Molecular Systematics of the Genus *Hygrophila* R.Br. (Acanthaceae) in India

Molecular Systematics of the Genus *Hygrophila* R.Br. (Acanthaceae) in India

Thesis submitted to
the University of Calicut in partial fulfilment of
the requirement for the degree of

DOCTOR OF PHILOSOPHY IN BOTANY

By

JASEELA V.T.

Research Supervisor
Dr. N.S. Pradeep

KSCSTE-Malabar Botanical Garden & Institute for Plant Sciences
Kozhikode, Kerala, India
2024

KSCSTE-MBGIPS

KSCSTE - MALABAR BOTANICAL GARDEN AND INSTITUTE FOR PLANT SCIENCES

(An Institution under Kerala State Council for Science, Technology & Environment)

GA College P.O, Kozhikode-673014, Kerala, India. Phone: +91 495 2430939

Email: malabarbot.garden@gmail.com, www.mbgips.in

CERTIFICATE

This is to certify that the thesis entitled "**Molecular Systematics of the Genus *Hygrophila* R.Br. (Acanthaceae) in India**" submitted to the University of Calicut by **Ms. Jaseela V.T.**, in partial fulfilment for the award of the degree of Doctor of Philosophy in Botany is a bonafide record of the research work carried out by her under my supervision and guidance. No part of the present work has previously formed the basis for the award of any other degree or diploma.

Kozhikode

Dr. N. S. Pradeep
Research Supervisor

DECLARATION

I hereby declare that the work presented in the thesis entitled "**Molecular Systematics of the Genus *Hygrophila* R.Br. (Acanthaceae) in India**" is based on the original work done by me under the guidance of **Dr. N.S. Pradeep** and has not been included in any other thesis submitted previously for the award of any degree. The contents of the thesis are undergone plagiarism check using iThenticate software at C.H.M.K. Library, University of Calicut, and the similarity index found within the permissible limit. I also declare that the thesis is free from AI generated contents.

Jaseela V.T.

Dr. N.S. Pradeep
Research Supervisor

Place: Kozhikode
Date:

ACKNOWLEDGEMENTS

First and foremost, I thank and praise the almighty God for his showers of blessings.

The successful completion of this research work would not have been possible without the support and assistance of many wonderful individuals. I would like to take this moment to acknowledge them and express my heartfelt gratitude for their help throughout my Ph.D. journey.

I am extremely grateful to my mentor Dr. N.S. Pradeep, Principal Scientist, KSCSTE - Malabar Botanical Garden and Institute for Plant Sciences, for his expert guidance, valuable suggestions and helpful criticisms.

I express my gratitude Dr. S. Pradeep Kumar, former Director in-charge, KSCSTE-Malabar Botanical Garden and Institute for Plant Sciences, for providing all the facilities and infrastructure to carry out this work.

I am indebted to Prof. P.V. Madhusoodanan and Dr. P.N. Krishnan, former Emeritus Scientists, and Prof. M. Sabu, Emeritus Scientist, KSCSTE-MBGIPS, for their valuable suggestions and unfailing encouragement.

I owe my sincere gratitude to Dr. Kanchi N. Gandhi, Senior Nomenclature Registrar, Harvard University Herbaria, Dr. A.K. Pradeep, Assistant Professor, University of Calicut. Dr. Gopal Krishna, Scientist, Botanical Survey of India, Kolkata and Dr. K.N. Nair, Ex-Chief Scientist, Plant Diversity, Systematics & Herbarium, CSIR-NBRI for their helpful suggestions in solving nomenclature and taxonomic problems during the course of this work. No words are adequate to express my gratitude to Prof. Arun K Pandey, Pro-chancellor, Mansarovar Global university, Madhya Pradesh and Dr. Mayank Dwivedi, Assitant Professor, Gurunanak Dev University, Punjab, for their guidance and help in the analysis of molecular data.

I express my gratitude to the Director, Botanical Survey of India (CAL) and Scientist-in-charges at various Botanical Survey of India (BSI) and Institutional herbaria for their kind help during my field visits, herbarium consultation and help in arranging stay facilities during field trips.

I wish to express my sincere gratitude to the Kerala Forest Department officials for allowing me to conduct field studies in reserve forests and protected areas.

I would also like to acknowledge the Council of Scientific and Industrial Research (CSIR), New Delhi, for providing financial assistance through Junior and Senior Research Fellowships.

I convey my immense sense of gratitude to Dr. M. M. Sardesai, Savitribai Phule Pune University; Dr. Mayur Nandikar, Scientist, Apt Research Foundation, Pune; Dr. Sujana K. A., Scientist, Southern Regional Centre Coimbatore; Dr. Deepu

Vijayan, Scientist, Central Botanical Laboratory Howrah; Dr. Vivek, BSI headquarters, Kolkata; Dr. Neelima, Bot. Asst. Central Regional Centre, Allahabad, Dr. R. Saravanan, Botanist, A J C Bose Indian Botanic Garden, Howrah; Sri Akshath Shenoy, Senior Preservation Assistant, Arunachal Pradesh Regional Centre; Dr. K M Prabhukumar, Senior Scientist, CSIR-National Botanical Research Institute, for their invaluable support, guidance, and assistance during my studies.

I owe my gratitude to Dr. V. Balakrishnan, Member Secretary of Kerala State Biodiversity Board; Dr E. S. Santhosh Kumar, Technical Officer, JNTBGRI; Dr. M.K. Ratheesh Narayanan, Assistant Professor, Payyanur College; Dr. C.N. Sunil, Retd. Associate Professor & Research Guide, SNM College, Maliankara; Dr. P.S. Udayan, Retd. Assistant Professor, Sree Krishna College, Guruvayoor, for their help, encouragement and valuable suggestions throughout the study.

I gratefully acknowledge Dr. Dhatchanamoorthy., Assistant Professor, TDU, Bengaluru; Salim P. M., Senior Technical Assistant and Satheesh, Staff., MSSRF, Puthoorvayal, for their invaluable assistance and help in plant collection. My deep sense of acknowledgement is also extended to Dr. Lakshmi Edison, Postdoctoral Associate, University of Florida, for her help in various ways.

I would also like to thank Dr. V.S. Hareesh, Dr. K.M. Manjula, and Dr. B. Ragupathi, Junior Scientists, KSCSTE-MBGIPS, for their help and support during my studies.

I am also thankful to my seniors, colleagues and friends, Dr. Anoop P. Balan, Dr. R.S. Neethu, Dr. Saranya Babu Jayaprakash, Dr. Sweta Tilak, Dr. Pavisha P., Dr. Rejilesh, Dr. Prajitha, Dr. Anoop K.P., Dr. Sojan Jose, Ms. Athulya Sadanand, Ms. Sinisha E.K., Mr. Bibin Joseph, Ms. Pooja Pushkaran, Mr. Mithun Venugoplal & Mr. Harshid P. for their kind support and help when needed.

I thank the field guides and technical Assistants at MBGIPS for their help during my collection trips and preparation of the herbarium. Thanks, are also due to the administrative and technical staff of MBGIPS for their help and support.

I express my deep sense of gratitude to my beloved fellow friends Dr. Dilsha, St. Joseph college, Devagiri; Dr. Jagdidh Dalavi and Dr. Rohit, Dept. of Botany, Shivaji University, Kolhapur; and Ms. Krishnapriya, Research Scholar, University of Calicut for their help and encouragement.

I am much indebted for the support and encouragement rendered by my family members, friends, my husband Faisal, who inspired me to realize my dreams and my kids Ameen & Ayan, who spent the best part of her childhood supporting my research. I would like to record my sincere thanks to all my teachers for their blessings and encouragement.

Jaseela VT

ABSTRACT

Hygrophila R.Br., a prominent genus within the tribe Ruellieae of the Acanthaceae family, is known for its diverse aquatic and semi-aquatic species, predominantly found in the Old-World tropics. Despite its ecological significance, the genus has been under-explored, particularly within India, where no comprehensive taxonomic studies have been conducted since the Flora of British India. This work addresses the taxonomic ambiguities and evolutionary relationships within the Indian species of *Hygrophila* through an integrative approach that combines morphological and molecular analyses.

Consultation of major herbaria and literature survey recorded 15 species of *Hygrophila* in India. Extensive fieldwork across India facilitated the collection and identification of 12 *Hygrophila* species. The 3 species of *Hygrophila* (2 in Flora of British India and 1 from Little Andaman) were documented only once from their type localities and have not been reported since, making it impossible for us to collect them. Detailed descriptions, phenological data, distribution maps, and taxonomic keys for Indian *Hygrophila* species are provided. The research revealed a high species diversity in Peninsular India, with approximately 40% endemism. The study reported the extended distribution of *Hygrophila phlomoides* Nees from South India. Notably, the study reinstated *Hygrophila phlomoides* Nees and clarified the nomenclature and identity of several species.

Morphological studies were complemented by molecular analyses using nuclear (ITS) and chloroplast (rbcL) genomes. This work represents the first molecular phylogenetic analysis of *Hygrophila* in India, contributing significantly to the global understanding of the genus. The phylogenetic data, supported by high bootstrap values, highlighted the non-monophyly of *Hygrophila*, suggesting the inclusion of the genus *Brillantaisia* P.Beauv. Based on this analysis, four species of *Brillantaisia* are proposed to be transferred to *Hygrophila*. These findings necessitate further global sampling to resolve taxonomic complexities within the genus.

This comprehensive treatment of *Hygrophila* enhances the documentation of India's plant diversity and paves the way for future research in phytochemistry, pharmacology, and conservation. The study's outcomes, including submitting DNA sequences to GenBank and developing an optimized DNA extraction protocol, provide a foundation for subsequent systematic and evolutionary studies in Acanthaceae.

Keywords: *Hygrophila,* Phylogeny, ITS, rbcL, Taxonomy

സംഗ്രഹം

അക്കാന്തേസിയെ എന്ന സസ്യ കുടുംബത്തിലെ റുഏലിയെ ഗോത്രത്തിലെ ഒരു പ്രമുഖ ജനുസ്സാണ് ഹൈഗ്രോഫില R.Br. ഈ വിഭാഗത്തിലെ വൈവിധ്യമാർന്ന ജല, അർദ്ധജല - സ്പീഷീസുകൾ പേരുകേട്ടതാണ്. ഇവ പ്രധാനമായും പഴയ ലോക ഉഷ്ണമേഖലാ പ്രദേശങ്ങളിൽ കാണപ്പെടുന്നപാരിസ്ഥിതിക പ്രാധാന്യം ഉണ്ടായിരുന്നിട്ടും ., ഈ ജനുസ്സ് പര്യവേക്ഷണം ചെയ്യപ്പെട്ടിട്ടില്ല, പ്രത്യേകിച്ച് ഇന്ത്യയ്ക്കുള്ളിൽ, ബ്രിട്ടീഷ് ഇന്ത്യയിലെ സസ്യ വിവരണങ്ങൾക്കുശേഷം ശേഷം സമഗ്രമായ സസ്യ വർഗ്ഗീകരണ പഠനങ്ങളൊന്നും ഈ ജനുസ്സിൽ നടന്നിട്ടില്ലരൂപഘടനകളും തന്മാത്രാ വിശകലനങ്ങളും സമന്വയിപ്പിക്കുന്ന ഒരു . ടെ ഹൈഗ്രോഫിലയിലെ ഇന്ത്യസംയോജിത സമീപനത്തിലൂൻ സ്പീഷീ സുകളിലെ വർഗ്ഗീകരണ അവ്യക്തതകളെയും പരിണാമ ബന്ധങ്ങളെയും ഈ പഠനം അഭിസംബോധന ചെയ്യുന്നു.

.ഇനം ഹൈഗ്രോഫില സ്പീഷീസുകളാണ് ഇന്ത്യയിൽ രേഖപ്പെടുത്തിയിട്ടുണ്ടായിരുന്നത് 15 ടെ ഹൈഗ്രോഫില സ്പീഷീസുകളു 12 ഇന്ത്യയിലുടനീളമുള്ള വിപുലമായ ഫീൽഡ് വർക്ക് ശേഖരണത്തിനും തിരിച്ചറ◌ിയലിനും വഴിതുറന്നുബ്രിട്ടീഷ്) ഇനം 3 ഹൈഗ്രോഫിലയുടെ . അവ കണ്ടെത്തിയ (ഉം 1 ഉം ലിറ്റിൽ ആൻഡമാനിൽ നിന്ന് 2 ഇന്ത്യയിലെ ഫ്ലോറയിൽ പ്രദേശങ്ങളിൽ നിന്ന് മാത്രമേ രേഖപെടുത്തിയിട്ടുണ്ടായിരുന്നുള്ളൂ, അതിനുശേഷം അത് മറ്റാരുംതന്നെ കണ്ടതായി പരാമർശങ്ങൾ ഇല്ല. പ്രസ്തുത പ്രദേശങ്ങളിൽനിന്നോ മറ്റേതെങ്കിലും സ്ഥലത്തുനിന്നോ ഞങ്ങൾക്കും അവയെ കണ്ടെത്താനും കഴിഞ്ഞില്ല. മറ്റ് 12 ഹൈഗ്രോഫില സ്പീഷീസുകളുടെ വിശദമായ വിവരണങ്ങൾ, ഫിനോളജിക്കൽ ഡാറ്റ, വിതരണ ഭൂപടങ്ങൾ, ഇന്ത്യൻ ഹൈഗ്രോഫില സ്പീഷീസുകൾക്കുള്ള വർഗ്ഗീകരണ പട്ടിക എന്നിവ ഈ പഠനത്തിന്റെ ഭാഗമായി നൽകിയിട്ടുണ്ട്പെനിൻസുലർ ഇന്ത്യയിൽ ഉയർന്ന . സ്പീഷിസ് വൈവിധ്യവും, ഇന്ത്യയിൽ ഏകദേശം 40% എൻഡിമിസവും ഗവേഷണം രേഖപ്പെടുത്തിഡ്സ്സിൻ◌്റെ വിപുലമായി ദക്ഷിണേന്ത്യയിൽ ഹൈഗ്രോഫില ഫ്ലോമോയ് . കാണപെടുന്നതായി ഈ പഠനം വിലയിരുത്തി. ശ്രദ്ധേയമായത് ഹൈഗ്രോഫില ഫ്ലോമോയ്ഡസ് നീസിനെ പുനഃസ്ഥാപിക്കപ്പെട്ടതും മറ്റ് നിരവധി സ്പീഷീസുകളുടെ നാമകരണവും തിരിച്ചറിയലും സാധ്യമാക്കി എന്നതുമാണ് .

ന്യൂക്ലിയർ (ITS), ക്ലോറോപ്ലാസ്റ്റ് (rbcL) ജീനോം എന്നിവ ഉപയോഗിച്ചുള്ള തന്മാത്രാ വിശകലനങ്ങളും, രൂപഘടന അനുസരിച്ചുള്ള കണ്ടെത്തലുകളും പരസ്പര പൂരകങ്ങളായ

വർത്തിച്ചുഇത് ഹ .ൈഗ്രോഫില എന്ന ജനസ്സിലെ ഇന്ത്യയിലെ ആദ്യത്തെ തന്മാത്ര വിശകലനത്തോടെയുള്ള സംയോജിത പഠനമാണ്. ആഗോളതലത്തിൽ ഈ ജനസ്സിനെക്കുറിച്ചുള്ള വിവരങ്ങൾ പ്രദാനം ചെയ്യുന്നതിൽ ഈ പഠനം ഗണ്യമായ പങ്കുവഹിച്ചു.

ബൂട്ട്സ്ട്രാപ്പ് മൂല്യങ്ങൾ പിന്തുണയ്ക്കുന്ന ഫൈലോജെനെറ്റിക് ഡാറ്റ, ഹൈഗ്രോഫിലയുടെ നോൺമോണോഫൈലി അടിവരയിട്ടു-, ഇത് ബ്രില്ലാൻ്റൈസിയ P.Beauv. നെ ഹൈഗ്രോഫില എന്ന ജനസ്സിൽ ഉൾപ്പെടുത്താൻ നിർദ്ദേശിക്കുന്നുഈ വിശകലനത്തെ . ഗ്രോഫിലയിലേക്ക് മാറ്റാൻ അടിസ്ഥാനമാക്കി നാല് ഇനം ബ്രില്ലാൻ്റൈസിയയെ ഹൈ ഇത്തരം കണ്ടെത്തലുകളു .ഈ പഠനം ശുപാർശ ചെയ്യുന്നടെ ആധികാരികത ഉറപ്പിക്കുന്നതിനായി ആഗോള തലത്തിൽ പഠനങ്ങൾ ആവശ്യമാണ്

ഹൈഗ്രോഫിലയുടെ ഈ സമഗ്രമായ പഠനങ്ങൾ ഇന്ത്യയുടെ സസ്യ വൈവിധ്യത്തിൻ്റെ പ്രമാണീകരണ ആവശ്യകത വർദ്ധിപ്പിക്കുകയും ഫൈറ്റോകെമിസ്ട്രി, ഫാർമക്കോളജി, സസ്യ സംരക്ഷണം തുടങ്ങിയ മേഖലകളിലെ ഭാവി ഗവേഷണങ്ങൾക്ക് വഴിയൊരുക്കുകയും ചെയ്യുന്നഡിഎൻഎ സീക്വൻസുകൾ ജെൻബാങ്കിന് സമർപ്പിക്കപ്പെട്ടതും ഡിഎൻഎ . ട്രാക്ഷൻ പ്രോട്ടോക്കോൾ വികസിപ്പിച്ചതും ഉൾപ്പെടെയുള്ള പഠന ഫലങ്ങൾസ്എക്, അക്കാന്തേസിയെ എന്ന സസ്യ കുടുംബത്തിലെ തുടർന്നുള്ള ചിട്ടയായതും പരിണാമപരവുമായ പഠനങ്ങൾക്ക് അടിത്തറ നൽകുന്നു.

സൂചകപദങ്ങൾ: ഹൈഗ്രോഫില, ഫൈലോജനി, ഐടിഎസ്, ആർബിസിഎൽ, വർഗ്ഗീകരണം

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

%	:	Percentage
µg	:	Microgram
µmol	:	Micro molar
^{0}	:	Degree
^{0}C	:	Centigrade
A	:	Absorbance
AFLP	:	Amplified Fragment Length Polymorphism
AMOVA	:	Analysis of Molecular Variance
APG	:	Angiosperm phylogeny group
BI	:	Bayesian Inference
BLAST	:	Basic Local Alignment Search Tool
bp	:	Base pair
BS	:	Boot Strap
BSI	:	Botanical Survey of India
CO1	:	Cytochrome c oxidase I
cm	:	Centimetre
comb. nov.	:	combination nova (new combination)
comb. superfl.	:	Combination superfluum
CTAB	:	Cetyl trimethyl ammonium bromide
DNA	:	Deoxyribo Nucleic Acid
cp DNA	:	Chloroplast DNA
mt DNA	:	Mitochondrial DNA
EDTA	:	Ethylene diamine tetra acetic acid
et al.	:	et aliorum (and of others)
g	:	Gram
GPS	:	Global Positioning system
h	:	Hour
IPNI	:	International Plant Name Index
ISSR	:	Inter Simple Sequence Repeat
ITS	:	Internal Transcribed Spacer
IUCN	:	International Union for Conservation of Nature
L	:	Litre
l.c.	:	loco citato (at the place cited)

M	:	Molarity
matK	:	Maturase K
MBGIPS	:	Malabar Botanical Garden & Institute for Plant Sciences
mg	:	Milligram
min	:	Minute
ml	:	Millilitre
ML	:	Maximum Likelihood
mm	:	Millimetre
MP	:	Maximum Parsimony
N	:	Normality
NaCl	:	Sodium chloride
nadhF	:	NADH dehydrogenase F
NaOH	:	Sodium Hydroxide
NCBI	:	National Center for Biotechnology Information
nm	:	Nanometre
nom. illeg.	:	nomen illegitimum (illegitimate name)
nom. inval.	:	nomen invalidum (invalid name)
nom. nov.	:	nomen novum
nom. nud.	:	nomen nudum (naked name)
nom. superfl.	:	nomen superfluum (name superflous)
NW	:	New World
OD	:	Optical Density
OW	:	Old World
PCR	:	Polymerase Chain Reaction
POWO	:	Plants of the World Online
p.p	:	pro parte (in part)
QGIS	:	Quantum Geographic Information System
pH	:	Hydrogen potential
RAPD	:	Random Amplified Polymorphic DNA
rbcL	:	ribulose-bisphosphate carboxylase
RFLP	:	Restriction Fragment Length Polymorphism
RNA	:	Ribonucleic acid
rpm	:	Rotations per Minute
RUBISCO	:	ribulose bisphosphate carboxylase oxygenase
s	:	Second
s.c.	:	sine collector (without collector’s name)

s.d.	:	Sine data (without date)
s.n.	:	sine numero (without collection number)
s.l.	:	sensu lato (in broader sense)
s.s.	:	sensu strict (in strict sense)
SCARs	:	Sequence Characterised Amplified Regions
SNP	:	Single Nucleotide Polymorphism
SSR	:	Simple Sequence Repeats
STRs	:	Short Tandem Repeats
syn. nov.	:	synonymum novum (new synonym)
TAE	:	Tris-Acetate-EDTA
UPGMA	:	Unweighted Pair Group mMthod with Arithmetic mean
UV	:	Ultra violet

CONTENTS

ABSTRACT

Hygrophila R.Br., a prominent genus within the tribe Ruellieae of the Acanthaceae family, is known for its diverse aquatic and semi-aquatic species, predominantly found in the Old-World tropics. Despite its ecological significance, the genus has been under-explored, particularly within India, where no comprehensive taxonomic studies have been conducted since the Flora of British India. This work addresses the taxonomic ambiguities and evolutionary relationships within the Indian species of *Hygrophila* through an integrative approach that combines morphological and molecular analyses.

Consultation of major herbaria and literature survey recorded 15 species of *Hygrophila* in India. Extensive fieldwork across India facilitated the collection and identification of 12 *Hygrophila* species. The 3 species of *Hygrophila* (2 in Flora of British India and 1 from Little Andaman) were documented only once from their type localities and have not been reported since, making it impossible for us to collect them. Detailed descriptions, phenological data, distribution maps, and taxonomic keys for Indian *Hygrophila* species are provided. The research revealed a high species diversity in Peninsular India, with approximately 40% endemism. The study reported the extended distribution of *Hygrophila phlomoides* Nees from South India. Notably, the study reinstated *Hygrophila phlomoides* Nees and clarified the nomenclature and identity of several species.

Morphological studies were complemented by molecular analyses using nuclear (ITS) and chloroplast (rbcL) genomes. This work represents the first molecular phylogenetic analysis of *Hygrophila* in India, contributing significantly to the global understanding of the genus. The phylogenetic data, supported by high bootstrap values, highlighted the non-monophyly of *Hygrophila*, suggesting the inclusion of the genus *Brillantaisia* P.Beauv. Based on this analysis, four species of *Brillantaisia* are proposed to be transferred to *Hygrophila*. These findings necessitate further global sampling to resolve taxonomic complexities within the genus.

This comprehensive treatment of *Hygrophila* enhances the documentation of India's plant diversity and paves the way for future research in phytochemistry, pharmacology, and conservation. The study's outcomes, including submitting DNA sequences to GenBank and developing an optimized DNA extraction protocol, provide a foundation for subsequent systematic and evolutionary studies in Acanthaceae.

Keywords: *Hygrophila,* Phylogeny, ITS, rbcL, Taxonomy

സംഗ്രഹം

അക്കാന്തേസിയെ എന്ന സസ്യ കുടുംബത്തിലെ റുഏലിയെ ഗോത്രത്തിലെ ഒരു പ്രമുഖ ജനുസ്സാണ് ഹൈഗ്രോഫില R.Br. ഈ വിഭാഗത്തിലെ വൈവിധ്യമാർന്ന ജല, അർദ്ധജല - സ്പീഷീസുകൾ പേരുകേട്ടതാണ്. ഇവ പ്രധാനമായും പഴയ ലോക ഉഷ്ണമേഖലാ പ്രദേശങ്ങളിൽ കാണപ്പെടുന്നുപാരിസ്ഥിതിക പ്രാധാന്യം ഉണ്ടായിരുന്നിട്ടും ., ഈ ജനുസ്സ് പര്യവേക്ഷണം ചെയ്യപ്പെട്ടിട്ടില്ല, പ്രത്യേകിച്ച് ഇന്ത്യയ്ക്കുള്ളിൽ, ബ്രിട്ടീഷ് ഇന്ത്യയിലെ സസ്യ വിവരണങ്ങൾക്കുശേഷം ശേഷം സമഗ്രമായ സസ്യ വർഗ്ഗീകരണ പഠനങ്ങളൊന്നും ഈ ജനുസ്സിൽ നടന്നിട്ടില്ലരൂപഘടനകളും തന്മാത്രാ വിശകലനങ്ങളും സമന്വയിപ്പിക്കുന്ന ഒരു . ടെ ഹൈഗ്രോഫിലയിലെ ഇന്ത്യസംയോജിത സമീപനത്തിലൂൻ സ്പീഷീ സുകളിലെ വർഗ്ഗീകരണ അവ്യക്തതകളെയും പരിണാമ ബന്ധങ്ങളെയും ഈ പഠനം അഭിസംബോധന ചെയ്യുന്നു.

.ഇനം ഹൈഗ്രോഫില സ്പീഷീസുകളാണ് ഇന്ത്യയിൽ രേഖപ്പെടുത്തിയിട്ടുണ്ടായിരുന്നത് 15 ടെ ഹൈഗ്രോഫില സ്പീഷീസുകളു 12 ഇന്ത്യയിലുടനീളമുള്ള വിപുലമായ ഫീൽഡ് വർക്ക് ശേഖരണത്തിനും തിരിച്ചറിയലിനും വഴിതുറന്നുബ്രിട്ടീഷ്) ഇനം 3 ഹൈഗ്രോഫിലയുടെ . അവ കണ്ടെത്തിയ (ഉം 1 ഉം ലിറ്റിൽ ആൻഡമാനിൽ നിന്ന് 2 ഇന്ത്യയിലെ ഫ്ലോറയിൽ പ്രദേശങ്ങളിൽ നിന്ന് മാത്രമേ രേഖപെടുത്തിയിട്ടുണ്ടായിരുന്നുള്ളൂ, അതിനുശേഷം അത് മറ്റാരുംതന്നെ കണ്ടതായി പരാമർശങ്ങൾ ഇല്ല. പ്രസ്തുത പ്രദേശങ്ങളിൽനിന്നോ മറ്റേതെങ്കിലും സ്ഥലത്തുനിന്നോ ഞങ്ങൾക്കും അവയെ കണ്ടെത്താനും കഴിഞ്ഞില്ല. മറ്റ് 12 ഹൈഗ്രോഫില സ്പീഷീസുകളുടെ വിശദമായ വിവരണങ്ങൾ, ഫിനോളജിക്കൽ ഡാറ്റ, വിതരണ ഭൂപടങ്ങൾ, ഇന്ത്യൻ ഹൈഗ്രോഫില സ്പീഷീസുകൾക്കുള്ള വർഗ്ഗീകരണ പട്ടിക എന്നിവ ഈ പഠനത്തിന്റെ ഭാഗമായി നൽകിയിട്ടുണ്ട്പെനിൻസുലർ ഇന്ത്യയിൽ ഉയർന്ന . സ്പീഷിസ് വൈവിധ്യവും, ഇന്ത്യയിൽ ഏകദേശം 40% എൻഡിമിസവും ഗവേഷണം രേഖപ്പെടുത്തിഡ്സ്സിൻ്റെ വിപുലമായി ദക്ഷിണേന്ത്യയിൽ ഹൈഗ്രോഫില ഫ്ലോമോയ് . കാണപെടുന്നതായി ഈ പഠനം വിലയിരുത്തി. ശ്രദ്ധേയമായത് ഹൈഗ്രോഫില ഫ്ലോമോയ്ഡസ് നീസിനെ പുനഃസ്ഥാപിക്കപ്പെട്ടതും മറ്റ് നിരവധി സ്പീഷീസുകളുടെ നാമകരണവും തിരിച്ചറിയലും സാധ്യമാക്കി എന്നതുമാണ് .

ന്യൂക്ലിയർ (ITS), ക്ലോറോപ്ലാസ്റ്റ് (rbcL) ജീനോം എന്നിവ ഉപയോഗിച്ചുള്ള തന്മാത്രാ വിശകലനങ്ങളും, രൂപഘടന അനുസരിച്ചുള്ള കണ്ടെത്തലുകളും പരസ്പര പൂരകങ്ങളായ

വർത്തിച്ചുഇത് ഹ .ൈഗ്രോഫില എന്ന ജനസ്സിലെ ഇന്ത്യയിലെ ആദ്യത്തെ തന്മാത്ര വിശകലനത്തോടെയുള്ള സംയോജിത പഠനമാണ്. ആഗോളതലത്തിൽ ഈ ജനസ്സിനെക്കുറിച്ചുള്ള വിവരങ്ങൾ പ്രദാനം ചെയ്യുന്നതിൽ ഈ പഠനം ഗണ്യമായ പങ്കുവഹിച്ചു.

ബൂട്ട്സ്ട്രാപ്പ് മൂല്യങ്ങൾ പിന്തുണയ്ക്കുന്ന ഫൈലോജെനെറ്റിക് ഡാറ്റ, ഹൈഗ്രോഫിലയുടെ നോൺമോണോഫൈലി അടിവരയിട്ടു-, ഇത് ബ്രില്ലാൻ്റൈസിയ P.Beauv. നെ ഹൈഗ്രോഫില എന്ന ജനസ്സിൽ ഉൾപ്പെടുത്താൻ നിർദ്ദേശിക്കുന്നുഈ വിശകലനത്തെ . ഗ്രോഫിലയിലേക്ക് മാറ്റാൻ അടിസ്ഥാനമാക്കി നാല് ഇനം ബ്രില്ലാൻ്റൈസിയയെ ഹൈ ഇത്തരം കണ്ടെത്തലുകളു .ഈ പഠനം ശുപാർശ ചെയ്യുന്നുടെ ആധികാരികത ഉറപ്പിക്കുന്നതിനായി ആഗോള തലത്തിൽ പഠനങ്ങൾ ആവശ്യമാണ്

ഹൈഗ്രോഫിലയുടെ ഈ സമഗ്രമായ പഠനങ്ങൾ ഇന്ത്യയുടെ സസ്യ വൈവിധ്യത്തിൻ്റെ പ്രമാണീകരണ ആവശ്യകത വർദ്ധിപ്പിക്കുകയും ഫൈറ്റോകെമിസ്ട്രി, ഫാർമക്കോളജി, സസ്യ സംരക്ഷണം തുടങ്ങിയ മേഖലകളിലെ ഭാവി ഗവേഷണങ്ങൾക്ക് വഴിയൊരുക്കുകയും ചെയ്യുന്നുഡിഎൻഎ സീക്വൻസുകൾ ജെൻബാങ്കിന് സമർപ്പിക്കപ്പെട്ടതും ഡിഎൻഎ . ട്രാക്ഷൻ പ്രോട്ടോക്കോൾ വികസിപ്പിച്ചതും ഉൾപ്പെടെയുള്ള പഠന ഫലങ്ങൾസ്എക്, അക്കാന്തേസിയെ എന്ന സസ്യ കുടുംബത്തിലെ തുടർന്നുള്ള ചിട്ടയായതും പരിണാമപരവുമായ പഠനങ്ങൾക്ക് അടിത്തറ നൽകുന്നു.

സൂചകപദങ്ങൾ: ഹൈഗ്രോഫില, ഫൈലോജനി, ഐടിഎസ്, ആർബിസിഎൽ, വർഗ്ഗീകരണം

Chapter 1

INTRODUCTION

Life on Earth is the most striking feature of the Earth. Biodiversity refers to the variety and variability of life on Earth, encompassing the diversity found within ecosystems, species, and genetic makeup. It is essential for maintaining the functioning of ecosystems and providing ecosystem services that are critical for human well-being. Human civilization has created a biodiversity crisis that has resulted in a drastic reduction and extinction of biodiversity due to habitat destruction, pollution, and overexploitation. This escalating threat to bio diversity calls for an urgent need for taxonomy. Taxonomy offers a fundamental perception of biodiversity's elements, essential for making informed decisions on conservation and sustainable use.

Taxonomy plays a critical role in documenting, understanding, and managing the biodiversity by providing a system for naming, describing, and classifying organisms, allowing scientists to identify and track species and their relationships to one another. By identifying and classifying species, researchers can better understand their ecological roles, as well as the threats and challenges they face. This information is critical for developing effective conservation strategies and for monitoring the health and status of ecosystems. The continued development and advancement of taxonomy are essential for supporting ongoing efforts to conserve and protect biodiversity, as well as for advancing our understanding of the natural world. In addition, it is essential to comprehend the evolutionary relationships between various groups of organisms. By studying the similarities and differences between species, researchers can gain insights into the evolutionary history of life on Earth and the processes that have led to the development of the different forms of life we see today.

The diversity of plants is important as plants are essential for living organisms on the earth. The intimate relationship between plants and humans has evolved over generations of experience and practices. There has been a parallel between plants and humans in so far as the evolutionary aspect is concerned. The rate at which plant species are turning extinct is frightening. The extinction of just one plant species can cause the loss of species that are linked to it, as ecosystems are finely balanced and angiosperms often have strong symbiotic ties with animals. The fact that numerous plant species remain unidentified and thus unaccounted within the ecosystem is a serious and urgent concern. Before we have a chance to even start to comprehend the potential effects of their disappearance, they might go extinct. In order to conserve these, identification and classification become necessary, which can necessitate the need for taxonomy and taxonomists.

Taxonomic research has been revolutionized in many ways by recent technological advances and their impact on scientific research. Plant systematics aims to reconstruct the evolutionary history of plant life but it also includes and encompasses traditional taxonomy. It divides plants into taxonomic groups, using morphological, anatomical, micromorphological, and molecular data. Modern taxonomists have ascertained the dearth of morphological characters to provide the genetic and evolutionary relationship between taxa in many cases.

Taxonomy

Taxonomy is the science dealing with the study of classification, principles, rules, and procedures. The term 'Taxonomy' is derived from the Greek *taxis* meaning '*arrangement*' and '*nomos'* meaning '*law*'. It is a fascinating multifaceted subject that acts as a foundation upon which biology is built and is the base of all branches of biology. It provides the primary basic essential requirement - the correct identity of the organism.

Modern taxonomy emerged with the arrival of Carl Linnaeus through his *Species Plantarum* (Linnaeus, 1753). Classical taxonomic work relied heavily on gross morphological data but modern taxonomists have shown that gross morphological characters cannot provide genetic and evolutionary relationships between taxa. Evidence from anatomy, biochemistry, cytology, ecology, embryology, palaeobotany, palynology, and very recently macromolecules helped trace this relationship of taxa.

In the past, classification systems for plants were typically produced by individual botanists or small groups, resulting in a number of diverse classification systems, especially with respect to different geographical regions. However, the landscape has changed in the last two decades with significant advancements in molecular phylogenetic studies, greatly enhancing our understanding of angiosperm evolution. Fossil records suggest that seed plants first appeared between 130-135 million years ago, during the early Cretaceous period. Similarly, the Angiosperm Phylogenetic Group (APG, 1998; 2003; 2009; 2016) has played a crucial role by consolidating data from various sources to give a broad definition of the classification of angiosperm. In the latest classification (APG IV), angiosperms are categorized into 64 orders and 416 families, providing a comprehensive overview of their taxonomy.

The family Acanthaceae (Asteridae; Lamiales) includes about 4000 species (Tripp *et al*., 2022) and encompasses a wide range of morphological forms, habitats, and biogeographic distributions. Despite being crucial elements of tropical and subtropical environments, comparative research has been impeded by inadequate comprehension of the evolutionary structure underlying the family. Though it has a practically global range and a high species richness, it is thought to have one of the lowest ratios of "scholars to species" actively working in the family (Tripp & Darbyshire, 2017). The circumscriptions of Acanthaceae have changed significantly over time and remains a topic of debate. This discrepancy made Acanthaceae one of the worst offenders in synthesizing remaining knowledge gaps for all angiosperms, making it difficult to reach all of the "Targets" outlined in the Global Strategy for Plant Conservation (Paton *et al*., 2008). The only way to reduce this deficiency is to pursue research on the taxonomy and phylogeny of the family. Keeping this goal in mind, present study was undertaken to resolve the taxonomic controversies and infer the relationships of Indian *Hygrophila* R.Br. of Acanthaceae, as taxonomy and relationships of the genus is not well understood.

Family Acanthaceae

Acanthaceae is one of the most diverse, widespread, and variable families of flowering plants in terms of taxonomy, geography, morphology, and ecology. Modern experts estimate there are over 4000 species globally, potentially exceeding 5000, making Acanthaceae one of the top 12 most diverse families of flowering plants (McDade *et al*., 2008; Tripp *et al*., 2022). A key feature of this diversity is its remarkable morphological variation, particularly in terms of flower forms, growth forms, and pollen types.

Acanthaceae, classified under the order Lamiales, is part of the Euasterids I group within the Core Eudicots (Chase & Reveal, 2009). This large, cosmopolitan family is primarily found in tropical and subtropical regions of globe, with a few species in temperate areas. Recent estimates (Karthikeyan *et al*., 2009) indicate that India hosts 593 Acanthaceae taxa, comprising 475 species and 118 varieties.

The family Acanthaceae was established by A.L. de Jussieu in 1789. The name of the family comes from '*acantho'*, which means '*thorn or spine-bearin*g' plants, although many genera lack spines. According to Mabberley (2008), the family Acanthaceae (including Avicenniaceae, Mendonciaceae, excluding Thomandersiaceae) consists of 212 genera and 3175 species, predominantly found in tropical regions, extending to open areas and deserts, and extend to Mediterranean zones, U.S. and Australia. The family Acanthaceae is studied and classified for its entire geographical region by Nees (1847), Bentham (1876), Lindau (1895), Bremekamp (1965) and Scotland and Vollesen (2000).

The plants of this family are mainly centred in Asia, Africa, Brazil, Central America, and Indo-Malaya. These plants are plentiful in plains and at high elevations in the mountains across central, southern, northern, eastern, and western India. They thrive in moist and shady environments, often in wastelands and grassy areas within forests. This family includes several genera unique to India, particularly its southern regions, the Malayan Peninsula, and the Indian Archipelago.

The majority of the members of the family are herbs or shrubs; while there are also twinning vines and trees. The family is represented by simple leaves arranged oppositely, bisexual flowers with bilateral symmetry, four to five petals and sepals that frequently form a tubular structure, two to four stamens and seeds born supported with retinacula in two-chambered capsules. The members of the family have remarkable diversity in the floral characteristics, pollen and seed sculpturing patterns, which are valuably used in delimiting the genera (Scotland and Vollesen 2000). With its beautiful flowers and ornamentation, the family is primarily known for horticulture interest and most of such species belong to the genera *Barleria* L., *Eranthemum* L., *Strobilanthes* Blume, *Ruellia* L., *Thunbergia* Retz., *Acanthus* L., etc. In addition, several species exhibit great medicinal value viz, *Adhatoda* Mill., *Andrographis* Wall. ex Nees, *Asystasia* Blume, *Barleria* L., *Hygrophila R.Br.*, *Justicia* L., etc.

The family can be identified by noticeable cystoliths, which appear as small, typically elongated calcium carbonate and oxalate concretions on the upper surfaces of leaves, young stems, inflorescence branches, and calyx when viewed under a hand lens. Other distinguishing features include the presence and structure of floral bracts and bracteoles, a bilabiate corolla with a bilocular ovary, generally bivalvate capsules that dehisce elastically, and usually curved retinacula supporting the seeds. The anthers and stamens have many traits that help distinguish different genera. In most genera, seeds are often forcefully ejected by jaculators on the funicle (retinacula) when the mature capsules dry or are moistened by rain. In opened capsules, the jaculators are visible as hook-shaped, apically forked projections. The members of this family inhabit a wide range of ecological environments, including many hygrophilous and xeromorphic species with spiny or needle-like leaves, lianas, and even mangrove plants (though arborescent forms are rare). Majority of the species are shrubs or herbs with opposite, typically decussate, entire, exstipulate leaves.

Tribe Ruellieae

The tribe *Ruellieae* was established by Nees (1847) in DC. *Prodromus systematis naturalis: regni vegetabilis*. Ruellieae consists of a diverse group of morphological, ecological, and geographical species. Species of the group inhabit a wide range of tropical and subtropical environments, covering rain forests to dry forests, arid shrublands, deserts and certain temperate habitats. In the New World (NW), centres of species richness include Mexico, Brazil, and Peru, whereas in the Old World (OW), places with a high species diversity include Tanzania, Madagascar, and southern to southeast Asia. Some Ruellieae are restricted endemics, whereas others are cosmopolitan. Most plant species are either herbs or woody shrubs, though some are also lianas or trees.

Ruellieae plants are among the dominating species and a crucial food source for herbivorous megafauna in certain African regions. In contrast, Ruellieae typically do not have such high population densities in the NW, despite the fact that their appearance in an extensive range of tropical and subtropical NW habitats, indicating their ecological importance. Many Ruellieae play significant roles in horticulture and are frequently used as street trees or living fences. Numerous species are also well-known for their role in regional folklore and medicine.

Given that derived characters shared by Ruellieae but absent in other Acanthaceae have been considered, a unanimity concerning conclusive synapomorphies has not yet been formed. This is largely because not all members of the tribe have been thoroughly studied. Aestivation of the left-contorted corolla, seeds with mucilaginous hygroscopic trichomes, the presence of a filament curtain, uneven stigma lobes, or other combinations of these characteristics has all been proposed as potential synapomorphies for the Ruellieae (Scotland *et al*., 1995; Manktelow, 2000; Manktelow *et al*., 2001; Moylan *et al*., 2004; Tripp, 2007). While some of these traits have been thoroughly investigated across several genera, other aspects are little understood, and character-state data for the entire Ruellieae have not been compiled.

Within Ruellieae, genera have been classified and organized into subgroups primarily based on pollen type (Scotland, 1992; Furness, 1994; Daniel, 1998; Tripp, 2007). Other factors include corolla limb symmetry (Bentham, 1876), the presence of style-retaining trichomes inside corollas (Bremekamp, 1944), the number of stamens (Nees, 1832), the presence or absence of anther appendages (Nees, 1832), the number of ovules (Anderson, 1867; Wood, 1994), projections on nectaries (Bremekamp, 1938), and trichome differences (Clarke, 1908). Pollen morphology has been particularly emphasized since Lindau's work (1893, 1895) across Acanthaceae. Scotland's (1993) comprehensive study of pollen variation within Ruellieae, featuring pollen from 24 genera, is notable. Additional studies have provided valuable pollen images from other Ruellieae, including works by Furness (1994), Furness and Grant (1996), Daniel (1998), Scotland and Vollesen (2000), Moylan and Scotland (2000), Vollesen (2006), Tripp (2007), Tripp *et al*. (2009) etc.

Nees (1847) documented 36 genera in his Ruellieae, with eight retained by Scotland and Vollesen (2000) in their Ruelliinae; the remaining genera are now considered synonyms or are classified in other tribes. Bentham (1876) identified 35 genera in his Ruellieae, with 21 retained by Scotland and Vollesen (2000). Lindau (1895) recognized 47 genera in his Contortae, with 27 retained by Scotland and Vollesen (2000). Bremekamp (1965) also treated many of the same genera as Lindau (1895) in his Ruellieae; with the remaining currently recognized elsewhere or considered synonyms. However, as Bremekamp and Nannenga-Bremekamp (1948) earlier acknowledged, ''as a large part of the genera belonging to the Ruellieae are still imperfectly known, a fully satisfactory subdivision of this tribe cannot yet be given''.

Genus *Hygrophila*

The genus *Hygrophila* R.Br. commonly known as '*swamp weeds*', is a member of the tribe Ruellieae of the family Acanthaceae. The species of the genus are aquatic or semiaquatic in nature. The genus comprises about 80 species (POWO, 2024), mainly distributed in tropical and subtropical regions of the world (Hu & Daniel, 2011). The species of genus primarily belong to the Old World but there are several taxa in the New World also. In India, the genus is represented by 16 species and 1 variety (Arisdason *et al*., 2020). It is one of the only two genera in the family

Acanthaceae that contains aquatic plants, the other being *Justicia* L. The genus name is derived from the Greek words *'hygro'*, meaning *'moist* or *wet*,' and *'phil*,' meaning *'loving*,' highlighting the species' preference for wet habitats.

Hygrophila is a complex genus with a broad taxonomic conception (Heine 1962), including *Asteracantha* Nees, *Cardanthera* Buch.-Ham. ex Voigt, *Hemiadelphis* Nees, *Synnema* Benth. and *Adenosma* Nees. This view was also supported by Cramer (1989), who considered *Hygrophila* complex as a natural ecological group. High morphological variability and overlapping characteristics of the taxa resulted in a number of closely related species. Many authors had a broad view and described many taxa based on mere variability in vegetative characters, while some others with a narrower view considered many of the names as synonyms. The generic circumscription and the specific status of *Hygrophila* has been a longstanding controversy.

Relevance of the Study

Hygrophila, the aquatic plant genus of the family Acanthaceae, remains a rather poorly studied genus. The taxonomy of the genus *Hygrophila* is controversial due to the variability in vegetative characters, wide distribution, and almost uniform floral characters. Most of the delimitations in *Hygrophila* were purely based on morphological characters, and the genus has considerable taxonomic confusion. Many taxonomists also raised confusion regarding the correct identity of the species in this genus. Due to the existing nomenclatural disputes about the circumscription of the genus, it is noted as being in need of revision at the genus level (Cook, 1996). Currently, the taxonomy of some species of *Hygrophila* is in a fluctuating state, and none has been so far attempted the study of the genus holistically after Clarke's (1885) treatment in Flora of British India. The great diversity, the incomplete taxonomic understanding, and the rarity of some taxa necessitated a study of the family *Hygrophila* in India. Molecular techniques together with taxonomy can overcome the issues in the genus *Hygrophila*. So here we aim to delimit and unravel the complexities in the genus by morphological and molecular phylogenetic analysis.

Objectives of the Study

1. Survey and collection of *Hygrophila* spp. from India
2. Identification of *Hygrophila* spp. using evident morphological characters
3. Molecular characterization of the genus *Hygrophila* of India
4. Analysis of morphological and molecular data and elucidation of phylogenetic relationships of *Hygrophila* spp. and related taxa
5. Establishment of live germplasm of *Hygrophila* at MBGIPS

Chapter 2

AREA OF STUDY

India boasts a rich heritage of natural diversity. It ranks fourth in Asia and tenth globally among the world's 17 megadiverse countries. The present study area includes the entire political boundary of India which is represented by 28 states and 8 union territories. The landmass of India is situated north of the equator between 8^0 4′ to 37^0 6′ N latitude and 68^0 7′ to 97^0 25′ E longitudes. The country occupies the largest Peninsula of Asia and has a total area of 3287263 km^2, a land frontier of about 15200 km, and a coastline of 7517 km t (Murthy *et al.*, 1996). The Indian mainland stretches from Kashmir in the north to Kanyakumari in the south (3214 km) and from Arunachal Pradesh in the east to Gujarat in the west (2933 km). Southern India lies within the Tropics, while the northern part falls in the sub-tropical or warm temperate zone, resulting in diverse landforms, climates, soil types, and natural vegetation. The northern side of India is bordered by Nepal, Bhutan, and China and North-West by Pakistan. North East is bordered by Myanmar and the Eastern face by Bangladesh. The Southern Peninsula extends into the Indian Ocean with the Bay of Bengal lying to the South-East and the Arabian Sea to the South-West.

Biogeographically, India is situated at the tri-junction of three realms: Afro-tropical, Indo-Malayan, and Palearctic (Udvardy, 1975). India's proximity to various distinct realms gives it a unique combination of biodiversity elements, making the country rich and unique in biological diversity.

Biogeography

India is divided into ten major biogeographic regions and 27 biogeographic provinces (Rodgers and Panwar, 1988), representing all the world's major ecosystems. Each of these regions encloses different ecosystems such as forests, grasslands, lakes, rivers, wetlands, mountains, and hills, each hosting specific plant and animal species (Fig. 01).

1. Trans Himalayan Zone

It is an extension of the Tibetan Plateau and includes the ranges immediately north of the Great Himalayan range, covering the districts of Ladakh and Kargil in Jammu and Kashmir, the Spiti Valley, Lingti plains (Lahaul Valley), Pooh tehsil in Himachal Pradesh, and small areas of the Nanda Devi range (Uttaranchal) and Kangchendzonga range (Sikkim). It comprises about 5.6 % of the country's landmass. The topography of this zone is irregular and intercepted by valleys and plateaus of various extents. This region mostly lies between 4,500 to 6,000 meters and is very cold and arid with sparse alpine steppe vegetation.

2. Himalayan Zone

The Himalayas are the youngest and highest mountain chains in the globe. Spanning up to 2400 km, the Himalayas have distinct biodiversity due to its high altitude, steep gradient, and rich temperate flora. It is among the richest zones in terms of species and habitat diversity. The Himalayas encompasses three biogeographical provinces: Northwest Himalayas, West Himalayas, Central Himalayas, and East Himalayas, comprising 6.4% of the country's landmass. Champian and Seth (1968) and Negi (1990) identified various forest types in the Himalayas: Sub-tropical semidesert forests, Montane sub-tropical forests, Montane wet temperate forests, Himalayan moist temperate forests, Himalayan dry temperate forests, Sub-alpine forests, Sub-alpine forests, and Dry alpine scrub forests.

3. Indian Desert Zone

The Great Indian Desert, or Thar Desert, lies to the northwest of the Aravalli hills and features undulating topography. It includes India's northwestern boundary and mainly covers the western and northwestern regions of Rajasthan, Southwestern Punjab, Haryana, and part of the Kachchh region of Gujarat in the southwest. It covers over 60% of the geographical area of Rajasthan and covers 6.8% of the country’s land mass. It has an arid climate, and the vegetation is scanty, mostly dominated by tree species. This zone is a highly fragile ecosystem and may lose its biodiversity richness rapidly.

4. Semi-Arid Zone

It is the transitional zone between the desert and the denser forests of the Western Ghats, covering 15.6 % of the country's landmass. This region is marked by discontinuous vegetation cover, open areas of bare soil, and a soil-water scarcity throughout the year. The natural vegetation chiefly consists of thorn-scrub forests, grasses, and bamboo. Some xerophytic herbs and a few ephemeral herbs are seen in this semi-arid tract.

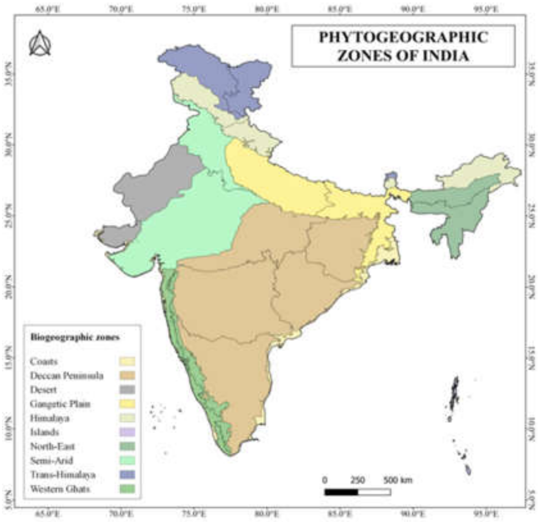


Fig. 1. Biogeographical zones of India

5. Western Ghats Zone

The Western Ghats or Sahyadri, is a narrow stretch of mountains along the west coast of Peninsular India, featuring an extremely diverse range of biotic provinces and biomes. It extends from the peninsula's southern tip (Kanyakumari in Tamil Nadu) northwards about 1600 to the mouth of the Tapti River Valley (Gujarat). It covers the states of Kerala, Tamil Nadu, Karnataka, Goa, Maharashtra, and Gujarat. This region is one of the 34 global biodiversity hotspots for conservation and one of the two on the Indian subcontinent, which covers about 5.8 % of the total land surface, of which one-third of the area is covered by forests (Vajravelu & Vivekananthan, 1996). About 4,500 species are found in the Western Ghats region. Out of the 17,000 angiosperms reported from India, 1720 (> one-third) species are endemic. The evergreen forest, semi-evergreen forest, moist deciduous forest, dry deciduous forest, dry scrub vegetation, shola forest, and grasslands are the major vegetation types of the Western Ghats. The varied climate and diverse topography create various habitats supporting unique plant and animal species.

6. Deccan Peninsula

The Deccan Peninsula, the largest part of Peninsular Plateau of India, lies in the rain shadow regions of the Western Ghats, covering around 43% of the country’s total land surface. The Deccan plateau extends south of the Satpura range to the southern tip of peninsular India. This region is surrounded by the western and the eastern ghats. It is typically semi-arid, being on the shielded side of both the Ghats, and is enclosed by Tropical thorn forests and Tropical dry and moist deciduous forests, spanning much of Maharashtra, Madhya Pradesh, Uttar Pradesh, Karnataka, Tamil Nadu, Andhra Pradesh, Orissa, and Bihar. Thorn scrub forest dominates much of this peninsula, interspersed with small regions of deciduous broadleaf forest. The Deccan plateau consists of three distinct physiographic subdivisions viz. North Deccan plateau: Maharashtra Plateau, South Deccan plateau, and East Deccan plateau. The Vindhya and Satpura hill ranges, Chhota Nagpur Plateau, Eastern Ghats, Tamil Nadu Plains, and Karnataka Plateau (Cherian, 2001) are the important biodiversity zones in these regions.

7. Gangetic Plain Zone

The Indo-Gangetic Plains, also known as the Great Plains, lies south of the Shiwalik and serve as a transitional zone between the Himalayas of the north and Peninsular India of the south. These are huge alluvial plains formed by alluvial deposits of three rivers, the Indus, Ganges, and Brahmaputra and their tributaries. These plains lie parallel to the Himalayas, from Jammu and Kashmir in the west to Assam in the east, draining most of northern and eastern India. The Gangetic Plain extends up to the Himalayan foothills in the northern area and covers 11% of the country's land mass. This agriculturally productive region extends from eastern Rajasthan, Uttar Pradesh, Bihar, to West Bengal, with rich soil, ample water supply, and a favorable climate. The main diversity of this area is represented as secondary forest patches.

8. Northeast India Zone

This significant biogeographic zone represents a transition among the Indian, Indo-Malayan, and Indo-Chinese biogeographic regions (Rao, 1997). This region is also known as the biogeographic gateway for plant migration as it forms the meeting place of the Himalayan Mountains with that of Peninsular India. This is the easternmost region of the country, covering eight states —Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Tripura and Sikkim. This zone is one of the highly diverse regions with respect to richness of species and endemism, comprising about 5.2 % of the country's geographical area. The main vegetation types found here are tropical evergreen and semi-evergreen forests, tropical deciduous forests, tropical bamboo forests, tropical grasslands, subtropical evergreen and semi-evergreen forests, subtropical pine forests, temperate forests, swamp vegetation, and sub-alpine forests. The Northeastern Indian region is characterised by high rainfall and humidity. The elevation ranges from near sea level to 4,500 meters, and the region represents one of the richest botanical regions in the Indian subcontinent.

9. The Coastal Zone

This zone stretches from Gujarat to Kanyakumari (Cape Comorin) in the West, covering Tamil Nadu, Kerala, Karnataka, Maharashtra, and Gujarat. The eastern side of the coastal line ranges from Cape Comorin to Sundarbans, covering Tamil Nadu, Andhra Pradesh, Odisha, and West Bengal. The west coast is characterised by backwaters, and the east coast is characterised by widespread deltas of the rivers Godavari, Krishna, and Kaveri. Large coastal plains have fertile soils suitable for cultivating different crops. Estuarine tracts with Mangrove vegetation are another characteristic feature of this coast.

10. The Islands Zone

This includes Lakshadweep (Arabian Sea islands) and the Andaman and Nicobar group of Islands (Bay Islands). This zone has a highly diverse set of biomes comprising 0.03 % of India’s landmass. Lakshadweep, situated in the Arabian Sea, consists of twelve atolls, three reefs, and five submerged banks, nearly 35 islands and islets rich in flora and fauna. The vegetation and floristic components are almost the same due to similar soil, climate, and rainfall conditions. The Andaman and Nicobar Islands are larger and comprise 572 islands and islets situated in the southeastern part of the Bay of Bengal. The islands form two broad groups: the Andamans and the Nicobar. It features a dense forest cover and an equatorial type of climate due to its proximity to the equator. About 81 % of the region is covered by forests, and the major forest types consist of tropical wet evergreen, tropical semi-evergreen, tropical moist deciduous, and littoral & swamp forests.

Physiographically, India has six major geographical regions: the Northern Mountains or the Himalayan Mountains, the Peninsular Plateaus, the Indo-Gangetic Plains, the Thar Desert, the Coastal Plains, and the Islands.

India possesses 11% of the world's flora despite making up only 2.4% of the planet's land area. It holds a remarkable position in the list of the world's biodiversity hotspots. Because they are all under severe stress and have lost at least 70% of their original natural vegetation, biodiversity hotspots are important for conservation. The Andaman and Nicobar Islands, the Eastern Himalayas, the Indo-Burma area, and the Western Ghats are India's four biodiversity hotspots.

Forest

India is one of the top ten nations in the world for forest cover. Indian forests encompass 67. 83 million hectares representing 20. 64 % of the country's total land areas. Different types of forests are seen in the Indian sub-continent, ranging from arid zone forests to Himalayan temperate forests. Chiefly, there are six major groups of forests: moist tropical, dry tropical, montane sub-tropical, montane temperate, sub-alpine, and alpine. They are further split into 16 main types of forests. There are moist, deciduous, and wet evergreen forests in the eastern part of India, thorny and dry deciduous forests in the western part, dry and moist deciduous forests in the northern and central areas, and the southern part possesses both western and central characteristics.

Rivers

India's rivers can be divided into two main categories. 1.) The Extra-Peninsula rivers or Himalayan rivers comprise 19 major rivers, of which six belong to the Indus system, nine are part of the Ganges system and four are part of the Brahmaputra system. 2.) The Peninsular rivers are often more or less dry in summer and entirely fed by monsoon. They fall into two groups: a. The coastal rivers, which are comparatively small streams that draw off the western side of the Western Ghats, pass through the narrow plains, and flow into the Arabian Sea; b. The inland rivers, which include the west-flowing Narmada and Tapti and the east-flowing Mahanadi, Godavari, Krishna, and Cauvery (Murthy *et al*, 1996).

Soil

There are mainly eight types of soils recognized in India, which include 1. Laterite or lateritic soils, 2. Black cotton soils (Regur), 3. Red soils, 4. Alluvial soils, 5. Alkali soils and saline soils, 6. Peaty and other organic soils, 7. Desert soils, and 8. Scanty soils of mountains and hills (Venu *et al*., 1996). Laterite soils, typically found in tropical areas with heavy rainfall are generally reddish or yellowish-red and turn black on exposure to sun. They are seen largely in the Western Ghats, Eastern Ghats, Vindhya, Satpuras, and Malwa Plateau. The black soil, believed to be derived from the volcanic activity in the Deccan Plateau, mainly occupies the Deccan lava region of Maharashtra and Gujarat. Red soils are mainly found in the peninsular regions and are low in humus and other organic nutrients. Red soil, derived from the weathering of ancient crystalline and metamorphic rocks, is mainly found in the eastern peninsular regions. They are low in humus and other organic nutrients. Alluvial soil, the largest and the most important soil group in India, constitutes 80% of the total geographic area. It is made of silt that has been left behind by several tributaries of the Indus, the Ganges, and the Brahmaputra rivers and is seen in the Great Northern plains from Punjab to the Assam valley. Alkali and saline soils have a higher percentage of salts and are developed in areas with dry climatic conditions and a lack of proper drainage. Peaty soils, found in regions of heavy rainfall and high humidity, are characterised by excessive organic matter that accumulates in poorly decomposed conditions. Here, the vegetation growth is much less due to high amount of humus/dead organic matter, making the soil alkaline. Desert soils, seen under arid and semi-arid conditions, are salty, contain a good amount of alkalis, and are deposited mainly by wind. Mountain soils, found in the mountain areas of the nation, are characterized by little humus and are thus acidic.

Climate

The climate of India is greatly influenced by three mountain ranges viz. The great Himalayas are at the northeast, the Western Ghats are along the southwest parts, and the Eastern Ghats are along the southeast regions. The Himalayan Mountain ranges serve as a significant climatic barrier, preventing southwest monsoon winds from dumping their moisture as snow on the mountains farther north and as heavy rain along submontane regions north of the Gangetic plains. They also obstruct the direct entry of extreme continental winds from the Tibet Plateau into India. The Western Ghats flanks the Indian peninsula along the West Coast and the Eastern Ghats along the East Coast. These two mountain ranges ensure rain on their windward slopes (Murthy *et al*., 1996).

Two seasonal winds affect India's climate: the South West Monsoon and North-East Monsoon. The South West Monsoon (Summer monsoon) moves from the sea to land after crossing the Indian Ocean, whereas North-East Monsoon (Winter monsoon) travels from land to Sea. Though India is often referred to as a tropical country, half of it is in the tropics and half in the North Temperate Zone. But due to the influence of the Himalayas and monsoons, there is some unity in the climatic conditions. The climate can be generally defined as tropical monsoon type. The country has four seasons: 1. Summer or Pre-monsoon (March to May), 2. Monsoon (June to September), 3. Post monsoon (October to November) and 4. Winter (December to February).

Rainfall

India experiences uneven and irregular rainfall that differs across various locations and seasons. Based on rainfall, there are four major climatic areas. The West Coast, which lies in the foothills of the Western Ghats, West Bengal, and Assam experience the heaviest annual rainfall of over 200 cm; Maharashtra, Madhya Pradesh, and Bihar, which are situated along the Vindhyan mountains, receive 100–200 cm; and the South Coastal Plains, North Western Deccan, and Upper Gangetic plains, which receive 50–100 cm. The Ladakh plateau in Kashmir and the Rajasthan desert that stretches to Kutch comprise the arid zone, which only receives a mere 15 cm of rain a year.

Chapter 3

REVIEW OF LITERATURE

Taxonomy

Acanthaceae

Nees (1847), Bentham (1876), Lindau (1895), Bremekamp (1965), Scotland and Vollesen (2000), and Manzitto-Tripp *et al.* (2022) studied and classified the family Acanthaceae for its whole geographical region.

Bentham & Hooker (1876) included Acanthaceae under the order Personales along with seven other families, namely, Scrophulariaceae, Orobancheaceae, Lentibulariaceae, Bignoniaceae, Pedaliaceae, Columelliaceae and Gesneriaceae. Engler & Prantl (1897) included all the above families under the order Tubiflorae. Bessey (1915) considered Scrophulariaceae as a primitive family of Scrophulariales. He treated Acanthaceae as the most advanced family among the Scrophulariales and considered that the members of the family should have been derived from Bignoniaceae. Engler and Diels (1936) placed Acanthaceae in the order Tubiflorae, while Benson (1957) positioned it in the order Scrophulariales. Hutchinson (1969) considered Acanthaceae family as the most advanced and included it under the order Personales. Cronquist (1981) included it under the order Scrophulariales. Many botanists retain Acanthaceae as a distinct taxon and they consider that it is derived from Scrophulariaceae or stocks ancestral to them. According to the APG IV (2016) these taxa are all currently positioned in Lamiales.

Jussieu (1789), in his *Genera Plantarum*, gave the name '*Acanthi'* for Acanthaceae after separating it from its related families. He classified Acanthaceae into two based on number of stamens. The appellation 'Acanthaceae' was used for the first time by Robert Brown (1810). Nees von Essenbeck (1832, 1847) after an in-depth study on the basis of the presence or absence of retinacula, established two major divisions of Acanthaceae such as Anechmatacanthaceae and Echmatacanthaceae.

Nees (1832) conducted the first extensive treatment of the Indian Acanthaceae and later produced a monograph for the entire family (Nees, 1847). He classified the family into two suborders: Anechmatacantheae (retinacula absent) and Echmatacantheae (retinacula present). Anechmatacantheae includes Thunbergieae and Nelsonieae, while Echmatacantheae comprises Hygrophileae, Ruellieae, Barlerieae, Acantheae, Aphelandreae, Gendarusseae, Eranthemeae, Dicliptereae and Andrographideae.

Anderson (1867) worked on cataloguing of Indian species of Acanthaceae. His classification of the family differed from Nees (1847), leading him to divide Acanthaceae into three suborders: Thunbergideae, Ruellideae, and Acanthieae. Bentham & Hooker (1876) classified Acanthaceae into 5 tribes — Thunbergieae, Nelsonioideae, Mendoncioideae, Thunbergioideae and Acanthoideae— on the basis of characters like aestivation and form of the corolla, seed number and absence or presence of retinacula, with a special focus on anther morphology. Clarke (1884-85), in Hooker's Flora of British India, treated the family Acanthaceae following Bentham and Hooker's classification recognizing five tribes.

Lindau (1895) based his classification on pollen morphology, particularly for tribal delimitation, and on the basis of pollen morphology. He recognized four subfamilies: Nelsonioideae, Mendoncioideae, Thunbergioideae, and Acanthoideae. The Acanthoideae subfamily, which comprises about 95% of Acanthaceae species and is characterized by explosive fruits with retinacula, was further divided into two groups based on corolla aestivation patterns: Imbricatae and Contortae.

Bremekamp (1965) transferred Lindau's Nelsonioideae to a new sub-family, Rhinanthoideae, under the family Scrophulariaceae, based on various palynological characters and he raised the rest of the three subfamilies to family status— Thunbergiaceae, Mendonciaceae and Acanthaceae. Bremekamp's Acanthaceae, having retinaculate fruits, are divided into two: monothecate anthers and colpate pollens of Acanthoideae and articulated anthers with cystoliths of Ruellioideae. The other two families Thunbergioideae and Mendoncioideae lack retinaculate fruits.

Scotland *et al*. (1995) conducted a parsimony analysis using ndhF and rbcL chloroplast gene sequences for Acanthaceae species. They found ndhF to have more informative characters and greater systematic resolution at the hierarchical level compared to rbcL. The classification of Acanthaceae by Scotland & Vollensen (2000) represents a synthesis of morphological and molecular data from 221 genera of Acanthaceae. Taxa were recognized based on a combination of morphological diagnostic features, the three-item analysis of morphological data, and published molecular sequence analysis. They presented a new classification of Acanthaceae and included a list of generic synonyms. Scotland & Vollesen (*l.c*) divided the family Acanthaceae into three subfamilies, two tribes, and four subtribes, leaving 20 genera unplaced. The three subfamilies are 1. Nelsonioideae, 2. Thunbergioideae, 3. Acanthoideae. McDade *et al*. (2000a) subdivided the subfamily Acanthoideae into four tribes: Acantheae, Barlerieae, Justiceae, and Ruellieae. Recent phylogenetic studies of Acanthaceae have resulted in the recognition of four subfamilies, viz., Nelsonioideae, Thunbergiodeae, Avicennioideae and Acanthoideae. Acanthoideae differs from the other three subfamilies in having reticulate and is further divided into seven tribes, Acantheae, Ruellieae, Justicieae, Barlerieae, Neurantheae, Andrographideae and Whitifieldieae (Reveal, 2012).

Building upon the foundation laid by Scotland & Vollesen (2000), Manzitto-Tripp *et. al.* (2022) revised the classification of Acanthaceae. This revision includes dichotomous keys to the major lineages and genera of Acanthaceae, along with geographically structured keys. They classified the family Acanthaceae into 4 subfamilies viz, Nelsonioideae, Avicennioideae, Thunbergioideae, and Acanthoideae. In previous works, such as McDade *et al*. (2008), have referred to these groups collectively as Acanthaceae (*s.l.*). Thunbergioideae are divided into two tribes: Mendoncieae and Thunbergieae. Within Acanthoideae, which exhibits the greatest taxonomic diversity, eight tribes are identified: Acantheae, Physacantheae, Barlerieae, Andrographideae, Whitfieldieae, Neuracantheae, Ruellieae, and Justicieae. A total of 19 subtribes and 191 genera are recognized in this treatment. This revised classification was accepted by Stevens (2001) and the Angiosperm Phylogeny Group (2016), and is comparable to the classification put forward by Reveal (2012). The reclassification of Acanthaceae denotes a synthesis of data from the last two decades of phylogenetic, floristic, taxonomic, and nomenclatural studies conducted in the family.

A number of species of Acanthceae have been described and discovered until now. Due to the complexity of synonymy, there is disagreement regarding the exact number of taxa in Acanthaceae. According to Arthur Cronquist (1981), the family comprises of around 250 genera and 2500 species. According to Takhtajan (1997) the family Acanthaceae (including Mendonciaceae and Thunbergiaceae of Bremekamp 1965, Nelsoniaceae of Sreemadhavan (1977) consists of 4400 species under 250 genera. Acanthaceae is a large family with nearly 250 genera and 2500 species (Mabberley, 1987), 230 genera and 4000 species (Hu *et. al.*, 2011), 2500 species in 250 genera (Willis, 1980). The family Acanthaceae (including Avicenniaceae, Mendonciaceae, excluding Thomandersiaceae) consists of 212 genera and 3175 species Mabberley (2008). The family Acanthaceae encompasses about 222 genera and *c*. 3565 species (Thorne and Reveal, 2007), 240 genera and 3250 species (Wasshausen and Wood, 2004).

Acanthaceae Studies in India

Van Rheede (1678–1693), in his monumental work '*Hortus Indicus Malabaricus*' (in 12 volumes), described 742 species, out of which 12 belong to the family Acanthaceae. The second half of the 19th century was remarked with the publication of *Flora of British India* by Hooker (1875). In volume 4 of this work, Clarke (1884-85) described 49 genera and 504 species and 127 varieties belonging to this family from the then-India region encompassing present India, Pakistan, Sri Lanka, Bangladesh, Nepal, Bhutan, Myanmar and Malayan peninsula. Since then, several new species have been discovered and numerous ornamentals and economically important plants have been introduced to India.

Nees Von Esenbeck was the first to give a comprehensive treatment of the Indian Acanthaceae in Wallich's *Plantae Asiaticae Rariores* (1832). The most important taxonomic work on Indian Acanthaceae is that of Santapau (1952), who published a detailed monograph on the Acanthaceae of Bombay, covering over 130 taxa over 42 genera. Panigrahi and Dubey (1983) solved some critical nomenclatural problems of Acanthaceae, especially on *Dicliptera* Juss. species. Anderson (1867) published *An Enumeration of Indian Species of Acanthaceae*, by examining large number of herbarium specimens. Cramer (1992) studied the nomenclatural changes of Acanthaceae with special reference to India and Ceylon. During the 20th century, intensive explorations occurred all over the country that resulted in many publications in Acanthaceae (eg:- Gamble, 1924; Ramamurthy, 1971; Madhusoodanan & Singh, 1992; Shendage & Yadav, 2010).

Karthikeyan *et al.* (2009) reported in *Flowering Plants of India* that India has 47 genera, 475 species, and 118 varieties of Acanthaceae. The family is considered as a complicated one by most of the taxonomists (Remadevi & Binojkumar, 2009). Classification of Acanthaceae is largely based on minute characters. It's possible that the different taxonomical characteristics that various species exhibit contribute to the incomplete understanding of the interrelationships among them. The identifying characters are often technical and many species appear to be so much similar that it becomes difficult to group into separate genera and tribes. The taxonomic status of this family is unclear and many taxa are not yet finally delimited.

Chemotaxonomy

A chemotaxonomic study of two species of Nelsonioideae by Narayana & Sarma (1980) showed that Nelsonioideae closely resemble Acanthaceae in their chemical characteristics. They discussed the systematic position of Acanthaceae and suggested that Nelsonioideae may be placed in Acanthaceae. Daniel & Sabnis (1987) reported the patterns of distribution of leaf phenolics and aucubins among 58 taxa of Indian Acanthaceae members. Among them, the genus *Hygrophila* was represented by *H. auriculata* (Shcum.) Heine, *H. ringens* (L.) R.Br. ex Steud. & *H. serpyllum* T.Anderson. *H. auriculata* was reported to contain proanthocyanins, flavons (Aspigenin) and phenolic acids (benzoic acid, vanillic acid, syringic acid, meliotic acid and coumaric acid). In *H. ringens*, no flavonoids were reported but contained phenolics (gentisic acid, hydroxy benzoic acid, vanillic acid, syringic acid, meliotic acid, coumaric acid and ferulic acid). *H. serpyllum* contained flavonoids (scutellarin and luteolin) and phenolic acids (hydroxy benzoic acid, syringic acid, benzoic acid, coumaric acid, sinapic acid and ferulic acid). On the basis of the study, they claim Nelsonioideae as an intermediate link between Thunbergiaceae and Acanthaceae and Thunbergiaceae relatively more primitive to Acanthaceae.

The chemotaxonomy of forty species of Acanthaceae was investigated by Jensen *et al*. (1988) by analysing iridoids and quaternary amines. Fourteen of 40 species investigated showed the presence of iridoid glucosides, including two species of *Hygrophila* viz., *H. triflora* (Roxb.) Fosberg & Sachet and *H. polysperma* (Roxb.) T.Anderson. Two compounds present in *Hygrophila* were precursors of *Thunbergia*, and from this, they indicated a link between the subfamilies Ruellioideae and Thunbergioideae. Positioning of the five subfamilies (Acanthoideae, Thunbergioideae, Mendoncioideae, Nelsonioideae and Ruellioideae) within the Acanthaceae was also confirmed by them due to the common occurrence of chemical compounds.

Anatomy

Cystoliths

One of the distinguishing characteristics of the Acanthaceae is the presence of cystoliths in the vegetative organs. According to Metcalfe and Chalk (1950) nature and distribution of cystoliths are important for the identification of genera and species. Cystoliths are outgrowths of the epidermal cell wall and are impregnated with calcium carbonate. They can also be defined as a form of silicified bodies with a cellulose skeleton or occasionally not encrusted. Cystoliths are primarily found in the leaves, and often the stems, petioles and bracts in the species of Acanthaceae, except for four genera in the subfamilies Thunbergioideae, Nelsonioideae and Acanthoideae.

Cystoliths in fresh material are best observed in the leaves with the use of a hand lens; in transmitted sunlight, they take the form of white streaks. They appear as white or occasionally black elevations or streaks in dried material, especially on the lower surface of the leaf. Even though cystoliths are not exclusive to Acanthaceae, no other closely related families have them, making their presence a useful tool for identifying Acanthaceae. They have been described in distantly related families like Cannabaceae, Urticaceae and Moraceae.

Several authors (Hobein, 1884; Lindau, 1895; Metcalfe & Chalk, 1950) have documented various cystolith types within Acanthaceae. Metcalfe and Chalk (*l.c.*) presented a systematic account of cystoliths in Acanthaceae, categorizing them into seven distinct groups. Hobein (*l.c.*) identified several taxon-specific types of cystolith within Acanthaceae. Rangaswamy (1941) placed *H. auriculata* in Hobein's classification. Ahmad (1976) studied the epidermal features of two genera viz., *Hygrophila* and *Dyschoriste* Nees and stated them as simple, spindle, sickle or cigar-shaped. Ahmad (1979) presented cystoliths in 82 species of the subfamily Acanthoideae and opined that cystoliths are absent in the subfamilies Nelsonioideae, Mendoncioideae and Thunbergioideae. Epidermal studies on some species of Acanthaceae (Selvaraj & Subramanian, 1983) reported cylindrical cystoliths in *H. auriculata* and *H. ringens* whereas conical type of cystoliths in *H. balsamica* (L.f.) Raf.

Inamdar *et al.* (1990) studied the occurrence, type, size and frequency of cystoliths of 46 species of Acanthaceae and recognized eleven types of cystoliths. Cystoliths differ in shape, size, nature, colour and occurrence throughout the family. Their size can vary even within the same species, but the length–breadth ratio is consistent for species within a genus, maintaining the same cystolith type for the genus. The subtribe Hygrophileae manifested mostly solitary, elongated, narrow cystoliths. Their work included two species of *Hygrophila* viz., *H. auriculata* and *H. serpyllum*, both showed solitary, elongated narrow cystoliths. Patil & Patil (2011) studied foliar anatomical features of 43 species of Acanthaceae in India, and they reported elongated cystoliths with obtuse ends in *H. auriculata*. Zakaria *et al*. (2020) conducted the foliar anatomical studies of 41 species of Acanthaceae from Peninsular Malaysia, and they distinguished 4 types of cystoliths. The species of *Hygrophila*, *H. pusilla* Blume was marked by solitary round-shaped cystolith.

Gabel *et al*. (2021) examined cystoliths in 28 species (5 in non-cystolith clade and 23 in cystolith clade) of Acanthaceae by means of light and scanning electron microscopy. All 23 species contained cystoliths within lithocysts, of which 19 possessed leaf epidermal impressions. Their study showed that leaf epidermal impressions are common on both surfaces of emergent leaves of *H. polysperma*.

There is some debate regarding the use of the term 'cystolith'. Earlier authors have referred to these structures in the Acanthaceae as "leaves lineolate" or leaves with 'raphides'. However, anatomically, cystoliths and raphides are quite different. Cystoliths are outgrowths of the epidermal cellulose cell wall impregnated with calcium carbonate, while raphides are aggregates of long calcium oxalate crystals in large cells that are dead at maturity and often filled with mucilage (Sreemadhavan *et al*., 1968). Due to these differences and the fact that cystoliths and raphides can only be distinguished anatomically in cleared leaves, Sreemadhavan *et al.* (*1.c*.) advocated for the use of the general term 'furuncle' by plant taxonomists. However, this term has not been widely adopted.

Stomata

Inamdar (1970) investigated the epidermal structure and ontogeny of stomata in few members of Acanthaceae. Their results supported the view of Metcalfe & Chalk (1950) that caryophyllaceous stomata have a pair of guard cells at right angles to the subsidiary cells, which are surrounded by two, three or four subsidiary cells. Pant & Mehra (1963) found that the development of caryophyllaceous stomata in *Asteracantha longifolia* Nees is characteristically syndetocheilic. The two crescentic subsidiary cells and the surrounding cells derive from the same meristemoid which finally forms the guard cells. They also noted that suppressing one or two divisions before guard cell formation results in incompletely amphicyclic or monocyclic stomata. Selvaraj & Subramanian (1983) studied the epidermal features of some species of Acanthaceae including three species of *Hygophila* (*H. auriculata*, *H. ringens* and *H. balsamica*). The amphistomatic condition was reported in all three species of *Hygophila* studied.

Flower

Rao (1953) and De (1967) studied the floral anatomy of Acanthaceae, and they investigated the presence of filament curtains in the members of the family. Their study showed that the species of *Hygrophila* also have a filament curtain with four corolla traces and five filament traces, with one being a redundant fifth trace. Rao (1953) also revealed the presence of an abaxial nectary protrusion in many Acanthaceae species including *Hygrophila*. Manktelow (2000) identified four types of filament curtain in Acanthaceae: phaulopsoid, corolla-fold, reduced and strobilanthoid. Manktelow (*l.c*) descibed the filament curtain as a complex structure developed within the synstapetal region of the corolla tube of Ruellieae *s.l.*, by the fusion of staminal filaments to form the curtain adnate against the corolla wall. Corolla fold filament type was observed in the species of *Hygrophila*.

Trichomes

The study of trichomes is an important taxonomic tool. The taxonomic and phylogenetic importance of trichome features like density, size, shape, surface texture, and orientation has been widely identified in Acanthaceae (Metcalfe & Chalk, 1950; Kumar & Paliwal, 1975; Singh & Jain, 1975; Ahmad, 1978; Bhatt *et al*., 2010; Patil & Patil, 2011).

Ahmad (1978) studied the structure and distribution of the foliar epidermal hairs of 111 taxa of Acanthaceae and recognized two major categories: glandular and non-glandular trichomes. The glandular hairs in *H. polysperma* were sub-sessile (short-stalked) with globular heads, and in *H. serpyllum*, it was long-stalked. The hairs in *H. auriculata* were non-glandular. He also observed two types of hair base in *H. serpyllum*:1–2-celled, small, slender hairs emerging from a single epidermal cell and 2–4-celled, longer, and stouter hairs with a multicelled hair base. Epidermal studies on some species of Acanthaceae by Selvaraj & Subramanian (1983) observed unicellular uniseriate non-glandular trichomes in *H. auriculata*.

Palynology

Historically, pollen characteristics have been crucial in defining genera and higher taxa within the family. Studies on the morphology of pollen from the Acanthaceae family have been carried out from time to time. Radlkofer (1883) conducted the first palynological investigation, highlighting the taxonomy significance of pollen grain characteristics within the Acanthaceae system and described a number of distinct pollen types. However, Lindau (1893, 1895) realized the taxonomical importance of the different pollen types in the Acanthaceae. He conducted a thorough investigation and identified 11 pollen types within the family Acanthaceae for the first time. Since then, several researchers have examined the pollen of different taxa of Acanthaceae (Natarajan, 1957; Raj, 1961; Huard, 1962; Moore & Webb, 1978; Scotland, 1992; Furness, 1990, 1994; Furness & Grant, 1996; McDade & Moody, 1999; Shamso, 2013). Bremekamp (1965) and Raj (1961 & 1973) accepted Lindau's classification but amended it in the light of new pollen examinations. McDade *et al.*, (2000b) employed palynological data to determine the phylogenetic relationships within the tribe Justicieae.

The Acanthaceae family is eurypalynous, exhibiting significant diversity in pollen morphology, including variations in pollen size, shape, aperture (colpi or pore, simple or compound), and tectum. This diversity has been noted by earlier researchers like Radlkofer (1883) and Lindau (1893, 1895). Lindau identified 11 pollen types, which were used in subfamilial classification. Similarly, Rizzini (1947) studied the pollen morphology of many Brazilian genera and employed pollen characteristics to delimit genera. Muller *et al*. (1989) identified 26 different pollen types within the family, including those from Madagascar and several islands of the Indian Ocean. Daniel (1998) also noted diversity in pollen morphology within the Acanthaceae family in Mexico, suggesting that this diversity was taxonomic rather than geographic, as the variation in Acanthaceous pollen was consistent or even greater in various parts of the world. Scotland and Vollensen (2000) provided detailed illustrations of the major pollen types of Acanthaceae sensu Bremekamp (1965).

Key pollen studies on the Acanthaceae family in India include those by Raj (1961), who examined 260 species across 103 genera, Raj (1973), studied 143 species from 63 genera, and Chaubal (1966), who investigated 154 species from 42 genera in Western India. Carine & Scotland (1998) focused on the pollen morphology of *Strobilanthes* sensu Anderson in Peninsular India and Sri Lanka, identifying 22 pollen types within two shape classes: spheroidal and prolate/subprolate. Wang & Blackmore (2003) identified eight pollen types of *Strobilanthes* from China, five of which matched those described by Carine & Scotland (1998).

Parveen & Qaiser (2010) examined the pollen morphology of 30 species from 11 genera of Acanthaceae using light and scanning electron microscopy. They categorized the family into eight distinct pollen types based on apertural type, shape class, and exine sculpturing: Pollen type-I: *Barleria cristata*-type, Pollen type-II: *Blepharis ciliaris*-type, Pollen type-III: *Hygrophila polysperma*-type, Pollen type-IV: *Justicia adhatoda*-type, Pollen type-V: *Lepidagathis incurva*-type, Pollen type-VI: *Peristrophe paniculata*-type, Pollen type-VII: *Ruellia patula* -type and Pollen type-VIII: *Strobilanthes atropurpureus*-type.

Furness (1994) examined the pollen morphology of 34 species of *Hygrophila* and 11 species of *Brillantaisia* P.Beauv. using light, scanning electron, and selectively with transmission electron microscopy. He recognized nine pollen types and six subtypes based on the sculpturing of the ectexine bands, the number and pattern of the pseudocolpi, and the nature of the endoapertures. Both genera exhibited 4-colporate pollen, with the ectexine seperated into bands by pseudocolpi (usually 3-4 between each colporus) and a continuous, thick endexine. Pollen morphology suggested a close relationship between *Hygrophila* and *Brillantaisia*, with only two *Brillantaisia* species having unique pollen types. The pollen morphology of *Hygrophila* indicated potential closely related species groups and differences between Old and New World species.

Cytology

Cytology has been an important element in evaluating relationships and deducing phylogenetic relationships in angiosperms (Raven, 1975). Karyological studies are critical in plants since they allow the detection of particular changes in chromosome number such as aneuploidy or diploidy processes (Briggs & Walters, 1997). The data derived from karyological studies could be effectively used in plant taxonomy for delimiting species. Therefore, the great diversity in chromosome numbers and their relative stability within populations and species are key characteristics for taxonomic classification in many plants.

Grant (1955) carried out cytogenetic studies in 101 collections of Acanthaceae. He studied three species of *Hygrophila* (*H. auriculata, H. lacustris, and H. polysperma*), all of which possessed a somatic chromosome number of 32. According to him, 8 is probably the chromosome number of the genus, and the genus appeared to be a natural group since the chromosome number estimations represented both the Old World and New World.

Many authors reported diploid chromosome number 2n = 32 in *H. auriculata* (Rangaswamy, 1941; Narayanan, 1951; Grant, 1955; Govindarajan and Subramanian, 1985; Saggoo & Bir, 1986; Sharan *et al*., 2016; Verma *et al*., 2018). Fedorov (1974) reported the basic chromosome number in *Hygrophila* as 12 and 16. Sugiura (1940) reported 12 spherical meiotic chromosomes in *H. auriculata* (2n=24).

Govindarajan and Subramanian (1983) conducted karyomorphological studies in South Indian Acanthaceae, in which they reported chromosome number of three species of *Hygophila* (*H. auriculata* 2n = 32, *H. ringens* 2n=44 & *H. balsamica* 2n=34). Detailed karyotype analysis revealed the presence of short type chromosomes in *H. auriculata*, medium & short in *H. ringens* and long to short in *H. balsamica*. They concluded that the various species of *Hygrophila* investigated have aneuploid chromosomal variations.

Embryology

The embryology of a number of species of the Acanthaceae has been investigated. Gigante (1929) studied the embryology of *Acanthus mollis* L., and Mauritzon (1934) has investigated species in 16 genera. Rangaswamy (1941) has studied the embryogenic characters of *Hygrophila spinosa* T.Anderson.

Phytochemistry

Phytochemistry has been recognized and applied in plant taxonomy several years ago. Acanthaceae has gained recognition with reference to biochemical and biological evaluation. Many phytochemicals have been extracted from different parts of *H. auriculata*. The plant comprises diverse groups of phytoconstituents such as polyphenols, phytosterols, proanthocyanins, tannins, alkaloids, amino acids, enzymes, carbohydrates, terpenoids, flavonoids, glycosides, steroids, saponins, mucilage and vitamins (Patra *et al.*, 2009; Hussain *et al.*, 2011; Sufian *et al.*, 2015).

The terpenoid lupeol is present in stems, leaves and roots and a hydrocarbon hetricontane is found in leaves and stems (Govindachari *et al*., 1957). Two alkaloids, asteracanthine and asteracanthicine are extracted from seeds (Parashar and Singh, 1965). The seeds of the plant primarily contain fatty acids (Quasim & Dutta, 1967). Flavaonoids such as apingenin-7-O-glucuronide along with traces of 7-O-glucosidase are extracted from flowers (Balraj and Nagarajan, 1982). Misra *et al.* (2001) isolated aliphatic esters such as 25-oxo-hentriacontyl acetate and methyl 8-n-hexyltetra cosanoate from aerial parts. An alkaloid trigonelline was reported in the plant by Ruchi (2014).

H. ringens contains flavonoids, alkaloids, steroids, amino acids, enzymes and stigma sterols. The flavonoid isoquercitrin was isolated from the plant by Bhatt and Shah (2019). The plant contains an alkaloid trigonelline (Ruchi, 2014). From the ethanol extract of the leaves of *H. pogonocalyx* Hayata, flavones, flavonols, phenylethanoid glycosides, alkylated glycosides and steroids were isolated (Ho *et al.*, 2013). The extract of *H. phlomoides* Nees shows the presence of alkaloids, reducing sugars, phenolic compounds, flavonoids, tannins, proteins, glycosides, saponins, steroids, terpenoids, and acidic compounds (Karmakar *et al*., 2022). Phytochemical analysis of the crude extract of *H. triflora* Fosberg. & Sachet identifies steroids and cardiac glycosides as the main phytoconstituents (Samanta *et al.*, 2012). Jensen and Nielsen

(1985) reported the presence of an iridoid glycoside in *H. triflora*. They isolated and determined the shape and termed it Hygrophiloside.

Ethnobotany & Pharmacognosy

Since time immemorial man has been using plants as medicine for curing ailments. Traditional medicines have been a boon in alleviating health issues on a global level. Numerous plants continue to offer beneficial therapeutic in modern and traditional medicine. Some species of *Hygrophila* are known for their ethnomedicinal properties and have been used in the traditional system of medicine.

Among the species of *Hygrophila*, *H. auriculata* is a promising medicinal plant with miraculous healing properties. The medicinal significance of *H. auriculata* is acknowledged in ancient medical texts. In Ayurvedic literature, it is referred to as Ikshura, Ikshugandha, and Kokilasha ('having eyes like the Indian cuckoo'). Ayurvedic treatises like 'Sushruta Samhita' and 'Charak Samhita' treat *H. auriculata* as Rasayan or rejuvenator (Kirtikar & Basu, 2005; Nadkarni, 2007). It is known as a diuretic, aphrodisiac, and renal tonic, and also for its health-promoting properties. The whole plant including roots, seeds, aerial parts and ashes of the plant are extensively used in traditional systems of medicine to cure diabetes, dysentery, jaundice, hepatic obstruction, rheumatism, inflammation, urinary infection, pain, gout, impotence, malaria, etc. (Chopra *et al.*, 1956, Jain, 1991; Kitty, 1988; Nadkarni, 2007).

Dried leaf powder of *Hygrophila auriculata* mixed with castor oil is applied to the affected areas to treat skin diseases (Jeeva *et al.*, 2007). The tribes of the Achanakmar-Amarkantak biosphere reserve in Central India use the ash of the whole plant (5g) mixed with honey twice a day for one month to cure kidney and urinary bladder stones (Tiwari *et al.,* 2014). Medicine men of West Bengal prescribe tender shoot and leaf juice half cup twice daily to treat severe anaemia and the seeds to cure gonorrhea (Saha *et al*., 2016)**.** Rural communities in West Bengal treat excessive menstrual bleeding by consuming fresh extract or juice from half-boiled leaves of the plant (Pattanayak *et al.*, 2016). Satpathy *et al*. (2018) reported that an antioxidant-enriched fraction from *H. auriculata* could serve as an alternative and affordable botanical drug for treating menopausal osteoporosis and cancer.

H. auriculata possesses antitumor (Mazumdar *et al*., 1997; Ahmed 2001; Pattanayak and Sunita, 2008; Anusha and Immanuel, 2019), antibacterial (Boily and Puyvelde, 1986; Valientic, 1995), antioxidant (Sunil Kumar and Klausmuller, 1999; Sawadogo *et al*., 2006, Vijayakumar *et al*., 2006; Hussain *et al*., 2009), anti-inflammatory, antipyretic (Patra *et al*., 2009), anti-nociceptive (Shanmugasundaram and Venkataraman 2005), antimotility (Patra *et al.,* 2008), antistress (Kannur *et al*., 2017), anthelmintic (Patra *et al*., 2008), aphrodisiac (Chauhan *et al*., 2009), hepatoprotective (Singh and Handa, 1995; Hewawasam 2003, Shailajan *et al*., 2005; Shanmugasundaram & Venkatraman 2005), hypoglycemic (Fernando *et al*., 1991; Vijayakumar *et al*., 2006), and hematopoietic (Gomes *et al*., 2001; Pawar *et al*., 2010; Sunita and Abhishek, 2008), diuretic (Hussain *et al*., 2009; Hussain *et al*., 2011) neuroprotective (Kanhere *et al*., 2013) activities.

Hygrophila ringens is known for its numerous medicinal applications in traditional medicine. Seeds and leaves are used as a poultice for inflammatory swellings, and the leaves have strong diuretic properties (Bhatt and Saha, 2016). It has anti-diabetic (Ramana *et al*., 2016), analgesic, anti-oxidant and hypoglycemic activities (Chowdhury and Begum, 2012). *H. phlomoides* exhibit analgesic, anti-inflammatory, laxative, and anthelmintic effects (Karmakar *et al*., 2022). Leaves of *H. pogonocalyx* exhibit anti-tyrosinase and neuro cytoprotective activity (Ho *et al.,* 2013)

H. triflora possesses anti-convulsant, anti-oxidant (Biswas, 2012) and anthelmintic (Samanta *et al*., 2012) activities. Chemical analysis of aerial parts of the plant revealed the presence of an iridoid glucoside, hygrophiloside (Jensen and Nielsen, 1985). CNS depression activity was found in the aerial part of the plant (Pal and Samanta, 2011).

Other Uses

H. auriculata, a common herb in India, is used as a vegetable in states like Assam, Odisha, Chhattisgarh, and West Bengal. In Sri Lanka, it is sold as a vegetable in local markets (Mathiventhan and Ramiah, 2015). The seed of the plant is sold commercially in markets. Plants like *H. balsamica*, H. *pinnatifida*, *H. polysperma*, *H. serpyllum* and *H. triflora* are used as aquarium plants.

Genus *Hygrophila*

Hygrophila R. Brown of the Acanthaceae family is a significant cosmopolitan genus that thrives in moist environments, consisting of about 77 species (POWO, 2024). It is predominantly found in tropical and subtropical regions, with many species being aquatic plants (Hu *et al*. 2011). According to APG III, the Acanthaceae family belongs to the order Lamiales, the clade Lamiids, and the core class Euasterids I of Core Eudicots (Chase & Reveal 2009).

In Linnean times species of *Hygrophila* were known by the name *Ruellia*. Later Robert Brown described the genus *Hygrophila* in 1810 in his *Prodromus Florae Novae Hollandiae*. He described the genus with only one species *H. angustifolia*, but he established the genus *Hygrophila* based on Linneaus's *Ruellia ringens*. He didn't make the combination *Hygrophila ringens*. Infrageneric delimitation within *Hygrophila* is challenging due to the lack of distinct morphological criteria, resulting in species being placed under various generic names. Morphological investigations have led to the synonymization of most names under *Hygrophila*.

Taxnomic Treatments

Van Rheede (1678–1693), Dutch Admiral of Malabar, described and illustrated several hundreds of medicinal plants of 'Malabar coast' in his *Hortus Malabaricus*. This twelve-volume botanical treatise is the oldest comprehensive work on plant wealth of Asia and tropics. In this monumental work, two species of the present day *Hygrophila* namely *'Bahel-Schulli'* (Hort. Malab. 2:87, t.45, 1679) (=*Hygrophila auriculata* (Schumach.) Heine, and *'Nir-Schulli'* (Hort. Malab. 2:89, t.46, 1679) (=*Hygrophila ringens* (L.) R. Br. ex Steudel) were described in Latin. Their vernacular names were given in four scripts viz., Latin, Malayalam, Arabic, and Devanagari. Rheede's work was later translated into English and Malayalam (Manilal 2003; 2008) with annotations about the identity and correct botanical nomenclature of all plants. Hortus Malabaricus laid the foundation for subsequent workers like Linnaeus, Hamilton, De Candolle, etc. They described several taxa of Indian plants based on Rheede's illustrations and also adopted the vernacular names into botanical nomenclature.

The Swedish botanist and zoologist Carl Linnaeus, who is regarded as the father of modern taxonomy, has played a vital role in the development of modern taxonomy and botanical nomenclature. It was Linnaeus's Species Plantarum (1753) that marked the beginning of taxonomy and the starting point of botanical nomenclature, in which he recorded many species of *Ruellia*. Some of the species of *Ruellia* are separated from it and are transferred into *Hygrophila*. He described the present day *Hygrophila* as *Ruellia ringens*, based on which Robert Brown described the genus *Hygrophila* in 1810. Linnaeus cited his own Flora Zeylanica (1747) and Rheede's Hortus Malabaricus, for the species.

Scottish botanist Robert Brown, after his two-year-long Australian voyage, first described the genus *Hygrophila* in his *Prodromus Florae Novae Hollandiae* (1810). The Linnean species *Ruellia ringens* was the base for his genus *Hygrophila*, but he did not make the combination *H. ringens*. There, he described only one species of *Hygrophila*, namely *H. angustifolia* R.Br.

Nathaniel Wallich (1828-49), who worked as superintendent at the Honourable East India Company's (EIC) Botanic Garden at Kolkata, started to amass a considerable herbarium of plant specimens. He enumerated the dried specimens of plants at EIC and published their numerical list, which is known as *Wallich's catalogue*. He catalogued 8 species of *Hygrophila*, many of which were nomen nudum.

German botanist Nees von Esenbeck (1847) carried out global treatment of the family Acanthaceae in de Candolle's *Prodromous Systematis Naturalis Regni Vegetabilis*. He described the genus *Hygrophila* and its allied genera under two different tribes, Hygrophileae and Nelsonieae both of which come under the retinacula present group Echmatacantheae. *Adenosma* Nees with seven species viz. *A. biplicata, A. balsamea, A. triflora, A. villosa, A. verticillata, A. thymus, A. uliginosa* and 3 varieties are described under the tribe Nelsonieae with papilliform retinacula, which were later transferred to *Hygrophila*. All others are grouped in the subtribe based on capsule-bearing seeds from the base under the tribe Hygrophileae. The allied genera under Hygrophileae which were later segregated into the genus *Hygrophila* are *Hemiadelphis* with 1 species (*H. polysperma*), *Physichilus* Nees with 3 species (*P. serpyllum*, *P. senegalensis* and *P. barbatus*), *Polychema* with 5 species (*P. caeruleum*, *P. abyssinicum*, *P. micranthum*, *P. odorum*, and *P. radicans*) and *Nomaphila* Blume with 4 species (*N. stricta*, *N. corymbosa*, *N. levis* and *N. petiolata*). The species of the genus *Hygrophila* were described in two sections based on inflorescence. 19 species of *Hygrophila* and 12 varieties were recorded under the whorled inflorescence group, and 4 species and 1 variety under dimidate inflorescence. Some of the names are synonyms now. Nees commenced the first comprehensive treatment of Indian Acanthaceae (1832) in Wallich's *Descriptions and figures of unpublished East Indian plants* (Plantae Asiaticae rariores). In this, he used the same classification and enumerated 12 species of *Hygrophila* and allied genera: *Adenosma* with three species, *Hemiadelphis* with 1 species, *Asteracantha* with one species and *Hygrophila* with 6 species. All the described species except one are new combiantion. All the species treated under the genus *Hygrophila* are transferred from *Ruellia*, listed in Wallich's catalogue.

Robert Wight (1850) accepted the view of Nees and illustrated four species of *Hygrophila* (*Hygrophila salicifolia*, *H. obovata*, *Physichilus serpyllum* and *Hemiadelphis polysperma*) in his *Icons Plantarum Indiae Orientalis* four species of *Hygrophila*.

Thomas Anderson made a substantial contribution to the family Acanthaceae during his work at Calcutta as Superintendent of the Royal Botanic Garden. The family Acanthaceae received his special attention, and he published works on Acanthaceae in different regions. Nees von Esenbeck's monograph of the family Acanthaceae in de Candolle's *Prodromus* formed the basis from which the deductions were drawn by him. Anderson (1864) carried out *An Enumeration of the Species of Acanthaceae from the Continent of Africa and the Adjacent Islands*. He recorded 11 species of present-day *Hygrophila* under 3 different genera: 1) *Adenosma* with *A. africana*, 2) *Nomaphila* with *N. laevis* and *N. ciliata*, 3) *Hygrophila* with *H. senegalensis*, *H. odora*, *H. caerulea*, *H. micrantha*, *H. abyssinica*, *H. barbata*, *H. lutea* and *H. spinosa*. Of the 11 species, 3 were new species and 6 were new combinations. *H. senegalensis* and *H. barbata* were transferred from *Physichilus* whereas *H. odora*, *H. caerulea*, *H. micrantha*, *H. abyssinica* from *Polychema*. Later in 1864, he recorded 6 species of *Hygrophila* (2 under *Hygrophila* and 4 under *Adenosma*) in the enumeration of Ceylon Acanthaceae in Thwaites Enumeration of Ceylon plants. Besides this, Anderson (1867) made an enumeration of Indian Acanthaceae in which 15 species of *Hygrophila* were listed. It was Anderson who transferred Nees's two genera *Physichilus* and *Hemiadelphis* to *Hygrophila* and he described 6 new species and proposed 9 new combinations in *Hygrophila*. Anderson (1864) examined Acanthaceae specimens of Linnean herbarium and delivered a note on its identity, in which out of 18 species of *Ruellia* L. was identified as *Hygrophila*. Bentham (1868), in *Flora Australiensis* followed Anderson's concepts in ascribing the Australian material to *H. salicifolia*, "a common Asiatic species".

Charles Baron Clarke (1885) enumerated 13 taxa (8 species and 5 varieties) under the genus *Hygrophila* from the then British India in Hooker's *Flora of British India*. Among them, 2 were not from the present political boundary of India. He categorized all the species of *Hygrophila* into two subgenera based on the presence of spines viz, *Euhygrophila* and *Asteracantha*. He also enumerated 10 species belonging to the present-day *Hygrophila* under two other genera viz, *Cardanthera* and *Nomaphila*, of these 4 species do not belong to the present Indian boundary. Among them, one is a synonym. Clarke (1908) treated the family Acanthaceae as the *Materials for the Flora of the Malayan Peninsula*, which was published posthumously. He described 10 species of *Hygrophila*: one species under *Cardanthera*, 7 under *Hygrophila* and 2 under *Nomaphila*. The genus *Hygrophila* was divided into 2 subgenera (*Hemiadelphis* and *Euhygrophila*). He strongly believed that the genus *Nomaphila* is identical to *Hygrophila* but treated it as a separate genus. He also reported two new species. Later Gamble (1924), in his comprehensive treatise *Flora of Presidency of Madras*, described 9 species of *Hygrophila* under *Asteracantha*, *Cardanthera* and *Hygrophila*.

Bremekamp (1955) described the position of the African "*Hemigraphis*" species in his *Notes on Some Acanthaceous Genera and Species of Controversial Position*. He believed that the three African species that Clarke in Flora of Tropical Africa (1899) had referred to as *Hemigraphis* Nees and the other two *Hemigraphis* added to African flora by S. Moore and Lindau do not either belong to the genus *Hemigraphis* nor to subtribe *Strobilanthinae*. He suggested that all these *Hemigraphis* species must belong to the subtribe *Hygrophilinae* and to the genus *Synnema*. He did not accept Anderson's wide view of the genus *Hygrophila* and advocated the restoration of the genera *Hemiadelphis* Nees, *Physichilus* Nees and *Nomaphila* and, if obligatory, the formation of one or more new genera.

Heine (1962), under the title *Tropical African Plants: Some West African Acanthaceae*, discusses in detail the reduction of *Asteracantha* Nees and *Synnema* Benth. to *Hygrophila*. The monotypic genus *Asteracantha* was retained as a separate genus based on the presence of axillary spines. Heine strongly believed that this character of axillary spines is not really constant as there are some plants in Africa without or with very few spines. So, he reduced the genus *Asteracantha* to *Hygrophila* and made a new combination *H. auriculata* (Schumach.) Heine.

The amendment of the genus *Hygrophila* was made by Heine (1971) as he found difficulty in maintaining the two genera *Hygrophila* (including *Nomaphila*) and *Synnema* as separate taxa, which shared very close similarities. He listed 19 species of the newly circumscribed genus and previously accepted within the genus *Synnema*. In this, he proposed 12 new combinations and 1 new name. *H. helodes*, the new name he proposed, is superfluous when published as there was *H. helodes* Nees. Heine disagreed with Livera's transfer of *Cardanthera thwaitessi* to *Plaesianthera* and he retained *Plaesianthera* in *Hygrophila*. Cramer (1991) re-established *Plaesianthera* and proposed merging of *Plaesianthera thwaitessi* Livera to *Brillantaisia thwaitessi* (T.Anderson) L.H.Cramer.

Barker (1986), in his revision of Australian Acanthaceae, examined the complex *H. ringens* and suggested that a revision of *Hygrophila* species from India to Malesia would clarify the status of all species discussed. Sidwell (1999) discusses that the monophyly of *Hygrophila* is not resolved, and her preliminary study highlighted the need for a complete revision of the genus. Cramer (1989) reassessed the genus *Hygrophila* in India and Ceylon, considering *Asteracantha*, *Cardanthera*, *Synnema*, *Hemiadelphis*, and *Adenosma*, which past Indian and Ceylon authors recognized as allied genera. The over emphasis on variable and overlapping characteristics by these authors has complicated the distinction between these genera and *Hygrophila*. Examination of more material from increased floristic activity in Africa and Asia shows that many characters are too variable to determine their generic status. The conclusion is that there are only two valid genera: *Hygrophila* and *Hemiadelphis*. Additional clarifications were also made to Heine's amendment of *Hygrophila*. He also studied the nomenclatural changes of Acanthaceae with special reference to India and Ceylon (Cramer, 1992).

Hu *et al*. (2011) in *Flora of China* described 6 species of *Hygrophila*. Santapau (1951) studied Acanthaceae of Bombay and listed 8 species and 1 variety of *Hygrophila* under *Cardanthera*, *Asteracantha* and *Hygrophila*. Cook (1996) described 7 species of *Hygrophila* in his aquatic and wetland plants of India. Mathew & George (2013) identified a new subspecies of *Hygrophila ringens* (*Hygrophila ringens* ssp. *longifolia*) from the Achankovil forest of southern Western Ghats, India. Sunojkumar and Prasad (2014) rediscovered *Hygrophila balsamica* var. *thymus* (Nees) Karthik. & Moorthy after a span of 180 years from Tirunelveli, South India. The authors reinstated its species status due to the unique character combination, and hence, they proposed a new combination, *H. thymus* (Nees) Sunojk. & M.G. Prasad.

Mathew *et al.* (2017) described three novelties in the family Acanthaceae from South Western Ghats of India, in which they proposed a new combination *H. auriculata* var. *alba* which is a new discovery to the specified region. New distributional record of threatened taxa *Hygrophila pogonocalyx* Hayata from India was reported by Sarma *et al.* (2017). Karthikeyan *et al.* (2009) listed 18 species of *Hygrophila* in their book *Flowering Plants of India'*. New combinations were also made. Contributions to the family Acanthaceae of Kerala was published by Remadevi & Binojkumar (2009) with a detailed account of five species of the genus *Hygrophila*. The authors also described a new variety of *Hygrophila ringens* (*Hygrophila ringens* var. *cochinensis*).

In addition to the classical treatments and revisionary works mentioned above, some state or regional floras prepared by many Indian workers were also helpful in understanding the species limits and distribution of *Hygrophila* in India. The treatment of *Hygrophila* species in various regional floras is given below.

Habit and Habitat

Hygrophila species have varying habits to adjust to different environmental conditions, showing a remarkable diversity of growth forms, including annual and perennial herbaceous forms. *Hygrophila* species are known to occur globally, mainly in tropical and subtropical regions. They are particularly abundant in regions with a monsoon climate, where seasonal rains create ideal conditions for their growth. They are found growing in all kinds of habitats where there is water or in wet areas like streams, lakes, ponds, canals, rice fields, marshes, etc.

Name of Author	Name of the Work and Year of Publication	Species Reported
Aitchison JET	A Catalogue of the Plants of Punjab & Sindh (1869)	2 spp: *H.spinosa, H.polysperma*
Clarke C B	The Flora of British India, Vol. IV (1885)	8 spp+5: *H. incana, H. phlomoides, H. polysperma, H. salicifoia, H. serpyllum, H. stocksii, H. spinosa* *Cardanthera uliginosa, C. balsamica, C. triflora, C. pinnatifida C. griffithi*
Prain D	Bengal Plants (1903)	6 spp: *H. spinosa, H. polysperma, H. serpyllum, H. quadrivalvis, H. phlomoides, H. salicifolia, H. phlomoides var rxburghi, H. salicifolia var assurgens*
Theodore Cook C I E	The Flora of Presidency of Bombay Vo II (1905)	3 spp+1+1: *H. angustifolia, H. polysperma, H. serpyllum* *Asteracantha longifolia* *Cardanthera pinnatifida*
Bamber CJ	Plants of the Punjab (1916)	3 spp: *H. polysperma, H. salicifolia, H. spinosa*
Gamble J S	Flora of the Presidency of Madras, Vol II (1924)	4 spp+1+ 4: *H. polysperma, H. serpyllum, H. angustifolia, H. quadrivalvis* *Asteracantha longifolia* *Cardanthera uliginosa, C. pinnatifida, C. balsamica, C. verticillata*
Kanjilal UN, A. Das, Kanjilal PC & R. N. De	Assam (1939)	1 spp: *H. polysperma*
Santapau	Khandala (1960)	3 spp+1: *H. polysperma, H. serpyllum, H. serpyllum var hookeriana* *Asteracantha longifolia*
Haines HH	Botany of Bihar & Orissa, repr. (1961)	*5 spp+1: H. polysperma, H. serpyllum, H. angustifolia, H. quadrivalvis, H. phlomoides* *Cardanthera triflora*

Maheshwari JK	Delhi (1963)	1 spp: *H. polysperma*
Ramamoorthy TP et al.	Hassan District (1976)	2 spp: *H. auriculata, H. salicifoia*
Babu C R	Herbaceous Flora of Dehra Dun (1977)	2 spp: *H. auriculata, H. polysperma*
Oommachan M	Bhopal (1977)	2+1 spp: *H. auriculata, H. serpyllum*
Nair NC	Flora of the punjab Plains (1978)	1 spp: *H. auriculata*
Bennet SSR	Howrah (1979)	3 spp: *H. auriculata, H. difformis, H. polysperma*
Manilal K S, Sivarajan V V	Flora of Calicut (1982)	3 spp: *H. auriculata, H. salicifoia, H. erecta*
Mathew KM	Flora of Tamil Nadu Carnatic vol 3 (1983)	4 spp: *H. auriculata, H.balsamica, H. quadrivalvis, H. salicifoia*
Deb DB	Flora of Tripura state (1983)	2 spp: *H. salicifolia, H. serpyllum*
Singh V	Banswara (1983)	2 spp: *H. auriculata, H. serpyllum*
Balakrishnan NP	Jowai and vicinity (1983)	1 spp: *H. salicifolia*
H. J. Chowdhery & B. M. Wadhwa	Himachal Pradesh Analysis (1984)	2 spp: *H. auriculata, H. polysperma*
Sharma BD, Singh NP, Raghava RS & Deshpande UR	Flora of Karnataka analysis (1984)	6 spp: *H. auriculata, H. balsamica, H. pinnatifida, H. polysperma, H. quadrivalvis, H. salicifoia*
Mukherjee AK	Pachmarhi (1984)	3 spp: *H. auriculata, H. polysperma, H. serpyllum*
Bakshi DNG	Murshidabad (1984)	2 spp: *H. auriculata, H. difformis*
Verma DM, Pant PC & Hanfi MI	Raipur, Durg & Rajnandgaon (1985)	4 spp: *H. auriculata, H. heinei, H. polysperma, H. salicifoia*
Rao RS	Goa, Daman Diu, Dadar, Nagarhaveli (1986)	2 spp: *H. auriculata, H. serpyllum*

Sesagriravu & Sreeramulu	Srikakulam Dt, AP (1986)	1 spp: *H. auriculata*
Henry *et al.*	Flora of Tamil Nadu (1987)	6 spp: *H. auriculata, H. balsamica, H. heinei, H. quadrivalvis, H. salicifoia, H. serpyllum*
Nair K K N & Nayar M P	Flora of Courtallum (1987)	1 spp: *H. salicifoia*
Henry	Tamil Nadu (1987)	6 spp: *H. auriculata, H. balsamica, H. heinei, H. quadrivalvis, H. salicifoia, H. serpyllum*
Chandrabose M & Nair NC	Coimbatore (1987)	1 spp: *H. auriculata*
Singh N P	Flora of eastern Karnataka, Vol II (1988)	1 spp: *H. auriculata*
Ramachandran V S, Nair V J	Flora of Cannanore (1988)	2 spp: *H. auriculata, H. erecta*
Kulkarni BG	Sindhurg (1988)	3 spp: *H. auriculata, H. pinnatifida, H. salicifolia*
Bole PV & Pathak JM.	Flora of Saurashtra. (1988)	*3 spp: H. auriculata, H. salicifoia, H. serpyllum*
Kamble SY & Pradhan SG	Akola (1988)	*2 spp: H. auriculata, H. serpyllum*
Panigrahi G & Murti SK	Bilaspur (1989)	2 spp: *H. schulli, H. polysperma*
Ellis JS	Flora of Nallamalais (1990)	1 spp: *H. auriculata*
Shetty BV & Singh V	Rajasthan (1991)	2 spp: *H. auriculata, H. serpyllum*
Naskar K	Aquatic & semi aquatic plants of lower ganga delta (1990)	3 spp: *H. auriculata, H. difformis, H. polysperma*
Vajravelu E	Flora of Palghat District, Including Silent valley National Park (1990)	2spp: *H. auriculata, H. salicifoia*
Almeida S M	Flora of Savantvadi, Maharashtra, India Vol 1 (1990)	3 spp: *H. auriculata, H. pinnatifida, H. serpyllum*

Murthy KRK & Yoganarasimhan SN	Coorg (1990)	2 spp: *H. auriculata, H. salicifoia*
Mathew K M	An excursion Flora of Central Tamil Nadu (1991)	6 spp: *H. schulli, H. balsamica, H. heinei, H. quadrivalvis, H. serpyllum, H. salicifoia*
Joseph, KT	Observation in the Aquatic Angiosperms of Malabar (North Kerala) (1991)	4 spp: *H. schulli, H. ringens, H. triflora, H. balsamica*
Lakshminarasimhan P& Sharma B.D.	Flora of Nasik district (1991)	2 spp: *H. auriculata, H. serpyllum*
Misra BK & Verma BK	Allahabad (1992)	2 spp: *H. auriculata, H. polysperma*
Malhotra AK & Moorthy S.	Flora of Taroba NP (1992)	2 spp: *H. auriculata, H. serpyllum*
Pullaiah T, Prasanna PV & Obulesu G.	Adilabad (1992)	2 spp: *H. auriculata, H. polysperma*
Chauduri AM	Forest Plants of Eastern India (1993)	2 spp: *H. polysperma. H. phlomoides,*
Kothari GR & Moorthy S.	Raigad (1993)	4 spp: *H. auriculata, H. quadrivalvis, H. pinnatifida, H. serpyllum*, 1 var. *H. serpyllum var hookeriana*
Naskar K	Plant Wealth of the Lower Ganga Delta (1993)	3 spp: *H. auriculata, H. phlomoides, H. polysperma*
Karthikeyan S & Kumar A	Flora of Yavatmal District. (1993)	1 spp: *H. auriculata*
LK Banerjee	Jaldapara Rhino Sanctuary (1993)	1 spp: H. salicifolia
Mohanan N, Henry A N	Flora of Thiruvananthapuram (1994)	2 spp: *H. auriculata, H. erecta*

Deshpande S, Sharma BD, and Nayar MP.	Mahabaleswar (1995)	2 spp: *H. auriculata, H. serpyllum*
Venkata Raju R R & T Pullaiah	Flora of Kurnool, Andra Pradesh (1995)	2 spp: *H. auriculata, H. salicifoia*
AK Sharma& JS Dhakre	Flora of Agra district (1995)	2 spp: *H. auriculata* *Hemiadephis polysperma*
Cook C D K	Aquatic and Wetland Plants of India (1996)	8 spp: *H. balsamica, H. difformis, H. heinei, H. pinnatifida, H. polysperma, H. quadrivalvis, H. schulli, H. serpyllum*
Sasidharan N, Sivarajan V V	Flowering Plants of Thrissur Forest (1996)	1spp: *H. schulli*
Oommachan M & Shrivastava J.	Jabalpur (1996)	2+1 spp: *H. auriculata, H. serpyllum* *Hemiadephis polyspermus*
Chauhan	Namdapha (1996)	2 spp: *H. erecta, H. salicifolia*
Khanna K.K., Tripathi A.K., and Mudgal V	Madhya Pradesh (1997)	8 spp: *H. auriculata, H. balsamica, H. erecta, H. heinei, H. phlomoides, H. polysperma, H. serpyllum, H. salicifoia*
Sivarajan V V, Mathew P	Flora of Nilambur (1997)	2 spp: *H. schulli, H. ringens*
Pullaiah T & Moulali A D	Flora of Andhra Pradesh 2 (1997)	4 spp: *H. auriculata, H. balsamica, H. heinei, H. salicifoia*
	Sriharikota island (1998)	1 spp: *H. balsamica*
Bhattacharya PK & Sarkar K.	Flora of West Champaran District (1998)	3 spp*: H. schulli, H. difformis, H. polysperma*
T. Pullaiah, C. Prabhakar, B. R. P. Rao	Medak district (1998)	2+1 spp: *H. auriculata* *Hemiadelphis polysperma*
Sinha BK, Hajra PK & Rao PSN	Great Nicobar (1999)	1 spp: *H. erecta*

Sesagriravu R, Sudhakar S, Venkanna P.	East Godavari dt (1999)	1 spp: *H. auriculata*
Gaur RD	District Garhwal North West Himalayas (1999)	1 spp: *H. polysperma*
Y N Seetaram, Kotresha K & Uplaonkar S B	Flora of Gulbargha District (2000)	1 spp: *H. auriculata*
Diwakar PG & Sharma BD	Buldhana District (2000)	2 spp: *H. auriculata, H. serpyllum*
T Pullaiah *et al.*	Guntur District (2000)	1 spp: *H. auriculata*
Singh NP *et al*	Flora of Maharashtra State. Dicotyledons Vol. II (2001)	6 spp: *H. heinei, H. phlomoides var roxburghii, H. pinnatifida, H. polysperma, H. ringens, H. schulli*
N. P. Singh *et al.*	Bihar (2001)	8 spp: *H. auriculata, H. difformis, H. erecta, H. heinei, H. phlomoides, H. polysperma, H. salicifoia, H. serpyllum*
Ramaswamy SN, Rao MR & Arekal GD	Shimoga district (2001)	3 spp: *H. auriculata, H. polysperma, H. salicifoia*
Daniel P & Umamaheswari P	Gulf of Mannar (2001)	1 spp: *H. schulli*
Yadav S R & Sardesai MM	Flora of Kolhapur District (2002)	5 spp: *H. schulli, H. ringens, H. pinnatifida, H. polysperma, H. serpyllum*
Sarma TK & Sarkar AK.	Flora of Palamau District (2002)	2 spp: *H. shulli, H. phlomoides var roxburghii*
Bhat K G	Flora of Udupi (2003)	2 spp: *H. schulli, H. ringens*
Suryanarayana B & Rao AS	Nellore (2002)	3 spp: *H. auriculata, H. balsamica, H. salicifolia*
Almeida	Flora of Maharashtra (2003)	8 spp: *H. anomala, H. erecta, H. pinnatifida, H.polysperma, H.schulli, H.ringens, H. serpyllum, H. stocksii* 1 var: *H.serpyllum* var *hookeriana*
Kumar A	Flora of Indravati Tiger Reserve (2003)	3 spp: *H. auriculata, H. balsamica, H. polysperma*

Bora PJ & Kumar Y	Floristic Diversity of Assam, Study of Pabitora WLS (2003)	2 spp: *H. polysperma. H. salicifolia*
Manilal KS	*Hortus Malbaricus* (Eng.), Vol. 2. (2003)	2 spp: *Bahel schulli, Nir schulli*
Manjunatha BK, Krishna V & Pullaiah T	Flora of Davanagere District (2004)	1 spp: *H. auriculata*
Sasidharan N	Biodiversity Documentation for Kerala (2004)	3 spp: *H. schulli, H. ringens, H. triflora*
Paria ND and Chattopadhyay SP	Hazaribagh District (2005)	4 spp: *H. auriculata, H. difformis, H. polysperma, H. salicifoia*
Kumar AN, Sivadasan M, Ravi N	Flora of Pathanamthitta. Western Ghats. Kerala. (2005)	2 spp: *H.schulli, H. ringens*
Pradhan SZG, Sharma BD & Singh NP	Sanjay Gandhi NP (2005)	5 spp (2 var): *H. phlomoides var roxburghii, H. polysperma, H. ringens, H. schulli, H. serpyllum, H. serpyllum var hookeriana*
Nayar T S, Beegam AR, Mohanan N, Rajkumar G	Flowering Plants of Kerala, A Hand Book (2006)	5 spp: *H. balsamica, H. heinei, H. ringens, H. schulli, H. triflora*
Simgh H & Sharma M	Chamba district (2006)	1 spp: *H. polysperma*
Eds. GS Giri, Pramanik KA, HJ Chowdhery	Materials for the flora of Arunachal Pradesh	4 spp: *H.erecta, H. phlomoides, H. polysperma, H. salicifolia,*
Kshirsagar S R & Patil D A,	Flora of Jalgaon District, Maharashtra (2008)	2 spp: *H.schulli, H.serpyllum*
Bhagat RB, Shimpale VB	Flora of Baramati (2008)	1 spp: *H. schulli*
Rao GVS & Kumari GR	Vishakhapattanam (2008)	3 spp: *H.auriculata, H.heinei, H.salicifoia*
Karthikeyan S, Sanjappa M, Moorthy S	Flowering Plants of India, Dicotyledons (Acanthaceae to	18 spp: *H. anomala, H. balsamica, H. difformis, H. erecta, H. griffithii, H. heinei, H. incana, H. madurensis, H. parishii, H. phlomoides H. pinnatifida, H. polysperma, H. pusilla, H. ringens, H. serpyllum, H. sculli,*

	Avicinniaceae) (2009)	*H. stocksii, H. triflora*
Remadevi S, Binojkumar M S	Contribution to the Flora of Kerala: The Family Acanthaceae (2009)	5 spp: *H. balsamica, H. difformis, H. erecta, H. salicifoia, H. schulli*
Sunil C N & Sivadasan M	Flora of Alappuzha District, Kerala, India (2009)	3 spp: *H. schulli, H. ringens, H. triflora*
Saini DC, Singh SK & Rai K	Biodiversity of aquatic and semi aquatic plants in eastern UP (2010)	3 spp: *H. auriculata, H. difformis, H. pinnatifida*
Punekar SA & Lakshminarasimhan	Anshi NP (2011)	1 spp: *H. pinnatifida*
Sinha GP, Singh DK & Singh KP	Mizoram (2012)	1 spp: *H. ringens*
Pal GD	Flora of Lower Subansiri District, Vol-2, Arunachal Pradesh (2013)	1 spp: *H. phlomoides*
Datar MN & Lakshminarasimhan P	Bhagwan Mahavir (Molem) NP & its adloinings, Goa (2013)	3 spp: *H. schulli, H. ringens, H. pinnatifida*
Manikandan R & Lakshminarasimhan P	Rajiv Gandhi NP, Karnataka (2013)	2 spp: *H. schulli, H. ringens*
S P Gaikwad K U Garad	Flora of Solapur District (2015)	2 spp: *H. schulli, H. ringens*
Pullaiah T	Telangana (2015)	2 spp: *H. schulli, H. polysperma*
Ansari R, Jeeja G, Prakashkuar R	Aquatic and Wetland Flora of Kerala: Flowering Plants (2016)	4 spp: *H. balsamica, H. difformis, H. ringens, H. schulli*
Jayanthi J	Campbell bay national park, Great Nicobar (2017)	1 spp: *H. erecta*
Karthigeyal K	Angiosperm Diversity of Mahatma Gandhi Marine National Park, S.	1 spp: *H. erecta*

	Andaman (2017)	
Singh PB	Flora of Mandi District, Himachal Pradesh (2018)	1 spp: *H. polysperma*
P Lakshminarasimhan *et al.*	West Bengal (2019)	6 spp: *H. heinei* var *birbhumensis, H. schulli, H. phlomoides, H. polysperma, H. ringens, H. triflora*
Singh P *et al.*	Plants of Indian Himalayan Region (2019)	5 spp: *H. auriculata, H. erecta, H. phlomoides, H. polysperma, H. ringens*
Karnataka BDB	Karnataka Flora Checklist2019)	8 spp: *H. balsamica, H. erecta, H. incana, H. pinnatifida, H. polysperma, H. ringens, H. schulli, H. serpyllum*
Sinha GP & Shukla AN	Uttar Pradesh (2020)	7 spp: *H. auriculata, H. difformis, H. erecta, H. salicifolia, H. pinnatifida, H. polysperma, H. serpyllum*
Gogoi *et al.*	Fl. Sikkim- A pictorial guide (2021)	3 spp: *H. auriculata*, *H. phlomoides, H.polysperma*
Narasimhan d & Irwin SJ	Flowering plants of Tamil Nade: A compendium (2021)	6 spp: *H. auriculata, H. balsamica, H. erecta, H. heinei, H. madurensis, H. ringens,*
Chowdhury M & Chowdhury A	Wetland Flora of West Bengal (2022)	7 spp: *H. auriculata, H. difformis, H. erecta, H. phlomoides, H. polysperma, H. ringens, H. quadrivalvis*

Table 1: Treatment of *Hygrophila* species in various state & regional floras

Molecular Phylogeny

The plasticity of plant morphologies and perplexing diversity have encouraged plant systematists to seek for more credible characters to use in evolutionary reconstruction. Phylogenetic reconstruction can often provide a basis for interpreting evolution and biogeography. The rapid advancement of DNA sequencing and polymerase chain reaction (PCR) technologies opens a multitude of questions and new opportunities for the study of plant molecular evolution and systematics. DNA Sequence data can infer phylogenies without making any assumptions about relationships (Kapli *et al*., 2023) since they are independent of other biological characters. By analyzing the similarities and differences in DNA sequences, molecular systematics can help researchers determine the evolutionary history of different groups of organisms and shed light on their origins, diversification, and relationships.

The development of polymerase chain reaction (PCR) and the emergence of DNA-based markers have equipped plant taxonomists with straightforward and dependable methods for examining the extent and distribution of variation within species' gene pools. This has allowed them to address evolutionary and taxonomic questions that were previously unresolvable with phenotypic methods alone. PCR-based DNA marker systems encompass Random Amplified Polymorphic DNAs (RAPDs), Amplified Fragment Length Polymorphisms (AFLPs), Microsatellites/Simple Sequence Repeats (SSRs), and Single Nucleotide Polymorphisms (SNPs). These techniques are widely accepted and their advantages and limitations have been listed (Agarwal *et al*., 2008; Primmer, 2009).

An earlier approach to inferring phylogenetic divergence employed morphological, cytological, and isozyme analyses. Molecular systematics has emerged as an indispensable tool for analyzing evolution, thereby having an enormous impact on our comprehension of the tree of life.

Molecular phylogenetics is a branch of evolutionary biology that uses molecular data, particularly DNA and RNA sequences, to infer the evolutionary relationships between different organisms or genes. This is done by combining molecular biology tools and statistical methods. By comparing the sequences of these molecules among various taxa, scientists can understand how closely or distantly related they are.

Some of the key techniques used in molecular systematics include DNA sequencing, phylogenetic analysis, and molecular clock analysis. DNA sequencing involves determining the sequence of nucleotides in a segment of DNA, which can then be compared to sequences from other organisms to infer evolutionary relationships. Phylogenetic analysis involves constructing a tree of life based on the similarities and differences between DNA sequences, while molecular clock analysis uses the rate of mutation to estimate the timing of evolutionary events.

Overall, molecular systematics has transformed our comprehension of life's evolution on Earth and has enabled us to reconstruct the evolutionary relationships between organisms with increasing accuracy and precision.

DNA Barcoding

DNA barcoding is an innovative method for identifying, characterizing, and classifying biological specimens or species by using a short, conserved DNA sequence from an organism's nuclear or organelle genome. Using DNA sequencing data, DNA barcoding identifies or "barcodes" an organism by identifying particular genetic regions in it and distinguishing it from other organisms.

DNA barcoding has significantly influenced biodiversity science. The DNA barcoding notion introduced by Hebert *et al.* (2003) marked a significant advancement in the field of DNA-based species identification. The method employed global community endeavours to build extensive public reference libraries, which enabled the accurate identification of species over wide areas of the living world. The selection of the standard barcodes has proven more difficult in plants. The application of COI was prohibited by the low replacement rates of plant mitochondrial DNA (Wolfe *et al.*, 1987). Consequently, two plastid DNA areas were selected through investigation into alternate barcoding regions: a 600 bp segment of the rbcL gene and an 800 bp fragment of the matK gene. It was suggested that these be complemented with trnH-psbA (Hollingsworth *et al.*, 2009) and the internal transcribed spacers (ITS) of the nuclear ribosomal DNA (Hollingsworth *et al.*, 2011; Li *et al.*, 2011).

It is possible to identify unknown species by storing the genetic barcodes in a digital library (Zahra *et al.*, 2016). Identification and biodiversity classification have improved since the advent of DNA barcoding (Hebert & Gregory, 2005). The fundamental idea of DNA barcoding is rather straightforward: differentiating biological entities based on differences in molecular markers (Khan *et al*., 2020).

Molecular Markers

In the realm of genetics, the advent of molecular markers has revolutionized our understanding of heredity, evolution, and biodiversity. These tools serve as molecular signposts, revealing intricate details about the genetic makeup of organisms. Molecular markers are DNA sequences with a known location which are easily detectable and whose inheritance can be easily traced. Molecular markers are employed based on naturally occurring DNA polymorphism, which serves as the foundation for developing strategies to take advantage of certain applications. It appears that DNA markers are the most promising options for effectively assessing and selecting plant material. DNA markers are not impacted by the environment and segregate as single genes, in contrast to protein markers. The first of these DNA markers to be used are restriction digestion fragments, namely the restriction fragment length polymorphism (RFLP) based gene marker.

The practice of molecular markers is becoming extensive for the identification of genotypes and also for quantifying the degree of genetic variation in a given population. Polymerase chain reactions (PCRs) have opened the door to an ever-expanding range of newer techniques (Saiki *et al*., 1988). PCR enables specific amplification of DNA sequences making it suitable for the identification of plant genotypes. Amplification of a genotype can benefit from some of the many characteristics of PCR like speed, simplicity, specificity, sensitivity and cost (Henry, 1997).

The first documented molecular marker technique for identifying DNA polymorphism was demonstrated by Botstein *et al*. (1980) in their work on the preparation of genetic maps using restriction fragment length polymorphism (RFLP). Many molecular approaches are currently available to identify variations between source plants and somaclones, as well as sequence variance between closely related organisms. Various molecular detection methods are employed in the analysis of the genetic diversity of plants, including RFLPs, isozyme, cytological techniques, and polymerase chain reaction-based techniques, including microsatellite markers, amplified fragment length polymorphism (AFLP), and random amplified polymorphic DNA (RAPD) (Bairu *et al*., 2011).

Variations in the coding and spacer regions of many chloroplast, mitochondrial and nuclear gene sequences are employed in many studies. The two most frequently used genes are coding ones called ribulose-bisphosphate carboxylase gene (rbcL) and MaturaseK gene (matK). Internal transcribed spacers (ITS) of the ribosomal DNA are also utilized to analyse the evolutionary relationships between different plant species. (Chase *et al*., 1993; Manhart, 1994; Alvarez and Wendel, 2003; Probojati *et al*., 2021).

Sequence data are cumulative, and the potential dimensions of informative data sets are vast. These data are accessible in public domains such as the National Center for Biotechnology Information (NCBI). Numerous academics and taxonomists have utilized and analyzed these data sequences to evaluate plant systematics and phylogeny at levels above the family or even genus (Donoghue *et al*., 1992; Duvall *et al*., 1993; German *et al*., 2009).

Allozyme Markers

Allozyme markers are a type of biochemical genetic marker based on the electrophoretic variation of enzymes encoded by different alleles at a locus. It represents different molecular forms of an enzyme catalyzed by the alleles of the same gene. These markers have been widely used in various fields of biological research, particularly in population genetics, evolutionary biology, and conservation genetics (Hoey *et al*., 1996; Krauss &Hopper, 2001).

Microsatellites or Simple Sequence Repeats (SSRs)

Microsatellites or simple sequence repeats (SSRs), are tandemly repeated DNA sequences known for their high variability and codominant inheritance (Ellegren, 2004). Microsatellite markers find applications in population genetics, forensics, and parentage analysis due to their polymorphic nature and ability to reveal fine-scale genetic structure (Selkoe & Toonen, 2006). However, their development can be labour-intensive, and issues such as null alleles and stutter bands may affect data interpretation (Dakin & Avise, 2004). SSRs are widely used for population studies, e.g., to evaluate genetic variation or homozygosity (Mukherjee *et al*., 2016; Li *et al*., 2022).

Restriction Fragment Length Polymorphism (RFLPs)

RFLP is a molecular technique used to identify variations in DNA sequences. It involves the use of restriction enzymes, which are proteins that can cut DNA at specific sequences, known as restriction sites. By comparing the lengths of the resulting DNA fragments after digestion with restriction enzymes, researchers can detect genetic differences among individuals. RFLP are broadly employed in genome mapping, genotyping, DNA fingerprinting, mapping of genes, and diagnosis of genetic disorders (Nam *et al*., 1989; Gawel *et al*., 1992).

Random Amplified Polymorphic DNA (RAPD)

Random Amplified Polymorphic DNA (RAPD) is a type of polymerase chain reaction (PCR) technique that amplifies random segments of genomic DNA using short, single primers of arbitrary nucleotide sequence. Introduced by Williams *et al.* in 1990, RAPD has become a popular method for genetic analysis due to its simplicity, cost-effectiveness, and ability to generate numerous polymorphic markers without prior knowledge of the genome being studied. RAPD markers are extensively used to assess genetic diversity within and between populations (Ismail *et al*., 2016), phylogenetic analysis (Lahshmi *et al*., 1997), breeding and Germplasm Characterization (Clarke and Bevan, 1997) etc.

Amplified Fragment Length Polymorphisms (AFLPs)

Amplified Fragment Length Polymorphisms (AFLPs) are a powerful DNA fingerprinting technique used in molecular genetics for the detection of genetic variation. Developed by Vos *et al*. in 1995, AFLPs combine the advantages of Restriction Fragment Length Polymorphism (RFLP) and Polymerase Chain Reaction (PCR) technologies, providing a highly reproducible and sensitive method for DNA analysis. Amplified Fragment Length Polymorphisms (AFLPs) remain a valuable tool in molecular genetics, offering high resolution and reproducibility for genetic analysis. AFLPs have been widely applied in various fields such as genetic mapping (Freyre *et al*., 1998), population genetics (Russell *et al*., 1997), phylogenetics (Rieseberg *et al*., 1999), and biodiversity studies (Prentice *et al*., 2003).

Inter Simple Sequence Repeat Markers (ISSR)

Inter Simple Sequence Repeat (ISSR) markers are a type of molecular marker used extensively in genetic research for their high reproducibility, cost-effectiveness, and simplicity. ISSR markers amplify regions between simple sequence repeats (SSRs), which are abundant throughout the genome. One of the primary advantages of ISSR markers is their ability to generate a high number of polymorphic loci, which is crucial for genetic diversity studies. ISSR markers provide a rich source of polymorphisms that are useful for distinguishing between closely related individuals and populations (Gupta *et al*., 1999). They have found applications in various fields, such as genetic diversity studies, phylogenetic analysis, and gene mapping. In plant genetics, ISSR markers have been widely utilized to assess genetic diversity, population structure, phylogenetic relationships and gene mapping and gene mapping (Fang and Roose, 1997; Reddy *et al*. (2002).

Internal Transcribed Spacers (ITS)

The ITS, nuclear ribosomal DNA (nrDNA) sequences hold significant utility in phylogenetic reconstruction and species delimitation (Álvarez & Wendel, 2019). ITS markers, comprising ITS1, 5.8S rRNA, and ITS2, exhibit both conserved and variable regions, allowing for discrimination at various taxonomic levels (Johnson et al., 2018). The ITS region separating 18S and 28S nrDNA and the 5.8S coding 19 sequences have been extensively studied across the inter-specific and inter-generic level divergence. The high copy number permits successful amplification of the region from the total DNA (Baldwin *et al*., 1995). Additionally, the availability of universal primers facilitates their widespread use across diverse taxa (Brown *et al*., 2022).

MaturaseK (matK)

The matK gene, previously known as orfK, is another widely used marker in plant molecular systematic and evolutionary studies (Johnson and Soltis, 1994; Steele and Vilgalys, 1994; Kathriarachchi *et al*., 2005). This gene, with approximately 1500 base pairs (bp), is located within the intron region of the chloroplast gene trnK, on the large single-copy section adjacent to the inverted repeat. The matK gene has been effectively used to address systematic problems in families such as Saxifragaceae (Johnson and Soltis, 1994), Polemoniaceae (Steele and Vilgalys, 1994), and Poaceae (Liang and Hilu, 1996).

Ribulose-bisphosphate Carboxylase Gene (rbcL)

Chloroplast DNA (cpDNA) is extensively used to understand plant phylogeny across different taxonomic groups (Clegg and Zurawski, 1992). Direct gene sequencing of polymerase chain reaction (PCR) products has rapidly expanded in the field of plant systematics and evolution. The rbcL gene, which encodes the large subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO), has been sequenced from numerous plant taxa and is extensively utilized in plant phylogeny studies (Palmer *et al*., 1988; Chase *et al*., 1993).

Molecular Studies in Acanthaceae

The Acanthaceae family has gained substantial taxonomic insight at familial, subfamilial, tribal, and subtribal levels. Over the past two decades, phylogenetic understanding of this large plant family has significantly advanced, revealing several major lineages that have been the focus of subsequent investigations. Molecular systematic studies addressing higher-level systematics of Acanthaceae have utilized genes such as rbcL, ndhF, trnL-trnF, and a combination of trnL-trnF with ITS (Hedren *et al*., 1995; Scotland *et al*., 1995; McDade *et al*., 1999 & 2000a). Although re-analysis of rbcL data provided limited resolution within Acanthaceae, the strict consensus tree confirmed Acanthaceae sensu as a group. These analyses showed no support for separating *Thunbergia* Retz. Acanthaceae, with the exception of *Elytraria* Michx. (Nelsonioideae), which remained unresolved.

Hedrén *et al*. (1995) studied the relationships in Acanthaceae and related families using rbcL nucleotide sequences. Parsimony analysis of the rbcL sequences largely agreed with Bremekamp's (1965) classification of the Acanthaceae, and Thorne (1992) supported Nelsonioideae as a sister group to the rest of the Acanthaceae. The examination of branch support indicated a weak upholding of several branches across the Bignoniales.

Scotland *et al*. (1995) undertook the work on phylogeny of the family Acanthaceae. He undertook a systematic study of Acanthaceae employing evidence from both morphological and molecular data. For the molecular study, they used the DNA sequence data from two chloroplast regions- ndhF and rbcL. They sampled 15 species and 9 outgroups of Acanthaceae for ndhF and 12 species and the same 9 outgroups for rbcL. Parsimony analyses of ndhF sequences were conducted with 15 species of Acanthaceae 9 putative representative outgroup species, while the parsimony analyses of rbcL sequences included 12 species of Acanthaceae and the same 9 outgroup species. Their results indicated that ndhF sequences provide more informative characters and greater systematic resolution at the hierarchical level compared to rbcL sequences. In comparison to 136 informative positions in rbcL sequences, a total of 421 nucleotide positions were found to be highly informative for ndhF sequences. The strict consensus cladogram of ndhF sequences proved that *Elytraria* and *Thunbergia* were successive sister taxa to the remaining Acanthaceae taxa. In addition, these data also demonstrated that taxa with both retinacula and explosive fruits could be further subdivided into two monophyletic groups that conform to taxa with and without cystoliths. Whereas the rbcL analysis resulted in less systematic resolution than ndhF and the cladogram did not support monophyletic Acanthaceae or Acanthoideae. It included a number of congruent arrangements of taxa within Acanthaceae and moreover, relationships within Acanthaceae are mostly unresolved. In this systematic analysis, the genus *Hygrophila* was represented by a single species (*H. corymbosa* (Blume) Lindau. In the parsimonious cladogram of both sequences, *Hygrophila* was grouped with the species of *Brillantaisia* P.Beauv.

McDade and Moody (1999) carried out a phylogenetic relationship among Acanthaceae based on sequence data from the intron and spacer of the trnL-trnF chloroplast region. Their results indicated that this chloroplast region should only be used to address the phylogenetic issued within the family but not within the genera. This study also not supported the monophyly of Acanthaceae but supported the monophyly of the *Ruellia* lineage, within which the genus *Hygrophila* is included.

McDade *et al*. (2000a) investigated the phylogenetic relationships within the Acanthaceae family using sequence data from the nuclear ribosomal internal transcribed spacer (nr-ITS) region, both alone and combined with data from the intron and spacer of the trnL-trnF chloroplast (cp) region. Their results revealed that the substitution rate in the nr-ITS region was nearly twice that of the trnL-trnF cp region, and there was no incongruence between the ITS and trnL-trnF results. Their study strongly supported the monophyly of Acanthaceae *s.s.* with the presence of four major lineages (*Acanthus*, *Barleria*, *Ruellia* and *Justicia* lineages). The genus *Hygrophila* comes within the *Ruellia* lineage and the consensus parsimonious tree links *Justicia* and *Ruellia* lineage as sister taxa.

McDade *et al*. (2000b) studied the phylogenetic analysis of tribe Justicieae (Acanthaceae), based on the data obtained from molecular, morphological and cytological studies. They employed DNA sequence data from the ITS of the nuclear ribosome and the intron and spacer of the trnL-trnF chloroplast region for the molecular studies. A total of 55 taxa were considered for phylogenetic analysis (49 species of Justicieae and 6 outgroups). Parsimony analyses revealed that the topologies derived from the two loci were congruent. Their result strongly supported the monophyly of tribe Justicieae.

Scotland & Vollensen (2000) put forth a classification of Acanthaceae based on previous molecular systematic studies (Hedren *et al*., 1995; Scotland *et al*., 1995; McDade & Moody 1999; McDade *et al*., 2000a), pollen morphology, corolla aestivation and other potentially informative morphological homologues.

Moylan *et al*. (2004) explored the classification of the Strobilanthinae using a morphologically and geographically distinct sample of taxa using molecular and morphological data. Two regions viz. nuclear (ITS) and chloroplast (trnL-F) were used for the analysis. Parsimony and maximum likelihood analyses of trnL-F demonstrated that the Strobilanthinae form a monophyletic group, whereas parsimony analysis of ITS indicated a non-monophyletic subtribe. However, maximum likelihood analysis of ITS supported the trnL-F findings and suggested that systematic error was affecting the ITS parsimony analysis. When combining ITS and trnL-F data, the analysis resolved a monophyletic subtribe. Consequently, the authors proposed a single expanded genus, *Strobilanthes* sensu lato, at the level of the well-supported and monophyletic Strobilanthinae.

For the analysis of the phylogenetic relationships within the tribe Acantheae (Acanthaceae), McDade *et al*. (2005) used the sequence data from nuclear region (nrlTS) and three chloroplast regions (rpsl6 intron, trnG-S spacer, and trnL-F intron and spacer). Maximum parsimony and Bayesian phylogenetic inference methods were employed in the study, and their results were entirely congruent and provided considerable resolution and support for relationships. The study strongly supported the monophyly of two sub-lineages ‘one-lipped corolla’ and ‘two-lipped corolla’ lineages) within Acantheae, revealing remarkable variations in the morphology of corolla. Unlike the one-lipped lineage, most genera in the two-lipped lineage were not monophyletic, and their relationships lacked strong support from molecular data or morphological characteristics. However, the basal lineages of both one- lipped and two-lipped groups prevented a more precise determination of the ancestral range. An overview of the biogeography of the Acantheae was also presented by the authors and their results pointed to an Old-World distribution for the common ancestor of Acantheae.

Tripp (2007) examined evolutionary relationships among species within the extensive genus *Ruellia* using molecular data from 196 samples sourced from the nuclear ribosomal ITS region and the chloroplast trnG-trnR region. Their objectives included assessing the monophyly of *Ruellia* compared to closely related genera in Ruellieae, reconstructing global phylogenetic relationships among *Ruellia* species, reassessing previous morphology-based classifications, and evaluating the effectiveness of morphological characters, particularly corolla morphology, for future sectional delineation. Bayesian and parsimony analyses indicated that four genera were phylogenetically linked to *Ruellia*. While *Ruellia s. l.*, incorporating *Blechum* P.Browne, *Eusiphon* Benoist, and *Polylychnis* Bremek., appeared monophyletic, support for this grouping was weak under parsimony. Their investigation encompassed two *Hygrophila* species (*H. brasiliensis* (Spreng.) Lindau and *H. costata* Nees & T.Nees), which were found to be monophyletic. Within *Ruellia*, Old World (OW) taxa formed a basal grade, with New World (NW) taxa forming a monophyletic group nested within the OW grade. Alternative hypotheses, such as the non-monophyly of NW *Ruellia*, were significantly less parsimonious and less plausible. Several clades within NW *Ruellia* were informally recognized, some aligning with previous taxonomic groupings to varying extents. The study concluded that corolla morphology has undergone convergent evolution, rendering it an unsuitable trait for sectional delineation, contrary to earlier assumptions.

McDade *et al.* (2008) examined the Acanthaceae lineages and their evolutionary relationships, utilizing molecular and morphological data to provide a clear understanding of the the lineages. Parsimony and Bayesian methods were used for analysis. DNA sequence data from the nuclear ITS gene and four chloroplast noncoding regions (trnL-F, rps16, trnS-G, and trnT-L) were used for molecular analysis. They sampled as many genera of uncertain relationship as possible as well as members of all known lineages of Acanthaceae, including Andrographideae. They examined the nuclear and chloroplast data independently, since the combined chloroplast and nrITS data presented a considerable discrepancy. Nearly all elements of the evolutionary framework of Acanthaceae were resolved with strong support, as well as the inclusion of Andrographideae sister to Barlerieae and the inclusion of the black mangroves (*Avicennia* L.) in the family Acanthaceae. *Ruellia* lineage was represented by 4 genera such as *Hygrophila* (*H. corymbosa*), *Strobilanthes*, *Sanchezia* and *Ruellia*. Ruellieae and Justicieae are each strongly supported as monophyletic and are sister taxa. Notably, no nonmolecular synapomorphies have been suggested for Ruellieae + Justicieae lineage. The results also suggested an Old-World origin with multiple dispersal events to the New World.

Borg *et al*. (2008) inferred extensive evolutionary relationships within the subfamily Thunbergioideae (Acanthaceae) and among main lineages of the family as a whole based on nucleotide sequences from three chloroplast DNA regions (rps16, rpl16, and trnT-trnL). The monophyletic status of Thunbergioideae was confirmed by Bayesian and Parsimony analyses. Relationships within the two largest genera, *Mendoncia* and *Thunbergia*, were substantially resolved, and most branches were strongly supported. Phylogenetic relationships also revealed that a twining nature is ancestral to the genus *Thunbergia*.

A study on phylogenetic placement, delimitation, and relationships among genera of the mysterious Nelsonioideae of the family Acanthaceae was conducted by McDade *et al*. (2012). Nelsonioideae's monophyly is supported by phylogenetic analysis of a molecular dataset (ndhF + trnL-F) for a wide sample of Lamiales. The clade is placed as sister to a lineage comprised of all other plants treated as Acanthaceae, with strong support. According to their findings, compared to other Acanthaceae lineages, Nelsonioideae has a convoluted history of intercontinental dispersals.

Tripp *et al*. (2013) investigated the phylogenetic relationships within the pantropical tribe Ruellieae using morphological and molecular data. The authors tested the placement of 48 genera in Ruellieae and explored the monophyly of currently recognized taxa using the molecular data from two nuclear (ITS+5.8S, Eif3E) and three chloroplast (trnG-trnR, trnG-trnS, psbA-trnH) markers. Bayesian and likelihood analyses were carried out to check the monophyly of Ruellieae. They were able to sample all but four of 48 genera, resolving all within Ruellieae except *Zygoruellia*. They identified seven major clades within Ruellieae and the existence of several monospecific and oligospecific genera nested within clades of larger genera. Among the seven subtribes, Erantheminae was the earliest diverging clade. A number of new generic synonyms were also proposed. The subtribe Hygrophilinae contains only two genera: *Hygrophila* and *Brillantaisia*. The genus *Hygrophila* was represented by 7 species viz. *H. cataracte* S.Moore, *H. pilosa* Burkill, *H. schulli* M.R.Almeida & S.M. Almeida, *H. costata* Nees & T.Nees, *H. difformis* (L.f.) Blume, *H. didynama* (Lindau) Heine and *H. salicifolia* (Vahl) Nees. Using a much-expanded taxon sample, their phylogenetic analyses revealed a close relationship between *Brillantaisia* and *Hygrophila*. The results demonstrated that *Hygrophila* is nonmonophyletic, with *Brillantaisia* being derived from and monophyletic within it. The species of *Hygrophila* formed two clades: one early diverging within Hygrophilinae, and one as a sister to *Brillantaisia*. Only the latter clade and its sister relationship with *Brillantaisia* were strongly supported. The three species in the second clade (*H. cataracte, H. pilosa*, and *H. schulli*) are African. In the other clade, *H. costata* and *H. difformis* are from the New World (NW), while *H. didynama* is African and *H. salicifolia* is Asian. The authors also identified four-colporate pollen grains and unappendaged anthers as diagnostic features for the strongly supported Hygrophilinae. Based on phylogenetic analyses, the authors attempted to reconstruct the geographical distribution histories of the various lineages within Ruellieae, noting that the subtribe Hygrophilinae occurs in both the Old World (OW) and New World (NW).

Tripp & McDade (2014) sought to reconstruct divergence times within Acanthaceae using fossils for calibration. To estimate phylogenetic relationships and divergence times, they utilized DNA sequence data from seven markers: one nuclear (ribosomal ITS+5.8S) and six chloroplast markers (intron: rps16; spacers: psbA-trnH, trnG-trnR, trnS-trnG, trnL-trnF, trnT-trnL). Most of the molecular data used were generated in previous studies (McDade *et al*., 2005; Tripp, 2007; McDade *et al*., 2008; Tripp, 2010), except for several sequences of trnG-trnR and psbA-trnH, and a few sequences of rps16, trnT-trnL, and trnL-trnF. The final dataset included 70 taxa (including one species of *Hygrophila*) from various Acanthaceae lineages, along with 2 outgroups. To determine the most appropriate placement for fossil constraints in their divergence time analyses, the authors first assessed each fossil for its phylogenetic affinity based on extensive study of the family. Their results indicated that long-distance dispersal events better explain the current distributions of Acanthaceae than the Gondwanan and northern land bridge hypotheses. The authors inferred at least 16 disjunction events in Acanthaceae *s.l.* between the Eastern and Western Hemispheres. Of these dispersal events, 13 were in the OW to NW direction, and one in the reverse direction (NW to OW). The other two were not able to differentiate because of cosmopolitan distributions of species.

Delimitation and phylogenetic relationships to test the monophyly of *Mirandea* Rzed. clade within *Tetramerium* Nees Lineage of tribe Justicieae were investigated by Kiel & McDade (2014), using DNA sequence data from two nuclear (nrITS and ncpGS) and three chloroplast (trnS-G, ndhF-trnL and trnT-L) regions. The authors presented micromorphological data from seeds and pollen to discuss the evolution of the traits. The results obtained from MP, ML, and Bayesian analyses were congruent and strongly supported the monophyly of the *Mirandea* clade.

Abdel-Hameed *et al*. (2014) analyzed the phylogenetic relationships among 29 Egyptian species of the family Acanthaceae using morphological data, including macromorphology, stomatography, and lamina architecture. Parsimony analysis, conducted with WinClada-NONA 1.6 software and based on 55 potentially informative morphological characters, indicated that these morphological criteria are likely useful for addressing phylogenetic questions among genera. The phylogenetic analysis provided strong support for the monophyly of the *Justicia*, *Pseuderanthemum*, and *Ruellia* lineages.

Abdel-Hameed *et al*. (2015) investigated the phenetic relationships and the role of morphological and molecular characters in the systematics of Acanthaceae. The study involved 30 species of Egyptian Acanthaceae from 17 genera, following a preliminary screening of 20 ISSR markers. Characters of macro micromorphology, leaf anatomy, and stomata studies were used for morphological investigations and for molecular investigations, five ISSR primers were selected. UPGMA method was used to find the appropriate character states among species. Using NTSYS-PC version 2.02 software, the phenetic analysis was based on 55 potentially informative morphological and molecular characters. The results suggested that both morphological and ISSR criteria are significant taxonomic traits. The analysis produced a phenogram that grouped the species into two series and highlighted the distinct segregation of the genus *Avicennia* from Acanthaceae.

Gao *et al*. (2019) determined the complete chloroplast genomes of four *Echinacanthus* Nees species from the tribe Ruellieae. Their findings revealed that all chloroplast genomes formed a circular structure, each comprising 113 unique genes, including 80 protein-coding genes, 29 tRNAs, and 4 rRNAs. Comparative analysis of the four chloroplast genomes indicated greater divergence between geographic groups. The six most divergent sequences identified were rrn16, ycf1, ndhA, rps16-trnQ (UUG), trnS (GCU)-trnG (UCC), and psaA-ycf3. Phylogenetic analysis using Bayesian and parsimony methods did not support the monophyly of *Echinacanthus*, although the relationships among the four species were clearly resolved.

Khan *et al.* (2020) investigated the phylogeny of the five different genera of Acanthaceae genera of Acanthaceae based on 34 macromorphological characters and three chloroplast markers (rbcL, matK and trnH-psbA). The molecular phylogenetic tree was constructed using sequences from NCBI GenBank in combination with the newly sequenced data. For morphological analysis, a tree was constructed using the UPGMA method in MVSP, based on 34 macro morphological traits of the selected species. The UPGMA analysis revealed two major clades, with *Ruellia* and *Justicia* grouped together in a single clade, consistent with phylogenies derived from flower and seed characters separately. The molecular data indicated that the genera *Barleria*, *Strobilanthes*, and *Ruellia* are monophyletic, but the genus *Justicia* is non-monophyletic. Results also supported the close relationship of *Ruellia* with *Hygrophila*. The authors concluded that rbcL is a more effective barcode, while the combination of matK and trnH–psbA could be employed for the identification and phylogenetic analysis of plants.

Kaewdaungdee (2022) conducted chloroplast genome sequencing of five *Barleria* species in Thailand for the purposes of species identification and authentication. The analysis included a short nucleotide sequence of genes, three plastid intergenic spacers (trnS-G, ndhF-rpl32-trnL), and the nuclear region (ITS). Phylogenetic studies were performed using maximum likelihood (ML) and Bayesian inference (BI) methods. The resulting phylogenetic tree showed that all *Barleria* species studied clustered within a single clade, with the outer outgroup clearly distinguished. The phylogenetic relationships among *Barleria* species were well-resolved, and the genomic data supported the species' morphological characteristics.

Molecular Studies in the Genus *Hygrophila*

Huang *et al*. (2001) examined population differentiation and phylogeography of *Hygrophila pogonocalyx* in two biogeographic regions of Taiwan using RAPD fingerprints. Samples were collected from northeastern and western Taiwan, which are divided by the central mountain range. Gene flow between populations is limited due to the migratory capacity of pollinators, primarily honeybees. Out of 50 primers tested, 25 produced 158 polymorphic bands between populations. The study found that genetic variation followed an "isolation by distance" model based on random RAPD primers. Hierarchical AMOVA analyses showed significant difference between geographical regions (93.4%), among populations (94.5%), and among populations within regions (16.9%). The authors suggested that the differentiation of geographic populations is likely due to long-term isolation since the formation of the Central Mountain Range around 1 million years ago. They also proposed that *H. pogonocalyx* may have developed genetic mechanisms to avoid selfing and that somatic mutations could help maintain variability within small populations.

Huang (2005) studied the genetic variation in the atpB–rbcL intergenic spacer region of chloroplast DNA (cpDNA) in *Hygrophila pogonocalyx* Hayata (Acanthaceae), an endangered species endemic to Taiwan. In this aquatic plant, seed dispersal from capsules is limited by gravity, leading to restricted gene flow within populations. The study sequenced 849 base pairs of the cpDNA atpB–rbcL spacer from eight populations of *H. pogonocalyx* and found low nucleotide diversity. Genetic variation among populations fit an "isolation-by-distance" model, with two geographically correlated groups, western and eastern, identified in a neighbor-joining tree and a minimum-spanning network. Phylogeographical analyses suggested that the differentiation between these groups resulted from historical fragmentation, likely due to the formation of the Central Mountain Range around 5 million years ago, aligning with molecular clock estimates of cpDNA.

Mukherjee *et al*. (2016) investigated the genetic diversity of *Hygrophila polysperma* using microsatellite and chloroplast DNA in both its native (India and Bangladesh) and invasive regions (Australia, Germany, Mexico, and the US) as part of a biocontrol initiative. They sampled 328 individuals and developed *Hygrophila*-specific microsatellite primers using next-generation 454 GS-FLX sequencing technology. These primers were selected for their ability to amplify *Hygrophila* DNA and produce clear, consistent profiles. The study found that the invasive populations were nearly genetically identical, indicating that they likely originated from a single clonal lineage. The lack of genetic variation and historical data suggested that *Hygrophila* was first introduced to the US, which then served as the source for its spread to Mexico and Australia.

Chapter 4

MATERIALS AND METHODS

Literature Survey

Relevant literature and data pertained to the present work were gathered from several resources such as institutional libraries and online data retrieval systems like JSTOR, Elsevier, Science Direct, Wiley, Springer link, UGC-INFONET Digital Library Consortium, INFLIBNET Centre (Information and Library Network Centre), Database of Indian Dissertation and PhD thesis repository, etc. Literature from institutional libraries was accessed directly or by personal communication. The literature retrieval systems of the Biodiversity Heritage Library (BHL) of the New York Botanical Garden (http://www.biodiversitylibrary.org), digitized historic botanical literature from the Missouri Botanical Garden Library. (http://botanicus.org), 'Hathi Trust Digital Library' (http://www.hathitrust.org), online libraries like JSTOR (https://www.jstor.org), internet archive (www.archives.com.), Gallica, the digital library of the Bibliothèque nationale de France (http://www.bnf.fr/en/ collections_and_services/digital_ libraries_ gallica.html), Index Nominum Genericorum (ING) of Smithsonian National Museum of Natural History (http://botany.si.edu/ing), Wallich catalogue details (http:/Wallich.rbge.info) of Royal Botanic Garden, Edinburgh and Wallich catalogue project details (http://stories.rbge.org.uk/archieves/865) of Royal Botanic Garden, Kew, etc. were also utilized.

Comprehensive information on the distribution, morphology, phenology, distinct features, and conservation status was gathered through referring research publications and national, state, and regional floras. To retrieve adequate information on the genus *Hygrophila* and the allied genera, floras of other countries (e.g., Flora of China; Hu & Daniel, 2011) were also consulted from the online database of eflora.org.

Herbarium Consultation

Specimens housed at 16 Indian herbaria (ASSAM, ARUN, BLAT, BSI, CAL, CALI, FRLH, JCB, KFRI, MBGH, MH, SUK, TBGT, AHMA, CMPR, DEV, herbarium of Goa University) were consulted to study the diversity, distribution, ecology, phenology and of specific and infraspecific variations of the genus in India. Collections from the online database of Global Plants on JSTOR, Wallich Catalogue and the virtual databases of 12 herbaria (B, BM, BR, C, E, G, GZU, K, L, LINN, M, P, US) were also consulted. A List of herbaria consulted with their details is provided at the end as Appendix I. Acronyms of herbaria are following the Index Herbariorum (Thiers, 2024, updated continuously).

Specimen Collection and Germplasm Conservation

Extensive field trips were conducted throughout India during 2019-2023 in different seasons to collect and document *Hygrophila* species. Fifty-five field trips were conducted, spending 142 days in the field. To study variations, different populations of each species were collected. Type localities of some of the taxa were visited for their collection, and multiple accessions were collected from different localities to analyse variations within and between species. Assessed the distributional status of the species, subspecies and varieties of the genus. Details from types and authentic specimens deposited at various herbaria were obtained in case of the unavailability of live specimens. The specimens collected were duly tagged and processed for further studies. The specimens were collected in polythene bags to prevent desiccation. Fresh and young leaves were collected in zip-lock covers and stored in a deep freezer. Also, the plant materials were dried and stored in silica gel crystals for future use. Specimens were collected while in both flowering and fruiting stages, and detailed field notes were recorded in the field book. Particular consideration was taken to document information on growth form, habitat, altitude, latitude, longitude, and other characteristics such as flower colour and fragrance, which cannot be determined from herbarium specimen examinations. During the field survey, the latitude and longitude of different populations were recorded using a handheld Global Positioning System (Garmin Montana GPS). During specimen collection, the photographs of the specimens and habitat were taken using SLR Digital Cameras (Canon EOS & Sony).

The plant materials collected from the natural habitat were introduced into 'Aquagene,' the aquatic plant conservatory of Malabar Botanical Garden & Institute of Plant Sciences (MBGIPS). All the accessions collected were properly labelled with metallic labels indicating the details such as the name of the taxon, collection number and collection locality. The variation under cultivation is also studied.

Herbarium Preparation

Herbarium preparations were made for each plant collected during the course of study which will be beneficial for further reference. Specimens of suitable size including key parts like flowers and fruits, were collected from the field and sealed in polyethylene bags. Herbaria were prepared using conventional methods (De Vogel, 1987; Forman & Bridson, 1989). The dried specimens were mounted on standard herbarium sheets of size 28 x 42 cm. The sheets were labelled with standard labels (14.5 × 11 cm) which contain details such as the name of the family, name of the genus and species, locality of collection, date of collection, description/ remarks/ notes and collector's name. The voucher number from the field book was tagged on the herbarium sheets. A full set of voucher specimens were deposited in MBG Herbarium for future reference.

Identification, Nomenclature, and Citation

The collected plant materials were identified using standard literature, including local/regional floras. The identity was confirmed by consulting authentic specimens housed at various national and international herbaria. The identities of the specimens were further confirmed by comparing them with type materials deposited in various herbaria and also with the protologue information of the taxa. The illustrations provided by various authors were also used for the identification.

Nomenclatural clarifications were made conforming to the Shenzhen International Code of Nomenclature for algae, fungi, and plants (ICN; Turland *et al*., 2018). The citations of taxa followed the database of the International Plant Names Index (http://www.ipni.org). World Flora Online Plant List (http://wfoplantlist.org/), Global Biodiversity Information Facility (GBIF.org - https://doi.org/10.15468/39omei), POWO (http://www.plants of the worldonline.org/) and The Plant List (http://www.theplantlist. org/) were also utilized. The generic citation and their type species were obtained mostly from 'Index Nominum Genericorum' (Farr *et al*., 1979). Titles of journals and periodicals were abbreviated as per 'Botanico-Periodicum-Huntianum' [B-P-H] (Lawrence *et al*., 1968). Titles of books were abbreviated in accordance with 'Taxonomic Literature' (ed. 2, vols. 1–7) by Stafleu and Cowan (1976–1988). Author citations followed Brummitt and Powell (1992).

Descriptions and Photographs

Specimens were carefully examined both in the field and in the laboratory. All field observed characters were noticed from the natural habitat, whereas in the lab, every plant part was critically observed under the Leica Stereo Microscope. Detailed morphological studies were made using all the available materials and variations were recorded. Descriptions were prepared after proper determination and examining a wide range of specimens. Data on phenology was mostly taken from field studies and rarely from the literature and herbarium sheets. For the description of taxa, the terminology followed Lawrence (1951), Stearn (1983) and Simpson (2019).

A data sheet was prepared for each population studied. The data sheet covered information on ecology and habitat, habit, nature of stem, nature of leaves, type of inflorescence, colour, size and shape of calyx, corolla, and other floral parts. A model of the data sheet used is provided at the end as an Appendix II.

Preparation of Distribution Maps and Conservation Status

The distribution maps were prepared based on specimens examined during the present investigation, herbarium references, and literature. The metric system was followed for all measurements. The distribution map was created using QGIS 3.28.4 (QGIS, 2023). The conservation status for each species was evaluated following the

IUCN Red List Categories and Criteria (IUCN, 2012) and IUCN guidelines (IUCN, 2022).

Presentation of Data

The systematic part begins with a treatment of the genus *Hygrophila*. This includes its correct name, citations, type details, detailed description, and geographical distribution. This is followed by a key to the species and species treatments. The species treatments are given with citations, type, detailed descriptions, and other relevant notes. The species are presented in alphabetic sequence. Details on etymology, vernacular names, distribution, ecology, flowering and fruiting period, nomenclatural notes, relationships, conservation status and variations were also provided for each taxon. All the specimens studied were cited under specimens examined. The details of specimens cited are given in the following sequence alphabetically: state, district, collection locality, date of collection, collector/collector's name, collection number, and acronym of the depository in parenthesis.

Molecular Phylogeny

Taxon Sampling

Forty-five accessions belonging to twelve species of Indian *Hygrophila* were analyzed in the study. During field exploration and specimen collection, leaf samples from multiple accessions were collected. The leaves were collected in zip lock covers and kept in the deep freezer, and they were dried in silica gel for future use. The herbarium vouchers are deposited in Malabar Botanical Garden & Institute of Plant Sciences Herbarium (MBGH), Kozhikode, Kerala, India. Outgroups were selected from the tribe Ruellieae itself. The sequences of *Ruellia simplex* C.Wright, *R. terminalis* (Nees) Wassh. and *Bravaisia integerrima* Standl. were included as outgroups for the analyses. The sequences of the outgroup taxa were retrieved from GenBank.

Equipment	Make/ model
Autoclave	Trueklav, India
Compound Microscope	Leica SAPO
Deep freezer (-20^{0}C)	Cellfrost, India
Deep freezer (-80^{0}C)	New Brunswick, U101, Germany
Electronic balance	Sartorius, Germany
Electrophoresis Power Unit	Bio-Rad Laboratories, USA
Gel Documentation	Bio-Rad gel doc XR+ System USA
Gel electrophoresis	Bio-Rad Laboratories,
GPS	Gramin montana
Hot Air Oven	Beston; Universal, India
Magnetic stirrer	Neuation iStir HP550
Micro Centrifuge	Eppendorf, Germany
Micro Wave Oven	LG, India
Micropipettes	Ependorf, Germany
pH Meter	Horiba, LAQUA pH-1100
Refrigerated Micro Centrifuge	Eppendorf, Germany
Stereo Microscope	Leica MSV 266
Thermal cycler (PCR)	Bio-Rad S1000
Nanodrop Spectrophotometer	Multiskan sky, Thermoscientific
Vortex Mixer	Neuation iswix VT
Water bath	Julabo, Germany
Water purification system	Millipore, France

Table 2: List of equipments used in the study

DNA Extraction

Total genomic DNA was extracted mainly from fresh leaves using the modified CTAB method (Jaseela *et al*., 2024). Various DNA isolation protocols (Murray & Thompson, 1980; Doyle & Doyle 1987; Rogers & Bendich 1989; Ahmad *et al*., 2000) as well as the utilization of a plant genomic DNA extraction kit and DNA Zol, were employed in an attempt to extract genomic DNA. But these methods proved to be ineffective, as indicated by the presence of sticky polysaccharides in the pellet, discoloration of the DNA, and the appearance of sheared DNA bands in gel image. So, we optimized a protocol for the isolation of genomic DNA from aquatic plants. List of equipments used and stock solutions required for DNA extraction are listed in Table 2 and 3 respectively.

The procedure used for genomic DNA isolation is as follows:

- Collect healthy & tender leaf samples. Wash thoroughly and dry using sterile filter paper
- Weigh 1 gm leaf sample and cut it into pieces into a precooled mortar
- Pre-warm CTAB extraction buffer (Table 2) and add polyvinylpyrrolidone (PVP) prior to extraction (0.2 g PVP for 0.5 ml extraction buffer)
- Pulverize the tissue in liquid nitrogen using a mortar and pestle and add 500 - 1000 µl extraction buffer, mix thoroughly to make it into a slurry and transfer into 2 ml centrifuge tube (the quantity of the buffer depends on the leaf sample)
- Incubate the tubes at 65^{0}C for 45 minutes in a water bath with occasional mixing at regular intervals
- Cool the mixture to room temperature and add 700 µl Chloroform: Isoamyl alcohol mixture (24:1), mix thoroughly to form an emulsion, and centrifuge at 12000 rpm for 15 minutes, at 25°C.

- Collect the upper aqueous phase to a fresh Eppendorf tube an equal amount of chloroform added and centrifuge at 12000 rpm for 10 minutes at 4°C
- Transfer the supernatant to a fresh Eppendorf tube and add 100% chilled isopropanol through the sides gently mix by inverting the tubes. Kept the mixture in -20°C for 2 hours or overnight
- Collect the pellets by centrifuging at 10000 rpm for 15 min at 4°C
- Wash the pellets twice with cold 70% ethanol a 10000 rpm for 5 minutes and air dry the pellets
- Suspend the pellets in 200µl TE buffer (Tris Buffer 10 mM, Na2 EDTA 1 M). Add 4 µl RNase and incubate at 37°C for 1-2 hr in a water bath
- Add 500 µl Phenol: Chloroform: Isoamyl mixture (24:25:1), mix gently, and centrifuge at 10000 rpm for 10 minutes at 4°C
- Collect the supernatant in a fresh Eppendorf tube and add 500 µl Chloroform, mix gently, and centrifuge at 10000 rpm for 10 minutes
- Collect the supernatant in a fresh 1.5 ml Eppendorf tube and add double volume chilled isopropanol and 1/10 volume 3 M Sodium Acetate. Kept the sample overnight at -20°C
- Collect the pellets by centrifugation at 10000 rpm for 15 minutes at 4°C
- Wash the pellet in cod 70% ethanol and air dry it. Resuspend the pellets in 50 µl TE buffer
- Keep in deep freezer for long-term storage

Solutions	Composition	Amount
Tris buffer (pH 8)	Tris 1M H_2O	12.11 gm 100 ml
EDTA	Na_2EDTA H_2O	18.61 gm 100 ml
CTAB Extraction buffer (pH 8.0) (stored at room temperature)	CTAB 2%W/V Tris buffer 100mM Na_2EDTA20mM PVP 1% NaCl 1.4 M H_2O	2 gm 10 ml 4 ml 1 gm 8.2 gm 100 ml
TE Buffer	Tris buffer 10 mM Na_2EDTA 1 M H_2O	1 ml 0.2 ml 100ml
Sodium acetate	Sodium acetate 3M H_2O	24.61 gm 100 ml

Table 3: Stock solutions required for genomic DNA extraction

Qualitative Analysis of DNA (Gel Electrophoresis & Imaging)

The isolated samples were subjected to electrophoresis. 0.8 % of agarose was used for genomic DNA gel electrophoresis (Table 4). 0.72 g of agarose powder was measured into a 250 mL conical flask, and 90 mL of TAE (1X) buffer was added to the flask and mixed properly. The agarose was melted by heating near boiling point in a microwave oven for several seconds until the gel solution was clear. The melted agarose was cooled sufficiently (about 50-55^{0}C). 2 µl ethidium bromide (10 mg/ml) was added to the gel before the gel was set and mixed well. The casting tray was prepared by sealing both ends and a comb of appropriate size was placed onto it. Molten agarose solution was poured into the casting tray carefully to avoid air bubbles and kept undisturbed (20-30 minutes) for solidification. Later, the solidified gel was placed into the electrophoresis tank after removing the comb and tape. 1X TAE buffer was poured so that the gel was submerged in the buffer. 5 µL of the sample was 1mixed with 1 µL gel loading dye and loaded into separate wells of the gel. The first well of the gel is filled with 6 µl of DNA ladder (Benchtop) to quantify the size of the isolated DNA. Electrophoresis was performed out at 100 V for 30 minutes or until the loading dye migrated ¾ of the gel. The migration distance of the DNA in the gel can be assessed by visually tracking the movement of the dyes. The gel was visualized under UV illumination, and images were captured using the Biorad XR+ gel documentation system. The DNA samples exhibiting clear bands were selected for further analysis.

Solution	Composition	Amount
TAE Buffer (10x) pH 8	Tris base Acetic acid Na2EDTA 0.5Mm H2O	21.6 gm 8 ml 11 gm 100 ml
Gel loading buffer	Bromo phenol blue 0.25% Xylene cyanole 0.25% Sucrose 40% (w/v) H2O	250 mg 250 mg 40 gm 100 ml
Ethidium bromide	Ethidium bromide H2O	1 gm 100 ml

Table 4: Stock solutions required for agarose gel electrophoresis

Quantitative Analysis of DNA

The purity and concentration of the samples were estimated using the Nanodrop Spectrophotometer (Thermo Scientific, USA). 1 µL blank (TE) and 1 µL DNA sample was placed on microplate of the spectrophotometer, which reads the absorbance of samples. DNA was quantified by measuring optical density (O.D.) at A_{260} and A_{280}. Purity is measured under the 260/280 column. Pure DNA has an A_{260}/A_{280} ratio of 1.8–2.0.

PCR Amplification

PCR amplification was carried out in 25 µl reaction mixture consisting of 50 -100 ng DNA, 1 µL each of forward primer (10 picomoles/µL) and reverse primer (10 picomoles/ µL), 12.5 µL of master mix (Takara Bio Inc., Japan - containing PCR buffer, dNTPs, Taq polymerase, MgCl2), and nuclease-free water to make it up to the final volume of 25 µL. The amplification reactions were performed in Biorad S1000 thermal cycler. PCR products were resolved on 1% of agarose gel with 0.5 mg/mL ethidium bromide in a 1X TAE buffer system and imaged using a gel documentation system. PCR amplification was done for 35 cycles. A coding region of the plastid genome (ribulose 1,5-biphosphate carboxylase, rbcL) and a non-coding locus of the nuclear genome (Internal Transcribed Spacer, nrITS) were used were used for amplification (Table 5). Primer details and reaction conditions used for the polymerase chain reaction (PCR) amplification are listed in Table 2. The purification and sequencing of the amplicon were done at Barcode Biosciences, Bengaluru.

Gene Marker	Gene region	Primer sequence 5'-3'	PCR conditions
rbcL	*rbcLa* (F)	ATGTCACCACAAACAGAGACTAAAGC	3 min at 94°C, 45 sec at 94°C, 45 sec at 55°C, 2 min at 72°C, 3 min at 72°C
	rbcLa (R)	GTAAAATCAAGTCCACCRCG	
ITS	*ITS1* (F)	TCCGTAGGTGAACCTGCGG	5 min at 97 °C, 1 min at 97 °C, 1 min at 48 °C, 2 min at 72 °C 72 °C for 5 min.
	ITS4 (R)	TCCTCCGCTTATTGATATGC	

Table 5: Primer details and PCR conditions

Purification of PCR Products

The amplified PCR products were purified using column purification (Genejet TM PCR purification kit, Thermo Fisher Scientific, Mumbai, India) as per manufacturer's guideline.

Sequencing of Amplicons

The purified amplicons were sequenced (sanger sequencing) for both strands (forward and reverse directions) using an ABI 3730XL DNA analyzer (Applied Biosystems Inc. USA) using the same primers used for amplification.

Sequence Analysis

Sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). The raw DNA sequences were edited to remove indecipherable sequences using the software Bio Edit sequence alignment editor version 7.2.6 (Hall, 1999). The similarity of the sequences was checked through the NCBI databank using the BLAST search tool (Altschul *et al*., 1990).

Phylogenetic Analysis

Homology search sequences obtained were performed using BLAST search algorithm. Alignment of forward and reverse sequences was performed using the CLUSTALW (Thompson *et al*., 1994) algorithm, and alignment was edited manually in Mesquite v.3.40 (Maddison and Maddison 2015). The alignments were checked thoroughly and corrected by trimming the low-quality reads at the ends of forward and reverse sequences in the chromatogram. The gaps were treated as missing data. The reverse sequence of each species matched with the forward sequence by reverse complimenting. The overlapping regions were joined together to obtain the contigs. The stability of the relationship was assessed from a bootstrap analysis of the neighbour-joining data. The ML analyses were conducted using the web server of IQ-TREE multicore version 1.6.12 (Minh *et al*., 2019). The tree was constructed by testing tree branches through SH-like approximate Likelihood Ratio Test (aLRT) with 1000 replicates and 693 iterations. The model finder utility of IQ-TREE suggested the 'TIM2+F+G4' model as the best fitting model for the ITS alignment. Similarly, 'K3Pu+F' and 'F81+F+G4' were selected as the best models for the phylogenetic analysis of ITS and rbcL, respectively. The resulting consensus tree was visualized through the iTOL web server (Letunic & Peer, 2021). Each DNA region was analysed separately at first, and then all the regions were combined for analysis. All well-supported clades (defined as ≥75% RAxML BS and ≥0.95 in the plastid and ITS tree) were compared to detect any topological conflicts. The outgroup taxa, *Ruellia simplex* C.Wright, *R. terminalis* (Nees) Wassh. and *Bravaisia integerrima* Standl. were selected from previous studies on the family Acanthaceae and retrieved from GenBank.

Chapter 5

RESULTS

TAXONOMIC TREATMENT

Hygrophila R.Br., Prodr. Fl. Nov. Holland. 479. 1810; Nees in Wallich, Pl. Asiat. Rar. 3: 80. 1832 & in A.DC., Prodr. 11: 85. 1847; T.Anderson, J. Linn. Soc. 9: 456. 1867; Aitch., Cat. Pl. Punjab & Sindh 111. 1869; Benth. in Benth. & Hook.f., Gen. Pl. 2: 1075. 1876; C.B.Clarke in Hook.f., Fl. Brit. India 4: 406. 1884; Lindau in Engl. & Prantl, Nat. Pflanzenfam. IV (3b): 296. 1895; Prain, Bengal Pl. 2: 800. 1903; T.Cooke, Fl. Bombay 2: 353. 1908; Lindl., Intr. Ed. 2: 285, Endl., Gen. n. 4039, Meisn., Gen. 296; Duthie, Fl. upper Gang. Pl. and adj. Siw. and sub-Him. tracts, 2: 185. 1911; Gamble, Fl. Pres. Madras 2: 1015. 1924; Santapau, Rec. Bot. Surv. Ind, 16(1): 219. 1953, J. Bom. Nat. Hist. Soc. 51(2): 350. 1953; Heine, Adansonia II, 11 (4): 656. 1971; Santapau, Fl. Khandala 16(1): 219. 1960; Haines, Bot. Bihar & Orissa 2: 702. 1961 (Repr. ed.); Maheshw., Fl. Delhi 269. 1963; K.M.Mathew, Fl. Tamilnadu Carnatic 2: 1177. 1983; B.D.Sharma *et al*., Fl. Karnataka Anal. 206. 1984; H.J.Chowdhery & B.M.Wadhwa, Fl. Himachal Pradesh Anal. 2: 550. 1984; A. K. Mukherjee, Fl. Pachmarhi and Bori Reserves 229. 1984; D.M.Verma *et al*., Fl. Raipur, Durg, and Rajnandgaon 280. 1985; R.S.Rao, Fl. Goa, Diu, Daman & Nagarhaveli 2: 325. 1986; M.Ahmed. & Nayar, Endemic Pl. Ind. Reg. 1: 149. 1986; A.N.Henry *et al*., Fl. Tamil Nadu 2: 148. 1987; Nicolson *et al*., Interpr. Van Rheede's Hort. Mal. 39. 1988; Bole & Pathak, Fl. Saurashtra 2: 182. 1988; N.P.Singh, Fl. East. Karnataka 2: 493. 1988; K.R.Naskar, Aq. Semi-aq, Pl. Lower Ganga Delta 202. 1990; J.L.Ellis, Fl. Nallamalais 2: 300. 1990; B.V.Shetty & V.Singh, Fl. Rajasthan 652. 1991; K.M.Matthew, Exc. Fl. Cent. Tamilnadu 646. 1991; Lakshmin. & B.D.Sharma, Fl. Nasik Dist. 368. 1991; A.K.Malhotra & S.Moorthy, Fl. Tadoba National Park. 105. 1992: K.R.Naskar, Pl. Weal. Low. Ganga Delta 2: 512. 1993; A.B.Chaudhuri, Fore. Pl. East. India 398. 1993; L.K.Banerjee, Pl. Resour. Jaldapara Rhino Sanct. 47. 1993; M.Mohanan & A.N.Henry, Fl. Thiruvanthapuram 347. 1994; C.D.K.Cook, Aquat. Wetl. Pl. Ind. 30.

1996; K.K.Khanna *et al*., Fl. Madhya Pradesh. 2: 320. 1997; Pull. & Moulali, Fl. Andhra Pradesh 2: 712. 1997; Sivar. & P.Mathew, Fl. Nilambur 502. 1997; B.K. Sinha *et al*., Fl. Great Nicobar Island 335. 1999; N.P.Singh *et al*., Fl. Bihar Anal. 385. 2001; M.R.Almeida, Fl. Maharashtra 4: 48. 2003; P.J.Bora & Y.Kumar, Fl. Diver. Assam, St. Pabitora Wildlife Sanctuary. 250. 2003; Anand Kumar, Fl. Indravati Tiger Reserve 220. 2003; Sasidh., Biodivers. Doc. Kerala. Part 6. Fl. Pl. Kerala. 344. 2004; Pradhan *et al*., Fl. Sanjay Gandhi NP 480. 2005; Anil Kumar *et al*., Fl. Pathanamthitta 380. 2005; T.S.Nayar *et al*., Fl. Pl. Kerala, A Hand Book. 20. 2006; G.S.Giri *et al*., Mat. Fl. Arunachal Pradesh 2: 242. 2008; Karthik. *et al*., Fl. Pl. India Dicot. 1: 20. 2009; Remadevi & Binoj., Fl. Kerala. Acanthaceae 80. 2009; Sunil & Sivadasan, Fl. Alappuzha Dist. 538. 2009; D.C.Saini *et al*., Biod. Aq. Semi Aq. Pl. Eastern U.P. 260. 2010; Punekar & Lakshmin., Fl. Anshi Nat. Park 354. 2011; G.P.Sinha *et al*., Fl. Mizoram. 239. 2012; Datar & Lakshmin., Fl. Bhagwan Mahavir Nat. Park & Adj. Goa185. 2013; R.Manik. & Lakshmin., Fl. Rajiv Gandhi NP 300. 2013; Pull., Fl. Telangana 2: 697. 2015; R.Ansari *et al*., Aq. Wetl. Fl. Kerala 55. 2016; P.Singh *et al*., Pl. Ind. Himal. Reg. 44. 2019; Karnataka Biodiversity Board, Fl. Karnataka A Checklist. 2: 36. 2019; Arisdason *et al*., Fl. Pl. India. Checklist 2: 284; 2020; Gogoi, Fl. Sikkim 39. 2021.

Type: *Hygrophila ringens* (L.) R.Br. ex Steud. (*Ruellia ringens* L.) Anon. *s.n*., Herb. Linnaeus No. 804.13 (LINN 804.13, digital image!) Fig. 2.

Fig. 2. *Hygrophila ringens* (L.) R.Br. ex Steud. (*Ruellia ringens* L.) – Type species of the genus *Hygrophila* R.Br.

Oryzetes Salisb., in Griffiths, Monthly Rev. 86: 304. 1818.

Nomaphila Blume, Bijdr. Fl. Ned. Ind. 14: 804. 1826.

Adenosma Nees N.Wallich, Pl. Asiat. Rar. 3: 75.1832, *nom. illeg.*

Asteracantha Nees, in Wall. Pl. As. Rar. 3: 75.1832; T.Cooke. Fl. Bombay 2: 352. 1908; J.S.Gamble, Fl. Pres. Madras 2: 1015. 1924; Santapau Fl. Khandala 16(1): 219. 1960.

Hemiadelphis Nees in Wall. Pl. As. Rar. 3: 75. 1832.

Tenoria Dehnh. & Giord., Cat. Pl. Horti Camald., ed. 2 24. 1832, *nom. illeg.*

Physichilus Nees, Companion Bot. Mag. 2: 310. 1837.

Polyechma Hochst., Flora 24(1): 376.1841.

Synnema Benth., Prodr. [A. P. de Candolle] 10: 538. 1846.

Eberlea Riddell ex Nees, in DC. Prod. 11: 85. 1847.

Cardanthera Buch.-Ham. ex Benth. & Hook. f. Gen. Pl. 2(2): 1074 (1876), *nom. illeg*.; T.Cooke. Fl. Bombay 2: 347. 1908.

Plaesianthera Livera, Ann. Roy. Bot. Gard. (Peradeniya) 9: 196. 1924.

Kita A.Chev., Rev. Int. Bot. Appl. Agric. Trop. 30: 266. 1950.

Santapaua N.P. Balakr. & Subr. J. Indian Bot. Soc. 42: 411. 1963.

Annual or perennial, aquatic or amphibious herbs to subshrubs. Stem erect or ascending, glabrous or pubescent, quadrangular, branching, sometimes armed at nodes; nodes swollen, hairy. Leaves homophyllous or heterophyllous, opposite, decussate, lanceolate, linear, elliptic, oblong or obovate, base attenuate or cuneate, margin entire, undulate, crenate or serrulate, tip obtuse, acute or acuminate, lamina usually lineate on the upper surface, sessile to petiolate. Flowers sessile or stalked, in axillary sessile cymes or whorls around the nodes or in terminal spikes; bracts elliptic or lanceolate, sometimes leafy; bracteoles linear or lanceolate, shorter than the calyx. Calyx tubular, 5-fid or rarely 4-lobed, subequal, basally united; teeth lanceolate or linear, acute or acuminate. Corolla white to purple, sympetalous, zygomorphic, glandular pubescent without; tube ventricose at the apex dilated above; limb 2-lipped, oblong, lobes twisted to the left in bud; upper lip 2-lobed, erect; lower lip 3-lobed, deflexed, palate crested, villous inside. Stamens 4, didynamous, included, the posterior pair sometimes smaller or rudimentary; filaments glabrous, filament pairs shortly united at base extending into a hirsute sheath; anthers oblong, the cells equal or unequal, muticous, divaricate or connate at the base. Ovary oblong-cylindric, glabrous or pubescent; ovules few or many in each cell; style slender, hairy, white to pale purples; stigma simple, linear or subulate. Capsule narrowly oblong opening by 2 valves, seed bearing from base. Seeds numerous, rarely few, discoid, orbicular, compressed, hygroscopically hairy. Retinacula minute and soft or prominent and hard, curved, acute or blunt.

Distribution: The genus consists of approximately 77 species (POWO, 2024), and its main distribution is throughout tropical and subtropical regions.

Key to the Species of *Hygrophila* in India

1. Plants armed with prominent axillary spines; calyx 4- partite; seeds 4–8. .... ***H. auriculata***

1. Plants unarmed, calyx 5- partite, seeds more than 8 2

2. Plants homophyllous 3

2. Plants heterophyllous...7

3. Flowers in terminal spikes 4

3. Flowers in axillary whorls 9

4. Plants succulent; bracts orbicular; retinacula soft, straight .... ***H. heinei***

4. Plants not succulent; bracts ovate-elliptic; retinacula hard, curved 5

5. Stamens 2, fertile, posterior pair reduced to teeth; seeds 20 or more ....***H. polysperma***

5. Stamens 4, fertile, anthers of the posterior half as long as those of the anterior; seeds 8 –10 6

6. Leaves hispid; flowers in dese leafy spike .... ***H. serpyllum***

6. Leaves glabrous; flowers in non-leafy spikes forming terminal panicle ....***H. griffithii***

7. Stem diffuse; leaves viscid; flowers pedicellate......................................***H. triflora***

7. Stem erect; leaves not viscid; flowers sessile 8

8. Flowers solitary; upper leaves lanceolate entire to serrate, lower leaves deeply lobed .... ***H. pinnatifida***

8. Flowers many; upper leaves orbicular crenate to serrate, lower leaves dissected . . ..***H. balsamica***

9. Herbs up to 100 cm high; stem thick..10

9. Herbs up to 30 cm high; stem slender 13

10. Lamina glabrous .... ***H. ringens***

10. Lamina pubescent 11

11. Leaves obovate, oblong, elliptic, apex obtuse to sub-acute; bracts in three ranks .. ..***H. phlomoides***

11. Leaves lanceolate, apex acute; bracts in one rank 12

12. Stem glabrous, lamina scabrous; bracts villous, acuminate .... ***H. pogonocalyx***

12. Stem white hairy, lamina greyish villous; bracts white hairy, acute .... ***H. incana***

13. Unbranched tufted herbs; flowers 1-3 ....***H. pusilla***

13. Branched herbs; flowers in cyme 14

14. Pyramidally branching herbs; leaves petiolate; lowers in axillary dichasial cyme becoming sympodial and unilateral .... ***H. madurensis***

14. Simple branching herbs; leaves sessile, flowers in axillary cyme ....***H. thymus***

Enumeration of Taxa

Hygrophila auriculata (Schum.) Heine in Kew Bull. 16(2): 172. 1962; Wight I.c. 2(2): 3. T. 449. 1841; C.B.Clarke in Hook.f., Fl. Brit. India 4: 408. 1884; Duthie, Fl. Gangetic plain 2: 184. 1911; Gamble, Fl. Pres. Madr. 1015. 1924 (Repr. Ed. 2: 712. 1957); Santapau, Bot. Mem Univ. Bomb. 2: 17. 1951 & Rec. Bot. Sur. India 16(1): 219. 1960; T.Cooke, Fl. Pres. Bombay 2: 428. 1958 (Repr. ed.); K.M.Mathew, Fl. Tamilnadu Carnatic 2: 1178. 1983; B.D.Sharma *et al*., Fl. Karnataka Anal. 206. 1984; H.J.Chowdhery & B.M.Wadhwa, Fl. Himachal Pradesh Anal. 2: 550. 1984; A.K.Mukherjee, Fl. Pachmarhi and Bori Reserves 229. 1984; D.M.Verma *et al*., Fl. Raipur, Durg, and Rajnandgaon 280. 1985; R.S.Rao, Fl. Goa, Diu, Daman & Nagarhaveli 2: 325. 1986; A.N.Henry *et al*., Fl. Tamil Nadu 2:148.1987; Nicolson *et al*., Interpr. Van Rheede's Hort. Mal. 39. 1988; N.P.Singh, Fl. Eastern Karnataka 2: 493. 1988; Bole & Pathak, Fl. Saurashtra 2: 182. 1988; K.R.Naskar, Aq. Pl. Lower Ganga Delta, Tax. Eco. Ec. Imp. 202. 1990; J.L.Ellis, Fl. Nallamalais 2: 300. 1990; B.V.Shetty & V.Singh, Fl. Rajasthan 652. 1991; A.K.Malhotra & S.Moorthy, Fl. Tadoba National Park 106. 1992: K.R.Naskar, Pl. Weal. Low. Ganga Delta 2: 513. 1993; Pull. & Moulali, Fl. Andhra Pradesh 2: 713. 1997; K.K.Khanna *et al*., Fl.

Madhya Pradesh. 2: 321. 1997; Anand Kumar, Fl. Indravati Tiger Reserve 220. 2003; Karthik. *et al*., Fl. Pl. India Dicot. 1: 23. 2009; D.C.Saini *et al*., Biod. Aq. Semi Aq. Pl. Eastern U.P. 260. 2010; P.Singh *et al*., Pl. Ind. Himal. Reg. 44. 2019; Gogoi, Fl. Sikkim 39. 202113.

Barleria auriculata Schum. in Schum. & Tonn., Beskr. Guin. Pl. 285. 1827.

Type:— Africa. Danish Guinea (presently Ghana), *s.d.*, *Thonning s.n.* (Lectotype designated here, C10003397 digital image!) Fig. 3.

Barleria longifolia L. In Torner Cent. II. Pl.22. 1756, Amoen. Acad. 4: 320. 1759, Sp. Pl. ed. 2. 2: 887. 1763; Wild. Sp. Pl. 3 (1): 375; Spr. Syst. Veg. 2: 827. n. 8.

Asteracantha longifolia (L.) Nees in Wall. Pl. As. Rar. 3: 90. 1832; T.Cooke. Fl. Bombay 2: 352. 1908; Duthie, Fl. Gangetic Plain 2: 184. 1911; J.S.Gamble, Fl. Pres. Madras 2: 1015. 1924; Haines, Bot. Bihar & Orissa 2: 704. 1961 (Repr. ed.); Wight, Icon 2(2): 3. t. 449. 1841; Santapau Fl. of Khandala 16(1): 219. 1960; Subramanyam, Aq. Ang. 39. 1961.

Hygrophila longifolia (L.) Kurz in J. Asiat. Soc. Bengal 39: 78. 1870, nom illeg. [Non Nees 1847].

Barleria macracantha R.Br., Voy. Abyss. [Salt] Append. 4: 64. 1814, *nom. nud*.

Bahel schulli Buch.-Ham., Trans. Linn. Soc. London 14: 289. 1825, *nom. superfl.*

Hygrophila spinosa T. Anders. In thw. Enum. Pl. Zeyl. 225. 1860; J.Linn.Soc.Bot. 7: 22. 1863; JET Aitchison. Cat. Pl. Punjab & Sindh 111. 1869; Clarke in Hook. f., Fl Brit. India 408. 1884; Prain, Bengal Pl. 2: 802. 1903; Haines, For. Fl. Chota Nagpur452. 1910; CJ Bamber, Pl. Punjab.624. 1916.

Tenoria undulata Dehnh., Cat. Pl. Horti Camald., ed. 2: 24. 1832.

Barleria hexacantha Bertol., in Ann. Stor. Nat. Bologn. fasc. 9: 445; Disq. Pl. Nov. 3: 1832.

Barleria hexacantha Moris, Mem. Reale Accad. Sci. Torino 36: 1833.

Asteracantha auriculata Nees, DC. Prod. 11: 248. 1847.

Barleria cornigera Very ex Nees DC. Prod. 11: 248. 1847.

Barleria glabrata Vahl ex Nees DC. Prod. 11: 248. 1847.

Barleria spinosa Hook. ex Nees DC. Prod. 11: 247. 1847.

Ruellia longifolia Roxb., Fl. Ind. 3: 50. 1832, *non Hygrophila longifolia* Nees 1847.

Asteracantha macracantha Hochst. ex A.Rich. Hochst. Tent. Fl. Ethiopia. 2: 146. 1850.

Asteracantha lindaviana De Wild. & T.Durand Compt. Rend. Soc. Bot. Belg. 38: 100. 1899.

Hygrophila lindaviana (De Wild. & T.Durand) Burkill Dyer, Fl. Trop. Africa 5: 509. 1900.

Hygrophila schulli M.R.Almeida & S.M.Almeida J. Bombay Nat. Hist. Soc. 83(Suppl.): 221. 1987; Nicolson *et al*., Interpr. Van Rheede's Hort. Mal. 40. 1988; K.M.Matthew, Exc. Fl. Cent. Tamilnadu 646. 1991; N.P.Singh *et al*., Fl. Bihar Anal. 385. 2001; M.R.Almeida, Fl. Maharashtra 4: 51. 2003; Sasidh. Biodivers. Doc. Kerala. Part 6. Fl. Pl. Kerala. 344. 2004; Pradhan *et al*., Fl. Sanjay Gandhi NP 482. 2005; T.S.Nayar *et al*., Fl. Pl. Kerala, A Hand Book. 21. 2006; Remadevi & Binoj., Fl. Kerala. Acanthaceae 86. 2009; Sunil & Sivadasan, Fl. Alappuzha Dist. 539. 2009; Datar & Lakshmin., Fl. Bhagwan Mahavir Nat. Park & Adj. Goa 185. 2013; R.Manik. & Lakshmin., Fl. Rajiv Gandhi NP 300. 2013; Pull., Fl. Telangana 2: 697. 2015; R.Ansari *et al*., Aq. Wetl. Fl. Kerala 58. 2016; Karnataka Biodiversity Board, Fl. Karnataka A Checklist. 2: 37. 2019; Arisdason *et al*., Fl. Pl. India. Checklist 2: 286; 2020. *nom. illeg*.

Hygrophila schulli var. *alba* Parmar, J. Econ. Taxon. Bot. 32(1): 149. 2008.

Hygrophila auriculata var. *alba* (Parmar) P.M.Salim, J.Mathew & Yohannan, Annals of Plant Sciences 1500. 2017.

Erect or decumbent-ascending, strigose-hispid, annual herbs to sub-shrubs, 30 cm–1 m long. Stems stout, quadrangular-sub quadrangular, strigose-hispid, greenish & reddish-purple, unbranched or less branched, branching from the base, thickened at nodes, node swollen with dense hispidous hairs. Leaves simple, chartaceous, opposite, sessile, in whorls of 6 intermixed with thorns, outer pair longer than the rest; margin undulate and minutely dentate, ciliate, apex acute to acuminate, narrowed at base, base cuneate, strigose on both sides, scabrous; larger leaves oblong-lanceolate or linear-lanceolate up to 10–20 × 2–6 cm; petioles up to 0.2 cm long; smaller leaves in variable number and size, up to 3.5–6 × 0.5–1.5 cm, sessile; each leaves with a sharp, straight, brownish yellow spine, spines 6 in whorls at nodes, slightly curved, 2.5–3.5 cm long. Flowers in dense clusters at leaf axils, sessile, 6–8 in each whorl; bracts foliaceous, involucrate, 2-seriate, lanceolate, 2.5–3.5 cm long, margin entire, ciliate, hispid, yellowish, hairy without; bracteoles smaller than bracts, leafy, 1.5–1.8 cm long, linear, hairy; margin membranous, ciliate. Calyx 4-partite, almost free up to the base, yellowish-green, unequal, posterior calyx segment longer, linear-lanceolate, hairy within, strigose without, margin entire, hyaline ciliate, apex acute with many hairs, 1.2–1.6 × 0.2-0.4 cm. Corolla purple-blue, sometimes pure white; widely bilipped, glandular hairy without, 2–3.5 cm long; tube white, 1.2–1.5 cm long, dilated above, abruptly woollen at the top; upper lip 2-fid with oblong lobes; lower lip 3-lobed with 2 longitudinal orange-yellow crested folds on the yellowish-white palate, deeply 3-lobed, lobes oblong or obovate and truncate or rounded at apex. Stamens four, didynamous, adnate to the corolla throat; filaments of each pair connate at base, flattened, glabrous, up to 0.3–0.5 & 0.6–0.8 cm long, pubescent, white; anthers bithecous, up to 0.3 cm long, hairy on back, anther cells parallel, unequal, oblong, purple. Ovary 2-celled, oblong, 2.5–6.5 mm long, apically ciliate; ovules 4 in each cell; style simple, filiform, puberulous, off white, *c.* 2.5 cm; stigma simple. Capsule shorter than sepals, 1 cm long, linear oblong, glabrous, pointed. Seeds supported on prominent hard retinacula; retinacula long and curved. Seeds orbicular, 4–8, ca 3 × 2mm, hygroscopic hairy, yellowish-brown (Plate 1).

Fig. 3. Lectotype of *Hygrophila auriculata* (Schum.) Heine (designated here). [*Thonning s.n.* (C10003397!)] © Museum Botanicum Hauniense, University of Copenhagen.

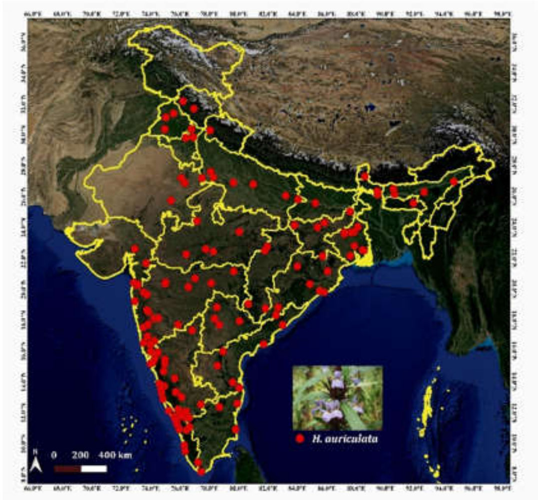
Flowering & fruiting: October – January.

Ecology: Common in marshy grounds, rice fields, moist ridges, around ditches, ponds and puddles, along the margin of lakes, rivers.

Distribution: Throughout India including tropical Himalaya. Myanmar, Nepal, Indo-China, Malesia.

Conservation Status: Least Concern (LC).

Etymology: The specific epithet '*auriculata'* from the Latin '*auriculatus'*= '*provided with ears'* in reference to the small, ear-like lobes of the corolla-lip.



Vernacular Name: Marsh Babel (English) Vayalchulli (Malayalam); Gokaṇṭa, Kokilaksha (Sanskrit); Gokshura, Kolavanke (Kannada); Gokula Kanta, Kantakalia (Hindi); Koilikhia (Punjabi); Kokilaksakamu (Telugu); Kolisa (Marathi); Kulekhara (Bengali); Neermulli (Tamil); Sangmora (Assamese).

Notes: It is the only spinescent species in the genus. Leaf size varies greatly and the flower colour also ranges from purple to white. Possess numerous medicinal properties. Seeds are edible and sold in markets.

Hygrophila auriculata is much known by its invalid name *Hygrophila schulli* M.R. Almeida & S.M. Almeida [J. Bombay Nat. Hist. Soc. 83(Suppl.): 221 (1987)]. Many botanists and also authors of floras continue to use this invalid name and the accepted valid name *H. auriculata* is rather less used.

M.R. Almeida & S.M. Almeida intended *H. schulli* as a new combination based on *Bahel schulli* Buch.-Ham. [Trans. Linn. Soc. London 14:289 (1825)] and referred to *Barleria longifolia* L., Amoen. Acad. 4: 320. 1759, *B. auriculata* Schumach., Beskr. Guin. Pl. 285. 1827, and *H. auriculata* (Schumach.) Heine, Kew Bull. 16: 172. 1962. as synonyms. Almeida & Almeida gave a clarification under the title 'Identity of *Bahel Schulli* in Hortus Malabaricus' in J. Bombay Nat. Hist. Soc. that 'the generic name *Bahel* of *Bahel schulli* is a later homonym of another distinct species, it is unavailable. However, the specific epithet '*schulli'* is available as per article 68.1'.

IPNI remarks, "Unfortunately, the status of *B. schulli* has been in dispute (invalid or illegitimate), and either way, it cannot serve as a basionym. Therefore, *H. schulli* was superfluous and illegitimate when published.” The status of *Bahel schulli* Buch.-Ham. is uncertain because of Buch.-Ham. is non-commital on accepting this name; it is either invalid or nom. superfl. & illegitimate for *Barleria longifolia* L. (1756).

Flower colour variation from purple to white is seen in different accessions of *H. auriculata*, collected from various regions of the study area. The variety, *H. auriculata* var *alba* is based on white flowered plants, but all range of colour from purple to white is observed in our study and thus we treat the variety as the synonym of *H. auriculata*.

Specimens Examined: INDIA, Andhra Pradesh, Chittoor district, Amudalakona gedda, 27.12.1975, *G.V.S. Rao* 46779 (CAL); Prakasam district, Kondaepi, 29.0910.1983, *R.K. Mohan* 0291 (CAL); Medak district, Narsapur, 28.09.1958, *K.M. Sebastine* 6808 (CAL); Warangal district, Pakhal, 28.11.1960, *K.M. Sebastine* 11657 (CAL); West Godavari district, Kollur lake road, 24.01.1958, *K. Subramanyam* 5099 (CAL). **Assam,** Cachar district, Halflong, 30.08.1968, *W.G. Crate* 552 (CAL). **Bihar,** Champaran district, Gobardhana, 14.11.1963, *B.V. Shetty* 309 (CAL); Bettia, 09.09.1965, *S.P. Banerjee* 363 (CAL). **Chhattisgarh,** Kondagaon district, 19.11.1958, *K. Subramanyam* 7200 (MH). **Dadra & Nagar Haveli,** Nagar Haveli district, Silvassa, 01.10.1963, *M.Y. Ansari* 94011 (BSI); Hazaribagh district, Hazaribagh, 26.02.1976, *R.N. Banirja* 3202 (CAL); N. Karanpura, 22.12.1993, *S. Chande* 20900 (CAL). **Gujarat,** Ahemedabad district, Kalol, *V. Singh* 5674 (CAL). **Himachal Pradesh,** Kangra district, Rait, 12.08.1997, *T.N. Srivastava & B.K. Kapahi* 17940 (BSI). **Jharkhand,** East Singhbhum district, Asna, 17.01.2001, *D. Das* 1931 (CAL); Ghatsila, 27.02.1983, *P. Basu* 13239 (CAL); Jamshedpur district, 16.11.2008, *C.R. Magesh* 43965 (CAL); Sahibganj district, Borrio, *G. Panigrahi* 11700 (ASSAM). **Karnataka,** Belgaum district, Anantapur, 02.01.2016, *R.K. Swamy* 289 (JCB); Asoga, 06.11.1996, *D. Raj* 48187 (FRLHT); Chamarajanagar district, BRT wildlife sanctuary, Yelandur check post, 19.11.2014, *J. Jayanti* 195799; Hassan district, Hassan-Kunigal Road, 14.01.1969, *C.J. Saldanha* 12167 (JCB); Mysuru district, Brahnuj valley, 23.09.1961, *A.S.Rao* 74558 (BSI). **Kerala,** Kannur district, Kadavathur, 14.12.2018, *E.K. Sinisha & V.T. Jaseela* 16514 (MBG); Muthiyalam, Payyannur, 20.10.2018, *V.T. Jaseela, & M.K.R. Narayanan* 16502 (MBG); Kozhikode district, Iringallur, 25.10.2018, *V.T. Jaseela* 16503 (MBG); Jaffarkhan colony road, near Planetarium, 13.11.2018, *V.T. Jaseela* 16513 (MBG); Idukki district, Erattayar, 15.10.1982, *C.N. Mohanan* 74633 (CAL); Vadakara. *E.K. Sinisha* 16520 (MBG); Thrissur district, Kodungallur, 25.09.1982, *K. Ramamoorthy* 74841 (CAL). **Madhyapradesh,** Balghat district, Lamta, 12.01.1961, *J.K. Maheswari* 4398 (CAL); Hoshangabad District, Near Bori area, 06.10.1960, *J. Joseph* 11299 (CAL); Bori, 23.12.1967, *G. Panigrahi* 2190 (BSA); Mandla district, Khari reserve forest, 30.11.1961, *J. Joseph* 13497 (MH); Mandla, 08.02.1961, *J. Joseph* 12240 (MH); Rewa district, Canal near Rewa college, 15.02.1959, K.M. *Sebastine* 7775 (MH); Sagar district, near Bina River, 03.11.1960, *N.P. Balakrishnan* 11460 (MH); Satna district, Anusuiya, 17.01.1987, *R. Prasad* 31766 (BSA); Sidhi district, Majhauli, 18.01.1964, *G. Panigrahi* 2190 (BSA). **Maharashtra,** Khandesh district, Khandesh, Nandurbar, 06.01.1957, *S.K. Jain* 11090 (BSI); Kolhapur district, Kolhapur, 08.08.2001, *D.M. Priti* 271 (SUK); Kolhapur, 11.11.1991, *M.M. Sardesai* 323 (SUK); Mumbai district, Madh island, 16.12.1957, *S.K. Jain* 9731 (BSI); Nagpur district, 13.11.1957, *K. Subramanyam* 4563 (MH); Pune district, Mahalunge, 02.11.1961, *K.R. Janardhanam* 72965 (BSI); Pune district, Sinhagad, 21.10.1964, *M.Y. Ansari* 101710 (BSI); Satara district, Dalmodi, 10.10.1989, *H.S. Patil* 2239 (SUK); Yavatmal district, Umarda forest, 12.12.1976, *S. Karthikeyan* 148413 (BSI). **Odisha,** Bargarh district, Amarakhol, 03.11.1959, *G. Panigrahi* 20784 (ASSAM); Koraput district, Chittarkanda, 26.04.1963, *D.C.S. Raju s.n.* (CAL); Nayagarh district, Daspalla, 21.11.2006, *S.K. Padhi* 6263 (RPRC); Puri distrct, Balukhand reserve forest, 21.04.1965, *V. Abraham* 178 (CAL); Sambalpur district, Hirakud, 24.08.1986, *S. Panda* 94 (CAL). **Puducherry,** Karaikal district, Tamanangudi, 03.03.2020, *V.T.Jaseela & N.S.Pradeep* 16579 (MBGH). **Rajasthan,** Bharatpur district, Ghana bird sanctuary, *s.d. P.J. Paramr* 8613 (BSJO); Chittogarh district, Bansi, 15.12.1963, *D.M. Verma*

1724 (BSA); Jhalavar district, Dug, 15.12.1964, *D.M. Verma* 6704 (BSA); Jodhpur district, Naga Dhari, 06.01.1975, *G.P.Ray* 1522 (BSJO); Sirohi district, Mt. Abu, 10.11.1959, *U.R. Deshpande* 60128 (BSI). **Tamil Nadu,** Coimbatore district, Devahalli, 30.01.1905, *C.E.C. Fischer* 738 (CAL); Shiruvani, 21.11.1956, *K. Subramanyam* 1389 (MH); Dharmapuri district, Tholuvebetta forest, 08.01.1998, *G. Balanathan* 65573 (FRLHT); Kanyakumari district, Vattakottai, 31.07.1977, *A.N. Henry* 49511 (CAL); Madurai district, Kallandari, 23.09.1957, *K. Subramanyam* 4345(MH); Pudukkottai district, Kottaipatnam, 31.01.1978, *K. Ramamurthy* 53689 (MH); Thanjavur district, Kodiakkarai, 03.02.1987, *S. Ragupathy* 231 (CAL). **Uttar Pradesh,** Rampur district, Bilaspur, 15.12.1964, *C.M. Arora* 3988 (BSA); Mirzapur district, Mirzapur, 10.03.1970, *G. Panigrahi* 9676 (BSA). **West Bengal,** Bankura district, Katiam hill, 26.12.1958, *J.C. Sengupta* 2073 (CAL); Cooch Bihar district, 09.03.1984, *B.C. Banerjee* 15359 (CAL); Hooghly district, Goghat, 23.11.1961, *P.K. Hajra* 159 (CAL); Midnapur district, Kankrijhore, 28.02.1975, *S. Maji* 1146 (CAL). 24-Parganas district, Gosaba Sundarban, 14.11.1984, *D.C. Pal & G.N. Tribedi* 1407 (CAL).

Hygrophila balsamica (L.f.) Raf., Fl. Tellur 4: 66. 1838; K.M.Mathew, Fl. Tamilnadu Carnatic 2: 1179. 1983; B.D.Sharma *et al*., Fl. Karnataka Anal. 206. 1984; A.N.Henry *et al*., Fl. Tamil Nadu 2:149. 1987; K.M.Mathew, Exc. Fl. Cent. Tamilnadu 647. 1991; Pull. & Moulali, Fl. Andhra Pradesh 2: 713. 1997; K.K.Khanna, *et al*., Fl. Madhya Pradesh. 2: 321. 1997; Anand Kumar, Fl. Indravati Tiger Reserve 220. 2003; T.S.Nayar *et al*., Fl. Pl. Kerala, A Hand Book. 20. 2006; Karthik. *et al*., Fl. Pl. India Dicot. 1: 20. 2009; Remadevi & Binoj., Fl. Kerala. Acanthaceae 81. 2009; R.Ansari *et al*., Aq. Wetl. Fl. Kerala 55. 2016; Karnataka Biodiversity Board, Fl. Karnataka, A Checklist. 2: 36. 2019; Arisdason *et al*., Fl. Pl. India. Checklist 2: 284; 2020.

Ruellia balsamica L. f., Suppl. Pl. 289. 1782.

Type:— Sri Lanka. 1777, *Koenig s.n.* (Lectotype designated here, LINN 804.21 digital image!) Fig. 4.

Cardanthera balsamica (L.f.) Bentham ex C.B.Clarke in Hook. f., Fl. Brit. India 4: 404. 1884; Gamble, Fl. Pres. Madras 1012. 1924; Nees in DC., Prodr. 11: 68. 1847.

Hygrophila balsamica (L.f.) E.Hossain, Bangladesh J. Bot., 3(1): 48.1974. *comb. superfl.*

Synnema balsamicum (L.f.) Alston in Trimen, Handbook Fl. Ceylon 4 (suppl.): 224. 1931.

Adenosma balsamea Spreng. in Syst. Veg., ed. 16. 2: 829. 1825.

Ruellia verticillata Wall., Cat. n. 2409. 1830.

Blepharis verticillata B.Heyne ex Wall., Cat. n. 2409. 1830.

Ruellia cernua B.Heyne ex Wall., Cat. n. 2380. 1830. *p. p.*

Ruellia verticilliflora Steud., Nom. ed. II. 2: 483. 1840.

Blepharacanthus verticillata Steud., Nom. ed. II. 1: 209. 1840.

Adenosma verticillata Nees in A.P.de Candolle, Prodr. 11: 69. 1847.

Synnema verticillatum (Nees) Kuntze, Rev. Gen. 500. 1891.

Hygrophila verticillata (Nees) E.Hossain Bangladesh J. Bot., 3(1): 48.1974, *nom. illeg.*

*Cardanthera verticillata (*Nees) Bentham ex C.B.Clarke in Hook. f., Fl. Brit. India 4: 404. 1884; Fl. Pres. Madras 2: 1012. 1924.

Synnema barbigera Kuntze in Revis. Gen. Pl. 2: 500. 1891.

Synnema barbigera Kuntze Rev. Gen. 500. 1891.

Hygrophila difformis (L.f.) Blume, Bijdr. Fl. Ned. Ind. 14: 804. 1826. ***syn. nov.***

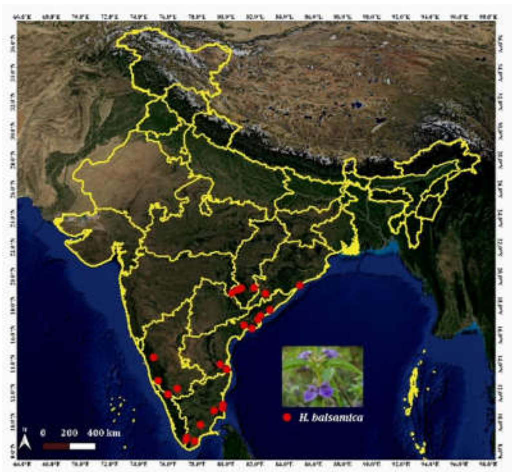
Ruellia difformis L.f., Suppl. Pl. 289. 1782.

Viscid, aromatic, aquatic annual herb. Stem erect or decumbent, much branched, slender, green, up to 60 cm tall, rooting at lower node; node swollen, hairy; branchlets tetragonous, sometimes show reddish colour, glandular pubescent; emergent part of the stem densely covered with glandular hairs of uniform length. Leaves opposite, decussate, heterophyllous; petiole short; aerial leaves obovate-elliptic to lanceolate, 1.5–6 × 0.5–1.5 cm, glandular hairy, cuneate at base, serrate-crenate at margin or almost entire, obtuse-subacute at apex, base densely hirsute; lower submerged leaves sometimes dissected, glabrous, pectinate; petiole 0.5–1.5 cm, glandular hairy. Flowers born in verticillate cymes, axillary, usually in clusters of 8–10; bracts obovate-oblong, entire, 3–5 × 1–1.5 mm, hairy; bracteoles linear, 1 × 0.3 mm; ovate, 2.1 mm long. Calyx tubular, companulate, sepal lobes 5, almost free, base white with green lobes, margin fringed with hairs, tip acute-subacute, hirsute, unequal, outer one larger, 7 × 3 mm, oblanceolate, hairy, green with a white midrib, 3–4 nerved, other four lobes lanceolate, acute or obtuse, 5 × 2 mm. Corolla purple, sometimes white, pubescent, 0.8–1.3 cm, tube 4–6 mm long, limb 2-lipped; lower lip reflexed, 2-lobed, mid lobe large with a 3-nerved palate, bullate with bluish and white hairs; upper lip 2-lobed, puberulous. Stamens 4, didynamous, dorsifixed; filaments linear, white, pubescent, longer pair about 5 mm long, shorter 2 mm long; anthers purple, obovate, cells equal, 1–2 mm long, blue, pubescent beneath, mucronate at base. Ovary oblong-obovate to globose, compressed, 2–4 × 1–3 mm pubescent with nectariferous disc at base, ovules many; style filiform, white to light purple, 6–8 mm, hairy; stigma subulate. Capsule 5–7 × 2–3 mm, oblong-ellipsoid, hairy. Seeds many, 4 × 3 mm, brown; retinacula minute, straight (Plate 2).

Fig. 4. Lectotype of *Hygrophila balsamica* (L.f.) Raf. (designated here), [*Koenig* 6087 (LINN. 804.21)] © Linn. Herb.

Flowering & fruiting: October – January.

Ecology: Plains, especially towards the coast in low-lying clayey moist ground, surrounded by watercourses, irrigation canals, swampy areas, flooded fallow fields, etc., are particularly abundant in harvested paddy fields.



Distribution: Peninsular India (Kerala, Tamil Nadu, Karnataka, Andhra Pradesh, Odisha) & Sri Lanka.

Conservation Status: *Hygrophila balsamica* is endemic to Sri Lanka and Southern part of India.

Etymology: The specific epithet '*balsamica'* means '*balsam like'*, in referece to the inflorescence of the plant.

Vernacular Name: Aromatic Swampweed

Notes: Hydrophyte with delicate and succulent stems and heterophyllous leaves. Lower leaves are pinnatifid (not often) and upper leaves are entire. It is easily distinguished from *Hygrophila triflora* Fosberg & Sachet by the much broader posterior calyx lobe, as against the equal narrow linear-lanceolate ones of the latter.

The species *Hygrophila difformis* was described by Blume (1826) in Bijdr. Fl. Ned. Ind, based on *Ruellia difformis* L.f. Linneaus (L.f.) in Suppl.Pl. (289:1781) described R. difformis from India. He gave a brief description and cited only one element "*Nir schulli*, Rheed. Mal. V2. P.89. t.46". Along with the citation he marks that "forte hius varietas". He quoted that Rheed's plant may be a variety of his species R. difformis. At first glance, *R. difformis* L.f. seemed to be based on Rheede's *Nir schulli*, but this plate shows a different species (*Hygrophila ringens* (L.) R.Br. ex Steud.).

Only one type of leaf (monophylly) is described in the protologue (Linneaus, 1781) and the type specimen also shows entire leaves. But our collections show both monophyllous and heterophyllous leaves. It is also evident in the collections made by others and in different herbarium specimens. It may vary according to the availability of water. The flower colour is given as yellow in the protologue but our accessions and literature show a purple flower for the plant.

There are two type specimens of *R. difformis* (one at LINN and the other at C herbaria). The specimen LINN 804.20, which was selected as the lectotype by Fosberg & Sachet (1981) displays two forms of leaves ie., dissected and normal leaves. Whereas specimen at C has only non-dissected leaves (Fig. 4). Scrutiny of the type specimens of *H. difformis* reveals that they are identical to *H. balsamica*. The only major difference which we can see in the protologue is that the former is homophyllous and the latter heterophyllous. There are conditions where *H. balsamica* shows heterophylly. When there is enough water, it may produce dissected leaves also. Both the names are published in the same protologue on the same page, one after the other (Fig).

The collector of both these species is Koenig, as evident from type specimens and protologue. Maybe he collected one (*H. difformis*) from water logged area and the other (*H. balsamica*) from an area where the water content is less. That may be the reason for the difference in heterophyllous leaves among these species. Thus, here we conclude that *H. difformis* is synonymous with *H. balsamica*.

Many authors considered *H. difformis* and *H. triflora* conspecific with each other. This may be because of the heterophyllous nature. But these two species are clearly two different entities. Fosberg and Sachet (1981) have made a detailed clarification in this regard.

Specimens examined: INDIA, Kerala, Malappuram, Tirurangadi, 01.01.2019, *Jincy* 169038 (CALI). **Madhyapradesh,** Bastar district, Indravati tiger reserve, Bijapur-Bhopalpatnam Road, 17.01.1988, *Anand Kumar* 15605 (CAL). **Odisha,** Koraput district, Malakangiri, 20.04.1963, *D.C.S. Raju* 690 (CAL). **Puducherry,** Karaikal district, Melaputhmangalam, 17.10.2009, *K. Sambandan & N. Dhathanamoorthy* 83; Tamanangudi, 04.03.2020, *V.T. Jaseela & N.S. Pradeep* 16580 (MBGH).

Hygrophila griffithii (T.Anderson) Sreem., Bull. Bot. Surv. India 10: 222. 1969; Kartik. *et al*., Fl. Pl. India Dicot. 1: 21. 2009; Arisdason *et al*., Fl. Pl. India. Checklist 2: 285; 2020.

Adenosma griffithii T.Anderson, J. Linn. Soc 9: 454. 1867.

Type:— India, Assam, 05.02.1836, *Griffith* 6087 (Lectotype designated here, K000882366, digital image!) Fig. 5.

Cardanthera griffithii (T. Anderson) Benth. ex C.B.Clarke in Hook.f., Fl. Brit. India 4: 404. 1885.

Synnema griffitthii (T.Anderson) O. Ktze, Revis. Gen. Pl. 2: 500. 1891.

Hygrophila griffithii (T.Anderson) E.Hossain, Bangladesh J. Bot., 3(1): 48. 1974.

Hygrophila griffithii (Benth. ex C.B.Clarke) Heine; Adansonia, 11(4): 658. 1971.

Annual diffuse herbs. Stem rounded, rooting, pubescent, branched; branches subtetragonus, 30 cm long, pubescent. Leaves opposite, ovate, spathulate, 2.5–7.5 × 1.25–3.75 cm, base attenuate, margin serrate, apex rounded, sparsely hirsute above, bristly on under side, sub-petiolate. Flowers axillary, sessile in spike inflorescence, interrupted at the base in fruit, and forming terminal panicles, 2.5–10 cm long, very rarely pedunculate. Lower bracts distant (at least in fruit), leaf like, more or less imbricate, solitary, ovate, serrate, often containing 2–3 flowers; proper bract 6–4 mm, elliptic. Bracteoles shorter than calyx lobes, 8 mm, narrowly oblong. Calyx 5 partite, divided almost to the base, 3.5 mm long, lobes linear lanceolate, acute, equal, short ciliate, equal to capsule, Corolla small, tubular, purple, bifid, puberulous, little longer than calyx; upper lip minute bifid; lower lip trifid. Stamens didynamous, included; anthers of the posterior pair smaller than those of the anterior pair, ovate. Ovary almost glabrous with a few gland-dipped hairs at its apex. Capsule linear, 5 mm long, acute, glabrous, many seeded, valves compressed furrowed.

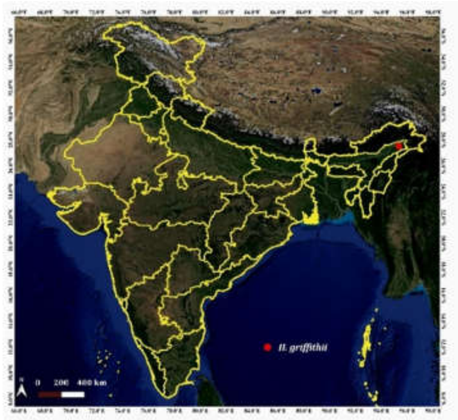
Fig. 5. Lectotype of *Hygrophila griffithii* (T.Anderson) Sreem. (designated here), [*Griffith* 6087 (C10003397)]
© The Board of Trustees of the Royal Botanic Gardens, Kew.

Ecology: Wet places.

Distribution: India (Assam), Myanmar.

Conservation Status: This taxon is recoreded only once from India. The status of the species is unknown.

Etymology: Specefic epithet after the collector.



Notes: T.Anderson (1867) established the genus *Adenosma griffithii* based on a specimen collected by Griffith from Bengal. He also cited Jenkins collection from Assam. Later Bentham (1876) transferred it to *Cardanthera* and Clarke (1884) validated the name *C. griffithii*. Sreemadhavan (1969) while describing the taxonomic status of *Cardanthera* made the combination *Hygrophila griffithii*.

The only report of the plant is in Flora of British India (1884). There is no later collection of this taxon from any part of the country. Herbaria like ASSAM and CAL also does not possess any collection other than the type.

Hygrophila heinei Sreem. Bull. Bot. Surv. Ind 10: 223. 1968; D.M.Verma *et al*., Fl. Raipur, Durg, and Rajnandgaon 280. 1985; A.N.Henry *et al*., Fl. Tamil Nadu 2:149. 1987; K.M.Matthew, Exc. Fl. Cent. Tamilnadu 647. 1991; Tripathi *et al*., Fl. Elem. Madhya Pradesh 75. 1994; C.D.K.Cook, Aquat. Wetl. Pl. Ind. 32. 1996; Pull. & Moulali, Fl. Andhra Pradesh 2: 714. 1997; K.K.Khanna, *et al*., Fl. Madhya Pradesh. 2: 322. 1997; N.P.Singh *et al*. Fl. Bihar Anal. 386. 2001; T.S.Nayar *et al*., Fl. Pl. Kerala, A Hand Book. 20. 2006; Karthik. *et al*., Fl. Pl. India Dicot. 1: 21. 2009; Arisdason *et al*., Fl. Pl. India. Checklist 2: 285; 2020.

Type:— India , 1777, *Koenig s.n.* (Lectotype designated here, LINN 804.22 digital image!) Fig. 6.

Ruellia uliginosa L.f., Suppl. Pl. 290.1782.

Adenosma uliginosa (L.f.) Nees in R.Brown, Verm. Bot. Schr. 3: 298.1827 & in Wallich, Pl. Asiat. Rar. 3: 79. 1832.

Cardanthera uliginosa (L. f.) Buch.-Ham. ex Nees in A.P.de Candolle, Prodr. 11: 69. 1847.

Synnema uliginosum (L.f.) Kuntze Revis. Gen. Pl. 2: 500. 1891.

Cardanthera uliginosa (L.f) Benth. *ex* C.B. Clarke in Hook.f. Fl. Brit. India 4: 403. 1884; J. S. Gamble, Fl. Pres. Madr. 1012. 1924.

Cardanthera uliginosa var. birbhumensis Guha J. Bot. 18: 6. 1967.

Hygrophila heinei var. birbhumensis (Guha) H.B.Naithani & S.Biswas in H.B.Naithani, Flow. Pl. India, Nepal & Bhutan: 333 (1990); Karthik. *et al*., Fl. Pl. India Dicot. 1: 20. 2009. ***syn. nov.***

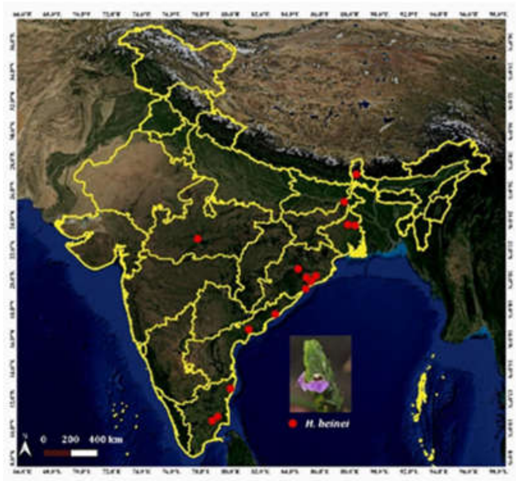
Hygrophila helodes Heine Adansonia, n.s., 11: 658. 1972, *nom. superfl.*

Hygrophila pseudopolysperma E.Hossain Bangladesh J. Bot. 3: 48. 1974, *nom. superfl.*

Fig. 6. Lectotype of Hygrophila heinei Sreem. (designated here), [*Koenig s.n.* (LINN 804.22)] © Linn. Herb.

Diffuse, hirsute, annual herbs up to 6 cm high, 30–50 cm high Stem erect or decumbent, much branched; branches slender, terete, lineolate, glabrous. Leaves simple, opposite, mostly decussate, oblong-obovate or spathulate, 2–4 cm long, 3–8 mm wide, glandular and sometimes also hairy, entire or crenate, sessile or nearly so; the lower leaves get dissected when submersed. Flowers in terminal tetragonal spikes, 3–8 (–12) cm long, scarcely interrupted at the base even in fruit; dense flowered; bracts in 4 ranks, foliaceous, broadly ovate to orbicular, 4–6 mm long, glabrous, glandular and/or hairy, imbricate closely packed; bracteoles bracteoles ovate broadly elliptical, 3–4 mm long. Calyx lobes 5, unequal, connate only at base, 0.4–0.5 cm long, *c.* 6 mm wide, attenuate above, glandular hairy without; linear-lanceolate. Corolla purple, 2-lipped, *c.* 8 mm long; petal tube funnel shaped, *c.* 5 mm long, yellow below, hairy within; petals pubescent without, villous on the palate, upper lip 2 lobed, lower lip 3 lobed. Stamens 4, didynamous; filaments up to 1.5 cm and 0.3 cm long, hairy below the attachment of the filaments to corolla tube; anther pairs up to 0.12 cm and 0.2 cm long. Ovary up to 0.15 cm long, glandular, linear-oblong, seated on a disc; style up to 0.45 cm long, hairy; stigma linear, bent. Capsules linear oblong, 0.45 × 0.1 cm, smooth, pubescent at apex; retinacula minute, straight. Seeds numerous, ovoid and flattened (Plate 3).

Flowering & fruiting: January – April.



Ecology: In swamps and rice fields, in shallow water, sugar cane fields etc.

Distribution: Endemic to India: Sikkim, Orissa, Arunachal Pradesh, Tamil Nadu.

Conservation Status: Least Concern (LC).

Etymology: In honor of H.H. Heine

Vernacular Name: Little Blue Swampweed

Notes: The variety*, Hygrophila heinei var. birbhumensis* (Guha) H.B.Naithani & S.Biswas was reported from Birbhum of West Bengal. The distinguishing character of this variety from *H. heinei* Sreem. are Stem ribbed longitudinally throughout and glabrous; leaves lanceolate; corolla bright orange yellow in colour; capsule copiously villose; seeds covered with mucilaginous hairs. All these characters, except orange yellow flower colour is seen in *H. heinei*. We visited the type locality, but were not able to found this taxon. Flower colour variation is noted in many *Hygrophila* species and only based on the flower colour variation it cannot assume a varietal status. So here we synonymise *Hygrophila heinei var. birbhumensis under H. heinei*.

Specimens examined: INDIA, Andhra Pradesh, West Godavari district, Kolleru lake, 23.01.1958, *K. Subramanyam* 5085 (CAL). **Odisha**, Ganjam district, Lathipoda-Rangana way, 27.02.1978, *H.O. Saxena* & *M. Brahmam* 3274 (JCB). **Tamil Nadu**, Chengalpettu district, Chengalpettu, *s.d.*, *Gamble* 19557 (MH); Nagapattinam district, Vedaranyam, 02.03.2020, *V.T. Jaseela* 18441 (MBGH); Pudukkottai district, Narthamali, 23.02.2022, *V.T. Jaseela* 18421 (MBGH).

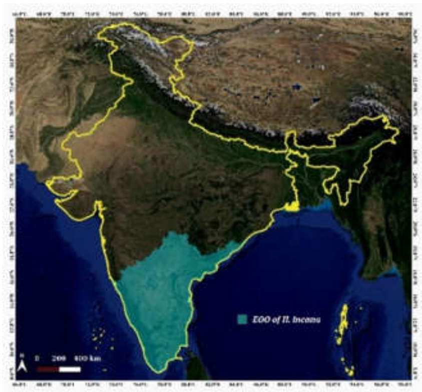
Hygrophila incana Nees in DC. Prodr. 11. 91 (excl. Syn. Roxb.); C.B.Clarke in Hook.f. Fl. Br. India. 4: 408. 1884; Karthik. *et al*., Fl. Pl. India Dicot. 1: 20. 2009; Karnataka Biodiversity Board, Fl. Karnataka A Checklist. 2: 36. 2019; Arisdason *et al*., Fl. Pl. India. Checklist 2: 285; 2020.

Fig. 7. Lectotype of Hygrophila incana Nees. (designated here), [Rottler s.n. (K00088237)] © The Board of Trustees of the Royal Botanic Gardens, Kew.

Ruellia incana B.Heyne ex C.B.Clarke in Hook. f., Fl. Brit. India 4: 408 (1884)

Ruellia mollissima Klein ex Nees, DC. Prod. 11: 91 (1847).

Stem erect and procumbent, quadrangular, closely soft white-hairy, mature stem glabrous, branches ascending. Leaves narrowly lanceolate, subsessile, 4 by 2/3 in., entire, acute at both ends, adult densely lineolate on both surfaces, greyish-hairy. Flowers in verticillate whorls; bracts proper, hhirsute. Calyx in flower divided more than half-way down, teeth linear softly white-hairy and hispid. Capsule 1/3 in., shortly exceeding the calyx. It is very distinct due to its soft, dense white hairiness, which is further characterized by scattered long, white, bristly hairs.



Note: Clarke documented this species in the *Flora of British India* (1885). Karthikeyan *et a*l. (2009) included it in their compilation *Flowering Plants of India solely* because it was mentioned in the Flora British India. The precise locality is not provided, with only "Deccan Plateaus/South India" being indicated. We were unable to collect this species, so our description relies on the protologue and type information. In his protologue, Nees identified the locality of *H. incana* as Chittagong, which was part of Bengal during British India but is now in Bangladesh.

Hygrophila madurensis (N. P. Balakr. & Subram.) Karthik. & Moorthy, Fl. Pl. India Dicot. 1: 22. 2009; Raja *et al*. in J. Threat. Taxa 7(9): 7582. 2015; Muthupandi *et al*. in Trop. Pl. research 6(1): 115. 2019; Arisdason *et al*., Fl. Pl. India. Checklist 2: 285; 2020. *Santapaua madurensis* N.P. Balakr. & Subram. in J. Indian Bot. Soc. 42: 411. 1963; A. N. Henry *et al*., Fl. Tamil Nadu 2: 160. 1987; N. P. Balakr. in Red Data Book Indian Pl. 2: 7. 1988.

Type: India, Nallakulam, Alagar hills, Madurai district, alt 200 m, 12.02.1958, *K.Subramanyam* 5286A (Holotype CAL 0000019444). Fig. 7.

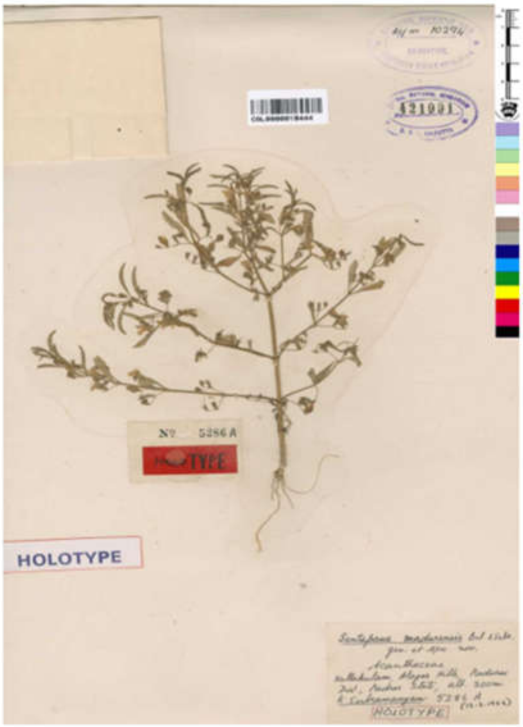
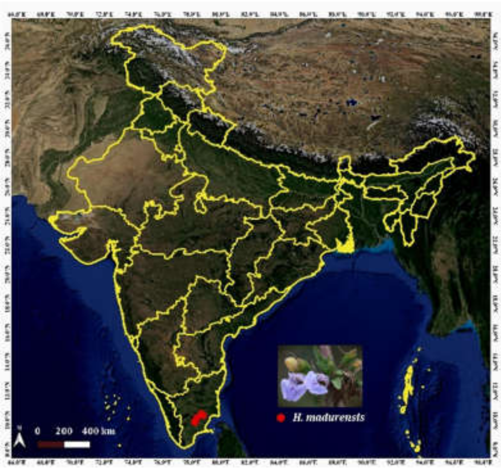


Fig. 8. Holotype of *Hygrophila madurensis* (N. P. Balakr. & Subram.) Karthik (designated here), [*Subramanyam* 5286 A (CAL0000019444)] © Botanical Survey of India, Central National Herbarium.

Erect herbs, 10–30 cm high; pyramidically branching, branches arising from base, decumbant. Stem green & reddish, sub-quadrangular with raphides arranged longitudinally, glabrous, swollen at nodes. Leaves thin, membranous, glabrous, 15–35 × 3–8 mm, raphides scattered on upper surface, ovate-oblong, lanceolate, oblanceolate, narrowed at base, base cuneate, margin minutely crenulate, acute or subacute at apex; lateral nerves 5–7 pairs, prominent below; petiole 2–2.5 mm long. Flowers in axillary open dichasial cymes becoming sympodial and unilateral; primary peduncle ±5 mm long; internodes *c.* 3 mm long; bracts green, linear, sessile, acute, 3–5 × 1 mm with raphides on upper surface; pedicels very short, ± 1 mm. long. Calyx 5-partite, lobes free almost to the base, subequal, linear, acute, 4–5 × 1.5 mm. Corolla purple, bi-lipped, 0.5–0.9 cm long; tube funnel shaped, broad, gradually widening to the mouth, ± 7 mm long, pubescent inside; upper lip bifid, emarginate, lobes rounded, tip white; lower lip trifid, lobes obtuse, rounded, hairy inside. Stamens 4, fertile, didynamous; the posticous pair smaller; filaments linear, filiform, glabrous, pubescent only at base, 2–4 mm long; anthers bithecous, oblong, ellipsoid, 1.0–1.3 mm long, hairy below. Ovary green, glandular hairy, oblong-elliptic, 2 mm long, 2-celled, nectariferous disc below; ovules many; style light purple, linear, 4 mm long, hairy; stigma simple, linear. Capsule brown, linear-oblong, narrow, flat, sessile, 6–7 × 1–2 mm; seed-bearing throughout the length of capsule; retinacula minute, conical, straight, slender, blunt. Seeds 20–40, small, ellipsoid or ovoid–ellipsoid, compressed, glandular puberulous when wet (Plate 4).



Flowering & fruiting: December – March.

Ecology: Marshy localities along edges of seasonal ponds.

Distribution: INDIA (Tamil Nadu), Endemic.

Conservation Status: *Hygrophila madurensis* is listed as Critically Endangered under criteria B1ab (ii, iii) + 2ab (ii, iii); D. Arisdason, W. 2011. *Hygrophila madurensis*. *The IUCN Red List of Threatened Species* 2011: T177037A 7363762. https://dx.doi.org/10.2305/ IUCN.UK.2011-1.RLTS.T177037 A736376 2.en.

The species has a narrow distribution and reported from very few locations. Therefore, it should be assessed periodically in all known localities in Tamil Nadu, as well as searching for other sites of occurrence of it is highly advised. This species is listed as critically endangered and endemic to Tamil Nadu, found along the foothills of the Eastern Ghats (Balakrishnan 1988, Walter & Gillett 1998, Reddy *et al*. 2006, Arisdason 2011). All the known population is prone to high human interference and over-grazing in the natural habitat. Hence, urgent conservation measures should be taken for the conservation of *H. madurensis*.

Hygrophila madurensis has been assessed for The IUCN Red List of Threatened Species in 2010. *Hygrophila madurensis* is listed as Critically Endangered under criteria B1ab(ii,iii)+2ab(ii,iii); D.

Etymology: Specefic epithet named after the type locality 'Madura'.

Vernacular Name: Madurai swampweed.

Specimens examined: INDIA, Tamil Nadu: Madurai district, Sakkimangalam, Ayyankulam, 08.03.2018, *C.P. Muthupandi* 65 (Thiagarajar College Herbarium); 10.03.2018, *C.P. Muthupandi & R. Kottaimuthu* 112 (Thiagarajar College Herbarium); Pudukkottai district, Kudumiyanmalai,17.12.2013, *Raja & Soosairaj* 3329 (RHT); Melathemuthupatti, 23.02.2022, *V.T. Jaseela* 18420 (MBGH).

Hygrophila phlomoides Nees in Wallich, Pl. Asiat. Rar. 3: 80. 1832 & in DC., Prodr. 11: 90. 1847; C.B. Clarke in Hook. f. Fl. Brit. India 4: 408. 1884; Prain, Bengal Pl. 2: 802. 1903; Haines, Bot. Bihar & Orissa 671. 1922 (Repr. ed., 2: 703. 1961); K.R.Naskar, Pl. Weal. Low. Ganga Delta 2: 513. 1993; A. M. Chaudhuri, For. Pl. East. India 398. 1993; K.K. Khanna *et al.*, Fl. Madhya Pradesh. 2: 322. 1997; N. P. Singh *et al.*, Fl. Bihar Anal. 386. 2001; G.S. Giri *et al.*, Mat. Fl. Arunachal Pradesh 2: 242. 2008; Karthik. *et al*., Fl. Pl. India Dicot. 1: 20. 2009; G. D. Pal, Fl. Lower Subansiri Dist. 2:187. 2013; P. Singh *et al.*, Pl. Ind. Himal. Reg. 44. 2019; Gogoi *et al*., Fl. Sikkim 39. 2021.

Ruellia phlomoides Wall., Numer. List [Wallich] n. 2376 (1830), *nom. nud*.

Type:—BANGLADESH, Sillet, *G. Gomez*, Wall Cat n. 2376 (Lectotype designated by Jaseela *et al*., , GZU000250031 digital image!). Fig. 8.

Ruellia hirsuta Roxb. Hort. Beng. 95. 1814.

Dipteracanthus ciliates Nees in Wall. Cat. 715. 1832.

Cardanthera longifolia Buch.-Ham. ex Nees DC. Prod. 11: 90. 1847.

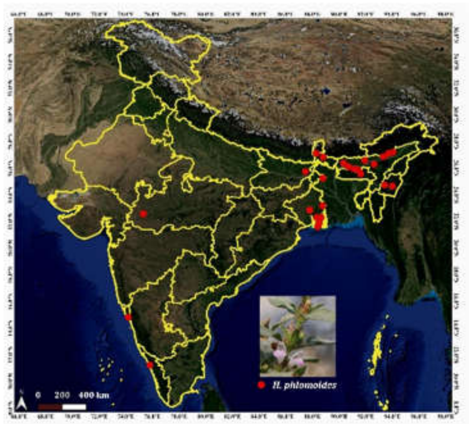
Hygrophila obovata Griff. Notul. 4: 134. 1854.

Fig. 9. Lectotype of *Hygrophila phlomoides* Nees (designated here), [*G. Gomez, Wall Cat n. 2376* (GZU000250031)] © The Board of Trustees for the Karl-Franzens-Universität Graz (GZU), Austria.

Perennial erect or decumbent herbs up to 1 m high. Stem thick, quadrangular, branched, striate, green to reddish brown, strigose, hirsute upwards. Internodes 4–6 cm. Node swollen, hairy. Leaves subsessile, homophyllous, opposite decussate, elliptic, obovate or elliptic-oblong, 2.5–5 × 1.5–2 cm, base cuneate, margin entire or slightly undulate, ciliate, apex obtuse to subacute, hirsute on both surfaces; midrib broad at base, conspicuous beneath; lateral nerves 8–13 pairs; cystoliths dense; petiole 0–4 mm long, hairy. Flowers axillary in dense villous whorls, sessile, 10–12; bracts in three ranks; outer bracts large, foliaceous, obovate, 1.5–1.8 × 0.5–0.8 cm, base cuneate, margin entire, apex obtuse, green, hairy; bracts in the second rank ovate-lanceolate, 1.1–1.3 × 0.4–0.5 cm, base cuneate, margin entire, apex sub-acute green, hirsute on both surfaces; inner bracts 0.8–1 × 0.25–0.3 cm, hirsute on both surfaces, green, margin entire, base cuneate, apex sub-acute; bracteoles linear to linear oblong *c.* 5 × 2 mm, as long as the calyx tube, hirsute. Calyx tubular, 5-lobed, 1.1 cm, reddish brown, white hirsute, divided halfway down, acuminate with hairs at apex, connate at base; teeth linear, hispid, unequal, one segment larger than others; persistent in fruit. Corolla bilabiate, 1.8–2 cm, pink-purple with dark dots or patches on palate, glandular pubescent without; tube 7–8 mm long, ventricose, glabrous; upper lip triangular, 2-lobed, margins entire, glabrous inside, glandular-pubescent outside; lower lip oblong, 3-lobed, middle lobe broader than the lateral lobes, suborbicular, margins entire, apex obtuse, glabrous inside except at centre of middle lobe, glandular- pubescent outside, dark purplestripes at centre. Stamens 4, didynamous, adnate to the base of the ventricose portion of corolla tube; filaments white, glabrous above, retrorsely pilose below; anterior (longer) filaments *c*. 10 mm, posterior (shorter) filaments *c.* 4 mm; anthers elliptic-oblong, dorsifixed, divergent, thecae ca. 2 mm, purple with white tinge, pubescent at the base of the slit, longitudinally dehiscent. Ovary oblong, 3 mm, glabrous, green, 2-loculed; ovules many in each locule, nectariferous disc cupulate; style filiform, *c.* 1.5 cm, white, antrorsely bristled-hairy with glands at base; stigma white, entire. Capsule linear-oblong, 1.5 cm, green, slightly compressed, exceeding the calyx. Seeds many, 15–20, orbicular ovoid, compressed, 2 × 1.5 mm, brownish, whitish woolly (Plate 5.).

Flowering & fruiting: October – March.

Ecology: The species is seen mostly in marshes, swamps and near banks of streams, ponds canal etc.



Distribution: India (Madhya Pradesh, Bihar, Arunachal Pradesh, Manipur, Meghalaya, West Bengal, Assam, Sikkim, Karnataka & Kerala), Bangladesh, Cambodia, China South-Central, East Himalaya, India, Laos, Malaya, Myanmar, Philippines, Thailand, Vietnam.

Conservation status: *Hygrophila phlomoides* is fairly common in its range of distribution and is widespread in many Indian states. Hence, it is evaluated here as of 'Least Concern [LC]' using the IUCN Red List Categories and Criteria version 3.1 (IUCN, 2022).

Etymology: '*phlomoides'*= 'like phlomis'

Vernacular Name: Sage Swampweed

Notes: The plant is allied to *Hygrophila ringens* (L.) R.Br. ex Steud. but can be differentiated by hispid hairs throughout; capsules longer than calyx.

Specimens examined: **INDIA**, **Arunachal Pradesh**, Darrang district, Aka hills, *s.d*, *N.L. Bor s.n.* Acc. No.21215 (ASSAM); Papum Pare district, Naharlagun, 00.04.1978, *G.D. Pal* 74639 (ARUN). **Assam**, Kamrup district, Rajapara Chandubhi Lake, 21.10.1965, *A.S. Rao* 42435 (ASSAM); Jarasal forest on way from Rajapara to Rani, 23.10.1965, *A.S. Rao* 42499 (ASSAM); Kokrajhar district, Chakrasila WLS, 04.11.2006, *R. Daimary* 111911 (ASSAM); Gorhatty hills, 00.10.1850, *Simons* 405 (CAL); Jaboea, Yongali Bam garden, 11.10.1898, *Hoex* 75 (CAL); Lakhimpur district, Satajan beel, 21.08.2018, *P. Gogoi, V.S. Ayam & A.P. Das* 027 (AHU); North Lakhimpur district, Naharbari, 17.05.1966, *D.M. Verma* 46404 (ASSAM); Sadiya district, On the way from Tinsukia to Digboi, 23.10.1960, *G. Panigrahi* 22105 (ASSAM); Sibsagar district, Nrigriting, 11.10.1885, *C.B. Clarke* 40737 (CAL); Southern range, 15.10.1941, 20683 (ASSAM); *s.l, s.d*, *Jenkins s.n.* (CAL); *s.l*, *s.d*, *Jenkins* 5438 (P03580844, image!). **Karnataka**, Karwar, 140 48.6760 N, 740 9.2420 E, 25.11.2021, *V.T. Jaseela & E.K. Sinisha* 18412 (MBGH). **Kerala**, Kozhikode, 10.11.2022, *V.T. Jaseela* 16519 (MBGH**)**. **Manipur**, Imphal, 9.11.1907, *s.coll*. 4818 (MH); Tamenglong district, Nungba, 13.11.1907*, A. Meebold* 6304 (CAL). **Meghalaya**, East Khasi district, Khasi, *s.d.*, *s.coll.* 69466 (MH); Burni, K & J Hills, *s.d*, *G.K. Deka* 22009 (ASSAM); Khasi hills, *s.d*, *J.D. Hooker & T. Thomson s.n.* (CAL, O-V2259613, US02854262, images!). **West Bengal**, Hooghly district, Arambug, 23.11.2022, *V.T. Jaseela* 18430 (MBGH); Jalpaiguri division, Gossaihat Beel, 18.03. 2012, *A. Chowdhury, R. Biswas & A.P. Das* 0994 (NBU); South 24 Parganas district, Bahusul, 25.03.1986, *G. N. Tribedi* 1465 (CAL); North 24 Parganas district, Canning, 10.11.1983, *G.N. Tribedi & D.C. Pal* 742 (CAL); Sundarbans, Gobordhanpur village, 02.11.2021, *V.T. Jaseela* & *N.S. Pradeep18407* (MBGH); Kisorimohapur village, 02.11.2021, *V.T. Jaseela* & *N.S. Pradeep* 18408 (MBGH); West Dinajpur district, Islampur, 03.11.1983, *R.N. Banerjee* 16154 (CAL).

Hygrophila pinnatifida (Dalzell) Sreem. Bull. Bot. Surv. India 10(2): 222. 1969**;** Heine, Adansonia sér. 2, 11(4): 659. 1972; E.Hossain, Bangladesh J. Bot. 3(1): 48. 1974; B.D.Sharma *et al*. Fl. Karnataka Anal. 207. 1984; M.Ahmed. & Nayar, Endemic Pl. Ind. Reg. 1: 149. 1986; C.D.K.Cook. Aquat. Wetl. Pl. India 33. 1996; Londhe in Singh *et al*., Fl. Maharashtra (Dicot.) 2: 633, 2001; M.R.Almeida. Fl.

Maharashtra 4: 49. 2003; Karthik. *et al*., Fl. Pl. India Dicot. 1: 20. 2009; D.C.Saini *et al*., Biodiversity of Aquatic and Semi-Aquatic Plants of Eastern U.P. (India). 261. 2010; Punekar & Lakshmin., Fl. Anshi Nat. Park 354. 2011; Datar & Lakshmin., Fl. Bhagwan Mahavir Nat. Park & Adj. Goa185. 2013; Karnataka Biodiversity Board, Fl. Karnataka, A Checklist. 2: 36. 2019; Arisdason *et al*., Fl. Pl. India. Checklist 2: 285; 2020.

Nomaphila pinnatifida Dalzell, Hooker's J. Bot. Kew Gard. Misc. 3: 38.1851; Hook. Ic. Pl. 9. t. 843(1852); Dalz. & Gibson Bombay Fl. 184, 1861.

Type:— India, Maharashtra, Bombay, 'near running stream at the foot of the ghats', *s.d*., *Dalzell*, *s.n*., (Lectotype designated here, K001392427, digital image!). Fig. 9.

Adenosma pinnatifida (Dalzell) T.Anderson. J. Linn. Soc., Bot. 9: 455.1867; Bedd. Icon. Pl. Ind. Or. t. 246. 1874.

Cardanthera pinnatifida (Dalzell) Bentham ex C.B.Clarke in Benth. & Hook.f., Gen.pl.2: 1074. 1876 & in Fl. Brit. India 4(11): 405.1884. Woodrow in J. Bombay Nat. Hist. Soc. 12: 355. 1899; T.Cooke. Fl. Bombay 2: 347. 1908; Santapau in Bot. Mem. Univ. Bombay 2: 13. 1952. (Plate - 83 g). Gamble, Fl. Madras 2: 1012. 1924.

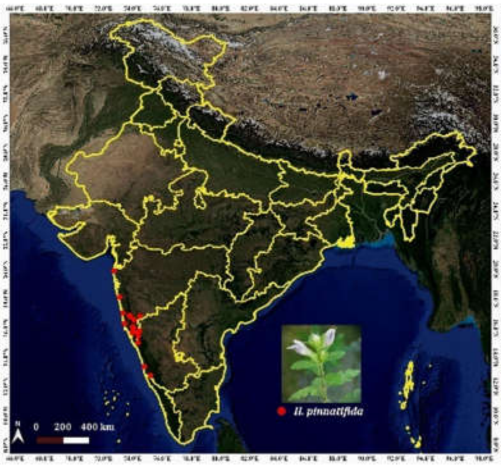
Synnema pinnatifidum Kuntze, Revis. Gen. Pl. 2: 500. 1891; Lindau in Pfam. 4(3B): 295. 1895.

A slender, erect, prostrate, aromatic annual aquatic herb, 30–60 cm high. Stem branched, obtusely tetragonous, grooved, hispid, glandular pubescent, swollen at nodes, rooting at lower nodes. Leaves opposite, heterophyllous, short petioled; petioles 2–9 mm in aerial leaves, 1.2–3.5 cm in submerged leaves, obscure due to decurrent leaf base, pubescent, glandular hairy; lamina of aerial leaves oblong-lanceolate, 1.3–3.8 × 0.5–1.7 cm, acute, attenuate at base, crenate-serrate, ciliate along margins, sub obtuse at apex, glandular pubescent, glandular hairy on both sides, mainly on nerves on abaxial surface, lamina of under-water leaves lanceolate-strap shaped, 7–13 × 1–1.7 cm long, leathery, acute, attenuate at base, deeply pinnatifid, lobes numerous, oblique, linear-oblong, obtuse, entire or serrulate. ciliate along margins, acute to acuminate at apex, glabrous above, sparsely hairy on nerves beneath, reddish-brown on abaxial surface, dark green on adaxial surface; midrib prominent, veins in 4–8 pairs, prominent. Flowers usually solitary in opposite axils (sometimes in lax spikes); peduncle *c.* 1 mm long, glandular pubescent; bracts pinnatifid elliptic-oblong, foliaceous, *c.* 6–8mm, sometimes with a serrate tooth near the apex, midrib prominent, glandular pubescent on both sides; bracteoles oblong, 5 mm long, obtuse, glandular hairy. Calyx 5-fid, sepal lobes linear, unequal, obtuse with pinnately lobed segments or if segments entire then wider above than below; lobes divided almost to the base, hairy on inside, glandular hairy on outside; segments *c.* 7 mm long. Corolla purple, bilipped, 1cm long, hairy without, tube enlarged below the limb; tube white, *c.* 4 mm long; upper lip 2-lobed, lobes oblong-obtuse; lower lip shortly 3-lobed, middle lobe larger than lateral lobes, bullate with transverse ridges, obtuse. Stamens 4, didynamous; filament glabrous, filament of longer pair c. 5 mm long, of shorter pair c. 3 mm long; anther cells parallel, oblong, equal, divaricate or connate apex, muticous at base. Ovary narrow, 2–2.5 mm long, ovate-oblong, glandular hairy at apex; style white, c. 8 mm long, minutely pubescent; stigma simple. Capsule *c.* 7 mm long, narrowly oblong, pointed, glabrous. Seeds numerous, small, ovoid, supported on soft, blunt, minute, conical retinacula (Plate 6).

Fig. 10. Lectotype of *Hygrophila pinnatifida* (Dalzell) Sreem. (designated here), [*Dalzell s.n.* (K001392427)]
© The Board of Trustees of the Royal Botanic Gardens, Kew

Flowering & fruiting: January – May.

Ecology: It grows in river beds, rocky and gravelly streams. In vegetative stage it is totally submerged in water. Commonly found growing in large or small flowing water bodies either floating or submerged.



Distribution: From the Northern Western Ghats of Maharashtra to the Central Western Ghats of Karnataka. Endemic to peninsular India.

Conservation status: Least Concern (LC).

Etymology: Owing to the dissected leaves of the plant.

Vernacular Name: Fern-Leaf Swampweed

Note: A delicate slender submerged herb with two types of leaves (heterophyllous); the submerged leaves are strap shaped, pinnately lobed, leathery, abaxial surface of leaves reddish-brown, dark green on adaxial side, whereas the aerial floating leaves are shorter, crenate-serrate along margins.

This aquatic plant is currently becoming popular as an aquarium plant.

Specimens examined: INDIA. **Goa**, North Goa district, Pernem, Querim, 16.03.1997, *M.K. Janarthanam & S. Rajkumar* 621 (GUH); Bicholim, Dodamarg, 12.04.1998, *M.K. Janarthanam & S. Rajkumar* 1506 (GUH); Saari, Tulsimala, Poryem, 12.03.2006, *M. E. Mascarenhas* 218 (GUH). **Karnataka**, Belgaum district, Belgaum, 18.03.2007, *V. M. Nilesh* 2324 (SUK); Davalli-Londa, 25.10.1978, *C. J. Saldanha & P. Prakash* 3492 (JCB); Londa-Anmod road, 22.05.1979, *C.J. Saldanha KFP* 7822 (CAL; JCB). Gavali, 28.04.2007, *V.M. Nilesh* 2878 (SUK); Jamboti, 27.04.2007, *V.M. Nilesh* 2885 (SUK); Shimoga district, Kilangur to Hulical road, 21.03.1964, *R.S. Raghavan* 97055 (CAL); Uttara Kannada, *s.l.*, *s.d.*, *s.c.* Acc. no. 37204 (MH); near Soongsal, 18.01.1885, *W.A. Talbot* Acc. no. 37205 (MH); Soongsal bed of river, Deviman Ghat, 04.01.1885, *W.A. Talbot* 1131 (BSI); Mullund, 1889, *W. A. Talbot* 1868 (BSI); Deviman Ghat, February 1873, *s.c., s.n.*

(BSI); Banks of Kali nadi, Londa, 21.04.1950, *H. Santapau* 10822 (BLAT); Katgal, 30.04.1956, Pouri & Party 1359 (BSI). **Maharashtra**, Concan, s.*l*., *s.d*., *Stocks*, Law Acc.no: 37203 (MH); Concan, *s.l*., *s.d*., *Stocks*, Law Acc.no: 335601; Kolhapur district, Barki, *s.d*., *M.M. Sardessai* 742 (SUK); Dajipur above Fonda Ghat, 04.06.1904, R.K. Bhide *s.n*. (BSI); Kolhapur, 21.12.1992, *M.M. Sardesai* 898 (SUK); Suleran, 28.01.2020, *V.T. Jaseela* 16563 (MBGH); Nuvendi near Ratnagiri, 25.05.1904, *R.K. Bhide s.n*. (BSI); Ramghat, 05.02.1992, *A.S. Norvekor 321* (SUK); Vasota, Kargaon, 00.11.1994*, M.P. Bachulkar & Cholekar* 6108 (SUK); Western Ghat, *S.R. Yadav* 324 (SUK); Nandurbar district, Kargaon, 19.11.1994, *M.P. Bachulkar 6108* (SUK); Pune district, Dajipur, 04.06.1904, *R.K. Bhide s.n.* (BSI). Ratnagiri district, Niwendi, 25.05.1904, *R.K. Bhide s.n.* (BSI); Ratnagiri district, Ramghat-Bhedshi, 10.02.1970, *B.G. Kulkarni* 120006 (BSI); Anandval, Malwan, 07.08.1991, *R.S. Rao* 131550 (BSI); Satara district, Koyna nagar, 14.04.1981, *M.R. Almeida s.n.* (BLAT); Sindhudurg district, Way to linga Sakal, 8 km from Chaukul, 30.04.1956, *B.G. Kulkarni* 108585 (BSI); Chaukul, 20.12.2019, *V.T. Jaseela* 16550 (MBGH); Dabhole, 20.12.2021, *V.T. Jaseela* 16600 (MBGH); Kesari, Sawantwadi, 24.04.1969, *M.R. Almeida* 1158 (BLAT); *s.l*., *V.D. Vartak* Acc. no: 13033 (AHMA); *s.l., s.d*., *M.N. Datar* Acc. no: 5715 (AHMA).

Hygrophila pogonocalyx Hayata, Icon. Pl. Formosan. 9: 81. 1920.

Type:— Taiwan, Toroku, June 1917, *Y.Shimada s.n.* (Holoype TI00213473, digital image!) Fig. 10.

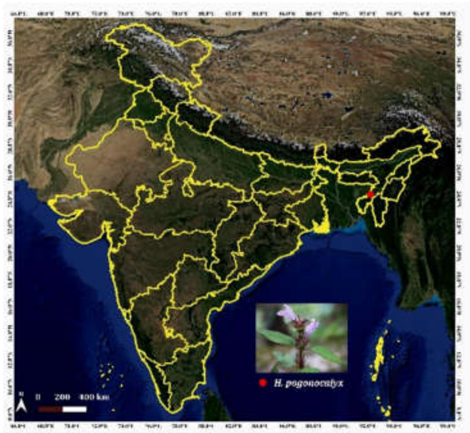
Erect annual herb. Stem quadrangular, hairy, base decumbent, 1–1.5 m, internodes subglabrous; nodes swollen, reddish, densely hairy. Leaves simple, opposite decussate, sessile, papery, linear-lanceolate, 5–9 × 0.7–1.2 cm, both sides scabrous-hirsute, base broadly attenuate, margins subentire, apex obtuse. Flowers arranged in clusters at nodes, sessile, multi bracteates; bracts foliaceous, ovate or lanceolate, 1.5 cm long 4–6 mm wide, apex caudate-acuminate, outwardly strigose, smooth inside; bracteoles foliaceous, pubescent. Calyx subcylindrical, 1.5–2 cm long, 5-fid up to half, lobes subaequal, linear, hairy, reddish colour, apex acuminate. Corolla blue, tubular-striated 2.5 cm long, 5 mm wide, limb 2-labiate, upper lip erect, 1 cm long, apex shortly 2-labiate, lower lip more or less open tip 3 labiate, lobes uniform, middle lobe oblong 3 mm long and 2 mm wide apex obtuse; corolla inside with numerous tufts of long and weak hairs. Stamens 4 didynamous, 5–10 mm; anthers 2 celled, oblong, 3 mm long and 1.5 mm wide, thecae basifixed, brown colour, glabrous, base sagitate. Ovary conical-cylindrical, bilocular, 2.5×2 mm, glabrous; Style glabrous. Disc cushion shaped. 2 ovules in each chamber (Plate 7).

Fig. 11. Type of *Hygrophila pogonocalyx* Hayata (designated here), [*Shimada s.n.* (TI00213473)] © The University Museum, The University of Tokyo.

Synonym: No synonym recorded for this species.

Flowering & fruiting: September – February.

Ecology: The plant is growing in moist or wet shady places.



Distribution: The species is globally distributed to the North-Eastern Taiwan and Western Taiwan. Presently it is recorded from Tripura, the North Eastern state of India which falls in Indo-Burma Bio-geographical hotspot region.

Conservation Status: The species *H. pogonocalyx* is an endangered species endemic to Taiwan (Hsieh and Huang 1974). Since 1996, *Hygrophila pogonocalyx* has been recorded in the Red List Categories as the criteria allotted by the International Union for Conservation of Nature and Natura Resources and is considered as a “critically endangered” (IUCN, 2022).

Etymology: Owing to the prominent spreading hairs on the abaxial surface of the sepals.

Notes: Hayata states that this is near to *H. salicifolia* (Vahl) Nees, but differs from it in the much more barbate bracts, and in the very scabrous hirsute leaves

Specimens Examined: INDIA, North Tripura, Dharmanagar Subdivision, Kameshwar, 29.12.2022, *V.T. Jaseela* 18434 (MBGH).

Hygrophila polysperma (Roxburgh) T. Anderson in J. Linn. Soc. 9: 456. 1867 & in DC l.c.p. 80; Aitch., Cat. Pl. Punjab & Sindh 111. 1869; C.B.Clarke in Hook.f., Fl. Brit. India 4: 406. 1884; Prain, Bengal Pl. 2:801. 1903; T.Cooke. Fl. Bombay 2: 353. 1908; C.J.Bamber, Pl. Punjab 623. 1916; Haines, Bot. Bihar & Orissa pt. 4: 670. 1922; Gamble, Fl. Pres. Madras 2: 1015. 1924; Santapau in Bot. Mem. Univ. Bombay 2: 19, 1951 & in JBNHS 51: 350, 1953 & Rec. Bot. Surv. Ind. 16 (1): 219, 1960; Haines, Bot. Bihar & Orissa 2: 702. 1961 (Repr. ed.); Maheshw., Fl. Delhi. 269.1963; B.D.Sharma *et al*., Fl. Karnataka Anal. 207. 1984; H.J.Chowdhery & B.M.Wadhwa, Fl. Himachal Pradesh Anal. 2: 550. 1984; A.K.Mukherjee, Fl. Pachmarhi and Bori Reserves 229. 1984; D.M.Verma *et al*., Fl. Raipur, Durg, and Rajnandgaon 281. 1985; K.R.Naskar K. Aq. Semi-aq, Pl. Lower Ganga Delta, Tax. Eco. Ec. Imp. 203. 1990; Naik, Fl. Marathwada 673, 1998; Misra BK & Verma BK. Flora of Allahabad District, Uttar Pradesh India.289-290.1992; Pull. *et al*., Fl. Adilabad Dist. 166. 1992; K.R.Naskar, Pl. Weal. Low. Ganga Delta 2: 513. 1993; A.B.Chaudhuri, Fore. Pl. East. India 398. 1993; C. D. K. Cook, Aquat. Wetl. Pl Ind. 33. 1996; K.K. Khanna, *et al*., Fl. Madhya Pradesh. 2: 324. 1997; P. K. Bhattacharya & K. Sarkar, Fl. West Champaran Dist. 337. 1998; N. P. Singh *et al*. Fl. Bihar Anal. 387. 2001; S. N. Ramaswamy *et al*., Fl. Shimoga Dist. 448. 2001; Londhe in Singh et, Fl. Maharashtra (Dicot.) 2: 035, 2001; S. R. Yadav & M. M. Sardesai, Fl. Kolhapur Dist. 359.2002; M. R. Almeida. Fl. Maharashtra 4: 51. 2003; P. J. Bora & Y. Kumar, Fl. Div. Assam, Stu. Pabitora Wildlife Sanctuary. 251. 2003; Anand Kumar, Fl. Indravati Tiger Reserve 220. 2003; N. D. Paria & S. P. Chattopadhyay. Fl. Hazaribagh Dist. 2: 762. 2005; Pradhan *et al*., Fl. Sanjay Gandhi NP 482. 2005;

GS Giri *et al*., Mat. Fl Arunachal Pradesh 2: 243. 2008; Karthik. *et al*., Fl. Pl. India Dicot. 1: 20. 2009; Pull., Fl. Telangana 2: 697. 2015; P. B. Singh, Fl. Mandi Dist. 405. 2018; P. Singh *et al*., Pl. Ind. Himal. Reg. 44. 2019; Arisdason *et al*., Fl. Pl. India. Checklist 2: 285; 2020; Karnataka Biodiversity Board, Fl. Karnataka, A Checklist. 2: 36. 2019.Gogoi R. Fl. Sikkim 39. 2021.

Justicia polysperma Roxb., Hort. Bengal. 3 (1814) *nom.inval*., *nom.nud*., & in Fl. Ind. (Carey & Wallich ed.) 1: 119. 1832.

Type:— India, Bengal, *s.d*., *Roxburgh*, *s.n.* (Lectotype designated here, BR0000006944551, digital image!) Fig. 11

Hemiadelphis polysperma (Heyne ex Roth) Nees in Wall. Pl. Asiat. Rar. 3: 80, 1832; Bremek. in Dens. Bot. Arkiv. 20: 60, 1961; Srinivasan & Agarwal, BuII. Bot Surv. Ind. 5(1): 83, 1963; Wight, Icon. t. 1492, 1849; Malhotra & Moorthy, BOBSI 13: 309, 1971;Ugemuge, Fl. Nagpur Dist. 285, 1986; AK Sharma & JS Dhakre. Fl. Agra Dist. 213. 1995; Pull. *al*., Flora of Medak Dist. 177. 1998.

Cardanthera uliginosa Ham. ex Nees in DC., Prodr. 11: 68, 1847.;

Adenosoma uliginosa Nees ex R. Br. In Shriften 3: 298, 1839.

Ruellia uliginosa Roxb., Fl. Ind. 3: 52, 1832. Wall. Cat. n. 2378 b, c, ex parte.

Hemidelphus polyspermus Nees var. *joshianus* Rao & Biswas Jn Indian For. 94: 657. 1968; Nayar & Raman. in Bull. Bot. Surv. India 15 :218. 1976.

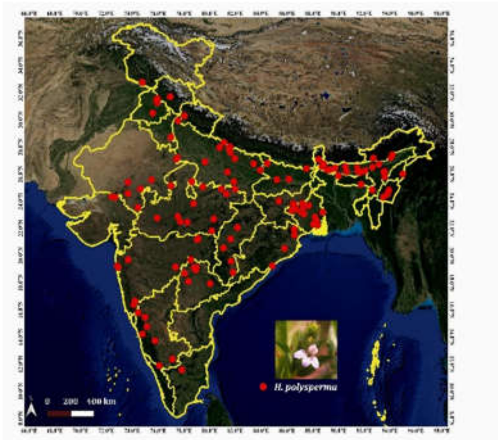
Hemigraphis tonkinensis Lindau, Bull. Herb. Boissier v. 649. 1897.

Small procumbent perennial herb with creeping stems, rooting at nodes. Stem diffuse, slender, terete ascending or erect, much branched, 15–30 cm tall, occasionally hairy towards apices, node swollen & hairy, when submerged not noticeably swollen. Leaves green, opposite, sessile or shortly stalked, mostly glabrous or pubescent, variable, entire or nearly so, base usually narrowed down, apex acute or sub-acute; lateral nerves 4 pairs, petiole 0–3 mm, elliptical to elliptic-lanceolate, lineolate, 0.5–5 × 0.3–l.5 cm long, upper gradually shorter, passing into bracts, with entire margins, 2-4 × 0.5–0.8 cm. Submerged leaves usually distinctly stalked, usually glabrous, elliptical to oblong, with entire margins, usually larger than the emersed ones. Flowers mostly in distinct terminal spikes, occasionally in axillary clusters or solitary in the axils of leaf like bracts; spikes dense, terminal, ca, 10 cm long, oblong or cylindric, 2.5–10 cm long, pubescent, usually imbricate; bracts foliaceous, single flowered, ovate-lanceolate, hairy, 6–8 × 2–3 mm elliptic-oblong, obtuse, pubescent on both sides and with a strong midnerve; the lower one broader a little than the two lateral. Bracteoles lanceolate to linear, ca 0.3–0.5 cm, nearly as long as the sepals, pubescent on both sides, margin ciliate. Calyx tubular, 5 fid, 0.5 cm long; segments linear, hairy, ciliate. divided rather more than half way down; teeth 5, narrowly linear, hairy, with scarious ciliate margins. Sepals 5, the lobes slightly unequal 3–3.5 mm long linear, with ciliate margins. Corolla pale purplish or white, 2-lipped, pubescent, up to 4–7 mm long; tube *c.* 5 mm long; lobes unequal, not deeply divided, upper 2 narrower, palate of the abaxial lip glabrous with upper lip bidentate, lower trilobed. Stamens 4, 2 with fertile anthers; adaxial filaments with an appendage towards the base; the adaxial stamens reduced to minute teeth, filaments filiform; anther-cells parallel, equal, muticous. Stigma unequally 2-lobed. Ovary green, cylindrical, 0.25 cm, hairy at apex; style hairy; stigma recurved. Capsule *c.* 7 × 2 mm. narrowly linear-oblong brown, furrowed, glabrous with apical tuft of hairs, tip shortly apiculate, valves recurved after dehiscence. Seeds subtended by retinacula. seeds discoid. Fruit narrow, up to 15 mm long. Seeds minute,15-25, orbicular, brown. 0.5 mm, hygroscopicaly hairy. Retinacula hard, slender and curved (Plate 8).

Fig. 12. Lectotype of *Hygrophila polysperma* (Roxburgh) T. Anderson (designated here), [*Roxburgh s.n.* (BR0000006944551)] © Meise Botanic Garden

Flowering & fruiting: December – February

Ecology: Common in wet places, forming mats on the grounds. Found in marshy places, drains, temporary pools and in slowly moving water in streams and canals, at the margin of paddy fields, channels, ditches and in other damp places.



Distribution: India to India China, introduced and established in some warmer parts of North America.

Etymology: polysperma= many seeded, owing to the large number of seeds produced by the plant.

Vernacular Name: Indian swampweed.

Notes: Very variable in its habit, pubescence, size and shape of leaves. In less moist areas the plant is mostly erect; but in marshy areas plants are diffuse and rooting at lower nodes.

Flower colour also varies much – violet tinged white or ashy-grey or light-bluish.

Hygrophila pusilla Bl. Bijdr. Fl. Ned. 804. 1826: Bremekamp in Verh. Nederl. Akad. Wctensch. Amst. Afd. Natuurk., sec. 2, 45(2): 14. 1948; Back. & Bakh. f. Fl. Java 2: 570. 1965. Arisdason *et al*., Fl. Pl. India. Checklist 2: 285; 2020.

Type:— Indonesia, Java, *s.d*., *Blume*, *s.n.* (Lectotype (designated here L2835411, digital image!) Fig. 12.

Hygrophila saxatilis Ridl. in Trans. Linn. Soc. 3: 333. 1893 et Fl. Mal. Pen. 2: 566. 1923.

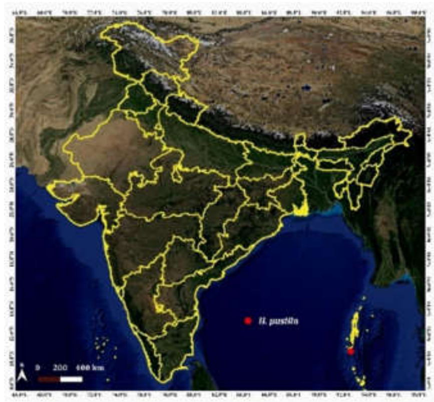
Tufted herbs up to 15 cm tall. Branches thin, quadrangular, nodes thickened and less than 12 mm apart. Leaves opposite, lanceolate (-spathulate), obtuse, 2–4 cm long, 4.5–5 mm broad, glabrous, entire or subcrenate; cystoliths numerous, slender, on both surfaces. Flowers 1–3, axillary, subsessile. Bracts linear-lanceolate, glabrous, ± 5 mm long; bracteoles oblong-lanceolate. Calyx 5–7 mm, 5-fid; segments unequal, acute, short hairy at apex, with numerous cystoliths, reflexed in capsule. Corolla 8–

12 mm long, bilab1ate, white with purple tinge; upper lip erect, shortly bifid, lower lip 3-lobed, palate, sparsely hairy. Stamens 4, attached at the middle of the corolla-tube, didynamous, connected pairwise by a membrane. Ovary glabrous, 2-celled, many ovuled, ± 2 mm long. Capsule oblong-linear, glabrous, about 1 cm long; seeds less than to (mostly 7) in each valve, borne on retinacula.

Fig. 13. Lectoype of *Hygrophila pusilla* Bl. (designated here), [*Blume s.n.* (L2835411)] © National Herbarium, Netherlands

Ecology: Occurring on rocks and crevices, near water-courses and swampy locations.

Distribution: Andaman Islands, Malaya and Java.



Etymology: 'pusilla' = 'very small, owing to the small size of the plant

Notes: It differs from *H. ringens*, in plants being shorter than 15 cm, internodes never longer than 12 mm, calyx lobes unequal with one segment longer than others and seeds less than 10 in each valve.

Hygrophila ringens (Linn.) R. Br. ex Steud. Nomencl. Bot. (ed. 1) 1: 418. 1821; Sprengel. Syst. Veg. 2: 828 (1825); Nicolson *et al*., Interpr.Van Rheede's Hort. Mal. 39. 1988; Babu, Fl. Malappuram Dist. 580. 1990; K. T. Joseph, Obs. Aq. Ang. Malabar 40. 1991; Sivar. & P.Mathew, Fl. Nilambur 502. 1997; V N. Naik, Fl. Marathwada 273, 1998; Londhe in Singh et al, Fl. Maharashtra (Dicot.) 2: 635, 2001; S. R. Yadav & M. M. Sardesai, Fl. Kolhapur Dist. 359.2002; M. R. Almeida. Fl. Maharashtra 4: 51. 2003; K. G. Bhat, Fl. Udupi 28: 2003; Sasidh. Biodivers. Doc. Kerala. Part 6. Fl. Pl. Kerala. 344. 2004; Anil Kumar *et al*., Fl. Pathanamthitta 380. 2005; Pradhan *et al*., Fl. Sanjay Gandhi NP 480. 2005; Datar *et al.* in Rheedea

15: 133. 2006, T. S. Nayar *et al*., Fl. Pl. Kerala, A Hand Book. 20. 2006; Karthik. *et al*., Fl. Pl. India Dicot. 1: 20. 2009; Sunil & Sivadasan, Fl. Alappuzha Dist. 538. 2009; Ratheesh Narayanan, Fl. Stud. Wayanad Dist. 614. 2009; G. P. Sinha *et al*., Fl. Mizoram. 239. 2012; Datar & Lakshmin., Fl. Bhagwan Mahavir NP & Adj. Goa 185. 2013; R.Manik. & Lakshmin., Fl. Rajiv Gandhi NP 300. 2013; S. P. Gaikwad & K. U. Garad, Fl. Solapur Dist. 467. 2015; R. R. Ansari *et al*., Aq. Wetl. Fl. Kerala: Flowering Plants. 57. 2016; P. Singh *et al*., Pl. Ind. Himal. Reg. 44. 2019; Karnataka Biodiversity Board, Fl. Karnataka, A Checklist. 2: 37. 2019; MC Naik *et al.* Fl. Div. Anal South Andaman Islands. 21(68): 402. 2020; Arisdason *et al*., Fl. Pl. India. Checklist 2: 285; 2020.

Ruellia ringens L., Sp. Pl. 635. 1753.

Lectotype (designated by Hansen): Herb. Linn. No. 804.13 (LINN) Fig. 13.

Hygrophila ringens var. *cochinensis* (Remadevi & Binojk.) Karthik. & Moorthy; Fl. Pl. India 23 (2009), ***syn. nov.***

Hygrophila ringens subsp. *longifolium* J.Mathew & Kad.V.George; Int. J. Advanced Res. 1(9): 132. 2013, ***syn. nov.***

Ruellia salicifolia Vahl. Sym. Bot. 3: 84. 1794.

Hygrophila salicifolia (Vahl) Nees in Wall., Pl. Asiat. Rar. 3: 81. 1832; Roxb., Fl. Ind.3: 50. 1832. Wight Ic. t. 1490. 1849; Daiz. & Gibs., Bombay Fl. 184, 1861; C.B.Clarke in Hook.f., Fl. Brit. India 4: 407. 1884; Prain, Bengal Pl. 2: 801. 1903; CJ Bamber, Pl. Punjab.623. 1916; Santapau in Bot. Mem. Univ. Bombay 2: 21, 1951 & in JBNHS 51: 351, 1953; Ramamoorthy in Sald. & Nicols., Fl. Hassan Dist. 550. 1976; R.R. Rao & Razi, Syn. Fl. Mysore Distr. 526. 1981; Manilal & Sivar., Fl. Calicut 224. 1982; K. M. Mathew, Fl. Tamilnadu Carnatic 2: 1180. 1983; D. M. Verma et al., Fl. Raipur, Durg, and Rajnandgaon 281. 1985; A.N. Henry *et al*. Fl. Tamil Nadu 2:149. 1987; K. K. N. Nair & M. P. Nayar, Fl. Courtallum 2: 294. 1987; Mohanan, Fl. Quilon Dist. 304. 1984; BG Kulkarni. Fl. Sindhdhurg 328. 1988; Bole & Pathak, Fl. Saurashtra 2: 183. 1988; Antony, Syst. Stud. Fl. Kottayam Dist. 302. 1989; E. Vajravalu, Fl. Palghat Dist. 350. 1990; Kesh.Murthy & Yogan., Fl. Coorg 333. 1990; K. M. Matthew, Exc. Fl. Cent. Tamilnadu 647. 1991; L. K. Banerjee, Pl. Resour. Jaldapara Rhino Sanct. 47. 1993; A. S. Chauhan, Contr. Fl. Namdapha 241. 1996; K.K. Khanna, *et al*., Fl. Madhya Pradesh. 2: 324. 1997; Pull. & Moulali, Fl. Andhra Pradesh 2: 715. 1997; Sasidh., Fl. Shenduruny WLS 233. 1997; Sasidh., Fl. Periyar Tiger Reserve 296. 1998; N. P. Singh *et al*. Fl. Bihar Anal. 387. 2001; S. N.

Ramaswamy *et al*., Fl. Shimoga Dist. 447. 2001; B. Suryanarayana & A. S. Rao, Fl. Nellore Dist. 416. 2002; Bora PJ & Yogendra Kumar Y. Fl. Diver Assam, St. Pabitora Wildlife Sanctuary. 250. 2003; B. K. Manjunatha *et al*., Flora of Devanagere District, Karnataka, India. 305. 2004; R. R. V. Raju & Pull. Fl. Kurnool. 359. 2005; N. D. Paria & S. P. Chattopadhyay. Fl. Hazaribagh Dist. 2: 764. 2005; GS Giri *et al*., Mat. Fl Arunachal Pradesh 2: 243. 2008; G. V. S. Rao & G. R. Kumari, Fl. Vishakapattanam Dist. Rao PSN (ed). 2: 20. 2008; Remadevi & Binoj., Fl. Kerala. Acanthaceae 84. 2009.

Hygrophila salicifolia var. *assurgens* (Nees) C.B.Clarke in Hook.f., Fl. Brit. India 4: 407 (1884); Prain, Bengal Pl. 2: 802. 1903.

Ruellia undulata Vahl. Sym. Bot. 3: 82. 1794.

Hygrophila undulata (Vahl) Nees, Wall. Pl. As. Rar. 3: 80. 1832.

Hygrophila angustifolia R. Br., Prodr. 1: 479. 1810; T.Cooke. Fl. Bombay 2: 354. 1908; Gamble, Fl. Pres. Madras 1016(713). 1924; Haines, Bot. Bihar & Orissa 2: 702. 1961.

Ruellia longifolia Roth, Nov. Sp. 306, 1821.

Nir-Schulli Rheede, Hort. Malab. 2: 89 – 90, t. 46. 1679.

B. angustifolia T.Cooke, Fl. Pres. Bombay 2 354, 1904 (nor R. Br. 1810).

Hygrophila quadrivalvis Nees in Wall., Pl. Asiat. Rar. 3: 80. 1832; C.B.Clarke in Hook.f., Fl. Brit. India 4: 408. 1884; Gamble, Fl. Pres. Madras 1016(714). 1924; Rani & Mathew in Fl. Tam. Car. 3. 1179. 1983; K. M. Matthew, Exc. Fl. Cent. Tamilnadu 647. 1991; G. R. Kothari & S. Moorthy, Fl. Raigad Dist. 294. 1993; C.D.K. Cook, Aquat. Wetl. Pl. Ind. 34. 1996.

Hygrophila salicifolia (Vahl) Nees var *cochinensis* Remadevi & Binojk. J. Econ. Taxon. Bot. 25(1): 233 (2001); Remadevi & Binoj., Fl. Kerala. Acanthaceae 84. 2009.

Ruellia obovata Roxb. (Hort. Beng. 47. 1814, nom. Nud.), ex. Hornem., Suppl. Hort. Bot. Hafn. 144. 1819., Roxb., Fl Ind. 3: 51. 1832.

Hygrophila obovata (Roxb. ex Hornem.) Ham., Tran. L. Soc.Lon. 14: 292. 1825; Nees in Wall., Pl. Asiat. Rar. 3: 81. 1832; Wt., Icon. t. 149. 1849.

Nir-Sculli Rheede, Hort. Malab. 2: 89-90, t. 46, 1689.

Dipteracanthus ringens (L.) Abeyw., Ceylon Journ. Sci., Biol. Sci., 2: 225. 1959

Hygrophila barbata Nees ex Steud., Nom. ed. II. 1: 783. 1840

Hygrophila ciliaris (Buch.-Ham. ex Wall.) Nees, DC. Prod. 11: 156. 1847

Hygrophila dimidiata Nees, Wall. Pl. As. Rar. 3: 81 (1832)

Hygrophila lancea (Thunb.) Miq., Ann. Mus. Bot. Lugd. Bat. 2: 123. 1865

Hygrophila malabarica Raf., Fl. Tellur. 4: 66. 1836

Hygrophila obovata (Roxb.) Nees, Wall. Pl. As. Rar. 3: 81. 1832

Hygrophila stricta Hassk., Cat. Hort. Bog. Alt. 148. 1844.

Hygrophila undulata (Vahl) Blume, Bijdr. 803. 1826.

Justicia lancea Thunb., Trans. L. Soc. 2: 338. 1794.

Ruellia angustifolia Poir., Encyc. 6: 338 (1804) non Sw.

Ruellia bracteata Willd. ex Nees, DC. Prod. 11: 155. 1847

Ruellia ciliaris Buch.-Ham. ex Wall., Cat. n. 2373 C 1830

Ruellia dimidiata (Nees) Nees, DC. Prod. 11: 157. 1847

Ruellia upudalii B.Vogel, Suppl. Pl. 62, t. 110. 1790

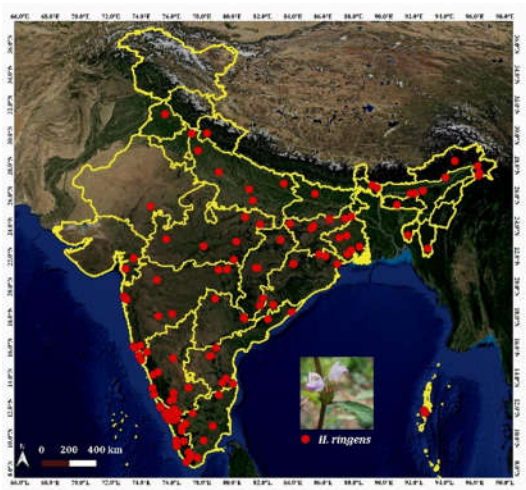
Hygrophila ringens var. *assurgens* (Nees) Karthik. & Moorthy; Fl. Pl. India 23. 2009

Hygrophila assurgens Nees, DC. Prod. 11: 90. 1847

Fig. 14. Lectotype of *Hygrophila ringens* (Linn.) R. Br. ex Steud. (designated by B. Hansen), (LINN 804.13) © Linnean Society of London

Perennial, erect or ascending herbs to subshrubs, 0.3–1.2 m high; rooting at lower nodes. Stems quadrangular, brached, glabrous or sparsely hairy sometimes. Leaves highly variable in shape and size, 3–15 × 1–3 cm, linear, linear lanceolate, oblong, linear oblong, oblong-lanceolate, base attenuate or cuneate, margins entire or undulate, apex acute to obtuse, lineolate, sometimes sparsely hairy on nerves below the lamina; petiole 0.4–1.5 cm long. Flowers in dense axillary whorls, sessile, pale purple; bracts 3–8 mm long, ovate, oblong, sub-obtuse, hairy without; bracteoles smaller, c. 4 mm long, linear; acute, hairy. Calyx tubular, 5-fid, *c.* 9 mm long, unequal, connate half way down; lobes linear, oblong, acuminate, hairy within. Corolla white to pale purple, 1–1.7 cm long, limb strongly bilipped, glandular pubescent without; tube slender, white, 5–7 mm long, ventricose above; the upper lip ovate-oblong, *c.* 1 cm long, shortly 2-lobed; the lower lip 5–6 mm long, oblong, shortly 3-lobed, lobed rounded, villous inside. Stamens 4 fertile, didynamous; filaments white, hairy at the base; anthers unequal, 1.5–4 mm long. Ovary oblong, 4 mm long, glabrous, green, 2-loculed, ovules many in each locule; style filiform, white, pubescent; stigma simple, entire. Capsule linear-oblong, 1–1.5 cm long, slightly compressed. Seeds many, ovoid, compressed, *c.* 1.5 mm across, hygroscopically hairy; retinacula long & curved (Plate 9).

Flowering & fruiting: October – March.



Ecology: Found in marshy areas, in and around the margins of small streams and other wet places along the foot hills and plains.

Distribution: Throughout India.

Conservation Status: Least Concern (L.C.)

Etymology: '*ringens'* = with a two lipped mouth.

Vernacular Name: Erect Hygrophila, Nir-schulli (Malayalam, Tamil), Sadu gobbi (Telugu).

Notes: While establishing the genus *Hygrophila*, Robert Brown assigned Linnean species *Ruellia ringens* for his genus. But Brown did not make the new combination *H. ringens* (so the name H. ringens R. Br. became invalid). Later Steudel validated the name *Hygrophila ringens* [*Hygrophila ringens* (L.) R.Br. ex Steud.] in Nomencl. Bot. 1:418,1821.

Linnaeus (1753) provided a brief diagnosis for his species *Ruellia ringens* collected from India. He described the flowers as "floribus solitaris". But in the case of

H.ringens, flowers are never solitary instead possessing an axillary inflorecence with more than two flowers. The type specimen at Linnean hebarium (LINN 804.13) seems to show more than one flower in an axil. So, the description 'solitary flower' may be an error.

Linnaeus quotes two references for *R. ringens* - "Flora zeylanica 234" and "upudali. Rheed. Mal. 10. 125. t.64". As we knew, flora zeylanica published before binomial nomenclature, contains genus number for each plant. Linnaeus describes *Ruellia* under the number 234 and refers Lychnis of Burm. zeyl. 144, Upudali of Rheed. T. 64 and Purucwael of Hermann Zeylanica 13. All these citation does not fit with *H. ringens*.

Upu-dali is mentioned in volume 9 of Hortus malabaricus (It may be wrongly typed as volume 10 instead of 9). The plant Upu-dali is *Dipteracanthus patulus*.

H. ringens subsp *longifolium* was reported from Achankovil forest of Kollam district, Kerala. The distinguishing characters were plant height (100-120 cm), solitary flower, shape and size of the leaves (lanceolate, 9-17 cm long). Accessions of *H. ringens* from different localities has shown that the plant height and size and shape of the leaves vary greatly. *H. ringens* is a very plastic species, which show variations according to environmental conditions. Number of flowers also varies from 4-12 and at the onset of flowering, there will be only single flower which later develop lateral buds, so these characters could not serve to treat the taxon the status of a subspecies. We visited the type locality and could not get plant with the above characteristics, but collected *H. ringens* of less height, less leave size and many flowers at axil, compared to the subspecies taxon. So here we synonymise the *H. ringens* subsp. *longifolium* under *H. ringens*. Also, it is noted that the authors mentioned type specimen of type specimens of *H. ringens* subsp. *longifolium* were deposited at Central National Herbarium (CAL- holotype) and Herbarium of the CMS college (isotype). But both the types are not available in the mentioned herbaria.

Hygrophila ringens var. *cochinensis* (Remadevi & Binojk.) Karthik. & Moorthy was reported from Alappuzha district. The distinguishing characters of this taxon are plant height about 1.5m, leaf margin serrate with hairs and pinkish white flowers. These characters are widely seen in *H. ringens* and thus it cannot serve as a variety. So, we conclude that *H. ringens* subsp *longifolium* is conspecefic to *H. ringens*.

Specimens examined: INDIA, Andhra Pradesh, Chittoor district, Japalathirtham, 03.03.1959, *K. Subramanyam* 7861(CAL); near Tabala temple, 30.12.1975, *G.V.S. Rao* 46870 (CAL); East Godawari district, Adalcula to Maradmally reserve forest, 15.01.20004, *S.K. Nazeeruddin* (CAL); Kurnool district, Gundlabrahmeswaram, 06.12.1980, *R.R.V. Raju* 2388 (CAL); Vishakapatanam district, Araku Valley, 16.09.1961, *N.P. Balakrishnan* 610 (CAL). **Assam,** Cachar district, Katakhal RF, 01.09.1957, *R.S. Rao* 9107 (CAL); Katakhal, 01.09.1957, *R.S. Rao* 9107 (CAL); Jirighat, 23.11.1907, *A. Meebold* 5733 (CAL). Sonitpur district, Baghmara reserve forest, 14.12.1960, *G. Panigrahi* 22540 (ASSAM). **Bihar,** Bonstri river, 12.12.1957, *G. Panigrahi* 12065 (CAL). **Jharkhand,** Dumka district, Silingi, 21.12.1957, *G. Panigrahi* 12065 (ASSAM); Singhbhum district, Massher, 22.12.1903, *H.H. Haines* 564 (CAL). **Karnataka,** Dakshina Kannada District, Karkala, 17.03.1915, *s.coll. s.n*. (MH); Hassan district, Halbag, 24.19.1971, *C.J. Saldanha & K.N. Gandhi* 2176 (CAL); Bisle ghat, 18.12.1969, *C.J. Saldanha* 15908 (JCB); Mandya district, Srirangapatna, 06.02.1979, *P. Prakash & K.P. Sreenath* 6012 (CAL); Mysore district, Mysore, *G. Thomson* 37323 (MH); Aglassi, 00.11.1908, *A. Meebold* 9330 (CAL); North Canara, *s.d*., *W.A. Talbot* 729 (CAL). **Kerala,** Alappuzha district, Punnamada, 16.04.1988, *M S. Swaminathan* 88218 (MH); Kumarakam, 28.12.1983, *Antony* 10 (MH); Punnamada, 28.12.1990, *M.S. Swaminathan & V.P .Prasad* 95610 (MH); Aroor, 02.01.1991, *M.S. Swaminathan & V.P. Prasad* 95617 (MH); Vembanad lake, 16.01.1988, *M.S. Swaminathan* 88218 (CAL); Eranakulam district, Cherai, 05.12.1976, *C.R. Suresh* 22362 (CALI); Cochin, 00.09.1881, *J.S.Gamble* 14898 (CAL); 00.11.1910, *A.Meebold* 12581 (CAL); Vembanad lake, 01.01.1991, *M.S. Swaminathan & V.P. Prasad* 95703 (MH); Idukki district, 17.10.1982, *C.N. Mohanan* 74641 (MH); Meenmutty, 14.02.1982, *V.S.Raju* 71247 (MH); Panamkutty, 17.10.1982, *C.N. Mohanan* 74641 (CAL); Uppupara, 26.09.1972, *B.D. Sharma* 41695 (MH); 17.12.1974, *K. Vivekananthan* 45334 (MH); Kannur district, Ezhimala, 17.12.1979, *V.S. Ramachandran* 65277 (CAL); Kadavathur, 14.12.2018, *E.K. Sinisha & V.T. Jaseela* 16515 (MBG); Mangad, 20.12.1980, *R. Ansari* 69911 (CAL); Mattannur, 21.01.1979, *R. Ansari* 59745 (CAL); Muthiyalam, Payyannur, 20.10.2018, *V.T. Jassela, & M.K.R. Narayanan* 16501 (MBG); Pazhassi dam site, 21.01.1979, *V.S. Ramachandran* 59061 (CAL); Kollam district, Adoor, 16.08.1980, *C.N. Mohanan* 63348 (CAL); Kollam back waters, 19.05.1979, *C.N. Mohanan* 63101 (CAL); Kulathupuzha, 20.02.1979, *C.N. Mohanan* 61159 (MH); Mannaraparai range, 15.11.1976, *M. Chandrabose* 49060 (CAL, MH); Pathanamthitta hills, 26.02.1979, *C.N. Mohanan* 61229 (MH); Panamkutty, 17.10.1982, *C.N. Mohanan* 74641 (MH); Kottyam district, 16.11.1984, *V.T. Antony* 986 (CAL); Kozhikode district, Iduvattilthazham, 30.10.2018, *V.T. Jaseela* 16507 (MBG); Iringallur, 25.10.2018, *V.T. Jaseela* 16505 (MBG); Jaffarkhan colony road, near Planetarium, 13.11.2018, *V.T. Jaseela* 16512 (MBG); Palazhi, 25.10.2018, *V.T. Jaseela* 16506 (MBG); Perumanna, 30.10.2018, *V.T. Jaseela* 16509 (MBG); Thikkodi, 12.11.2018, *V.T. Jaseela* 16511 (MBG); Malappuram district, Feroke, 27.10.1970, *V.V. Sivarajan* 726 (CALI); Kalikavu, 11.03.1984, *P. Bhargavan* 81199 (MH); Muttippalam, 23.09.1910, *C.E. Fischer* 2226 (CAL); Palakkad district, Anamari, 30.11.76, *E. Vajravelu* 44765 (MH); Kallamalai, 12.11.76, *E. Vajravelu* 44756 (MH); Kanjarumpuzha, 26.10.1964, *K.M. Sebastine* 22315 (MH); Karivara forest, 28.11.1973, *E. Vajravelu* 44869 (MH); Padagiri, 25.12.1980, *M.C. Nair* 69796 (MH); Pottikkal forest, 24.01.1980, *P. Bhargavan* 65685 (MH); Thunakkadavu fall, 11.01.1916, *C.E.C. Fischer* 3857 (CAL); Thrissur district, Parambikulam, 14.02.1963, *K.M. Sebastine* 15653 (MH). Wayanad district, Lakkidi, 10.10.1976, *C.R. Suresh* 22283 (CALI); Lakkidi, 21.10.1985, *R.T. Balakrishnan* 41738 (CAL); Niravilppuzha, 18.12.2018, *V.T. Jaseela* 16518 (MBG); **Madhya Pradesh**, Bastar district, Dharba, 27.08.1959, *K. Subramanyam* 8648 (CAL). **Maharashtra,** Ratnagiri district, Deobag, 01.10.1970, *B.G. Kulkarni* 121349 (BSI). **Meghalaya**, East Khasi hills: Khasi hills, *Griffith* (CAL); Jaintia Hills district, K & J Hills, 17.08.1968, *N.P. Balakrishnan* 46138 (ASSAM). **Odisha,** Keonjhar district, Sanaghagara, 01.11.1995, *R.K. Moharanan* 5313 (RPRC); Kendrapara District, Rajnagar, 03.02.1961, *G. Panigrahi* 23683 (ASSAM); Mayurbhanj district, Gurguria, 16.02.1958, *G. Panigrahi* 12456 (ASSAM); Simlipahar, Gurguria, 19.10.1983, *H. Saxena* & *M. Brahmam* 5207 (JCB); Sambalpur district, Rairakhol, 02.12.1987, *S. Panda* 931(CAL). **Tamil Nadu,** Kanyakumari district, Thengapatnam, 25.01.1978, *A.N. Henry* 53287 (MH). **West Bengal,** Alipurduar district, Maliakalguri, 24.10.1891, *E.A. Beawood* 112 (CAL); Jalpaiguri district, Gossaihat Beel, 18.03. 2012, *A. Chowdhury, R. Biswas & A.P. Das* 0988 (NBU); Jaldapara, 27.09.1975, *J.K.Sikdar* 731 (CAL); North-24-Parganas district, Dhamakhali, 25.03.1985, *G.N. Tribedi* 1465 (CAL); Nalbon, 24.03.1962, *M.K. Ghosh* 1302 (CAL); Rampura, 06.08.1902, *D. Prain s.n.* (CAL); Sudhanyakhali, 14.11.1983, *G.N. Tribedi* & *D.C. Pal* 1411 (CAL); 24 Parganas district, 01.05.1813, *s.c.* 33524; West Dinajpur district, Islampur, 03.11.1983, *R.N. Banerjee* 16154 (CAL).

Hygrophila serpyllum (Nees) T. Anders. in Journ. Linn. Soc. 9: 456.1867; C.B.Clarke in Hook.f., Fl. Brit. India 4: 406. 1884; Birdwood, Cat. Fl. Matheran & Mahabaleshwar 21, 1897; T.Cooke. Fl. Bombay 2: 354. 1908; Woodrow in Journ. Bombay nat. Hist. Soc. 12: 355, 1899; Prain, Bengal Pl. 2: 801. 1903; Duthie, FL Gangetic Plain 2: 186. 1911; J. S. Gamble, Fl. Pres. Madras 2: 1016. 1924; Santapau in Univ. Bombay Bot. Mem 2: 19. 1951; Puri & Mahajan, BOSSI 2(1&2): 129, 1960; Haines, Bot. Bihar & Orissa 2: 703. 1961 (Repr. ed.); Santapau, Fl. Khandala ed. 3, 193, 1967; Santapau H. Flora of Khandala on the westernghats of India in Records of BSI. 16(1): 219. 1960; Malhotra & Moorthy BOSSI 13: 309, 1971; Oommachan Fl. Bhopal 296.1977; Singh V. Fl. Banswara. 181.1983; A. K. Mukherjee, Fl. Pachmarhi and Bori Reserves 229. 1984; R. S. Rao. Fl. Goa, Diu, Daman & Nagarhaveli 2: 325. 1986; A.N. Henry *et al*. Fl. Tamil Nadu 2:149. 1987; Bole & Pathak, Fl. Saurashtra 2: 183. 1988; SY Kamble & SG Pradhan. Fl. Akola Dist. 170. 1988; S. M. Almeida, Fl. savantwadi 322, 1990; Kamble & Pradhan, fl. Akola 170, 1988; B. V. Shetty & V. Singh, Fl. Rajasthan 652. 1991; K. M. Matthew, Exc. Fl. Cent. Tamilnadu 647. 1991; Lakshmin. & B.D. Sharma, Fl. Nasik Dist. 368. 1991; A. K.Malhotra & S. Moorthy, Fl. Tadoba National Park. 106. 1992: G. R. Kothari & S. Moorthy, Fl. Raigad Dist. 295. 1993; S. Deshpande et al., Fl. Mahabaleshwar and adjoinings 2: 446. 1995; M. Oommachan & J. Shrivastava, Fl. Jabalpur 194. 1996; C. D. K. Cook, Aquat. Wetl. Pl Ind. 35. 1996; K.K. Khanna, *et al*., Fl. Madhya Pradesh. 2: 325. 1997; Diwakar PG & Sharma BD. Flora of

Buldhana District, Maharashtra State.245.2000; N. P. Singh *et al*. Fl. Bihar Anal. 387. 2001; Londhe in Singh *et al*., Fl. Maharashtra *(Dicot.)* 2: 637, 2001; S. R. Yadav & M. M. Sardesai, Fl. Kolhapur Dist. 358.2002; M. R. Almeida. Fl. Maharashtra 4: 52. 2003; Pradhan *et al*., Fl. Sanjay Gandhi NP 483. 2005; Karthik. *et al*., Fl. Pl. India Dicot. 1: 20. 2009; Karnataka Biodiversity Board, Fl. Karnataka, A Checklist. 2: 37. 2019; Arisdason *et al*., Fl. Pl. India. Checklist 2: 287; 2020.

Physichilus serpyllum Nees in Hook. Comp, Bot. Mag 311. 1836; Wight, lc. 4(3): 18. t. 1493. 1849; Daiz. & Gibs., Bombay Fl. 184, 1861.

Type:— India, Mysore, 1.7.1835, *Campbell*, *s.n.* (Holotype E00273442, digital image!) Fig. 14

Ruellia polysperma Roth Nov. Sp. 305.
Adenosoma polysperma Spreng. Syst. ii. 829.

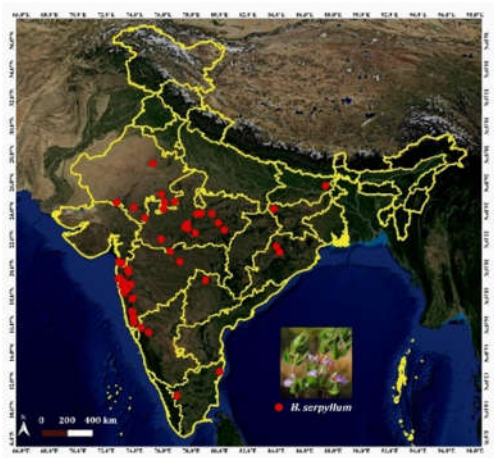
Hygrophila stocksii T.Anderson ex C.B.Clarke, Fl. Brit. India [J. D. Hooker] 4(11): 407 1884, ***syn. nov.***

Hygrophila serpyllum var *hookeriana* Clarke, C.B.Clarke in Hook.f., Fl. Brit. India 4: 407. 1884; Santapau H. Flora of Khandala on the westernghats of India in Records of BSI. 16(1): 220. 1960; G. R. Kothari & S. Moorthy, Fl. Raigad Dist. 294. 1993; M. R. Almeida. Fl. Maharashtra 4: 53. 2003; Pradhan *et al*., Fl. Sanjay Gandhi NP 483. 2005; Karthik. *et al*., Fl. Pl. India Dicot. 1: 20. 2009; Arisdason *et al*., Fl. Pl. India. Checklist 2: 286; 2020, ***syn. nov.***

Procumbent perennial herbs with woody rootstock. Stems creeping and rooting at nodes, with numerous ascending or erect flower-bearing shoots; creeping stems branched, often woody or wire like, not noticeably swollen when submerged, mostly glabrous or nearly so; erect shoots usually simple up to 25 cm tall but usually less, usually densely clothed in whitish hairs above. Submerged leaves usually distinctly stalked; petiole 0.3–1.5 cm long; blades of submerged leaves elliptical to oblong or

Fig. 15. Holotype of *Hygrophila serpyllum* (Nees) T. Anderson [*Campbell s.n.* (E00273442)] © The Board of Trustees of the Royal Botanic Garden, Edinburgh.

orbicular, up to 2.5 cm long and *c.* 2 cm wide, usually glabrous, with entire margins; blades of emergent leaves sessile or shortly stalked, lanceolate to elliptical or ovate, 1–2 (–4) cm long, rarely glabrous, mostly pubescent, with long white hairs and short glandular hairs, margins entire. Flowers mostly in distinct terminal spikes, at the beginning of the flowering period developing solitary or in axillary clusters in the axils of leaf like bracts; spikes distinctly interrupted below, becoming dense above. Bracts leaf-like to elliptical, 0.5–1 cm long, pubescent with long white hairs and short glandular hairs, imbricate only at the tip of the spike. Bracteoles oblanceolate to linear, as long as the sepals. Calyx 5, divided almost to the base; the lobes all alike, linear with ciliate margins, 4.5 mm long. Petal bright blue, tube *c.* 5 mm long, pubescent; abaxial lip *c.* 4 mm long, the palate bright blue with whitish swellings, glabrous. Stamens 4, all with fertile anthers; anthers deep purple to black, the adaxial pair *c.* 7 mm long, the abaxial pair *c.* 4 mm long. Seeds 10–16; retinacula hard, slender and curved (Plate 10).



Flowering & fruiting: January – March.

Ecology: In marshy places, drains, temporary pools and in slowly moving water in streams and rivers.

Distribution: Endemic to peninsular India.

Conservation Status: Least Concern (LC).

Etymology: '*serpyllum*' = 'to creep', owing to the habit of the plant.

Vernacular Name: Marsh carpet.

Notes: *H. serpyllum* is a very plastic species. During and shortly after the monsoon, it grows prostrate with relatively large, almost round, petiolate leaves. In this phase, the plant is dark green and produces some flowers in the axils of leaf-like bracts. This form has been named *Hygrophila serpyllum* var. *hookeriana* C.B.Clarke. As winter approaches and water recede, the shoots grow upright with narrower, shortly petiolate or sessile leaves covered in whitish hairs, developing flowers in terminal spikes. The entire plant becomes whitish-green, appearing very different from its wet-season form. So, it is concluded that *H. serpyllum* var. *hookeriana* is only an ecoform of *H. serpyllum* and hence synonymise it under *H. serpyllum*.

The name *Hygrophila stocksii* was proposed (unpublished mss) by T. Anderson, which was validated by C.B. Clarke in Fl. Brit. India (1884). Clarke cited the herbarium specimen '*Physichilus* n. 4, Herb. Ind. Or. H. F. & T', which was collected by Stocks from Concan. Anderson remarks that "this is evidently a variable plant and is much influenced by the nature of the soil in which it is found. Some luxuriant forms of it were distributed in the Indian herbarium of Dr. Hooker & Thompson under the numbers *Physichilus* sp. 2, 3 and 4". There is only one sheet of *Hygrophila stocksii* T. Anderson ex Clarke at Kew. Cooke remarks that it is only luxurient form of *H. serpyllum*. We have collected *H. serpyllum* from different localities of Maharashtra. The plant shows variations in size of the plant, leaf size, shape. The herbarium specimens also show degrees of variations in plant size, leaf size and shape. So, we conclude that *H. stocksii* is only a morphological variation of *H. serpyllum* and hence it is treated as conspecific to *H. serpyllum* (Nees) T. Anderson.

Specimens examined: INDIA, Daman, 04.05.1963, *M.Y. Ansari* 88992 (CAL). **Karnataka**, Hassan district, Salagame-Halebid, 26.04.1980, *C.J. Saldnah & S. R. Ramesh* 11228 (JCB); Udupi district, Kollur, Mookambika wildlife sanctuary, 17.10.2007, *P.G. Diwakar & R. K. Singh* 193261 (BSI). **Madhyapradesh,** Indoor district, Kalakund, choral river bed, 19.04.1963, *A.R.K. Shastry* 84252 (BSI); Mandla District, Khari reserve forest, 04.04.1962, *J. Joseph* 13994 (MH); Sagar district, near Bina River, 01.03.1960, *K. Subramanyam* 10120 (MH); Hoshangabad district, Bori reserve forest, 28.04.1961, *J. Joseph* 12431 (MH). **Maharashtra**, Buldhana district, Gaumal, 15.12.1982, *P.G.* Diwakar163074 (BSI); Dhulia district, Maudvi Khurd, Dhadgain, 06.03.1965, *R.D. Pataskar* 104145 (CAL); Kolhapur district, Amba, 02.02.1997, *M.M. Sardesai* 78 (SUK); Nasik district, Saptashringi, 15.02.1983, *P.C. Narasimhan* 165473 (BSI); Pune district, Ambavane, 31.01.1964, *B. V. Reddi* 95848 (BSI); Bhimashankar, 10.02.1999, *S.R. Yadav* 5896 (SUK);

Tungarli, Lonavala, 06.05.1956, *S.K. Jain* 967 (BSI); Mulshi, 22.03.1963, *R. R. Rao* 87328 (BSI); Paud, 19.10.1956, *Y.S. Narayan* 8294 (BSI); Sakarpathar, 13.03.1964, *B.V. Reddi* 68341 (BSI); Thangaon, 15.02.1958, *S.D. Mahajan* 32084 (CAL); Raigad district, Alibag, 22.11.1957, *D.K. Kamath 27010* (BSI); Matheran, 04.04.1957, *G. S. Puri* 10999 (BSI); Satara district, Bamnoli, 09.12.1994, *M. P. Bachulkar* 5912 (SUK); Khandala, 21.03.1961, *A.K. Jain* 35 (BSI); Khandala, 21.03.1903, *G.A. Gamble* 16145 (BSI); s.d.T.Cooke 22964 (BLAT); *s.d. Santapau* 9103 (BLAT); Sindhudurga district, Shirshinge, 19.03.1995, *M.P. Bachulkar 20953* (SUK); Thane district, Utwada hill, 20.10.1967, K.V. *Billore* 112955 (CAL). **Rajasthan**, Banswara district, Danpur village, 26.03.1977, *V. Singh* 4259 (BSJO); Sirohi district, Mount Abu, *Kanodia* 62182 (BSI); Udaipur district, Jaisamand, *s.d. Verma* 4252 (BSA).

Hygrophila thymus (Nees) Sunojk. & M.G.Prasad, Rheedea 24(1): 13. 2014

Adenosma thymus Nees in Wall., Pl. Asiat. Rar. 3: 79. 1832; Benth. in DC. Prodr. 11: 69. 1847, excl. syn.; T. Anders., J. Linn. Soc. Bot. 9: 454. 1867.

Type:— India, B. Heyne ex Wall. Cat. No. 2380B (Lectotype first step designated by Sunojk. & M.G. Prasad, 2014; second step designated here, K000882371] digital image!) Fig. 15

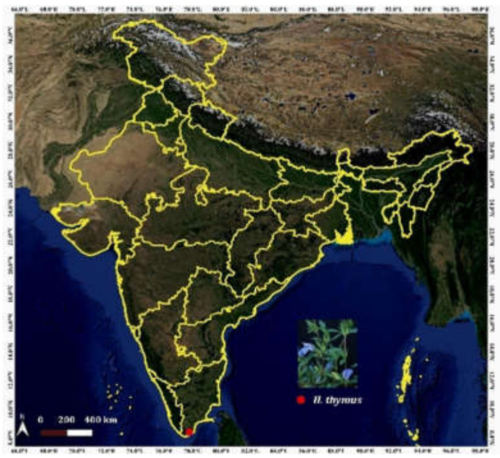
Cardanthera balsamica (Linn.f.) Benth. ex C.B. Clarke var. *thymus* (Nees) C.B. Clarke in Hook.f., Fl. Brit. India 4: 404. 1884.

Hygrophila balsamica (Linn.f.) Raf. var. *thymus* (Nees) Karthik. & Moorthy Fl. Pl. India 1: 21. 2009.

A slender, diffuse, trailing herb, spreads up to 25 cm long; branches ascending. Stem subquadrangular, glabrous, reddish & green in colour; nodes slightly pulvinous, pubescent; internodes 1.5–1.8 cm long. Leaves opposite, decussate, homophyllous, sessile; blades oblong-linear, 1.5–3 × 0.3–0.6 cm, adaxial glabrous, hairs prominent on midrib on abaxial surface, apex acute-obtuse, base tapering, margins entire at base, shallowly serrate towards apex, ciliate; veins 6–7 on each side. Flowers in axillary cymes, seen towards apex, white-purple, sessile, 8–9 mm

Fig. 16. Lectotype of *Hygrophila thymus* ((Nees) Sunojk. & M.G.Prasad (first step designated by Sunojk. & M.G. Prasad, 2014; second step designated here), [*B. Heyne* ex Wall. Cat. No. 2380B. (K000882371)] © The Board of Trustees of the Royal Botanic Gardens, Kew.

long; peduncles c. 1 mm long, glabrous; bracts 3, one large & two small, smaller than calyx, linear. 0.3–0.6 cm, ovate-oblong, base cuneate, margin ciliate, apex obtuse, hairy on both surfaces; bracteoles 2, *c.* 3.5 × 1 mm, hairy. Calyx *c.* 5 mm long, deeply 5-lobed, glandular pubescent, lobes unequal, divided almost to the base, lanceolate, margin glandular ciliate, apex obtuse. Corolla blipped; tube small, greenish, 4–5 mm long, funnel-shaped; hairy without, glabrous inside except some glandular hairs on the lower lip, upper lip longitudinally 3-nerved, slightly emarginate at apex, c. 4 × 3 mm, dull white, lower lip c. 4 × 4 mm, 3-lobed at apex, pale violet with dark pink cross lines, glandular hairs towards base. Stamens 4, didynamous; filaments white, upper two shorter, *c.* 0.03 cm long; lower two longer, *c.* 0.45 cm long; anthers white, 0.15 mm long, base sagitate, longitudinally splits open. Ovary oblong-elliptic, *c.* 0.2cm long, style white, 5 mm long, pubescent; stigma simple. Capsule 6 mm long, oblong, acute at apex, sparsely minute hairy towards upper side, slightly depresses. Seeds minute, ovate, flat, 0.4 × 0.2 mm, glabrous, brown (Plate 11).



Flowering & fruiting: January – April.

Distribution: Endemic to South India (Tamil Nadu).

Conservation status: Based on IUCN Red List criteria (IUCN, 2001), *Hygrophila thymus* can be given the conservation status of Critically Endangered (CR B1ab [ii,iii]) due to its restricted distribution, habitat loss and population size.

Note: Nees (1832) described *Adenosma thymus* based on a specimen collected by Benjamin Heyne from Deccan peninsula, India (Wall. Cat. No. 2380 B). Bentham (1847) and Anderson (1867) accepted the name *Adenosma thymus* after Nees (*l.c.*). Later, Clarke (1884) reduced *Adenosma thymus* as a variety of *Cardanthera balsamica* and remarked "it is certainly a starved specimen of *C. balsamica*". It seems that no more specimens of this taxon were mentioned in any of the literature, hence Clarke's observations about the differences are most likely based on a single herbarium sheet. Sunojkumar & Prasad (2014) rediscovered this species from Tamil Nadu and reinstated the species following Nees (1832) and Bentham (1847). They proposed the new combination *Hygrophila thumus*.

Specimens examined: **INDIA. Tamil Nadu**, Thirunelveli district, Vijayanarayanam, 09.03.2011, Sunojkumar & M.G. Prasad 88157 (CALI); 22.03.2022, *V.T. Jaseela* 18422 (MBGH).

Hygrophila triflora (Roxb.) Fosberg & Sachet, Baileya 21(3): 147. 1981; N. Sasidharan, Biod. Doc. Kerala. Part 6. Fl. Pl. Kerala. 344. 2004; T.S.Nayar *et al*., Fl. Pl. Kerala, A Hand Book. 21. 2006; Karthik. *et al*., Fl. Pl. India Dicot. 1: 20. 2009; Sunil & Sivadasan, Fl. Alappuzha Dist. 538. 2009; Arisdason *et al*., Fl. Pl. India. Checklist 2: 285; 2020.

Ruellia triflora Roxb. Hort. Beng. 46. 1814 & in Fl. Ind. 3: 52. (ed. Carey) 1832.

Type:— India, *s.d*., *Roxburgh s.n*. (Lectotype designated by Fosberg & Sachet, K000882353, digital image!) Fig. 16

Adenosma triflora (Roxb.) Nees in Wall., Pl. Asiat. Rar. 3: 79. 1832 & in DC., Prodr. 11: 68. 1847.

Cardanthera triflora (Roxb.) Buch.-Ham. ex Benth. in Benth. & Hook. f., Gen. Pl. 2: 1074. 1876; C.B.Clarke in Hook.f., Fl. Brit. India 4: 405. 1884.

Synnema triflorum (Roxb.) Kuntze Rev. Gen. 500. 1891.

Cardanthera triflora Buch.-Ham., Voigt, Hort. Suburb. Calcutt. 482. 1845, ex Nees, DC. Prod. 11: 68. 1847, ex Benth. in Benth. & Hook. f., Gen. Pl. 2: 1074. 1876; ex C.B.Clarke in Hook. f., Fl. Brit. Ind. 4: 405 (1884); Haines, Bot. Bihar & Orissa 2: 702. 1961 (Repr. ed.).

Cardanthera glutinosa Nees Buch.-Ham. ex Nees, DC. Prod. 11: 68. 1847.

Ruellia uliginosa Mart. ex Nees DC. Prod. 11: 68. 1847.

Ruellia tanacetifolia Vahl ex Nees , DC. Prod. 11: 68. 1847. not validly publ.

Ruellia triflora Sessé & Moc., Fl. Mexic., ed. 2 147. 1894.

Viscid aromatic perennial herbs. Stem green, ascending, subquadrangular, glandular pubescent with long and short hairs, rooting at nodes. Leaves opposite, decussate, subsessile, dimorphic, submerged leaves 5–7 × 2.5–3.5 cm, pinnately or bipinnately dissected, minutely hairy above, glabrescent beneath; aerial leaves 4.5–5.5 × 2–3 cm, obovate to suborbicular, base cuneate or rounded, margins serrate-dentate, apex obtuse, lateral nerves 6–7 pairs, densely glandular hairy above, sparsely hairy on nerves beneath; petiole 0.1–0.3 cm. Flowers in axillary cymes, 1.5–1.8 cm, 2–3 flowers in each axil; at first single sessile flower arises on axil, later 2 flowers arise on either side of it, at that time the inflorescence become stalked; peduncle 0.5 cm; bracts 2, ovate, 0.5–0.9 cm, sessile, margin serrate towards tip & entire towards base, apex acute, both surfaces glandular hairy. Calyx tubular, 5-partite, 8 mm long; lobes linear-lanceolate, equal, divided almost to the base, margin entire, apex acute, glandular hairy. Corolla pale blue with a purple palate, glandular hairy without, bilabiate; tube white, 0.5 cm; lower lip pubescent, shortly 3-lobed at apex, upper lip shortly 2-lobed, each lobe minutely 2-fid, obtuse. Stamens 4, didynamous; filaments white, hairy at base, 9 mm long; anthers 2.5 mm long, purplish. Ovary 3–4 mm; style filiform, pubescent, 0.8–1 cm long; stigma linear,

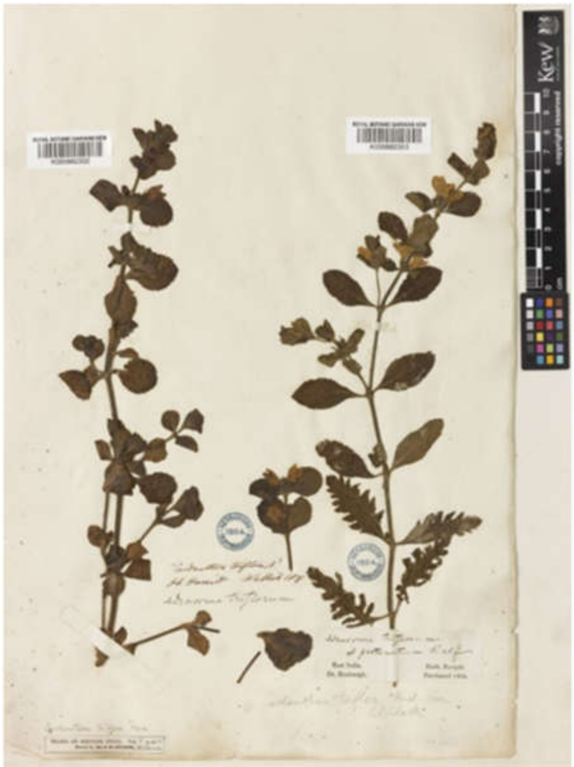
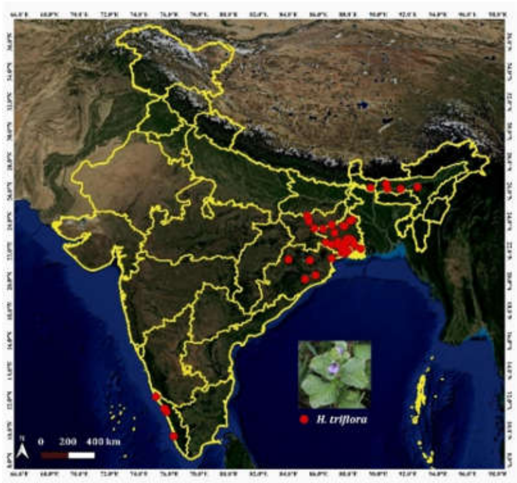


Fig. 17. Lectotype of *Hygrophila triflora* (Roxb.) Fosberg & Sachet (designated by Fosberg & Sachet), [*Roxburgh s.n.* (K000882353)] © The Board of Trustees of the Royal Botanic Gardens, Kew.

bifid. Capsule loculisidal, 6–8 mm, smooth. Seeds many, small, hairy, brownish. Retinacula soft, blunt (Plate 12).

Flowering & fruiting: September – March.

Ecology: Along the sides of water courses, in flooded paddy fields, marshy banks of ponds and canals etc.



Distribution: India (Assam, Kerala, Odisha, West Bengal), Myanmar, N. Australia.

Conservation Status: Least Concern (LC).

Etymology: '*triflora'* = three flowered, owing to the number of flowers at the axil.

Notes: Being an aquarium plant, this has been introduced to several parts of the world and got naturalized. It spreads very fast in ponds, lakes, streams and marshes.

This species is treated as a synonym of *H. difformis* (L.f) Blume. Fosberg and Sachet have extensively discussed the systematic and nomenclatural status of this species. They clearly represented that both are two different species and our study also confirms the same.

Specimens examined: Kerala, Kozhikode district, Iringallur, 25.10.2018, *V.T. Jaseela* 16504 (MBG); Palazhi, 17.12.2018, *V.T. Jaseela* 16517 (MBGH). **West Bengal,** Hooghly district, Aarambug, 24.12.2022, *V.T. Jaseela* 16573 (MBGH); Howrah district, Mourigram, 25.04.1963, *S.S.R. Bennet* 134 (CAL); Jalpaiguri district, Gorumara national park, Klouria Tower, 23.09.2000, *V. Ranjan & A. Kumar* 44650 (CAL); Purba Medinipur, Mecheda, 25.12,2022, *V.T. Jaseela* 18432 (MBGH).

Excluded Species

Hygrophila anomala (Blatt.) Almeida MR. Fl. Maharashtra 4A: 48. 2003; Sant. in Univ. Bombay Bot. Mem. 2: 14. 1952 & in Rec. Bot. Surv. Ind. 16: 173. 1960; Karthik. *et al*., Fl. Pl. India Dicot. 1: 20. 2009; Arisdason *et al*., Fl. Pl. India. Checklist 2: 284; 2020. *Cardanthera anomala* Blatt., J. Proc. Asiat. Soc. Bengal 26: 350. 1931.

Cardanthera anomala (Blatter) Sant. in Bot.Mem. Univ. Bombay 2: 14, 1951.

Synnema anomala (Blatt.) Santapau in Rec. Bot. Surv. Ind. 16 (1): 173, 1960 & in Rec. Bot. Surv. Ind. 16 (1): the flora of Khandala on the western ghats of India 3:194. 1967; Raizada, Ind. For. 94: 451. 1968; Raghavan & Singh in Jain & Sastry (eds.), Pl. Cons. Bull. 3: 5. 1983 et in J. Econ. Taxon. Bot. 5(1): 161. 1984; Singh & Raghavan in ibid. 8(1): 33. 1986; M.Ahmed. & Nayar, Endemic Pl. Indian Reg. 1: 153. 1987; Mishra & Singh. End. Threat. Fl. Pl. Maharashtra: 192. 2001; Singh et al., Fl. Maharashtra Dicot. 2: 675. 2001.; M. R. Almeida. Fl. Maharashtra 4: 48. 2003.

The species *Hygrophila anomala* was first described by Blatter (1931) as *Cardanthera anomala* Blatt. He described the species based on a collection from Maharashtra by Halberg in 1916. Blatter was not entirely persuaded to classify this species as *Cardanthera anomala*; but, since Clarke had already classified *Cardanthera* (*Cardanthera thwaitesii* Benth.) as an unusual species, he did so as well. Santapau (1951) had not seen the type of this species (due to the absence of type), he did not want to change its nomenclature. Santapau sees it as a very strange plant and he was in very doubt that the plant has been placed in the wrong genus.

The name *Cardanthera* Voigt is an invalid name since Voigt had not described the genus. A description of *Cardanthera* was published by Nees (1847) while *Synnema* Benth. was published in 1846. Thus, *Cardanthera* becomes a synonym and a superfluous name and the name *Synnema* became the valid name for the genus. Consequently, Santapau (1967) transferred the name to *Synnema anomalum* (Blatt.) Santapau. Raizada (1968) supported the view of Santapau and included the species under *Synnema* and he corrected the specific epithet from '*anomalum'* to '*anomala'*.

The one character that helps to differentiate *Cardanthera* and *Hygrophila* is the papilliform retinacula in *Cardanthera* and the hook-shaped one in *Hygrophila* (Heine, 1962), a view shared also by Sreemadhavan (1968). Blatter (l.c.) described the seeds to be supported on slightly upcurved retinacula. Therefore Almeida (2003) changed *Synnema anomalum* (Blatt.) Santapau into *Hygrophila anomala* (Blatter) Almeida, which is not accepted during the present study.

Prof. Halberg who found this species was in favour to make a new genus. But Blatter did not feel confident about the proposal as the stamen characters remain constant though there may occur variations in other parts. The number of stamens in Blatter's species is two, in which only one is fertile and the other is barren and the filament is adnate to the entire length of the tube. In the genus *Hygrophila*, there is no such number of stamens having only two and the filaments are not fully adnate to the corolla. There are always four stamens in which all four are fertile and in some cases two are fertile and the other two are sterile. Hence its placement in *Hygrophila* is not justified.

Hallberg collection no.9766 type from Vehar Lake, Salsette and Hallberg collection no. 9767 cotype from Tardeo, Bombay Island and Khandala are not available at Blatter herbarium (BLAT). There are no type specimens at Blatter herbarium and the status of the plant is extinct.

During the field exploration, we were not able to collect the plant. Due to the unavailability of the type specimen, we were not able to settle the position of *H. anomala*, but it cannot be retained in *Hygrophila*.

Hygrophila parishii (T. Anderson) Karthik. & Moorthy, Fl. Pl. India Dicot. 1: 22. 2009; Arisdason *et al*., Fl. Pl. India. Checklist 2: 285; 2020.

Nomaphila parishii T.Anderson, Journ. L. Soc. 9: 455. 1867.

Nomaphila pubescens Kurz, Journ. As. Soc. Beng. 40: II. 74. 1871.

Karthikeyan & Moorthy (2009) made the combination *Hygrophila parishii* based on Anderson's *Nomaphila parishii*. Anderson (1867) in his enumeration of Indian Acanthaceae, identified the species *N. parishii* with locations listed as Pegu, Tenasserim, and Maratban—all in Myanmar. The only record of this species in India, other than *Flora of British India*, is from *Flora of Madhya Pradesh* (Khanna *et al*., 1997), where it was found in Balaghat and noted to be rare. There has been no recent report of this species in India. Moreover, examination of a specimen from Madhya Pradesh housed at Central National Herbarium revealed that it does not match with the type of *N. parishii*.

Molecular Phylogeny

Sequence Analyses

77 DNA sequences from 12 species of Indian *Hygrophila* were newly generated in the present study and have been deposited in GenBank. Amplification success was highest for the ITS region (45 sequences for 12 species).

Alignment

The alignment of ITS region was found to be more informative and as compared to *rbc*L. The alignment for the study has been submitted in treeBASE (http://purl.org/phylo/treebase/phylows/study/TB2:S31407?x-access-code=46442b28993177fd98787218e7b9d3f6&format=html). Other information from ITS, rbcL and ITS+*rbc*L has been summarised in the Table 6.

Characteristics of the ITS Region in *Hygrophila*

The multiple alignment of the Internal Transcribed Spacer (ITS) region, including the 5.8S gene, encompassed a total of 667 sites, accounting for insertions and deletions (INDELS). Within this alignment, the ITS region (comprising ITS1, 5.8S, and ITS4) exhibited 254 unique patterns, with 152 being parsimony-informative, 46 being singleton sites, and 469 being constant sites. The best-fit model for this alignment was determined to be TIM2+F+G4, selected based on the Bayesian Information Criterion (BIC). This model is a variant of the Time-Reversible model (TIM) that incorporates a proportion of invariable sites (F) and a gamma distribution of rate variation across sites (G4). During the analysis, a total of 693 iterations were performed to optimize the model parameters and estimate the phylogenetic tree. Overall, these results highlight the comprehensive analysis conducted on the ITS region, emphasizing the diversity of patterns, informative sites, and the selection of an appropriate model for phylogenetic inference.

Alignment information	ITS (ITS1+5.8s+ITS4)	*rbc*L	ITS+*rbc*L
No. of sequences	64	48	63
Characters/columns	667	555	1215
Distinct pattern sites	254	36	270
Parsimonious informative site	152	13	163
Singleton sites	46	11	38
Constant sites	469	531	1014
Best Fit Model	TIM2+F+G4	K2P+I	TIM2+F+G4
Log-likelihood of consensus tree	-3238.259	-956.090	-4097.902

Table 6: Alignment characteristics for the two analysed DNA regions and the combined plastid and nuclear ribosomal ITS matrices.

Characteristics of the rbcL Region in *Hygrophila*

The multiple alignment of the rbcL region consists of 48 sequences, each containing 555 columns of data. Within this alignment, there are 36 distinct patterns, with 13 of these patterns being parsimony-informative, indicating variability that is phylogenetically informative. Additionally, there are 11 singleton sites, which are unique to a single sequence, and 531 constant sites that remain unchanged across all sequences. The best-fit model for this alignment, determined using the Bayesian Information Criterion (BIC), is the Kimura 2-Parameter (K2P) model. The K2P model is a commonly used substitution model in phylogenetic analysis that considers transitions and transversions in nucleotide sequences. By selecting the K2P model based on the BIC criterion, it suggests that this model provides the best balance of model complexity and goodness of fit to the data among the models considered.

This information about the alignment characteristics and the selected model can guide further phylogenetic analysis and help in accurately inferring evolutionary relationships among the sequences.

Characterstics of Combined ITS+*rbc*L Alignment

The combined alignment consists of 63 sequences, each containing 1215 columns of data. This alignment exhibits a high degree of variability, with 270 distinct patterns observed across the sequences. Of these patterns, 163 are parsimony-informative, meaning they contain phylogenetically relevant information that can be used to infer evolutionary relationships. Additionally, the alignment includes 38 singleton sites, which are unique to a single sequence, and 1014 constant sites that remain unchanged across all sequences.

The best-fit model for this alignment, as determined by the Bayesian Information Criterion (BIC), is TIM2+F+G4. This model takes into account the specific substitution patterns observed in the data and incorporates a gamma distribution to account for rate variation among sites. The selection of the TIM2+F+G4 model suggests that this model provides the optimal balance between model complexity and goodness of fit to the observed data, making it a suitable choice for further phylogenetic analysis and evolutionary inference.

Phylogenetic Analysis

The optimal Maximum Likelihood (ML) tree was obtained through the analysis of 112 sequences, with 77 newly generated accessions for 12 species. The phylogenetic analysis revealed that the *Hygrophila* clade is non-monophyletic (Figure 18-20), attributed to the inclusion of *Brillantaisia* spp. originating from Africa and Madagascar. Following the divergence of *Hygrophila* spp. from their last common ancestors, the *Hygrophila s.l.* clade is strongly supported with a bootstrap value of 100.

Hygrophila didynama (Lindau) Heine is basal to all *Hygrophila* species in the presented phylogeny, with a native range from Southwest Tanzania to South Tropical Africa. It is a perennial or rhizomatous geophyte primarily found in the wet tropical biome. Another species, *Hygrophila costata* Nees & T.Nees (=*Hygrophila brasiliensis* Spreng.), native to Tropical and Subtropical America, is a hydro subshrub or subshrub primarily found in the seasonally dry tropical biome. It is utilized for medicinal purposes and as a food source.

Brillantaisia species, namely *B. grottanellii* Pic.Serm., *B. madagascariensis* T. Anderson ex Lindau, *B. pubescens* T.Anderson ex Oliv., and *B. vogeliana* (Nees) Benth., were retrieved from GenBank and incorporated into molecular phylogenetic analyses. Notably, *Brillantaisia* species form a monophyletic group with strong support (BS 100), creating a polytomy with accessions of *H. auriculata* (Schum.)

Heine collected from Kerala, Tamil Nadu, and Karnataka. Within this polytomy, accessions of *H. auriculata* var. *alba* (Parmar) P.M.Salim, J. Mathew & Yohannan also groups together, indicating a complex relationship among these species and varieties.

The sequence data of *Hygrophila schulli* M.R.Almeida & S.M.Almeida, retrived from GenBank and collected in Maharashtra, reveals a clear affiliation with accessions of *H. auriculata*. This provides compelling evidence supporting its classification as a synonym of *H. auriculata*. Extending our understanding from both literature sources and personal field observations, it is evident that the native range of *H. schulli* spans from Tropical and Southern Africa, across the Indian Subcontinent to Indo-China. This annual or subshrub species thrives primarily in the seasonally dry tropical biome.

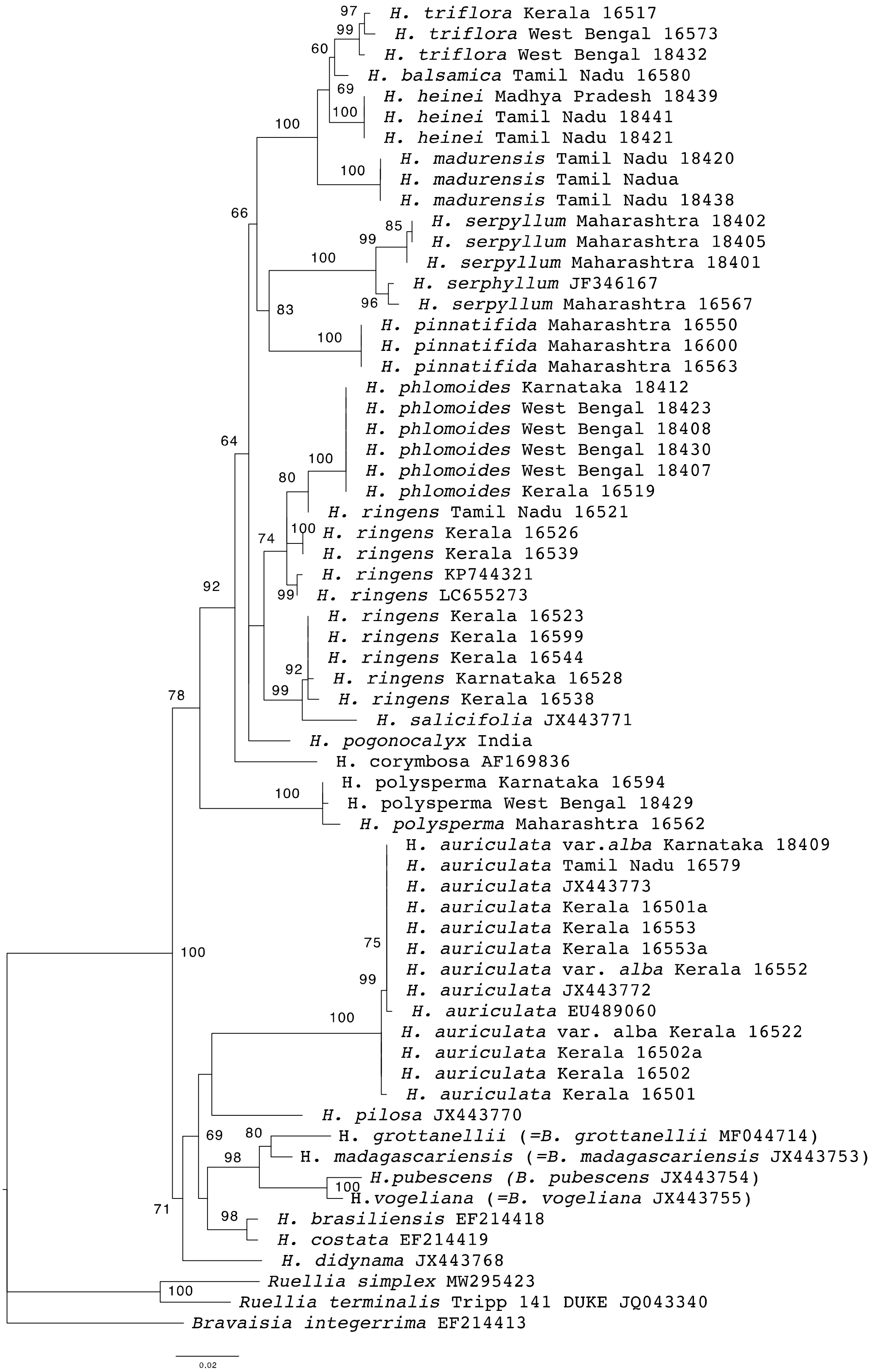


Fig. 18. Best ML tree of the genus *Hygrophila* R.Br. based on ITS sequence data.

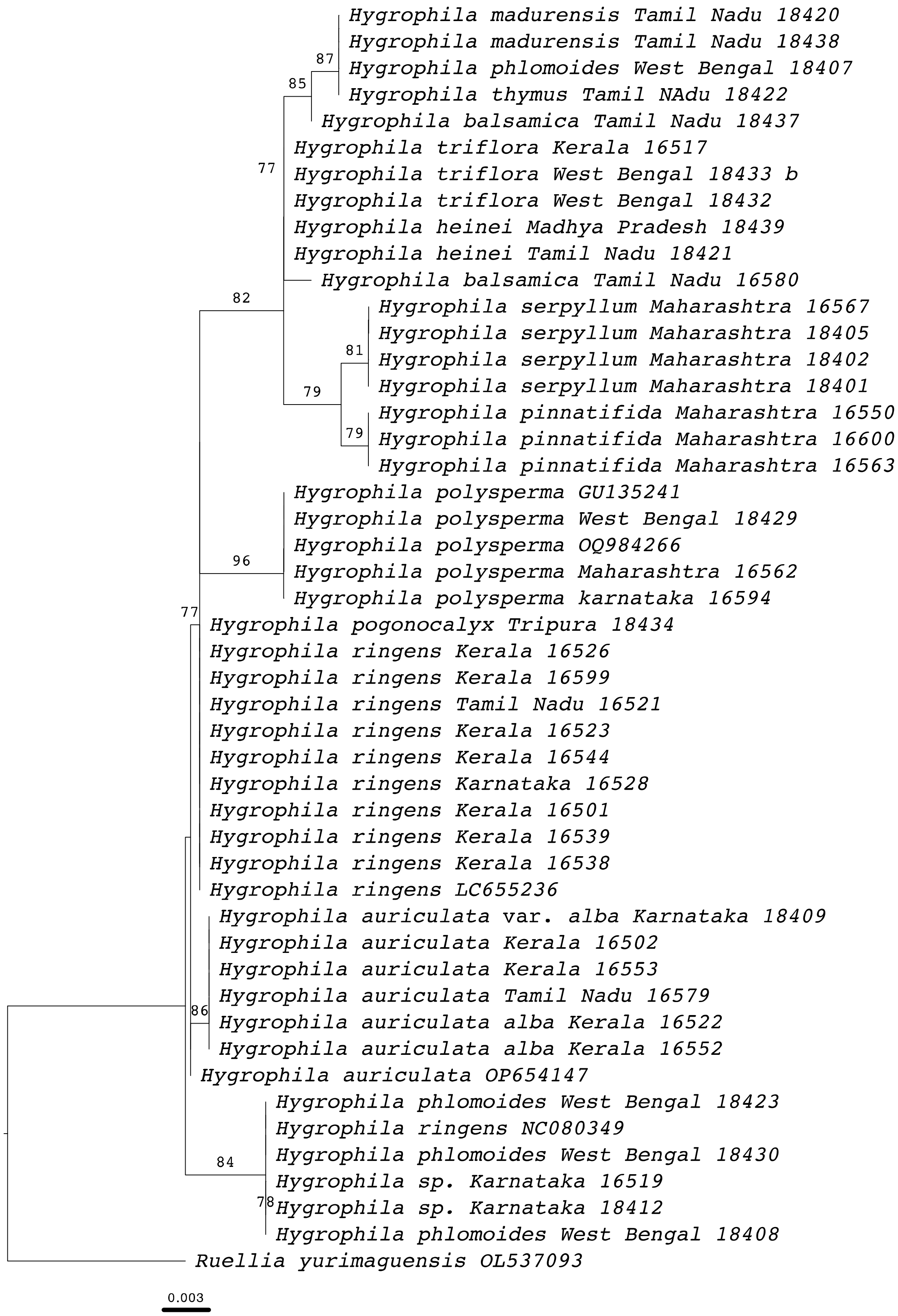


Fig. 19. Best ML tree of the genus *Hygrophila* R.Br. based on *rbc*L sequence data.

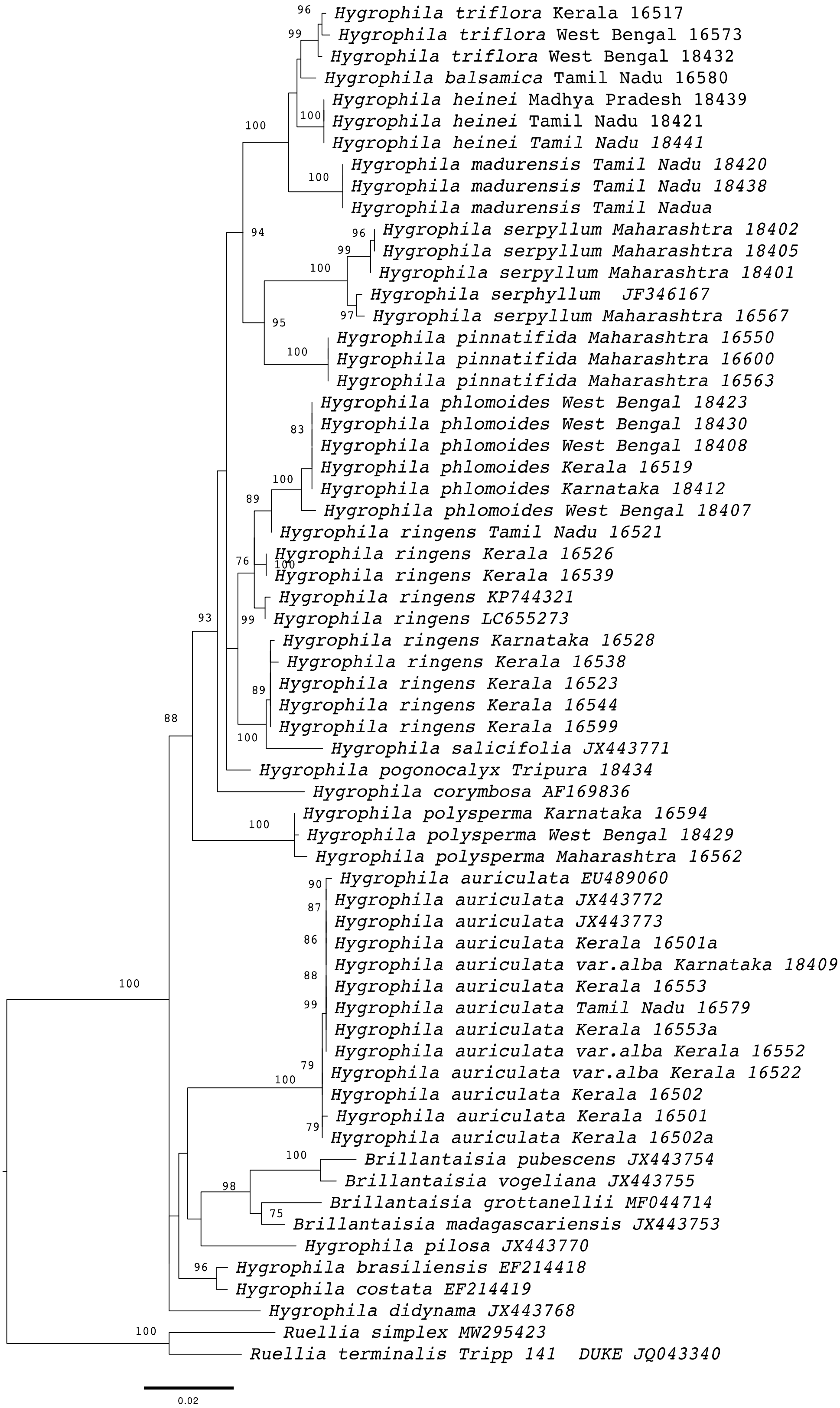


Fig. 20. Best ML tree of the genus *Hygrophila* R.Br. based on ITS+*rbc*L sequence data.

The accessions of *H. polysperma* collected from Maharashtra, Karnataka, and West Bengal exhibit a robust monophyletic grouping with strong support (BS 100). This species, native to Afghanistan, South China, Peninsula Malaysia, and Taiwan, is an annual or helophyte, predominantly thriving in the seasonally dry tropical biome.

H. heinei Sreem. presents itself with a native range extending from India to Sri Lanka. Accessions from Madhya Pradesh and Tamil Nadu, representing the subtropical biome, are recovered as monophyletic (BS 100) and form the sister to the monophyletic clade (BS 98) of *H. balsamica* (L.f.) Raf. The natural habitat of *H. heinei* spans Peninsular India to Sri Lanka, where it flourishes as a helophyte primarily in the wet tropical biome.

H. triflora Fosberg & Sachet., accessions from Kerala and West Bengal form a monophyletic group (BS 99) and are recovered as the sister group to *H. balsamica* and *H. heinei* (BS 96). Three accessions of the critically endangered *H. madurensis* (N.P.Balakr. & Subram.) Karthik. & Moorthy, indigenous to Tamil Nadu, India, display monophyletic grouping (BS 100) and are recovered as the sister clade (BS 96) to *H. auriculata*, *H. heinei*, *H. balsamica*, and *H. triflora*. *H. madurensis* primarily thrives in the wet tropical biome.

The accessions of *H. serpyllum* (Nees) T.Anderson form a monophyletic group (BS 100). The species is native to India growing seasonally in dry tropical biome. Five accessions of *Hygrophila serpyllum* collected from Maharashtra is sister (BP 85) to six accessions of monophyletic *H. pinnatifida* (Dalzell) Sreem. (BS 100), native to India growing primarily in wet tropical biomes is grouped together with *H. serpyllum*.

Six accessions of *H. phlomoides* Nees collected from West Bengal, Kerala, Karnataka and Tamil Nadu form a monophyletic group (BS 100). The native range of species extends from E. Himalaya to China (Yunnan) and West and Central Malesia. It is a perennial and grows primarily in the wet tropical biome. The species is sister to *H. pogonocalyx* Hayata (BS 78) which is having native range in Taiwan. It is a perennial and grows primarily in the subtropical biome. The presence of these species in India is indication of extended range of both the species. The accessions collected from Kerala and Karnataka of *Hygrophila ringens* is monophyletic (BP 100) and is sister to *H. salicifolia* (Vahl) Nees (BP 99). *Hygrophila phlomoides* is found imbedded in the *H. ringens* (L.) R.Br. ex Steud. clade.

Hygrophila corymbosa (Blume) Lindau whose native range is Indo-China, Philippines. It grows primarily in the wet tropical biome is recovered basal to all the above species elucidated.

Several accessions of *H. auriculata* collected from various localities in India is grouping along with its synonym *H. schulli* (GB accession embedded within the *H. auriculata* clade) (BS 100). Four species of *Brillantaisia* P. Beauv. (BP 99) which is native taxa of Tropical Africa, Madagascar, Sri Lanka is grouping along with *H. auriculata* making it as sister species with moderate BP support. Grouping of *Brillantasia* within the *Hygrophila* species renders the latter as non-monophyletic. The remaining species of *H. pilosa* Raf., *H. barasiliensis*, *H. costata* and *H. didynama* are form early branching lineages of the phylogeny retrieved.

These phylogenetic insights contribute valuable information to our understanding of the evolutionary relationships within the genus *Hygrophila*, shedding light on the genetic connections and ecological preferences of the studied species.

New Combinations in *Hygrophila* R.Br. (Based on phylogenetic analysis)

1. ***Hygrophila grottanellii*** (Pic.Serm.) V.T.Jaseela, N.S.Pradeep, M.D.Dwivedi & A.K.Pandey, *comb. nov*. *Brillantaisia grottanellii* Pic.Serm. Miss. stud. Lago Tana 7 (1): 254. 1951.

Lectotype (designated here:) AFRICA, Ethiopia, Tucur Dinghia forest, 24.01.1937, *Pichi-Sermoli* 2099 [FT003141, digital image!; isolectotype FT003140, digital image!]

Specimens examined:— Africa, Ethiopia, 27 km N of Mizan, en route to Tepic, secondary roadside forests, 7°03'20", 35°26' 27.1"E, 12.04.2010, E. Tripp & E. Kelbessa 924 (US!); Illubabor region: c. 35 km from the Tepi-Goré road towards Goddare mission, 7°17', 35°13', Friis et al. 9788 (O!, WAG!); Mt. Karkarha, c. 10

miles SSE of Mezan Tefari,18.02.1976, Ash 3402 (MO!, UPS!); *c.* 57 km from Jimma on the Sheki-Gogeb river track, 08.12.1972, Friis et al. 1677 (BR!).

Distribution and habitat:— The native range of this species is Ethiopia. It grows beside streams in shade, in montane forest, in disturbed ground and occasionally collected from plantations.

Notes: *Brillantaisia grottanellii* was described by Pichi Sermolli based on his collection during Lake Tane expedition. Main set of Pichi Sermolli's Lake Tane expedition specimens were received at Erbario Tropicale (FT). We could locate two sheets of type collection by Pichi Sermolli at FT (FT003140, FT003141). Sidewell (1998) in the revision of *Brillantaisia*, cited holotype at FT, but did not specify what sheet she intended to be the holotype. Hence, both specimens represent syntypes according to Art. 40.2 & Note1 (Turland et al. 2018). Both the specimens agree with the protologue and here we designate FT003141 as the lectotype of *B. grottanelli*. Sidewell l.c. also mentioned an image seen at BM. But we could not trace any specimen of *B. grottanellii* at BM herbarium.

2. ***Hygrophila madagascariensis*** (T.Anderson ex Lindau) V.T.Jaseela, N.S.Pradeep, M.D.Dwivedi & A.K.Pandey, ***comb. nov***. *Brillantaisia madagascariensis* T. Anderson ex Lindau, in Bot. Jahrb. Syst. 17: 103 (1893)

Type: Madagascar, Betsileo, in the forest of Nandihizana, Hildebrandt 3901 (B† holotype; BM-lectotype; G!, JE!, M!, P!, US!, W!, WU!, Z!- isolectotypes).

Brillantaisia bagshawei S.Moore in J. Bot. 46: 312 (1908); Type:Uganda, Bugoma forest, Bagshawe 1387 (BM!-holotype).

Brillantaisia spicata Lindau Bot. Jahrb. Syst. 20: 4. 1894.

Syntypes: Tanzania, Usambara Mountains, Bangarra Lutindi, Holst 3316 (COI, K!, HBG, M!); Usambara Mountains, Gonja, Handei, Holst 4216 (COI, G!, K!, KFTA!, M!, P!,W!, WAG!, US!, Z!)

Brillantaisia verruculosa Lindau in Bot. Jahrb. Syst. 22: 113 (1895) Type: Cameroon, Yaounde, Zenker & Staudt 166 (B†- holotype).

B. majestica Wernham in J. Bot. 54: 229 (1969). Type: Cameroon, Buea, Mount Cameroon, Bates 817 (K!-holotype).

Specimens examined:— AFRICA, Cameroon, de Ninton, 05.01.1973, Letouzey 11784 (WAG!); Congo, Nord-Kivu, Forestier Central Kamatembe, 14.041934, de Witte 1536 (B!); Ethiopia, In valley below Seventh Day Adventist Mission station, Ghimbie, Wollega Province, F.G.Meyer 8131 (US!); Illubabor region, 21.12.1972, Friis et al. 1908 (BR!); Madagascar, Andohahely For. Res. Res. Nat. 11, Tanatan, A.J.M Leeuwenberg 13967 (B!, US!, WAG!); Ankaizinana, 26.04.1923, R. Decary 2066 (P); D' Andapa, Bassin de la Lokoho, 25.11.1948, Humbert & Capuron 21957 (B!); Sudan, Talanga, 01.12.1980, Friis & Vollensen 613 (BR); Tanzania, Kilimanjaro, Moshi District, Kidia, 21.10.1989, Hemp 30 (UBT!); Morogoro Nguru South Forest Reserve, E-facing slopes near Mafuta Village, 09.08.2004, M.A. Mwangoka & B. Elnest 3220 (MA!);

Distribution and habitat:— This species is distributed throughout West Tropical Africa through the Congo basin to Ethiopia and south into Tanzania, also in Madagascar. It grows in montane forest, damp places, recently logged areas and path sides.

3. ***Hygrophila tanzaniensis*** V.T.Jaseela, N.S.Pradeep, M.D.Dwivedi & A.K.Pandey, ***nom. nov.*** *Brillantaisia pubescens* T.Anderson ex Oliv. Trans. Linn. Soc. London 29: 125. 1875.

Lectotype (designated by K. Vollensen & R.K. Burmmitt): Tanzania, Khutu, Kirengwe, *s.d., Grant s.n.* (K000393851, digital image!)

Brillantaisia rutenbergiana Vatke in Abh. Naturwiss, Vereine, Bremen 9: 131. 1885. Type: Madagascar, Andranovaka, Rutenberg *s.n.* (P-holotype).

Ruelliola grevei Baillon in Bull. Mens. Soc. Linn. Paris 2: 852. 1890. Type: adagascar, Greve 26 (P!-holotype; P!-isotype).

Brillantaisia anomala Lindau in Pflanzenw. Ost-afr. C: 366. 1895. Type: Mozambique, Villa Gouveio, Carvalho *s.n.* (COI-holotype).

B. pubescens var. *rutenbergiana* (Vatke) Benoist in Cat. pi. madag. 13. 1939.

Hygrophila pubescens (T. Anderson ex Oliv.) Benoist in Fl. Madag. fam. 182 1: 36. 1967, non Nees 1847. *nom. illeg.*

Notes:— The existence of the previously published *Hygrophila pubescens* Nees (1847) necessitates a new name for this species. Since the type locality Kirengwe, is in Tanzania, we propose *tanzaniensis* as the new specific epithet.

Specimens examined:— Africa, Congo, Simama s[ous-]riv[ière] Dikuluwe terr[itoire] Mitwaba, 07.09.1955, J. Brynaert 406 (BR!); Madagascar, Gorges de la Tsiribihina, 09.09.1940, R. Decary 15526 (P!); Malawi, Machinga District, Liwondo National Park, 17.071985, LaCroix IF 3122 (WAG!); Mozambique, Gorongosa National Park, 11.06.2014, Würsten B. & Castigo T. 913 (BR!); Tanzania, Ostfrika, Lindi Bez, Lutambasee, 09.12.1934, H.J. Schlieben 5323 (B!, US!); Zambia, Choma river, NW of Mweru-wa-Ntipa, P.J. Tyrer 339 (BR!).

Distribution and habitat:— This species is known from East and Central Africa, Malawi, Zambia, Zimbabwe, Mozambique and Tanzania, west to Zaire, Madagascar. It grows in river beds, sandy dry places, shade on forest floor or in savannah woodlands.

4. ***Hygrophila vogeliana*** (Benth.) V.T.Jaseela, N.S.Pradeep, M.D.Dwivedi & A.K.Pandey, ***comb. nov***. Leucorhaphis vogeliana Nees in A.P.de Candolle, Prodr. 11: 97. 1847*; Brillantaisia vogeliana* (Nees) Benth. in Hooker, Niger Fl. 477. 1849.

Lectotype (designated here): Fernando Po, *s.d*., Vogel 179 (Lectotype, K000393867, digital image!; Isolectotype K000393865, digital image!; K000393866, digital image!)

Brillantaisia molleri Lindau in Bot. Jahrb. Syst. 17(1-2): 99 (1893); Type: Sao Tome, Moller 33 (87) (B†-holotype; COI!, K!-isotypes).

Brillantaisia preussii Lindau in Bot. Jahrb. Syst. 17: 100 (1893). Syntypes: Cameroon, Barombi, Preuss 320 (K!-isosyntype) Buea, Preuss 998 (BM!, K!-isosyntypes).

Brillantaisia schumanniana Lindau in Bot. Jahrb. Syst. 17: 102 (1893). Type: Cameroon, Braun 47 (B†-holotype).

Brillantaisia soyauxii Lindau in Bot. Jahrb. Syst. 17: 101 (1893). Type: Gabon, Munda, Sibange Farm, Soyaux 454 (B†-holotype; E!, H!, US!,WAG!-isotypes).

Notes:— Bentham (1849) described *B. vogeliana* based on Nees's *Leucorhaphis vogeliana*, which was based on a collection by Vogel from Fernando Po (now Bioko) during his Niger expedition. Sidewell (1998), in the revision of *Brillantaisia* P. Beauv., designated the type as "Type: Fernando Po, Vogel 179 (K!-holotype; K!-isotype). While searching for the original materials, the authors could locate three sheets (K000393865, K000393866, K000393867) of this species at Kew herbarium. All these sheets bear the collection number '179', collected during Niger expedition. Only one sheet can serve as the type according to Art. 9.6 of ICN (Turland *et al*., 2018). So, the sheet with the barcode K000393867 is designated here as the lectotype, as it bears the collector's name, collection number and location.

Specimens examined:— Africa, Cameroon, Mungo River, near bridge in Koumba-Loum road, 03.11.1972, Leeuwenberg 10628 (WAG!); Victoria, by river. 01.10.1929, T.D. Maitland 757 (B!); Mone forest reserve, 02.12.2008, Tah et al. 660 (K); South West, Kupe-Muanenguba, Kupe Village, 11.11.1995, Sidewell 438 (BR!, MA!); Congo, Maniema Province, Namoya, North of Kibiswa, 14.08.2008, Q. Luke 12533 (BR!); 14.04.2005, Gabon, Ogooué-Maritime, Valkenburg et al. 3015 (WAG!); Monts Doudou, Campagne, 18.09.2000, Bourobou et al. 299 (WAG!); Woleu-Ntem, Chantier Oveng, 02.05.1986, Louis 2077 (WAG!); Ghana, Begoro, 01.11.1953, J.K. Morton 36 (WAG!); Sao Tome and Principe, road from Nova Moka to the waterfall of Manuel Jorge River, 15.10.1993, E. Figueiredo & P.I. Arriegas 33 (MA!); Uganda, South Maramagambo C.F.R., N. Kigezi, 08.06.1969, J.M. Lock 69/159 (BR!).

Distribution and habitat:— The native range of this species is West Tropical Africa and on the islands of the Gulf of Guinea across Central African Repulic and Congo to Sudan, Uganda and Kenya. It grows in wasteland, disturbed ground, beside streams in shade, along roadsides and is occasionally collected from plantations.

Chapter 6

DISCUSSION

***Hygrophila* R.Br.**

The genus *Hygrophila* R.Br., with around 80 species distributed in the tropical and subtropical regions of the world, consists of aquatic or semiaquatic herbs. The genus has a long botanical history dating back to the period of Robert Brown. It is one of the significant genera in both the Old and New World's history, with a distribution that ranges from sea level to 1600 m. The members of the genus are found in wet habitats and exhibit variations according to ecological conditions. The genus is complex, and it has not been thoroughly studied. It has only received casual attention from botanists, particularly in India. Some species within the genus exhibit overlapping characteristics, leading to the description of new taxa based on these phenotypic traits. This has resulted in taxonomists struggling to reach a consensus on the correct identity and number of species within the genus. Hence, the current study focuses on morphological and molecular characteristics to delineate the actual species within the genus based on field explorations and specimen collections from various regions across India.

An examination of the genus *Hygrophila* R.Br. within the Indian context reveals that Clarke (1885) provided the initial comprehensive account in Hooker's *Flora of British India*, detailing 18 species: 8 species in *Hygrophila*, 8 in *Cardanthera* and 2 in *Nomaphila* (*Cardanthera* and *Nomaphila* were later merged with *Hygrophila*). However, the geographical area covered by British India was significantly reduced following India's independence in 1947. Despite this, no exhaustive taxonomic work focused solely on *Hygrophila* R.Br. has been conducted in India. Not only in the Indian scenario but also in Any part of the world, there are no comprehensive taxonomic studies of the genus *Hygrophila*. This study aims to address this gap by examining various *Hygrophila* species in India, utilizing taxonomic and molecular tools to produce an updated account of this complex. This work will significantly contribute to the comprehensive review of *Hygrophila* R.Br. in India.

The current broad taxonomic understanding of *Hygrophila* R. Br. considers it a composite genus comprising various elements previously authors had classified under related genera. Heine (1962, 1971) initiated a re-examination of African and Asian material, including the genera *Adenosma*, *Asteracantha*, *Cardanthera*, *Hemiadelphis* and *Synnema* and reduced them to the synonymy of *Hygrophila*. Cramer (1989) also reassessed the *Hygrophila* complex, and his views agreed with Heine, except for *Hemiadelphis*. Cramer disagreed with the amendment of the monotypic genus *Hemiadelphis* to *Hygrophila* and opined that it should be retained in *Hemiadelphis*, as it has only two fertile stamens (now *Hygrophila polysperma*). Some authors of floras continue to treat *Hygrophila polysperma* under *Hemiadelphis*. But the current study agrees with Heine, treating the taxa (*Hemiadelphis polysperma*) with only two fertile stamens under *Hygrophila*, as there is no other character to delineate it from *Hygrophila*.

Hygrophila auriculata (Schumach) Heine, an OW species, has a wide distribution covering most of the states in India. The species was placed in the monotypic genus *Asteracantha* (L.) Nees. The main dispute for distinguishing the genus *Asteracantha* was based on the presence of axillary spines in the inflorescence of this species, A. longifolia (L.) Nees. Anderson (1860, 1867) had a broader view of the genus *Hygrophila* than Nees. He considered *Asteracantha* a true *Hygrophila*, reclassifying the species as *Hygrophila spinosa* T. Anders, without providing taxonomic justification. This viewpoint was supported by many authors like Bentham & Hooker (1876), Clarke (1885), and Trimen (1895). However, Lindau (1895) reinstated *Asteracantha* as a monotypic genus based on the diagnostic axillary spines of the inflorescence, which was supported by Alston (1931) and Santapau (1951), although their findings were based on limited material from Ceylon and India. In contrast, Heine (1971) found that the axillary spines were inconsistent in the African species, significantly reduced in some specimens and absent in others. Given that the axillary spines were the only distinguishing feature and were variable, Heine concluded that *Asteracantha* could not be reliably established as a distinct genus from *Hygrophila*. *Hygrophila auriculata* has a variety named *H. auriculata* var. *alba*, based on white-flowered plants. However, *H. auriculata* exhibits a range of colour variations from deep purple to white, and we collected all these ranges of colour-varied plants. So, the variety cannot stand as a distinct taxon from *H. auriculata* and we merge it under *H. auriculata*.

Hygrophila balsamica (L.f.) Raf., native to India and Sri Lanka is a heterophyllous plant. Linneaus fil. (1826) while establishing the species described only monomorphic leaves. But our collections and also many herbarium specimens show dimorphic condition of leaves. The species *Hygrophila difformis* was described by Blume (1826) based on *Ruellia difformis* L.f. from India. At first glance *H. difformis* seemed to be based on *H. ringens* (L) R.Br. ex Steud., as Linneaus fil. cited only one element '*Nir schulli*, Rheed. Mal. V2. P.89. t.46' for *H. difformis*. Based on our observations from field collections and critical review of protologue and type specimens we synonymise the name *H. difformis* (L) Blume under *H. balsamica*, as both the species are same and Linneaus fil. has mistaken for the heterophyllous plant as a new species. Many authors considered *H. difformis* and *H. triflora* (Roxb.) Fosberg & Sachet conspecific with each other. This may be because of the heterophyllous nature. But these two species are clearly two different entities. Fosberg and Sachet (1981) provided detailed clarification in this regard.

Hygrophila serpyllum (Nees) T. Anderson exhibits significant morphological plasticity. The species exhibit variations in habit according to ecological conditions. During and shortly after the monsoon season, it adopts a prostrate form with relatively large, nearly orbicular, petiolate leaves. This phase, characterized by its dark green colouration, produces some flowers in the axils of leaf-like bracts and is referred to as *Hygrophila serpyllum* (Nees) T.Anderson var. *hookeriana* C.B. Clarke. As the winter season approaches and water levels recede, the shoots become erect, bearing narrower leaves that are either shortly petiolate or sessile and covered with whitish hairs. The flowers develop in terminal spikes, and the entire plant has a whitish-green appearance, distinctly different from its wet season form. *Hygrophila* *serpyllum* (Nees) T.Anderson var. *hookeriana* C.B. Clarke is an ecoform of the proper species. Numerous intermediate forms have been observed in the field and various Indian herbaria. Thus, here we synonymise the name *Hygrophila serpyllum* var. *hookeriana* under *Hygrophila serpyllum*. The species *Hygrophila stocksii* T.Anderson ex C.B.Clarke, was originally established by T. Anderson in his mss. Later C.B. Clarke in Fl. Brit. India (1884) validated the name *H. stocksii*. This is also a variable plant of *serpyllum* with luxurious growth. So, we synonymise *H. stocksii* T.Anderson ex C.B.Clarke under *H. serpyllum*.

H. ringens (L.) R. Br. Ex Steud. is a highly polymorphic plant which has a wide distribution in India. The plant exhibits significant variation mainly in terms of habit, leaf shape and size, and hence has numerous synonymies assigned to it. A subspecies *H. ringens* subsp. *longifolia* J.Mathew & Kad.V.George described from Kollam district of Kerala. The distinguishing characters of the subspecies are plant height (1m), leaf size (13 cm) and number of flowers (solitary). However, *H. ringens* generally ranges from 30 cm to 1m in height, with leaves varying from 3-15 cm, and typically has 4-12 flowers per axil. Collections from the type locality revealed plants with diverse dimensions in height, leaf size, and flower number. Due to the polymorphic nature of *H. ringens* and also based on field observations, we synonymise *H. ringens* subsp. *longifolia* under *H. ringens*. Additionally, *H. ringens* var. *cochinensis* (Remadevi & Binojk.) Karthik. & Moorthy, initially established for *H. ringens*, is synonymized under *H. ringens* due to overlapping distinguishing characteristics.

Two species, *H. griffithii* (T.Anderson) Sreem. and *H. incana* Nees, were reported from Assam and South India, respectively. There is no report of both species after type collection. Field visits to the respective localities failed to collect the plants. *H. pusilla* Blume, mainly distributed in Malayan archipelago was reported from Little Andaman. We were not able to collect the plant. Study of these three plants in the present work are based on protologue information and herbarium specimens.

During the work period, the area of study was extensively explored, and detailed field notes were carefully made. The collected specimens were critically studied. To ensure the correct identification, specimens from various herbaria were consulted. The current treatment includes 15 species. In general, vegetative morphology shows a high degree of variation in many species, while floral morphology is more or less homogenous in many taxa.

Comparative Morphology

Habitat

Hygrophila thrives in moist, wet-humid, aquatic or semi-aquatic conditions in the tropical and subtropical regions. Species of the genus are found in a wide variety of habitats such as paddy fields, swamps, ditches, canals, banks of streams & ponds, forest floors, rock crevices and found to occur from sea level to 1600 m in India. Of the 15 species recognized from the study area, most inhabit places of low altitude (0–300 m). Though there is apparent variation in the morphology of plants collected from varying localities, it does not show a consistent correlation with the habitat (Plate 13).

Habit

Species of Indian *Hygrophila* are annuals or perennials, with the majority being annual herbs. Simple forms to highly branched forms are seen among the species of *Hygrophila*. They are erect, ascending, decumbent, creeping, or prostrate (*H. polysperma, H. serpyllum, H. heinei*). Few species like *H. auriculata*, *H. phlomoides*, *H. pogonocalyx*, and *H. ringens* may also grow as large herbs or under shrubs.

Stem thickness and branching pattern can vary between species and growth conditions. Most species have quadrangular stems, but in a few, it is terete (*H. thymus, H. triflora*). Stem in *H. heinei* is succulent. Nodes are swollen in most cases. Indumentum varies from glabrous to pubescent, with or without glandular hairs.

Most members of *Hygrophila* often have variable habits. Plant size, leaf size, and shape may vary according to environmental conditions. So, such plastic characters are not effective in differentiating the species of *Hygrophila*.

Leaves

Leaves are variable in size, shape, base, margin, apex, pubescence, etc., depending on the species and environmental factors. Leaves are usually simple, opposite, decussate, subsessile or petiolate, chartaceous. They are mostly monomorphic or sometimes dimorphic (*H. balsamica, H. pinnatifida, H. triflora*). The shape of the lamina varies between species and also within species. It may be lanceolate, linear-lanceolate, obovate, elliptic, orbicular or oblong. In most species, the leaves base is cuneate or attenuate, and the apex is acute or obtuse. In the majority of the species, leaf margins are entire or undulate, but in a few, they are serrate or crenate (*H. pinnatifida, H. triflora*). Leaves are either glabrous or pubescent. The colour of the leaves can range from green to reddish-brown, with variations in intensity and hue. Veins prominent with 6-14 pairs of lateral veins.

Inflorescence

The inflorescence morphology of *Hygrophila* reveal that most of the species have axillary, sessile cymose inflorescence (*H. auriculata, H. incana, H. madurensis, H. phlomoides, H. pogonocalyx, H. pusilla, H. thymus, H. triflora*). Few species show terminal spike inflorescence (*H. griffithii, H. heinei, H. polysperma, H. serpyllum*). In *H. pinnatifida,* the flowers are solitary (Plate 14).

Flowers

Flowers of *Hygrophila* species typically have five petals and vary in colour from white to purple. Flower size and arrangement can also differ between species. In *Hygrophila*, flowers are borne in terminal spikes or axillary whorls; fassicled in whorls at nodes. In most species, flowers are sessile but sometimes pedicellate (*H. triflora*). Flower colour varies from white-purple. Flower size ranges from 0.5–3.5 cm. Smallest flower is noted in *H. polysperma* and largest in *H. auriculata,* among the Indian members of *Hygrophila* (Plate 15).

Bracts and Bracteoles

Flowers of *Hygrophila* are subtended by bracts and bracteoles. The shape of the bracts may be elliptic or lanceolate. Foliaceous bracts are seen in *H. auriculata, H. phlomoides, H. heinei* and *H. triflora*. Bracts are entire, ciliate and green in colour. Abaxial surface villous, hispid or glandular pubescent. Bracteoles oblong or lanceolate, bracteoles linear-lanceolate or oblong, shorter than the calyx (Plate 16).

Calyx

Tubular, 5-fid in all the species except in *H. auriculata*, in which it is 4-fid. Calyx lobes equal or subequal, lanceolate or linear, basally united, acute or acuminate. Calyx is glabrous in *H. balsamica*, *H. madurensis* and *H. thymus* and for the rest of the species, it is pubescent, but the nature of hairiness varies. In *H. pinnatifida*, calyx lobes may be pinnatifid also (Plate 17).

Corolla

Corolla shortly tubular bilabiate; bluish-purple, glandular-pubescent outside, glabrous inside; limb deeply 2-lipped, oblong, straight; lobes twisted to the left in the bud. Upper lip 2-lobed, erect, lower lip 3-lobed, deflexed, palate crested; lower lip deflexed, 3-lobed, crested; lobes twisted to the left in the bud. Corolla tube ventricose at the apex & dilated above, whitish; length varies in size. Tube short in *H. balsamica, H. heinei, H. thymus, H. triflora* and for the other species tube is long.

Stamens

Stamens 4, exserted, didynamous, adnate at the throat of the corolla tube. Stamens of the posterior pair similar to the anterior, or smaller, or rudimentary. In *H. polysperma* out of the 4 stamens, 2 are fertile and 2 are barren. Filaments glabrous; filaments united in pairs at base and forming a hirsute sheath. Anthers oblong, thecae equal or subequal, divaricate or connate at the base, muticous (Plate 18).

Pistil

Pistils are more or less homogenous in nature. Ovary 2-locular, ovules few or many in each locule, oblong-cylindric, green, glabrous or pubecent, tip acute or obtuse.

Style long, slender, whitish, hairy. Stigma is simple, linear, and unequal. Nectariferous discs are present below the ovary in all species, and their shape is always uniform (cup-shaped) (Plate 19).

Capsule

Capsule vary in shape, size and hairiness. Capsules are narrow, linear, oblong or cylindric. Capsule open by 2 valves, bearing seeds from the base. They are glabrous (*H. phlomoides*, *H. pogonocalyx, H. ringens*), pubescent at apex (*H. auriculata*, *H. pinnatifida*, *H. polysperma*, *H. serpyllum*) or pubescent throughout (*H. balsamica, H. heinei, H. madurensis, H. thymus, H. triflora*). Apex are acute, mucronate or obtuse (Plate 20).

Seed

Seeds brownish 4–many, orbicular or ovoid, compressed, hygroscopically hairy, white hairy when wet. Seeds are supported on retinacula. Retinacular are either minute and soft or prominent and hard. Retinacula minute, soft and blunt in *H. balsamica, H. heinei, H. madurensis, H. thymus* and for the rest of the species they are prominent and hard, curved, acute (Plate 21).

Distribution of in *Hygrophila* India

The distribution of *Hygrophila* is widespread, occurring primarily in tropical and subtropical regions around the world with about 80 species (POWO, 2024). In India, the genus *Hygrophila* is distributed across a wide range of geographical regions, from the northeastern wetlands to the coastal plains of the south. The genus is well-represented with 15 species in India, of which 13 occur in Peninsular India (Plate 22).

Molecular Phylogeny

The present work aimed to solve the generic circumscription of genus *Hygrophila* and its species delineation using molecular phylogeny. Molecular characterization involves DNA extraction, PCR amplification and sequencing of amplicons. Fresh samples were collected during field visits and from the conservatory for DNA extraction. Different protocols were tried for the isolation of DNA. When several methods were used to isolate DNA, we encountered numerous issues, starting with the initial stage and continuing through the entire process. Pellets that were highly viscous and sticky were hard to confront, and their brown colour implied that they were contaminated by phenolic compounds. The quantity of DNA isolated using these methods was meagre, and most samples had subpar DNA quality. The DNA was unusable for molecular studies since the A260/A280 ratio was less than 1.6, below the ideal limit of 1.8. Hence, we reported an improved protocol for DNA isolation from young and fresh leaves of aquatic plant species using CTAB, based on the protocols we previously tried to extract DNA.

A total of 12 taxa of *Hygrophila* were sequenced for the phylogenetic analysis. Available DNA sequences of *Hygrophila* and its allies were downloaded from NCBI by referring to previous molecular phylogenetic works. These existing works used nuclear (ITS) and chloroplast regions for phylogenetic analysis. We used nrITS and cpDNA marker rbcL for the present phylogenetic analysis. Altogether 111 sequences were used in the phylogenetic reconstruction, and separate analyses were conducted for each gene, followed by a concatenated data analysis. There is no incongruence between trees obtained from Bayesian analysis and Maximum likelihood analysis. This study represents the first molecular systematic analysis utilizing ITS and rbcL sequences to assess the phylogenetic relationships within Indian *Hygrophila* R.Br.

The analysis, based on ITS and rbcL sequence data, clearly resolves the phylogenetic relationships within the genus *Hygrophila*. In comparison to the outgroup taxa employed in this investigation, the genus *Hygrophila* is a monophyletic group with strong support.

Numerous well-supported conclusions about the relationships within the genus can be drawn from the phylogenetic study. *H. auriculata* has a variety named *H. auriculata* var. *alba*, based on flower colour. *H. auriculata* shows a varying range in flower colour from white to dark purple. During our field examination we collected different accessions of *H. auriculata*, showing variation in flower colour. Arisdason *et al*. (2020) recently synonymised the variety under *H. auriculata*. Our morphological and phylogenetic analysis supports the same as the white-flowered accessions grouped with the purple-flowered accessions of *H. auriculata*.

Heine (1962) reduced the monotypic genus *Hemiadelphis* to the synonymy of *Hygrophila*. Cramer (1989) did not support *Hemiadelphis* to *Hygrophila* as *Hemiadelphis ploysperma* has only two fertile stamens and its lower corolla lip is not bullate as in *Hygrophila* species. Many authors of floras also followed Cramer's view and treated the species as *Hemiadelphis ploysperma*. Different accessions of *H. polysperma* included in the study formed a well-supported clade with other species of *Hygrophila*. Our morphological and phylogenetic analysis supports Heine's view.

Arisdason *et al*. (2020) treated *Hygrophila phlomoides* Nees as a heterotypic synonym of the highly variable species *H. ringens* (L.) R.Br. ex Steud. However, many characters distinguish the former from the latter. The phylogenetic analysis also shows it is a very distinct species from the latter, as different accessions of *H. phlomoides* and *H. ringens* formed two distinct groups. Therefore, we recognize both species as distinct from one another.

H. serpyllum exhibits variations according to ecological conditions. The leaf size and shape, flower colour, and habit of the plant may vary according to the environment. Thus, a variety was accepted for *H. serpyllum* (*H. serpyllum* var. *hookeriana*). Different accessions of *H. serpyllum* showing morphological variation analysed in the study were grouped together, indicating no varietal status for *H. serpyllum*.

Sequences of *Brillantaisia* species retrieved from GenBank constitute a monophyletic group with robust support (BS 100), forming a polytomy alongside accessions of *H. auriculata*. All *Brillantaisia* species and *H. auriculata* are OW species, which formed a single clade along with the other African species of *Hygrophila*.

The subtribe Hygrophilinae consists of only two genera: *Hygrophila* and *Brillantaisia*. Their relationship has been recognized for a long time (Nees, 1847;

Bentham, 1876; Lindau, 1895, Furness, 1994) owing to their polyspermous capsules, bilabiate corollas, and four-colporate pollen grains. Sidwell (1998) specifically investigated the hypothesis of a close relationship between *Hygrophila* and *Brillantaisia* using morphological data and a limited selection of outgroups. She found a clade that included both genera, with *Hygrophila* forming a basal grade from which *Brillantaisia*, as a monophyletic group, was derived. Using a much-expanded taxon sample, phylogenetic analyses of Tripp *et al*. (2013) also support a close relationship between *Brillantaisia* and *Hygrophila*, confirming that *Hygrophila* is non-monophyletic and that *Brillantaisia* is monophyletic and derived from *Hygrophila*. The results of this study confirm these findings.

Chapter 7

SUMMARY

The present study of the genus *Hygrophila* R.Br. (Acanthaceae) in India is the first comprehensive study of the taxonomy, morphology and phylogeny of the genus in India. The genus *Hygrophila* in India is analysed based on extensive field studies and consultation of herbarium specimens housed at major herbaria. The molecular phylogeny of the genus is also analysed using the sequences of the nuclear (ITS) and chloroplast (rbcL) genomes. During the current study, 15 species of *Hygrophila* are recognized with correct names, updated nomenclatural citations with type details, detailed descriptions, colour photographs and details of phenology and distribution in India. A taxonomic key is provided to identify Indian taxa. Phylogenetic relationship of *Hygrophila* and related taxa were also elucidated. The significant findings of the study are presented here.

Taxonomy

Field trips were conducted in different regions of the study area and explored to collect live samples and observe habitat diversity and infraspecefic variations. The distributional and conservation status of each taxon was assessed. Different populations of each taxon were collected from different localities for studying variations. The type localities of the respective taxa were visited and collected 13 out of 15 taxa. Details of habitat, flowering fruiting and other relevant features were recorded from the field itself.

All the specimens collected were identified in consultation with protologues, types and other authentic literature & collections. The nomenclature of each species is updated with the latest literature and in consultation with protologues and types. Detailed description, phenology, distribution maps, photo plate, ecology and habitat were provided for each taxon. Specimens housed at Indian herbaria (ASSAM, ARUN, AHMA, BLAT, BSI, CAL, CALI, JCB, KFRI, MBGH, MH, RHT, SUK, TBGT, Goa University herbarium) and the virtual database of the overseas herbaria (B, BM, BR, C, E, G, GZU, K, L, P, TI, US and W) were consulted.

The present study revealed that six out of fifteen taxa (40%) occurring in India are endemic. The highest diversity of Hygrophila occurs in the western ghats. The study reported an extended distribution of H. chlorides Nees from south India (Karnataka and Kerala).

Hygrophila phlomoides Nees was reinstated from the synonymy of *H. ringens* L.) R.Br. ex Steud. The following names were synonymized in the present study. Their corresponding correct names are given in brackets.

- *Hygrophila difformis* (L.f.) Blume [**Hygrophila balsamica** (L.f.) Raf]
- *Hygrophila heinei* var. *birbhumensis* (Guha) H.B.Naithani & S.Biswas [**Hygrophila heinei** Sreem.]
- *H. stocksii* T. Anderson [**Hygrophila serpyllum** T.Anderson]
- *Hygrophila ringens* var. *cochinensis* [Remadevi & Binojk.) Karthik. & Moorthy [**Hygrophila ringens** (L.) R.Br. ex Steud.]
- *Hygrophila ringens* subsp. *longifolium* J.Mathew & Kad.V.George [**Hygrophila ringens** (L.) R.Br. ex Steud.]

Types were designated for the following names

- *Hygrophila auriculata* (Schumach.) Heine
- *Hygrophila balsamica* (L.f.) Raf.
- *Hygrophila griffithii* (T.Anderson) Sreem.
- *Hygrophila heinei* Sreem.
- *Hygrophila incana* Nees
- *Hygrophila phlomoides* Nees

- *Hygrophila pinnatifida* (Dalzell) Sreem.
- *Hygrophila polysperma* (Roxburgh) T. Anderson
- *Hygrophila.pusilla* Bl.
- *Hygrophila thymus* (Nees) Sunojk. & M.G. Prasad

Live plants of twelve out of fifteen taxa were collected. All the collected samples were planted in the aquatic plant conservatory ("Aquagene") at MBGIPS. Multiple accessions were collected from different localities to analyse variation under cultivation. Most of the plants successfully established but a few exhibited difficulties in growth and flowering in ex-situ conditions.

Molecular Phylogeny

This study represents the first phylogenetic analysis of the genus *Hygrophila* R.Br. Utilizing one nuclear region (ITS) and one chloroplast region (rbcL), the work investigates the phylogenetic relationships within the genus *Hygrophila* and its allied genera. Separate analyses were conducted for each gene followed by a concatenated data analysis. The results indicated no incongruence between the tree topologies of the maximum likelihood (ML) and Bayesian inference (BI) analyses using the concatenated data. The divergence of *Hygrophila* species from their last common ancestors resulted in a strongly supported *Hygrophila* clade, with a bootstrap value of 100. The phylogenetic analysis revealed that *Hygrophila* is non-monophyletic due to the inclusion of the genus *Brillataisia* P.Beauv. Sequences from 77 accessions of the genus in India were analyzed, many of which were sampled for phylogenetic study for the first time. Four species of the genus *Brillantaisia* P. Beauv. is proposed to transfer to the genus *Hygrophila* based on the study. The study concludes that increased sampling of *Hygrophila* species from across the globe is necessary to resolve the complexities within the genus. Consequently, three new combinations and a new name are proposed within *Hygrophila*, as detailed below (with older names in brackets).

- *Hygrophila grottanellii* (Pic.Serm.) V.T.Jaseela, N.S.Pradeep, M.D.Dwivedi & A.K.Pandey, ***comb. nov*** (*Brillantaisia grottanellii*)
- *Hygrophila madagascariensis* (T.Anderson ex Lindau) V.T.Jaseela, N.S.Pradeep, M.D.Dwivedi & A.K.Pandey, ***comb. nov*** (*Brillantaisia madagascariensis*)
- *Hygrophila vogeliana* (Benth.) V.T.Jaseela, N.S.Pradeep, M.D.Dwivedi & A.K.Pandey, ***comb. nov*** (*Brillantaisia vogeliana*)
- *Hygrophila tanzaniensis* V.T.Jaseela, N.S.Pradeep, M.D.Dwivedi & A.K.Pandey, ***nom. nov.*** (*Brillantaisia pubescens*)

Chapter 8

RECOMMENDATIONS

Hygrophila R.Br., one of the largest genera in the tribe Ruellieae of the family Acanthaceae, is predominantly found in Old World tropics and comprises around 80 taxa of aquatic and semiaquatic herbs. The genus is highly diverse and prevalent in the tropical and subtropical regions. Due to its complexity and broad taxonomic conception, the classification and specific status of *Hygrophila* have been subjects of longstanding controversy. There have been limited morphological and taxonomic studies on the genus, particularly in the Indian context. No comprehensive studies have been conducted since its treatment in the *Flora of British India*. The current study aims to address the taxonomic controversies and clarify the relationships within Indian *Hygrophila* R.Br. of Acanthaceae, as the taxonomy and relationships of the genus remain poorly understood. Although molecular phylogenetic studies have been conducted on other genera within the tribe, similar research has not yet been undertaken for *Hygrophila* in India or elsewhere in the other part of the world. In this context, an integrated approach combining morphological and molecular evidence has been employed to delineate species and infer systematic and evolutionary relationships within the genus *Hygrophila* in India.

The present work completed a taxonomic study of the genus, based on both morphological and molecular characteristics, encompassing 15 species. Extensive fieldwork was conducted across India, live collections were established, and thorough morphological and molecular analyses were performed. The study revealed that the highest species diversity is in Peninsular India, with around 40% of species being endemic. This is the first comprehensive treatment of morphology and taxonomy of the genus *Hygrophila* R.Br. in India. It will contribute to the documentation of plant diversity in the country, and the correctly identified plants will open new avenues for research in phytochemistry, pharmacology, and ethnobotany. This research also marks the primary phylogenetic study of the genus, with the generated species sequences submitted to GenBank to support future evolutionary studies of *Hygrophila* and other genera of Acanthaceae. The phylogeny produced for Indian species is a crucial first step towards prioritizing species and areas for conservation. Additionally, the optimized DNA extraction protocol developed during this work will enable the extraction of high-quality DNA from aquatic plants that is suitable for downstream analysis.

This research is a significant contribution to the global understanding of the phylogeny of the genus. By sharing our data and resources, we can pave the way for more robust and comprehensive studies, thereby enhancing the global knowledge of plant biodiversity and conservation. Our findings will guide future studies, which should aim to include all remaining *Hygrophila* species and foster worldwide collaborations to enhance the understanding of the evolution and phylogeny of the genus.

Expanding the geographical scope of our study is crucial. It will not only help to clarify the taxonomy of species with overlapping characteristics and distribution but also aid in understanding the biogeographical patterns and evolutionary history of the genus. Therefore, extensive fieldwork and research across broader regions in Asia are necessary to study *Hygrophila* species that closely resemble each other. Pollen morphology has been successfully used in other Acanthaceae studies to clarify species delineations. Hence, investigating the pollen characteristics of *Hygrophila* species will help resolve taxonomic ambiguities. This approach will complement the current morphological and molecular data, offering a more comprehensive taxonomic framework.

Human activities and climate change are increasingly threatening aquatic habitats. Protecting these environments is crucial for the survival of *Hygrophila* species and other aquatic flora and fauna. Conservation efforts should include habitat restoration, pollution control, and the establishment of protected areas. Implementing conservation strategies will help protect the aquatic habitats where endangered *Hygrophila* species thrive.

Abdel-Hameed, U. K., Salim, M. A., Mourad, M. M., Ishak, F. I., & Tantawy, M. E. (2014). Phylogenetic Analysis of Acanthaceae in Egypt Based on Morphological Criteria. *Vegetos*, 27(2), 29-39.

Abdel-Hameed, U.K., Tantawy, M.E., Salim, M.A., Mourad, M.M., &Ishak, I.F. (2015) Phenetic Analysis of Morphological and Molecular Traits in Acanthaceae Juss. *Journal of Biosciences and Medicines*, 3(3), 18-34.

Agarwal, M., Shrivastava, N., & Padh, H. (2008). Advances in molecular marker techniques and their applications in plant sciences. *Plant cell reports*, *27*, 617-631.

Ahmad, K. J. (1978). Epidermal hairs of Acanthaceae. *Blumea: Biodiversity, Evolution and Biogeography of Plants*, 24(1), 101-117.

Ahmad, K. J. (976). Epidermal studies in some species of *Hygrophila* and *Dyschoriste* (Acanthaceae) *Journal of Indian Botanical Society*, 55, 42-52.

Ahmad, K. J. (1979). Taxonomic significance of epidermal characters in Acanthaceae. *Progress in plant research: silver jubilee publication, National Botanical Research Institute/editors*, TN Khoshoo, PKK Nair. 160.

Ahmad, S. M., Ganaie, M. M., Qazi, P. H., Verma, V., Basir, S. F., & Qazi, G. N. (2004). Rapid DNA isolation protocol for angiospermic plants. *Bulgarian Journal of Plant Physiology*, 30, 25-33.

Ahmed, S., Rahman, A., Mathur, M., Athar, M., & Sultana, S. (2001). Antitumor promoting activity of *Asteracantha longifolia* against experimental hepatocarcinogenesis in rats. *Food and Chemical Toxicology*, 39(1), 19–28.

Almeida, M.R. (2003). *Flora of Maharashtra*. Blatter Herbarium, St. Xavier's College, Mumbai, 4A: p.48.

Alston, A.G.H. (1931). *Handbook to the Flora of Ceylon*, London. 6 (Suppl.): 232

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. Journal of molecular biology, 215(3), 403-410.

Álvarez, I. J. F. W., & Wendel, J. (2003). Ribosomal ITS sequences and plant phylogenetic inference. *Molecular phylogenetics and evolution*, 29(3), 417-434.

Anderson, T. (1864). An Enumeration of the Species of Acanthaceae from the Continent of Africa and Adjacent Islands. *Journal of the Linnean Society*, Botany, 7, 13-54.

Anderson, T. (1864). Acanthaceae In: G.H.K. Thwaites, *Enumeratio Plantarum Zeylaniae*, London. Dulau & Co. p. 223.

Anderson, T. (1867). An Enumeration of the Indian Species of Acanthaceae. *Journal of the Linnean Society*. Botany, 9, 425-526.

Anusha, P., & Immanuel, S. R. (2019). Anticancer activity and GCMS analysis of methanol leaves extract of *H. auriculata* (Schumach.) Heine. *Journal of Pharmacognosy and Phytochemistry*, 8(5), 1453-1457.

APG (1998). An ordinal classification for the families of flowering plants. *Annals of the Missouri Botanical Garden,* 85, 531–553.

APG II. (2003). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society*, 141(4), 399-436.

APG III. (2009). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. APG III. *Botanical Journal of the Linnean Society*, 161, 105–121.

APG IV. (2016). An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society*, 181, 1–20.

Arisdason, W., Lakshminarasimhan, P., Karthigeyan, K., Krishna, G., Albertson, D. W., Venu, P., Panja D., Ghosh, T., Debnath, H. S., Gnanasekaran, G., Murthy, G.V. S., & Roy, D. K. (2020). Acanthaceae. In: Mao A.A. & S.S. Dash (eds.), *Flowering plants of India an annotated checklist* (Dicotyledons). Botanical Survey of India, Kolkata, 1, 270–319.

Bairu, M. W., Aremu, A. O., & Van Staden, J. (2011). Somaclonal variation in plants: causes and detection methods. *Plant Growth Regulation*, 63, 147-173.

Baldwin, B. G., Sanderson, M. J., Porter, J. M., Wojciechowski, M. F., Campbell, C. S., & Donoghue, M. J. (1995). The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri botanical garden*, 247-277.

Balraj, P., & Nagarajan, S. (1982). Apigenin 7-O-glucuronide from the flowers of *Asteracantha longifolia* Nees. *Indian Drugs* 19(4), 150–152.

Barker, R. M. (1986). A Taxonomic Revision of Australian Acanthaceae, *Journal of the Adelaide Botanic Gardens*, 9, 1-286.

Benson, L. (1957). *Plant Classification*. Oxford and I B H Publishing Co. New Delhi.

Bentham, G. (1868). *Flora Australiensis*. Lovell Reeve & Co., London. 4, 544.

Bentham, G. & Hooker, J. D. (1876). *Genera Plantarum*. Reeve, London, 1, 57-102.

Bessey, C. E. (1915). The phylogenetic taxonomy of flowering plants. *Annals of the Missouri Botanical Garden,* 2(1/2), 109-164.

Bhatt, A., Naidoo, Y., & Nicholas, A. (2010). The foliar trichomes of *Hypoestes aristata* (Vahl) Sol. ex Roem. & Schult. var. *aristata* (Acanthaceae) a widespread medicinal plant species in tropical sub-Saharan Africa: with comments on its possible phylogenetic significance. *Biological Research*, 43, 403-409.

Bhatt, M., & Shah, N. (2016). Acute toxicity, in-vitro urolithiatic and diuretic evaluation of methanolic extract of *Hygrophila salicifolia*, whole herb in rat. *International Journal of Pharmaceutical Science and Drug Research*, 8(2), 111–116

Bhatt, M. K., & Shah, N. (2019). Isolation and structure elucidation of isoquercitrin from *Hygrophila salicifolia* (Acanthaceae). *International Journal of Pharmaceutical Science and Drug Research*, 11, 51–55.

Biswas, A., Bhattacharya, S., Mahapatra, S. D., Debnath, M. D., & Biswas, M. (2012). The Antioxidant Effects of *Eupatorium triplinerve*, *Hygrophila triflora* and *Pterocarpus marsupium*—A Comparative Study. *European Journal of Applied Sciences*, *4*, 136-139.

Blatter, E. (1931). *Journal and Proceedings of the Asiatic Society of Bengal*. Calcutta, 26, 350.

Blume, C. L. (1826). Bijdragen tot de Flora van Nederlandsch Indie. 14, 804.

Boily, Y., & Van Puyvelde, L. (1986). Screening of medicinal plants of Rwanda (Central Africa) for antimicrobial activity. *Journal of Ethnopharmacology*, 16(1), 1-13.

Borg, A. J., McDade, L. A., & Schönenberger, J. (2008). Molecular phylogenetics and morphological evolution of Thunbergioideae (Acanthaceae). *Taxon*, 57(3), 811-822.

Botstein, D., White, R. L., Skolnick, M., & Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American journal of human genetics*, 32(3), 314.

Bremekamp, C. E. B. (1965). Delimitation and subdivision of the Acanthaceae. *Bulletin of Botanical Survey of India*, 7, 21–30.

Bremekamp, C. E. B. (1938). Acanthaceae. *Recueil des travaux Botaniques Néerlandais*,3(5), 160–164.

Bremekamp, C. E. B. (1944). Materials for a monograph of the Strobilanthinae (Acanthaceae). *Verh K Ned Akad Wet Afd Natuurkd Sect,* 2(41), 1–305.

Bremekamp, C. E. B., & Nannenga-Bremekamp N.E. (1948). A preliminary survey of the Ruelliinae (Acanthaceae) of the Malay Archipelago and New Guinea. *Proceedings of the Koninklijke Nederlandse Akademie van Wetenschappen*, 45,1–39.

Bremekamp, C. E. B. (1955). Notes on some acanthaceous genera and species of controversial position. *Acta Botanica Nederlandica,* 4(5), 644–655.

Briggs, D., & Walters, S.M. (1997). *Plant Variation and Evolution* (3rd ed). Cambridge University Press.

Brown, E. A., Chain, F. J. J., Crease, T. J., MacIsaac, H. J., Chariton, A. A., Bailey, S. A., ... & Cristescu, M. E. (2022). Development of standardized internal transcribed spacer and small subunit rRNA gene primer sets for environmental DNA metabarcoding studies to investigate rare aquatic biodiversity. *Conservation Genetics Resources*, 1-13.

Brown, R. (1810). *Prodromus florae Novae Hollandiae et insulae VanDiemen*. R Taylor, London, p. 479

Brummitt, R.K. & Powell C. E. (1992). *Authors of Plant Names*. Royal Botanic Gardens, Kew.

Carine, M. A., & Scotland, R. W. (1998). Pollen morphology of *Strobilanthes* Blume (Acanthaceae) from Southern India and Sri Lanka. *Review of Palaeobotany and Palynology*, 103, 143-165.

Champian, H.G., & Seth, S.K. (1968). *A revised survey of forest types in India*. Delhi.

Chase, M. W., & Reveal, J. L. (2009). A phylogenetic classification of the land plants to accompany APG III. *Botanical Journal of the Linnean Society*, 161(2), 122-127.

Chase, M. W., Soltis, D. E., Olmstead, R. G., Morgan, D., Les, D. H., Mishler, B. D., ... & Albert, V. A. (1993). Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene rbcL. *Annals of the Missouri Botanical Garden*, 528-580.

Chaubal, P. D. (1966). *Palynological studies on the family Acanthaceae*. Thesis, Poona University, India.

Chauhan, N. S., Sharma, V., & Dixit, V. K. (2009). Effect of *Asteracantha longifolia* seeds on sexual behavior of male rats. *Natural Product Research*, 14, 1–9.

Cherian, P.T. (2001). Deccan Peninsula. In: Alfred, J.R.B., Das, A.K. & Sanyal, A.K. (eds.), *Ecosystems of India*. ENVIS, Government of India, Kolkata, p. 387–410.

Chopra, R. N., Nayer, S. L., & Chopra, I. C. (1956). *Glossary of Indian Medicinal plants*, National Institute of science and Communication. C.S.I.R Publication, New Delhi, India, p. 330-332.

Chowdhury, M. I. & Begum, A. (2012). Chemical and Biological Investigations of "*Hygrophila ringens* (L.)". LAP Lambert academic publishing.

Clarke, C. B. (1899). In: Thiselton-Dyer's Flora of Tropical Africa 5, 162.

Clarke, C. B. (1908). Family LXXXIX.—Acanthaceae. P. 628–698 In: G King, ed. Materials for the flora of the Malayan Peninsula. *Journal of Asiatic Society of Bengal*, 74.

Clarke, C.B. (1885). Acanthaceae. In: Hooker, J.D., Ed., *Flora of British India*, Vol. 4, L. Reeve, London, 387-558.

Clarke, J. B., & Bevan, J. R. (1997). Optimizing random amplified polymorphic DNA (RAPD) analysis for detection of somaclonal variation in sugar beet. *Plant Cell, Tissue and Organ Culture*, 47(3), 215-221.

Clegg, M.T., & Zurawski, G. (1992). *Chloroplast DNA and the Study of Plant Phylogeny: Present Status and Future Prospects*. In: Soltis, P.S., Soltis, D.E., Doyle, J.J. (eds) Molecular Systematics of Plants. Springer, Boston, MA.

Cook, C.D.K. (1996). Aquatic & Wetland Plants of India. Oxford University Press, p. 30.

Cramer, L. (1989). The *Hygrophila* complex (Acanthaceae) in India and Ceylon. *Nordic Journal of Botany,* 9(3), 261–263.

Cramer, L. H. (1991). *Brillantaisia* P. Beauv. (Acanthaceae), A new generic record for Asia. *Kew bulletin*, 335-338.

Cramer, L.H. (1992). Name changes in the Acanthaceae of India and Ceylon (SriLanka). *National Science Council Sri Lanka*, 20 (1), 59 – 69.

Cronquist, A. (1981). *An Integrated System of Classification of Flowering Plants*. Columbia University Press, New York, 248-250.

Dakin, E. E., & Avise, J. C. (2004). Microsatellite null alleles in parentage analysis. Heredity, 93(5), 504-509.

Daniel, M., & Sabnis, S. D. (1987). Chemosystematics of some Indian members of the Acanthaceae. Proceedings: Plant Sciences, 97, 315-323.

Daniel, T.F. (1998). Pollen morphology of Mexican Acanthaceae: Diversity and systematic significance. *Proceedings of the California Academy of Sciences*, 50, 217–256.

de Jussieu, A. L. (1789). *Genara Plantarum*. Secundum, Ordines Naturales, Disposita. Parisiis, p.307

De Vogel, E. F. (1987). *Manual of herbarium taxonomy*: Theory and practice.

Donoghue, M. J., Olmstead, R. G., Smith, J. F., & Palmer, J. D. (1992). Phylogenetic relationships of Dipsacales based on rbcL sequences. *Annals of the Missouri Botanical Garden*, 333-345.

Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical bulletin*, 19,11-15.

Duvall, M. R., Learn Jr, G. H., Eguiarte, L. E., & Clegg, M. T. (1993). Phylogenetic analysis of rbcL sequences identifies *Acorus calamus* as the primal extant monocotyledon. *Proceedings of the National Academy of Sciences*, 90(10), 4641-4644.

Ellegren, H. (2004). Microsatellites: simple sequences with complex evolution. *Nature Reviews Genetics*, 5(6), 435-445.

Engler, A., & L. Diels. (1936). Syllabus der Pflanzenfamilien. Verlag von Gebrüder Borntraeger, Berlin.

Engler, H. H., & Prantl, K. (1897). *Pflantzenfamilien*. Guillaume Engelmann.

Fang, D. Q., & Roose, M. L. (1997). Identification of closely related citrus cultivars with inter-simple sequence repeat markers. *Theoretical and Applied Genetics*, 95(3), 408-417.

Farr, E. R., Leussink, J. A., & Stafleu, F. A. (Eds.). (1979). *Index Nominum Genericorum* (Plantarum) 3 Vols.

Fedorov, A. N. A. (1974). *Chromosome Numbers of Flowering Plants*. Reprint by OHO Koeltz Science Publishers, n-624 Koenigstein, West Germany.

Fernando, M. R., Wickramasinghe, S. N., Thabrew, M. I., Ariyananda, P. L., & Karunanayake, E. H. (1991). Effect of *Artocarpus heterophyllus* and *Asteracanthus longifolia* on glucose tolerance in normal human subjects and in maturity-onset diabetic patients. *Journal of ethnopharmacology*, 31(3), 277-282.

Forman, L., & Bridson, D. (1989). *The herbarium handbook*. Royal Botanic Gardens, Kew

Fosberg, F., R. & Sachet, M. (1981). *Baileya* 21(3): 147.

Freyre, R., Skroch, P. W., Geffroy, V., Adam-Blondon, A. F., Shirmohamadali, A., Johnson, W. C., ... & Gepts, P. (1998). Towards an integrated linkage map of common bean. *Genome*, 41(5), 738-745.

Furness, C. A. (1994). The pollen morphology of *Hygrophila* and *Brillantaisia*. *Acta Botanica Gallica*. 141:267–278.

Furness, C. A. (1990). Pollen morphology of *Crossandra* Salisbury and *Crossandrella* C. B. Clarke (Acanthaceae: Acantheae). *Grana*, 29, 161-176.

Furness, C. A., & Grant, M. C. (1996). The pollen morphology of some *Ruellia* species (Acanthaceae) from Africa and Madagascar. *Grana*, 35, 231-239.

Gabel, N. H., Wise, R. R. & Rogers, G. K. (2021). Distribution of cystoliths in the leaves of Acanthaceae and its effect on leaf surface anatomy. *Blumea* 65 (3), 224 – 232.

Gamble, J. S., & Fischer C.E.C. (1924). *The Flora of the Presidency of Madras*. Adlard & Sons Ltd., London.

Gao, C., Deng, Y., & Wang, J. (2019). The complete chloroplast genomes of *Echinacanthus* species (Acanthaceae): phylogenetic relationships, adaptive evolution, and screening of molecular markers. *Frontiers in Plant Science*, 9, 411624.

Gawel, N.J., Jarret, R.L., & Whittemore, A.P. (1992). Restriction fragment length polymorphism (RFLP)-based phylogenetic analysis of *Musa*. *Theoretical and Applied Genetics*, 84, 286–290.

German, D. A., Friesen, N., Neuffer, B., Al-Shehbaz, I. A., & Hurka, H. (2009). Contribution to ITS phylogeny of the Brassicaceae, with special reference to some Asian taxa. *Plant Systematics and Evolution*, 283, 33-56.

Gigante, R. (1929). Embriologia dell' *Acanthus mollis* L, *Nuovo Gior. Bot*, Ital, 36, 1-33.

Gomes, A., Das, M., & Dasgupta, S. C. (2001). Haematinic effect of *Hygrophila spinosa*.T Anderson on experimental rodents. *Indian Journal of Experimental Biology*, 39(4), 381–382.

Govindachari, T. R., Nagarajan, K., & Pai, B. R. (1957). Isolation of lupeol from the root of *Asteracantha longifolia* Nees. Journal of Indian Science and Research, 16B, 72.

Govindarajan, T., & Subramanian, D. (1985). Karyomorphological Studies in South Indian Acanthaceae. *Cytologia*, 50, 473-482.

Govindarajan, T., & Subramanian, D. (1983). Karyomorphological studies in south Indian Acanthaceae. *Cytologia*, *48*(3), 491-504.

Grant, W. F. (1955). A cytogenetic study in the Acanthaceae. *Brittonia* 8 (2): 121-149.

Gupta, M., Chyi, Y. S., Romero-Severson, J., & Owen, J. L. (1999). Amplification of DNA markers from evolutionary diverse genomes using single primers of simple-sequence repeats. *Theoretical and Applied Genetics*. 89(7-8), 998-1006.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In: *Nucleic acids symposium series*, 41(41), 95-98

Hebert, P. D., & Gregory, T. R. (2005). The promise of DNA barcoding for taxonomy. *Systematic biology*, 54(5), 852-859.

Hebert, P. D., Cywinska, A., Ball, S. L., & DeWaard, J. R. (2003). Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: *Biological Sciences*, 270(1512), 313-321.

Hedrén, M., Chase, M. W., & Olmstead, R. G. (1995). Relationships in the Acanthaceae and related families as suggested by cladistic analysis of rbcL nucleotide sequences. *Plant Systematics and Evolution*, 194, 93-109.

Heine, H. (1962). Tropical African Plants: XXVI. Some West African Acanthaceae. *Kew Bulletin*, 16(2), 161–183.

Heine, H. (1971). Notes sur Les Acanthacees Africaines: *Hygrophila* R. Br. *Adansonia*, ser. 2(4), 656-659.

Henry, R. J. (1997). *Practical applications of plant molecular biology*. Garland Science.

Hewawasam, R. P., Jayatilaka, K. A. P. W., Pathirana, C., & Mudduwa, L. K. B. (2003). Protective effect of Asteracantha longifolia extract in mouse liver injury induced by carbon tetrachloride and paracetamol. *Journal of Pharmacy and Pharmacology*, 55(10), 1413-1418.

Ho, C. W., Lin, R. D., Lee, T. H., Lin, C. H., Wen, C. L., Tseng, Y. T., & Lee, M. H. (2013). Chemical and pharmacological investigation of micropropagated *Hygrophila pogonocalyx* produced from leaf explants. *Botanical Studies*, *54*, 1-10.

Hobein, M. (1884). Uber den systematischen Wert des Cystolithen bei den Acanthaceen. Botanische Jahrbücher für Systematik, *Pflanzengeschichte und Pflanzengeographie* 5, 422–440

Hoey, B. K., Crowe, K. R., Jones, V. M., & Polans, N. O. (1996). A phylogenetic analysis of Pisum based on morphological characters, and allozyme and RAPD markers. *Theoretical and Applied Genetics*, 92, 92-100.

Hollingsworth, P. M., Forrest, L. L., Spouge, J. L., Hajibabaei, M., Ratnasingham, S., ... & Little, D. P. (2009). A DNA barcode for land plants. *Proceedings of the National Academy of Sciences*, 106(31), 12794-12797.

Hollingsworth, P. M., Graham, S. W., & Little, D. P. (2011). Choosing and using a plant DNA barcode. *PLoS ONE*, 6, e19254.

Hooker, J. D. (1875–1897). *Flora of British India*. Vols. 1–7. L. Reeve, London.

Hu, C. C., Deng, Y. F., Wood, J. R. I. & Daniel, T. F. (2011). Acanthaceae. pp. 369–477 In: Wu, Z.Y., Raven, P., & Hong, D.Y. (eds.), Flora of China, vol. 19. Beijing, Science Press; St. Louis: Missouri Botanical Garden Press.

Huang, J. C., Wang, W. K., Hong, K. H., & Chiang, T. Y. (2001). Population differentiation and phylogeography of Hygrophila pogonocalyx based on RAPDs fingerprints. *Aquatic Botany*, 70(4), 269-280.

Huang, J. C., Wang, W. K., Peng, C. I., & Chiang, T. Y. (2005). Phylogeography and conservation genetics of Hygrophila pogonocalyx (Acanthaceae) based on atpB–rbcL noncoding spacer cpDNA. *Journal of plant research*, 118, 1-11.

Huard, D. (1962). *Orgie de quelques anomalies observees chez des pol Pollen et Spores*, 7, 19-26.

Hussain, M. S., Ahamed, K. F., Ravichandiran, V., & Ansari, M. Z. H. (2009). Evaluation of in vitro free radical scavenging potential of different fractions of *H. auriculata*. Asian Journal of Traditional Medicines, 4(5), 179–187.

Hussain, M.S., Fareed, S. & Ali, M. (2011). Preliminary phytochemical and pharmacognostical screening of the Ayurvedic drug *Hygrophila auriculata* (K. Schum) Heine. *Pharmacognosy* Journa,l 3(23), 28-40.

Hutchinson, J. (1969). *Evolution and phylogeny of flowering plants*. Academic Press, London, 1-717.

Inamdar, J. A. (1970). Epidermal structure and ontogeny of caryophyllaceous stomata in some Acanthaceae. *Botanical Gazette*, 131(4), 261-268.

Inamdar, J. A., Chaudhari & Rao, R. (1990). Studies of the cystoliths of Acanthaceae. *Fedds Repert*. 101 (7/8), 417- 424.

Ismail, N. Z., Arsad, H., Samian, M. R., Ab Majid, A. H., & Hamdan, M. R. (2016). Evaluation of genetic diversity of *Clinacanthus nutans* (Acanthaceaea) using RAPD, ISSR and RAMP markers. *Physiology and Molecular Biology of Plants*, 22, 523-534.

IUCN. (2012). *IUCN Red List Categories and Criteria*. Version 3.1. Second edition. IUCN, Species Survival Commission, Gland and Cambridge.

IUCN. (2022). *Guidelines for using the IUCN Red List Categories and Criteria*. Version 15.1. Prepared by the Standards and Petitions Committee.

Jain, S. K. (1991). *Dictionary of Indian Folk Medicine and Ethnobotany*. Deep Publications, New Delhi, 311.

Jaseela V. T., Sinisha E. K. & N. S. Pradeep. (2024). A modified CTAB method for extracting high-quality genomic DNA from aquatic plants. *Plant Science Today*. 11(2), 125–132.

Jeeva, G. M., Jeeva, S. & Kingston. (2007). Traditional treatment of skin diseases in south Travancore, southern peninsular India. *Indian Journal of Traditional Knowledge*, 6(3), 498–501

Jensen, H. F. W., Jensen S. R., & Nielsen, B. J. (1988). Chemotaxonomy of the Acanthaceae. Iridoids and quaternary amines. *Phytochemistry*, 27(8), 2581–2589.

Jensen, S. R., & Nielsen, B. J. (1985). Hygrophiloside, an iridoid glucoside from *Hygrophila difformis* (Acanthaceae). *Phytochemistry*, 24, 602-603.

Johnson, L. A., & Soltis, D. E. (1994). matK DNA sequences and phylogenetic reconstruction in Saxifragaceae s. str. *Systematic botany*, 143-156.

Johnson, M. T., Carpenter, E. J., Tian, Z., Bruskiewich, R., Burris, J. N., Carrigan, C. T., ... & Weller, S. G. (2018). Evaluating methods for isolating total RNA and predicting the success of sequencing phylogenetically diverse plant transcriptomes. *PLoS ONE*, 13(10), e0205056.

Kaewdaungdee, S., Sudmoon, R., Tanee, T., Lee, S. Y., & Chaveerach, A. (2022). Chloroplast genome analysis for genetic information and authentication in five Barleria species. *Genes*, 13(10), 1705.

Kanhere, R., Anjana, A., Anbu, J., Sumithra, M., Nazeer Ahemad, K. F. H. (2013). Neuroprotective and antioxidant potential of terpenoid fraction from *Hygrophila auriculata* against transient global cerebral ischemia in rats. *Pharmaceutical biology*, 51(2), 181-189.

Kannur, D., & Nandanwadkar, S., Dhawane, S., Phulambrikar, S. & Khandelwal, K. (2017). Experimental evaluation of *Hygrophila Schulli* seed extracts for antistress activity. *Ancient Science of Life*, 37, 267.

Kapli, P., Kotari, I., Telford, M. J., Goldman, N., & Yang, Z. (2023). DNA sequences are as useful as protein sequences for inferring deep phylogenies. *Systematic Biology*, 72(5), 1119-1135.

Karmakar, U. K., Akter, S., Bokshi, B., & Sadhu, S. K. (2022). Exploration of analgesic, antiinflammatory, laxative, and anthelmintic activities of *Hygrophila phlomoides*. Int. J. Pharm. Sci. Rev. Res., 77(2), 38–43.

Karthikeyan, S., Sanjappa, M., & Moorthy, S. (2009). *Flowering Plants of India*, Vol. 1. Botanical Survey of India, Kolkata.

Kathriarachchi, H., Hoffmann, P., Samuel, R., Wurdack, K. J., & Chase, M. W. (2005). Molecular phylogenetics of Phyllanthaceae inferred from five genes (plastid atpB, matK, 3′ ndhF, rbcL, and nuclear PHYC). *Molecular phylogenetics and evolution*, 36(1), 112-134.

Khan, I., Shinwari, Z. K., Zahra, N. B., Jan, S. A., Shinwari, S., & Najeebullah, S. (2020). DNA barcoding and molecular systematics of selected species of family Acanthaceae. *Pakistan Journal of Botany*, 52(1), 205-212.

Khanna, K. K., Tripathi A. K., & Mudgal, V. (1997). Acanthaceae. In: Mudgal V., Khanna K.K. & and Hajra P.K. (eds), *Flora of Madhya Pradesh*, Botanical Survey of India, Calcutta, 2, 320-325.

Kiel, C. A., & McDade, L. A. (2014). The Mirandea clade (Acanthaceae, Justicieae, Tetramerium Lineage): Phylogenetic signal from molecular data and micromorphology makes sense of taxonomic confusion caused by remarkable diversity of floral form. *Systematic Botany*, 39(3), 950-964.

Kirtikar, K. R., & Basu, B. D. (2005). *Indian Medicinal Plants*, Vol. III, International Book Distributors, Dehradun, p 1863.

Kitty, C. A. (1988). *Complete guide to maintaining health and treating illness with plants*. London: Leopard Book, Random House, p 9-12.

Krauss, S. L., & Hopper, S. D. (2001). Genetic diversity and conservation of *Grevillea scapigera* (Proteaceae), a critically endangered shrub with a limited distribution in southwestern Australia. *Molecular Ecology*, 10(11), 3171-3183.

Kumar, S., & Paliwal, G. S. (1975). Foliar anatomy of the family Acanthaceae. *Acta Botanica Indica*, 3, 121-131.

Lakshmi, M., Rajalakshmi, S., Parani, M., Anuratha, C. S., & Parida, A. (1997). Molecular phylogeny of mangroves I. Use of molecular markers in assessing the intraspecific genetic variability in the mangrove species *Acanthus ilicifolius* Linn. (Acanthaceae). *Theoretical and Applied Genetics*, 94, 1121-1127.

Lawrence, G. H., Buchheim, A. G., Daniels, G. S., & Dolezal, H. (1968). *Botanico-Periodicum-Huntianum* (BPH). Botanico-Periodicum-Huntianum (BPH).

Lawrence, G.H.M. (1951). *Taxonomy of Vascular Plants*. The Macmillan Co., New York.

Letunic, I., & Bork, P. (2021) Interactive Tree of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic acids research* 49(W1), W293-W296.

Li, D. Z., Gao, L. M., Li, H. T., Wang, H., & Ge, X. J. (2011) Comparative analysis of a large dataset indicates that internal transcribed spacer (ITS) should be incorporated into the core barcode for seed plants. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 19641–19646.

Li, X., Qiao, L., Chen, B., Zheng, Y., Zhi, C., Zhang, S., ... & Cheng, Z. (2022). SSR markers development and their application in genetic diversity evaluation of garlic (*Allium sativum*) germplasm. *Plant Diversity,* 44(5), 481-491.

Liang, H., & Hilu, K. W. (1996). Application of the mat K gene sequences to grass systematics. *Canadian Journal of Botany*, 74(1), 125-134.

Lindau, G. (1893). *Beiträge zur Systematik der Acanthaceen*. Botanische Jahrbüchen. 18, 36-64.

Lindau, G. (1895). Acanthacaeae. In: Engler, A. & Prantel, K., *Die Naturalichen Pflanzenfamilien*, 4, 274–354.

Linnaeus, C. (1747). Flora Zeylanica. Stockholm, Laurentius Salvius.

Linnaeus, C. (1753). *Species Plantarum*. Stockholm. Laurentii Salvii.

Mabberley, D. J. (1987). The Plant Book: A Portable Dictionary of the Higher Plants. Cambridge: Cambridge University Press.

Mabberley, D. L. (2008). *The Plant Book*. Cambridge, UK: Cambridge University Press.

Maddison, W. P., Maddison, D. R. (2015). *Mesquit*: A modular system for evolutionary analysis. Mesquite v.3.40, http://mesquiteproject.org

Madhusoodanan, P. V., & Singh, N. P. (1992). A new species of *Lepidagathis* (Acanthaceae) from South India. *Kew Bulletin*, 301-303.

Manhart, J. R. (1994). Phylogenetic analysis of green plant rbcL sequences. *Molecular phylogenetics and evolution*, 3(2), 114-127.

Manilal, K. S. (2003). *Van Rheed's Hortus Malabaricus. (English Ed.) with Annotations and Modern Botanical nomenclature*. University of Kerala. Thiruvananthapuram

Manilal, K. S. (2008). *Van Rheed's Hortus Malabaricus. (Malayalam Ed.*). University of Kerala. Thiruvananthapuram

Manktelow, M., McDade, L. A., Oxelman, B., Furness, C. A., & Balkwill, M. J. (2001). The enigmatic tribe Whitfieldieae (Acanthaceae): Delimitation and phylogenetic relationships based on molecular and morphological data. *Systematic Botany*. 26, 104–119.

Manktelow, M. (2000) The filament curtain: a structure important to systematics and pollination biology in the Acanthaceae. *Botanical Journal of Linnean Society*, 133, 129–160.

Mathew, J., Yohannan, R., Salim, P. M., & George, K. V. (2017). Novelties in the family Acanthaceae from south Western Ghats, India. *Annals of Plant Sciences*. 6.01, 1499–1503.

Mathew, J., & George, K. V. (2013). *Hygrophila ringens* ssp. *longifolium* (Acanthaceae): A new taxa from southern Western Ghats, India. *International Journal of Advanced Research*, 1(9),132-134.

Mathiventhan, U., & Ramiah, S. (2015). Vitamin C content of commonly eaten green leafy vegetables in fresh and under different storage conditions. *Tropical Plant Research*. 2(3), 240-245.

Mauritzon, J. (1934). Die Endosperm-und Embryo-Entwicklung einiger Acanthaceen, von Johan Mauritzon. H. Ohlssons buchdruckerei.

Mazumdar, U. K., Gupta, M., Maiti, S. & Mukherjee, D. (1997). Antitumor activity of *H. spinosa* on Ehrlich ascites carcinoma and sarcoma-180 induced mice. *Indian Journal of Experimental Biology*, 35, 473-477.

McDade, L. A., Masta, S. E., Moody, M. L., & Waters, E. (2000a). Phylogenetic relationships among Acanthaceae: Evidence from two genomes. *Systematic Botany*, 25, 106–121.

McDade, L. A., & Moody, M. L. (1999). Phylogenetic relationships among Acanthaceae: evidence from noncoding trnL-trnF chloroplast DNA sequences. *American Journal of Botany*, 86(1), 70-80.

McDade, L. A., Daniel, T. F., Kiel, C. A., & Borg, A. J. (2012). Phylogenetic placement, delimitation, and relationships among genera of the enigmatic Nelsonioideae (Lamiales: Acanthaceae). *Taxon*, 61(3), 637-651.

McDade, L. A., Daniel, T. F., Kiel, C. A., & Vollesen, K. (2005). Phylogenetic relationships among Acantheae (Acanthaceae): major lineages present contrasting patterns of molecular evolution and morphological differentiation. *Systematic Botany*, 30(4), 834-862.

McDade, L.A. and Moody, M.L. (1999) Phylogenetic Relationships among Acanthaceae: Evidence from Non-Coding trnL-trnF Chloroplast DNA Sequences. *American Journal of Botany*, 86, 70-80.

McDade, L.A., Daniel, T.F., & Kiel, C.A. (2008). Towards a comprehensive understanding of phylogenetic relationships among lineages of Acanthaceae s.l. (Lamiales). *American Journal of Botany*, 95, 1136–1152.

McDade, L.A., Daniel, T.F., Masta, S.E., & Riley, K.M. (2000b). Phylogenetic relationships within the tribe Justicieae (Acanthaceae): Evidence from molecular sequences, morphology, and cytology. *Annals of Missouri Botanic Garden*, 87, 435–458.

Metcalfe, C.R., & Chalk, L. (1950). Anatomy of The Dicotyledons. Clarendron Press, Oxford.

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., Von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Molecular biology and evolution*, 37(5), 1530-1534.

Misra, T.N., Singh, R.S., Pandey, H.S., Singh, B.K., & Pandey, R.P. (2001): Constituents of *Asteracantha longifolia*. *Fitoterapia*, 72(2), 194-196.

Moore, P. D., & Webb, J.A. (1978). *An Illustrated Guide to Pollen Analysis*. Hodder and Stoughton, London.

Moylan, E. C. & Scotland, R. W. (2000). *Hemigraphis neocaledonica* Heine from New Caledonia is transferred to *Brunoniella* Bremek. *Kew Bulletin*, 55, 477–481.

Moylan, E. C., Bennett, J. R., Carine, M. A., Olmstead, R. G., & Scotland, R. W. (2004). Phylogenetic relationships among *Strobilanthes* s.l. (Acanthaceae): evidence from ITS nrDNA, trnL-F cpDNA, and morphology. *American Journal of Botany*, 91, 724–735.

Mukherjee, A., Williams, D., Gitzendanner, M. A., Overholt, W. A., & Cuda, J. P. (2016). Microsatellite and chloroplast DNA diversity of the invasive aquatic weed *Hygrophila polysperma* in native and invasive ranges. *Aquatic Botany*, 129, 55-61.

Muller, J., Schuller, M., Straka, H., & Friedrich, B. (1989). Palynologia Madagassica et Mascarenica. Fam. 182. Acanthaceae. *Tropische und Subtropische Pflanzenwelt*, 67, 138-187.

Murray, M. G., & Thompson, W. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic acids research*, 8(19), 4321-4326.

Murthy, G. V. S., & Sanjappa, M. (1996). Physiography. In: Hajra, P.K. *et a*l. (eds.), *Flora of India*, Introductory volume. Botanical Survey of India, Kolkata. p.1-16.

Nadkarni, A. K. (2007). Indian Materia Medica, Vol I, Popular Prakashan, Mumbai, p668.

Nam, H. G., Giraudat, J., Den Boer, B., Moonan, F., Loos, W. D., Hauge, B. M., & Goodman, H. M. (1989). Restriction fragment length polymorphism linkage map of Arabidopsis thaliana. *The Plant Cell*, 1(7), 699-705.

Narayana, L. L & Sarma, V. V. L. N. (1980). Chemotaxonomy of Acanthaceae. *Journal of Indian Botanical Society*, 59, 48–52.

Narayanan, C. R. (1951). Somatic chromosomes in Acanthaceae. *Journal of Madras University*, 21(B), 220–231.

Natarajan, A.T. (1957). Studies in the morphology of pollen-Tubiflorae. *Phyton* (Buenos Aires), 8, 21-42.

Nees Von Esenbeck, C. G. (1832). Acanthaceae. In: Wallich, *Plantae Asiaticae Rariores*. London, 3, 70–117.

Nees Von Esenbeck, C.G. (1847). Acanthaceae. In: de Candolle, A., (ed). *Prodromus Systematis Naturalis Regni Vegetabilis*. Sumptibus Sociorum Treuttel & Wurtz, Paris, 11, 46-519.

Negi, S.S. (1990). *A handbook of the Himalay*a. Indus Publishing Company, New Delhi, New Forest, Dehradun, pp. 350.

Pal, D. K., Samanta, K. (2011). CNS activities of ethanol extract of aerial parts of *Hygrophila difformis* in mice. *Acta Poloniac Pharmaceutica-Drug Research*, 68, 75-81.

Palmer, J. D., Jansen, R. K., Michaels, H. J., Chase, M. W., & Manhart, J. R. (1988). Chloroplast DNA variation and plant phylogeny. *Annals of the Missouri Botanical Garden*, 1180-1206.

Panigrahi, G., & Dubey, A. K. (1983). *Dicliptera bupleuroides* var. *roxburghiana*, a new name for D. roxburghiana CB Clarke, non Nees (Acanthaceae). *Taxon*, 32(2), 286-288.

Pant, D. D., Bharati, M. (1963). Development of Caryophyllaceous Stomata in *Asteracantha longifolia* Nees, *Annals of Botany*, 27(4), 647–652.

Parashar, V. V., Singh, H. (1965). Investigation of *Astercantha longifolia* Nees. Indian Journal of pharmacology, 27(4), 109-113.

Parveen, A., & Qaiser M. (2010). Pollen Flora of Pakistan – LXVII: Acanthaceae. Pakistan Journal of Botany, Special Issue (S.I. Ali Festschrift) 42, 175–191.

Patil, A. M., & Patil, D. A. (2011). Investigations on foliar epidermal characteristics in some Acanthaceae. *Current Botany*, 2, 1-8.

Patil, A. M., & Patil, D. A. (2011). Occurrence and significance of cystoliths in Acanthaceae. *Current Botany*, *2*(4).

Paton, A.J., Brummitt, N., Govaerts, R., Harman, K., Hinchliffe, S., Allkin, B., & Lughadha, E.N. (2008). Towards Target 1 of the Global Strategy for Plant Conservation: A working list of all known plant species—progress and prospects. *Taxon*, 57, 602–611.

Patra, A, Jha, S., Murthy, N., Roy, D., & Sahu, A. (2008). Analgesic and antimotility activities of leaves of *Hygrophilia spinosa* T. Anders. *Pharmacology online*, 2(1), 821-828.

Patra, A., Jha, S., Murthy, P. N., Aher, V. D., Chattopadhyay, P., Panigrahi, G. & Roy, D. (2009). Anti-inflammatory and antipyretic activities of *H. spinosa* T. Anders leaves (Acanthaceae). *Tropical Journal of Pharmaceutical Research*, 8, 133-137.

Pattanayak, S., Mandal, T., & Bandyopadhyay, S. (2016). Ethno-gynecological study on the medicinal plants traditionally used in southern districts of West Bengal, India. *Indian journal of traditional knowledge*. 15, 482-486.

Pattanayak, S. P., & Sunita, P. (2008). Antitumor potency and toxicology of an Indian Ayurvedic plant, *Hygrophila spinosa*. *Pharmacologyonline*, 2(1), 361-371

Pawar, R. S., Jain, A. P., Lodhi, S. & Singhai A. K. (2010). Erythropoietic activity of *Asteracantha longifolia* (Nees.) in rats. *Journal of Ethnopharmacology*, 129(2), 280-282.

POWO. (2024). Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. [accessed 2024 Jan15] http://www.plantsoftheworldonline.org

Prentice, H. C., Malm, J. U., & Hathaway, L. (2003). Genetic variation in the rare and endemic plant Scabiosa columbaria var. pratensis (Dipsacaceae). *Biological Conservation*, 113(2), 257-266.

Primmer, C. R. (2009). From conservation genetics to conservation genomics. *Annals of the New York Academy of Sciences*, *1162*(1), 357-368.

Probojati, R. T., Listyorini, D., Sulisetijono, S., & Wahyudi, D. (2021). Phylogeny and estimated genetic divergence times of banana cultivars (*Musa* spp.) from Java Island by maturase K (mat K) genes. *Bulletin of the National Research Centre*, 45, 1-13.

QGIS.ORG (2023). *QGIS Geographic Information System*. Open-Source Geospatial Foundation Project. http://qgis.org

Quasim, C., & Dutta, N.L. (1967). Chemical investigation of *Asteracantha longifolia* Nees. *Journal of the Indian Chemistry Society*, 44 (1), 82.

Radlkofer, L. (1883). Ueber den systematischen Werth der Pollenbeschaffenheit bei den Acanthaceen. Sitzungsber. Math. Phys. K1. Bayer. Akad. Wiss. Miinchen, 13, 256-314.

Raizada, M.B. (1968). Indian Forester, 94, 451.

Raj B. (1973). Further contribution to the pollen morphology of Acanthaceae. *Journal of Palynology*, 9, 91-141.

Raj, B. (1961). Pollen morphological studies in Acanthaceae. *Grana Palynologica*, 3, 3-108

Ramamurthy, K. (1971). A new genus of Acanthaceae from Kerala State, South India. *Nelumbo*, 13(1-2), 153-155.

Ramana, T. V., Rathor, O. S., & Gyananath, G. (2016). “Exploring the anti-diabetic potency & acute toxicity of aqueous leaf extracts of (L.) R. Br Ex (Steud) in experimental mice”, *International Journal of Current Research*, 8, (12), 43450--43455.

Rangaswamy, K. (1941). Cytomorphological studies in *Asteracantha longifolia* (*Hygrophila spinosa*). In *Proceedings of the Indian Academy of Sciences*, 14(B), 149–165.

Rao, V. S. (1953). The floral anatomy of some Bicarpellatae. 1. Acanthaceae. Journal of University of Bombay, 21(5), 1–34.

Rao, R.R. (1997). Diversity of Indian flora. *Proceedings of Indian National Science Academy*, B63, 127–138.

Raven, P. H. (1975). The bases of angiosperm phylogeny: cytology. *Annals of the Missouri Botanical Garden*, 724-764.

Reddy, M. P., Sarla, N., & Siddiq, E. A. (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica*, 128(1), 9-17.

Remadevi, S., & Binoj Kumar M. S. (2009). *Contribution to the Flora of Kerala: The Family Acanthaceae*. Bishen Singh Mahendra Pal Singh, Dehradun, India, pp. 33–39.

Reveal, J. L. (2012). An outline of a classification scheme for extant flowering plants. *Phytoneuron*, 12(37), 1–221

Rheede Tot Drakenstein, H.A. van (1678–1693). *Hortus Indicus Malabaricus*, Vols.1–12, Someren & Dyck, Amsterdam.

Rieseberg, L. H., Carter, R., & Zona, S. (1999). Molecular tests of the hypothesized hybrid origin of two diploid Helianthus species (Asteraceae). *Evolution*, 53(3), 650-660.

Rizzini C.T. (1947). Estudos Sobre as Acanthaceae. *Bol. Mus. Nac. Riode Janeiro, Bot.,* 8, 1-38.

Rodgers, W.A., & Panwar, S.H. (1988). *Biogeographical classification of India*. New Forest, Dehradun.

Rogers, S. O., & Bendich, A. J. (1989). Extraction of DNA from plant tissues. *Plant molecular biology manua*l, 73-83.

Ruchi. P. (2014). In vivo and in vitro studies on phytoceuticals of *Hygrophila species*- PhD thesis. Laboratory of Medicinal Plants Biotechnology Department of Botany, University of Rajasthan.

Russell, J. R., Fuller, J. D., Macaulay, M., Hatz, B. G., Jahoor, A., Powell, W., & Waugh, R. (1997). Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs, and RAPDs. *Theoretical and Applied Genetics*, 95(4), 714-722.

Saggoo, M.I . S., & Bir, S. S. (1986). Meiotic Studies in Certain Members of Family Acanthaceae from South India. *Journal of Indian Botanical Soc*iety, 65, 310–315.

Saha, D., Sarma, T. K., & Mukherjee, S. K. (2016). Some medicinal plants of North 24 parganas district of West Bengal (India). *International Journal of Pharmacy and Biological Sciences*, *6*(3), 191-206.

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G.T., Mullis, K. B., & Erlich, H. A. (1988). Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*, 239, 487–491

Samanta, K., Hossain, E., & Pal, D. K. (2012). Anthelmintic activity of *Hygrophila difformis* Blume. *Journal of Buffalo Science*, *1*(1), 35.

Santapau, H. (1967). The flora of Khandala on the western ghats of India. In: *Records of Botanical Survey of India*, 16 (1), 194.

Santapau, H. (1952). The flowering of *Strobilanthes*. Bombay Natural History Society, 50, 430 -431.

Santapau, H. (1951). *The Acanthaceae of Bombay*. University of Bombay, 18–22

Sarma, D., Debnath, A., Paul, C., & Debnath, B. (2017). *Hygrophila pogonocalyx* (Acanthaceae), new distributional record of a threatened taxa from India: Its ecology and proposal for conservation. *Journal on New Biological Reports,* 6 (1), 1 – 5.

Satpathy, S., Patra A., Hussain, M., & Ahirwar, B. (2018). Amelioration of postmenopausal osteoporosis and anticancer properties of an antioxidant enriched fraction from *Hygrophila spinosa* T. Anders. *South African Journal of Botany*, 117, 247-255.

Sawadogo, W. R., Meda, A., Lamien, C. E., Kiendrebeogo, M., Guissou, I. P., & Nacoulma, O. G. (2006). Phenolic content and antioxidant activity of six Acanthaceae from Burkina Faso. *Journal of Biological Sciiences*, 6, 249-252.

Scotland, R. W. (1992). Systematics, similarity, and Acanthaceae pollen morphology. *Botanical Journal of Linnean Society*, 109, 529–541.

Scotland, R. W. (1993). Pollen morphology of Contortae (Acanthaceae). *Botanical Journal of Linnean Society*, 111, 471–504.

Scotland, R. W., Sweere, J. A., Reeves, P. A., & Olmstead, R. G. (1995). Higher level systematics of Acanthaceae determined by chloroplast DNA sequences. *American Journal of Botany*, 82, 266–275.

Scotland, R. W. & Vollesen, K. (2000). Classification of Acanthaceae. *Kew Bulletin*, 55(3), 513–589.

Scotland, R. W., Sweere, J. A., Reeves, P. A., & Olmstead, R. G. (1995). Higher-level systematics of Acanthaceae determined by chloroplast DNA sequences. *American Journal of Botany*, 82(2), 266-275.

Selkoe, K. A., & Toonen, R. J. (2006). Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology Letters*, 9(5), 615-629.

Selvaraj, R., & Subramanian, D. (1983). Epidermal studies in some species of Acanthaceae. *Journal of Indian Botanical Society*, 62, 253-258.

Semagn, K., Bjørnstad, Å., & Ndjiondjop, M. N. (2006). An overview of molecular marker methods for plants. *African Journal of Biotechnology*, 5(25).

Shailajan, S., Chandra, N., Sane, R. T., Menon, S. (2005). Effect of *Asteracantha longifolia* Nees against CCl4 induced liver dysfunction in rat. *Indian Journal of Experimental Biol*ogy, 43(1), 68-75.

Shamso, E. M. (2013). A Palynological Study of Acanthaceae in Egypt and its Systematic Implication. *Egyptian Journal of Botany*, 53, 257-272.

Shanmugasundaram, P. & Venkataraman, S. (2005). Antinociceptive activity of *H. auriculata* (Schum) Heine. *African Journal of Traditional, Complementary and Alternative Medicines*, 2, 62-69.

Sharan, S., Singh, A. K., & Sharma, S. S. (2016). Studies in *Astercantha longifolia* L. Syn. *Hygrophila spinosa* T. Anders. *Int. J. Mendel*, 33(1-2), 33-37.

Shendage, S. M., & Yadav, S. R. (2010). Revision of the genus *Barleria* (Acanthaceae) in India. *Rheedea*, 20(2), 81-130.

Sidwell, K. (1999). The Taxonomic Position of the Sri Lankan Species *Brillantaisia thwaitesii* (T. Anderson) L. H. *Kew Bulletin*, 54(1), 215–219.

Sidwell, K. (1998). A revision of *Brillantaisia* (Acanthaceae). *Bulletin of the Natural History Museum*, London, Botany series, 28, 67–113.

Simpson, M. G. (2019). *Plant systematics*. Academic press.

Singh, V., & Jain, D. K. (1975). Trichomes in Acanthaceae. 1. General structure. *Journal of Indian Botanical Society*, 54, 116-127.

Singh, A., & Handa, S.S. (1995). Hepatoprotective activity of Apium graveolens and *Hygrophila auriculata* against paracetamol and thioacetamide intoxication in rats. *Journal of Ethnopharmacology*, 49(3), 119-126.

Sreemadhavan, C. P., Henry, A. N., &Subramanyam K. (1968). Descriptive terminology for cystolith and raphid–bearing plant organs. *Taxon*, 17, 17– 18.

Sreemadhavan, C. P. (1977). Diagnosis of some new taxa and some new combinations in Bignoniales. *Phytologia* 37, 413–416.

Stafleu, F. & Cowan, R.S. (1976-1988). Taxonomic literature: A selective guide to botanical publications and collections with dates, commentaries and types. Scheltema & Holkema Publishers, Hague, pp.991.

Stearn, W.T. (1983). *Botanical Latin-History*, Grammar, Syntax, Terminology and Vocabulary. David & Charles, London.

Steele, K. P., & Vilgalys, R. (1994). Phylogenetic analyses of Polemoniaceae using nucleotide sequences of the plastid gene matK. *Systematic Botany*, 126-142.

Stevens, P.F. (2001). *Angiosperm Phylogeny Website*. Version 14 July 2017 [and more or less continuously updated since]. http://www.mobot.org/MOBOT/research/APweb/ (accessed Jan 2023).

Sufian, M. A., Begum, F., Haque, M., Hasan, C., & Rashid, M. (2015). Sterol and triterpenoids from *Hygrophila schulli* Buch.-Ham. *Bangladesh Journal of Botany*. 44, 319-321.

Sugiura, T. (1940). Studies on the chromosome numbers in higher plants. IV. *Cytologia*, 10(3), 324-333.

Sunil Kumar, K. C., & Klausmuller. (1999). Medicinal plants from Nepal; II. Evalution of inhibitors of lipid preoxidation in biological membranes. *Journal of Ethanopharmacology*, 64(2), 135-139.

Sunita, S. & Abhishek, S. (2008). A comparative evaluation of phytochemical fingerprints of *Asteracantha longifolia* Nees using HPTLC. *Asian Journal of Plant Sciences*, 7, 611-4.

Sunojkumar, P., & Prasad, M. G. (2014). Taxonomic reinstatement of an endemic *Hygrophila* (Acanthaceae) subsequent to its rediscovery after 180 years from India. *Rheedea*, *24*(1), 12-15.

Takhtajan, A. L. (1997). *Diversity and Classification of Flowering Plants*. Columbia University Press, New York.

Thiers B. (2024, updated continuously). *Index Herbariorum*. A global direction of public herbaria and associated staff (online), Available at: http://sweetgum.nybg.org/science/ih

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic acids research*, 22(22), 4673-4680.

Thorne, R.F. (1992). An updated phylogenetic classification of the flowering plants. Aliso: *A Journal of Systematic and Evolutionary Botany*,13, 365–389.

Thorne, R. F., & Reveal, J. L. (2007). An updated classification of the class Magnoliopsida ("Angiospermae"). *The Botanical Review*, 73(2), 67-181.

Tiwari, A. P., Sikarwar, R. L. S., & Dubey, P. C. (2014). Documentation of ethnomedicinal knowledge among the tribes of Achanakmar-Amarkantak biosphere reserve, central India. *Indian Journal of natural products and resources*, 5(4), 345-350.

Trimen, H. (1895). *A Handbook to the Flora of Ceylon*, London. Dulau & Co. 4, 292–296.

Tripp, E. A., Darbyshire, I., Daniel, T. F., Kiel, C. A., & McDade, L. A. (2022). Revised classification of Acanthaceae and worldwide dichotomous keys. *Taxon*. 71 (1), 103–153.

Tripp, E. A., Daniel, T. F., Lendemer, J. C., & McDade, L. A. (2009). New molecular and morphological insights prompt transfer of *Blechum* to *Ruellia* (Acanthaceae). *Taxon*, 58, 893–906.

Tripp, E. A. (2010). Taxonomic revision of *Ruellia* section Chiropterophila (Acanthaceae): a lineage of rare and endemic species from Mexico. *Systematic Botany*, 35(3), 629-661.

Tripp, E. A. (2007). Evolutionary relationships within the species rich genus *Ruellia* (Acanthaceae). *Systematic Botany*, 32, 628–649

Tripp, E. A., & Darbyshire, I. (2017). Phylogenetic relationships among Old World *Ruellia* L.: a new classification and reinstatement of the genus *Dipteracanthus* Schinz. *Systematic Botany*, 42(3), 470-483.

Tripp, E. A., & McDade, L. A. (2014). A rich fossil record yields calibrated phylogeny for Acanthaceae (Lamiales) and evidence for marked biases in timing and directionality of intercontinental disjunctions. *Systematic Biology*, 63(5), 660-684.

Tripp, E. A., Daniel, T. F., Fatimah, S., & McDade, L. A. (2013). Phylogenetic relationships within Ruellieae (Acanthaceae) and a revised classification. *International Journal of Plant Sciences*, 174(1), 97-137.

Turland, N. J., Wiersema, J. H., Barrie, F. R., Greuter, W., Hawksworth, D. L., Herendeen, P. S., ... & Smith, G. (2018). *International Code of Nomenclature for algae, fungi, and plants* (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Koeltz botanical books.

Udvardy, M.D.F. (1975) A classification of the biogeographical provinces of the world. IUCN Occasional Paper 18. *International Union of Conservation of Nature and Natural Resources*, Morges, Switzerland.

Vajravelu, E. & Vivekananthan, K. (1996). Southern Western Ghats-south of Goa. In: Hajra, P.K. et al. (eds.), *Flora of India*- Introductory Volume (Part 1). Botanical Survey of India, Kolkata.

Valientic, A. J., Vanhoof, L., Totte, J., Lasure, A., Vanden Berghe, D., Rwangaboo, P. C., & Mvukiyuwami, J. (1995). Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. *Journal of Ethanopharmacology*, 46(1), 31-47.

Venu, P., Murthy, V.S. & Sanjappa, M. (1996). Geology. In: Hajra, P.K. et al. (eds.), *Flora of India* Introductory Volume (Part 1). Botanical Survey of India, Kolkata.

Verma, A. K., Mishra, M., Singh, H., & Bharati, K. A. (2018). Database on chromosome count of some Indian plants. *Chromosome Botany*, *13*(1), 37-60.

Vijayakumar, M., Govindarajan, R., Rao, G. M., Rao, C. V., Shirwaikar, A., Mehrotra, S., *Pushpangadan, P.* (2006). Action of *Hygrophila auriculata* against streptozotocin-induced oxidative stress. *Journal of Ethanopharmacology*, 104(3), 356-61.

Vollesen, K. (2006). A taxonomic revision of the genus *Duosperma* (Acanthaceae). *Kew Bulletin*, 61, 289–351.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., ... & Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, 23(21), 4407-4414.

Wallich, N. (1828–1849). *A numerical list of dried specimens of plants, in the East India Companys Museum collected under the superintendence of Dr. Wallich of the Company's Botanic Garden at Calcutta*. London.

Wang, H. & S. Blackmore. (2003). Pollen morphology of *Strobilanthes* Blume (Acanthaceae) in China and its taxonomic implications, *Grana*, 42, 82-87.

Wasshausen, D. C. & Wood, J. R. I. (2004). *Acanthaceae of Bolivia*. Contributions from the United States National Herbarium 49, 1–152.

Wight, R. (1850). *Icones Plantarum Indiae Orientalis or Figures of Indian Plants*. Vol. 4. Messrs Franck & Co., Madras. p. 18.

Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A., & Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic acids research*, 18(22), 6531-6535.

Willis, J.C. (1980). *A Dictionary of Flowering Plants and Ferns*, 8th ed. Cambridge Univ. Press, Cambridge,

Wolfe, K H., Li, W.H., & Sharp, P M. (1987). Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. *Proceedings of the National Academy of Sciences*, 84(24), 9054-9058.

Wood, J. R. I. (1994). Notes relating to the flora of Bhutan: XXIX Acanthaceae, with special reference to Strobilanthes. *Edinburgh Journal of Botany,* 51 (2), 175–273.

Zahra, N.B., Shinwari, Z.K., & Qaiser, M. (2016). DNA barcoding: a tool for standardization of herbal medicinal products (HMPS) of Lamiaceae from Pakistan, *Pakistan Journal of Botany*, 48(5), 2167-2174.

Zakaria, S.M., Amri, C.N., Talip, N., Latiff, A., Juhari, A.A., Shahari, R., Tajudin, N.S., & Rahman, M.Y. (2020). The Variation of Cystoliths and Its Taxonomic Significance in Acanthaceae of Peninsular Malaysia. *Malaysian Applied Biology*, 49 (5), 25-31.

I. List of herbaria consulted with their details

1.	Agarkar Research Institute, Pune (AHMA).
2.	Andaman and Nicobar Regional Centre, Port Blair (PBL).
3.	Arunachal Pradesh Regional Centre, Itanagar (ARUN).
4.	Blatter herbarium, Saint Xavier's College, Mumbai (BLAT).
5.	Botanical Survey of India, Southern Regional Centre, Coimbatore (MH).
6.	Botanical Survey of India, Western Circle, Pune, Maharashtra, India (BSI)
7.	Calicut University, Calicut, Kerala (CALI).
8.	Central National Herbarium, Kolkata, West Bengal (CAL).
9.	Eastern Regional Centre, Shillong (ASSAM).
10.	Foundation for Revitalisation of Local Health Traditions, Scientific and Research Organisation, Bangalore (FRLH).
11.	Jawaharlal Nehru Tropical Botanical Garden and Research Institute, Thiruvananthapuram, Kerala (TBGT).
12.	Kerala Forest Research Institute, Thrissur, Kerala (KFRI).
13.	Shivaji University, Kolhapur, Maharashtra (SUK).
14.	St. Joseph's College Herbarium, Devagiri, Kozhikode (DEV)
15.	St. Joseph's College Herbarium, Bengaluru (JCB)
16.	The Rapinat Herbarium, St. Joseph's college, Tiruchirappalli, Tamil Nadu (RHT).

The virtual databases of European herbaria

1.	Botanic Garden and Botanical Museum Berlin-Dahlem, Germany (B)
2.	Calicut University Herbarium, Department of Botany, University of Calicut, Kerala, India (CALI).
3.	Conservatory and Botanical Garden of the City of Geneva, Geneva Herbarium, Switzerland. Genève (G).
4.	Linnean herbarium, Department of Phanerogamic Botany Swedish Museum of Natural History (LINN)
5.	Meise Botanic Garden, Belgium. Meise (BR).
6.	National Museum of Natural History, Smithsonian Institution, Washington, USA (US).
7.	Natural History Museum, Vienna (W).
8.	Naturalis Biodiversity Center, Leiden University, Netherlands (L).
9.	Naturalis Biodiversity Center, The Netherlands. Leiden (U).
10.	Royal Botanic Garden Edinburgh, U.K. Scotland. Edinburgh (E).
11.	Royal Botanic Gardens, U.K. England. Kew (K).
12.	Staatliche Naturwissenschaftliche Sammlungen Bayerns, Munich, Germany (M).
13.	The Herbarium of the Muséum national d'Histoire Naturelle (P)
14.	The Natural History Museum, U.K. England. London (BM).
15.	The University Museum, University of Tokyo (TI)
16.	University of Copenhagen Denmark. Copenhagen (C).
17.	University of Graz, Austria (GZU)

II. Model Datasheet of *Hygrophila*

<u>*Hygrophila* R.Br</u><u>. (Acanthaceae)</u>

Coll. No: .... Date: ....

Locality: .... Collector: ....

Species Name: ....

Habitat: ....

Habit: ....

Biotic association: ....

<u>STEM</u>

Type:. ....

Nature:...

Plant height: ....

Shape: ....

Branching: ....

Surface: ....

Colour: ....

Hair: Present/Absent, If present nature of hair: ....

Any other characters: ....

<u>LEAF</u>

Dimorphic / Monomorphic ....

Phyllotaxy: ....

<u>LAMINA</u>

Nature: Simple/Compound ....

Leaf attachment: Sessile/Petiolate ....

If petiolate, petiole colour:................ surface: .... size ....

Shape:...Size: ....

Margin:................................Apex: .... Base: ....

Surface: Adaxial Abaxial:..

Venation: ....…… Texture: ....

Hair: Present/Absent, If present nature of hair: ....

Any other characters: ....

<u>INFLORESCENCE</u>

Type: ....

Position: Axillary/Terminal/Both .... No. Of flower: ....

Peduncle: Present / Absent..

Any other characters: ....

<u>FLOWER</u>

Floral symmetry: ....

<u>Bract</u>: Ebracteate / bracteate ....

If bracteate:No. of bracts :................ Shape: .... Size: ....

Margin: .... Apex: .... Base: ....

Surface: Abaxial .... Adaxial ....

Colour ....

<u>Bracteole</u>: Ebracteolate/bracteolate

If bracteolate: No. of bracteols: ....Shape:................. Size: ....

Margin: .... Apex: .... Base: ....

Surface: Abaxial .... Adaxial ....

Colour ....

Attachment of flower: Sessile/ Subsessile / Pedicellate

If Pedicellate: Pedicel Size: .... Surface .... Colour ....

Any other characters: ....

CALYX

No. of sepals:.................. Cohesion: Polysepalous / Gamosepalous............................

Size: .... Shape: ....

Margin: .... Apex: ....

Surface: Adaxial .... Abaxial ....

Colour: .... Hair: Present/Absent, If present nature of hair: ....

Aestivation: .... Form: ....

Any other characters: ....

COROLLA

No. of petals: .... Cohesion: Polypetalous / Gamopetalous

Form: .... If bilipped, upper .... lower ....

Size: .... Shape: ....

Margin: .... Apex: ....

Surface: Adaxial .... Abaxial ....

Colour: .... Hair: Present/Absent, If present nature of hair: ....

Aestivation: ....

Any other characters: ....

ANDROECIUM

No. of Stamen: ....

Filament: Length: .... Adhesion ....

Surface: .... Colour: ....

Anther: Length .... Cohesion ....

Anther lobe: Shape: .... Colour: ....

Surface: .... Dehiscence: ....

GYNOECIUM

Ovary position: .... Carpel: ....

Placentation: .... Colour: ....

Size: .... Shape: ....

Hair: Present/Absent, If present nature of hair: ....

Style: Length.................. Surface: .... Colour: ....

Hair: Present/Absent, If present nature of hair: ....

Stigma: Entire/Lobed(bilobed/trilobed)/Bifid/Trifid/Any other ....

Length: .... Surface: .... Colour: ....

Any other characters: ....

FRUIT

Type: .... Colour: ....

Shape: .... Size: ....

Hair: Present / Absent ....Texture: ....

Any other characters: ....

SEED

No. of seeds: ....

Shape: .... Surface: .... Colour: ....

Hair: Present/Absent ....

Any other characters: ....

SpecialNotes:...
....
....
....

Additional Specimens Examined:...

....
....
....