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Research article

Taxonomic delimitations in five species of genus *Barleria* (Acanthaceae) using scanning electron microscopy

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Abstract: In some complex families like Acanthaceae macromorphological features are not sufficient for delimitation of various taxa. The role of leaf epidermal details have now proven to play a key role in the identification and delimitation of such taxa. Present work was undertaken to study the leaf epidermal details of five species of genus *Barleria i.e. B. cristata*, *B. prionitis*, *B. gibsonii*, *B. strigosa* and *B. prattensis* using Scanning Electron Microscope (SEM). Based on various epidermal features (type, size, arrangement of stomata; epidermal cell features; types, presence and absence of cystoliths; type, size and abundance of various types of trichomes; etc.) these species were differentiated and delimited. This will add a significant contribution to taxonomic studies of the family Acanthaceae.

Keywords: Acathaceae - *Barleria* - Cystoliths - Leaf epidermal details - Stomata - Trichomes.

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INTRODUCTION

The family Acanthaceae is an ecologically important family as it is an important constituent of tropical floras. The family Acanthaceae is considered as a complicated one by most taxonomists as the taxonomical characters amongst species are most diverse. The genus *Barleria* L. is the third largest genera of Acanthaceae and was dealt by Linnaeus (1753) based on specimens from India. It comprises approximately 300 species (Balkwill & Balkwill 1997, Darbyshire 2010). It is a pantropical in distribution but predominantly an Old World genus, with its greatest centre of species diversity in tropical East Africa, followed by South Africa and Asia (Balkwill & Balkwill 1998).

Balkwill & Balkwill (1997) reported 32 species from India. Whereas Karthikeyan *et al*. (2009) enumerated 29 species, one subspecies and six varieties. Shendage & Yadav (2010) revised the genus *Barleria* L. in India and recognises 26 species, one subspecies and one variety in India. Genus Barleria L. was first studied in detailed by Nees (1847) and it can be differentiated from other genera on the basis of its distinguishing characteristics: Calyx 4-partite, 2 large outer segments, 2 smaller inner ones, spheroidal, pollens honey combed, presence of double cystoliths in epidermal cells.

The taxonomic status of genera of the family Acanthaceae is very complicated and many taxa are yet to be finally delimited. Delimitation of taxa based on only morphological characters are tough task in certain genera of many families. Therefore implications of branches of taxonomy in contrast to many reports of palynological, embryological, and cyto-taxonomic are nowadays giving new hope to the taxonomist. Nowadays leaf epidermal biology is gaining great importance for its role in the delimitation of various complex taxa in various families. And now a days they are gaining importance as DNA sequencing (Edeoga & Ikem 2001). These epidermal micro-features are helpful in solving the taxonomic problems in both generic and species level, and in near future it will pay its usefulness in lower levels, if studied. The distinguishing characters of stomata, subsidiary cells and epidermal cells are very helpful tools (Cutler 1984). Genus *Barlaria* L. have very diverse characteristics and it is very hard to delimit species within this genus based on only morphological characteristics. in the present study, an attempt has been made to examine the taxonomic significance of micro-morphology of leaf surface for delimitation of 5 species of *Barleria* L. in central India *i.e. B. cristata* L., *B.*

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prionitis L., *B. gibsonii* Dalzell, *B. strigosa* Willd and *B. prattensis* Santapau.

Table 1. Some information about the *Barleria* L. species used in present study.

	***B. cristata* L.**	***B. prionitis* L.**	***B. gibsonii* Dalzell**	***B. strigosa* Willd**	***B. prattensis* Santapau**
Native range	South China to Tropical Asia	Tropical Asia	India	Indian subcontinent to Thailand	India
First published in	Sp. Pl.: 636 (1753)	Sp. Pl.: 636 (1753)	Hooker's J. Bot. Kew Gard. Misc. 2: 339 (1850)	Sp. Pl., ed. 4, 3: 379 (1800)	Kew Bull. 3: 487 (1948 publ. 1949)
Synonyms	1. *Barleria alba* G.Lodd. 2. *Barleria ciliata* Roxb. 3. *Barleria dichotoma* Roxb. 4. *Barleria indica* L. ex T.Anderson 5. *Barleria laciniata* Wall. 6. *Barleria lactea* Desf. ex Steud. 7. *Barleria napalensis* Nees 8. *Barleria nuda* Nees 9. *Barleria prinoides* Nees 10. *Barleria venulosa* Nees 11. *Lepidagathis laciniata* Wall. ex Nees	1. *Barleria echinata* St.-Lag. 2. *Barleria hystrix* L. 3. *Barleria prionitis* var. *pubescens* Kuntze 4. *Barleria quadrispinosa* Stokes 5. *Barleria spicata* Roxb. 6. *Prionitis hystrix* (L.) Miq.	1. *Barleria gibsonioides* Blatt.	1. *Barleria caerulea* Roxb. 2. *Barleria polytricha* Wall. 3. *Pseudobarleria caerulea* (Roxb.) Oerst. 4. *Pseudobarleria hirsuta* Oerst. 5. *Pseudobarleria polytricha* (Wall.) Oerst.	-

MATERIAL AND METHODS

Plant material

Dried plant materials used in this study was obtained from BSA herbaria at Botanical Survey of India, Central Regional Centre, Allahabad, Uttar Pradesh, India as follow: *Barleria cristata* L., *B. prionitis* L., *B. gibsonii* Dalzell, *B. strigosa* Willd and *B. prattensis* Santapau. Taxa were preliminarily identified with the help of published literature, floras and comparisons with type material on the Jstor Global Plants website.

Leaf sample preparation

Sample of leaves were taken from the middle intercostal regions of both surface of leaves. Cleaned leaf segments of approximately 10 mm^2 from each plant samples were mounted on aluminium stubs using two-sided

adhesive carbon tapes. These samples were cleaned using fine quality brushes. The samples used in the study were coated with a very thin layer of gold in a sputtering gold coater and then observed in the scanning electron microscope (Hitachi Table Top 3000). Electron images were recorded for each sample using a digital image processor.

Scanning electron microscopic study

Data were taken for each sample of all five species and photographs were recorded. For each species, three samples were thoroughly studied. Study of different epidermal details on dorsal and ventral side of leaves, such as type of stomata, arrangement of stomata, frequency of stomata, features of epidermal cells, presence and absence of different types of trichomes, detailed study of trichomes on both surfaces of leaves, observations for the presence of cystoliths and its characteristics, etc.

Terminology

The description of the stomatal type studied in this work is based on Melcalfe & Chalk (1950). Terminology for leaf epidermal morphology micromorphology was based on the previous description of Ahmad (1978).

RESULTS

Present work intends to utilize this details of leaf epidermal features such as stomata, trichomes and cystoliths for delimitation of certain taxa in genus *Barleria* L. Table 1 depicts the information about the native places, synonyms and reports where these species were first published (from the website www.theplantlist.org). Table 2, 3, 4 & 5 represents detailed information about the comparative and characteristic features of stomata, non-glandular trichomes, glandular trichomes and cystoliths, respectively. Based on these descriptive features all five species can be easily delimited and identified. Features of the adaxial and abaxial epidermis, stomata, non-glandular trichomes, glandular trichomes and cystoliths of each species have been shown in figures 1–5.

Table 2. Comparative account of details of stomata and epidermal cell in five species of *Barleria* L. used in study.

Characteristics	***B. cristata* L.**	***B. prionitis* L.**	***B. gibsonii* Dalzell**	***B. strigosa* Willd**	***B. prattensis* Santapau**
Stomata type	Diacytic	Diacytic	Diacytic	Diacytic	Diacytic
Presence	Hypostomatic	Hypostomatic	Hypostomatic	Hypostomatic	Hypostomatic
Stomatal arrangement	Irregular	Irregular	Irregular	Irregular	Irregular
Stomata density	Good	Good	Dense	Dense	Good
Stomata length range	25–32 µm	31–34 µm	17–25 µm	26–32 µm	26–32 µm
Shape of epidermal cell	Wavy	Wavy tiles like	Wavy tiles like	Wavy	Wavy

Table 3. Comparative account of details of Non-glandular trichomes in five species of *Barleria* L. used in study.

Non-glandular trichome	***B. cristata* L.**	***B. prionitis* L.**	***B. gibsonii* Dalzell**	***B. strigosa* Willd**	***B. prattensis* Santapau**
Lower (abaxial) epidermis					
Cell count	Multicellular, uniseriate, unbranched	-	-	-	-
Frequency	Dense	-	-	-	-
Size range	268–601 µm	-	-	-	-
Upper (adaxial) epidermis					
Cell count	Multicellular, uniseriate, unbranched	-	-	Multicellular, uniseriate, unbranched	-
Frequency	Good	-	-	Few	-
Size range	291–961 µm	-	-	579–1000 µm	-

Stomata and epidermal cells

The analysis of adaxial and abaxial leaf epidermis in all five species showed that all are hypostomatic in nature *i.e.* stomata are present only on lower or abaxial surface of leaves. The arrangement of stomata was also similar in all species *i.e.* irregular arrangement (Figs. 1B, 2A, 3A, 4A & 5A). Likewise, type of stomata was also found similar in all five species (Figs. 1C, 2B, 3B, 4B & 5B) and all are of diacytic type (The stoma is enclosed by a pair of subsidiary cells whose common wall is at right angles to the guard cells). The size of stomata does not vary significantly and stomata was comparatively denser in *B. gibsonii* Dalzell and *B. strigose* Willd among all five species. The ranges of stomatal lengths have been shown in table 2. The epidermal cell was irregular in

shape in all species (Table 2).

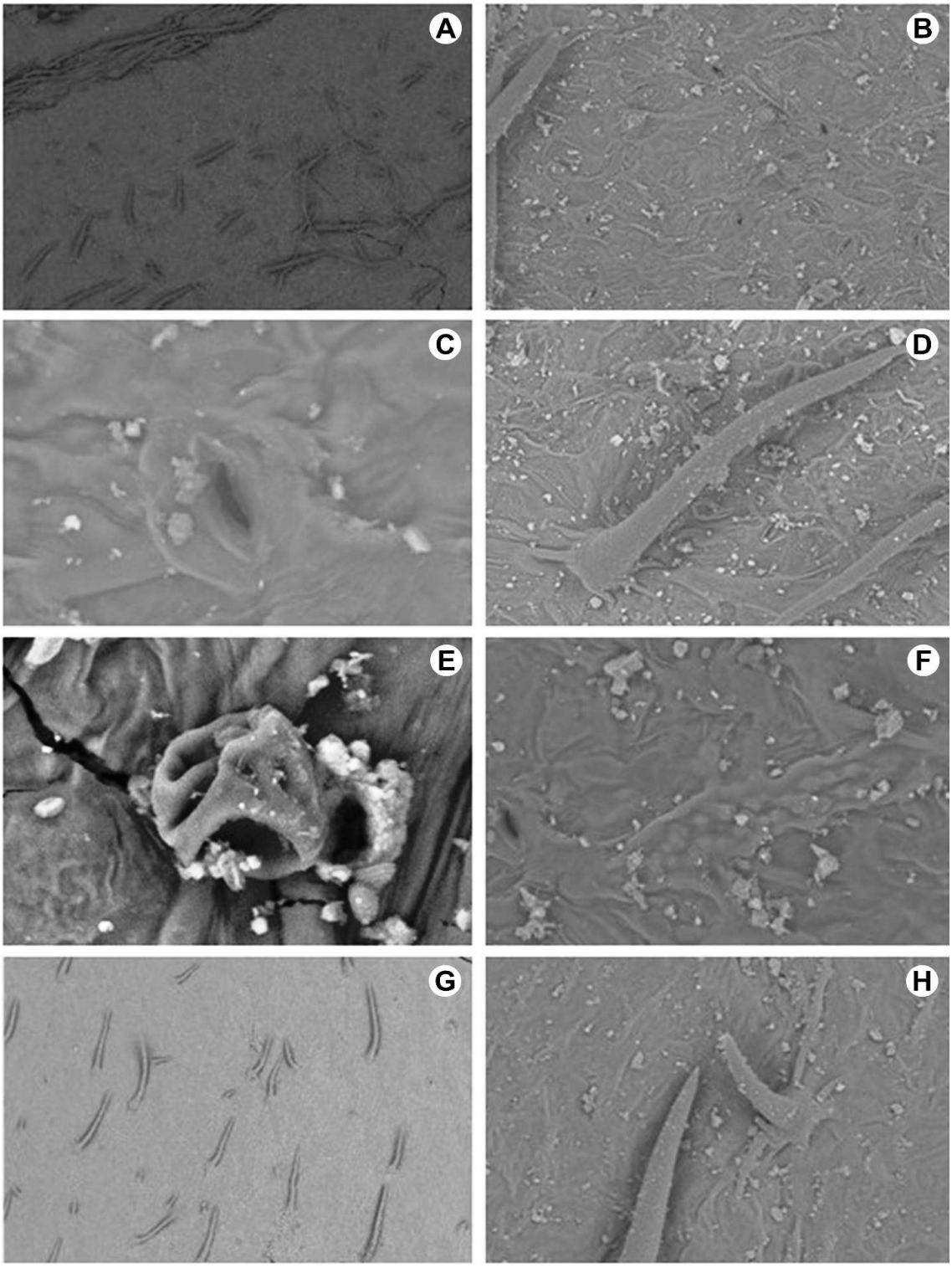

Figure 1. *Barleria cristata* L. [Abaxial epidermis of leaf: A, B, C, D, E & F (**A**- Distribution of non-glandular trichomes, **B**- Stomatal arrangement, **C**- Single diacytic stoma, **D**- Enlarged non-glandular trichomes, **E**- Single glandular trichome, **F**- Single cystoliths); Adaxial epidermis of leaf: G & H (**G**- Distribution of nonglandular trichomes, **H**- Details of non-glandular trichome)]

Non-glandular trichomes

Non-glandular trichomes were observed only in two species *viz. B. cristata* L. and *B. strigosa* Willd. In *B. cristata* L. Non-glandular trichomes were observed on both abaxial (Fig. 1A) and adaxial surface (Fig. 1G) of leaf while in *B. strigose* Willd. non-glandular trichomes were present only on the adaxial surface of leaf (Fig. 4E). In *B. cristata* non-glandular trichomes were denser on the abaxial surface than the adaxial surface of the leaf. These Non-glandular trichomes were multicellular and with pointed ends in both the species. The ranges of size of these non-glandular trichomes have been shown in table 3.

Table 4. Comparative account of details of glandular trichomes in five species of *Barleria* L. used in study.

Glandular trichome	***B. cristata* L.**	***B. prionitis* L.**	***B. gibsonii Dalzell***	***B. strigosa* Willd**	***B. prattensis* Santapau**
Presence	On both surface of leaf	On both surface of leaf	On both surface of leaf	On both surface of leaf	On both surface of leaf
Frequency	****	*****	***	****	**
Diameter range	20–24 µm	25–35 µm	19–22 µm	27–32 µm	21–23 µm
Features	Erupted like bead	Sunken within rim	Somewhat erupted	Erupted above the level	Erupted above the level

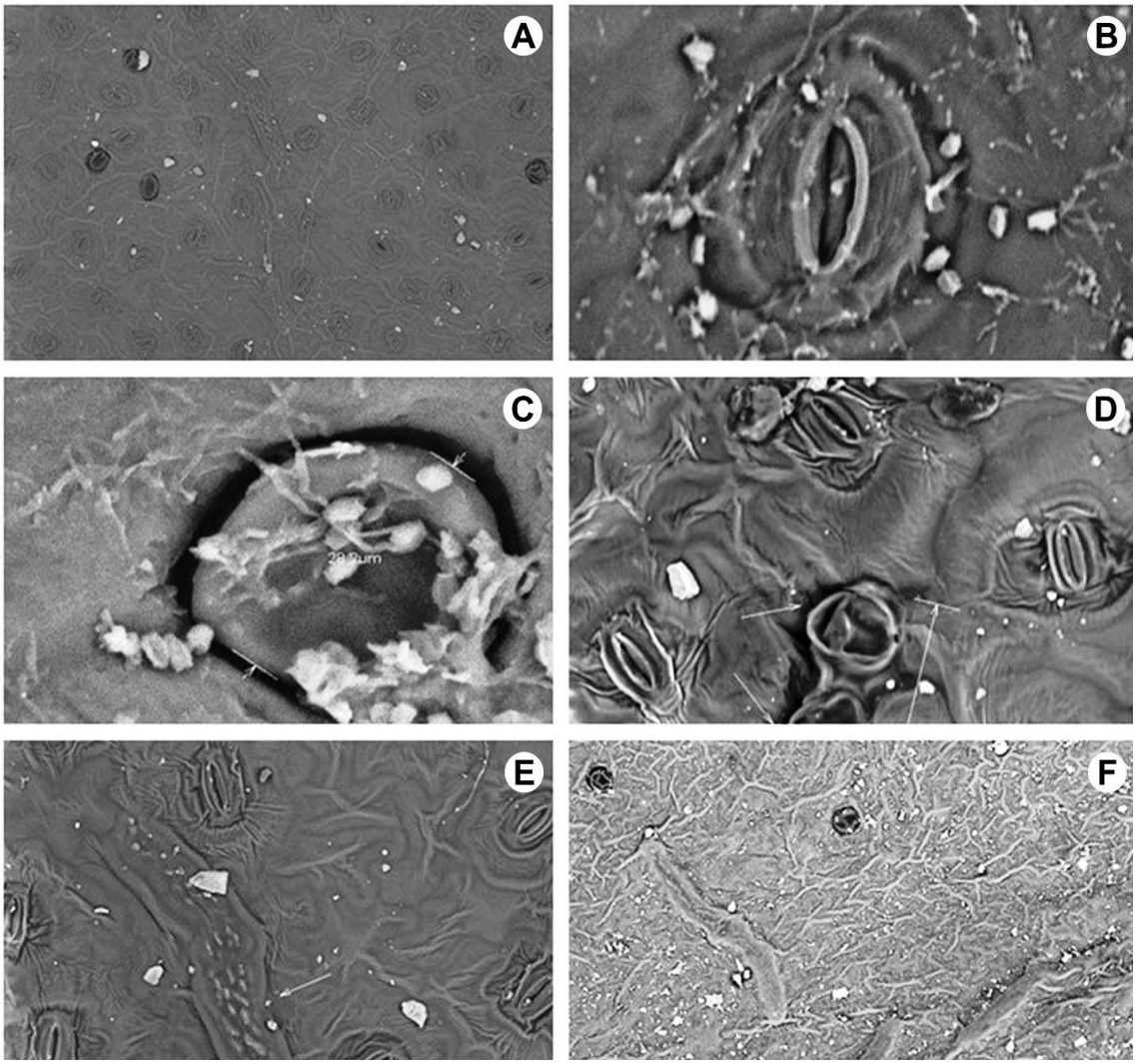

Figure 2. *Barleria prionitis* L. [Abaxial epidermis of leaf: A, B, C, D & E (**A-** Stomatal arrangement, **B-** Single diacytic stoma, **C-** Single glandular trichome, **D-** Double glandular trichomes, **E-** Single cystoliths); Adaxial epidermis of leaf: F (**F-** Glandular trichomes and cystoliths)]

Table 5. Comparative account of details of cystoliths in five species of *Barleria* L. used in study.

Cystoliths	***B. cristata* L.**	***B. prionitis* L.**	***B. gibsonii Dalzell***	***B. strigosa* Willd**	***B. prattensis* Santapau**
Presence	Lower and upper epidermis both	Lower and upper epidermis both	Lower and upper epidermis both	Lower and upper epidermis both	Lower and upper epidermis both
Frequency	***	*****	*****	***	**
Length (mean)	115 µm	230 µm	55 µm	38 µm	280 µm
Features	Erupted and beaded, paired or single	In level, single or paired	Broad and single or paired	Very small, paired or single	Appeared Like rope, very long, paired or single

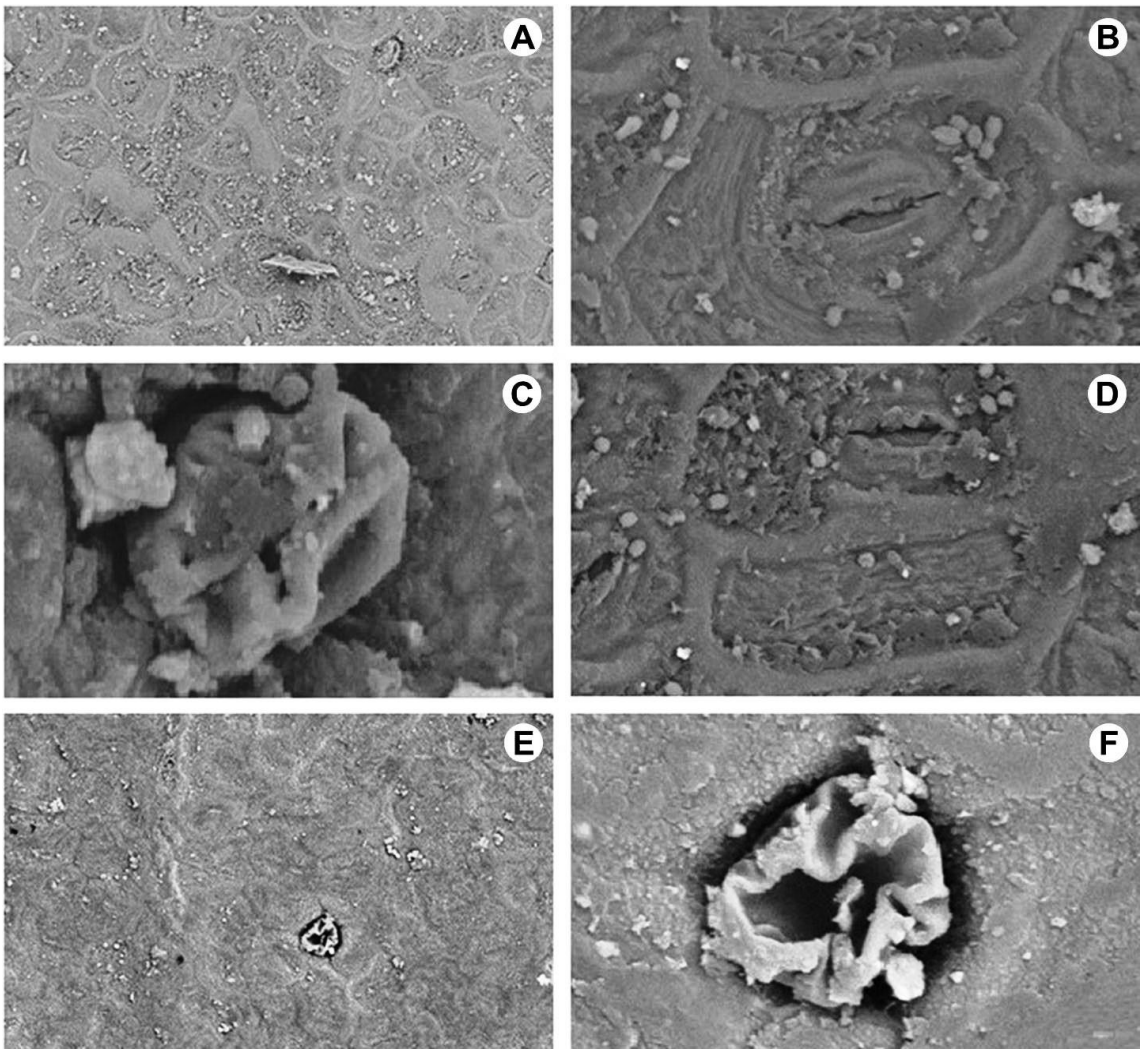

Figure 3. *Barleria gibsonii* Dalzell [Abaxial epidermis of leaf: A, B, C & D (**A**- Stomatal arrangement, **B**- Single diacytic stoma, **C**- Single glandular trichome, **D**- Details of cystoliths); Adaxial epidermis of leaf: E & F (**E**- Glandular trichomes, **F**- Glandular trichome)]

Glandular trichomes

Glandular trichomes were present on the abaxial as well as the adaxial surface of leaves of all five species studied. In all five species studied glandular peltate trichomes were rounded in shape. However, their morphology, frequency, size and characteristics vary in many ways. In *B. cristata* L. Glandular trichomes were erupted and appears like bead (Fig. 1E) on the surface. In *B. prionitis* L. they were sunken within a rim like structure (Fig. 2C). In *B. gibsoni* Dalzell these glandular trichomes were somewhat erupted (Fig. 3C). In other two species (*B. strigosa* Willd. and *B. prattensis* Santapau) these were erupted above the level of epidermis (Fig. 4C & 5C). The highest density of these glandular trichome was observed in *B. prionitis* L. while they were lowest in density in *B. prattensis* Santapau. Glandular trichomes were peltate in nature and consists of small basal cell and a multicellular head. The diameter of these peltate glandular trichomes in different species have been depicted in table 4.

Cystoliths

Cystoliths were present on both the adaxial and abaxial epidermis of leaves in all five species (Figs. 1F, 2E, 3D, 4D & 5D). The most characteristic feature that is observed in all species is the dominance of double cystoliths. The frequency and length of cystoliths vary in each species. The maximum density of cystoliths was observed in *B. prionitis* L. and *B. gibsonii* Dalzell and the lowest was in *B. prattensis* Santapau. The size of cystoliths varies significantly in all five species. In *B. prattensis* mean length of cystoliths was 280 µm which is maximum in all five species, while the smallest cystoliths were observed in *B. strigose* Willd. Cystoliths were epidermal in nature.

DISCUSSION

Recent studies of leaf epidermal details have been found useful in solving taxonomic problems in many

families. These micro-morphological characters of leaves are also useful in phylogenetic studies in these many families like Solanaceae, Acanthaceae and Urticaceae, etc. (Gangadhara & Inamdar 2005, Adedeji *et al*. 2007, McDade *et al*. 2008). Till date there is no literature available for solving taxonomic problems in the whole family Acanthaceae based on leaf epidermal characters. Although there are a few genera and some species in which epidermal studies have been carried out and among these studies based on scanning electron microscopy are still rare. Therefore, the present study was undertaken to delimit the taxa in genus *Barleria* L. based on leaf epidermal details only.

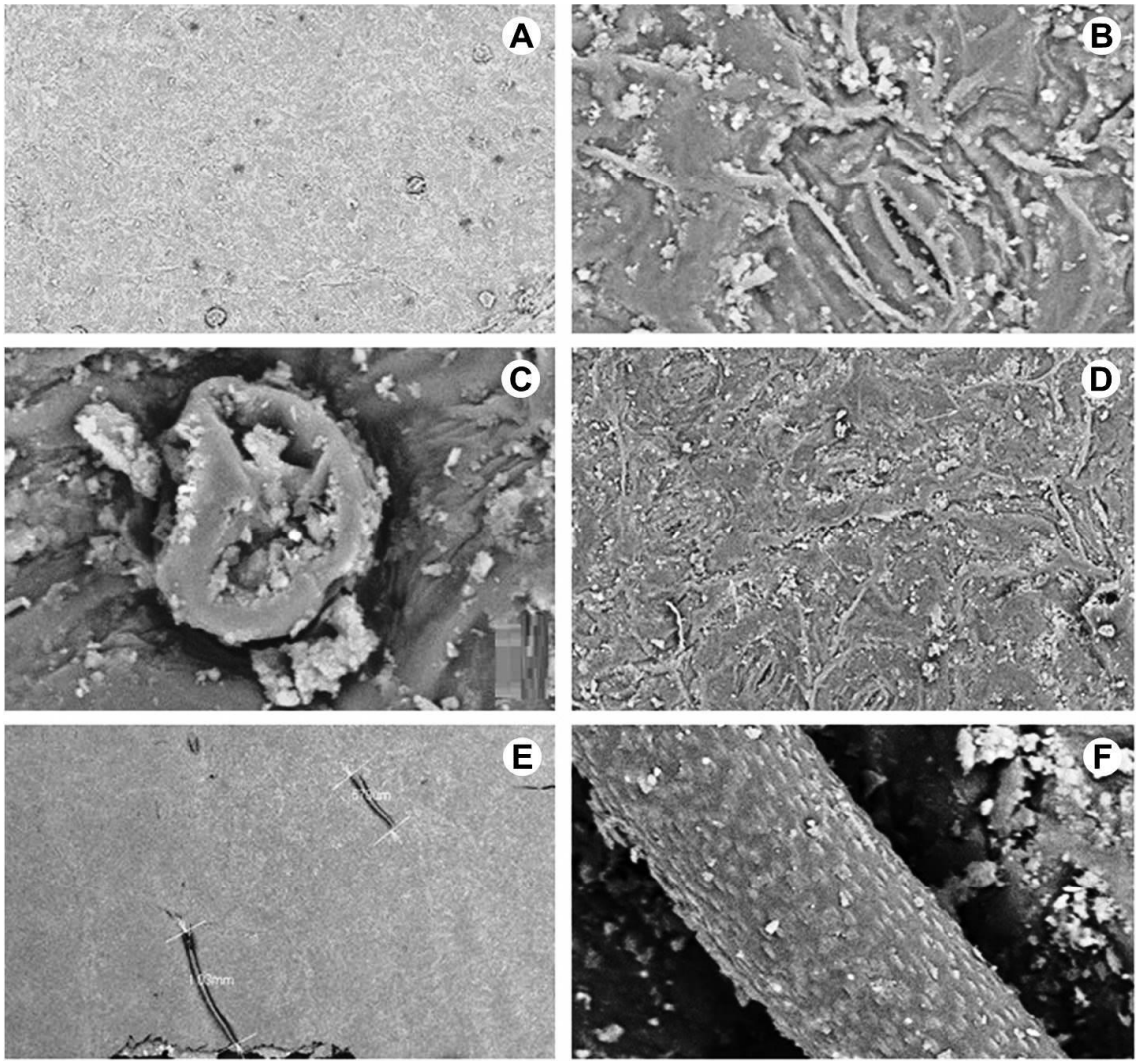

Figure 4. *Barleria strigosa* Willd [Abaxial epidermis of leaf: A, B, C & D (**A**- Stomatal arrangement, **B**- Single diacytic stoma, **C**- Single glandular trichome, **D**- Cystolith); Adaxial epidermis of leaf: E & F (**E**- Non-glandular trichomes, **F**- Non-glandular trichome)]

As the results shown that these five species of *Barleria* L. *i.e. B. cristata* L., *B. prionitis* L., *B. gibsonii* Dalzell, *B. strigosa* Willd and *B. prattensis* Santapau are quite different from each other in terms of leaf epidermal details. The epidermal cells were more or less similar in all five and they have wavy margins. Arrangement of stomata was irregular in all five species and type of stomata was also similar if all five species *i.e.* diacytic type which is a common characteristic of family Acanthaceae (Melcalf & Calk 1950). Pant & Mehra (1963) first traced the ontogeny of diacytic type of stomata in Acanthaceae. It is clear that stomatal characters are more or less similar in all five species studies, they were different only in terms of their density and size but not significantly, to be used as delimiting character to differentiate these species. Further, it is trichomes and cystoliths, which are differentiating and delimiting characters for these species. Earlier works on the trichomes of many genera of Acanthaceae also suggests similar results *i.e.* trichomes are used as aid for identification.

Trichomes are unicellular or multicellular hair-like outgrowths originated from the aerial epidermis of plant parts and they vary in morphology, location and mode of secretion (Werker 2000). Two types of trichomes were observed in the present study and glandular peltate trichomes were found in all five species studied. Glandular

trichomes are associated with the secretion of chemicals that provide a defence mechanism against herbivores and pathogens. The morphology and size of glandular trichomes vary in each species.

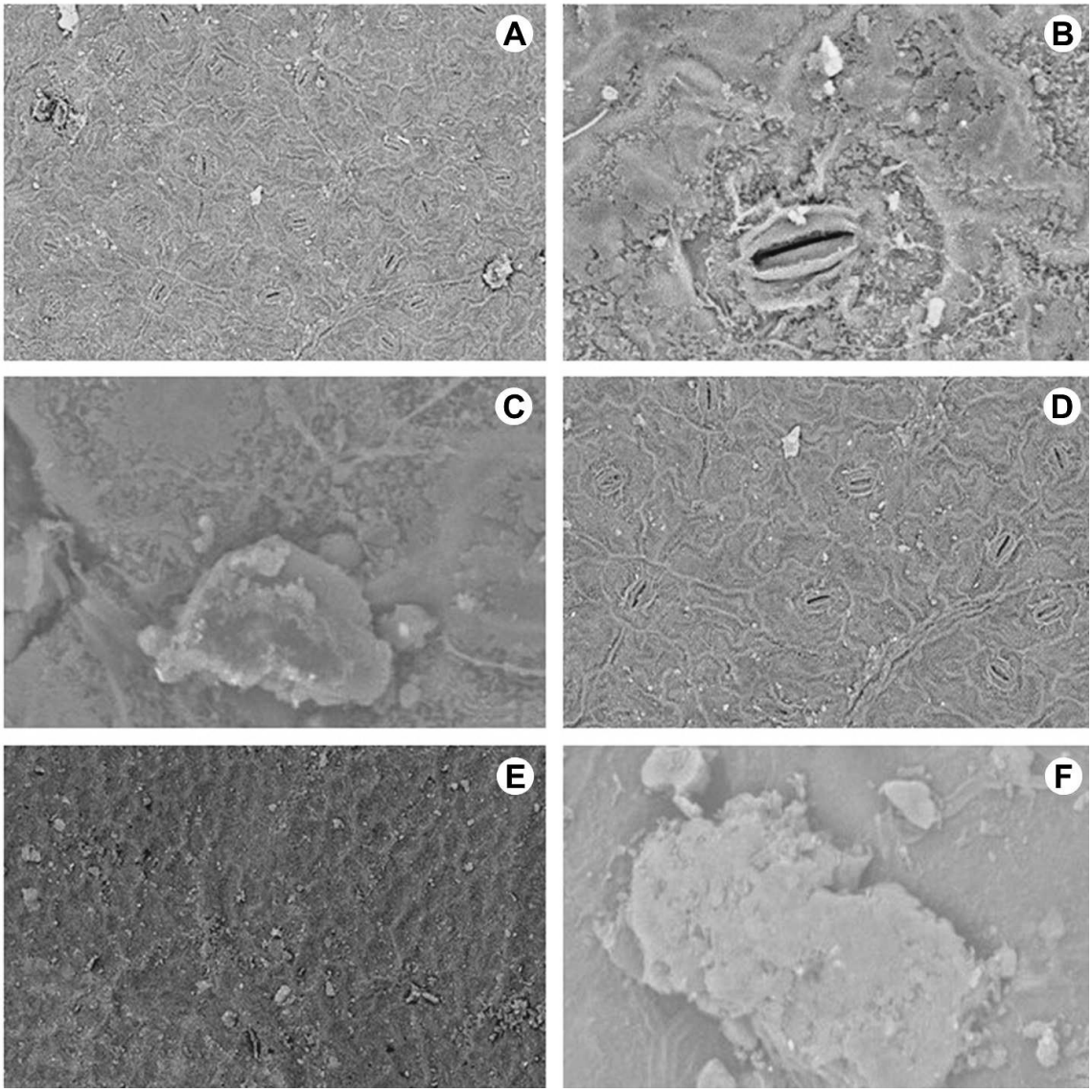

Figure 5. *Barleria prattensis* Santapau [Abaxial epidermis of leaf: A, B, C & D (**A**- Stomatal arrangement, **B**- Single diacytic stoma, **C**- Single glandular trichome, **D**- Cystolith); Adaxial epidermis of leaf: E & F (**E**- Epidermal cell, **F**- Double non-glandular trichomes)]

Glandular trichomes were present on both the adaxial and abaxial surfaces of leaves in each species. However, Non-glandular trichomes were observed in only two species *i.e. B. cristata* L. and *B. strigosa* Willd. and in *B. cristata* it was present on both adaxial and abxial side of leaf epidermis while in *B. strigosa* Willd it was observed only on adaxial side of leaf epidermis. The wall ornamentation of nonglandular trichomes was generally round, oval, elliptic tubercles in both *B. cristata* L. and *B. strigosa* Willd (Fig. 4F). It is the general observation that the non-glandular trichomes were denser on the veins of leaves. A similar observation was also noticed by Oppenheimer (1959). Non-glandular trichomes play a significant role in the protection of leaves. Non-glandular trichomes were covered with cuticular warts which have protective values towards the dust like a 'Lotus effect' (Nosonovsky & Bhushan, 2007). Non-glandular trichomes were supported by a basal pedestal like structure in both species *i.e. B. cristata* L. (Fig. 1D) and *B. strigosa* Willd. These pedestal like structure are the point where non-glandular trichome joints with the epidermis and it also provide mechanical support (Ascensao *et al*. 1999). The structure and distribution of trichomes in a few other species of *Barleria* L. was studies by Ahmad (1968) with the use of light microscopy. Arumugam & Natesan (2015) studies trichomes in *Barleria noctifolra* and *Barleria prionitis.*

The dominance of double cystoliths in *Barleria* L. is an interesting feature. These are also present in other genera of Acanthaceae (Melcalfe & Chalk 1950). Cystoliths are epidermal growths filled with calcium carbonate. Cystoliths were present in all five species and all cases it seen on both the adaxial and abaxial

surfaces of leaves. While the size, density and morphology of these cystoliths appeared as distinguishing features in these studied species (Table 5). If these features of cystoliths are combined with features of trichomes then it will be an important character for the identification of a particular species. In *B. prionitis* L. and *B. gibsoni* Dalzell were denser as compared to the other three species. During the study, it has been found that the size of cystoliths are a distinguishing feature for each studied species. In *B. gibsonii* Dalzell and *B. strigosa* Willd cystoliths were very small in comparison to other three species *i.e.* 55 µm and 38 µm, respectively.

Studies on the leaf epidermal details using light microscopy has been available for some genera of Acanthaceae while studies based on scanning electron microscopy is still rare. With the help of SEM we can easily expose the microcharacters upto the micrometer level and these characters could be utilized in solving many taxonomic problems and phylogenetic studies. Hence, the present study will definitely utilize the micromorphological characters of leaves as tool for taxonomic problems in the family Acanthaceae and add a database of information for micromorphological characters.

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