Introduction

1. Introduction

1.1. Morphometrics

"Nothing is measured,
features are observed and
compared to the definition of our terminology"

The examination and comparison of morphological characteristics of organisms have long been a fundamental aspect of the field of biology, spanning many years. Numerous inquiries in the field of biological research revolve around the concept of morphology. What is the typical morphology of an organ or structure within a given population? What is the observed pattern of variation within a population in relation to the average shape? What are the variations in shape among different groups? What is the functional significance of these differences? (**Budd, 2006; Lauder, 1990**). Historically, the taxonomic classification of species and the comprehension of biodiversity relied mainly on the characterization of physical characteristics. Morphometrics is a discipline that encompasses the necessary methodologies to systematically investigate and effectively tackle such queries.

Morphometrics, the term was firs introduced by the biologist Julian Huxley in his book "Problems of Relative Growth" (**Huxley, 1950**). The term 'Morphometrics' refers to the quantitative characterization, analysis, and understanding of shape and its variations in the field of biology (**Bookstein, 1982**). It is an interdisciplinary field that has arisen from the integration of biology, anthropology, statistics, and mathematics (**Mitteroecker & Schaefer, 2022**). This methodology measures the shape and size of living organisms and their various components. It serves as a potent instrument for comprehending the complex interplay of natural forms and patterns. This methodological approach serves to establish a connection between the field of biology and other related disciplines, offering valuable insights into various aspects of evolution, taxonomy, ecology, and beyond. This comprehensive investigation delves into the historical and evolutionary aspects of morphometrics, charting its progression from its early stages to its current utilization, with a particular emphasis on its implementation in the field of plant research (**Lawing et al., 2010**).

1.1.1. Early Foundations: Roots of Morphometrics

The field of morphometrics as we know it today had modest beginnings. The origins of this field can be traced back to the mid-20th century, during which scientists endeavoured to develop techniques for quantifying and analysing the morphology and dimensions of biological structures. One individual who played a significant role in this domain was D'Arcy Thompson (1945), whose influential publication "On Growth and Form" established the foundation for the measurement and analysis of biological structures. Although Thompson's work mostly consisted of descriptive analysis and did not include the quantitative rigor characteristic of contemporary morphometrics, it played a vital role in paving the way for the advancement of more accurate methodologies.

The discipline of morphometrics has experienced substantial expansion and enhancement of methodologies throughout the course of several decades. Prominent scholars such as Fred L. Bookstein (**1982**) and Rohlf & Archie (**1984**) have made significant contributions to the field of form analysis by introducing groundbreaking procedures that integrate statistical approaches. The initial endeavours laid the foundation for the advancement of landmark-based morphometrics, a methodology that entails the recognition and digitalization of distinct anatomical landmarks on biological specimens. Within the field of plant studies, the discipline of morphometrics has evolved as a highly effective instrument for the characterization and quantitative analysis of various plant structures. Scholars initiated the utilization of morphometric methodologies to various plant components, including leaves (**Cope et al., 2012**), flowers (**van der Niet, 2010**), seeds (**Venable et al., 1998**), and fruits (**Lo Bianco & Mirabella, 2018**). The primary objective of these investigations was to provide a comprehensive understanding of the intricate differences in plant morphology, which are frequently influenced by ecological conditions and evolutionary adaptations.

1.1.2. Types of Morphometrics:

Morphometrics refers to the scientific investigation of form variation and its correlation with other variables (**Bookstein, 1982**). Historically, morphometrics has been employed to apply multivariate statistical analyses to collections of quantitative data, including but not limited to measurements of length, width, and height. During the late 1980s and early 1990s, a notable change took place in the way morphological structures were measured and the methods employed for data analysis (**Kuhl & Giardina, 1982; Rohlf & Archie, 1984; Rohlf & Bookstein, 1990**). This

paradigm changes prioritized techniques that effectively captured the geometric aspects of the morphological structures under investigation and maintained the integrity of this information throughout the analytical processes. This advancement gave birth to several sub type of the branch morphometrics.

1.1.2.1. Traditional morphometrics:

This comprehensive suite of multivariate statistical techniques encompasses a wide range of methods that are employed to effectively characterize and analyse patterns of shape variation both within and between distinct groups. The aforementioned methodology, commonly referred to as classic morphometrics (**Marcus, 1990**) or multivariate morphometrics (**Reyment, 1990**), involved the utilization of multivariate statistical techniques on collections of morphological characteristics. Traditionally, linear distance measurements such as length, width, and area were commonly employed, occasionally supplemented by counts, ratios, and angles. The quantification of covariation in the morphological measurements was accomplished using these methodologies, allowing for the assessment of patterns of variation within and among samples. Due to the strong correlation between linear distance measurements and size, significant efforts have been dedicated to the development of techniques for size correction (**Bookstein & Mitteroecker, 2014**). This enables the extraction of size-independent shape variables and facilitates the elucidation of patterns of shape variation (**Jungers et al., 1995; Sundberg, 1989**).

Although the integration of multivariate statistics and quantitative morphology in multivariate morphometrics was a significant advancement, some challenges persisted. Numerous approaches for size correction were suggested; however, a consensus over the preferred way remained elusive (**McCoy et al., 2006; Revell, 2009**). The significance of this matter lies in the fact that various approaches of size adjustment typically produce marginally divergent outcomes. Furthermore, the evaluation of the homology of linear distances posed challenges due to the absence of homologous sites defining some distances, such as maximal width. Furthermore, it should be noted that the absence of information regarding the relative locations at which the distances were measured in relation to one another could result in the same set of distance measures being derived from two distinct forms. For example, in the scenario when the maximum length and maximum width are assessed for both an oval and a teardrop, it is possible for both objects to exhibit identical

height and breadth measurements **(Stegmann & Gomez, 2002)**. However, it is evident that they possess distinct shapes. Hence, it is anticipated that the statistical ability for discriminating shapes will be much less compared to its expected level. In general, the generation of graphical representations of shape from linear distances was often impractical due to the lack of preservation of geometric relationships among variables. A mere set of linear distances typically fails to adequately retain the original object's geometry. Consequently, several elements pertaining to the form were not retained **(Mitteroecker & Bookstein, 2011; Pietrusewsky, 2018)**.

1.1.2.2. Geometric Morphometrics (GM/GMM):

Due to the difficulties in measuring traditional morphometrics, researchers have looked at alternative methods of quantifying and analysing morphological shape. Data on the geometric properties of the morphological structure was studied, leading to analytical methods. The methodology included outline and landmark data. With these advances, Kendall (**1989**) and other statisticians established a complete shape analysis statistical framework **(Rohlf, 1990a; Slice, 2005)**. This theoretical framework allowed multivariate statistical methods to be integrated with direct imaging of biological forms. Bookstein (**1996**) called this "morphometric synthesis" Since the 1990s, geometric morphometrics has been a popular form analysis tool. This method emphasises form analysis by overlaying landmark data and minimising size. This method provides a broader view of form differences than linear measures.

Geometric morphometrics relies on "Procrustes superimposition." To align landmark configurations in a shared coordinate system, translation, rotation, and scaling are used. Shape variables that accurately express landmark positions without size or orientation elements are the result **(Goodall, 1991)**. Geometric morphometrics works best for studying complicated shape variations. Researchers can visualise shape changes, analyse shape data, and find trends. This approach is useful for studying complex biological forms including leaves, flowers, skulls, and bones **(Rohlf, 1999)**. Geometric morphometrics is used in evolutionary biology, ecology, anthropology, and developmental biology **(Gurgel-Gonçalves et al., 2011; Lawing et al., 2010; Mitteröcker, 2021)**. This tool helps study how shapes evolve, adapt, or change in response to external stimuli. Geometric morphometrics, a cutting-edge shape analysis tool, allows researchers to discover detailed structure in living organisms while minimising size and orientation biases.

1.1.2.3. Landmark-Based Morphometrics

Landmark-based morphometrics is a fundamental technique within the discipline of morphometrics. The process entails the identification and digitalization of distinct structural markers on biological materials. The selection of these landmarks depends upon their biological significance and is frequently determined by their ability to epitomise crucial characteristics of an organism. The utilisation of these coordinates as variables for direct analysis would be deemed unsuitable due to the persistent presence of variations in the position, orientation, and scale of the specimens (**Webster & Sheets, 2010**). Hence, it is imperative to eliminate non-shape variation by mathematical procedures before doing an analysis of those variables. After eliminating non-shape variation, the remaining variables can be considered as shape variables. These shape variables can then be utilised for statistical comparison between samples, and graphical representations of shape can be constructed for the purpose of comparison. After being converted into digital format, the coordinates of these landmarks are utilised as data points for the purpose of assessing their shape and size. The efficacy of landmark-based morphometrics is in its capacity to record variations objectively and accurately in shape. Through the examination of spatial interactions among landmarks, scholars can explore the variations in physical characteristics among creatures. Significantly, this methodology additionally enables the differentiation between shape and size, rendering it particularly advantageous in cases when size is not the central objective of the investigation (**Bookstein, 1991**).

The utilisation of landmark-based morphometrics includes a diverse array of disciplines. In the past, morphometric procedures primarily relied on analysing sets of distances or angles. However, advancements in theory, computation, and other areas have led to a shift in focus towards utilising Cartesian coordinates of anatomical points to define the more conventional measurements. The investigation of landmark coordinates necessitates specialised methodologies, although it produces robust, succinct, and all-encompassing evaluations. In the field of evolutionary biology, the study of form divergence contributes to our comprehension of how species undergo differentiation (**Chitwood & Otoni, 2017**). Anthropologists employ this method to examine craniofacial characteristics within different populations. Palaeontologists utilise this method to examine fossilised remains and reconstruct the evolutionary lineage of species that are no longer extant. In essence, landmark-based morphometrics offers a reliable and adaptable framework for quantifying

and assessing shape variations in biological entities. The objectivity and precision of this technology render it an essential instrument in numerous scientific fields (**Bookstein, 2021**).

1.1.2.4. Outline analysis

Outline methods were among the initial techniques employed in geometric morphometrics. The boundaries of a structure or region may be same in multiple specimens, but the points utilised to sample these curves may not be the same. Digitising points along an outline and fitting them with a mathematical function, usually Fourier analysis, is the standard procedure in outline analysis. In multivariate analysis, function coefficients are used as form variables to compare curves. The points inside the multivariate parameter space, namely the Fourier coefficient space, have the capability to undergo a transformation that allows them to be represented in the physical space of the organism. Consequently, these changed points can be visually depicted as outlines. The early methods fit lengths of equally spaced radii from a central point, which can be a prominent feature or the entity's centroid. The previous methodology had drawbacks; thus, alternatives were recommended. These included using variations in tangent angles at each outline point, analysing Ax and Ay values in relation to curve distance, or treating outline coordinates as complex numbers (**Rohlf, 1990b**). These procedures were effective, however the statistical analyses yielded different results, which was a major issue. While all these methods "worked", the problem was that statistical analyses based on the different methods gave different statistical results and there was no agreed upon theory that would enable a researcher to select the best approach (**Rohlf, 1986**).

1.1.2.5. Elliptic Fourier Analysis (EFA)

A mathematical technique called elliptic Fourier analysis (EFA) is used to depict a shape's contour as a collection of harmonically connected ellipses. It is especially helpful for analysing closed contours, such as the outlines of biological organisms, objects, or patterns, because it effectively captures shape information. The shape is broken down by EFA into a collection of Fourier harmonics, each of which describes a different feature of the geometry of the object. The characteristics of these harmonics include amplitude, frequency, and phase. Together, they provide a mathematical representation of the shape that is close to its original (**Kuhl & Giardina, 1982**). Researchers can capture both the general shape and specific details by considering many harmonics, enabling a thorough description of complex shapes. A wide variety of applications can benefit from

EFA's usefulness. It can be used in botany to examine leaf forms and record changes in leaf outlines among various plant species. In ecology, EFA aids in the study of organism shapes, illuminating adaptations and ecological interactions (**Neto et al., 2006**).

1.1.2.6. Principal Component Analysis (PCA)

The primary objective of statistical analysis is to calculate a concise set of values or variables that effectively encapsulates a larger dataset. In the field of morphometrics, the typical procedure involves the estimation of average forms and the covariance pattern of the sample in relation to that average. Typically, such estimations do not serve as standalone outcomes, but rather serve as a first stage in the analysis of more intricate inquiries. One statistical approach that is particularly noteworthy is principal components analysis (**Abdi & Williams, 2010**).

PCA can be regarded as an estimator of a parametric structure characteristic of the population when using a single homogenous sample. PCA is a dimension-reduction method that aims to identify orthogonal linear combinations of the original variables. These combinations are selected in such a way that they effectively capture the variability present in the samples. The latter application has significant importance in morphometric research due to the substantial amount of form variables that need to be assessed, often surpassing feasible sample sizes. It is a viable approach to tackle this issue, as it involves utilising scores on a limited set of principal components for further analysis. However, it is crucial to consider that these variables are derived based on a criterion that maximises variance and may not necessarily align with the pertinent variation related to the biological question(s) under investigation (**Polly et al., 2013**).

1.1.3. Advancements and Future prospects:

As of the year 2022, the domain of morphometrics research exhibits ongoing growth and development. Recent technological advancements, such as the integration of 3D scanning techniques and image processing software, have facilitated the investigation of plant morphology with unparalleled accuracy. Currently, scholars possess the capability to examine inquiries pertaining to the field of developmental biology, functional morphology, and even the genetic underpinnings of morphological diversity. In addition, the incorporation of molecular data alongside morphometrics has provided novel opportunities for comprehending the genetic foundations of form and size variations in plant species. The utilisation of an interdisciplinary

strategy holds the potential to uncover the specific genes and regulatory pathways that are accountable for the observed variations in morphology.

In summary, the field of morphometrics has made significant progress since its inception, transitioning from a qualitative characterization of shapes to a quantitative and empirically based profession. The utilisation of this approach within the examination of plant families has significantly enhanced our comprehension of plant morphology, evolutionary processes, and adaptive mechanisms. The ongoing advancements in technology will inevitably lead to the significant involvement of morphometrics in deciphering the complex patterns inherent in the natural world's structure. This will facilitate scientific exploration and contribute to the preservation of biodiversity.

1.2. 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"

-   Webster, A. D.

The members of the family Convolvulaceae are called 'Morning Glories' where they occur, their foliage merge 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 depart them when their flowers wither by noon. The lyrical name 'Morning Glory' hence suits them well. They possess bell shaped flowers. Flowers come in high profusion and have exceedingly delightful colours and surprisingly outlined geometrical dimensions. Even a casual observer will find it hard to miss the aesthetic ecstasy that these bindweed flowers exude. Although forest is their natural habitat, most of the taxa prefer hedges and waysides and associated with crops hence it is also known as 'Bind Weed family'. The pleasing shape and the range of colours make morning glory an ideal ornamental plant for tropics and subtropics.

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. In world the family comprises 1600 species that belongs to 55 genera (**Mabberley, 2017**), while in India 24 genera and 162 species are reported (**Wood et al., 2022**). In India the members of family are predominantly distributed in Peninsular India.

The formal classification of the Convolvulaceae family began with Carl Linnaeus in the 18th century. Linnaeus included many Convolvulaceae species in his seminal work "Species Plantarum" (**1753**), where he described several genera that are still recognized today, such as *Ipomoea* and *Convolvulus*. Linnaeus's classification was primarily based on morphological characteristics, such as floral structure and leaf arrangement, which laid the groundwork for subsequent taxonomic studies.

Most classification systems treat family Convolvulaceae in the order Solanales (**Cronquist, 1968; Dahlgren, 1989; Thorne, 1992**). Convolvulaceae are distinct from other families in order Solanales by characters including latex cells and canals, intraxylary phloem, unique seed and pollen morphology. Considering the said characters Takhtajan (**1980**) recognized a separate order Convolvulales. In pre-cladistics period several authors have contributed to the classification of Convolvulaceae including Choisy (**1833**) and Bentham and Hooker (1876). The most influential classification was given by Hallier (**1893**) based on pollen morphology characters which was later adopted by subsequent authors. He divided family into two classes: Echinoconieae, comprises genera with spiny pollen grains and Psiloconiae group consist of genera with smooth pollen grains.

Austin (**1975b**) and Woodson et al. (**1975**) has given most comprehensive classification system circumscribing Cuscutaceae under Convolvulaceae. He has recognized eight tribes in the family *viz* Erycibeae, Cresseae, Convolvuleae, Merremioids, Ipomoeeae, Argyreieae, Dichondreae, Cuscuteae. Later, three families were segregated from the Convolvulaceae; monotypic Humbertiaceae, Dichondraceae to include genus Dichondra and Cuscutaceae because of unique parasitic habit. But now in APG IV (**2016**) Cuscutaceae is merged into Convolvulaceae, so the family is a natural assemblage including above said families.

A first detailed description of family Convolvulaceae is given in the book '*Convolvulaceae Orientalis*' (**Choisy, 1833**). The advent of molecular techniques in the late 20th century revolutionized the taxonomy and systematics of Convolvulaceae. DNA sequencing and phylogenetic analysis allowed researchers to reassess relationships within the family, leading to the reclassification of several genera and species. These molecular studies provided new insights into the evolutionary history and biogeography of Convolvulaceae. A recent study provided a comprehensive overview of the current classification status of the Convolvulaceae family, using nuclear genomic data to resolve phylogenetic uncertainties (**Pisuttimarn et al., 2023; Simões et al., 2022**). Convolvulaceae includes approximately 2000 species, classified into around 60 genera and 12 tribes (**POWO, 2024; Simões et al., 2022; Stefanović et al., 2002**) with significant economic importance due to crops like sweet potato and ornamental plants such as morning glories and bindweeds. The study confirms the monophyly of Convolvulaceae and provides a strongly supported backbone for the family. It addresses unresolved phylogenetic relationships, such as the placement of the parasitic genus Cuscuta. The paper reveals that the traditional tribal classification,

particularly the tribe "Merremieae," is non-monophyletic. The tribe needs revision due to the unexpected placement of some genera. Six informal subfamilies are identified: Convolvuloideae, Dicranostyloideae, Cuscutoideae, Eryciboideae, Cardiochlamyoideae, and Humbertioideae.

1.2.1. General Characters of family Convolvulaceae:

The members are mostly herbs, usually with twining stems, or prostrate or creeping, or even rarely erect arborescent shrubs, very rarely trees, often with milky sap. The rootstocks are sometimes large, otherwise fibrous. The leaves are alternate, simple or palmately or pinnately lobed, entire or toothed to sometimes lobed, usually petiolate and exstipulate, but pseudo-stipules (leaves of axillary shoot) are rarely present; petioles are sometimes with extra-floral nectaries. Inflorescences are dichasial cymes, mostly axillary, sometimes terminal, 1- to many-flowered, or may be solitary, or sometimes paniculate cymes with mostly opposite or subopposite bracts at the base of the cymes or under the solitary flower, sometimes enlarged. Flowers are generally hermaphrodite, hypogynous, actinomorphic, rarely slightly zygomorphic, pentamerous. various in size and colour often showy but mostly wilting quickly. The sepals are usually free, or rarely connate only at the base, imbricate with quincuncial aestivation, equal or unequal, persistent, occasionally accrescent in fruits. Corolla is sympetalous, of various shapes, often funnel-shaped, infundibuliform or campanulate, sometimes rotate, salver-shaped or rarely urceolate; the limb with 5 lobes or teeth or almost entire with plicae and interplicae; in bud petals are mostly induplicate-valvate, contorted plicate or valvate. Stamens are isomerous, alternipetalous, with usually slender, filiform as well as usually unequal filaments inserted on the base of corolla tube (epipetalous). Anthers are mostly linear or oblong, 2-celled, opening longitudinally, sometimes finally twisted, introrse or extrose, having smooth or spinulose pollen grains. Carpels are mostly 2, syncarpous with superior ovary, 2- or 1-celled, sometimes 4-celled by development of accessory partitions, rarely of 3-carpels and 3-celled. Discs are mostly present, annular or cupular, sometimes 5-lobed, occasionally absent. Ovules are mostly 2 or rarely 4 or 6 in each carpel, in axile placentation, sessile, erect, anatropous or apotropous, covering with massive single integument. Styles are filiform, simple or bifid, either free or united or with 2 distinct styles, rarely very short or even absent Stigmas are 2-4 of various shapes, capitate or bilobate, or rarely 3-lobed, linear, ellipsoid, globular or branched, sometimes aplanate, rarely peltate, reniform, conical or funnel-shaped. Fruits are capsules, dehiscing by valves or circumsessile, or irregularly dehiscing, or indehiscent baccate, or nut-like; seeds are 1-4, as many as ovules or fewer, glabrous or

pubescent with the scanty, cartilaginous endosperm. Cotyledons are generally folded, straight or curved, with 2 plicate, or often bifid and greenish, embedded within the endosperm.

Leaf shapes among Convolvulaceae species are spectacularly diverse and this diversity in leaf shape are profound changes due to the patterning during development stages. The topology of the vasculature and outline of the leaf blade are distinct geometric phenomena that require different morphometric approaches to quantify. Members of the family Convolvulaceae were collected from different localities in their respective seasons. However, the collected species were first identified based on other characters mainly flower and form characteristics and available literature. Landmarks and EFDs are ideal methods to analyse the distinct features of leaves contributing to their shape. The use of traditional taxonomy has caused confusion in the identification of various species due to its resemblances in morphology. Reproductive parts of the Convolvulaceae members were often discriminatory in species identification but absent most of the time in different seasons. 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. The members of morning glory family were selected because they possess much variation in leaf shape.

With these hypotheses in mind the following objectives were defined for the present study.

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.

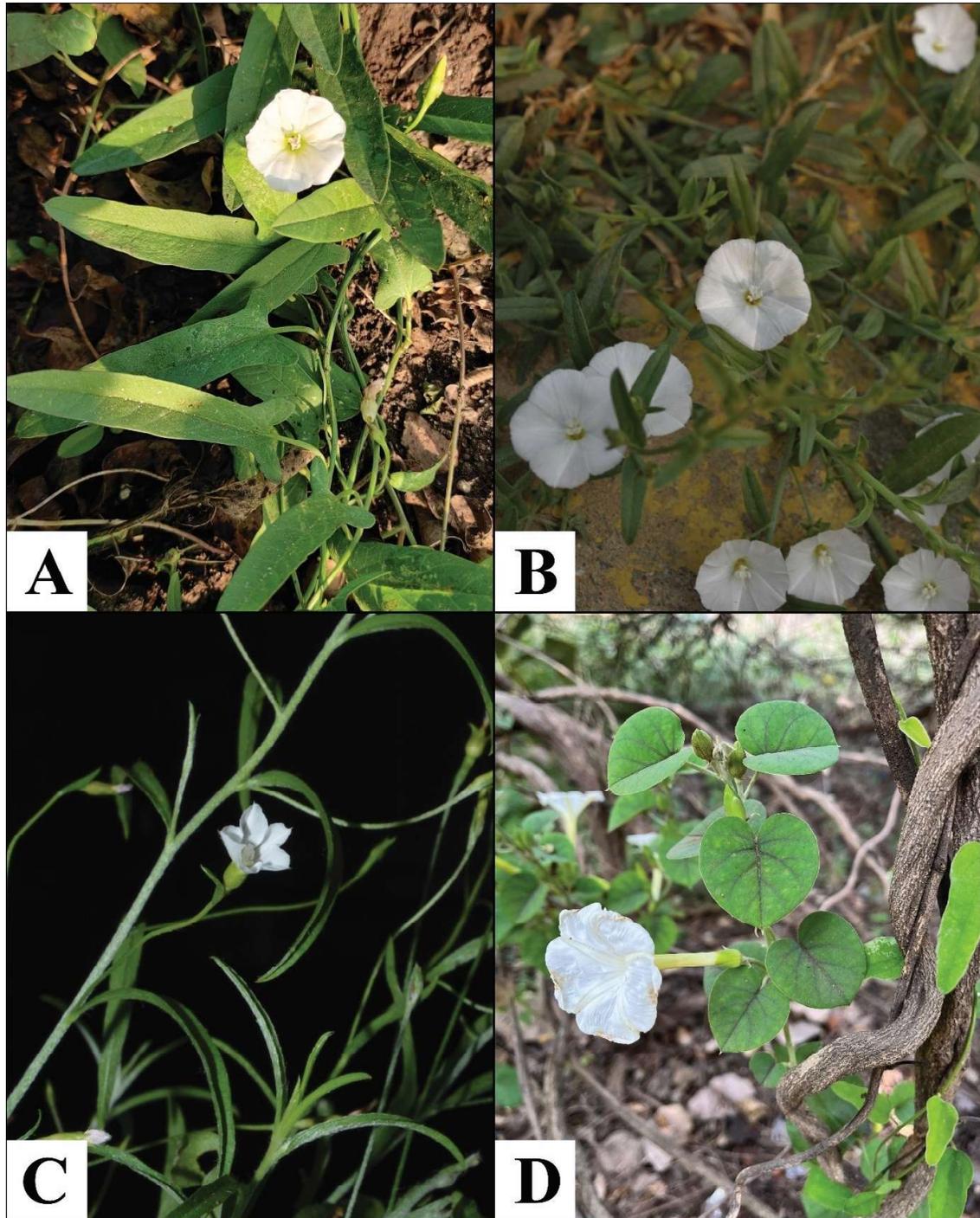4. To envisage and understand developmental aspects if any at population and species level.

Plate 1 - Collected plants of family Convolvulaceae (**A**- *C. arvensis*; **B**- *C. prostratus*; **C**- *C. stocksii*; D- *R. hypocrateriformis*)
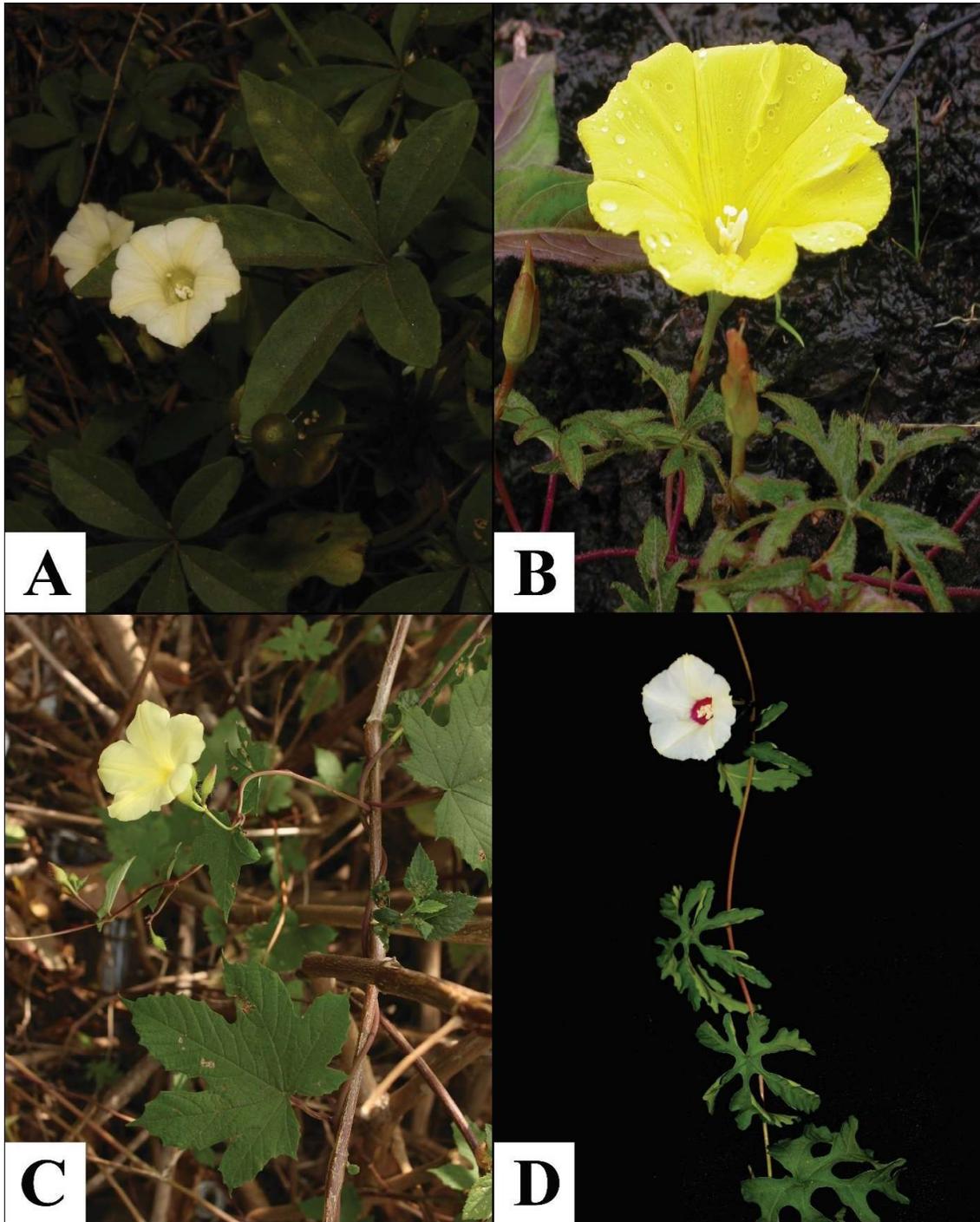
Plate 2 - Collected plants of family Convolvulaceae (**A**- *D. quinquefolia*; **B**- *D. rhyncorhizus*; **C**- *D. vitifolius*; **D**- *D. dissectus*)
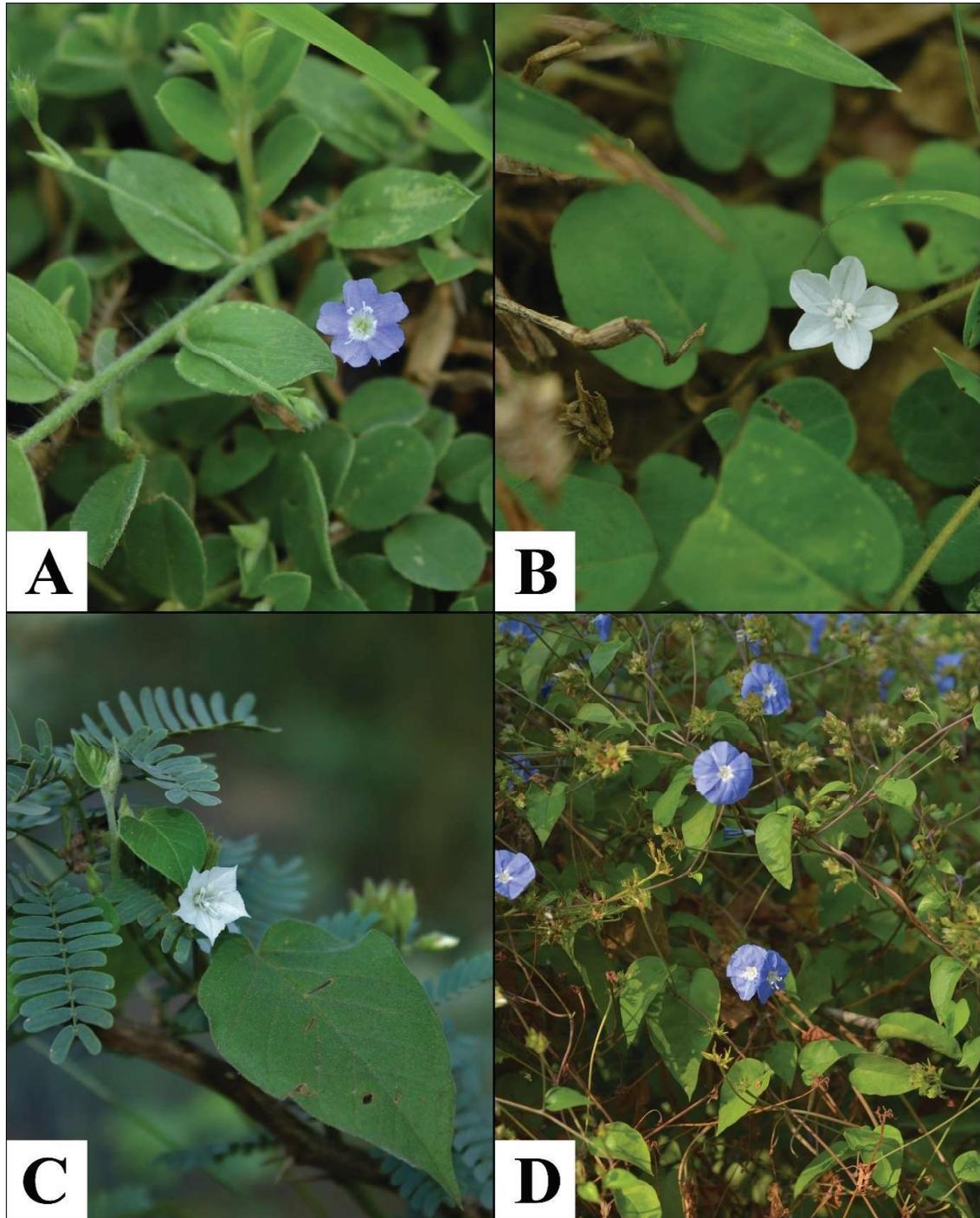
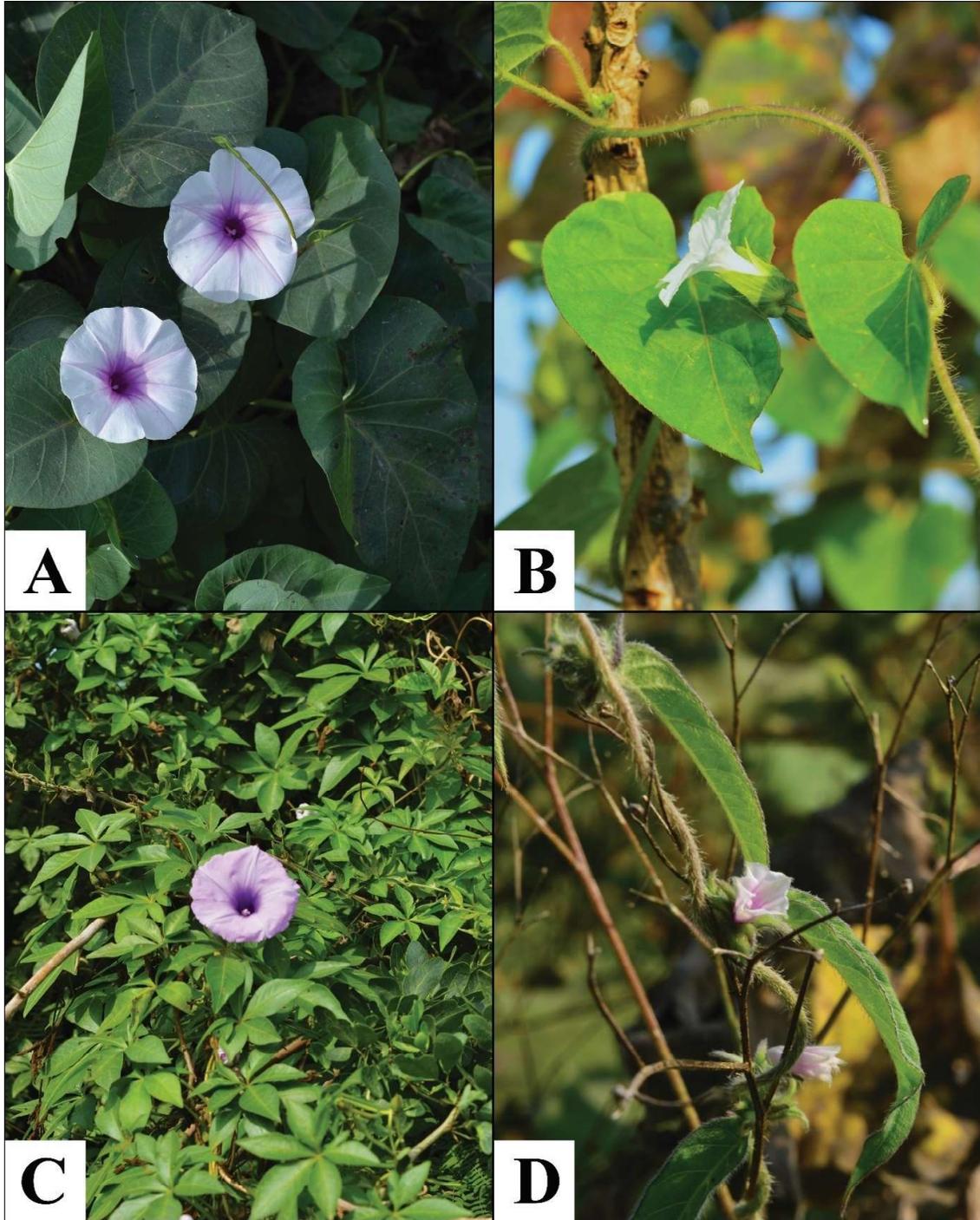Plate 3 - Collected plants of family Convolvulaceae (**A**- *E. alsinoides*; **B**- *E. nummularius*; **C**- *J. peniculata*; **D**- *J. pentanthos*)

Plate 4 - Collected plants of family Convolvulaceae (**A**- *I. aquatica*; **B**- *I. biflora*; **C**- *I. cairica*; **D**- *I. eriocarpa*)
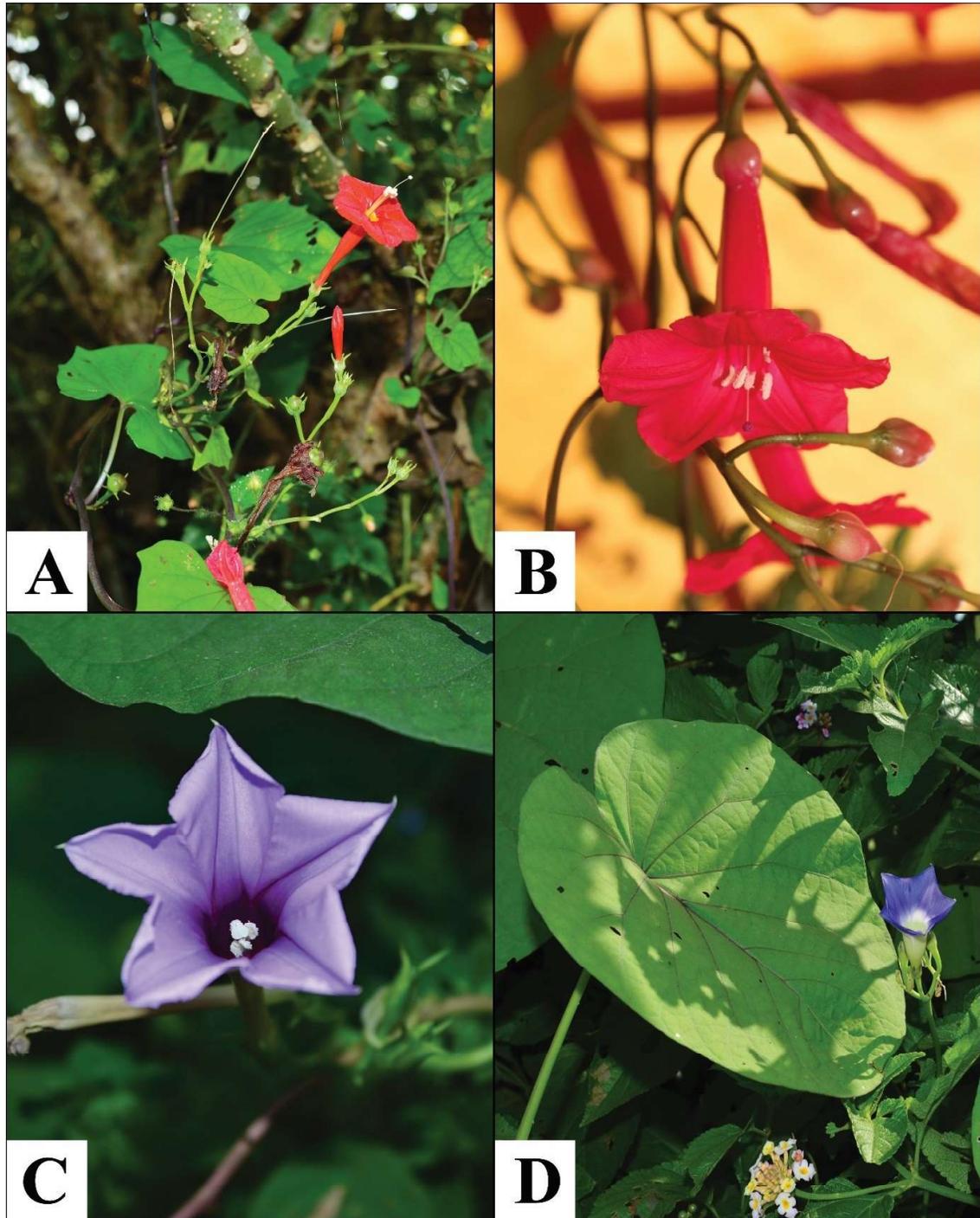
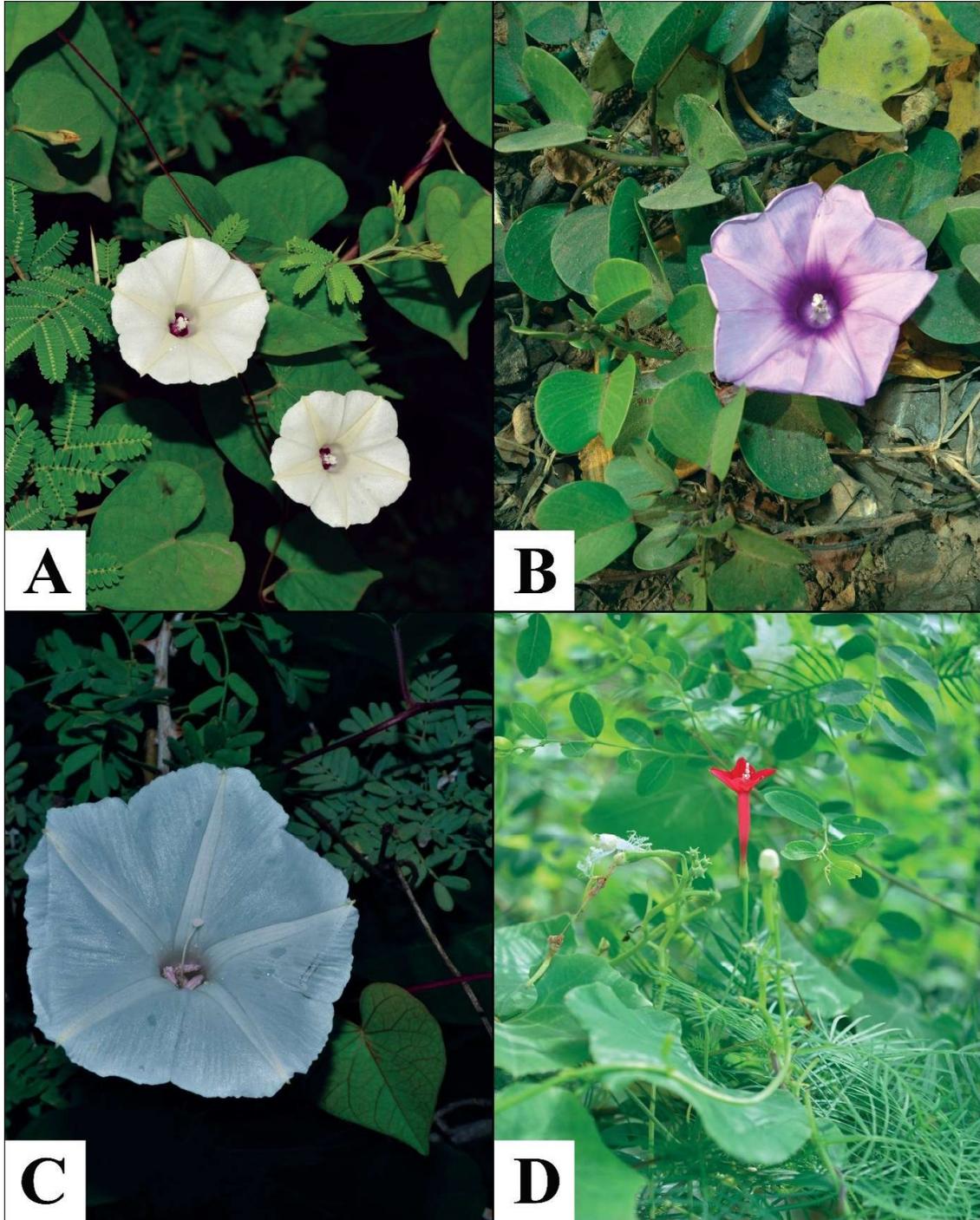Plate 5 - Collected plants of family Convolvulaceae (**A**- *I. hederifolia*; **B**- *I. horsfalliae*; **C**- *I. muricata*; **D**- *I. parasitica*)

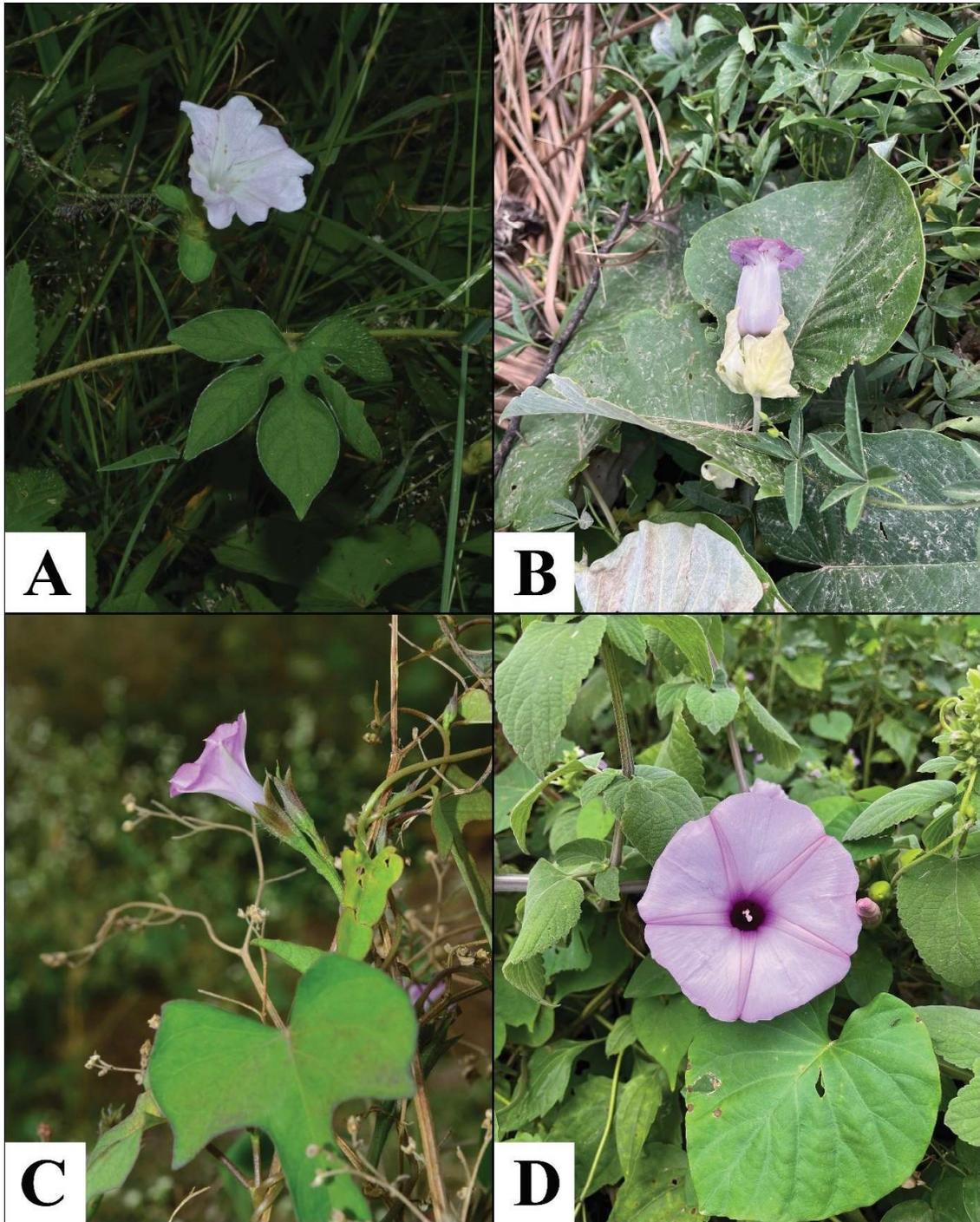Plate 6 - Collected plants of family Convolvulaceae (**A**- *I. obscura*; **B**- *I. pes-caprae*; **C**- *I. marginata*; **D**- *I. quamoclit*)

Plate 7 - Collected plants of family Convolvulaceae (**A**- *I. pes-tigridis*; **B**- *A. nervosa*; **C**- *I. triloba*; **D**- *S. tiliifolia*)

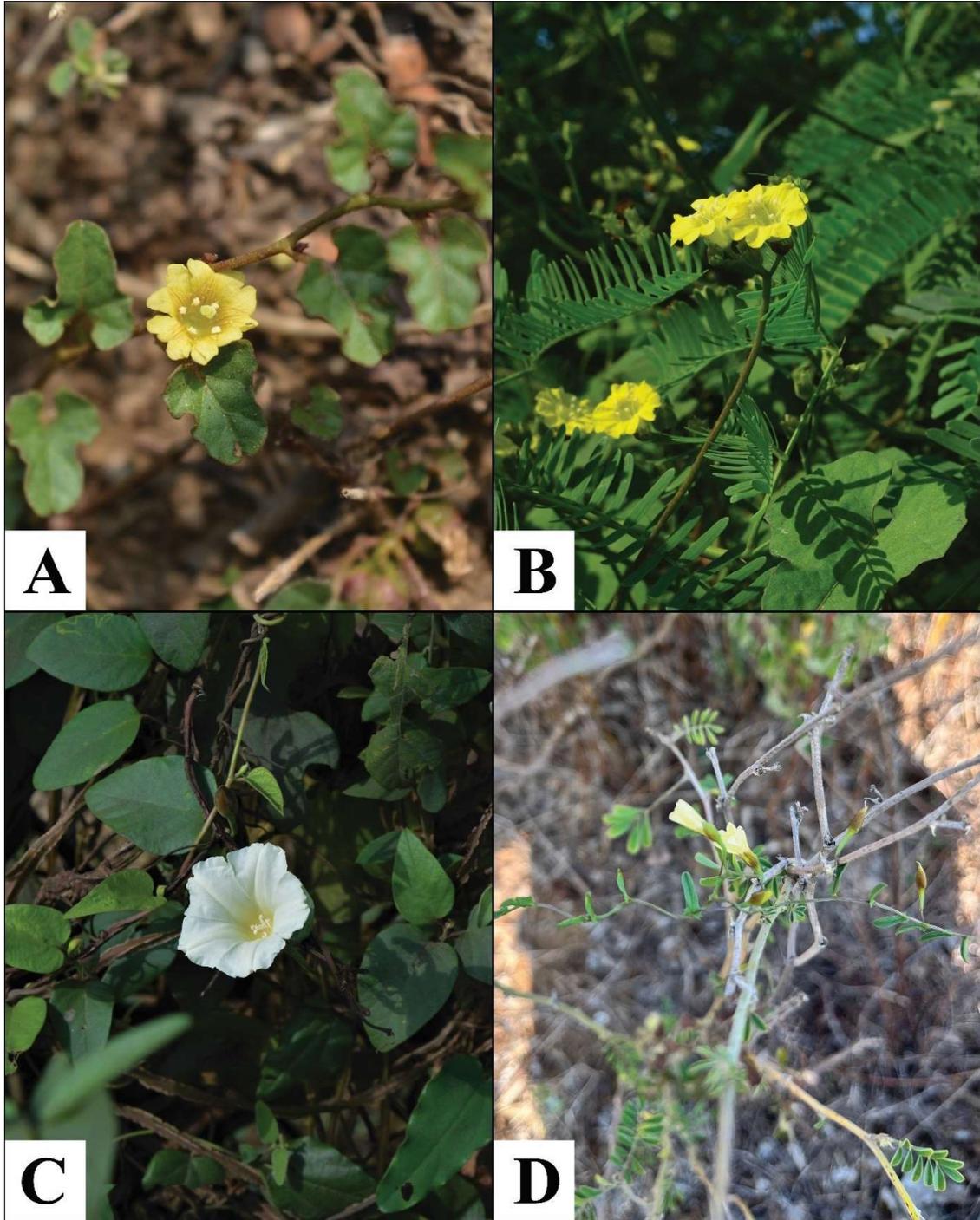Plate 8 - Collected plants of family Convolvulaceae (**A**- *M. emarginata*; **B**- *M. hederacea*; **C**- *O. turpethum*; **D**- *X. tridentata*)