COMPARATIVE MORPHOLOGY, ANATOMY AND MOLECULAR PHYLOGENY OF *AMMANNIA* L., *ROTALA* L. AND *NESAEA* KUNTH (LYTHRACEAE) IN SOUTH INDIA

Thesis submitted to the
University of Calicut in partial fulfillment of the
requirements for the award of the degree of

DOCTOR OF PHILOSOPHY

IN

BOTANY

by

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CERTIFICATE

This is to certify that the thesis entitled **Comparative Morphology, Anatomy and Molecular Phylogeny of *Ammannia* L., *Rotala* L. and *Nesaea* Kunth (Lythraceae) in South India, s**ubmitted to the University of Calicut by Mrs. Lemiya. K. M., in partial fulfillment of the award of the degree of Doctor of Philosophy in Botany is a *bona fide* record of the research work carried out by her under my supervision and guidance. No part of the present work has formed the basis for the award of any other degree or diploma previously.

Calicut University

Dr. A.K. Pradeep
(Supervising Teacher)

DECLARATION

The thesis entitled **Comparative Morphology, Anatomy and Molecular Phylogeny of *Ammannia* L., *Rotala* L. and *Nesaea* Kunth (Lythraceae) in South India,** submitted by me in partial fulfillment of the requirement for the award of the degree of Doctor of Philosophy in Botany of the University of Calicut is an original research work carried out by me in the Department of Botany, University of Calicut. No part of the work formed the basis for the award of any other degree or diploma of any University.

Calicut University **Lemiya K. M.**

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Lemiya K.

Dedicated to My Son
Omar

CONTENTS

ABBREVIATIONS

^{o}C	:	Degree Celsius
µg	:	Microgram
µl	:	Micro litre
µm		Micro meter
μm^2		Micro meter square
µM	:	Micro molar
1U	:	1 unit
APG	:	Angiosperm Phylogeny Group
BLAST	:	Basic Local Alignment Search Tool
bp	:	Base pair
cm	:	Centimeter
CALI	:	Calicut University Herbarium
cpDNA	:	Chloroplast DNA
CTAB	:	Cetyl tri methyl ammonium bromide
CUBG	:	Calicut University Botanical garden
DNA	:	Deoxyribonucleic acid
DNase	:	Deoxyribonuclease
dNTP	:	Deoxynucleotide Phosphate
Na-EDTA	:	Ethylene diamine tetraacetic Acid Disodium Salt
HCl	:	Hydrochloric acid
hrs	:	Hours
ITS	:	Internal transcribed spacer
M	:	Molar
MEGA	:	Molecular Evolutionary Genetics Analysis
mg	:	Milli gram

min.	:	Minutes
ml	:	Milli litre
mM	:	Milli molar
mm	:	Milli meter
MtDNA	:	Mitochondrial DNA
NaCl	:	Sodium chloride
NCBI	:	National Centre for Biotechnology Information
PAUP	:	Phylogenetic Analysis Using Parsimony
PVP	:	Poly vinyl pyrrolidone
*rbc*L	:	Ribulose Bisphosphate Carboxylase Large
RNA	:	Ribonucleic acid
RNase	:	Ribonuclease
rpm	:	Rotations Per Minute
sec	:	Second
T_A	:	Annealing temperature
UV	:	Ultraviolet
V/cm	:	Volt per centimeter

EQUIPMENTS USED

Item	Brand	Country
-20 ^{0}C Refrigerator	Vestfrost solutions	Denmark
-80 ^{0}C Refrigerator	Haier	India
Autoclave	Rotek	India
Desktop Centrifuge	Eppendorf	Germany
Digital pH Meter	Systronics	India
Double distillation unit	Borosil	India
Electrophoresis unit	GE Healthcare Bio-Sciences KK	Japan
Fume hood	Kemi	India
Gel Documentation system	Cell Biosciences/ ProteinSimple	USA
Hot air oven	Kemi	India
Magnetic stirrer	IKA	India
Nanodrop spectrophotometer	Thermo Fischer Scientific	USA
PCR Machine	Eppendorf	Germany
Refrigerated Centrifuge	Sigma	Germany
Refrigerator	LG	India
UV transilluminator	Crescent	New York
Water bath incubator shaker	Kemi	India
Weighing balance	Sartorius	Germany

Introduction

The family Lythraceae J. St. Hil. is relatively large comprising about 31 genera and 600 species distributed in all major continents except Antartica. The limits of the family and relationships among 31 genera is relatively little known inspite of earlier phylogenetic studies. Lythraceae as traditionally circumscribed (Koehne, 1903), comprises 28 genera and is easily recognized by a combination of characters: simple opposite entire leaves, a persistant, perigynous, campanulate to tubular calyx tube with crinkled petals inserted at the rim, stamens inserted deep in the tube and many seeded capsular fruit. Eight genera of this family are herbaceous, the remaining are either woody shrubs or small trees. The genera are clearly delimited and are regarded as monophyletic with the exception of the following three pairs: *Ammannia-Nesaea*, *Lythrum-Peplis*, and *Ginoria-Haitia* (Graham *et al*., 2005). 17 genera (61% of the family) are mono typic or ditypic. A few genera such as *Lagerstroemia*, the cultivated crepe myrtle; *Lawsonia,* the source of henna dye; and *Cuphea*, the popular garden plant are of economic importance.

The geographical distribution of the family is divided between the Old World (18 genera) and the New World (13 genera) with greatest concentration of genera in tropical America and Africa and poor representation in the northern latitudes (Graham *et al.,* 2005). Cross continental sister relationships have been hypothesized on morphological grounds for a few genera (Koehne, 1886; Graham, 2002). The family Lythraceae, are currently placed under the order Myrtales of Malvids of Eudicot clade in APG III system. It was monographed by Koehne (1881, 1903) who considered families such as Duabangaceae, Punicaceae and Sonneratiaceae characterized by semi inferior or inferior ovary outside the limits of perigynous flowered Lythraceae. However the current consensus is to include Punicaceae, Sonneratiaceae and Trapaceae under Lythraceae. Koehne divided the family into two tribes, tribe

Lythreae and tribe Nesaeeae and each with two subtribes. This classification is highly artificial as the tribes and subtribes are delimited mainly based on a few non exclusive characters. Tobe *et al.* (1998) held defining tribes on the basis of presence or absence of complete septal walls in the ovary as erroneous. Studies on palynology (Lee, 1979) and anatomy (Baas & Zweypfenning, 1979) did not support Koehne's (1903) division. A morphological cladistic analysis of Lythraceae by Graham *et al*. (1993a) did not recover clades equivalent to tribal or subtribal groupings or produce well-supported alternative relationships (Graham *et al.,* 2005).

It was Clarke (1879) who gave an account on Indian Lythraceae in India in Hooker's *Flora of British India.* It deals with 250 species, under two tribes, *viz.,* Ammannieae and Lythreae. The first phylogenetic treatment of the family based on modern cladistic methods was provided by Graham *et al*. (1993a), in which they divided the family Lythraceae into two major clades, on the basis of analyses of 26 characters such as anatomy, floral morphology, pollen, and seed morphology. Detailed phylogenetic studies based on molecular evidences (Huang & Shi, 2002) from the region of chloroplast and internal transcribed spacer region of nucleus, combined genera of different monophyletic groups from tribes and the subtribes that were proposed by Koehne (1903). Paraphyly in subfamily Lythroideae was observed by Graham *et al*. (1993*a*).

Fossil and molecular evidences interpret the early development of Lythraceae, which is succeeded by rapid expansion, extensive diversification and widespread distribution (Graham *et al*., 2005). The extreme variation in habit found within the family combined with a fairly generalized floral morphology has made determining the relationships among these genera very difficult.

Ammannia L., *Nesaea* Kunth and *Rotala* L. are three amphibious or aquatic herbaceous genera of this family which are distributed in subtropical to tropical Africa, Asia and Australia. The taxonomic limits of three closely related genera are poorly understood as they share closely similar habit, floral morphology and seed structure (Graham *et al.,* 2011). Inadequate knowledge of characters that are considered to be diagnostic of three genera have led to various generic and infrageneric delineations and a multiplicity of species transfers among *Ammannia*, *Nesaea* and *Rotala.*

Ammannia L.

The genus *Ammannia*, is named by Linnaeus in honour of Paul Amman, Professor of Botany, Leipzig, Germany (Raus, 1997; Stafleu & Cowan, 1976). It consists of *c.* 30 species distributed in both temperate and tropical zone. It is mostly reported from Africa with a maximum of seven species from each continent. The members of this genus are usually annual glabrous herbs with opposite, sessile, entire leaf and small flowers that are arranged in axillary subsessile clusters or small cymes (Koehne, 1903). It is traditionally distinguished from the members of the genus *Nesaea* by the nature of the placenta and the septa of the ovary. Some species of *Ammannia* are well-defined while many others are distinguished from one another by seemingly minor qualitative differences that are difficult to recognize in practice. In some cases, species limits are based more on geographical disjunctions than on morphological differences. Relationships of morphologically similar species occurring in different continents are still unknown. The genus still pose numerous unresolved taxonomic problems. Many nomenclatural transfers of species have occurred among genera in the absence of universally accepted generic limits. Many species of this genus are being used in traditional medicinal system. *Ammannia baccifera* was found to have hypothermic, hypertensive, antiurolithiasis, antibacterial,

seminal weakness, fever, flatulence and CNS depressant activities (Tripathy *et al.*, 2010). *Ammannia multiflora* and *A. auriculata* were reported to possess antimalarial and anti oxidative properties respectively (Upadhyay, 2012; Nawwar *et al.,* 2014).

***Rotala* L.**

The genus *Rotala* comprises more than 55 species of aquatic and amphibious plants distributed in tropical and subtropical regions of the world. Generally these show greater adaptability and vegetative plasticity than any other herbaceous genera of this family. In Africa and Asia in the genus is represented by equal number of species, while species of Southern Asia display greater morphological diversity than the African taxa (Graham *et al.,* 2011). The genus *Rotala* was closely allied with genus *Ammannia* in having remarkable degree of similarity in habit. Clarke (1879) in his account of Indian species of this group considered *Ammannia* as a larger, more inclusive taxon including *Rotala* as a subgenus, in it. Currently *Rotala* is treated as distinct genus based mainly on the dehiscence of capsules and structure of pericarp. Cook (1979) in his monograph of this genus recognized 44 species all over the world which includes 20 Indian species. He considered, southern Asia to be the probable centre of origin of the genus. Joseph and Sivarajan (1989) revised the genus for Peninsular India and revised 16 species. Until now, 29 species are known from India which includes nine new species, described from Peninsular India (Prasad *et al*., 2012; Sunil *et al*., 2013; Gaikwad *et al*., 2013; Prasad & Raveendran, 2013a; Prasad & Raveendran 2013b; Yadav *et al.,* 2010; Anto *et al*., 2014; Ratheesh Narayanan *et al.,* 2014; Lemiya & Pradeep, 2015). Among them, seven species are endemic to Kerala.

Not many, still some of the species were reported for their medicinal properties. *Rotala rotundifolia* is known for its antipyretic, detoxication, anti

swelling and diuresis properties, and is also useful in the treatment of cirrhosis ascetic fluids, gonorrhea, menstrual cramps and piles in the south of China (Han *et al.,* 2004). Leaves and flowers of *Rotala indica* are used for respiratory diseases and stomach disorder (Kumar & Narain, 2010). A survey on ethno medicinal wetland plants reports the use of *Rotala rotundifolia* to cure cough, cold and fever (Panda & Misra, 2011).

Nesaea Kunth

The genus *Nesaea* comprises *c.* 50 species most of which distributed in Africa and Madagascar with a few species in tropical Asia, Australia, North and Central America. But in India only two species were reported so far. Koehne (1882, 1903) who described a number of new species and transferred many species from the genus *Ammannia* to *Nesaea*. Traditionally, they have been separated from *Ammannia* by a questionable difference in the mode of capsule dehiscence which is very difficult to determine and to differentiate these two genera. Recent studies based on morphological and molecular evidences resulted in the accomplishment of new combinations and nomenclatural changes for *Nesaea* (Graham & Gandhi, 2013).

Area of study

Species of *Ammannia, Rotala* and *Nesaea* from south Indian states were collected for morphological, anatomical and molecular studies. South Indian region encompasses 5 states *viz.,* Kerala, Tamil Nadu, Karnataka, Andhra Pradesh and Telungana occupying 19.31% of India's area. There is a wide diversity of plants and animals in South India, resulting from its varied climates and geography. The main land of South India extends between latitudes 8°4′N and 19°6′N and longitudes 74°37′E and 81°7′E. The geography of the region is diverse with two mountain ranges- the Western and Eastern Ghats, bordering the plateau heartland and the sloping coastal strips.

The region has a tropical climate with a minimum mean temperature of 18°C and depends on monsoons for rainfall. The most humid tropical monsoon climate is characterized by moderate to high year-round temperatures and seasonal heavy rainfall above 2,000 mm (79 in) per year. The important mountain systems in South India are the Western Ghats and Eastern Ghats. Western Ghats forms a boundary at western edge of Deccan Plateau and separate it from coastal strip along the Arabian Sea and it passes from Gujarat through three South Indian states *viz.,* Karnataka, Kerala, and Tamil Nadu to tip of the Peninsular India. The Eastern Ghats are a discontinuous range of mountains along India's eastern coast and run through Tamil Nadu and some part of Karnataka. Generally Western Ghat intercept the rain bearing monsoon winds which causes heavy rain fall (south-west monsoon) from June to September and form an important watershed for South India. Tamil Nadu and southeast Andhra Pradesh receive rains from the northeast monsoon from about November to February. As a result, large number of wetlands such as lakes, reservoirs, ponds, puddles, lateritic bogs, wet grasslands, marshes are present, harboring variety of wetland plants. And these areas, which are occupied by aquatic and shore vegetation establishd a strong association between aquatic and terrestrial ecosystems. Western Ghats is one of the world's ten "Hottest biodiversity hotspots" and inhabits over 7,402 species of flowering plants (Nayar *et al.,* 2014). Recently some new species of *Rotala* were reported from various lateritic hills of Western Ghat mainly from the state of Karnataka. Regarding the percentage of area under aquatic vegetation within the South Indian states, Tamil Nadu has the highest percentage (59%) and Kerala has lowest percentage (10%) of wetland area under aquatic vegetation (Bassi *et al.*, 2014). Irrigated and rain fed low lands in South India constitutes around 7.44 million ha, among this area Andhra Pradesh posses largest low land area (Krishna, 2010).

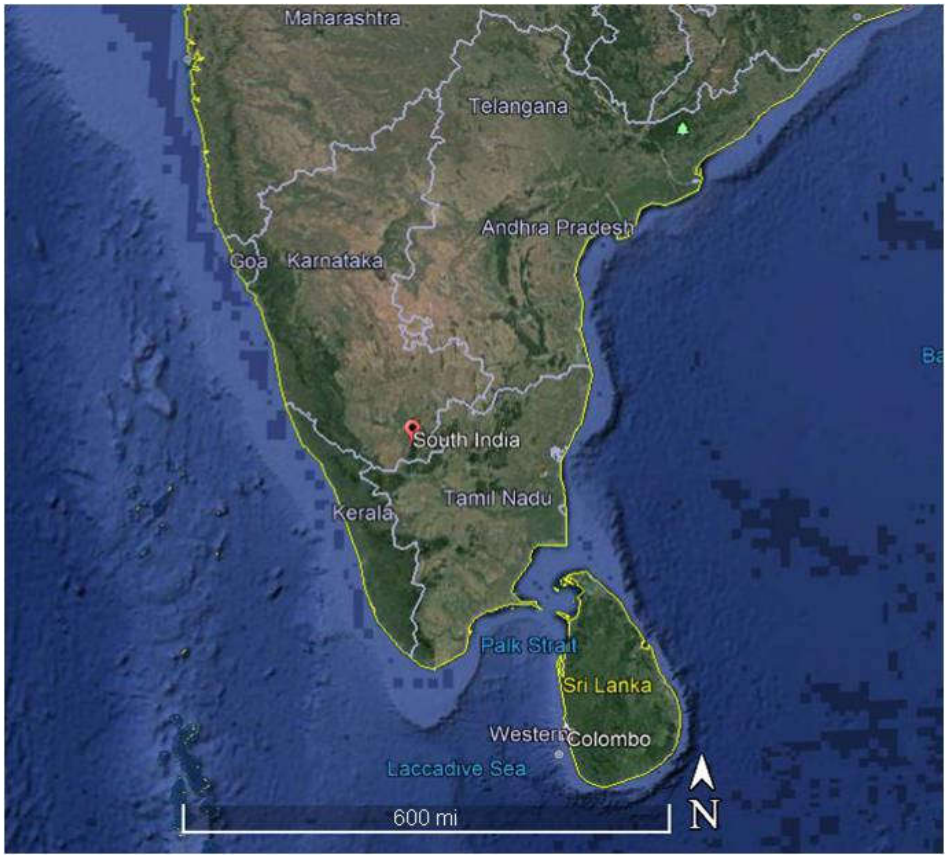

Figure 1. Map of South India showing the study area.

Delineation of the problems

The herbaceous genera *Ammannia, Rotala* and *Nesaea* shares closely similar habit, floral morphology, seed structure and highly plastic floral merosity. Historically, these three genera *viz.*, *Ammannia, Rotala, Nesaea* have long been confused due to their close similarity. Koehne was the first person to delimit these three genera by merging 13 generinc names to *Rotala*, 6 generic names to *Ammannia* and 2 others with *Nesaea* (Koehne, 1903). He considered *Nesaea* as the 'primaeval' genus central to all subsequent evolution in the family, while *Ammannia* and *Rotala* as independently diverged genera directly from *Nesaea* (Koehne, 1886). Later Blatter and Hallberg (1918) and Gamble (1915) who recognized three genera in Indian region followed Koehne. The distinction between three

genera was mainly based on nature of dehiscence of capsule. But in1954, Keay grouped *Ammannia* and *Nesaea* together in one tribe until which the former was grouped with *Rotala*. The grouping by Keay was based on flowers in cymes or umbels as opposed to *Rotala* having flowers solitary or sub-solitary, sessile or sub sessile in leaf axils and fruit with septicidal capsule. As the separation of the three genera on the basis of complete and incomplete septa in ovary recognized as very difficult, Graham *et al.,* (2011) proposed a key based on several other characters such as shape of upper and lower leaves, arrangement of flowers in the leaf axil and nature of capsule wall along with capsule dehiscence in order to distinguish *Ammannia* from *Rotala*. Later on many authors (De Wilde & Duyfjs, 2014; Graham & Graham, 2014) added more characters and proposed keys based on these characters. Still the sturdy character that most of them emphasized in order to distinguish *Ammannia* from *Rotala* was nature of capsule dehiscence and presence of transverse striations on pericarp. And there were many nomenclature changes particularly from *Ammannia* to *Rotala* by various authors from 1960's. Cook (1974) treated the African genus *Hionanhera* congeneric with *Ammannia* and proposed two alternate, dichotomous keys to distinguish three genera *Ammannia, Rotala* and *Nesaea*. A multi-diamensional study or biosystematic study was carried out by Panigrahi which included gross morphology, anatomy of stem and leaves, vascularisation of inflorescence, palynology and seed biology of these genera along with *Hionanthera* in order to understand the interrelationship and evolutionary tendencies between them (Panigrahi & Panigrahi, 1977). In accordance with his study he grouped out *Rotala-Hionanthera* from *Ammannia-Nesaea*.

Recent morphological and molecular studies in some African species of *Ammannia*, *Nesaea, Rotala* and *Hionanthera* best supports the formation of monophyletic clade of *Ammannia* and *Nesaea* and thus can be included in

single genus (Graham *et al.,* 2011). Based on these studies a number of new combinations and other nomenclatural changes were accomplished which resulted in the transfer of many species of *Nesaea* to *Ammannia* (Graham & Gandhi, 2013). Still now, not much studies were carried out on South Indian endemic species of *Ammannia* L.*, Nesaea* Kunth and *Rotala* L. whereas South Asia is considered as centre of origin of genus *Rotala* (Panigrahi, 1979). The last one decade witnessed the publication of several new taxa in the genus *Rotala.* Some of recently described species were found to be so cryptic with the previously described species. Thus in the present scenario, it has become necessary to document different taxa of the three genera in South India and to find out relationship among them based on molecular diversity studies along with comparative morphological and anatomical studies. The present studies on South Indian taxa representing the above three genera focus on evolutionary and phylogenetic relationship within the genus and possible links to the African lineage. The present investigation also to elucidate the taxonomic limits of South Indian species of three closely similar genera, *Ammannia, Nesaea* and *Rotala*.

Objectives of the study

- Exploration, collection and documentation of species diversity and preparation of herbarium specimens for future reference.
- The establishment and maintenance of germplasm in the University Botanical Garden.
- Morphological and anatomical characterization of South Indian taxa of *Ammannia*, *Rotala*, and *Nesaea*.
- To elucidate taxonomic limits of the three closely similar genera of Lythraceae- *Ammannia*, *Rotala* and *Nesaea* in South India based on gross morphology, anatomy and seed morphology.

- Molecular phylogenetic studies of three genera with a far better representation of this lineage using chloroplast and nuclear DNA regions.
- Evaluation and assessment of possible links between the two disjunctive centers of diversity, north eastern Africa and the Indian subcontinent.

Review of Literature

Generic or species delimitation, a process by which boundaries are determined and new species or genera are discovered, is of central importance in modern systematics. In fact different biological disciplines are being used to delimit species as an operational taxonomic unit that represents the fundamental entity of study (Peterson & Navarro-Siguenza, 1999). Species recognition and delimitation has long been based on morphological characters that are highly polymorphic in nature (Duminil & Michele, 2009). But in many cases, different individuals of the same species may exhibit variation in their morphology either naturally or in response to local conditions. This intra-species variation could be at the origin of incipient species (Pratt & Clark, 2001). Alternatively, the taxonomic group may contain cryptic species in which individual with identical morphology categorized as separate taxonomic entities (Shaw, 2000; Chan *et al.,* 2002; Whittall *et al*., 2004). Another drawback of morphological characters for the delimitation is based on their accessibility. Indeed it is often difficult to have access to the vegetative part of adult woody individuals and also in a situation, when the diagnostic morphological characters are reproductive traits that are absent during most of the year (Duminil *et al.*, 2006). By the establishment of the International Organization of Plant Biosystematics (IOPB), the relationship between orthodox and experimental taxonomy (Biosystematics) have become much clear. Presently the classical taxonomy is transformed to a multidisciplinary approach, to aid the species delimitation truthful. Recent trends in systematic studies are entirely based on combination of different disciplines such as external morphology, foliar anatomy, leaf architecture, palynology, seed morphology, cytogenetics, ecology, phytochemistry, taximetrics, and molecular methods. This multidisciplinary approach towards

species delimitation was recently acclaimed as “integrative taxonomy” (Padial *et al*., 2010; Schlick-steiner *et al*., 2010; Rajaei, 2015).

The family, Lythraceae has received considerable scientific attention both taxonomic and economic prospective since long. It comprises 31 genera and more than 600 species (Huang & Shi, 2002) with a worldwide distribution especially in tropical region. The family has been traditionally placed in the order of Myrtales and closely allied with Onagraceae based on morphological, anatomical and embryological evidences (Dahlgren & Thorne, 1984). Members of Lythraceae show extreme variation in habit, ranging from tall trees and woody shrubs to small aquatic herbs. The Lythraceae are found today on all major continents except Antarctica, raising biogeographic questions about place and time of origin of the family and paths of radiation and generic diversification (Graham *et al*., 2005).

The relationships and taxonomic limits of three closely similar herbaceous genera of Lythraceae- *Ammannia* L., *Rotala* L. and *Nesaea* Kunth have long been poorly understood. All the three genera inhabit similar lowland habitats such as flooded paddy fields, shallow ponds and other seasonally wet areas. Their fairly generalized floral morphology makes generic and species delimitation, based on traditional morphological characters problematic. Further, the relationship of morphologically similar species occurring in different continents is still unknown and poses numerous unresolved taxonomic problems. Amongst all the herbaceous genera of Lythraceae, the genus *Rotala* shows considerable amount of phenotypic plasticity (Joseph & Sivarajan, 1989) and greater generic adaptability which is evidenced by increasing rate of speciation especially in Peninsular India. Nine new species were described from this region in the last decade. *Rotala* may be supposed to be neoendemic as they occur in the areas that are prone to climatic and environmental stresses, and Peninsular India can be considered

as one of the centre of active speciation of the genus (Rijuraj *et al*., 2017). Some of the newly emerged species are still perplexing which leads to the requirement of a multidisciplinary approach to delimit species. The details of recently described new species are given in the Table 1.

Table 1. Recently described new species from different regions of South India from 2010 onwards.

Sl. No	Taxa	Type locality	Year of Report
1	***Rotala belgaumensis*** S. R. Yadav, Malpure & Chandore	Belgaum, Karnataka	2010
2	***Rotala tulunadensis*** K. S. Prasad, P. Biju, C. Ravi & K.G. Bhat	Permude, Kasargode (Dist.), Kerala.	2012
3	***Rotala khaleeliana*** Sunil, M.K.R.Narayanan & Nandakumar	Kanayikanam, Kannur (Dist.), Kerala.	2013
4	***Rotala meenkulamensis*** *K. S. Prasad & K. Ravi*	Meenkualam, Kannur (Dist.), Kerala.	2013
5	***Rotala kasaragodensis*** K.S.Prasad & Raveendran	Mugu, Kasargode (Dist.), Kerala.	2013
6	***Rotala sahyadrica*** S.P.Gaikwad, Sardesai & S.R.Yadav	Satara, Maharashtra	2013
7	***Rotala dhaneshiana*** Sunil, Ratheesh & Sivadasan	Muthanga , Wayanad (Dist.), Kerala.	2014
8	***Rotala cheruchakkiensis*** Anto, Devikrishna, Pulickal, C.D.Vargheseet I. Antony	Mangad, Thrissur (Dist.), Kerala.	2014
9	***Rotala anamika*** Lemiya	Parapanangadi, Malappuram (Dist.), Kerala.	2015

Historical Survey of Generic Delimitation

The voluminous literature on systematic studies on the delimitation of three genera *viz., Ammannia, Rotala, Nesaea* have been accumulated since the middle of 19th century. The genus *Ammannia* was first established by Linnaeus in 1753, to include three species, *A. latifolia*, *A. ramosior* and *A.*

baccifera. Britton and Brown (1913) designated *A. latifolia* L. as lectotype of the genus. Subsequently, in 1771, Linnaeus described the genus *Rotala* to include a single species *R. verticillaris,* which in turn was based on Rheede's account in *Hortus Malabaricus* (Rheede, 1689). Kunth (1825) described *Nesaea* Kunth based on *Lythrum triflorum* L.f. presumably from Mauritius. Among the genera described in Lythraceae during the period from 1764 to 1866, 22 were found to be either congeneric with *Rotala, Ammannia* or *Nesaea.*

In 1828 Candolle in his *Prodromus Systematis Naturalis* divided Lythraceae into two tribes, Tribe I Salicarieae and Tribe II Lagerstroemieae. He has recognized 22 genera in the first tribe Salicarieae. *Rotala, Ammannia* and *Nesaea* were placed in Salicarieae and occupied first, sixth and fifteenth position respectively in this tribe. Wight and Arnott (1834) in their prodromus *Florae Peninsulae Indiae Orientalis* followed Candolle (1828) and divided Lythraceae (Order LVIII Salicarieae) into two tribes, Tribe 1. Lythreae comprising seven genera and Tribe 2 Lagerstroemiae comprising two genera. The former tribe includes *Rotala, Ammannia* and *Nesaea*. In the genus *Rotala* he included one species, *R. verticillaris* while *Ammannia* is divided into five subgenera *viz.,* Deplostemon (DC.) Wight & Arnott, *Tritheca, Ditheca, Hapalocarpum* and *Mirkooa.* They lecto-typified the subgenus Diplostemone by *Ammannia octandra* which was included earlier under Diplostemoneae by Candolle (1828). Since then, Wight (1840) classified his concept of three genera, based on the number of petals, stamen and cells of ovary. He limited *Ammannia* to most species having 4 petals, 4 stamens and a 2- celled ovary, *Rotala* to those species with 3-5 cleft calyx with equal number of petals and stamens and a 3-valved capsule and *Nesaea* to species with 4-6 cleft calyx with accessory teeth in the sinuses, 4-6 petals and double the number of stamens and 3-4 celled ovary. Within this revision, he merged *Ammannia pentandra* Roxb. in *Rotala* and the genera *Mirkooa* and *Nimmonia* in the

genus *Ameletia* DC. He also included some species of *Ammannia* (especially from the subgenus *Diplostemon*) in *Nesaea.* Bentham and Hooker (1867) included *Rotala* under section I of genus *Ammannia* L. which in turn included in the tribe Ammannieae and *Nesaea* as a genus within the tribe Lythreae of the family Lythraceae.

Subsequently, Clake (1879) subdivided Lythraceae in to two tribes, tribe I, Ammannieae and tribe II Lythreae. The former comprising two genera *Ammannia* and *Hydrolythrum*. The genus *Ammannia* is subdivided in to two subgenera, subg. *Ammania* and subg. *Eu-Ammannia*. *Rotala* is placed under *Ammannia* as a subgenus with ten species while subg. *Eu-Ammannia* comprises 8 species of *Ammannia*. Baillon (1877) treated different species of Lythraceae into three series and treated the genus *Nesaea* in the series named *Salicariees* and the other two genera are *Rotala* and *Ammannia* in the series named *Ammaniees.* But according to Heirn (1871), *Ammannia* was closely allied with *Nesaea* than to *Rotala.*

Koehne was the first, to monograph Lythraceae (1881, 1903) in which he divided Lythraceae into two tribes *viz.*, Lythreae and Neseeae. *Rotala* and *Ammannia* were placed in the former tribe and *Nesaea* in the latter. The genera included in the Neseeae is said to have ovary with septa complete and placenta continuous with the style and for those of Lythreae, septa was said to be as incomplete, ceasing below the apex of placenta which in turn was not attached to the base of the style. He merged 14 other generic names which were described after 1771 as congeneric with *Rotala*, 6 genera proposed after 1753 as congeneric with *Ammannia* L. and two others with *Nesaea.* He treated two subgenera *viz*., *Eu-ammannia* Clarke and *Cryptotheca* (Bl.) Koehne followed by various sections, subsections and series in *Ammannia* and various sections, subsections and series within the genera *Rotala* and *Nesaea.* He also proposed *Nesaea* as the ‘primaeval’ genus central to all

subsequent evolution in the family suggesting that *Ammannia* and *Rotala* independently diverged directly from *Nesaea* (Figure 2). This was illustrated as a type of nearest neighbour diagram in his monograph (Koehne, 1885).

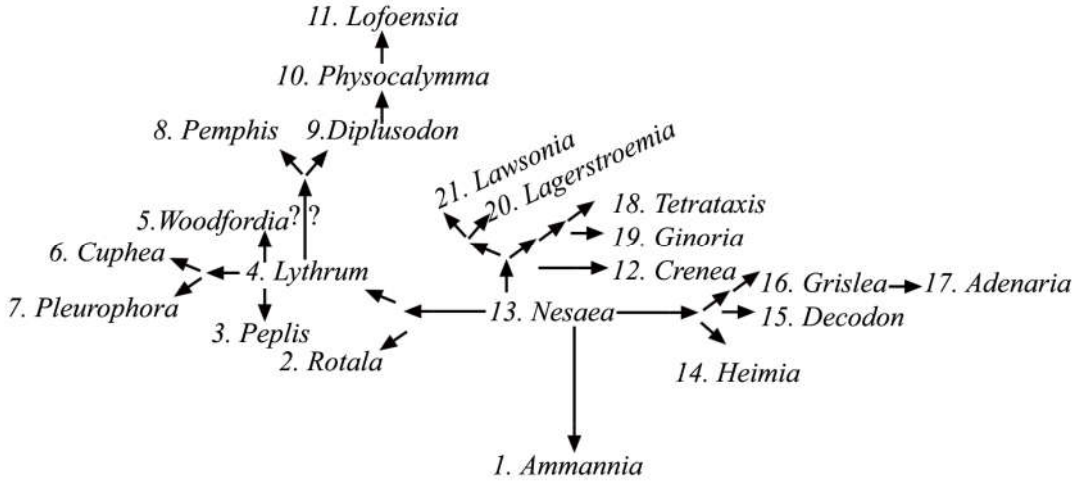

Figure 2. Neighbour diagram showing *Nesaea* as the primaeval geneus (reproduced from the Monograph of Koehne, 1885).

In agreement with Koehne (1903), Gin (1909) also treated the three genera as various sections and series. Blatter and Hallberg in 1918 and Gamble (1915) treated the three genera- *Ammannia* L., *Rotala* L., and *Nesaea* Kunth from India. They distinguished genera based on the nature of dehiscence of capsule. Later, Hutchinson and Dalziel (1927), treated these genera from African region, but he opposed Koehne in considering style as a diagnostic character in distinguishing sections *Eu-stylia* and *Astylia* within the sub genus *Eu-ammannia*. Thus, they distinguished *A. auriculata* Willd., *A. prieureana* Guill *et* Perr., *A. baccifera* L., *A. gracilis* Guill *et* Perr. and *A. senegalensis* L. primarily based on the relative length of style and ovary in their *Flora of West Tropical Africa*.

In agreement with Heirn, Keay (1954) grouped two genera *Ammannia* and *Nesaea* together on basis of presence of flowers in cymes or umbels or crowded into bracteate heads, in contrast to *Rotala* having flowers solitary,

sessile or sub sessile in leaf axils with a septicidal capsule. But, he considered length of style as one of the key characters which is in corroboration with the observations made by Hutchinson and Dalziel (1927). Webb (1968) also used relative length of styles for distinguishing different species in *Ammannia*. Graham (1964) considered the septa in the ovary which was used by Koehne in order to distinguish two tribes of Lythraceae, is highly variable and difficult to ascertain for some specimens. In his studies on different genera of Lythraceae in southeastern United States, he distinguished two genera *Ammannia* and *Rotala* based on a combination of different characters including leaf base, nature of capsule dehiscence, pattern of capsule wall and presence of solitary or many flowers on the leaf axils. He also pointed out the heteromorphism of this family and recognized a few species of *Nesaea* with trimorphic flowers and some species of *Rotala* and *Nesaea* with dimorphic flowers. Cleistogamous flowers have also been reported in *Ammannia* and were thought to be occurred in apetalous *Rotala* and *Nesaea* also (Graham, 1964).

Many authors (Leeuwen, 1974; Cook *et al*., 1974) distinguished the genus *Ammannia* from *Rotala* on the basis of structure of capsule. Leeuwen (1974) along with the nature of capsule dehiscence, also described a discrete anatomical difference in the structure of pericarp. According to him both genera are characterized by two main layers of parenchymatous cells, the inner of which is slightly more lignified than outer one. In *Ammannia* cells of both layers are similar in shape while in *Rotala*, cells of inner layers are almost linear with fine microscopic transverse line. In order to distinguish different species of *Rotala* he considered a number of characters together, *viz*., arrangement of leaves, number of calyx lobes, petals and stamens, presence or absence of calyx appendages between the calyx lobes, presence/absence and colour of bracteoles, insertion of stamens in the calyx tube, presence or absence of style and number of valves of the fruit. Based on the presence of

transverse striations on the surface of capsule, Rajagopal and Ramayya (1969) transferred *Ammannia pygmaea* Kurz to the genus *Rotala*.

Fernandes and Diniz (1955), in their treatment of African Lythraceae, established *Hionanthera* as a genus positioned intermediate between *Ammannia* and *Rotala*. They also described a few new species of *Rotala* and *Nesaea* from Africa. Their illustrations and description suggest that many of the morphological characters are comparable with *Rotala* and *Ammannia*. Cook *et al*. (1974) provided altenative keys to recognize three genera *viz*., *Ammannia, Rotala* and *Nesaea* and merged the genus *Hionanthera* with *Ammannia*. Subsequently Panigrahi (1976), recapitulated the description of 30 species belonging to four genera, based on gross external morphology and their geographical range. He has taken comprehensive approach for the generic delimitation by relating anatomical characteristics of stem and leaves (Panigrahi, 1976), vasculature of inflorescence (Panigrahi & Panigrahi, 1977), palynology (Panigrahi, 1979) and seed surface morphology. A key based on characters derived from these multi-directional evidences were also provided. Panigrahi and Panigrahi (1983), carried out comparative analysis of character states and suggested that rate of evolutionary adaptive advance or adaptive modification of stems, leaves, inflorescences, pollen grains and seeds have not always synchronous either in the four genera (*Ammannia, Rotala, Nesaea* and *Hionanthera*) or within infra-generic categories. Giving importance to the analysis of reproductive structures especially pollen grains as they are more conservative than vegetative body, they brought natural affinity between the four genera as: *Rotala-Hionenthara* vs *Ammannia-Nesaea* in contrast to *Nesaea* vs *Rotala-Hionanthera-Ammannia* based on vegetative anatomy alone. They also concluded that the genus *Rotala* shows a greater genetic adaptability and vegetative plasticity than *Ammannia* and *Nesaea*.

Panigrahi in 1986 studied surface characteristics of dry and germinating seeds of some species of three genera along with genus the *Hionanthera* using SEM. He considered *Ammannia* as more advanced genus than *Rotala* as it shows 'investing' type of seed which are found to be most advanced condition of some genera having higher seed type. Regarding the structure of capsule wall, the genus *Rotala* posses transverse striations on it, where as *Ammannia* and *Nesaea* posses polygonal or isodiametric striations. The mucilaginous hairs protruded from germinating were found to be released from intercellular marginal cracks along the epidermis of outer convex surface only and concave surfaces are free from these protrusions. Many of the characters such as length, shape of apex and base and surface pattern of hairs are found to be diagnostic at the species level.

In 1991, Immeleman, in his synopsis of *Ammannia* and *Nesaea* in South Africa, pointed out the difficulty in considering state of capsule dehiscence as one of the major characteristics to separate two genera. Hence he treated different species of these two genera in a single combined key. He also reported heterostyly in some African species of *Nesaea* and identified two dimorphic and two trimorphic species within this genus. Verdcourt (1994) in his '*Flora of Tropical East Africa'*, recognized *Rotala* and *Hionanthera* but could not satisfactorily distinguish *Ammannia* and *Nesaea*. He also noticed the presence of more or less circumscissile and irregularly bursting capsules in *Ammannia* and *Nesaea,* and hence considered the distinction based on the capsule dehiscence as untenable.

Tobe *et al.* (1998), found the distinction between two tribes (Lythreae and Nesaeeae) which was proposed by Koehne (1881, 1903), based on presence or absence of complete septal wall, to be erroneous and it no longer provide a basis for the separation of *Ammannia, Rotala* and *Nesaea* at tribal

level. Anatomical studies also revealed that incomplete septal walls are present at the apex of the placenta in all genera.

A multidisciplinary approach towards Lythraceae systematics can be seen in the last decade. Significant contributions have been made by Graham *et al*. (2005; 2011) in this regard. This mode of approach not only facilitated extensive collection of evidences from all fields of botany but also helped in solving the problems in taxonomy and to improve the classification and phylogeny. The combination of molecular technique along with other traditional methods is being followed as a means of complete solution to the problem regarding the delimitation and further nomenclature of the constituent genera of the family. The earlier works on various disciplines were critically reviewed and presented as follows.

Morphology

Generally used morphological structures are leaf, stem, roots, flower, fruit and seed. The variations in these morphological structures are fundamental to understand the origin of evolutionary diversification existing among the members of any group. Changes in the related timing of events during development, is the most general explanation for evolutionary shifts in morphology (Gould, 1977; Lord & Hill, 1987; Raff & Kaufman, 1991).

Ammannia, Nesaea and *Rotala* are annual or perennial amphibious herbs of which *Rotala* is more aquatic with floating or submerged vegetative stems and submerged or emergent inflorescences. Stems are usually four angled and glabrous in three genera, sometimes terete mostly in *Rotala* and *Nesaea* (Koehne, 1903; Panigrahi, 1976; Graham *et al*., 2011). All three genera possess aerenchymatous spongy cortex (Schrenk, 1889) similar to several other herbaceous genera of Lythraceae (Lempe *et al*., 2001). The leaves of all three genera are usually simple and the phyllotaxy is usually

decussate but verticillate or alternate type is also present in some species of *Rotala* and *Nesaea* (Koehne, 1903; Graham, 1964; Graham *et al*., 2011). Shape of lamina was reported as linear, oblanceolate or oblong in all three genera and sometimes ovate in a few species of *Rotala*. Leaf bases in these three genera range from attenuate to cordate or auriculate and attenuate to truncate.

Ammannia and *Nesaea* have clustered cymes and rarely solitary axillary flowers on long pedicels. In a very few species of *Nesaea* and in *Rotala*, flowers are racemose or solitary and never in cymes (Cook, 1979; Graham *et al*., 2011). Generally, calyx tubes were described as small in all genera (not longer than 6 mm) and were campanulate, urceolate, turbinate or subglobose. (Panigrahi, 1976; Koehne, 1881; Koehne, 1903). The general structure of flower, size and shape vary considerably in these three genera, often making the distinction of individual genera impossible (Graham *et al.*, 2011). Flowers are predominantly tetramerous for *Ammannia* (Panigrahi, 1976; Graham *et al*., 2005; Graham *et al*. 2011) and *Rotala* infrequently penta- to octamerous in *Ammannia* and tri or penta-merous in *Rotala* (Cook, 1979). In *Nesaea*, flowers are reported to be hexamerous with variation from tetra- to octa-merous. Cleistogamous flowers have been reported in *Ammannia* and are found to occur in the apetalous species of *Rotala* and *Nesaea* (Graham, 1964).

Stamens may originate from the base to middle level in the calyx tube. But some authors used this feature as one of the diagnosing character for separating the genus *Ammannia* and *Nesaea*. With respect to previous studies, heterostyly was recognized in *Rotala* and *Nesaea*, but was unknown in *Ammannia*. Koehne (1903) described six possibly trimorphic species and ten dimorphic species in *Nesaea*. Immelman (1991) verified two trimorphic and two dimorphic species of *Nesaea* in Southern Africa. According to Cook

(1979), *Rotala* has four dimorphic species, the remainder being monomorphic. Pleisomorphic staminanl arrangement in most of the species of the genus *Nesaea* was observed to be diplostemonous, but in a few species it was reported to be haplostemonous (stamens in front of the sepals only) or obdiplostemonous (stamens in front of the petals only). *Ammannia* and *Rotala* were described as haplostemonuos with a few exceptions of hexa- and octamerous flowers of *Ammannia* (Graham *et al*., 2005; Graham *et al.*, 2011).

The very significant morphological features which have major role in distinguishing three genera are capsule wall structure, nature of septa and placenta, type of dehiscence, number of locules. Koehne (1881) included the genus *Nesaea* within tribe Nesaeeae due the presence of ovary with septa complete and placenta continuous with style and *Rotala* and *Ammannia* in the tribe Lythreae by the presence of incomplete septa, ceasing below the apex of placenta which in turn was not attached to the base of the style. Later Tobe *et al.* (1998) recognized these distinguishing characters mistaken since some anatomical studies revealed the presence of incomplete septal walls at the apex of placenta in all genera. And in some species of *Nesaea*, were described as having septa extending fully to the top of the ovary and others not. In addition, the tribe and sub tribe level concept based on ovary or seed morphology was not supported by any molecular evidences (Graham *et al.*, 2005). The continuity of placenta with style was also found to be varied with age, expansion and final shape of placenta (Graham *et al.*, 2011). In some species of these three genera particularly in most species of *Nesaea* the placenta become elongated, ovoid or somewhat compressed with maturity and the connection to the style persist. While in others especially in *Ammannia* placental connection with the apex of ovary observed to be broken with maturity as the placenta enlarges and becomes globose and thus ultimately the placenta appears as free central (Graham *et al.*, 2011). A recent morphological study by Graham and Graham (2014) indicated that a few

species of *Ammannia* have globose ovary with free basal placentation and complete septa extending to near ovary apex whereas the remaining species of *Ammannia* and all species of *Rotala* possess similar type of ovaries but with incomplete septa, ceasing approximately at the distal end of the placental lobes or below.

Earlier literature best supports seed morphology as a valuable taxonomic information along with other distinguishable characters. According to studies of Barthlott and Voit (1979), anticlinal undulations in the seed exine could be considered as one of high taxonomic significance and often characterize difference at the species and genus level. Barthlott (1981) reported that characters of cell boundaries in seed coat can often be used to characterize groups of related species, genera or taxonomic categories up to the family level. Generally seeds of Lythraceae were described as well suited to aquatic dispersal by means of wing- like expansions of the seed coat and/or buoyant spongy tissue on the outer seed coat (Graham, 1964). A comprehensive study on seed surface patterns of three genera was first conducted by Panigrahi in 1986. She examined the surface characteristics of both dry and germinating seed as both character found to be reflect some species specific characters. Striations on the dry seed surface in SEM is found useful to distinguish *Rotala* from *Ammannia* and *Nesaea* as *Rotala* posses transverse striations while in other two genera, polygonal striations are prominant. In accordance with various studies on moistened seeds in several genera, wet seeds found to emerge unique, spirally grooved, inverted hairs through the epidermal layer of the seed coat and slowly uncoil to a length of 2 to 3 millimeters. These hairs are observed as slightly mucilaginous and considered to serve in seed dispersal by attaching the seed to passing objects (Graham, 1964) and also supposed to increase the flow of water into the seed and speeding up the germination (Panigrahi, 1986). Significant variations in surface pattern, length and shape of these mucilaginous hairs proved to be a

potential character of taxonomic significance (Panigrahi, 1986). In accordance with the study of Turki (2007), SEM analysis of spermoderm indicated differences in texture and reticulation of their anticlinal (radial) walls, the appearance of the outer periclinal walls and the persistency of the primary cell walls among a few species of *Ammannia*. Based on the surface texture of the seed, he described *A.baccifera* as smooth with a few striations, *A. auriculata*, *A. multiflora* and *A. aegyptiaca* as striated, whereas *A. senegalensis* as highly striated. He also took an account of relief of cell boundaries either as normal (*A. auriculata* and *A. senegalensis)* or channelled (*A. baccifera A. multiflora* and *A. aegyptiaca*).

Palynology

Previous studies (Erdtman, 1952; Nair, 1966) indicate that different pollen characters such as pollen units, polarity, symmetry, shape, size, apertures and nature of exine could be used as an execellent tool. Among pollen characters, 'aperture' is considered as primary and most conservative, 'exine ornamentation' as secondary and other features as tertiary characters in the order of importance. Exine is the outermost layer of the wall of pollen grains and the morphological characterestics of the pollen grain is marked in this outer layer. Apertures are represented by thin area of this exine (in some cases thicker than nonapertural area) through which pollen tube emerges during germination (Walker & Doyle, 1975). The second major function of aperture is to allow for volume change accommodation in pollen grains in accordance with the changes in humidity and this phenomenon is known as harmomegathy (Payne, 1972). The stresses exerted on pollen grain wall (Blackmore & Barnes, 1986), during harmomegathic movements are depend on whether cytoplasmic volume changes are mostly accommodated by apertural or mesocorpial areas of exine (Crane, 1986) and this phenomenon further contributed to the diversity of exine substructure in various taxa.

According to Blackmore and Barnes (1986), it is important to consider the harmomegathy, in the context of an overview of all functions, phylogenetic history and ontogenetic constrains. In agreement with Erdtman (1969), almost all palynological discussion of plant relationship and palynology depended on aperture form, their number and distribution and position which could be designated as NPC (N= Number; P= position and C= Character). On the basis of number of apertures, pollen can be classified as inaperturate (without any aperture), mono- aperturate (having one aperture), di- aperturate (with two apertures), tri- aperturate (with three apertures) and poly- aperturate (with more than three). In a number of taxa it has been observed that there is a linear relationship between ploidy level and size of aperture (Nair & Ravikumar, 1984). Beaulieu *et al*. (2008) found that there is a strong positive relationship between genome size and cell size, further suggesting that genome size may partly be correlated with pollen size. Knight *et al.* (2010) studied the correlation between the pollen size and genome size in 464 species, but found only a weak relationship between the two in their analysis. In many contexts the palynological evidences were found to help to place taxa of uncertain affinities to suggest rearrangement, withdrawal and separations as well as collaborating other lines of evidence (Davis & Heywood, 1963).

Palynologicaly, Lythraceae is found to be one of the most diverse families of Myrtales at both sculptural and structural levels (Graham *et al.*, 1985; Graham *et al*., 1987; Graham *et al.*, 1990). Preliminary studies on Lythraceae indicated that pollen characters can provide more information about the relationship and distinction among different genera (Graham *et al.*, 1968; Graham & Graham, 1971). Erdtman in 1952 reported Lythraceae as europalynous family. Previous studies showed that the family Lythraceae is generally characterized by heterocolpate pollen grains (Patel *et al.*, 1984; Muller, 1969). In various genera of Lythraceae, subsidiary colpi (pseudocolpi) and intercolpar concavities were found to be present, and in *Ammannia* and

Nesaea, twice the number of subsidiary colpies as the number of apertures were observed. Graham *et al*, (1985), performed a detailed palynological survey including LM, SEM and TEM of 10 genera of Lythraceae which also include *Ammannia*. According to this study, the pollen data were observed as consistent with cytology, anatomy and embryology, and suggested that subgeneric classification into tribes Lythraea and Nesaeeae that was introduced by Koehne was not valid.

Studies on *pollen flora of Pakistan* by Perveen and Qaisser (2005) indicate most striking variation in exine ornamentation and aperture types. He recognized two distinct pollen types based on apertural types viz. *Lagerstroemia indica*-type (with tricolpate pollen grains) and *Ammannia baccifera*-type (with heterocolpate pollen grains). They included *Woodfordia fruticosa* and *Lagerstroemia indica* in the former type distinguishing each by means of polar length and exine types, each representing single species. Three genera *viz.*, *Ammannia*, *Lythrum* and *Lawsonia* were included in *Ammannia baccifera*-type represented by three species of *Ammannia* and single species of *Lythrum* and *Lawsonia* on the basis of exine ornamentation and pollen shape.

According to the palynological studies in the family Lythraceae, Graham *et al.* (1990), placed 26 genera into four groups based on the pollen shape, type of aperture and presence or absence of pseudocolpi. They included *Ammannia* and *Nesaea* in Group II, as they are characterized by the presence of tricolporate pollen with 6 pseudocolpi, moderately developed to distinct mesocolpal ridges, very distinct pores, and faint annuli and unique sculpture with fine, interlaced striations. The genus *Rotala* was incorporated into Group III as it was characterized by six faint pseudocolpi and regulated exine bordering colpus. In conclusion, they confirmed the close relationship of *Ammannia* and *Nesaea* than with *Rotala*. A review study on fossil evidence

of Lythraceae (Graham, 2013) pointed out, pollen and seed as the most common kinds of the fossil remains. She concluded Pollen of the *Lythrum/Peplis* from the Late Cretaceous of Wyoming was the oldest evidence of Lythraceae. Pollen of *Ammannia* from Thailand is listed by Watanasak (1990) as one of the several markers for the early to middle Miocene in Thailand. Pollen of *Ammannia* from the late middle Miocene of Thailand was also listed by Songtham *et al*. (2005). But the fossil record regarding genus *Rotala* is found to be scarce or extinct. Still some subfossil records (*c.*14,000 years old) from North East Thailand was listed for this genus (Penny, 2001). Turki in 2007 conducted a palynological study in five species of *Ammannia* in Egypt and he revealed that the pollen shape varies in 5 species namely, *A. auriculata, A. multiflora, A. senegalensis, A. baccifera* and *A. aegyptiaca*, from subprolate, prolate to perprolate. The exine sculpturing variation was also found to be significant and it is striated in *A. auriculata, A. multiflora* and *A. senegalensis* where as regulate to reticulate in *A. baccifera* and palisate in *A. aegyptiaca.*

Anatomy

There has been a tremendous progress in past 40 years in the investigation of vascular anatomy and its use in plant systematics (Stace, 1991). These Anatomical characters of vegetative and floral parts of flowering plants have been successfully employed to solve various taxonomic problems and for the elucidation of phylogenetic relationships. It was Bureau (1872), who, for the first time used anatomical characters in plant classification for the delimitation of taxa of various levels, within the family Bignoniaceae. Thus there evolved a term ‘Systematic anatomy’. In recent years large number of papers on the systematic anatomy in the hierarchic order from order to species level have been published. Anatomical characters of vegitative organs vary depending on species and the habitat in which they thrive. Thus

classifications based on anatomical characters alone often pose difficulties than those based on pure morphological characters. But in many cases where the distinction among different species found to very difficult due to negligible differences in morphological characters, even trifling anatomical characters along with the morphological characters may provide larger barrier in between the species.

Solereder (1908) as well as Metcalfe and Chalk (1950) were considered to be the first to list and summarize different anatomical works by various scientists in the family Lythraceae. In particular Metcalfe and Chalk (1950) have observed intraxylary phloem in various genera of the family. A tentative phylogenetic classification of Lythraceae was proposed by Baas and Zweypfenning (1979) based exclusively on wood anatomy of about twenty genera. They could construct a phylogenetic scheme depending on the monophyletic nature of chambered crystalliferous fibers and fiber-dimorphism, as well as classical transformation series for types of ray tissue. According to Kribs (1935), anatomical characters of the stem were of limited value in determining the relationships among all herbaceous members of Lythraceae. But the herbaceous genera *viz*., *Ammannia, Rotala, Nesaea, Hionanthera, Didiplis* and *Peplis* of this family were not included in their studies. A few isolated studies were reported in one or two species of the genus *Rotala* by some authors (Gunther, 1905; Datta & Maiti, 1968). It was Panigrahi who first to carried out a detailed anatomical survey of three herbaceous genera *viz., Ammannia, Nesaea* and *Rotala* (Panigrahi, 1981; Panigrahi, 1986; Panigrahi, 1988). This study were carried out in 8 species of *Ammannia*, 11 species of *Nesaea* and 10 species of *Rotala* and presented a 'key' for each genus based on anatomy. It appear anatomical adaptation to aquatic habitat is more predominant in *Rotala* than the other two genera. The range of variation in cortex/xylem ratio was found to be rather extreme in the case of genus *Rotala* from 0.312: 1 to 16.235: 1, when compared to *Nesaea*

(between 1.15:1 and 7.5: 1). In *Ammannia* the ratio varies between 225:1 for *A. verticillata* and 0.658:1 for *A.coccinia* (Panigrahi & Panigrahi, 1983)

A few attempts have been made to study the diversity of vessel elements in the dicotyledons (Bailey & Tupper, 1918; Bailey, 1944; Inamdar & Murthy, 1977; Aleykutty & Inamdar, 1978; Inamdar, 1981). As per these studies the diversity of vessel elements relies on their structure, shape, size, distribution and number of perforation plates in the same species or in different species, or in different organs of the same species. Bailey (1957) was the first to point out the taxonomic importance of the study of xylem elements, especially the vessel members of various genera and species of the angiosperms. Baas *et al.* in 2004 described the vessel elements as simple and pitted in woods of Angiosperms. Within the family Lythraceae, some earlier reports revealed the presence of vessel elements with simple perforation plates (Solereder & Scott, 1908; Metcalfe & Chalk, 1950; Bass & Zweypfenning, 1979). Cronquist (1981) also mentioned such vessel elements in stem are simple pitted with simple perforation plate. The presence of vestured pits in vessels is observed in all families of Myrtales including Lythraceae (Van vliet & Baas, 1984; Jansen *et al.*, 2008). Only very few studies were reviewed in detail about the vessel elements of different species within Lythraceae. According to the studies of Kshirsagar and Vikos (2012b), the vessel elements in the stem of *Cuphea, Woodfordia, Lawsonia* and *Lagerstroemia* exhibit variation in their length and breadth in the vessels and perforation plates were observed to be mostly simple, except in certain taxa (e.g. *Lagerstroemia microcarpa* & *L. parviflora*) where, both simple and scalariform perforation plates are occurs. Kshirsagar and Vikos (2012a) investigated the vessel elements of four species of *Ammannia* and nine species of *Rotala* from India. It showed that these species exhibit a variation in length and breadth of vessel elements and minimum length and breadth was reported in *Rotala* where the same for maximum is in *Ammannia.* The

commonly shared characterestics are simple perforation plate and presence of vestured pits in the lateral wall. Many species are separated by position of perforated plates (terminal or sub terminal), presence or absence of spur like projection otherwise called as tail, and nature of end wall (oblique or transverse).

Previous review studies already reported the presence of calcium deposits, predominantly calcium oxalate, in different anatomical structures especially in stems and roots of various plant species. These may commonly exist in various shapes like druses, raphides, styloides, prismatics and crystal sands. Kuo-Huang *et al.* (1994) reported the presence of CaO druses in the form of crystal idioblasts in spongy tissues and fundamental parenchymatous cells of *Rotala indica*, *R. rotundifolia*, *R. hippuris* and *R. wallichi*. Turki (2007), in his studies on a few Egyptian species of *Ammannia*, he interpreted anatomical characters of leaf and stem in order to distinguish five species. He separated these five species based on width of vascular strands and pith of stem and midrib width, vascular strand number and Palisade/spongy tissue ratio in leaf cross section.

Comprehensive studies on anatomical structure of shoot apex of heterophyllus hydrophytes were conducted by many researchers in order to find out the difference between vegetative shoot apex of submerged and/or terrestrial plants and inflorescence apex as heterophylly is due to the response to emergence or submergence (Hagemann, 1963; England & Tolbert, 1964). In many plant species it was studied that there is no disparity in size or organization of submerged and terrestrial apices, while some other species show variations. De Vos (1974) described comparative anatomical structure of vegetative and inflorescence shoot apex of both submerged and terrestrial plants of *R. rotundifolia* and he concluded as three types of apex for this species. All three types of apices were characterised by superficial lining of

double layered tunica (from which the leaves are originated) and corpus, three concentric layered (outer and an inner layer of phloem, and a middle layer of xylem) mature vascular cylinder and a few cylindrical pith cells.

Kshirsagar (2012) comprehended the complete aspect of anatomical features of leaves of four species of *Ammannia viz., Ammannia baccifera* subsp. *baccifera, A.baccifera* subsp. *aegyptiaca, A. multiflora and Ammannia desertora* from India. He reported mucilaginous cell in epidermis, presence of both palisade and spongy tissue in mesophyll, collenchymatous hypodermis in the midrib and large arc-shaped vascular bundle in the centre of midrib region. He also noted some unique characters like striated cuticle and one layered palisade in *A. multiflora* and presence of palisade in both sides in *A. baccifera* subsp. *baccifera* and *A. baccifera* subsp. *aegyptiaca,* which help to distinguish from one another.

Various reports proved that different epidermal anatomical features such as stomata and trichome were found to support in plant systematics, as variations in these characters have been experimentally shown to be gene-dependent (Cutler & Brandham, 1977; Barthlott, 1981; Oladele, 1983; Adegbite, 1995). Thus, their proven genetic stability and high structural diversity have been the basis for their use in identification and classification of many groups (Pant & Verma, 1974; Gill & Nyawaume, 1990; Egbedo, 1990, 1991; Oladele, 1991; Nwokeocha, 1996; Abubakar & Yunusa, 1998; Ogunkunle & Oladele, 2000; Ayodele & Gbadebo, 2000; Ogundipe & Ayodele, 2000). The foliar epidermal characters are now widely employed as an important taxonomic character in various plant groups (Essiett *et al*., 2012). However a very few studies have been available regarding leaf epidermal morphology in the family Lythaceae. Rajagopal (1979) brought out the distributional pattern and taxonomic significance of foliar stomata. In this study he pointed out the hypostomatic or amphistomatic nature of leaves in

Lythraceae. Rajagopal and Ramayya (1980) further discussed the occurrence of stomatal ledges in angiosperms and made an attempt to group the families into four categories based on their structure. They included Lythraceae in group I, which was characterized with ledges on the outer or inner walls of the guard cells. Thanki *et al*. (2000) reported the common occurrence of anomocytic and haplocyctic stomata in Lythraceae when he was studying the structure and development of stomata in a few species of this family. Kshirsagar and Vikos (2013) conducted a detailed epidermal diversity study in nine Indian species of *Rotala* namely *R. densiflora* (Roth. *ex*. Roem. & Schult.) Koehne, *R. fimbriata* Wight, *R. floribunda* (Wight) Koehne, *R. indica* (Willd.) Koehne, *R. malampuzhensis* R.Vasudevan Nair *ex* Cook, *R. occultiflora*, Koehne, *R. rotundifolia* (Buch.-Ham. *ex* Roxb.) Koehne, *R. rosea* (Poir.) Cook and *R. serpyllifolia* (Roth) Bremek. According to his study, the nine species shared some common characterestics like amphistomatic leaves, larger epidermal cells on upper side than in lower side and wavy or sinuous anticlinal cell wall. Many of the species are distinguished by stomatal types. The species such as *R. densiflora, R. floribunda, R. indica, R. occultiflora* and *R. serpyllifolia* were found to be characterized by the presence of anomocytic stomata while anisocytic stomata are present in *R. fimbriata, R. malampuzhensis, R. rotundifolia,* and *R. rosea*. He also noticed 2-celled, glandular trichome in *R. malampuzhensis* and scale-like trichome in *R. floribunda*.

Earlier reviews bring to light that the anatomical features could not be considered as superior to other criteria that were used for taxonomic purposes. These are claimed as additional characters along with other disciplinary approaches which were together helpful for discerning evolutionary trends and interrelationships of taxa at and above the species level. Also in some cases when morphological characters proved to be perplexing in the

preliminary identification, then the anatomical characters proved to be significant.

Molecular systematics

In the age of genomics, "Plant systematics" seeks its application and links to various molecular techniques in order to trace the evolutionary relationships among different taxa at various hierarchal levels. Along with describing, naming and classifying organisms, one has to know the evolutionary relationship since an organism is an ultimate result of evolutionary process (mutation). From 1960's onwards Molecular biologists have tried to merge evolutionary studies with their methodology and tools inorder to promote the cladistical approach to systematization of living organisms (Hennig, 1965).

The idea of phylogenetic analysis arose from the seminal article of evolutionary implications of macromolecular sequence data by Zuckerkandl and Pauling (1965). Within this article they put forward two suggestions; first one was about the molecular change, which might occur at a rate which is proportional to the clock time (molecular clock hypothesis). Secondly, about the topology of evolutionary branching that could be deduced from the pattern of molecular change. These two suggestions formed the basics of science of molecular evolution. Proteins were the earliest biomolecules used to study phylogenetics as Zuckerkandl and Pauling (1965) estimated the number of differences between homologous protein sequences of different hemoglobin to estimate the time since divergence. This drift continued throughout 1970's with some noteworthy achievements like phylogenetic reconstruction of cytochrome C aminoacid by Fitch and Margoliash (1967), estimation of the time of human-Chimpanzee- Gorilla split by Sarich and Wilson (1967) and the development of neutral theory of molecular evolution by Kimura (1968).

After 1980 there was a bloom of molecular systematics as a resolution for evolutionary problems

In molecular phylogeny, the relationships among organisms or genes are studied by comparing homologues of DNA or protein sequences (molecular markers). Dissimilarities among the sequences indicate genetic divergence as a result of molecular evolution during the course of time. By comparing homologous molecules from different organisms it is possible to establish their degree of similarity and thereby revealing a hierarchy of relationship in phylogenetic tree. A combination of phenetic methods and molecular analysis based methods thus strengthens the exercise of the determination of phylogenetic relationships of organisms to a great extent.

Molecular Markers

Generally the molecular phylogenetic analysis relies on a group of biomolecular markers. Use of single marker gene or protein sequence reflects only the evolution of that particular gene. It may lead to interpretation problems, as other genes in the organism may show different rates of evolution or even different evolutionary history. Hillis and Dixon (1991) provided detailed review on the importance of molecular markers in systematic studies. As per various studies, an ideal marker particularly marker genes should possess the following characters (Joshi *et al*., 1999; Hwang & Kim, 1999).

- Single copy gene is preferred to multi copy gene
- Frequent occurrence in genome and high reproducibility
- For investigating phylogenetic relationships at higher levels, highly conserved molecular markers found to be useful, while in *vice versa* the hyper variable molecular markers.

- Still, too much base variation is not preferable as it will not reflect true ancestry.
- In the case of DNA based marker, primer should be available in order to amplify marker gene and these primers should not be too universal so that the amplification of non specific gene can be omitted.
- Marker gene with optimum substitution rate or evolutionary rate should be selected so as to provide enough informative sites.

Some of the very popular markers being used widely in phylogenetic studies are described below in some details.

Biochemical markers

Isozymes: Isozymes are multi-molecular forms of enzymes that share a common substrate but differ in electrophoretic mobility. They appear on electrophoretic gels as multiple bands, which are coded by more than one gene locus (Moss, 1982). Markert and Moller (1959) proposed the term isozyme ‘‘to describe the different molecular forms in which proteins may exist with the same enzymatic specificity’’. This functional definition was intended to be broad, to cover all molecular forms of enzymes. This new wealth of variation would come to be of considerable evolutionary interest. The isozyme analysis is frequently used for taxonomic purpose especially when a taxon is morphologically diverse or plastic (Labhane & Dongarwar, 2013). The technique involved is simple band counting methodology after getting the zymogram using specific enzyme substrate. The use of this technique to resolve a variety of problems in plant systematics was introduced and explained by Gottlieb (1971a, 1973a, b, 1977). Gottlieb (1982) reported that diploid plants have a highly conserved minimal number of isozymes for many of the enzymes which are routinely included in electrophoretic studies

and hence, plants with additional genes specifying these enzymes are of phylogenetic interest. Further Crawford (1983) suggested that electrophoretic data might be of great help to predict evolutionary relationship of different taxa. Nearly 57 enzymes were generally used for studying phylogenetics, biodiversity and conservative studies. Most common enzymes that were used in phylogenetic analysis were reported to be aspartate amino tansferase (AAT), acid phosphatases and alcohol dehydrogenases. Various plant phylogenetic investigations based on isozyme studies were reported by different authors (Elisens & Crawford, 1988; Purdy & Bayer, 1995; Lange & SchifinoWittmann, 2000). Very few isozyme studies were reported in Lythraceae. Most of the studies were focussed on the genus *Lythrum* (Strefeler, 1996).

DNA based markers:

In the present scenario, molecular DNA based data sets are reported to be important resources for phylogenetic reconstruction and this area has progressed rapidly with *in-vitro* DNA amplification and direct sequencing methods. In angiosperm systematics, this molecular approach has been effective in addressing many phylogenetic questions that had not been solved only by using phenotypic characters (Chase *et al.*, 1993). Now DNA characters become more predominant than protein sequences as nucleotide sequences of a pair of homologous genes having higher information content than the amino acid sequences of the corresponding proteins which in turn is due to the mutations that result in non-synonymous changes that alter the DNA sequence but do not affect the amino acid sequence. As DNA markers are based on unique nucleotide sequences and are not affected by environmental factors or physiological conditions, their analysis can provide accuracy. An obvious advantage of molecular assays is the immense number

of characters that they reveal (Karp *et al.,* 1996). Not all genes are suitable phylogenetic markers and not all marker molecules are useful for the analysis of a given group of organisms. For phylogenetic analysis, a reasonable amount of variation in a particular gene sequence in between the OTUs being studied is desirable and reaction must be "primed" using small oligonucleotide that are identical to regions of the gene in all OUTs of interest. The solution to this challenge is that PCR for evolutionary analysis can be used for those genes or elements that, in all of the samples of interest, have regions that vary in sequence and are flanked by regions that are highly conserved and the primers so called "universal primers" can be targeted for these highly conserved sequence and the variable region can be amplified and sequenced. Thus primers designed against this region can be annealed universally to different organisms and screened for known sequences for variation. The method of screening molecular sequences for their ability to resolve relationships within a particular group include studies which assess the ability of a gene to recover well-established phylogenetic relationships within clades of similar age and the construction of fossil-based pair wise difference curves, which estimate the rate of potentially informative character changes during the geological interval when a clade underwent phylogenetic divergence (Mindell & Honeycutt, 1990; Graybeal, 1994). Usually, the phylogeny inferred from a single marker gene or protein sequence only reveals evolution of that particular gene. But use of a single marker can lead to interpretation problems, because other genes in the organism may show different rates of evolution or even show different evolutionary history if horizontal gene transfer (gene transfer between unrelated organisms) has taken place. Some of the well known markers being used widely in phylogenetic studies of angiosperms are described below in some details.

Nuclear Ribosomal gene

Ribosomal ribonucleic acid (rRNA) is the central component of ribosomes - small intracellular particles, which convert the information carried in the genetic code into protein molecules (Gilbert, 1986) and are most widely exploited nuclear marker gene in the field of molecular phylogenetic construction due to four factors such as critical function, ubiquity, variability and abundance (Hollingsworth *et al*., 1999). In angiosperm, genes encoding for rRNA is arranged to two distinct set of tandem arrays. First set include 5S rRNA genes and intergenic spacers in tandem arrays while second set 18S-5.8S-26S rRNA cistron also in tandem arrays and both sets are located at one or more chromosomal loci. But of these two sets, 18S-5.8S-26S rRNA arrays have been used in systematic studies more frequently than 5S. The tandem arrays of rRNA genes consist of hundreds to thousands copy per array and the repetitive structure of these arrays promotes the process of homogenization (Zimmer *et al*., 1980; Baldwin *et al.*, 1995; Cronn *et al.*, 1996; Soltis *et al*., 1997). The three rDNAs 18S (*c*. 1.8 kb), 5.8S (163-165 base pairs) and 26S (3.5 kb) are most conserved and used for the most ancient stages. Other transcribed but non- coding RNA spacers are situated within (internal transcribed spacers, ITS) and at the ends (5'& 3' external transcribed spacers, ETS) of the cistron. These spacers are studied to evolve fastly and these highly variable sequences are reported to employ for studying phylogenetic relatioship. Within this angiosperm, sometime 5.8S referred to as 17S whereas 26S as 25S, 27S or 28S based on some non angiospermous homologues which have slightly different sedimentation rate. The variability of non transcribed spacers (Non Transcribed Spacer, NTS) separating tandemly arranged ribosomal cistron is comparatively higher and thus can be used to study close relationships including within-population (Hribova *et al*., 2011; Grechko, 2002). The variability in these spacers together with conserved flanking region allows the construction of universal primers.

Among these spacers, ITS is found as one of the most attractive nuclear markers for phylogenetic analyses at lower systematic ranks due to rapid concerted evolution, promoting intragenomic uniformity of the copies and a sequence length of approximately 700 base pair (suitable for PCR amplification of the entire ITS region, including 5.8S rDNA) (Baldwin, 1992; Baldwin *et al*., 1995).

Mitochondrial genes

Plant mitochondrial DNA (mtDNA) is large and variable in size (104 kb- 11.3 Mb) when compared to animal and fungal mtDNA (Sloan *et al*., 2012). This size variation among species is mainly related to differences in the size of intergenic regions (introns, intergenes, repeated sequences and alien sequences of chloroplast, and nuclear origin (Marienfeld *et al.*, 1999). Gene arrangement of mtDNA in higher plants varies enormously due to the presence of repeated regions, source of recombination within and between mtDNA genomes (Schuster & Brennicke, 1994). Fortunately, mtDNA coding sequences are highly conserved, facilitating the identification of conserved regions within which universal primers can be defined (Demesure *et al.*, 1995; Duminil *et al.*, 2002). Still, evolutionary rate is estimated at 3-4 times lower in plant mtDNA than in cpDNA, 12 times lower than in plant nuclear DNA, and 40-100 times lower than in animal mtDNA (Sloan *et al*., 2012). This low rate of base substitution in plant mitochondrial genes makes them potentially valuable in phylogenetic reconstruction at higher taxonomic levels. Very few mitochondria genes have recently been studied and found to have potential for inferring phylogeny at higher levels. *CoxI* and *atpA* were two such genes for which dePamphilis and coworkers (pers. comm.) have developed amplification and sequencing primers. Phylogenetic analyses of over 50 diverse species of gymnosperms and angiosperms for *coxI* and for a dozen diverse angiosperms for *atpA* indicate that these genes may be

applicable over a wide taxonomic range from suprafamilial through ordinal and subclass. But the sequence divergences within the families were reported to be less, which limits the resolution at this level (Soltis & Soltis, 1998). All the deficiencies of these mitochondrial genes are due to the presence of RNA editing sites that causes special problem for phylogenic estimation. So it is beneficial to identify and remove this RNA editing site prior to phylogenetic analysis (Bowe & dePamphilis, 1996; Malek *et al*., 1996). Sequence data were also derived from some other mitochondrial genes such as *coxIII* (Malek *et al*., 1996), *atp*9*, nad*2*,* and *cob* and are found to be applicable in phylogenetic analysis. Although the extensive use of mtDNA markers are questionable due to the difficulty in searching of more variable regions, the current trend is to test as many mtDNA loci as possible using available universal primers (Jeandroz *et al.*, 2002; Dumolin-Lapegue *et al.*, 1997; Jaramillo-Correa *et al.*, 2003; Froelicher *et al.*, 2011). Universal primers are based on the conserved nature of the exonic sequences of mtDNA across species, enabling the identification of consensus regions within coding sequences (Duminil, 2014).

Chloroplast genes

Chloroplast DNA is the smallest when compared to mitochondrial and nuclear DNA. This circular molecule is characterized by two inverse repeats which in turn separate the remaining segment into large and small and large single copy number. It is supposed to be conserved in its evolution with regard to nucleotide substitution with very little rearrangements which permits the molecule to be used in resolving phylogenetic relationships especially at deep levels of evolution (Zurawski & Clegg, 1987). The advantages of the chloroplast genome for phylogeny reconstruction chiefly depend on the fact that the chloroplast genome is small (typically between 120 and 200 kb), making it relatively easy to examine the entire genome via restriction site analysis (Soltis *et al.*, 1992). Chloroplast genome is considered

structurally conservative and evolves fairly slowly at the nucleotide sequence level (Palmer, 1985, 1991; Downie & Palmer, 1992). However, various studies proved that different portions of the chloroplast genome evolve at different rates (Soltis *et al*., 1992; Liston, 1992; Ferris *et al.,* 1993; Patwardhan *et al*., 2014). Numerous species are documented for a few coding and non-coding regions, and most commonly used markers for phylogenetic inference and genetic barcoding are *rbcL* (Duvall, 1993), *atpB* (Soltis *et al.,* 2002), *trnL-F* (Taberlet *et al.*, 1991), *matK*, *psbA-trnH*, *rpoC1*, *rpoB-trnC*, *psbK-psbI*, *atpF-atpH*, *atpH-atpI* (Tamura *et al.*, 2004; Kress *et al*., 2005; Chase *et al.*, 2007; Hollingsworth, 2009; Seberg & Petersen, 2009). Some of them are given below.

***rbc*L:** Ribulose 1, 5-bisphosphate carboxylase/oxygenase (rubisco) is the most abundant on the planet and central to the global carbon cycle and is the first enzyme of C3 cycle (Chase *et al.*, 1993). *rbcL* gene which is located in chloroplast genome as a single copy gene is about 1428 base pairs long and universal to all plants. It was one of the first plant genes to be sequenced (Zurawski *et al*., 1981). The extensive phylogenetic utility of *rbcL* sequences has been emphasized and reviewed previously (Clegg, 1993; Palmer *et al*., 1988; Chase *et al*., 1993). Doebley *et al.* (1990), Soltis *et al*. (1990) and Les *et al*. (1991) were the first to elucidate the relationship using *rbc*L sequences. Instead of using PCR, this study was entirely based on cloned products for sequencing. With the introduction of PCR, the *rbc*L sequencing outburst began (Zurawski *et al*., 1981). Zurawski was the first, who made available at no cost a complete set of sequencing primers. These primers were designed based on angiosperm sequences and experiments throughout the flowering plants, as well as seed plants in general. At family level and above, *rbcL* has, by far, been the preferred gene for inferring phylogeny. The lower limit of applicability of *rbcL* sequences typically extends to the generic level, but in some groups reaches the specific level. Analyses of *rbcL* sequences have been used to resolve generic relationships within several families of flowering plants (Morgan *et al*., 1994; Xiang *et al*., 1993; Conti *et al*., 1993; Kim &

Jansen, 1996). In a few cases, *rbcL* sequence analyses have even clarified relationships among congeneric species: in *Comus* (Xiang *et al*., 1993), *Saxifraga* (Soltis *et al*., 1996), and Droseraceae (Williams *et al*., 1994). The primers for amplification and sequencing vary among taxa. In most of the green plants amplification primer Z1- the first 30 bases of *rbc*L of *Zea mays* (forward), is widely used. At the 3' end of *rbcL,* the amplification primer "3' *rbcL*", which is downstream of the *rbcL* terminus, is widely used in angiosperms (Olmstead *et al*., 1992). For the study of lower taxonomic level this 3' *rbcL* is actually located roughly 80 bp downstream of the terminus of *rbcL.*

atp*B*: *AtpB* gene, which encode for the β subunit of ATPase, is located in large single copy region of chloroplast genome just downstream of *rbcL.* These have closely similar properties (Hoot, 1995) and also similar sequence length of about 1,497 bp. In his study, Hoot (1995) gave a set of amplification and sequencing primer sets that work broadly across the angiosperms thus given the conservative nature of this gene. Savolainen *et al.* (2000) confirmed that *atpB* and *rbcL* have similar rate of evolution in angiosperm but *atpB* seemed to provide better resolution of relationship.

***matK*:** *matK* encodes a maturase involved in splicing type II introns from RNA transcripts (Neuhaus & Link, 1987; Wolfe *et al*., 1992) and is considered to be one of the most rapidly evolving chloroplast genes. It is in large single copy region and has a sequence length of about 1.5 kb. In all photosynthetic land plants so far examined, *mat*K is located within an intron, positioned between the 5' and 3' exons of the transfer RNA gene for lysine, *trnK* (Fig. 1.4). The gene *mat*K, as well as the noncoding regions that flank it, are easily amplified using the highly conserved flanking coding regions that include the *trnK* exons and the genes *rpsJ6* and *psbA* (Soltis & Soltis, 1998). The Number of nucleotide differences per site in pairwise comparisons for *matK* is 3.2, 2.4, and 3.4 times higher, respectively, than for *rbcL. MatK*

sequences may therefore be informative at the generic and even species levels (Johnson & Soltis, 1995; Soltis *et al*., 1996). Previous studies proved that, combination of *rbcL* and *matK* data will provide enhanced resolution and internal support in several angiosperm families when compared to either gene alone (Soltis *et al.,* 1996; Xiang *et al*., 1998; Plunkett *et al*., 1997). To date, phylogenetic analyses using the *trnK* region have concentrated on *matK* only. However, the *trnK* intron regions flanking *matK* may provide additional useful phylogenetic information (Soltis & Soltis, 1998).

***ndh*F:** The gene *ndhF* encodes the subunit of chloroplast NADH dehydrogenase (Kim & Jansen, 1995; Olmstead & Reeves, 1995) and is located in the small single-copy region of the chloroplast genome close to the junction with the inverted repeat. The gene *ndhF* consists of two very different regions-5' region of the gene (1,380 bp) which is more similar to *rbcL* in both rate and pattern of nucleotide substitution and the 3' region (855 bp) which is more A + T rich, has higher levels of non synonymous base substitutions, and shows a greater transversion bias at all codon positions (Kim & Jansen, 1995). Kim and Jansen (1995) suggested that the distinct evolutionary patterns in the 5' and 3' portions of *ndhF* likely reflect different functional constraints. They also noted that the presence of these two different patterns of evolution within the same gene may be advantageous for phylogenetic reconstruction. The conserved and more variable portions may be useful for inferring relationships in older and more recently evolved groups, respectively.

***trnL- F* intergenic spacer:** The non coding region including the *trnL* (UAA) intron and the intergenic spacer between the *trnL* (UAA) 3' exon and the trn*F* (GAA) gene (Taberlet *et al.,*1991; Gielly & Taberlet, 1994) found to have Phylognetic potential. These regions may be evolved at a rate similar to *rbcL* or as much as three times faster than *rbcL*. But the sequence length is small

when compared to *rbcL* and were easily amplified and sequenced (Taberlet *et al*., 1991). Size variation ranges from 350-600 bp for *trnL* intron and 120 to 350 bp for *trnL-F* spacer in the monocots and dicots. Several recent studies have employed both the *trnL* (UAA) intron and the *trnL-F* (GAA) intergenic spacer. The level of variation in this *trnL-trnF* data set is comparable to that of *rbcL*. Till now, two sets primers were designed by Taberlet *et al.* (1991) to amplify the complete set of *trnL- F* region like the *trnL* (UAA) intron, and intergenic spacer between the *trnL* (UAA) 3' exon and *trnF* (GAA). These two sets of primers were observed to work for most species tested including bryophytes, pteridophytes, gymnosperms and angiosperms.

Table: 2. Primers designed by Taberlet *et al*., 1991

Name	Code	Sequence 5'- 3'
a	B48557	CATTACAAATGCGATGCTCT
b	A49291	TCTACCGATTTCGCCATATC
c	B49317	CGAAATCGGTAGACGCTACG
d	A49855	GGGGATAGAGGGACTTGAAC
e	B49873	GGTTCAAGTCCCTCTATCCC
f	A50272	ATTTGAACTGGTGACACGAG

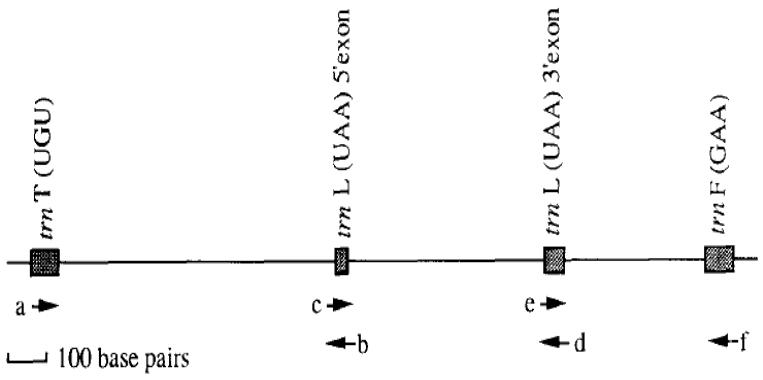

Figure 3: Positions and directions of universal primers used to amplify three non-coding regions of cpDNA. Tips of arrows indicate the 3' ends of the primers (reproduced from Taberlet *et al*., 1991).

Sequence-based phylogenetic studies

Nowadays, the application of molecular sequence based Phylogenetic analysis become routine as a solution for various systematic problems. Majority of molecular data used for phylogenetic studies are chloroplast DNA and nuclear rDNA while low copy nuclear gene have not been used due to greater difficulty in isolation and characterization. In 1998, the ground-breaking classification-the APG system of classification consolidate sequence based molecular phylogenetics as the best available method. It can be considered as the first attempt to realign the orders and families of flowering plants into a phylogenetic system based on the analysis of molecular data. A summary of this phylogenetic system is presented in the form of a phylogenetic tree or cladogram in figure 4. (Daly *et al.*, 2001).

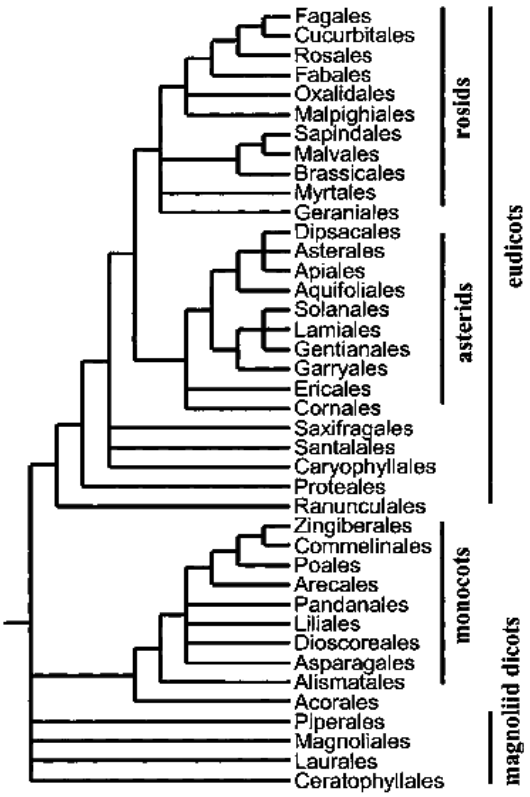

Figure 4. A conservative estimate of phylogenetic relationships among the orders of flowering plants (Modified from The Angiosperm Phylogeny Group, 1998).

Martin and Dowd (1986), elucidated the phylogenetic relationship of about 9 families in the order Myrtales, including two genera (*Lythrum salicaria* L. & *Woodfordia fruticosa* L.) of Lythraceae based on amino acid sequence from the small subunit of the enzyme RUBISCO. Conti *et al*., (1997) employed a detailed study of phylogenetic relationship among fourteen families in the order Myrtales based on *rbcL* sequences. Within this analysis, the family Lythraceae was represented by five genera (*Lawsonia inermis* L., *Nesaea aspera* (Guill. & Perr.) Koehne, *Lythrum hyssopifolia* L., *Cuphea llavea* Lex., *Duabanga grandiflora* (DC.) Walp.). In short, the *rbcL* topology supports the interpretation of familial boundaries for Lythraceae to include *Duabanga, Lawsonia, Nesaea, Lythrum* and *Cuphe*a which was originally delimited on the basis of morphological data. A comprehensive study on Lythraceae based on Chloroplast *rbcL* gene, *psaA- ycf3* Spacer, and Nuclear rDNA Internal Transcribed Spacer (ITS) Sequences was done by Huang and Shi (2002). Analysis of genera, produced topologies which are roughly congruent with each other and exhibited four well supported clades such as clade I (*Ammannia-Nesaea-Lawsonia*), clade II (*Cuphea-Woodfordia*), clade III (*Lythrum-Peplis)* and clade IV (*Pemphis-Punica*). This was further succeeded by a comparative phylogenetic analysis of 31 genera of Lythraceae, based on four gene region (*rbcL* gene, the *trnL-F* region, and *psa*A-*ycf*3 intergenic spacer of the chloroplast and the ITS of the nucleus) and morphological data by Graham *et al*. (2005). According to this study, the species within this genus is separated to six clades and was found to be odd with the relationships inferred by Koehne's classification (1903). Also except clade I, others exhibited some morphological diversity, and thus were not completely congruent with morphological data. Still, two noticeable upshots were about the generic status of *Ammannia, Nesaea* and *Rotala*. First one was the possibility of congeneric status of *Ammannia* and *Nesaea* was confirmed by *N. aspera* by *Ammannia* clade in *rbcL* and *trnL-F* analysis.

Second one, confusing close relationship of *Ammannia* and *Rotala* (Koehne, 1903; Cook *et al.,* 1974) was annulled as the position of *Rotala* strongly supports the close relation to American shrub, *Heimia*. Morris (2007) utilized two newly sequenced chloroplast regions (*atpB-rbcL* intergenic spacer and the *trnK-matK* region) along with previous sequence data (*rbcL*, *trnL-trnF*, *psaA-ycf*3, and ITS) and provided a better resolved result in 28 genera of Lythraceae. Topologies resulted were found to be congruent with the earlier analysis except that the *Didiplis* was observed as sister to *Rotala* and closely related to *Heimia*, a relationship never previously suggested before. Also among the two newly sequenced data sets *trnK-matK* found to resolve better the basal relationships with greater number of parsimony-informative characters, and the topology produced is more similar to that found in the overall combined analyses than any of the other individual data sets.

First attempt to construct a phylogenetic framework for a single genus *Cuphea*, based on nuclear ITS sequences for 53 species and four out group taxa were analyzed by Graham *et al.* (2006). Thus it was the first survey to demonstrate the utility of ITS data for the single genus of Lythraceae, to test the phylogenetic relationship predicted by morphology. This was further followed by a detailed pylogenetic studies on molecular basis (Barber *et al.*, 2010) with an expanded number of species from 52 to 70 and with additional two chloroplast sequence *trnL- F* spacer and *rpl16* intron along with ITS in order to generate a more complete and robust phylogeny.

Graham *et al.* (2011) focused on four confounding herbaceous genera, *Ammannia*, *Rotala, Nesaea* and *Hionenthara* and followed a multidisciplinary analysis including molecular studies. The first molecular phylogeny of Lythraceae included *Ammannia*, *Nesaea* and *Rotala* and utilized sequence data from the nuclear rDNA internal transcribed spacer (ITS) and plastid regions *rbcL*, *trnL–trnF*. All molecular analysis best support A/H/N clade

(*Ammannia/ Hionenthara/ Nesaea*) with high bootstrap value. *Ammannia* was found to nested within *Nesaea*, and *Hionanthera* is sister to the widespread African/Madagascan species *N. radicans* Guill. & Perr. In accordance with previous studies, the three sequence data support a close relationship of *Rotala* with *Heimia* and found far distant from A/H/N clade. Based on this molecular study, Graham and Gandhi (2013) concluded the congeneric status of *Ammannia, Nesaea* and *Hionanthera* leading to nomenclatural transfer of many species of *Nesaea* and *Hionanthera* to *Ammannia.*

Cytology

Various karyotypical data were being used in plant systematics. Karyotype provide a phenotypical view of genotype and its comparative analysis found to have a crucial role in analysis of genetic relationship among the species or population and also provide information about the understanding the way they diverged from each other. Their Mendelian pattern of inheritance is considered to be useful to detect the synapomorphies (character states that are shared due to common ancestry) and identify sister-group relationships among taxa (Hennig, 1966). It further contributed to make chromosomal structural mutations as powerful markers in modern phylogenetic investigations (Rokas & Holland, 2000). Chromosome number, arm ratios, secondry constrictions and chromosome satellites contribute important karyotypical features which are aided for taxonomic and phylogenetic investigations (Jauhar, 2012). Avdulov (1931) and Levitsky (1931) initialized the use of cytological features in the investigation of the phylogenetic relationship among the species and genera. Levitsky used karyological data for the tribe Helleborae in the family Ranunculaceae and concluded a general parallelism of symmetrical karyotype as primitive character and of asymmetrical character as a specialized one. Chromosome number is the most commonly used karyotype feature that provides

substantial information about the genome in quickest, cheapest and easiest way. Generally karyotypical features are not influenced by external conditions, developmental phases and age (Guerra, 2008).

Very few cytological surveys were reported related to Lythraceae. Graham (1979) used cytological evidence (chromose number) along with morphological data to investigate the relationship between of *Ammannia auriculata* and *A. coccinea* and as a result a chromosome number of a 33 (haploid number) was observed in *A. coccinea*, which in turn obviously as a result of their derived status as amphidiploids of *A. auriculata* (n= 16) and *A. robusta* (n=17). A detailed chromosome study in Lythraceae was reported by Tobe *et al*. (1986) where they investigated the chromosome number of 12 genera and concluded that, except two (*Lythrum* and *Peplis*), others have a base chromosome number x= 8 which in turn confirm the earlier report of the same base number for Lythraceae (Raven, 1975). *Rotala indica* and *Nesaea triflora* were two representative species for the genus *Rotala* and *Nesaea* respectively and chromosome number observed to be 2n=32 for *R. indica* and 2n= 46 for *N. triflora*. A distinctly different chromosome base number of x=12 were reported by various authors in *Sonneratia* and *Daubanga* (Graham *et al*., 1993b) in contrary to base number x=8 generally accepted for Lythraceae. Chromosome counts for 78 species of the genus *Cuphea* are available among which most common base number were found to be as x=8 and second most common was reports as x=12. Of the total number of species, 46% were reports as polyploids and 26% were comprised of aneuploids. A latest reports on investigation of chromosome counts completely based on meiotic figures of pollen mother cells in the family Lythraceae resulted in 88 chromosome number from 62 species of 9 genera (Graham & Cavalcanti, 2001). Among this, eight genera maintained original diploid number of x= 8 while another 12 genera with secondary basic numbers of 15, 16, 24, 28 and 32. Graham and Cavacanti (2001), observed the

herbaceous perennials such as species of *Ammannia, Nesaea, Cuphea*, and *Lythrum* as the most chromosomally diverse genera in this family. Morphologically diverse, herbaceous genus *Rotala* was also found to be equally complicated but is poorly understood to confirm this assumption. In their attempt to delimit the herbaceous genera- *Ammannia, Nesaea, Rotala* and *Hionenthara*, Graham *et al.* in 2011, employed chromosome count. It corroborates with previous studies and concluded a high chromosomal diversity for three genera while chromosome numbers could not be counted for *Hionenthara*. Hence, they suggested that both autogamy and polyploidy through hybridization or unreduced gametes results in changes in chromosome number that leads to morphological changes and speciation and also about the irrelevancy of using these chromosome number data in delimiting these highly diverse genera.

Collection and documentation

The present study is confined to 24 species belonging to three genera *Ammannia, Nesaea* and *Rotala* from South India. Among these 8 species of *Rotala* are endemic to South India. South Indian states were surveyed for the collection of specimens, 52 accessions of 24 species were selected. Based on the pattern of distribution, the number of accession per species varied. Minimum of two accessions (except for newly described species) were selected with wider range of variation from distant localities.

Collection and establishment

Extensive explorations were carried out in four South Indian states for the collection of plant specimens. Altogether 25 field trips of 3-5 days duration were made and 50 accessions belonging to *Ammannia, Nesaea* and *Rotala* were made for further studies. Field data and plant data of all collected samples were recorded in the field book following Bridson and Forman (1999). Collection number was also assigned to each accession. Live materials were collected separately to grow at Calicut University Botanical Garden for future observations.

Identification and documentation

Populations of all the three genera were carefully observed in the field and variation shown by each taxa is taken into account. Voucher specimen of each taxa were deposited in Calicut University Herbarium (CALI). Living specimens were maintained at CUBG providing habitat simulating its original habitat. Collected specimens were determined in consultation with revisions and monographs available for the above genera in India, and also in consultation with protologues and types. Voucher specimens cited in local floras, deposited in various herbaria were also examined.

Table 3. List of species of *Ammannia, Nesaea* and *Rotala* species collected from different places in South India

Sl. No:	Name of taxa	Collection number	Location of collection
1	*Ammannia baccifera* subsp. *aegyptiaca* (Willd.) Koehne	132945	Chitoor, Palakkad Dist., Kerala
2	*Ammannia baccifera* L. subsp. *baccifera*	132944	Kumarapalayam, Tamil Nadu
		132947	Chitoor, Palakkad Dist., Kerala
		132993	Hasan, Karnataka
3	*Ammannia multiflora* Roxb.	132972	Kumarapalayam, Tamil Nadu
		132946	Chitoor, Palakkad Dist., Kerala
		132970	Erode, Tamil Nadu.
4	*Ammannia octandra* L. f.	132978	Myla, Puthuchery
5	*Nesaea brevipes* Koehne	132927	Chaliyam, Calicut Dist., Kerala
		133000	Anthannur, Tamil Nadu
		132996	Hasan, Karnataka
6	*Nesaea prostrata* (Buch.- Ham. *ex* Dillwyn) Suresh	132918	Calicut University, malappuaram Dist., Kerala
7	*Rotala anamika* Lemiya	132916	Parappanagadi, Malappuram Dist., Kerala
8	*Rotala cheruchakkiensis* Anto, Devikrishna, Pulickal, C.D.Varghese & I. Antony	132939	Nelliampathy, Palakkad Dist., Kerala.
		148307	Mayannur, Thrissur Dist., Kerala
9	**Rotala cookii* Joseph *et* Sivar.	38967	Parappanagadi, Malappuram Dist., Kerala
10	*Rotala densiflora* (Roth *ex* Roem. & Schult.) Koehne	132960	Dakshina Karnataka Dist., Karnataka
		132961	Irikkur, Kannur Dist., Kerala
		132908	Thirunelli, Wayanad Dist., Kerala
		132948	Pattambi, Malappuram Dist., Kerala

11	*Rotala fimbriata* Wight	132992	Bommanahalli, Mysore Dist., Karnataka
		132997	Idukki, Kerala
12	*Rotala indica* (Willd.) Koehne	132910	Poovattuparamba, Calicut Dist., Kerala
		132910	Mannarkad, Palaghat Dist., Kerala
		132995	Hasan, Karnataka
13	*Rotala juniperina* A. Fernandes	4090	Muthanga, Wayanad Dist., Kerala
		132990	Muthanga, Wayanad Dist., Kerala
14	*Rotala macrandra* Koehne	132911	Poovatuparamba, Calicut Dist., Kerala
		132933	Thalappara, Malappuram Dist., Kerala
		132934	Thirunelli, Wayanad Dist. Kerala
15	*Rotala kasaragodensis* K.S. Prasad & Raveendran	03120	Mugu, Kasaragod Dist., Kerala
16	*Rotala malampuzhensis* R.V. Nair *ex* C.D.K. Cook	132915	Madayipara, Kannur Dist. Kerala
		132965	Mugu, Kasaragod Dist., Kerala,
		132980	Neriyamangalam, Eranamkulam Dist., Kerala
		132985	Sagara, Shimoga Dist., Karnataka
17	*Rotala malabarica* Pradeep, K.T.Joseph *et* Sivar.	132914	Madayipara, Kannur Dist., Kerala
		132968	Mugu, Kasaragod Dist., Kerala
18	*Rotala mexicana* Cham. & Schldtl.	132902	Poovatuparamba, Calicut Dist., Kerala
		132950	Periya, Wayanad Dist. , Kerala
19	*Rotala occultiflora* Koehne	132905	Calicut University, Malappuram Dist., Kerala
		132984	Asarmugh, Dakshina Karnata Dist., Karnataka

		132986	Makairahalli, Karnataka
20	*Rotala rosea* (Poiret) C.D.K.Cook	132949	Alappuzha, Kerala
		132953	Mannarkkad, Palaghat Dist., Kerala
		132971	Kumarapalayam, Tamil Nadu
		132987	Mangaluru, Dakshina Karnataka Dist., Karnataka
21	*Rotala rotundifolia* (Buch. - Ham. *ex* Roxb.) Koehne	132909	Thirunelli, Wayanad Dist., Kerala
		132932	Ooty, Nilgiri Dist., Tamil Nadu
		132963	Kodaikanal, Dindigul Dist., Tamil Nadu
		132979	Agumbe, Shimoga Dist., Karnataka
22	*Rotala tulunadensis* K.S. Prasad, P.Biju, C.Ravi & K.G. Bhat	132974	Permude, Kasaragode Dist. Kerala
23	*Rotala verticillaris* L.	127624	Bhuvaneswar, Orissa
24	**Rotala vasudevanii* Joseph & Sivar.	3997	Aluva, Eranamkulam Dt., Kerala

* Attempts to locate this specimen in its type locality and other possible areas failed hence the present morphological analysis is based on herbarium specimens.

Morphological characterization

Morphology has been considered as one of the major criteria for classification over many centuries. Both vegetative and reproductive characters need to be given equal emphasis as both of them are subjected to different types of selection pressures, to which they react in different ways (Mondal, 2005). Some of the vegetative characters that are employed in plant taxonomy include growth, habit, underground organs, stem and leaves. The inflorescence characters are among most noticeable and their evolution has received relatively much attention in the recent past. The inflorescence characters vary between species, genera and families. The investigation of inflorescence architecture and its systematic importance in various plant groups have been reported by different authors (Panigrahi, 1986; Immelman, 1991; Graham *et al*., 2011).

The morphological characters that are focused for systematic studies of three closely similar herbaceous genera *Ammannia, Nesaea* and *Rotala* are mainly habit, stem characters or leaves and inflorescence characters. The genus *Rotala* shows more variability chiefly in their habit and leaf characteristics when compared to *Ammannia* and *Nesaea*. Various inflorescence characters such as presence or absence of petals, shape and number of petals number of calyx lobes, shape and size of bracteoles, size of style etc. found to play an important role in determining both generic and species levels among these genera. The present chapter involves a detailed investigation of morphological characters including SEM analysis of seeds of *Ammannia*, *Nesaea* and *Rotala.*

Materials and Methods

Vegetative morphology was studied from plants of all 22 species from three genera and the data were collected from the living populations both in

the natural habitat and under cultivation and also from a few herbarium specimen. All specimen collected were studied critically in the laboratory study using stereo microscope. The data sheets were prepared based on information on habit, type of pubescence, nature of inflorescence, color of petals, structure, and position of stamens and staminoides and other features that are not faithfully represented in herbarium specimens critically in the laboratory study using stereo microscope. Botanical illustration of selected species, depicting important characters was also made with the aid of camera lucida.

SEM analysis of Seed

Seeds were washed, fixed in alcohol (70%) and dried at 50°C for 3−6 hrs. Observations of seed shape, size and testa ornamentation were made using a scanning electron microscope JSM-6390LV/JED-2300 at 20 kV after critical-point drying and sputter coating with gold−palladium.

Result

Habit and Stem characteristics

***Ammannia* L.**

Erect or decumbent, terrestrial or amphibious, annual glabrous herbs. Stem 20−80 cm long, with maximum height in *Ammannia baccifera* subsp. *aegyptiaca,* often green and sometimes red (*Ammannia baccifera* and *Ammannia baccifera* subsp. *aegyptiaca*) 4-angular, often branched, rooting at base, internodes *c.* 1−2 cm apart, decreasing near the top. Less area of spongy aerenchyma**.** **Figure 5. A**

***Nesaea* Kunth**

Erect or decumbent, terrestrial, annual glabrous or pubescent herbs. Stem 10−30 cm long, often green, 4-angular, often branched, creeping and

rooting below, internodes *c.*1−2 cm apart, decreasing near the top. Less area of spongy aerenchyma. **Figure 5. B**

***Rotala* L.**

Erect or decumbent, annual or perineal, glabrous, more amphibious or aquatic herbs with floating or submerged vegetative stems. Stems highly variable in size, 2−30 cm long, often green sometime red, slender, 4- angular or terete, simple or winged or profusely branched, very few tuft forming (*Rotala mexicana*), rooting below, sometimes creeping and rooting at nodes, large area of spongy aerenchymateous spaces. **Figure 5. C**

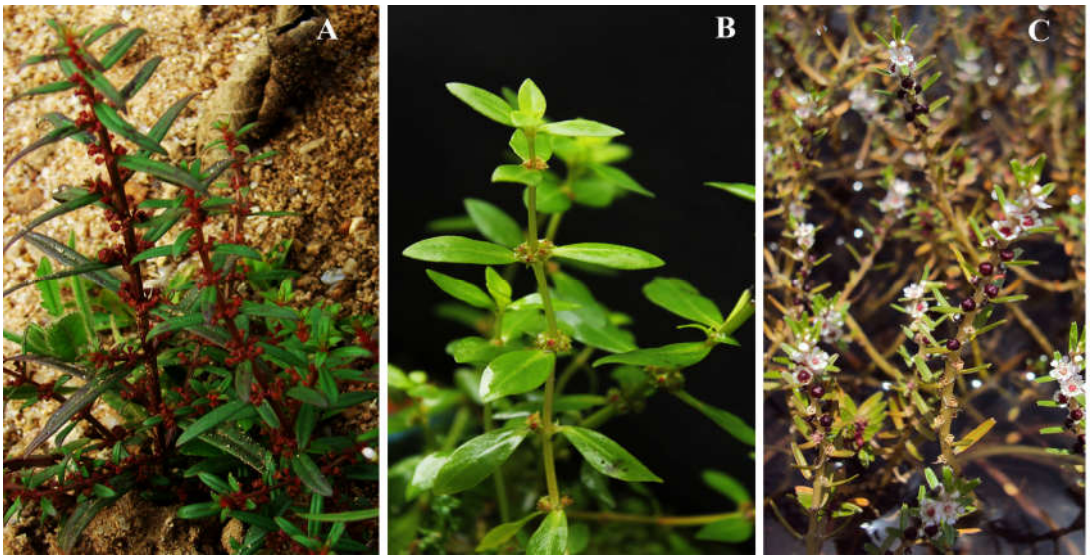

Figure 5. A. *Ammannia baccifera* subsp. *baccifera*; **B**. *Nesaea brevipes*; **C**. *Rotala malabarica*.

Foliar Morphology

***Ammannia* L.**

In all species leaves are simple, decussate and sessile, glabrous, linear or oblong to lanceolate or lanceolate to oblanceolate, or linear to elliptic, often green, sometimes red, 2−10× 0.3−3 mm for the leaves in branches, 8−40×0.5−

7 mm in the main axis, leaf margin entire, with a distinct mid vein, base often sub cordate or auriculate, sometimes cuneate (*Ammannia baccifera*), apex often acute. **Figure 6. A-F**

***Nesaea* Kunth**

Leaves are generally simple, decussate and sessile, glabrous, elliptic or lanceolate/linear to ovate, often green, size varies from 10−20 × 3−10 mm, leaf margin microscopically serrate or crenulated, with distinct mid vein, base sub cordate to sub amplexicaulated or attenuated, apex acute or acute to mucronate. **Figure 6. G- L**

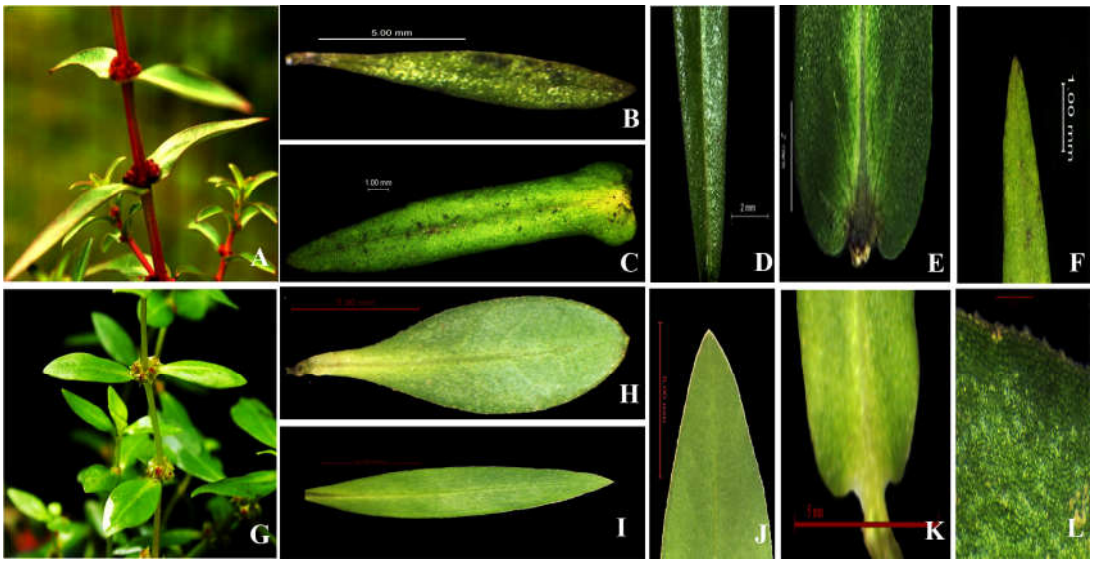

Figure 6. Foliar morphology of *Ammannia* and *Nesaea*. **A-F.** *Ammannia;* **A.** *A. baccifera* subsp. *aegyptiaca*; **B & C.** Leaves; **B.** *A. baccifera* subsp. *baccifera;* **C.** *A. multiflora*; **D & E.** Leaf bases; **D.** *A. baccifera* subsp. *baccifera*; **E.** *A. baccifera* subsp. *aegyptiaca*; **F.** *A. multiflora*-Leaf apex; **G-L.** *Nesaea*; **G.** *N. brevipes*; **H & I.** Leaves; **H.** *N. prostrata*; **I.** *N. brevipes*; **J.** *N. brevipes*-Leaf apex; **K.** *N. prostrata*- Leaf base; **L.** *N. brevipes*- Leaf margin.

***Rotala* L.**

Leaves are highly variable both in shape and size, simple, decussate, whorled (*R. mexicana, R. occultiflora, R. verticillaris, R. cookii*), sessile or

rarely shortly petiolate, sometimes dimorphic, linear or lanceolate or elliptic to ovate for submerged leaves, linear or lanceolate or elliptic or ovate or obovate or sub orbicular for aerial leaves, 5−20 × 2−20 mm, leaf margin entire, base rounded or cuneate or cuneate to cordate or obtuse, apex acute or obtuse or round or rarely bimucronate (*R. malabarica*). **Figure 7.**

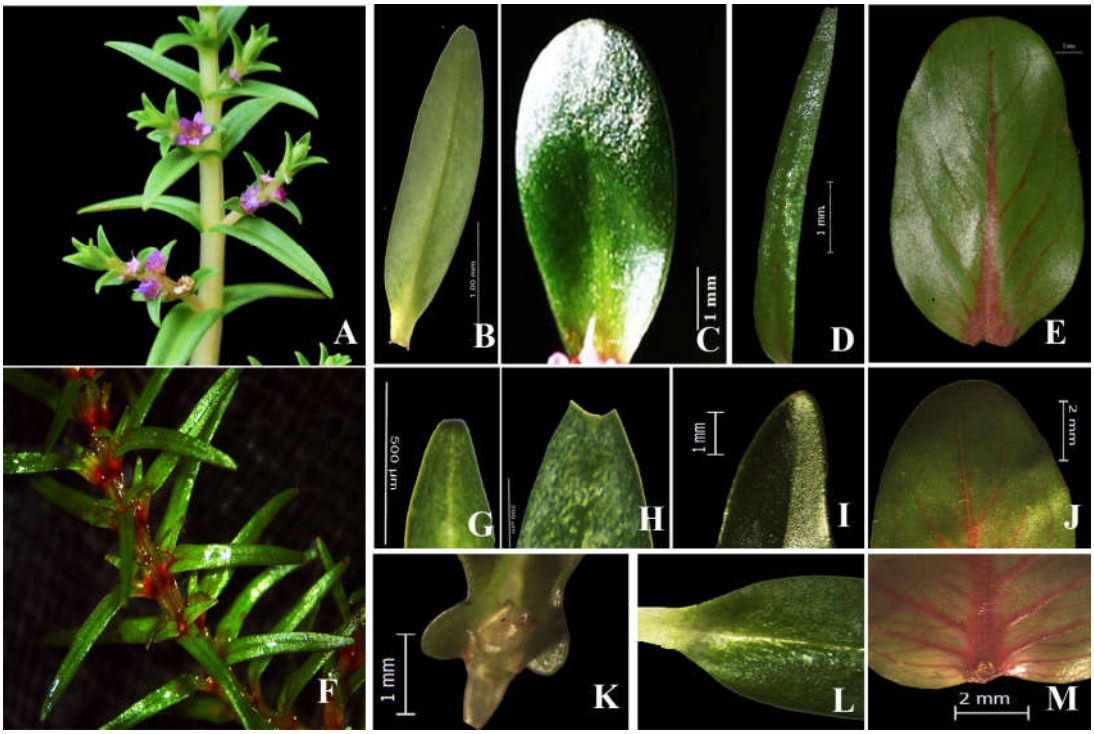

Figure 7. Foliar morphology of *Rotala*. **A.** *R. densiflora*; **B-E.** Leaves; **B**. *R. malampuzhensis*; **C.** *R. indica*; **D.** *R. occultiflora*; **E.** *R. macrandra*; **F.** *R.occultiflora*; **G-J.** Leaf apex; **G.** *R. malampuzhensis*; **H.** *R. malabarica*; **I.** *R. densiflora;* **J.** *R. macrandra;* **K-M.** Leaf bases; **K.** *R. fimbriata*; **L.** *R. densiflora*; **M.** *R. macrandra*

Infloresence morphology

Ammannia L.

Flowers bisexual, actinomorphic, monomorphic, 3 or many in axillary cymes, subsessile, peduncle occasionally present, bracteoles 2, very short, calyxtube campanulate, glabrous, free from but often enclosing ovary,

hypanthial, calyx lobes often 4, triangular, calyx appendages absent, petals absent or occasionally present and often 4, showy with distinct mid vein, often orbicular. Stamen often equal to the number of calyx lobes, rarely double the number of calyx lobes (*A. octandra*), inserted at the middle of the calyx tube, appearing free, anthers dorsifixed, ovary superior, 4 locules, often globose, placentation free central at maturity, style simple, stigma often capitate, irregularly dehiscent capsule, seeds numerous or a few.

Figure 8. A-E

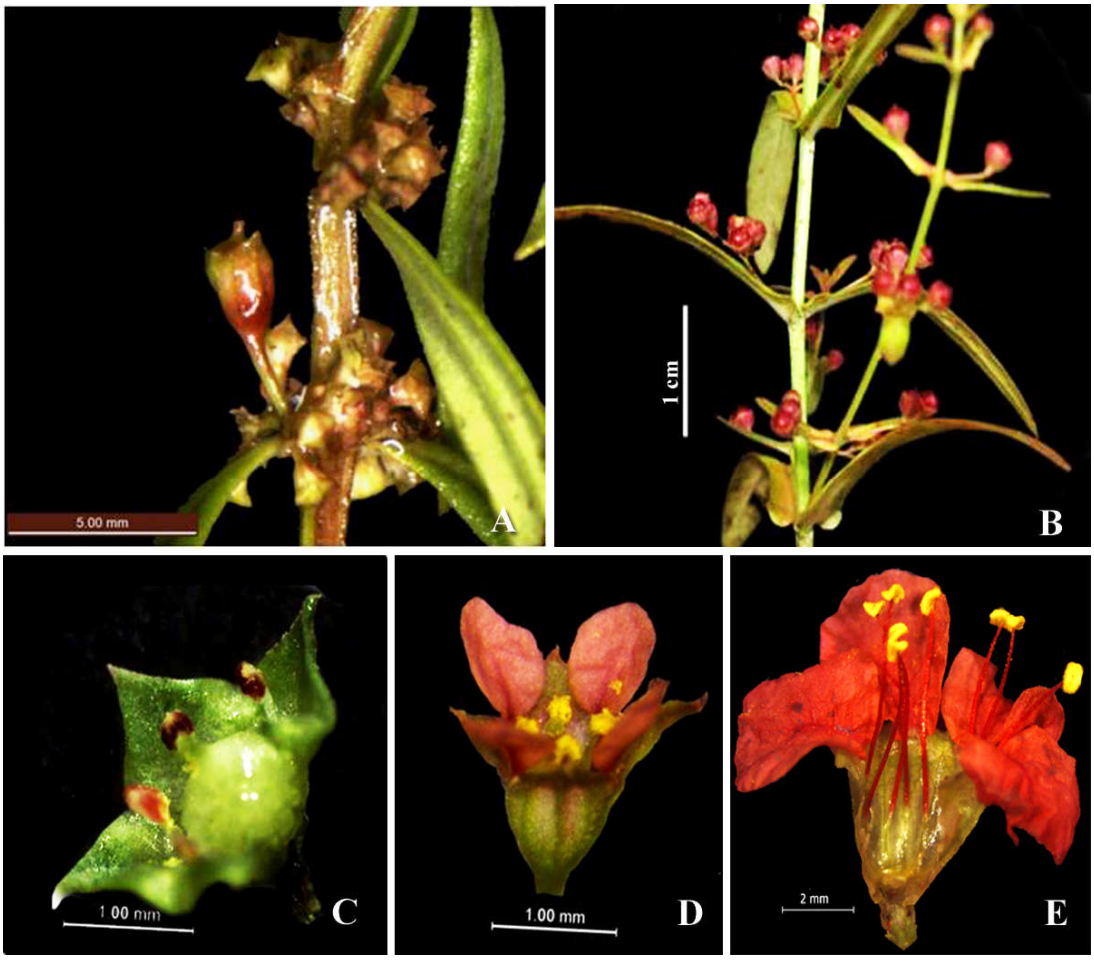

Figure 8. Inflorescence and flowers in *Ammannia*: **A.** *A.baccifera* subsp. *baccifera*- Showing subsessile flowers in axillary cymes; **B.** *A. multiflora*- Showing pedunculate flowers; **C, D & E.** Flowers in *Ammannia*: **C.** *A. baccifera* subsp. *baccifera*- Apetalous flower; **D.** *A. multiflora*- Petaliferous flower; **E.** *A. octandra*- Petaliferous flower with exerted stamens.

***Nesaea* Kunth**

Flowers bisexual, actinomorphic, monomorphic, 3 in axillary cymes, subsessile, peduncle absent, bracteoles 2, very short, calyxtube campanulate, glabrous rarely pubescent (*N. prostrata*), free from but often enclosing ovary, hypanthial, calyx lobes often 4, triangular, very small calyx appendages present, glabrous rarely pubescent, petals 0 or 4, showy petals, often orbicular. Stamen often equal to the number of calyx lobes, inserted below the middle of the calyx tube, appearing free, anthers dorsifixed, ovary superior, 4 locules, often globose, placentation free central at maturity, style simple, stigma often capitate, irregularly dehiscent capsule, seeds numerous or a few.

Figure 9. A-F

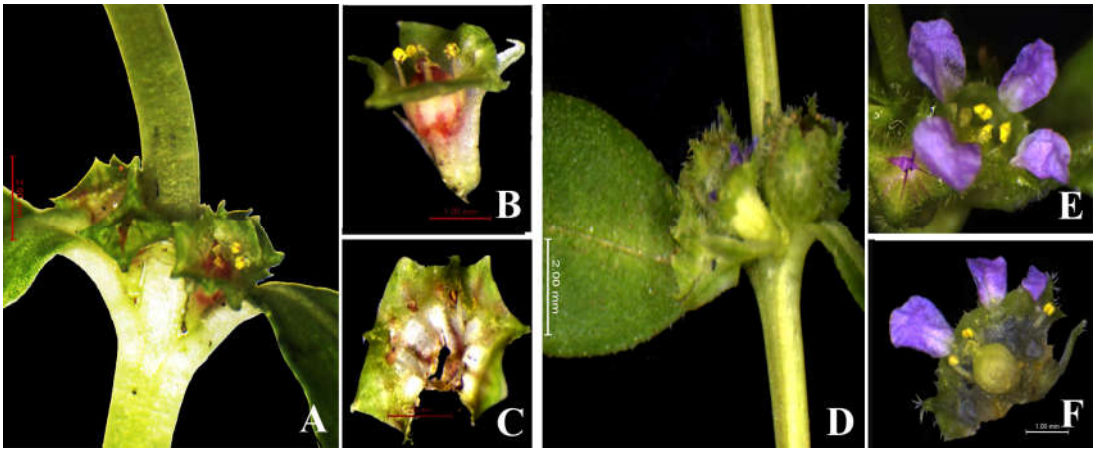

Figure 9. Inflorescence morphology of *Nesaea*. **A, B & C.** *N. brevipes;* **A.** Subsessile flowers in axillary cymes; **B.** Apetalous flower; **C.** Apetalous flower-calyx tube opened. **D, E & F.** *N. prostrata*; **D.** Single node showing inflorescence; **E.** Petaliferous flower; **F.** Flower-split opened.

***Rotala* L.**

Flowers bisexual, actinomorphic, mono or dimorphic, occasionally cleistogamous, solitary in the axils of bracts of main axis or lateral or terminal racemes, bracteoles 2, calyx tube often campanulate, occasionally suburceolate or tubular, free from but often enclosing ovary, hypanthial, calyx lobes 3− 5, triangular or acuminate, calyx appendages or small interjected

folds occasionally present between calyx lobes, nectar glands are rarely present at the base of the calyx tube in between the stamen, petals 0−5, inserted at the top of the calyx tube in between calyx lobes, showy or non showy petals, entire, erose or pinnately divided in *R. fimbriata,* elliptic or ovate or obovate or orbicular. Stamen 1−5, never more than the number of petals or calyx tube, inserted at the lower half or occasionally at the base of the calyx tube and appearing free, anthers dorsifixed, ovary superior, 2−4 locules, placentation free central at maturity, style simple, stigma often capitate, fruit septicidally dehiscent capsule, opening by 2−4 valves, seeds numerous or a few**.** **Figure 10.**

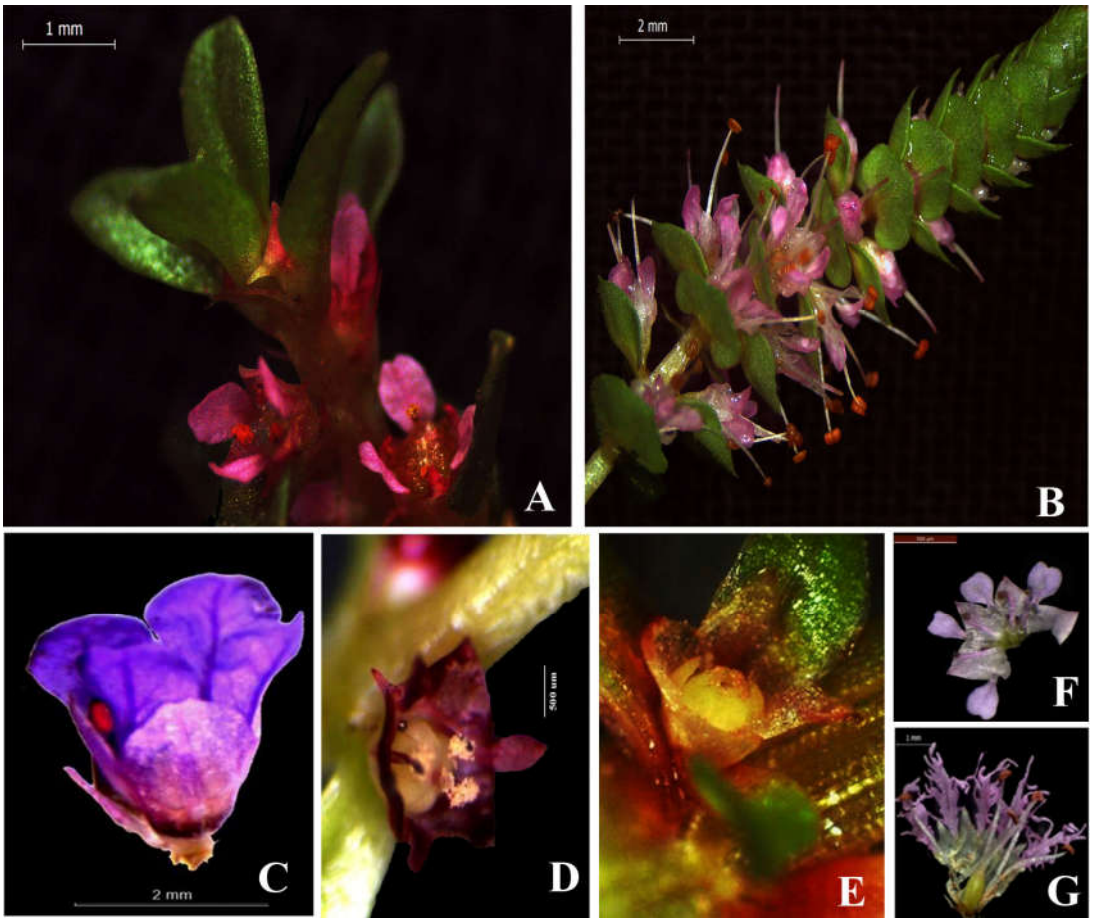

Figure 10. Inflorescence and flowers in *Rotala*. **A.** *R. anamika*- axillary solitary flowers; **B.** *R. macrandra*-flowers in terminal racemes; **C. & D.** petaliferous flower; **C.** *R. rotundifolia*; **D.** *R. malampuzhensis*; **E.** *R. occultiflora*-apetalous flower; **F.** *R. malabarica*-calyx tube split open. **G.** *R. fimbriata*-flower showing fimbriate petals.

Seed morphology

Ammannia L.

Seeds ovoid or semiovoid, 0.3−0.5×0.25−0.4 mm. The testa cells on the convex surface often reticulate (*A.baccifera, A. multiflora* and *A. octandra*), composed of irregular rows of rectangular cells arranged end-to-end, cell boundries raised and bulged out or ridged over the entire length on the outward surface . Occassionaly, testa with longitudinal rows of striations of oblong rectangular cells (*A. baccifera* subsp. *aegyptiaca*). **Figure 11. (A1-C2) & 12. (D1 & D2).**

Nesaea Kunth

Seeds, ovoid, 0.3− 0.5× 0.3− 0.4 mm. The testa cells on the convex surface often irregularly reticulate, composed of irregular rectangular cells arranged end to end, ridged over the entire length. Cells are longer in the middle comparing at ends. **Figure 12. E1-F2**

Rotala L.

Seeds semi ovoid, occasionally semi-ellipsoid, 0.3− 1.5× 0.25− 0.35 mm. Testa mostly cells reticulate in appearance, some composed of distinct rectangular or hexagonal cells arranged very close together all over the surface with cell boundaries raised to form a ridged surface (*R. malabarica, R. tulunadensis, R. macrandra, R. rosea, R. kasaragodensis, R. juniperina*), while others with indistinct long rectangular cells all over the surface with medium thickened ridged margin (*R. cookii, R. cheruchakkiensis, R. anamika*), or rarely reticulate with transverse striations of thick ridges or costae in the middle (*R. verticillaris* and *R. densiflora*).

Occasionally testa with either longitudinal rows of striations of oblong rectangular cells (*R. indica, R. occultiflora*) or longitudinal rows of striations with distinct ridges (*R. mexicana*) or with ridges which are least prominent (*R. malampuzhensis*) and with a noticeable distance between longitudinal rows.

R. fimbriata posses striated seeds with papilla in the middle of testa and thus more or less colliculate in appearance. In *R. rotundifolia*, testa consists of distinct longitudinal and transverse rows of striations separately at opposite ends. Both types of rows are very close to each other. **Figure 13-18.**

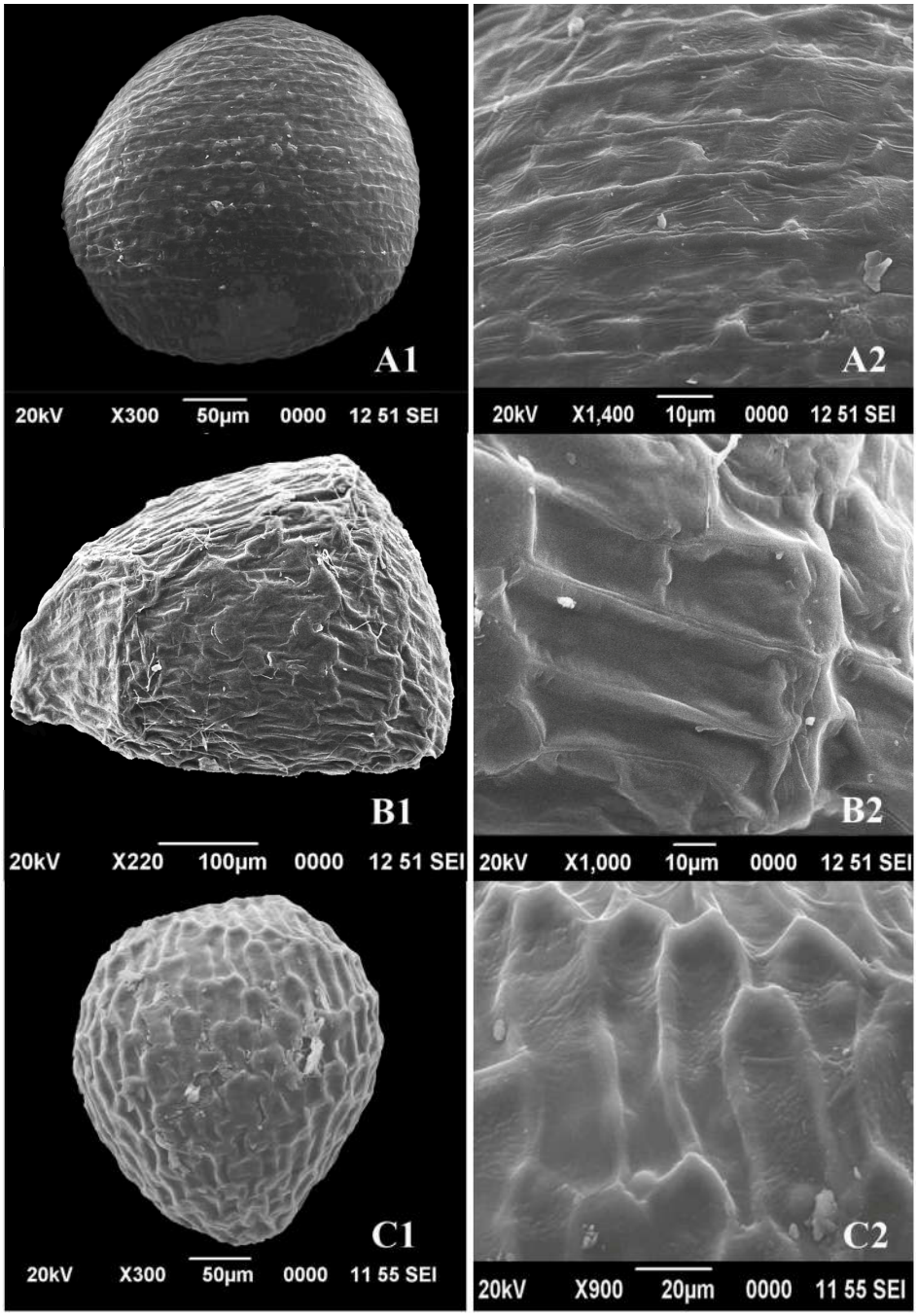

Figure 11. A1-C2. Seed surface morphology of *Ammannia*. **A1 & A2.** *A. baccifera* subsp. *aegyptiaca*; **B1 & B2.** *A. baccifera* subsp. *baccifera*; **C1 & C2.** *A. multiflora*.

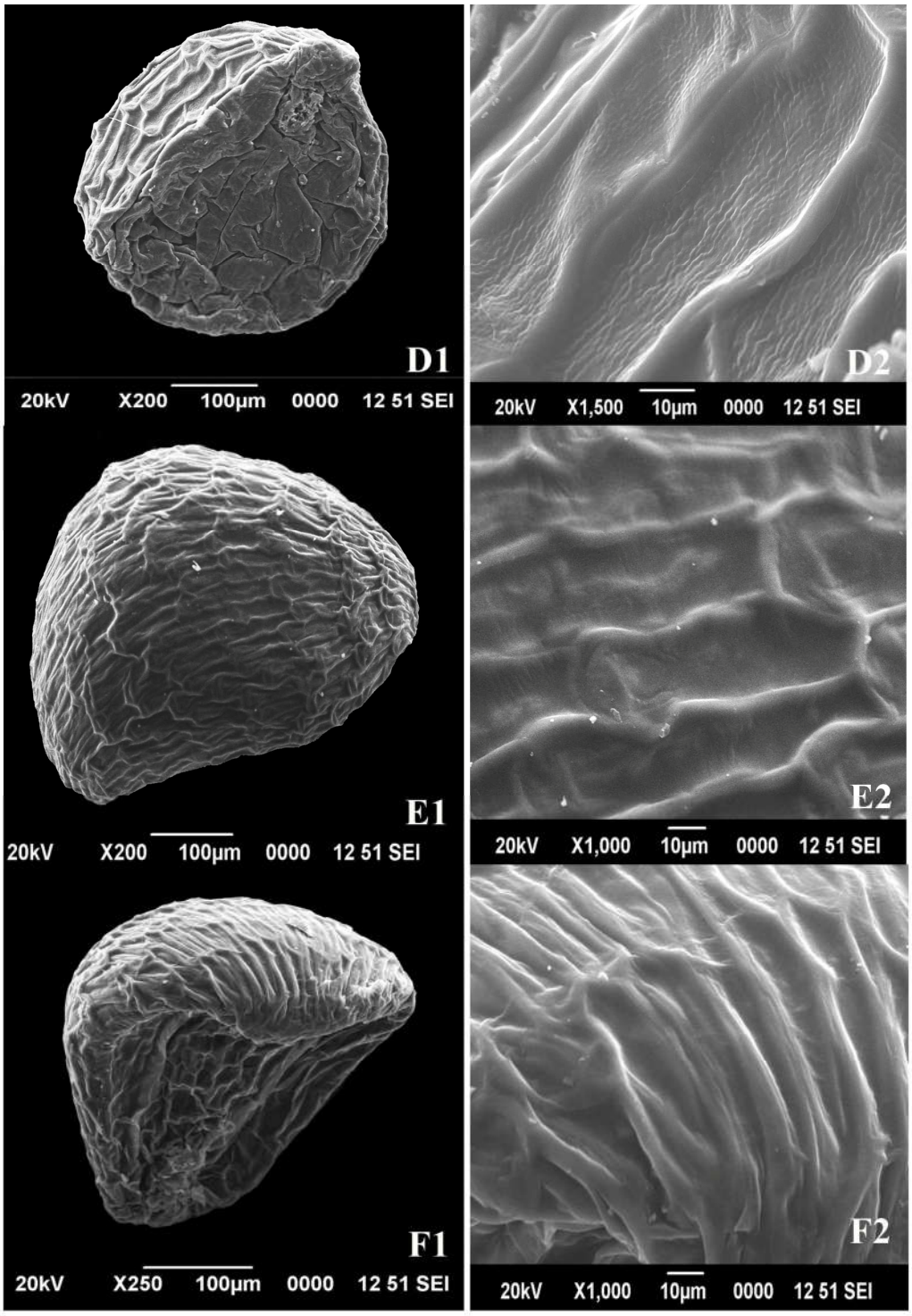

Figure 12. D1 & D2. Seed surface morphology of *A. octandra*. **E1-F2.** Seed surface morphology of *Nesaea*. **E1 & E2.** *N. brevipes*; **F1 & F2.** *N. prostrata.*

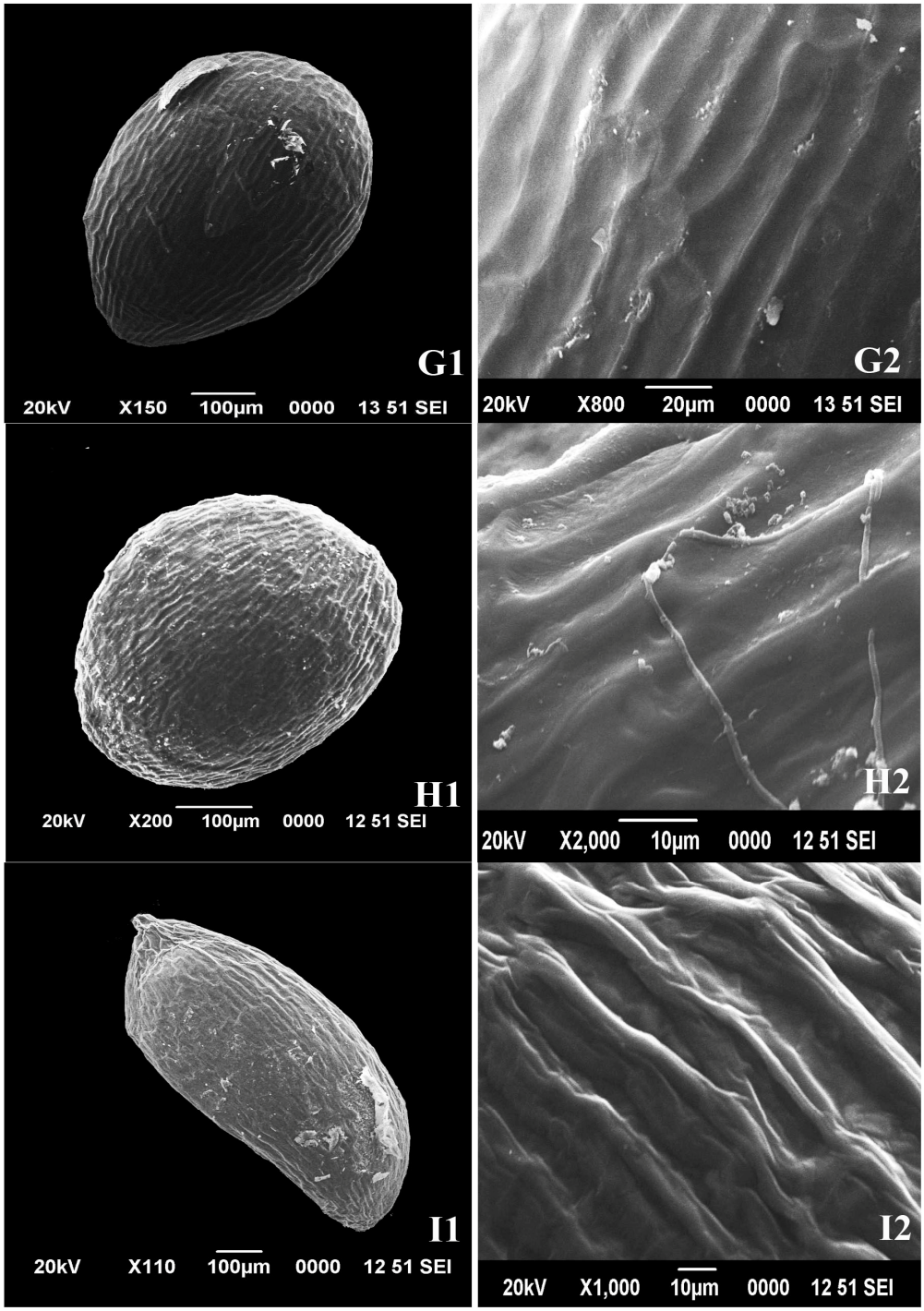

Figure 13. G1-I2. Seed surface morphology of *Rotala*. **G1 & G2.** *R. anamika*; **H1 & H2.** *R. cheruchakkiensis*; **I1 & I2.** *R. cookii*.

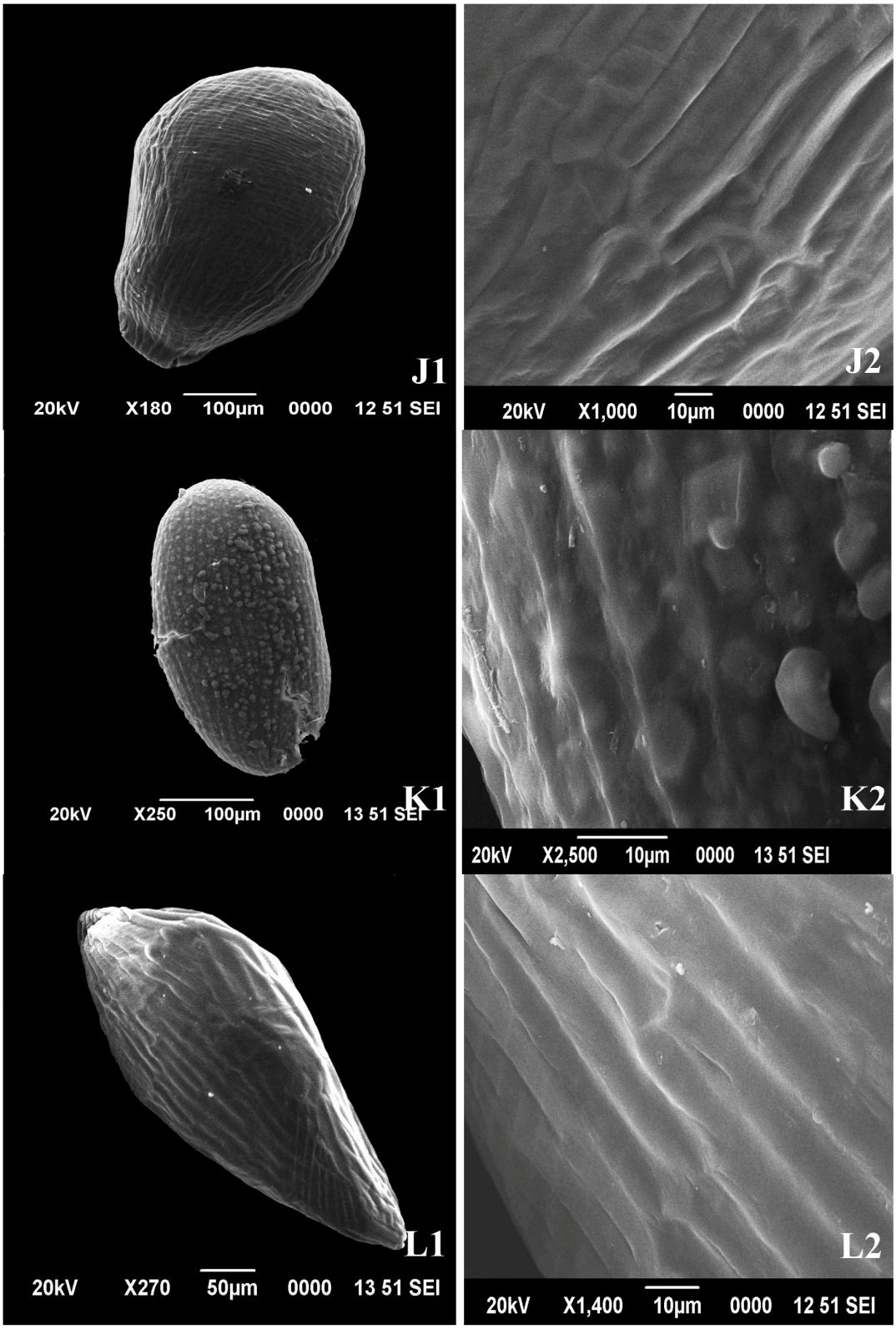

Figure 14. J1-L2. Seed surface morphology of *Rotala*. **J1 & J2.** *R. densiflora*; **K1 & K2.** *R. fimbriata*; **L1 & L2.** *R. indica*

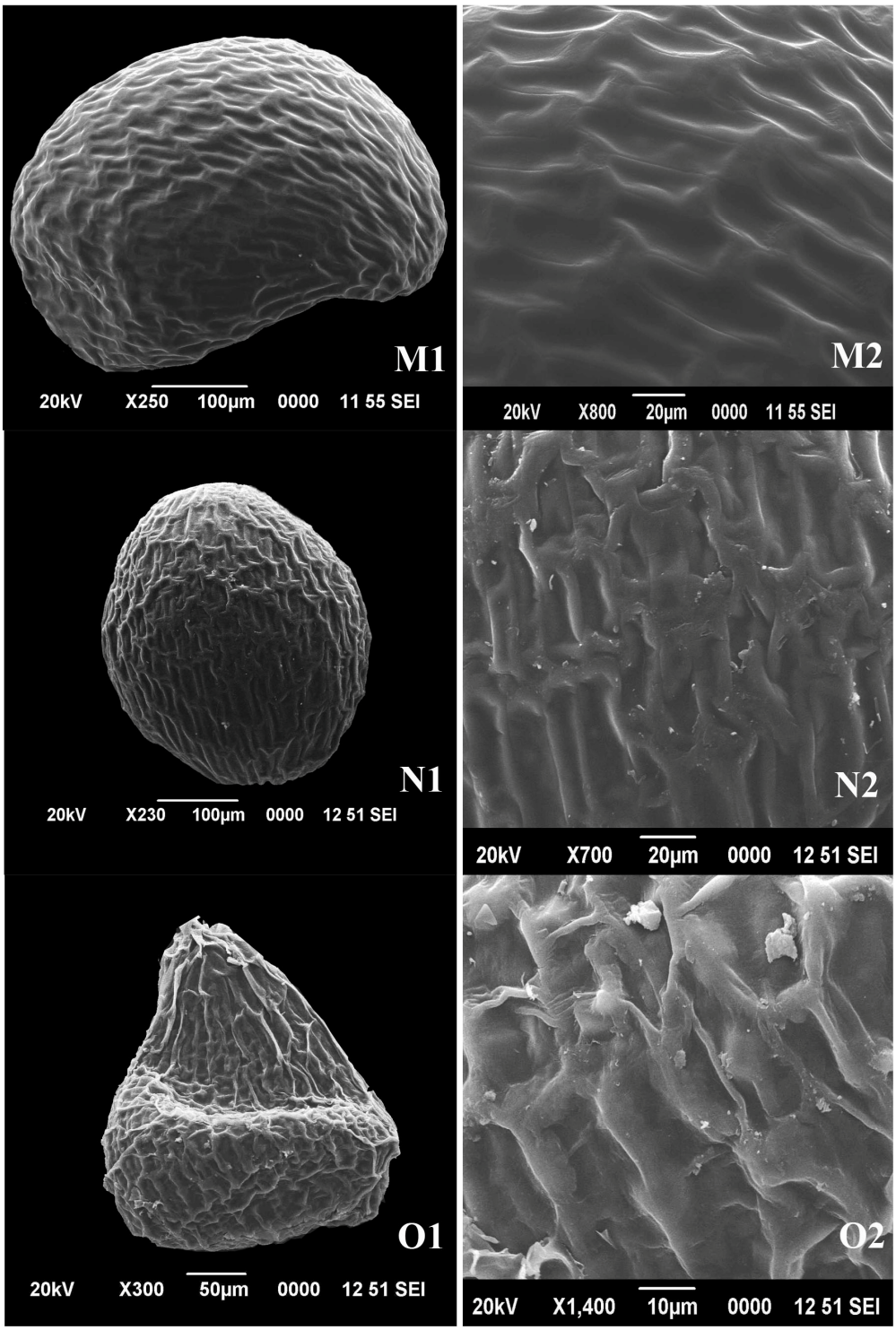

Figure 15. M1-O2. Seed surface morphology of *Rotala*. **M1 & M2.** *R. juniperina*; **N1 & N2.** *R. kasaragodensis*; **O1 & O2.** *R. macrandra*

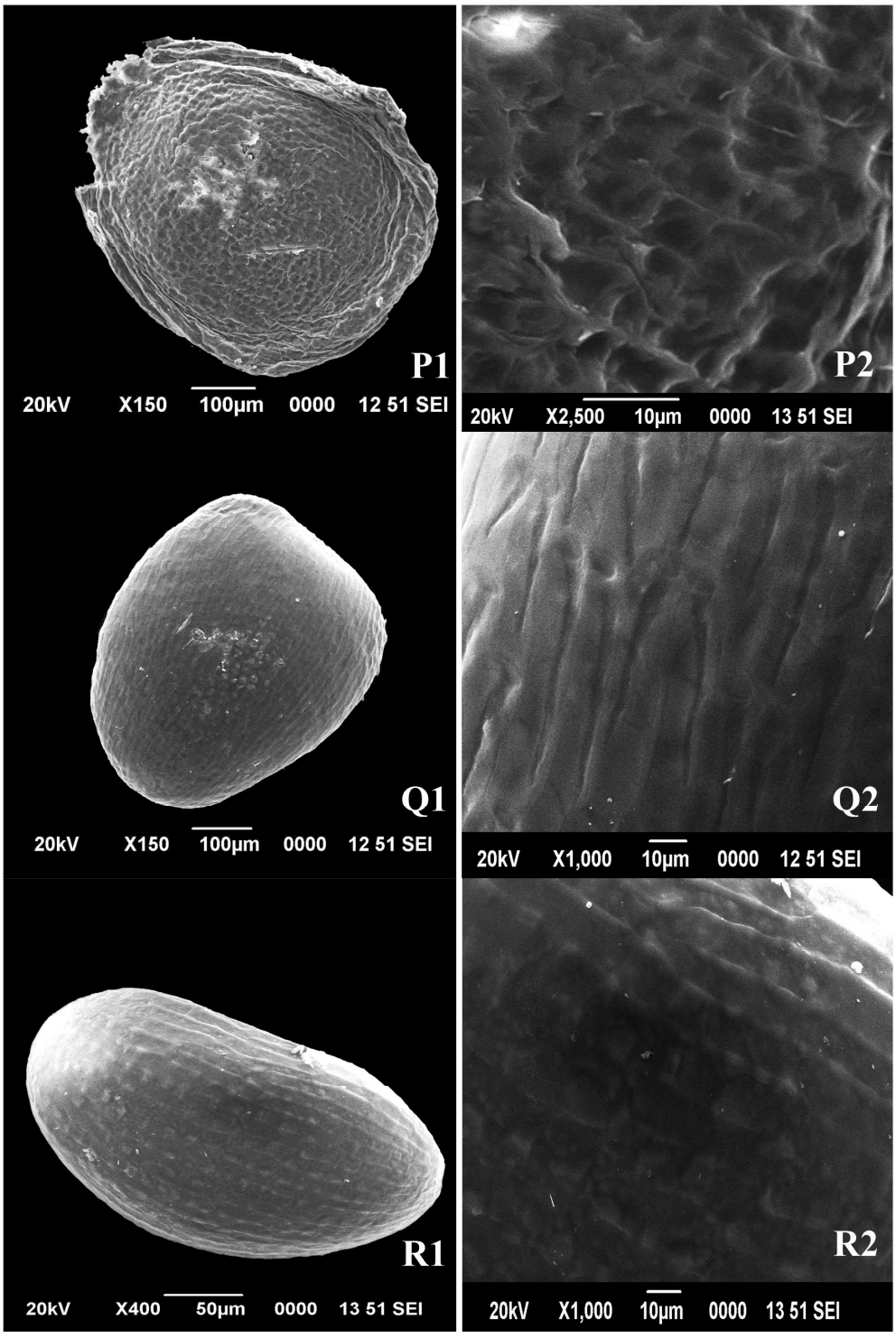

Figure 16. P1-R2. Seed surface morphology of *Rotala*. **P1 & P2.** *R. malabarica*; **Q1 & Q2.** *R. malampuzhensis*; **R1 & R2.** *R. mexicana.*

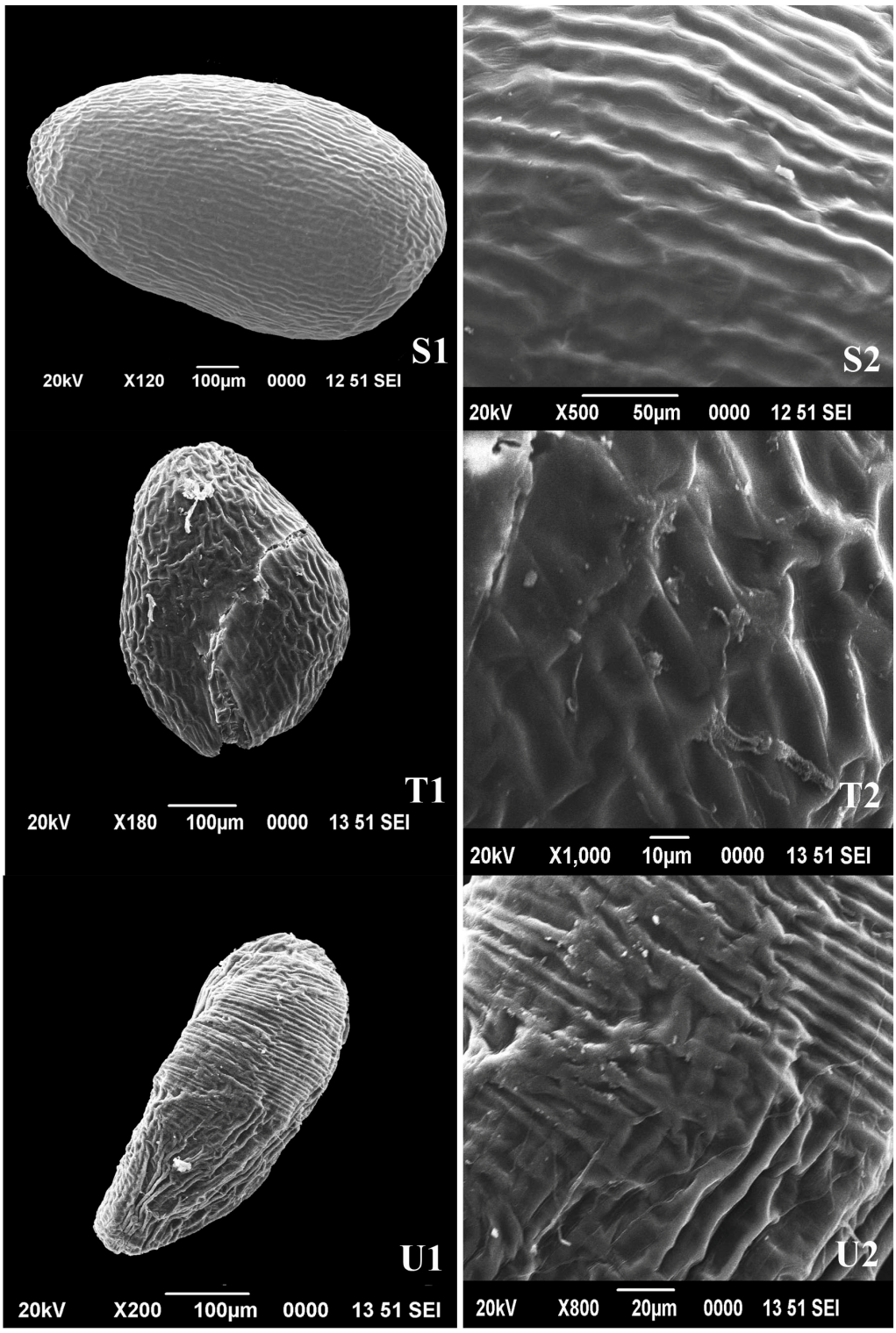

Figure 17. S1-U2. Seed surface morphology of *Rotala*. **S1 & S2.** *R. occultiflora*; **T1 & T2.** *R. rosea*; **U1 & U2.** *R. rotundifolia.*

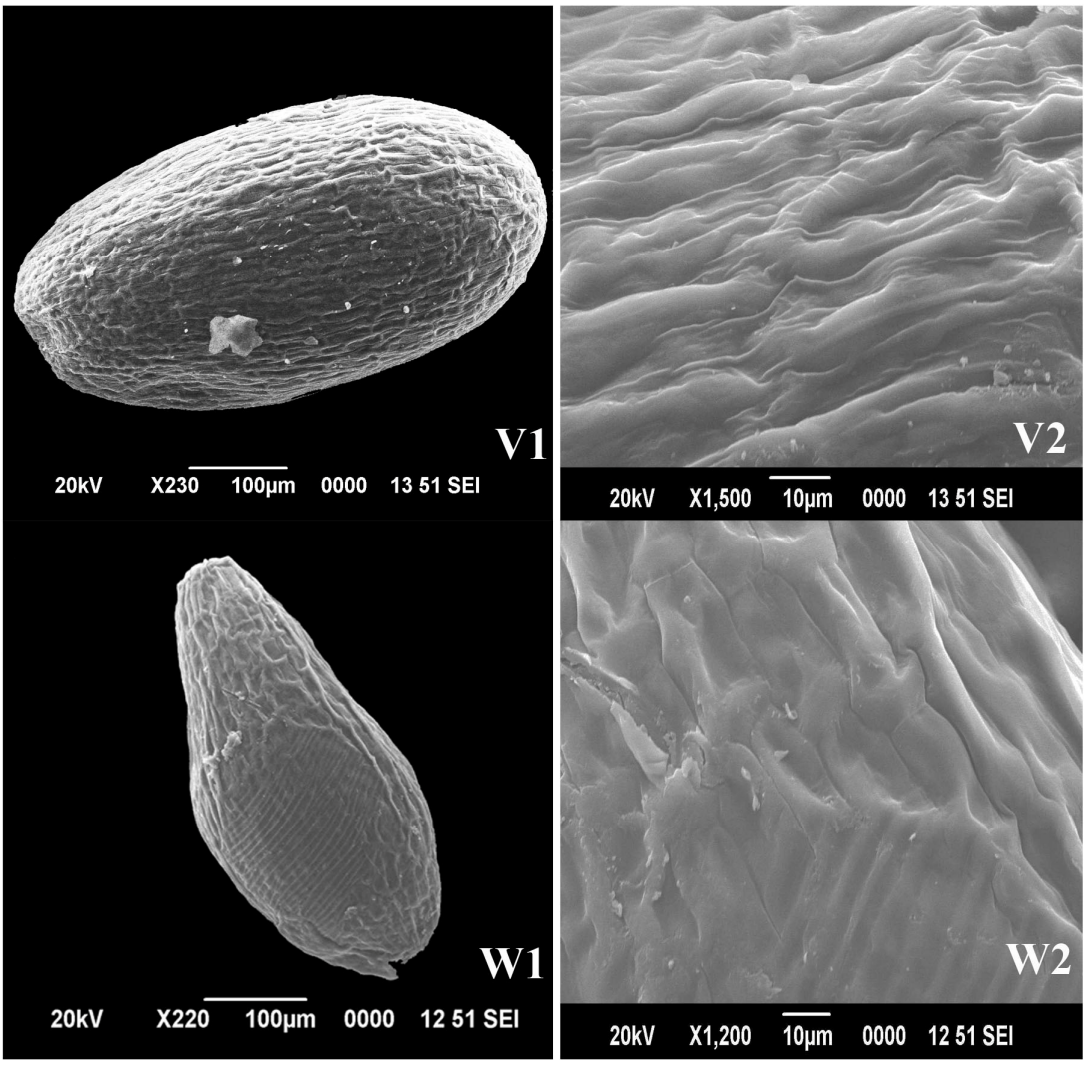

Figure 18. V1-W2. Seed surface morphology of *Rotala*. **V1 & V2.** *R.tulunadensis*; **W1 & W2.** *R. verticillaris.*

Discussion

General morphological characters support a distant relationship of the genus *Rotala* with other two genera *Ammannia* and *Nesaea.*The characters traditionally used to distinguish these genera are the inflorescence type and the nature of the capsule. *Ammannia* and *Nesaea* share some common characteristics such as cymose inflorescence with tightly clustered flowers and smooth capsule. Even very few characters were analyzed, that are studied

to link these three genera and they are similar habitat, amphibious (with a few exception for the genus *Rotala*) glabrous plant parts, simple entire margined primarily decussate leaves and aerenchymatous stem. Very few of these mutual morphological characters among these three genera are also described in some unrelated amphibious and aquatic members of other family. Such shared similarities were explained as 'convergent adaptation to ecologically specialized habitats with seasonally fluctuating water levels' (Cook, 1979).

The three genera inhabit almost similar kinds of inundated areas such as rice fields, ditches, low swales and seasonally wet places. Amphibious to strictly aquatic species are found in the genus *Rotala*, although *Ammannia* and *Nesaea* prefer to growing wet on seasonally inundated fields. These aquatic species are usually characterized with floating or submerged vegetative stems and submerged or emergent inflorescences. Usually *Ammannia* and *Nesaea* are annual and *Rotala* is either annual or perennials. *Ammannia* and *Rotala* are found to be very common in South India where as only two species of *Nesaea* could be described from South Indian states. Southern Asian species of these three genera display greater morphological diversity than the African taxa (Graham *et al.,* 2011).

The genus *Rotala* shows greater generic adaptability and vegetative plasticity than other herbaceous genera of Lythraceae. Variation in leaf and stem characterstics of different species of *Ammannia* and *Nesaea* are found to be very limited. Range of variation is found to be high in the genus *Rotala*, where noticeable variations were observed in both size and shape of these characterestics. Leaf base character was found to be useful in distinguishing New world species of *Ammannia* and *Rotala.* Cordate to auriculate leaf bases are usually found in *Ammannia* while attenuate to truncate leaf bases in *Rotala* (Graham *et al*., 2011). Dimorphism of leaf also is a perceptible character in *Rotala*, particularly in aquatic species as dimorphic character of leaves of aquatic plants is proved to be due to some environmental condition

such as relative nutritional requirements and cellular water requirement (Allsopp, 1965; Sculthorpe, 1967).

Inflorescence type is considered as a distinguishable character for the recognition of genera. The inflorescence in *Ammannia* and *Nesaea* is always clustered axillary cyme, while in *Rotala* it never be cyme, instead they form solitary receme, occasionally terminal spikes. The shape and size of flowers overlap considerably and thus it could not be taken as distinguishable character at generic level. All the three genera include both petalous and apetalous species and are useful in recognizing species. In the genus *Rotala* floral merism varies from trimerous to pentamerous, where as in *Ammannia* and *Nesaea* it was always found to be tetramerous. Graham *et al.* (2005; 2011) recorded less frequent penta to octa-merous and tetra to octa-merous African species of *Ammannia* and *Nesaea* respectively. Heterostyly is not reported from any of the species belonging to the genera under study. However, it occures in some African taxa (Koehne, 1903). Koehne (1903) described six trimorphic species and ten dimorphic species of *Nesaea* followed by description of four dimorphic species of *Rotala* by Cook (1979). Later Immelman (1991) recorded heterostyly in *Nesaea* and Graham *et al*. (2005, 2011) reported the same in both *Nesaea* and *Rotala.* Position of stamens varies among different species in three genera from base to middle of the floral tube and this character is not useful for treatment at generic level. Also in all three genera the staminal arrangement is observed as haplostemonous.

Transversly striated capsular wall is found to be unique to the genus *Rotala*. Various anatomical studies (Leeuwen, 1974; Panigrahi, 1976) have shown these striations are constituted by inner layer of bilayered pericarp in which the cells are narrowly elongated and lignified. In *Ammannia* and *Nesaea* these striations are absent and the capsules are appeared as smooth walled. Septicidal dehiciscence of capsule is also considered as a character to distinguish *Rotala* from other two genera. In *Ammannia* the dehiscence is

irregular and in *Nesaea* it is initially circumscissile dehiscence and then split irregularly. But the mode of dehiscene as a diagnostic character is often found to be perplexing since circumscissile capsules and those bursting irregularly were present both in *Ammannia* and *Nesaea* (Verdcourt, 1994; Immelman, 1991). The distinction based on continuity of placenta with style or lack of continuity, which was initially used by Koehne (1903) to separate two tribes Nesaeeae and Lythreae, was found to be erroneous. Studies have shown that (Tobe *et al*., 1998; Graham *et al*., 2011) connection between placenta and style varies with the age, expansion and final shape of placenta.

It has been pointed out by many authors that seed characterstics such as seed size, shape and testa properties can be used in distinguishing taxa (Gunn, 1970, 1971 & 1982; Perrino, 1984). The only significant work on the seed surface, morphology of the three genera was that of Panigrahi (1986). However she has included only a few taxa represented in South India. In the present study, we observed that seed shape and testa properties have a major role in determining taxa. Most of the species belonging to these three genera have semi ovoid seed with regularly or irregularly reticulate testa surface. While several others had unique shape or testa ornamentation. Generally, seed surface morphology plays an important role in distinguishing taxa at species and intraspecific level.

Considering various morphological features, it is evidenced that, the genus *Rotala* is far distinct from *Ammannia* and *Nesaea*. *Ammannia* and *Nesaea* share many features in common, often posing problems in generic delimitation. The present study based on morphological characteristics support merging of *Nesaea* with *Ammannia.*

Anatomical characterization

Anatomical characters are found to be a useful source of information for the systematic elucidation and inferring phylogenetic inferences. These have been observed to be useful from lower specific level to higher levels and are reported to be valuable when morphological characters are perplexing in some instances. Anatomical description of different vegetative structures like leaf and stem plays a crucial role in the systematic treatment. The anatomical characters of vegetative organs are known to undergo considerable variation not only based on the habitat but also according to species. Constancy of these anatomical features can be expected in species when all the individuals of which live under absolutely uniform condition.

Several authors discussed the application of anatomical characters in describing three closely similar species *Ammannia, Nesaea* and *Rotala* of Lythraceae. Panigrahi (1976), employed vegetative anatomical character of stem to delimit four genera, *Ammannia, Rotala, Nesaea* and *Hionanthera*. Further she described some species of the genus *Ammannia* (1986) and the genus *Rotala* (1988) in detail with the aid of anatomy of stem, leaf and cuticle. Later very few researchers discussed the vegetative anatomical features regarding the genus *Ammannia* and *Rotala* separately. The present chapter gives an account of anatomical characters *viz.,* stem anatomy, leaf midrib anatomy and cuticular studies useful to delimit three South Indian genera *Ammannia, Rotala* and *Nesaea*.

Materials and Methods

The present study is based on fresh materials of 18 species belonging to *Ammannia, Rotala* and *Nesaea*, which were collected from different regions of South India. Samples for anatomy of the stem and mature leaves were chosen from three accessions (except for *R. tulunadensis*). All

assessments were made on all plants at almost similar developmental stages. Stem and leaves from living plants were fixed immediately after collection in formalin-acetic acid-alcohol (FAA) solution for a minimum of 24 hours and washed in 70% alcohol. Leaves from herbarium specimens were fully rehydrated. For the stem anatomy, hand sections of stem were taken transversally. For the mid rib anatomy, midrib from the basal most part of the lamina were excised and subjected to hand sectioning. For the cuticular study, epidermal layers were isolated either by simple hand peeling or by maceration in 40% nitric acid. Both transverse sections and epidermal peelings were stained in 1% aqueous solution of saffranin for about 3 minutes. Excess stain was rinsed off with distilled water. The specimen was then placed in droplets of glycerin on a glass slide and covered with a cover glass for microscopic study to determine various characters related to stem coss section (cross section area, nature of cortex, pith etc.), midrib cross section and epidermal peeling (stomatal complex types, stomatal density, stomatal index, and stomatal size). All these reading were based on average obtained from observations of three microscopic fields for each accession, under Leica DM2500 Phase contrast microscope (40X). Photomicrographs of good preparations were taken at a magnification of ×400 objective. The length, breadth and size measurements were measured with micrometer eyepiece graticule. To analyze the important cuticular character, stomatal index (SI), the formula described by Wilkinson (1979) was used.

Results

Leaf midrib anatomy

Ammannia baccifera subsp. *aegyptiaca* (Willd.) Koehne

Midrib 0.5−1.5 mm thick. Adaxial side less flat, glabrous, while the abaxial surface is convex. Epidermal cells on both adaxial and abaxial surface equal

in size. The ground tissue is parenchymatous with druses. The vascular bundle, bicollateral, arc or crescent shaped, 0.14−0.2 mm thick. Number of vascular strands varies from 7−14. Pith is parenchymatous with a few druses.

Figure 19. A1 & A2.

***Ammannia baccifera* subsp. *baccifera* L.**

Midrib 0.25−0.35 mm thick. Adaxial side slightly concave, glabrous, while the abaxial surface is convex to rounded. Epidermal cells on both adaxial and abaxial surface equal in size. The ground tissue is parenchymatous with druses. The vascular bundle bicollateral, arc or crescent shaped 0.045− 0.056 mm thick. Number of vascular strands varies from 6−12. Single layer of sclerenchyma cells surrounding the vascular structure. Pith is parenchymatous with a few druses. **Figure 19. B1 & B2**

***Ammannia multiflora* Roxb.**

Midrib 0.2−0.4 mm thick. Adaxial side deeply cleft, glabrous, while the abaxial surface is convex to rounded. Epidermal cells on both adaxial and abaxial surface equal in size. The ground tissue is parenchymatous with druses. The vascular bundle bicollateral, arc or crescent shaped 0.04−0.07 mm thick. Number of vascular strands varies from 9−12. Pith is parenchymatous with a few druses. **Figure 19. C1 & C2.**

***Ammannia octandra* L. f.**

Midrib 0.5− 0.9 mm thick. Adaxial side, slightly concave, glabrous, while the abaxial surface is rounded. Epidermal cells on adaxial surface slightly larger than the cells on abaxial surface. The ground tissue is parenchymatous with druses. The vascular bundle, bicollateral, arc or crescent

shaped 0.06−0.08 mm thick. Number of vascular strands varies from 12−17. Single layer of sclerenchyma cells surrounding the vascular bundle forms a continuous ring. Pith parenchymatous with very few druses.

Figure 20. D1 & D2.

***Nesaea brevipes* Koehne**

Midrib 0.5−0.6 mm thick. Adaxial side slightly cleft, glabrous, while the abaxial surface is convex to rounded. Epidermal cells on cells on both adaxial and abaxial surface equal in size. The ground tissue is parenchymatous without druses. The vascular bundle, bicollateral, arc or crescent−shaped 0.05−0.1 mm thick. Number of vascular strands varies from 7− 13. Sclerenchyma cells surrounding the vascular bundle absent. Pith Parenchymatous with very few druses. **Figure 20. E1 & E2.**

***Nesaea prostrata* (Buch.-Ham. ex Dillwyn) Suresh**

Midrib 0.3− 0.6 mm thick. Adaxial side slightly concave, glabrous, while the abaxial surface is convex. Epidermal cells on cells on both adaxial and abaxial surface equal in size. The ground tissue is parenchymatous without druses. The vascular bundle, bicollateral, arc or crescent−shaped 0.02−0.03 mm thick. Number of vascular strands varies from 6−8. Sclerenchyma cells surrounding the vascular bundle absent. Pith is parenchymatous with a few druses. **Figure 20. F1 & F2**

Various diagnostic characters analysed in midrib sections are given in the table 4.

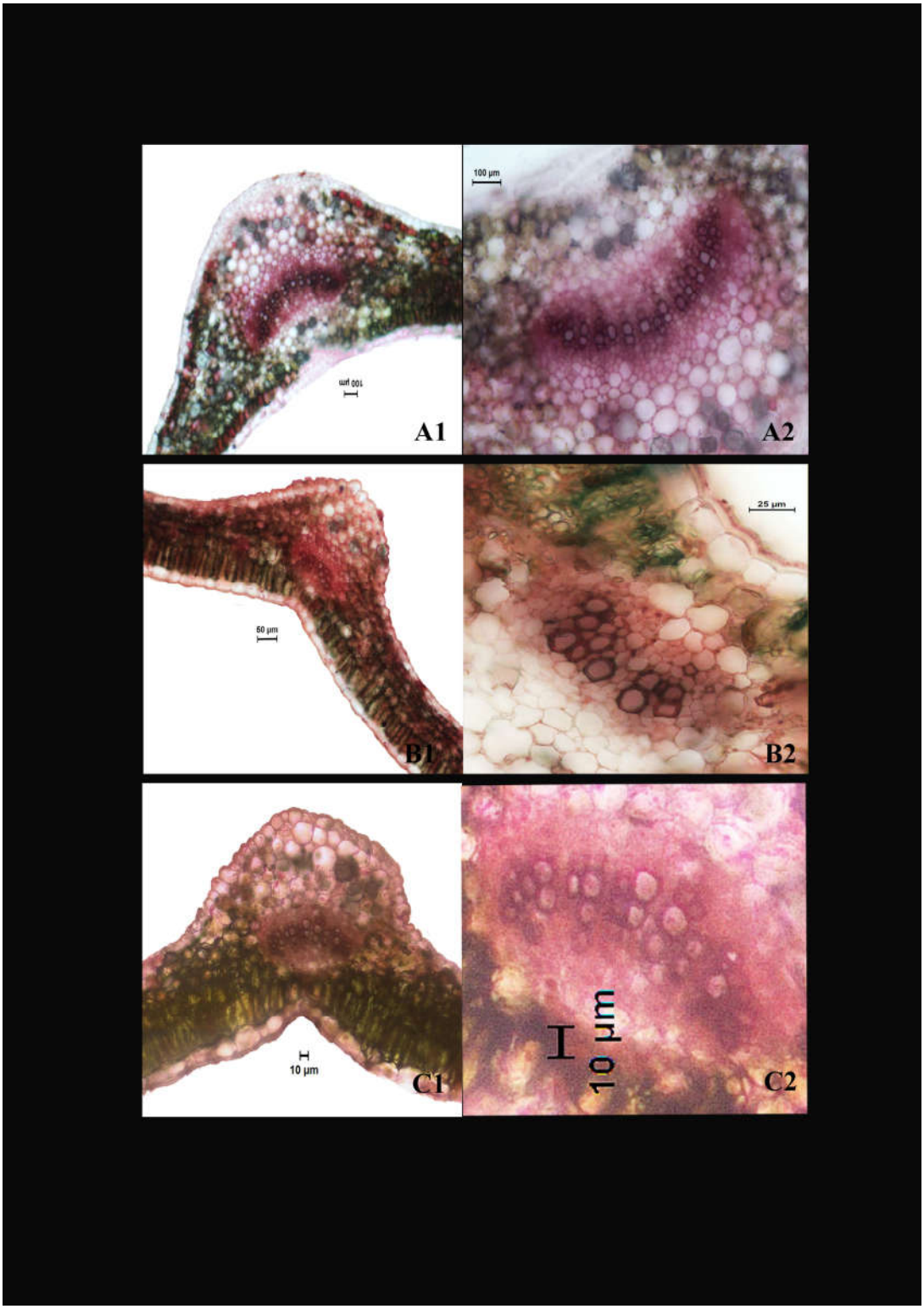

Figure 19. *Ammannia*-midrib transverse section**.** (**A1, B1 & C1.** Entire view; **A2, B2 & C2.**Vascular bundles); **A1 & A2.** *A. baccifera* subsp. *aegyptiaca*; **B1 & B2.** *A. baccifera* subsp. *baccifera*; **C1 & C2.** *A. multiflora*

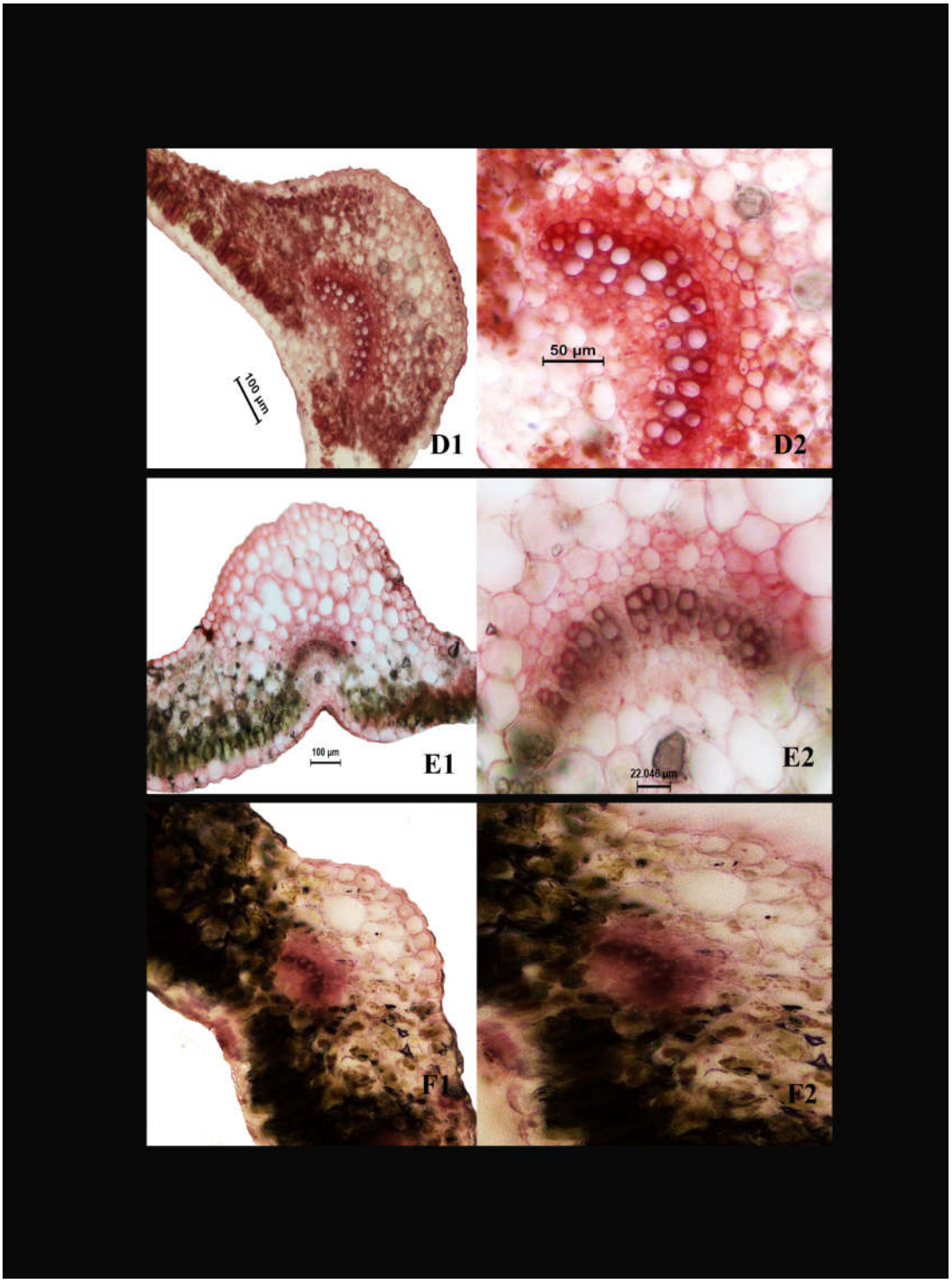

Figure 20. *Ammannia* & *Nesaea*-midrib transverse section**. D1, E1 & F1-** Entire view; **D2, E2 & F2.** Vascular bundles; **D1 & D2.** *A. octandra*; **E1 & E2.** *N. brevipes*; **F1& F2.** *N. prostrata*

***Rotala densiflora* (Roth *ex* Roem. & Schult.) Koehne**

Midrib 0.2−0.3 mm thick. Adaxial side deeply cleft, glabrous, while the abaxial surface is convex to rounded. Epidermal cells on cells on adaxial surface is larger than that on abaxial surface. The ground tissue is parenchymatous with druses. The vascular bundle bicollateral, arc or crescent−shaped 0.015−0.03 mm thick. Number of vascular strands varies from 6−8. Sclerenchyma cells surrounding the vascular bundle absent. Pith is parenchymatous with very few druses. **Figure 21. G1 & G2.**

***Rotala fimbriata* Wight**

Midrib 0.3− 0.5 mm thick. Adaxial side shallowly clefted, glabrous, while the abaxial surface is rounded. Epidermal cells on both adaxial and abaxial surface equal in size. The ground tissue is parenchymatous without druses. The vascular bundle, bicollateral arrangement, arc or crescent−shaped 0.03− 0.05 mm thick. Number of vascular strands varies from 7− 9. Sclerenchyma cells surrounding the vascular bundle absent. Pith is parenchymatous without druses. **Figure 21. H1 & H2**

***Rotala indica* (Willd.) Koehne**

Midrib 0.09−0.3 mm thick. Adaxial side, less flat to slightly curved, glabrous, while the abaxial surface is convex. Epidermal cells on both adaxial and abaxial surface equal in size, thick walled. The ground tissue is parenchymatous with much druses. The vascular bundle, bicollateral, arc or crescent−shaped 0.03−0.04 mm thick. Number of vascular strands varies from 8−10. Single or double layer of sclerenchyma cells surrounding the vascular bundle. Pith parenchymatous with druses. **Figure 21. I1& I2**

***Rotala juniperina* A. Fernandes**

Midrib 0.2− 0.3.mm thick. Adaxial side, flat to slightly undulate, glabrous, while the abaxial surface is rounded. Epidermal cells on adaxial surface slightly larger than that of the abaxial surface. The ground tissue is parenchymatous without druses. The vascular bundle bicollateral, arc or crescent−shaped 0.01−0.03 mm thick. Number of vascular strands varies from 5− 6. Sclerenchyma cells surrounding the vascular bundle absent. Pith is parenchymatous with druses. **Figure 22. J1 & J2**

***Rotala macrandra* Koehne**

Midrib 0.2− 0.4 mm thick. Adaxial side, flat, glabrous, while the abaxial surface is convex. Epidermal cells on both adaxial and abaxial surface equal in size, thick walled. The ground tissue is parenchymatous with druses. The vascular bundle, bicollateral, arc or crescent−shaped 0.01−0.03 mm thick. Number of vascular strands varies from 8−10. Sclerenchyma cells surrounding the vascular bundle. Pith is parenchymatous without druses.

Figure 22. K1 & K2

***Rotala malabarica* Pradeep, K.T. Joseph *et* Sivar.**

Midrib 0.13− 0.15 mm thick. Adaxial surface, less flat to slightly concave, glabrous, while the abaxial surface is slightly ridged. Epidermal cells on adaxial surface slightly larger than that on the abaxial surface. The ground tissue is parenchymatous without druses. The vascular bundle, bicollateral, less flat, 0.015−0.02 mm thick. Number of vascular strands, 5 or 6. Sclerenchyma cells surrounding the vascular bundle is absent. Pith is parenchymatous without druses. **Figure 22. L1 & L2**

***Rotala malampuzhensis* R.V. Nair *ex* C.D.K. Cook**

Midrib 0.1− 0.3 mm thick. Adaxial side, slightly concave, glabrous, while the abaxial surface is abruptly rounded. Epidermal cells on both adaxial and abaxial surface equal in size. The ground tissue is parenchymatous without druses. The vascular bundle, bicollateral, arc or crescent−shaped 0.01− 0.03 mm thick. Number of vascular strands varies from 6−7. Sclerenchyma cells surrounding the vascular bundle absent. Pith is parenchymatous with druses. **Figure 23. M1 & M2**

***Rotala mexicana* Cham. & Schldtl.**

Midrib 0.1− 0.2 mm thick. Adaxial side, slightly undulate, glabrous, while the abaxial surface is convex. Epidermal cells on abaxial surface much larger than that on the adaxial surface. The ground tissue is parenchymatous without druses. The vascular bundle, bicollateral, arc or crescent−shaped 0.01− 0.015 mm thick. Number of vascular strands, 1or 2. Sclerenchyma cells surrounding the vascular bundle absent. Pith is parenchymatous without druses. **Figure 23. N1 & N2**

***Rotala occultiflora* Koehne**

Midrib 0.05− 0.085 mm thick. Adaxial surface, less flat to slightly convex, glabrous, while the abaxial surface is obscurely ridged. Epidermal cells on adaxial surface slightly larger than that on the abaxial surface. The ground tissue is parenchymatous without druses. The vascular bundle, bicollateral, less flat, 0.004−0.008 mm thick. Number of vascular strands, 1 or 2. Sclerenchyma cells surrounding the vascular bundle absent. Pith is parenchymatous without druses. **Figure 23. O1 & O2**

***Rotala rosea* (Poiret) C.D.K. Cook**

Midrib 0.3−0.7 mm thick. Adaxial side sinuate, glabrous, while the abaxial surface is abruptly rounded. Epidermal cells on adaxial surface is larger than that on abaxial surface. The ground tissue is parenchymatous with druses. The vascular bundle, bicollateral, arc or crescent-shaped 0.03−0.07 mm thick. Number of vascular strands varies from 6−7. Sclerenchyma cells surrounding the vascular bundle absent. Pith is parenchymatous with druses.

Figure 24. P1 & P2

***Rotala rotundifolia* (Buch.- Ham. *ex* Roxb.) Koehne**

Midrib 0.25− 0.4 mm thick. Adaxial side, less flat to shallowly furrowed, glabrous, while the abaxial surface is convex. Epidermal cells on adaxial surface slightly larger than that of the abaxial surface, thick walled on abaxial surface. The ground tissue is parenchymatous with very few druses. The vascular bundle, bicollateral arrangement, arc or crescent-shaped 0.015−0.02 mm thick. Number of vascular strands varies from 7−9. Sclerenchyma cells surrounding the vascular bundle. Pith is parenchymatous without druses.

Figure 24. Q1 & Q2

***Rotala tulunadensis* K.S. Prasad, P. Biju, C. Ravi & K.G. Bhat**

Midrib 0.18−0.2 mm thick. Adaxial side, abruptly concave, glabrous, while the abaxial surface is convex. Epidermal cells on both adaxial and abaxial surface equal in size, thick walled. The ground tissue is parenchymatous with much druses. The vascular bundle bicollateral, arc-shaped 0.02−0.03 mm thick. Number of vascular strands varies from 8−10. Sclerenchyma cells surrounding the vascular bundle absent. Pith is parenchymatous with druses. **Figure. 24. R1 & R2**

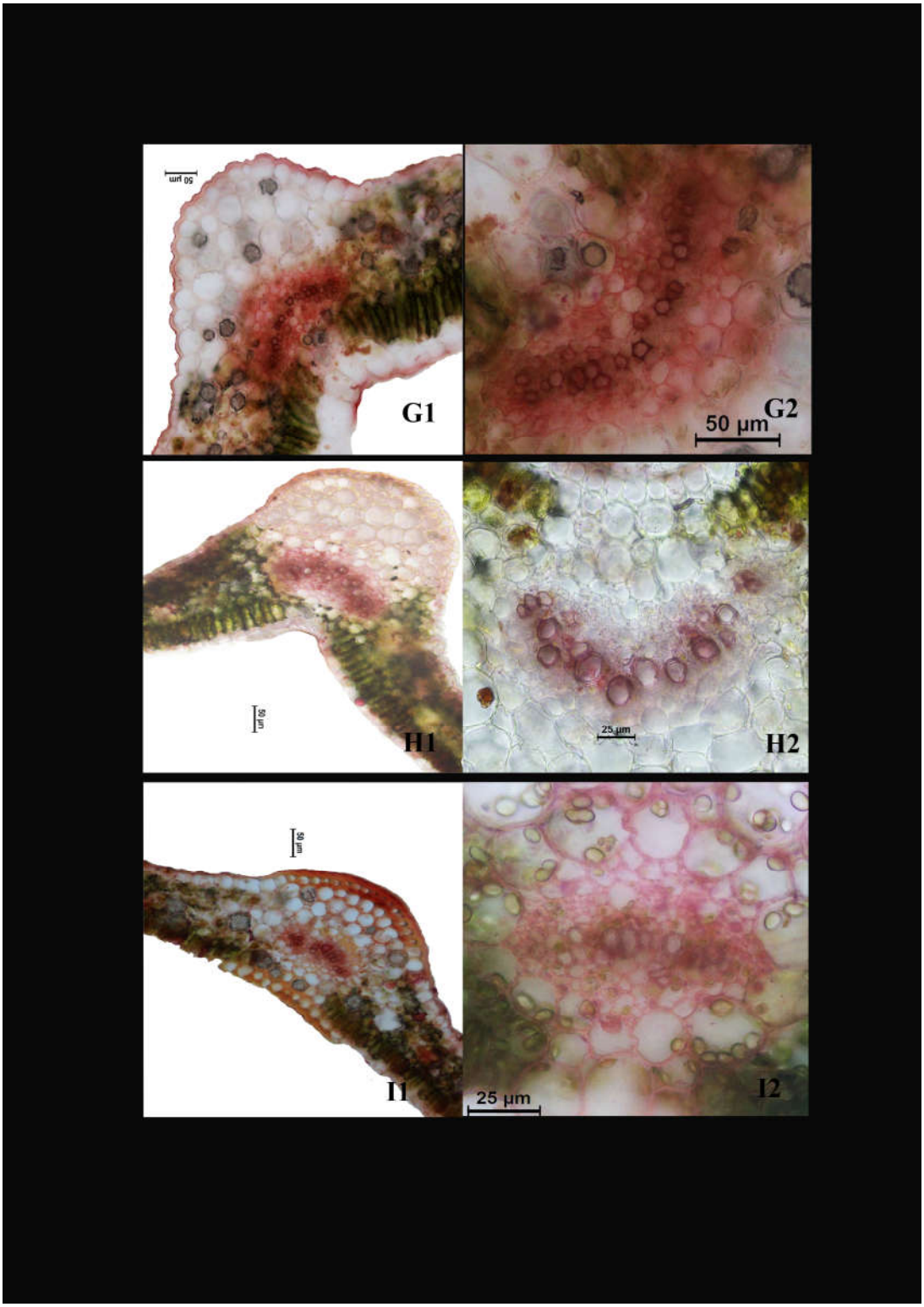

Figure 21: *Rotala*-midrib transverse section**.** (**G1, H1 & I1.** Entire view; **G2, H2 & I2.** Vascular bundles); **G1 & G2.** *R. densiflora*; **H1 & H2.** *R. fimbriata*; **I1 & I2.** *R. juniperina*

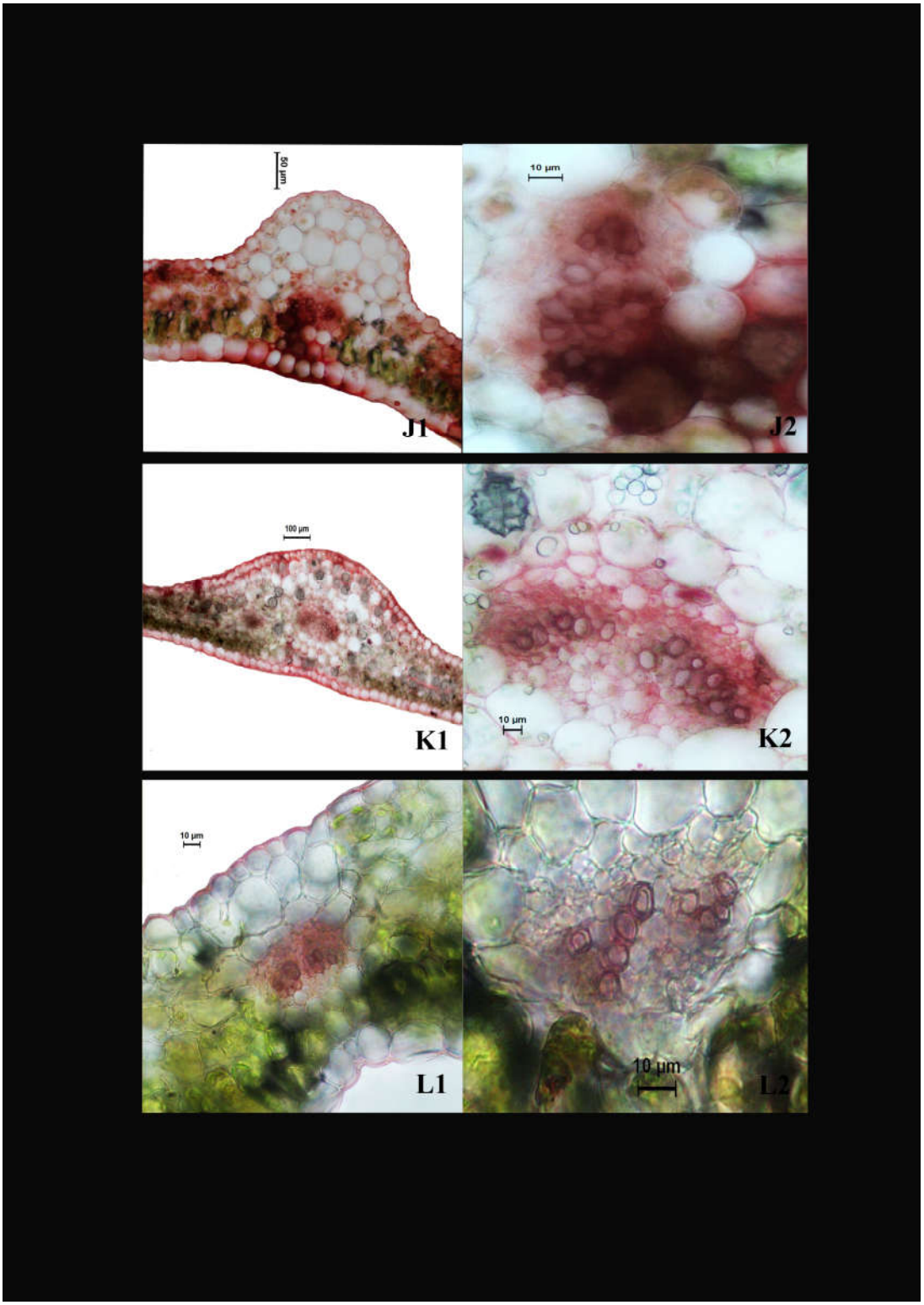

Figure 22. *Rotala*-midrib transverse section. (**J1, K1 & L1.** Entire view the stem transverse section; **J2, K2 & L2**; Vascular bundles); **J1 & J2.** *R. juniperina*; **K1 & K2.** *R. macrandra*; **L1 & L2.** *R. malabarica*

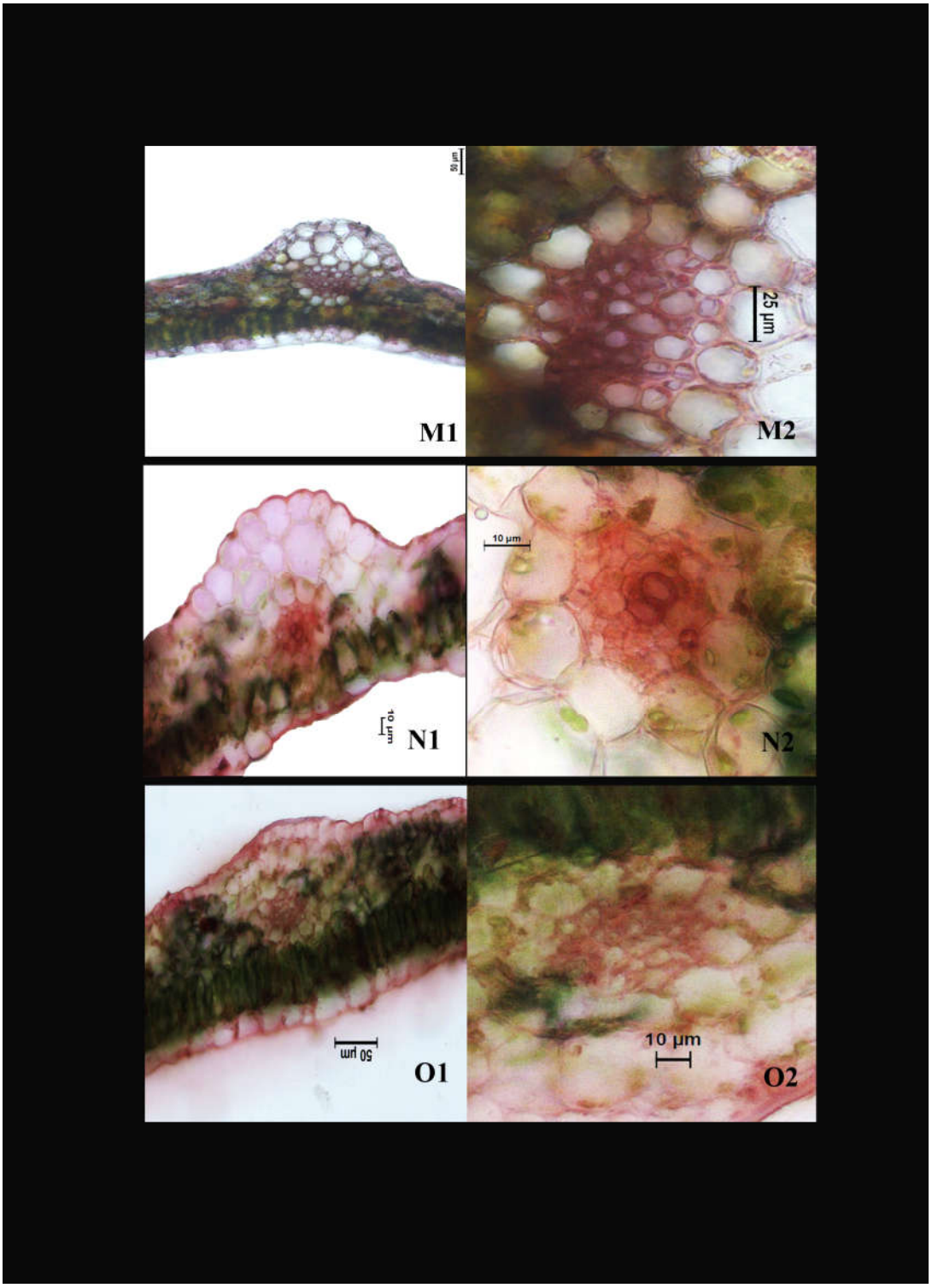

Figure 23. *Rotala*-midrib transverse section. (**M1, N1 & O1.** Entire view; **M2, N2 & O2.** Vascular bundles); **M1 & M2.** *R. malampuzhensis*; **N1 & N2.** *R. mexicana*. **O1 & O2.** *R. occultiflora*

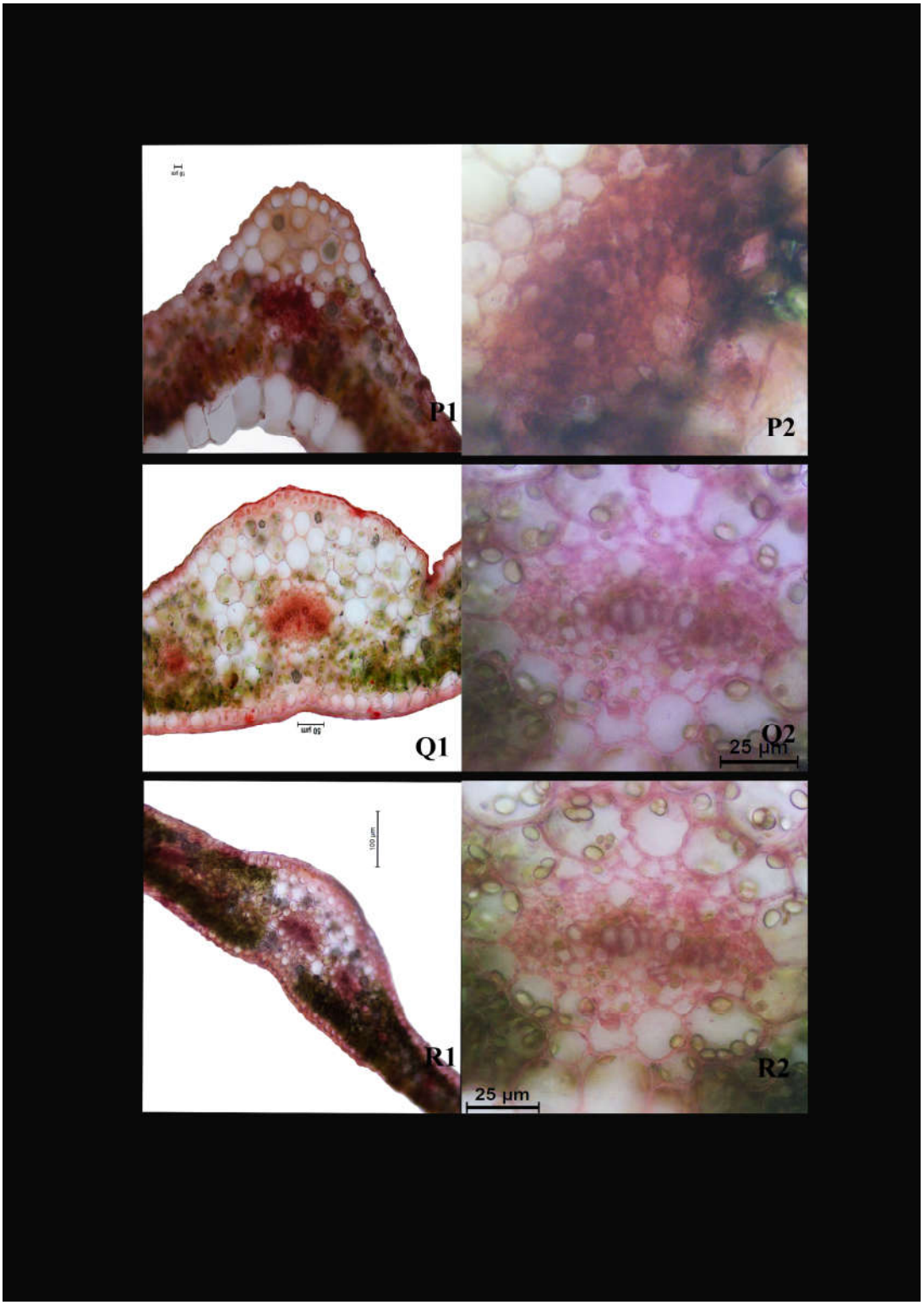

Figure 24. *Rotala* midrib transverse section. **(P1, Q1 & R1.** Entire view; **P2, Q2 & R2.** Vascular bundles); **P1 & P2.** *R. rosea;* **Q1& Q2.** *R. rotundifolia*; **R1& R2.** *R. tulunadensis*

Table 4. Diagnostic characters of leaf midrib anatomy

Sl No.	Name of species	Mid rib shape- adaxial surface	Mid rib shape- abaxial surface	Midrib thickness (mm) ± S.E	Number of vessel strands	Shape of vascular tissue	Width of vascular bundle (mm) ± S.E	Sclerenchyma surrounding vascular tissue	Epidermis thickness	
									Adaxial	Abaxial
1	*A. baccifera* subsp. *baccifera*	Slightly concave	Convex to round	0.28±0.024	6-12	Arc or crescent	0.052±0.002	N	E	E
2	*A. baccifera* subsp. *aegyptiaca*	Less flat	Convex	0.8±0.24	7- 14	Arc or crescent	0.15±0.015	N	E	E
3	*A.multiflora*	Deeply cleft	Convex to round	0.34±0.05	9- 12	Arc or crescent	0.045±0.008	N	E	E
4	*A.octandra*	Slightly concave	Rounded	0.7±0.07	12- 17	Arc or crescent	0.06±0.004	Y	L	S
5	*N. brevipes*	Slightly cleft	Convex to round	0.5 ±0.02	7- 13	Arc or crescent	0.07±0.01	N	E	E
6	*N. prostrata*	Slightly concave	convex	0.45±0.07	6- 8	Arc or crescent	0.02±0.002	N	E	E
7	*R. densiflora*	Deeply cleft	Convex to round	0.25±0.025	6- 8	Arc or crescent	0.02±0.004	N	L	S
8	*R. fimbriata*	Shallowly cleft	Round	0.5±0.09	6- 7	Arc or crescent	0.03±0.005	N	E	El
9	*R. indica*	Curved	Convex	0.25±0.05	7- 9	Arc or crescent	0.035±0.004	Y	E	E
10.	*R. juniperina*	Flat to undulate	Round	0.2±0.02	8- 10	Arc or crescent	0.017±0.004	N	L	S
11	*R. macrandra*	Flat	Convex	0.3±0.04	7- 9	Arc or	0.02±0.008	N	E	E

				0.3±0.05		crescent				
12	*R. malabarica*	Flat to slightly concave	Slightly ridged	0.13±0.005	8-10	Less flat	0.015±0.0005	N	L	S
13	*R. malampuzhensis*	Slightly concave	Abruptly round	0.15±0.05	6-7	Arc or crescent	0.02±0.005	N	E	E
14	*R. mexicana*	Slightly undulate	Convex	0.2±0.03	5- 6	Arc or crescent	0.015±0.001	N	S	L
15	*R. occultiflora*	Flat to slightly convex	Obscurely ridged	0.065±0.008	1- 2	Less flat	0.005±0.0009	N	L	S
16	*R. rosea*	Sinuate	Abruptly round	0.45±0.04	1- 2	Arc or crescent	0.06±0.01	N	L	S
17	*R. rotundifolia*	Flat to shallowly furrowed	Convex	0.3±0.04	5- 6	Arc or crescent	0.015±0.0005	N	L	S
18	*R. tulunadensis*	Abruptly concave	Convex	0.18±0.004	8- 10	Arc shaped	0.025±0.0006	N	E	E

Y- Present; N – Absent; L- larger; S- smaller; E- Equal

Stem anatomy

***Ammannia baccifera* subsp. *aegyptiaca* (Willd.) Koehne**

The outline of the stem in cross section is tetragonal to round, obscure ridges or without, 2.5−3.5 mm in diameter. Epidermis single layered, with barrel-shaped cells, covered with thin cuticle. Hypodermis collenchymatous. Cortex of irregular aerenchyma cells with druses; 0.45−0.55 mm thickness. Endodermis single layered with barrel-shaped cells. Pericycle schlerenchymatous in the form of a discontinuous layer. Phloem 0.012−0.027 mm thick, xylem 0.035− 0.05 mm thick. Pith more or less isodiametric, parenchymatous with druses, 1.2−1.3 mm wide. **Figure 25. A1, A2 & A3**

***Ammannia baccifera* subsp. *baccifera* L.**

The outline of the stem in cross section is tetragonal with elongated ridges, 1− 2 mm in diameter. Epidermis single layered with barrel-shaped cells, covered with thin cuticle; Hypodermis chlorenchymatous. Cortex of irregular aerenchyma cells with druses. 0.1− 0.2 mm thickness. Endodermis single layered with barrel shaped cells. Pericycle schlerenchymatous in the form of a discontinuous layer. Phloem 0.005−0.01 mm thick, xylem 0.01−0.02 mm thick. Pith more or less isodiametric, parenchymateous with druses, 0.5− 0.6 mm wide. **Figure 25. B1, B2 & B3**

***Ammannia multiflora* Roxb.**

The outline of the stem in cross section is tetragonal with short wings, 0.8− 1mm across. Epidermis with barrel-shaped cells, covered with thin cuticle; Hypodermis chlorenchymatous. Cortex of irregular aerenchyma cells some with druses, 0.05−0.08 mm thicknesses. Endodermis single layered with barrel-shaped cells. Pericycle sclerenchymatous cap-like in the form of a discontinuous layer. Phloem 0.009−0.02 mm; xylem 0.02−0.04 mm thick. Pith more or less isodiametric, parenchymatous, 0.12− 0.16 mm wide.

Figure 25. C1, C2 & C3

***Ammannia octandra* L. f.**

The outline of the stem in cross section is tetragonal with rounded to angular ridges, 2.5− 2.7 mm across. Epidermis with barrel-shaped cells, covered with thick cuticle. Hypodermis parenchymatous; Cortex of irregular aerenchyma cells with druses, 0.2− 0.4 mm thickness. Endodermis single layered with large barrel− shaped cells. Pericycle sclerenchymatous in the form of a discontinuous layer. Phloem 0.005−0.008 mm wide, xylem 0.02−0.04 mm wide. Pith more or less isodiametric, parenchymatous with druses, 0.9−1.0 mm wide. **Figure 26. D1, D2 & D3**

***Nesaea brevipes* Koehne**

The outline of the stem in cross section is tetragonal without ridges, 1−2 mm in diameter. Epidermis single layered, with barrel-shaped cells, covered with thin cuticle. Hypodermis chlorenchymatous. Cortex of irregular aerenchyma cells with druses, 0.3− 0.45 mm thickness. Endodermis single layered with barrel-shaped cells. Pericycle sclerenchymatous in the form of a discontinuous layer. Phloem 0.005− 0.01 mm thick, xylem 0.01− 0.03 mm thick. Pith more or less isodiametric, parenchymatous with druses; 0.25−0.35 mm wide. **Figure 26. E1, E2 & E3**

***Nesaea prostrata* (Buch.- Ham *ex* Dillwyn) Suresh**

The outline of the stem in cross section is tetragonal without ridges, 0.9−1.2 mm in diameter. Epidermis single layered, with barrel-shaped cells, covered with thin cuticle. Hypodermis chlorenchymatous. Cortex of irregular aerenchyma cells with druses, 0.1− 0.25 mm thickness. Endodermis single layered with barrel-shaped cells. Pericycle sclerenchymatous in the form of a discontinuous layer. Phloem 0.005− 0.01 mm thick, xylem 0.015− 0.02 mm thick. Pith more or less isodiametric, parenchymatous with druses; 0.23−0.3 mm wide. **Figure 26. F1, F2 & F3**

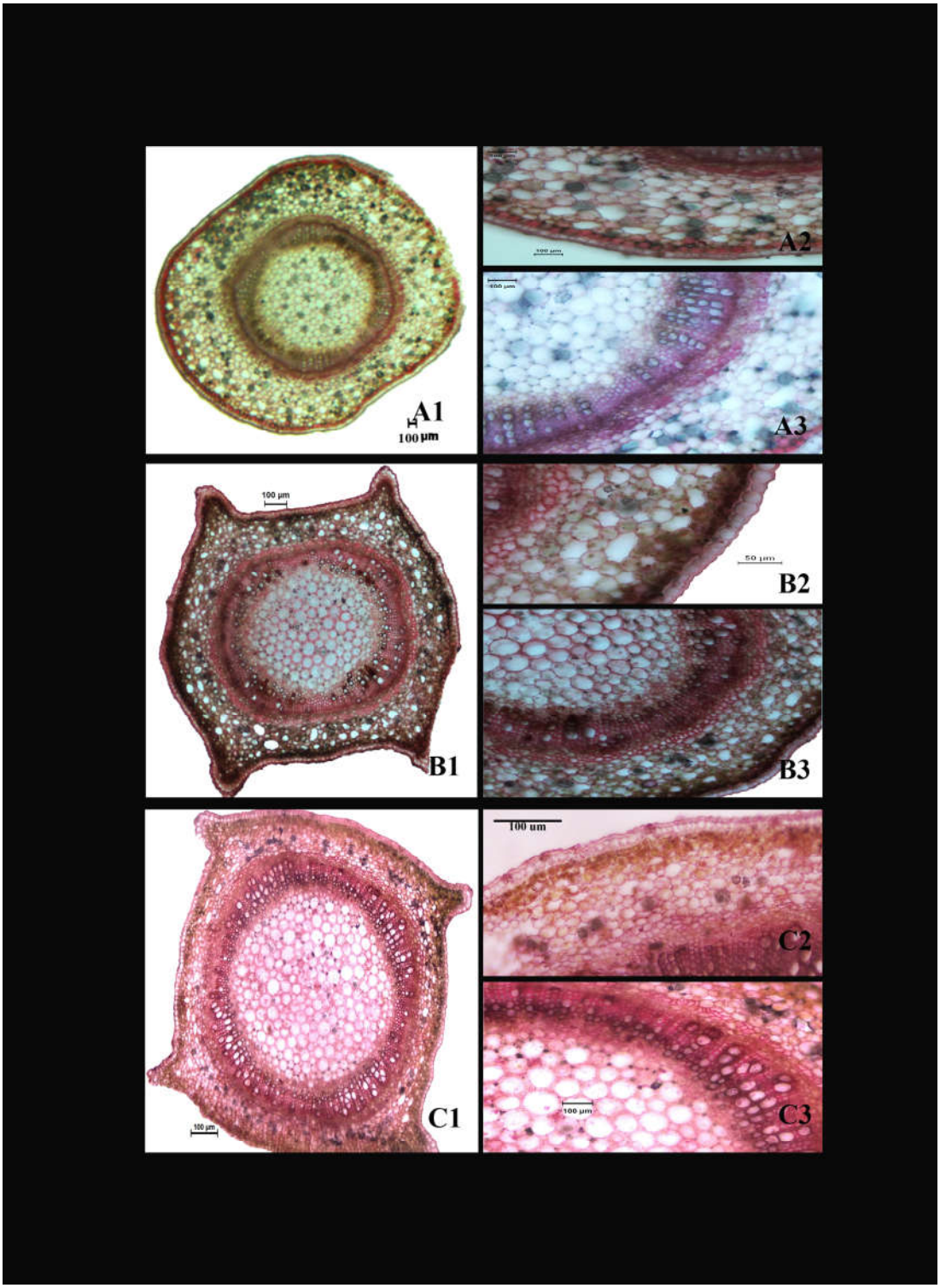

Figure 25. Stem transverse section of *Ammannia*. **A1, B1 & C1.** Entire view; **A2, B2 & C2.** Cortical portion of tranverse section; **A3, B3 & C3.** Pith with vascular bundles; **A1, A2 & A3.** *A. baccifera* subsp. *aegyptiaca* **B1, B2 & B3.** *A. baccifera* subsp. *baccifera* . **C1, C2 & C3.** *A. multiflora*

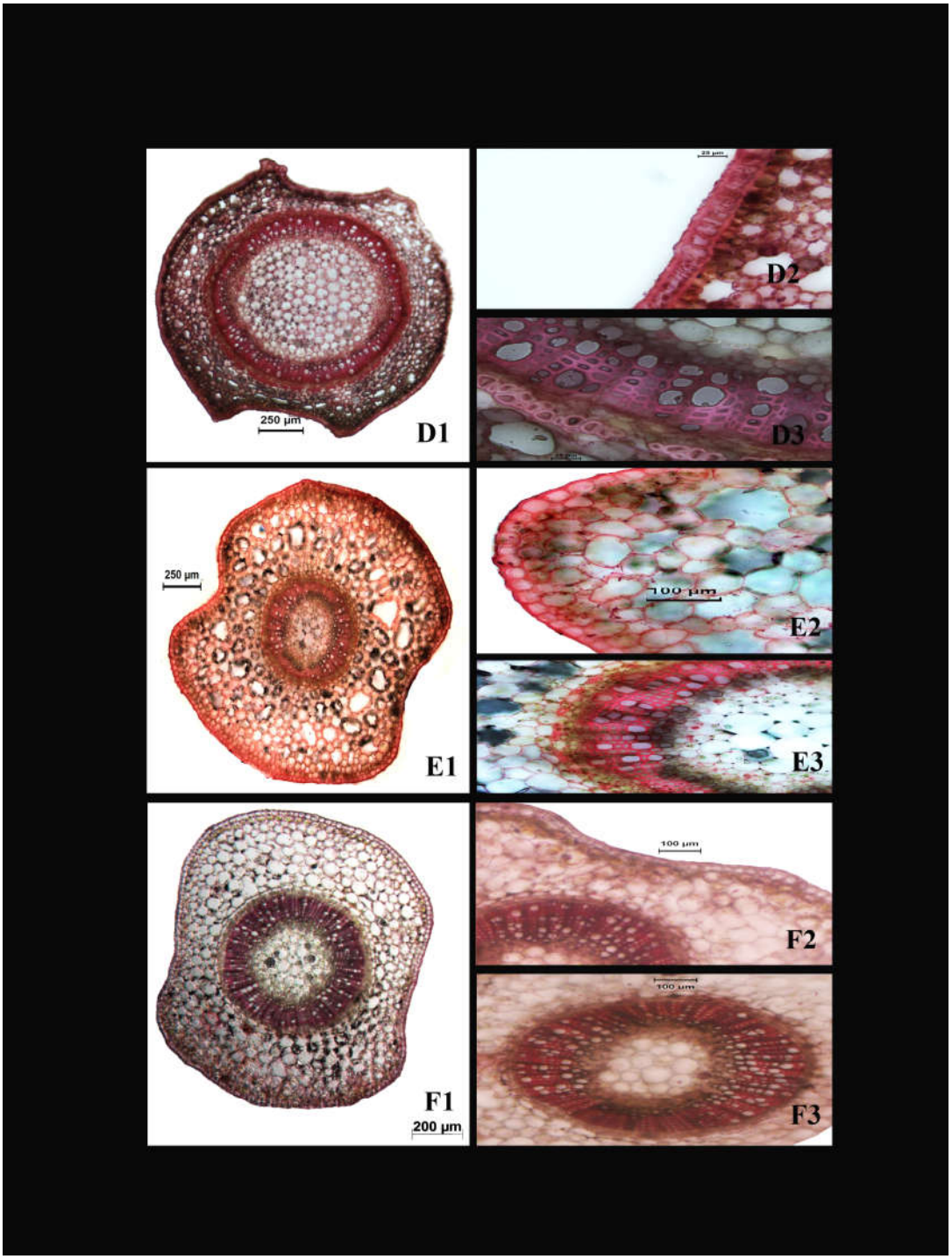

Figure 26. Stem transverse section of *Ammannia & Nesaea*. **D1, E1 & F1.** Entire view; **D2, E2 & F2.** Cortical portion of tranverse section; **D3, E3 & F3.** Pith with vascular bundles; **D1, D2 & D3.** *A. octandra*. **E1, E2 & E3.** *N. brevipes*; **F1, F2 & F3.** *N. prostrata*

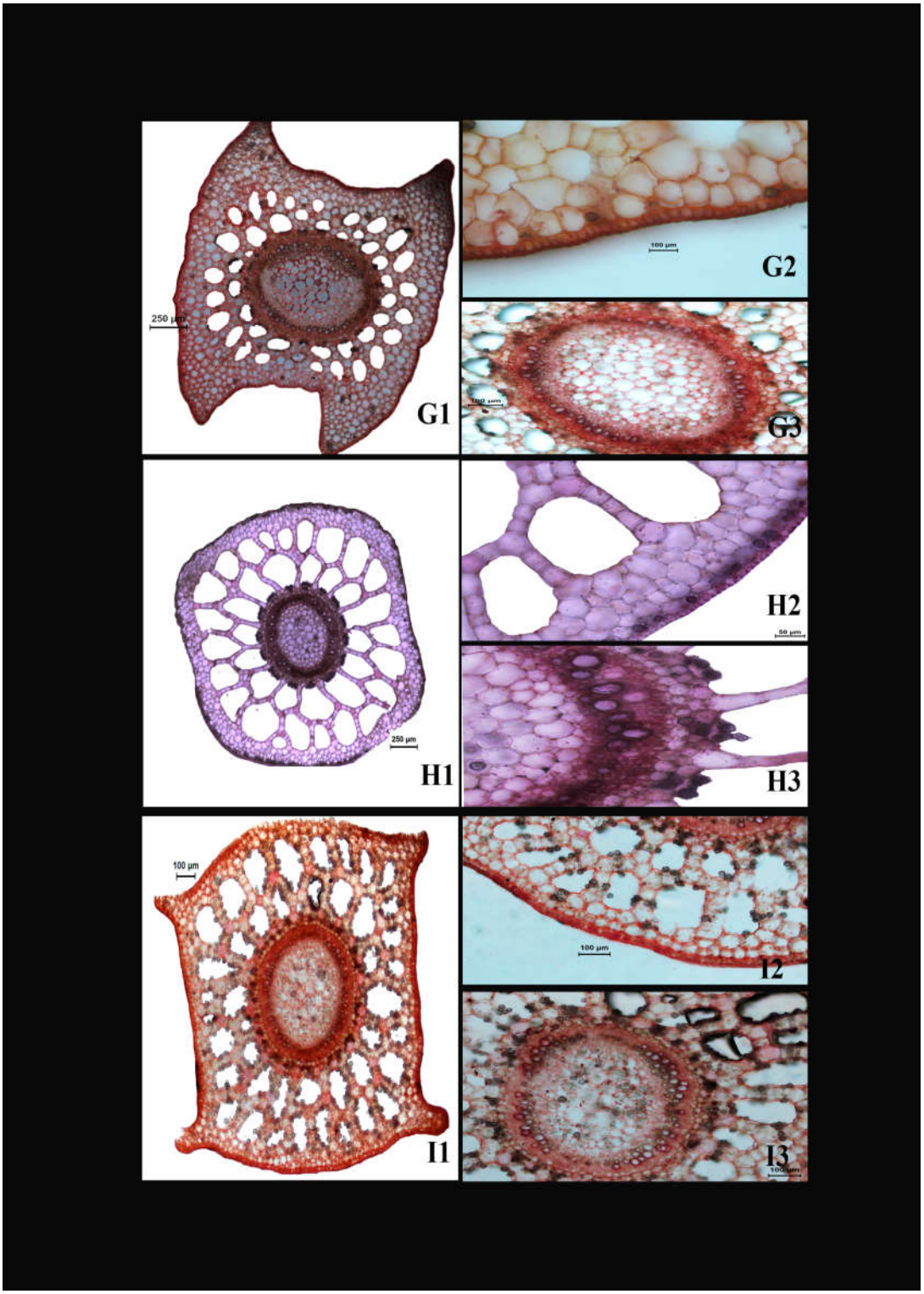

Figure 27. Stem transverse section of *Rotala*. **G1, H1 & I1**. Entire view; **G2, H2 & I2.** Cortical portion of tranverse section; **G3, H3 & I3.** Pith with vascular bundles; **G1, G2 & G3.** *R. densiflora*. **H1, H2 & H3.** *R. fimbriata*. **I1, I2 & I3**. *R. indica*

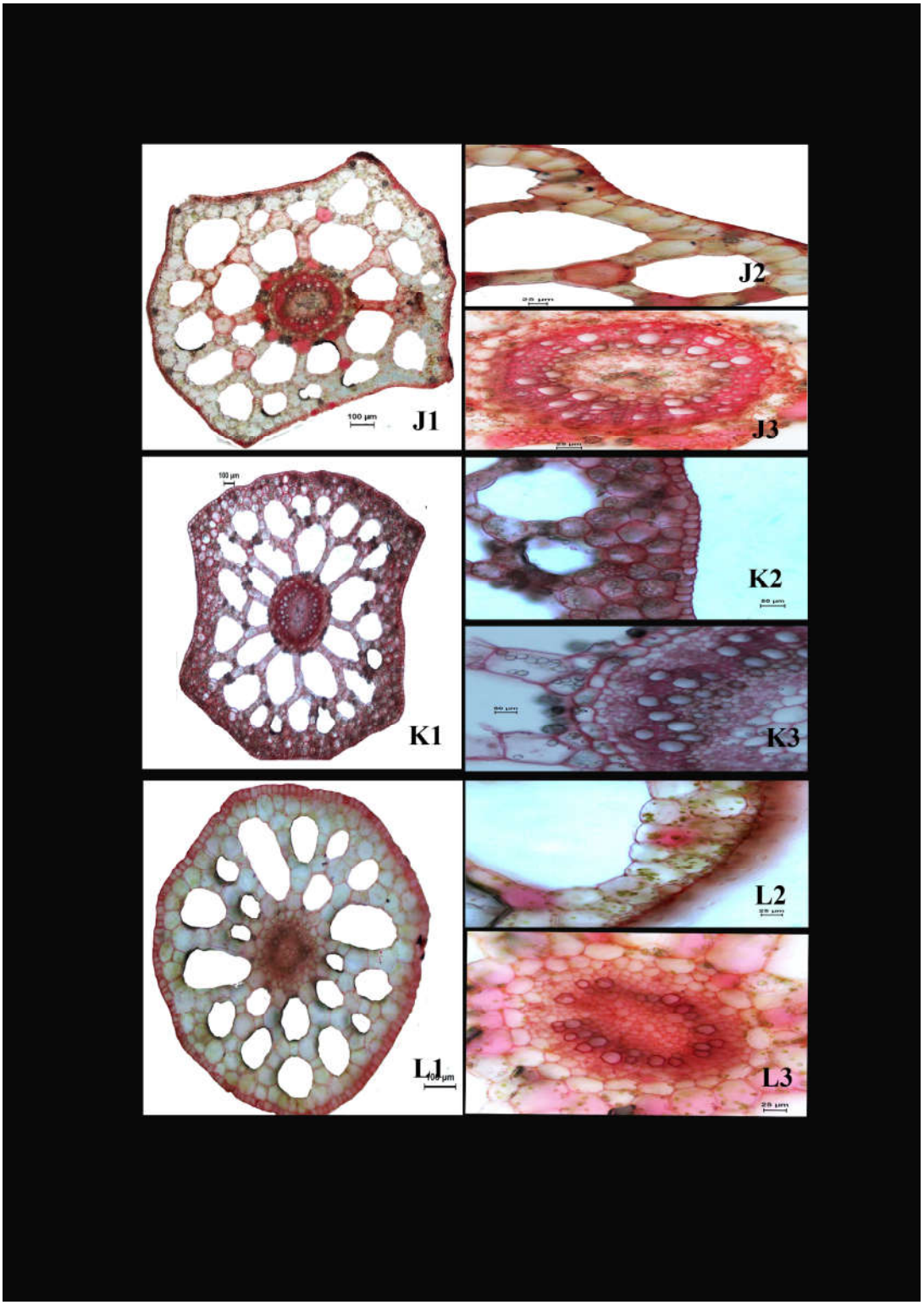

Figure 28. Stem transverse section of *Rotala*. **J1, K1 & L1.** Entire view; **J2, K2 & L2.** Cortical portion of tranverse section; **J3, K3 & L3.** Pith with vascular bundles; **J1, J2 & J3.** *R. juniperina*; **K1, K2 & K3.** *R. macrandra*; L1**, L2 & L3.** R. *malabarica*.

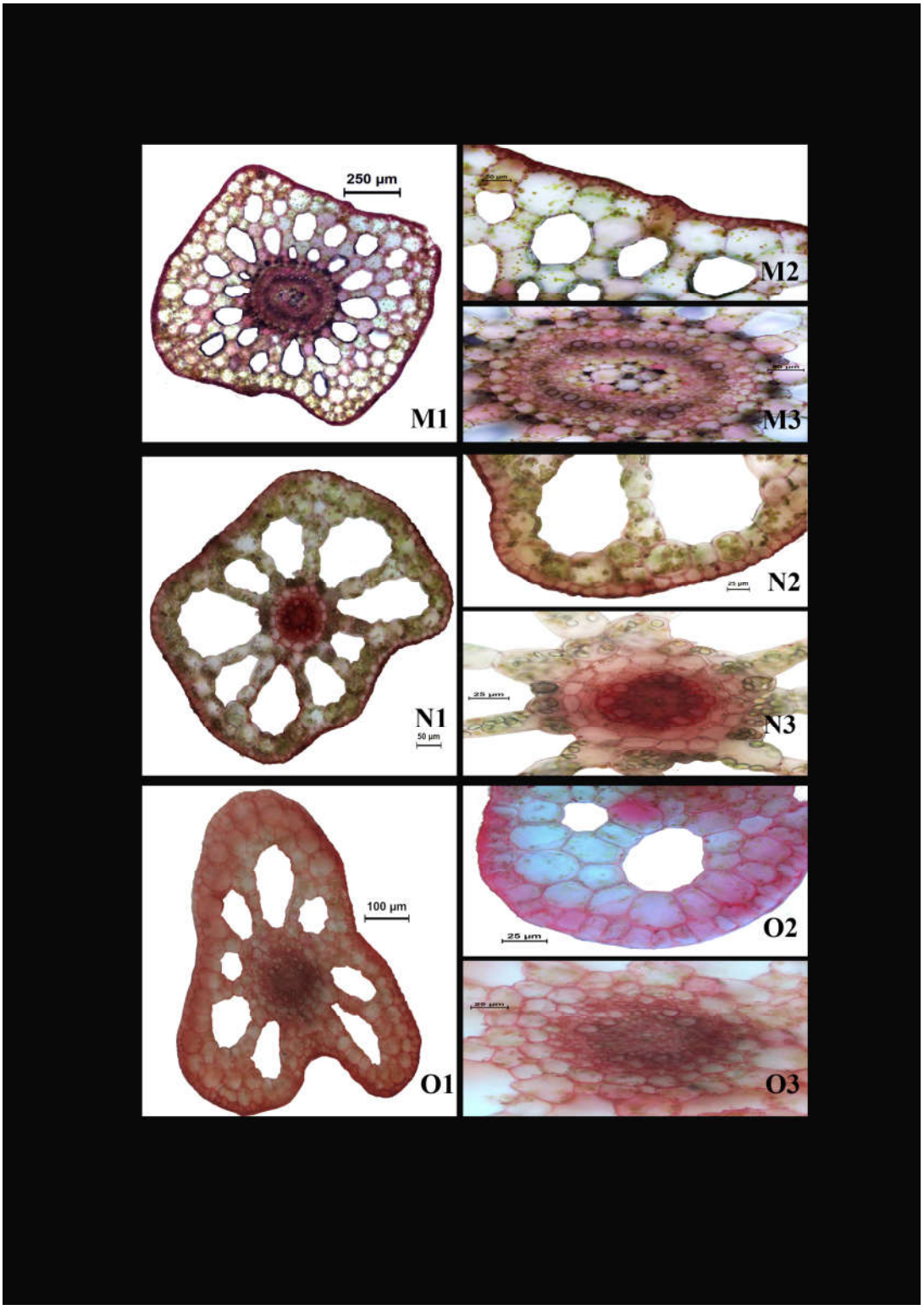

Figure 29. Stem transverse section of *Rotala*. **M1, N1 & O1.** Entire veiw; **M2, N2 & O2.** Cortical portion of tranverse section; **M3, N3 & O3.** Pith with vascular bundles; **M1, M2 & M3.** *R. malampuzhensis*. **N1, N2 & N3.** *R. mexicana*. **O1, O2 & O3.** *R. occultiflora*.

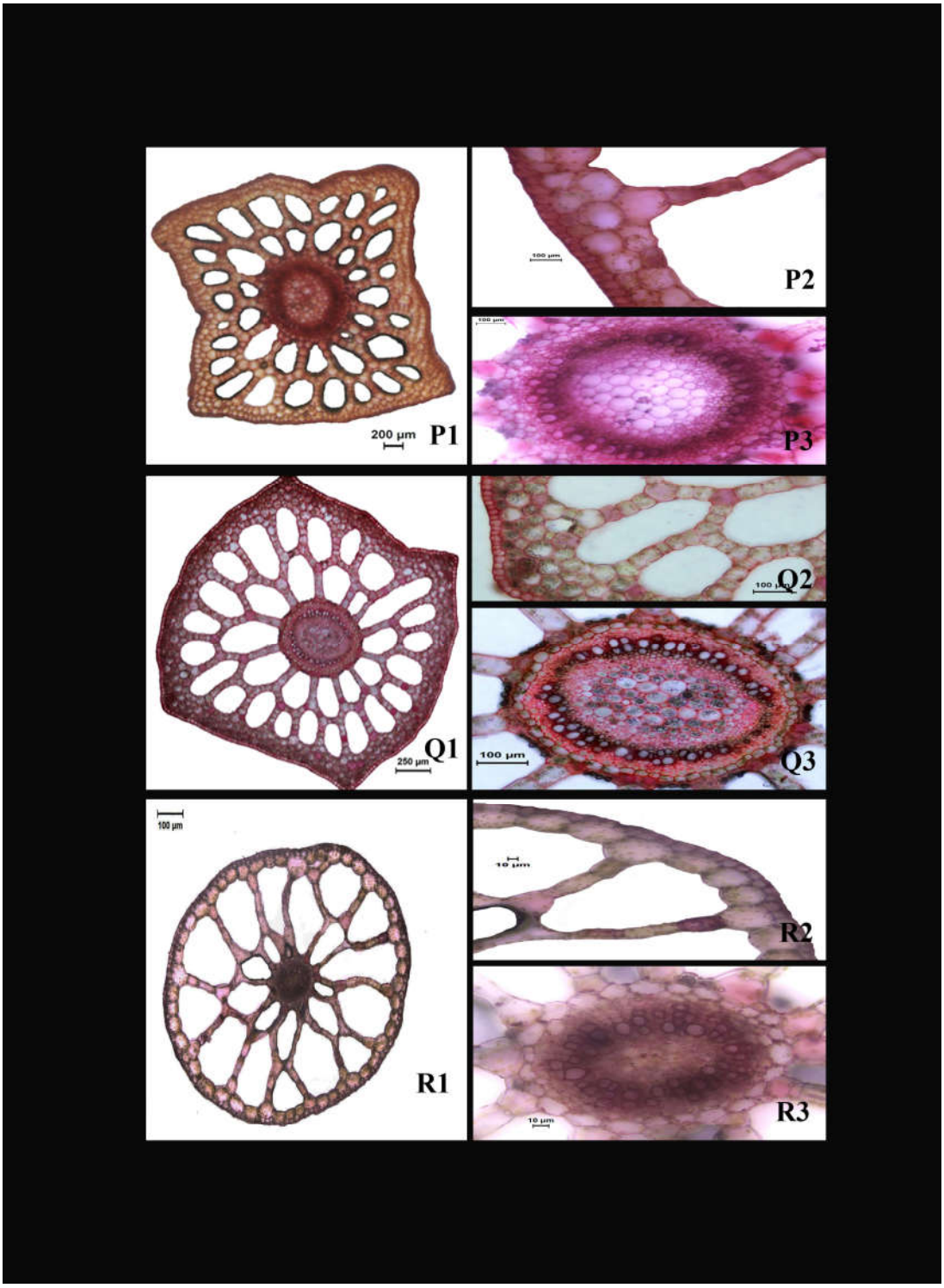

Figure 30. Stem transverse section of *Rotala*: **P1, Q1 & R1.** Entire view; **P2, Q2 & R2.** Cortical portion of tranverse section; **P3, Q3 & R3.** Pith with vascular bundles; **P1, P2 & P3.** *R. rosea*; **Q1, Q2 & Q3.** *R. rotundifolia*; **R1, R2 & R3.** *R. tulunadensis*

***Rotala densiflora* (Roth *ex* Roem. & Schult.) Koehne**

The outline of the stem in cross section is tetragonal with prominent ridges: 1.5−2.5mm in diameter. Epidermis single layered with barrel-shaped cells, covered with thin cuticle. Hypodermis parenchymatous. Cortex of aerenchyma cells with a few druses. 2.8− 0.35mm thickness. Endodermis single layered with barrel-shaped cells, druses are found to be more on the surface of cortical parenchymatous cells that lines the endodermis. Pericycle parancymatous in the form of a continuous layer. Phloem 0.003−0.005 mm, xylem 0.01−0.03 mm thick. Pith more or less isodiametric, parenchymatous with druses, 0.5−0.7 mm width. **Figure 27. G1, G2 & G3**

***Rotala fimbriata* Wight**

The outline of the stem in cross section is tetragonal without ridges: 2.1−2.4 mm in diameter. Epidermis single layered with barrel-shaped cells, covered with thin cuticle. Hypodermis parenchymatous with druses. Cortex of aerenchyma cells with druses, 0.45−0.55 mm thickness. Endodermis single layered with barrel-shaped cells. Pericycle sclerenchymatous in the form of a discontinuous layer; accumulation of druses as a discontinuous layer on the surface of cortical parenchymatous cells that lines the endodermis. Phloem 0.009-0.015 mm, xylem 0.025− 0.036 mm thick. Pith more or less isodiametric, parenchymatous with druses, 0.45−0.54 mm wide.

Figure 27. H1, H2 & H3

***Rotala indica* (Willd.) Koehne**

The outline of the stem in cross section is tetragonal without ridges: 0.8−2.0 mm in diameter. Epidermis single layered with barrel-shaped cells, covered with thin cuticle. Hypodermis parenchymatous. Cortex of aerenchyma cells with druses; 0.35−0.45 mm thickness. Endodermis single

layered with barrel shaped cells, accumulation of druses as a discontinuous layer on the surface of cortical parenchymatous cells that lines the endodermis. Pericycle parencymatous in the form of a continuous layer. Phloem 0.005− 0.008 mm, xylem 0.02− 0.035 mm thick. Pith more or less isodiametric, parenchymatous with druses, 0.4−0.5 mm width.

Figure 27. I1, I2 & I3

***Rotala juniperina* A. Fernande**s

The outline of the stem in cross section is tetragonal with obscure to without ridges: 1.2−2 mm in diameter. Epidermis single layered with barrel-shaped cells, covered with thin cuticle. Hypodermis parenchymatous. Cortex of aerenchyma cells without druses, 0.3− 0.45 mm thickness. Endodermis single layered with barrel-shaped cells, druses are found to be more on the surface of cortical parenchymatous cells that lines the endodermis. Pericycle parenchymatous in the form of a continuous layer. Phloem 0.006−0.01 mm, xylem 0.01− 0.025 mm thick; more or less isodiametric parenchymatous pith with a few druses, 0.1− 0.15 mm width. **Figure 28. J1, J2 & J3**

***Rotala macrandra* Koehne**

The outline of the stem in cross section is tetragonal with elongated ridges: 3.0−4.0 mm in diameter. Epidermis single layered with barrel-shaped cells, covered with thin cuticle. Hypodermis parenchymatous. Cortex of aerenchyma cells, 0.60−0.70 mm thickness. Endodermis single layered with barrel-shaped cells, accumulation of druses as a discontinuous layer on the surface of cortical parenchymatous cells that lines the endodermis. Pericycle parencymatous in the form of a continuous layer. Phloem 0.005−0.008 mm, xylem 0.02−0.04 mm thick. Pith more or less isodiametric parenchymatous, 0.20−0.25 mm width. **Figure 28. K1, K2 & K3**

***Rotala malabarica* Pradeep, K.T. Joseph *et* Sivar.**

The outline of the stem in cross section is tetragonal to round, 0.86−1.0 mm in diameter. Epidermis single layered with barrel-shaped cells, covered with thin cuticle. Hypodermis parenchymatous or chlorenchymatous without druses. Cortex of aerenchyma cells without druses, 0.25−0.35mm thickness. Endodermis single layered with barrel-shaped cells, accumulation of druses as on the surface of cortical parenchymatous cells that lines the endodermis absent. Pericycle parenchymatous in the form of a continuous layer; phloem 0.007−0.013 mm, xylem 0.01−0.02 mm thick. Pith more or less isodiametric, parenchymatous without druses, 0.1−0.16 mm wide.

Figure 28. L1, L2 & L3

***Rotala malampuzhensis* R.V. Nair *ex* C.D.K. Cook**

The outline of the stem in cross section is tetragonal or round without ridges: 0.75− 1mm in diameter. Epidermis single layered with barrel-shaped cells, covered with thin cuticle. Hypodermis parenchymatous. Cortex of aerenchyma cells without druses, 0.3−0.5 mm thickness. Endodermis single layered with barrel-shaped cells, accumulation of druses as on the surface of cortical parenchymatous cells that lines the endodermis. Pericycle parenchymatous in the form of a continuous layer. Phloem 0.003−0.006 mm, xylem 0.007−0.01 mm thick. Pith more or less isodiametric, parenchymatous with druses, 0.05−0.065 mm width. **Figure 29. M1, M2 & M3**

***Rotala mexicana* Cham. & Schldtl.**

The outline of the stem in cross section is tetragonal without ridges: 0.2− 0.3mm in diameter. Epidermis single layered with barrel-shaped cells, covered with thin cuticle. Hypodermis parenchymatous. Chlorenchymatous cortex of *c.* 9 distict aerenchyma cells without druses, 0.1−0.2 mm thickness.

Endodermis single layered with barrel-shaped cells, very few druses are found to be more on the surface of cortical parenchymatous cells that lines the endodermis. Pericycle parancymateous in the form of a continuous layer. Phloem 0.001−0.002 mm, xylem 0.005−0.01 mm thick. Pith more or less isodiametric, parenchymatous without druses, 0.04−0.05 mm width.

Figure 29. N1, N2 & N3

***Rotala occultiflora* Koehne**

The outline of the stem in cross section is triagonal without ridges: 0.3−0.4mm in diameter. Epidermis single layered with barrel-shaped cells, covered with thin cuticle. Hypodermis parenchymatous. Cortex of 6 distict aerenchyma cells without druses, 0.0625−0.125 mm thickness. Endodermis single layered with barrel-shaped cells, accumulation of druses as on the surface of cortical parenchymatous cells that lines the endodermis absent. Pericycle parancymatous in the form of a continuous layer. Phloem 0.002−0.004 mm, xylem 0.008−0.01 mm thick. Pith more or less isodiametric, parenchymatous without druses, 0.04−0.05 mm width.

Figure 29. O1, O2 & O3

***Rotala rosea* (Poiret) C.D.K. Cook**

The outline of the stem in cross section is tetragonal with obscure to without ridges: 2−3mm in diameter. Epidermis single layered with barrel-shaped cells, covered with thin cuticle. Hypodermis parenchymatous. Cortex of aerenchyma cells without druses, 0.5−0.75 mm thickness. Endodermis single layered with barrel shaped cells, druses are found to be more on the surface of cortical parenchymatous cells that lines the endodermis. Pericycle parencymatous in the form of a continuous layer. Phloem 0.015−0.02 mm,

xylem 0.04−0.08 mm thick. Pith more or less isodiametric, parenchymatous without druses, 0.4−0.5 mm width. **Figure 30. P1, P2 & P3**

***Rotala rotundifolia* (Buch.- Ham. *ex* Roxb.) Koehne**

The outline of the stem in cross section is tetragonal without ridges: 0.7− 0.8mm in diameter. Epidermis single layered with barrel-shaped cells, covered with thin cuticle. Hypodermis parenchymatous. Cortex of aerenchyma cells with druses, 0.25− 0.3 mm thickness. Endodermis single layered with barrel-shaped cells, accumulation of druses on the surface of cortical parenchymatoeus cells that line the endodermis. Pericycle parenchymatous in the form of a continuous layer. Phloem 0.002−0.0035 mm, xylem 0.01−0.02 mm thick. Pith more or less isodiametric, parenchymatous with druses, 0.09−0.1 mm width. **Figure 30. Q1, Q2 & Q3**

***Rotala tulunadensis* K.S. Prasad, P. Biju, C. Ravi & K.G. Bhat**

The outline of the stem in cross section is round, 0.74− 0.9 mm in diameter. Epidermis single layered with barrel-shaped cells, covered with thin cuticle. Hypodermis parenchymatous or chlorenchymatous without druses. Cortex of aerenchyma cells without much druses, 0.27− 0.3mm thickness; Endodermis single layered with barrel-shaped cells, accumulation of druses on the surface of cortical parenchymatous cells that lines the endodermis absent. Pericycle parenchymatous in the form of a continuous layer. Phloem 0.003−0.004 mm, xylem 0.006−0.02 mm thick. Pith more or less isodiametric parenchymatous without druses, 0.05−0.08 mm wide.

Figure 30. R1, R2 & R3

Detailes of diagnostic characters, which are analysed in stem transverse section are given in table 5.

Table 5. Various diagnostic characters analysed in stem transverse section

Sl No	Name of species	Nature of hypodermis	% of Cortex ± S.E	(% of Cambium +Pith) ± S.E	Nature of pericycle	Druses		
						Parenchymatous distribution	Endodermis deposition	pith
1	*A. baccifera* subsp. *baccifera*	CL, Double layered	58±0.015	42±0.02	Sc	Y	N	N
2	*A. baccifera* subsp. *aegyptiaca*	C, single layered	43±0.007	58±0.005	Sc	Y	N	Y
3	*A. multiflora*	CL, 2-3 layered	21±0.004	79±0.006	Sc	Y	N	Y
4	*A. octandra*	P, single layered	28±0.012	72±0.020	Sc	Y	Y	Y
5	*N. brevipes*	P, single layered	85±0.007	16±0.005	Sc	N	N	N
6	*N. prostrata*	CL, single layered	75±0.020	24.7±0.019	Sc	N	N	N
7	*R. densiflora*	P, 2-3 layered	90.2±0.015	9.72±0.02	P	Y	Y	Y
8	*R. fimbriata*	P, 2-3 layered	60±0.010	36±0.015	Sc	Y	Y	Y
9	*R. indica*	P, 1-2 layered	83.5±0.003	16.5±0025	P	Y	Y	Y
10	*R. juniperina*	P, single layered	70±0.020	30±0.015	P	Y	Y	Y
11	*R. macrandra*	P, double layered	93.2±0.002	6.8±0.002	P	Y	Y	Y
12	*R. malabarica*	Cl, single layered	72±0.005	18.9±0.004	P	N	N	N
13	*R. malampuzhensis*	P, single layered	69±0.04	31±0.03	P	N	Y	N
14	*R. mexicana*	P, single layered	80±0.007	20±0.0075	P	N	Y	N
15	*R. occultiflora*	P, single layered	68±0.012	32±0.010	P	N	N	N
16	*R. rosea*	P, single layered	78±0.03	22±0.02	P	Y	Y	Y
17	*R. rotundifolia*	P, double layered	95±.008	5±0.007	P	Y	Y	Y
18	*R. tulunadensis*	CL, single layered	81±0.02	15±0.015	P	N	N	N

CL- chlorenchyma; P- Parenchyma; C- Collenchyma; Sc- Sclerenchyma; Y- Present; N- Absent

Cuticular studies

***Ammannia baccifera* subsp. *aegyptiaca* (Willd.) Koehne**

Amphistomatic; largely isotricytic, often tetracytic and occasionally anisotricytic on both abaxial and adaxial surface. Stomatal size ranges fom 585.8 to 713.6 μm^2 on abaxial surface and from 803.9−853.8 μm^2 on adaxial surface. Stomatal index is 14.3 % on abaxial and 10.3 % on adaxial surfaces. Epidermal cells polygonal, iso and anisodiametric on abaxial surface, 22−63 μm long and 16 − 37 μm wide; Polygonal, iso or anisodiametric on adaxialsurface, 29−77 μm long and 20−47 μm wide. Anticlinal cell wall thin and wavy to sinuate on abaxial surface and thick, straight to curved on adaxial surface **Figure 31. A1 & A2**

***Ammannia baccifera* subsp. *baccifera* L.**

Amphistomatic; largely isotricytic, often tetracytic and occasionally staurocytic and diacytic on abaxial and largely isotricytic, often tetracytic and staurocyctic and occasionally anisotricyctic on adaxial surface. Stomatal size ranges from 485.1 to 706.4 μm^2 on abaxial surface and from 545.2 to 783.6 μm^2 on adaxial surface. Stomatal index is 19.5% on abaxial and 11.3% on adaxial surfaces. Epidermal cells polygonal or irregular, iso and anisodiametric on abaxial surface, 25− 73μm long and 17− 46 μm wide; Polygonal, with 5−7 sides,iso or anisodiametric on adaxial surface, 38−94μm long and 32− 51μmwide. Anticlinal cell wall thick and wavy to sinuate on abaxial surface, thick and straight to curvedon adaxial surface

Figure 31. B1 & B2

***Ammannia multiflora* Roxb.**

Amphistomatic; largely isotricytic, often tetracytic and anomocytic and occasionally staurocytic and on abaxial and largely isotricytic, often tetracytic

and anomocytic and occasionally staurocytic and anisotricytic on adaxial surface. Stomatal size ranges from 343.2 to 572.6 μm^2 on abaxial surface and from 544.8 to 790.2 μm^2 on adaxial surface. Stomatal index is 14.7% on abaxial and 10.3% on adaxial surfaces. Epidermal cells irregular, iso and anisodiametric on both abaxial and adaxial surfaces, 31−78μm long and 14−36 μm wide on abaxial and 34−97μm long and 19−56 μm wide. Anticlinal cell wall thin and sinuate on both abaxial and adaxial surfaces

Figure 31. C1& C2

***Ammannia octandra* L. f.**

Amphistomatic; largely Isotricytic, often anomocyctic and staurocytic, occasionally diacytic and anisotricytic on abaxial and largely isotricytic often tetracytic and occasionally staurocytic on adaxial surface. Stomatal size ranges from 334.8 to 504.9 μm^2 on abaxial surface and from 617.7 to 1175μm^2 on adaxial surface. Stomatal index is 20.4% on abaxial and 11.5% on adaxial surfaces. Epidermal cells polygonal or irregular , iso and anisodiametric on abaxial surface, 36−79 μm long and 17− 35 μm wide; Polygonal, iso or anisodiametric on adaxial surface, 29−77 μm long and 30−63μm wide. Anticlinal cell wall, thick and wavy to sinuate on abaxial surface, thick and curved to wavy on adaxial surface. **Figure 32. D1 & D2**

***Nesaea brevipes* Koehne**

Amphistomatic; largely Isotricytic, often anomocyctic and tetracytic, occasionally staurocytic and anisotricytic on abaxial surface and largely isotricytic and often tetracytic on adaxial surface. Stomatal size ranges from 494.8 to 1004.4 μm^2 on abaxial surface and from 309.4 to 405.9 μm^2 on adaxial surface. Stomatal index is 14.3% on abaxial and 15.7% on adaxial surfaces. Epidermal cells polygonal or coarsely irregular, iso and anisodiametric on both abaxial and adaxial surfaces, 31−78 μm long and 22−

42 μm wide on abaxial surface, 35−105 μm long and 22−42 μm wide on adaxial surface. Anticlinal cell wall thick and wavy to sinuate on both abaxial and adaxial surfaces. **Figure 32. E1 & E2**

***Nesaea prostrata* (Buch.-Ham. *ex* Dillwyn) Suresh**

Amphistomatic; largely Isotricytic, often tetracytic and occasionally staurocytic on both abaxial and adaxial surfaces. Stomatal size ranges from 258.7 to 464.5 μm^2 on abaxial surface and from 373.5 to 570.9 μm^2 on adaxial surface. Stomatal index is 10% on abaxial and 22.3 % on adaxial surfaces. Epidermal cells irregular, iso and anisodiametric on both abaxial and adaxial surfaces, 23−73 μm long and 17−41μm wide on abaxial and 23−87 μm long and 15−34 μm wide on adaxial surfaces. Anticlinal cell wall thin and sinuate on both abaxial and adaxial surfaces. **Figure 32. F1 & F2**

***Rotala densiflora* (Roth *ex* Roem. & Schult.) Koehne**

Hypostomatic to amphistomatic (with a reduced amount of stomata on adaxial surface); largely isotricytic and tetracytic often anomocyctic on abaxial surface. Stomatal size ranges from 488 to 577 μm^2, Stomatal index 21% on abaxial surface. Epidermal cells polygonal or rectangular, iso or anisodiametric with 4-8 sides on adaxial surface, 53−94μm long and 35−60μm wide; irregular and anisodiametric on abaxial surface, 30−52μm long and 24−36 μm wide. Anticlinal cell wall, thick and curved on adaxial surface, thin and wavy to sinuate on abaxial surface. **Figure 33. G1 & G2**

***Rotala fimbriata* Wight**

Amphistomatic; largely isotricytic often tetracytic occasionally staurocytic on both abaxial and adaxial surface. Average stomatal size ranges from 450.5 to 670 μm^2 on abaxial surface and from 417.5 to 594.9 μm^2 on adaxial surface. Stomatal index is 13.4% on abaxial and 8.3% on adaxial surfaces. Epidermal cells polygonal, iso and anisodiametric on abaxial surface, 25−48 μm long and 18−35 μm wide; Polygonal or rectangular or

squarish, iso or anisodiametric on adaxial surface, 25−48μm long and 18−35 μm wide. Anticlinal cell wall thin and wavy to sinuate on abaxial surface, thick and wavy on adaxial surface. **Figure 33. H1 & H2**

***Rotala indica* (Willd.) Koehne**

Amphistomatic; largely isotricytic, often tetracytic and occasionally staurocytic and anomocyctic on both abaxial and adaxial surfaces. Stomatal ranges from 260 to 367 μm^2 on abaxial surface and from 208.8 to 382.5 μm^2 on adaxial surface. Average stomatal index is 24.3% on abaxial and 14.3% on adaxial surfaces. Epidermal cells polygonal, iso and anisodiametric on abaxial surface, 28−69 μm long and 15− 33.5 μm wide; Polygonal with 5−7 sides, iso or anisodiametric on adaxial surface, 25.7−54μm long and 18− 33.5μm wide. Anticlinal cell wall thick and wavy to sinuate on abaxial surface, thick and straight to curved on adaxial surface **Figure 33. I1 & I2**

***Rotala juniperina* A. Fernandes**

Amphistomatic; largely isotricytic, often tetracytic and occasionally anomocytic and anisocytic on both abaxial and adaxial surfaces. Stomatal size ranges from 605.6 to 640.0 μm^2 on abaxial surface and 615.4− 690μm^2on adaxial surface. Stomatal index is 15.6% on abaxial and 17.9% on adaxial surfaces. Epidermal cells polygonal, iso and anisodiametric on abaxial surface, 25−67μm long and 14−28μm wide; Irregular, iso or anisodiametric on adaxial surface, 24 −69μm long and 14−36μm wide Anticlinal cell wall thick and wavy to sinuate on abaxial surface, thick and sinuate on adaxial surface.

Figure 34. J1 & J2

***Rotala macrandra* Koehne**

Amphistomatic; largely isotricytic, often tetracytic and occasionally staurocytic and anomocyctic on both abaxial and adaxial surfaces. Stomatal size ranges from 447 to 665.2 μm^2 on abaxial surface and from 562.8 to 622.1 μm^2 on adaxial surface. Stomatal index is 14.3% on abaxial and 12.3% on

adaxial surfaces. Epidermal cells polygonal, iso and anisodiametric on abaxial surface, 28−70µm long and 16- 35µm wide; Polygonal with 5- 7 sides, iso or anisodiametric on adaxial surface, 25.7−54 µm long and 18−33.5 µm wide. Anticlinal cell wall thick and wavy to sinuate on abaxial surface, thick and straight to curved on adaxial surface. **Figure 34. K1 & K2**

***Rotala malabarica* Pradeep, K.T. Joseph *et* Sivar.**

Amphistomatic; largely tetracytic and isotricytic often anomocyticand staurocytic on abaxial and largely isotricytic, often anomocytic, tetracytic and staurocytic and on adaxial surfaces. Stomatal ranges from 194.5−479.3 µm^2 on abaxial surface and 306−375.4 µm^2 on adaxial surface. Stomatal index is 18.2% on abaxial and 12.3% on adaxial surfaces. Epidermal cells polygonal iso and anisodiametric on abaxial and adaxial surface, 29−53 µm long and 12−18 µm wide on abaxial and 34−45 µm long and 14−19µmwide. Anticlinal cell wall thick and curved to wavy on both abaxial and adaxial surface.

Figure 34. L1 & L2

***Rotala malampuzhensis* R.V. Nair *ex* C.D.K. Cook**

Amphistomatic; largely tetracytic and isotricytic often anomocytic and occasionally staurocytic and anisocytic on abaxial and largely isotricytic, often anomo and tetracytic and occasionally staurocytic and anisocytic on adaxial surfaces. Stomatal size ranges from 342.8 to 620.8 µm^2 on abaxial surface and from 621.8 to 732.2 µm^2 on adaxial surface. Stomatal index is 19.5% on abaxial and 14.1% on adaxial surfaces. Epidermal cells polygonal or irregular, iso and anisodiametric on abaxial surface, 21−56 µm long and 14−32 µm wide; Polygonal or rectangular with 4−6 sides, iso or anisodiametric on adaxial surface, 27−82 µm long and 14-40 µm wide. Anticlinal cell wall thick and wavy to sinuate on abaxial surface, thick and wavy on adaxial surface.

Figure 35. M1 & M2

***Rotala mexicana* Cham. & Schldtl.**

Amphistomatic; largely tetracytic, often anomocytic and isotricytic, occasionally staurocytic on abaxial and largely isotricytic and staurocytic, often anomocytic and isotricytic on adaxial surfaces.Stomatal size ranges from 485.3 to 504 μm^2 on abaxial surface and from 378 390 μm^2 on adaxial surface. Stomatal index is 10.8% on abaxial and 6.1% on adaxial surfaces. Epidermal cells polygonal, iso and anisodiametric on both abaxial and adaxial surfaces, 23 − 64.3μm long and 9.5−20.8μm wide on abaxial surface; 27−50 μm long and 10−28.6 μm wide. Anticlinal cell wall thick and curved to wavy on both abaxial surface and adaxial surfaces. **Figure 35. N1 & N2**

***Rotala occultiflora* Koehne**

Hypostomatic, largely tetracytic, often isoticytic and anomocyctic and occasionally staurocytic on abaxial surface. Stomatal size ranges from 304.3 to 668.9 μm^2. Stomatal index 20.2% on abaxial surface. Epidermal cells polygonal on both abaxial and adaxial surfaces, 20.8− 71μm long and 13− 21μm wide; 14−34.1μm long and 9.2−15μm wide. Anticlinal cell wall, thick and and wavy to sinuate on abaxial and straight to curved on adaxial surface.

Figure 35. O1 & O2

***Rotala rosea* (Poiret) C.D.K. Cook**

Amphistomatic; largely tetracytic and isotricytic often anomocytic and occasionally staurocytic on abaxial and largely tetracytic often anomo and isotricytic and occasionally staurocytic on adaxial surface. Stomatal size ranges from 382.5 to 628.1 μm^2 on abaxial surface and from 328 to 618.9 μm^2 on adaxial surface. Stomatal index is 13.3% on abaxial and 10% on adaxial surfaces. Epidermal cells polygonal or irregular, iso and anisodiametric on abaxial surface, 25.9− 59 μm long and 15− 20 μm wide; Polygonal or rectangular, iso or anisodiametric on adaxial surface, 42−66 μm long and 16−

42µm wide. Anticlinal cell wall thick and wavy to sinuate on abaxial surface, thick and wavy on adaxial surface. **Figure 36. P1 & P2**

***Rotala rotundifolia* (Buch.- Ham. *ex* Roxb.) Koehne**

Amphistomatic; largely isotricytic and tetracytic, often anomocytic and occasionally anomocytic on abaxial and largely isotricytic, occasionally tetracytic on adaxial surfaces. Stomatal size ranges from 497.7 to 612.9 μm^2 on abaxial surface and from 497.8 to 628.6 μm^2 on adaxial surface. Stomatal index is 9.5% on abaxial and 8.3% on adaxial surfaces. Epidermal cells polygonal, iso and anisodiametric on abaxial surface, 29− 63µm long and 13 − 42 µm wide; Polygonal with 5− 7 sides, iso or anisodiametric on adaxial surface, 36− 69 µm long and 15-41µm wide. Anticlinal cell wall thick and wavy to sinuate on abaxial surface, thick and curved to wavy on adaxial surface. **Figure 36. Q1 & Q2**

***Rotala tulunadensis* K.S. Prasad, P. Biju, C. Ravi & K.G. Bhat**

Amphistomatic; largely tetracytic and isotricytic often anomocytic on abaxial and largely isotricytic, often staurocytic and tetracytic and occasionally anomocytic on adaxial surfaces. Average stomatal size ranges from 400 to 773.6 μm^2 on abaxial surface and from 362.1 to 538.36 μm^2 on adaxial surface, Average stomatal index is 8.9% on abaxial and 9.0% on adaxial surfaces. Epidermal cells polygonal, iso and anisodiametric on abaxial surface, 30− 83 µm long and 14− 49µm wide; Polygonal or irregular, iso or anisodiametric on adaxial surface, 22− 91 µm long and 12− 42 µm wide. Anticlinal cell wall thick and wavy to sinuate on both abaxial and adaxial surfaces. **Figure 36. R1 & R2**

The details of the epidermal characters of three genera are given in table 6 and table 7.

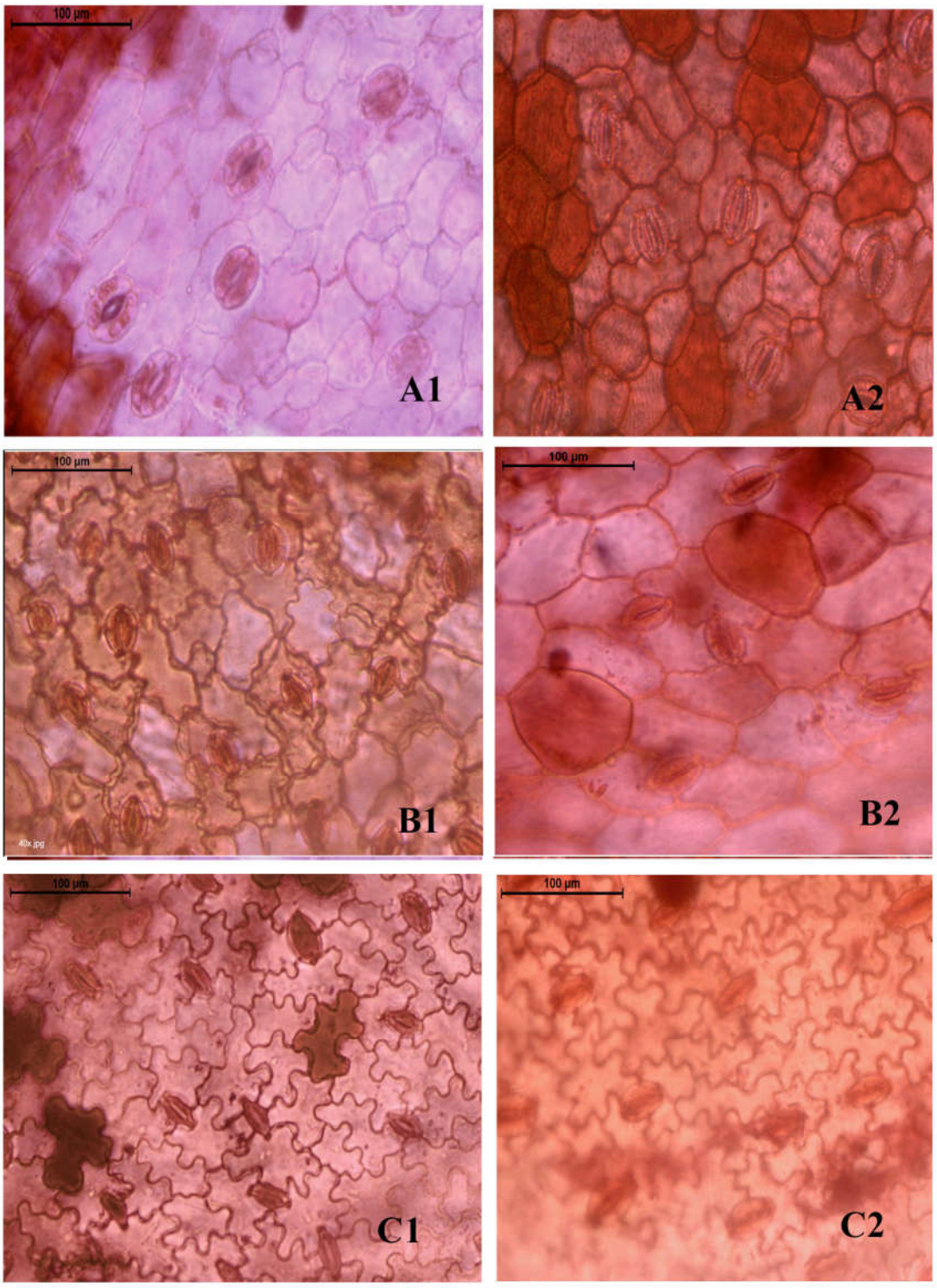

Figure 31. Epidermal features in *Ammannia.* **A1, B1 & C1.** Abaxial surface; **A2, B2, & C2**. Adaxial surface; **A1 & A2.** *A. baccifera* subsp. *aegyptiaca* **B1 & B2.** *A. baccifera* subsp. baccifera; **C1 & C2.** *A. multiflora*

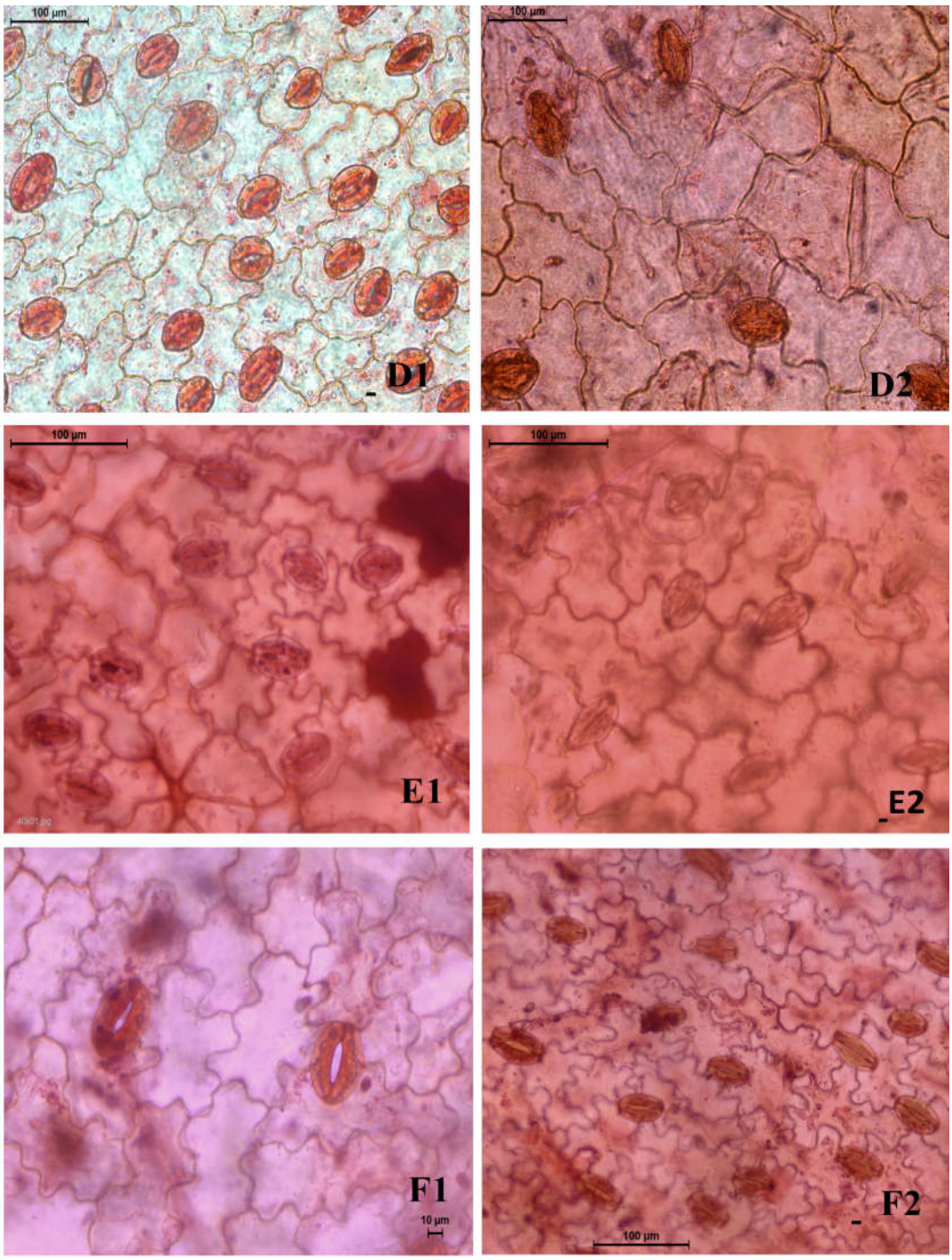

Figure 32. Epidermal features in *Ammannia* & *Nesaea.* **D1, E1 & F1-** Abaxial surface; **D2, E2, & F2.** Adaxial surface; **D1 & D2.** *A. octandra*; **E1 & E2-** *N. brevipes*; **F1 & F2.** *N. prostrata*

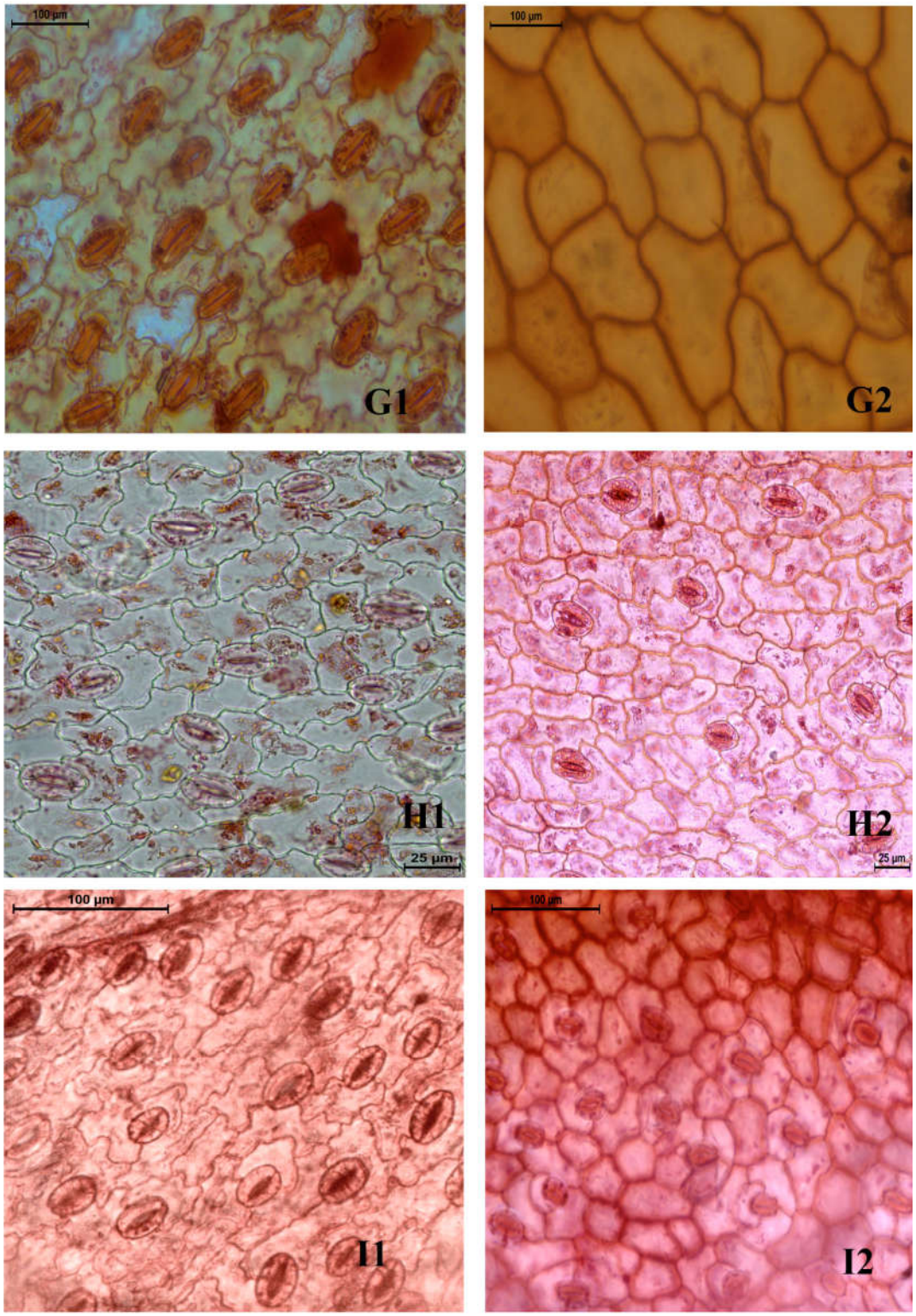

Figure 33. Epidermal features in *Rotala.* **G1, H1 & I1.** Abaxial surface; **G2, H2, & I2.** Adaxial surface; **G1 & G2.** *R. densiflora*; **H1 & H2.** *R. fimbriata*; **I1 & I2.** *R. indica*.

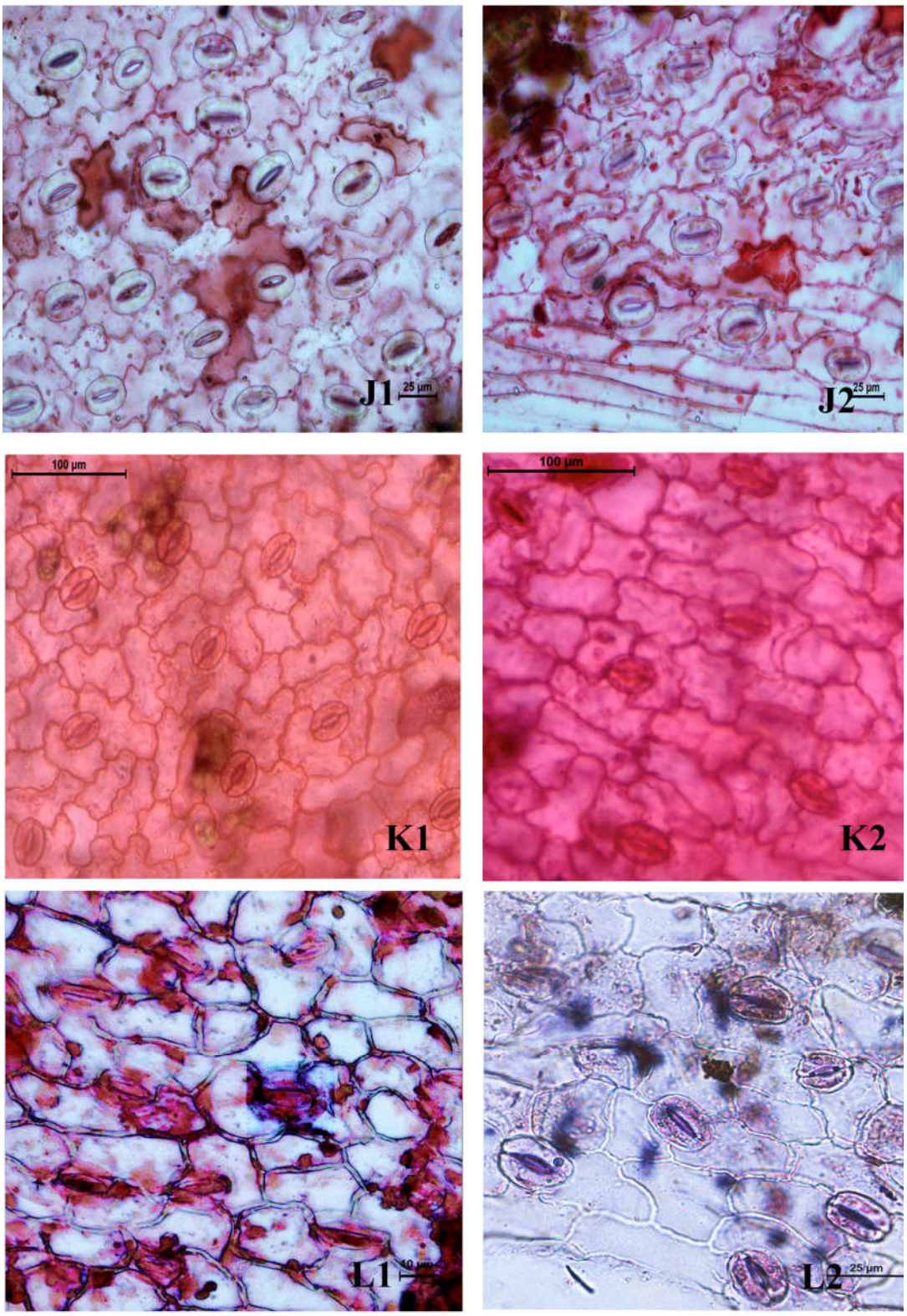

Figure 34. Epidermal features in *Rotala.* **J1, K1 & L1.** Abaxial surface; **J2, K2, & L2.** Adaxial surface; **J1 & J2.** *R. juniperina;* **K1 & K2.** *R. macrandra*; **L1 & L2.** *R. malabarica*.

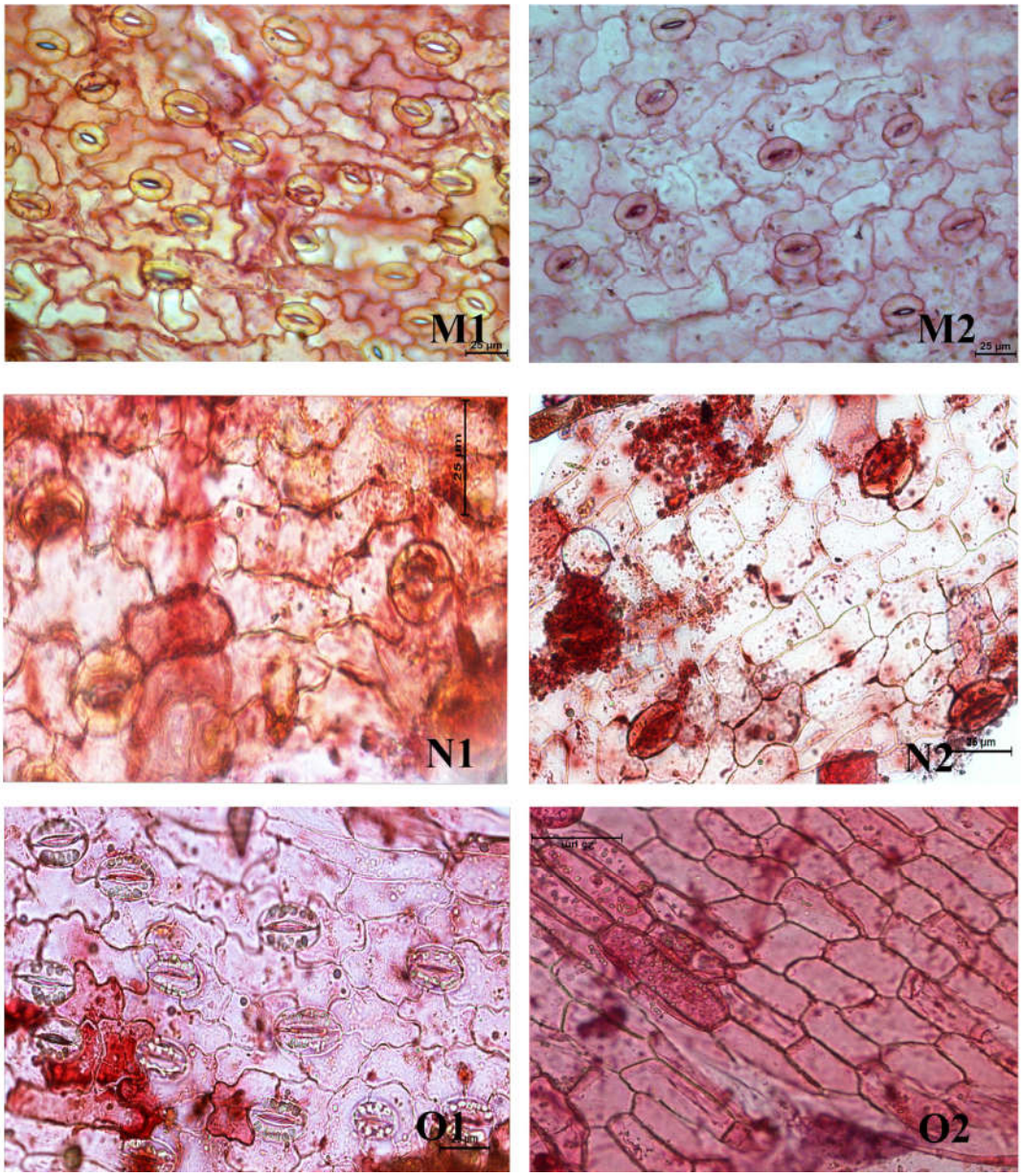

Figure 35. Epidermal features in *Rotala.* **M1, N1 & O1.** Abaxial surface; **M2, N2, & O2.** Adaxial surface; **M1 & M2.** *R. malampuzhensis*; **N1 & N2.** *R. mexicana*; **O1 & O2.** *R. occultiflora*.

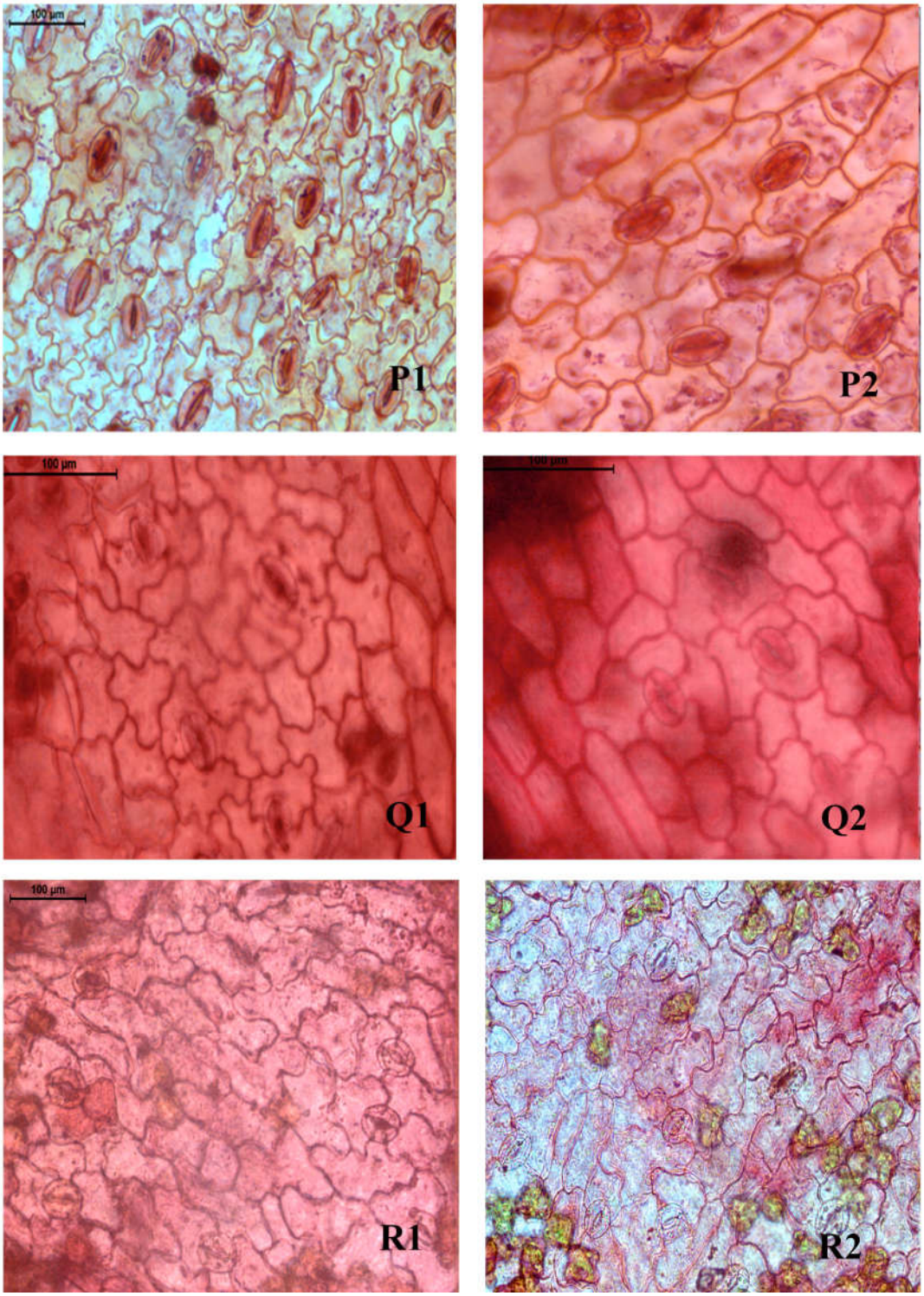

Figure 36. Epidermal features in *Rotala.* **P1, Q1 & R1.** Abaxial surface; **P2, Q2, & R2.** Adaxial surface; **P1 & P2.** *R. rosea*; **Q1 & Q2.** *R. rotundifolia*; **R1 & R2.** *R. tulunadensis*.

Table 6. Leaf epidermal characteristics on abaxial surface of *Ammannia, Nesaea* and *Rotala*

Sl No:	Taxa	Average stomatal size (μm^2) ± S.E	Average stomatal index (%) ± S. E	Stomatal type	Average epidermal size (μm^2) ± S. E	Epidermal cell shape	Anticlinal cell wall type
1	*A. baccifera* subsp. *aegyptiaca*	674±36.95	14.3±0.294	ISO	1042.5±38.9332	P	WS
2.	*A. baccifera* subsp. *baccifera*	571.3±15.41	19.5±0.047	ISO	1406±64.18452	P	WS
3.	*A. multiflora*	475±48.78	14.7±0.286	ISO	1196.3±120.1575	I	Si
4.	*A. octandra*	397.8±34.34	20.4±0.205	ISO	1240±95.82488	P	WS
5.	*N. brevipes*	693.5±79.12	14.3±0.726	ISO	1871.4±118.1661	P	WS
6.	*N. prostrata*	384.4±49.88	10±0.471	ISO	1161.±81.43107	I	Si
7.	*R. densiflora*	558±31.68	21±1.699	ISO	1264.±289.1847	P	WS
8.	*R. fimbriata*	574.2±24.02	13.4±0.711	ISO	894.6±61.83968	I	WS
9.	*R. indica*	326.7±15.41	24.3±1.901	ISO	854.4±67.56819	P	WS
10	*R. juniperina*	613.1±10.55	15.6±1.389	ISO	887.1±35.96341	P	WS
11	*R. macrandra*	544±11.39	14.3± 1.152	ISO	1092.2±31.09157	P	WS
12	*R. malabarica*	337.5±68.06	18.2±1.152	ISO, TET	581.4±139.9431	P	CW
13.	*R. malampuzhensis*	512.6±5.93	19.5±0.787	TET, ISO	766.4±43.00001	P	WS
14.	*R. mexicana*	478.7±9.94	10.8±0.748	TET	707.7±72.52955	P	CW
15.	*R. occultiflora*	478.7±37.13	20.2±0.356	TET	703.1±62.97152	P	WS
16.	*R. rosea*	512.8±37.78	13.3±0.355	TET, ISO	805.1±73.7831	P	WS
17.	*R. rotundifolia*	606.5±17.75	9.5±1.042	ISO,TET	1030.7±50.41743	P	WS
18.	*R. tulunadensis*	561.3±43.44	8.9±0.638	ISO,TET	1275.1±239.975	P	WS

ISO- Isotricytic; TET- tetracytic; P- Polygonal; I- Irregular; WS- wavy to sinuate; Si- Sinuate; CW- Curved to Wavy

Table 7. Leaf epidermal characterestics on adaxial surface of *Ammannia, Nesaea* and *Rotala*

Sl No:	Taxa	Average stomatal size(µm^2)± SE	Average stomatal index (%)± SE	Stomatal type	Average epidermal size (µm^2) ± SE	Epidermal cell shape	Anticlinal cell wall type
1.	*A. baccifera* subsp. *aegyptiaca*	840±8.74	10.3±0.721	ISO	1570±177.14	P	SC
2.	*A. baccifera* subsp. *baccifera*	635±51.07	11.3±0.51	ISO	2627±389.46	P	SC
3.	*A. multiflora*	659.1±28.09	10.3±1.97	ISO	1872.6±409.52	I	Si
4.	*A. octandra*	866±96.49	11.5±0.21	ISO	2345.2±445.93	P	CW
5.	*N. brevipes*	749±14.95	15.7±0.541	ISO	1663.2±76.18	P	WSi
6.	*N. prostrata*	460.4±12.06	22.3±0.532	ISO	1296.4±147.88	I	Si
7.	*R. densiflora*	1782±56.79	1.7±0.356	ISO	3328.4±933.26	P	C
8.	*R. fimbriata*	536.3±8.50	8.3±0.726	ISO	874.6±58.99	P	W
9.	*R. indica*	271.2±43.39	14.3±0.821	ISO	964.6±81.684	P	SC
10.	*R. juniperina*	661.3±18.81	17.9±0.364	ISO	1010.2±28.38	I	Si
11.	*R. macrandra*	564.3±7.08	12.3±0.961	ISO	1170±41.76	P	SC
12.	*R. malabarica*	340.1±16.48	12.3±1.90	ISO	668.1±84.43	P	CW
13	*R. malampuzhensis*	610.6±20.84	14.1±1.16	ISO	1211.7±56.54	P	W
14.	*R. mexicana*	387.04±16.72	6.1±1.327	TET, STAU	748.7±67.36	P	CW
15.	*R. occultiflora*	0	0	0	307.3±24.93	P	SC
16	*R. rosea*	458.7±47.23	10±0.236	TET	1612.1±403.31	P	W
17	*R. rotundifolia*	591.7±34.28	8.3±0.356	ISO	1359.7±145.53	P	CW
18	*R. tulunadensis*	455.7±6.061	9±0.707	ISO	1329.3±61.05	P	WSi

ISO- Isotricytic; TET- tetracytic; P- Polygonal; I- Irregular; CW- curved to wavy; C- Curved; Si- sinuate; SC- straight to curved; WSi- Wavy to sinuate

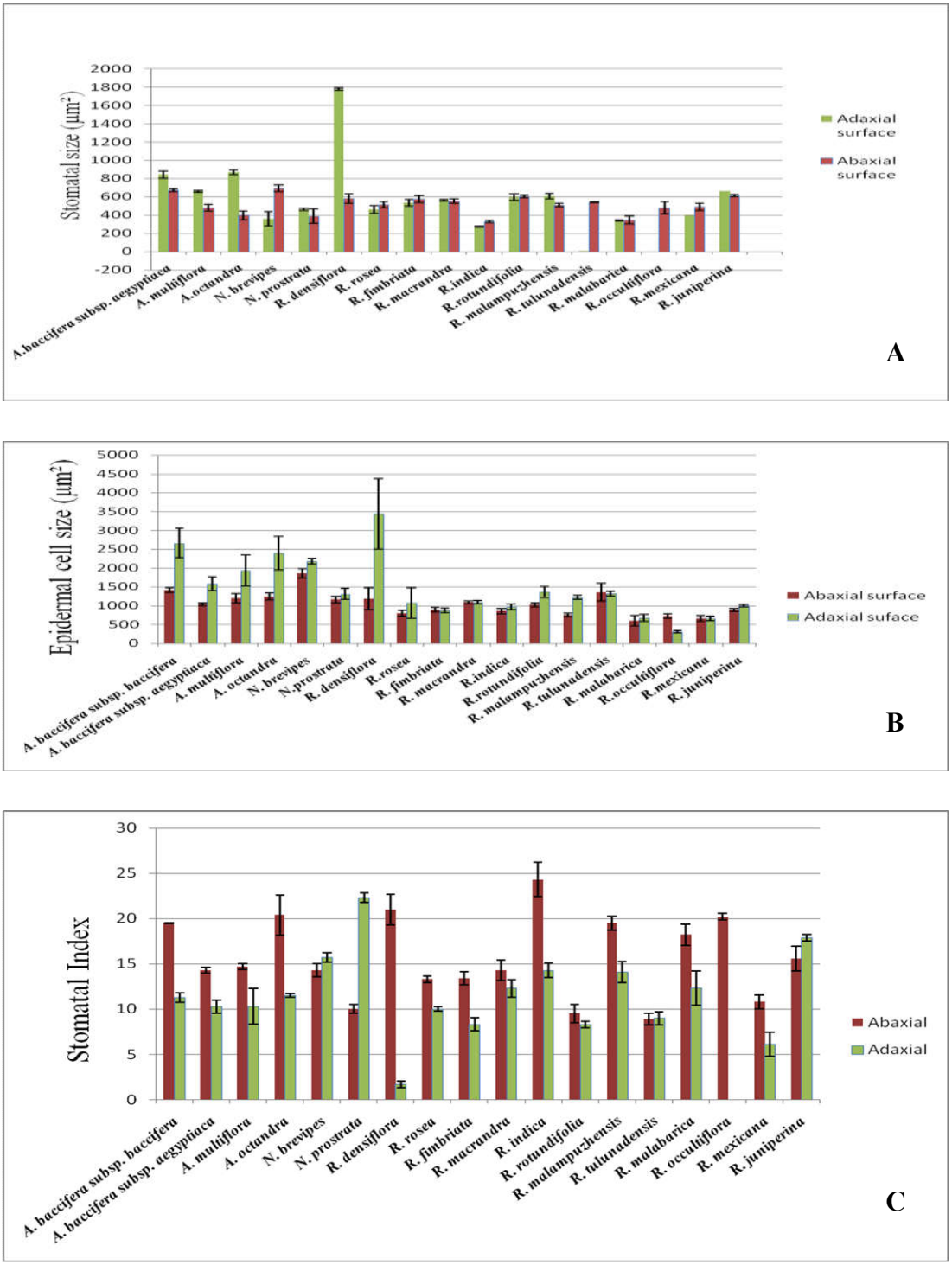

Figure 37. Graphical representation of variation in different species of *Ammannia*, *Nesaea* and *Rotala*. **A.** Stomatal size; **B.** Epidermal size; **C.** Stomatal Index.

Discussion

The present chapter provided a detailed description of various anatomical features *viz.,* stem anatomy, leaf midrib anatomy and cuticular features of *Ammannia, Rotala* and *Nesaea.* A very few characters especially cortical characters of stem, were found to be useful for the taxonomic delimitation at generic level. Characters such as the presence of intraxylary phloem, bicollateral vascular system, amphistomatic stomata, deposition of druses, very small cork cells that arose from pericycle and the occurrence of aerenchyma in the primary cortex are common in all members. All these observations corroborates with the studies by Metcalf and Chalk (1950) on Lythraceae.

Anatomical characters of stem such as nature of cortex and pericycle in stem were primarily found to afford generic characters in agreement with the studies of Panigrahi (1976) according to which stem anatomical characters are subjected to less variation owing to external condition. The cortex of the stem of all genera were found to be aerenchymatous where the genus *Rotala* is differed in their area of cortex and number of aerenchyma from other two genera *Ammannia* and *Nesaea*. The presence of aerenchymatous cortex was reported earlier in various aquatic or amphibious plants, where these structures were developed in response to poor aeration (Justin & Armstrong, 1987; Jackson & Armstrong, 1999). The amount of aerenchyma in the cortex was found to be more in genus *Rotala* due to their more aquatic nature compared to *Ammannia* and *Nesaea*. This study shows the stability of aerenchyma pattern in the genus level, in agreement with the previous studies of Jung *et al*. (2008) and Panigrahi (1976), further leading to apply this as a tool for taxonomic purposes.

A detailed study of hypodermal characters for the herbaceous species of Lythraceae was conducted for the first time in this comparative analysis.

Metcalf and Chalk (1950) have reported the presence of hypoderm only in *Ginora* and *Lagerstroemia* among the family Lythraceae. Hypodermal characters were exhibited uniformity in almost all species of *Rotala* where as the specific level separation of taxa was reported for *Ammannia* and *Nesaea.* Parenchymatous hypodermis was reported in most of the species of the *Rotala* except for *R. malabarica* where it is found to be chlorenchymatous. Chlorenchymatous hypodermis was described in *A. baccifera* subsp. *baccifera, A. multiflora* and *N. brevipes.* Collenchymatous hypodermis was reported only in *A. baccifera* subsp. *aegyptiaca*, thus can be used as a key character in order to delimitate these species.

The occurrence of either sclerenchymatous or parenchymatous pericycle was found tobe useful in recognizing taxa at generic level. Parenchymatous pericycle is restricted to the genus *Rotala* while sclerenchymatous discontinuous patches in the pericycle are common to both *Ammannia* and *Nesaea.* Presence of sclerenchymatous pericycle was also recorded in some of the earlier studies (Metcalf & Chalk, 1950; Solereder, 1908; Turki, 2007).

Crystals, in the form of 'druses' were very common in *Ammannia* and in most species of *Rotala*, where as druses were absent in both the species of *Nesaea.* Various previous studies (Nakata & McConn, 2006; Ilarslan *et al*., 2001; Franceschi & Nakata, 2005) have highlighted the importance of morphology and distribution of druses in species, genus and even family level which suggested a genetic control on their existence. Occurrence of druses in *Rotala* and *Ammannia* were also reported by Kuo-Huang *et al*. (1994) and Turki (2007) respectively. Within the genus *Rotala*, the pattern of deposition of druses on the surface of the endodermis was observed as a distinguishable systematic feature which was reported for the first time.

Various leaf midrib anatomical characters such as midrib surface shape (abaxial and adaxial) and relative epidermal cell thickness on both surfaces exhibited high variation among species and were employed as key characters at the species level. Hence these characters are of potential value for the circumscription of some species of *Ammannia* and *Rotala.* The midrib characters that observed as very common in all three genera with a very few exceptions was the shape of vascular bundle and presence or absence of sclerenchyma surrounding the vascular bundle.

The cuticular studies revealed the presence of amphistomatic leaves in South Indian species of *Rotala, Ammannia* and *Nesaea* except in *Rotala occultiflora* which is hypostomatic and may be due to the adaptation to water loss in xeric conditions (Metacalf & Chalk, 1950; Mbagwu & Edeoga, 2006). Presence of hypostomatic leaf in *R. occultiflora* is the first report, as earlier workers (Kshirsagar & Vaikos, 2013) reported amphistomatic leaf for the same. Here, we described *R. densiflora* as hypostomatic to amphistomatic, as some population is found to be strictly hypostomatic, while a very few others to be amphistomatic with maximum of 10 number of stomata per unit area. Earlier studies of Rajagopal (1979) also highlighted amphistomatic or hypostomatic nature in different species of Lythraceae. This hypostomatic population is observed to posses largest stomata on their adaxial surface among all species considered here, which is in corroboration with some previous studies, where an inverse relationship between stomatal size and and stomatal number were reported (Muenscher, 1915; Camargo & Marenco, 2011; Ajayan *et al.,* 2015; Zoric *et al.,* 2009). In all species, isotricytic stomata are distributed in large proportion on both abaxial and adaxial surfaces with a very few exception. In earlier studies (Panigrahi 1981; Thanki *et al.,* 2000), ‘anomocytic’ stomata were reported in Lythraceae which is now considered as synonym of ‘Isotricytic and tetracytic’ type according to latest classification of Prabhakar (2004). Strangely, staurocytic stomata were found

to be distributed in major proportion along with tetracytic stomata in *R. mexicana*. Presence of trifling percentage of diacytic stomata was observed to be species specific to *A. baccifera* subsp. *baccifera* and *A. octandra*.

Ahmad *et al*. (2010) noted valuable intergeneric and interspecific variations in the pattern of epidermal cells that can be used to as an important taxonomic tool to identify many species. Majority of species of three genera possess polygonal epidermal cells with either wavy to sinuate or straight to curved anticlinal cell wall. Relatively larger epidermal cells are distributed on adaxial surface compared to abaxial surfaces of most of the species studied here (Figure 37. B).

Stomatal index is considered as one of the useful tools in order to distinguish species, since it is fairly constant for a particular species (Salisbury, 1928) than stomatal density. There was a great variation in stomatal index, in between different species of *Ammannia*, *Rotala* and *Nesaea*. Highest index was observed in abaxial surface of *R. indica* and lowest in the adaxial surface of *R. densiflora*. Generally high stomatal index was observed on abaxial surfaces of leaf compared to adaxial surface in all species of *Ammannia* studied and in most species of *Rotala*, which is reported to be the characteristic to herbaceous species (Willmer & Fricker, 1996), but the same was observed on adaxial surface in the case of genus *Nesaea*. However in *R.tulunadensis* and *R. juniperina*, stomatal index is slightly more on the adaxial surfaces than abaxial surface which is the characteristics of floating leaves of aquatic plants. Considering the genus *Rotala*, the amphibious or terrestrial species show a noticeable difference between abaxial and adaxial stomatal index, while in *R. macrandra* and *R. rotundifolia*, which are more aquatic in nature, showed negligible difference between adaxial and abaxial stomatal index (Figure 37. C).

Significant comparison of stomatal size between abaxial and adaxial leaf surfaces has been reported by Zoric *et al*. (2009). Similar types of comparative studies in this group revealed, species of *Ammannia* possess relatively larger stomata on their adaxial surfaces compared to abaxial surface. Besides, in most of the species of *Rotala* (*R.macrandra*, *R. malampuzhensis, R. densiflora* and *R. juniperina*) and in *Nesaea prostrata* the similar condition is observed. When Compared to *Ammannia* and *Nesaea*, different species of *Rotala* shows more diversity in stomatal size between abaxial and adaxial surfaces. The stomatal size is observed maximum for *Rotala densiflora* on its adaxial surface and minimum for *Rotala indica* on its abaxial surface. Stomatal size is not taken in into consideration in the delimitation of species among three genera as stomatal size range shown (except *R. densiflora*) by the numbers exhibit continuous variation (Figure 37. A).

Molecular Phylogeny

The application of molecular biology and bioinformatics for the elucidation of evolutionary patterns and exploration of genetic diversity has revolutionized the plant taxonomic studies in the last decade of the 20th century. Originally used molecular markers (RAPD, RFLP etc.) suffered from their anonymities which bring uncertainty and limitation in their application. The development of DNA sequencing techniques and various universal primers helped the rapid accumulation of sequence information of various taxa in phylogenetic analysis that inspired taxonomists to consider identifying species using DNA sequence information.

For the morphologically, most perplexing three herbaceous genera, *Ammannia, Rotala* and *Nesaea*, it would be fair to conduct molecular phylogenetic analysis in order to get a resolution of both generic and species relationship. Subsequent to various molecular studies in two major disjunctive area of diversity such as Africa and China (Huang & Shi, 2002; Graham *et al*., 2006; Graham *et al*., 2011), the present analysis is the first molecular analysis for the South Indian species of *Ammannia, Nesaea* and *Rotala*. The current study included maximum number of the species from genus *Rotala,* comparing to studies in Africa and China. Also increasing number of newly describing species of *Rotala* in recent years from South India recommends the requirement of molecular analysis in order to confirm the distinctiveness of these newly coming species.

Materials and Methods

The major steps of the molecular analysis involved were: 1) isolation of the genomic DNA from the selected taxa, 2) PCR amplification of selected genes using specific primers and sequencing of the amplified gene, 3) BLAST

analysis and sequence submission and 4) phylogenetic analysis using suitable software.

Isolation of genomic DNA

Fresh leaves of 19 species from *Ammannia, Nesaea* and *Rotala* were used for extraction following the modified CTAB method of Doyle and Doyle. The procedure is as detailed below:

The leaves (100 mg of each sample) were ground to a fine powder in a pre chilled mortar and pestle using liquid nitrogen. 5 mg of polyvinyl pyrolidone (PVP) was added at the time of grinding. The powdered tissue was transferred in to pre-warmed 500 µl of extraction buffer (2% CTAB, 1.4M NaCl, 20mM EDTA and 100 mM Tris-HCl). 2% of β-mercapto ethanol and 5 µl of proteinase K were added immediately after the mixing of tissue in extraction buffer. The whole mixture is made up to 800 µl with extraction buffer and mixed gently. Then these were incubated for 45 minute at 65^0C. Equal volume of 24:1 chloroform: Isoamyl alcohol was added, mixed by inversion and centrifuged for 10 min at 10,000 rpm at room temperature. Repeated the extraction with supernatant. The supernatant was added by 2/3 volume of ice-cold isopropanol and inverted the mixture gently several times in order to precipitate DNA. Incubated the prepared sample at 4^0C for 30 min. to 1 hr and centrifuged at 12000 rpm for 20 min at 4^0C in order to pellet the DNA. The pellet was dissolved in 50 to 100 µl of TE buffer. 5µl of RNases was added and incubated for 30 min at 37^0C. Then RNases was removed by chloroform: isoamyl alcohol extraction as described earlier and again precipitated the DNA using 0.5 volume of Ammonium acetate and 2 volume of ice cold absolute ethanol. Mixed gently by inversion and incubated at -20^0C for 30 min. to overnight. To pellet the DNA, Centrifuged the mixture at 12000 rpm for 20 min.at 4^0C. The obtained DNA was washed with 70% ethanol and re suspended in 50µl TE buffer. The final DNA was incubated at

65^0C for 2 min. to distort any DNase present. The obtained DNA samples were stored in -20^0C for further use. The concentration and purity of (ratio of absorbance at 260 to that of 280) DNA was assessed by uv-spectrophotometry using UV scanning Nanodrop^{TM} 2000 spectrophotometer (Thermo scientific, Germany). The intactness of the DNA was examined by Agarose gel electrophoresis using 0.8% agarose.

Table 8. Reagents and buffers used for total DNA extraction and Agarose gel electrophoresis.

1M Tris HCl: 60.55 g Tris base is dissolved in 300 ml of sterilized double distilled water. Adjusted the pH to 8.0 by adding conc. HCl and finally bought the volume to 500 ml.
0.5M Na-EDTA: 93. 05 g of EDTA – di sodium salt was dissolved in 300 ml of sterilized double distilled water. Adjusted the pH to 8.0 using NaOH pellets and made the volume up to 500 ml.
5 M NaCl: 58.44 g of NaCl was dissolved in 100 ml of sterilized double distilled water
Extraction buffer: Composition: 2% Cetyl Trimethyl Ammonium Bromide (CTAB), 1.4M NaCl, 20mM Na-EDTA (pH 8.0) and 100 mM Tris-HCl (pH 8.0) Preparation: Measured 1ml of 1M Tris, 4 ml of 0.5 M Na- EDTA and 5.6 g of NaCl. Mixed with approximately 30 ml of hot sterilized double distilled water and 2 g of CTAB was mixed slowly to this. Finally, the volume was adjusted to 100 ml.
24:1 Chloroform: Isoamyl alcohol Mixed 96 ml of chloroform with 4 ml of Isoamyl alcohol and stored in a brown bottle at 4°C in dark
7.5M Ammonium acetate : Dissolved 57.8 g of ammonium acetate in 100 ml of double distilled water. Adjusted the pH to 7 with acetic acid

Proteinase K buffer : Composition: 100 mM Tris HCl, 50 mM EDTA and 500 mM NaCl. Preparation 10 ml of 1 M Tris HCl was mixed with 10 ml of 0.5 M NaCl and made the final volume to 100 ml.
Proteinase K: 100 mg of proteinase K was added to 10 ml of 10 X proteinase K buffer in a screw cap bottle and stored in −20°C.
50 X TAE buffer (pH 8.0): Composition: Tris, 0.5 M EDTA, Glacial acetic acid Preparation: 24 g of Tris base was dissolved sterilized double distilled water and 100 ml of 0.5 M EDTA and 57.1 ml of Glacial acetic acid were added. The final volume was made up to 1L with sterilized double distilled water.
TE Buffer (pH 8.0): Composition: 10 mM Tris, 1mM EDTA Preparation: Measured 1 ml of 1 M Tris, 0.2 ml of 0.5 M EDTA and made to 100 ml.
Ethidium bromide (10mg/ml): 100 mg of Ethidium bromide was dissolved in 10 ml of double distilled water.

PCR amplification and Sequencing

Two chloroplast DNA sequences- i) the large subunit of ribulose bis phosphate carboxylase oxygenase (*rbcL*) ii) *trnL-F* Region and iii) nuclear ribosomal DNA sequences of the internal transcribed spacers (ITS1, 5.8S rRNA gene, ITS2) were used for the present phylogenetic analysis. PCR reactions were performed using primers i) ITS A and ITS B (Blattner, 1999), ii) *trnL-F* c and *trnL-F* f (Taberlet *et al*., 1991) and *rbcL* forward and *rbcL* reverse (Fay *et al*., 1998), in order to amplify corresponding region of gene of interest. Amplification reactions were carried out in 96 well thermal cycler with gradient block (Eppendorf Mastercycler Pro S, Germany) thermal cycler. The amplification reaction mixture (final volume 25 µL) contained the following: 1 X Buffer , 0.2 mM dNTP Mix, 0.4 mM of each Forward and

Reverse primer (0.8 mM for *trnLF*), 0.2 µg-2µg of Template DNA, 0.125 µl of Taq Polymease(5U/µl). Thermal profile of PCR was set as initial denaturation at 95°C for 2 min, 34 times cycle of 20 sec at 95°C, 40 min at T_A°C (55.7,°C for *rbcL,* 56.9 °C for *trnLF*, 56 °C for ITS), 1 min at 72°C and a final extension step of 5 min at 72°C. Amplified products were subjected to Agarose gel electrophoresis (1% agarose in TAE- Tris Acetate EDTA- buffer) along with commercially available 100 bp DNA ladder (HiMedia Labs Ltd.). The intense band is selected and size was determined by comparing with DNA ladder. The obtained amplified products were purified using the commercially available gel extraction kit (Origin). The sequencing of purified products were performed at commercial facility (SciGenome, Cochi), using the same primers used for PCR.

Table 9. Base composition and citation information for all PCR and sequencing primers used for these analyses.

Sl No.	Primer	Sequence 5'-3'	Citation
1	*rbcL* forward	ATG TCA CCA CAA ACA GAA ACT AAA GC	Fay *et al.* (1998)
2	*rbcL* reverse	CTT TTA GTA AAA GAT TGG GCC GAG	Fay *et al*. (1998)
3	*trnL-FC*	GGG GAT AGA GGG ACT TGA AC	Taberlet *et al*. (1991)
4	*trnL-F F*	ATTTGAACTGGTGACACGAG	Taberlet *et al.* (1991)
5	ITS A	GGA AGG AGA AGT CGT AAC AAG	Blattner (1999)
6	ITS B	CTT TTC CTC CGC TTA TTG ATA TG	Blattner (1999)

Agarose gel analysis

Agarose was weighed appropriately so that the final concentration of agarose gel is either 0.8% (for the separation of genomic DNA) or 1% (for the separation of PCR product) and mixed with 100 ml of 1X TAE buffer. The mixture was heated and brought to a boil with occasional gentle mixing until the agarose dissolved completely. This solution was cooled to 50°C

temperature and then added ethidium bromide (EtBr) (10mg/ml) to a final concentration of approximately 0.2 − 0.5 µg / ml. EtBr binds to the DNA and allows to visualize the DNA under ultraviolet (UV) light. Poured the gel slowly into the tank and removed the bubbles away using a disposable tip. The comb was Inserted and left further to set for 30 min. to 1 hr. 1X TAE buffer was added to gel tank to submerge the gel to 2− 5 mm depth. Appropriate amount of each samples were transferred to a fresh micro centrifuge tube and mixed with 0.2 volumes of commercially available loading buffer in which bromophenol blue was used as major dye component. The samples were loaded in to each well, closed the tank, switched on the power source and run the gel at 5V/ cm. Switched off the gel when the bromophenol blue has run 3/4 the length of the gel and disconnected the electrode from power source further carefully removed the gel from the gel tank. Finally visualized the DNA fragment under UV trans illuminator.

BLAST search and Sequence submission

The DNA sequences were then used separately for similarity searches using BLAST (Basic Local Alignment Search Tool) online tool in the NCBI GenBank DNA database (www.ncbi.nlm.nih.gov) in order to check similarity to the specified gene sequence region (*rbc*L or *trnL- F* or ITS region) of related plant species. The *rbcL, trnL-F* and ITS sequences which we determined have been submiteed in the GenBank database.

Phylogenetic Analysis

The newly generated *trnL- F* and ITS sequences of 19 species and *rbc*L sequences of 18 species from *Ammannia, Nesaea* and *Rotala* along with some additional sequences (represented from other genera of Lythraceaea) which are retrieved from GenBank were aligned using ClustalW in MEGA v.6 (Tamura *et al.,* 2013). The ingroup taxa in the aligned sequences for *rbc*L

data set include 18 genera, *trnL–F* data set included 19 genera and ITS data set included 20 genera. The outgroup taxa for all analyses selected were *Fuchsia magellanica* and *Ludwigia hyssopifolia* from the sister family, Onagraceae. The phylogenetic analyses were conducted for all three sequence regions, both separately and in combination. Before combining the data sets, the partition homogeneity test was performed by PAUP4.0b10 (Farris *et al*., 1995; Swofford, 1998), to assess data congruence. One thousand replicates were performed, and the resulting P value was used to confirm the suitability of combined data sets for phylogenetic reconstruction

Phylogenetic reconstruction with maximum parsimony (MP) was performed with PAUP4.0b10 (Swofford, 2003). Heuristic searches were used in MP analysis with 1000 replicates of random stepwise addition sequences, tree bisection-reconnection (TBR) branch swapping and MulTrees option in effect. Equal weights were assigned for the character states at all nucleotide positions. The gaps of the sequence matrix were treated as missing data. Strict consensus trees were constructed from all most parsimonious trees for each analysis. Bootstrap probabilities (BP) were calculated from 1,000 replicates using a heuristic search, to examine the relative level of support for individual clades. The Maximum parsimony (MP) analysis was also conducted in Molecular Evolutionary Genetics Analysis MEGA 6 (Tamura *et al.,* 2013). The stability of relationships was assessed by conducting bootstrap analyses based on 1,000 resamplings.

Result

Total genomic DNA from 19 species representing three genera of Lythraceaea *viz., Ammannia, Rotala* and *Nesaea* was successfully isolated without much contamination. Nuclear ribosomal ITS gene sequences (size varies from 600 to 700 bp nucleotides) and chloroplast *trnL- F* gene sequences (size ranges from 700 to 1000 bp nucleotides) were amplified for

19 species, where as *rbcL* sequences could be generated, only from 18 species (Figure 37). Despite repeated efforts to optimize the PCR condition, *rbc*L sequences could not be generated for *Rotala malabarica*. The *rbcL, trnL-F* and ITS sequences which we determined have been deposited in the GenBank database and most sequences have been assigned GenBank accession numbers. The details regarding newly generated sequences with their accession numbers are given in the table 10.

Table 10. Information plant samples from South India included in this study, including taxon name, gene region and GenBank accession numbers,

Sl. No:	Taxa	GenBank Accession Numbers		
		trnL-F	***rbcL***	**ITS**
1	*A. baccifera* subsp. *aegyptiaca*	Submitted	KX807170.1	MH071604
2	*A. baccifera* subsp. *baccifera*	Submitted	MF356450	MH173266
3	*A. cordata*	KX570644.1	KX639505.1	MF372515.1
4	*A. multiflora*	KX807169.1	KR133275.1	MF 599386
5	*A. octandra*	KX570644.1	KU883365.1	MH071605
6	*A. prostrata*	Submitted	KX663334.1	MF 5993888
7	*R. cheruchakkiensis*	KY021931	MF374636	MH071603.
8	*R. densiflora*	KY462183	KU997675.1	MF287651
9	*R. fimbriata*	KX964288.1	KU979011.1	MG518325
10	*R. indica*	KY462184	KX869915.1	MF599387
12	*R. juniperina*	KX964287.1	KU997675.1	MG517612
12	*R. macrandra*	MF374637	KX869917.1	MH071598
13	*R. malabarica*	MF374635	Not genereated	MF372514.1
14	*R. malampuzhensis*	Submitted	KU866300.1	MH071601
15	*R. mexicana*	KY462182	MF142802	MG991825
16	*R. occultiflora*	Submitted	KX869914.1	MH071599
17	*R. rosea*	Submitted	KX869918.1	MF352034
18	*R. rotundifolia*	Submitted	MF356451	MH071600
19	*R. tulunadensis*	KX943031	KX156835.1	MH071602

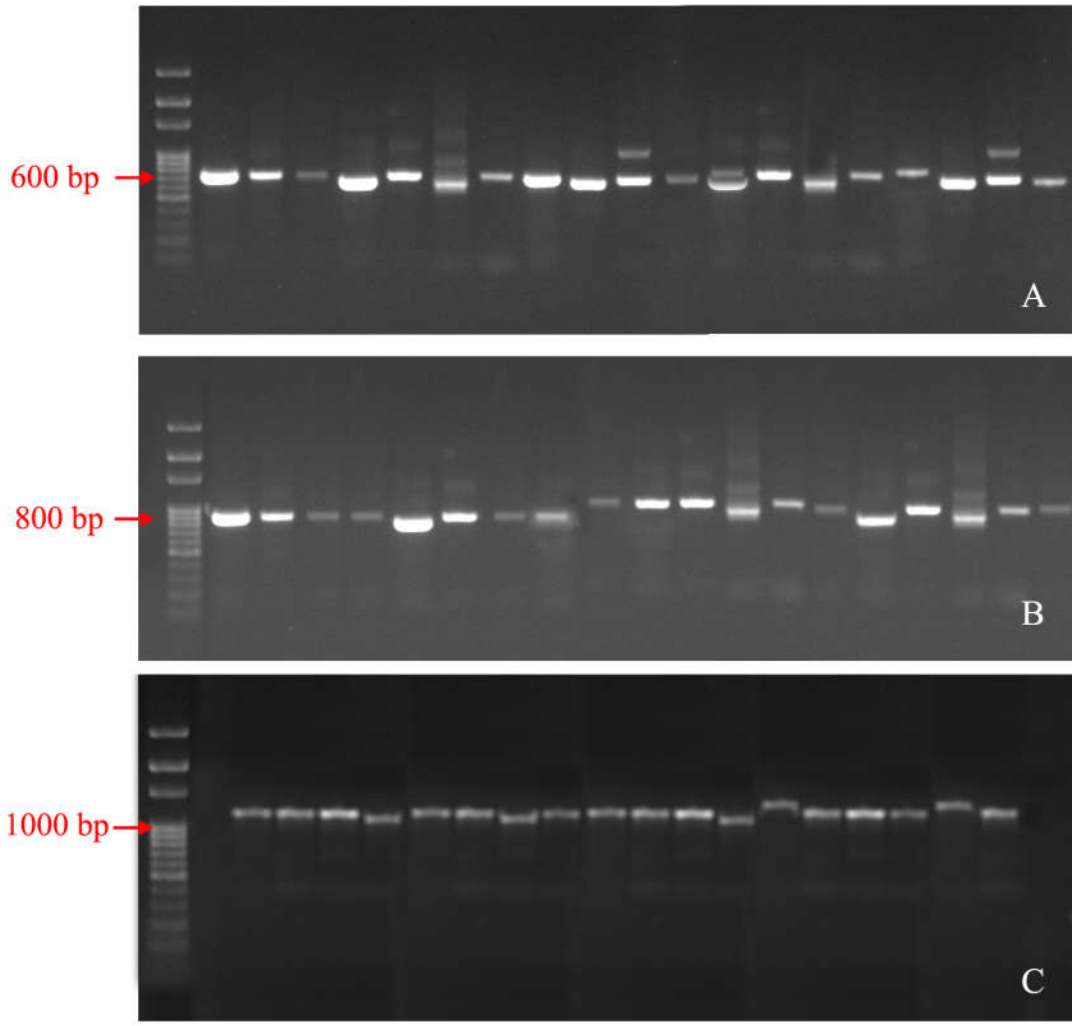

Figure 38. PCR Amplified products: A**.** ITS; B**.** *trnL-F*; C**.** *rbcL*

Both the MEGA v.6 and PAUP4.0b10 resulted in a phylogenetic tree of similar topology, but PAUP4.0b10 produced phylogenetic tree with higher bootstrap support. Hence further intepretaion was based on trees those are generated by PAUP4.0b10. Partition homogeneity tests (P=0.01) indicated that, different data partitions were significantly incongruent; hence the datasets should be analyzed separately. The total length of the aligned sequences is 3712 nucleotides for combined analysis, and the same for *rbc*L, *trnL-F* and ITS are 2553, 657 and 912 nucleotides respectively. *TrnL-F* and ITS sequences provided higher percentage of (53% and 45% respectively)

informative positions, while combined sequences of ITS, *rbcL* and *trnL-F* (32%) and *rbcL* (37%) were found to be less parsimony informative. Descriptive values resulting from the MP analyses for the trees of the separate and combined data sets are listed in Table. These values clearly showed that the datasets for ITS sequences have shortest tree length and thus said to be most parsimonius tree (Barton *et al.,* 2007).

Table 11. Comparative statistics for maximum parsimony analyses of individual DNA regions, and combined analyses (The analysis included total aligned length, number and % of parsimony informative characters, number and length of most parsimonious trees, consistency index (CI), and retention index (RI)).

DNA region	Number of nucleotide sequences	Aligned length	most parsimonious tree length	%of informative sites	CI	RI
rbcL	47	2553	2085	37	0.821	0.924
trnL-F	46	657	2151	53	0.704	0.725
ITS	47	912	704	45	0.399	0.693
Combined	36	3712	2108	32	0.616	0.716

Analysis of both ITS and combined data matrix using *Ludwigia hyssopifolia* and *Fuchsia magellanica* as outgroups, supported the monophyly of the genus *Rotala* with high bootstrap value (>95%) and also clearly increased resolution and branch support in the resulting trees. Number of branches with >90 % BS was found to be more in combined, ITS and *trnL-F* tree while for rbcL tree, these number was found to be less than 10. Thus chloroplast region *rbcL* region as a separate analysis was found to be insufficient due to lower resolution and bootstrap value.

Analysis of combined sequences

The combined tree show three groups which are Group I, Group II and Group III. Group III represented *Rotala* clade in which single clade of *Nesaea brevipes* and *Nesaea prostrata* is included near to the *Ammannia-Hionenthera- Nesaea* clade. *N. brevipes*/ *N. prostrata* clade in the cluster A is found to be sister to monoclade of *Rotala tulunadensis* (BS=94%). *R. macrandra, R. juniperina, R. indica* and *R. rotundifolia* exist as a independent monoclades within this cluster. Cluster B consist of two clades i) clade of *R. fimbriata* and *R. rosea* ii) clade of *R. densiflora* and *R. cheruchakkiensis* (BS=66%) and a monoclade of *R. malampuzhensis*. Cluster C included a single clade of *R. occultiflora* and *R. mexicana* and this clade has high branching support (BS=100%).

Group II can be described as *Ammannia- Nesaea-Hionenthera (A/H/N)* clade (BS=100%) which is sister to *Lawsonia inermis.* Cluster A within this group consist of three separate monoclade namely *A. octandra, A. baccifera* and *N. schiznii* and two clades i) calde of *A. multiflora* and *A. baccifera subsp.aegyptiaca* ii) clade of *A. auriculata* and *A. prieuriana* which in turn is found to sister to *N. aspera*.

Analysis of ITS

ITS data set comprised of largest number of taxa representing 49 species of 20 genera. Group I best supports (with 100% BS) the *Ammannia-Nesaea* a calde which is sister to monoclade of *Hionenthera*. This group is also devided into different clusters such as Clusters A, B, C and D. Cluster A represented petaliferous *Ammannia* in which South Indian species of *Ammannia multiflora* exist as a monocalde and exhibited a sister relationship to the clade of *A. prieuriana* and *A. auriculata* with the bootstrap value of 80%. Cluster B represented apetaliferous *Ammannia,* in which clade of *A. baccifera* (South Asian species) is found to be sister to monoclade of *A. baccifera* subsp. *aegyptiaca* with high branching support (BS=99%). Cluster

C represented different African species of *Nesaea*. Cluster D stood for morphologically perplexing single clade of *Nesaea brevipes* and *N. prostrata* (BS=100%) and is found to clade with *N. radicans. A. octandra* is found to be exist as a separate monoclade within this cluster with a moderate boot strap value of 85%.

Group II represented the monophyletic clade of *Rotala*, in which early diverging, 4 clusters *viz*. cluster A, B, C, D comprised fully of different subclades of *Rotala*. Cluster A consist of single clade of *Rotala rotundifolia* and *Rotala indica* (represented from China) which is sister to mono clade of south Indian species of *R. indica* and this relationship was found to be supported by high bootstrap value (100%). Cluster B is divided into two subclades*, Rotala fimbriata* and *R. rosea* with one clade (BS=100%), while *R. juniperina* and *R. malabarica* form the second clade which in turn clusterd with two separate mono clades of *R. cheruchakkiensis* and *R. densiflora*. Cluster C consist of single clade of *R. occultiflora* and *R. mexicana* with 100% bootstrap value. In cluster D, *Rotala malampuzhensis* form a monoclade and is clustered with a clade of *R. macrandra* and *R. rotundifolia*. But the new species *R. tulunadensis*, form separate monoclade and exhibited a sister relationship to other clusters of this group.

Analysis of *trnL- F* region

trnL-F tree consist of 5 groups, in which group II is found to be *Ammannia- Hionenthera- Nesaea* clade and group V is described as *Rotala* clade. Within group V, in cluster A, the clade formed by *R. densiflora, R. malampuzhensis* and *R. cheruchakkiensis* (BS=85%) is sister to monoclade of *R. juniperina*. The sister clade of the same cluster formed by *R. fimbriata* and *R. rosea* is supported by high bootstrap value. Cluster B consist of a clade of South Indian species *R. macrandra* and *R. rotundifolia* which exhibited sister relationship to monoclade of African species of *R. rotundifolia.* Cluster C best support the clade of *R. occultiflora* and *R. mexicana* with 100% bootstrap value. *R.indica* and *R. tulunadensis* exist as two distinct monoclades.

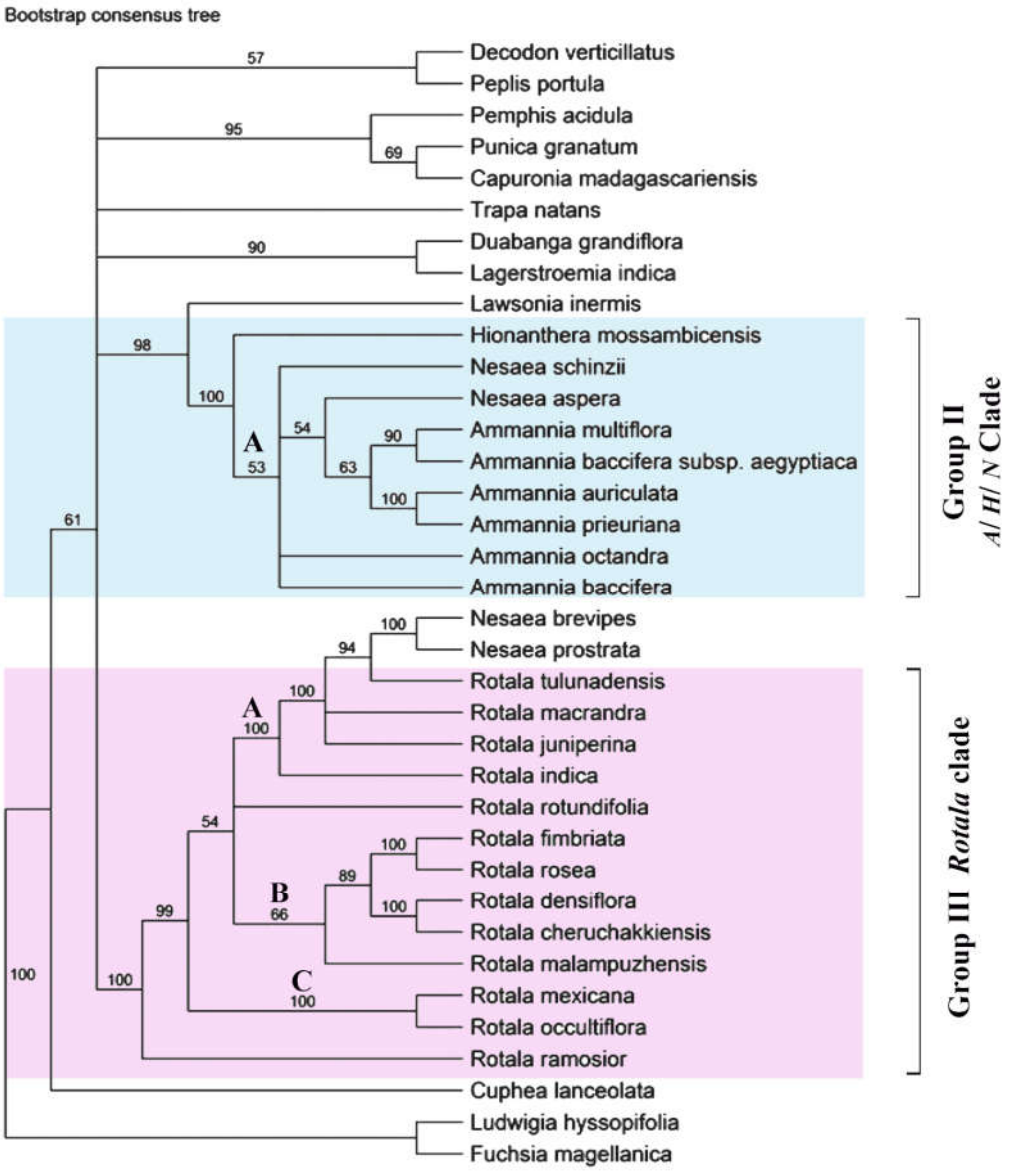

Figure 39: Maximum parsimony (MP) tree for Lythraceae generated from combined sequence data of nuclear rDNA internal transcribed spacer (ITS) and the chloroplast *rbc*L and *trn*L–*trn*F regions. Numbers on the branches represent ML bootstrap proportions > 50%. *Ludwigia* and *Fuchsia* (Onagraceae) represent the outgroup.

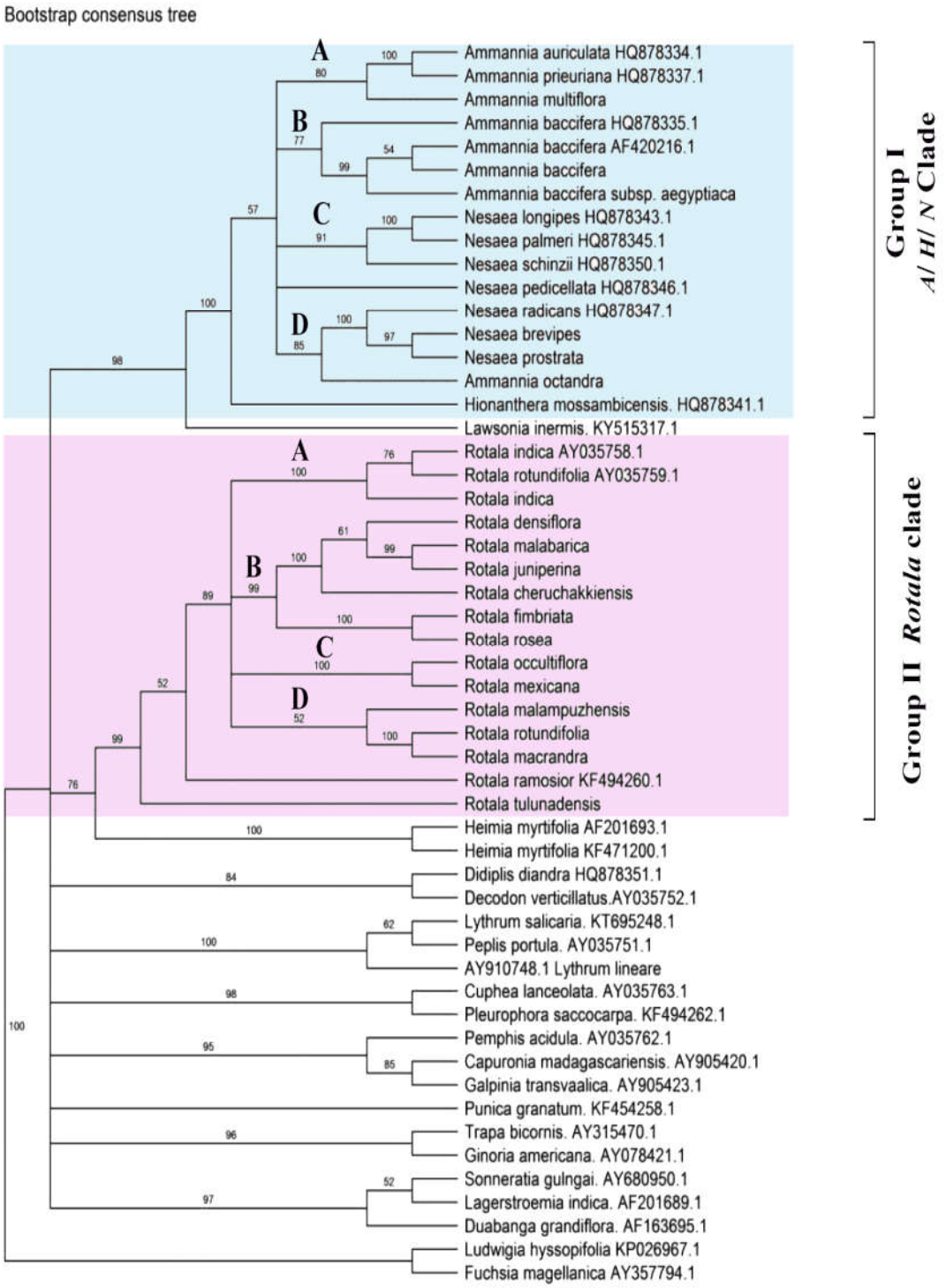

Figure 40: Maximum parsimony (MP) tree for Lythraceae generated from nuclear rDNA internal transcribed spacer (ITS) regions. Numbers on the branches represent ML bootstrap proportions > 50%. *Ludwigia* and *Fuchsia* (Onagraceae) represent the outgroup.

The placement of *N. brevipes* and *N. prostrata* as a single clade with a sister relationship to mono clade of *N. aspera* within the group II is moderately supported by 60 % bootstrap value. Other two south Indian taxa of *A. octandra* and *A. baccifera* were placed as an early diverging, separate monoclades within group. Still *A. multiflora* and *A. baccifera* subsp. *aegyptaica* are not included in A/H/N clade, rather placed in group I (BS=80%) in which *R. malabarica* is also included. *A. baccifera* subsp. *aegyptiaca* formed a clade with *R.malabarica* and sister clade to *A. multiflora* (BS=100%).

ITS, *rbcL* and *trnL-F* data sets for South Indian species of *Ammannia, Rotala* and *Nesaea* are included here in both combined and separate phylogenetic analysis for the first time. Phylogenetic analysis based on the combined and ITS data set resulted in trees with better resolution and improved bootstrap support than *trnL- F* and *rbcL* data set. The topology of these data sets supported an expanded interpretation of generic boundaries for Lythraceae to include *Rotala* as a distinct clade and far distant from *Ammannia/ Nesaea/ Hionanthera* clade (A/N/H clade). *Ammannia* and *Nesaea* formed as strongly supported clade along with *Hionanthera* and within this clade *Ammannia* and *Nesaea* were found to be paraphyletic. These observations correspond to the previous studies of Huang and Shi (2002) and Graham *et al*., (2005, 2011). The most important phylogenetic inferences that emerged from this analysis are discussed below.

Discussion

Ammannia- Nesaea- Hionanthera clade

Ammannia, Nesaea, Hionanthera clade is strongly supported for highly resolved ITS data sets (BS=100%) and to a greater extent for combined data sets (BS=98%) and *trnL-F* data sets (BS=57%). *Ammannia* is nested completely within *Nesaea* in ITS and *trnL-F*, while fairly (except for *N. brevipes*/ *N. Prostrata*) in combined analyses, which are in congruent with morphological data, further lead to infer on congeneric status of these two genera.

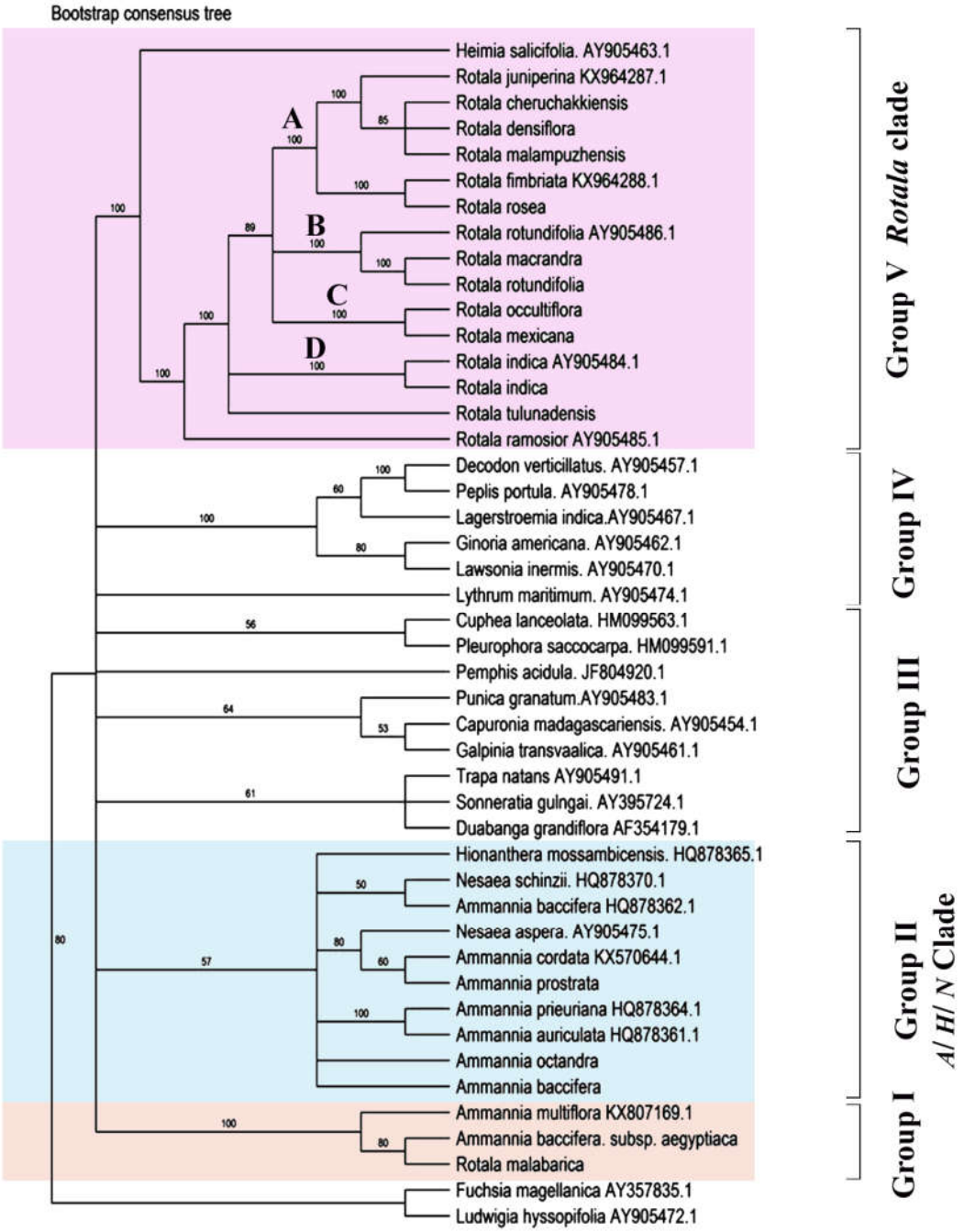

Figure 41. Maximum parsimony (MP) tree for Lythraceae generated from *trnL–trnF* regions. Numbers on the branches represent ML bootstrap proportions > 50%. *Ludwigia* and *Fuchsia* (Onagraceae) represent the outgroup.

Based on the previous molecular studies of Graham *et al.* (2011) in combination with comparative morphological studies, Graham & Gandhi (2013) carried out nomenclatural transfer of many African species of *Nesaea* and *Hionanthera* to *Ammannia.* Present analysis corroborates recent phylogenetic studies on these three genera (Graham *et al*., 2011; Graham & Gandhi, 2013). Inclusions of two South Indian species of *Nesaea* in the A/N/H clade in the present analysis also support the transfer of *Nesaea* to *Ammannia*. The transfer of *Nesaea brevipes* and *Nesaea prostrata* to the genus *Ammannia* is once again substantiated. Thus, this phylogenetic analysis provided a supporting data to merge morphologically variable and confusing *Ammannia* and *Nesaea* into a single genus *Ammannia*. Also Koehne's hypothesis of "*Nesaea* as 'primaeval' genus from which *Ammannia* and *Rotala* are independently diverged" is put to rest by the present phylogenetic study since many of them share more recent common ancestor.

Highly resolved ITS topology exhibit congruence with the morphological character at the species level. Placement of petaliferous (*A. auriculata, A. prieuiana* and *A. multiflora*) and apetalous *Ammannia* (*A. baccifera* subsp. *baccifera* and *A. baccifera* subsp. *aegyptiaca*) taxa in separate clade with a moderate bootstrap support of 76% is congruent with similar clade in the combined analysis of Graham *et al.* (2011). Primitive trait, shows a sister relationship to both petaliferous and apetalous clade and found to be congruent in all analysis.

***Rotala* clade**

Monophyly of *Rotala* is strongly supported by both ITS and combined data analysis. The *Rotala* lineage is distantly allied to *Ammannia/ Nesaea/ Hionanthera* clade. ITS analysis exhibit a sister relationship between *Rotala* and *Didiplis* while *Heimia* is sister to both these genera. This relationship is in accordance with the phylogenetic study of Morris (2007) and Graham *et al.*

(2011). *Heimia-Rotala* sister relationship is also supported by both *rbcL* and *trnL-F* analysis. In a previous morphological cladistic analysis (Graham *et al*., 1993a), it was descibed that these two genera share characters such as 'compressed seed, internal epidermal straight hairs and stamens in one whorl' which were considered as unique syapomorphies to identify this clade. In the present study, we could not retrieve the data set for all three gene region corresponding *Didiplis* and *Heimia* and hence a detailed relationship among these three genera was not able to resolve within combined data analysis.

The ITS data set best explained the delimitation at both the generic level, and interspecific level. Both the combined and partitioned analysis best support the relationship between *R. mexicana* and *R. occultiflora* with 100 % bootstrap value. Morphologically these two species share characters such as apetalous flower, whorled phyllotaxy, trilocular ovary leading to congruence between molecular and morphological analysis for the two species. Morphologicaly closely similar *R. rotundifolia/R. macrandra* were also found to be congruent with *trnL-F* and *ITS* analysis where these two species show sister relationship. There is a polytomy in a relationship between *R. cheruchakkiensis*, *R. malampuzhesis* and *R. densiflora* in *trnL-F* analysis. But this relationship is fully resolved in combined analysis where *R. malampuzhensis* shows sister relationship to both *R. fimbriata/R. rosea* clade and *R. densiflora/R. cheruchakkiensis* calde. Morphologically recently described *R. cheruchakkiensis* is more closely related to *R. malampuzhensis* but in contrast to this, ITS and combined analysis proved a close relationship to *R. densiflora* with lowest sequence divergence. The recently described *R. tulunadensis* separated as distinct monoclade in *trnL-F* and ITS analysis and could be confirmed as early diverged species without much evolutionary change. All these relationships are supported by high boot strap value. A detailed interpretation of South Indian species was conducted first the time in the present analysis.

Links between disjunctive centers of diversity

The current phylogenetic analysis gives an answer to two disjunctive centres (North East Africa and Indian subcontinent) of diversity of the three widespread genera. The population distributed in these two centres shows morphological and sequence similarities that maintain species or genus identity. Further our molecular evidence supported a well differentiation of these taxa in two centres though together formed distinct unambiguous clade for the same species or genus.

The highly resolved ITS tress obviously pointed out this relationship. When we consider the clade of apetalous *Ammannia*, it is observed that the two South Asian species of *A. baccifera* formed a clade and form sister clade to South Indian *A. baccifera* subsp. *aegyptiaca*. Clade of *A. baccifera*/*A. baccifera* subsp. *aegyptiaca* was found to form sister relationship to Tanzanian species of *A. baccifera*. Within the petalous *Ammannia* clade *A. multiflora* from South India formed a distinct clade and formed sister clade to African species of *A. auriculata* and *A. prieuriana*. The only two South Indian *Nesaea* species *N. brevipes* and *N. prostrata* were claded together and placed as sister to other African species of *Nesaea*. Similar condition is found to be repeated in the clade of *Rotala* also, though the analysis involved a few African Species of *Rotala* and the majority of species diversity in *Rotala* is found in South Asia. Within *R. indica/ R. rotundifolia* clade, two Chinese species of *R. indica* and *R. rotundifolia* claded together and South Indian species of *R. indica* is found to be distinct and showed sister relationship to the former clade. In conclusion phylogenetic analysis revealed that the same species that dispersed in two disjunct centres like North East Africa and Indian subcontinent followed a diversification and closely relative species or the same species within the same region showed close sequence similarity. This might have occurred following one or more migration events between the continents as reported in various previous studies (Qiu *et al*., 1995; Wen & Shi, 1999; Stanford *et al*., 2000).

Systamatic treatment

The delimitation of three closely allied Lythraceaea genera (*Ammannia* L., *Rotala* L., and *Nesaea* Kunth) have been problematic since long. This is due to their remarkable degree of similarity in habit, floral and fruit characters and shared habitats. Most of the earlier authors (Linnaeus, 1753; Bentham & Hooker, 1867) considered *Ammannia* as a larger, more inclusive taxon, including *Rotala* in it (Joseph & Sivarajan, 1989). Bentham and Hooker (1867) recognized two subgenera, subg. *Rotala* and subg. *Eu-Ammannia* and this was followed by Clarke (1879) in Hooker's *Flora of British India*. Historically *Ammannia* and *Nesaea* have long been confused due to their closely similar vegetative morphology and floral structure. The two genera have decussate leaves, cymose infloresences, perigynous, tetramerous flowers, caducuous petals and thin walled capsules. Traditionally, they have been distinguished based on mode of capsule dehiscence (Graham & Gandhi, 2013). *Rotala* in turn is distinguished from *Ammannia* by its axillary solitary flowers or spicate inflorescence and striated capsule walls.

Recent molecular studies (Graham *et al*., 2011) on African taxa of these three genera based on the sequence data from the nuclear rDNA internal transcribed spacers (ITS) and plastid *rbcL* and *trnL-F* regions, revealed *Ammannia* and *Nesaea* constitute a monophyletic assemblage. The present combined analysis based on vegetative floral, seed surface morphology, anatomical and molecular studies support considering *Ammannia* and *Nesaea* as congeneric, which is in corroboration with Graham *et al.* (2011) findings based on African taxa. The current treatment provides a systematic account on 18 South Indian species of *Rotala* and 6 species of *Ammannia.* The merging of *Nesaea* under *Ammannia* is also accepted in the light of present investigation.

Key to genera

1. Flowers in axillary cymes; capsule wall smooth.....***Ammannia***

2. Flower solitary in axils or in axillary spikes; capsule wall striated .. ***Rotala***

Ammannia L.

Sp. Pl. 1: 119. 1753.

Type species: *Ammannia latifolia* L. (Britton & Brown 2: 577. 1913).

Nesaea Comm. *ex* Kunth in Humboldt, Bonpland & Kunth, Nov. Gen. Sp. 6: ed. folio. 151. 6 Aug. 1823, *nom. cons*. TYPE: *N. triflora* (L.f.) Kunth. *Hionanthera* A.Fern. & Diniz, Bol. Soc. Brot., sér. 2. 29: 90. 1955. TYPE: *Hionanthera mossambicensis* A.Fern. & Diniz.

Amphibious or terrestrial, annual, herbs. Stems erect or decumbent, simple or branched, glabrous, rarely pubescent, usually tetragonus. *Leaves* decussate, sessile, entire, 1-nerved; bracteoles 2, small, membranous, whitish. Flowers 3 to many in dense axillary cymes, subsesile to pedicelled, peduncle to 1 mm or absent, 4 (5–8)-merous. *Calyx tube* campanulate or urn shaped, 8-ribbed; appendages absent or very short. *Petals* absent to 8, very caducous, obovate, spathulate or rounded. *Stamens* as many as or twice the number of calyx lobes; filaments inserted at the middle of the calyx tube, anthers didymous. *Ovary* incompletely 2−4 – locular, sometimes unilocular; stigma simple, capitates; ovules numerous, placentation axile or on the septa. *Fruit* dediscing irregularly, capsule globose or ellipsoid, included or exceeding the calyx, capsule wall smooth; seeds numerous, very small, ovoid or semi ovoid.

Distribution and habitat*:* Widely distributed in tropical and subtropical areas, mainly in Africa and Asia *c.* 30 species were reported worldwide which include 4 or 5 species in Indian subcontinent.

Key to the species

1a. Flowers petaliferous 2

1b. Flowers apetalous 4

2a. Calyx tube glabrous 3

2b. Calyx tube pubescent ..... ***A. prostrata***

3a. Stamens 4 ..... ***A. multiflora***

3b. Stamens 8 ..... ***A. octandra***

4a. Flowers distinctly pedicelled, interfold between two calyx lobes without any appendages 5

4b. Flowers subsessile, interfold between two calyx lobes with small bristle-like appendages ..... ***A. cordata***

5a. Leaf base cuneate ..... ***A. baccifera* subsp. *baccifera***

5b. Leaf base subcordate ..... ***A. baccifera* subsp. *aegyptiaca***

Ammannia baccifera subsp. aegyptiaca (Willd.) Koehne, Engl., Bot. Jahrb. Syst. 1: 260. 1881; Manilal & Sivar., Fl. Calicut 112. 1982; Sharma *et al.,* Fl. Karnataka 107. 1984; Fl. Palghat Dist.. 205. 1990; Matthew, Fl. Palani Hills 1: 510. 1999. **Figure: 42**

Ammannia aegyptiaca Willd., Hort. Berol. 1(1): t. 6. 1803.

Iconotype: Hortus Berolinensis 1(1): t.6.1803 (B- WILLD. photo in PRE)

Ammannia salicifolia Hiern in Oliver, Fl. Trop. Africa 2: 478. 1874; Hook. f., Fl. Brit. India 2: 569. 1879.

Erect, annual, 25− 90 cm tall. *Stem* 4-angular, glabrous, reddish tinged, branched, rooting at base; winged. *Leaves* sessile, decussate, linear-lanceolate to oblanceolate, 8− 10 × 0.3−0.5 mm for leaves in the branches, 20−40 × 0.5−1 mm for leaves in main axis; base sub-cordate; apex acute. *Bracts* similar to

foliage leaves; bracteoles elliptic to lanceolate, *c.* 1 mm long, much shorter than calyx tube. *Flowers* 3 to many in dense axillary cymes; pedicels subsessile to 1 mm long, peduncle absent to 1 mm long. *Calyx tube* campanulate, 1 mm long, calyx lobes 4, triangular, deltate, sometimes reddish tinge at the tip, *c.* 0.5mm long; calyx appendages absent. *Petals* 0. *Stamens* 4, inserted at the middle of the calyx tube, exserted. *Ovary* globose, 4-locular; style *c*. 0.3 mm long; stigma capitate; capsule globose, half or lower exserted, *c*. 1.5mm in diam., 4- valved; seeds semi ovoid, about 0.5mm long.

Fl. & Fr.: Dec.− Mar.

Distribution and habitat: Widely distributed in tropical Africa, S. Europe, C. Asia, Himalaya, India to China, Malaysia and Australia. It grows in low lying marshy rice fields and along river sides in South India.

Specimens examined: **Karnataka:** Mysore Dist., Srirangapatana, 11 Mar.1964, *K. M Sebastine 187728* (MH). **Kerala:** Palaghat Dist., Chitoor, 24 Sept. 2013, *Lemiya K. M 132945* (CALI). Horsely Hills, *Ranga Charyulu 1124,* (MH). **Tamil Nadu:** Coimbatore Dist., Perubupathi, 27 Jan. 1931, *S. R. Rafi & Naganathan 4983* (MH). Dharmapuri Dist., Hogainakkal, 14 mar. 1965, *E. Vajravelu 23547* (MH). Madurai Dist., Poyakarai, 12 Jun. 1957, *K. Subramanyam 3440* (MH). Nilgiri Dist., Kalhatti Falls, 29 Nov. 1971, *N. C Radhakrishnan 319068* (MH)

Note: *Ammannia baccifera* subsp. *aegyptiaca* (Willd.) Koehne is based on Willdenow's illustration in *Hortus Berolinensis.* He has not cited any specimen in the protologue. Koehne (1881) reduced Willdenow's species to a subspecies (A. baccifera subsp. *aegyptiaca*) under *A. baccifera* since the name *A. aegyptiaca* is based on a single element Willdenow's illustration (t.6) in *Hort. Berol*. (1803) is designated as the Iconotype for the subspecies (B-WILLD. Photo in PRE).

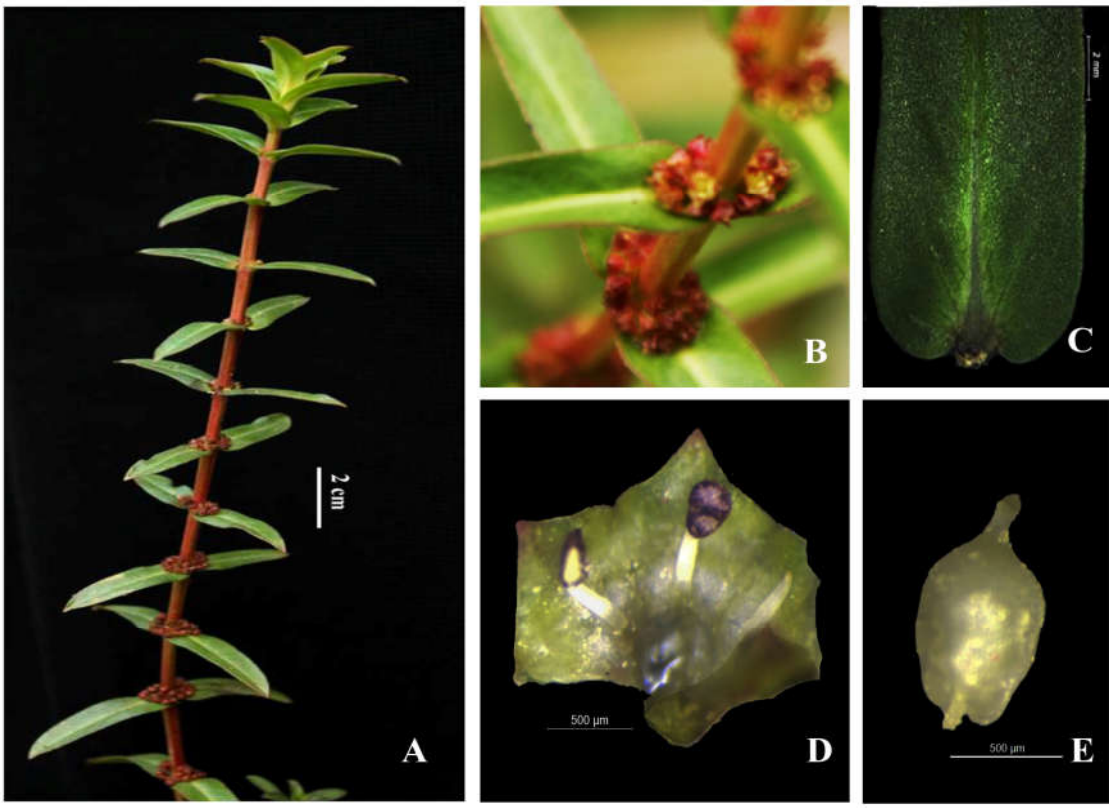

Figure 42: *Ammannia baccifera* susp. *aegyptiaca* (Willd.) Koehne. **A.** Habit**; B.** Inflorescence**; C.** Leaf base**; D.** flower split open; **E.** Gynoecium.

Ammannia baccifera subsp. baccifera L., Sp. Pl. 120. 1753, Hiern, Fl. Trop. Afr. 478. 1871; Koehne, Bot. Jahrb. 259. 1880; Koehne, Engl.,Pflanzenr. 53. fig. 5M. 1903; Cooke, Fl. Bombay 1: 509. 1903; Gamble, Fl. Pres. Madras. 1. 510. 1919; Manilal & Sivar., Fl. Calicut 111. 1982; Matthew, Fl. Tamil Nadu. Carnatic. 606. 1983; Sharma *et al.,* Fl. Karnataka 107. 1984; Ramach. & V.J. Nair, Fl. Cannanore Dist. 191. 1988; Singh, Fl. Eastern Karnataka. 1. 314. 1988; Vajr., Fl. Palghat Dist. 204. 1990; M. Mohanan & Henry, Fl. Thiruvanthapuram 202. 1994; Matthew, Fl. Palani Hills 1: 510. 1999; Sasidh., Fl. Parambikulam WLS 131. 2002; Anil Kumar *et al*., Fl. Pathanamthitta 227. 2005; Sunil & Sivadasan, Fl. Alappuzha Dist. 298. 2009. **Figure: 43**

Type**:** Lectotype: China (LINN-156.4) LT designated by Graham (1985).

Ammannia apiculata Koehne, Bot. Jahrb. Syst. 1(3): 254. 1880.

A.indica Lam., Illus. Gen. Pl. 1: 311, n. 1555. 1792.

A. vesicatoria Roxb., Fl. Indica, ed. Carey & Wallich, 1: 447 (1832)

A. attenuata A. Rich., Tent. Fl. Abyss. 1: 278. 1848.

Erect, annual, upto 60 cm tall. *Stem* 4-angular, glabrous, often redish, branched, rooting at base; winged. *Leaves* sub sessile, decussate, elliptic or lanceolate to linear, 2−3 × 0.5−1 mm for leaves in the branches, 8−20 × 2−7 mm for leaves in main axis; base cuneate or sub-cordate; apex acute or acuminate. *Bracts* similar to foliage leave; bracteoles, elliptic to lanceolate, *c.* 1 mm long, much shorter than calyx tube. *Flowers* 3 to many in dense axillary cymes; pedicels subsessile to 1 mm, peduncle absent to 1 mm. *Calyx tube* campanulate, 1 mm long, calyx lobes 4, triangular, deltate, sometimes reddish tinge at the tip, *c.* 0.5 mm long; calyx appendages absent. *Petals* 0. *Stamens* 4, inserted at the middle of the calyx tube, exserted. *Ovary* globose,4-locular; style *c*. 0.3 mm long; stigma capitate; capsule globose, half or lower exserted, *c*. 1.5 mm in diam., 4-valved; seeds semi ovoid, about 0.5 mm long.

Fl. & Fr.: Dec.− Mar.

Distribution and Habitat: A widespread species that is found across the world from tropical Africa, Afghanistan, Pakistan, India, Southeast Asia, Philippines, New Guinea, Caribbean islands, the Mediterranean basin and Australia. It is a variable species of swampy areas, paddy fields and water courses of low elevation across the South India.

***Specimens examined*: Andhra Pradesh:** Cuddapah Dist. Balapelle, 17 Jul. 1962, *J. L Ellis 14222* (MH). Warangal Dist., Etturnagaram, 30 Mar. 1999, *R. K Premanath 110833* (MH). **Karnataka:** Hasan Dist., Vaddarahalli, 29 Sept. 2015, *Lemiya K. M 132993* (CALI). **Kerala:** Alappuzha Dist., Pallithode, 1 Jan. 1992, *C. N Sunil 1093* (CALI). Palakkad Dist., Chitoor, 21 Sept. 2013, *Lemiya K. M 132947* (CALI). Malappuram Dist., Changaramkulam, 15 Nov.

2013, *Lemiya K. M 132957* (CALI). **Tamil Nadu:** Coimbatore Dist., Konnamalai, 20 Feb. 1963, *C. P Sreemadhavan 440* (MH). Velliangiri Hills, 12 Apr. 1857, *K. M. Sebastian 2752* (MH). Pollachi, 21 Sept. 2013, *Lemiya K. M 132944* (CALI). Namakkal Dist., Kumarapalayam, 26 Dec. 2014, *Lemiya K. M 132973* (CALI). Nilgiri Dist., Anaikkatty, 15 Mar. 1972, *M. V Subharao 40250* (MH).

Figure 43: *Ammannia baccifera* subsp. *baccifera* L. **A.** Habit; **B.** Inflorescence; **C.** Single flower; **D.** flower-split open; **E.** Leaf base.

Ammannia cordata Wight & Arn., Prodr. Fl. Penin.Ind. Orient. 1: 304. 1834; Graham & Gandhi, Harv. Pap. Bot. 18(1): 74. 2013. **Figure 44.**

Type: (Lectotype designated by Graham & Gandhi, 2013): INDIA, *R. Wight 1021* (K); Isolectotypes: (E, GZU).

Nesaea brevipes Koehne, Bot. Jahrb. Syst. 3: 326. 1882; Koehne in Engler, Pflanzenr. IV. 216 (Heft 17); 226.1903; Gamble, Fl. Pres. Madras 510. 1919;

Sharma *et al.,* Fl. Karnataka 108. 1984; Ramach. & V.J. Nair, Fl. Cannanore Dist. 193. 1988; Sunil & Sivadasan, Fl. Alappuzha Dist. 300. 2009.

N. cordata (Wight & Arn.) M. R. Almeida, Fl. Maharashtra 2: 87. 1998, *nom. illeg*.

Erect, annual, up to 10−30 cm tall. *Stem* 4-angular, winged, branched, glabrous, rooting from the basal nodes; winged. *Leaves* subsessile, decussate, lamina microscopically serrate, elliptic or lanceolate to ovate, 10−15 × 3−7 mm; base truncate or sub-cordate to obscurely *amplexicaulated;* apex acute; bracts elliptic to ovate, apiculate to subulate at apex; bracteoles, linear, *c*. 0.5 mm long, shorter than calyx tube, up to the middle of calyx tube. *Flowers* 3 in axillary cymes; subsessile, peduncle absent. *Calyx tube* broadly campanulate, with longitudinal reddish ribs alternating the stamens, 1−1.5 mm long, *c*. 1.5 mm across, calyx lobes 4, triangular, apiculate at apex small bristle-like appendages at the interfolds between two calyx lobes. *Petals* 0. *Stamens* 4, inserted below the middle of the calyx tube, *c.* 1mm length, slightly exserted. *Ovary* globose, 3-locular, *c.* 0.8 × 0.7 mm; style short, less than 0.25 mm long; stigma capitate; capsule globose, , *c*. 1.5 mm in diameter, 4- valved not exceeding the calyx tube; seeds, reddish, semi ovoid, less than 0.5 mm long.

Fl. & Fr.: Dec.− Mar.

Distribution and habitat: It is endemic to the Indian subcontinent - Bangladesh, Sri Lanka and India. It is commonly seen at an altitude of 250 to 800 m along the Western Ghats of Kerala, Tamil Nadu, and Karnataka. Its distribution also extends to coastal districts of Gujarat and Goa. It grows in marshy area, seasonally flooded places, rice fields, river banks and on wet soil

Specimens examined*:* **Andhra Pradesh:** Cuddapah Dist., Balayapalle, 21 Feb. 1963, *J. L. Ellis 15729* (MH). **Karnataka:** Mysore Dist., Nanjangud, 29 Sept. 2015, *Lemiya K. M 132996* (CALI). **Kerala:** Alappuzha Dist., Angadikkal, 6 Oct 1993, *C. N Sunil 1333* (CALI). Malappuram Dist., Calicut

University campus, 10 Sept. 2012, *Lemiya K. M 132917*. Calicut Dist., Chaliyam, 14 Nov. 2012, *Lemiya K. M* 132927 (CALI). Kannur Dist., Tholpetty, 8 Feb. 1978, *V. S Ramachandran 52391*(MH). **Tamil Nadu:** Chengalpettu Dist., *s. loc*., 26 Jan 1976, *A. N Henry 47051* (MH). Erode Dist., Anthiyur, 5 Jan. 2015, *Lemiya K. M, 133000* (CALI).

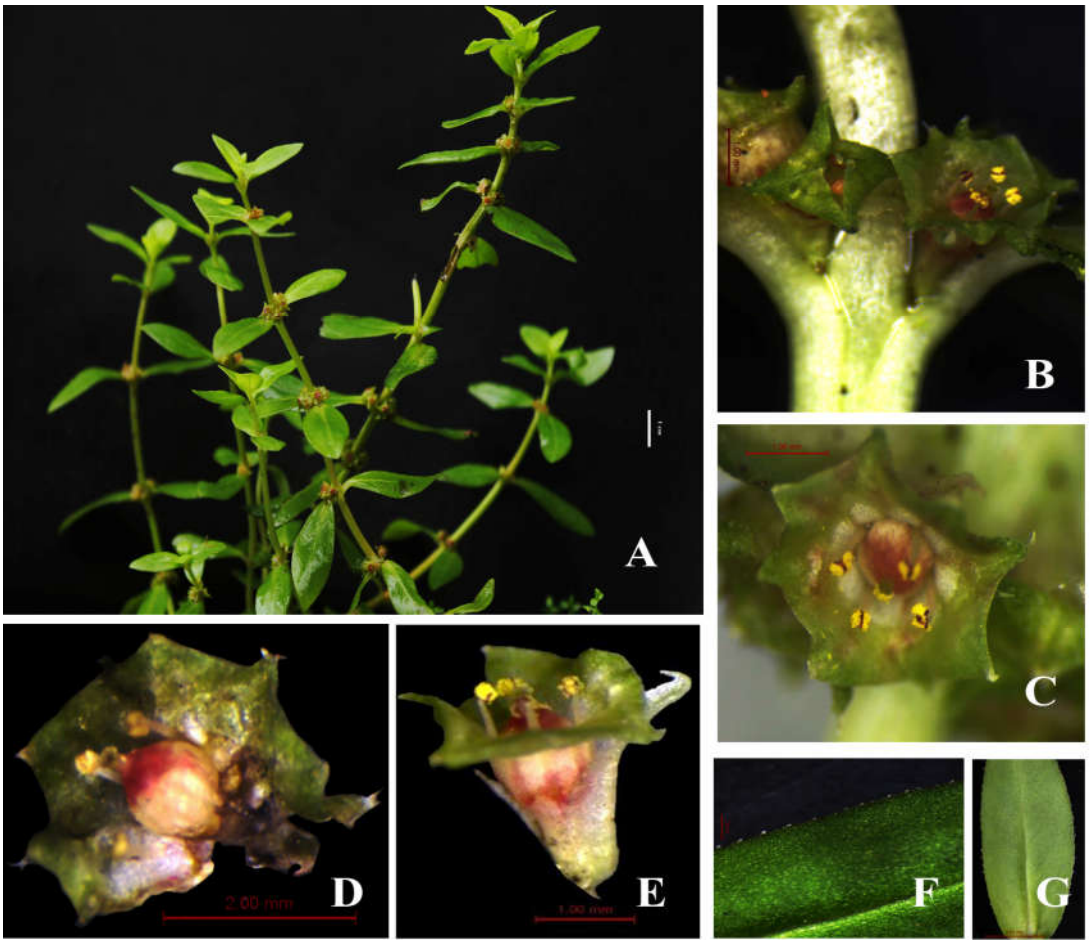

Figure 44. *Ammannia cordata* Wight & Arn. **A.** Habit; **B.** Inflorescence; **C.** Single flower; **D.** Flower-split open; **E.** Calyx tube. **F.** Leaf showing margin; **G.** Single leaf.

Ammannia multiflora Roxb. Fl. Ind. 1: 447. 1820; Hook. f., Fl. Brit. India 2: 570. 1879; Gamble, Fl. Pres. Madras 509. 1919; Singh, Fl. Eastern Karnataka 1: 315. 1988; Sharma *et al.,* Fl. Karnataka 107. 1984; Matthew. Excursion. Fl. Central Tamil Nadu. 198. 1991; Matthew, Fl. Palani Hills 1: 510. 1999;

Pullaiah, Biodiversity in India 2: 146. 2003; Pullaiah, Fl. Eastern Ghats: Hill Ranges of South East India 3: 63. 2007. **Figure 45.**

Type**:** Roxburgh *s.n.* (iso. K, barcode K000729678, right-hand specimen), East India.

Ammannia australasica F.Muell. in Trans. Phil. Soc. Vict. I: 41. 1855.

Ammannia japonica Miq., Ann. Mus. Bot. Lugduno-Batavi II. 261. 1866.

Ammannia madagascariensis Boivin *ex* Tul., Ann. Sci. Nat., Bot. ser. 4, 6: 129. 1856.

Ammannia multiflora f. *deceptions* Koehne, Bot. Jahrb. Syst. 1: 248 1880.

Ammannia senegalensis var. *multiflora* Hiern, Fl. Trop. Afr. 2: 477 1871.

Erect, annual herb, to 40 cm tall. *Stem* 4-angular, glabrous, branched, rooting at base. *Leaves* sessile, decussate, elliptic or lanceolate to linear, 5−10 × 2−3 mm for leaves in the branches, 25−30 × 5−7 mm for leaves in main axis; base auriculate or subcordate; apex acute; bracteoles linear, very short, much shorter than calyx tube. *Flowers* 3 in each pedicel in axillary cymes; pedicels *c.* 1mm long; peduncle 2− 3 mm long; 8-ribbed. *Calyx tube* campanulate, *c.* 1.5 mm long, calyx lobes 4, triangular, acuminate at the apex, *c.* 0.5 mm long; calyx appendages absent. *Petals* 4, with dark mid vein, pinkish, orbicular, *c.* 1 mm long. *Stamens* 4, inserted at the middle of the calyx tube, slightly exserted. *c.* 0.8 mmlong. *Ovary* globose, 4-locular; style *c*. 0.5 mm long; stigma capitate; capsule globose, slightly exerted, *c*. 1.5 mm in diam., 4-valved; seeds ovoid, about 0.5mm long.

Fl. & Fr.: Jan.−Dec.

Distribution and habitat: This species is widespread from Asia, tropical and subtropical Africa and Australia. It grows in shallow water, damp heavy soils and rice fields in South India. In Kerala the distribution is restricted to Palakkad district especially in Kerala-Tamil Nadu border.

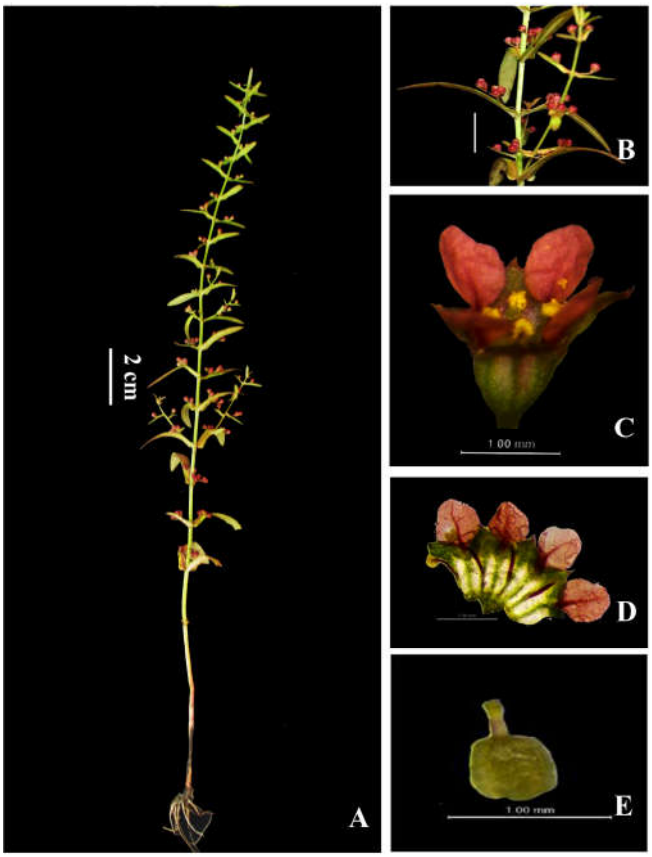

Figure 45. *Ammannia multiflora* Roxb**. A.** Habit; **B.** Inflorescence; **C.** Single flower; **D.** Flower split-open; **E.** Gynoecium.

***Specimens examined*:** **Kerala:** Palakkad Dist., Chitoor, 21 Sept. 2013, *Lemiya K.M 132946* (CALI). **Tamil Nadu:** Erode Dist., Mullampatty, 25 Dec. 2014, *Lemiya K. M 132970* (CALI). Namakkal Dist., Kumarapalyam, 26 Dec. 2014, *Lemiya K. M 132972* (CALI). Ramanathapuram Dist., *s.loc*., l22 Aug. 1964, *K. Ramamurthy 21027* (MH).

Note: Some authors (De Wilde & Duyfjes, 2014) consider *A. auriculata* and *A. multiflora* as conspecific. An examination of types and protologues of both the taxa is not in support of the above treatment. The present investigation on the molecular analysis of the two species is in favour of treating them as two different species. *A. multiflora* differs from *A. auriculata* having style half as long as or shorter than the ovary.

Ammannia octandra L.f., Suppl. Pl. 127. 1782; Roxb., Pl. Coromand.133, 1800 & Fl. Ind. 1, 447. 1820; Wight & Arn. Prodr. Fl. Ind.Orient. 304, 1834;

Hook. f., Fl. Brit. India 2, 571. 1879; Koehne in: Engl., Pflanzenr. IV 216: 50. 1903; Gamble, Fl. Pres. Madras 510. 1919; Mathew, Fl.Tam. Carn. 1: 606. 1983; Matthew, Excursion. Fl. Central Tam. 198, 1991. **Figure 46.**

Type: Koenig *s.n.* (holo BM (typ. cons.); iso C, LINN 156.5), India, Madepala.

Ammannella linearis Miq. Fl. Ned. Ind. 1(1): 619. 1855.

Diplostemon octandrus Miq. Fl. Ned. Ind. i. I. 615.

Erect, annual herbs, up to 30 cm tall. *Stem* 4-angular, branched, glabrous, rooting at base. *Leaves* sessile, decussate, lamina miutely serrate, linear/oblong to lanceolate, 10−35 × 3−5 mm; base auriculate to sub-cordate; apex acute, bracteoles, linear, very short, *c.* 1mm long. *Flowers* short peduncled, up to 6 mm long, 3-flowered axillary cymes, peduncle 3 mm, pedicel *c.* 2 mm long; subsessile. *Calyx tube* campanulate, *c*. 5 mm long, *c*. 2 mm across, calyx lobes 4, triangular, acute at apex, 4 -angled with sharp margin. *Petals* 4, orbicular, crumpled, flame coloured, *c.* 3.5 × 4 mm, dark midvein up to the top of petals. *Stamens* 8, inserted in the middle of the calyx tube, *c.* 4.5 mm long, dark red, exserted. *Ovary* globose, 4-locular, *c.* 1.8 × 1.5 mm; style *c.* 4 mm long; stigma capitate; capsule globose, *c*. 2.5 mm in diameter, 4-valved, included in the calyx tube, seeds turgid, *c.* 0.3 mm long.

Fl. & Fr.: Dec.−Mar.

Distribution and habitat: It is distributed in South and South East Asia (Myanmar, Sri Lanka and India). In India it is found in Andhra Pradesh, Maharashtra and Tamil Nadu. It grows in low lying moist places, edge of tanks, marshes and rice fields.

Specimens examined: **Puthuchery:** Myla, 09 Dec. 2015, *Prasad M. G, 132978* (CALI). Tamil Nadu: Chengalpettu Dist., Ennore, 24 Feb. 1986, *D.*

Narasimhan 997 (MH). Coimbatore Dist., Valayar, 6 May 1957, *K. Subr*amanyan *3021* (MH). Ramanadapuram Dist., *s.loc*., 7 Feb. 1987, *V. Balasubramaniam 1170* (MH).

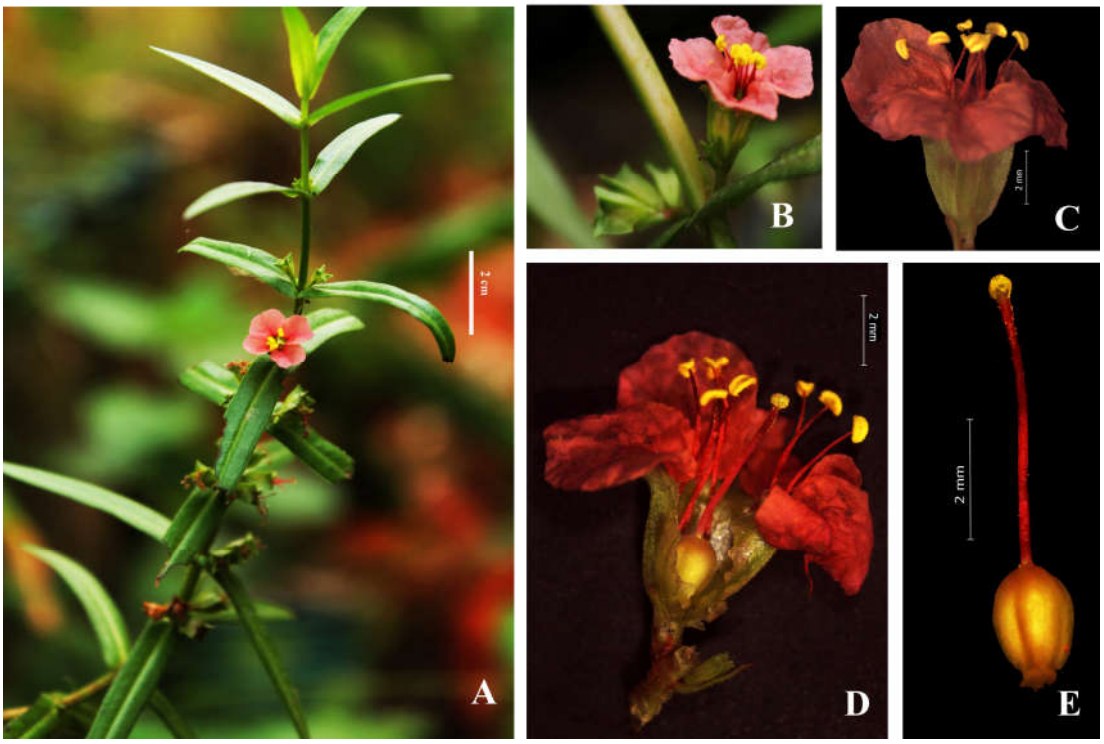

Figure 46. ***Ammannia octandra*** **L. f. A.** Habit; **B.** Inflorescence; **C.** Single flower; **D.** Flower-split open; **E.** Gynoecium.

Ammannia prostrata (Buch.-Ham. *ex* Dillwyn) Suresh, Rev. Hort. Malab. 47. 1839; Graham & Gandhi, Harv. Pap. Bot. 18(1): 80. 2013.

Figure 47.

Lectotype: INDIA. Rheede's plate of Belitsjira, Hort. Malab. 9: 165, t. 84. 1689 (vide Mabberly 1977: 531-533).

Nesaea prostrata (Buch.-Ham. *ex* Dillwyn) Suresh in Nicolson *et al*., Interpret. Rheede's Hort. malab. 168. 1988; Sunil & Sivadasan, Fl. Alappuzha Dt. 304. 2009.

Ammannia lanceolata B.Heyne *ex* C.B.Clarke in Hook. f., Fl. Brit. Ind. 2: 570. 1879.

Nesaea lanceolata (B.Heyne *ex* C.B.Clarke) Koehne, Bot. Jahrb. Syst.3: 325. 1882.

Nesaea lanceolata var. *stricta* Koehne, Bot. Jahrb. Syst.3: 326. 1882.

Erect or 160ecumbent, annual herb, to 10− 15 cm high. *Stem* 4-angular, glabrous, branched, creeping and rooting at basal nodes; winged. *Leaves* subsessile, decussate, margins crenulate, lamina linear to ovate, 10−15 × 5−10 mm; base attenuate to subcordate; apex acute to mucronate. *Bracts* elliptic to oblong hairy, acute at apex; *c*. 1 mm × 0.5 mm; bracteoles, linear, *c*. 1 mm long, up to calyx tube, hairy at apex. *Flowers* in axillary cymes; subsessile, peduncle absent. *Calyx tube* campanulate, pubescent, 1−1.5 mm long, *c*. 1 mm across, calyx lobes 4, hairy, triangular, acuminate at apex, calyx appendages present bristle like; *Petals* 4, purple, orbicular, 0.4 mm long. *Stamens* 4, inserted below the middle of the calyx tube, *c*. 0.7 mm long, included. *Ovary* globose, hairy, 3-locular, *c*. 1 × 1 mm; style short, less than 0.25 mm long; stigma capitate; capsule globose, *c*. 1.5 mm in diameter, enclosed in calyx tube; seeds, redish, semi ovoid, less than 0.5 mm long, glabrous.

Fl. & Fr.: Aug.− Nov.

Distribution and habitat: *Ammannia prostrata* is found in Andhra Pradesh, Kerala, Lakshadweep, Karnataka, Rajasathan, Tamil Nadu, Uttar Pradesh and West Bengal. It is also distributed in Sri Lanka and Australia. It grows in wet low-lying areas, banks of ponds, along streams, fallow rice fields (Cook, 1996) and coastal regions.

Specimens examined: **Kerala:** Alappuzha Dist., Thaikkattussery, 6 Oct. 1993, *Sunil C. N 1331* (CALI). Malappuram Dist., Calicut University Campus, 18 Sep. 2012, *Lemiya K. M 132918* (CALI). Thrissur Dist., Parambikulam, 22 Nov. 1962, *K. M Sebastian 15314* (MH). **Tamil Nadu:** Puthukotai Dist., *s. loc.,* 15 Mar. 1986, *Arulappan 737* (MH). Ramanadapuram Dist., *s. loc.,* 8 Dec. 1977, *N. C Nair 53194* (MH). South Arcot Dist., Parangipettai, 10 Feb. 1979, *K. Ramamurthy 58178* (MH).

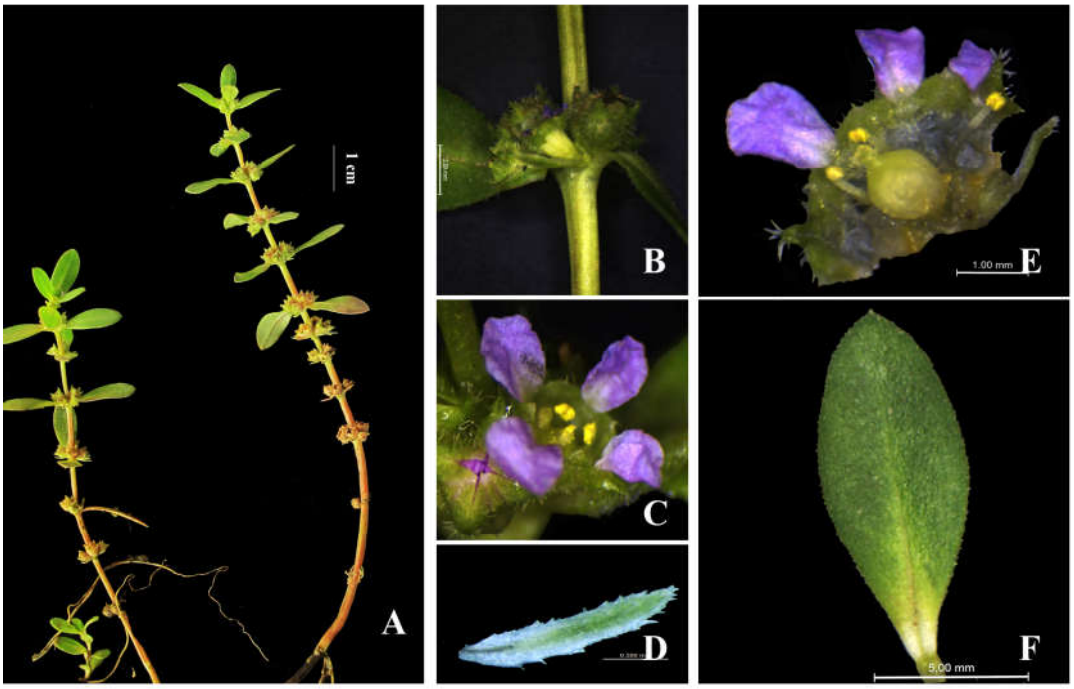

Figure 47. *Ammannia prostrata* (Buch.-Ham. *ex* Dillwyn) Suresh **A.** Habit; **B.** Inflorescence; **C.** Single flower; **D.** Bract**; E.** Flower-split open; **F.** Leaf.

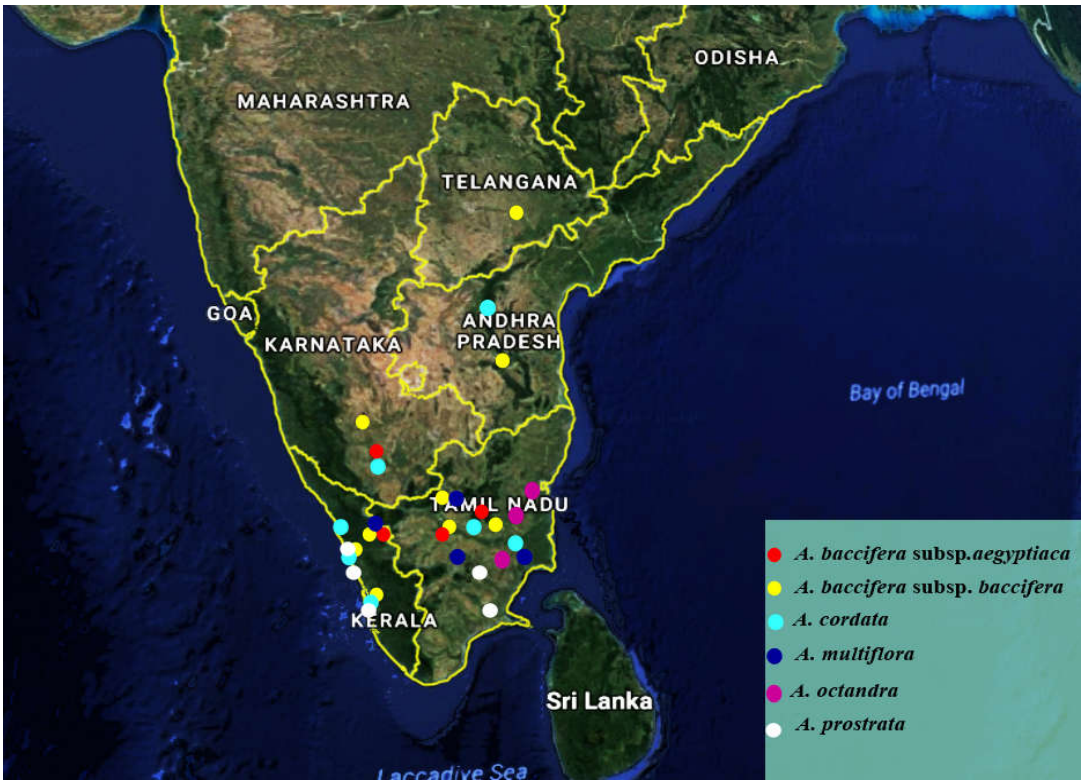

Figure 48. Distribution map of different species of *Ammannia* in South India.

***Rotala* L.**

Mantissa Pl. 143. 1771

Type species**:** *R. verticilaris* L., Mantissa Pl. 2: 175.1771; non Hiern in Oliver, Fl. Trop. Africa 2:467.1871.

Ammannia L., Sp. Pl. 119.1753, *p. p*.

Peplis L., Sp. Pl. 332. 1753, *p. p*.

Suffrenia Bellardi, Mem. Acad. Sci. Lit. Beaux-Arts Turin "pour es anneess 10:1802, *et* 11: 1803.

Sellowia Roth *ex* Roem. & Schult., Syst. Veg. 5(31): 407. 1819.

Winterlia Sprengel, Syst. 1: 519. 1824, non Moench 1794.

Boykiana Rafin., Neogenyton 2. 1825, nom. rej.

Ameletia DC., Mem. Soc. Phys. Geneve 2(2):82. 1826.

Nimmonia (*Nimmoia* orth. Mut.) Wight, Madras J. Lit. Sci. 5: 312.1837.

Nexilis Rafin., New Fl. 4: 9. 1838.

Hypobrichia M. A. Curtis *ex* Torrey & Gray, Fl. N. Amer. 1: 479. 1840.

Rhyacophila Hochst., Flora (Regensburg) 24: 659. 1841.

Quartinia Endl., Gen. Suppl. 2: 94. 1842, non A. Rihard 1840.

Hydrolythrum Hooker f., Ic. Pl. ser. 2, t. 1007. 1843.

Tritheca (Wight & Arn.) Miq., Fl. Ind. Bat. 1(1): 614. 1855.

Ditheca (Wight & Arn.) Miq., Fl. Ind. Bat. 1(1): 615. 1855.

Aquatic, amphibious or terrestrial, annual or perennial, glabrous herbs. *Stems* creeping, ascending, erect or floating, simple or branched. *Leaves* decussate, whorled or rarely alternate, simple, entire, sessile or rarely shortly petiolate. *Bracts* leaf-like or scale-like; bracteoles 2. *Flowers* bisexual, actinomorphic, monomorphic or dimorphic (heterostylous), occasionally cleistogamous, solitary in the axils of bracts, born along the main axis or on lateral or terminal racemes. *Calyx tube* free from but often enclosing the

ovary, hypanthial; calyx lobes 3−6, valvate, persistent; tooth-like calyx appendages or small interjected folds occasionally present between the calyx lobes; nectaries often present at base of calyx tube. *Petals* 0−5, minute or large and showy, inserted at the top of the calyx tube, usually crumbled in bud, entire, erose or pinnately divided in *R. fimbriata*. *Stamens* 2−6, episepalous, never more in number than the calyx lobes, inserted on the inner surface of the calyx tube on the lower half or occasionally basal and appearing free, occasionally replaced by nectar glands; anthers dorsifixed, introrse. Pollen tricolporate, occasionally heterocolpate, isopolar or occasionally heteropolar often dimorphic, pseudocolpi absent or if present 3 in number, or generally in the middle of the principle colpi amb triangular or circular or angulo-circular, columellae present, sexine granular, reticulate or granulo-reticulate. *Ovary* superior, 2−4 locules: placentation axile becoming free central at maturity; style simple; stigma capitate; fruit septicidally dehiscent capsule opening by 2−4 valves; the valves with microscopic, horizontal striations; one valve is crowned with persistent style; seeds numerous or few, semi-ovoid to ellipsoidal.

Distribution and habitat: Within the whole genus *Rotala*, only very few are wide spreaded viz., *R. indica, R. mexicana*, *R. occultiflora.* Twenty one species are confined to Africa and Malagsy and twenty species are confined to S. and E. Asia and Australia. Species in S. Asia exhibit maximum morphological diversity. Most annual species occures in wet lands like marshy areas, paddy fields, water logged moist soil in lateritic plains, river side, fresh water ponds etc.

Key to the species

1a. Flower apetalous 2

1b. Flower petaliferous 4

2a. Leaves in whorls of 3; leaf base often sheathing; bracteoles leaf- like, partly or completely enclosing the flower 3

2b. Leaves in whorls of 3-8 or decussate; leaf base not sheathing; bracteoles scarious, not enclosing the flower .....***R. mexicana***

3a. Calyx lobes 5 .....***R. occultiflora***

3b. Calyx lobes 4 ..... .***R. kasaragodensis***

4a. Plants emergent aquatics 5

4b. Plants amphibious or terrestrial 8

5a. Nectar scales present 6

5 b. Nectar scales absent..... 7

6a. Leaves monomorphic .....***R. vasudevanii***

6b. Leaves dimorphic… .....***R. cookii***

7a. Submerged leaves elliptic or ovate; aerial leaves obovate, rounded at apex….....***R. tulunadensis***

7b. Submerged leaves capillary; aerial leaves linear, minutely bifid at apex…….....***R. verticillaris***

8a. Calyx appendages present 9

8b. Calyx appendages absent…..... 13

9a. Nectar scales present. 12

9b. Nectar scales absent…..... 10

10a. Bracteoles as long as or exceeding calyx tube… 11

10 b. Bracteoles shorter than calyx tube.....……***R. rosea***

11a. Petals 3, elliptic…… ..... ……***R. juniperina***

11b. Petals 4 or 5, obovate.....……***R. densiflora***

12a. Leaf apex acute to shortly truncate; petals elliptic to oblong, acute at apex…….....***R. malampuzhensis***

12b. Leaf apex subtruncate to slightly bimucronate; petals broadly obovate, shallowly sinuate at apex…..... …….***R. anamika***

13a. Petals fimbriate…… ....***R. fimbriata***

13b. Petals entire..... ……...14

14a. Nectar scales present… 15

14b. Nectar scales absent……..... 16

15a. Leaves linear, leaf apex bimucronate, petals sub orbicular..... ……..... .... `***R. malabarica***

15b. Leaves obovate to ovate; leaf apex acute to truncate, petals elliptic ….. .... ***R. cheruchakkiensis***

16a. Stamens inserted at the middle of the calyx tube, petals elliptic…… …… ..... ***R. indica***

16b. Stamens inserted at the base of the calyx tube; petals obovate..... .17

17a. Stamens as long as or shorter than calyx tube; bracteoles almost equaling the calyx tube…..…..... ***R. rotundifolia***

17 b. Stamens much longer than calyx tube; bracteoles much shorter than the calyx tube ……..... ***R. macrandra***

Rotala anamika Lemiya, Rheedea. 25(2): 159-163. 2015. **Figure 49 & 50**

Type: INDIA, **Kerala,** Malappuram District, Parappanangadi, Koottumoochi, 11°05.802’ N, 75°52.068’ E, 3.4 m, 25. 08. 2012, *Lemiya K. M 13291* (Holo-MH; Iso- CALI).

Erect, annual herb, 7–10 cm tall. *Stem* 4-angular, glabrous, not winged, rooting from lower nodes. *Leaves* sessile, lamina elliptic-ovate, 2–4 × 1–2 mm, glabrous, broader towards base, rounded, apex subtruncate to slightly bimucronate, 1-nerved, leaves often deciduous while in fruiting. *Bracts* similar to foliage leaves; bracteoles linear, 1–1.5 mm long, as long as calyx tube, glabrous. Flowers sessile, axillary, solitary, 3, 4 or 5-merous. *Calyx tube* 1–2 mm long, glabrous, bright pink at anthesis, lobes 3–5, broadly triangular, 0.5–0.8 mm long, acute, calyx appendages alternating with calyx lobes, 0.3–0.5mm long, linear or capillary, as long or slightly longer than the calyx lobes. *Petals* 3–5, bright pink, spreading, broadly obovate, 0.75–1 mm long, shallowly sinuate at apex, margin entire. Stamens 3–5, bright pink, *c.*1mm long; filaments inserted slightly below the middle of the calyx tube. Anthers up to the level of calyx tube, not exserted. *Staminodes* 3, lanceolate, *c*. 0.25

mm long, bright pink, alternating with stamens, shorter than half way by the persistent calyx. *Ovary* globose to elliptic or slightly trilobed, 1–1.3 mm long, glabrous, trilocular. Style short, *c*. 0.25 mm long, often persistent in fruit, stigma capitates, capsule 3-valved, enclosed more than ovary, (depressed) globose, *c*. 1 mm long, seeds pale green, up to 15, plano-convex, *c*. 0.5 mm long.

Fl & Fr.: Sept.−.Dec.

Distribution and habitat: Endemic to Kerala. The plant is known only from the type locality in Malappuram district of Kerala. It grows in marshy areas and in rice field.

Specimens examined: **Kerala**: Malappuram Dist., Calicut University Botanical Garden (CUBG), 45 m, 13.09. 2014, *Lemiya K. M 132969* (CALI). Parappanangadi, Koottumoochi, 11°05.802' N, 75°52.068' E, 3.4 m, 25. 08. 2012, *Lemiya K. M 132916* (MH).

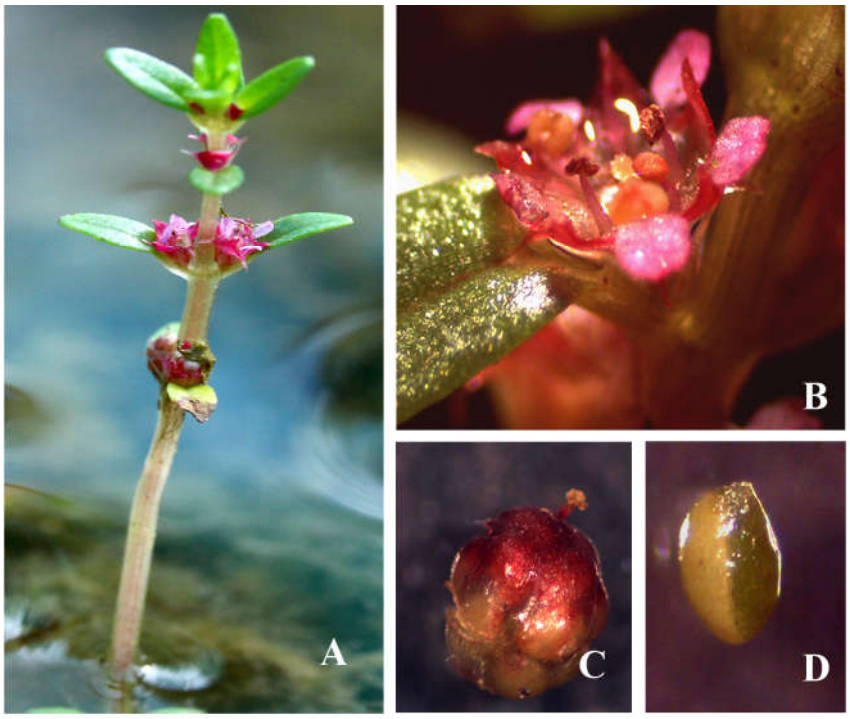

Figure 49. *Rotala anamika* Lemiya **A.** Habit; **B.** Single flower; **C.** Gynoecium; **D.** Seed.

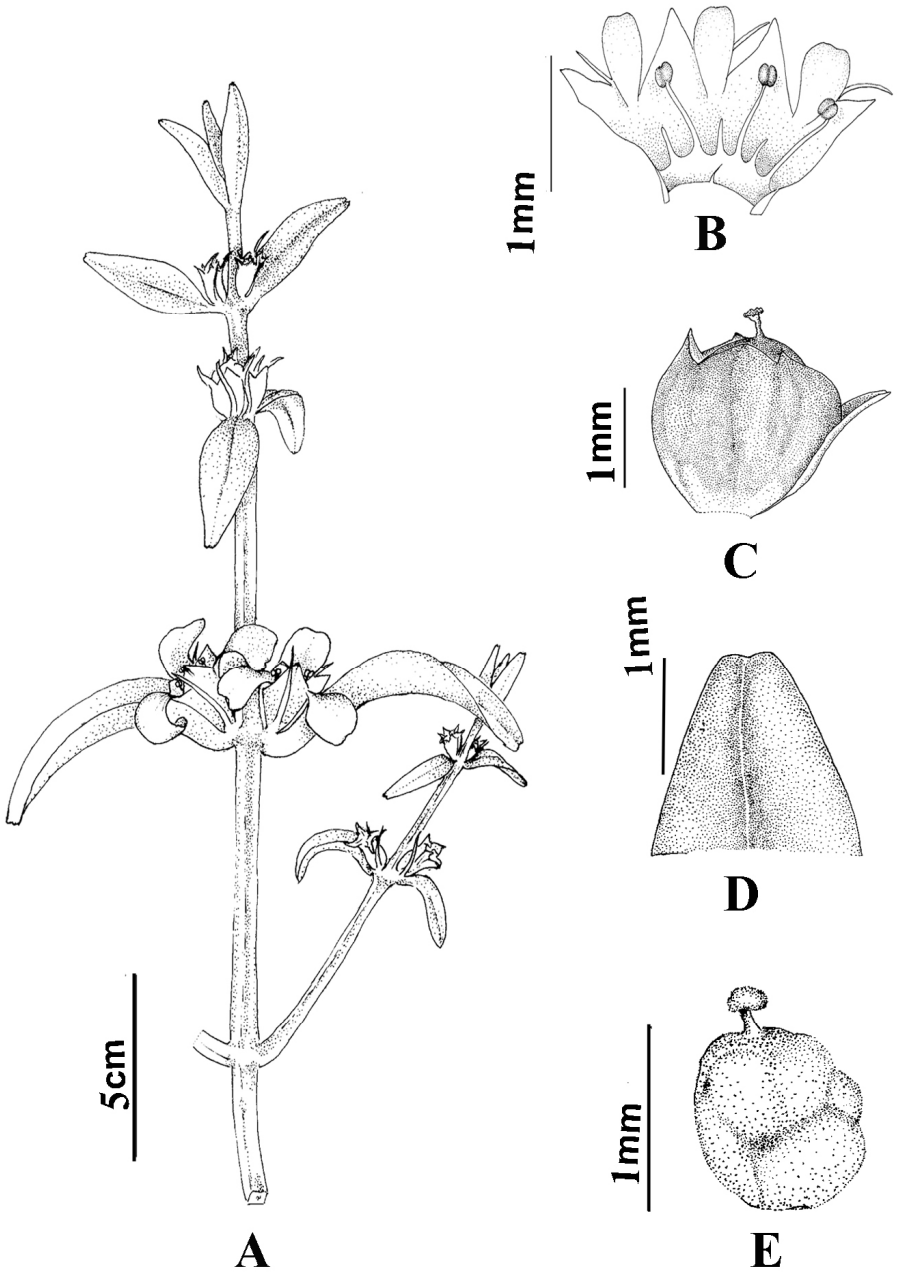

Figure 50. *Rotala anamika* Lemiya **A.** Habit; **B.** Flower-split open; **C.** Fruit with calyx; **D.** Leaf tip enlarged; **E.** Gynoecium

Rotala cheruchakkiensis Anto, Devikrishna, Pulickal, C.D. Varghese & I.Antony, Int. J. Advanced Res. 2(11): 532, 2014. **Figure 51**

Type: India, Kerala, Thrissur District, Mangad, 10°42' 30" N, 76°11' 43" E, alt. 200 m, 15/07/2014, Anto P.V. 1001(Holotype: CAL. Isotypes: BSI, MH, CATH).

Erect, annual herb, to 2.5−3.5 cm tall. *Stem* terete, glabrous, unbranched, rooting from lower nodes. *Leaves* obovate to ovate, 1.5−2.5 mm × 1−1.5 mm; cuneate at base, acute to shortly truncate at apex. *Bract* leaf like on main stem; bracteoles colourless, subulate, *c.* 0.5 mm long, not exeeding calyx tube, slightly rose coloured. Flowers sessile, axillary, solitary white to pale pink in colour. *Calyx tube* campanulate, glabrous, white to rose, *c.* 1 mm long; calyx lobes 3, ovate acuminate, calyx appendages absent. *Petals* 3, light rose, elliptic, 0.5−0.8 mm long, acuminate, dentate at apex, margins entire. *Stamens* 3, *c.* 0.7mm long, filaments below the middle of the calyx tube. *Staminodes* 3, greenish, lanceolate, very short, *c.* 0.25 mm long, alternating with stamens, shorter. *Ovary* globose, green with rose tinge, trilocular; style short not up to 0.25 mm long; stigma capitate; capsule, slightly exceeding calyx tube, *c*. 1.2 mm long; seed reddish, 0.15−2 mm wide, often persistent in fruits.

Fl & Fr.: July−Sept.

Distribution and habitat: It is found in Palakkad and Thrissur districts and is endemic to Kerala. It grows in temporary pools in the rocks.

Specimens examined: **Kerala**: Palakkad Dist., Nelliampathy, 6 Aug. 2013, *Lemiya K. M & Thoiba K 132939* (CALI). Thrissur Dist., Mayannur, 21 Sep. 2016, Anto *P. V 148307* (CALI).

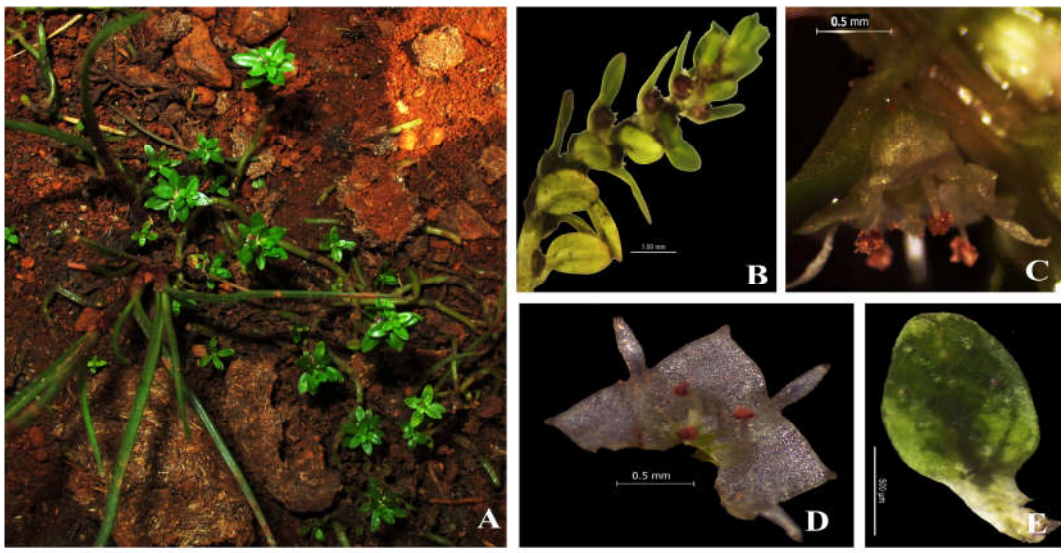

Figure 51. *Rotala cheruchakkiensis* Anto, Devikrishna, Pulickal, C.D. Varghese & I. Antony **A.** Habit; **B.** Enlarged portion of twig; **C.** Single flower; **D.** Flower-split open; **E.** Leaf.

Rotala cookii Joseph & Sivar., Pl. Syst. Evol. 159: 1988; Joseph *et* Sivarajan, Proc. Ind. Acad.Sci (Plant Sci.) 99 (3): 181, 1989. **Figure 52.**

Type: **Kerala**: Malappuram Dist., Parappanangadi, 40 km south of Calicut, 12 Dec. 1983, Joseph 38967 (Holo.- CAL; Iso.- CALI, MH, Z).

Aquatic, annual, often submerged, up to 40 cm long. *Stem* profusely branched, glabrous,floating, erect above, submerged creeping and rooting below. *Leaves* in whorls of 7−10, dimorphic, when submerged capillary, up to 15 mm long, aerial leaves are broader, spreading, linear, up to 6 mm long; bimucronate at apex; narrowed towards base. *Bracts* similar to foliage leaves; bracteoles linear, shorter than the calyx tube. *Flowers* sessile, 2−4 in each nodes, those on submerged shoot cleistogamous. *Calyx tube* campanulate, up to 1 mm long, disintegrating as fruit ripens; calyx lobes 4, deltate, up to 0.5 mm long; calyx appendages absent. *Petals* 4, white, obovate- obtuse, up to 0.8 mm long. *Stamens* 4, inserted just below the middle of the calyx tube, slightly exserted; nectar scales 4, small, greenish yellow or pink, minute, apex obtuse

or truncate. *Ovary* globose; style short; stigma capitate; capsule globose, up to 1 mm in diam. 3- valved; seeds semi-ellipsoidal, up to 1 mm long.

Fl & Fr.: Jun. – Aug.

Distribution and habitat: Endemic to Kerala. It occures in fresh waters of low land paddy fields during mansoon months (Jun – Aug.). Once abundant in the type locality, this species is now not seen in its type locality and adjoining paddy fields.

Specimen examined: **Kerala**: Malappuram Dist., Parapanangadi, 10 Dec. 1983, *K. T Joseph 38967* (CALI)

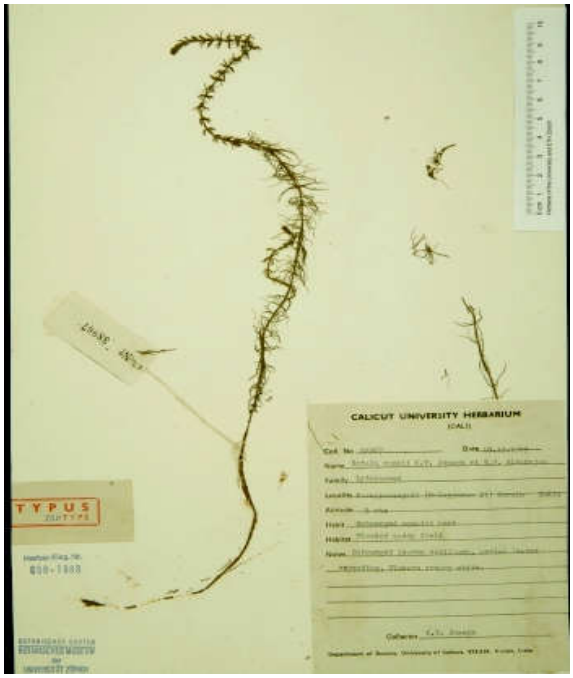

Figure 52. Holotype of *Rotala cookii* Joseph & Sivar. at CALI

Rotala densiflora (Roth *ex* Roem. & Schult.) Koehne, Bot. Jahrb. 1: 164. 1880, & in Engl., Pflanzenr. IV 216: 35. 1903; Gamble, Fl. Pres. Madras 1: 508. 1919; Van Leeuwen, Blumea 19: 55. 1971; Sald. & Nicols., Fl. Hassan. 274. 1976; Cook, Biossiera 29: 82. 1979; Mani & Sivar. Fl. Calicut. 113. 1982; Sharma *et al.,* Fl. Karnataka 108. 1984; Singh, Fl. Eastern Karnataka 1: 317. 1988; Matthew, Fl. Tam. Carnatic 3: 612. 1983; Sharma *et al.,* Fl. Karnataka 108. 1984; Joseph et Sivarajan, Proc. Ind. Acad. Sci (Plant Sci.) 99(3): 181. 1989; Matthew, Excursion. Fl. Central Tamil Nadu. 201. 1991; Pullaiah, Biodiversity in India 2: 146. 2003; Sunil & Sivadasan, Fl. Alappuzha Dist. 302. 2009. **Figure 53.**

Type: India orientali, *B. Heyne* (Isotype: L).

Ammannia densiflora Roth *ex* Roem. & Shult., Syst. Veg. 3: 304. 1818.

Rotala roxburghiana Wight, Ic. Pl. Ind. Or. 1: t. 260 B. 1840, *nom. Illeg.*

Ammannia pentandra sensu Clarke in Hook. f., Fl. Brit. Ind. 2: 568. 1879; Blatt, & Hallb., J. Bombay Nat. Hist. Soc. 25: 707. 1918.

Erect, annual or perennial, upto 45 cm tall. *Stem* 4-angular, glabrous, simple or branched, rooting from lower nodes; narrowly winged. *Leaves* sessile, decussate below, alternate above, variable in size and shape, linear-lanceolate to oblong; base cordate to obtuse; apex acute or acuminate. *Bracts* like foliage leaves; bracteoles pinkish, lanceolate-oblong, 1.5-2 mm long, as long as or exceeding calyx tube. *Flowers* sessile, axillary, solitary. *Calyx tube* campanulate, 1 mm long, calyx lobes 5, triangular, pink, 0.5mm long; calyx appendages present. *Petals* 5, obovate, 2-lobed at apex, 1mm long, bright pink. *Stamens* 5, inserted just below the middle of the calyx tube, exserted. *Ovary* globose, trilocular; style 0.5 mm long; stigma capitate; capsule globose, about as long as calyx tube, 1.5 mm in diam., 3-valved; seeds semi ovoid, *c.* 0.5 mm long.

Fl. & Fr.: Aug.– Jan.

Distribution and habitat: It is widely distributed in tropical and subtropical Asia, East Africa and Australia. It grows in shallow water at the edges of tanks, ditches in floating mats of vegetation, in marshes and in rice fields.

***Specimens examined*: Andhra Pradesh**: Anantapur Dist., Kalasamudram, 8 Feb.1983, *N Yesoda 1182* (MH). Karim Nagar Dist., Godavari, 26 Dec. 1964, *G. V Subbran 22476* (MH). **Karnataka**: Dakshina Karnataka Dist., Mangalore, 17 Nov. 2011, *Lemiya K. M 132960* (CALI). **Kerala:** Kannur Dist., Manantoddy, 11 Feb. 1978, *V. S Ramachandran 53870* (MH). Irikkur,

29 Nov. 2013, *Pramod C 132961*(CALI). Wayanad Dist., 9 Jan. 2012, *Lemiya K. M 132908* (CALI). Malappuram Dist., Pattambi, 22 Oct. 2013, *Lemiya K. M 132948* (CALI).

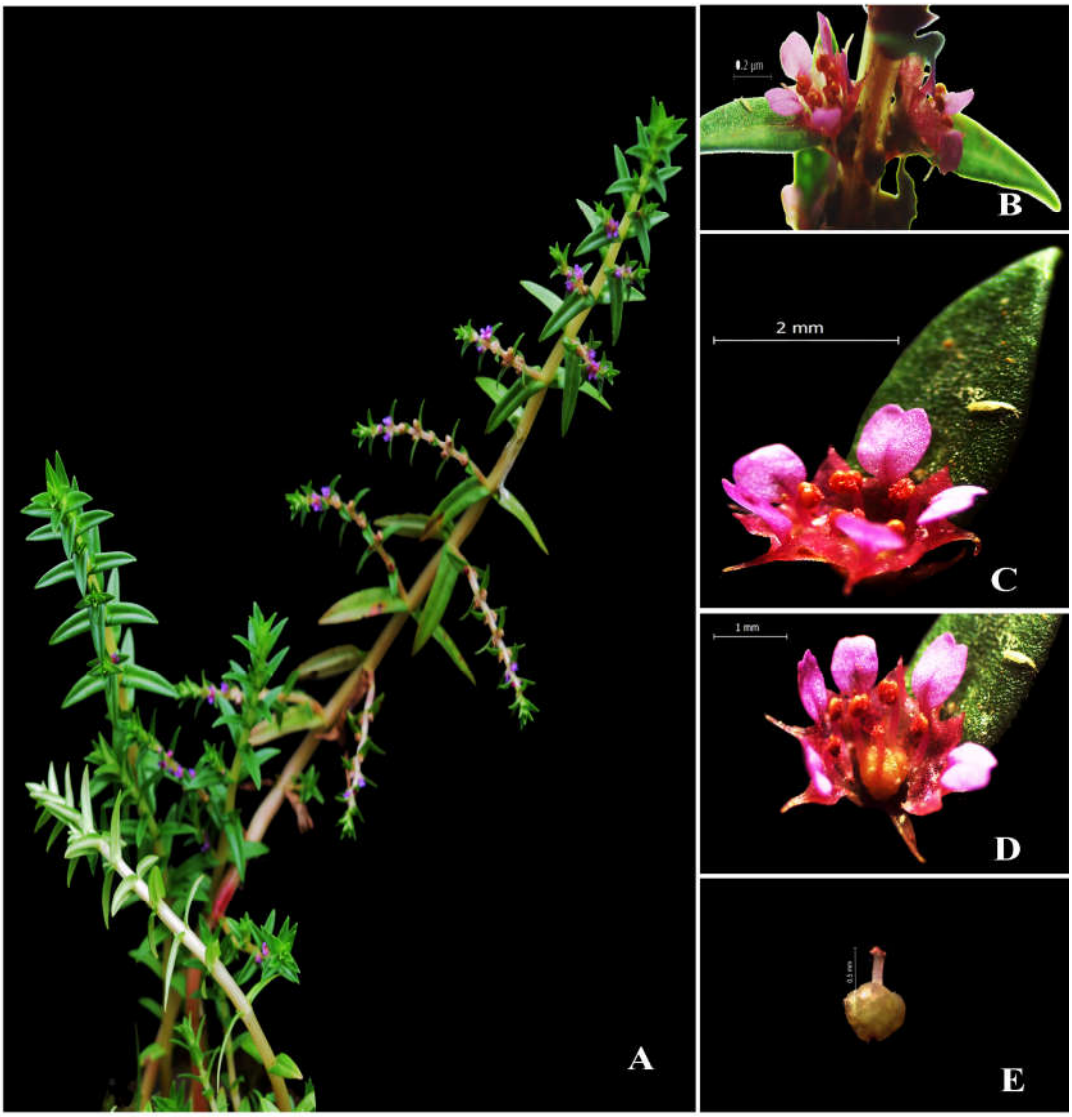

Figure 53. *Rotala densiflora* (Roth *ex* Roem. & Schult.) Koehne **A.** Habit; **B.** Inflorescnce; **C.** Single flower; **D.** Flower-split open; **E.** Gynoecium.

Rotala fimbriata Wight, Ic. Pl. Ind. Or. 1 t. 217, 1840; Koehne, Bot. Jahrb. 1: 166. 1880 & in Engl., Pflanzenr. IV 216: 37. 1903; Blatt. & Hallb., J. Bombay Nat. Hist. Soc. 25: 710. 1918; Gamble., Fl. Pres. Madras 1**:** 508.

1919; Cook, Biossiera 29: 108. 1979; Matthew, Fl. Tam. Carnatic 3: 612. 1983; Sharma *et al.,* Fl. Karnataka 108. 1984; Singh, Fl. Eastern Karnataka 1: 317. 1988; Joseph *et* Sivarajan, Proc. Ind. Acad. Sci (Plant Sci.) 99(3): 182. 1989; Matthew, Excursion. Fl.Central Tam. 201. 1991; Matthew, Fl. Palani Hills 1: 513 (1999); Pullaiah, Biodiversity in India 2: 146. 2003. **Figure 54.**

Type: Wight, Ic. Pl. Ind. Or. 1: t. 217. 1840.

Ammannia pentandra Roxb. var. *fimbriata* (Wight) Clarke in Hook. f., Fl. Brit. India 2: 569. 1879.

Ammannia heyneana Wall., Cat. 2104, 1828. *nom. nud*.

Ammannia hexandra Panigrahi, Indian Forester 102**:** 766. 1976, non Wall. *ex* Koehne, 1880.

Erect, amphibious annual, 30−35cm tall, *Stem* weakly 4-angular, glabrous, simple or branched, rooting at base; narrowly winged. *Leaves* sessile, decussate below, alternate above, internodes very close towards the apex, linear or lanceolate to oblong; base cordate to auriculate; apex acute to obtuse. *Bracts* like foliage leaves; bracteoles transparent, linear to subulate, very small, less than1 mm, not up to the middle of the calyx tube. *Flowers* sessile, axillary, solitary; calyx tube campanulate to funnel form, 2− 2.5 mm long, calyx lobes 5, deltate, transparent, 0.5 mm long; calyx appendages absent. *Petals* 5, pinnately divided into linear segments, *c*. 2 mm long, bright violet. *Stamens* 5, attached at the base of the calyx tube, filaments 2−2.5 mm; anthers exserted. *Ovary* ellipsoidal, style *c.* 2 mm long, trilocular; stigma capitate; capsule elongated*, c.* 3 mm long, about as long as calyx tube, 1.5 mm diam., 3-valved; seeds semi-ellipsoial, *c.* 0.5 mm long.

Fl. & Fr.: Aug.− Dec.

Distribution and habitat: It is endemic to Southern India. It is widely distributed throughout its known range. It grows in waterlogged areas especially rock outcrops with ephemeral pools, ditches.

Specimens examined: **Karnataka:** Bangaluru Dist., Bommanahalli, 29 Nov. 2015, *Lemiya K. M 132992* (CALI). **Kerala**: Idukki Dist., Amballur, 13 Dec. 2015, *A. K Pradeep 132997* (CALI).

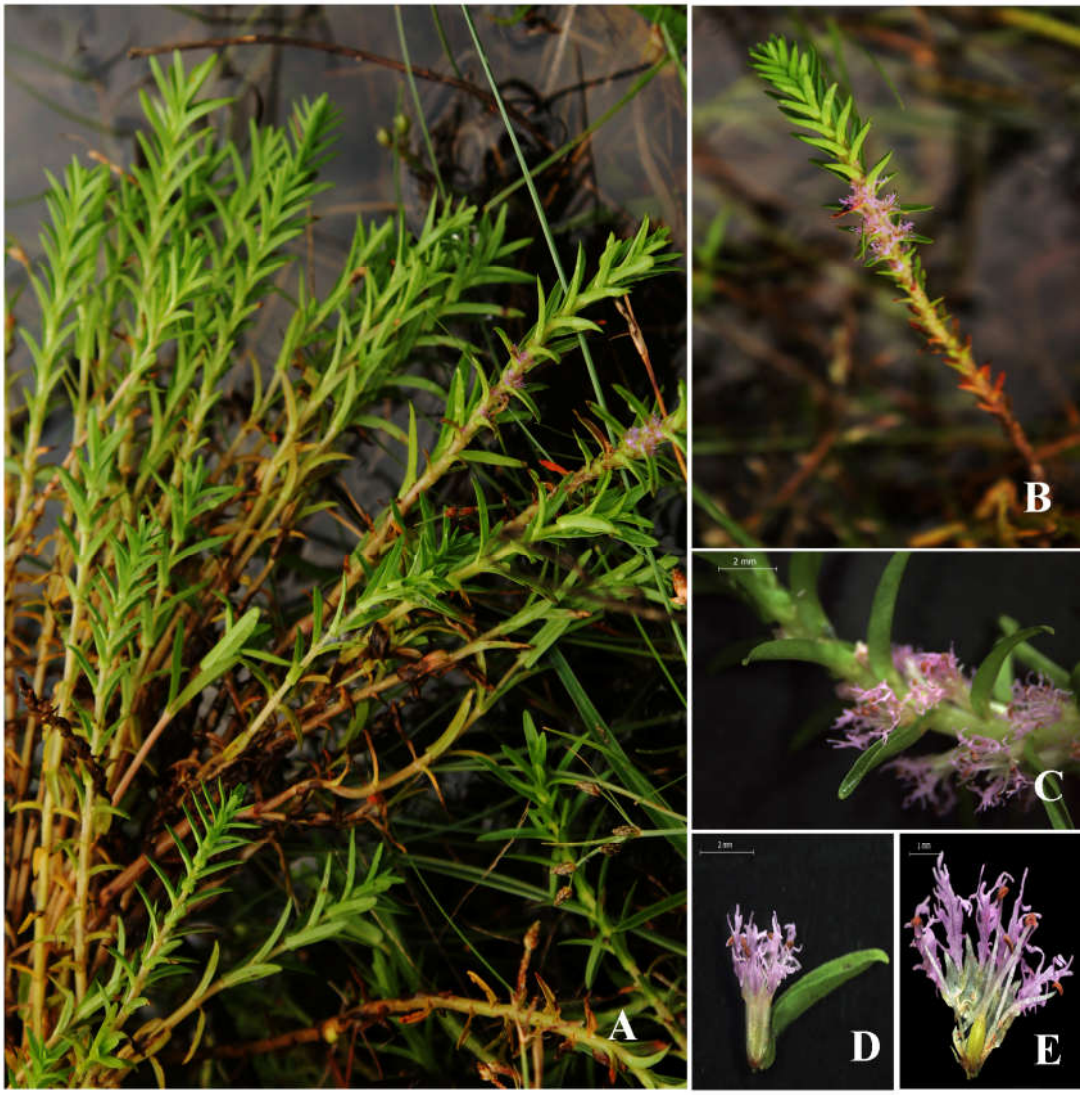

Figure 54. *Rotala fimbriata* Wight. **A.** Habit; **B.** Enlarged portion of twig; **C.** Inflorescnce; **D.** Single flower; **E.** Flower-split open.

Rotala indica (Willd.) Koehne**,** Bot. Jahrb. 1: 172, 1880, & Engl., Pflanzenr. IV 216: 40. 1903; Blatt. & Hallb., J. Bombay Nat. Hist. Soc. 25: 711. 1918; Gamble, Fl. Pres. Madras 1: 508. 1919; Van Leeuwen, Blumea 19: 54. 1971; Panigrahi, Indian Forester 102: 766. 1976; Sald. & Nicols., Fl. Hassan Dist.; 274. 1976; Cook, Biossiera 29: 108. 1979; Mani. & Sivar. Fl. Calicut. 112. 1982; Matthew, Fl. Tam. Carnatic 3: 613. 1983; Sharma *et al.,* Fl. Karnataka 108. 1984; Joseph et Sivarajan, Proc. Ind. Acad.Sci (Plant Sci.) 99(3): 184. 1989; Matthew, Excursion. Fl. Central Tamil Nadu. 201. 1991; Matthew, Fl. Palani Hills 1: 513. 1999; Pullaiah, Biodiversity in India 2: 147. 2003; Sunil & Sivadasan, Fl. Alappuzha Dist. 305. 2009. **Figure 55.**

Type: India, *Klein* 546 (holo: B- Herb. Willdenow 814/ 7001).

Peplis indica Willd., Sp. Pl. 2(1): 244. 1799.

Ameletia indica (Willd.) DC., Mem. Soc. Phys. Nat. Hist. Geneve 3(2): 82, t, 3, f. A.1826.

Ammannia nana Roxb., Fl. Ind. (ed. 1) 1: 448, 1820, *non Wall.*

Ameletia polystachya Wall. *ex* Wight. and Arn., Prodr. 1: 304, 1834.

Ammannia peploides Spreng., Syst. Veg. 1 444, 1825; Clarke in Hook.f., Fl. Brit. India 2:566. 1879.

Erect, annual, 25–30 cm tall. *Stem* 4-angular, glabrous, winged, creeping and rooting from lower nodes. *Leaves* sessile; lamina obovate- spathulate or sub orbicular, 5−20 × 0.7−1.4 mm, glabrous; base obtuse-cuneate; apex acute-obtuse. *Bracts* dimorphic, leaf like on main stem and smaller elliptic–oblong on flowering stem; bracteoles linear- lanceolate, 0.7−0.78 mm long, almost equaling calyx tube, glabrous, purple coloured. *Flowers*, sessile, axillary, solitary. *Calyx tube* 1.5−2 mm long, glabrous, purple at anthesis, lobes 4, narrowly campanulate, triangular-acute, calyx appendages absent. *Petals* 4, purple, elliptic to narrowly ovate, 0.32–0.37mm long, acuminate at apex, margin entire. *Stamens* 4, purple, filaments at about the middle of the calyx

tube. *Ovary* ellipsoidal, trilocular; style short, up to 0.5−0.8 mm long, often persistent in fruit; stigma capitate; capsule ellipsoidal, 2 mm long; seeds ellipsoid, 2 mm long.

Fl. & Fr.: Oct.– Mar.

Distribution and habitat: It is widely distributed in Tropical Asia, Europe, Africa and America. In India it is distributed in all South Indian states. It is usually found growing in paddy fields and wetlands.

Specimens examined: **Chattisgurh:** Raithum, 10 Nov. 2012, *Prasad M. G 132925* (CALI). **Kerala**: Alappuzha Dist. Bharanikkavu, *Sunil C.N 2438* (CALI). Calicut Dist., Poovattuparambu, 9 Jan. 2012, *Lemiya K. M 132910* (CALI). Idukki Dist., 6 Feb. 1981, *N. C Nair 69868* (MH). Kottayam Dist., Vanikammana, 26 Aug. 1984, *V. T Antony 659* (MH). Palakkad Dist., Mannarkkad, 6 Nov. 2013, *Lemiya K. M 132952* (CALI); Thiruvizham Kunnu, 27 Jan 1919, *E. Vajravelu 33356* (MH).

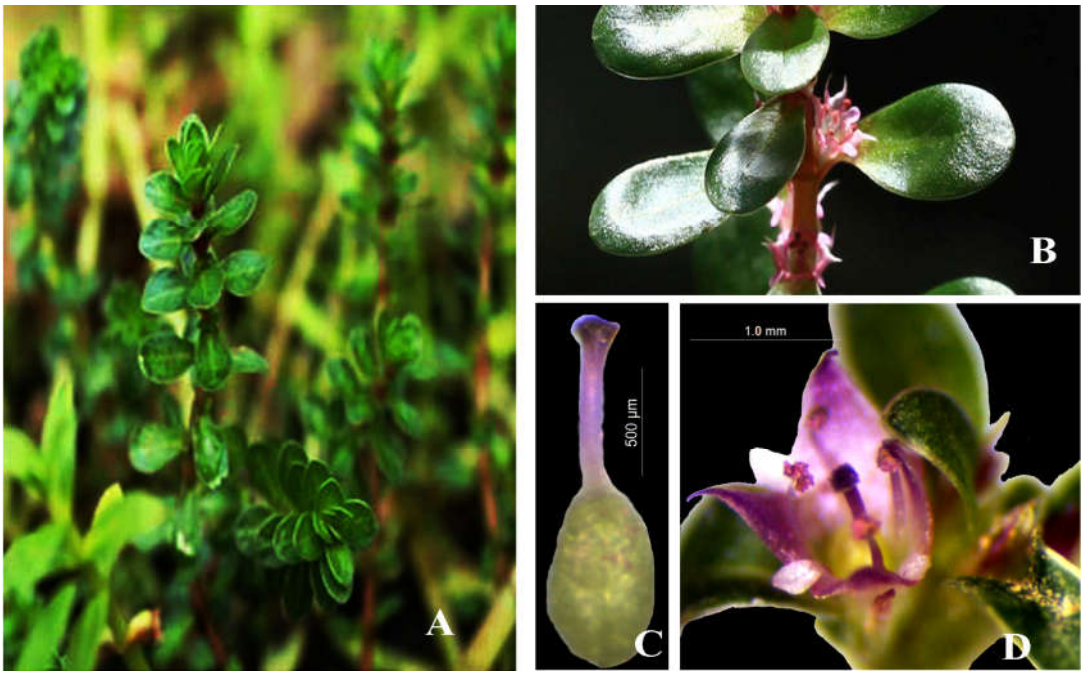

Figure 55. *Rotala indica* (Willd.) Koehne. **A. Habit**; **B.** Enlarged portion of stem with inflorescence; **C.** Gynoecium; **D.** Single flower.

Rotala juniperina A. Fernandes, Bol. Soc. Brot. Ser. 2 48: 126, 1974. Cook, Biossiera 29: 97. 1979. **Figure 56.**

Type: Zambia, Kabulamwanda Dam, 108 km N. of Choma, *c.* 1150 m, 24 April 1954, *Robinson 723* (holotype: K).

Type: Malawi, Mlanje Distr., 16 km N. W. of Likabula Forest Depot, 700 m,15 June 1962, *Robinson 5353* (holo: SRGH n. v.; isotypes: BR, K, M)

Rotala decumbens A. Fernandes, Bol. Soc. Brot. Ser. 2 (48): 127. 1974.

Erect amphibious annual or perennial, up to 20 cm tall. *Stem* 4-angular, glabrous, simple or branched, rooting at base; narrowly winged. *Leaves* sessile, decussate below, lanceolate to ovate; *c.* 20 mm main axis but *c.* 1 mm on lateral branches. base cuneate to cordate, apex acute. *Bracts* like foliage leaves on main axis, smaller and oblong acuminate on lateral brances. Bracteoles 2, pale pinkish, linear, *c.* 1.5 mm long, as long as or slightly exceeding calyx tube. *Flowers,* sessile, axillary, solitary; calyx tube campanulate, *c.* 1 mm long, calyx lobes 3, narrowly triangular, pink, 0.5mm long; calyx appendages 3, spreading at anthesis, subulate in fruit. *Petals* 3, elliptic, acute at apex, *c.* 0.5 mm long, pink. *Stamens* 3, inserted below the middle of the calyx tube, anthers born at the top of the calyx tube. *Ovary* subglobose, trilocular; style *c.* 0.25 mm long; stigma capitate; capsule subglobose, slightly exceeding calyx tube, 1.5 mm in diam., 3-valved; seeds semi-ovoid, *c.* 0.5 mm long.

Fl. & Fr.: Apr.– July

***Distribution and habitat*:** This species was restricted to Africa. But recently this species was found in Wayanad Wildlife Sanctuary, inside Muthanga forest. It prefers marshy areas in moist deciduous forest, water logged areas, and shallow ponds.

***Specimens examined*: Kerala:** Wayanad Dist., Muthanga, 30 Nov. 2007, *C. N Sunil 4090. Ibid*., 28 Sep. 2015, *Lemiya K. M 132990* (CALI).

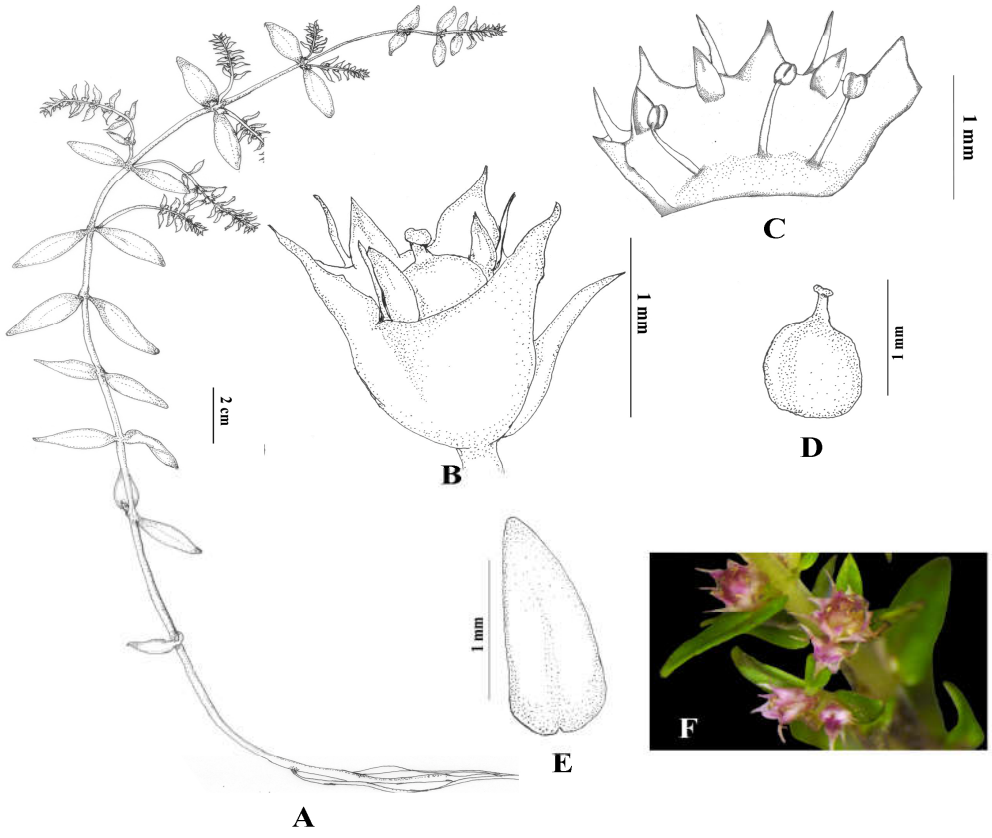

Figure 56. *Rotala juniperina* A. Fernandes**. A**. Habit; **B.** Single flower; **C.** Flower- split open; **D.**Gynoecium. **E.** Bract; **F.** Enlarged portion of a twig.

Rotala kasaragodensis K.S. Prasad & Raveendran, Edinburgh J. Bot. 70 (3): 451–454. 2013. **Figure 57.**

Type: India, **Kerala,** Kasaragod Dist., Mugu, 12°36 ′ 56 ″ N, 75°1 ′ 40 ″ E, 150 m, on wet soil, 6 ix 2012, *K.S. Prasad 03120* (holo- CAL; iso- BSI, MBGS, MH).

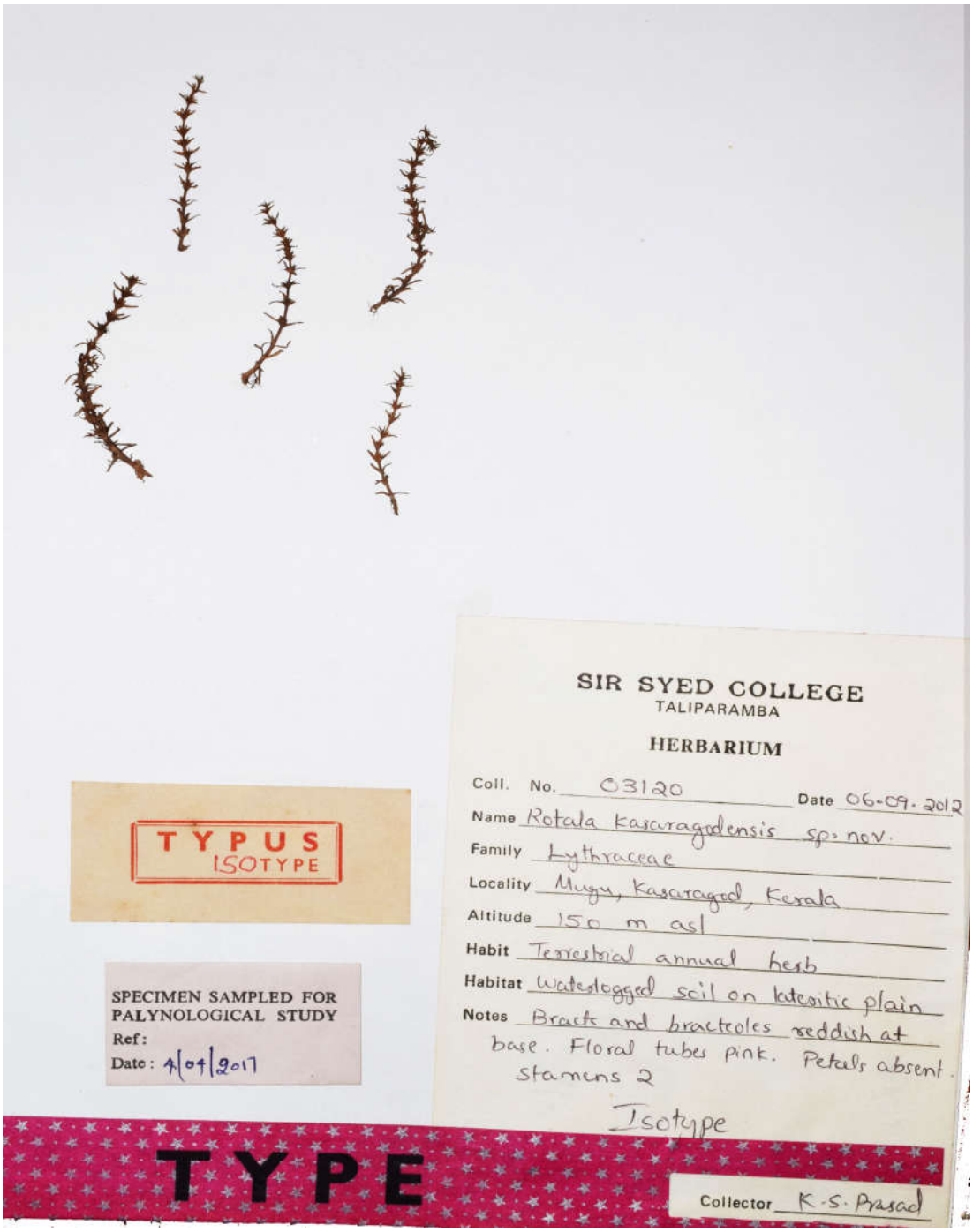

Figure 57. Isotype of *Rotala kasaragodensis* K.S.Prasad & Raveendran at CALI.

Erect, annual, 2.5−6 cm tall. *Stem* 3-angular, glabrous, creeping and rooting from lower nodes. *Leaves* in whorls of 3; aerial leaves linear to ovate 3−5 mm long; apex acute; base dialated, boat shaped enclosing the flowers 9 mm long;

Bracts leaf-like; bracteoles 2, bract-like, 0.5− 1mm long. *Flowers*, sessile, axillary, solitary. *Calyx tube* suburceolate, membraneous, 1−1.2 mm long; calyx lobes 4, narrowly triangular; calyx appendages absent. *Petals* 0. *Stamens* 2, filaments near the base of the calyx tube. *Ovary* ellipsoidal, trilocular; style *c.* < 0.25 mm; stigma capitate; capsule ellipsoid, 1.5 mm long; seeds semiovoid, *c.* 0.3 mm long.

Fl. & Fr.: Aug. – Oct.

***Distribution and Habitat*:** It is known only from Kasargod dsitrict of Kerala and Dakshina Kannada district of Karnataka. It grows in waterlogged soil in lateritic plains.

Specimen examined: Kerala: Kasaragode Dist., Mugu, 06 Sep. 2012, *K. S Prasad 03120* (CALI)

Rotala macrandra Koehne, Bot. Jahrb. 1: 176. 1880, & Engl.,Pflanzenr. IV 216: 41. 1903; Gamble, Fl. Pres. Madras 1**:** 509. 1919; Cook, Biossiera 29**:** 54. 1979; Mani & Sivar. Fl. Calicut. 112. 1982; Joseph et Sivarajan, Proc. Ind. Acad. Sci (Plant Sci.) 99 (3): 185. 1989; Sasidh. & Sivar., Fl. Pl. Thrissur For. 196. 1996; Sunil & Sivadasan, Fl. Alappuzha Dist. 305. 2009. **Figure 58.**

Type: India, *Wallich* 2095 H (Lectotype K-W, Isolecto. CGE, LE).

Ameletia rotundifolia Wight., Ic. Pl. Ind. Or. 1: t. 258. 1840, non Buch.-Ham. Ex Roxb. 1820.

R. rotundifolia sensu Blatler & Hallb., J. Bombay Nat. Hist. 25: 718. 1918, *p. p.* non Koehne, 1880.

Ammannia rotundifolia Wight. & Arn., Prod. 1. 306. 1834; Clarke in Hook. f., Fl. Brit. India 2: 566. 1879.

Erect, annual or perennial herb, 35−40 cm tall. *Stem* 4-angular, glabrous, creeping and rooting from lower nodes. *Leaves* sessile, decussate or occasionally in whorls of 3 near the apex; submerged leaves linear-lanceolate to orbicular, pink to purple, aerial leaves obovate to orbicular with a reddish tinge on abaxial surface; base obtuse to cuneate; apex acute to rounded; red leaf nerves distinct. *Bracts* ovate to acute; bracteoles linear- lanceolate, 0.45−0.55 mm long, half as long as calyx tube, glabrous. *Flowers* sub sessile, axillary and solitary. *Calyx tube* 1 mm long, white or tinged with pink above; calyx lobes 4, campanulate, triangular, pink, 0.7mm long, calyx appendages absent. *Petals* 4, obovate, 2 mm long, bright rose. *Stamens* 4, inserted near the base of the calyx tube, exserted. *Ovary* globose, trilocular; style 3 mm long, often persistent in fruit; stigma minutely capitate; capsule globose, *c*. 1.5 mm diam., 4-valved; seeds semi-ellipsoidal, *c.* 0.5 mm long.

Figure 58. *Rotala macrandra* Koehne**. A.** Habit; **B.** Enlarged portion of tip; **C.** Flowers in terminal racemes; **D.** Flower- split open; **E.** Bract.

Fl. & Fr.: Sept. – Jan.

Distribution and habitat: It is endemic to southern India and is known from the states of Karnataka, Kerala, Tamil Nadu and Maharashtra. It is a common

weed in streams, temporary ponds and flooded paddy fields. In submerged condition the plant is very flexuous, leaves are very thin and more or less translucent, shape varies from lanceolate to orbicular and are pale green, pink or red. It usually set flowers and fruits when the surrounding water. It resembles *R. rotundifolia* in general appearance but differs in having exserted stamens and also usually restricted to the wet low lands in the coastal region.

Specimens examined: **Kerala,** Alappuzha Dist., Chenganoor, 30 Mar. 1989, *N. Anilkumar 1655* (MH). Calicut Dist., Poovatuparamba, 9 Jan. 2012, *Sreejith P. E 132911* (CALI). Malappuram Dist., Thalappara, 15 Jan. 2013, *Lemiya K. M & Thoiba K 132933* (CALI). Palakkad Dist., Peringothukavu, 23 Nov. 1973, *Vajravelu 44811* (MH). Wayanad Dist., Thirunelli, 12 Jan. 2015, *Pramod C 132934* (CALI).

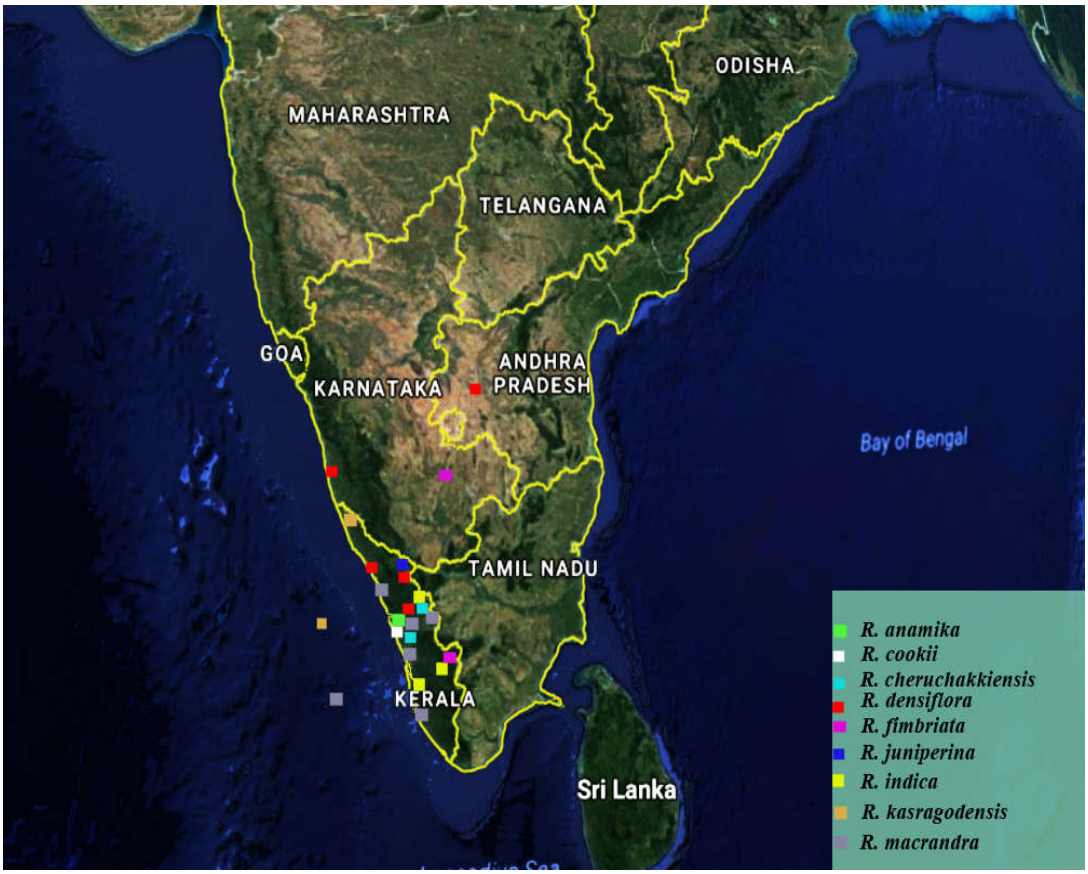

Figure 59. Distribution map of different species of *Rotala* in South India.

Rotala malabarica Pradeep, K.T.Joseph *et* Sivar., Bot. Bull. Acad. Sin. 31(1): 59. 1990. **Figure 60.**

Type: India: Kerala, Kannur Dist., Payangadi, Aduthila. *Pradeep 5139* (holotype: MH; Isotype: CALI).

Erect, annual herb, to 17 cm tall. Stem 4-angular, glabrous, much branched, creeping and rooting below. *Leaves* sessile, simple, decussate, linear, *c.* 10 × 1 mm, bimucronate at apex, cuneate at base. *Bracts* leaf-like; bracteoles 2, linear, scarious, upto calyx tube*, c.* 0.7 mm long. *Flowers* sessile, axillary, solitary. *Calyx tube* campanulate, light pink, *c.* 1mm long; calyx lobes usually 5 or 4, triangular acute; *c.* 0.5 mm, calyx appendages absent. *Petals* usually 5 rarely 4, pink, 0.5 mm long, clawed at base, limb suborbicular, retuse to obcordate at apex. *Stamens* usually 5 or 4; filaments, *c.* 1mm long, near the base of the calyx tube. *Nectar scales* present, ovate oblong, light purple in colour with dark tinge at the tip, up to the half of the length of the stamen. *Ovary* globose, greenish or reddish, trilocular. Style to the length of the ovary, slightly exceeding calyx tube; stigma capitate; capsule globose, 1.5 mm across; seeds ovoid, 0.5 mm long.

Fl. and Fr.: Jul. – Sept.

***Distribution and habitat*:** It is only known from northern region of Kerala. It usually grows in waterlogged soil in lateritic plains along with *R. malampuzhensis*.

Specimens examined: **Kerala:** Kannur Dist., Payangadi, *Pradeep A. K 5139* (CALI). Kannur Dist., Madayipara, 13 Aug. 2012, *Pramod C* 132914 (CALI). Kasaragode Dist., Mugu, 13 Sept. 2014, *Lemiya K. M 132968* (CALI).

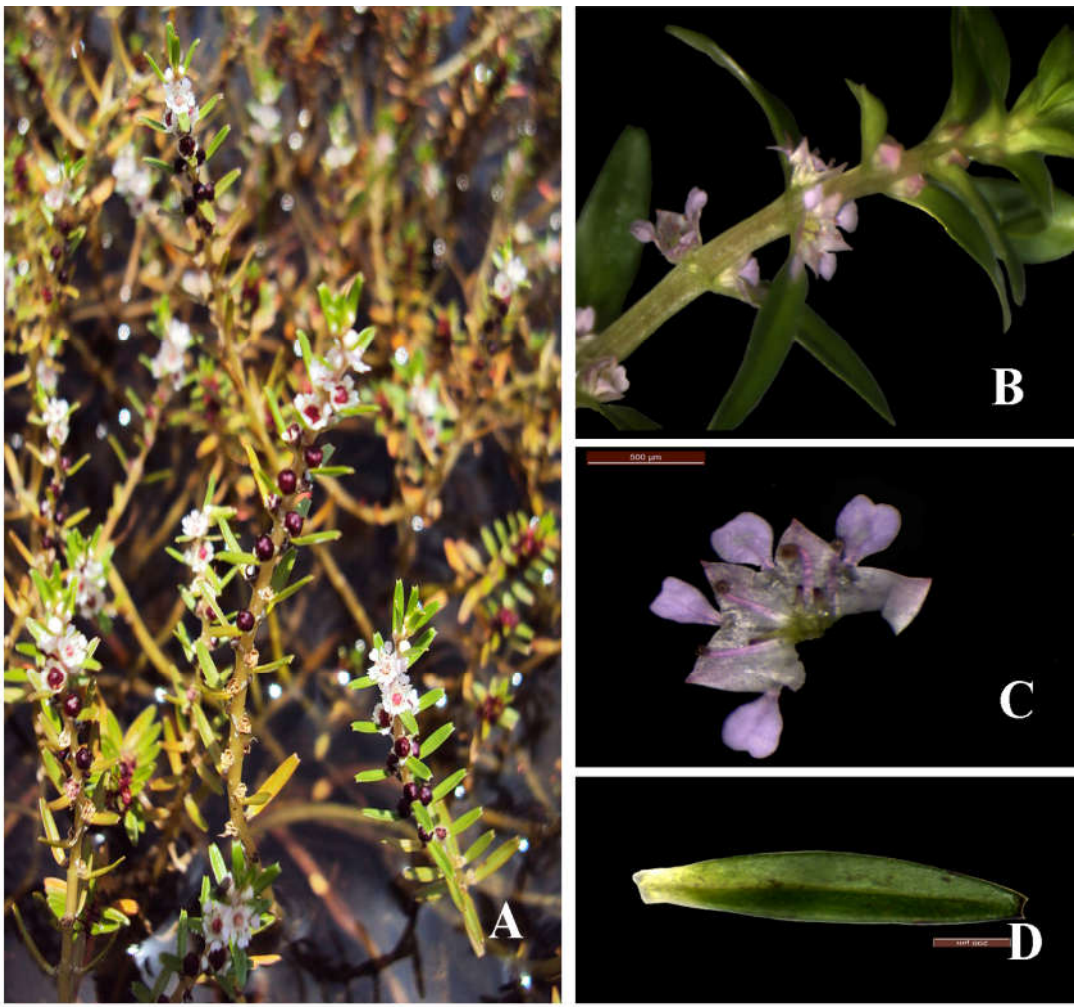

Figure 60. *Rotala malabarica* Pradeep, K.T. Joseph *et* Sivar. **A.** Habit; **B.** Enlarged portion of stem with inflorescence; **C.** Flower- split open; **D.** Leaf.

Rotala malammpuzhensis R. V. Nair (J.Bombay Nat. Hist. Soc. 72 57, 1975. Nom invalid.) *ex* C. D. K. Cook, Boissiera 29: 98. 1979; Mani. & Sivar., Fl. Calicut 114, 1982; Panigrahi and Nicols., Taxon 32: 120. 1983; Joseph *et* Sivarajan, Proc. Ind. Acad. Sci (Plant Sci.) 99 (3): 186, 1989; Pullaiah, Biodiversity in India 2:147, 2003; Sunil & Sivadasan, Fl. Alappuzha Dt. 306. 2009. **Figure 61.**

Type: India, Kerala, Palaghat Dist. Malampuzha River, July 1971, *R. Vasudevan Nair 89602* (holotype: MH).

Erect, annual tuft forming herb, up to 10 cm tall. *Stem* 4-angular, glabrous, scarcely branching, creeping and rooting from lower nodes. Submerged *leaves* scale-like to orbicular, aerial leaves lanceolate to linear glabrous, 15mm × 4mm; cuneate at base, acute to shortly truncate at apex. *Bract* leaf-like on main stem and smaller elliptic–oblong on flowering stem; bracteoles linear-lanceolate, 0.7−0.78 mm long, almost equaling calyx tube, glabrous, purple coloured. *Flowers* sessile, axillary, solitary. *Calyx tube* 1.5−2 mm long, glabrous, purple at anthesis; calyx lobes 4, narrowly campanulate, triangular-acute, calyx appendages short. *Petals* 3-5, purple, elliptic to narrowly ovate, 0.32−0.37mm long, acuminate at apex, margins entire. *Stamens* 4, purple, filaments at about the middle of the calyx tube; *Nectar scales* 3, purple, lanceolate, 0.25 mm long, alternating with stamens, shorter than ovary. *Ovary* ellipsoidal, trilocular; style short, to 0.5−0.8 mm long, often persistent in fruit; stigma capitates; capsule ellipsoid, *c.* 2 mm long; seeds hemispherical, *c.* 1 mm long.

Fl. & Fr.: July − Sept.

Distribution and habitat: *Rotala malampuzhensis* is endemic to the Western Ghats occurring in all five states. It grows in waterlogged areas especially as rock outcrops, around temporary pools, ditches, rice fields. It shows high variability in its size according to nature of habitat.

Specimens examined: **Karnataka:** Dakshina Karnataka Dist., Mangaluru, 8 Oct. 2015, *Lemiya K. M & Drisya V 132985* (CALI). **Kerala:** Malappuram Dist., Nilambur, 15 Oct. 1986, *Laxmana K. M 3545* (CALI). Kannur Dist., Madayipara, 13 Aug. 2012, *Pramod C 132915* (CALI). Kasaragode Dist., Mugu, 10 Sept. 2014, *Lemiya K. M 132965* (CALI). Eranakulam Dist., Neryamangalam, 24 Aug. 2015, *Drisya 132980* (CALI).

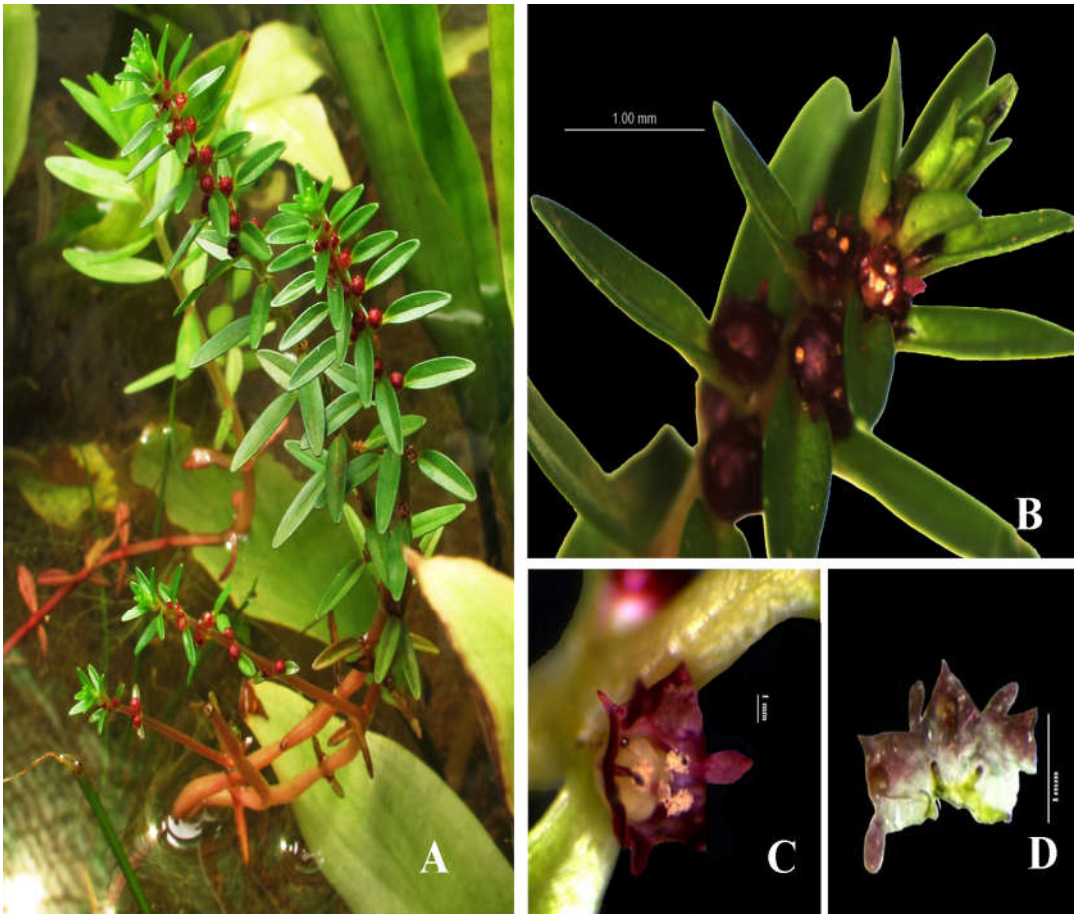

Figure 61. *Rotala malampuzhensis* R. V. Nair *ex* C.D.K. Cook. **A.** Habit; **B.** Inflorescnce; **C.** Single flower; **D.** Flower-split open.

Rotala mexicana Cham. & Schldtl., Linnaea 5: 567. 1830; Koehne**,** Bot. Jahrb. 1: 150. 1880. & Engl.,Pflanzenr. IV. 216: 29. 1903; Blatt. & Hallb., J. Bombay Nat. Hist. Soc. 25: 702. 1918; Van Leeuwen, Blumea 19: 54. 1971; C. D. K.Cook, Biossiera 29: 33. 1979; Philox, Kew Bull. 41: 43. 1986; Joseph *et* Sivarajan, Proc. Ind. Acad. Sci (Plant Sci.) 99 (3): 187. 1989; Sasidh. & Sivar., Fl. Pl. Thrissur For. 196. 1996; Pullaiah, Fl. Eastern Ghats: Hill Ranges of South East India 3: 71. 2007. **Figure 62.**

Ammannia mexicana (Cham. & Schltdl.) Baill., Hist. Pl. Madag. Atl. t. 363. 1895.

Type: Mexico, prope Hacienda de la Laguna, Oct., Schiede & Deppe 566: (Holo.-HAL n. v; Iso.- LE, MO).

Rotala apetala F. v. Muller, Fragm. Phyt. Austral. 3 108, 1862.

Ammannia pygmaea S. Kurz, Seeman's J. Bot. 5: 376. 1867; R. V. Nair & Nambiar, J. Bombay Nat. Hist. Soc. 63: 784. 1965.

Rotala pygmaea (S. Kurz) Rajagopal & Ramayya, Kew Bull. 23: 465. 1969.

Erect, annual herb, up to 3 cm tall. *Stem* 4-angular, glabrous, profusely branched, creeping and ascending rooting at nodes. *Leaves* in whorls of 3−8 when submerged, linear, up to 15 mm long, aerial leaves are decussate, linear to lanceolate, up to 10 mm long; base cuneate; apex acute or acuminate. *Bracts* like foliage leaves; bracteoles linear, as long as or exceeding calyx tube. *Flowers* sessile, axillary, solitary. *Calyx tube* semi globose, 0.5−0.75 mm long, red or pink; calyx lobes 3−5, usually 4, triangular, 0.25−0.5 mm long; calyx appendages absent. *Petals* 0. *Stamens* 2, included within the calyx tube, but occasionally exserted. *Ovary* globose, trilocular; style short, up to 0.3 mm long; stigma capitate; capsule globose, 0.5−0.75 mm in diam. 3-valved; seeds semi ovoid, c. 0.3 mm long.

Fl. & Fr.: July – Nov.

Distribution and habitat: It is commonly found in shallow water or on wet mud following rain. A widespread and variable species found usually in shallow water or wet or moist open grounds during monsoon. It is a widespread cosmopolitan species. It can be seen throughout the warmer parts of the world except in Congo basin, N. E. Africa, Arabia and Pacific Islands. In India it is commonly seen in Andhra Pradesh, Assam, Bihar, Kerala, Karnataka, Madhya Pradesh, Orissa, Rajasthan, Sikkim and Uttar Pradesh.

Specimens examined: **Andhra Pradesh**: Kurnool Dist., Gundlabrahmeswaram, 25 Oct. 1964, *J. L Ellis 22196* (MH). **Kerala**: Calicut Dist., Poovattuparamba, 24 Sep. 2011, *Lemiya K.M 132902* (CALI). Wayanad Dist., Periya, 11 Oct. 2013, *Pradeep A. K. & Pramod C 13250* (CALI).

Figure 62. *Rotala mexicana* Cham. & Schldtl**. A.** Habit; **B.** Enlarged portion of stem; **C.** Single flower; **D.** Gynoecium.

Rotala occultiflora Koehne, Bot. Jahrb. 1: 152. 1880, & Engl., Pflanzenr. IV. 216: 30. 1903; Blatt. & Hallb., J. Bombay Nat. Hist. Soc. 25: 705. 1918; Gamble, Fl. Pres. Madras 1: 508. 1919; C. D.K. Cook, Biossiera 29**:** 41. 1979;

Mani & Sivar. Fl. Calicut 112. 1982; Sharma *et al.,* Fl. Karnataka 108. 1984; Singh, Fl. Eastern Karnataka 1: 318. 1988; Joseph *et* Sivarajan, Proc. Ind. Acad.Sci (Plant Sci.) 99(3): 191. 1989; Pullaiah, Biodiversity in India 2: 147. 2003. **Figure 63.**

Type: India, Malabar, Concan, *Laws, Hooker f. & Thomson* (holotype: K; isotype: L).

Amphibious, annual prostrate herbs. *Stem* 3-angular, glabrous, winged, 3−6 cm long, creeping and rooting from lower nodes. *Leaves* in whorls of 3; submerged leaves linear, 4−6 mm long; aerial leaves 3−5 mm long; apex obtuse to bifid; base dialated, boat shaped enclosing the flowers. *Bracts l*eaf-like; bracteoles 2, bract-like, apex bifid to truncate, much longer than calyx tube, 2− 3mm long. *Flowers,* sessile, axillary, solitary. *Calyx tube* suburceolate, membranous, 1−1.2 mm long; calyx lobes 4 or 5, narrowly triangular; calyx appendages absent. *Petals* 0. *Stamens* 3, sometimes 2, filaments near the base of the calyx tube. *Ovary* ellipsoid, trilocular; style short; stigma capitate; capsule ellipsoid, 1.5 mm long; seeds ellipsoid, 0.5 mm long.

Fl. & Fr.: Aug. – Oct.

Distribution and habitat: It is distributed in northern Australia and India. In India it is found in Goa, Kerala, Karnataka and Maharashtra. It occurs in marshes and temporary wet places, such as the margins of pools and streams. Mostly found along the sea coast, sometimes in brackish water.

***Specimens examined*: Karnataka**: Dakshina Karnataka Dist., Asarmukh, 8 Oct. 2015, *Lemiya K. M & Thoiba K 132984* (CALI). Mysore Dist., Makanahalli, 8 Oct. 2015, *Lemiya K. M. & Thoiba K 132986* (CALI). **Kerala:** Malappuram Dist., Calicut University Campus, 13 Oct. 2011, *Lemiya K. M. 132905* (CALI).

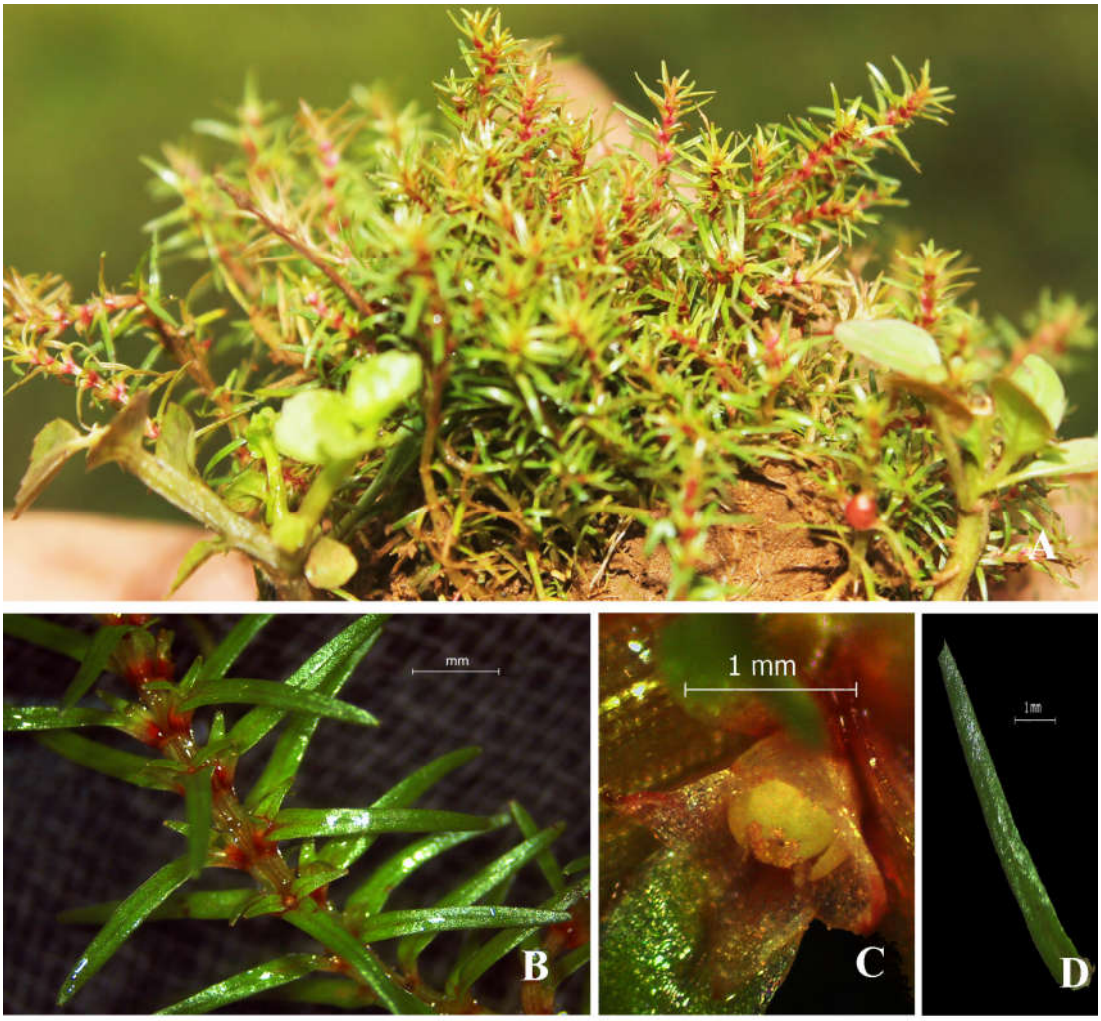

Figure 63. *Rotala occultiflora* Koehne. **A.** Habit; **B.** Enlarged portion of stem; **C.** Single flower; **D.** Leaf.

Rotala rosea (Poiret) C.D.K. Cook, Boissiera 29: 86. 1979; Mani. & Sivar., Fl. Calicut 113. 1982; Matthew, Fl. Tam. Carnatic 3: 614. 1983; Sivarajan, Proc. Ind. Acad.Sci (Plant Sci.) 99(3): 192. 1989; Matthew, Fl. Palani Hills 1: 513 (1999); Pullaiah, Biodiversity in India 2: 148, 2003; Pullaiah, Fl. Eastern Ghats: Hill Ranges of South East India 3: 71. 2007; Sunil & Sivadasan, Fl. Alappuzha Dist. 306. 2009. **Figure 64.**

Ammannia rosea Poiret in Lamarck, Encycl. Meth. Bot. Suppl. 1: 329. 1810.

Type: Indes orientales, herb. *Desfontaines* (holotype: FT).

Ammannia pentandra Roxb., Fl. Ind. (ed. 1): 448, 1820; Clarke in Hook. f. Fl. Brit. India 2: 568. 1879. (excl. var. *fimbriata*); Cooke, Fl. Pres. Bombay 1: 507. 1903.

Rotala pentandra (Roxb.) Blatt. And Hallb., J. Bombay Nat. Hist. Soc. 25: 707. 1918. *p. p*.

Ammannia leptopetala Blume, Mus. Bot. Lud. Bat. 2: 134. 1856.

Rotala leptopetala (Blume) Koehne, Bot. Jahrb. 1: 162. 1880, *p. p*. emend. Koehne, Bot. Jahrb. 3: 388. 1883; *nom. illeg*.

Erect, annual, 20−30 cm tall. *Stem* 4-angular, glabrous, winged, rooting at base. *Leaves* sessile, decussate; lamina linear- lanceolate to ovate, 4.5−20 × 1.5−6 mm, glabrous, base cuneate, apex obtuse. *Bracts* like foliage leaves; bracteoles linear, 0.5−1 mm long, shorter than calyx tube. *Flowers* axillary, sessile, solitary. *Calyx tube* 1.5 mm long, campanulate at anthesis; calyx lobes 5, shallowly triangular, 0.25 mm long; calyx appendages subulate, usually towards the apex of calyx lobes. 0.5 mm long. *Petals* 5 or rarely 4 or rarely rudimentary, not persisting in fruit, 0.35mm long, obovate, dentate at apex. *Stamens* 5 or rarely 4, filaments inserted just below the middle of the calyx tube. *Ovary* globose, trilocular; style short, 0.2 mm long; stigma capitate; capsule globose, 2 mm in diam, exceeding the calyx; seeds semi- ovoid, 0.3 mm long.

Fl. & Fr.: Aug.− Feb.

***Distribution and habitat*:** It is widespread in India, Thailand, Viet Nam, Philippines, Indonesia, and China. It grows in wetlands and rice fields, near sea level to 1,000-1,800 m elevation.

Specimens examined: **Karnataka**: Dakshina Karnataka Dist., Mangaluru, 8 Nov. 2015, *Lemiya K. M 132987* (CALI). Thanjavur Dist., Perallum, 12 Sept. 1988, *Raguthey 852* (MH). Tambaram Dist., Ponne, 5 Apr. 1984, *D Narasimham 397* (MH). **Kerala:** Alappuza Dist. Nedumudi, 11 Oct. 2013,

Lemiya K. M 132949 (CALI). Kannur Dist., Manantoddy, 11 Feb. 1978, *V. S Ramachandran 53870* (MH). Elangad, 21 Dec. 1979. *V. S Ramachandran 65367* (MH). Palakkad Dist., Mannarkkad, 6 Nov. 2013, *Lemiya K. M 132953* (CALI). **Tamil Nadu**: Kumarapalayam, 26 Dec. 2014, *Lemiya K. M 132971* (CALI).

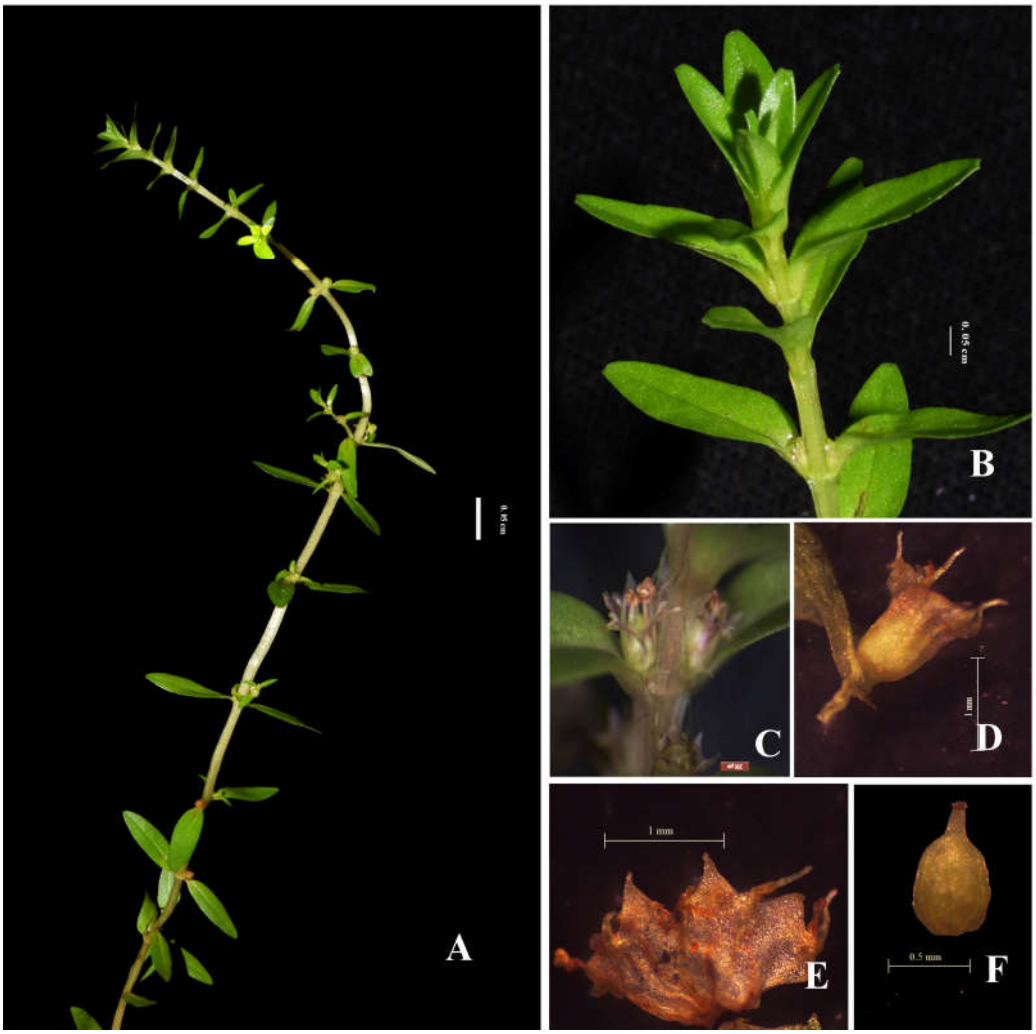

Figure 64. *Rotala rosea* (Poiret) C.D.K. Cook. **A.** Habit; **B.** Enlarged portion of tip; **C.** Inflorescence; **D.** Single flower; **E.** Flower-split open; **F.** Gynoecium.

Rotala rotundifolia (Buch. - Ham. *ex* Roxb.) Koehne, Bot. Jahrb. 1:175. 1880 & in Engl. Pflanzenr. IV. 216: 41. 1903; Blatt. & Hallb., J. Bombay Nat. Hist. Soc. 25: 718. 1918; Gamble, Fl. Pres. Madras 509. 1919; Sald. &

Nicols., Fl. Hassan Dist 274, 1976; C. D. K. Cook, Biossiera 29: 49. 1979; Mani. & Sivar., Fl. Calicut 113. 1982; Matthew, Fl. Tam. Carnatic 3: 616. 1883; Sharma *et al.*, Fl. Karnataka 108. 1984; Joseph et Sivarajan, Proc. Ind. Acad. Sci (Plant Sci.) 99(3): 193. 1989; Matthew, Excursion. Fl. Central Tamil Nadu. 202. 1991; Matthew, Fl. Palani Hills 1: 514. 1999; Pullaiah, Fl. Eastern Ghats: Hill Ranges of South East India 3: 73. 2007. **Figure 65.**

Ammannia rotundifolia Buch.- Ham. *ex* Roxb., Fl. Ind. Ed. 1. 1:446. 1820.

Type: India. Roxburgh, Fl. Indica Pl. No. 1344 (K, vide C. D. K. Cook, 1979).

Ameletia rotunundifolia (Ham. *ex* Roxb.) Dalz. Gibson, Bombay Fl. 96. 1861, non wight, Ic. Pl. Ind. Or. 1: t. 258. 1840.

Ammannia subspicata Bentham in Hooker, London J. Bot. 1: 484. 1842.

Erect, amphibious, perennial herb. *Stem* 4-angular, glabrous, 25−30 cm high, branched, creeping and floating, rooting from lower nodes. *Leaves* sessile, decussate or in whorls of 3 in above; submerged leaves linear to orbicular, *c.* 2 cm long, variable in color, green or red, aerial leaves obovate or orbicular, 0.5−2 × 0.7−2 cm, glabrous, occasionally red on abaxial surface, leaf nerves distinct, green or white, base obtuse-cuneate, apex acute to round or obtuse. *Bracts* ovate, leaf like; bracteoles linear- lanceolate, 0.7−1 mm long, almost equaling calyx tube, glabrous, purple coloured. *Flowers*, sessile, axillary, solitary, Inflorescence, many flowered, dense pedunculate receme, elongate. *Calyx tube* 1.5−2 mm long, narrowly campanulate, glabrous, light purple at anthesis; calyx lobes 4, triangular-acute, *c.* 1 mm long; calyx appendages absent. *Petals* 4, purple, obovate, *c.* 2 mm long, midnerve distinct, margin entire. *Stamens* 4, purple, inserted at the base of the calyx tube, *c.* 1.5 mm long; anthers level with the middle of the calyx lobes**.** *Ovary* globose, 4-locular; style *c.* 0.5 mm long; stigma capitate, relatively massive; capsule globose, *c.* 1.5 mm long; seeds ellipsoid, *c.* 0.5 mm long.

Fl. & Fr.: Sept.−Mar.

Distribution and habitat: It is widespread in South and South East Asia and also in Japan. In India it is commonly found in Kerala, Tamil Nadu, Karnataka and Maharashtra. It grows in the paddy fields, marshy areas, stream sides and waterlogged grass lands. It is closely similar to *R. macrandra,* but occurs only in high lands while *R. macrandra* grows in low land areas.

Specimens examined: **Andhra pradesh:** Adilabad Dist., Sevgudavagu, 22 Jan. 1987, *T. Ravishankar 85116* (MH). Visakhapatanam Dist., Douvaput, 19 May 1964, *G. V Subbarao 19698* (MH). **Karnataka:** Chikmagalur, *s. loc* 17 Feb. 2013, *Prasad M. G 132937* (CALI). Shimoga Dist; 22 Jan. 2015, *Shinoj 132979* (CALI). **Kerala,** Wayanad Dist., Thirunelli, 9 Jan. 2012, *Lemiya K. M 132909* (CALI). **Tamil Nadu:** Nilgiri Dist., Ooty, 24 Dec. 2012, *Lemiya K. M 132932* (CALI). Dindigul Dist., Kodaikanal, 28 Dec. 2013, *Lemiya K. M 132963* (CALI).

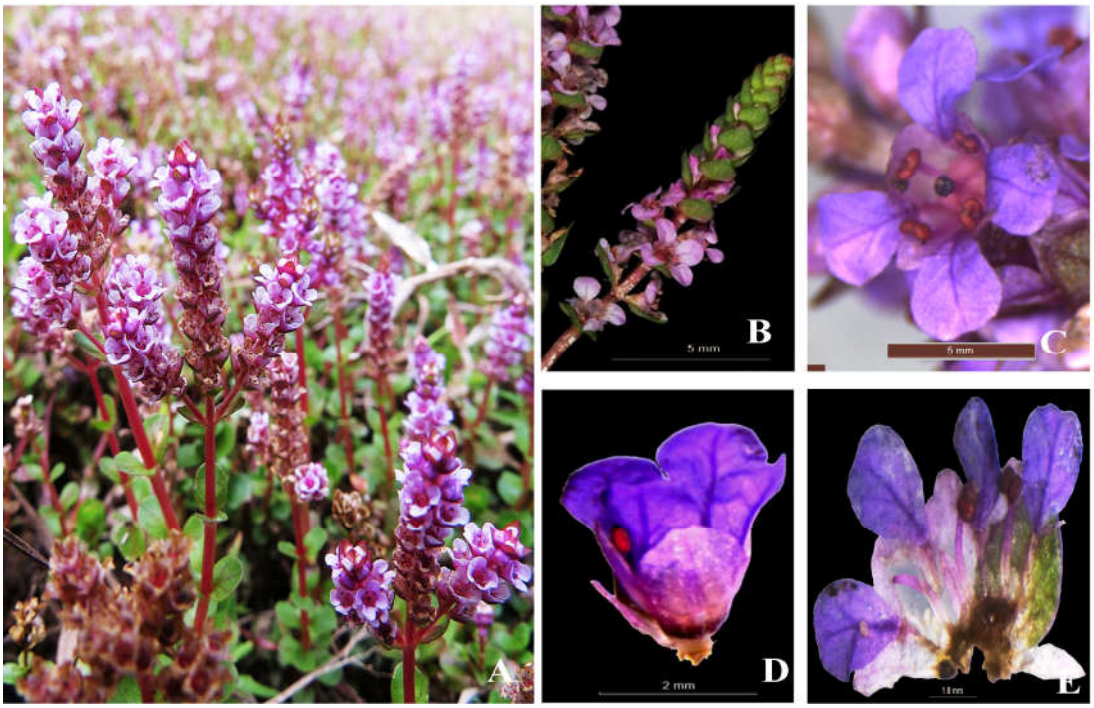

Figure 65. *Rotala rotundifolia* (Buch.- Ham. *ex* Roxb.) Koehne. **A.** Habit; **B.** Inflorescence; **C.** single flower; **D.** Calyx tube; **E.** Flower- split open.

Rotala tulunadensis K.S. Prasad, P. Biju, C. Ravi & K.G. Bhat, Nordic J. Botany 30: 58. 2012. **Figure 66.**

Type: India, Kerala, Kasaragod District, Permude lateritic plateau, 12 ° 39′ 25 ″ N, 75 ° 0′ 47 ″ E, 150 – 200 msl ., 29 Dec. 2010, *K. S. Prasad* 02651 (holotype: CAL, isotypes: MH, BSI).

Perennial spreading herbs, *c.* 15 cm tall. *Stem* terete, glabrous, branched, creeping and rooting below, floating and erect above. *Leaves* sessile; dimorphic, submerged leaves elliptic to ovate rounded at base, obtuse or retuse at apex, 10 × 20-4 × 20 mm, aerial leaves obovate, rounded at both ends, 3− 4 × 2− 3 mm. *Bracts* leaf-like, obovate, smaller towards apex; bracteoles subulate, 0.7−1 mm long. *Flowers* sessile, axillary, solitary, pedicellate, 3−3.5 mm long; bracteoles, subulate, *c.* 1 mm long. *Calyx tube* 2.5−3 mm long, 0.5−0.7mm wide, tinged red, glabrous, constricted below the apex, with distinct wings on the angle and running whole length of the tube; calyx lobes 4, triangular; calyx appendages absent. *Petals* 4, rose, ovate to suborbicular *c.* 1mm long, acuminate at apex. *Stamens* 4, purple, filaments at about the middle of the calyx tube. *Ovary* ellipsoid, 4- locular; style *c*. 0.5 long, often persistent in fruit; stigma capitate; capsule ellipsoid, *c*. 4.5 mm long; seeds ellipsoid *c.* 0.4 mm long.

Fl. & Fr.: Dec. – Mar.

Distribution and habitat: It is only known from its type locality in Kasaragod district. It grows in a temporary, shallow pool on lateritic rocks.The plant remains submerged during rainy seasons and emerges out as water level recedes during winter and summer.

Specimen examined: **Kerala:** Kasaragode Dist., Permude, 23 Dec. 2014, *Lemiya K. M 132974* (CALI).

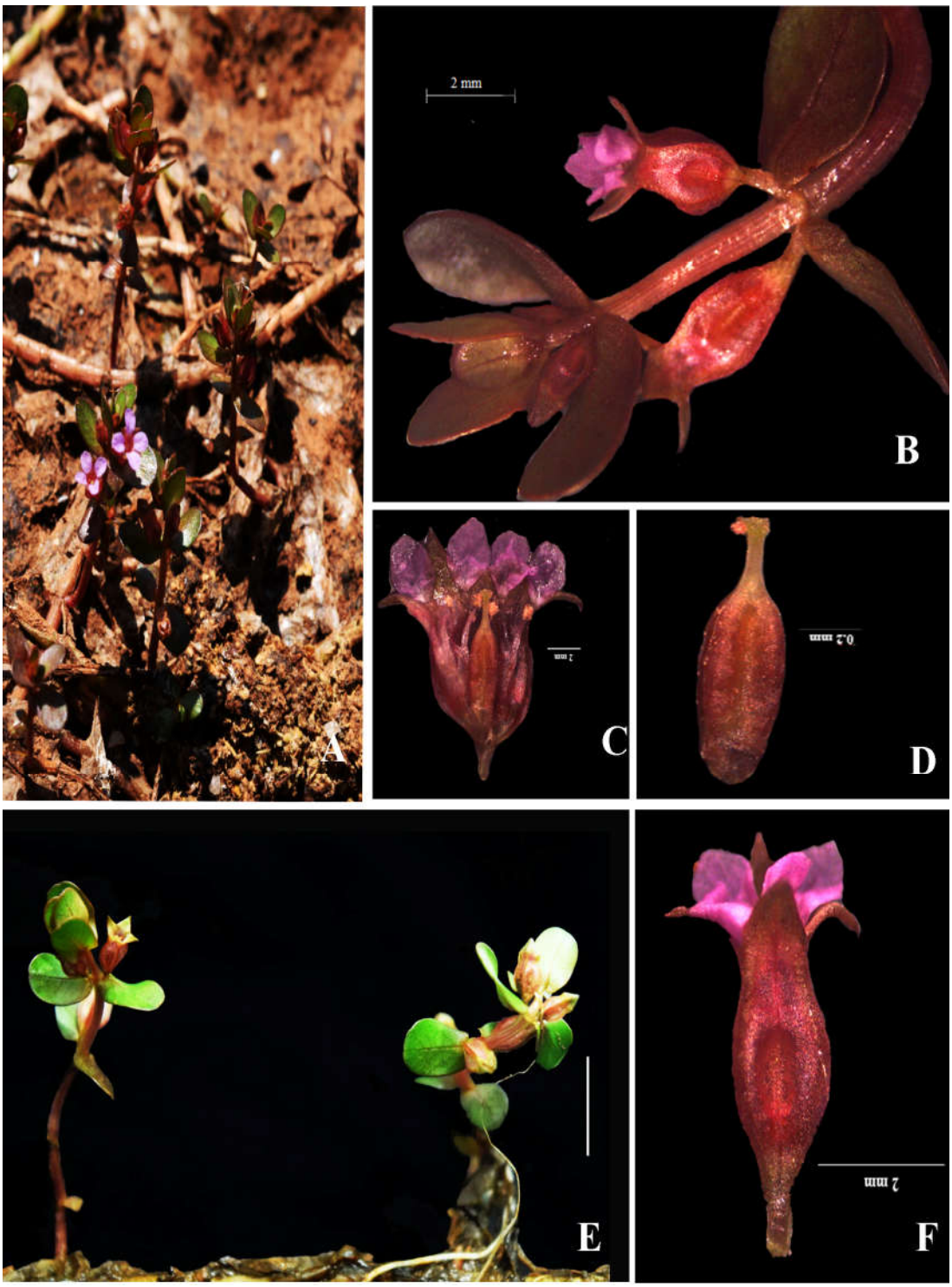

Figure 66. *Rotala tulunadensis* K.S. Prasad, P. Biju, C. Ravi & K.G. Bhat. **A.** Habit; **B.** Inflorescence; **C.** Flower split open; **D.** Gynoecium; **E.** A flowering branch.**F.** Single flower.

Rotala vasudevanii Joseph & Sivar., Proc. Indian Acad. Sci. (Pl. Sci.) 99: 195. 1989. **Figure 67.**

Type: India, Kerala, Alwaye, *Vasudevan Nair 3997* (Holo.- Z, Iso- CALI).

Hydrolythrum wallichii sensu R.V. Nair, Journ. Bombay Nat. Hist. Soc. 61: 718. 1965, *non* Hook. f. 1867, *non Rotala wallichii* (Hook.f.) Koehne, 1880.

Erect, annual, aquatic, submerged, only flowering branch apices emerging out, glabrous, up to 50 cm tall. *Stem* 4-angular, sparsely branched. *Leaves* sessile, in whorls of 9−12, monomorphic, linar; acute at apex; narrowed at base; *Bracts* leaf-like; bracteoles transparent, more than thalf as long as calyx tube. *Flowers* towards branch tips, sessile in the axils of bracts. *Calyx tube* campanulate; calyx lobes 4, triangular- acute, 0.4 mm long, calyx appendages absent. *Petals* 4, obovate, pink. *Stamens* 4, inserted near the base of the calyx tube. nectar scales 4, small, shortly bilobed. *Ovary* globose; style short, persistent; stigma capitate, minutely papillose; capsule subglobose, *c*. 1.5 mm in diameter., 2-valved; seeds ellipsoid.

Fl. & Fr.: Sept.−Nov.

Distribution and habitat*:** It was known only from the type locality in Aluva, Kerala. No recent collection of this taxon is available in any Indian herbaria. We were not able to locate this taxon in its original locality or adjoining areas.It is reported to occur in shallow ponds and waterlogged areas. ***Specimen examined: **Kerala:** Eranakulam Dist., Aluva, Sept. 1965, *R. Vasudevan Nair 3997* (CALI).

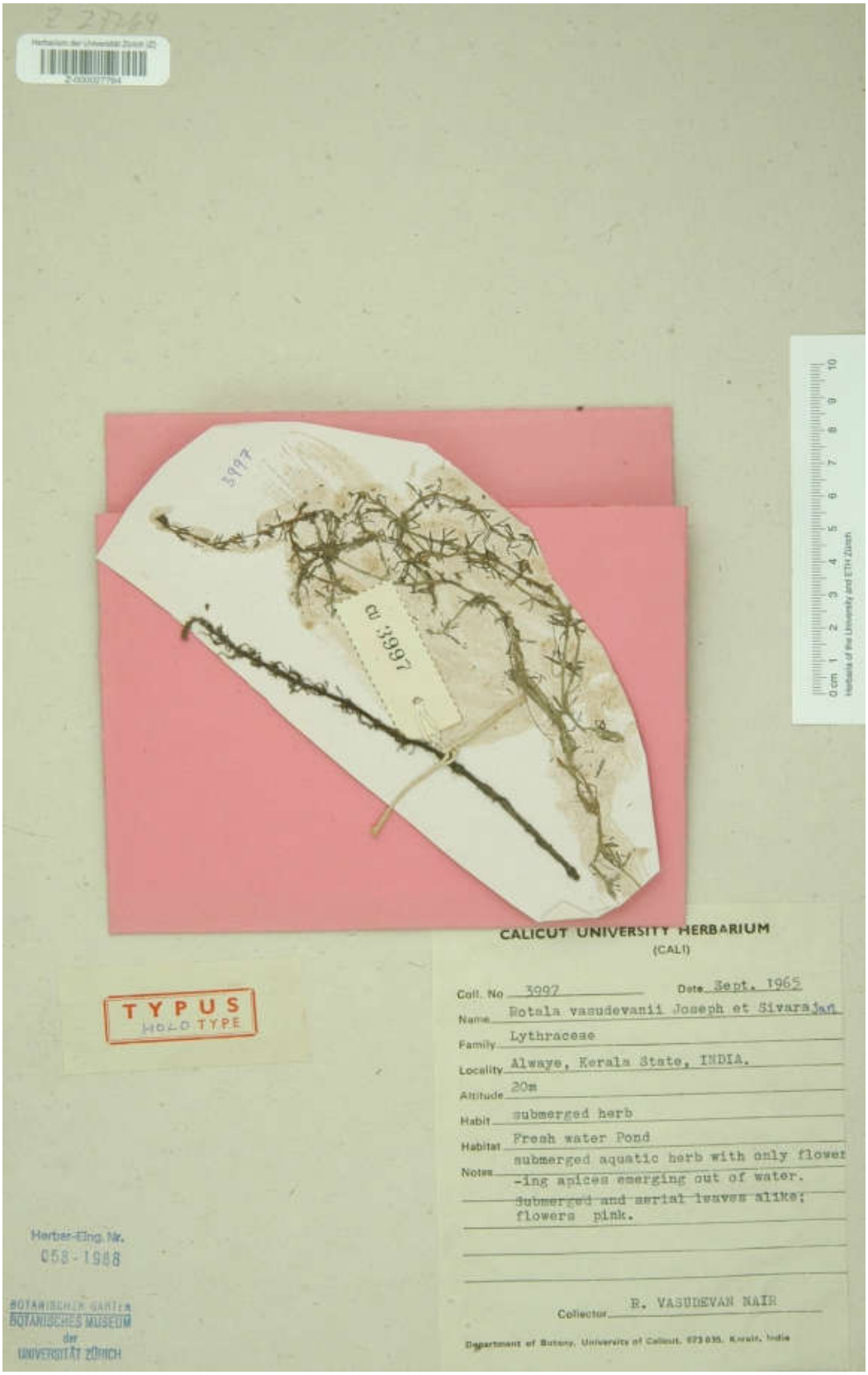

Figure 67. Holotype of *Rotala vasudevanii* Joseph & Sivar. at CALI

Rotala verticillaris L., Mantissa Pl. 2: 175. 1771; Dalz. & Gibz., Bombay Fl. 96.1861; Engl., Pflanzenr. IV 216: 30. 1903; Blatt. & Hallb., J. Bombay Nat. Hist. Soc. 25: 705. 1918; Gamble, Fl. Pres. Madras 1: 508. 1919; Sald. & Nicols., Fl. Hassan Dist. 274. 1976; C. D. K. Cook, Biossiera 29: 23. 1979; Matthew, Fl. Tam. Carnatic 3: 613. 1983; Sharma *et al.,* Fl. Karnataka 109. 1984; Joseph *et* Sivarajan, Proc. Ind. Acad. Sci (Plant Sci.) 99(3): 196. 1989; Matthew, Excursion. Fl. Central Tamil Nadu 202. 1991; Pullaiah, Fl. Eastern Ghats: Hill Ranges of South East India 3: 75. 2007. **Figure 68.**

Ammannia verticillaris (L.) Baillon, Hist. Pl. 6 439, f. 423, 424. 1877.

Type: India orientalis (holotype: LINN, Savage Cat. No. 52- 1).

Ammannia rotala Clarke in Hooker f., Fl. Brit. India 2: 567. 1879; Cooke, Fl. Pres. Bombay 1: 507. 1903, non F. V. Muller, 1862.

Prostrate, perennial herb. *Stem* simple or branched, glabrous, 10−20 cm high, terete below, 4- angular above, creeping, rooting from lower nodes. *Leaves* usually in whorls of 4, linear to oblong, 5−10 × 1−2.5 mm; cuneate at base, apex truncate or shortly bifid. *Bract* leaf-like on main stem, usually in whorls of 4; bracteoles 2, colorless, capillary, very short, *c.* 0.5 mm long, not up to the middle of calyx tube. *Flowers* sessile, axillary, solitary, monomorphic. *Calyx tube* sub urceolate, glabrous, slightly constricted at the mouth, *c.* 2 mm long; calyx lobes 3, deltate, *c.* 0.5 mm long, calyx appendages absent, small interjected fold between the calyx lobe present. *Petals* 3, pink, ovate to cordate, *c.* 0.5 mm long, margin entire. *Stamens* 3, *c.* 0.7mm long, inserted above the middle of the calyx tube. Not up to the calyx lobes, 4−5 mm long. *Ovary* ellipsoid, c. 1.2 mm long, trilocular; style very short, less than 0 0.25 mm long; stigma capitate; capsule globose -ellipsoid, slightly exceeding calyx tube; Seeds semi ovoid, 0.35−0.5 mm long.

Fl. & Fr.: Jan.– Mar.

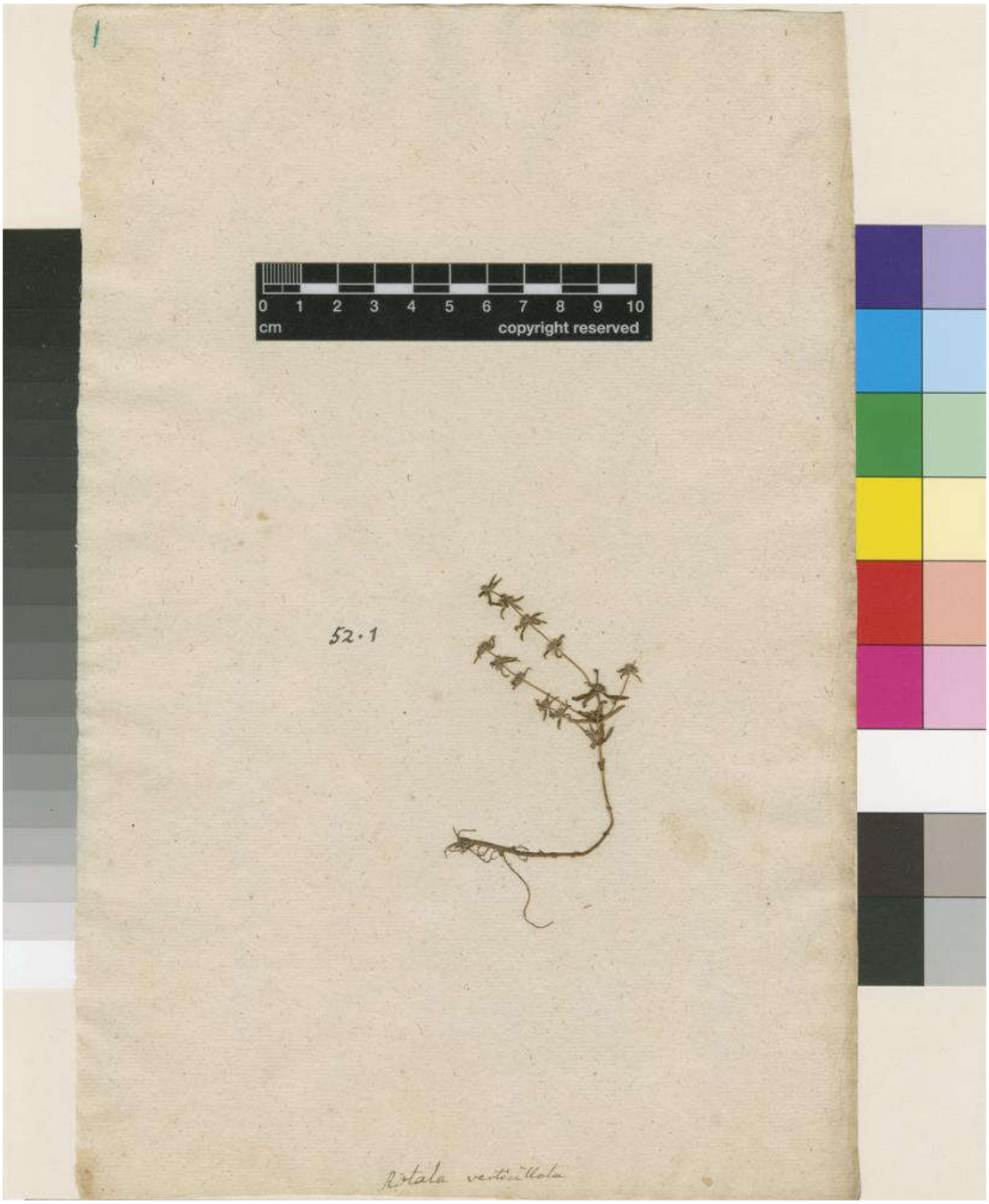

Figure 68. Holotype of *Rotala verticillaris* L .at Linn.

Distribution and habitat: It is found in pools and along the marshy margins of ponds. It flowers as the water recedes. It is endemic to Southern India and Sri Lanka. In India, this species is confined to Andhra Pradesh, Madhya Pradesh and Tamil Nadu, Karnataka and Maharashtra.

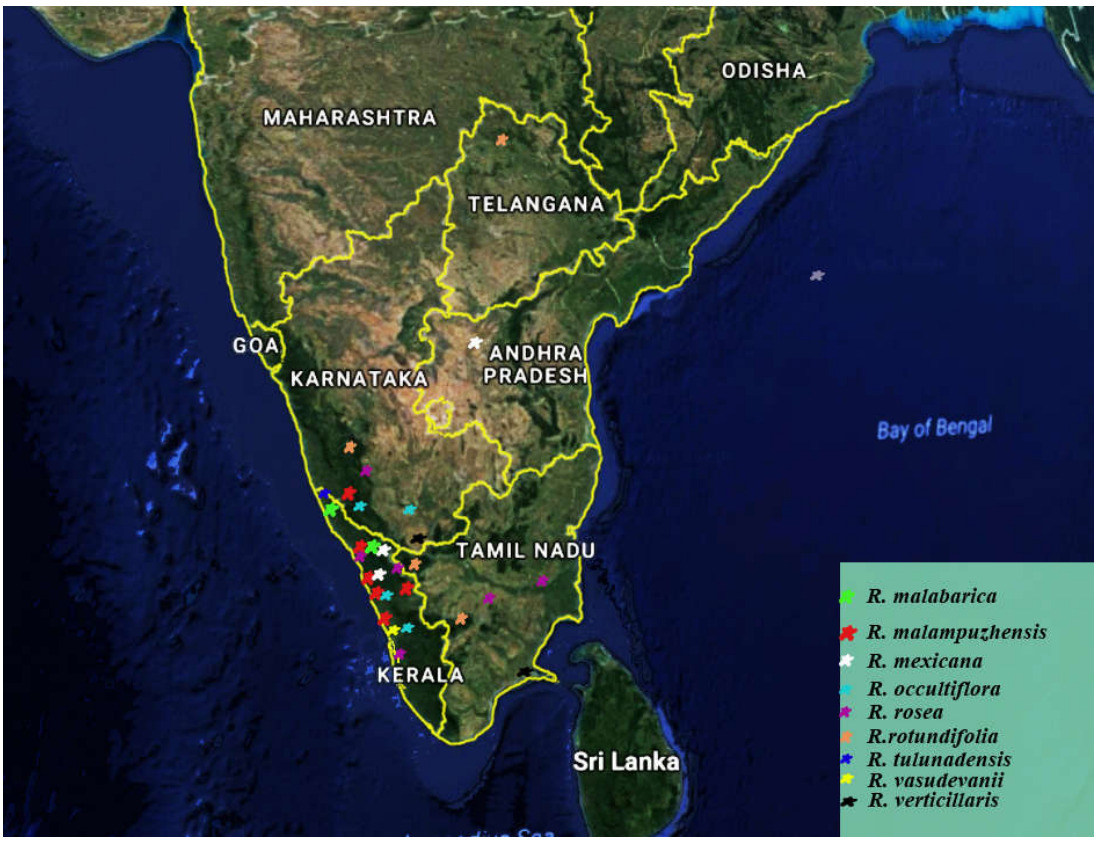

Figure 69. Distribution map of different species of *Rotala* in South India.

Specimens examined: **Karnataka:** Chamarajanagar Dist., K. Kudi BR Hills, 25 Mar. 2004, *C. Murugan 117666* (MH). (CALI). **Tamil Nadu**: Ramanathapuram Dist., Ilayangudi, 25 Feb. 1988, *V. Balasubramanyam 1680* (MH). **Odisha:** Bhubaneswar, 8 Dec. 2011, *A. K. Pradeep & Pramod C 127624*

Summary and Conclusison

The present investigation is aimed to elucidate the taxonomic limits of South Indian species of three closely related genera, *Ammannia, Nesaea* and *Rotala*. This is the first comprehensive study based on morphological, anatomical and molecular analysis of South Indian species three genera. 24 species were documented in the present study and the herbarium sheets were prepared for future reference. A live germplasm including maximum available species was maintained in Calicut University Botanical Garden (CUBG). One new discovery, *Rotala anamika* Lemiya and one new report *Rotala juniperina* A. Fern. from India, based on morphological characteristics, are significant contribution of the study. In addition, morphological characterization found to supply valuable contributions in both specific and generic level systematics. Among the morphological characters, Inflorescence type and surface pattern of capsule wall were considered as the important in generic delimitation. Other morphological characters such as foliar morphology, seed morphology and floral morphology were found to have a key role in the species delimitation. The overall morphological differences were observed to be confusing and marginal, between the two genera *Ammannia* and *Nesaea* which lead to the merging of genus *Nesaea* with *Ammannia.* The present morphological study also supported the distant relationship of the genus *Rotala* with newly merged genus *Ammannia*. Seed morphology also play a crucial role in determining species identity, as in the case of *Rotala kasaragodensis,* since most of floral characters were often found to be overlapping and confusing with that of *R. occultiflora*. But seed surface pattern was observed to be highly useful in differentiating *R. kasaragodensis* from *R. occultiflora*.

Various anatomical characters, related to stem, midrib and cuticular features found to be more significant for characterizing taxa within the

species level. However anatomical interpretation showed some incongruence in the relationship among the species when compared to that related to morphological data interpretation. Different anatomical features can be used as key characters in order to distinguish taxa at species level. But the assessment of variation of these characters at generic level was inferred to be very difficult especially in distinguishing *Ammannia* from *Nesaea.* Some of the species delimiting characters such as presence or absence of sclerenchyma in the midrib cross section, nature of hypodermis in stem cross section and pattern of distribution of druses in stem cross section were observed to be employed as diagnostic characters for at the species level.

The present molecular diversity study is the first molecular analysis regarding the South Indian species of these herbaceous genera and also included largest number of species of *Rotala* worldwide. Molecular data based on ITS, *rbc*L and *trn*L− F regions were analyzed and sequences generated for 19 species corresponding to three regions were deposited in NCBI. Of the 57 sequences, 37 sequences have been assigned GenBank accession numbers. Phylogenetic analysis revealed congruence with morphological analysis in the generic level. Both exhibited distant relationship of *Rotala* with *Ammannia*/ *Nesaea* clade and a close relationship of *Nesaea* and *Ammannia* with high sequence similarity and leads to assign both genera under the generic name *Ammannia*. The present phylogenetic analysis also facilitated to confirm the distinctiveness of two recently described species, *R. cheruchakkiensis* and *R. tulunadensis*. There is congruence with morphological analysis at the specific level except for *R. malampuzhensis, R. cheruchakkiensis, R. malabarica, R. densiflora* and *R. rosea.* The molecular data also helped to assess link between two the centres of diversity for these genera, Indian sub continent and North Eastern Africa. The present data revealed that the same species which are dispersed in two disjunctive centers showed diversification whereas the same species or even

very closely related species in the same area showed close evolutionary relationship.

While taking into account of three analyses, morphological and molecular data exhibit more congruence than anatomical evidences. All the three analysis proved existence of two genera *viz*., *Ammannia* and *Rotala.* The genus *Nesaea* is reduced into the synonymy of *Ammannia*. This supports the work of Graham and Gandhi (2013), in which they presented congeneric status of *Ammannia* and *Nesaea* by conducting nomenclature transfer of a number of African species of *Nesaea* to *Ammannia*. Based on the outcome of the combined molecular, anatomical and morphological analysis, a key based on morphological characters was provided to distinguish South Indian Species of two genera. For further molecular analysis at specific level, assessment of more sequence regions is suggested using additional universal primers.

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