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Research Article

CHARACTERIZATION OF SOME COMMON MEMBERS OF THE FAMILY MALVACEAE *S.S.* ON THE BASIS OF MORPHOLOGY OF SELECTIVE ATTRIBUTES: EPICALYX, STAMINAL TUBE, STIGMATIC HEAD AND TRICHOME

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ABSTRACT
Epicalyx, staminal tube, stigma and trichome morphological characters have been used to characterize some common members of Malvaceae *s.s*. These characters have been analyzed following a recent molecular phylogenetic classification of Malvaceae *s.s*. Stigmatic character is effective for segregation of the tribe Gossypieae from other tribes. But precise distinction of other two studied tribes, viz. Hibisceae and Malveae on the basis of this character proved to be insufficient. Absence of epicalyx in *Malachra* has indicated an independent evolutionary event within Hibisceae. Distinct H-shaped trichome of *Malvastrum* has pointed out its isolated position within Malveae. Staminal tube morphological similarities of *Abutilon* and *Sida* have suggested their closeness. A key to the genera has been provided for identification purpose.

Keywords: *Malvaceae s.s., Epicalyx, Staminal Tube, Stigma, Trichome*

INTRODUCTION
Epicalyx and monadelphous stamens are considered as key characters of the family Malvaceae *s.s*. Epicalyx was recognized as an important character for taxonomic value by several authors (Fryxell, 1988; Esteves, 2000) since its presence or absence was employed to determine phylogenetic interpretation within the tribes of Malvaceae *s.s.* (Tate *et al.,* 2005). There have been substantial variations within staminal tube morphology to segregate taxa at species level (Esteves, 2000). Heel (1966) described morphology of the staminal tube in Malvaceae. Esteves (2000) critically examined the variation in morphology of both androecium and epicalyx of Brazilian species of *Pavonia*. Fryxell (1988) used stigma-mericarp ratio for distinction of the tribes of Malvaceae *s.s*. In this regard, stellate trichome is considered to be a key attribute of the family Malvaceae *s.s.* Variation of trichome morphology was studied in some specific taxa (Celka *et al.*, 2006). Nevertheless, all these attributes studied previously in respect of Malvaceae are relatively scanty as to reach any conclusion; further detailed study needs to be established.
Many traditional circumscriptions of taxa have been rearranged based on the molecular phylogenetic analyses. For example, Malvaceae *s.s.* is now treated as a subfamily Malvoideae which includes four tribes (Bayer and Kubitzki, 2003; Tate *et al.*, 2005). Therefore, analyses of morphological characters are to follow phylogenetic classification for better conclusion.
The present study deals with the use of attributes such as epicalyx, staminal tube, stigmatic head and trichome for characterization of some common members of Malvaceae *s.s.* following a phylogenetic grouping as proposed by Bayer and Kubitzki (2003). These characters are further utilized to prepare taxonomic key.

MATERIALS AND METHODS
A total of 14 taxa of Malvaceae *s.s.* were examined. In all cases fresh materials were studied. A list of worked out specimens are given in table 1. The identifications of the taxa were confirmed following the publications of Prain (1903), Flora of West Bengal (1997) and Ya *et al.,* (2007).
Morphology of epicalyx and staminal column was studied under a stereoscopic microscope. The morphology was drawn by free hand drawing. For measurement purpose, fresh materials with a

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millimeter scale were scanned under a scanner. The scanned photos (jpg format) were then measured using TPS Dig2 software (Rohlf, 2010).

Trichomes from all stems, leaves, calyx and epicalyx (when present) were studied by applying nail polish on the surface for few minutes until it was dry. Then the epidermal peels were removed slowly by a forcep. Peels containing trichomes were studied under a compound microscope and drawings were made using a drawing prism. Measurements were taken using micrometers (Erma-Japan).

A key to the genera of Malvaceae *s.s.* was prepared using staminal tube, stigma and trichome morphological characters. Similarly a key to the species of *Sida* was also prepared.

Table 1: List of studied taxa

Sl. No.	Name of the taxa
1	*Abelmoschus esculentus* (L.) Moench
2	*Abutilon indicum* (L.) Sweet
3	*Alcea rosea* L.
4	*Fioria vitifolia* (L.) Mattei
5	*Gossypium herbaceum* L.
6	*Hibiscus rosa-sinensis* L.
7	*Malachra capitata* (L.) L.
8	*Malvastrum coromandelianum* (L.) Garcke
9	*Malvaviscus arboreus* var. *penduliflorus* (Moc. & Sessé ex DC.) Schery
10	*Sida acuta* Burm.f.
11	*Sida cordata* (Burm.f.) Borss. Waalk.
12	*Sida rhombifolia* L.
13	*Thespesia populnea* (L.) Sol. ex Corrêa
14	*Urena lobata* L.

RESULTS AND DISCUSSION

Morphology of Epicalyx

The morphology of epicalyx is varied in relation to number of segments, degree of connation, shape, size, number of nerves and type of hairs on the surface. Another character that is important to separate the genera from one another is the ratio of length of the epicalyx to that of the calyx. The epicalyx characters of studied taxa are described below.

Abelmoschus esculentus (Figure 1A)

Epicalyx segments 8-11, free, linear-lanceolate, pubescent, 5 nerved, ca. 9 mm long and ca. 1 mm wide at middle; hairs simple, long; epicalyx-calyx length ratio ca. 0.3.

Alcea rosea (Figure 1G)

Epicalyx segments 6-8, connate at middle, ovate-lanceolate, pubescent, 5-6 nerved, ca. 14 mm long and ca. 5 mm wide at middle; hairs simple, long, dense; epicalyx-calyx length ratio ca. 0.7.

Fioria vitifolia (Figure 1D)

Epicalyx segments 8-10, free, linear, pubescent, 2-3 nerved, ca. 4 mm long and ca. 0.5 mm wide at middle; hairs stellate and simple, dense; epicalyx-calyx length ratio ca. 0.3.

Gossypium herbaceum (Figure 1I)

Epicalyx segments 3, slightly connate at base, cordate, deeply lobed, pubescent, numerous minute black spots [gossypol glands (Fryxell, 1988)] visible on the surface, 13-15 nerved, ca. 46 mm long and ca. 31 mm wide at middle; hairs simple; epicalyx-calyx length ratio ca. 4.6.

Hibiscus rosa-sinensis (Figure 1B)

Epicalyx segments 5-7, shortly connate at base, linear, pubescent, single nerved, ca. 8 mm long and ca. 2 mm wide at middle; hairs simple, minute; epicalyx-calyx length ratio ca. 0.4.

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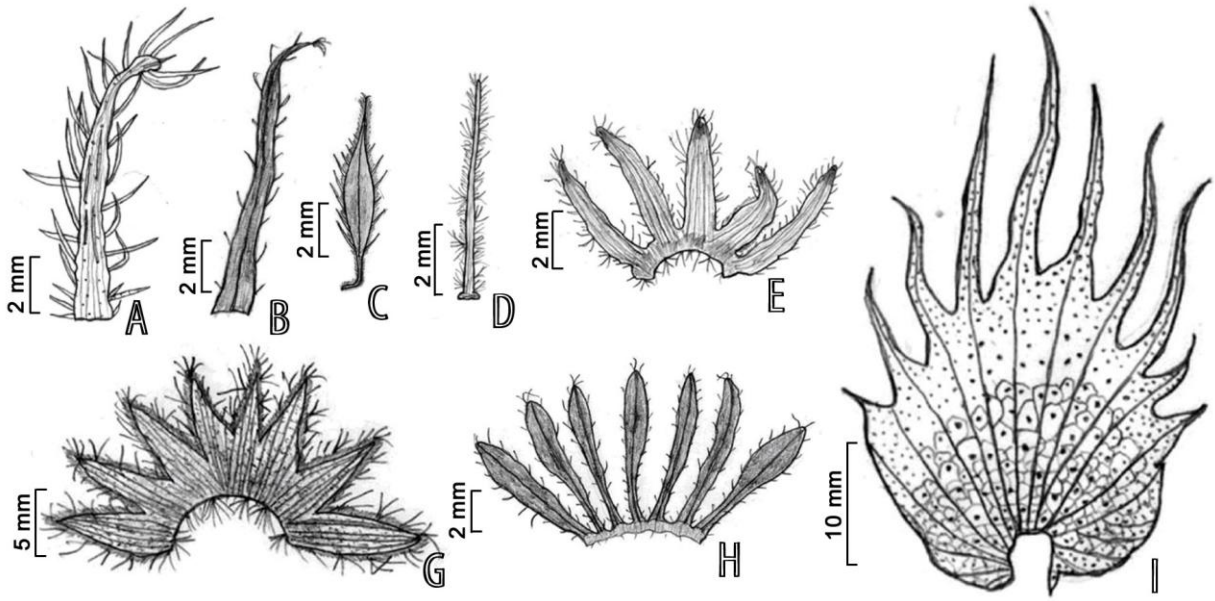

Figure 1: Epicalyxes of Malvaceae *s.s.* A- *Abelmoschus esculentus*; B- *Hibiscus rosa-sinensis*; C- *Malvastrum coromandelianum*; D- *Fioria vitifolia*; E- *Urena lobata*; G- *Alcea rosea*; H- *Malvaviscus arboreus* var. *penduliflorus*; I- *Gossypium herbaceum*

Malvastrum coromandelianum (Figure 1C)
Epicalyx segments 3, free, linear-lanceolate, pubescent, single nerved, ca. 7mm long and ca. 1 mm wide at middle; hairs simple, long, multicellular; epicalyx-calyx length ratio ca. 0.9.
Malvaviscus arboreus var. *penduliflorus* (Figure 1H)
Epicalyx segments 5-7, shortly connate at base, spathulate, pubescent, single nerved, ca. 9 mm long and ca. 4 mm wide at middle; hairs simple, small; epicalyx-calyx ratio length ca. 0.6.
Urena lobata (Figure 1E)
Epicalyx segments 5, connate at base, lanceolate, pubescent, 3-5 nerved, ca. 5 mm long and ca. 1 mm wide at middle; hairs simple, multicellular; epicalyx-calyx length ratio ca. 1.

Morphology of Staminal Tube and Stigma

In Malvaceae *s.s.* numerous stamens are united below in a stamina tube and separating above into distinct antheriferous filaments. The stamina tube shape, length and breadth; position and length of antheriferous filaments; and number of anthers are noted in this study. Likewise, the stigmatic head morphology, such as, number, union and length are also noted. The stamina tube and stigmatic head morphology are described below.
Abelmoschus esculentus (Figure 2A)
Staminal tube conical, length ca. 22 mm, breadth ca. 7 mm at base and ca. 1 mm at apex; antheriferous filaments confined to the upper ½ of the staminal tube, sparsely arranged, ca. 2 mm long; anthers 40. Stigma 5, closely arranged, ca. 1 mm long.
Abutilon indicum (Figure 2B)
Lower half of the staminal tube ovoid, upper half tubular, length ca. 7 mm, breadth ca. 4 mm at base and ca. 1.5 mm at apex; antheriferous filaments confined to the apex of the staminal tube, densely arranged, ca. 2.5 mm long; anthers 55. Stigma 20, ca. 4 mm long.
Alcea rosea (Figure 2C)
Staminal tube truncate, lower portion wider, length ca. 9 mm, breadth ca. 6 mm at base and ca. 1.5 mm at apex; antheriferous filaments confined to the upper ½ of the staminal tube, densely arranged to form a ball like appearance, ca. 2 mm long; anthers numerous, ca. 110. Stigma 25-30, ca. 7 mm long.

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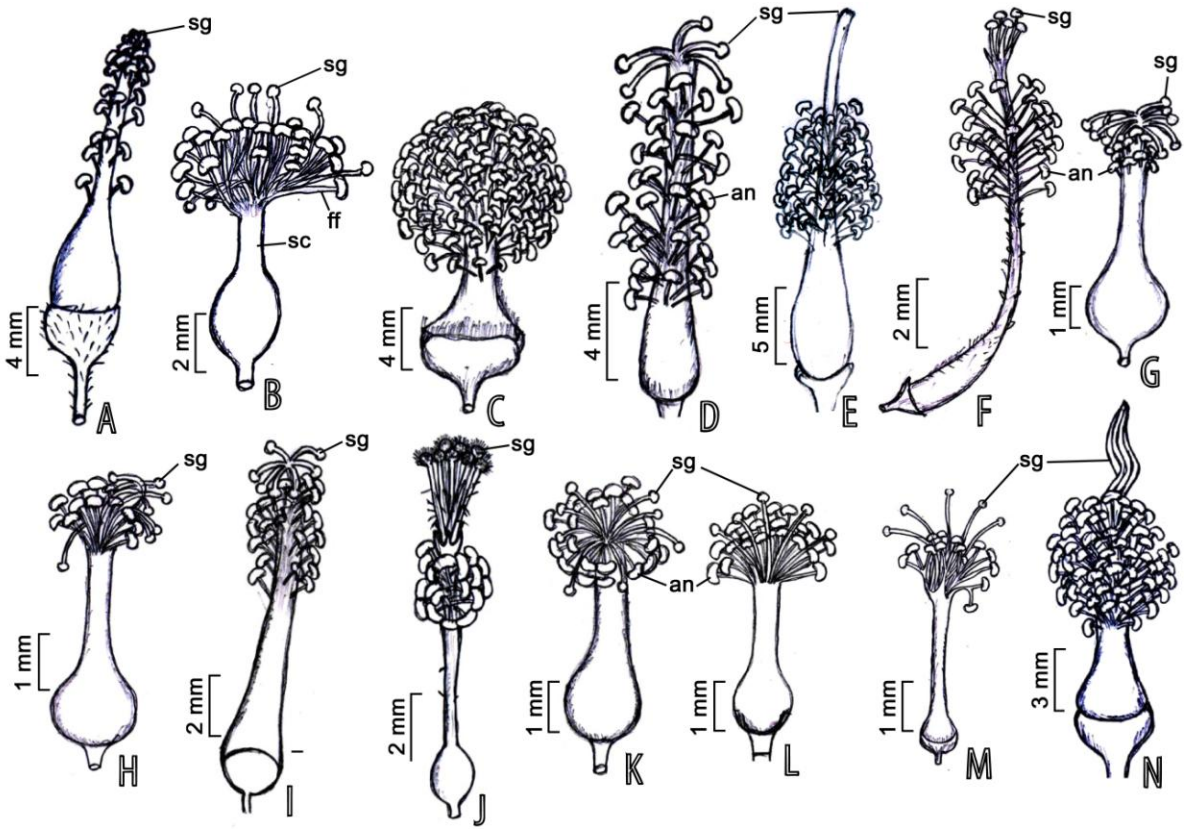

Figure 2: Staminal tubes with antheriferous filaments and stigma of Malvaceae *s.s.* A- *Abelmoschus esculentus*; B- *Abutilon indicum*; c- *Alcea rosea*; D- *Fioria vitifolia*; E- *Gossypium herbaceum*; F- *Hibiscus rosa-sinensis*; G- *Urena lobata*; H- *Malachra capitata*; I- *Malvastrum coromandelianum*; J- *Malvaviscus arboreus* var. *penduliflorus*; K- *Sida acuta*; L- *Sida cordata*; M- *Sida rhombifolia*; N- *Thespesia populnea*. an- anther, ff- free part of filament, sc- stamina column, sg- stigmatic head.

Fioria vitifolia (Figure 2D)
Staminal tube tubular, wider at base, length ca. 14 mm, breadth ca. 3 mm at base and ca. 1.5 mm at apex; antheriferous filaments confined to the upper ¾ of the staminal tube; sparsely arranged, ca. 3 mm long; anthers 50-60. Stigma 5, ca. 3 mm long.
Gossypium herbaceum (Figure 2E)
Staminal tube tubular, wider at base, length ca. 15 mm, breadth ca. 4 mm at base and ca. 1.5 mm at apex; antheriferous filaments confined to the upper ½ of the staminal tube, densely arranged, ca. 8 mm long; anthers numerous, ca. 92. Stigma united, tubular, ca. 8 mm long.
Hibiscus rosa-sinensis (Figure 2F)
Staminal tube truncate, wider at base, length ca. 80 mm, breadth ca. 4 mm at base and ca. 2 mm at apex; antheriferous filaments confined to the upper 1/3 of the staminal tube, more or less densely arranged, ca. 5 mm long; anthers 60-80.Stigma 5, ca. 4 mm long.
Malachra capitata (Figure 2H)
Staminal tube tubular, base ovoid, length ca. 9 mm, breadth ca. 2 mm at base and ca. 1 mm at apex; antheriferous filaments confined to the apex of the stamina tube, sparsely arranged, ca. 1 mm long; anthers 20. Stigma 10, ca. 2 mm long.
Malvastrum coromandelianum (Figure 2I)
Staminal tube tubular, slightly wider at base, length ca. 4 mm, breadth ca. 1.5 mm at base and ca. 1 mm at apex; antheriferous filaments confined to the upper ½ of the staminal tube, sparsely arranged, ca. 1 mm long; anthers 25. Stigma 10, ca. 1.5 mm long.

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Malvaviscus arboreus var. *penduliflorus* (Figure 2J)
Staminal tube ovoid at base and tubular at apex, length ca. 20 mm, breadth ca. 2 mm at base and ca. 1 mm at apex; antheriferous filaments confined to the upper ¼ of the staminal tube, densely arranged, ca. 4 mm long; anthers 25. Stigma 10, pubescent, ca. 3 mm long.
Sida acuta (Figure 2K)
Staminal tube ovoid at base and tubular at apex, length ca. 3 mm, breadth ca. 1.5 mm at base and ca. 1 mm at apex; antheriferous filaments confined to the apex of the staminal tube, densely arranged to form a ball like appearance, ca. 1.5 mm long; anthers 25-30. Stigma 10, ca. 1.5 mm long.
Sida cordata (Figure 2L)
Staminal tube ovoid at base and tubular at apex, length ca. 3 mm, breadth ca. 1.5 mm at base and ca. 1 mm at apex; antheriferous filaments confined to the apex of the staminal tube, densely arranged to form a ball like appearance, ca. 1.5 mm long; anthers 10-15. Stigma 5, ca. 2 mm long.
Sida rhombifolia (Figure 2M)
Staminal tube slightly swollen at base and tubular at apex, length ca. 2 mm, breadth ca. 1.5 mm at base and ca. 1 mm at apex; antheriferous filaments confined to the apex of the staminal tube, form 4-5 bunches, densely arranged to form a ball like appearance, ca. 1.5 mm long; anthers 15-30. Stigma 5-10, ca. 2 mm long.
Thespesia populnea (Figure 2N)
Staminal tube tubular, wider at base, length ca. 20 mm, breadth ca. 6 mm at base and ca. 3 mm at apex; antheriferous filaments confined to upper ½ of the staminal tube, densely arranged to form a ball like appearance, ca. 5 mm long; anthers numerous, 80-100. Stigma united, clavate, 5-grooved, ca. 6 mm long.
Urena lobata (Figure 2G)
Staminal tube tubular, base ovoid, length ca. 6 mm, breadth ca. 2 mm at base and ca. 1 mm at apex; antheriferous filaments confined to upper ¼ of the staminal tube, sparsely arranged, ca. 1 mm long; anthers 10. Stigma 10, ca. 1 mm long.

Trichome Morphology

Trichome morphology of the studied specimens is presented in Table 2 and Figure 3.

Table 2: Trichomes of Malvaceae *s.s.*

Taxa	Figure No.	Trichome description
Abelmoschus esculentus	3A	Simple trichomes 2-3 celled, filiform, length- 70-77 µm. Stellate trichomes 6-8 armed, diameter- 43-46 µm.
Abutilon indicum	3B	Stellate trichomes 3-9 armd, diameter- 9-32 µm.
Alcea rosea	3C	Simple trichomes 1-2 celled, whip like, length- 61-70 µm. Stellate trichomes 6-10 armed, diameter- 20-46 µm.
Fioria vitifolia	3D	Stellate trichomes 5-8 armed, basal cells large, diameter- 9-40 µm.
Gossypium herbaceum	3E	Simple trichome 1-2 celled, filiform, length- 23-49 µm.
Hibiscus rosa-sinensis	3F	Simple trichome 1-celled, filiform, basal cell prominent, length- 15-19 µm. Stellate trichomes 4-8 armed, basal cells short, diameter- 25-35 µm.
Malachra capitata	3G	Simple trichomes 1 or 2-3 celled, filiform, length- 20-26 µm. Stellate trichomes 4-6 armed, basal cells short, diameter- 24-29 µm.
Malvastrum coromandelianum	3H	Simple trichomes 2-3 celled, whip like, length- 25-48 µm. Stellate trichome H-shaped, length- 35-62 µm, diameter- 35-62 µm.
Malvaviscus arboreus var. *penduliflorus*	3I	Simple trichomes 1-2 celled, filiform, length- 35-41 µm. Stellate trichomes 4-6 armed, diameter- 17-26 µm. Some 4 armed trichomes take H- shape, length- 41-47 µm.
Sida acuta	3L	Simple trichomes 1- celled, whip like, length- 37-49 µm. Stellate trichomes 5-7 armed, diameter- 17-28 µm.
Sida cordata	3N	Simple trichomes 1-2 celled, whip like, length- 30-38 µm. Stellate trichomes 4-5 armed, diameter- 28-90 µm.
Sida rhombifolia	3M	Simple trichomes 1- celled, whip like, length- 35-48 µm. Stellate trichomes 5-10 armed, diameter- 19-34 µm.
Thespesia populnea	3K	Stellate trichomes compound (indumentum) with many closely attached arms, diameter- 13-25 µm.
Urena lobata	3J	Simple trichomes 2-3 celled, basal cells short, length- 18-30 µm Stellate trichomes 5-7 armed, diameter- 21-28 µm.

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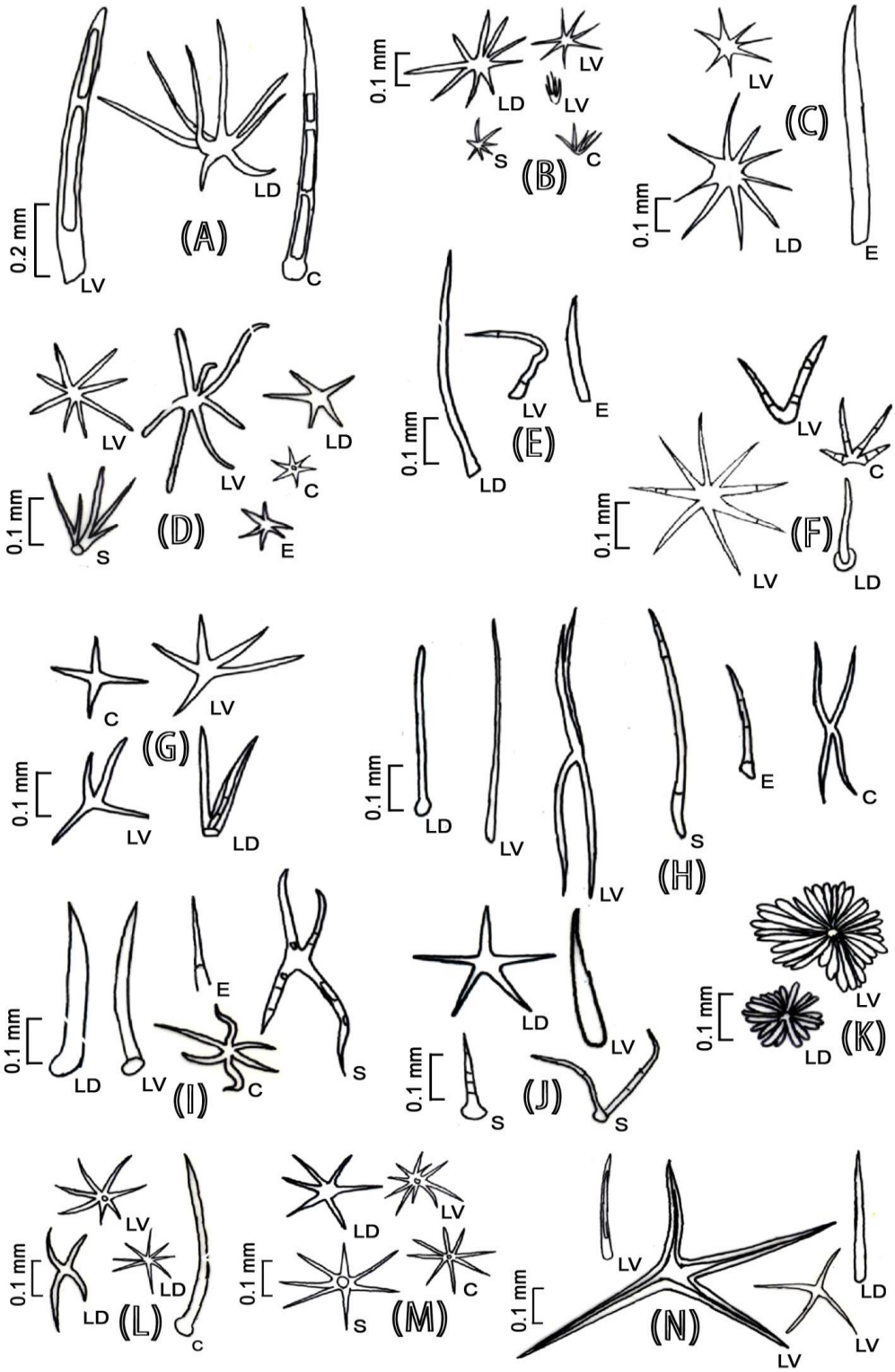

Figure 3: Trichome Morphology of Malvaceae s.s. (A) *Abelmoschus esculentus*; (B) *Abutilon indicum*; (C) *Alcea rosea*; (D) *Fioria vitifolia*; (E) *Gossypium herbaceum*; (F) *Hibiscus rosa-sinensis*; (G) *Malachra capitata*; (H) *Malvastrum coromandelianum*; (I) *Malvaviscus arboreus* var. *penduliflorus*; (J) *Urena lobata*; (K) *Thespesia populnea*; (L) *Sida acuta*; (M) *Sida rhombifolia* and (N) *Sida cordata*. S- stem; C- calyx; E- epicalyx; LD- leaf dorsal; LV- leaf ventral

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In molecular phylogenetic treatments, Malvaceae *s.s.* is considered to be a subfamily Malvoideae under the family Malvaceae *s.l.* (Bayer and Kubitzki, 2003; Tate *et al.*, 2005). Malvoideae is divided into four tribes: Gossypieae, Hibisceae; Kydieae and Malveae. In this study, both *Gossypium* and *Thespesia* belong to the tribe Gossypieae; *Abelmoschus*, *Fioria*, *Hibiscus*, *Malachra*, *Malvaviscus* and *Urena* to Hibisceae; and *Alcea*, *Abutilon*, *Malvastrum* and *Sida* to Malveae.

Gossypium shows the largest epicalyx which consists of 3 free segments, whereas *Thespesia* is devoid of any epicalyx. Both the taxa, however, resemble since they exhibit characters like free parts of the stamens confined occupying 50% of stamina tube towards upper part, numerous anthers (ranging from 80 to 100) and united stigma. The united stigma distinguishes them from all other studied taxa. The presence of indumentums type of trichome on the leaf surface of *Thespesia*, discrete it from *Gossypium* and other studied taxa. Indumentum is probably helpful to reduce stomatal conductance and an adaptive feature for saline habitat. *Thespesia* is considered as a mangrove associate flora (Naskar, 2004), though it can also grow well in non-saline regions.

A clear distinction is observed among the members of the tribe Hibisceae. While *Abelmoschus*, *Fioria* and *Hibiscus* show free linear to lanceolate epicalyx segments with the epicalyx-calyx ratio of ca. 0.3, on the contrary *Malvaviscus* and *Urena* show epicalyx segments connate at base. However, the epicalyx-calyx length ratio is 0.6 in *Malvaviscus* and 1.0 in *Urena*. Interestingly the devoid of epicalyx in *Malachra* indicates an independent evolutionary event within the tribe Hibisceae. Androecium morphology is highly variable within this tribe. It is difficult to categorize the members of Hibisceae on the basic of androecium morphology. Similarly, trichome morphological characters are also insufficient for categorization. Only in *Malvaviscus* some stellate trichome look more or less like 'H', might be an identifying character.

Except, *Alcea* and *Malvastrum* all other worked out members of the tribe Malveae lack epicalyx. Epicalyx morphology is completely distinct in both these taxa. In *Malvastrum* the epicalyx is characterized by 3 lanceolate, free epicalyx segment, whereas, in *Alcea* 6-8 epicalyx segment are united near the middle. The only similarity is the near values of epicalyx-calyx ratio. The antheriferous stamens at the apex of staminal tube indicate the closeness of *Abutilon* and *Sida*. Staminal tube morphology is different in *Alcea* and *Malvustrum* and with that of *Abutilon-Sida* group. The *Abutilon-Sida* relationship is also supported by recent molecular phylogenetic study where these taxa have been treated under *Abutilon* Alliance (Tate *et al.*, 2003). To some extend the trichome morphology proved to be categorized the taxa under the tribe Malveae. The distinct 'H' shaped trichomes in *Malvastrum* is adequate to discrete it from all other studies taxa of Malveae. Similarly, more than twice larger stellate trichome of *Sida cordata* distinguishing it from *S. acuta* and *S. rhombifolia*.

A key to the genera of Malvaceae *s.s.* and a key to the species of *Sida* are prepared using the characters from epicalyx, staminal tube, stigma and trichome.

Key to the Genera of Malvaceae s.s.-

1a. Stigma united ……..…… **2**
1b. Stigma free ……..……. **3**
2a. Epicalyx large; stigma tubular, grooves not prominent; leaf bears simple trichomes..... ***Gossypium***
2b. Epicalyx absent; stigma clavate, distinctly 5-grooved; leaf bears compound stellate trichomes (indumenta) …… ***Thespesia***
3a. Stigma 5 or 10 ……..……..…….... **4**
3b. Stigma 20 or 25 or 30 ……..……..……..…….. **5**
4a. Epicalyx absent; antheriferous filamens confined to the apex of staminal tube.....…… **6**
4b. Epicalyx present; antheriferous filamens absent on the staminal tube apex ……..…… **7**
5a. Epicalyx absent, atheriferous filaments confined to the apex of the staminal tube ……..… ***Abutilon***
5b. Epicalyx present, antheriferous filaments absent on the staminal tube apex …..…..……. **8**
6a. Length of the staminal tube > 8 mm ……..……..……. ***Malachra***
6b. Length of the staminal tube < 5 mm ……..……..…… ***Sida***
7a. Anthers 40; stigma closely arranged, <1.5 mm long ……..……..….. ***Abelmoschus***

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7b. Anthers 50-80; stigma diffusely arranged, >2.5 mm long ……..…….... **9**
8a. Epicalyx segments free; leaf bears distinct H- shaped trichomes ….. ……..…..….. ***Malvastrum***
8b. Epicalyx segments united; distinct H- shaped trichomes absent on leaf surface ……..…... **10**
9a. Epicalyx < 5 mm long; staminal tube truncate; antheriferous filaments confined to the upper ¾ of the staminal tube ……... ***Fioria***
9b. Epicalyx > 7 mm long; staminal tube tubular; antheriferous filaments confined to the upper 1/3 of the staminal tube.……..…..…… ***Hibiscus***
10a. Epicalyx segments connate at middle; antheriferous filaments confined to the upper ¾ of the seminal tube and form a ball like structure; anthers > 100 … ……..…….. ***Alcea***
10b.Epicalyx segments connate at base; antheriferous filaments confined to the upper ¼ of the staminal tube; anthers 10 or 25 ……..…….. **11**
11a. Epicalyx segments lanceolate; anthers 10; stigma ca. 1 mm long, glabrous ……..….... ***Urena***
11b. Epicaly segments spathulate; anthers 25; stigma ca. 3 mm long, pubescent ……..... ***Malvaviscus***

Key to the Species of Sida-

1a. Stellate trichomes diameter 28-90 µm …….....…….....……. ***S. Cordata***
1b. Stellate trichome diameter 17-34 µm …….....….....…….....……. **2**
2a. Distinct bunches of 4-5 antheriferous filaments visible on staminal tube apex...……... ***S. rhombifolia***
2b. Bunches of antheriferous filaments is not clear ……..…….…… ***S. acuta***

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