–24.3 µm diameter. *Individual grain size (d),* 10.2 µm–11.7 µm diameter. Individual grain possesses no distinct furrows. *D/d = 2.1. Exine* tectate, 2.6–2.8 µm thick, surface uneven and rugged, primary exine sculpture finely rugulate, the rugulae faintly striate. *Colpi* distinct, 7 µm–9 µm long, width 1.4 µm–1.6 µm. Ratio of colpus length (2f) to tetrad diameter (2f/D), 0.31 µm–0.37 µm, colpus margin distinct, significantly wider at middle, bluntly acute towards ends. *Septum thickness* 1.2 µm–1.4 µm (Fig. 7A-D).

Discussion

Among three phytogeographical regions (Eastern Himalayas (EH), North eastern India (NE) and Western Ghat (WG) of *Gaultheria fragrantissima* Wall., NE populations show some minor variations in leaf-stomata and pollen morphology, while EH and

Table-3. Comparative account of leaf-stomata and pollen morphological data of *Gaultheria fragrantissima* Wall. among EH, NE and WG populations:-

Characters	Eastern Himalayan population	North eastern Indian population	Western Ghat population
Leaf-stomata			
Type	Amphi- and brachyparacytic common.	Mostly paracytic, rarely brachyparacytic.	Mostly paracytic and amphiparacytic, rarely brachyparacytic.
Dimension	26.7 µm – 39.6 µm × 22.6 µm – 36.3 µm.	22.6 µm – 31.7 µm × 25.3 µm – 36.3 µm.	22.6 µm – 36.3 µm × 16.5 µm – 29.7 µm.
Dimension of guard cell	13.2 µm – 29.7 µm × 4.9 µm – 6.6 µm.	19.8 µm – 21.4 µm × 4.9 µm – 6.6 µm.	14.9 µm – 25.8 µm × 4.9 µm.
Shape of epidermal cells (abaxial leaf)	Quadrangular, penta-, hexa- to polygonal in shape	Mostly polygonal, rarely quadrangular, penta- to hexagonal in shape	Quadrangular, penta- to polygonal in shape.
Dimension of epidermal cells	11.6 µm – 39.6 µm × 6.6 µm – 15.2 µm.	14.9 µm – 39.6 µm × 7.1 µm – 14.9 µm.	11.6 µm – 33 µm × 8.3 µm – 21.4 µm.
Abaxial epidermal wall	Straight (surface view)	Straight (Surface view)	Straight (Surface view)
Pollen morphology			
Tetrad	Tetrahedral to rhomboidal tetrads.	Tetrahedral to rhomboidal tetrads.	Tetrahedral to decussate tetrads.
Tetrad size (D)	23.4 – 24.1 µm diameter.	20.7 – 25.2 µm diameter.	21.9 µm – 24.3 µm diameter
Individual grain size (d)	11 – 13 µm.	7.2 – 12.6 µm.	10.2 – 11.7 µm.
Exine	3.9 – 5.2 µm thick; surface coarsely to faintly rugulate.	2.2 – 3.6 µm thick; surface finely rugulate to finely granular.	2.6 – 2.8 µm thick; surface finely rugulate.
D/d	1.99	2.03 – 2.31	2.1
Colpi	7 – 12.6 µm.	10.8 – 12.2 µm.	7 – 9 µm.
2f/D	0.2 – 0.5 µm	0.42 – 0.54 µm	0.31 – 0.37 µm
Septum thickness	1.2 – 2.8 µm	1.2 – 2.8 µm	1.2 – 1.4 µm

EH = Eastern Himalaya; NE = North eastern India; WG = Western Ghat

WG populations show mostly similar characters in leaf-stomata but some minor variations in pollen morphology. NE populations are characterized by lacking amphiparacytic stomata, smaller stomatal dimension (22.6 µm–31.7 µm) and polygonal epidermal cells in leaf-stomata, finely granular exine and more 2f/D (0.42–0.54 µm) value in pollen morphology. Meanwhile, WG populations are differentiated from EH and NE populations in possessing pollen decussate tetrads, smaller exine thickness (2.6–2.8 µm), shorter colpi (7–9 µm) and shorter septum thickness (1.2–1.4 µm) which are all minor quantitative data. Therefore, this work on leaf-stomata and pollen morphology of *G. fragrantissima* Wall. on six different populations from three distinct phytogeographical regions in India shows all these populations belong to the same species, *G. fragrantissima* Wall., although these populations are exomorphologically variable to some extent.

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