Morphometric Analysis in Some Members of Family Convolvulaceae Juss.

Ph. D Synopsis submitted to

The Maharaja Sayajirao University of Baroda

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For the degree of

Doctor of Philosophy (Ph. D.)

In

Botany

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Introduction

1. Morphometrics:

> "***The fundamental observation of biology is morphology. Morphological data form the basis of virtually all systematic descriptions.***"
>
> - MacLeod, 2002.

This statement is so reinforced by the fact that many systematic studies begin by grouping organisms on the strength of morphological dissimilarity. Once they are so grouped and segregated, we begin to study relationships among the groups, often by careful examination of variation in morphological features, but increasingly more often by using these morphologically-defined groups as the basis for conducting studies of molecular variation at every level.

Leaf Morphology is central to plant taxonomy and systematics (Bell and Bryan, 2008). Moreover, leaf morphology has mostly been studied using traditional descriptors and morphometrics in recent years (Marcus, 1990). There are many approaches to digital leaf shape morphometrics, including contour signatures, landmark analysis, shape features, fractal dimensions, Principal component analysis, and texture analysis. Among these analyses, landmark and Fourier analysis together perform exceptionally well at distinguishing between groups among leaf shapes (McLellan and Endler, 1998). Landmark analysis is ideal for capturing aspects of shape that are consistent among all leaves within a given dataset. The selection of landmarks should include points that are biologically homologous and adequately represent the morphology of the leaf. Landmarks can then be analyzed using Generalized Procrustes Analysis (GPA), which normalizes shape data (annotated by landmarks) at equal scale, allowing for an accurate comparison of shapes regardless of their size. Shape features are an additional, simple method of outline analysis that can include the perimeter to area ratio, aspect ratio, and circularity measurements. Thus, Digital morphometrics can be a powerful tool for assessing leaf shape variation among species, genotypes, and clones under common conditions and suggests biotic factors such as pests and pathogens as important drivers influencing leaf shape (Klein et. al., 2017)

This analysis, which first appeared in Bookstein (1991), has been reproduced in many later publications, including several of the standard textbooks, and the data set it visualizes has served as exemplar for diverse advanced GMM analyses, such as integration and factor analysis (Dryden and Mardia 2016; Bookstein 2015, 2018, 2021)

2. Family - Convolvulaceae:

"In all fair hues from white to mingled rose,
Along the hedge the clasping bindweed flowers,
Along the hedge, besides the trodden lane
Where day by day we pass and pass again."

- A. D. Webster

The family Convolvulaceae is named after the genus (Type Genus) '*Convolvulus*' (*Sp. Plantarum* 1: 1753), it is because of as majority of species showed climbing habit (*Convolving* = to roll together). The type species of the family is *Convolvulus arvensis* L. The members of the family Convolvulaceae are called '**Morning Glories**' where they occur, their foliage merges with the landscape making no visual impact. But when they manifest themselves in flowers, they blossom to glory with the rising sun. This ephemeral glory slowly dwindles down and departs when their flowers wither by noon. The lyrical name 'Morning Glory' suits them well. Forest is their natural habitat but more they prefer hedges and waysides spreading the landscape with spots of glory.

Objectives

1. Collection and Identification of members of family Convolvulaceae from various localities.
2. To study the Morpho-variability of vegetative organs of different populations of selected taxa for understanding any specific relationship and interrelationship.
3. Data processing through various available morphometric methods.
4. Artificial key will be prepared on the basis of vegetative and reproductive organs to suggest proper identification.
5. To envisage and understand developmental aspects if any at population and species level.

Review of Literature

1. Morphometrics:

In the last decade there has been an increasing interest in the use of modern geometric morphometrics (GMM) to study the form of leaves and its variations. GMM analyzes the relative positions of morphoanatomical landmarks and sets of points used to approximate curves (outlines) and surfaces to quantify size and shape (Jensen, 1990). The geometric information of shape differences is preserved, statistical power is increased (Rohlf and Marcus, 1993), and patterns can be visualized using image rendering and a variety of other diagrams (Adam et. al., 2004). The increase in the number of publications using GMM within (Sanfilippo et. al., 2009) and from outside (Frieß and Baylac, 2003; Buchanan and Collard, 2010) biology has been exponential and pays testament to the success of this set of methods. Taxonomists and botanists have recognized the potential of GMM in their field: ‘‘If the systematist is really interested in focusing on shape, separately from size, and/or on testing hypotheses about shape differences, then traditional approaches are not adequate; landmark methods are clearly superior, especially when the landmarks represent well-defined, biologically homologous points ‘‘… there is no information in the context of a set of landmarks that cannot be extracted by application of the … approach’’ (Jensen, 2003). Leaf shape variability has been investigated using analyses of landmarks and outlines to accurately discriminate species and their hybrids. For instance, using GMM on leaves, Jensen (1990) and Jensen et al. (1993) detected hybridization in black and red American oaks and Pen˜aloza-Ramirez et al. demonstrated that oak hybrids and backcrosses have intermediate morphology. Viscosi et al. (2009) also applied GMM and found evidence that in European white oaks leaf shape correlates strongly with the taxonomy of species and hybrids inferred using molecular data. In taxonomy and other fields, genetics and morphometrics can fruitfully interact as complementary tools to understand the origin of phenotypic differences (Klingenberg, 2010).

A large percentage of leaf shape and venation patterning exhibits high heritability Interestingly, complex shape phenotypes correlate with epidermal features, such as hirsuteness, and reproductive traits, revealing that disparate, potentially adaptive and economically relevant phenotypes co-segregate. Leaf morphology also correlates with population structure, and geography (Chitwood et al. 2014). The cordate-shaped leaves of lianas have been hypothesized to play structural roles by balancing the blade atop the petiole (like a cantilever) to allow light foraging through leaf positioning (Givnish and Vermeij, 1976).

2. Family - Convolvulaceae:

The taxonomic account of the family Convolvulaceae was first done by A.L. de Jussie (1789) treating it as a family and that had been accepted by the later workers. Choisy (1845) in DC. “Prodromus” had broadly accepted to include the genus *Cuscuta* L. within Convolvulaceae.

On the other hand, *Erycibeae* is considered as a separate Natural Order based on the genus *Erycibeae* Roxb. Later Bentham (1876) in Bentham and Hooker's Genera Plantarum had accepted this natural order which includes the genus *Cuscuta* L. and *Erycibeae* Roxb. Peter (1891, 1897) in Engler and Prantl's *Die Natiirlichen Pflanzenfamilien* also accepted the concept of the family Convolvulaceae. Afterwards Hallier (1893), van Ooststroom (1953) and, Melchior (1964) had accepted the conventional family Convolvulaceae. On the other hand, Roberty (1952, 1964) had delimited the family Convolvulaceae leaving out the family Cuscutaceae Dumortier, the Dodder family, as a separate one. However, he had included Erycibeae Roxb. Within the family Convolvulaceae.

Convolvulaceae is a cosmopolitan family with approximately 2000 accepted species categorized into 60 genera and 12 tribes all over the world (Simões et al., 2022). It is most diverse in the tropics, sub-tropical and then also in temperate regions of both hemispheres. The estimations of the species and the number of genera is different as presented by different authors, to be 50 genera and 1500 species by Cronquist (1981, 1988), 55 genera and 1650 species by Willis (1973), 58 genera and 1700 species by Takhtajan (1997), and about 55 genera and 1930 species by Judd et al. (2002). The estimation of genera is based on the taxonomic discretion of different authors and the maximum number of genera is stated as 62 by Roberty (1964). However, in account of the generic name there are as good as 88 genera treated by different workers at different times in the taxonomic account of this family and the count is less due to consideration of some genera as synonyms of others (Wood et. al., 2020). The earliest report on the family Convolvulaceae and its members of India has been given by Hooker (1885) in Flora of British India. Subsequently, Cooke (1908) described the distribution and diversity of Convolvulaceae in the Flora of Bombay Presidency. In India, the family Convolvulaceae is represented by 20 genera and around 1800 species (Santapau and Henry, 1973).

Methodology

Plant sampling:

For collection of plant material as per flowering and fruiting seasons intensive and extensive field survey were conducted in different phytogeographical regions of India. Since, January 2021 more than 50 filed visits were conducted in different regions in different seasons and different species were collected. During plant collection stress was given to collect and study the plants in flowering and fruiting stages that helped us for correct identification of the species. Collected samples were identified with the help of available taxonomic literature (Cooke, 1908; Hooker, 1885; Shah, 1978). The vouchers of the collected specimens were prepared following the standard methodology as described by Jain and Rao (1976) and deposited at the BARO herbarium of the Maharaja Sayajirao University of Baroda, Vadodara.

Leaf Sampling:

Leaf samples of various developmental stages (young to mature) were collected. For each species, approximately 50 leaves free from pest incidence, disease attacks or any other kind of damage were picked up for every species. Leaf samples were dried using the bloat drying method and kept in bloating papers to keep them flat (Babu et al., 2018). For the convenience of analysis minimum of 20 leaves per species were scanned after careful selection so that each species was represented equally in the sampling size. A Canon CanoScan LiDE 120 flatbed scanner was used to scan the leaf samples at a resolution of 300 DPI.

Image Analysis:

The images obtained after scanning the leaf samples were converted into BMP format by using MS Paint software. The bitmap images were then processed with SHAPE v.1.3d, image processing software (Iwata & Ukai, 2002) to implement an Elliptic Fourier Analysis and Principal Component Analysis (EF-PCA). Each colour image was converted into a binary image, from which the outline was traced and then transcribed in chain-code using the ChainCoder (Chc2Nef) software program. Outlines were then reduced to the coefficients of Elliptic Fourier descriptors (EFDs) of 30 harmonics for simple leaves and 50 harmonics for palmate/finely dissected leaves. Then the size and orientation of each contour were standardized, whereupon the coefficients effectively become shape variables. These coefficients are mathematical descriptors of forms that can be statistically analysed by routine methods (Kuhl & Giardina, 1982).

Data analysis:

The procedure for calculating EFDs from the original digitized image reconstructs the outlines to an approximate level and the decision to set the number of harmonics was arbitrary to some extent (Rohlf & Archie, 1984). In this aspect, the coefficients of the Elliptic Fourier descriptors were calculated so that the score on each principal component was equal to the mean with ±2 SD (standard deviation), and the scores of the remaining components were kept zero (Iwata et al., 1998; Yoshioka et al., 2004). The resulting data matrix of normalized EFDs was explored by principal component analysis (PCA) using a variance-covariance matrix described in the procedure of Yoshioka et al. (2004). The PCA of the coefficient matrices reduced the data dimensionality of uncorrelated shape descriptor variables to a smaller number. Variations in the leaf shape were analysed using a one-way analysis of variance (ANOVA) of principal component (PC) scores at $p=0.05$.

Plant Collection:

More than 55 species belonging to 12 genera of family Convolvulaceae from different localities (Table-1).

Table 1 - List of Collected Species

Sr. No.	Scientific name	Sr. No.	Scientific name
1	*Argyreia boseana* Santapau & V. Patel	30	*Ipomoea laxiflora* H.J. Chowdhery & Debta
2	*Argyreia cuneata* Ker Gawl.	31	*Ipomoea marginata* f. *candida* (Naik & Zate) Das & Lakshmin.
3	*Argyreia elliptica* (Roth) Choisy	32	*Ipomoea marginata* f. *marginata* L.
4	*Argyreia nervosa* (Burm.f.) Bojer	33	*Ipomoea muricata* (L.) Jacq.
5	*Argyreia pilosa* Wight & Arn.	34	*Ipomoea nil* (L.) Roth
6	*Argyreia sericea* Dalzell	35	*Ipomoea obscura* (L.) Ker Gawl.
7	*Argyreia setosa* (Roxb.) Sweet	36	*Ipomoea parasitica* (Kunth) G. Don
8	*Argyreia sharadchandrajii* Lawand & Shimpale	37	*Ipomoea pes-caprae* (L.) R.Br.
9	*Camonea kingii* (Prain) A.R. Simões & Staples	38	*Ipomoea pes-tigridis* L.
10	*Convolvulus arvensis* L.	39	*Ipomoea quamoclit* L.
11	*Convolvulus prostratus* Forssk.	40	*Ipomoea sindica* Stapf
12	*Convolvulus rottlerianus* subsp. *stocksii* (Boiss.) J.R.I. Wood & Scotland	41	*Ipomoea triloba* L.
13	*Evolvulus alsinoides* (L.) L.	42	*Ipomoea tuberculata* Ker-Gawl.
14	*Evolvulus nummularius* (L.) L.	43	*Jacquemontia paniculata* (Burm.f.) Hallier f.
15	*Ipomoea aculeata* Blume	44	*Jacquemontia pentanthos* (Jacq.) G.Don
16	*Ipomoea aquatica* Forssk.	45	*Merremia aegyptia* (L.) Urb.
17	*Ipomoea biflora* (L.) Pers.	46	*Merremia dissecta* (Jacq.) Hallier f.
18	*Ipomoea cairica* (L.) Sweet	47	*Merremia emarginata* (Burm.f.) Hallier f.
19	*Ipomoea carnea* subsp. *fistulosa* (Mart. ex Choisy) D.F. Austin	48	*Merremia hederacea* (Burm.f.) Hallier f.
20	*Ipomoea clarkei* Hook.f.	49	*Merremia quinquefolia* (L.) Hallier f.
21	*Ipomoea coptica* (L.) Roth.	50	*Merremia rhyncorhiza* (Dalzell) Hallier f.
22	*Ipomoea deccana* D.F. Austin	51	*Merremia tridentata* (L.) Hallier f.
23	*Ipomoea diversifolia* R.Br.	52	*Merremia tuberosa* (L.) Rendle
24	*Ipomoea eriocarpa* R.Br.	53	*Merremia vitifolia* (Burm.f.) Hallier f.
25	*Ipomoea hederifolia* L.	54	*Operculina turpethum* (L.) Silva Manso
26	*Ipomoea horsfalliae* Hook.	55	*Rivea hypocrateriformis* (Desr.) Choisy
27	*Ipomoea indica* (Burm.) Merr.	56	*Stictocardia tilifolia* (Desr.) Hallier f.
28	*Ipomoea involucrata* F. Dietr. ex Choisy	57	*Turbina corymbosa* (L.) Raf.
29	*Ipomoea kotschyana* Hochst. ex Choisy		

Results

More than 3000 leaf samples were scanned using A Canon CanoScan LiDE 120 flatbed scanner. A 30 x 30 mm scale was positioned at the top of each scanned sheet as a size maker. The leaves were placed directly onto the scanner, abaxial surface faced down, and scanned at a resolution of 300 DPI (Iwata & Ukai, 2002).

The calculated elliptic Fourier coefficients from the leaves were used to perform a principal component analysis (PCA) based on the variance-covariance matrix. The resulting PCA summarizes the information derived from the coefficients of the leaf contour. Although during our analysis we could record 11 PC out of which only four were utilized for further analysis as rest seven were showing minor variations. The four effective principal components considered in the analysis are composed of 85.06% cumulative variance. Principal component 1 (PC1) contributed 42.66% of the explained variance, this was followed by PC2 with 22.78% variance, PC3 with 12.55% variance and PC4 with 7.07% variance accounted for (Table -2).

Table 2 - Eigenvalues and accounted variation explained by the significant principal components.

Principal Component	Eigenvalue	Proportion (%)	Cumulative (%)
PC1	0.04988	42.66	42.66
PC2	0.02659	22.78	65.44
PC3	0.01719	12.55	77.99
PC4	0.01128	7.07	85.06

The variation of the leaf shape was illustrated using the accounted variation of each principal component (Figure-1). The contour of the leaf shape can be reconstructed with the estimated coefficients for the corresponding principal components. The estimated contour shape of the leaves is illustrated in Figure-2 along with the variation of the first to fourth principal component scores. The influence depicted by PC1 was largely on the size of the lamina and this constituted the largest accounted variation, when positive or negative, respectively conferring, and an ovate shape to the leaf. The PC2 represented the roundness of the leaf base and some degree of tapering towards the tip. The effect accounted for by PC3 was distortions at the lower leaf base and the right distal part of the leaf. The variation contributed by PC4 was roundness along the leaf margin.

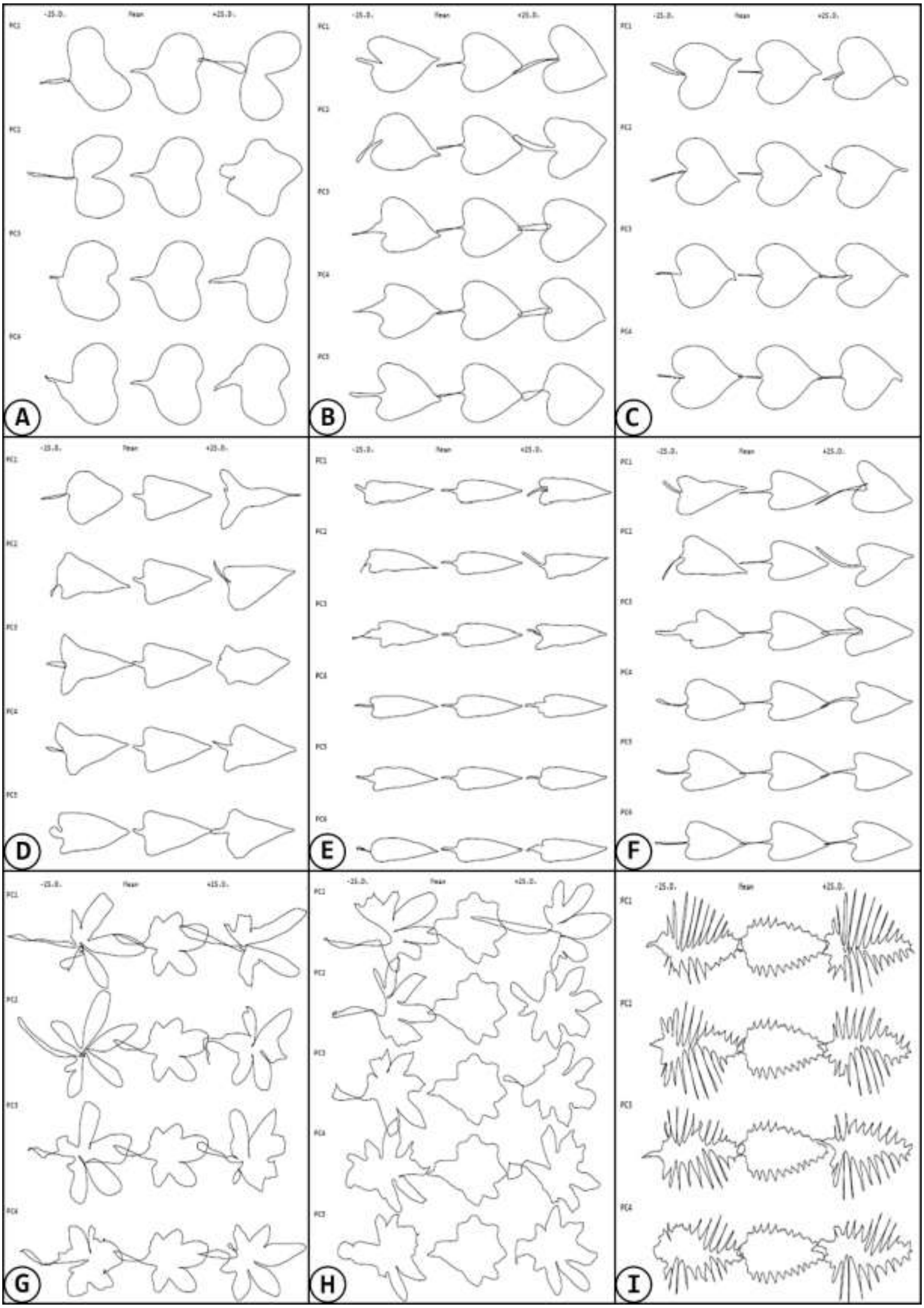

Figure 1 - Outline analysis of leaf samples (A- *I. pes-caprae*, **B-** *I. biflora*, **C-** *I. alba*, **D-** *I. aquatica*, **E-** *I. eriocarpa*, **F-** *I. sindica*, **G-** *I. cairica*, **H-** *I. pes-tigridis*, **I-** *I. quamoclit***)**

Discussion

This study applies an image based interspecific digital geometric morphometric method (GMM analysis). The potential effectiveness of these methods if applied to a larger group can be observed in the differentiation reported between the selected species. All the processed species studied showed leaf shapes ranging from simple to finely dissected (Figure-1), and the shape variations were visibly separated into four distinct groups. Our analysis suggests that different species of family Convolvulaceae are in accordance with the existing species delimitation by traditional classification. Our results are further supported by evidence obtained from the GMM analyses of the leaf variation in four species of Quercus L., (Viscosi et al., 2009), morphometric studies in Passiflora leaves with the help of EFD (Chitwood & Otoni, 2017), and leaf outline evaluation in Hoya species by using EFD (Jumawan & Buot, 2021).

When quantitatively determining the placement of each species into the designated correct group. Each of the PC variables contributed to this separation is an important and informative factor The first principal component (PC1) of form variables thus is typically dominated by a combination of size variance and allometric shape variance, and the PC1 scores may serve as a measure of “allometric size” (Bookstein, 1992, 2021). In a sample of multiple groups of species that differ both in size and shape, PC1 can also be influenced by these group differences. This should not, however, be used in isolation, but given the same weightage as traditionally used characters (e.g., leaf type, apex, base, and venation) in understanding the evolution between different species.

It is possible that a GMM-based clustering would show identical groupings to a gene-based phylogeny, as some level of success was achieved in revealing the evolution of shapes within *Oxalis* L. when geomorphometric data were combined with molecular data (Morello et al., 2018). However, GMM-based clustering contributes to a more robust result regarding the evolutionary and functional significance of the variations in leaf shape across plant groups. These variations may be caused by climatic variables and/or genetic modifications, with different shapes and sizes representing the effects of temperature, rainfall, exposure to sunlight, and mutations, providing scientists with reasons to hypothesize the evolutionary importance of leaf shape (Edwards et al., 2017; Gallaher et al., 2019; Kidner & Umbreen, n.d.; Nicotra et al., 2011).

Conclusion

In the present study, I have tried to investigate the leaf shape and development of naturally growing species of family Convolvulaceae with a different leaf shape. A utility of landmark-free morphometric methods for biometric analyses beyond mere discrimination and classification yet to be explored. We have recorded the various divergent leaf shape depicting highly variable pattern which defines the shape of the lamina. A combination of homologous point locations with semi-landmarks on curves and surfaces in geometric morphometrics enables a wide range of analyses and biological interpretations. When this method is applied to more species within the family, the species may or may not be cluster into reliable groups. Although, well- supported phylogeny and evidence from the leaf shape development aspect can help us to explain and understand the morpho-variability of other families as well as stability in the family in perticular. Based on the results, it appears that the quantitative variations which are likely to be overlooked are the most important for segregating closely related plant species.

As the shift from traditional morphometrics to digital morphometrics becomes more widespread, there will be more applications and technological solutions that will allow mass botanical digitization and overcome difficulties in plant identification. The ease of use and efficiency of digital morphometrics should encourage botanists to collect a comprehensive pool of data, thus allowing them to draw more accurate inferences, as they seek to answer their very many research questions in other allied fields. By integrating systematics and digital morphometrics, taxonomists and herbarium curators can leverage the availability of additional data generated from modern technologies in the digital identification of plants.

Bibliography

- Adams, D. C., Rohlf, F. J., & Slice, D. E. (2004). Geometric morphometrics: ten years of progress following the ‘revolution’. Italian journal of zoology, *71*(1), 5-16.
- Babu, A. K., Kumaresan, G., Raj, V. A. A., & Velraj, R (2018) Review of leaf drying: Mechanism and influencing parameters, drying methods, nutrient preservation, and mathematical models. Renewable and sustainable energy reviews, 90, 536-556.
- Bell, A. & Bryan, A. (2008). Plant Form: An Illustrated Guide to Flowering Plant Morphology. London: Timber Press. 432 p.
- Bentham, G. & Hooker, J.D. (1876). Genera Plantarum. Vol. 1, Reeve, London, 57-102.
- Bookstein, F. L. (1991). Morphometric Tools for Landmark Data: Geometry and Biology. Cambridge: Cambridge University Press.
- Bookstein, F. L. (2015). Integration, disintegration, and self-similarity: characterizing the scales of shape variation in landmark data. Evolutionary Biology, 42, 395–426.
- Bookstein, F. L. (2018). A Course in Morphometrics for Biologists. Cambridge: Cambridge University Press.
- Bookstein, F. L. (2021). Centric allometry: Studying growth using landmark data. Evolutionary Biology, 48(2), 129-159.
- Buchanan, B., & Collard, M. (2010). A geometric morphometrics-based assessment of blade shape differences among Paleoindian projectile point types from western North America. Journal of Archaeological Science, 37(2), 350-359.

- Chitwood, D. H., & W. C. Otoni (2017) Morphometric analysis of Passiflora leaves: The relationship between landmarks of the vasculature and elliptical Fourier descriptors of the blade. GigaScience 6: giw008
- Chitwood, D. H., Ranjan, A., Martinez, C. C., Headland, L. R., Thiem, T., Kumar, R., ... & Sinha, N. R. (2014). A modern ampelography: a genetic basis for leaf shape and venation patterning in grape. Plant physiology, 164(1), 259-272.
- Choisy, J. D. (1845). Convolvulaceae. Pp. 323-465. – in De Candolle, A. (ed.) Prodromus systematis naturalis regni vegetabilis, 9.
- Cooke, T. (1908). The Flora of the Presidency of Bombay Vol-2:222. Taylor and Francis, London.
- Cronquist, A. (1981). An Integrated System of Classification of Flowering Plants. Columbia University Press, New York.
- Cronquist, A. (1988). The evolution and classification of flowering plants. Bronx: The New York Botanical Garden.
- Dryden, I. V., & Mardia, K. V. (2016). Statistical shape analysis (2nd ed.). Hoboken, NJ: Wiley.
- Edwards, E. J., Chatelet, D. S., Spriggs, E. L., Johnson, E. S., Schlutius, C., & Donoghue, M. J. (2017). Correlation, causation, and the evolution of leaf teeth. American Journal of Botany, 104(4), 509-515.
- Friess, M., & Baylac, M. (2003). Exploring artificial cranial deformation using elliptic Fourier analysis of Procrustes aligned outlines. American Journal of Physical Anthropology, 122(1), 11-22.
- Gallaher, T. J., Adams, D. C., Attigala, L., Burke, S. V., Craine, J. M., Duvall, M. R., ... & Clark, L. G. (2019). Leaf shape and size track habitat transitions across forest–grassland boundaries in the grass family (Poaceae). Evolution, 73(5), 927-946.
- Hallier, H. (1893). Versuch einer naturlichen Gliederung der Convolvulaceen auf morphologisher und anatomischer Grundlage. Bot. Jahrb. Syst. 16: 453-591.
- Hooker, J. D. (1885). The Flora of British India. Asclepiadeae to Amaranthaceae. Vol-4:179. London, L. Reeve.
- Iwata, H. & Ukai, Y. (2002). SHAPE: a computer program package for quantitative evaluation of biological shapes based on elliptic Fourier descriptors. Journal of Heredity, 93(5), 384-385.
- Iwata, H., Niikura, S., Matsuura, S., Takano, Y., & Ukai, Y. (1998). Evaluation of variation of root shape of Japanese radish (Raphanus sativus L.) based on image analysis using elliptic Fourier descriptors. Euphytica, 102(2), 143-149.
- Jain, S. K., & Rao, R. R. (1976). A Handbook of field and Herbarium methods. Today & Tomorrow Publishers, New Delhi.
- Jensen, R. J. (1990). Detecting shape variation in oak leaf morphology: a comparison of rotational-fit methods. American Journal of Botany, 77(10), 1279-1293.
- Jensen, R. J., Hokanson, S. C., Isebrands, J. G., & Hancock, J. F. (1993). Morphometric variation in oaks of the Apostle Islands in Wisconsin: evidence of hybridization between Quercus rubra and Q. ellipsoidalis (Fagaceae). American Journal of Botany, 80(11), 1358-1366.
- Judd, W. S., Campbell, C. S., Kellogg, E. A., Stevens, P. F. & Donoghue, J. M. (2002). Plant Systematics: A Phylogenetic Approach. 2nd ed. Sinauer Associates, Sunderland, Massachusetts, pp. 440-442.
- Jumawan, J. H., & Buot Jr, I. E. (2021). Leaf Outline Evaluation in Selected Philippine Hoya R. Br. Species (Apocynaceae) Using Elliptic Fourier Analysis. The Thailand Natural History Museum Journal, 15(1), 21-30.
- Jussieu, A. L. de 1789. Genera plantarum: secundum ordines naturales disposita, Parisiis, apud viduam Herissant et Theophilum Barrois, Paris.
- Kidner, C. A., & Umbreen, S. (2010). Why is leaf shape so variable. International Journal of Plant Developmental Biology, 4(1), 64-75.
- Klein, L. L., Caito, M., Chapnick, C., Kitchen, C., O'Hanlon, R., Chitwood, D. H., & Miller, A. J. (2017). Digital morphometrics of two North American grapevines (Vitis: Vitaceae) quantifies leaf variation between species, within species, and among individuals. Frontiers in plant science, 8, 373.
- Klingenberg, C. P. (2010). Evolution and development of shape: integrating quantitative approaches. Nature Reviews Genetics, 11(9), 623-635.
- Kuhl, F. P., & Giardina, C. R. (1982). Elliptic Fourier features of a closed contour. Computer graphics and image processing, 18(3), 236-258.
- MacLeod, N. (2002). Geometric morphometrics and geological shape-classification systems. Earth-Science Reviews, 59(1-4), 27-47.

- Marcus, L. F. (1990). Traditional morphometrics. In: Rohlf FJ, Bookstein FL, eds. Proceedings of the Michigan morphometrics workshop. Ann Arbor: University of Michigan press. 380 p.
- McLellan, T., & Endler, J. A. (1998). The relative success of some methods for measuring and describing the shape of complex objects. Systematic Biology, 47(2), 264-281.
- Melchior, H. (1964). Convolvulaceae. In - Engler’s Syllabus der Pflanzenfamilien. Vol. 2: 438pp. Berlin-Nikolassee, Gebr. Borntraeger, Berlin.
- Morello, S., Sassone, A. B., & López, A. (2018). Leaflet shape in the endemic South American Oxalis sect. Alpinae: An integrative approach using molecular phylogenetics and geometric morphometrics. Perspectives in Plant Ecology, Evolution and Systematics, 35, 22-30.
- Nicotra, A. B., Leigh, A., Boyce, C. K., Jones, C. S., Niklas, K. J., Royer, D. L., & Tsukaya, H. (2011). The evolution and functional significance of leaf shape in the angiosperms. Functional Plant Biology, 38(7), 535-552.
- Peter, A. (1891). Convolvulaceae. In Engler, A. and Prantl, K. (Eds.), Die Naturlichen Pflanzenfamilien. 4(3a): 1-40. Engelmann, Leipzig.
- Roberty, G. (1952). Genera Convolvulacearum. Candollea, 14, 11-65.
- Roberty, G. (1964). Les genres de Convolvulac'ees (esquisse). Boissiera 10: 129-156.
- Rohlf, F. J., & Archie, J. W. (1984). A comparison of Fourier methods for the description of wing shape in mosquitoes (Diptera: Culicidae). Systematic Zoology, 33(3), 302-317.
- Rohlf, F. J., & Marcus, L. F. (1993). A revolution morphometrics. Trends in ecology & evolution, 8(4), 129-132.
- Sanfilippo, P. G., Cardini, A., Hewitt, A. W., Crowston, J. G., & Mackey, D. A. (2009). Optic disc morphology-Rethinking shape. Progress in retinal and eye research, 28(4), 227-248.
- Santapau, H., & Henry, A. N. (1973). A dictionary of the flowering plants in India. New Delhi: Publications & Information Directorate, Council of Scientific & Industrial Research.
- Savriama, Y. (2018). A Step-by-Step Guide for Geometric Morphometrics of Floral Symmetry. Frontiers in Plant Science, 9, 1433.
- Shah, G. L. (1978). Flora of Gujarat state. Part-1:459. University Press, Sardar Patel University, Vallabh Vidhyanagar, Gujarat, India.
- Simões, A. R. G., Eserman, L., Zuntini, A. R., Chatrou, L. W., Utteridge, T., Maurin, O., Rokni, S., Roy, S., Forest, F., Baker, W. J. & Stefanović, S. (2022). A bird’s eye view of the systematics of Convolvulaceae: novel insights from nuclear genomic data. Frontiers in Plant Science, 2313.
- Takhtajan, A. (1997). Diversity and Classification of Flowering Plants. Columbia University Press, New York.
- Thomas, J. G., & Geerat, J. V. (1976). Sizes and shapes of liane leaves. The American Naturalist, 110(975), 743-778.
- van Ooststroom, S. J. (1953). Convolvulaceae. In van Steenis, C.G.G.J. (Ed.), Flora Malesiana, Noordhoff-Kolff N.V, Batavia. Ser. 1(4), 388-512.
- Viscosi, V., Fortini, P., Slice, D. E., Loy, A., & Blasi, C. (2009). Geometric morphometric analyses of leaf variation in four oak species of the subgenus Quercus (Fagaceae). Plant Biosystems, 143(3), 575-587.
- Willis, J. C. (1973). A Dictionary of the Flowering Plants and Ferns. 8th ed. (Revised by H.K. Airy Shaw). Cambridge University Press, London. 1245 pp.
- Yoshioka, Y., Iwata, H., Ohsawa, R. Y. O., & Ninomiya, S. (2004). Analysis of petal shape variation of Primula sieboldii by elliptic Fourier descriptors and principal component analysis. Annals of Botany, 94(5), 657-664.

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