*Indian Journal of Plant Sciences ISSN: 2319–3824*
*An Open Access, Online International Journal Available at http://www.cibtech.org/jps.htm*
*2022 Vol.11, pp.1-7/Naskar*
***Research Article***

# ANALYSIS OF MORPHOMETRIC DATA FROM LEAF RADIANS OF SOME MEMBERS OF THE FAMILY MALVACEAE *SENSU STRICTO* FOR TAXONOMIC SIGNIFICANCE

***Saikat Naskar**
*Department of Botany, The University of Burdwan, Burdwan- 713104, West Bengal, India*
**Author for Correspondence: saikatnaskar@rediffmail.com*

**ABSTRACT**
Ratio transformation leaf radian data from ten species of Malvaceae *s.str.* have been considered as variables for Principal Component Analysis. Plotting of mean leaf shape data in a 2D plot considering the scores of ratio transforming radians with PC1 and PC2 shows that the species can be classified by leaf shape data but its reliability in tribal classification of Malvaceae *s.str.* is limited. The leaf shape variability is significant in *Sida rhombifolia* and *Fioria vitifolia*. It has been found that *Sida cordata* is positioned intermediate between *Abutilon* and other species of *Sida*. The distinctness of the tribe Gossypieae from other tribes of Malvaceae *s.str.* is also proven by the isolated position of *Thespesia* in the 2D plot.

***Keywords*****:** *Malvaceae s.str., Morphometrics, PCA, Ratio Transformation Radians, Taxonomy*

**INTRODUCTION**
Morphometrics is the quantitative description, analysis and interpretation of shape and shape variation in biology (Rohlf, 1990). Different morphometric techniques are employed to interpret shapes of structures. The shape of organisms has a tremendous taxonomic value as it is considered heritable and independent from environmental factors. Shape variation can be, therefore, utilized in taxonomy. Leaf shape is considered as a rich source of systematic data and its variation occurs at every hierarchical level: within and between individuals, populations, and taxa (Dickinson *et al.*, 1987). In morphometrics, shape is characterized as a configuration of landmarks. Landmarks are defined by features of the structure at the point where they are located (Bookstein *et al.*, 1985). Shape is described by measuring the distance between these points. The ratio of these distances or leaf dimensions can provide a summary of shape relationships that is independent of size differences (Dickinson *et al.*, 1987).
Malvaceae *sensu lato* is an economically important family that comprises 249 genera and ca. 5400 in the tropic and temperate regions of both the hemisphere of the world (World Plants, 2022+). Presently this family consists of nine subfamilies of which the subfamily Malvoideae is equivalent to the traditionally recognized family Malvaceae *s.str.* (Cvetković *et al.*, 2021). Judd and Manchester (1997) supported the monophyly of Malvoideae (= Malvaceae *s.str.*) based on morphological, anatomical, palynological and phytochemical characters. No traditionally recognized diagnostic feature of Malvaceae *sensu stricto* was established as apomorphic, rather “Sterculiaceae+Bombaceae+Malvaceae” and “Bombaceae+Malvaceae” clades were supported by monadelphous androecium and monoloculate anthers respectively. “Bombaceae+Malvaceae” clade was named as Malvatheca by Baum *et al.* (1998). Three tribes, Hibisceae, Malveae and Gossypieae form the core Malvoideae (Baum, 2004). Morphological, phytochemical and molecular studies have enriched our understanding on the systematics of this economically important family. But the use of the different forms of morphological data can give us more opportunities to explain interrelationship among the taxa of the family Malvaceae *s.str.* Till data

morphometric analysis of leaf shape variation among different taxa of Malvaceae *s.str.* has not been done. So application of this tool would be novel in this family.

## MATERIALS AND METHODS

### *Leaf samples*

A total of 150 fully developed leaves from ten species of the Malvaceae *s.str.* were studied. Each species represents five plants, so a total of 50 plants were collected from different places of the North 24 Parganas district of West Bengal, India (Table 1). Plants were identified following the standard methodologies of taxonomy.

### *Preparation of Leaves for Measurements*

Fresh leaves were fixed on herbarium paper with synthetic adhesive and kept for a day under herbarium pressure. The pressed leaves with scale were scanned under a Scanner. Outlines were drawn around the images of leaf margins to convert serrated margins into entire (Fig. 1A) using photo-editing software.

### *Measurement of leaf radians*

At first two landmarks (L1 and L2) were marked at the base and apex of a leaf (Fig. 1B). The third landmark point (L3) was marked on the L1–L2 line where the lamina was widest. A series of landmark points (from L4 to L20) were marked one by one on the right side of the leaf margin at 10˚ intervals considering the L1–L3 line as the base. Then leaf radians were drawn by connecting the landmarks of leaf margin and L3. The lines were further extended up to the left side margin of the leaf. In this way, a total of 36 leaf radians were drawn for analysis. TPS Util and TPS Dig software (Rohlf, 2021) were used to generate landmark points and radians on leaf images. Within the radians, the right side radians such as L3-L4 were equivalent with the left side radian L3-L4'. Similarly, the lines L3-L5, L3-L6, L3-L7, etc were equivalent with respective lines L3-L5', L3-L6', L3-L7' etc. The mean values of equivalent radians of two sides of leaves were considered.

### *Data normalization*

Data-normalization for multivariate analysis was achieved by conversion of radian measurement data into ratio. For the ratio transformations, the equivalent radians were divided by the lamina length or L1-L2 or *L*. These ratio transformation radian data were named as *R4*, *R5*, *R6* up to *R20* (Table 2).

### *Test of correlation of ratio transformation radian data*

Pearson correlation test by ratio transformation radian (*R*) data was performed to identify the highly correlated *R*s. Highly correlated shape variables have lesser discriminatory power in a multivariate analysis. The highly correlated *R*s ($r \geq 0.9$) were identified and some were excluded. Justification for exclusion is given in table 3. Only 10 *R*s were finally selected from 20 *R*s for multivariate analysis.

### *Principal Component Analysis*

Principal Component Analysis (PCA) was performed on the ratio transformation data as variables using Statistica 8.0 software. Species were served as categorical variables of ratio transformation data in the PCA. The result of PCA was visualized by plotting species in a 2-D plot considering scores of PC1 on the x-axis and PC2 on the y-axis to show the closeness of the species.

## RESULTS & DISCUSSION

### *Principal Component Analysis of Leaf Shape Variability*

The first two principal components (PC) contribute 43.99% and 28.91% variability i.e. more than 70% of the total variability of leaf shapes. Leaf shapes are plotted based on their respective scores with PC1 and PC2 in a 2D scattered plot (Fig. 2A). *S. rhombifolia* and *F. vitifolia* show maximum variability as the leaf shapes of these two species are plotted more scattered than other species (Fig. 2B). The least variability in leaf shape is observed in *H. rosa-sinensis*, *S. cordata* and *T. populnea*. In all other species, the leaf shape variability is moderate. The mean leaf shapes of all species are plotted against PC1 and PC2 (Fig. 4) to show the degree of closeness of the species.

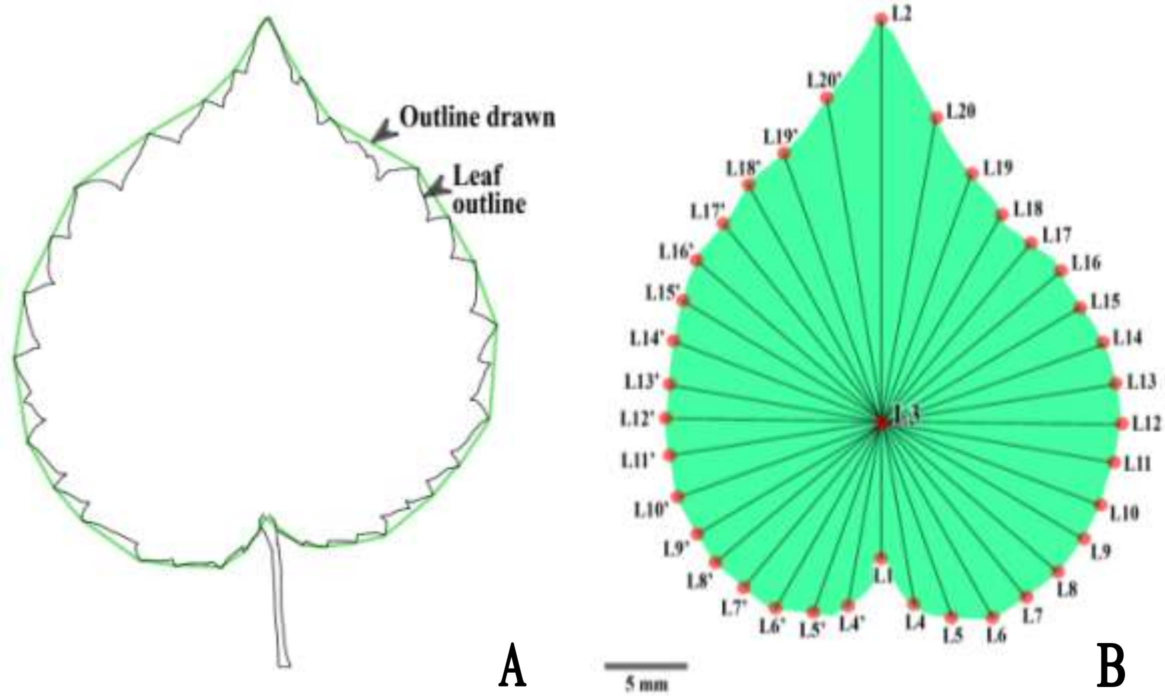


**Figure 1: A. Serrated leaf margin is converted into entire by outlining the leaf. B. Radians are drawn within a leaf image by connecting the centre landmark 3 (L3) with other landmarks of the leaf margin.**

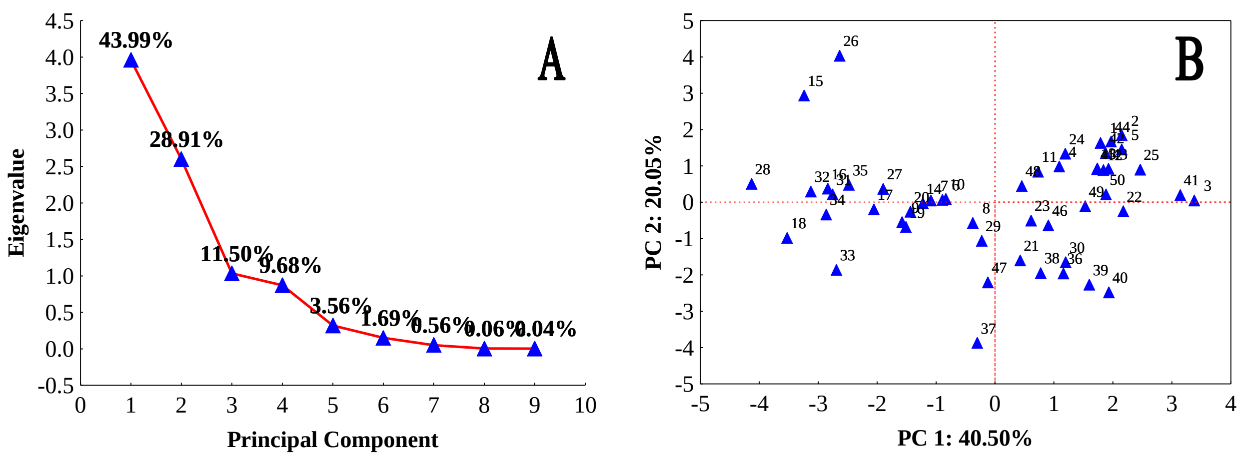


**Figure 2: A: Contribution of principal components in total leaf shape variations. B: Leaf shapes are plotted according to their scores with PC1 and PC2. Symbols: 1–5, *S. acuta*; 6–10, *S. cordata*; 11–15, *S. rhombifolia*; 16–20, *A. indicum*; 21–25, *M. coromandelianum*; 26–30, *F. vitifolia*; 31–35, *U. lobata*; 36–40, *T. populnea*; 46–50, *H. rosa-sinensis*.**

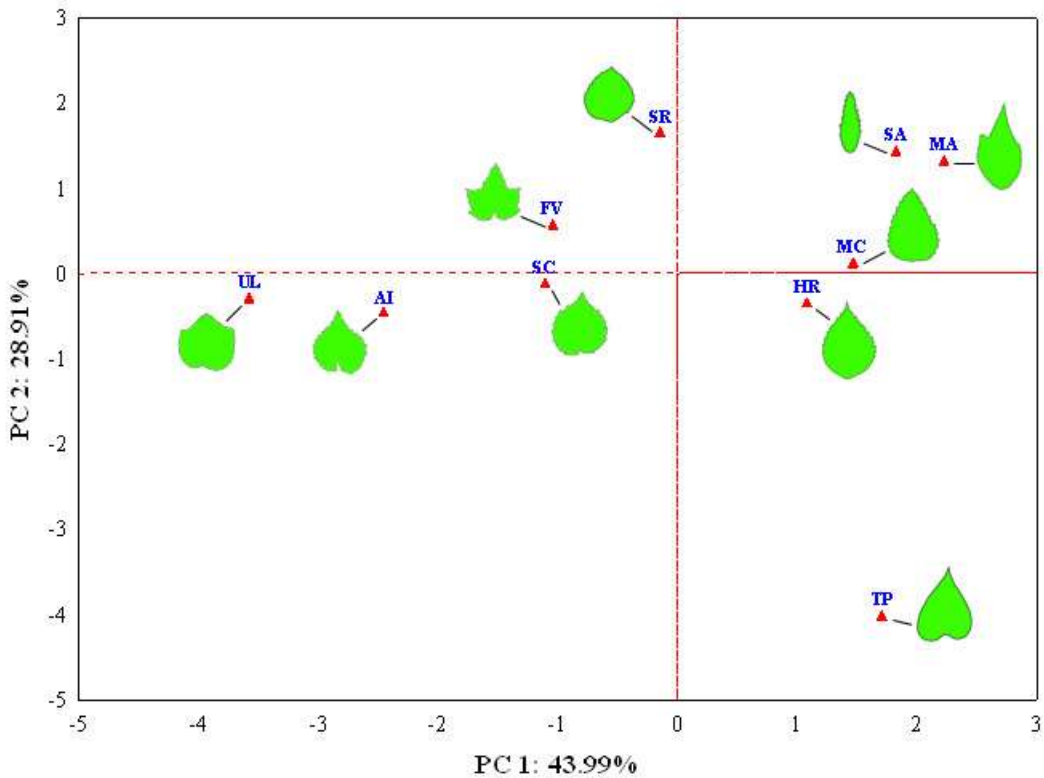


**Figure 3: Mean leaf shapes are plotted against PC1 and PC2 to show closeness of the species. UL- *U. lobata*; Al- *A. indicum*; SC- *S. cordata*; FV- *F. vitifolia*; SR- *S. rhombifolia*; HR- *H. rosa-sinensis*; MC- *M. coromandelianum*; SA- *S. acuta*; MA- *M. arboreus*; TP- *T. populnea.***

**Table 1: List of studied taxa of Malvaceae *s.str.* and traditional tribes of the taxa**

| Sl. no. | Taxa | Tribe |
|---|---|---|
| 1 | *Abutilon indicum* (L.) Sweet | Malveae |
| 2 | *Fioria vitifolia* (L.) Mattei | Hibisceae |
| 3 | *Hibiscus rosa-sinensis* L. | Hibisceae |
| 4 | *Malvastrum coromandelianum* (L.) Garcke | Hibisceae |
| 5 | *Malvaviscus arboreus* Cav. | Malvavisceae |
| 6 | *Sida acuta* Burm. f. | Malveae |
| 7 | *S. cordata* (Burm. f.) Borss. Waalk. | Malveae |
| 8 | *S. rhombifolia* L. | Malveae |
| 9 | *Thespesia populnea* (L.) Sol. ex Corrêa | Gossypieae |
| 10 | *Urena lobata* L. | Malvavisceae |

**Table 2: Leaf radian measurements and ratio transformation of radian data by lamina length**

| Acronym | Measurement | Ratio transformation |
|---|---|---|
| *L* | Overall leaf length | L1-L2 |
| *R4* | Toward leaf base | (L3-L4 + L3-L4') / *L* |
| *R5* | Toward leaf base | (L3-L5 + L3-L5' / *L* |
| *R6* | Toward leaf base | (L3-L6 + L3-L6') / *L* |
| *R7* | Toward leaf base | (L3-L7 + L3-L7') / *L* |
| *R8* | Toward leaf width | (L3-L8 + L3-L8') / *L* |
| *R9* | Toward leaf width | (L3-L9 + L3-L9') / *L* |
| *R10* | Toward leaf width | (L3-L10 + L3-L10') / *L* |
| *R11* | Toward leaf width | (L3-L11 + L3-L11') / *L* |
| *R12* | Width of the leaf | (L3-L12 + L3-L12') / *L* |
| *R13* | Toward leaf width | ( L3-L13 + L3-L13') / *L* |
| *R14* | Toward leaf width | (L3-L14 + L3-L14') / *L* |
| *R15* | Toward leaf width | (L3-L15 + L3-L15') / *L* |
| *R16* | Toward leaf width | (L3-L16 + L3-L16') / *L* |
| *R17* | Toward leaf apex | (L3-L17 + L3-L17') / *L* |
| *R18* | Toward leaf apex | ( L3-L18 + L3-L18') / *L* |
| *R19* | Toward leaf apex | (L3-L19 + L3-L19') / *L* |
| *R20* | Toward leaf apex | (L3-L20 + L3-L20') / *L* |

**Table 3: Step by step selection procedure of ratio transformation radians (*R*s) for subsequent multivariate analysis. Less correlation means $r < 0.9$ and high correlation means $r \geq 0.9$.**

| All *R*s | Justifications for selection/exclusion | Selected *R*s |
|---|---|---|
| *R4* | Less correlation with any other *R*, therefore selected | *R4* |
| *R5* | High correlated with R6, therefore only *R5* is selected | *R5* |
| *R6* | High correlated with *R5*, *R7* & *R8*, therefore not selected | - |
| *R7* | High correlated with *R6*, *R8*, *R11* & *R12* but less correlation with *R5*, therefore selected | *R7* |
| *R8* | High correlated with *R7*, *R11* & *R12*, therefore not selected | - |
| *R9* | Less correlation with any other *R*, therefore selected | *R9* |
| *R10* | Less correlation with any other *R*, therefore selected | *R10* |
| *R11* | High correlated with *R7*, *R8*, *R12* & *R13*, therefore not selected | - |
| *R12* | High correlated with *R7*, *R8*, *R11* & *R13*, therefore not selected | - |
| *R13* | High correlated with *R11*, *R12*, *R15* & *R17* but less correlation with *R10*, therefore selected | *R13* |
| *R14* | Less correlation with any other *R*, therefore selected | *R14* |
| *R15* | High correlated with *R13* & *R17*, therefore not selected | - |
| *R16* | Less correlation with any other *R*, therefore selected | *R16* |
| *R17* | High correlated with *R13*, *R15* & *R19*, therefore not selected | - |
| *R18* | Less correlation with any other *R*, therefore selected | *R18* |
| *R19* | High correlated with *R17* & *R20* but less correlation with *R18*, therefore selected | *R19* |
| *R20* | High correlated with *R19*, therefore not selected | - |

***Taxonomic significance of leaf shape variability***

This study indicates that the leaf shapes of the members of Malvaceae *s.str*. is unique to the species and can be useful for species discrimination if a large number of leaf samples are used. The PCA shows that

*R7* is responsible for maximum leaf variance. Since *R7* was generated from a radian of leaf base, it indicates that the leaves are highly variable at base region than the other regions. The leaves of studied taxa show cuneate, obtuse, rounded and cordate leaf bases. It has been observed that leaves with similar base types were not closely placed in the 2D plot (Fig. 3). So, solely based on gross leaf base character any classification within Malvaceae *s.str.* would be inappropriate. Traditionally, Malvaceae *s.str*. has five well-recognized tribes, viz. Decaschistieae, Gossypieae, Hibisceae, Malvavisceae and Malveae (Duke and Doebley, 1995). In the present study, except for the first tribe, members of the other four tribes are considered. Discrimination of the tribes based on leaf shape data is not proved to be sufficient even though the discriminatory power at the species level is sufficient enough. It indicates that leaf shape can be useful data at the species level but above the species level its reliability is limited. Within the genus *Side*, huge variability is observed. Also, the high variability within *S. rhombifolia* and *F. vitifolia* may indicate the existence of some infraspecific taxon within the species. *S. rhombifolia* is often called a species complex not only for leaf variability but also in variability in other morphological features. Therefore, the identification of *S. rhombifolia* is problematic. Analysis of leaf shapes from a large number of samples of *S. rhombifolia* from different locations can give important information to resolve the taxonomic problem of the species complex. Duke and Doebley (1995) stated that the *Abutilon* alliance is the ancestor of the trib. Malveae. The closeness of the leaf shape of *Abutilon* and *S. cordata* may indicate that the latter species is the ancestor of other species of *Sida*. The high variability in leaf shape of *F. vitifolia* is reported here for the first time and it needs more critical study from a large number of samples from different localities. The variability of leaf shapes of *H. rosa-sinensis* is also prominent. Sampling from different ornamental varieties of the species is probably the reason for the high variability in leaf shape. Widely scattered position of the leaf shapes of the tribe Hibisceae and Malvaviscеae in the 2D plot again proves its non-reliability for classification at the tribal level. However, the plotting of leaf shape of *T. populnea* (tribe Gossypieae) in an isolated place in the 2D plot indicates its wide dissimilarity with the other tribes of Malvaceae *s.str*. *Thespesia* has been shown as a sister of all other tribes of Malvaceae *s.str.* (Judd and Manchester, 1997; Baum *et al.,* 2004).

## CONCLUSION

Though leaf radian data are not too reliable in the tribal classification of Malvaceae s.str., its reliability to explain evolutionary trends within the species of a genus is proven. Application of this method may give some novel interspecific relationships within a genus. Based on leaf shape analysis, the present study highlights that *S. cordata* is a link between the ancestor *Abutilon* and other species of *Sida*.

## REFERENCES

**Baum DA, Alverson WS and Nyffeler R (1998).** A durian by any other name: taxonomy and nomenclature of the core Malvales. *Harvard Papers in Botany,* **3** 315–330.

**Baum DA, Smith SD, Yen A, Alverson WS, Nyffeler R, Whitlock BA and Oldham RL (2004).** Phylogenetic Relationships of Malvatheca (Bombacoideae and Malvoideae; Malvaceae sensu lato) as Inferred from Plastid DNA Sequences. *American Journal of Botany,* **9** 1863–1871.

**Bookstein FL, Chernoff B, Elder RL, Humphries JM, Smith GR and Strauss RE (1985).** Morphometrics in evolutionary biology. *Academy of Natural Sciences of Philadelphia*, Philadelphia, PA.

**Cvetković T, Areces-Barazain F, Hinsinger DH, Thomas DC, Wieringa JJ, Ganesan SK and Strijk JS (2021).** Phylogenomics resolves deep subfamilial relationships in Malvaceae *s.l.*. *Genes, Genomics & Genetics,* **11** jkab136.

**Dickinson TA, Parker WH and Strauss RE (1987).** Another approach to leaf shape comparisons. *Taxon,* **36** 1-20.

**Duke JCL and Doebley J (1995).** A Chloroplast DNA Based Phylogeny of the Malvaceae. *Systematic Botany,* **20** 259-271.

**Judd WS and Manchester SR (1997).** Circumscription of Malvaceae (Malvales) as determined by a preliminary cladistic analysis of morphological, anatomical, playnological, and chemical characters. *Brittonia* **49** 384–405

**Rohlf FJ (1990).** Morphometrics. *Annual Review of Ecology and Systematics* **21** 299-316.

**Rohlf FJ (2021).** *SB Morphometrics*. *Available: http://www.sbmorphometrics.org*

**World Plants (2022 [continuously updated]).** *Available: https://www.worldplants.de/world-plants-complete-list. [Accessed on 20.02.2022]*