Discussion

5. Discussion

The field of systematics should be recognised as a scientific discipline that can maintain its relevance in the modern information age, rather than being seen as a traditional activity of collecting stamps **(Koch & German, 2013)**. This understanding should also be communicated to the public and experts in the field. To build up a natural system of classification of plants, it is necessary to compare one form with another such parts like stem, leaf, root, flower, fruits, and seeds. These examinations are helpful to a certain extent in identifying and classifying the plants. The phenotype of each taxon is unique, and this uniqueness itself is a clear identifying feature for a taxon **(Pires & Dolan, 2012; Sattler & Rutishauser, 2022)**.

Morphological studies are an effective method employed to characterize and generate information regarding the systematic and ecological aspects **(McLellan & Endler, 1998)**. Morphometrics can be used to examine the complete shape of an object, from simple shapes like ovals to more intricate ones like irregular leaf outlines **(Adebowale et al., 2014; Viscosi & Fortini, 2011)**. Leaves exhibit a wide range of anatomical and morphological diversity, which can differ even among species that are closely related. Nevertheless, leaves share numerous characteristics. The findings from our study can be seen as a reflection of this dual nature.

The main objective of the present study was to evaluate the success of digital morphometric methods in the segregation of plant species based on leaves. For that the present study employed outline-based morphometric techniques, specifically Elliptic Fourier Analysis (EFA) and Principal Component Analysis, to investigate leaf shape variations among selected species within the Convolvulaceae family. These quantitative methods have proven valuable in capturing the entire outline of leaf structures, providing objective data for species segregation, and exploring potential ecological and evolutionary implications (**Cope et al., 2012; Proietti et al., 2021**). The findings of this research illustrated how mathematical analysis of leaf outlines utilizing elliptical Fourier descriptors can be employed to distinguish individuals within the Convolvulaceae family at the species level.

Elliptic Fourier Analysis (EFA) is a mathematical method used to analyse and describe intricate closed curves or shapes **(Kuhl & Giardina, 1982)**. Shape analysis and pattern identification are areas where it holds great importance, providing advantages in various fields like biology, image processing, and computer vision **(Andrade et al., 2008; Furuta et al., 1995; Iwata & Ukai, 2002).** The EFA methodology was initially devised by **Kuhl and Giardina in 1982** and has since gained extensive adoption and recognition due to its adaptable range of uses. EFA decomposes the leaf outline into a series of elliptic Fourier descriptors, which represent the outline's shape in a quantitative and mathematically concise manner. It is a powerful method for evaluating differences in the morphology of leaves, offering great insights on the distinctive form characteristics of groups of species. **Viscosi and Cardini (2011)** combined leaf morphometrics with climatic data and molecular phylogenies in the Myrtaceae family, revealing associations between leaf shape, precipitation patterns, and evolutionary divergence. Similarly, **Klingenberg et al. (2012)** integrated leaf morphometrics with habitat information and phylogenetic relationships in the Rosaceae, providing insights into the potential adaptive significance of leaf shape variations. The significance of leaf shape analysis extends to the identification and classification of species, making it a valuable method for viewing and assessing aspects of leaf shape **(Andrade et al., 2008; Hâruta, 2011; Viscosi & Fortini, 2011)**. Furthermore, it was shown that the range of leaf shapes within species is continuous, suggesting that traditional approaches may not fully capture the complete range of leaf shape variation within or between groups. The EFA is an effective method for understanding the changes in organisms, especially when it comes to evaluating the differences between populations (**Pessoa et al., 2020**).

Principal Component Analysis (PCA) is a widely used multivariate technique that has been combined with EFA to explore patterns of leaf shape variation and identify the most significant shape features contributing to species segregation (**Andrade et al., 2008; Furuta et al., 1995; Klingenberg et al., 2012**). In the present study, PCA revealed distinct clustering patterns among the studied Convolvulaceae species based on their leaf shapes, suggesting potential ecological or evolutionary drivers of morphological divergence. The interpretation of the principal component (PC) axes and their associated shape changes provided insights into the specific leaf shape characteristics that contribute to species segregation. For instance, the first PC axis might represent variations in leaf width-length ratio or the degree of leaf lobing, while subsequent axes could capture differences in leaf base symmetry or apex shape. These shape features may be associated with functional adaptations related to light interception, water use efficiency, or herbivory resistance, as suggested by previous studies **(Chitwood & Sinha, 2016; Royer et al., 2009).**

In the present leaf shapes of 58 taxa of family Convolvulaceae were quantified using different metrices and found to be significantly differ although leaves of many species appear to be superficially very similar in appearance.

The effective use of outlines for shape description and image reconstruction is beneficial in cases where landmark-based analysis is not feasible. According to McLellan and Endler (**1998**), elliptical Fourier analysis is a reliable method for reconstructing the outline of complex objects and understanding the overall intricacy of their form. Previous research has shown the utilization of leaf shape characteristics to categorize species **(Andrade et al., 2008; Jensen, 2003; Viscosi & Fortini, 2011)**. In their study, Jensen, et al. (**2008**) examined the leaf outlines of two *Acer* species and their hybrid. They were able to identify two distinct groups corresponding to the two species, but they were unable to separate the hybrid. Viscosi and Fortini (**2011**) discovered that elliptical Fourier analysis was an effective method for examining the leaf morphology of three oak species. They also determined that this methodology was a beneficial tool for distinguishing between species within the subgenus *Quercus*.

The utilization of Elliptical Fourier descriptors on the leaf outline of Convolvulaceae members offered a prompt approach to examine the variety in shape and outline, as well as to distinguish between different species within the family. The leaf outline methodology is a quick and handy strategy that can assist other researchers in their investigations. The current work demonstrates that the sole consideration of leaf outline form is a valid taxonomic criterion, enabling accurate grouping of populations within their respective hierarchical taxonomic order. This classification method relies on the utilization of fully developed leaves. The quantitative analysis of leaf outlines can be a useful technique for making taxonomic comparisons, even when examining populations. Those familiar with the extensive array of leaf variations among members of the Convolvulaceae family would likely find this quite astonishing.

More quantitative features are needed to characterize morphology. Leaf form affects taxonomy in the taxa we analysed. Thus, our discovery that leaf shape distinguishes species may seem circular. We identified vegetative individuals in the field using habitat, stem, leaf, and flower characteristics. This study also measured leaf shape variability quantitatively. It provides proof of the grouping tendencies observed in morphological classification, which alpha taxonomy automatically uses (**Belo et al., 2024**). Field-collected digital photos were practical and successful for gathering large samples and obtaining quantitative data semi-automatically. This technology could enable population-scale morphological analysis accessible to a wide variety of academics by applying it to other measurable qualities from photos. This would allow huge samples to be studied without collecting and preserving enormous amounts of plants in herbariums. Scientists investigating small, endangered groups are becoming concerned.

Our approach using morphometrics has assessed leaf shape variation in a non–qualitative way, as it is commonly studied in traditional morphometrics (**Chen et al., 2018**). Our study supports the utility of GM on testing systematic affinities in species of family Convolvulaceae. Successful differentiation between and within species groups such as *Argyreia, Ipomoea, Merremia* and *Distimake* employing leaf shape sets a landmark for future taxonomic studies in a genus where its general morphology is typically affected by homoplastic processes.

The results of EFA and PCA analysis comparing all the taxa under study showed that leaf shape, at least in the way that it was captured in this study, does not differentiate them sufficiently to allow identification. Some examples of systematic and taxonomic implications derived from morphometrics have been previously done in Liu et al. (**2018**) with Chinese oaks leaves, Terral et al. (**2012**) with the seeds of *Phoenix* genera species, or Van der Niet et al. (**2010**) assessing flower shape variation. These studies agreed in the application of GM as a useful approach for providing detailed information on the morphological variation of the plant structures with taxonomic value. In addition, research on plant organ shapes and its relationship with other organisms or environmental factors might shed additional light on other fields such biogeography, ecology, and genetics. Despite this, the comparisons showed a significant difference in their mean leaf shapes. We can therefore conclude that a statistical difference in leaf shape does exist but that this is largely due to other factors.

The PC scores extracted from the four effective principal components were utilized to perform cluster analysis. A similarity matrix using Euclidean distance was utilized in the principal component scores. The resulting matrix was employed to create a cluster analysis using single linkage or nearest neighbour clustering method. The data was displayed in the form of dendrogram.

The dendrogram of genus *Argyreia* revealed three major groups among all the analysed species. The first group which was separated consisted of four species i.e., *A. pilosa, A. nervosa, A. boseana* and *A. sharadchandrajii.* Second group consisted of *A. setosa, A. sericea* and *A. elliptica. A. cuneata* were segregated from other species forming a single clad. Considering the results, *A. pilosa, A. nervosa, A. boseana* and *A. sharadchandrajii* and group of *A. setosa, A. sericea* and *A. elliptica* appears to share most of the same biometric characteristics, excluding their size. The climatic and environmental changes in Argyreia may have caused a divergent morphological evolution. Separation of *A. cuneata* from the rest of the species was due to variation in PC1 while other two clades got separated based on the values of PC2, PC3 and PC6. Species level segregation was due to minor variation in the rest of the PCs (Plate – 57).

The dendrogram of *Convolvulus, Evolvulus and Jacquemontia* genus was constructed. The dendrogram separated Jacquemontia and other genus into three clades. Further the major clad consists of Convolvulus, Evolvulus and Jacquemontia species based on their leaf shape. *C. arvensis* got separated first and formed a single species clade which possesses unique sagittate leaves. This separation was due to the variation depicted by PC1, PC7-PC10 Two species of Jacquemontia got separated from the first clade and form a monophyletic clade with *J. paniculata* and *J. pentanthos*. This clade formation was based on the variation among PC6 and PC7. Rest of the four species got separated based on their leaf shape. Remaining species *C. prostratus, C. stocksii,* and *E. alsinoides* have lanceolate leaves and formed a one clade. While the *E. nummularius* got separated from the third clade based on circular leaf shape and cordate leaf base. Reason behind the separation of E. nummularius was mainly the variation in PC8 and PC2 (Plate – 58).

Cluster dendrogram of 31 Ipomoea species was constructed. The dendrogram revealed two major clades which separates simple and palmate/dissected leaves among the species of Ipomoea. Palmate leaved species and highly dissected leaved species separated first and formed five sub-clades. The separation between palmate/dissected leaved and simple leaved Ipomoea species was due to the variation in PC1-PC5. Rest of the PCs were responsible for the separation of simple leaved species. All the species having simple leaves got separated in a single clade except three species *I. hederifolia, I. triloba* and *I. laxiflora* which got nested with palmate leaved species. Formation of this clade needs further investigation and analysis combining other aspects. There is a weak indication of leaf shape differentiation between species with similar leaf shape e.g. *I. triloba* and *I. laxiflora, I. marginata* and *I. marginata* f *candida, I. campanulata* and *A. sericea* and many more. which suggests a tendency for pairwise comparisons of populations from the same species to be less distant than comparisons of populations from different species (Plate – 59).

Dendrogram of tribe Merremieae was constructed with R. hypocrateriformis as outgroup species. The tribe now includes 12 genera **(Simões & Staples, 2017)**. The dendrogram revealed two major groups among the analysed species of tribe Merremieae. First clade included species with palmate leaves and the other included species with simple leaves. Clade with the simple leaved species further divided into two subclade segregating species of *Merremia, Camonea, Operculina* and *Rivea*. Two species of Operculina formed a single clade and got separated from *Merremia* and *Camonea*. M. emarginata and R. hypocrateriformis formed a sub clade as they possess almost similar leaf shape with circular leaf shape and cordate leaf base. Other simple leaved species formed a sub clade which separates *X. tridentata* as a single monophyletic genus from the rest other species. C. pilosa got nested with *M. hederacea* as they have somewhat similar leaf shape with acute leaf apex and rounded leaf base. The genus *Distimake* got separated from all the simple leaved species which is in the favour classification based in leaf shape. All the species of genus Distimake exhibit palmate and dissected leaves. Our result also supports the recent development in the classification of tribe Merremieae. Simoes and staples (**2017**) in their paper dissolved the old genus Merremieae and circumscribed new genera from already existing species. Our result also shows similar pattern and supports the new classification of the tribe (Plate – 60).

Separation of genus *Distimake* and other simple leaved species of tribe Merremieae was mainly because of PC1, PC2, PC3 and PC4. Separation of *Rivea* and *M. emarginata* from the rest of the species was based on the variation of PC1 and PC2. While Operculina species separated from other species based on the variation of PC8 and PC9.

Combined Dendrogram was constructed the same was for all the analysed species of family Convolvulaceae. There was no evident separation of every genus in combined dendrogram which suggests that there is overlapping in leaf shape characters and hence in the data. The dispersion of the typical leaf form observed in natural populations of Convolvulaceae species indicates that the shape delineated in the protologue does not consistently predominate and that the morphological signature acquired may potentially represent infrequent morphotypes (Plate – 61).

There were two major clades formed. Despite there was no clear separation in the combined dendrogram, species of *Ipomoea* with palmate and dissected leaves got separated from rest of the species forming two different clades, one separate clade and other nested with all the other species. While four species of genus *Distimake* were nested between the dendrogram separated from the palmate/dissected leaved *Ipomoea* species which could have formed a single clade based on the leaf shape. There was clear distinction between simple leaved species forming several small clades despite their different leaf shape.

For gaining an understanding of the role that each PC plays in the separation of the species that were analysed in this study, a two-way dendrogram was generated. PC1 played a role in separating the first two major clades, which includes species with palmately dissected leaves, from the second major clade. PC10 differentiates the remaining species of *Ipomoea* that have palmate or dissected leaves from the remainder of the species that have simple leaves. Both PC3 and PC4 are responsible for the separation of species belonging to the genus *Distimake* and a few species belonging to the genus *Ipomoea* that have simple leaves. To a certain extent, the residual PCs were responsible for the differentiation that occurred between all the remaining species (Plate – 62).

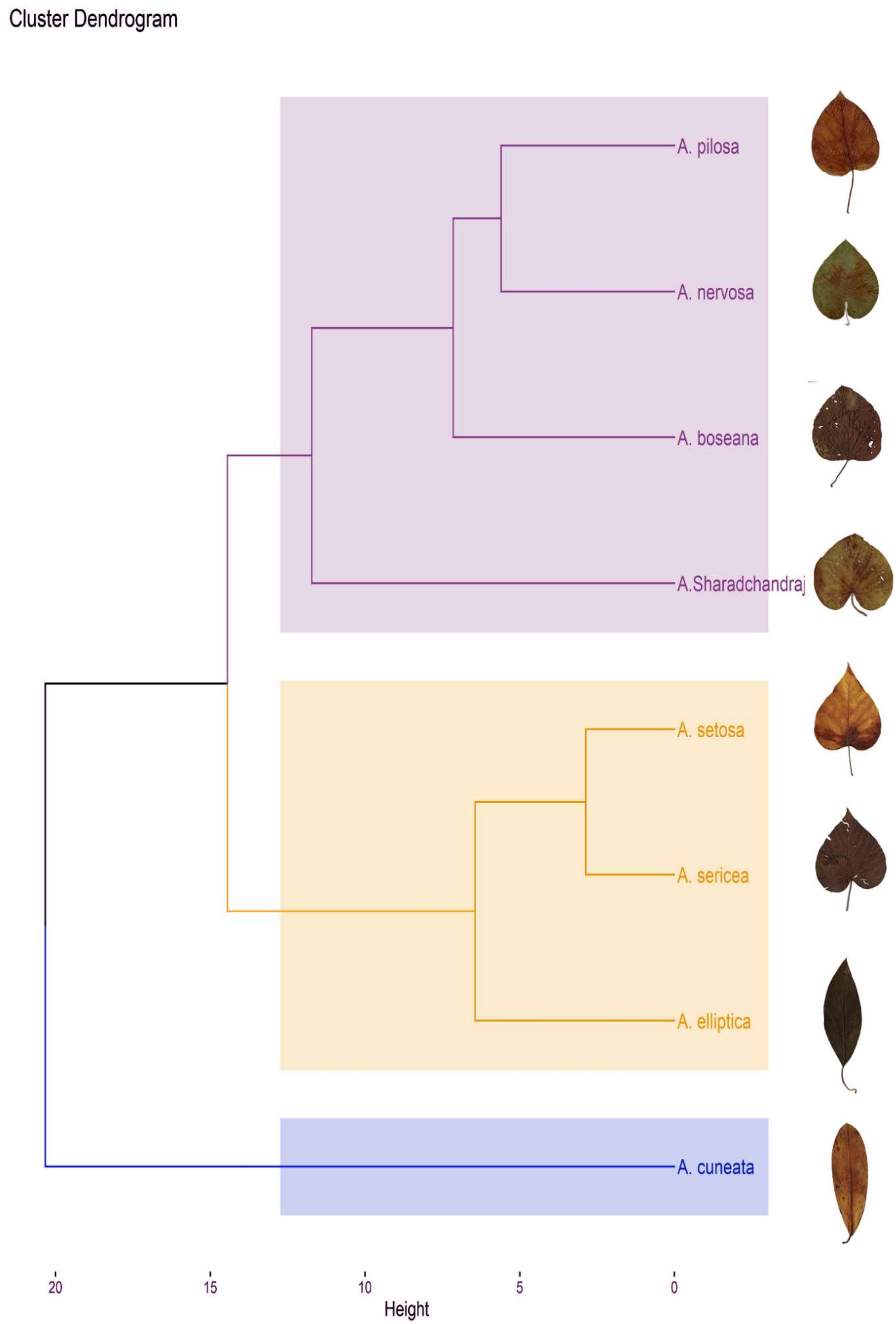

Plate 57 - Cluster dendrogram of genus *Argyreia*

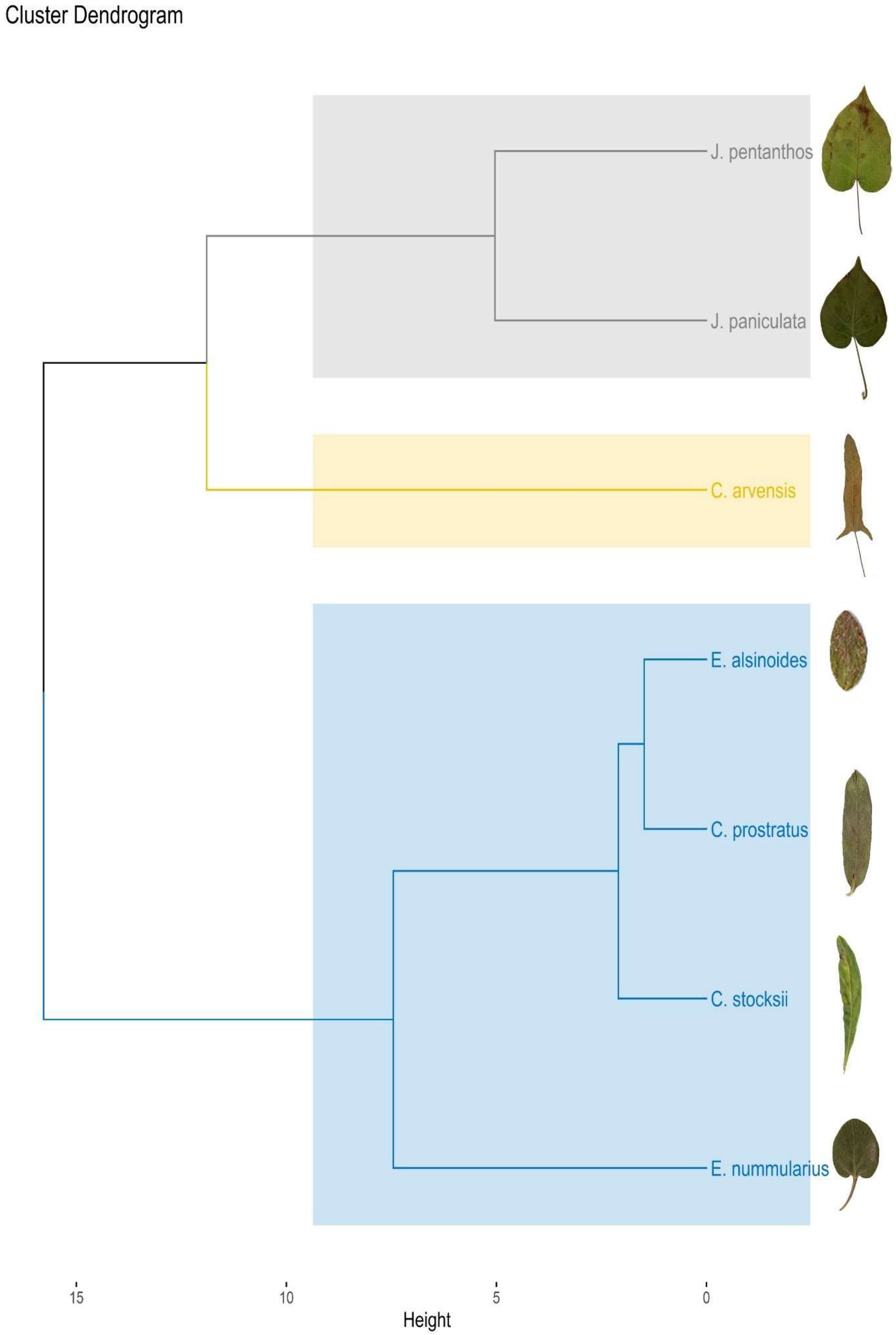

Plate 58 - Cluster dendrogram of *Convolvulus*, *Evolvulus* and *Jacquemontia*

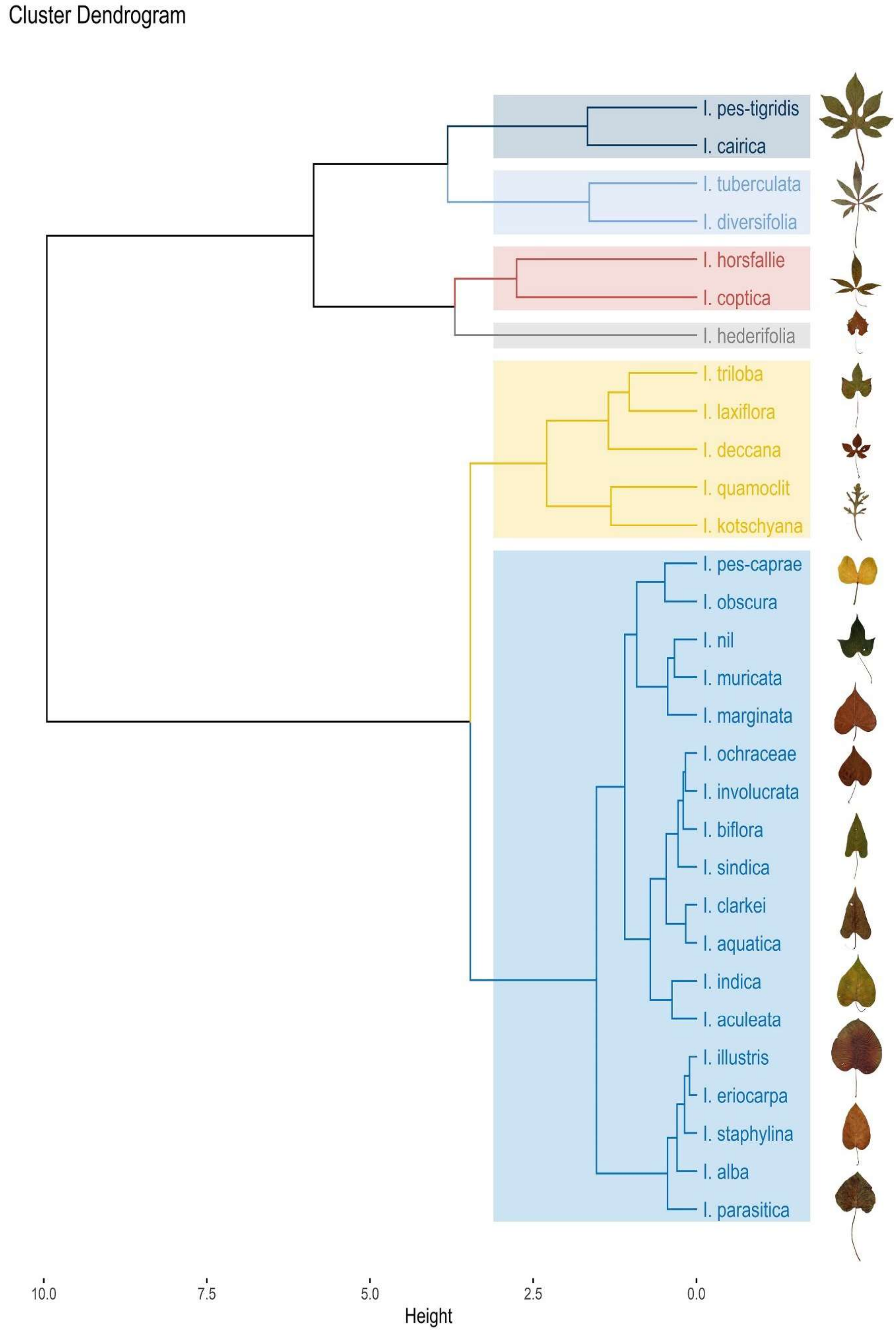

Plate 59 - Cluster dendrogram of genus *Ipomoea*

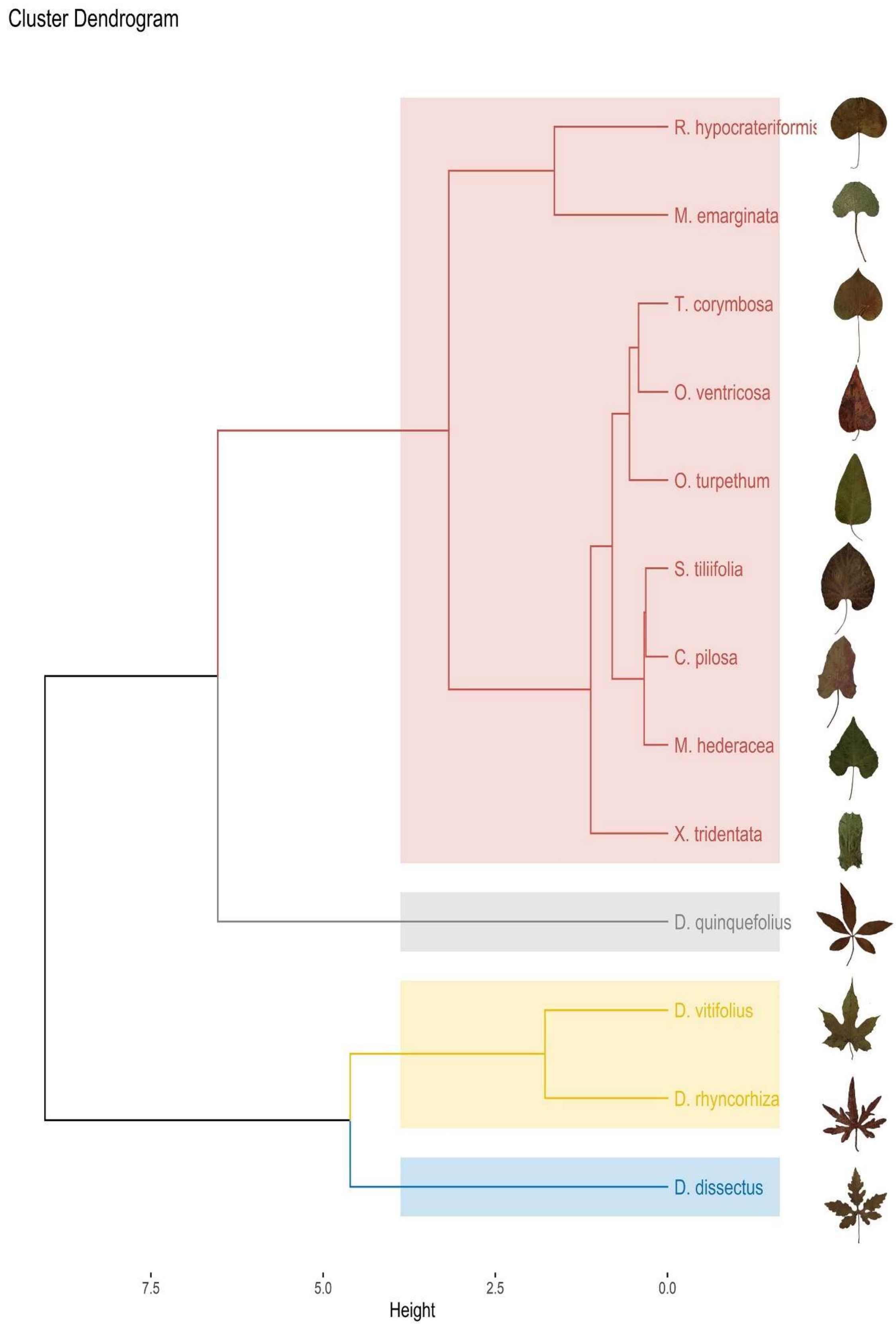

Plate 60 - Cluster dendrogram of tribe *Merremieae* and other species

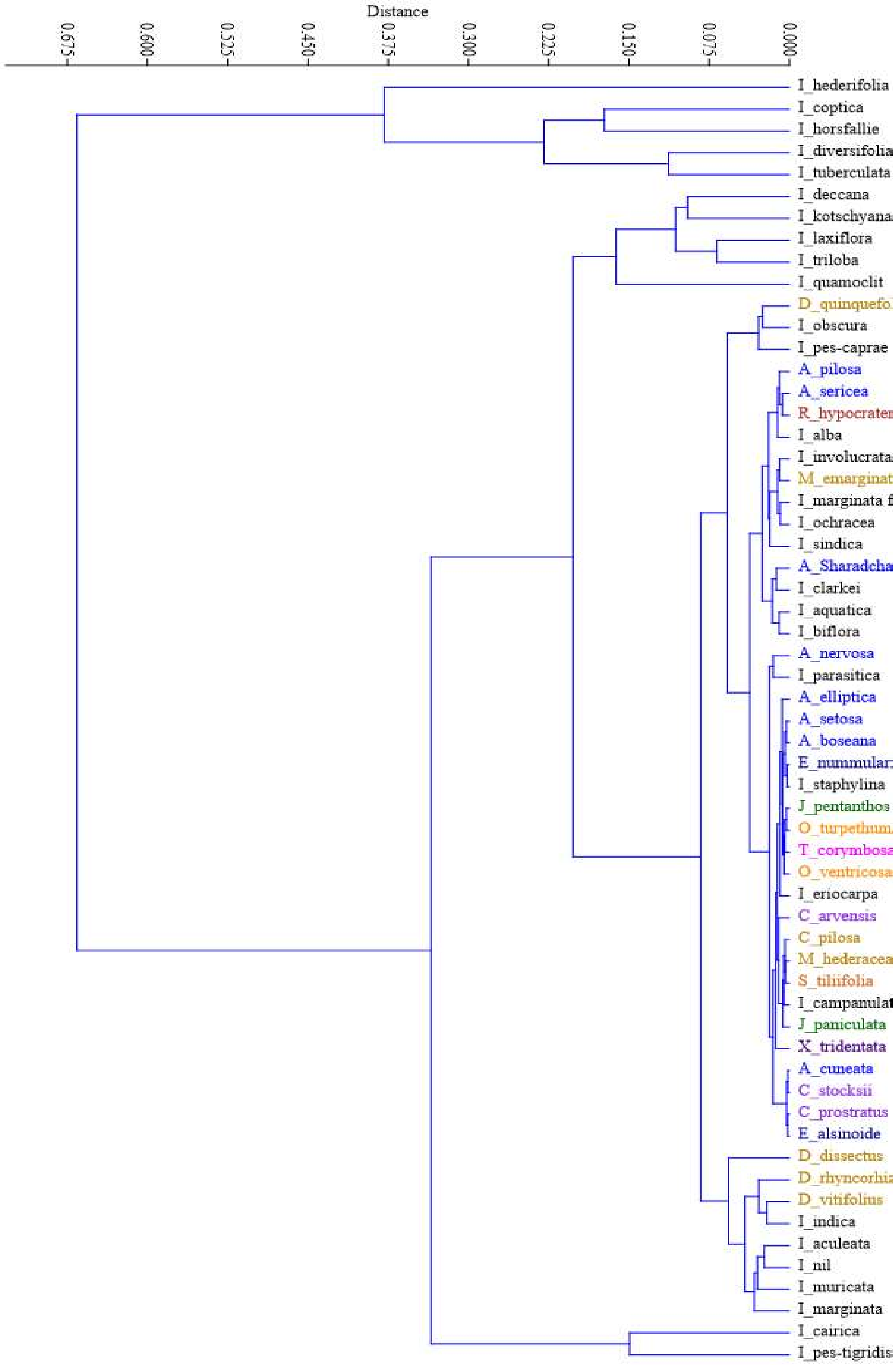

Plate 61 - Cluster dendrogram of all analysed species of family Convolvulaceae

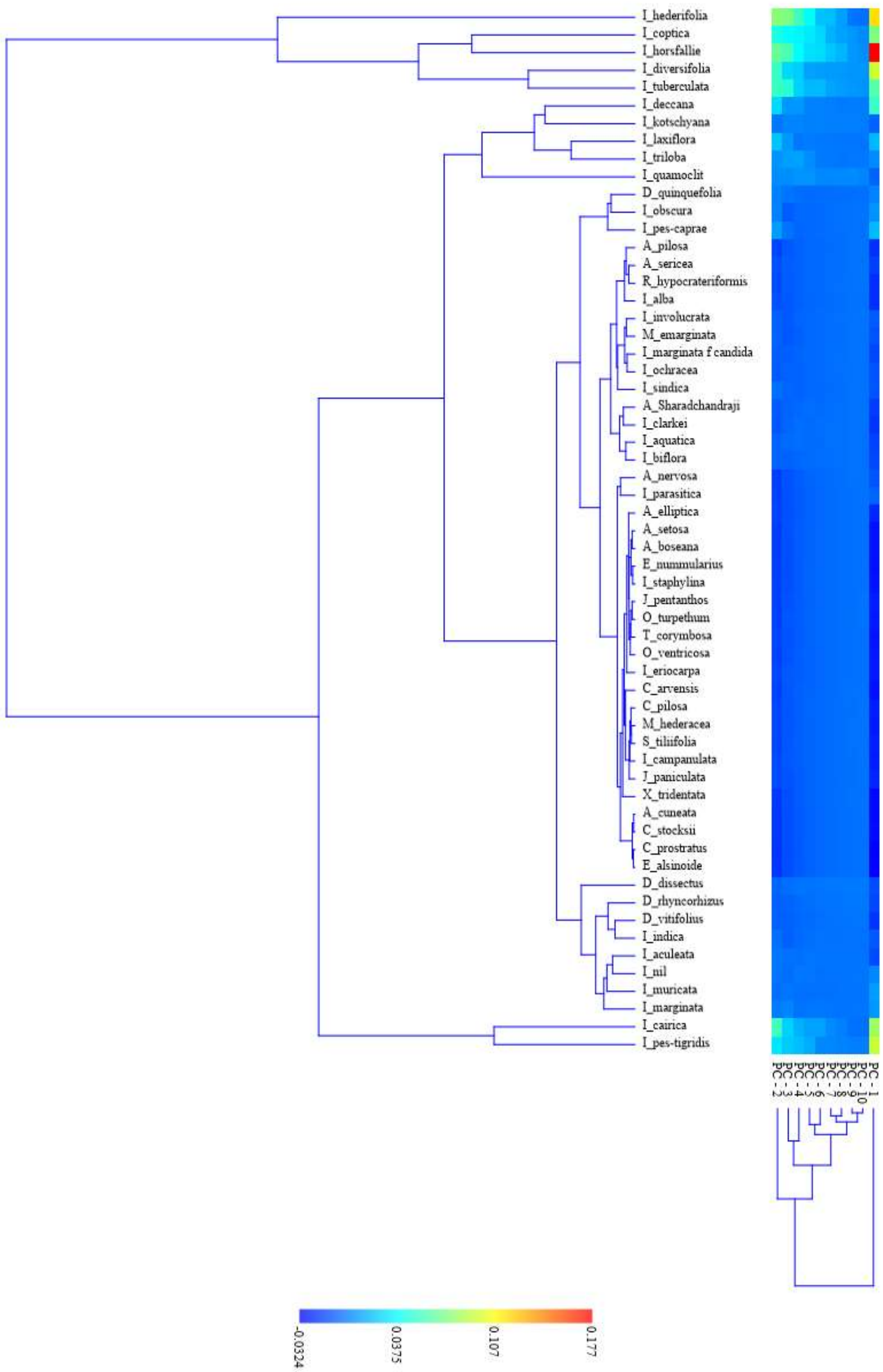

Plate 62 - Two-way graph showing species separation based on morphometric analysis and role of each PC in separation

Morphometric approaches are essential for distinguishing shape characteristics that distinguish species regardless of developmental context **(Chitwood & Otoni, 2017)** from purely heteroblastic changes in leaf shape that are common among closely related species (**Klein et al., 2017**). This research examines the discriminatory ability of geometric morphometric methods compared to other morphological measurements and analyses the morphological links between species.

We emphasize the value of utilizing EFA in conjunction with multivariate approaches to enhance the comprehension of regularly used taxonomic interpretations. The findings of our analysis demonstrated significant variation in the physical characteristics of the species we studied, as evidenced by both continuous and categorical data. However, only EFA has the capability to quantify the impact of alterations in leaf asymmetry (**Benítez et al., 2020; Savriama et al., 2012**). As stated in the Principal Component Analysis (PCA), there is a gradient resulting from many diverse forms of shape alterations, with a minor gap existing between different groups. Practically, the difference between qualitative and quantitative data strengthens the debate on the definition of taxa, as it allows for the proposal of novel morphological relationships.

The current study demonstrates that species belonging to the Convolvulaceae family can be differentiated, to some extent, by quantitatively comparing their leaf shapes. The PCA and EFA analyses both concurred in identifying shape variables 1 and 2 as the most significant characteristics in distinguishing the species. The findings are promising, considering that only the contour of the leaves was utilized. However, this study was restricted to comparing individuals from a solitary population of each species in the same geographic area. The results are thus just provisional due to the requirement for more comprehensive comparisons across the species' ranges.

The study proposes that a combination of qualitative and quantitative methods may be advantageous for semiautomated identification systems to be employed on recently collected samples in or around field locations. Species possessing a palmate or dissected leaf blade can be readily differentiated from other species with simple leaves due to their distinct leaf form. An opportunity for further enhancement of this methodology lies in the exploration of methods to measure additional characteristics of the leaf blade that skilled individuals rely on to differentiate species instinctively, such as texture, colour (both dried and fresh), venation, and glossiness.

Individually, none of these variables are enough to reliably differentiate, but the task is to determine if analysing combinations can yield considerably improved results **(Wäldchen & Mäder, 2018)**. It is necessary to examine juvenile leaves, as they have the potential to be valuable for differentiation purposes. It is necessary to search for and document new characters, both in terms of quality and quantity, especially from recently obtained material (**Migicovsky et al., 2018**).

In the present study, EFA effectively captured leaf shape variations among the studied Convolvulaceae species, as evidenced by the clear separation of species in the multivariate space defined by the Fourier coefficients. Previous studies have demonstrated the efficacy of EFA in discriminating plant species based on leaf morphology across various taxonomic groups, including Myrtaceae **(Viscosi & Cardini, 2011)** and Rosaceae (**Klingenberg et al., 2012**). The ability of EFA to capture subtle shape differences, even among closely related species, highlights its potential for resolving taxonomic uncertainties and identifying cryptic species.

While outline-based methods like EFA and PCA have proven effective in capturing leaf shape variations, it is important to consider potential limitations and areas for improvement. One limitation of EFA is its sensitivity to outline starting points, which can lead to inconsistencies in the resulting Fourier coefficients **(Rohlf & Archie, 1984)**. To address this issue, future studies could employ alternative outline-based approaches, such as Elliptical Fourier Descriptors (EFDs) or Procrustes superimposition of outlines, which are less sensitive to outline starting points (**Andrade et al., 2008; Iwata et al., 1998**).

Taxonomists are increasingly using digitized images for specimen identification by visual feature matching or for extracting morphological traits that can be standardized and utilized for phylogenetic, morphometric, or other analyses **(Alagumariappan et al., 2020; Belhumeur et al., 2008; Bisen, 2021; Pryer et al., 2020**). Due to the availability of abundant of image databases and the scarcity of taxonomic experts, there is a significant need for the application of computer vision to specimen images **(Du et al., 2007; Wäldchen & Mäder, 2018)**.

Leaf morphological traits may be influenced by genetics, development, and biotic or abiotic factors (**Fleming, 2005**). For example, the leaf venation length and distribution and lamina leaf area may represent the response to water availability, light exposure, and other environmental factors **(Sack & Scoffoni, 2013)**. Moreover, viruses or fungal infections may cause leaf deformation **(Klein**

& Svoboda, 2017). Thus, it is mandatory to consider biotic and abiotic conditions when comparing inter-individual variation of leaf morphological traits of different accessions, choosing appropriate experimental units. of the leaf is influenced by genetic and environmental factors (and their interaction)(**Fleming, 2005**), such as temperature (**Moles, 2018**), soil fertility **(Reich & Oleksyn, 2004)**, water availability (**Moles et al., 2014**) and solar radiation (**Ren et al., 2021**).

GMMs separate the leaf shape from the leaf size and carry out the statistical analysis **(Savriama & Klingenberg, 2011).** GMMs retain the shape variables, and the shape difference not related to size can be obtained by coordinate transformation analysis (**Igual et al., 2014; Viscosi et al., 2012**). In addition, GMMs use the overlap of the transformation grid and wireframe graphs to simulate the changing trends of leaf morphological characteristics **(Klingenberg, 2011; Viscosi & Cardini, 2011).** Thus, the main differences in leaf morphology among all the analysed species could be seen directly.

Our results confirm, after the analysis, the clear separation of simple leaved species and palmately leaved species of family Convolvulaceae in leaf geometric morphometry. In our case, we lack a precise genetic characterization to determine the genetic background of Species. However, a complete genetic analysis is needed to discriminate the discrepancies of genetic assignment using morphological traits in a group as complex. We also need to consider the necessary samples sizes to better understand this process, related to the inter- and intra-specific variability.