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Research Article

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Foliar epidermal micromorphology of genus *Glochidion* J.R.Forst. & G.Forst. (Phyllanthaceae) by using light and electron microscopy

Priyanka Brahma, Sanjib Baruah*

Department of Botany, Bodoland University, Kokrajhar-783370, Assam, India

ABSTRACT

The present study was conducted to compare both qualitative and quantitative characteristics of foliar epidermal micromorphology on some members of *Glochidion* in Assam. As taxonomic attributes, the foliar epidermal micromorphology study of nine taxa of both abaxial and adaxial surfaces was performed by using light microscopy (LM) and field emission scanning electron microscopy (FESEM). The result showed both amphistomatic and hypostomatic types of leaf surfaces. On the same surface of the leaf, multiple types of stomata were observed such as anomocytic, anisocytic, hemiparacytic, and paracytic types. Significant diversity and variations were observed in stomatal number, size, area, epidermal cell number, subsidiary cells, and trichomes. The stomatal index, stomatal shape, epidermal cell shape, length and width of the stomata, and trichomes showed variation among the studied taxa. Glands were absent in all studied members. Papillae and epicuticular wax crystals were observed in some taxa. In addition, a taxonomic key was also provided based on foliar leaf epidermal characteristics using qualitative and quantitative data from LM and FESEM. Based on quantitative data of foliar leaf micromorphology, principal component analysis (PCA) and cluster analysis were carried out to authenticate the micromorphological data. These would aid in the identification of taxa as well as in taxonomic delimitation.

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***Corresponding author:**
Sanjib Baruah
E-mail: sanjibbaruah9@gmail.com

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INTRODUCTION

The genus *Glochidion* is a large member of the family Phyllanthaceae and the genus consists of c 320 species across the world and c 22 species and 8 varieties in India (Balakrishnan & Chakrabarty, 2007; Balakrishnan *et al*., 2012; Chakrabarty & Balakrishnan, 2018). A total of 16 species from erstwhile Assam were described in "Flora of Assam" by Kanjilal *et al*. (1940). Earlier the genus was placed under the family Euphorbiaceae (Bentham & Hooker, 1862-1863; Hooker, 1890; Kanjilal *et al*., 1940). According to Hoffmann *et al*. (2006), the genus was closely connected to the type of Phyllanthus as a result of the genus *Glochidion*, which includes *Breynia* J.R. & G.Forst., *Flueggea* Willd., and *Margaritaria* L.f., were classified under the family Phyllanthaceae and the tribe Phyllantheae. Additionally, a recent classification assigned the genus to the Phyllanthaceae family (The Angiosperm Phylogeny Group *et al*., 2016). The biovulate ovary and lack of latex distinguish the family Phyllanthaceae from Euphorbiaceae (Chakrabarty & Balakrishnan, 2018).

The members of *Glochidion* are mainly shrubs or small trees and large trees. They can be found primarily along roadsides, in damp or humid deciduous areas, tropical, primary, and secondary forests, sal woods, mountainous terrain, and occasionally swampy places. The genus can be identified by its drooping branches, axillary and supra-axillary inflorescence, arrangement of male and female flowers, and lobed and unlobed capsules. Taxonomic studies of *Glochidion* based on morphological characters were conducted in many regions of the world (Robinson, 1909; Beille, 1927; Backer & van den Brink, 1963; Shaw, 1981; Li, 1994; Chakrabarty & Gangopadhyay, 1995; Nguyen, 2007; Li & Gilbert, 2008; Chakrabarty & Balakrishnan, 2018; Yao *et al*., 2020). However, the micromorphological or leaf epidermal study is still lacking. Only a few species i.e., *G. hohenckari* and *G. neilgherrense* of the foliar epidermal study were covered by some workers (Thakur & Patil, 2011, 2014).

Micromorphological traits can often remain seen to be adequately varied to be used as a tool for taxonomy and species identification (Vislobokov *et al*., 2021). The Epidermal features

are extremely useful in identification at specific and generic levels (Stace, 1965). The taxonomic value of trichome types and epicuticular wax has been successfully utilized in many flowering plants (Barthlott *et al*., 1998; Moon *et al*., 2009; Song & Hong, 2017). Hairs and stomata types can provide valuable taxonomic and phylogenetic traits (Aldhebiani & Jury, 2013). Morphology as a technique fails to distinguish between such small variations that separate very similar species, i.e., the closely related ones with the naked eye. Owing to this, the micromorphological study was considered one of the traits for plant identification, resolving taxonomic issues, and advancing plant categorization (Blair & Turner, 1972). Electron microscopy has been used to solve taxonomic data obtained from various sources. SEM produces a high-resolution image that has been used as a probable taxonomic tool (Davis & Heywood, 1963; Mukherjee & Acharya, 2014). Based on the morphological characters, some taxa i.e., below the species level make it difficult to identify the members of the genus *Glochidion*. Thus, the present study aims to work out the foliar epidermal study on the lower and upper surface of the leaves, their stomata characters, leaf epidermal characters, trichomes, papillae, and epicuticular wax crystals using both light microscopy (LM) and field emission scanning electron microscopy (FESEM). Generally, the members of *Glochidion* can be identified based on their reproductive morphological characteristics but some infraspecific taxa or lower the taxa of species level are difficult to identify only based on their morphological characteristics. So, the current study of foliar epidermal micromorphological characters based on both light microscopy (LM) and field emission scanning electron microscopy (FESEM) would help in the identification and classification of the infraspecific taxa also. Furthermore, principal component analysis (PCA) and cluster analysis were carried out to assess the correlation or similarities among the taxa.

MATERIAL AND METHODS

Collection, Identification, and Preservation of the Specimen

A total of 9 members of the genus *Glochidion* were collected from different places in Assam from March 2020 to May 2022. All the collected specimens were identified based on their morphological characteristics concerning some literature like Flora of Assam, Flora of British India (Hooker, 1890; Kanjilal *et al*., 1940; Chakrabarty & Balakrishnan, 2018), digital herbaria (Kew, CAL) and some online taxonomic databases (eFloras, 2008; IPNI, 2021; POWO, 2021). For authentication of the specimen, the collected specimen was prepared as herbarium specimens according to Jain and Rao (1977) method and poisoned using 4% mercuric chloride ($HgCl_2$) and ethanol (C_2H_5OH) solution (Clark, 1986) and submitted to BSI, Shillong, India. A list of the specimens with their locality and the accession number is enlisted in Table 1.

Foliar Leaf Epidermal Study through LM (Light Microscopy)

Different methods were adopted to study stomata and epidermal cells due to the thick and hard leaf surface of some members of *Glochidion*. Upper and lower epidermal peelings were made either mechanically or scrapped with the help of a blade using a 10% aqueous solution of nitric acid (HNO_3). The peels were stained with 1% aqueous safranin solution (Boulos & Beakbane, 1971). Fresh or preservative leaf samples were kept at room temperature in a Petri dish solution containing 5% NaOH until they became colourless. When the leaf sample became completely colourless, then epidermal cells were peeled using a needle, washed with distilled water, stained with 1% of Safranin, and observed under the light microscope, model: LEICA DM750 (Radford *et al*., 1974; Lersten & Curtis, 2001; García-Gutiérrez *et al*., 2020).

The leaf samples were placed in a Petri dish filled with 5% sodium hypochlorite (NaClO) solution at room temperature for 2-24 hours. Sodium hypochlorite is used as a softener which makes it easy to scrape the leaf surface using a sharp scalpel or blade onto a slide from both the adaxial and abaxial surfaces of the leaf (Kong & Hong, 2019).

Leaf samples were kept in a Petri dish containing Franklin's solution (1:1 ratio of 35% hydrogen peroxide and glacial acetic acid) for 50-60 ºC in an oven until they became colourless. After becoming whitish translucent it was washed with distilled water and removed the epidermal layer using a needle. It was stained with 1% of safranin, put in one drop of glycerine, and covered with a cover slip. The slide was observed under the light microscope (Model: LEICA DM750) and photographs and all the measurements were taken from the software LAS EZ

Table 1: List of the studied taxa with their locality and accession number

S. No.	Taxa	Locality	Accession No.
1	*G. ellipticum* Wight	Kokrajhar District, Assam	98605
2	*G. heyneanum* (Wight & Arn.) Wight	Kokrajhar District, Assam	98606
3	*G. lanceolarium* (Roxb.) Voigt	Chakrashila Wildlife Sanctuary, Kokrajhar District, Assam	98608
4	*G. multiloculare* (Rottler ex Willd.) Voigt var. *multiloculare*	Kokrajhar District, Assam	98604
5	*G. multiloculare* var. *pubescens* Chakrab. & M.Gangop.	Orang National Park, Udalguri District, Assam	98610
6	*G. sphaerogynum* (Mull.Arg.) Kurz	Chakrashila Wildlife Sanctuary, Kokrajhar District, Cachar, Assam	98609
7	*Glochidion zeylanicum* var. *arborescens* (Blume) Chakrab. & M.Gangop.	Ultapani Forest Range, Kokrajhar District, Assam	98603
8	*G. zeylanicum* var. *tomentosum* Trimen	Ultapani Forest Range, Kokrajhar District, Assam	98607
9	*G. zeylanicum* (Gaertn.) A.Juss. var. *zeylanicum*	Nokpakghat, Karbi Anglong District, Assam	98611

Version 3.4.0 at 50 µm (da Silva *et al*., 2017; García-Gutiérrez *et al*., 2020).

Terminology of the epidermal cell, stomata, and trichomes was followed by methods given by Metcalfe and Chalk (1950), Stace (1965), Cotthem (1970) and Ayodele and Olowokudejo (2005).

Foliar Leaf Epidermal Study through FESEM (Field Emission Scanning Electron Microscopy)

The foliar leaf epidermal study was carried out on both adaxial and abaxial surfaces using a field emission scanning electron microscope (Model: SIGMA VP FESEM, ZEISS). A small section of both surfaces of the dried leaf specimen was cut and mounted with double adhesive tape on stubs, coated with gold-palladium, and observed under FESEM (Pathan *et al*., 2010; Ensikat *et al*., 2011; Traiperm *et al*., 2017).

Quantitative Analysis

The Stomatal index was calculated using the formula given by Metcalfe & Chalk (1950) and the stomatal area was also calculated (Eberly, 2008).

$$\% \text{ Stomatal Index (S.I.)} = \frac{S}{S+E} \times 100, \text{ S = Number of stomata per vision, E = Number of epidermal cells per vision}$$

$$\text{Stomatal Area (S.A.)} = \pi \times (SW/2) \times (SL/2), \text{ SW = Width of stomata, SL= Length of stomata}$$

The value of stomata size (length and width), stomatal density, epidermal cell density, stomatal index, stomatal area and trichome size (length and width) were calculated as the average of three experiments, i.e., Mean and standard deviation (Mean±SD).

Principal component analysis (PCA) was performed (Hammer *et al*., 2001) based on the quantitative data of foliar leaf micromorphology and subjected to classical cluster analysis (hierarchical clustering) using Past software version 4.60b.

RESULTS

The present study was carried out to study foliar leaf epidermal characteristics of 9 taxa. The results of qualitative data and statistical data on stomata type, size, number, area, stomatal index, epidermal cell shape, density, and trichome size of foliar leaf epidermal characteristics using LM and FESEM were presented in Tables 2 and 3, corresponding to Figures 1 to 4. The result of PCA and cluster analysis was also denoted in Table 4 and Figures 5 and 6 respectively.

Stomata Position, Types, Shape, Size, and Area

During the study of light microscopy and field emission scanning electron microscopy, both amphistomatic i.e., stomata present on both surfaces and hypostomatic leaves i.e., stomata present only on the abaxial surface of the leaf were observed. Among the taxa, hypostomatic leaves were observed in *G. ellipticum*, *G. heyneanum*, *G. lanceolarium*, *G. sphaerogynum*,

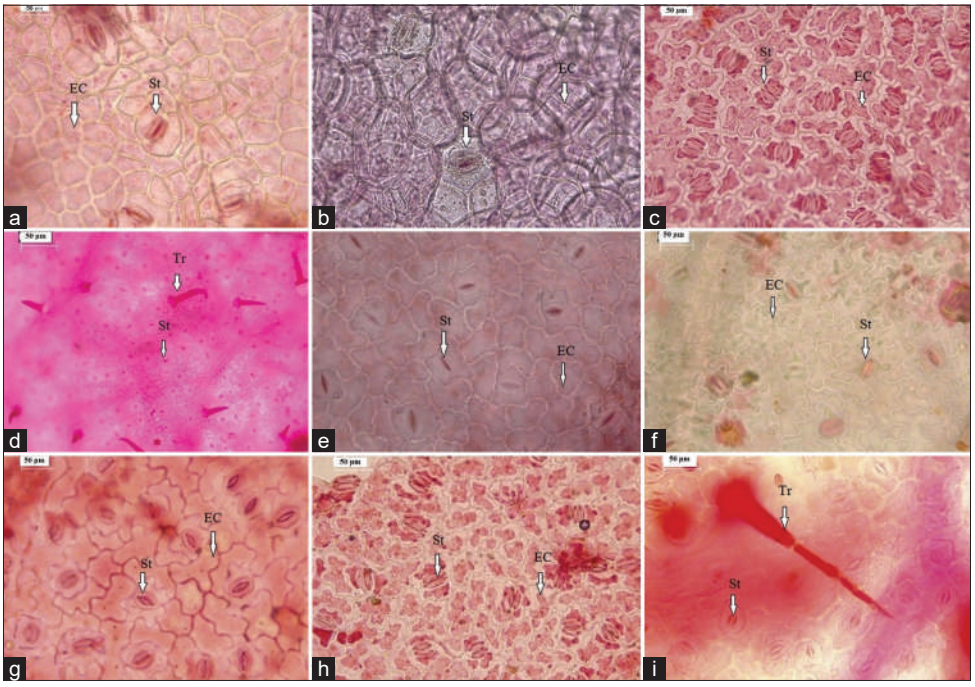

Figure 1: Light micrographs (LM) of abaxial surface of foliar leaf epidermal features of *Glochidion* a) *G. multiloculare* var. *multiloculare*, b) *G. multiloculare* var. *pubescens*, c) *G. ellipticum*, d) *G. heyneanum*, e) *G. lanceolarium*, f) *G. sphaerogynum*, g) *G. zeylanicum* var. *zeylanicum*, h) *G. zeylanicum* var. *arborescens* and i) *G. zeylanicum* var. *tomentosum*. St = Stomata, EC = Epidermal Cell, Tr =Trichome. Scale bars = 50 µm (a-i).

Table 2: Qualitative leaf epidermal micromorphology characteristics of some members of genus *Glochidion* based on a light microscopy (LM) and field emission scanning electron microscopy (FESEM) study

Name of taxa	Stomatal position	Stomatal type		Stomatal shape	Epidermal cell shape	Anticlinal cell wall	Papillae	Epicuticular wax crystals	Trichome types
G. ellipticum	Hypostomatic	Ab	Anomocytic, Anisocytic	Elliptic, oval	Jigsaw, rectangular	Sinuous	–	–	–
		Ad	–	–	Jigsaw, rectangular	Sinuous	–	–	–
G. heyneanum	Hypostomatic	Ab	Anomocytic, Anisocytic	Elongated	Isodiametric, pentagonal, hexagonal, polygonal	Sinuous, rounded, smooth, angular	–	–	Uncinate, hooked, multicellular, unbranched, non-glandular
		Ad	–	–	Isodiametric, pentagonal, hexagonal, polygonal	Sinuous, rounded, smooth, angular	–	–	Uncinate, hooked, multicellular, unbranched, non-glandular
G. lanceolarium	Hypostomatic	Ab	Anomocytic, Anisocytic, Hemiparacytic	Elongated	Undulate, jigsaw	Sinuous	–	–	–
		Ad	–	–	Undulate, jigsaw	Sinuous	–	–	–
G. multiloculare var. *multiloculare*	Amphistomatic	Ab	Anomocytic, Anisocytic, Hemiparacytic	Elongated, elliptic	Isodiametric, pentagonal, hexagonal, polygonal	Smooth, angular, rounded, irregularly thickened	Rounded	Thick waxes at the papillae, smooth, upright around stomata	–
		Ad	Anomocytic, Anisocytic	Elongated, elliptic	Isodiametric, pentagonal, hexagonal, polygonal	Smooth, angular, rounded, irregularly thickened	–	–	–
G. multiloculare var. *pubescens*	Amphistomatic	Ab	Anomocytic, Anisocytic, paracytic	Elliptic	Isodiametric, pentagonal, hexagonal, polygonal	Rounded, smooth, angular	Rounded	Thick waxes at the papillae and trichomes, smooth, upright around stomata	Uniseriate, multicellular, unbranched, non-glandular
		Ad	Anomocytic, Anisocytic	Elliptic	Isodiametric, pentagonal, hexagonal, polygonal	Rounded, smooth, angular	–	–	Uniseriate, multicellular, unbranched, non-glandular
G. sphaerogynum	Hypostomatic	Ab	Anomocytic, Paracytic	Elliptic	Undulate, jigsaw	Sinuous	Slightly present around stomata	–	–
		Ad	–	–	Undulate, jigsaw	Sinuous	–	–	–
G. zeylanicum var. *arborescens*	Hypostomatic	Ab	Anomocytic, Paracytic	Elliptic	Jigsaw, pentagonal, hexagonal, polygonal, undulate	Sinuous	–	–	Uniseriate, multicellular, unbranched, non-glandular
		Ad	–	–	Jigsaw, pentagonal, hexagonal, polygonal, undulate	Sinuous	–	–	Uniseriate, multicellular, unbranched, non-glandular
G. zeylanicum var. *tomentosum*	Hypostomatic	Ab	Anomocytic, Anisocytic	Elliptic	Isodiametric, pentagonal, hexagonal, polygonal, jigsaw, undulate	Rounded, smooth, irregular, sinuous	–	–	Uniseriate, multicellular, unbranched, non-glandular
		Ad	–	–	Isodiametric, pentagonal, hexagonal, polygonal, jigsaw, undulate	Rounded, smooth, irregular, sinuous	–	–	Uniseriate, multicellular, unbranched, non-glandular
G. zeylanicum var. *zeylanicum*	Hypostomatic	Ab	Anomocytic, Anisocytic, Hemiparacytic, Paracytic	Elliptic, oval	Jigsaw, pentagonal, hexagonal, polygonal, rectangular, undulate	Sinuous, buttressed	–	–	–
		Ad	–	–	Jigsaw, pentagonal, hexagonal, polygonal, rectangular, undulate	Sinuous, buttressed	–	–	–

Ab=Abaxial, Ad=Adaxial, – = Absent

Table 3: Quantitative data of leaf epidermal micromorphology characteristics of some members of the genus *Glochidion*

Name of taxa	Leaf surface	No. of stomata per area	Stomatal Density (SD)	Epidermal Cell Density (ECD)	Stomatal Index (%) (SI)	Stomatal Length (μm) (SL)	Stomatal Width (μm) (SW)	Stomatal Area (μm²) (SA)	Trichome Length (μm) (TL)
G. ellipticum	Ab	67–79	72.667±6.027	319.667±26.857	22.06	43.14±2.340	24.756±1.432	838.1562	–
	Ad	–	–	–	–	–	–	–	–
G. heyneanum	Ab	110–156	133.333±23.007	718±137.502	15.66	24.336±0.293	12.99±1.535	248.0966	144.473±23.618
	Ad	–	–	–	–	–	–	–	114.033±27.881
G. lanceolarium	Ab	62–90	77±14.106	278.333±51.052	21.66	25.843±2.437	18.01±2.78	365.322	–
	Ad	–	–	–	–	–	–	–	–
G. multiloculare	Ab	13–16	14.333±1.527	109.333±10.503	11.56	35.81±2.506	21.016±1.651	590.6089	–
var. *multiloculare*	Ad	7–11	9.333±2.081	84.333±4.041	10.02	33.46±2.180	20.406±1.304	535.8284	–
G. multiloculare	Ab	16–28	21.667±6.027	150.667±43.730	12.57	34.15±1.04	20.603±1.499	552.2396	131.336±7.170
var. *pubescens*	Ad	14–18	16.333±2.081	144±37.403	15.25	37.31±2.192	20.867±1.979	611.2478	127.823±5.480
G. sphaerogynum	Ab	95–108	101.333±6.506	438.667±35.345	18.77	24.06±2.442	9.696±2.850	183.0159	–
	Ad	–	–	–	–	–	–	–	–
G. zeylanicum var.	Ab	111–140	124±14.730	270±27.221	31.47	11.70±1.112	6.21±0.504	57.0357	195.033±7.374
arborescens	Ad	–	–	–	–	–	–	–	151.023±5.450
G. zeylanicum var.	Ab	80–92	86.333±6.027	212.333±8.326	28.89	14.367±0.529	7.713±1.015	86.9722	176.486±22.977
tomentosum	Ad	–	–	–	–	–	–	–	115.196±24.205
G. zeylanicum var. *zeylanicum*	Ab	80–100	91.333±10.263	246±54.442	27.08	17.507±1.847	9.34±0.598	128.30825	–
	Ad	–	–	–	–	–	–	–	–

Ab=Abaxial, Ad=Adaxial, – = Absent, data were evaluated three times, n=3 and represented as Mean±SD

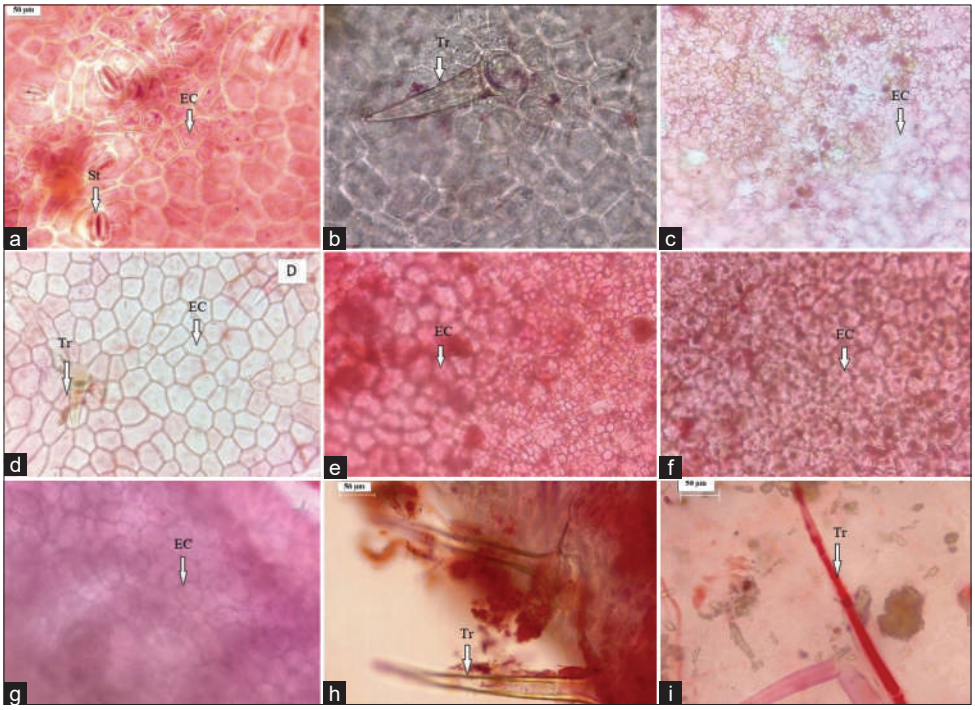

Figure 2: Light micrographs (LM) of adaxial surface of foliar leaf epidermal features of *Glochidion* a) *G. multiloculare* var. *multiloculare*, b) *G. multiloculare* var. *pubescens*, c) *G. ellipticum*, d) *G. heyneanum,* e) *G. lanceolarium*, f) *G. sphaerogynum*, g) *G. zeylanicum* var. *zeylanicum*, h) *G. zeylanicum* var. *arborescens* and i) *G. zeylanicum* var. *tomentosum*. St = Stomata, EC = Epidermal Cell, Tr = Trichome. Scale bars = 50 μm (a-i).

G. zeylanicum var. *zeylanicum*, *G. zeylanicum* var. *arborescens*, *G. zeylanicum* var. *tomentosum* (Figures 1c–1i & 2c–2i) and hypostomatic type was dominant among the studied taxa. Amphistomatic leaves were observed only in *G. multiloculare* var. *multiloculare* (Figures 1a & 2a) and *G. multiloculare* var. *pubescens* (Figures 1b & 2b).

The study confirmed four different types of stomata i.e., anomocytic, anisocytic, paracytic and hemiparacytic. Among them, anomocytic was the most common type of stomata recorded in all the taxa. The stomatal shape varied from elliptic to oval and elongated. Stomatal density and epidermal cell density were calculated. The stomatal index was also calculated based on

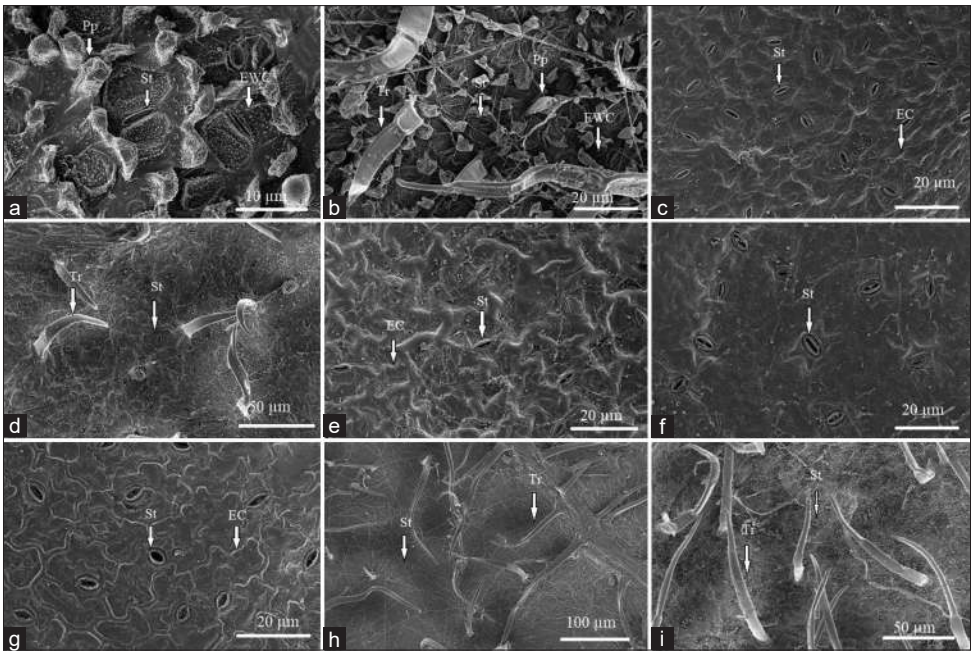

Figure 3: Field emission scanning electron micrographs (FESEM) of abaxial surface of foliar leaf epidermal features of *Glochidion* a) *G. multiloculare* var. *multiloculare*, b) *G. multiloculare* var. *pubescens*, c) *G. ellipticum*, d) *G. heyneanum*, e) *G. lanceolarium*, f) *G. sphaerogynum*, g) *G. zeylanicum* var. *zeylanicum*, h) *G. zeylanicum* var. *arborescens* and i) *G. zeylanicum* var. *tomentosum*. St = Stomata, EC = Epidermal Cell, EWC = Epicuticular Wax Crystal, Pp = Papillae, Tr = Trichome. Scale bars = 10 µm (a), 20 µm (b, c, e, f, g), 50 µm (d, i), 100 µm (h).

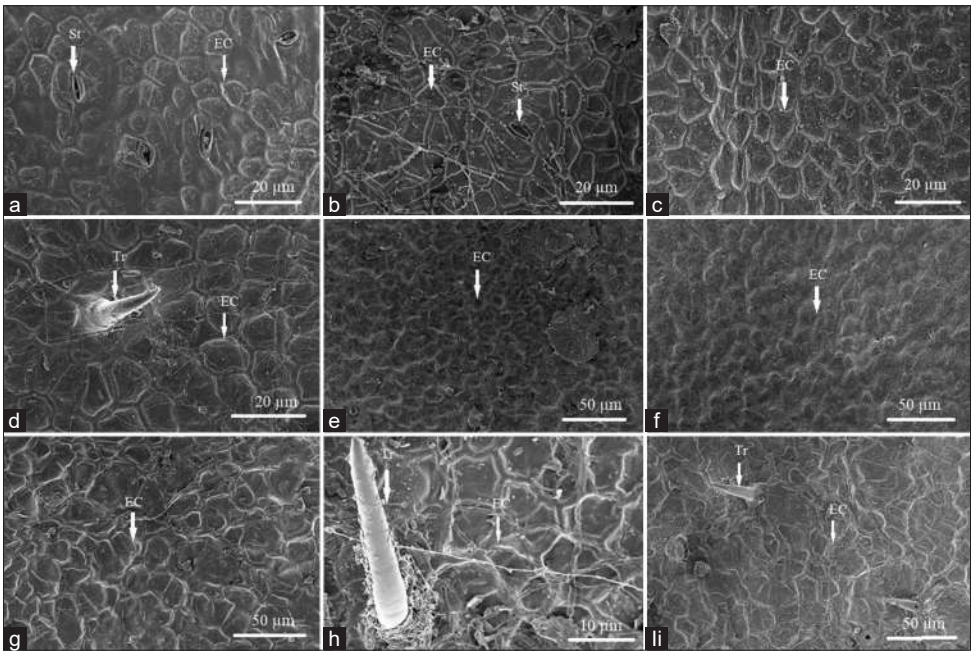

Figure 4: Field emission scanning electron micrographs (FESEM) of adaxial surface of foliar leaf epidermal features of *Glochidion* a) *G. multiloculare* var. *multiloculare*, b) *G. multiloculare* var. *pubescens*, c) *G. ellipticum*, d) *G. heyneanum*, e) *G. lanceolarium*, f) *G. sphaerogynum*, g) *G. zeylanicum* var. *zeylanicum*, h) *G. zeylanicum* var. *arborescens* and i) *G. zeylanicum* var. *tomentosum*. St = Stomata, EC = Epidermal Cell, Tr = Trichome. Scale bars = 10 µm (H), 20 µm (a, b, c, d), 50 µm (e, f, g, i).

the number of stomata and the number of epidermal cells per unit area and the stomatal area was calculated based on stomatal length and width (Table 3). The stomatal size measurement was taken at 50 µm. The stomatal length varied from 11.70±1.112 µm in *G. zeylanicum* var. *arborescens* (Table 3) to 43.14±2.340 µm on the abaxial surface in *G. ellipticum* (Table 3) and the width varied from 6.21±0.504 µm in *G. zeylanicum* var. *arborescens* (Table 3) to 24.756±1.432 µm on the abaxial surface in *G. ellipticum* (Table 3). The highest stomatal index (31.47%) was observed in *G. zeylanicum* var. *arborescens* (Table 3) and the lowest stomatal index (11.56% on the abaxial surface, 10.02% on the adaxial surface) was observed in *G. multiloculare* var.

Table 4: PCA analysis of some members of genus *Glochidion* based on quantitative data

PC	Eigenvalue	% variance
1	77966.7	67.874
2	30643.9	26.677
3	5909.99	5.1449
4	345.037	0.30037
5	2.40281	0.002092
6	1.88273	0.001639
7	0.196663	0.000171

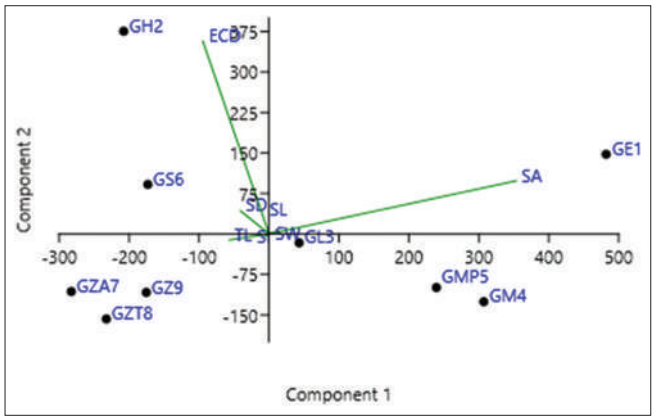

Figure 5: Multivariate PCA analysis of different members of genus *Glochidion* based on their quantitative data of foliar epidermal micromorphology

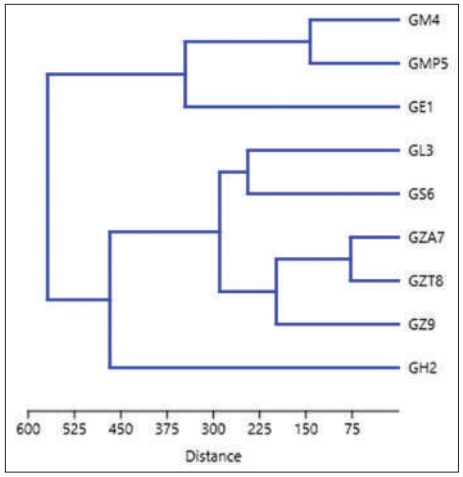

Figure 6: Dendrogram using hierarchical cluster analysis of different members of genus *Glochidion* based on their quantitative data of foliar epidermal micromorphology

multiloculare (Table 3). In *G. multiloculare* var. *multiloculare*, the stomata were randomly distributed and a very less number of stomata were observed on the adaxial surface compared to the abaxial surface of the leaf.

Epidermal Cells Shape

The studied taxa exhibited different shapes of epidermal cells i.e., they varied from hexagonal, and pentagonal to polygonal, isodiametric, rectangular, jigsaw, and undulate (Table 2). Jigsaw shapes of epidermal cells were observed in the taxa like *G. ellipticum* (Figures 1c, 2c, 3c & 4c), *G. lanceolarium* (Figures 1e, 2e, 3e & 4e), *G. sphaerogynum* (Figures 1f, 2f, 3f & 4f), *G. zeylanicum* var. *zeylanicum* (Figures 1g, 2g, 3g & 4g), *G. zeylanicum* var. *arborescens* (Figures 1h, 2h, 3h & 4h) and *G. zeylanicum* var. *tomentosum* (Figures 1i, 2i, 3i & 4i).

Anticlinal Cell Wall

Anticlinal cell walls were distinguished from smooth, angular, rounded, and irregular to sinuous. The smooth, angular, rounded anticlinal cell was observed in *G. multiloculare* var. *multiloculare* (Figures 1a, 2a, 3a & 4a) and *G. multiloculare* var. *pubescens* (Figures 1b, 2b, 3b & 4b), and smooth, angular, rounded, irregular to sinuous, buttressed were observed in *G. ellipticum* (Figures 1c, 2c, 3c & 4c), *G. heyneanum* (Figures 1d, 2d, 3d & 4d), *G. lanceolarium* (Figures 1e, 2e, 3e & 4e), *G. sphaerogynum* (Figures 1f, 2f, 3f & 4f), *G. zeylanicum* var. *zeylanicum* (Figures 1g, 2g, 3g & 4g), *G. zeylanicum* var. *arborescens* (Figures 1h, 2h, 3h & 4h), *G. zeylanicum* var. *tomentosum* (Figures 1i, 2i, 3i & 4i).

Papillae

Papillae were observed clearly through FESEM on the lower surface of *G. multiloculare* var. *multiloculare* (Figure 3a) and *G. multiloculare* var. *pubescens* (Figure 3b), and they were almost rounded in structure. The rest of the taxa exhibited no papillae.

Epicuticular Wax Crystals

Epicuticular wax crystals were observed through the FESEM study, particularly on the lower side of the leaf. They were thick at the papillae, smooth, and upright around the stomata in *G. multiloculare* var. *multiloculare* (Figure 3a) and smooth, thick at the papillae, and upright around the stomata and trichomes in *G. multiloculare* var. *pubescens* (Figure 3b). In *G. sphaerogynum* (Figure 3f) they were slightly present around the stomata.

Trichomes Types and Size

Trichomes were either present or absent in the studied taxa; if they were present, they were densely distributed on the abaxial surface and sparsely distributed on the adaxial surface. Trichomes were completely absent or glabrous on both the surfaces of the leaf in *G. multiloculare* var. *multiloculare* (Figures 1a, 2a, 3a & 4a), *G. ellipticum* (Figures 1c, 2c, 3c & 4c), *G. lanceolarium* (Figures 1e, 2e, 3e & 4e), *G. sphaerogynum* (Figures 1f, 2f, 3f & 4f), *G. zeylanicum* var. *zeylanicum* (Figures 1g, 2g, 3g & 4g). Basically, two types of trichomes were observed: uniseriate, multicellular, unbranched, non-glandular, and uncinate, hooked, multicellular, unbranched, non-glandular trichomes. Uniseriate, multicellular, unbranched, non-glandular trichomes were observed in *G. multiloculare* var. *pubescens* (Figures 1b, 2b, 3b & 4b), *G. zeylanicum* var. *arborescens* (Figures 1h, 2h, 3h & 4h), *G. zeylanicum* var. *tomentosum* (Figures 1i, 2i, 3i & 4i), and uncinate, hooked, multicellular, unbranched, non-glandular trichomes were

observed in *G. heyneanum* (Figures 1d, 2d, 3d & 4d). The length of the trichome varied from 131.336±7.170 µm in *G. multiloculare* var. *pubescens* (Table 3) to 195.033±7.374 µm in *G. zeylanicum* var. *arborescens* (Table 3) on the abaxial surface and 114.033±27.881 µm in *G. heyneanum* (Table 3) to 151.023±5.450 µm in *G. zeylanicum* var. *arborescens* (Table 3) on the adaxial surface.

Key to Species and Varieties of *Glochidion* based on Foliar Leaf Epidermal Characters through LM and FESEM

1a. Leaves amphistomatic.....2

1b. Leaves hypostomatic.....3

2a. Stomata are anomocytic, anisocytic, hemiparacytic in the abaxial surface and anomocytic, anisocytic in the adaxial surface..... *G. multiloculare* var. *multiloculare*

2b. Stomata are anomocytic, anisocytic, paracytic in the abaxial surface and anomocytic and anisocytic in the adaxial surface..... *G. multiloculare* var. *pubescens*

3a. Epicuticular wax crystal slightly present around stomata on abaxial side.....*G. sphaerogynum*

3b. Epicuticular wax crystal completely absent.....4

4a. Stomatal index is 22.06 % present on abaxial surface.....*G. ellipticum*

4b. Stomatal index is 21.66% present on the abaxial surface.....*G. lanceolarium*

5a. Stomatal shape elongated on both abaxial and adaxial surfaces.....*G. heyneanum*

5b. Stomatal shape elliptic to the oval on both abaxial and adaxial surface.....*G. zeylanicum* var. *zeylanicum*

6a. Trichome length varies from 195.033±7.374 µm on the abaxial surface to 151.023±5.450 µm on the adaxial surface..... *G. zeylanicum* var. *arborescens*

6b. Trichome length varies from 176.486±22.977 µm on the abaxial surface to 115.196±24.205 µm on the adaxial surface.....*G. zeylanicum* var. *tomentosum*

Principal Component Analysis (PCA) and Cluster Analysis

Based on PCA, the correlations among the nine taxa concerning the quantitative data of foliar leaf micromorphology were analyzed (Table 4 & Figure 5). The multivariate data of the first PC showed the highest variance of 67.874% and an eigenvalue of 77966.7 compare to other PC. PC1 derived the correlation among the taxa based on quantitative data of stomatal density, PC2 for epidermal cell density, PC3 for stomatal index, PC4 for stomatal length, PC5 for stomatal width, PC6 for stomatal area and PC7 for trichome length. In component 1 total 4 taxa were observed i.e., *G. ellipticum* (GE1), *G. lanceolarium* (GL3), *G. multiloculare* var. *multiloculare* (GM4), *G. multiloculare* var. *pubescens* (GMP5). In component 2, taxa like *G. heyneanum* (GH2), *G. sphaerogynum* (GS6), *G. zeylanicum* var. *arborescens* (GZA7), *G. zeylanicum* var. *tomentosum* (GZT8), *G. zeylanicum* var. *zeylanicum* (GZ9) were observed.

Based on their quantitative data, hierarchical cluster analysis (Figure 6) was performed for the nine taxa. The tree was mainly divided into three clusters cluster 1 and cluster 2. Cluster 1 exhibited *G. heyneanum* (GH2), *G. lanceolarium* (GL3), *G. sphaerogynum* (GS6), *G. zeylanicum* var. *arborescens* (GZA7), *G. zeylanicum* var. *tomentosum* (GZT8) and *G. zeylanicum* var. *zeylanicum* (GZ9). Cluster 2 exhibited *G. ellipticum* (GE1), *G. multiloculare* var. *multiloculare* (GM4) and G. multilocuare var. *pubescens* (GMP5). Taxa present on the same cluster specified more correlation among the taxa.

DISCUSSION

The micromorphological investigation of foliar leaf epidermal study gives important information regarding the delimitation of the genera and species studied and many plant groups use stomata distribution, shape, and size for taxonomic purposes (Shah *et al*., 2018). Both qualitative and quantitative foliar leaf epidermal characters by LM and FESEM can help to generate taxonomic keys and overcome the problem of similarity among the taxa. In the present study, it was observed that the characters of the taxa were significantly distinguishable from each other. The qualitative study provides stomatal features, shape, epidermal cells, types, anticlinal cell walls, papillae, epicuticular wax crystals, and trichomes types and quantitative analysis provides stomatal density, length, width, area, stomatal index, trichomes length, and width. The taxonomic key was made based on the characteristics studied under light microscopy (LM) and field emission scanning electron microscopy (FESEM).

In the present study, two types of stomatal positions were observed i.e., hypostomatic leaves and amphistomatic leaves. Almost all the taxa possessed hypostomatic leaf surfaces except *G. multiloculare* var. *multiloculare* and *G. multiloculare* var. *pubescens* which have amphistomatic leaf surfaces. The diversity of stomata is beneficial at the higher taxonomic level (Cotthem, 1970). Anomocytic type of stomata was observed dominantly in all the taxa. Anisocytic, paracytic and hemiparacytic types of stomata were also observed in studied taxa. The stomatal shape varied from elliptic, and oval to elongated. The stomatal size was also found to be a significant character in studied taxa. They play an essential role in phylogenetic relationship studies and their physical characteristics are important for plant origin and classification (Khan *et al*., 2014; Razzaq *et al*., 2021). In

the quantitative study, we found the highest stomatal length in *G. ellipticum* (43.14±2.340 µm) and lowest in *G. zeylanicum* var. *arborescens* (11.70±1.112 µm) and the highest stomatal width were observed in *G. ellipticum* (24.756±1.432 µm) and lowest in *G. zeylanicum* var. *arborescens* (6.21±0.504 µm). The highest percentage of the stomatal index was observed in the variety *G. zeylanicum* var. *arborescens*, and the lowest percentage of the stomatal index was observed in *G. multiloculare* var. *multiloculare* and their stomata were randomly distributed with the lowest number in the adaxial surface compared to the abaxial surface (Table 3). The shape of epidermal cells varied from isodiametric, pentagonal, and hexagonal to polygonal and some taxa like *G. ellipticum*, *G. lanceolarium*, *G. sphaerogynum*, *G. zeylanicum* var. *zeylanicum*, *G. zeylanicum* var. *arborescens*, *G. zeylanicum* var. *tomentosum* have undulate and jigsaw shape of the epidermal cell. In the present study, the anticlinal cell wall was mostly sinuous and sometimes smooth, rounded, and angular. Rounded papillae were observed on the abaxial side of *G. multiloculare* var. *multiloculare* (Figure 3a) and *G. multiloculare* var. *pubescens* (Figure 3b). There were no papillae present in the rest of the taxa. Under light microscopy, identifying papillae's exact location, size, and shape is practically impossible (Duarte-Silva *et al*., 2013). Therefore, scanning electron microscopy was used to identify those traits on the leaf surfaces.

Epicuticular wax acts as a barrier to plant cuticles and protects the plant against uncontrolled water loss, and reflection of solar radiation from UV to visible light (Adams *et al*., 1990; Barthlott *et al*., 1998; Koch & Barthlott, 2006). Epicuticular wax crystals were clearly observed on the lower surface of *G. multiloculare* var. *multiloculare* (Figure 3a) and *G. multiloculare* var. *pubescens* (Figure 3b). They were mostly smooth, thick, and generally present either around the stomata or trichomes. Epicuticular wax crystal was slightly observed in *G. sphaerogynum* (Figure 3f). Micromorphology of cuticular waxes is useful in taxa delineation at several taxonomic levels within flowering plants (Barthlott *et al*., 1998). After becoming the importance of SEM, nowadays many characteristics of the leaf surface have been discovered. Papillae and epicuticular wax have been observed mostly under SEM (Duarte-Silva *et al*., 2013).

In the studied taxa, trichomes were either present or absent. Trichomes were observed in the taxa such as *G. multiloculare* var. *pubescens* (Figures 1b, 2b, 3b & 4b), *G. heyneanum* (Figures 1d, 2d, 3d & 4d), *G. zeylanicum* var. *arborescens* (Figures 1h, 2h, 3h & 4h), and *G. zeylanicum* var. *tomentosum* (Figures 1i, 2i, 3i & 4i). They were densely present on the abaxial surface rather than the adaxial surface. They were uniseriate, multicellular, unbranched, and non-glandular types. In the case of *G. heyneanum* (Figures 1d, 2d, 3d & 4d), they were an almost hooked shape, uniseriate, multicellular, unbranched, and non-glandular. Glands were absent in all the taxa. The absence of glandular trichomes on the leaf suggests that some other tissues on the leaf are responsible for the secretion of secondary metabolites (Ndam *et al*., 2015). Quantitatively the highest length of the trichomes was observed in *G. zeylanicum* var. *arborescens* (195.033±7.374 µm) and the lowest in *G. multiloculare* var. *pubescens* (131.336±7.170 µm) on the abaxial surface and in the adaxial surface highest trichome length was observed in *G. zeylanicum* var. *arborescens* (151.023±5.450 µm) and lowest in *G. heyneanum* (114.033±27.881 µm). Trichomes have been used to resolve taxonomic conflicts as well as to understand the evolutionary relationship among the species (Payne, 1978; Solihani *et al*., 2015; El-Taher *et al*., 2020).

Principal component analysis (PCA) is an analytical method that seeks to quantify the variation observed in the dataset (Granato *et al*., 2018). The result showed the variation and correlation among the studied taxa. Eigenvalues of stomatal density, epidermal cell density and stomatal index showed more significant values compared to other micromorphological traits (Table 4). Hierarchical cluster analysis of the quantitative data revealed the relationships among the taxa. *G. multiloculare* var. *multiloculare* (GM1) and *G. multiloculare* var. *pubescens* (GMP2) grouped in the same cluster and same clade (Figure 6) indicated that these two taxa had adjacent relationships. In the present study, two varieties were found under *G. zeylanicum* i.e., *G. zeylanicum* var. *arborescens* (GZA7) and *G. zeylanicum* var. *tomentosum* (GZT8) along with *G. zeylanicum* var. *zeylanicum* (GZ9) were signified close distance among each other (Figure 6).

Qualitative and quantitative foliar leaf epidermal characters revealed by LM and FESEM can help generate a taxonomic key that will be used to confirm the identity of selected taxa and overcome the problem of similarity among the species and variety of different members of the genus *Glochidion*. This is the first detailed work on the foliar epidermal micromorphology of the genus *Glochidion*.

CONCLUSION

The present study summarizes the importance of foliar epidermal micromorphology study for the identification of taxa. These characters show the diversification among the taxa. The taxa can be distinguished based on their stomatal position, shape, size, area, stomatal index, epidermal cell shape, anticlinal cell wall, papillae, epicuticular wax crystals, trichomes types, and sizes. The construction of an artificial key signifies the identification of the species and varieties of the genus *Glochidion*. The present study demonstrated the importance of foliar leaf epidermal characters in the identity of taxa up to the variety level. Both qualitative and quantitative data provide diagnostic features to distinguish among the studied taxa. Besides, PCA and cluster analysis define the establishment of the taxonomic boundaries and species delimitation of micromorphological traits.

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