TAXONOMIC AND PALYNOLOGICAL STUDIES ON MEMBERS OF GESNERIACEAE FAMILY OF MIZORAM, INDIA

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Submitted

In partial fulfillment of the requirement of the Degree of Doctor of Philosophy in Botany of Mizoram University, Aizawl.

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CERTIFICATE

This is to certify that Ms. **Margaret Lalhlupuii** has submitted the thesis entitled "**Taxonomic and Palynological studies on members of Gesneriaceae family of Mizoram, India**" under my supervision, for the requirement of the award of the Degree of Doctor of Philosophy in the Department of Botany, Mizoram University, Aizawl. The work is authentic, content of the thesis is the original work of the Research Scholar, and the nature and the presentation of the work are the first of its kind in Mizoram.

It is further certified that no portion(s) or part(s) of the content of the thesis has been submitted for any degree in Mizoram University or any other University or Institute.

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DECLARATION

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DECEMBER, 2024

I **MARGARET LALHLUPUII**, hereby declare that the subject matter of this thesis is the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to do the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/ Institute.

This is being submitted to the Mizoram University for the **Degree of Doctor of Philosophy in Botany.**

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Date: (MARGARET LALHLUPUII)

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
&	And
°	Degree
′	Minute
<	Less than
>	Greater than
=	Equal to
APG	Angiosperm Phylogeny Group
Ma	Mega annum
et al.	“et alia”: and others
RET	Rare, Endangered, Threatened
IUCN	International Union for Conservation of Nature
NCBI	National Center for Biotechnology Information
BSI	Botanical Survey of India
WWF	World Wildlife Fund
N	North
E	East
sq	Square
km	Kilometer
FSI	Forest Survey of India
m	Meter
C	Celsius
mm	millimeter
DSLR	Digital Single Lens Reflex
GPS	Global Positioning System
POWO	Plants of the World Online
WFO	World Flora Online
ASSAM	BSI, Shillong
CAL	BSI, Kolkata
K	Kew

E	Edinburgh
MZUH	Mizoram University Herbarium
µm	Micrometer
SEM	Scanning Electron Microscope
NEHU	North-Eastern Hill University
DNA	Deoxyribonucleic acid
CTAB	Cetyltrimethylammonium bromide
PCR	Polymerase Chain Reaction
ITS2	Internal transcribed spacer 2
rbcL	Ribulose 1,5-bisphosphate carboxylase large chain
MEGA X	Molecular Evolutionary Genetics Analysis, X
BIC	Bayesian Information Criterion
K2	Kimura-2-Parameter
IPNI	International Plant Names Index
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
PCA	Principal Component Analysis
P/E	Polar/Equatorial view
BLAST	Basic Local Alignment Search Tool
LC	Least Concern
NT	Not Threatened
EN	Endangered
NE	Not Evaluated
CR	Critically Endangered
VU	Vulnerable
UV	Use Value
IVs	Importance Value
RFC	Relative Frequency Citation
NA	Not Available
O	Ornamental
M	Medicine
F	Food
FD	Fodder

U	Unknown
EH	Epiphytic herb
SS	Subshrub
PH	Perennial herb
LH	Lithophytic herb

Chapter 1

1. Introduction

1.1 Taxonomy of Gesneriaceae

The family Gesneriaceae Rich. & Juss. was first recognized by Richard and Jussieu in 1804, although they did not formally named at that time. Later, in 1816, A.P. de Candolle formally named the family as "Gesnerieae". Known as the African violet family, named after the attractive African violet plants, the family Gesneriaceae is based on the genus *Gesneria*, which honors Swiss scholar Conrad Gessner (Weber & Skog, 2007). The first detailed study of the Gesneriaceae family in India was conducted by Clarke in 1874, presented in his book Commelynaceae et Cyrtandraceae Bengalenses. This was followed by his second major work in 1883, Monographiae Phanerogamarum Prodromi. Later, on 1954 Burtt significantly revised and updated the information on this family through a series of studies. Bentham & Hooker (1876) placed Gesneriaceae in the cohort Personales, while Cronquist (1988), Dahlgren (1989), and Takhtajan (1997) classified it in the order Scrophulariales, under the subclass Asteridae and class Magnoliopsida. In the APG IV (2016) classification, the family was placed in the order Lamiales, within the Asterid clade of the Superasterid group. In 1954, Burtt began a pivotal series titled "Studies in the Gesneriaceae of the Old World" published in Notes from the Royal Botanic Garden, Edinburgh. By 1963, Burtt had recognized two subfamilies based on cotyledon characteristics: (i) the Anisocotylous Cyrtandroideae, mainly from the Old World, and (ii) the isocotylous Gesnerioideae, predominantly from the New World. He further divided Cyrtandroideae into five tribes: Cyrtandreae, Trichosporeae, Klugieae, Loxonieae, and Didymocarpeae. This classification was based on characteristics such as fruit structure, mode of dehiscence, the nature of the fruit wall, seed appendages, inflorescence traits, ovary shape, and the habitat of the plants. Most members of Cyrtandroideae are distributed in the Old World, except for *Rhynchoglossum* and *Epithema*, which also have New World distributions. The plants develop unequal-sized cotyledons shortly after germination, with one cotyledon enlarging while the other withers, and they possess a superior ovary.

Gesneriaceae is a medium-sized family, predominantly consisting of perennial herbs, though it also includes shrubs, small trees, annuals, and climbers. They can grow in a variety of ways as geophytes, epiphytes, and lithophytes. Approximately 20% of its species are epiphytic, placing Gesneriaceae among the top ten plant families in terms of the number of epiphytic species (Madison, 1977; Kress, 1986). Typically, small and perennial, these plants have showy flowers and are characterized by opposite decussate leaves (sometimes anisophyllous or alternate), pair-flowered cymes, and pentamerous, zygomorphic bisexual flowers. The flowers usually have four or two stamens, an annular or one-sided disk, and a 1-locular ovary with parietal placentation. Although related to other families in Lamiales, such as Scrophulariaceae, Acanthaceae, and Bignoniaceae, Gesneriaceae can be distinguished by features like five-lobed corollas, parietal placentation, and the presence of endosperm in the seeds of most taxa (Cronquist, 1981). The fruits are either fleshy (e.g., *Alloplectus*, *Drymonia* and *Nematanthus*), likely dispersed by animals, or dry and dehiscent capsules (e.g., *Aeschynanthus* and *Lysionotus*), with seeds spread by gravity, wind or rain (Weber, 2017).

1.2 Habitat of Gesneriaceae

The family occupies a wide range of habitats, leading to significant morphological diversity and adaptive traits (Wiehler, 1983; Möller & Cronk, 2001; Zimmer et al., 2002; Roalson et al., 2003). The species are found from sea level up to nearly 5,000 meters, as in *Corallodiscus kingianus* (Weber, 2004a). The members of the family display an outstanding morphological diversity related to the colonization of a wide range of habitats and the evolution of specialized plant–animal interactions (Wiehler, 1983; Weber & Skog, 2007). Gesneriaceae are particularly abundant at mid-elevation in the mountain forests preferably in undisturbed habitats where they grow as epiphytes or on rock outcrops. Some of them have high ornamental value due to their perennial nature with attractive flowers. Identifying specific ecophysiological factors for the family is challenging, though most Gesneriaceae species require high humidity. They are often found near streams, waterfalls, or in shaded, humid areas such as rock crevices and shallow caves. One of their most notable features is the ability to survive periods of desiccation, where the leaves shrivel during dry spells but recover when moisture returns, earning them the name "resurrection plants".

1.3 Origin, phylogeny and evolution

The Gesneriaceae family is believed to have originated around the Cretaceous-Palaeogene boundary, approximately 67.23 million years ago (Ranasinghe et al., 2024). According to Burtt (1998), the Gesneriaceae family had its origins in Gondwana and then moved to Australia, South America and Asia. Woo et al. (2011) however, disproved this Gondwanan origin, arguing that several long-distance dispersal events throughout the Miocene provide a more plausible explanation for the present distribution of some of the tribe of the family throughout South America and the Southwest Pacific. Based on molecular dating and biogeographical analyses, Perret et al. (2013) found that the ancestors of Gesneriads were likely originated in South America during the Late Cretaceous. The presence of Gesneriaceae in the Palaeotropics and Australasia is thought to result from two independent long-distance dispersal events during the Eocene and Oligocene. Around 34 million years ago, the ancestors of Gesnerioideae rapidly colonized various Neotropical regions, including the tropical Andes, Brazilian Atlantic forests, Cerrado, Central America, and the West Indies (Perret et al., 2013). The diversification of Gesneriads, since their origin approximately 70 million years ago, was strongly influenced by their adaptation to diverse habitats and the development of specialized plant-animal interactions for pollination and seed dispersal (Roalson & Roberts, 2016; Serrano-Serrano et al., 2017; Ogutcen et al., 2021). The diversification patterns of the two main clades in the Gesneriaceae family have been found to differ significantly, reflecting distinct evolutionary trajectories and ecological adaptations. The contemporary members of the primarily New World Gesnerioideae did not start to diversify until the late Eocene (39.34 Ma), while the primarily Old World Didymocarpoideae did so considerably earlier in the Early Eocene (56.29 Ma). The wide variation in habit and floral morphology within Gesneriaceae, along with high levels of trait convergence, initially caused significant confusion in understanding the diversity in the group (Jong & Burtt, 1975). Subsequent diversification primarily occurred *in situ*, particularly in the mountainous regions of the Andes, Central America, and the Brazilian Atlantic rainforest.

1.4 Species diversity and distribution

The Gesneriaceae is one of the 23 families of the order Lamiales (Weber, 2004a), which has an equal distribution in the Neotropics and Paleotropics, with some outliers in temperate South America and Europe (Weber, 2004a). In Asia-Malesia and the Americas, the family comprises around 60 genera, while Africa has 9 genera (approximately 160 species), and Europe has 3 genera (6 species). Nine genera (20 species) are primarily distributed in the southern hemisphere and India is home to 28 genera, including 156 species, 1 subspecies, and 5 varieties and a total of 28 species belonging to 12 genera have been reported in Mizoram (Janeesha, 2017; Sinha, 2012). Globally, the family comprises 3,525 species across 147–151 genera (Skog & Boggan, 2007; Möller & Clark, 2013; Weber et al., 2013; Lakshminarasimhan et al., 2023). India is home to native Gesneriaceae members in 3 out of 4 subtribes of tribe Epithemateae and 6 out of 10 subtribes in tribe Trichosporeae, i.e., 9 out of 14 in subfamily Didymocarpoideae which highlights the high level of diversity in India (Moller et al., 2017).Many species of Gesneriaceae are cultivated as ornamental plants, and a few have ethnobotanical significance.

1.5 Palynology

The family Gesneriaceae are comparatively less frequent in the lowlands where they predominantly occur on slopes, riverbanks and moist rocks in rainforest. Flowers of Gesneriaceae are highly diversified, reflecting adaptation to a wide range of pollinators including birds, bats and several types of bees (Sanmartin-Gajardo &Vianna, 2010). Pollen morphology has often provided characters useful for reliable taxonomic subdivisions (Schlag-Edler & Kiehn, 2001). Information about pollen morphology of the Gesneriaceae is still scarce and its bearing on the infrafamilial classification is little known (Fritze & Williams, 1988). The extensive morphological diversification coupled with high levels of convergence complicated the assessment of relationships in Gesneriaceae and caused considerable confusion in the early taxonomy of this family (Clark et al., 2012).

1.6 Seedcoat Morphology

Seeds provide important taxonomic information because of their complex and great morphological and micromorphological variety. Their general dimensions, colour, and shape, as well as their internal structure, which includes ultrastructural features, can be of high systemic importance (Skvortsov & Rusanovitch, 1974). Seed surface analysis has been used to classify several taxa and determine their evolutionary relationships (Bhattacharya & Saha, 1990, 1992). Features of the seed surface appear to represent genetic and phylogenetic differences in the plants and are not significantly impacted by environmental factors.

1.7 Molecular Study

Molecular studies suggest that Gesneriaceae is monophyletic, with the family occupying a basal position in Lamiales, and it is a sister group to most other families within this order. Origins of the molecular phylogenetics can be traced back to Linnaeus's 18th-century comprehensive use of the traditional approach of categorizing species based on their similarities and differences. In the family Gesneriaceae, the first use of molecular data in phylogenetic reconstruction was exploratory and did not lead to classification modifications (Möller & Cronk, 1997, 2001; Smith & Carroll, 1997; Smith et al., 1997). Because of the use of molecular data and phylogenetic analysis, there is a tremendous progress in understanding connections within the family. This has resulted in significant modifications to the circumscription of infrafamilial taxa and the delimitation of genera.

1.8 Endemic and RET

An endemic species is a species of plant or animal that is native to a specific geographic area, such as a country, state, or island. Endemic species are often isolated in some way, making it difficult for them to spread to other areas. They may also be adapted to the unique conditions of their habitat, making it difficult for them to survive in other environments. These species have adapted to specific local conditions, such as climate, topography, or habitat type, over long periods of time. Endemic species are vulnerable to environmental changes, habitat loss, pollution and human activities. Many endemic species are found in areas known as biodiversity hotspots, which are areas that have lost at least 70% of their original natural vegetation and are home to more than 1,500 species of endemic vascular plants.

1.8.1 RET (Rare, Endangered, Threatened) Species

RET species are categorized into three primary categories based on their risk of extinction, with the classifications determined by organizations such as the International Union for Conservation of Nature (IUCN, 1997) as follows:

1. **Rare Species:** Rare Species is a species that is uncommon, scarce, or found in isolated geographical locations. Rare species are generally considered threatened because their small populations are less likely to recover from ecological disasters.

2. **Endangered Species:** This category includes species facing a high risk of extinction in the wild. The IUCN defines it as a population of organisms that is either insufficient in number or at risk of going extinct. Factors such as habitat loss, pollution, climate change, and over-exploitation contribute to their perilous status.

3. **Threatened Species:** Threatened species, designates a species that is probably going to become endangered in the near future (Nayar & Sastry, 1990). This term encompasses both rare and endangered species, serving as a broader classification for species at risk. Threatened species face substantial decline in numbers and may be pushed towards endangerment or extinction if conservation

actions are not implemented promptly. The identification and assessment of the conservation status of RET species are critical for directing conservation efforts effectively. The IUCN Red List of Threatened Species is a widely recognized global database that provides information on the extinction risk status of various species.

1.9 Ethnobotany

In 1896, J.W. Harshberger coined the term ethnobotany, which reflects the intricate interactions between humans and their surrounding plant environments, encompassing the traditional knowledge, cultural practices and uses of plants in various societies (Trivedi & Sharma, 2011). This information includes how local people use, classify, and care for plants in their environment (Martin, 1995). By investigating and conserving traditional knowledge, the field of "ethnobotany" has grown to be vital, particularly in recent years (Kunwar & Bussmann, 2008). It has made a substantial contribution to healthcare development and conservation initiatives. Understanding indigenous knowledge of plants, their cultural relationships, and how plant resources are incorporated into regional customs and religions forms the basis of these research (Balick & Cox, 1997). The World Health Organization's Global Centre for Traditional Medicine (2023) estimates that over 88% of people worldwide, especially those living in rural regions, rely on herbal medicine for their basic medical needs. Remarkably, a number of successful drug screening studies have been introduced using ethnobotanical expertise based on ethnomedicinal plants (Wyk et al., 1997).

1.10 Relevance of the present studies

In recent years, due to various factors like habitat destruction, climate change, introduction of invasive species, overexploitation of biological resources and many anthropogenic factors lead to vulnerability of biodiversity in many parts of the world. The state Mizoram being a part of Indo-Burma hotspot region is also facing the same problem leading to threatening of many plants from extinction. Due to the physiographical condition of the state, many parts of the states remain inaccessible and unexplored from taxonomic point of view. The selected family Gesneriaceae has large representation from North Eastern States of India including Mizoram. Although some detailed taxonomic work has been done on Gesneriaceae of Indian part of Eastern Himalayas and from other North Eastern States of India, most of the studies or reports in context of Mizoram region was based on earlier collections which dates back 80-90 years ago (Sinha et al., 2012). These historical studies lack updated information on the current distribution, diversity and characteristics of the family.

To address this gap, the present study focuses on the comprehensive exploration of Gesneriaceae members in Mizoram. The research involves detailed examinations of their taxonomic characters, geographic distribution, micromorphological characters based on pollen. The addition of seed micromorphology along with molecular phylogenetic studies has significantly gain a more comprehensive understanding of plant families. Overall, these studies aims to provide a clearer understanding for the identification of the members of the family and their evolutionary relationship, contributing to broader knowledge of the family and supporting conservation efforts in the region.

1.11 Objectives of the present studies

The study includes the following objectives:

1. Survey, collection and identification of members of Gesneriaceae from Mizoram.
2. Palynological studies on all the plants collected under Gesneriaceae from the state.
3. Preparation of an identificationkey based on morphological and palynological data.
4. Biogeographical distribution data of the family members in Mizoram.
5. To document the ethnobotanical uses of Gesneriaceae members in Mizoram.

Chapter 2

2. Review of Literature

2.1 Brief History

Linnaeus (1753) in his *Species Plantarum* treated the species of the Gesneriaceae under *Diandria Monogynia* wherein he recognized *Henckelia barbata, Henckelia aromatica, Henckelia urticaefolia, Henckelia pumila, Boe amagellanica* and others in his publication (Roy, 2017). In 1804, the French botanists Richard and Jussieu were the first to think of the family Gesneriaceae. According to their findings, *Gesneria* L., *Besleria* L., *Columnea* L., *Achimenes* Pers., *Gloxinia* L'Her., and *Jussieu's* P. Brown are unique and belong to a new family because of their unilocular fruit, fleshy disk at the ovary's base, and stamen insertion on the corolla. Richard andJussieu, however, made no mention of naming the new family. A.P. de Candolle (1816) formally recognized the family as Gesnerieae twelve years later, which solely includes Neotropical species. This was confirmed by citing Jussieu's study, although A.P. de Candolle did not provide an explanation.

Martius (1829) was the first to discuss the link between the Old World Cyrtandraceae and the New World Gesneriaceae. Despite their strong relationship, he chose to keep them separate. Subsequently, Don (1838) and Brown (1839) formally combined these two groups into a single family under Gesneriaceae, concluding that the differences were insufficient to justify separate family classifications.

Bentham (1876) was the first to provide a comprehensive account of Gesneriaceae in a broad sense, using the unified concept of Gesneriaceae from Brown and Don. Clarke (1883) later made revisions to the paleotropical Cyrtandraceae. The entire family was revised by Fritsch (1893–1894), who also offered a thorough intrafamilial categorization. He recognized a number of tribes under the Cyrtandroideae and Gesnerioideae sub-families, which he split into two groups according to the position of ovary. Anisocotylous seedlings were a noticeable and unusual trait that Paleotropical Gesneriaceae shared (Burtt, 1963). The family

Gesneriaceae was expanded by Ivania (1966, 1967) and Wang etal. (1998). The tribes Klugiae and Loxonieae were combined by Weber (1975, 1976a, 1976b, 1978) to form the tribe Epithemateae (Burtt, 1997). The Paleotropical sub-family Cyrtandroideae did not include certain genera from Australia and the South Pacific. Together with the temperate South American tribes, these genera were assigned to the Coronanthereae tribe. Based on the characteristics of the nectary, Wiehler (1983) combined the Coronanthereae and Mitrarieae into a new subfamily called Coronantheroideae.

2.2 Classification

The classification of Gesneriaceae family was started with Don (1838), dividing the "order Gesneriaceae" into two tribes Gesnerieae and Cyrtandriae (published as Cyrtandraceae) and seven subtribes, according to the geographic range of the species. The tribe Gesnerieae all occurring in New World was divided into three sub-tribes: Gloxinieae, Conradieae and Beslerieae. The tribe Cyrtandriae, all occurring in Old World was divided into four sub-tribe: Trichosporeae, Didymocarpeae, Cyrtandreae, Loxotieae. A year later, in 1839, Endlicher put up a classification that was comparable but less thorough. The family was split up into sub-orders by him. The family received its current form when George Don (1838) and Robert Brown (1839) formally united the Old World Cyrtandraceae/Didymocarpaceae with the New World Gesneriaceae after concluding that the differences between these two were insufficient to justify familial separation (Weber, 2004b).

Brown (1839) divided the family into three tribes: Beslerieae, Gesneriae and Cyrtandreae which primarily comprised Old World genera. A.P. de Candolle (1839) divided the Gesneriaceae into two tribes at the same time, naming them Gesneriae (New World) having ovary fully or partly inferior and Besleriae (Old World) having superior ovary. Didymocarpae and Cyrtandreae are the two tribes into which A.P. de Candolle (1845) split Cyrtandraceae six years later. Bentham provided a more thorough analysis of the family in 1876. He prioritized morphological traits over phytogeographical relationships. Bentham (1876) classified the Gesneriaceae family into two tribes: Cyrtandreae with five sub-tribe: Columneae, Eucyrtandreae, Aeschynantheae, Beslerieae and Didymocarpeae. Tribe Gesnereae with four sub-tribe: Bellonieae, Gloxinieae, Eugesnereae and Pentaraphieae.

In 1883, Clarke provided a thorough analysis of the family under the name "Cyrtandreae". In 1893–1894, Fritsch made revisions to the family. A categorization based on cotyledon character was published by Burtt in 1963. He separated the family into two subfamilies: (i) Cyrtandreae, which includes Old World Gesneriaceae with anisocotyl, and (ii) Gesnerioideae, which includes New World Gesneriaceae with isocotyl. Burtt (1963) classified the Gesneriaceae family into two sub-family. The sub-family Cyrtandroideae further divided into five tribes: Cyrtandreae, Trichosporeae, Didymocarpeae, Klugieae and Loxonieae. The sub-family Gesnerioideae was divided into two tribe: Mitrarieae and Coronanthereae.

Ivanina (1967) classified the entire family four years later, and her work was published as "The family Gesneriaceae – a carpological review" in Russian. She divided the entire family into three sub-families: Cyrtandroideae, Episcioideae (a new sub-family), and Gesnerioideae, based on the characteristics of the fruit and seeds. Tribes and sub-tribes are further categorized into each sub-family. Gesneriaceae in the New World and Southern Hemispheres were divided into two sub-families by Wiehler (1983), including Gesnerioideae and Coronantheroideae. The five tribes that make up the Gesnerioideae are Gloxinieae, Episcieae, Beslerieae, Napeantheae and Gesnerieae. The Coronantheroideae family also includes the sole tribe Coronanthereae.

Burtt & Wiehler (1995) recently published a classification of the family Gesneriaceae, primarily based on morphology, with additional information from breeding studies and chromosome counts for the neotropical members. Unlike the previous categories, the following three sub-families were identified as Gesnerioideae, Coronatheroideae and Cyrtandroideae.

A preliminary classification was proposed by Weber (2004a) based on molecular systematic studies. His approach combined both formal and informal groupings, identifying four main informal groups: (1) Coronantheroid Gesneriaceae, (2) Gesnerioid Gesneriaceae, (3) Epithematoid Gesneriaceae and (4) Didymocarpoid Gesneriaceae. While Coronantheroid and Epithematoid Gesneriaceae were not further subdivided, the Gesnerioid Gesneriaceae were organized into traditional tribes: Beslerieae, Napeantheae, Gloxinieae, Gesnerieae, and Episcieae. The Didymocarpoid Gesneriaceae were further divided into informal groups, including: (1) Basal Asiatic genera, (2) European genera, (3) African and Madagascan genera, and (4) Advanced Asiatic and (5) Malesian genera.

A new formal classification of Gesneriaceae developed by Weber et al. (2013) on molecular phylogenetic studies was applied in the present study. According to their classification, the family is divided into three sub-families: Sanangoideae (monospecific with *Sanango racemosum*), Gesnerioideae and Didymocarpoideae. The sub-family Gesnerioideae is divided into five tribes: Titanotricheae (monospecific, found in East Asia), Napeantheae (monogeneric), Beslerieae (with two subtribes: Besleriinae and Anetanthinae), Coronanthereae (with three subtribes: Coronantherinae, Mitrariinae and Negriinae; found in the southern hemisphere), and Gesnerieae (with five sub-tribes: Gesneriinae, Gloxiniinae, Columneinae, formerly Episcieae, Sphaerorrhizinae, formerly Sphaerorhizeae, monogeneric, and Ligeriinae, formerly Sinningieae). In Didymocarpoideae, which is predominantly found in the Old World, especially in East and Southeast Asia/Malesia, two tribes are recognized by Weber etal. (2013): Epithemateae with four distinct sub-tribes: Loxotidinae (monogeneric with *Rhynchoglossum*), Monophyllaeinae, Loxoniinae, Epithematinae (monogeneric) and Trichosporeae. Trichosporeaeis further subdivided into ten subtribes: Jerdoniinae (monospecific), Corallodiscinae (monogeneric), Tetraphyllinae (monogeneric), Leptoboeinae, Ramondinae (found in Europe), Litostigminae (monogeneric), Streptocarpinae (found in Africa and Madagascar), Didissandrinae, Loxocarpinae, and Didymocarpinae. Didymocarpinae is the largest sub-tribe, comprising approximately 30 genera and around 1600 species. According to Möller et al. (2017), there are 23 genera of Gesneriaceae identified in India, and specifically 9 of these genera are found to be distributed in Mizoram. Based on Weber et al. (2013) classification, current taxonomic studies of Gesneriaceae in the northeastern region of Mizoram comprises 2 sub-families, 3 tribes, 6 sub-tribes, and 18 species under 9 genera (Figure 5.1).

2.3 Global Distribution

Wang et al. (1998) described about 56 genera and 442 species from China of which 25 genera and 354 species are endemic to China. Puglisi & Middleton (2017) mentioned that the didymocarpoid Gesneriaceae colonised Southeast Asia from west to east but only 3 genera have reached up to the Solomon Islands or Australia. The Gesneriaceae family in Brazil includes 32 genera and over 232 species, including 155 endemic species (Hinoshitaet al., 2024). In Thailand**,** *Middletonia* which belongs to subtribe Loxocarpinae, tribe Trichosporeae of the family Gesneriaceae have been identified with 4 species by Weber et al. (2013). From Sumatra of Indonesia, a total of 17 genera with *Cyrtandra* the most diverse one with 61 species, followed by *Aeschynanthus* were recorded by Tjitrosoedirdjo et al. (2009). The genus *Didymocarpus* Wall. has varied considerably over time and presently about 70 species is found to be distributed from northwest India, eastwards through Nepal, Bhutan, northeast India, Burma (Myanmar), to southern China, Vietnam, Laos, Cambodia, Thailand, the Malay Peninsula and northwards to Sumatra (Weber et al., 2000). So far, 5 species have been recorded in the genus *Didymocarpus* from Vietnam. *Cyrtandra* comprises at least 600 species distributed throughout Malesia, where it is known for many local endemics and in Polynesia and Micronesia.Close relationships of Gesneriaceae with Asia and Australia have been proposed for some taxa (Knox et al., 1993; Wright et al., 2000; Jaramillo & Manos, 2001; Howarth et al., 2003).

2.4 Distribution in India

In India, the distributions of many of the northern species of Gesneriaceae have strong links to the countries like China and Southeast Asian countries neighbouring India (Moller et al., 2017). Gesneriads are distributed throughout the Western Himalaya, Eastern Himalaya, Jammu and Kashmir, Uttarakhand (Garhwal

Himalaya), Himachal Pradesh, Maharashtra, Western Ghats, Kerala, Karnataka, Tamil Nadu, Bihar and Orissa, Andaman and Nicobar Islands, West Bengal, Sikkim, Assam, Meghalaya, Arunachal Pradesh, Nagaland, Manipur, Mizoram and Tripura. India is home to 28 genera, including 156 species, 1 subspecies, and 5 varieties. In South India, the family comprises 7 genera with 24 species and one variety with one to few species for the rest genera. Of them, *Henckelia* has the largest range, with 14 species and one variety (Janeesha, 2017). Sinha & Datta (2016) identified 80 species in 22 genera from the northeastern region of India, with *Aeschynanthus* (16 species) exhibiting the widest range, followed by *Didymocarpus* and *Henckelia* with 15 species respectively. The family Gesneriaceae from the Indian part of the Eastern Himalaya has 103 species in total, divided into 22 genera. The most common genus found in the Indian part of the Eastern Himalaya is *Aeschynanthus* Jack, which has 27 species. *Didymocarpus* Wall; with 21 species, and *Henckelia* Spreng, with 16 species (Roy, 2017). In Arunachal Pradesh, taxonomic research has identified 78 species of Gesneriaceae flora from 19 genera, with the genus *Henckelia* having the most species 22 recorded as the dominating genus and *Aeschynanthus* comes in second with 14 species (Taram, 2023). In Mizoram, there are 28 species grouped into 12 genera (Sinha, 2012).

2.5 Palynological Study

Several studies on the pollen morphology of the Gesneriaceae family have been published over time, with Erdtman(1952) being the first to make significant contributions. However, these studies typically focus on only a small number of species (Melhem & Mauro, 1973; Nowicke, 1974; Palee et al., 2003) or regional studies (Campos, 1962; Gasparino et al., 2013). Araujo et al. (2021) described pollen from natural hybrids, while Campos (1962), Melhem & Mauro (1973), Fourny et al. (2010), Gasparino et al. (2011, 2014), Souza et al. (2018), and Gasparino et al. (2021) conducted some studies on the morphology of Gesneriaceae pollen, particularly for the native Brazilian species. In a study involving thirty neotropical species, Williams (1978) reported a variety of pollen types for Gesnerioideae, which led to additional research on the family's pollen grains. Smith et al. (1997) state that the size of the pollen grains supports the monophyly of the Gesnerioideae, but the size of the pollen grains in Gesneriaceae taxa has little taxonomic relevance. The physical characteristics of pollen grains of the Gesneriaceae family, according to Melhem and Mauro (1973), allow for the differentiation of genera and in certain situations, even species. Gesneriaceae pollen grains are distinguished by their microreticulate to reticulate ornamentation and 3-colpate or 3-colporate apertures (Gasparino et al., 2021).

Palynology has become an indispensable tool in various applied sciences, such as systematics providing phylogenetic evidence important to plant systematics (Doyle & Endress, 2010; Dransfield et al., 2008; Hesse & Blackmore, 2013). It focuses on the taxon-specific patterns of the pollen wall, how they developed and evolved. Additionally, it provides insights into the evolutionary mechanisms behind different types of corollas (Aftab & Perveen, 2006). Although The Plant List currently recognizes 3,50,699 species, only about 10% have been studied for pollen grain morphology, with even fewer investigated in terms of pollen ultrastructure (Halbritter et al., 2018). Therefore, it is essential to continue both traditional and advanced palynological research. Despite the long-standing tradition and its diverse applications of palynology, its current relevance and future direction warrant consideration. In the twenty-first century, whether palynology remains a fundamental scientific field or evolves into a collection of applied disciplines, expanding our knowledge of pollen grains and refining pollen terminology will be important. Online pollen databases, which efficiently handle data storage, transmission, and dissemination, will become increasingly important for exchanging pollen and spore information that include PalDat (Weber & Ulrich, 2017). While journals are now published in both print and electronic formats, each with its own pros and cons, illustrated monographs based on pollen morphology will continue to serve as valuable resources for detailed information and long-term documentation.

The study of pollen grains provides critical morphological characteristics that aid in the identification and classification of many species.Very less work has been done on the palynological studies in context to Gesneriaceae taxa worldwide where few works reported from the Brazil and Thailand were provided (Pranee et al., 2003; Gasparino et al., 2013). Palynological work on Gesneriaceae species in India is an important gap in botanical research. Despite the ecological richness and biodiversity of the region in Mizoram, limited research has been conducted on the pollen morphology and seed micromorphology of Gesneriaceae species found there. This study is being undertaken to address the significant gap in palynological data as well as seed micromorphological related to the distribution of Gesneriaceae family in Mizoram, India.

2.6 Seedcoat Morphology

Before the late 1960s, gesneriad seed illustrations in publications were created using the light microscope (Fritsch, 1894; Brouwer & Stahlin, 1955). The seeds of the Gesneriaceae are so small that they are nearly invisible to the naked eye. The general shape of the seeds may usually be seen under a light microscope, but the surface morphology can only be seen via a scanning electron microscope. Including the hair-like appendages and length of Trichosporeae members, the seeds range in size from 0.2 mm to 4 cm and are remarkably plentiful per fruit (Beaufort-Murphy, 1985). According to a study by Ivanina (1966), the seed coats of the Gesnerioideae are thin, lined either longitudinally or obliquely, and the seeds of some of the more advanced genera have aril-like appendages. In contrast, the seed coats of the Cyrtandroideae are thick and hard, with surfaces that are either reticulate, pitted, prickly, or tuberculate. Ivanina further mentioned that the seeds can be arranged in groups based on size, with larger seeds being proportionate to fewer seeds in the fruit. The general morphological pattern of the seeds seemed to match the tribal level family classification in Beaufort-Murphy's study. Primary surface reticulation patterns, however, seemed to match the recognized classification at the generic level. Within the Old World subfamily Cyrtandroideae, which is distinguished by the rapid development of uneven cotyledons following germination, the Trichosporeae tribe exhibits seeds that have at least one, frequently hair-like, appendage at each end, to which the name refers (Mendum et al., 2001). Fruits of *Aeschynanthus* are long, thin capsules with many anatropous ovules inside. The apical end of the capsule points toward the base, while the hilar end points toward the apex. Using the quantity and kind of hilar appendages, the genus has been divided into different divisions.

2.7 Molecular Phylogeny

Molecular studies, such as those by Zimmer et al. (2002), suggest that Gesneriaceae is a monophyletic family. It occupies abasal position within the Lamiales order and is considered a sister group to most other families within this order (Oxelman et al., 1999, 2005; Backlund et al., 2000; Olmstead et al., 2001; Bremer & Struwe 1992; APG III, 2009; APG IV, 2016). In 2004a, Weber developed a preliminary classification based on molecular systematics. Weber's treatment was provided on a combination of formal and informal groups due to the lack of sufficient molecular evidence for the Didymocarpoid Gesneriaceae. At present day, molecular phylogenetics is the most important systematics method (Bremer & Struwe, 1992) which uses a combination of statistical and molecular methods to infer evolutionary relationships (Dowell, 2008). Molecular studies have shed light on the phylogenetic relationships of numerous genera and tribes, supporting the following: (1) the existence of a distinct tribe, Epithemateae, sister to the other tribes within the sub-family Cyrtandroideae (Mayer et al., 2003); (2) the discrepancy between traditional classification and tribal relationships suggested by molecular data within sub-family Cyrtandroideae, excluding Epithemateae (Moller et al., 2009); and (3) the internal relationships and monophyly of several tribes (Episcieae: Clark et al., 2006; Gloxinieae: Roalson et al., 2008) as well as genera (*Columnea*: Smith & Sytsma, 1994a, 1994b; *Saintpaulia*: Moller & Cronk, 1997; *Streptocarpus*: Moller & Cronk, 2001; *Aeschynanthus*: Denduangboripant & Cronk, 2001; *Achimenes*: Roalson et al., 2003).

According to Moller et al. (2009), the classifications which is currently in use for Old World Gesneriaceae are artificial and do not reflect any natural relationships. *Rhynchotechum* was discovered to be farther from Cyrtandra and at a more basal position than the majority of Asian Gesneriaceae. Based on ITS and trnL-F, Wei et al. (2010) produced a similar phylogeny that supported *Boeica* as the genus closest to

Rhynchotechum. Afterwards, Weber et al. (2013) used molecular phylogenetic analysis to suggest a new official classification for the Gesneriaceae family. Sanangoideae, Gesnerioideae, and Didymocarpoideae were the three sub-families they identified and are further separated into sub-tribes and tribes.

Roalson& Robert (2016) used the sequences found in NCBI-GenBank to investigate the diversification in various Gesneriaceae clades. According to their research, the Gesneriaceae have a high rate of diversification in both Old World and New World clades. According to them, one significant factor contributing to the higher pace of diversification in New World taxa compared to Old World taxa is ornithophily.

Molecular studies on members of Gesneriaceae in Mizoram and from other parts of Northeast India are relatively sparse. This limits the understanding of genetic diversity, phylogenetic relationships and species delineation based on molecular data. Molecular phylogenetic methods such as DNA sequencing (*rbcL* and ITS) can help to clarify the taxonomic relationships between different taxa of Gesneriaceae of Mizoram which could significantly contribute to global database about them.

2.8 Endemic and RET (Rare, Endangered, Threatened) Species

Endemic and RET species are crucial components of biodiversity, and their presence is necessary for a number of ecosystem functions (Dee et al., 2019). Significant contributions to the well-being and operation of ecosystems are made by endemic and RET species. Local food webs and ecosystem dynamics depend heavily on them due to their distinct ecological roles and adaptations. The loss of an endemic species can have cascading effects. Global climate change, environmental degradation and human disturbance can cause changes in the distribution of RET species, making them more restricted or fragmented (Namgail et al., 2009). When populations decline, the risk of extinction increases, particularly for specialist species Therefore, one crucial strategy for preserving biodiversity is to identify, map, evaluate, monitor, and manage the habitat, niche, and distribution of Endemic and RET species (Jamwal et al., 2020). Effective conservation strategies also require international collaboration, sustainable resource management, and community-based conservation efforts (Brooks et al., 2006).

2.8.1 Endemic Species

Tan et al. (2020) reported the richness of Gesneriaceaein Southwest China, with Hengduan Mountains and the Guizhou-Yunnan-Guangxi border regions identified as the primary hotspots for species diversity and endemism. *Tribounia, Billolivia,* and *Chayamaritia* are among the endemic genera found on the Indo-China Peninsula, which has emerged as an exceptional hub for diversification. These are relatively new genera with fewspecies. The species density is lowest on the Indian subcontinent, where there are only two endemic genera: *Championia* (Sri Lanka) and *Jerdonia*. Papua New Guinea and the Solomon Islands became the endemic centers of *Boea,* which had previously been widespread in tropical Asia (Puglisi et al., 2018; Puglisi & Middleton, 2017).

India has a large number of endemic plant species, including 5,725 angiosperms, 10 gymnosperms, 193 pteridophytes, 678 bryophytes, 260 liverworts, 466 lichens, 3,500 fungi, and 1,924 algae (Sanjappa, 2005). A total of 40 species of *Henckelia* which belongs to the Gesneriaceae family are recognized. Of these, twenty-five are endemic, with fourteen species restricted to southern India (mostly in the southern Western Ghats). Ten of these are found in the Eastern Himalayas (Arunachal Pradesh), while one species, *H. khasiana* (Kanthraj et al., 2023; Nampy et al., 2021), is found in northeastern India (Meghalaya). About 25 species of *Didymocarpus* are known to exist in India with the majority are narrow endemics that are only found in comparatively undiscovered regions of Northeast India. Among these, the state of Mizoram is home to the indigenous species *Didymocarpus adenocarpus, D. lineicapsa, D. parryorum,* and *D. Wengeri* (Prasanna et al., 2020).

2.8.2 RET (Rare, Endangered, Threatened) Species

A rare species is one with a small population that is not currently endangered but is at risk, an endangered species is one that faces extinction across all or a significant portion of its range and a threatened species is one that is most likely to become endangered in the near future (Nayar & Sastry, 1990).On a global basis, the IUCN have estimated that between 20,000 and 25,000 vascular plant species, or roughly 10% of the world's total, are threatened to varied degrees. With an estimated 50,000 plant species, 15,000 of which are blooming plants, India boasts a very diverse flora. About 5,000 of these species are native to India, and roughly 1500 of them—almost 10% of all flowering plants—are threatened to some extent. It is believed that 75 vascular plant species, which are found in the Indian states of Orissa, Andhra Pradesh, Tamil Nadu, and Karnataka, are native to the Eastern Ghat region. BSI released a three-volume book titled "Red Data Book of Indian Plants" that included a thorough analysis of rare, endangered, and threatened species in India (Nayar & Sastry, 1987, 1988, 1990). A recent census found that Orissa State alone is home to 56 rare and endangered plant species, 12 apparent endemic species, and 17 endemic species.

According to Skog & Kvist (1997), there are about 210 species of Gesneriaceae in Ecuador, 107 of which are or were indigenous to the western region of the country at elevations lower than 1000 meters. Of the 107 species, 23 are unique to Ecuador's western lowlands, and nearly half are typically epiphytic. More than a third of the Gesneriaceae flora in western Ecuador is already threatened (Kvist et al., 2004). These species were primarily found in moist lowland forests and low-elevation cloud forests, which are now primarily used for agriculture.

Endemism is extremely high among the Gesneriaceae members present in Mizoram where majority of these species are having a narrow distributional range that shows specific habitat preferences. The narrow endemism of many species within this family makes them particularly vulnerable and susceptible to extinction thus underscoring the need for targeted conservation strategies. Research findings can inform conservation approaches to protect these fragile ecosystems for long term conservation of Gesneriaceae taxa.

2.9 Ethnobotany

Research in ethnobotany usually focuses on indigenous peoples and minorities. For planners and scientists trying to improve rural communities, as well as for the cultures they come from, indigenous knowledge systems are crucial (Bhattarai, 1992). The first comprehensive ethnobotanical research on Gesneriaceae was conducted by Kvist & Holm-Nielsen (1987) from Ecuador. Traditional treatments have utilized certain species of Gesneriaceae, mostly to treat fever, cough, colds, snakebite, pain, and inflammatory and viral disorders. A total of 300 chemicals, including quinines, lignans, xanthones, phenolic glucosides, simple phenolic, flavonoids, terpenes, steroids, and compounds with unique skeletons, have been identified in many Gesneriaceae species (Verdan & Stefanello, 2012). Because of their striking and frequently vibrant flowers with varying shapes, *Sinningia* sp. and *Saintpaulia* sp. are widely grown as ornamental plants (Nishii et al., 2015). Native to Sri Lanka, *Chirita moomii* Gardn. is a pretty plant with decorative appeal (Herath, 2013).

Northeastern India has a rich and varied culture, including traditional knowledge systems among 145 tribal people, in addition to its abundant floristic diversity (Ali & Das, 2003). The indigenous tribes employ a wide variety of plant species as food. In addition to being edible, some species of the Gesneriaceae family are used in traditional medicine to cure snake bites, fevers, inflammation, and coughs and colds (Roy, 2017). According to Taram et al. (2021), these plants are prized for their aesthetic features and have magico-religious significance. Known for its rich traditional knowledge and abundant biodiversity, Mizoram is a hotspot in the Indo-Burma area that highlights a special relationship between indigenous inhabitants and flora. Sino-Tibetan in origin, the Mizo tribes populate the state (Lewis, 2009). Other cultural and ethnic groups that call Mizoram home include the Lushai, Mara, Lai, Chakma, Bru/Riang, Pang, Bawm, and Mog/Magh. The indigenous people employed a total of 304 species from 96 families, which were recognized as medicinal herbs (Rai & Lalramnghinglova, 2011). The link between the local indigenous population and the vegetation is thought to have both positive and negative effects on the plant diversity of Mizoram. The Mizo people engage in a number of ethnobotanically significant practices involving members of the Gesneriaceae family. For example, the stem of *Aeschynanthus parviflorus* (D.Don) Spreng. is used to cure skin inflammation (Ralte et al., 2024), and its blossoms are consumed with meat (Roy, 2017). Furthermore, *Rhynchotechum ellipticum* (Wall. ex D.Dietr.) A.DC. is commonly consumed as a part of traditional foods (Lalmuanpuii et al., 2021).

Chapter 3

3. Study Area

3.1 Mizoram

Mizoram is one of the Northeastern states of India and is a part of the Indo-Burma biodiversity hotspot which is recognized globally for its rich flora and fauna. The region is home to diverse ecosystems, including tropical and subtropical forests, bamboo thickets, and moist evergreen forests, creating varied habitats that support a wide range of plant species, including members of the Gesneriaceae family.The region is characterized by its rugged hilly terrain, deep valleys, and subtropical climate. The state offers a unique and biodiverse landscape that makes it an ideal location for studying the Gesneriaceae family.

3.1.1 Geographical location

The state, which shares two international borders, is a region of blue-green hills that spans 21,087 square kilometers. It is located between 21° 58′ N and 24° 35′ N latitudes and 92° 16′ E and 93° 29′ E (Anonymous, 2001) and is bordered by Assam in the north, Manipur in the north–east, Myanmar in the east and south, and the west by Bangladesh and the state of Tripura. The state is divided into eleven districts: Aizawl, Lunglei, Saiha, Champhai, Kolasib, Serchhip, Lawngtlai, Mamit, Saitual, Khawzawl and Hnahthial. Aizawl, the capital city, has an area of 3,575 sq. km. Lunglei District is the largest, covering an area of 4,536 sq. km, while Kolasib District is the smallest, with an area of 1,382 sq. km.

According to the State Forest Report, Mizoram's total geographical area is 21,081 sq. km, forest covers about 86.27% of the geographical area (FSI, 2017). This forest cover includes 138 sq. km of very dense forest, 5,858 sq. km of moderately dense forest, and 12,752 sq. km of open forest (Anon, 2015).

3.1.2 Physiography

The physiography of Mizoram comprises hills and valleys. The hills are categorized into high hills (average altitude above 1300 m above mean sea level), medium hills (altitudes ranging between 500 m and 1300 m), and low hills (altitudes below 500 m). The hills run parallel to each other in the north-south direction, with valleys between them. The western part of the state is characterized by dissected hills and hillocks in most river valleys.

3.1.3 Geology

Mizoram is predominantly made up of sedimentary rocks located in a sedimentary basin and has the potential for mineral resources like sandstone, clay, limestone, coal, and petroleum. However, minerals like metal, diamonds, marble, and granite are not likely to be found in the state. The geology of Mizoram is characterized by repetitive sediments forming longitudinal plugging anticlines and synclines. Two prominent geological groups, Surma and Barail, are distinguished (Munshi, 1964). The Surma group in the western part exhibits ridge and valley features with a trell is drainage pattern. The Barail group in the eastern part shows a dendritic drainage pattern and denuded hills oriented differently. In the northeastern corner along the border with Myanmar, north-linear trends and sub-parallel mountain ranges with valley topography are observed due to the alteration of hard stone and soft shale beds under the Barail group.

3.1.4 Drainage

Mizoram is drained by numerous rivers, streams, and rivulets with various patterns and lengths. Most drainage lines originate in the central part of the state and flow north or south, directed by north-south trending ridges. All the rivers in Mizoram are Monsoon fed and they attain maximum volume in the Monsoon and post-monsoon period. The entire area is under the regular influence of monsoons. It rains heavily from May to September and the average rainfall is 254 cm, per annum (Verma, 2011). Valleys are narrow, carved out in softer formations, and rivers often form deep gorges, cutting across ridges to create water gaps. The drainage patterns include trellis, dendritic, and parallel patterns. The northern part is drained by large rivers like Tlawng, Tuivawl, Tuirial, Langkaih, and Tuivai, eventually flowing into the Tuiruang River in Cachar plains of Assam. The southern part is drained by prominent rivers like Chhimtuipui (Kolodyne) with its tributaries - Mat, Tuichang, Tiau, and Tuipui, along with the Khawthlangtuipui river draining the southwestern part, eventually flowing into Bangladesh. Mizoram has a total of 25 watersheds.

3.1.5 Climate

Mizoram experiences a moderate climate all year round, with yearly temperatures ranging from 10°C to 32°C due to its tropical position. The yearly average temperature is 22 °C, with minimal fluctuations. The temperature is highest during May, June, and July, with monsoon onset bringing a decline. Winters are minimized in December and January, with temperatures between 11°C to 23°C. Autumn temperatures range between 18°C to 25°C, while summer temperatures range from 21°C to 31°C. The state experiences considerable rainfall for eight months of the year (2000–3500 mm) and high humidity (up to 100%) due to its tropical, subtropical, and temperate climates (Anonymous, 2008). As temperatures have risen and rainfall has decreased during the past three decades, there has been a noticeable shift in the climate (Sati, 2017).

3.1.6 Soil

Mizoram's soil is typically sandy, young and immature. Sedimentary rocks are seen in some locations along the base lines and in watersheds. Sarker & Nandy (1976) divided Mizoram's soils into three categories of soil taxonomy: (i) Entisols (ii) Inceptisols and (iii) Ultisols. The surface soils have low pH values between 4.5 to 6.0, or averagely acidic, are dark, extensively leached, low in bases, and rich in iron. Mizoram soils range from sandy loam to clayey loam with loamy skeletal, mixed, hyperthermic, typical dystrochrepts, and are acidic in character with low base saturation (Kumar et al., 2008).

3.1.7 Vegetation/Forest

The forests of Mizoram are broadly classified into three categories based on the classification of Champion and Seth (1968):

i) Tropical Wet Evergreen Forest: Abundant in the western and northwestern parts of the state, characterized by tall evergreen trees like *Dipterocarpus turbinatus* C.F.Gaertn., *Terminalia myriocarpa* Van Heurck & Mull.Arg., *Mangifera indica* L., and bamboo species.

ii) Tropical Semi-Evergreen Forest: Found in the central part, featuring deciduous and evergreen species like *Artocarpus chama* Buch.-Ham., *Schima wallichii* (DC.) Korth., *Bombax insigne* Wall.and various bamboo species.

iii) Montane Sub-tropical Pine Forest: Located in higher elevations, dominated by *Rhododendron* sp., *Lithocarpusde albatus* (Hook.f. & Thomson ex Miq.) Rehder, *Prunus cerasoides* Buch.-Ham.ex D.Don, *Pinus kesiya* Royale and other species. The rich vegetation of the state is influenced by its diverse physiography and climatic conditions.

3.2 The People and Culture

In Mizoram, there are 37 Sub-tribes, 14 Scheduled Tribes, and 16 Scheduled Castes (Anonymous, 1991). Shakespeare (1912) classified the Lushai (Mizo) into several sub-tribes, clans, and sub-clans. Dutta (1992) has identified fifteen ethnic groups or populations in Mizoram, including the Pawi (Lai), Mara (Lakher), Lusei, Paihte, Hualngo, Tlau, Thado, Ralte, Hmar, Bawm, Pang, Chakma, Riang, Biate, and Mog. Formerly known as Lushais, the Mizo ethnic group speak a language known as Lushai language or Duhlian, which has been changed to "Mizo language" (Phukan, 1992; Thanga, 1992). Others languages spoken by ethnic communities include Chakma, Bru and Hmar.

The people of Mizoram celebrate a number of fairs and festivals. Mim Kut, Chapchar Kut and Pawl Kut are the three most notable festivals. These celebrations are mostly connected to environment and agricultural pursuits. Mizo’s enjoy dancing and listening to music. Cheraw, sometimes known as Bamboo Dance, is one of their unique dances. The other dances are Tlanglam, Chheihlam, Chailam, and Khuallam Sarlamkai/Solakia. Men and women wear puan, the traditional Mizo garment, in the same way. People at the grassroots level weave Puan's textiles. These traditional handwoven garments come in a variety of hues and patterns.

Rice is Mizoram's staple food, often served with dishes like ‘Mizo Bai,’ a blend of seasonal vegetables mixed with ‘saum’ (fermented pork fat), and simple boiled dishes like ‘tlak’ or ‘mung’ without salt.

The most common wild edible plant consumed by the indigenous people included species like *Clerodendrum colebrookianum*, *Colocasia esculenta*, *Eryngium foetidum, Hibiscus sabdarifa*var.*sabdarifa, Musa balbisiana*, *Parkia roxburghii, Solanum gilo, Spilanthes clava*, *Bruinsmia polysperma*, *Gnetum gnemon*, *Livistona chinensis, Maranta dichotoma, Marsdenia maculata, Persea americana* and *Zingiber officinale* (inflorescence) having high market value and demand (Kar et al., 2013). The utilization of these wild edible plant can make a positive impact in enhancing food security and offers a rich variety of nutrition.

Chapter 4

4. Materials and Methods

4.1 Field Surveys and Data Collection

The taxonomic field studies were conducted across various sites within the state of Mizoram, India. Extensive field surveys were conducted during 2018 to 2022 to collect the members of Gesneriaceae specially during the flowering and fruiting season. The COVID-19 pandemic had a profound impact on field-based research, including plant sample collection. The inability to carry out fieldwork during 2020 and 2021 has delayed critical studies based on sample collection due to restricted travel, and imposed quarantines, which hindered access to remote or protected natural areas where plant sample collection was needed. Study sites covered five protected areas: Phawngpui National Park and Murlen National Park, Ngengpui wildlife sanctuary, Tawi wildlife sanctuary and Dampa tiger reserve. In addition to this, field surveys were conducted in 31 villages across 11 districts of Mizoram, focusing state forest reserve areas, parks and local forest areas. These surveys were conducted in a variety of places, such as those close to streams, waterfalls, rocky ledges, and moist, shaded environments. High-quality photographs of the plants were taken, including close-ups of leaves, flowers, fruits, and overall habitat using DSC-W610 Digital camera (Sony, Tokyo, Japan) and D5300 DSLR camera (Nikon, Tokyo, Japan), the GPS coordinates were noted down for each collection site to document precise locations.

4.2 Plant Collection

During fieldwork, specimens were collected carefully by selecting healthy plants. Detailed field notes on each specimen were recorded, including observations on habitat, associated plant species, and any distinguishing features. These specimens are then pressed and dried to preserve their structure for further processing.

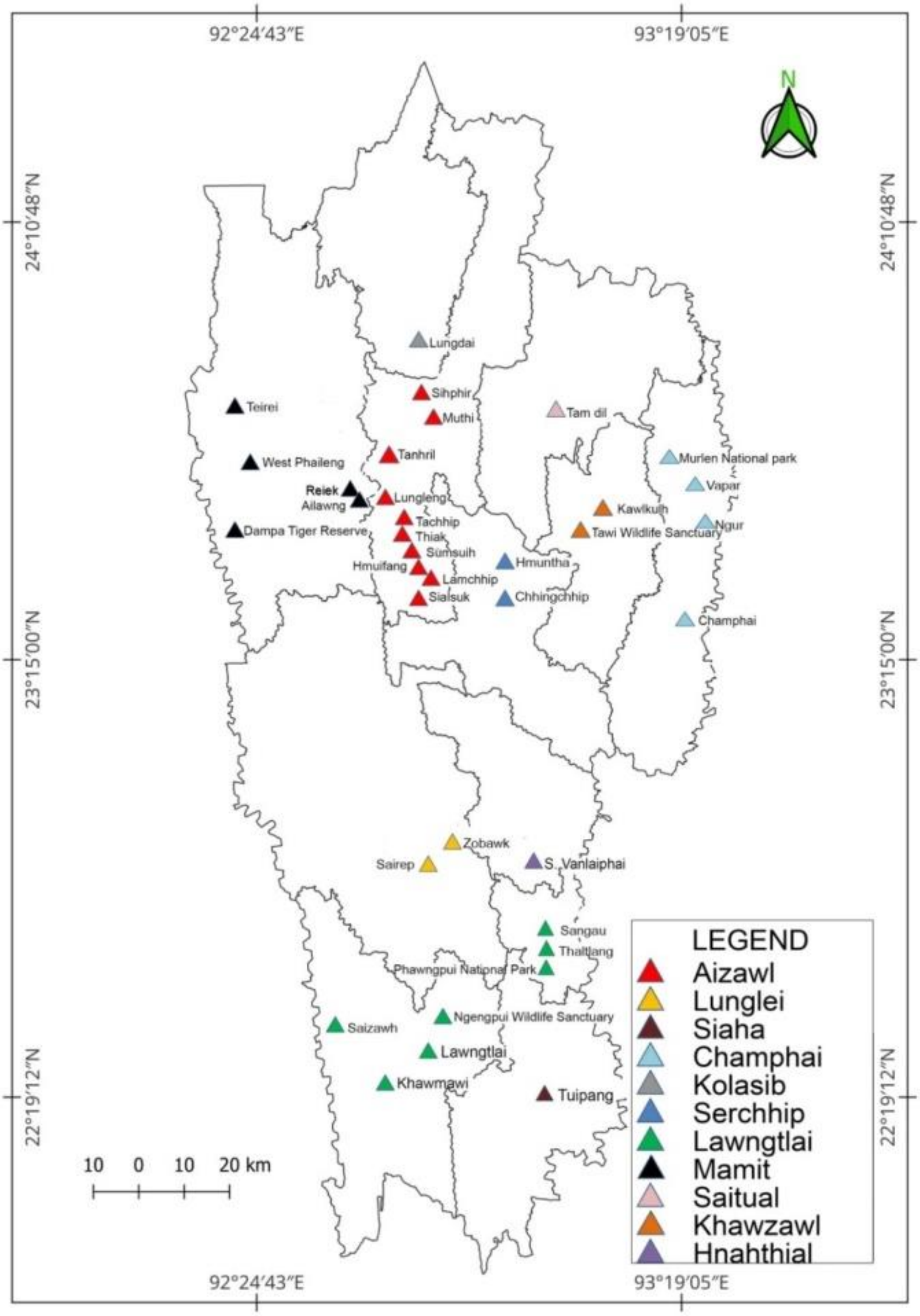

Figure 4.1: A map of Mizoram showing the location of the study sites.

4.3 Plant Identification

All the morphological characteristics such as plant habit, height, colour, arrangement, and type of inflorescence, were carefully noted. The flowers were dissected using a dissection tools, with more attention to the number, size, texture, shape, and arrangement of petals, calyx, stamens, and pistils. Microscopic features like trichomes, pollen, and seeds were examined using a simple microscope, light microscope, stereo microscope, and scanning electron microscope. The length and width of each part were measured accurately with a ruler. All observations were meticulously documented with detailed descriptions, drawings, and photographs. Living samples collected from individual plants were used to gather all morphological data. Observations and descriptions of the species were studied, compared, and identified with relevant literature like Sinha et al. (2012), Sinha & Datta (2016), Bhattacharya & Goel (2015), Taram (2023), Roy (2017), Moller et al. (2017) and online consultation including Plants of the World Online (POWO, 2023), Tropicos (2023), World Flora Online (WFO, 2023) and the Gesneriad Reference Web. For authentication of the identified specimens, herbarium specimens deposited at ASSAM (BSI, Shillong) and CAL (BSI, Kolkata) were consulted along with the digital herbarium databases from Royal Botanic Garden, Kew (K) and Royal Botanic Garden, Edinburgh (E). The collected specimens were processed following standard herbarium methods (Jain & Rao, 1977), and herbarium vouchers were deposited at the Mizoram University Herbarium (MZUH), Department of Botany.

4.4 Herbarium Methodology

A herbarium serves as a repository for plant specimens collected from various locations, systematically arranged on sheets and classified according to a recognized system.

(a) Plant Collection: Plant specimens were gathered during flowering and fruiting season from the study area, including twigs with flowers or fruits from various species. For herbs, the entire plant, including its underground parts, was collected. The specimens were carefully prepared and preserved following the methodologies of Jain & Rao (1977) and Womersley (1981). Photographs were also taken to enhance the study and aid in species identification.

(b) Field notes and field numbers: Field notes were meticulously recorded in a specialized notebook designed for plant labelling and on-site observations. The pages of the notebook were numbered and featured detachable tags, each tied to a specimen for tracking and identification.

(c) Preservation of Plants before Drying: The collected specimens were spread on regular old newspapers and bundled together. Each bundle was encased in a large polythene bag. A 30% paraformaldehyde solution is prepared by dissolving 300g of paraformaldehyde in 3000ml of lukewarm water. This solution is poured over the bundles, thoroughly soaking them. The bags are then securely tied to create an airtight seal. Upon arrival at the laboratory, the bundles are opened, allowing the specimens to be exposed to the air to dissipate any excess paraformaldehyde fumes. This meticulous preservation process ensures the integrity of the plant specimens for further study and analysis.

(d) Pressing and Drying Plant Specimens: Plant specimens are pressed flat, typically not exceeding 11 x 16 inches. If necessary, specimens larger than these dimensions are either folded or cut into sections. Larger fruits or bulbs are often sliced or halved before pressing. Each specimen includes a stem with attached leaves, flowers, or fruits, and herbaceous plant roots are also included. Careful arrangement in the press maximizes the preservation of diagnostic features, with leaves, flowers, and fruits spread out to prevent overlap and enable observation from various angles.

The collection number is clearly written on the outside of the newspaper containing each plant specimen. Keeping the plant press tight prevents shrinkage and wrinkling, producing specimens that can be securely mounted on herbarium paper. Thorough drying is essential before storage and mounting. The plant press is maintained in an oven with steady bottom heat between 95°F and 113°F, ensuring low ambient humidity and adequate airflow for rapid and thorough drying. As specimens dry, straps on the press are further tightened to minimize shrinkage and wrinkling.

(d) Poisoning: To safeguard against insect damage and fungal pests. Specimens may be poisoned by dipping or painting them with an alcoholic solution of mercuric chloride. Another method employs used of Naphthalene balls as a repellent, strategically placed in the boxes containing mounted specimens approximately every three months. This comprehensive approach ensures the preservation and protection of plant specimens in the herbarium collection.

(e) Mounting and Stitching: Mounting is the essential process of attaching a dried, pressed plant along with its label to a sturdy sheet of heavy paper. Mounting sheets, made from durable white card measuring 28 x 42 cm (± 1 cm), are utilized. Before attachment, the specimen and its label are carefully arranged on the sheet to allow optimal observation of diagnostic features, emphasizing reproductive structures and the range of variation in vegetative components, including both sides of the leaves.

Once the specimen has been pressed, dried, and treated, fevicol (adhesive) is applied to the underside of the plant. The specimen, along with its collection number, is then affixed to the mounting sheet. These sheets, with specimens adhered using fevicol, are placed in a press for one day to ensure proper sticking and drying. In cases where items are large or bulky, they are securely sewn onto the sheet with a sturdy thread. The objective is to firmly secure the specimen to the mounting paper while allowing certain plant parts to remain loose for potential removal if needed.

(f) Labelling: Labelling is a crucial step in completing a plant specimen, providing essential field data that must be accurate. Following the mounting of specimens on herbarium sheets, each sheet is appropriately labelled, with the label affixed to the lower right-hand corner. Herbarium labels play a vital role in the completeness of the specimen, and the standard label size is 4” x 2.5”.

The labels typically include the following data: (i) Collection Number and Date, (ii) Name of the Family, (iii) Name of the Genus and Species, (iv) Locality of Collection, (v) Phenology, (vi) Distribution, (vii) Notes and (viii) Collector's Name and Number.

4.5 Classification and Systematic treatment

Based on the classification system developed by Weber et al. (2013), systematic treatment of each of the species were examined. The accepted names for each of the Gesneriad species have been sourced from Plants of the World Online (POWO) and the International Plant Names Index (IPNI). The morphological descriptions and terminology of species in this study have followed the work of Beentje (2010). The systematic treatment included author citation, synonym and type locality for each of the species. A detailed morphological description including flowering and fruiting characteristics, ecology and distribution information, field-examined specimens, taxonomic illustrations, distribution map and dissection photo plate were provided. Specimen details are listed in the following format: distribution, collection locality, elevation, collection date, collector's name and herbarium voucher number.

4.6 Palynology and Seed Coat Anatomy

Palynological studies were conducted using fresh pollen samples of all the Gesneriaceae taxacollected during the study (Schlag-Edler and Kiehn, 2001). The sizes of pollen grains were expressed as Polar axis (P) × Equatorial axis (E) in micrometre (µm). Measurements included the polar length, equatorial diameter, colpi length, pore diameter, and exine thickness. The terminology of Punt et al. (2007) and Halbritter et al. (2018) was employed to describe pollen characteristics. For seed coat analysis, parameters such as seed size, shape, appendages, apical features, cell structure, and cell orientation were noted using the terminology provided by Beaufort-Murphy (1985). Scanning Electron Microscopy (SEM) images were taken to examine the micromorphological features of seeds and pollen grains. Fresh pollen and seed samples were dried in a desiccator and placed on brass stubs with double-sided adhesive tape. The specimens were sputter-coated with a fine layer of gold using a Fine Coat Ion Sputter JFC-1100. Images were captured using a field emission scanning electron microscope (JEOL JSM-6360, Freising, Germany) at Cyto-Gene Research and Development, Lucknow, and SAIF, NEHU, Shillong.

4.7 Cluster analysis of Pollen grains and Seed coat characters

Hierarchical cluster analysis was employed to group species based on their resemblance in terms of pollen characteristics, and seed coat morphology. A data matrix was created from the recorded micromorphological features of the Gesneriaceae family, focusing on pollen grains, and seed coat structures. This matrix was used for cluster analysis through the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method, utilizing EUCLIDEAN distance in R software V.4.1.2 (R Core Team, 2021). A distance metric was used to measure similarity, and the results are illustrated in a dendrogram. A principal component analysis (PCA) was performed using R-Studio (V.4.1.2) statistical software. The packages 'ggplot2' (Wickham, 2016), 'factoextra' (Kassambara & Mundt, 2020) and 'FactoMineR' (Sebastien et al., 2008), 'corrplot' (Wei & Simko, 2024) and 'ggrepel' (Slowikowski, 2024) were used for plotting graphs and using R-Studio V.4.1.2 (R Core Team, 2021).

4.8 Molecular Studies

4.8.1 Collection, Extraction and Amplification of Plant Sample

Fresh young leaves were collected from the study area for the complete genomic isolation of DNA. The extraction of the DNA was then performed using the standard CTAB method proposed by Doyle & Doyle (1987). Approximately, 100 mg of leaf from each sample was used for the extraction of DNA. The extracted DNA were then amplified using ITS2 and *rbcL* primers with the details given in Table 4.1.

4.8.2 PCR Amplification

PCR amplification for both the primer ITS2 and *rbcL* were performed in a volume of 25 µL. The PCR amplifications were carried out in a Veriti 96 Well, Thermal Cycler

(Fischer, Massacgysetts, U.S.) and Bio-Spectrometer (Eppendorf, Hamburg, Germany). The PCR conditions follow a denaturation of 1 min at 94°C, 35 cycles of 1 min at 94°C, 30 seconds at 55°C of annealing, and a final extension of 10 min at 72 °C. The PCR products were visualized in 1.5% agarose gels and goodquality PCR products of DNA were then outsourced to Eurofins Genomic India Pvt. Ltd., Bangalore for sequencing. The determined ITS2 and *rbcL* for each specific sequences have been submitted to the GenBank databases and accession number were allotted for the submitted taxa.

Sequences obtained for the submitted taxa and closely related species were aligned using the ClustalW algorithm implemented in MEGA X software (Cheng et al., 2022). Ambiguous regions and gaps were manually adjusted to ensure accurate alignment. Construction of phylogenetic tree was carried out using MEGA X (Molecular Evolutionary Genetics Analysis, X). Phylogenetic model test was carried out to determine the best-fit nucleotide substitution model with the lowest BIC scores (Bayesian Information Criterion). Then, Maximum Likelihood (ML) phylogeny with Kimura-2-Parameter (K2) model with discrete Gamma Distributed (G) rate with a bootstrap support of 1000 was used for the phylogenetic analysis. Reference sequences from the GenBank database were used for the phylogenetic tree construction and two sequences from Acanthaceae (*Justicia adhatoda* for *rbcL* and *Andrographis paniculata* for ITS2) were used as an outgroup. Then, the sequences were analysed for their placement within the phylogenetic tree and compared with known sequences from related taxa.

Table 4.1 List of the barcoding primers used for PCR of the isolated DNA of the studied Gesneriaceae family

Sl.No.	**Region**	**Primer**	**Sequences (5′ to 3′)**
1	ITS2	Forward	ATGCGATACTTGGTGTGAAT
		Reverse	GACGCTTCTCCAGACTACAAT
2	*rbcL*	Forward	ATGTCACCACAAACAGAGACTAAAGC
		Reverse	GAAACGGTCTCTCCAACGCAT

Chapter 5

5. Results and Discussion

5.1 General characters of Gesneriaceae

Members of the Gesneriaceae are terrestrial (e.g.,*Didymocarpus adenocarpus*), epiphytic (e.g., *Aeschynanthus* sp.), lithophytic (e.g., *Epithema carnosum*), annual or perennial herbs, shrubs (*Rhynchotechum ellipticum*) or small trees with proper rooting. Many of the epiphytic taxa are usually facultative epiphytes (rarely obligate), with quadrangular or terete, herbaceous or pendent stems; leaves opposite or alternate, fleshy to leathery texture; and brightly coloured corollas, often subtended by bright bracts.

5.1.1 Root

Fibrous roots or with rooting above or underground stems, rootstocks either present or absent. Rhizomes, scaly rhizomes (*Chrysothemis pulchella*) or tubers may be present.

5.1.2 Stem

Woody (e.g., *Rhynchotechum ellipticum*) or herbaceous (e.g., *Henckelia pumila*), erect, ascending, creeping, pendulous (e.g., *Aeschynanthus reiekensis*) or absent. Glabrous or pubescent with moderate secondary growth, quadrangular or cylindrical in cross section, and often just a few meters long, however some can grow up to 15 meters and have a diameter of 2–3 cm. Cross section display typical vascular structure with xylem forming a continuous cylinder with narrow to wide vessels and inconspicuous to wide rays.

5.1.3 Leaves

Opposite (e.g., *Henckelia anachoreta*), alternate (e.g., *Rhynchoglossum obliquum*) or sometimes in whorls (e.g., *Aeschynanthus mannii*) of three or four, isophyllous or anisophyllous pairs, simple, usually unlobed, coriaceous to fleshy usually petiolate;

apex acute, acuminate or round; base oblique, cuneate, cordate, subcordate or round; margin entire, serrate, dentate or crenate, stipules absent; lamina usually undivided, rarely lobed or pinnately dissected, indumentum of stem and leaves of glandular and eglandular hairs, rarely absent.

5.1.4 Inflorescence

Axillary, usually in cymes, rarely racemes, often near apex or towards terminal, sometimes reduce to a solitary flower; pedunculate or non-pedunculate; bracteolate or ebracteolate. Flowers showy, zygomorphic (e.g. *Aeschynanthus* and *Didymocarpus*) or actinomorphic, protandrous and perfect.

5.1.5 Calyx

Calyx usually five, free or united, tubular, campanulate (*Henckelia anachoreta*) or divided to the base. Lobe of the calyx lanceolate, triangular, narrowly triangular, elliptic to broadly ovate, reflexed or not reflexed, and with glandular or eglandular hairs; margin entire to serrate or sub-entire.

5.1.6 Corolla

Tubular (e.g., *Aeschynanthus parasiticus*), infundibuliform or funnel-shape, campanulate (e.g., *Rhynchotechum ellipticum*), red, white, blue, violet, orange, yellow, green or various colours combined, with or without markings.

5.1.7 Androecium

Stamens 4 (e.g., the genus *Aeschynanthus*) or 2 (i.e., the genus *Didymocarpus*); staminodes usually present, 1 to 3; filaments inserted at the base, middle or near mouth of the corolla; anther dorsifixed, dithecal, opening by the longitudinal slits, cohering in pairs or all four, inserted or exserted.

5.1.8 Gynoecium

Ovary syncarpous, superior or inferior; style well-developed, glabrous or pubescent; stigma flabellate (e.g., *Henckelia pumila*) peltate (e.g., *Didymocarpus adenocarpus*), bilobed (e.g., *Achimenes longiflora*) or capitate (e.g., *Aeschynanthus mannii*), ovules numerous. Disk either free or adnate to ovary, annular, sometimes absent.

5.1.9 Fruits

Usually capsular (e.g., *Aeschynanthus*) and rarely berry (e.g., *Rhynchotechum ellipticum*), indehiscent or dehiscent. Seeds numerous, minute, ellipsoid or ovoid, sometimes with appendages at 1 or both ends, with or without endosperm

5.2 Systematic treatment

The systematic arrangement of species in this study was based on the classification system developed by Weber et al. (2013) (Figure 5.1). A total of 18 species within genera (9 genera) and subtribes (6 sub-tribes) were identified and are arranged in alphabetic sequence. The genus *Aeschynanthus* and *Didymocarpus* represent five species each. *Henckelia* constitute two species. *Achimenes longiflora, Chrysothemis pulchella, Epithema carnosum, Lysionotus serratus, Rhynchoglossum obliquum* and *Rhynchotechum ellipticum* represent single species. Key to the genus were prepared and for each genus having two or more species, a specific species key was provided.

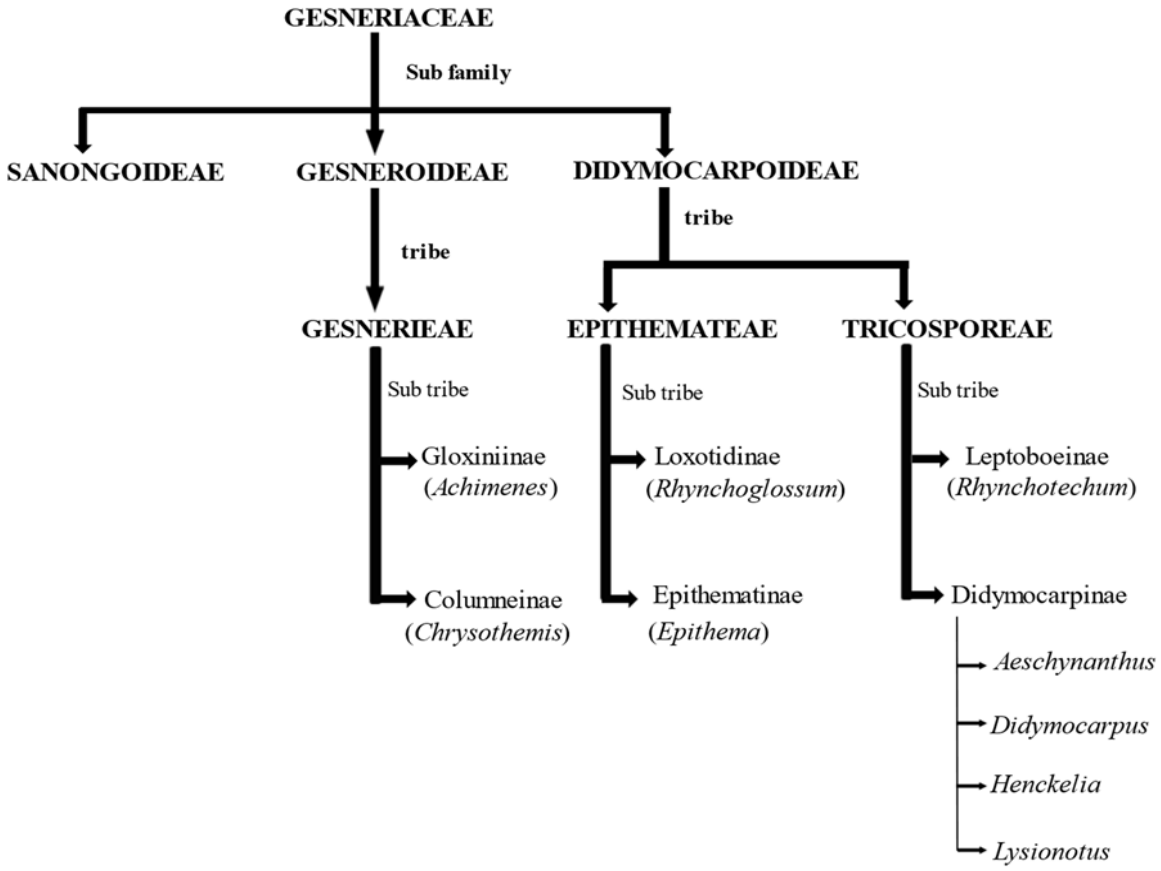

Figure 5.1 Genera of Gesneriaceae of Mizoram, India in accordance with the new classification of Weber et al. (2013).

Key to the genera

1a. Shrubs to sub-shrubs……………………………………………………………….……2

1b. Herbaceous………………………………………………………………….…....3

2a. Corolla tubular; fertile stamens 2; pedicel glabrous; exine ornamentation microreticulate…………………………….….…..……………………**7. *Lysionotus***

2b. Corolla campanulate; fertile stamens 4; pedicel pubescent; exine ornamentation nanoreticulate………………………………….....................**9. *Rhynchotechum***

3a. Seed appendage present ………………………….………….……**2. *Aeschynanthus***

3b. Seed appendage absent…………………………………………..….…..………..4

4a. Leaf arranged in whorl; peduncle absent……….……….…………**1. *Achimenes***

4b. Leaf arranged in opposite; peduncle present……………….……………….....5

5a. Ubisch bodies present in intine of pollen……………………..….**3. *Chrysothemis***

5b. Ubisch bodies absent…………………………………………..………………6

6a. Leaf arrangement alternate ………………….……………….…**8. *Rhynchoglossum***

6b. Leaf arrangement opposite…………………………………….…………………7

7a. Ovary inferior ………………………………………….…….…………**5. *Epithema***

7b. Ovary either superior or linear…………………………….……...………8

8a. Stigma bilobed or flabellate …………………………..………...……**6. *Henckelia***

8b. Stigma is either peltate, oblique or capitate………….….……..…**4. *Didymocarpus***

5.2.1 Systematic Treatment of the Genera

1. *Achimenes* Pers.

Achimenes longifora DC., Prodr. 7(2): 536. 1839; T.S. Nayar & al., Fl. Pl. Western Ghats 1 (Dicots): 530. 2014; B.K. Sinha & Su.Datta, Nelumbo 58: 3. 2016 **(Figure No. 5.2; Plate No. 1)**

Synonym: *Trevirana longiflora* (DC.) D.Dietr., Pl. 3: 568. 1843.

Type: Mexico. Pavon, Sesse and Mocifio, s.n. (holotype: G DC, illustration!)

Taxonomic description: Perennial herbs up to 70 cm tall, brown to maroon stem, pubescent. Leaves arranged in whorl, 4.3–9.5 × 2.8–4.4 cm long, adaxial surface of the leaves is green and abaxial surface having maroon colour, pubescent, margin serrate. Petiole 1.3–1.7 cm long, pubescent, green. Peduncle absent. Pedicel 1.3–1.5 cm long, densely pubescent, maroon. Calyx 1.5–1.8 × 0.2–0.3 cm long, divided up to base, lanceolate, pubescent, re-curved at tip. Inflorescence 2–4 flowered, axillary. Corolla 38–48 mm long, obliquely infundibuliform, tube glabrous, yellow to maroon, 5 lobes, purple in colour, inner corolla having dark brown patches. Stamen 4, glabrous; filament 18–20 mm long, anther inserted, all 4 anthers fused at one point. Staminode white, one in number, *c*. 1 mm long. Pistil 25–28 mm long; ovary inferior, *c*. 1mm long; stigma densely pubescent, bilobed white, *c*. 1 mm long, style 7 mm long, densely pubescent. Capsule ovoid.

Phenology: Flowering from June to September and fruiting from August to December.

Habitat and Ecology: Growing on moist and shady environments, soft soil, forest floor or along stream banks where conditions are consistently humid. In their natural habitat, they often form clusters or colonies, especially on shaded walls or in areas protected from direct sunlight. In cultivation, it can be grown in a range of temperatures, but it prefers warm, humid conditions and will not tolerate frost.

Distribution: Mizoram (Lungleng, Reiek; **Figure 5.3**), Cultivated in most places as ornamental plant. India (Meghalaya, Sikkim, Uttarakhand and West Bengal); cultivated in Jharkhand and Western Ghats. (Central America, Caribbean). Native range: Tropical regions of Mexico and Central America.

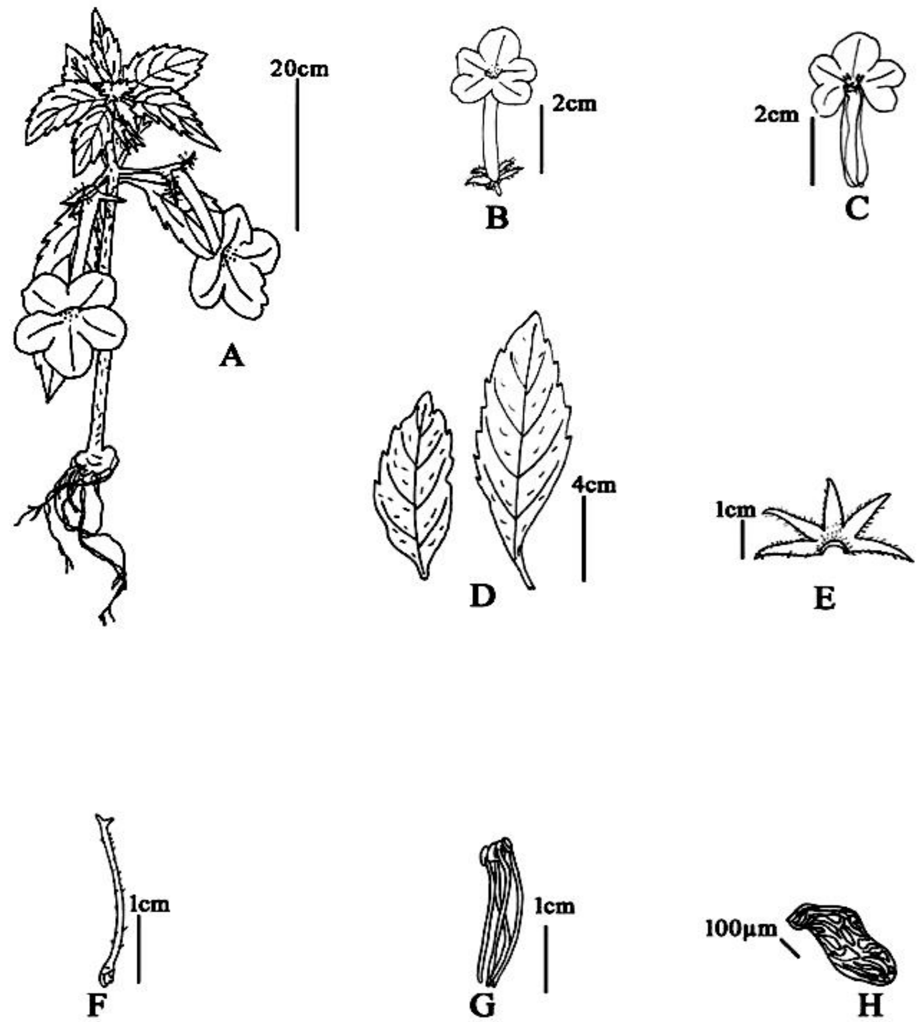

Figure 5.2: llustration of *Achimenes longiflora* DC.A. Habit; B. Flower; C. Flower opened; D. Leaves; E. Calyx; F. Pistil; G. Stamens; H. Seed.

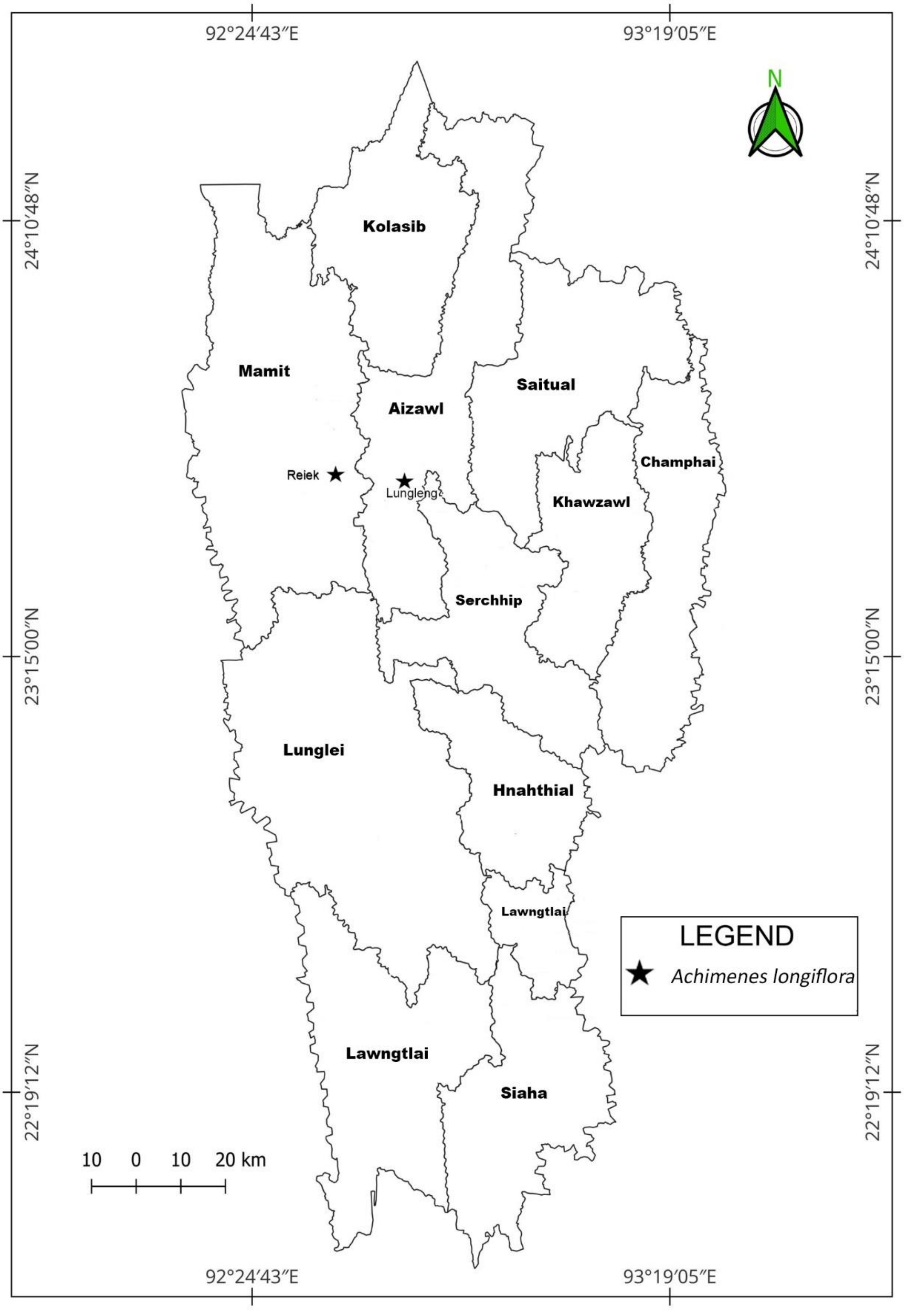

Figure 5.3: Distribution map of *Achimenes longiflora* DC. in Mizoram.

Specimens examined: INDIA. Mizoram, Aizawl District, Lungleng village, 23°66′56.0″ N 92°66′35.0″ E, 980 m elevation, 07 November 2022, Margaret Lalhlupuii 128817 (MZUH129815); Rajbhawan, Shillong, Meghalaya, 24.06.1964, G.

Aeschynanthus Jack

Epiphytic herbs or under shrubs, non-rhizomatous. Stems often pendent, creeping, branched or unbranched. Leaves opposite or arranged in whorls, entire, fleshy or leathery, glabrous. Cymes axillary, 1 to 15 flowered, showy, zygomorphic. Peduncle absent or present. Calyx 5 lobed, free at base or above the tube, glabrous or pubescent. Corolla tubular, red, labiate. Stamen 4, exserted, didynamous. Staminode one. Stigma peltate or capitate. Pollen monad, isopolar and tricolporate. Seed numerous having hilar appendages.

Key to the species

1a. Leaf arranged in whorls; calyx pubescent; seed cell shape is narrowly lineate polygonate andcell character depressed andverrucate…………………….……………………….………………….…**1. *A. mannii***

1b. Leaf in opposite arrangement; calyx glabrous; seed cell shape straight, irregular, elevated, auriculate…………….……..……..……..…………………………...….2

2a. Peduncle absent; 2 or >2 hilar appendages………………………...…………..3

2b. Peduncle present; 1 hilar appendage……………………….…………**5. *A. superbus***

3a. >2 upto 4 hilar appendages; leaves linear, small, scars prominent with vinaceous dots …………………………………………..…….….…………..**4. *A. reiekensis***

3b. 2 hilar appendages; leaves lanceolate, scars and vinaceous dots absent…..……4

4a. Inner corolla tube with tufts of hairs: pollen shape prolate………**2. *A. parasiticus***

4b. Inner corolla tube without tufts of hairs; pollen shape perporate……...……………………………………………………**3. *A. parviflorus***

1. *Aeschynanthus mannii* Kurz ex C.B. Clarke in A. DC. & C. DC., Monogr. Phan. 5(1): 29. 1883 & in Hook.f., Fl. Brit. India 4: 341. 1884; C.E.C. fisch., Rec. Bot. Surv. India 12(2): 117. 1938; Kanjilal et al., Fl. Assam 3: 390. 1939; G.P. Sinha in G.P. Sinha & Fl. Mizoram 2: 198. 2012; U.C. Bhattach. & Goel, Phytotaxonomy 14: 10. 2015; B.K. Sinha & Su.Datta, Nelumbo 58: 7. 2016; Su.Datta & al., J. Jap. Bot. 91: 233. 2016. (**Figure No. 5.4; Plate No. 2**)

Synonym: *Trichosporum mannii* Kuntze, Revis. Gen. pl. 2: 478. 1891.

Type: Burma (Myanmar), Patkoi Mts. Griffith 3816 (K, photo at CAL no. 6520A!).

Taxonomic description: Epiphyte, erect, unbranched, 30 cm along with roots and 24–26 cm. Stem erect, unbranched, straight with 4–8 internodes, rough, glabrous, brown in colour, leaves scar clearly visible at eachnode, mosses and lichens grow on part of stem. Leaves thick, succulent, glabrous, margin entire, maroon colour dotted of around 8 dots per leaflet, apex acute, deep midrib with poor venation, light green to yellow colour, leaves arrange in whorl in opposite-decussate manner with 6 number per whorl, 3 to 5whorls of leaves are present in one stem, leaves measure 2.5–3.2 × 0.8–1.2 cm long, leaves scar are clearly visible in each node, leaves cluster at the terminal of the stem. Petiole 2 mm long, glabrous, light green to yellow in colour, basal rounded. Inflorescence cyme axillary, 1 to 8 flowered. Peduncle absent. Pedicel 4 mm long, yellow in colour, densely pubescent. Bract 1mm long, maroon colour, two in number, densely pubescent, slender. Calyx divided up to the base, basal is yellow and upper part is off-white with maroon colour pointed, densely pubescent, slender, 5–7 × 1–2 mm long. Corolla red colour at maturity and green at bud stage, tubular, obscure, funnel shape, 25–27 mm long, densely hairy, mouth oblique with 10 mm long opening from upper to lower lip and 1–12 mm long, 5 lobed, 3 upper lobes have a maroon streak and 2 lower lobes lack maroon streak,

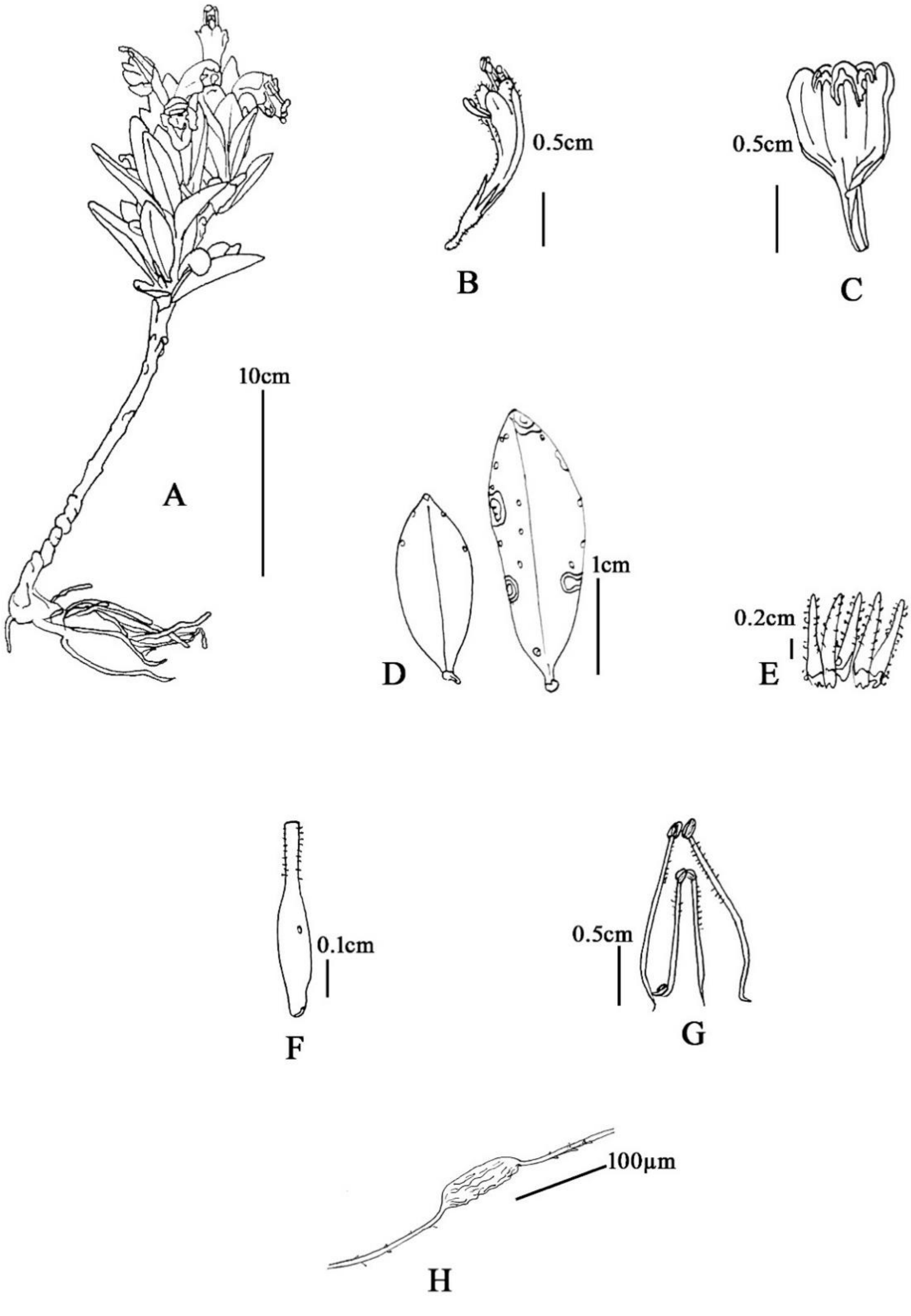

Figure 5.4: Illustration of *Aeschynanthus mannii* Kurz ex C.B.Clarke A. Habit; B. Flower; C. Flower opened; D. Leaves; E. Calyx; F. Pistil; G. Stamens; H. Seed.

corolla lobes 3–5 × 4–5 mm long; corolla tube is 20–22 mm long. Stamen 4, exserted, didynamous, epipetalous, pubescent, lower half is white colour and upper half is maroon in colour; filament 15–19 mm long in which 5–7 mm long is exserted above the corolla; anther 1 × 2 mm long, anther gold in colour, fused at the terminal apex. Staminode present at the centre, white colour 1mm long. Pistil 3–5 mm long; ovary 1mm long, light yellow, glabrous; style off-white, glabrous, 3 mm long; stigma 1mm long, capitate, maroon pointed at apex. Capsule 10–20 cm long.

Phenology: Flowering from February to March and fruiting from April to July.

Habitat and Ecology: An epiphytic herb with shallow roots spreading widely. Often found in clusters with mosses and lichens, this plant thrives at high altitudes ranging from 1500 to 2000 meters.

Distribution: Mizoram (Phawngpui; **Figure 5. 9**); India (Meghalaya); Myanmar.

Specimens examined: INDIA. Mizoram, Lawngtlai District, Phawngpui National Park, 22°41′47.4″ N 92°02′96.8″ E, 1663m elevation, 22 February 2019, Margaret Lalhlupuii128818 (MZUH000036); Mann 99 (ASSAM); K000096771 (K).

2. *Aeschynanthus parasiticus* (Roxb.) Wall., Numer. List: n. 796. 1829; G.P. Sinha in G.P. Sinha& al., Fl. Mizoram 2: 199. 2012; Giri & al., Mat. Fl. Arunachal Pradesh 225. 2008. U.C. Bhattach. & Goel, Phytotaxonomy 14: 14. 2015.**(Figure No. 5.5; Plate No. 3)**

Synonym: *Incarvillea parasitica*Roxb., Pl. Coromandel 3(4): 88. 1820; *Aeschynanthus andamanensis* Goel, Vasudeva Rao & Mehrotra in Bull. Bot. Surv. India 31: 154.1992; *Aeschynanthus deleiensis* C.E.C.Fisch. in Bull. Misc. Inform. Kew 1935: 320.1935; *Aeschynanthus mimetes* B.L. Burtt, Bot. Mag. 162: t. 9595. 1940.

Type: Plate of *Incarvillea parasitica* in Pl. Corom. 3: 88, t. 291. 1820

Taxonomic description: Epiphytic, creeping, 28 cm tall. Stem brown, terete, glabrous. Leaves opposite, thick textured, 7–12.6 × 2.6–3 cm long, adaxial surface

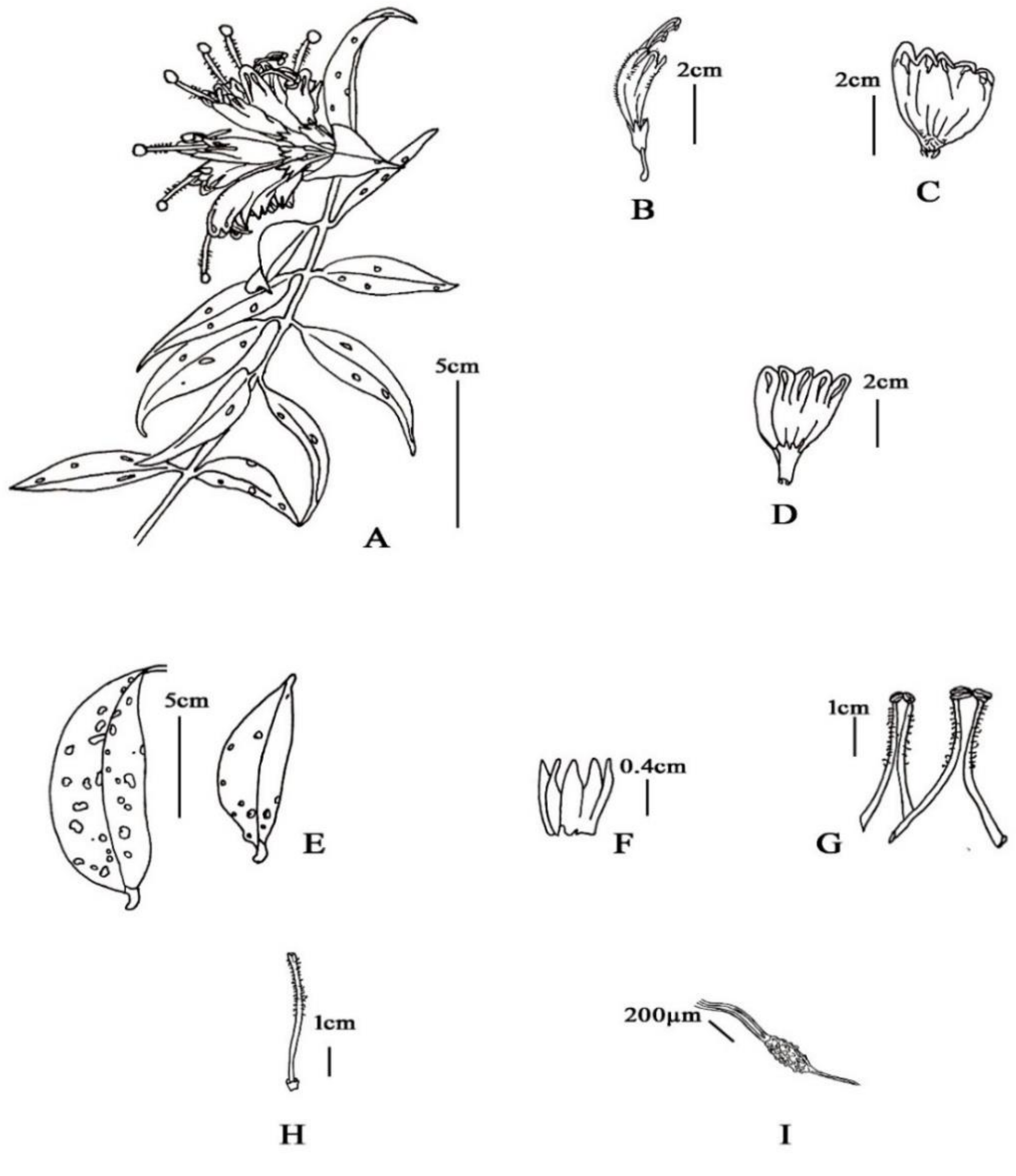

Figure 5.5: Illustration of *Aeschynanthus parasiticus* (Roxb.) Wall. A. Habit; B. Flower; C. Flower opened; D. Leaves; E. Calyx; F. Outer flower; G. Pistil; H. Stamens; I. Seed.

dark green and abaxial surface light green, obtuse lanceolate, base rounded, margin entire, vein visible but not much clear, glabrous, smooth. Petiole brown, rounded at the base, 1 cm long. Inflorescence cyme axillary, 2–15 flowered, not mature at the same time. Peduncle absent. Bract 7–9 × 3–4 mm long, obtuse lanceolate, light green. Pedicel green, 10–13 mm long, terete. Calyx 5 lobed, 17 mm long, tube 10–15 mm long, lobe 6–8 × 3–5 mm long, green, not divided up to the base, lanceolate, glabrous, apex acuminate. Corolla 41–5 mm long, tubular tube 35–37 mm long, lobes 6–8 × 5–7 mm long, lower tube yellow, upper tube orange red in colour, pubescent, denser hair at margin of lobes, lower inner tube having tufts of hairs. Stamen 4, exserted, didynamous; filament 40 mm long, epipetalous, white at the basal region and pink colour at the upper which is 1/3rd of the filament length, pubescent, anther fused at two points, dorsal anther yellow and ventral greyish. Staminode present in 1, white measuring 2 mm long. Pistil 40–45 mm long, pale yellow at half the length from base and upper white in colour, pubescent at upper white region; ovary white in colour with 1 mm long; style 38–40 mm long; stigma capitate, 1–2 × 2–3 mm diameter. Capsule 10–30 cm long.

Phenology: Flowering from February to March and fruiting from April to July.

Habitat and Ecology: An epiphytic herb found in forests at elevations ranging from 800 to 2000 meters.

Distribution: Mizoram (Lungleng, Murlen, Sairep, Tawi, Thiak, Zobawk; **Figure 5.9**); India (Andaman and Nicobar Islands, Arunachal Pradesh, Assam, Myanmar, Meghalaya, Nagaland, Odisha, Sikkim, Tripura and West Bengal); Nepal, Bangladesh, Myanmar, China, Vietnam.

Specimens examined: INDIA. Mizoram, Aizawl District, Thiak village, 22°84′42.3″ N 92°82′01.3″ E, 1181m elevation, 24 September 2018, Margaret Lalhlupuii128819 (MZUH000037);11 km from Tarain to Dawki Rd., K and Hills, Assam, 15.11.1962, N.P. Balakrishnan 49827 (ASSAM); Garo Hills, 6.9.1975, K. S. Rao 61583 (ASSAM 52118); Muntaii, Tripura, 1000 ft (304.8 m), 31.3.1941 K. Biswas 4988

(CAL); Jowai river bank, Assam, 27.1.57, G. Panigrahi 5224 (CAL).K000096744 (K).

3. *Aeschynanthus parviflorus* (D. Don) Spreng., Syst. Veg., ed. 16, 4(2, Cur. Post.): 238. 1827; G.P. Sinha in G.P. Sinha & al., Fl. Mizoram 2: 200. 2012; A.DC., Prodr. 9: 261. 1845; Giri & al., Mat. Fl. Arunachal Pradesh 226. 2008; U.C. Bhattach. & Goel, Phytotaxonomy 14: 15. 2015; B.K. Sinha &Su.Datta, Nelumbo 58: 10. 2016. **(Figure No. 5.6; Plate No. 4)**

Synonym: *Aeschynanthus consobrinus* Kraenzl in Repert. Spec. Nov. Regni Veg. 24: 215. 1928; *Aeschynanthus maculatus* Lindl. in Edwards's Bot. Reg. 27: t. 28 .1841; *Aeschynanthus ramosissimus* Wall. in Pl. Asiat. Rar. 1: 55.1830; *Aeschynanthus sikkimensis* (C.B.Clarke) Stapf in Bot. Mag. 148: t. 8938.1922; *Trichosporum maculatum* (Lindl.) Kuntze in Bot. Mag. 148: t. 8938.1922; *Trichosporum parviflorum* D.Don in Edinburgh Philos. J. 7: 85.1822.

Type: Nepal, Wall. Cat. Num. List no.799 (K)

Taxonomic description: Under-shrubs, epiphytic, creeping, 15–50 cm tall, rooting, with spreading hair. Stem green, glabrous, terete. Leaves opposite, thick textured, 7–13 × 1.5–2 cm long, lanceolate, base rounded, apex acute, margin entire, veins invisible, glabrous, adaxially dark green and abaxially light green. Petiole glabrous, 6–8 mm long, terete. Inflorescence cyme axillary, 1–10 flowered. Bract 3–5 mm long, dark brown. Peduncle absent. Calyx 5 lobed, divided up to the base, 7–10 × 1–3 mm long, lobes lanceolate (very slender), maroon, apex acuminate, glabrous. Pedicel 6–8 mm long, maroon, pubescent. Corolla 26–30 mm long, tubular, tube 19–25 mm long, corolla lobe 7–10 × 4–6 mm long, pubescent, maroon streak on all the outer five lobes. Stamen 4, exserted, didynamous epipetalous, filament pubescent at the upper surface which is pink in colour and lower region is pale yellow in colour; filament is 22–25 mm long; anther fused at twopoints. Staminode present, 1 at the centre of tube, 3 mm long and white in colour. Pistil 26–30 mm long; ovary 3mm long, yellow in colour; style white in colour measuring 22–24 mm long; stigma capitate, 2–3pink to maroon. Capsule 10–25 cm long.

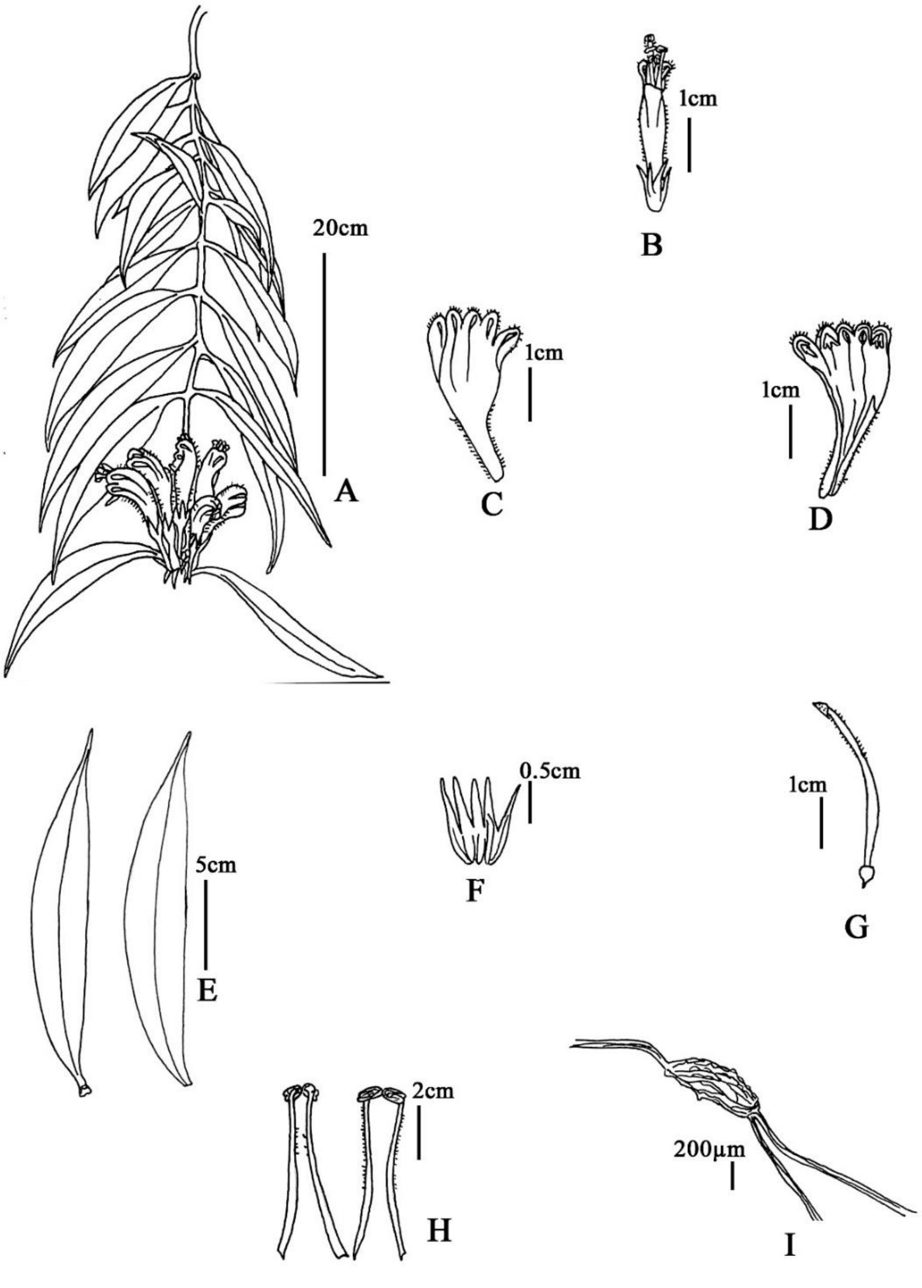

Figure5.6: Illustration of *Aeschynanthus parviflorus* (D.Don) *Spreng*. A. Habit; B. Flower; C. Flower opened; D. Leaves; E. Calyx; F. Outer flower; G. Pistil; H. Stamens; I. Seed.

Phenology: Flowering from July to September and fruiting from September to December.

Habitat and Ecology: An epiphytic herb naturally occurring in forests at altitudes between 1000 to 2000 meters. For ornamental purposes, they can also be cultivated in soil mixed with coco peat and dead wood.

Distribution: Mizoram (Hmuifang, Reiek, Sialsuk; **Figure 5.9**); India (Arunachal Pradesh, Meghalaya, Nagaland, Sikkim and West Bengal); Bhutan, China, Myanmar, Thailand and Vietnam.

Specimens examined: INDIA. Mizoram, Aizawl District, Hmuifang village, 22˚84'42.3" N 92˚82'01.3" E, 1181m elevation, 25 September 2018, Margaret

Lalhlupuii 128820 (MZUH000038); K and J Hills, 28.5.1932, G. K. Deka 10217 (ASSAM); Latpancher, Darjeeling, 8.3.95, S. Chandra and Party 21527(CAL); Kabi, Sikkim, 5500 ft (1676.82 m), N.C. Mazumder and R. H. Datta 84 (CAL); K000196664 (K).

4. *Aeschynanthus reiekensis* Lalhlupuii, S.D.Khomdram & S.D.Yumkham, Taiwania 68: 2 (2023).

Type: INDIA. Mizoram, Mamit District, Reiek Tlang, 23°40′58.2″ N 92°36′22.0″ E,1391.70 m elevation, 17 July 2018. **(Figure No. 5.7; Plate No. 5)**

Taxonomic description: Epiphytic sub-shrubs, pendulous and branched. Stems 11 to 50 cm long, glabrous, smooth, rounded, green with a purple tinge when young, become straw coloured when old; nodes 3 to 8 in each branchlets, swollen; leaf scars prominent; internodes 2–4 cm long. Leaves opposite; petiole 4–8 mm long, glabrous; leaf blade 8–13 × 0.3–0.6 cm, linear, coriaceous, green above, pale beneath with vinaceous dots, glabrous, acuminate at apex, cuneate at base, entire to sub-entire at margins, sometimes recurved back and revolute when drying; midrib slightly sunken, lateral veins obscure. Cymes axillary, pseudo-terminal, 1–3 flowered; peduncle absent. Bracts 2, 2 × 1 mm, triangular, maroon, glabrous. Pedicels 8–16 mm long, green at base, maroon towards apex, glabrous. Calyx lobes 3–7 × 1–13 mm, lobes free or sometimes slightly fused of calyx tube (1.5–2.5 mm) at base, narrowly triangular to linear, glabrous, green flushed with maroon, acute-acuminate at apex, entire at margins. Corolla 20–29 mm long, tubular, strongly oblique mouth, inflated at middle; tube 1 mm broad at base, gradually widened towards the throat with 5–6 mm , orange to red, whitish yellow at base, sparsely to densely glandular puberulent except at base, yellow to pale red with a tuft of multicellular glandular hairs just above the base internally; lobes yellowish-orange to orange, arched with red on the rim internally, with a claret streak running down on the lower lobes, upper lobes un-streaked, each lobe 3 × 4 mm, not spreading or reflexed. Stamens 4, exserted at anthesis; anthers fused in 2 pairs, 1.5–3 × 1 mm, purple or grey; pollen grey; anterior filament 30–33 mm long, inserted at 5–7 mm from corolla base; posterior filaments 25–28 mm long, inserted at 6–8 mm from corolla base; white at base and light purple higher up, glandular hairy. Staminode 1, c. 1 mm long. Disk 1–2 mm high, 5-crenate. Pistil 2–4.1 cm long, white, sparsely to densely glandular pubescent; stigma capitate, pink or purple, c. 2 mm across. Capsules 10–23 cm.

Phenology: Flowering from July to November and fruiting from September to December.

Habitat and ecology: Reiek Tlang is a part of community protected forest and a tourist spot located in the Northwest of Mizoram state in Mamit district. It covers an area of 25 km^2 and is situated about 29 km from Aizawl. The area is part of tropical wet semi-evergreen forest, with an average altitude of 900 m above sea level. The rainfall averages between 200–250 cm annually and the temperature varies between 20°C to 28°C. In Mizoram, epiphytes represent about 10.56% of the total recorded plants (Lalzarzovi & Lalnuntluanga, 2017). A small population of *A. reiekensis* was growing at altitudes between 1288–1391.70 m above sea level, as an epiphyte on *Castanopsis tribuloides* (Sm.) A.DC. (Fagaceae) which is locally known as Thingsia.

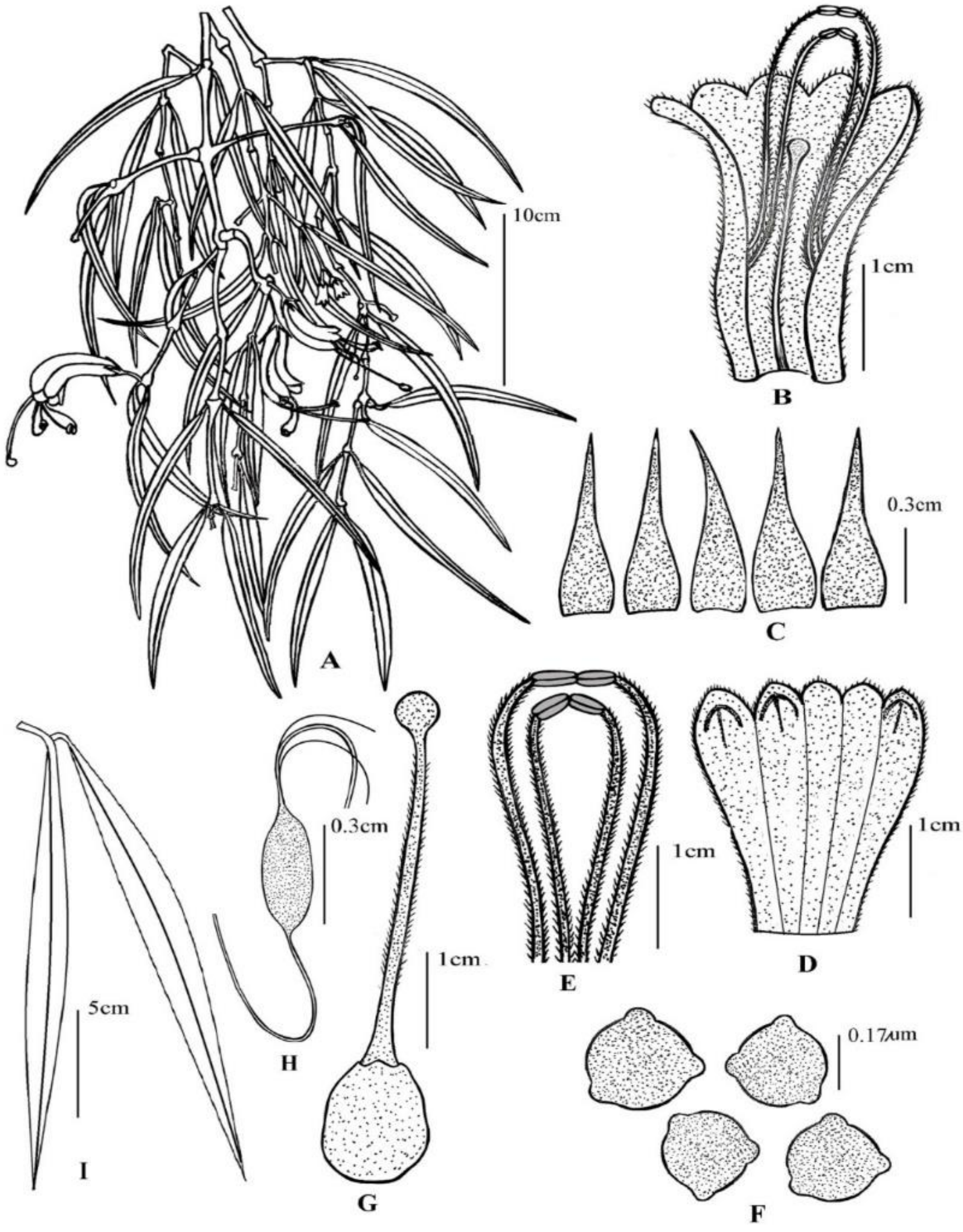

Figure 5.7: Illustration *Aeschynanthus reiekensis* Lalhlupuii, S.D.Khomdram & S.D.Yumkham A. Habit; B. Flower opened; C. Calyx; D. Outer flower; E. Stamens; F. Pollen grains; G. Pistil; I. Leaves 41 mm long; stipe 1–2 mm long, glabrous; ovary 5–15 mm long, linear, white to greenish white, glabrous, with sessile glands or minutely papillose; style 8–20 mm.

Distribution: So far collected only from the type locality, Reiek Tlang (**Figure 5.9**).

Specimens examined: INDIA. Mizoram, Mamit District, Reiek Tlang, 23°40′58.2″ N 92°36′22.0″ E, 1391.70 m elevation, 17 July 2018, Margaret Lalhlupuii128821 (MZUH0000512).

5. *Aeschynanthus superbus* C.B.Clarke, Commelyn. Cyrtandr. Bengal.: 73, t. 46. 1874 & in Hook.f., Fl. Brit. India 4: 342. 1884; G. P. Sinha in G.P. Sinha & et. al., Fl. Mizoram 2:201.2012; Bhattacharyya and Goel, Phytotaxonomy 14: 17. 2014; Sinha and Datta, Nelumbo 58: 12. 2016. (**Figure No. 5.8; Plate No. 6**)

Synonym: *Trichosporum superbum* (C.B.Clarke) Kuntze in Revis. Gen. Pl. 2: 478.1891.

Type: Pundua, N. Wallich 795 (BM)

Taxonomic description: Epiphytic herb, stout, under shrub. Stem 100 to 300 cm long, glabrous, smooth, terete, green to brown, branching and branches sub-woody. Leaves opposite, 4.5–14 × 10–23 cm long, leathery, green adaxially, pale green abaxially, glabrous, base cuneate to rounded, margin entire, apex acuminate, thick mid-rib, lateral veins 4–11 pairs. Petiole 0.5–1 cm long, glabrous. Inflorescence cyme axillary, 4–9 flowered. Peduncle 7–9 mm long, glabrous, green to red. Bract red, glabrous, 75–80 × 40–50 mm long, elliptic to ovate. Pedicel 1cm long, red, terete, glabrous. Calyx red, glabrous, five lobed, divided up to base, 27–30×8–10 mm long, linear to lanceolate. Corolla red, tubular, 60–78 mm long, tubular, having distinct five lobes, glabrous in the outer surface, strong maroon streak on inner lobes, pubescent at the margin of the lobes, mouth slightly oblique, tube 58–60 mm long, upper two lobes with orbicular, oblong or ovate and lower three lobes elliptic or ovate. Stamen 4, exserted, didynamous, epipetalous, pubescent; filament 47–60 mm long, yellowish green; anther basifixed, 4–5×2–2.5 mm long. Staminode 1, white, 2 mm long. Pistil 67–70 mm long, yellowish green, glabrous; stigma peltate, yellow, 2–3 ×3–3.5 mm long, pubescent at upper half; style 15–40 mm long; ovary18–44 mm long, glandular pubescent or with sessile glands. Capsule 20–50 cm long.

Phenology: Flowering from August to September and fruiting from November to January

Habitat and Ecology: Growsin evergreen forests, typically found at elevations ranging from 600 to 2000 meters. Unlike to other *Aeschynanthus parasiticus*, this variety is notably larger and taller, featuring a stout stem, and is classified as a shrub.

Distribution: Mizoram (Ailawng, Zobawk; **Figure 5.9**) India (Arunachal Pradesh, Meghalaya and Nagaland); Bhutan, Myanmar, China, Laos, Vietnam.

Specimens examined: INDIA. Mizoram, Lunglei District, Zobawk village, 22˚84’42.3” N 92˚82’01.3” E, 1181m elevation, 07 September 2022, Margaret Lalhlupuii128822 (MZUH000040); K and J Hills, 13.9.1931, S. R. Barma 9422 (ASSAM); Jowai River bank, Assam, 26.1.57, G. Panigrahi 5231 (ASSAM); E00096763 (E).

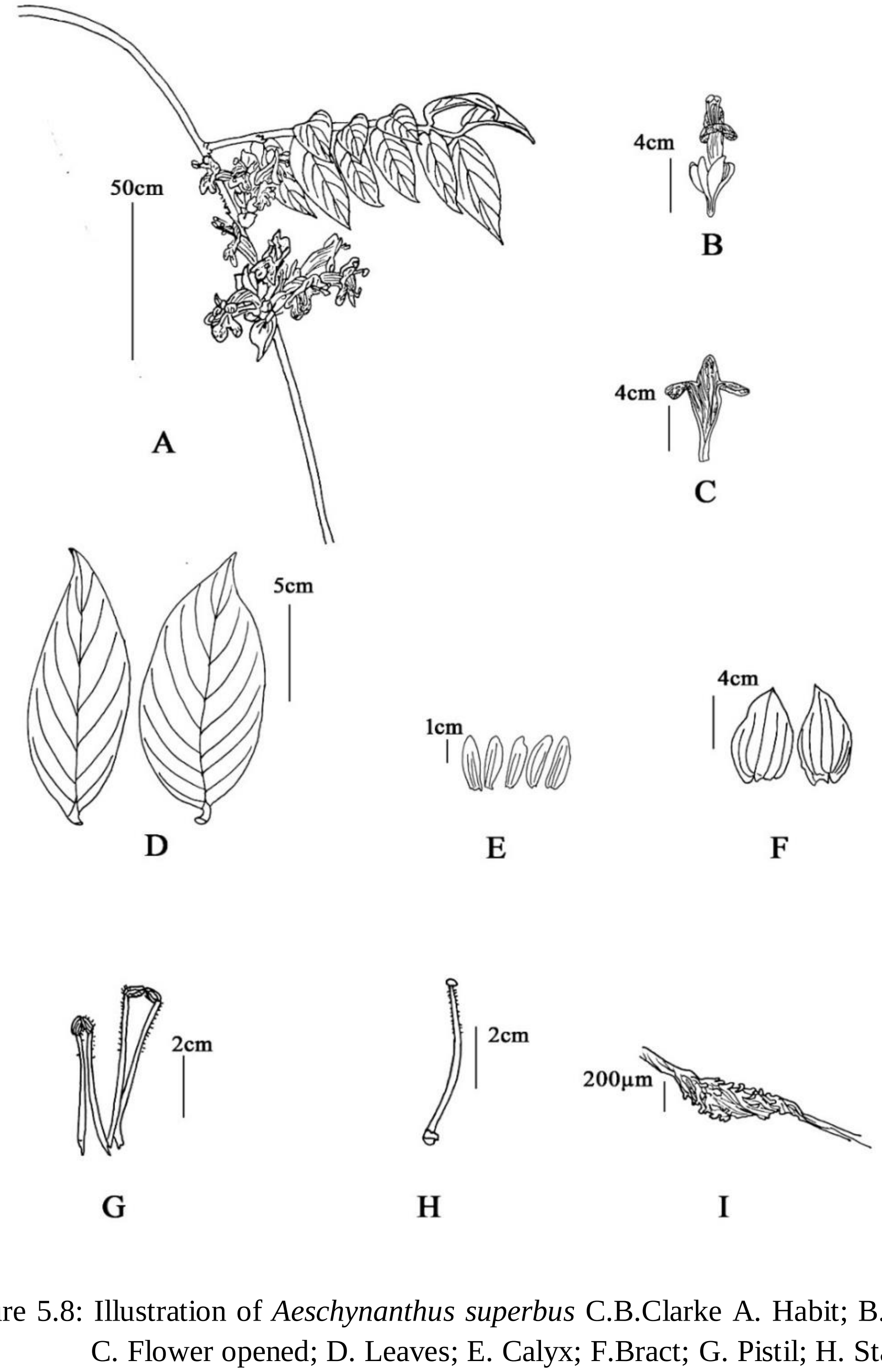

Figure 5.8: Illustration of *Aeschynanthus superbus* C.B.Clarke A. Habit; B. Flower; C. Flower opened; D. Leaves; E. Calyx; F.Bract; G. Pistil; H. Stamens; I. Seed.

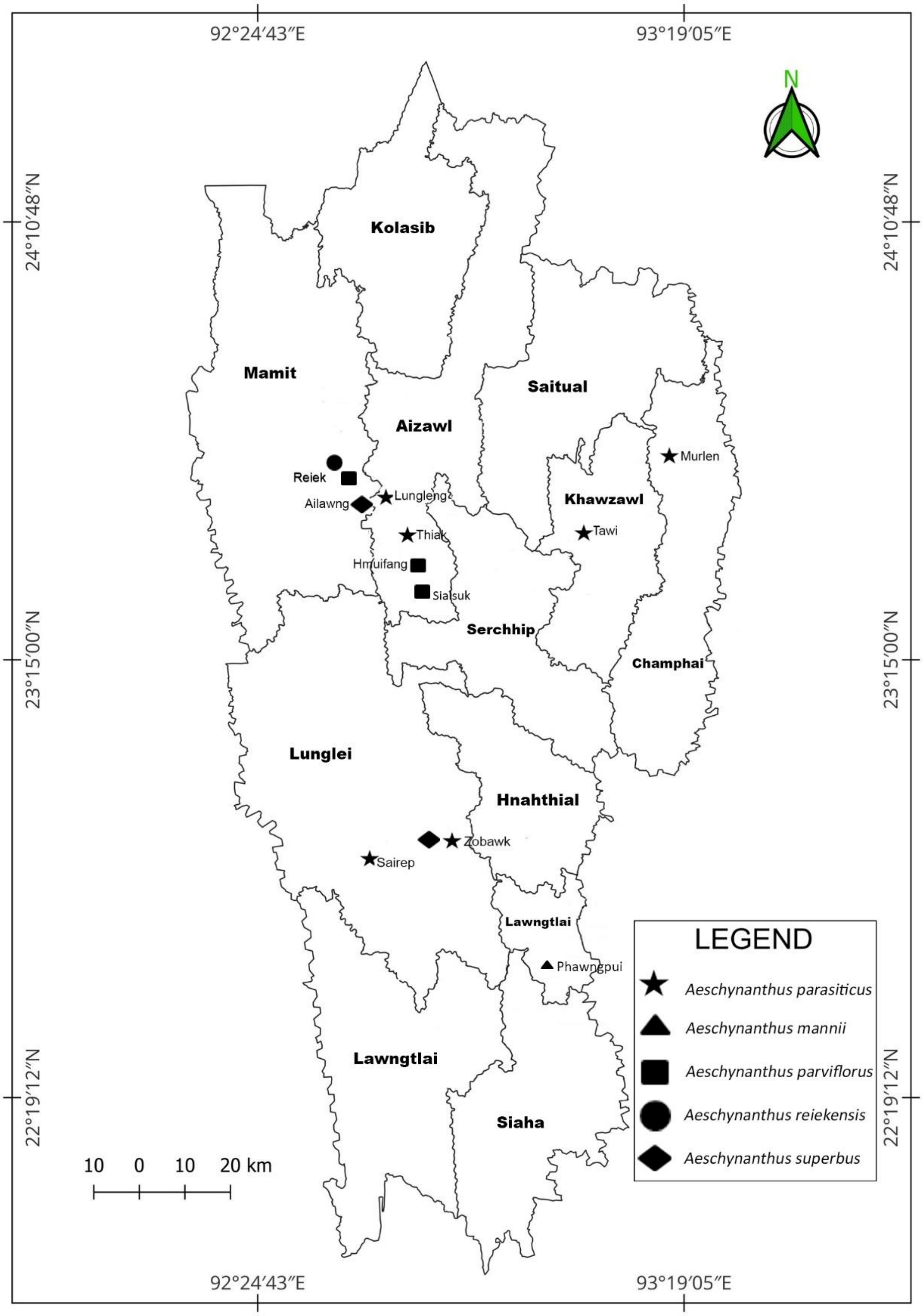

Figure 5.9: Distribution map of *Aeschynanthus* Jack species in Mizoram.

3. *Chrysothemis* Decne.

Chrysothemis pulchella (Donn ex Sims) Decne. Rev. Hort. (Paris), sér. 3, 3: 242 (1849).

Synonym*:* *Besleria pulchella* Donn ex Sims in Bot. Mag. 28: t. 1146 (1808); *Episcia pulchella* (Donn ex Sims) Mart. in Nov. Gen. Sp. Pl. Bras. 3: 39 (1829); *Skiophila pulchella* (Donn ex Sims) Hanst. in Linnaea 26: 207 (1854); *Tussacia pulchella* (Donn ex Sims) Rchb. ex Walp. in Repert. Bot. Syst. 6: 740 (1847). (**Figure No. 5.10; Plate No. 7**)

Type: Jan, 1990 Schomburgk (s.n.) K000450083

Taxonomic description: Perennial, 20–80 cm long, stem sparsely pubescent, succulent, green. Leaves opposite decussate, 11–24 × 4.6–11.2 cm long, pubescent on upper surface but glabrous on lower surface, margin serrate, base oblique, secondary venation 6–9, rough. Petiole 5–30 mm long, succulent, green. Bract pubescent, 10 mm long. Pedicel 10 mm long, pubescent. Calyx 5, up to 20 mm long, brick red, teeth pubescent, serrate. Inflorescence corolla 2.5 cm long, tubular, pubescent at lower tube, yellow, 5 lobes, inner lobe having maroon streaks. Stamen 4, 12 mm long, glabrous; filament 12 mm long, coil at the upper end. Staminode absent. Pistil glabrous, 15 mm long, ovary inferior, *c*. 3 mm long, stigma bilobed, 3 mm long, style 9 mm long. Capsule ovoid.

Phenology: Flowering from July to October and fruiting from September to November.

Habitat and Ecology: The plant is often cultivated for ornamental and decorative uses. It is also commonly found to be naturalized in proximity to gardens, alongside roads and near houses as an escapee.

Distribution: Mizoram (Bethlehem, Lungleng, Tanhril; **Figure 5.11**); India (Kerala, Manipur); Central America and South America

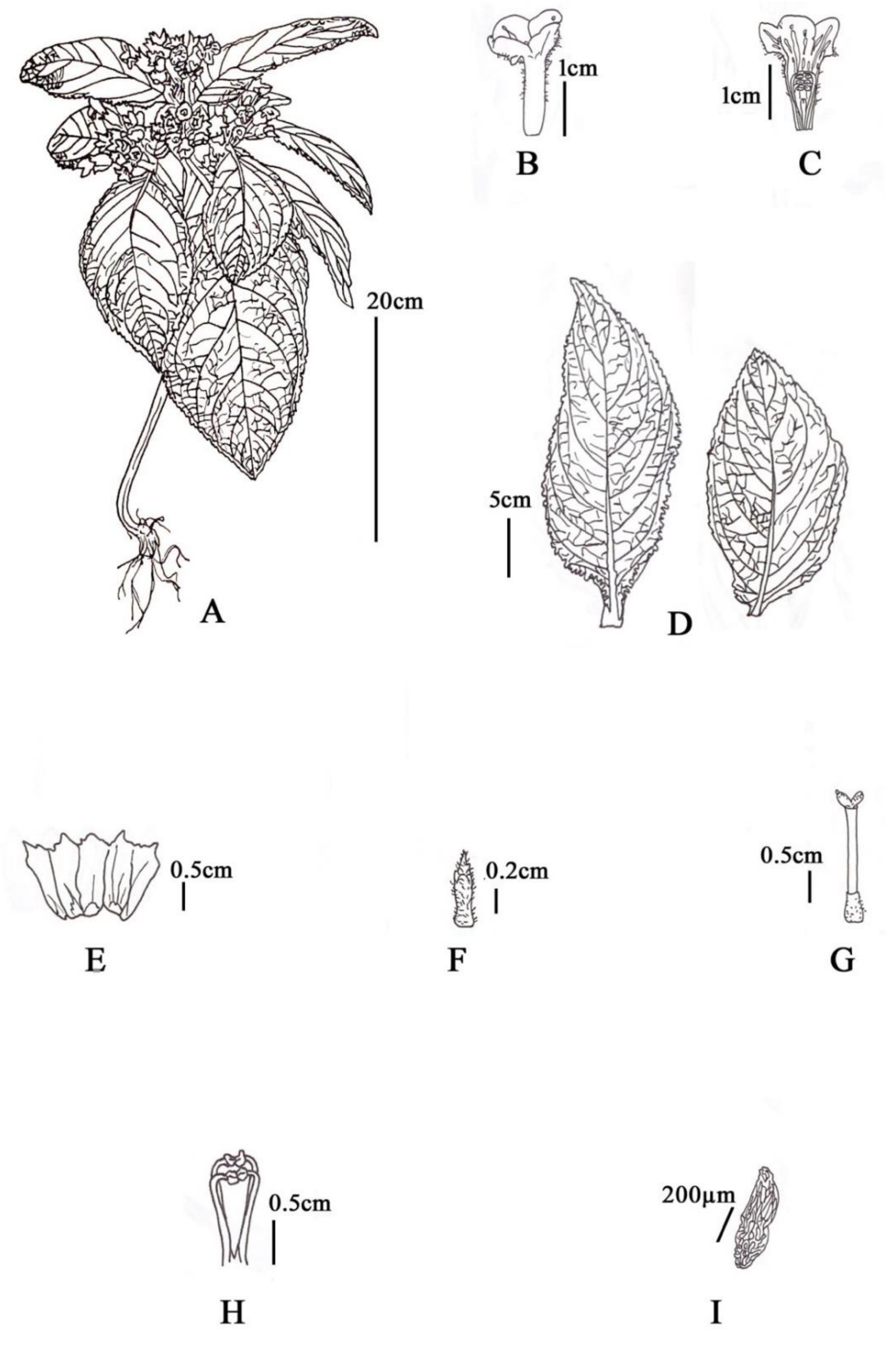

Figure 5.10: Illustration of *Chrysothemis pulchella* (Donn ex Sims) Decne. A. Habit; B. Flower; C. Flower opened; D. Leaves; E. Calyx; F. Bract; G. Pistil; H. Stamens; I. Seed.

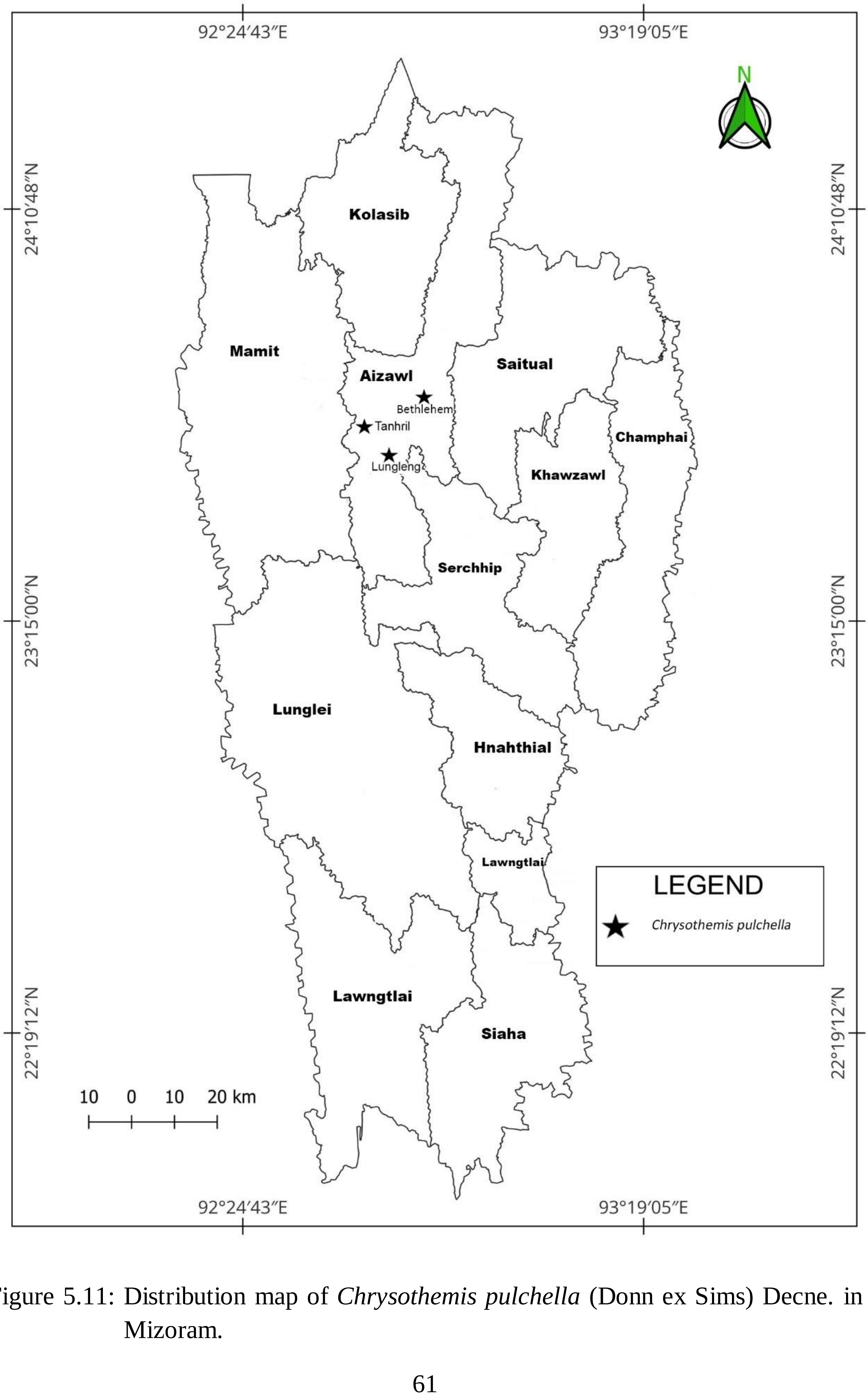

Figure 5.11: Distribution map of *Chrysothemis pulchella* (Donn ex Sims) Decne. in Mizoram.

Specimens examined: India, Mizoram, Aizawl District, Tanhril (Mizoram University), 23°73′86.0″N, 92°67′01.0″E, 890 m elevation, 23 September 2021, Margaret Lalhlupuii 128823 (MZUH129812); P00587406 (P).

4. *Didymocarpus* Wall.

Epiphytic or lithophytic herbs, caulescent or acaulescent. Stem pubescent, terete. Leaves opposite, decussate, anisophyllous to subequal. Inflorescence cyme,3–22flowered. Calyx 3–partite to 5–partite, glabrous or pubescent. Corolla funnel, tubular or infundibuliform, purple, white to pale pink, yellow to orange in colour. Stamens 2. Staminode absent, 2 or3. Stigma peltate or capitate. Ovary linear. Capsule linear up to Pollen monad, isopolar and tricolpate. Seed numerous.

Key to the species

1a. Epiphytic herbs, acaulescent…………………….…………..…**5. *D. vickifunkiae***

1b. Lithophytic herbs, caulescent………………………………..…………………**2**

2a. Calyx 3-partite, exine ornamentation nano-reticulate…….…...…**2. *D. lineicapsa***

2b. Calyx 5-partite, exine ornamentation micro-reticulate………………......………**3**

3a.Stigma capitate……………..……,,……………………...……**4. *D. wengeri***

3b. Stigma peltate……………………….………………………………………**4**

4a. Corolla white; seed cell character auriculate.............................**1. *D. adenocarpus***

4b. Corolla orange; seed cell character tuberculate…………..……....**3. *D. parryorum***

1. *Didymocarpus adenocarpus* C.E.C.Fisch., Kew Bull. 1929: 253. 1929 & Rec. Bot. Surv. India 12 (2): 118. 1938; Kanjilal & al., Fl. Assam 3: 396. 1939. G. P. Sinha in G.P. Sinha & et. al., Fl. Mizoram 2: 206. 2012; B.K. Sinha & Su.Datta, Nelumbo 58: 17. 2016. (**Figure No. 5.12; Plate No. 8**)

Type: India. Assam (= Mizoram): Southern Lushai Hills, 4500 ft., Sept. 1928, Rev. W.J.L. Wenger 239, K (K000820546!).

Taxonomic description: Herbs, terrestrial, lithophytic, 10–26 cm tall. Stem pubescent, 9–12 cm long, terete, green. Leaves opposite decussate, anisophyllous, 2–5 pairs, opposite arrangement, base cordate to oblique, margin serrate to crenulate, pubescent on both upper and lower surface, adaxially green, abaxially pale green, 15–21.8 × 4–11 cm long, lateral vein 8 to 9 pairs. Petiole 10–30 mm long, pubescent, green, terete. Inflorescence 3–22 flowered, axillary. Peduncle 9–11 mm long, glabrous, green, terete. Secondary peduncle 28 mm long, pubescent, green, terete. Pedicel 4–7 mm long, glabrous, green, terete. Calyx 8–10 mm long, glabrous, campanulate, white, transparent, blunt short broadly, triangular five lobes, narrowly funnel shape. Bract two, white, glabrous, transparent, 5–7 × 6–8 mm long, sub-orbicular, obtuse. Corolla 25–35 mm long, five lobes 4–5 × 4–5 mm long, whitish at corolla tube and purple at upper tube lobes, glabrous, tubular and slightly bending, infundibulum towards mouth. Stamen two, inserted at one third of the corolla tube length; anther dorsifixed; filament 5–7 mm long, white, glabrous. Staminode two, white, *c*. 2 mm long. Pistil 15–18 mm long, ovary linear 2 mm long, glabrous; stigma 2–4 mm long, peltate, glabrous; style 12–12 mm long. Capsule linear, brown, 3–4 cm long.

Phenology: Flowering from August to September and fruiting from September to November.

Habitat and Ecology: Grows on rock and shaded wall, at high altitude ranging from 1200 to 1500 m.

Distribution: Mizoram (Reiek, Ailawng; **Figure 5.17**)**,** Endemic.

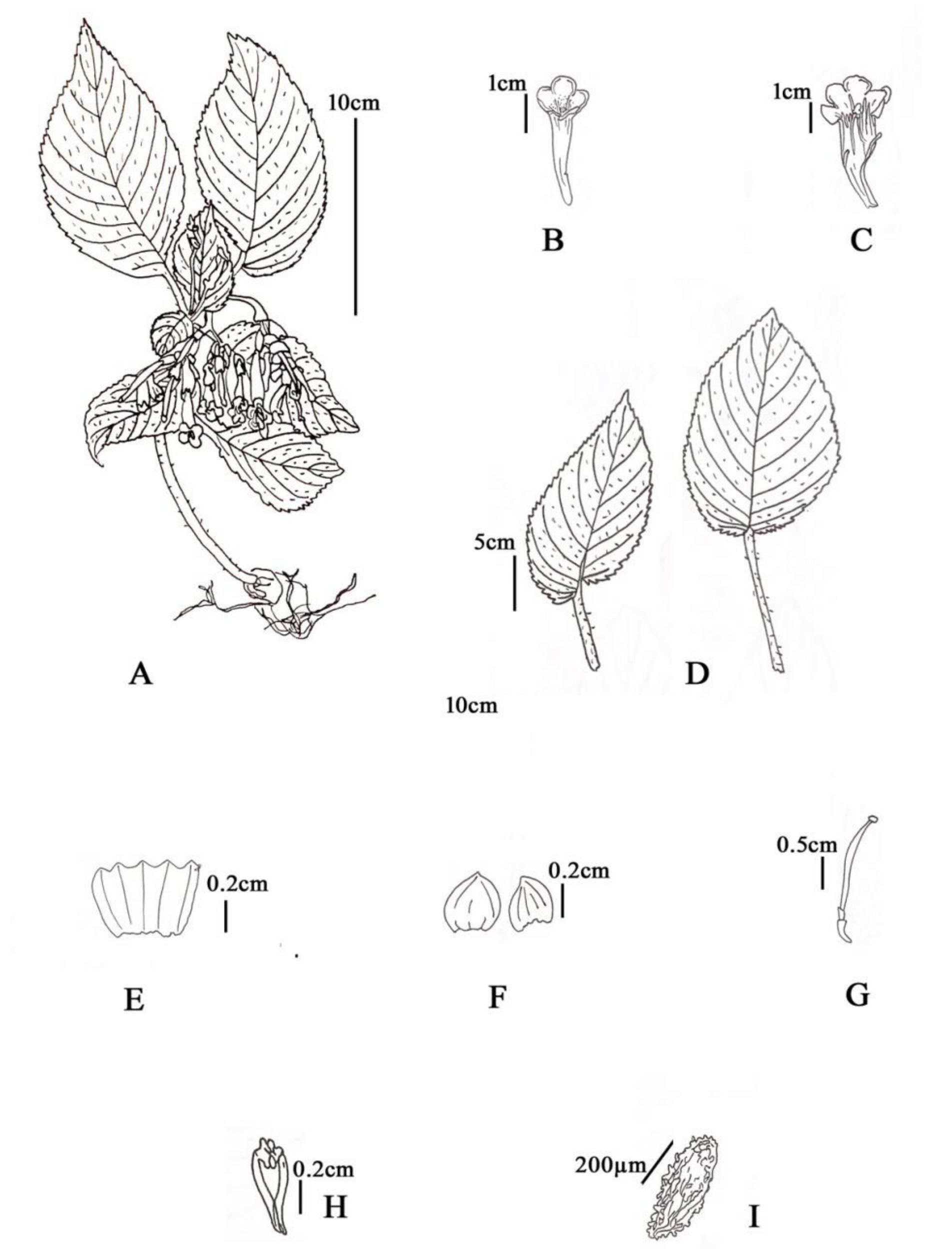

Figure 5.12: Illustration of *Didymocarpus adenocarpus* C.E.C.Fisch. A. Habit; B. Flower; C. Flower opened; D. Leaves; E. Calyx; F. Bract; G. Pistil; H. Stamens; I. Seed.

Specimens examined: INDIA. Mizoram, Mamit District, Reiek village, 23°68′35.1″ N 92°60′78.7″, 1384 m elevation, on 12th August 2022, Margaret Lalhlupuii128824 (MZUH000041); K000820547 (K).

2. ***Didymocarpus lineicapsa*** (C.E.C.Fisch.) B.L.Burtt, Notes Roy. Bot. Gard. Edinburgh 21(4): 187. 1954. G.P. Sinha in Sinha & et. al., Fl. Mizoram 2: 206. 2012; B.K. Sinha & Su.Datta, Nelumbo 58: 17. 2016. (**Figure No. 5.13; Plate No. 9**).

Synonym: *Trisepalum lineicapsa* C.E.C.Fisch., Bull. Misc. Inform. Kew 1928 (7): 276 (1928).

Type: India. Assam (= Mizoram): Lushai Hills, Aijal (= Aizawl), 1225 m, September 1927, Mrs N.E. Parry No.79, K (K000820539!).

Taxonomic description. Herbs, lithophytic, terrestrial, up to 26 cm tall, 1 to 3stemsarising from the same rhizome. Stem 2–11.5 cm long, pubescent, terete, erect, green. Leaves 2–3 pairs, opposite decussate, anisophyllous, often whorled at top, 6.5–17 × 3.5–10 cm long, margin serrate to dentate, base oblique, apex acute, lanceolate to narrowly elliptic, lateral veins 6 to 9 pairs. Petiole 50–28 mm long, pubescent, terete, green. Inflorescence axillary, 1 to 14 flowered. Calyx 6–10 mm long, maroon, tripartite, linear lanceolate, pubescent, free to base, third segment tridentate. Peduncle 15–17 × 30–35 mm long, pubescent, green to pink. Corolla 20–30 mm long, tubular, five lobed, purple, light purple at base and dark purple towards the throat and lobes, pubescent at lower surface of lobes and tube glabrous. Stamen two; filament inserted at one third of the corolla tube, 5–7 mm long, glabrous. Staminode absent. Pistil 1.2–2 cm long, glabrous; stigma dark purple, capitate; style 10–15 mm long, glabrous. Disc 2 mm, ovary linear, glabrous, white. Capsule upto 3.1 cm.

Phenology: Flowering from August to September and fruiting from September to December.

Habitat and Ecology: Lithophytic herb growing on moist surface of the rock and on steep clayey banks along the roads in shaded.

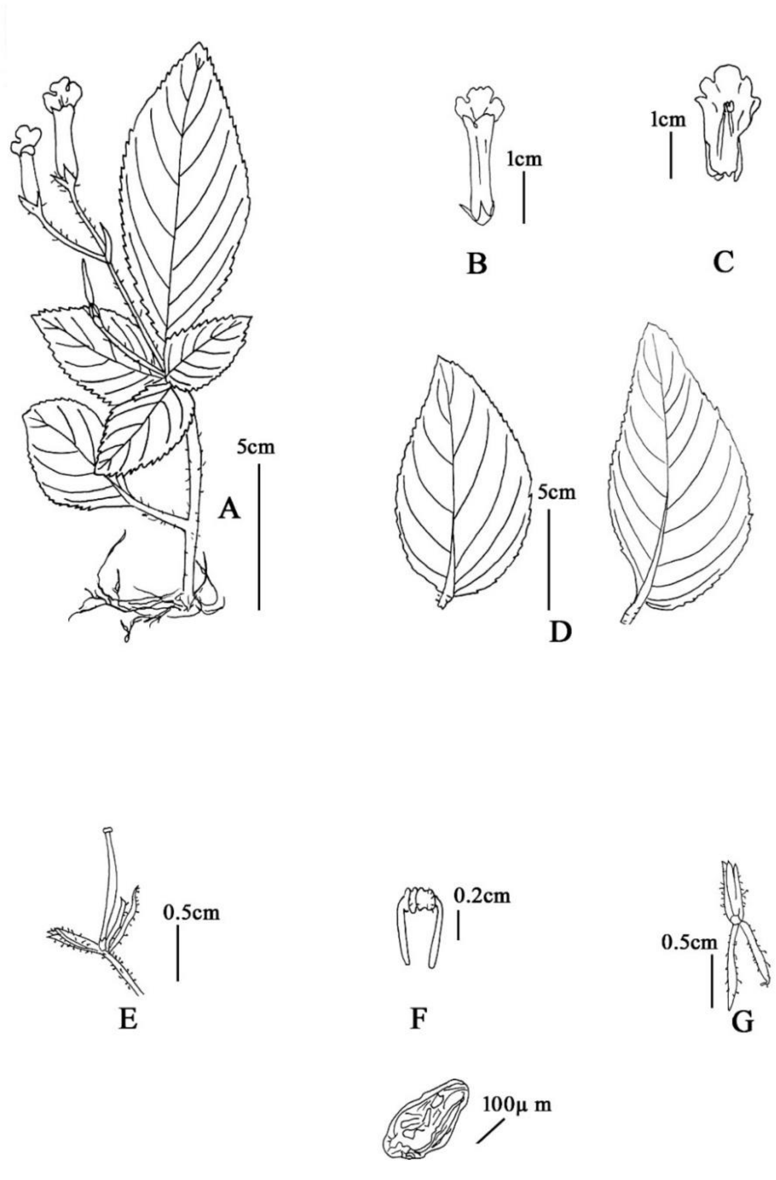

Figure 5.13: Illustration of *Didymocarpus lineicapsa* (C.E.C.Fisch.) B.L.Burtt A. Habit ; B. Flower; C. Flower opened; D. Leaves; E. Calyx; F.Bract; G. Pistil; H. Stamens; I. Seed.

Distribution: Mizoram (Muthi, Reiek, Tamdil, Tanhril; **Figure 5.17**). Endemic.

Specimens examined: INDIA. Mizoram, Aizawl District, Muthi Park, 23°76′68.3″ N 92°75′96.2″ E, 1090 m elevation, on 12th September 2022, Margaret Lalhlupuii128825 (MZUH000043); K00O820541 (K)

3. *Didymocarpus parryorum* C.E.C.Fisch., Kew Bull. 1928: 141. 1928 et Rec. Bot. Surv. India 12 (2): 118. 1938. G.P. Sinha in G.P. Sinha & et. al., Fl. Mizoram 2: 207. 2012; B.K. Sinha & Su.Datta, Nelumbo 58: 19. 2016. (**Figure No. 5.14; Plate No. 10**

Type: India, Mizoram (previously Assam), Lushai Hills at Sairep, 1700 m, July 1926, Parry 7 (K, photo!)

Taxonomic description: Terrestrial herbs, lithophytic, 7 to 30 cm tall. Stem sparsely pubescent, erect, terete. Leaves opposite, subequal in pairs, arranged in whorl, sessile, cordate, pubescent, margin serrate, 7.5–13 × 6.5–10 cm long, lateral venation 4 to 6 pairs. Petiole 15–60 mm long, sparsely pubescent, terete. Inflorescence 5 to 21 flowered. Peduncle 15–40 mm long, glabrous, terete. Secondary peduncle 70–76 mm long, glabrous, terete. Pedicel 4–5 mm long, glabrous, terete. Secondary pedicel 18–30 mm long, glabrous, green, terete. Calyx 5, lanceolate, 12–20 × 3–7 mm long, yellow, glabrous, divided up to base. Bract yellow, glabrous, 6–10 × 3–5 mm long. Secondary bract yellow, glabrous, 5–10 × 40–50 mm long. Corolla orange glabrous, tubular, tip infundibuliform, 22–28 × 25–30 mm long, five lobes, inner corolla having dark orange streak, epipetalous, glabrous. Stamen 2, filaments glabrous, 40–50 mm long staminodes 2, short, linear. Pistil l0 mm long, ovary linear, *c*. 1 mm long; stigma peltate. Capsule linear, 15 – 30 mm long. Seeds ellipsoid, glabrous.

Phenology: Flowering from August to September and fruiting from September to November.

Distribution: Mizoram (Sairep and Lawngtlai; **Figure 5.17**). Endemic.

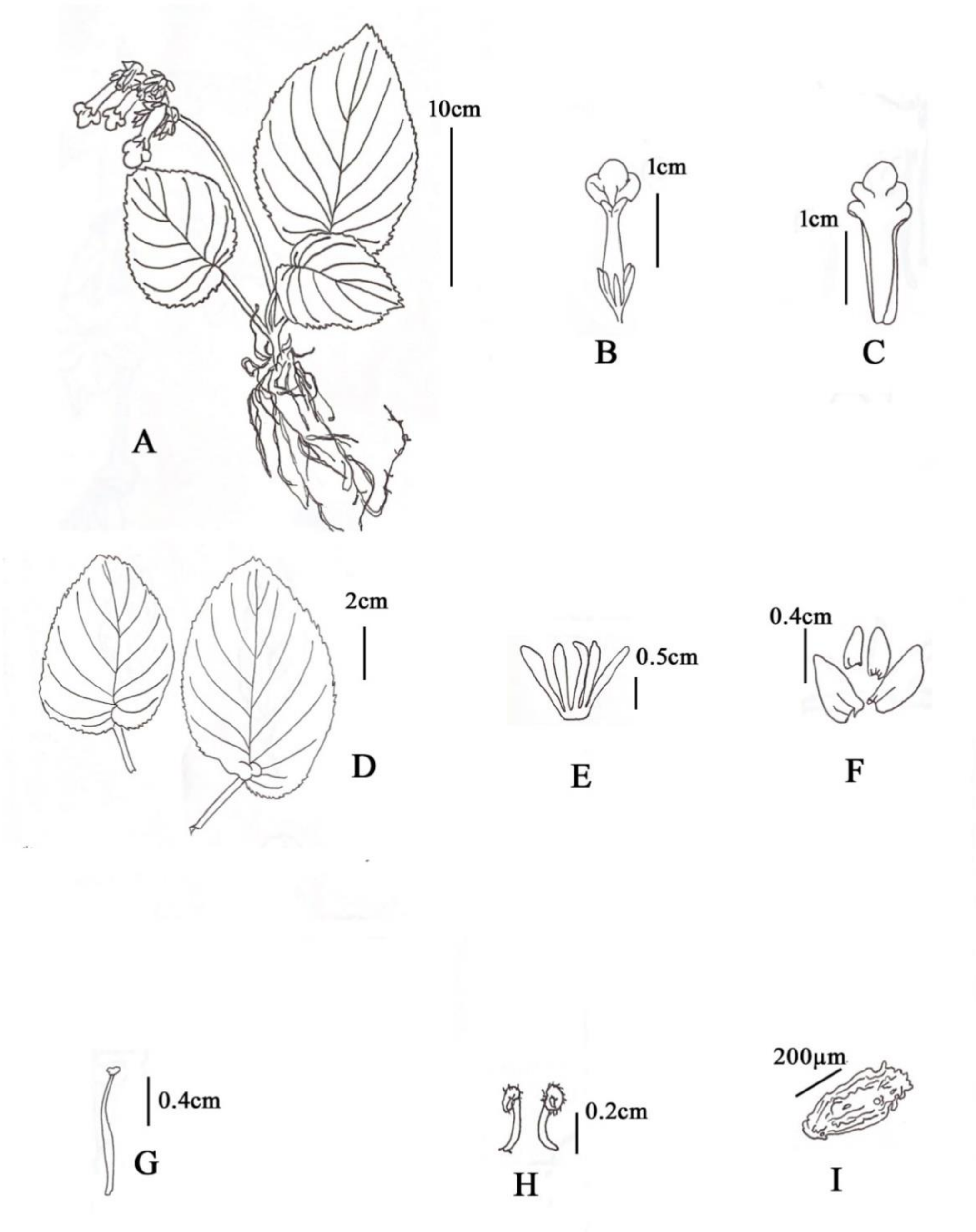

Figure 5.14: Illustration of *Didymocarpus parryorum* C.E.C.Fisch. A. Habit; B. Flower; C. Flower opened; D. Leaves; E. Calyx; F. Bract; G. Pistil; H. Stamens; I. Seed.

Habitat and Ecology: This plant is generally found growing on moist loamy banks in partially shaded tropical wet evergreen forests.

Specimens examined: INDIA. Mizoram, Lunglei District, Sairep village, 22°83′84.0″ N 92°81′67.4″, 1349 m elevation, on 8th September 2022 Margaret Lalhlupuii128826 (MZUH000044).

4. *Didymocarpus wengeri* C.E.C. Fisch., Kew Bull. 1928: 74. 1928; et Rec. Bot. Surv. India 12 (2): 118.1938. G.P. Sinha in G.P. Sinha & et. al., Fl. Mizoram 2: 208. 2012; B.K. Sinha & Su.Datta, Nelumbo 58: 21. 2016. (**Figure No. 5.15; Plate No. 11**)

Type: India, Mizoram (previously Assam), South Lushai Hills, 2500 ft., Sept. 1927, Wenger (K, photo!)

Taxonomic description: Terrestrial herbs, lithophytic, 15 cm tall. Stem 2 to 5 cm long, sub-acaulescent, terete, green, pubescent. Leaves 1 to 4 pairs, opposite decussate, anisophyllous, terminal pairs are smaller in size, 7–11.5 × 5–10.5 cm long, margin serrate, pubescent, abaxial leave whitish green and adaxial leave green. Petiole 20–50 mm long, terete, pubescent, brownish green. Inflorescence 5–8 cm long, hirsute. Peduncle 70–90 mm long, glandular hirsute. Bracts 1–2 mm in diameter, reddish brown, orbicular, sparsely hirsute on upper surface, glandular hirsute on lower surface. Pedicel 2–4 mm long, glandular hirsute. Calyx 5-sect from base, free, segments 3–4 mm long, linear lanceolate, glabrous. Corolla lobe yellow and tube orange,glabrous to capitates glandular hairy, tube infundibuliform, 8–10 mm long; lobes orbicular, 4–5 mm wide. Stamens 2, glabrous, filament 4–5 mm. Pistil 7–8 mm long, Ovary linear elliptic, glabrous; style 4 mm, equals the length of the ovary; stigma capitate, 2–lobed. Capsule 1.5 cm long, brown.

Phenology: Flowering during September and fruiting from September to November.

Distribution: Mizoram (Tuipang; **Figure 5.17**). Endemic.

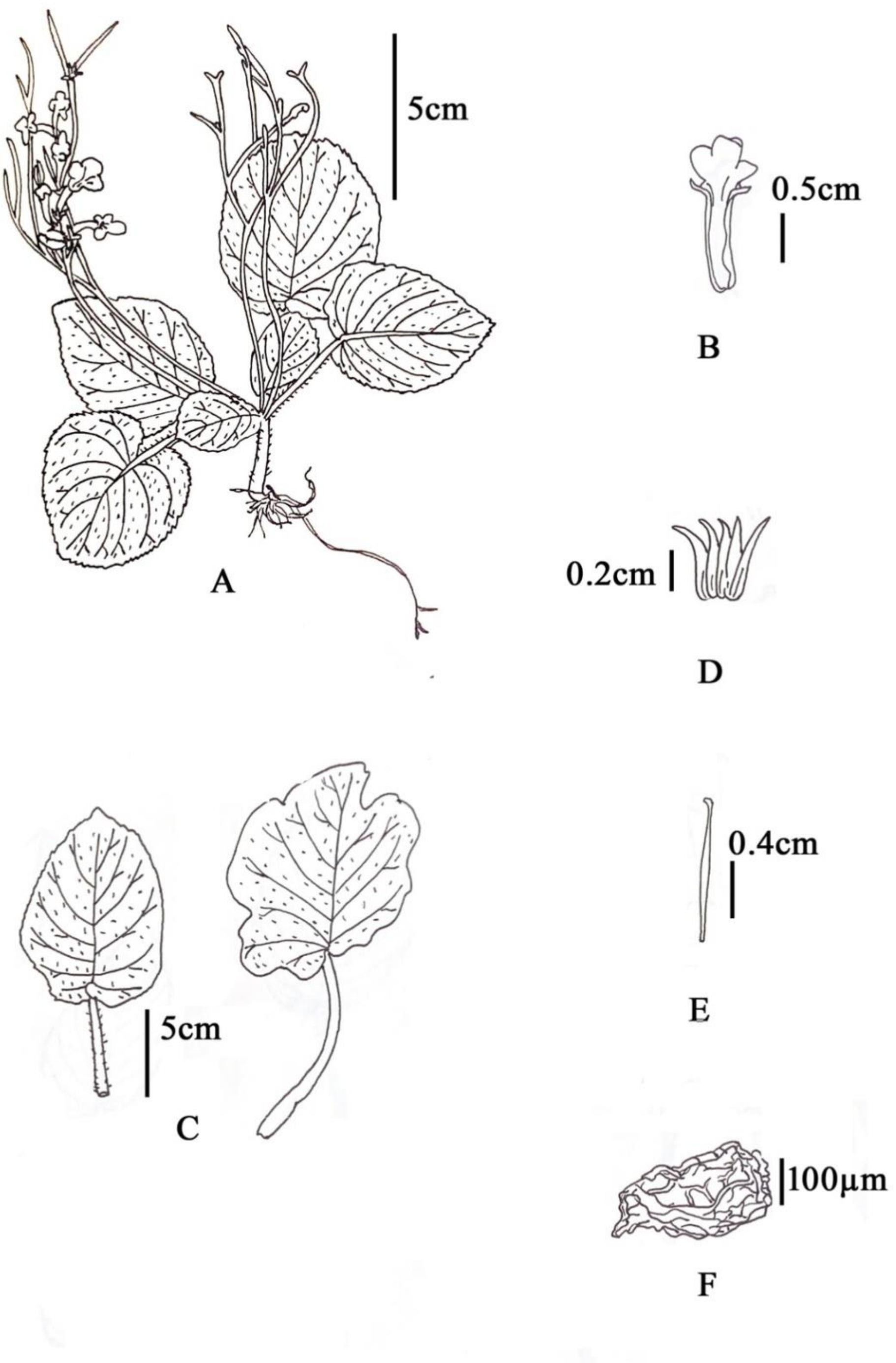

Figure 5.15: Illustration of *Didymocarpus wengeri* C.E.C.Fisch. A. Habit; B. Flower; C. Leaves; D. Calyx; E. Pistil; F. Seed.

Habitat and Ecology: Growing on steep clay banks along the roads in partially shaded, tropical wet evergreen forests.

Specimens examined: INDIA. Mizoram, Siaha District, Tuipang village, 22°30′48.4″ N 93°02′70.9″, 1426 m elevation, on 30th September 2022, Margaret Lalhlupuii128827 (MZUH000045); K000820530 (K).

5. *Didymocarpus vickifunkiae* V.Gowda & N.S.Prasanna, sp.nov.

Type: INDIA. Mizoram: Mamit district Reiek Tlang, 1278 m, 23°40048.240N, 92°37037.230E, 07 August 2018, N.S. Prasanna VG2018MZ2490 (holotype: BHPL!; isotypes: VG2018MZ2491, ASSAM!; VG2018MZ2492, BHPL!). (**Figure No. 5.16; Plate No. 12**)

Taxonomic description: Terrestrial herbs, epiphytic, up to 23 cm tall. Root fibrous, thin, whitish. Acaulescent, no branching. Leaves 2–4 pairs, 10–17.5 × 6–9 cm long, opposite, symmetrically ovate, base oblique, margin shallowly serrate, pubescent, deep midrib, lateral veins 7 pairs, apex obtuse, adaxial light green and abaxial white. Petiole terete, 20–35 mm long, light green, pubescent. Inflorescence cyme 3 to 6 flowered. Peduncle 22–50 mm long, white, pubescent, terete. Pedicle 4–10 mm long, pubescent, white. Bracts two, symmetric, 4–6 × 2–3 mm long, pubescent, lanceolate. Calyx lobed, divided up to the base, symmetrically lanceolate, curve at the apex, pubescent at outer surface and inner surface glabrous, white in colour, 80–12 × 2–40 mm long, apex acute. Corolla funnel form, 35–40 mm long, pubescent, white to pale pink in colour with maroon streaks, tube 13–20 mm long, anterior lip 3–lobed, orbicular, posterior lip– 2 lobed. Stamen 2 inserted at the middle of tube, long, glabrous on the lower-part, upper-part pubescent, light-yellow in colour, epipetalous; filament 3–5 mm long, glabrous; anther 2 mm long, bearded, white. Staminodes 3 in number. Pistil pubescent, 20–25 mm long, white; style 15–20 mm long; ovary linear, 3–4 mm long, stigma peltate.

Phenology: Flowering from August to September and fruiting from September to November.

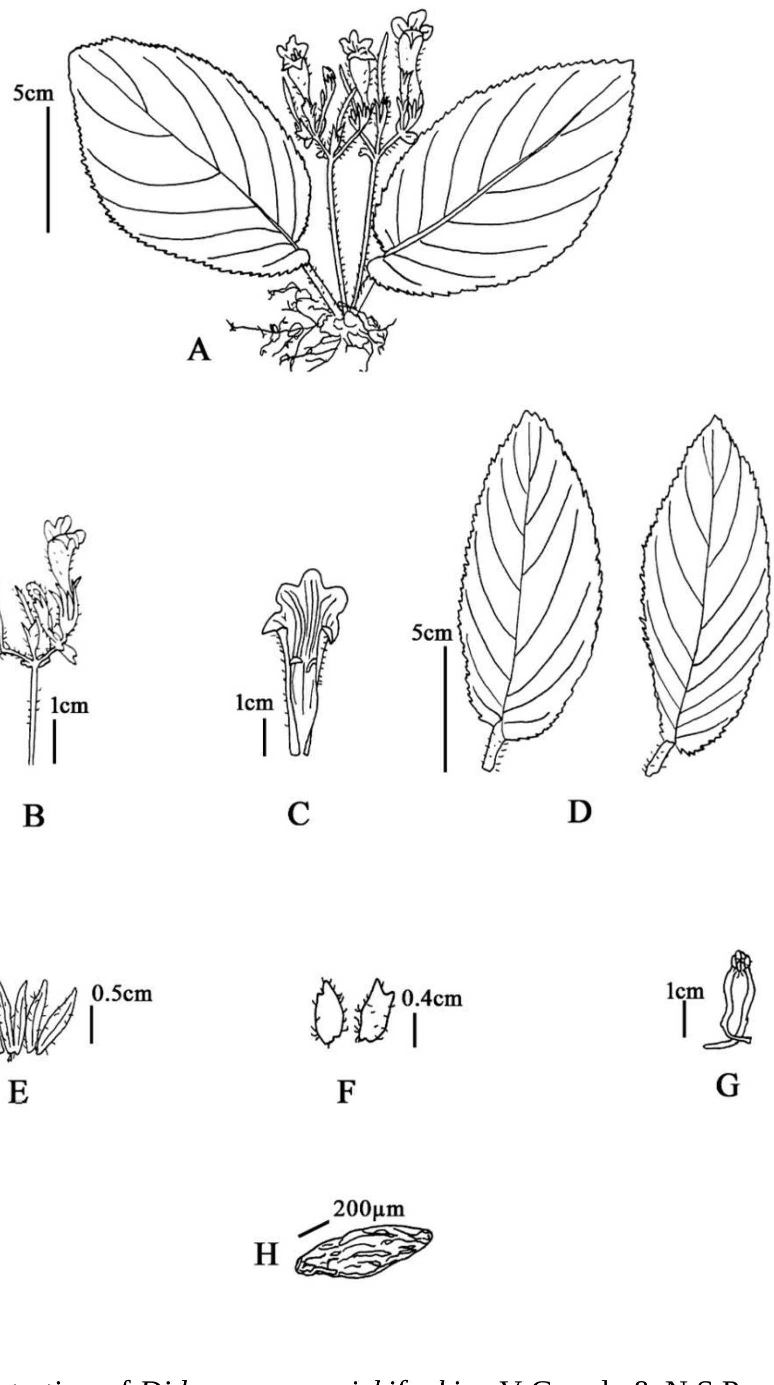

Figure 5.16: Illustration of *Didymocarpus vickifunkiae* V.Gowda & N.S.Prasanna, A. Habit; B. Inflorescence; C. Flower opened; D. Leaves; E. Calyx; F. Bract; G. Stamen; H. Seed.

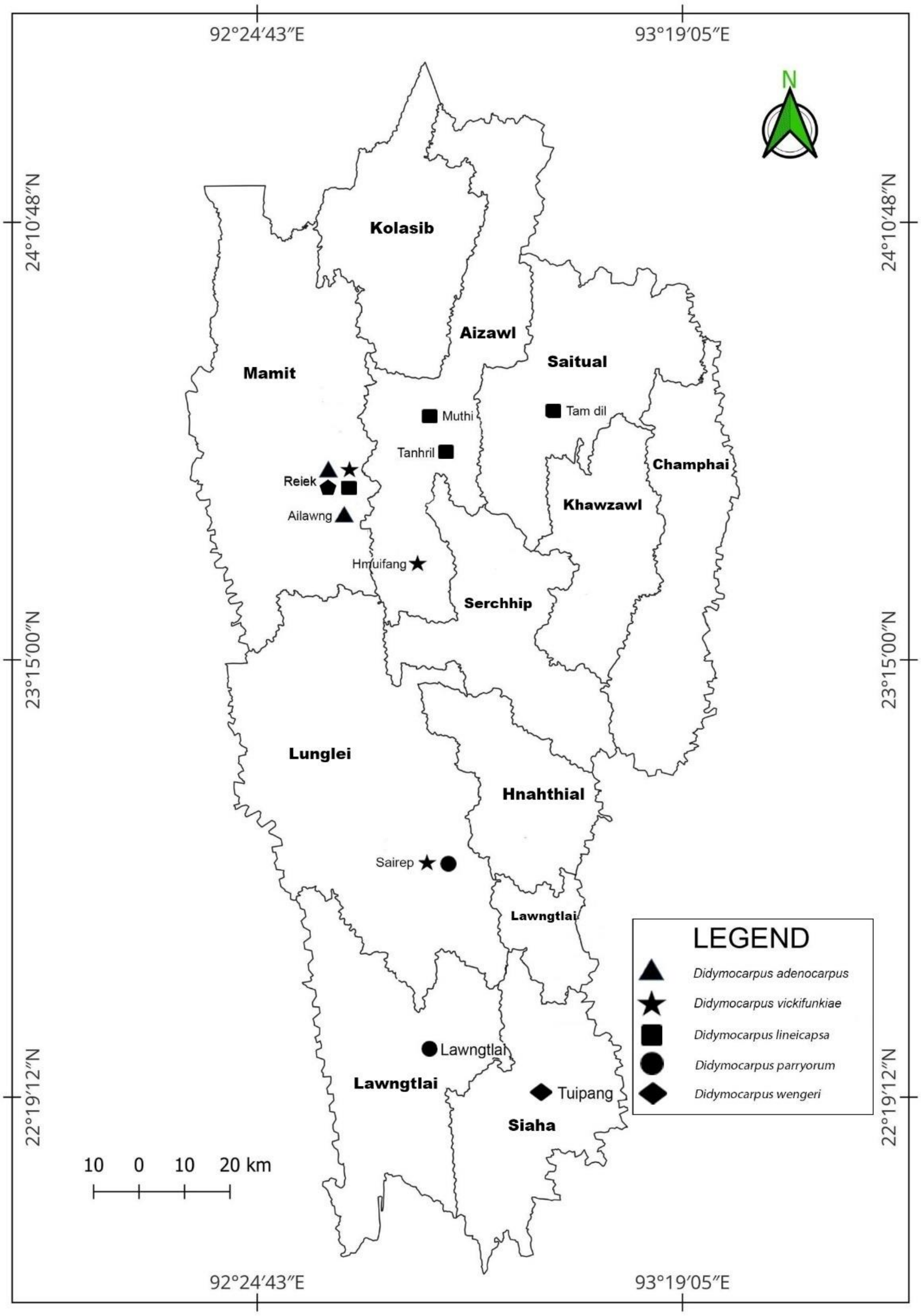

Figure 5.17: Distribution map of *Didymocarpus* Wall.species in Mizoram.

Habitat and Ecology: Epiphytic herb,grows on tree trunk and branches along with mosses.

Distribution: Mizoram (Sairep, Hmuifang, Reiek; **Figure 5.17**).

Specimens examined: INDIA. Mizoram, Lunglei District, Sairep village, 22°83′84.0″ N 92°81′67.4″, 1349 m elevation, on 8th September 2022, Margaret Lalhlupuii128828 (MZUH000042); Mamit district, Reiek Tlang, 07 July 2018, N.S. Prasanna VG2018MZ2480 (BHPL!); Mamit district, Reiek Tlang, 07 July 2018, N.S. Prasanna VG2018MZ2493 (BHPL!)

5. *Epithema* Blume

Epithema carnosum Benth., Scroph. Ind. 57. 1835; C.B. Clarke, Commelyn. Cyrtandr. Bengal 129, t. 90.1874 & in Hook.f., Fl. Brit. India 4: 369. 1884; Kanjilal & al., Fl. Assam 3: 399. 1939; G.P. Sinha in G.P. Sinha & et. al., Fl. Mizoram 2: 208. 2012; B.K. Sinha &Su.Datta, Nelumbo 58: 21. 2016. (**Figure No. 5.18; Plate No. 13**)

Synonym: *Aikinia carnosa* G. Don Gen.Syst. 4: 665. 1838

Type: Napalia, 1832, Wall. Cat. Num. List no. (K, n.v.) Herbs.

Taxonomic treatment: Perennial herbs, lithophytic, terrestrial, 21cm tall. Stem pubescent, succulent, green to maroon. Lower leaves large, pubescent, opposite, 7.5−11×5.5−11cm long, ovate to cordate, base oblique, margin serrate, lateral venation 4 to 6 pairs. Upper leaves small, sessile to subsessile, 2−4 × 2−3 cm long. Petiole 52 mm long, pubescent, succulent. Inflorescence crowded in curled, 1- sided scorpioid cymes, 1 to 4 flowered. Peduncle 18 mm long, pubescent, green. Pedicel 3 mm long, glabrous. Bract obovate, green, 8−10 × 12−14 mm long, covering a part of inflorescence, margin serrate, pubescent. Calyx pubescent, 5 lobed, whitish green, 3.5–4 × 1–2 mm long. Corolla purplish white, tubular, 8–11 mm long, five lobed, corolla tube 7–8 mm long, corolla lobe 2–3 mm long. Stamen 2; filaments 2–3 mm

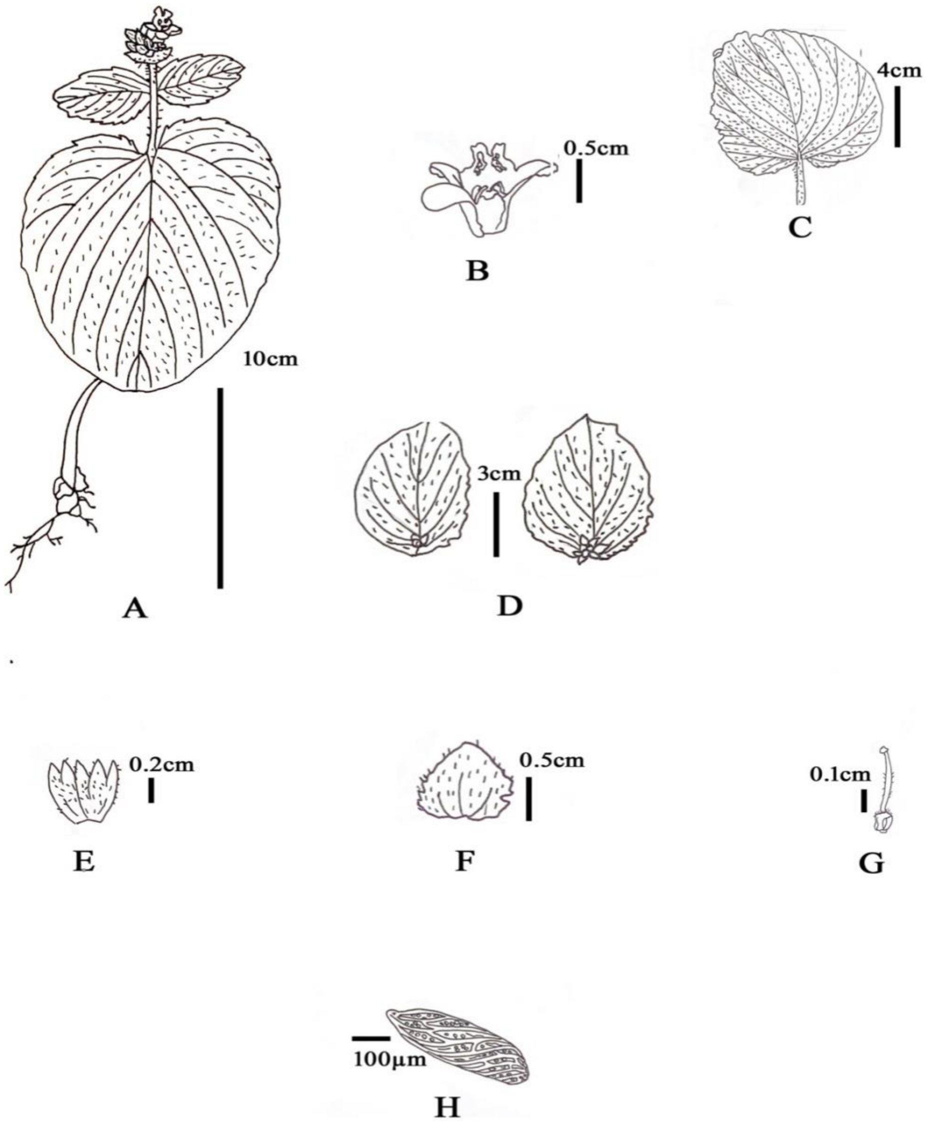

Figure 5.18: Illustration of *Epithema carnosum* Benth. A. Habit; B. Flower; C. Larger leaves; D. Smaller leaves; E. Calyx; F. Bract; G. Pistil; H. Seed.

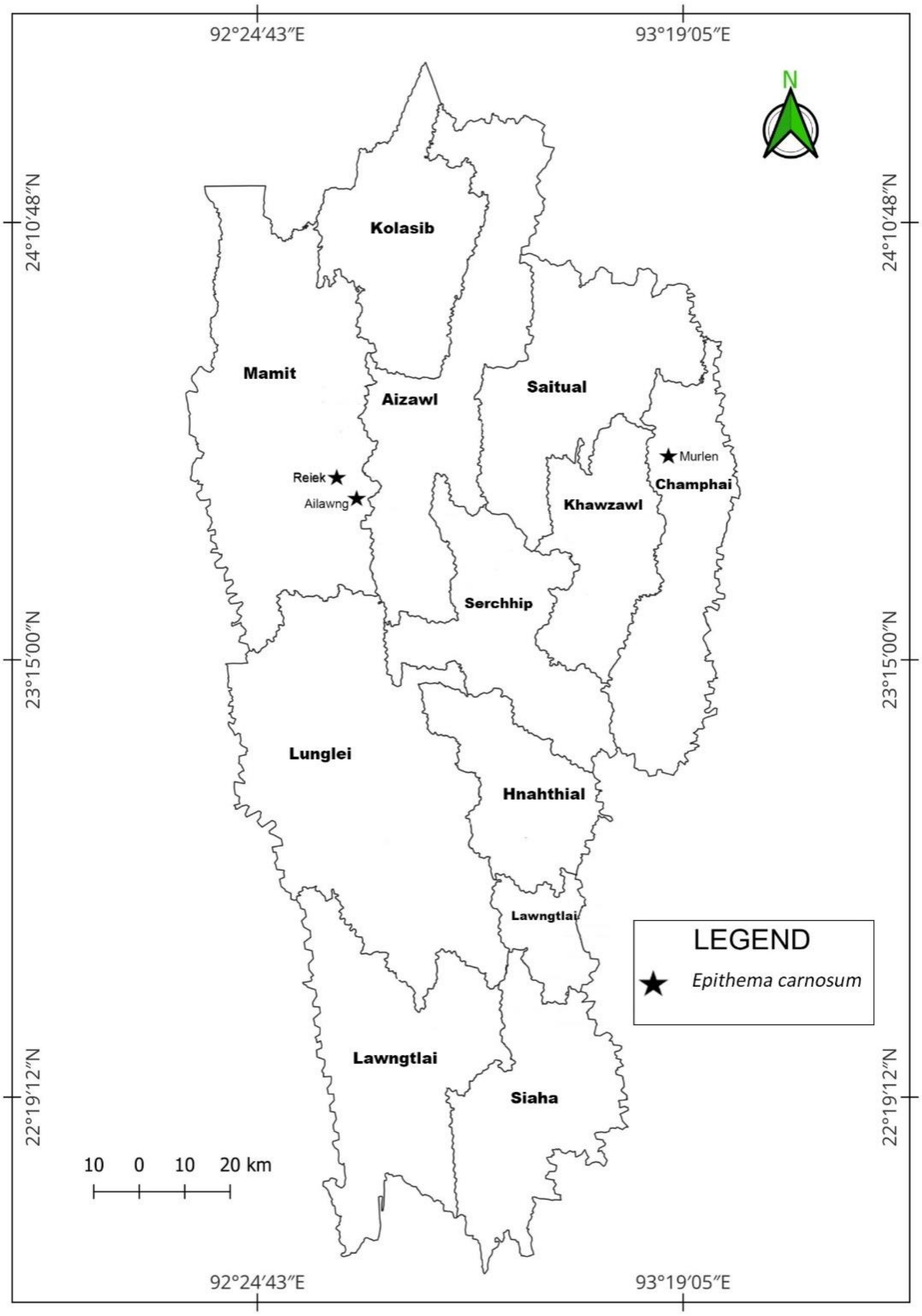

Figure 5.19: Distribution of *Epithema carnosum* Benth. in Mizoram.

long, glabrous; anther coherent, c. 1 mm long. Staminode 2, *c.* 1 mm long. Pistil 4 mm long; ovary inferior, *c.*1 mm long, ovoid to globose; style 3 mm long, pubescent; stigma *c.* 1 mm long, sub-capitate. Capsules globose to subglobose.

Phenology: Flowering from August to September and fruiting from September to November.

Habitat and Ecology: Grows in moist and rocky surface near waterfall or stream in association with *Begonia hatacoa* and *Impatiens tripetala.*

Distribution: Mizoram (Ailawng, Murlen, Reiek; **Figure 5.19**); India (Andaman and Nicobar Island, Andhra Pradesh, Arunachal Pradesh, Maharashtra, Meghalaya, Karnataka, Kerala, Sikkim, Tamil Nadu and West Bengal); Bhutan, China, Laos, Myanmar, Nepal, Sri Lanka, Thailand and Vietnam.

Specimen examined: INDIA. Mizoram, Champhai District, Murlen National Park, 23°66′49.7″ N 93°26′60.5″, 1262 m elevation, on 29th August 2022, Margaret Lalhlupuii128829(MZUH000046); 2nd Aug. 1870, C. B. Clarke12300C (CAL); 8th Aug. 1872; Khasi, Jaintia Hills (Syndai – Muktapur), 17th Aug. 1968, N. P. Balakrishnan 46147 (CAL); K001089588 (K).

6. *Henckelia* Spreng.

Annual herbs, non-rhizomatous. Stem erect, terete, pubescent or glabrous. Leaves opposite decussate. Inflorescence cyme axillary, 1–19 flowered. Calyx free to campanulate. Corolla white or purple colour. Stamen 2. Staminode 2. stigma bilobed or flabellate. Pollen monad, isopolar and tricolpate. Seed numerous, elliptic to narrowly elliptic.

1a. Stem glabrous; calyx campanulate; stipule absent; stigma bilobed; pollen grain is of small size, prolate; seed cell character tuberculate………..…..…**1. *H. anachoreta***

1b. Stem pubescent; calyx free; stipule present; stigma flabellate; pollen grain is medium size, perporate; seed cell character ocellate……………………**2. *H. pumila***

1. *Henckelia anachoreta* (Hance) D.J. Middleton & Mich. Möller in A. Weber &al.,Taxon60 : 774. 2011. B.K. Sinha &Su.Datta, Nelumbo 58: 24. 2016. (**Figure No. 5.20; Plate No. 14**)

Synonym: *Chirita anachoreta* Hance, Ann. Sci. Nat., Bot., sér. 5. 5: 231. 1866; Wang & al., Flora of China 18: 343. 1998.

Type: China, 27 Jul. 1864, G. Theophilus Sampson s.n. (BM, photo!)

Taxonomic description. Annual herbs, non-rhizomatous. Stem erect, glabrous, 10–40 cm, terete, succulent. Leaves 6.5–12.5 × 2.8–5 cm long, opposite, decussate and unequal usually 2–6 pairs, blade oblique, lanceolate to ovate, sparsely pubescent, base oblique, margin dentate to serrate, apex acute, lateral vein 6–8 pairs. Petiole 5–12 × 2–3 mm, glabrous, reddish brown. Cymes axillary, 1–5 flowered. Peduncle 45–80 mm long, sparsely pubescent, scaly, green, terete. Bract 2, lanceolate to ovate 8 × 6 mm long, green, pubescent. Pedicel 8 × 12 mm long, glabrous, light green, terete. Calyx campanulate, glabrous, 20–24 mm long, 5 lobes, free above the middle, calyx tube 15–17 × 4–5 mm long, tube lobe 7 mm long, white, margin entire, apex acuminate. Corolla 40–48 mm long, tubular, white to off-white, purple at upper lobe, glabrous both side, corolla tube 35–37 mm long, lobes 6–11 × 4–5 mm, five lips, inner corolla having yellow streak. Stamen 2, filament 10 mm long, white, glabrous, anther fused, 2–3 mm. Pistil 3 cm long, pale green to white, ovary *c.* 2 mm, style pubescent, stigma bilobed, 3 mm long. Staminode 2, glabrous, 4 mm long, white and yellow at the tip. Capsule erect, glabrous, 4–12 cm long.

Phenology: Flowering from August to September and fruiting from September to October.

Habitat and Ecology: The plant grows on moist shaded areas, rocks in forests, valley streamside and damp shaded areas.

Distribution: Mizoram (Reiek,Sairep, Sihphir,Thiak; **Figure 5.22**); India (Nagaland, Arunachal Pradesh); China, Laos, Myanmar, Thailand, Vietnam.

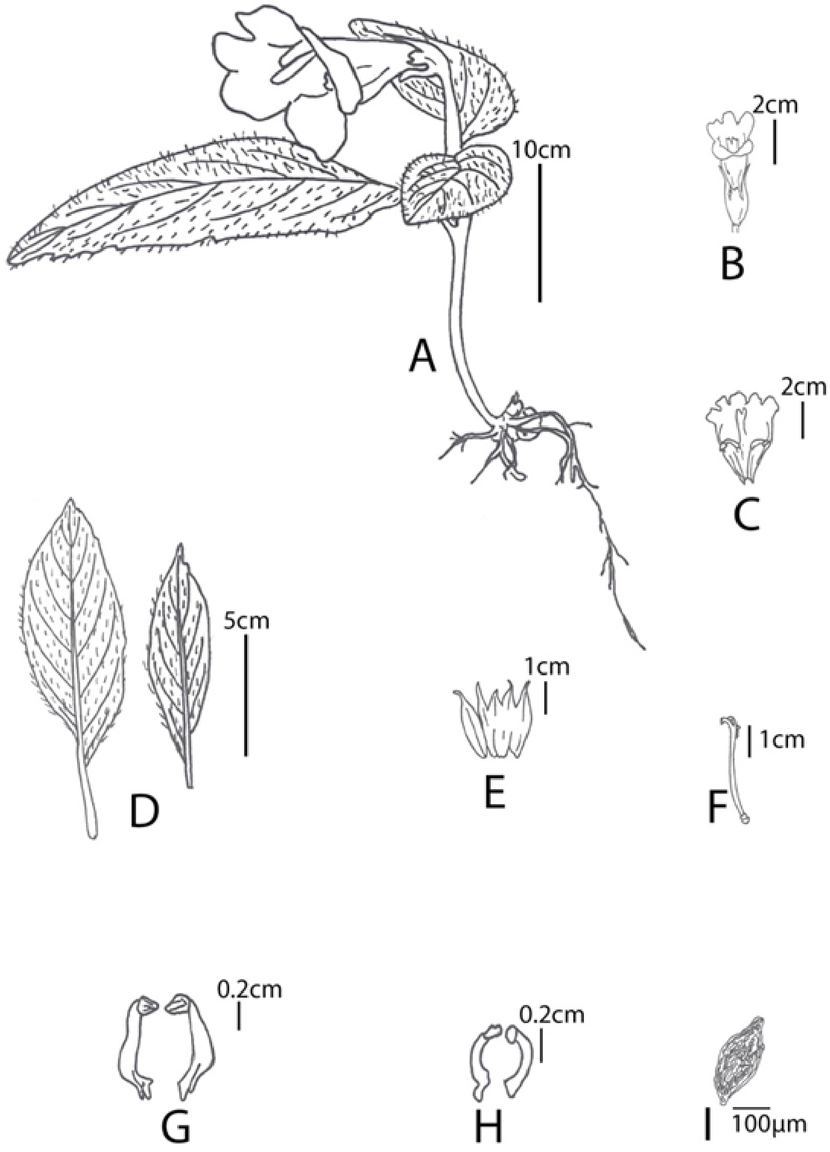

Figure 5.20: Illustration of *Henckelia anachoreta* (Hance) D.J.Middleton & Mich.Möller A. Habit; B. Flower; C. Flower opened; D. Leaves; E. Calyx; F. Pistil; G. Stamen; H. Staminode; I. Seed.

Specimens examined: INDIA. Mizoram, Aizawl District, Thiak village, 23˚46’90.9” N 92˚71’77.2” E, 1051 m elevation, on 24th September 2022, Margaret Lalhlupuii128830 (MZUH000047); K000858407 (K).

2. *Henckelia pumila* (D. Don) A. Dietr., Sp. Pl., ed. 6, 1: 574.1831; C.B.Clarke in Hook.f., Fl. Brit. India 4: 357. 1884.G.P. Sinha in G.P. Sinha & et. al., Fl. Mizoram 2: 205. 2012. (**Figure No. 5.21; Plate No. 15**)

Synonyms: *Chirita pumila* D.Don, Prodr. Fl. Nepal.: 90.1825; C.B.Clarke in Hook.f., Fl. Brit. India 4: 357. 1884; Giri& al., Mat. Fl. Arunachal Pradesh 2: 230. 2008.

Type: Nepal, Wallich 801 (K, photo!)

Taxonomic description. Annual herbs, non-rhizomatous, 23 cm tall. Stem 14.5 cm, erect, pubescent, terete, green, basal maroon. Leaves opposite, decussate, lanceolate to ovate, base oblique, margin serrate, purple to brown spotted on upper surface, pubescent, 17–21 × 8.5–11 cm long.Smallleaves 11.7–12 × 6.6 cm, margin serrate, pubescent, lateral veins 7–9 pairs. Stipule 1.5–2.5 × 0.8–1.2 cm, margin serrate, green, pubescent. Petiole 1.5–2 cm. Cyme axillary, 1–19 flowered. Bract two, green, pubescent, serrate margin, 7 × 4 mm. Peduncle 30–40 mm, green, glabrous. Pedicel 10 mm, glabrous, terete, green. Calyx free, densely pubescent, 10–15 mm long, lanceolate, 5 lobes, divided half the base, green, calyx tube 3 mm, lobe 12 mm. Corolla 40–48 mm, pubescent, funnel, five lobes, tube 34 mm, white, tube 14 mm, purple, inner corolla have yellow streak. Pistil 25 mm, pubescent, stigma flabellate, white, 3 mm. Staminode two, 5 mm, tip green. Stamen two, 10 mm long, anther basifixed 3 mm. Capsule erect, glabrous, 3–12 cm long.

Phenology: Flowering from July to September and fruiting from September to November.

Habitat and Ecology: The plants adapted to grow in high altitude region, mainly on streamside, river bank, moist rocks, cliffs, tree trunk or among grassy clumps.

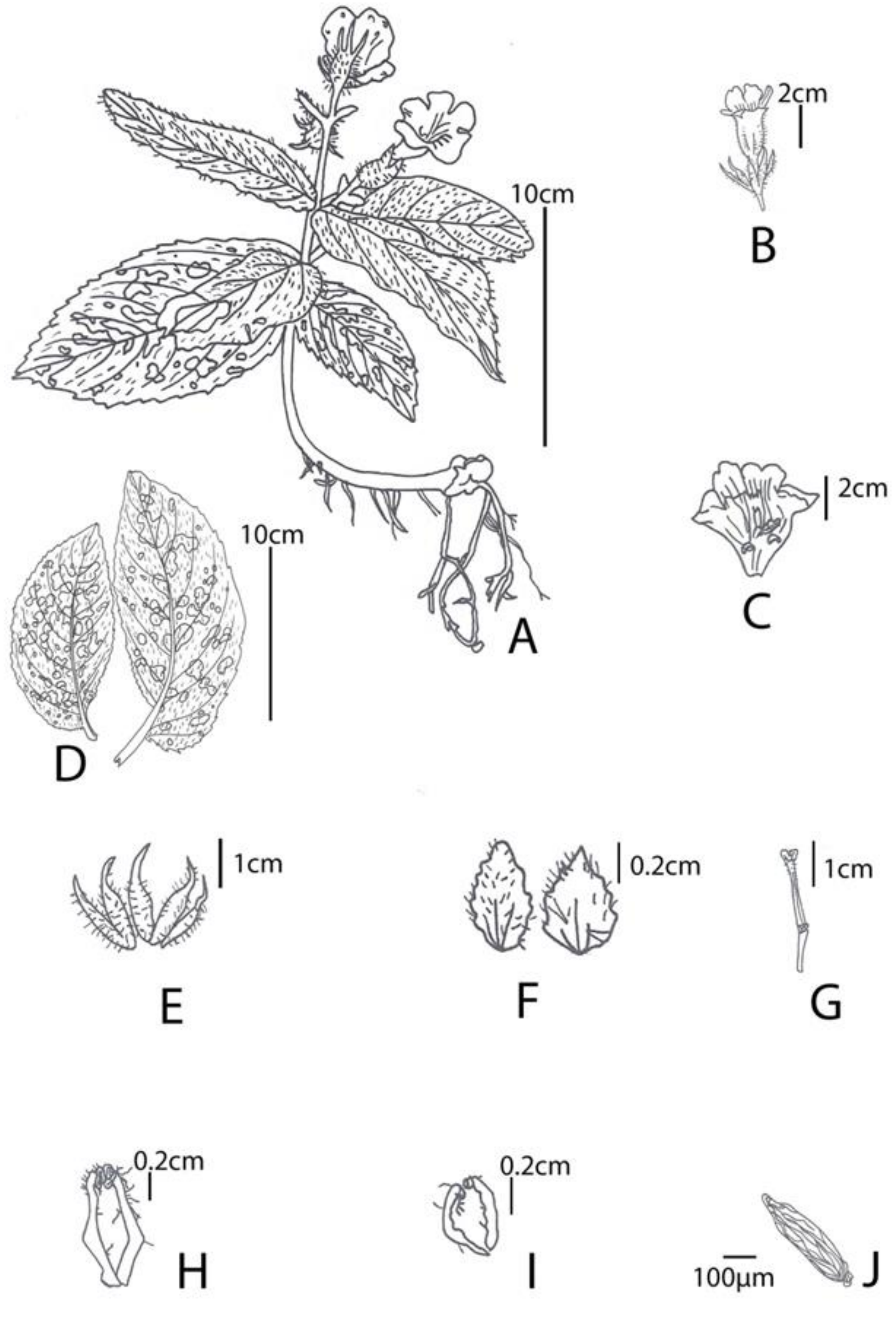

Figure 5.21: Illustration of *Henckelia pumila* (D.Don) A.Dietr.A. Habit; B. Flower; C. Flower opened; D. Leaves; E. Calyx; F. Bract; G. Pistil; H. Stamen; I. Staminode; J. Seed.

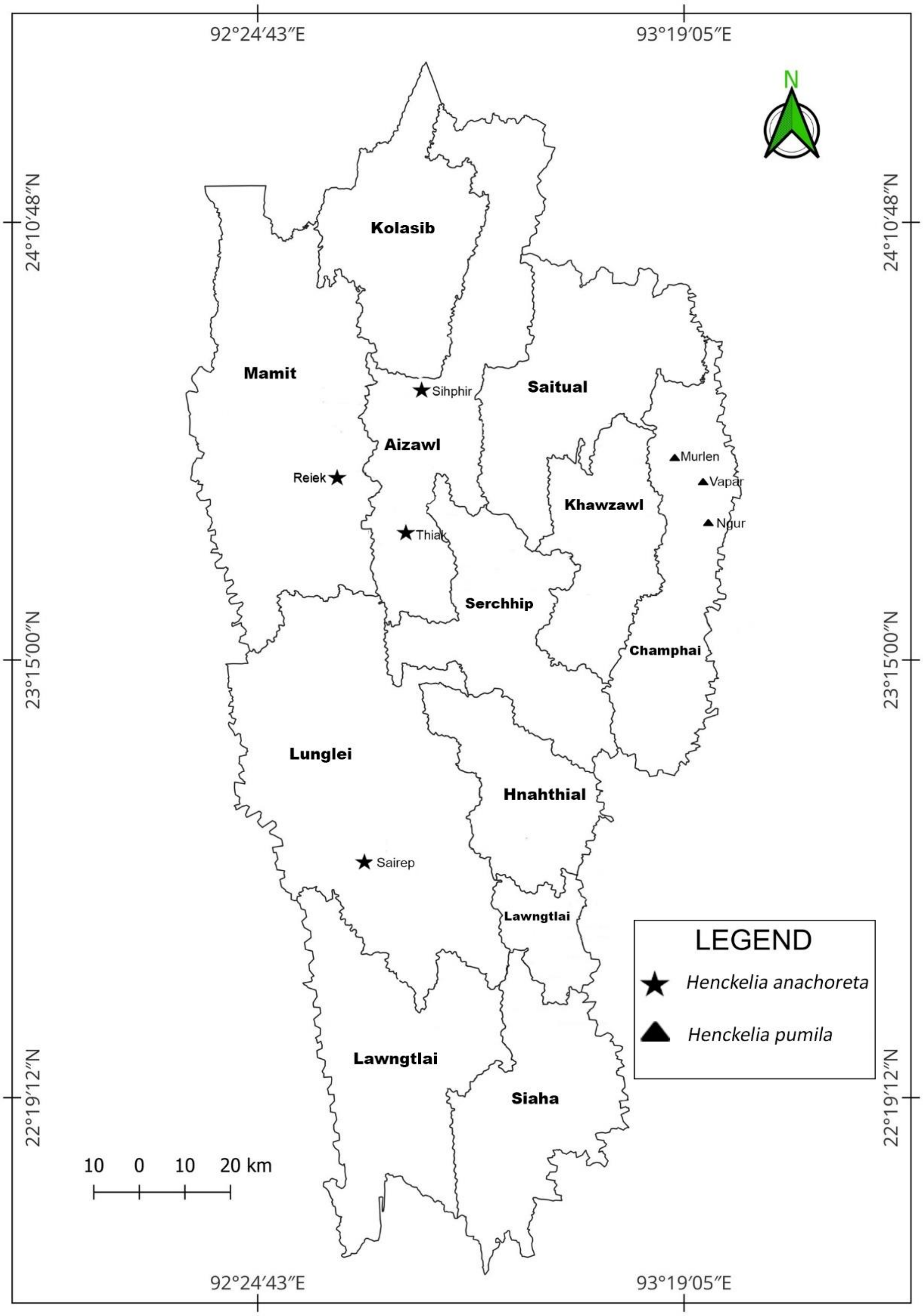

Figure 5.22: Distribution map of *Henckelia* Sprengs. species in Mizoram.

Distribution: Mizoram (Murlen, Ngur, Vapar; **Figure 5.22**); India (Assam, Himachal Pradesh, Meghalaya, Nagaland, Sikkim, Uttarakhand, West Bengal); Bhutan, Myanmar, Nepal, Thailand, Vietnam, China.

Specimens examined: INDIA. Mizoram, Champhai District, Murlen National Park, 23˚64'72.9" N 93˚29'13.7" E, 1638 m elevation, on 26th July 2022, Margaret Lalhlupuii128831 (MZUH000048); P.K. Hajra 54307 (ASSAM); 28.07. 1870, C. B. Clarke 12258 (CAL); K000858346 (K).

7. Genus *Lysionotus* D. Don

Lysionotus serratus D. Don, Edinburgh Philos. J. 7 (13): 86. 1822; Hook.f., Bot. Mag. 107.t. 6538. 1881; C.B. Clarke in A.DC.& C.DC., Monogr. Phan. 5: 58. 1883 & in Hook.f., Fl. Brit. India 4: 344. 1884; G.P. Sinha in G.P. Sinha & et. al., Fl. Mizoram 2: 209. 2012; B.K. Sinha &Su.Datta, Nelumbo 58: 32. 2016. (**Figure No. 5.23; Plate No. 16**)

Synonyms: *Lysionotus ternifolius* Wall., Pl. As. Rar.2: 20, t 118.1831; *Hemiboea himalayensis* Levl. In Rep. Sp. Nov 9: 329. 1910; *Lysionotus himalayensis* (Levl.) W. T. Wang and Z Y Li in Acta Phytotaxa. Sin 30: 481.1992; *Chirita polycarpa* Wall. In Numercl. Bot., ed. 2, 1:351. 1840; *Chirita ternifolia* Buch.-Ham.Ex D.Don in Prodr. Fl. Nepal.: 124. 1825; *Calosacme polycarpa* Wall. in Nomencl. Bot., ed. 2, 1: 351. 1840.

Type: Nepal, Wallich N., 1822, Wallich, N. 804 (K000831981)

Taxonomic description. Sub-shrubs, epiphytic, erect, 5 to 70 cm tall. Stem glabrous, terete, having maroon patches. Leaves ternate, opposite decussate, elliptic to narrow lanceolate, base oblique, apex acuminate, margin serrate, having leathery texture, 5–21.8 × 1.3–6 cm long, adaxial leaves green, abaxial leaves whitish green, lateral venation 4–5 pairs, base cuneate to rounded. Petiole 2–20 mm long, glabrous, green. Inflorescence terminal to sub-terminal, 2 to 17 flowered. Peduncle 10–17 mm long, green, glabrous, terete. Secondary peduncle 25 mm long, glabrous, green, terete. Pedicel 10–15 mm long, terete, green, glabrous. Bract 2, green, 4–8 × 2–4 mm long,

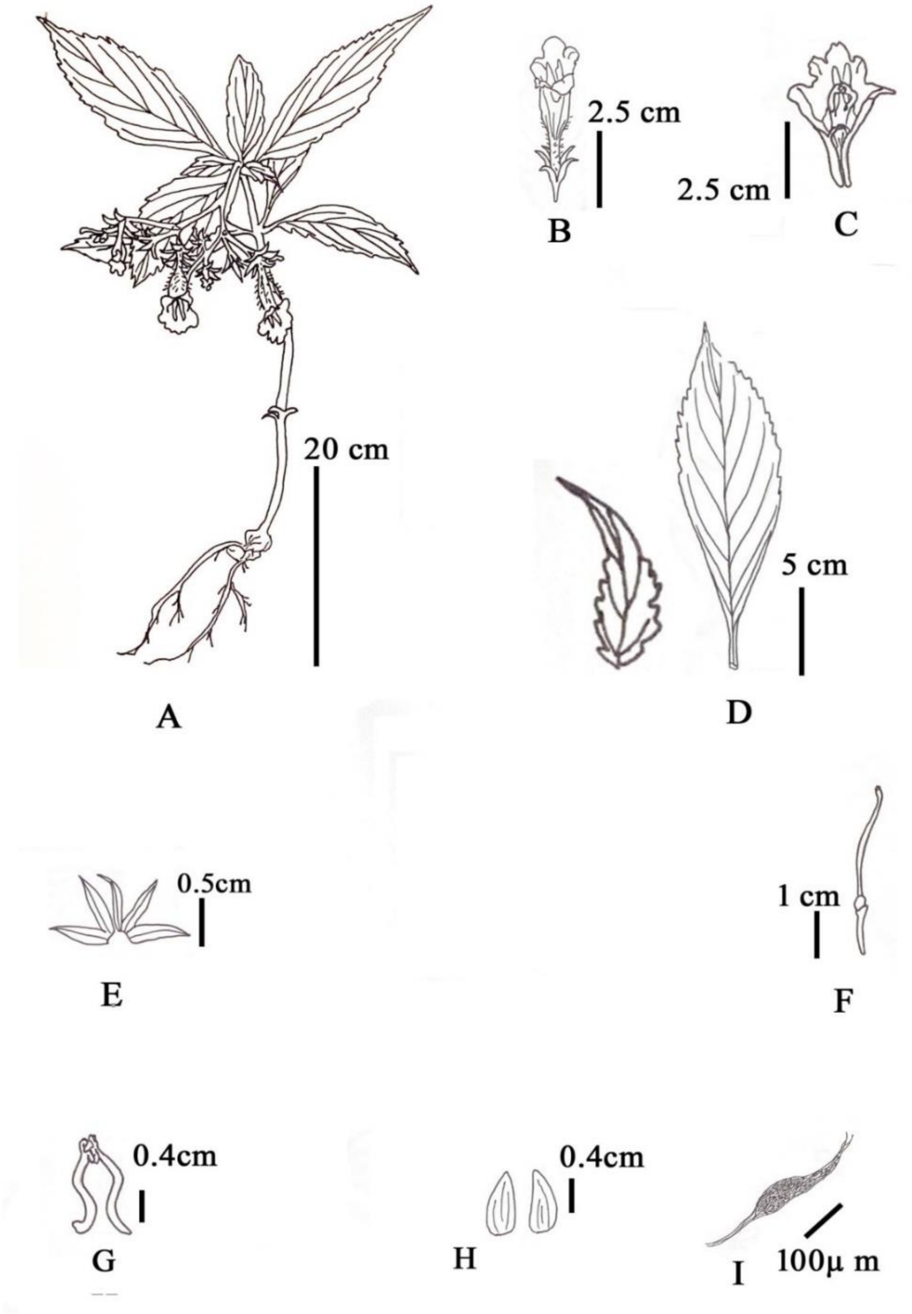

Figure 5.23: Illustration of *Lysionotus serratus* D.Don A. Habit; B. Flower; C. Flower opened; D. Leaves; E. Calyx; F. Pistil; G. Stamen; H. Bract; I. Seed.

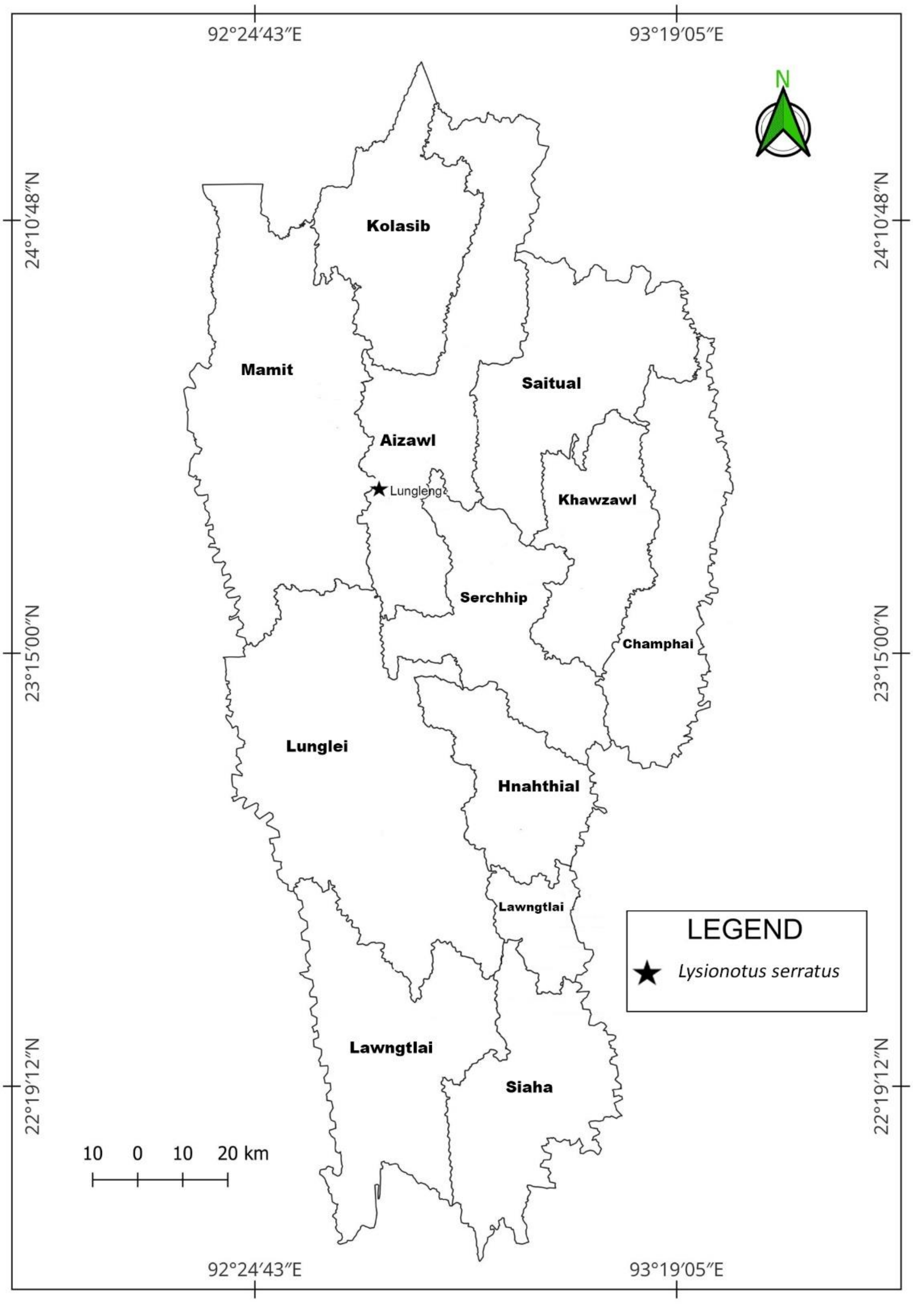

Figure 5.24: Distribution map of *Lysionotus serratus* D.Don in Mizoram.

glabrous. Calyx 5, divided up to base, lobe lanceolate, 8–12 × 2–4 mm long, glabrous. Corolla purple to white with purplish nerve, tubular, inflated in middle, pubescent, 30–45 mm long, five lobes, corolla tube slender funnel form, 32–35 mm long, corolla lobe ovate, 10–13 × 10–15 mm long, inner corolla having yellowish twolines streak.Stamen 2; anther connective with conspicuous lateral appendage hornlike, *c.* 1 mm long; filament white, 10–15 mm long, glabrous; anther globose. Staminode 2, glabrous, white 5 mm long. Pistil 25–40 mm long, glabrous; stigma peltate; style 2 – 3.5 mm long, glabrous; Ovary 8–10 × c. 2 mm, glabrous. Capsule 7–15 cm long.

Phenology: Flowering from July to August and fruiting from September to November.

Distribution: Mizoram (Lungleng; **5.24**); India (Assam, Arunachal Pradesh, Manipur, Meghalaya, Nagaland, Sikkim, Tripura, West Bengal); Bhutan, Myanmar, China, Nepal, Thailand, Vietnam.

Habitat and Ecology: An epiphytic sub-shrub growing on tree trunk along with *Aeschynanthus parasiticus* in forest and stream sides.

Specimens examined: INDIA. Mizoram, Aizawl District, Lungleng village, 23˚66'66.0" N 92˚66'23.0" E, 1055 m elevation, on 26th July 2022, Margaret Lalhlupuii128832 (MZUH000049); Dzuko valley and Surr Hills, 1500 m, 5.09.2006, A. A. Mao and R. Gozo 109234 (ASSAM); Meghalaya, Khasi hills, Mairang to Nongkhlor, 22 Aug. 1978, G.M.Bhaumik 60352 (ASSAM); Mahananda Wild Life Sanctuary, West Bengal,6.5.90.,S.Chandra 20796 (CAL).

8. *Rhynchoglossum* Blume

Rhynchoglossum obliquum Blume, Bijdr. Fl. Ned. Ind. 14: 741. 1826; C.B. Clarke in A.DC.& C.DC., Monogr. Phan. 5: 161. 1883 & in Hook.f., Fl. Brit. India 4: 367. 1884; Kanjilal & al., Fl. Assam 3: 398. 1939; G.P. Sinha in G.P. Sinha & et. al., Fl. Mizoram 2: 211. 2012; B.K. Sinha & Su.Datta, Nelumbo 58: 36. 2016. (**Figure No. 5.25; Plate No. 17**)

Type: Indonesia, Java, Goha, Unknown collector 506 A (L, n.v.).

Taxonomic description: Perennial herbs, 20−80 cm tall. Stem glabrous, terete. Leaves alternate, 7−11×3.2−4.5cm long, base oblique, abaxial and adaxial surface glabrous, margin entire, apex acuminate, lateral venation 7 to 13 pairs. Petiole 1−3.2 cm long. Inflorescence raceme, axillary, 1 to 15 cm long, 5 to 22 flowered. Peduncles 2−7 cm long. Bract 1 or absent, 0.5−2 mm long, subulate. Pedicel 3−4 mm long, glabrous. Calyx 4−6 mm long, glabrous, transparent, lower basal green and upper lobes purple to blue, lobes 2−3 mm long, margin entire. Corolla 10−13 mm long, tubular, purple to violet, glabrous, corolla tube 7−10 mm long, corolla lobe 4−6 mm long, lower lip 2−3 mm long, upper lip 4−6 mm long. Stamen 2; filaments 4−5 mm long, glabrous; anthers 0.8−1 mm long. Staminode 2, *c.*1mm long. Pistil 8−10 mm long, glabrous. ovary superior, 1−3 mm long, glabrous; style 3.5−5 mm long, glabrous; stigma capitate, 2−3 mm long. Capsule ovoid, 3−5 mm long.

Phenology: Flowering from August to September and fruiting from September to December.

Habitat and Ecology: Grows in roadside, in moist, shaded forests. The plant thrives in humid environments, often near streams or in areas with high rainfall.

Distribution: Mizoram (Ailawng, Chaltlang, Lungleng, Murlen, Muthi, Reiek, Sihphir, Tanhril, Zobawk; **Figure 5.26**); India (Andhra Pradesh, Assam, Arunachal Pradesh, Assam, Bihar, Goa, Himachal Pradesh, Jharkhand, Karnataka, Kerela, Madhya Pradesh, Maharastra, Manipur, Meghalaya, Nagaland, Odisha, Sikkim, Tamil Nadu, Tripura, Uttarakhand and West Bengal); Bangladesh, Bhutan, Cambodia, China, Indonesia, Malaysia, Myanmar, Nepal, Philippines, Sri Lanka, Thailand, Vietnam

Specimens examined: INDIA. Mizoram, Aizawl District, Lungleng village, 23°66′56.0″ N 92°66′35.0″ E, 980 m elevation, 21th September 2022, Margaret

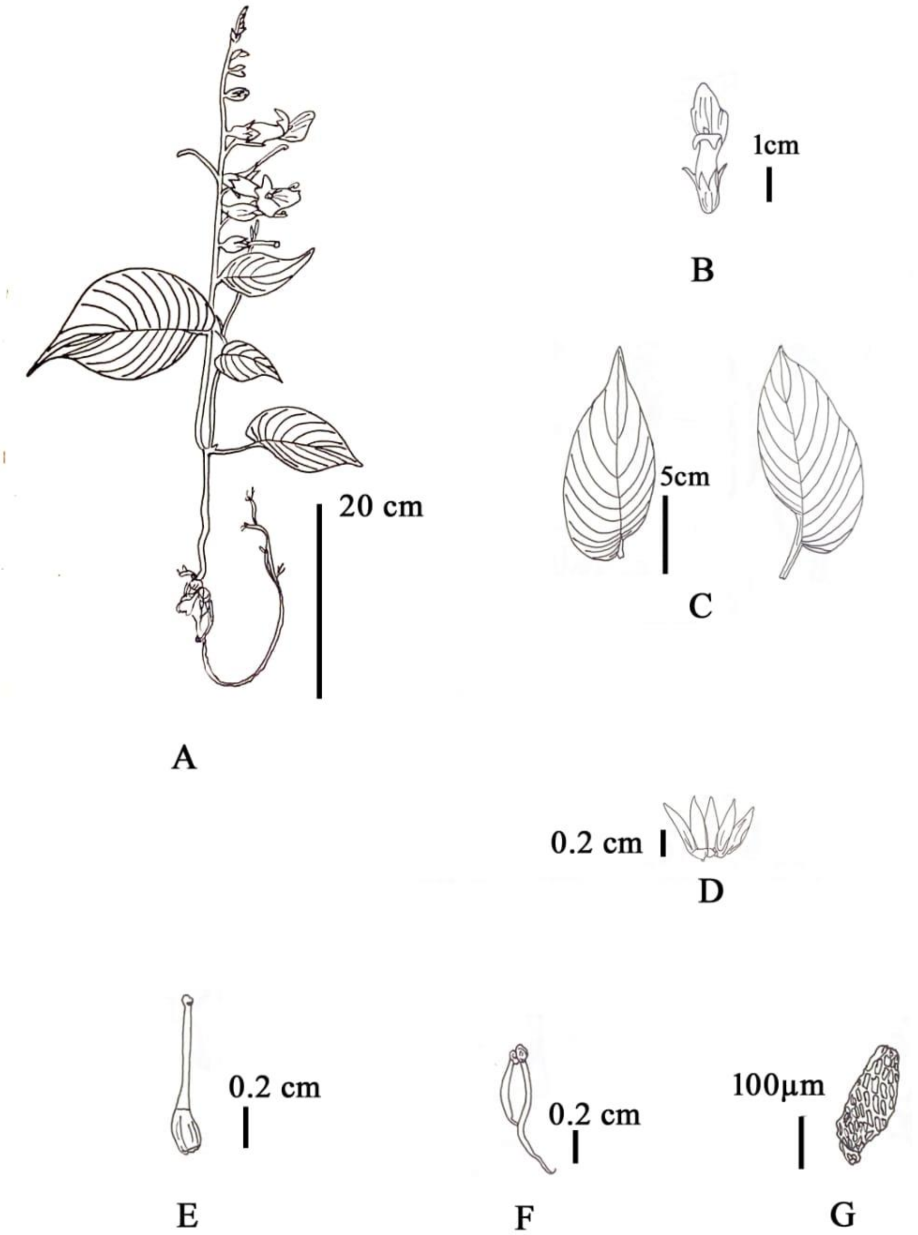

Figure 5.25: Illustration of *Rhynchoglossum obliquum* Blume A. Habit; B. Flower; C. Leaves; D. Calyx; E. Pistil; F. Stamen; G. Seed.

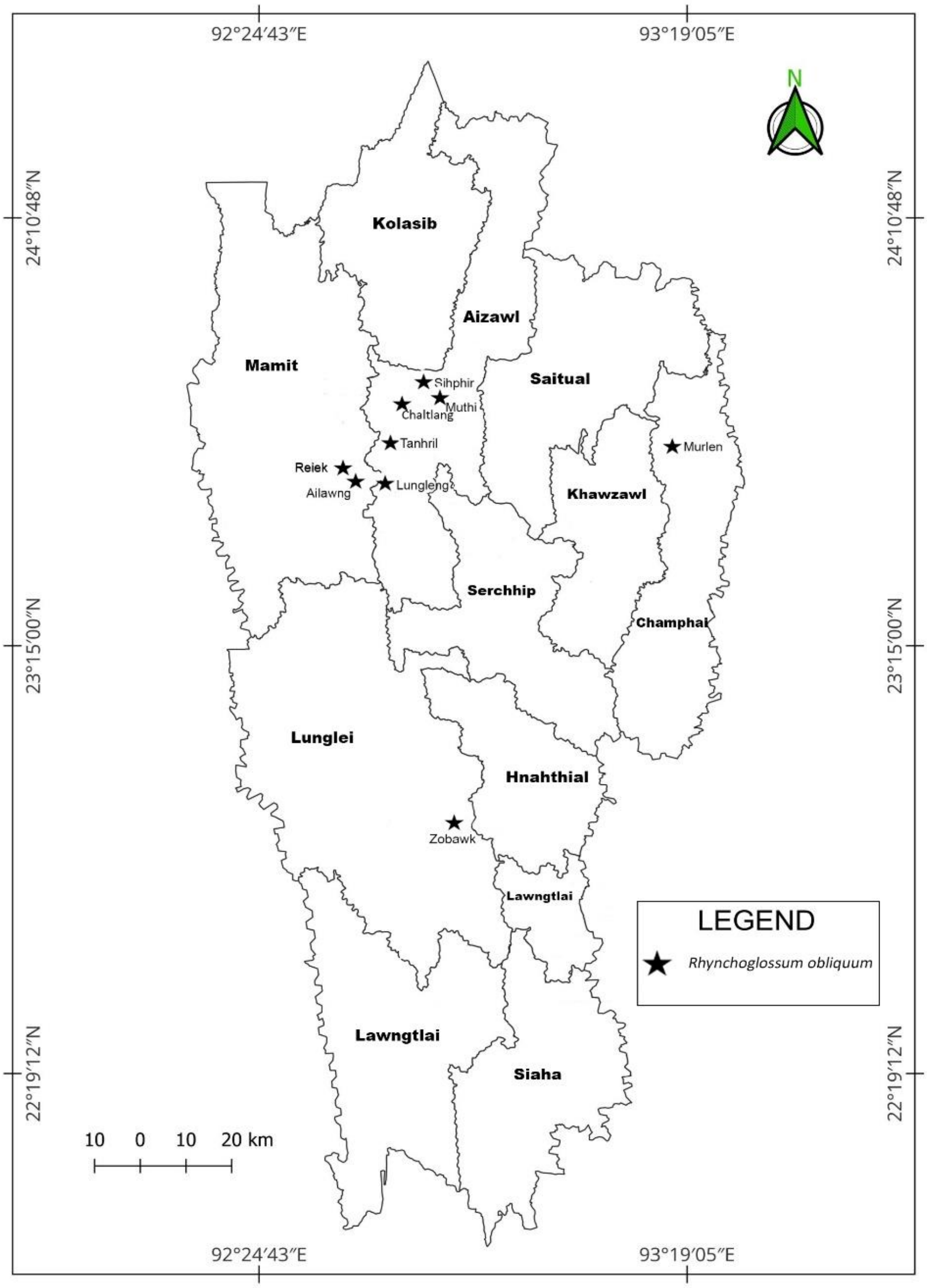

Figure 5.26: Distribution of *Rhynchoglossum obliquum* Blume inMizoram

Lalhlupuii 128833 (MZUH000050); Dampa T. R., 920 m, 21.9.2006, B. K. Sinha and Odyuo 112749 (ASSAM); Nagaland, 12.10.1977 C. L. Malhotra 75227 (ASSAM); Darjeeling, 1300 m, G. King 13722 (CAL); Mahananda Wildlife Sanctuary, Darjeeling, 24.09.07 T. K. Paul and Anant Kumar A2545 (CAL); K000858044 (K).

9. *Rhynchotechum* Blume

Rhynchotechum ellipticum (Wall. ex D.Dietr.) A.DC. in DC., Prodr. 9: 285. 1845; C.B.Clarke, Commelyn. Cyrtandr. Bengal. 131, t. 41. 1874, & in A.DC.& C.DC., Monogr. Phan. 5: 196. 1883; et in Hook.f., Fl. Brit. India 4: 373. 1884; Kanjilal& al., Fl. Assam 3: 399. 1939;G.P. Sinha in G.P. Sinha & et. al., Fl. Mizoram 2: 211. 2012; B.K. Sinha & Su.Datta, Nelumbo 58: 38. 2016. (**Figure No. 5.27; Plate No. 18**).

Synonym: *Chiliandra obovata* Griff. Not Pl. Asiat 4: 150. 1854. *Corysanthera elliptica* Wall. Ex D. Dietr Syn. Pl. 3: 582. 1843. *Rhynchotechum latifolius* Hook. f., et Thomson ex C. B. Clarke Commelyn. Cyrtandr. Bengal. t. 94.1874.

Type: Mts Sylhet, F. de Silva, W. Gomez & H. Bruce in Wallich 6411 (K)

Taxonomic description: Subshrub, 50–200 cm tall. Stem glabrous, rush-brown to brown. Leaves opposite decussate, 9–34 × 3.2–13 cm long, serrate margin, leaf blade oblanceolate or elliptic, base cuneate to attenuate, glabrous, apex acute to acuminate, midrib pubescent, margin denticulate to serrulate; lateral vein pairs 11–25. Petiole 25–40 mm long, rush brown to brown. Inflorescence 5 to 24 flowered. Peduncle 15–30 mm long, terete, maroon, pubescent. Pedicel 3–13 mm long, pink to white. Bract 4–5 mm long, pubescent, maroon, lanceolate. Calyx 3–5 × 1–1.5 mm long, pubescent, divided below half the base. Corolla pink, 8–10 mm long, campanulate, inner corolla glabrous, outer corolla pubescent. Stamen 4, epipetalous, arcuate; filament 4–5 mm long; anthers purple, 1–1.5 mm diameter, slightly puberulent; Staminode 1, *c.* 1 mm long, maroon. Pistil 9 mm long, glabrous; ovary superior, *c.* 2 mm long, glabrous; stigma apex globose to truncate, *c.* 2 mm long; style 5 mm long. Fruit broadly ovoid, 3–4.5 × 3–4.5 mm, glabrous.

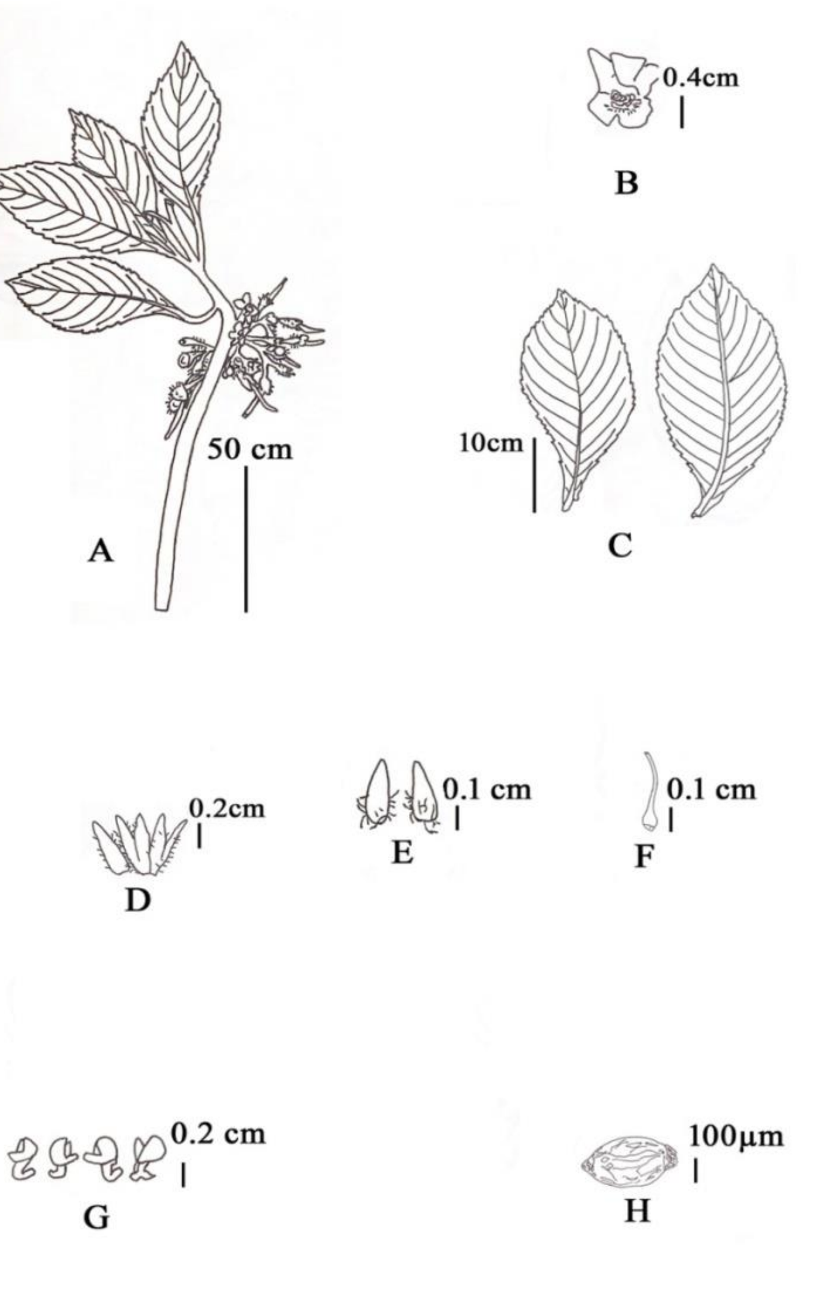

Figure 5.27: Illustration of *Rhynchotechum ellipticum* (Wall. ex D.Dietr.) A.DC.A. Habit; B. Flower; C. Leaves; D. Calyx; E. Bract; F. Pistil; G. Stamen; H. Seed.

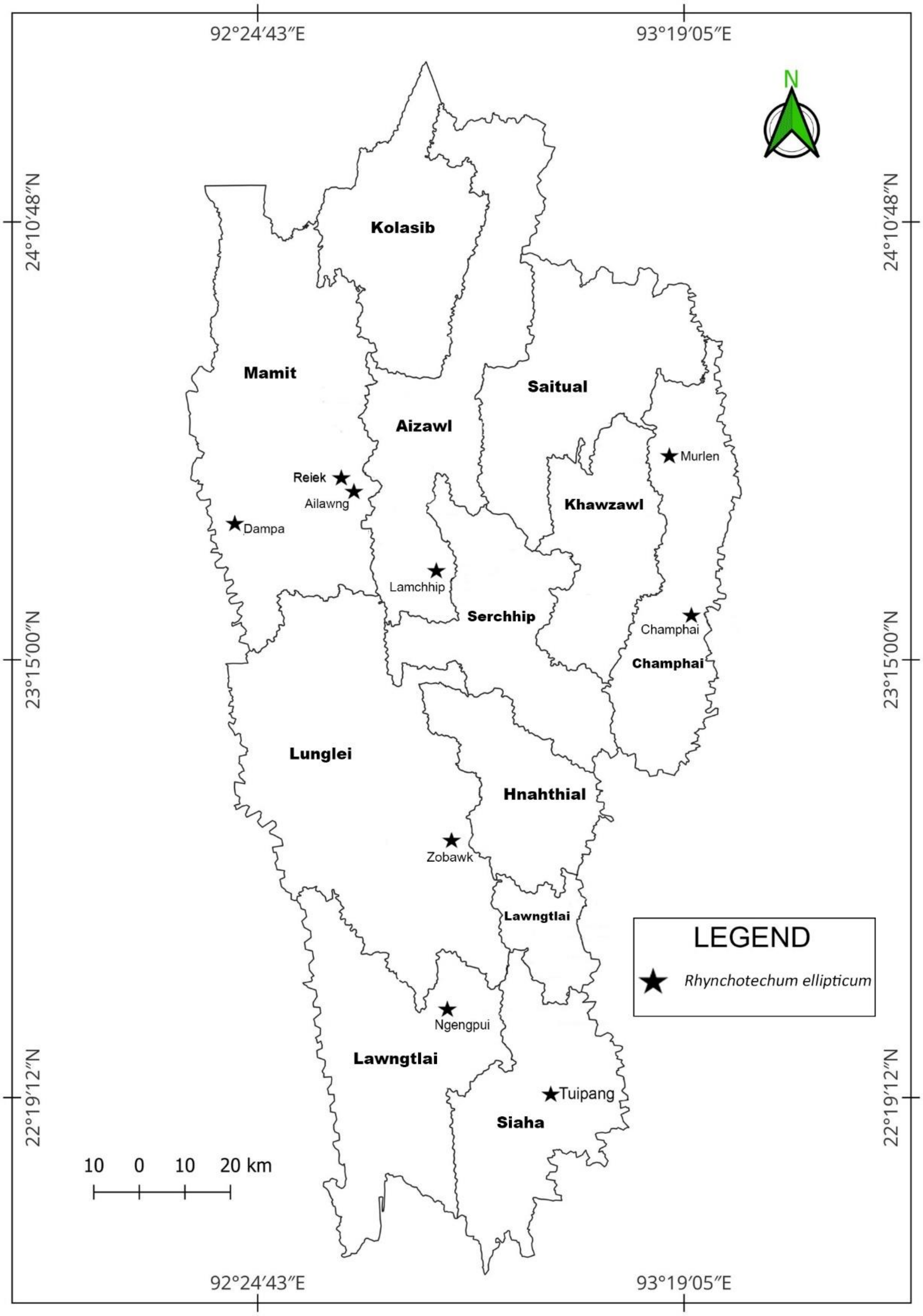

Figure 5.28: Distribution map of *Rhynchotechum ellipticum* (Wall. ex D.Dietr.) A.DC.in Mizoram.

Phenology: Flowering from August to September and fruiting from September to December.

Habitat and Ecology: Growing in primary and secondary forests, typically in shady and moist conditions along road side, stream side and on rocky places.

Distribution: Mizoram (Ailawng, Champhai, Dampa, Lamchhip, Murlen, Ngengpui, Reiek, Tuipang, Zobawk; **Figure 5.28**); India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Nagaland, Sikkim, Tripura, West Bengal); Bangladesh, Bhutan, Cambodia, China, Laos, Malaysia, Nepal and Vietnam.

Specimens examined: INDIA. Mizoram, Lunglei District, Zobawk village, 22°84′51.8″ N 92°81′93.2″, 1136 m elevation, on 7th September 2022, Margaret Lalhlupuii128834 (MZUH000051); Kokrajhar District, Assam, 25.3.2009, Ranjit Daimary 118034 (ASSAM); K and J Hills, 28.10.1915, Upendranath Kanjilal, 6153 (ASSAM); Mongpoo, 1000 m, C. Biswas 1411 (CAL).

5.2.2 Discussion

The morphological characters observed in the Gesneriaceae family of Mizoram serves as the basis and framework for taxonomy forming a large indicator for visually identifying plants. As per Woodger (2014), the morphological characteristics are compared, measured, numbered, and documented to evaluate the differences or similarities among the collected plant species and helps to solve taxonomic difficulties. Kirchoff (2008) mention the usefulness of plant morphology in the identification of plants as mentioned by which have been extensively used in the preparation of classification systems. The morphology of a plant species is a feature that is easy to observe (Hadiyanti et al., 2018), the studied Gesneriaceae were classified and diagnostic keys were provided along with the characters obtained from the micromorphological studies of pollens and seeds.

From the analyses of the result, the plants were either shrubs or herbs, sometimes epiphytic or lithophytic. The unusual morphological features of Epithemateae having strong anisophylly in most taxa (Burtt, 1977) support our findings where *Rhynchoglossum obliquum* and *Epithema carnosum* were have a strong anisophyllous leaves with unilateral racemes in *Rhynchoglossum obliquum*. The capsule of the tribe Tricosporeaehaving anelongated dehiscing usually loculicidally, were observed in *Lysionotus serratus, Henckelia anachoreta*, *H. pumila*, *Didymocarpus adenocarpus*, *D. lineicapsa*, *D. parryorum*, *D. vickifunkiae* and *D. Wengeri* species, rarely berry in *Rhynchotechum ellipticum* and sometimes seed end with appendage (Moller et al., 2017) in the genus *Aeschynanthus* were seen in the seed of *A. mannii, A. parasiticus, A. parviflorus, A. reiekensis* and *A. superbus*. All the studied corolla consists of five fused petals which exhibits a wide range of forms resulting from the different proportionsand shapes of the tube forming funnel type, tubular or infundibuliform. According to Weber (2017), colouration of the corolla is extremely variablein Gesneriaceae, where the corolla was found to be red, orange, purple, yellow, white or pink in these studies.

The documentation of the taxa collected in these studies were named with the latest accepted name of the Gesneriad species consulted from the POWO and IPNI following the latest classification prpposed by Weber et al., 2013. The revised generic name *Chirita* Buch.-Ham. ex D. Don were moved to *Henckelia* Spreng. (Weber et al., 2011).*Chirita anachoreta* Hance was renamed to *Henckelia anachoreta* (Hance) D.J.Middleton & Mich.Möllerand *Chirita pumila* D.Don was renamed into *Henckelia pumila* (D.Don) A.Dietr.

The study also revealed that some species documented in the Flora of Mizoram over a century ago were no longer found during recent surveys. This finding is a stark indicator of the impact of climatic variation and modernization on the natural vegetation of Mizoram. The absence of these species in their historical locations suggests that changes in climate, along with increased human activity, have significantly altered the natural habitats and distribution patterns of the Gesneriaceae family. This emphasizes the importance of continued monitoring and conservation efforts to protect the remaining biodiversity in the region.

5.3 Palynology

5.3.1 Collection of pollen and preparation

The pollen materials employed in this study were fresh samples collected from the study area during their respective flowering seasons. Healthy flowers that are fully opened and have matured anthers were selected as these will produce viable pollens. Immature or unopened flowers may not have released pollen or may have less pollen. The anthers were carefully detached from the mature flowers and gently brush with a soft brush on the brass stub with double-sided adhesive tape. This will release the pollen from the anthers. The specimens were sputter-coated with a fine layer of gold using a Fine Coat Ion Sputter JFC-1100. Images were captured and the pollen data obtained were based on observations using SEM.

5.3.2 Investigation of pollen morphological features using SEM

The Scanning electron micrographs were mainly used for the studying the shape, size, aperture, exine ornamentation and elements to collect information about the detailed pollen characters. Size of pollen grain was expressed as Polar axis (P) × Equatorial axis (E) in micrometre (µm). The shape of a pollen grain was determined by the ratio of polar length to the equatorial diameter (P/E). Measurements included the polar length, equatorial diameter, colpi length, pore diameter, and exine thickness.

Common pollen terminology used is in accordance with Punt et al. (2007), Halbritter et al. (2018), Erdtman (1952); Faegri & Iversen (1964); Kremp (1965) and Walker & Doyle (1975) are given below.

Monads: A pollen unit that consists of a single pollen grain.

Isopolar: Pollen grains that have the same structure or morphology at both poles.

Tricolporate: Pollen grains with three elongated apertures that have colpi (furrows) and pores.

Tricolpate: Pollen grains with three colpi but without pores.

Microreticulate: The exine has a fine reticulate pattern, with meshes smaller than 1 µm.

Nanoreticulate: The exine has a very fine reticulate pattern, with meshes smaller than 0.5 µm.

Cristatum: The surface of the pollen crested or comb-like.

Psilate: The surface of the pollen grain is smooth without any projections.

Fossulate: The surface has pits or grooves.

Granulate: The surface has small granules.

Gemmae: The surface has small, pointed projections.

Ubisch body: Polymorphic sporopollenin-element produced by the tapetum.

Columellae: The surface is supported by rod-like structures beneath the outer layer.

Nanoechini: The surface has very small spiny projections.

5.3.3 Pollen morphological description

The analysis of studied Gesneriaceae family in 17 species show diverse palynotaxonomic variations. The detailed micromorphology characters of each species are given below in an alphabetical order with their photoplate no. (SEM pictures) as follows:

Achimenes longiflora DC.**(Plate 19: 1a & 1b)**

Pollen grains monad, polarity with an isopolar type, tricolporate with long aperture, colpus having pores. The grains are of medium size, with a polar length ranging from 27 to 30 µm and an equatorial width of 13 to 16 µm, prolate (P/E ratio 1.9), exine ornamentation nanoreticulate (0.2–0.3 µm), reticulum cristatum with small gemmae (suprasculpture).

Aeschynanthus mannii Kurz ex C.B.Clarke **(Plate 19: 2a & 2b)**

Pollen grains monad, polarity with an isopolar type, tricolporate with long aperture, colpus having pores. The grains are of small size, with a polar length ranging from 19 to 21 µm and an equatorial width of 19 to 21 µm, isodiametric (P/E ratio 1), exine ornamentation microreticulate (0.5–0.6µm), granulate.

Aeschynanthus parasiticus (Roxb.) Wall.**(Plate 19: 3a & 3b)**

Pollen grains monad, polarity with an isopolar type, tricolporate with long aperture, colpus having pores. The grains are of medium size, with a polar length ranging from 28 to 29 µm and an equatorial width of 16 to 18 µm, prolate (P/E ratio 1.6), exine ornamentation microreticulate (0.5–0.6 µm).

Aeschynanthus parviflorus (D. Don) Spreng. **(Plate 20: 1a & 1b)**

Pollen grains monad, polarity with an isopolar type, tricolporate with long aperture, colpus having pores. The grains are of medium size, with a polar length ranging from 28 to 29 µm and an equatorial width of 13 to 15 µm, perporate (P/E ratio 2), exine ornamentation microreticulate (0.5–0.7 µm).

Aeschynanthus reiekensis Lalhlupuii, S.D.Khomdram & S.D.Yumkham **(Plate 20: 2a & 2b)**

Pollen grains monad, polarity with an isopolar type, circular to subangular amb, tricolporate with long aperture and tapering, colpus having pores. The grains are of small size, with a polar length ranging from 28 to 29 µm and an equatorial width of 13 to 15 µm, prolate (P/E ratio 1.4–1.9), exine ornamentation microreticulate (0.5–0.6 µm).

Aeschynanthus superbus C.B.Clarke **(Plate 20: 3a & 3b)**

Pollen grains monad, polarity with an isopolar type, tricolporate with long aperture, colpus having pores. The grains are of small size, with a polar length ranging from 19 to 20 µm and an equatorial width of 12 to 13 µm, prolate (P/E ratio 1.5), exine ornamentation microreticulate (0.5–0.6 µm), granulate.

Chrysothemis pulchella (Donn ex Sims) Decne. **(Plate 21: 1a & 1b)**

Pollen grains monad, polarity with an isopolar type, tricolporate with long aperture, colpus having pores. The grains are of medium size, with a polar length ranging from 22 to 28 µm and an equatorial width of 10 to 11 µm, prolate (P/E ratio 1.5), exine ornamentation fossulate. Ubisch bodies present.

Didmymocarpus adenocarpus C.E.C.Fisch. **(Plate 21: 2a & 2b)**

Pollen grains monad, polarity with an isopolar type, tricolpate with long aperture, colpus having pores. The grains are of small size, with a polar length ranging from 21 to 25 µm and an equatorial width of 13 to 16 µm, prolate (P/E ratio 1.6), exine ornamentation microreticulate (0.5–0.8 µm).

Didymocarpus lineicapsa (C.E.C.Fisch.) B.L.Burtt **(Plate 21: 3a & 3b)**

Pollen grains monad, polarity with an isopolar type, tricolpate with long aperture, colpus having pores. The grains are of small size, with a polar length ranging from 16 to 17 µm and an equatorial width of 11 to 12 µm, prolate (P/E ratio 1.4), exine ornamentation nanoreticulate (0.3–0.4 µm).

Didymocarpus parryorum C.E.C.Fisch. **(Plate 22: 1a & 1b)**

Pollen grains monad, polarity with an isopolar type, tricolpate with long aperture, colpus having pores. The grains are of small size, with a polar length ranging from 16 to 18 µm and an equatorial width of 12 to 13 µm, prolate (P/E ratio 1.3), exine ornamentation microreticulate (0.4–0.6 µm), granulate.

Didymocarpus vickifunkiae V.Gowda & N.S.Prasanna **(Plate 22: 2a & 2b)**

Pollen grains monad, polarity with an isopolar type, tricolpate with short aperture length, colpus having pores. The grains are of small size, with a polar length ranging from 20 to 23 µm and an equatorial width of 10 to 11 µm, perporate (P/E ratio 2), exine ornamentation nanoreticulate (0.08–0.2 µm).

Epithema carnosum Benth. **(Plate 23: 1a & 1b)**

Pollen grains monad, polarity with an isopolar type, tricolporate with long aperture, colpus having pores. The grains are of small size, with a polar length ranging from 22 to 23 µm and an equatorial width of 12 to 17 µm, prolate (P/E ratio 1.8), exine ornamentation nanoreticulate (0.2–0.4 µm), fossulate.

Henckelia anachoreta (Hance) D.J.Middleton & Mich.Möller **(Plate 23: 2a & 2b)**

Pollen grains monad, polarity with an isopolar type, tricolpate with long aperture, colpus having pores. The grains are of small size, with a polar length ranging from 22 to 26 µm and an equatorial width of 12 to 19 µm, prolate (P/E ratio 1.5), exine ornamentation microreticulate (0.5–0.7 µm), granulate.

Henckelia pumila (D.Don) A.Dietr. **(Plate 23: 3a & 3b)**

Pollen grains monad, polarity with an isopolar type, tricolpate with long aperture, colpus having pores. The grains are of medium size, with a polar length ranging from 26 to 29 µm and an equatorial width of 12 to 14 µm, perporate (P/E ratio 2.1), exine ornamentation microreticulate (0.6–0.9 µm), granulate.

Lysionotus serratus D.Don **(Plate 24: 1a & 1b)**

Pollen grains monad, polarity with an isopolar type, tricolpate with long aperture, colpus having pores. The grains are of small size, with a polar length ranging from 19 to 25 µm and an equatorial width of 11 to 14 µm, prolate (P/E ratio 1.7), exine ornamentation microreticulate (0.5–0.8 µm).

Rhynchoglossum obliquum Blume **(Plate 24: 2a & 2b)**

Pollen grains monad, polarity with an isopolar type, tricolpate with intermediate aperture length, colpus having pores. The grains are of medium size, with a polar length ranging from 27 to 28 µm and an equatorial width of 14 to 15 µm, prolate (P/E ratio 1.9), exine ornamentation microreticulate (0.5–0.9 µm), free standing columellae.

Rhynchotechum ellipticum (Wall. ex D.Dietr.) A.DC. **(Plate 24: 3a & 3b)**

Pollen grains monad, polarity with an isopolar type, tricolpate with long aperture, colpus having pores. The grains are of small size, with a polar length ranging from 16 to 18 µm and an equatorial width of 10 to 13 µm, prolate (P/E ratio 1.4), exine ornamentation nanoreticulate (0.3–0.5 µm), reticulum cristatum with nanoechini (suprasculpture).

Pollen size ranges: very small (<10 µm); small (10–25 µm); medium (26–50 µm), P/E ratio: oblate (<1); isodiametric (=1); prolate (>1); perporate (≥2), Exine ornamentation ranges; reticulate prefix micro- (1–0.5 µm); nano- (0.5–0.1 µm).

Table 5.1: Pollen characters and character states for the cluster analysis of Gesneriaceae. The numbers in brackets are the codes for character states.

Sl.No.	Characters	Character states
1.	Pollen unit	Monads = 0, Dyads = 1, Polynads = 2
2.	Polarity	Apolar = 0, Isopolar = 1, Heteropolar = 2
3.	Aperture	Tricolporate = 0, Tricolpate = 1, Colporate = 2
4.	Polar length	<10 µm = 0, 10–25 µm = 1, 26–50 µm = 2
5.	Equatorial view	<10 µm = 0, 10–25 µm = 1, 26–50 µm = 2
6.	P/E ratio	<1 = 0, 1 = 1, >1 = 2
7.	Exine ornamentation	Non-reticulate=0, Microreticulate=1, Nanoreticulate=2
8.	Exine sculpturing elements	Psilate=0, Fossulate=1, granulate=2, Gemmae=3, columellae=4, nanoechini=5

Table 5.2: Data matrix used in cluster analysis of Gesneriaceae species based on pollen features.

Sl.No	Taxa	1	2	3	4	5	6	7	8
1.	*Achimenes longiflora* DC.	0	1	0	2	1	1.9	2	3
2.	*Aeschynanthus mannii* Kurz ex C.B.Clarke	0	1	0	1	1	1	1	2
3.	*Aeschynanthus parasiticus* (Roxb.) Wall.	0	1	0	2	1	1.6	1	0
4.	*Aeschynanthus parviflorus* (D.Don) Spreng.	0	1	0	2	1	2	1	0
5.	*Aeschynanthus reiekensis* Lalhlupuii, S.D.Khomdram & S.D.Yumkham	0	1	0	1	1	1.7	1	0
6.	*Aeschynanthus superbus* C.B.Clarke	0	1	0	1	1	1.5	1	2
7.	*Chrysothemis pulchella* (Donn ex Sims) Decne.	0	1	0	2	1	1.5	0	1
8.	*Didymocarpus adenocarpus* C.E.C.Fisch.	0	1	1	2	1	1.6	1	0
9.	*Didymocarpus vickifunkiae* V.Gowda & N.S.Prasanna	0	1	1	2	1	2	2	0
10.	*Didymocarpus lineicapsa* (C.E.C.Fisch.) B.L.Burtt	0	1	1	1	1	1.4	2	0
11.	*Didymocarpus parryorum* C.E.C.Fisch.	0	1	1	1	1	1.3	1	2
12.	*Epithema carnosum* Benth.	0	1	0	2	1	1.8	2	1
13.	*Henckelia anachoreta* (Hance) D.J.Middleton & Mich.Möller	0	1	1	2	1	1.5	1	2
14.	*Henckelia pumila* (D.Don) A.Dietr.	0	2	1	2	1	2.1	1	2
15.	*Lysionotus serratus* D.Don	0	1	1	2	1	1.7	1	0
16.	*Rhynchoglossum obliquum* Blume	0	2	1	2	1	1.9	1	4
17.	*Rhynchotechum ellipticum* (Wall. ex D.Dietr.) A.DC.	0	1	1	1	1	1.4	2	5

5.3.4 Pollen cluster analysis using dendrogram

Hierarchical cluster analysis was employed to group species based on their resemblance in terms of pollen characteristics through the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method, utilizing EUCLIDEAN distance. On the cluster analysis, a total of eight pollen characters viz., pollen unit, polarity, aperture, polar length, equatorial view, P/E ratio, exine ornamentation and exine sculpturing elements were used.

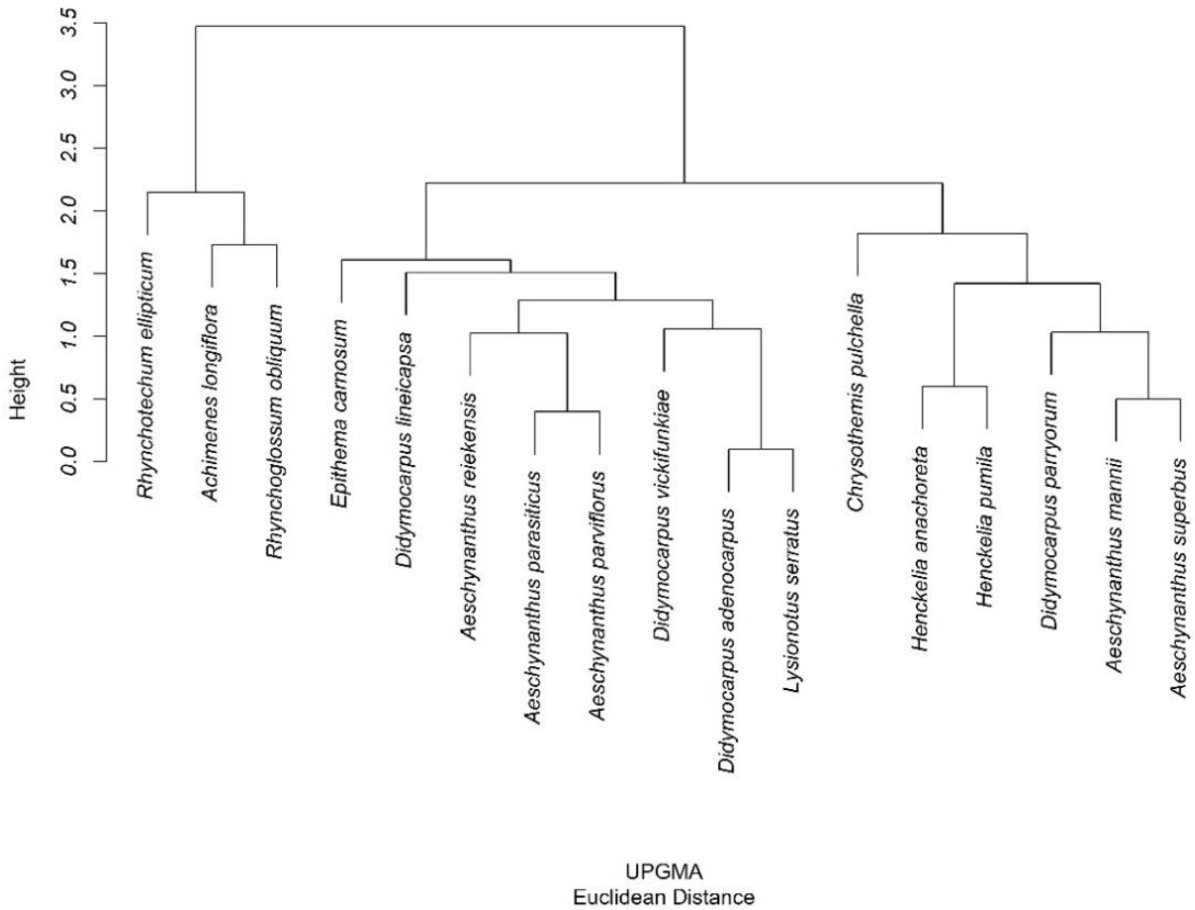

Figure 5.29: Dendrogram based on cluster analysis of pollen morphological characters Using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) with Euclidean Distance.

The constructed dendrogram produced three major clades. The Clade 1 (left major clade) includes the species *R. ellipticum, A. longiflora* and *R. obliquum*. The Clade 2 (middle major clade) includes the *E. carnosum, D. lineicapsa, D. vickifunkiae, D. adenocarpus, A. reiekensis, A. parasiticus, A. parviflorus* and *L. serratus*. The Clade 3 (right major clade) includes *Chrysothemis pulchella*, *H. anachoreta*, *H. pumila*, *D. parryorum*, *A. mannii* and *A. superbus*.

The Gesneriaceae species in the dendrogram are arranged according to the similarities in their pollen morphological traits. By merging branches, three major clades are created, with closely related species initially forming a clade before uniting with more distantly related species or groups. When species similarity is measured using the Euclidean distance, the closer the species is to one another, the more similar they are. The initial cluster that formed between *D. adenocarpus* and *L. serratus* demonstrates a notable degree of resemblance in their medium size pollen. These two species are members of the same subfamily, Didymocarpoideae, tribe Tricosporeae and sub-tribe Didymocarpinae, from different genera. Pollen grain size is one of the quantitative traits that have been found to be significant for the classification of subfamilies(Smith et al., 1997), and the grouping of this distinct genera due to medium pollen size supports this finding.Species of the genus *Aeschynanthus* were group together due to their uniform characters like being monad, isopolar, tricolporate and small size, with a microreticulate exine ornamentation. This grouping suggests that they have similar evolutionary pressures or genetic origins.

In the dendrogram, *Henckelia* species were grouped together in a single clade. However, several *Aeschynanthus* and *Didymocarpus* species were positioned separately represented at both Clades 2 and 3 (Figure 5.29). These could suggest that the ability of quanlitative (i.e., size, polar axis, equatorial axis, P/E ratio) and quantitative (i.e., pollen type, pollen shape, aperture features, exine sculpturing) palynological features to species relationships at the genus level is limited.

5.3.5 Principal Component Analysis of Pollen

The results of the Principal Component Analysis (PCA) reveal insightful patterns in the pollen characteristics across different samples. The Eigenvalues computed for PCA components indicate that the first principal component (PCA1) explains a substantial portion of the total variance, accounting for 39.9% of the variability observed, while the second principal component (PCA2) contributes an additional 21.3%. Collectively, PCA1 and PCA2 account for 61.2% of the total variance, underscoring their significance in capturing the underlying structure of the dataset (Table 5.3 & Figure 5.31).

The cluster analysis of PCA divided these samples into three groups (Figure 5.31). Group 1 consisted of *A. longiflora*, *D. vickifunkiae* and *E. carnosum*, Group 2 consisted of *A. mannii*, *A. reiekensis*, *A. superbus*, *D. parryorum* and *R. ellipticum*, Group 3 consisted of *A. parasiticus*, *A. parviflorus*, *C. pulchella*, *D. adenocarpus*, *H. anachoreta*, *H. pumila*, *Lysionotus serratus* and *R. obliquum*.

Table:5.3: Eigenvalues, variance explained % and cumulative proportion of total variance from principal component analysis (PCA) components for pollen characteristics of different samples.

PCA	***Eigenvalue***	**% of variance**	**Cumulative %**
1	1.99	39.90	39.90
2	1.06	21.3	61.17

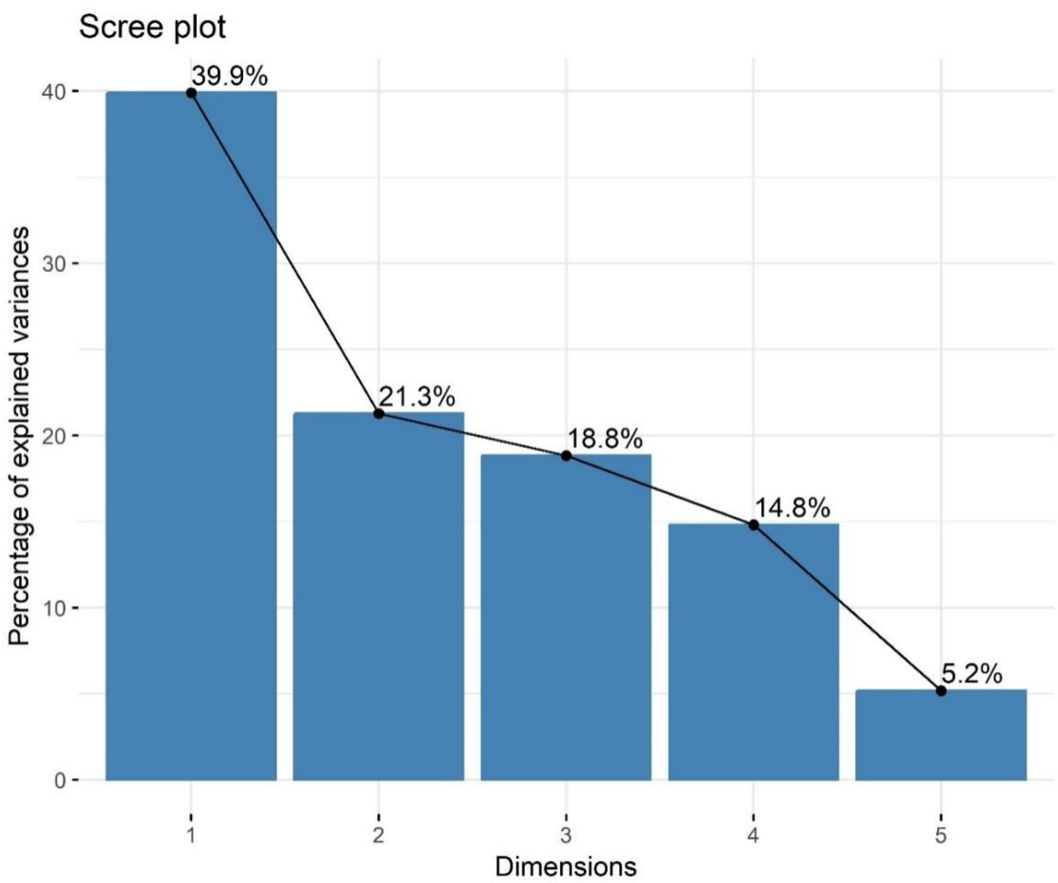

Figure 5.30:Principal component analysis (PCA) for Pollen contribution of each principal component to total variance.

Upon examining the correlations between the original variables and the PCA components, it becomes evident that certain pollen characteristics exhibit strong associations with the principal components (Table 5.3). Variables such as polar length, P.E. ratio, aperture, and exine ornamentation display positive correlations with PCA1, but negatively correlated with exine elements. PCA2 had positively correlated with all variables except with polar length, indicating their substantial contributions to the variation explained by these components. (Figure 5.31 & Figure 5.32).

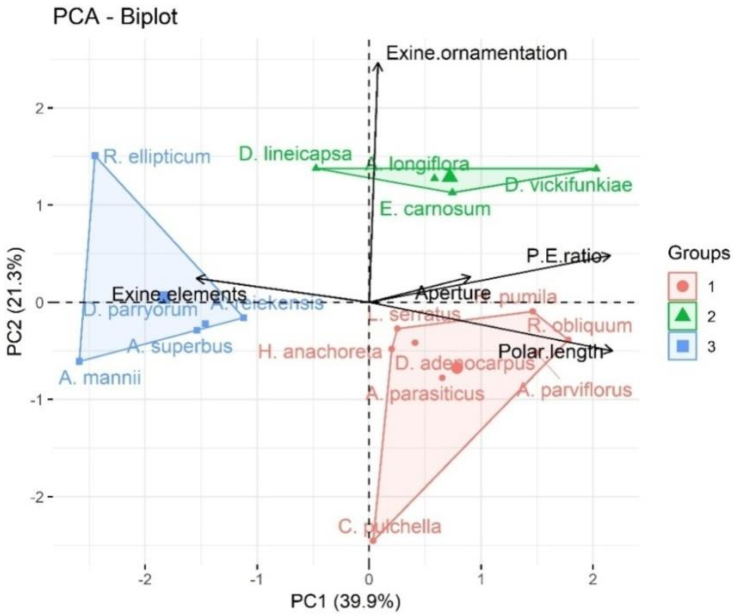

Figure 5.31: Principal component analysis (PCA) for pollen characteristics of different samples: Biplot of individuals and variables (PC1 & PC2).

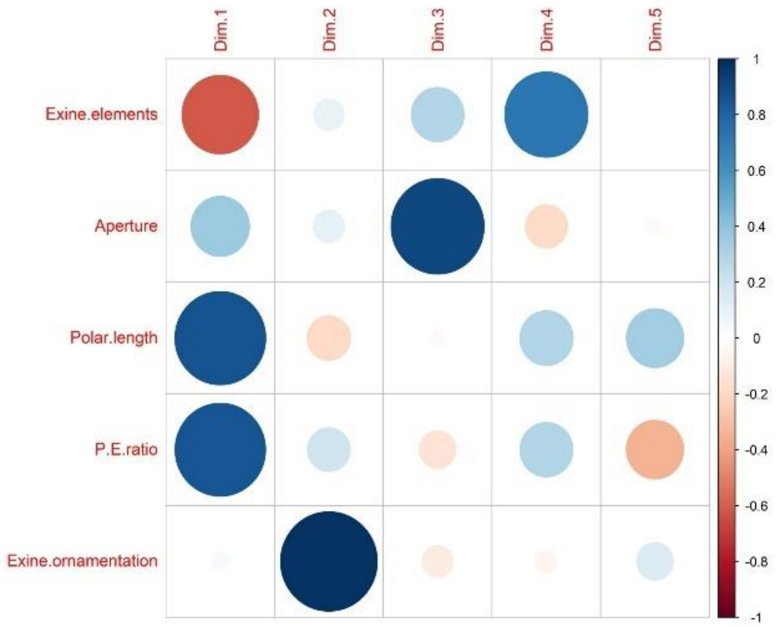

Figure 5.32: Heatmap of Principal component analysis (PCA) for pollen characteristics of different samples: Principal components and their relation with variables.

Further analysis of the contribution of variables to the PCA components highlights the influential role of specific pollen characters in shaping the observed patterns. Variables such as polar length, P/E ratio, exine elements, aperture, and exine ornamentation significantly contribute to the variation captured by PCA1, indicating their pronounced impact on the overall pollen characteristics. In contrast, exine ornamentation emerge as key contributors to the variation elucidated by PCA2, underscoring their importance in delineating distinct pollen characteristics across the study samples.

5.3.6 DISCUSSION

The pollen of the studied Gesneriaceae species were all monad and isopolar, consistent with findings by Pranee (2003) on the pollen morphology of certain Thai Gesneriaceae. The pollen characteristics exhibited distinct variation in shape, size, aperture and pattern of exine sculpturing. Majority of the pollen falls under medium size in 12 species out of the total 17 species.The exine ornamentation are of microreticulate and nanoreticulate types, although the work done by Pranee (2003) on Thai Gesneriaceaedid not report the presence of nanoreticulate exine ornamentation. In the present study the nanoreticulate exine ornamentation were found in five species (*A. longiflora, D. vickifunkiae, D. lineicapsa, E. Carnosum* and *R. ellipticum*) and the remaining 12 species have microreticulate ornamentation. It can be noted that several pollen ornamentations in Gesneriaceae might have originated independently multiple times from various character states as reported in other plant families (Sannier et al., 2009). This suggest that transitions between these states can occur flexibly and are not restricted to specific evolutionary pathways. Tricolporate pollen types were present in all the 5 taxa of genus *Aeschynanthus* along with *A. longiflora*, *C. pulchella* and *E. carnosum* and tricolpate pollen in the remaining 10 species. The ratio of polar axis and equatorial axis (P/E) ranges from 1 µm (*A.mannii*) to 2.1 µm (*H. pumila*). The shape of pollen grains ranges from isodiametric in *A.mannii,* prolate in 14 species and the perporate shape in *A. parviflorus, D. vickifunkiae* and *H. pumila*. Regarding sculpturing of exine, the elements present are classified in six categories namely: Psilate (Plate19: 3b; 20: 1b;

20: 2b; 21:2b; 21:3b; 22:2b; 24:1b) Fossulate (Plate 21: 1b; 23: 1b), Granulate (Plate 19:2b; 20: 3b; 22:1b; 23:2b:3b), Columellae (Plate 24:2b), Gemmae (Plate 19: 2b) and Nanoechini (Plate 24:3b).

Pollen characteristics in the family Gesneriaceae provides important information into species delimitation and phylogeny, establishing these traits as excellent taxonomic marker. It has been demonstrated to be helpful for evaluating phylogenetic relationships at higher levels (Claxton et al., 2005; Banks et al., 2008) as well as taxonomic positions at lower levels within the family (Chodat, 1896; Simpson & Skvarla, 1981; Paiva, 1998). The current study offers new insights into the relationships between closely related species and useful information on the pollen morphology of the Gesneriaceae family. Detailed comparative study of micromorphological pollen characters of the family Gesneriaceaeprovides an information for their distinction and phylogeny between the species recognized as an excellent taxonomic marker. Species within the same genus may form different groups in the dendrogram (Figure 5.29) due to variation in key pollen morphological traits. This may be influenced by environmental adaptation, phenotypic plasticity, or non-monophyletic relationships (Mc Culloch et al., 2022).Even the evolution of some pollen morphology can sometimes result in the development of progressively degenerative structure (Hayat et al., 2010). From the PCA analysis it was seen that the characters such as pollen size, shape, aperture, and exine ornamentation are likely the most influential in determining the structure of the dendrogram in the cluster analysis forming the different clades.

Main pollinators of the Gesneriads include insects, bees, butterflies, bats, and birds (Weber, 2017). Flower colour plays a key role in attracting pollinators and acts as a visual cue forpollinators to associate certain visual signals with reward (Chittka and Menzel, 1992; Schiestl and Johnson, 2013). Bird-pollinated flowers frequently have a distinctive orange/red colour, which is better detected by birds than by bees especially against a green vegetation background which can be seen in all the taxa of *Aeschynanthus* from the present study. It was also reported that the southeast Asian species of the genus *Aeschynanthus* have vibrant orange to red colour corolla have long been considered to be pollinated by sunbirds and spider hunters (Weber, 2017). The existence of a general relationship between pollen morphology and the pollination through pollen vectors like anemophilous and entomophilous species was suggested by Wodehouse (1935). The genus *Didymocarpus*, *H.* anachoreta, *H. pumila* and *R. ellipticum* are most probably pollinated by the bee as they possessed the flowers which are broad-tubed, infundibuliform or campanulate, with a wide opened mouth flowers and of light colours (white, blue, violet, pink, orange and yellow in various shades and combinations). As per Golam (2007), entomophilous plants have highly sculptured, oily pollen grains, whereas wind-pollinated species have smooth, dry pollen. The afore mentioned Gesneriads appear to be entomophilous plants, as evidenced by their highly sculptured exine ornamentation, including features such as granulate patterns, columellae, nanoechini, and fossulate structures. Species of the *A. longiflora and C. pulchella* may be pollinated by the butterflies as it was been reported in *Achimenes* species by Weber (2017). There are just a few species ofGesneriaceae that are known or suspected to be self-pollinated, which may be true for *E. carnosum* and *R.* obliquum because of their inconspicuous small flowers (Weber, 2017).

A clear correlation exists between floral morphology, pollen characters and pollination mechanism in the Gesneriaceae family as observed in other plant families (Lens et al. 2005). According to Fenster et al. (2004), the selection of pollinator functional groups is influenced by a variety of floral characteristics, including colour, morphology and fragrance.This factors likely contribute to diverse pollinator in the members of the family Gesneriaceae including the studied taxa from Mizoram.

5.4 Seed Micromorphology

5.4.1 Collection of seeds and preparation

Fruits of the studied taxa were collected during their fruiting season from the study area for the analysis of seed micromorphological characters. Seeds were carefully placed on the brass stub with double-sided adhesive tape usinga fine forceps. For the *Aeschynanthus* species, extra care was taken to avoid the hilar appendages from overlapping. The specimens were sputter-coated with a fine layer of gold using a Fine Coat Ion Sputter JFC-1100. Images were captured and the seed micromorphological data obtained were based on observations using SEM. The photographs were used to describe the seed shape, size, appendages, apical character, cell orientation, cell shape and cell character.

5.4.2 Seed characters of Gesneriaceae

Seed micromorphological characters of 18 species were studied and investigated for its seed shape, surface texture, and epidermal cell patterns. Micromorphology of seed coat plays a crucial role in dispersal mechanisms and ecological strategies, revealing a significant diversity in seed structure and adaptation.

The seedcoat morphology glossary of terminology based on Beaufort-Murphy (1985), and Mendum (2001) are given below.

Acute: terminating gradually to a point.

Constrictate: terminating as an abruptly constricted region that is not undulate as in Coronate.

Filiform: having hairlike projections at either or both apices.

Obtuse: terminating gradually in a rounded apex.

Straight: cell orientation parallel with the seed axis.

Slightly spiral: spiral irregular.

Spiral: cell orientation distinctly helical with the seed axis.

Irregular: cell orientation lacking an apparent pattern.

Narrowly lineate polygonate: very narrow cells, the edges of which are contiguous or nearly so.

Lineate-polygonate: approximately 2: 1 - 3: 2 ratios.

Polygonate: approximately 6: 5 ratios.

Ocellate: an elevated ringlike projection surrounding a depressed area.

Papillate: small rounded projections.

Rough: an uneven surface that lacks distinctively shaped features.

Smooth: lacking surface texture.

Tuberculate: rounded to distinctly tubelike projections (larger than Papillate).

Auriculate: rounded projections closely spaced and covering the entire surface.

Verrucate: with irregular warty projections.

Achimenes longiflora DC. **(Plate 25: 1a & 1b)**

The seed size ranges from 398–402 × 149–151 µm, seed shape is an elliptic, seed appendage absent, apical character is constrictate, cell orientation slightly spiral, cell shape is narrowly lineate polygonate and cell character depressed and smooth.

Aeschynanthus mannii Kurz ex C.B.Clarke **(Plate 25: 2a & 2b)**

The seed size ranges from 1624–1626 × 424–426 µm, seed shape is narrowly elliptic, seed appendage present having 1 hilar appendage, apical character is filiform type, cell orientation straight, cell shape is narrowly lineate polygonate and cell character depressed and verrucate.

Aeschynanthus parasiticus (Roxb.) Wall. **(Plate 25: 3a & 3b)**

The seed size ranges from 1039–1041× 239–241 µm, seed shape is narrowly elliptic, seed appendage present having 2 hilar appendage, apical character is filiform type, cell orientation straight, cell shape is an irregular and cell character elevated and auriculated.

Aeschynanthus parviflorus (D.Don) Spreng. **(Plate 26: 1a & 1b)**

The seed size ranges from 934–936× 305–307 µm, seed shape is narrowly elliptic, seed appendage present having 2 hilar appendage, apical character is filiform type, cell orientation straight, cell shape is an irregular and cell character elevated and auriculated.

Aeschynanthus reiekensis Lalhlupuii, S.D.Khomdram & S.D.Yumkham **(Plate 26: 2a & 2b)**

The seed size ranges from 1158–1162× 249–251 µm, seed shape is narrowly elliptic, seed appendage present having 2-4 hilar appendage, apical character is filiform type, cell orientation straight, cell shape is an irregular and cell character elevated and auriculated.

Aeschynanthus superbus C.B.Clarke **(Plate 26: 3a & 3b)**

The seed size ranges from 798–802× 199–201 µm, seed shape is narrowly elliptic, seed appendage present having 1 hilar appendage, apical character is filiform type, cell orientation spiral, cell shape is an irregular and cell character elevated and auriculated.

Chrysothemis pulchella (Donn ex Sims) Decne. **(Plate 27: 1a & 1b)**

The seed size ranges from 504–508 × 127–129 µm, seed shape is narrowly elliptic, seed appendage absent, apical character is obtuse type, cell orientation slightly spiral, cell shape is narrowly lineate polygonate and cell character elevated and rough.

Didymocarpus adenocarpus C.E.C.Fisch. **(Plate 27: 2a & 2b)**

The seed size ranges from 419–421 × 159–161 µm, seed shape is an elliptic, seed appendage absent, apical character is obtuse type, cell orientation slightly spiral, cell shape is irregular and cell character composite and auriculate.

Didymocarpus lineicapsa (C.E.C.Fisch.) B.L.Burtt **(Plate 27: 3a & 3b)**

The seed size ranges from 295–297 × 147–149 µm, seed shape is an elliptic, seed appendage absent, apical character is obtuse type, cell orientation slightly spiral, cell shape is lineate polygonate and cell character depressed and smooth.

Didymocarpus parryorum C.E.C.Fisch. **(Plate 28: 1a & 1b)**

The seed size ranges from 418–422 × 191–193 µm, seed shape is an elliptic, seed appendage absent, apical character is obtuse type, cell orientation spiral, cell shape is irregular and cell character composite and tuberculate.

Didymocarpus vickifunkiae V.Gowda & N.S.Prasanna **(Plate 28: 3a & 3b)**

The seed size ranges from 688–692 × 157–159 µm, seed shape is narrowly elliptic, seed appendage absent, apical character is constrictate type, cell orientation slightly spiral, cell shape is polygonate and cell character depressed and smooth.

Didymocarpus wengeri C.E.C.Fisch. **(Plate 28: 2a & 2b)**

The seed size ranges from 404–406 × 167–169 µm, seed shape is an elliptic, seed appendage absent, apical character is an acute type, cell orientation slightly spiral, cell shape is polygonate and cell character composite and smooth.

Epithema carnosum Benth. **(Plate 29: 1a & 1b)**

The seed size ranges from 542–546 × 189–191 µm, seed shape is an elliptic, seed appendage absent, apical character is an acute type, cell orientation spiral, cell shape is narrowly lineate polygonate and cell character composite and tuberculate.

Henckeliaanachoreta (Hance) D.J.Middleton & Mich.Möller **(Plate 29: 2a & 2b)**

The seed size ranges from 348–352 × 149–151 µm, seed shape is an elliptic, seed appendage absent, apical character is constrictate type, cell orientation straight, cell shape is polygonate and cell character elevated and tuberculate.

Henckelia pumila (D.Don) A.Dietr. **(Plate 29: 3a & 3b)**

The seed size ranges from 598–602 × 149–151 µm, seed shape is narrowly elliptic, seed appendage absent, apical character is constrictate type, cell orientation slightly spiral, cell shape is polygonate and cell character composite and ocellate.

Lysionotus serratus D.Don **(Plate 30: 1a & 1b)**

The seed size ranges from 535–539 × 162–164 µm, seed shape is narrowly elliptic, seed appendage present having 1 hilar appendage, apical character filiform, cell orientation is straight, cell shape lineate polygonate and cell character elevated and papillate.

Rhynchoglossum obliquum Blume **(Plate 30: 2a & 2b)**

The seed size ranges from 292–296 × 105–107 µm, seed shape is narrowly elliptic, seed appendage absent, apical character constrictate, cell orientation is slightly spiral, cell shape lineate polygonate and cell character depressed and verucate.

Rhynchotechum ellipticum (Wall. ex D.Dietr.) A.DC. **(Plate 30: 3a & 3b)**

The seed size ranges from 434–436 × 234–236 µm, seed shape is an elliptic, seed appendage absent, apical character is an acute type, cell orientation is straight, cell shape polygonate and cell character papillate and verucate.

Seed shape ranges: narrowly elliptic 6:1 - 3:1; elliptic 2:1 – 3:2; broadly elliptic 6:5; circular 1:1

Table 5.4: Seed characters and character states for the cluster analysis of Gesneriaceae. The numbers in brackets are the codes for character states.

Sl. No.	Characters	Character states
1.	Seed shape	Elliptic = 0, Narrowly elliptic = 1, Circular = 2
2.	Seed appendages	Absent = 0, one hilar = 1, two hilar = 2, two or more = 3
3.	Apical character	Acute = 0, obtuse = 1, constrictate = 2, filiform = 3
4.	Cell orientation	Spiral = 0, slightly spiral = 1, straight = 2
5.	Cell shape	Narrowly lineate polygonate = 0, lineate polygonate = 1, irregular = 2, polygonate = 3
6.	Cell character	Verrucate = 0, auriculate = 1, tuberculate = 2, rough = 3, smooth = 4, papillate = 5, ocellate = 6

Table 5.5: Data matrix used in cluster analysis of Gesneriaceae species based on seed features.

Sl. No.	Taxa	Seed shape	Seed appendage	Apical character	Cell orientation	Cell shape	Cell character
1	*Achimenes longiflora* DC.	0	0	2	1	0	4
2	*Aeschynanthus mannii* Kurz ex C.B.Clarke	1	1	3	2	2	0
3	*Aeschynanthus parasiticus* (Roxb.) Wall.	1	2	3	2	2	1
4	*Aeschynanthus parviflorus* (D.Don) Spreng.	1	2	3	2	2	1
5	*Aeschynanthus reiekensis* Lalhlupuii, S.D.Khomdram & S.D.Yumkham	1	3	3	2	2	1
6	*Aeschynanthus superbus* C.B.Clarke	1	1	3	0	2	1
7	*Chrysothemis pulchella* (Donn ex Sims) Decne.	1	0	1	1	0	3
8	*Didymocarpus adenocarpus* C.E.C.Fisch.	0	0	1	1	2	1
9	*Didymocarpus vickifunkiae* V.Gowda & N.S.Prasanna	1	0	2	1	3	4
10	*Didymocarpus lineicapsa* (C.E.C.Fisch.) B.L.Burtt	0	0	1	1	1	4
11	*Didymocarpus parryorum* C.E.C.Fisch.	0	0	1	0	2	2
12	*Didymocarpus*	0	0	0	1	3	4

	wengeri C.E.C.Fisch.						
13	*Epithema carnosum* Benth.	0	0	0	0	0	2
14	*Henckelia anachoreta* (Hance) D.J.Middleton & Mich.Möller	0	0	2	2	3	2
15	*Henckelia pumila* (D.Don) A.Dietr.	1	0	2	1	3	6
16	*Lysionotus serratus* D.Don	1	1	3	2	1	5
17	*Rhynchoglossum obliquum* Blume	1	0	2	1	1	0
18	*Rhynchotechum ellipticum* (Wall. ex D.Dietr.) A.DC.	0	0	0	0	0	0

5.4.3 Seed cluster analysis using dendrogram

On the cluster analysis, the six primary seed morphological characters studied viz., seed shape, seed appendages, apical character, cell orientation, cell shape and cell character either individually or collectively have produced two major clades with different taxa. The Clade 1 (left major clade) includes all the species of *Aeschynanthus* namely *A. mannii*, *A. reiekensis*, *A. parasiticus*, *A. parviflorus* and *A. superbus*. Apart from this *Rhynchoglossum obliquum, Rhynchotechum ellipticum, Henckelia anachoreta*, *D. adenocarpus* and *D. parryorum* are clustered. The Clade 2 (right major clade) includes the *Epithema carnosum, C. pulchella, A. longiflora, D. lineicapsa, D. vickifunkiae, D. wengeri, L. serratus* and *H. pumila*.

The dendrogram provides valuable insights into the taxonomic relationships within the Gesneriaceae family based on seed micromorphological data. Species in the genus *Aeschynanthus* with hilar appendages are group in the same major clade, maintaining genus-level consistency. However, *A. superbus* characterised by seed cell arranged in spiral orientation stands out as distinct character from other *Aeschynanthus* species, highlighting intrageneric variation. Interestingly, an initial clade is formed between *A. superbus* and *R. obliquum*, both of which share a narrowly elliptic seed shape. Together, *C. pulchella* and *A. longiflora* sharing similar cell orientation and cell shape are in the same clade, supporting the placement of both genera under the subfamily Gesneroideae within the Gesneriad tribe as per systematic classification of the family Gesneriaceae by Weber et al. (2013). The distinct separation between clusters suggests that seed coat micromorphological data is a reliable tool for distinguishing species and genera within the Gesneriaceae family underscoring its value for taxonomic refinement. Variation in branch lengths and Euclidean distances reflect the extent of evolutionary divergence among species. However, the placement of different taxa from the same genus may indicate significant divergence due to evolutionary or ecological pressures.

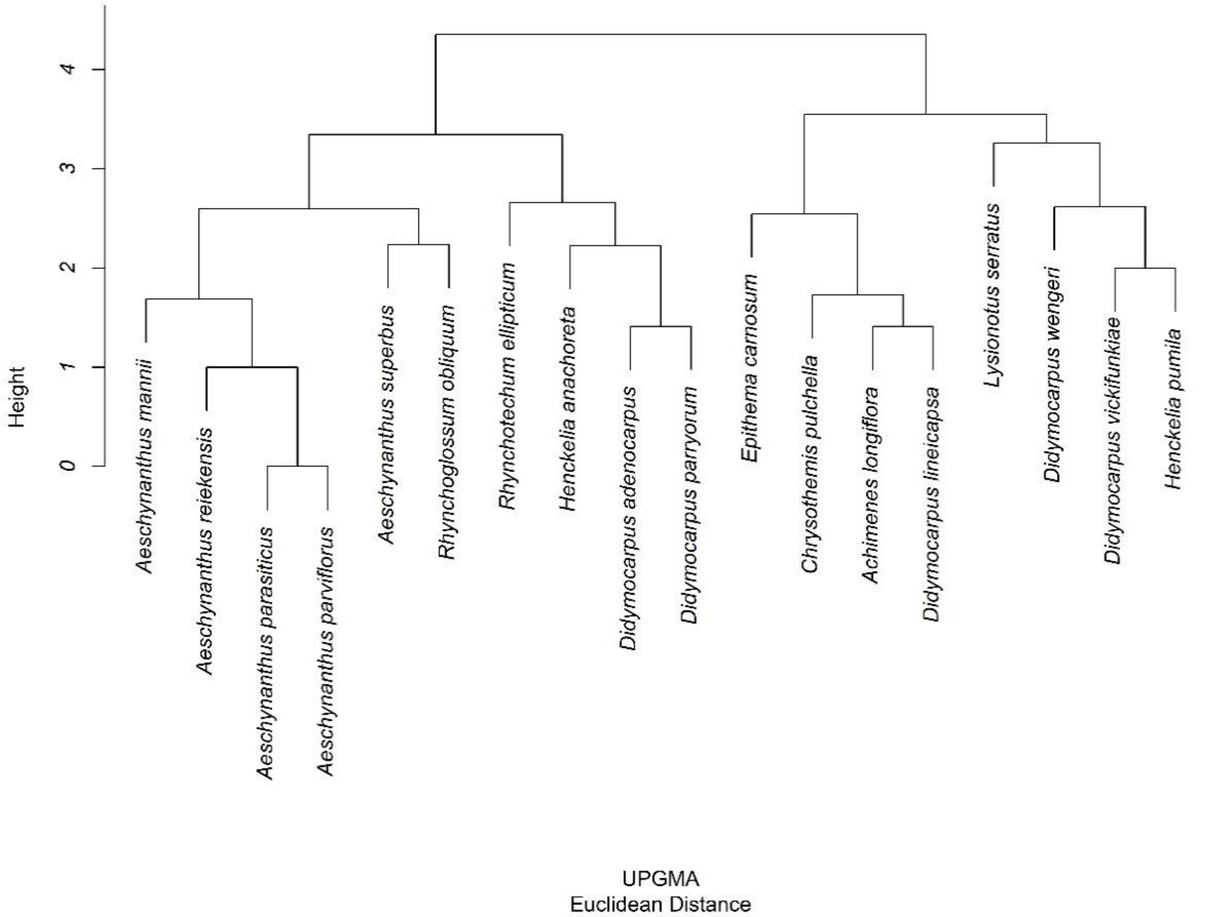

Figure 5.33: Dendrogram based on cluster analysis of Seed morphological characters of different species of the family Gesneriaceae Rich. & Juss. Using UPGMA (Unweighted Pair Group Method with Arithmetic Mean).

5.4.4 Principal Component Analysis of Seed

To ascertain the optimal number of principal components in seed micromorphology of Gesneriaceae family, we assessed the variance percentages from the scree plot (Figure 5.34). Ultimately, we chose to focus on the first two principal components in the PCA, which collectively accounted for 65.7% of the data variation; the first component explained 49%, while the second explained 16.7%. Eigenvalues of 2.94 and 1.00 were recorded (Table 5.6).

Table 5.6: Eigenvalues, variance explained % and cumulative proportion of totalvariance from principal component analysis (PCA) components for seed characteristics of different species of Gesneriaceae of Mizoram.

PCA	***Eigenvalue***	**% of variance**	**cumulative %**
1	2.94	49	49.03
2	1.00	16.7	65.69

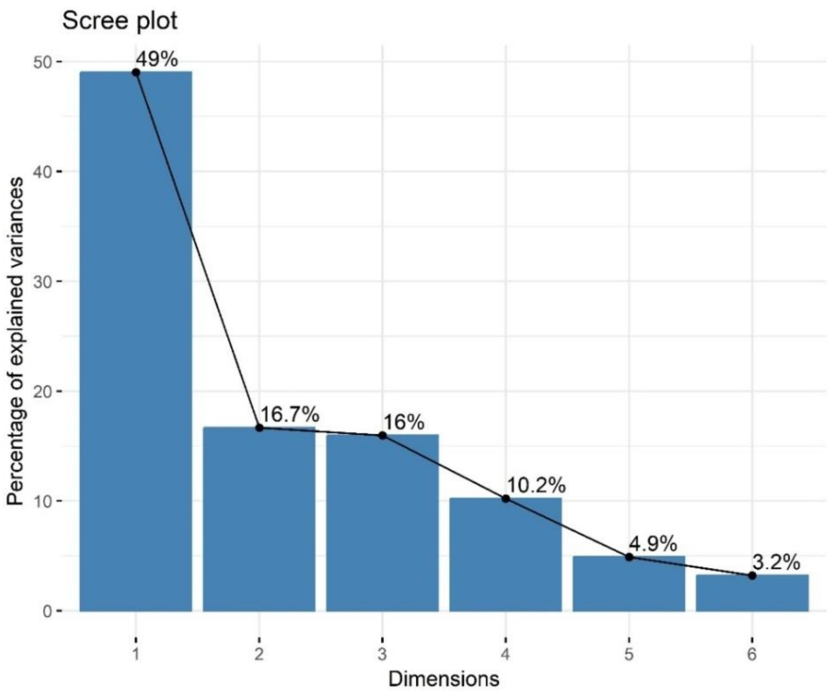

Figure 5.34: Principal component analysis (PCA) for Seed contribution of each principal component to total variance.

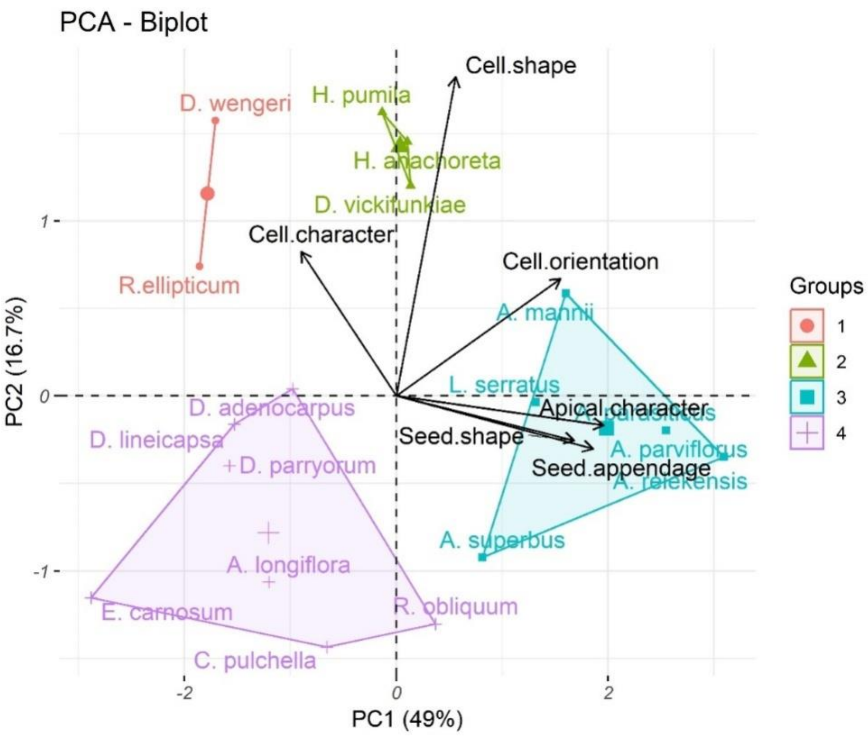

Figure 5.35: Principal component analysis (PCA) for seed characteristics of different samples: Biplot of individuals and variables (PC1 & PC2).

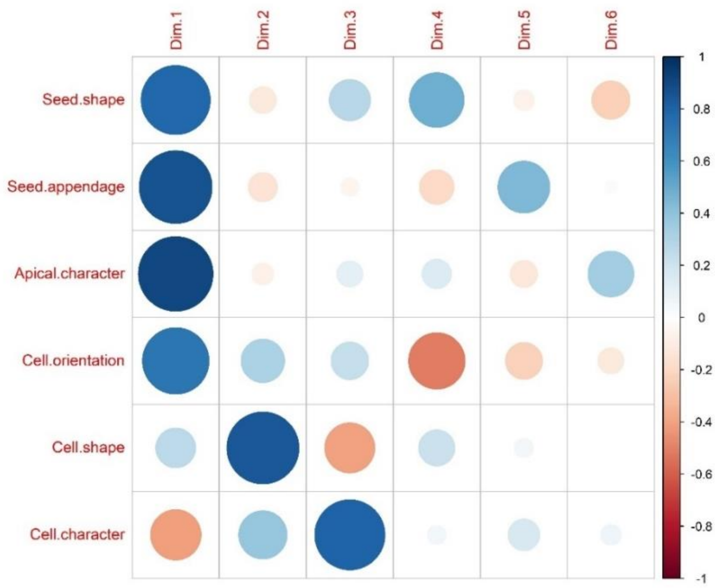

Figure 5.36: Heatmap of Principal component analysis (PCA) for seed characteristics of different samples: Principal components and their relation with variables.

The cluster analysis divided these samples into four groups (Figure 5.35). Group 1 consisted of *D. wengeri* and *R. ellipticum*, Group 2 consisted of *D. vickifunkiae*, *H. anachoreta* and *H. pumila*, Group 3 consisted of *A. mannii*, *A. parasiticus*, *A. parviflorus*, *A. reiekensis* and *A. superbus*, and lastly, Group 4 comprises of *A. longiflora*, *C. pulchella*, *D. adenocarpus*, *D. lineicapsa*, *D. parryorum*, *E. carnosum* and *R. obliquum*. In terms of variable correlations, the first component exhibited strong positive associations with apical character, seed appendage, seed shape, cell orientation and cell shape, while a negative correlation was observed with cell character. Conversely, the second component displayed direct relationships with cell shape, cell character and cell orientation, with negative correlations observed for seed appendage, seed shape and apical character (Table 5.6 & Figure 5.34).

Among the variables, apical character made the highest contribution to the first component (28.2), whereas cell shape contributed the least (2.30). Conversely, in the second component, cell shape had the highest contribution among all variables (71.70), while apical character had the lowest (0.61).

Seed characteristics such as seed shape, seed appendage, apical character, cell orientation, cell shape and cell character, have been shown to be important for the classification among the speciesof Gesneriaceae genera. The five species of *Aeschynanthus* and the *L. serratus*, which have filiform apical structures, were all grouped together in a single group. The length and number of these hilar appendages function as a key innovation allowing biogeographic transgression of main clades area (Denduangboriant et al., 2001). Similar to this, *H. anachoreta*, *H. pumila* and *D. vickifunkiae* which have constrictate apical character are also found in a single group, supporting the analysis in Figure 5.36, that apical character made the highest contribution to the first component (28.2) among the variables and highly consistent, indicating that they were relatively constant within species. The results of the PCA also supported the former view that populations within the same species cluster together, while the distribution of samples of different species does not show a certain regularity. Furthermore, the PC1 of the PCA provided a highly dominant variability of 49% and thePC2 of the PCA variability of 16.7% of the total variance. These results suggest that even though the seed micromorphologycal characters are highly variable within species, each traitstill play an important role in systematic analysis in Gesneriaceae members.

5.4.5 Discussion

The seeds of the Gesneriaceae have not been studied very extensively, possibly because of their small size (Beaufort-Murphy, 1985). These small seeds are produced in large numbers and have different mode of dispersal based on variations in the surface of seed and appendages. Thepresent studies shows that thesize of the seeds varies from 294–1160 × 146–425 µm, with *R. obliquum* having the smallest seeds and *A. mannii* having the largest. Species in the genus *Aeschynanthus* exhibit significantly larger seeds, reaching upto 4 cm in length including the hilar appendage, making them several times larger than those of other species. The observed seed shapes are predominantly elliptic or narrowly elliptic. Specifically, *A. longiflora, D. adenocarpus, D. lineicapsa, D. parryorum, D. wengeri, Epithema carnosum, Henckelia anachoreta* and *Rhynchotechum ellipticum* have elliptic seed shape.

The surface morphology of seeds is used extensively in taxonomic and biosystematics studies (Beaufort-Murphy, 1985). In addition, adherence to the surface of rocks and mosses is significantly influenced by the existence of seed ornamentation such as verrucate (Plate 26:2b; 31:2b:3b), auriculate (Plate 25:3b; 26:1b:2b:3b; 27:2b), tuberculate (Plate 28:1b; 29:1b:2b), rough (Plate 27:1b), smooth (Plate 25:1b; 27:3b; 28:3b), papillate (Plate 30: 1b), and ocellate (Plate 29:3b). As per Brochmann (1992) the seed coat of species from various habitats differed significantly, indicating seed size and weight can be closely related to habitat ecology.According to Davis & Heywood (1963), a significant degree of natural selection may be indicated by the consistency of many of these seed characteristics which may reflect the high diversityin seed coat ornamentation observed across specieslinked to these observed dispersion strategy and environmental adaptations.

Variability in seed coat ornamentation may represent an adaptation to specific ecological condition and dispersal mechanism, potentially in adding in seed attachment to animals for effective dissemination.

The number and type of hilar appendages has been used to classify species within the genus *Aeschynanthus*. This study identified three types of hilar appendages aligned with classification proposed by Bentham (1876). The haplotrichium, with a single appendage found in *A. mannii* (Plate 25:2a) and *A. superbus* (Plate 26: 3a), diplotrichium, with two appendages at the hilar end found in *A. parasiticus* and *A. parviflorus* (Plate 25:3a; 26:1a) and polytrichium with many appendages at the hilar end isfound in *A. reiekensis* (Plate 26:2a). *A. superbus* can be distinguished from other *Aeschynanthus* species in the clade by its spiral cell orientation, as seen in the dendrogram (Figure 5.33). These distinctive features provide valuable taxonomic characters and can contribute to the development of an identification key for the genus. Different seed characters including the hilar appendage and the cell orientations offer a set of characters useful for the taxonomy and it is useful for construction of an identification key.

The differences in size and morphology of the Gesneriaceae seeds are nicely correlated with the differences in their type of fruit and reflect different adaptation to seed dispersal. In the Didymocarpoideae subfamily, species of *Aeschynanthus* and *Lysionotus serratus*, produced long pod-like capsule that dehisced primarily by longitudinal slits, allowing the fruit to open with one or both shape of valves. Seed dispersal is often non-specific with seed falling into the ground or being shaken out by wind, rain, or passing animals. Due to their small size and light weight, seeds are also dispersed by wind, often landing on nearby rocks and mosses. Small seeds with long filiform appendages are particularly suited for wind dispersal but less adapted in wet conditions, suggesting an adaptation to seasonal climates such as those found in Mizoram. Shorter appendages, by contrast result in faster seed falls and reduce the likelihood of being carried by the light air currents.The follicular fruit found in *Henckelia anachoreta* and *H. pumila*, is another distinctive kind, where dehiscence occurs solely along the median carpel bundle in the upper carpel. In contrast, species like *Epithema carnosum* and *Rhynchoglossum obliquum* which have short, ovoid, or globose capsules embedded in a funnel-shaped calyx, are dispersed by rain-splash.

This study reveals that seed characteristics like the seed shape and seed coat ornamentation were found to be possibly related to the seed dispersal and environmental adaptation among the Gesneriaceae taxa. These features provide valuable insights for systematic evaluations as well as taxonomic evolutionary and ecological studies among the Gesneriaceae members of Mizoram.

5.4 Molecular Studies

In order to study the interspecies relationship among the studied members of Gesneriaceae, a phylogenetic analysis was further conducted using the concatenated sequences of ITS2 and *rbcL* primer.

5.4.1 Bioinformatics Analysis

The forward and the reverse sequences obtained from all the PCR products amplified with ITS2 and *rbcL* primers were trimmed. The contig of the forward and the reverses sequences was then submitted in the Basic Local Alignment Search Tool (BLAST) of the National Centre for Biotechnology Information (NCBI) for homology search. GenBank databases and accession number were allotted for the submitted taxa.

Table 5.7: List of the *rbcL* and ITS2 GenBank accession no. of the Gesneriaceae taxa of Mizoram

Sl no.	Taxa	ITS2 Accession No.	*rbcL* Accession No.
1.	*Achimenes longiflora* DC.	OR758858	PP502405
2.	*Aeschynanthus mannii* Kurz ex C.B.Clarke	OR759953	PP479926
3.	*Aeschynanthus parasiticus* (Roxb.) Wall.	OR759969	PP479927
4.	*Aeschynanthus parviflorus* (D.Don) Spreng.	OR759968	PP479928
5.	*Aeschynanthus reiekensis* Lalhlupuii, S.D.Khomdram & S.D.Yumkham	PQ142802	PP502407
6.	*Aeschynanthus superbus* C.B. Clarke	OR759953	PP479929
7.	*Chrysothemis pulchella* (Donn ex Sims) Decne.	OQ940523	PP502404
8.	*Didymocarpus adenocarpus* C.E.C.Fisch.	OR759955	PP479933
9.	*Didymocarpus vickifunkiae* V.Gowda & N.S.Prasanna	OQ940527	PP479930
10.	*Didymocarpus lineicapsa* (C.E.C.Fisch.) B.L.Burtt	OR759956	PP479932
11.	*Didymocarpus parryorum* C.E.C.Fisch.	OR759954	PP479934
12.	*Didymocarpus wengeri* C.E.C.Fisch.	OR759957	PP479931
13.	*Epithema carnosum* Benth.	OR759958	PP502406
14.	*Henckelia anachoreta* (Hance) D.J.Middleton & Mich.Möller	OR758860	PP502400
15.	*Henckelia pumila* (D.Don) A.Dietr.	OR758860	PP479935
16.	*Lysionotus serratus* D.Don	OQ940524	PP502402
17.	*Rhynchoglossum obliqum* Blume	OQ940526	PP502401
18.	*Rhynchotechum ellipticum* (Wall. ex D.Dietr.) A.DC.	OQ940525	PP502403

5.4.2 Phylogenetic Tree Construction

Construction of phylogenetic tree was carried out using MEGA X (Molecular Evolutionary Genetics Analysis, X). Phylogenetic model test was carried out to determine the best-fit nucleotide substitution model with the lowest BIC scores (Bayesian Information Criterion). Then, Maximum Likelihood (ML) phylogeny with Kimura-2-Parameter (K2) model with discrete Gamma Distributed (G) rate with a bootstrap support of 1000 was used for the phylogenetic analysis.

5.4.3 Phylogeny Studies

Phylogenetic analysis using a maximum likelihood tree (MLT) in a K2P model with bootstrap-1000 of Gesneriaceae species showed a high similarity in the BLAST search to the available sequences in the NCBI database. For the *rbcL* and ITS2 sequences, 18 species within 9 genera of the family Gesneriaceae were used to construct the ML tree in this study. All nodes were supported with bootstrap values up to 99%. The *Didymocarpus* species clustered into a clade with 44% bootstrap values supporting the monophyly of *Didymocarpus*. *Achimenes* clustered with *Chrysothemis* with bootstrap value 77%. *Rhynchotechum ellipticum* and *Rhynchoglossum obliquum* form a clade, and then sequentially formed clades with *Henckelia anachoreta* and *H. pumila*. In another major small clade, the clade formed by *Aeschynanthus reiekensis, A. mannii, A. parasiticus* and *A. parviflorus*, clustered with *A. superbus*. Among the clade formed within the genus *Didymocarpus*, *D. wengeri* shared a sister relationship with *D. anningensis*. For ITS2 sequences all the nodes were supported with the bootstrap values ranging between 37 to 100%. The *Rhynchoglossum obliquum* clustered into a clade with the genera *Henckelia*, *Lysionotus*, *Chrysothemis*, *Achimenes* at 100% bootstrap values, supporting the monophyly of the family. According to clustering analysis, the species *H. pumila* and *H. anachoreta* form a cluster with a bootstrap value 64%. At a bootstrap value 51%, *Lysionotus* and *Henckelia* exhibit their closeness. The bootstrap value 84 % for *Achimenes* and *Chrysothemis*. The genera *Rhynchotechum*, *Epithema, Didymocarpus* and *Aeschynanthus* clustered with bootstrap values of 82% closeness. *A. parviflorus* forms a clade with *A. pachytrichus*, and then sequentially formed clades with *A. parasiticus*, *A. mannii, A. superbus* and *A. reiekensis*. The bootstrap values for the genus *Didymocarpus* were 50%. *D. vickifunkiae* formed a distinct clade from the other species in the *Didymocarpus* group.

5.4.4 Discussion

The investigated part of the *rbcL* coding region shows very little variation and seems to be strictly conserved by selective pressure. Every genus in the phylogenetic tree formed coherent clades by tightly clustering with sequences from other Gesneriaceae genera. This grouping of sequences by genus indicates both evolutionary divergence and phylogenetic coherence among genera within the family. The monophyly among the species of eight genera was confirmed and the evolutionary relationships within the Gesneriaceae family were established in the phylogenetic tree. *Justicia adhatoda*, of the Acanthaceae family, was placed in an outgroup. In the ITS2 tree analysis, a tight evolutionary link between species of different genera within this family is seen by this clustering pattern. Similar to this, each genus in the phylogenetic tree strongly clustered with sequences from other genera in the Gesneriaceae family to form cohesive clades. Phylogenetic coherence and evolutionary divergence among the various genera within the family are indicated by this genus-by-genus grouping of sequences. This helped to establish the evolutionary relationships within the Gesneriaceae family and validated the closeness among the species of eight genera. The outgroup taxa, *Andrographis paniculata*, belonging to the Acanthaceae family, was placed outside the Gesneriaceae clades.

At the genus level, both the ITS2 and *rbcL* phylogenetic tree provide a clear representation of the relationship between species provided by both the ITS2 and *rbcL* phylogenetic tree constructions. This study supports the findings of Zimmer et al. (2002), which concluded that the Gesneriaceae family is monophyletic. However, this investigation reveals that the data base submission for both ITS2 and *rbcL* sequences are limited, suggesting a need for more comprehensive data to fully represent the diversity within the family. The present study will make a significant contribution to fill the gap by providing valuable data and insights, providing ITS2

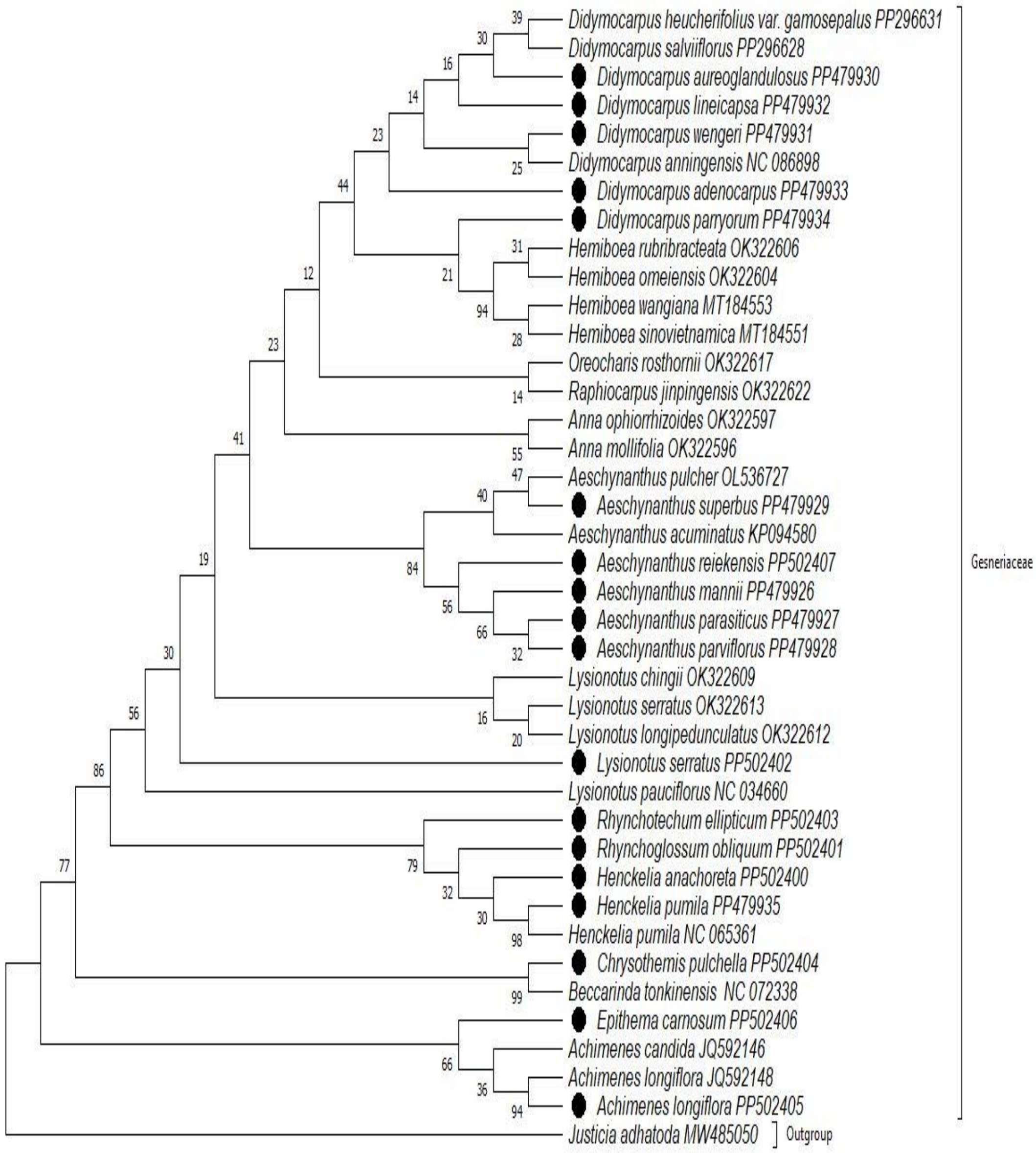

Figure 5.37: Maximum Likelihood (ML) Based Phylogenetic Tree analysis of *rbcL* Sequences using MEGA X (Bootstrap value:1,000).

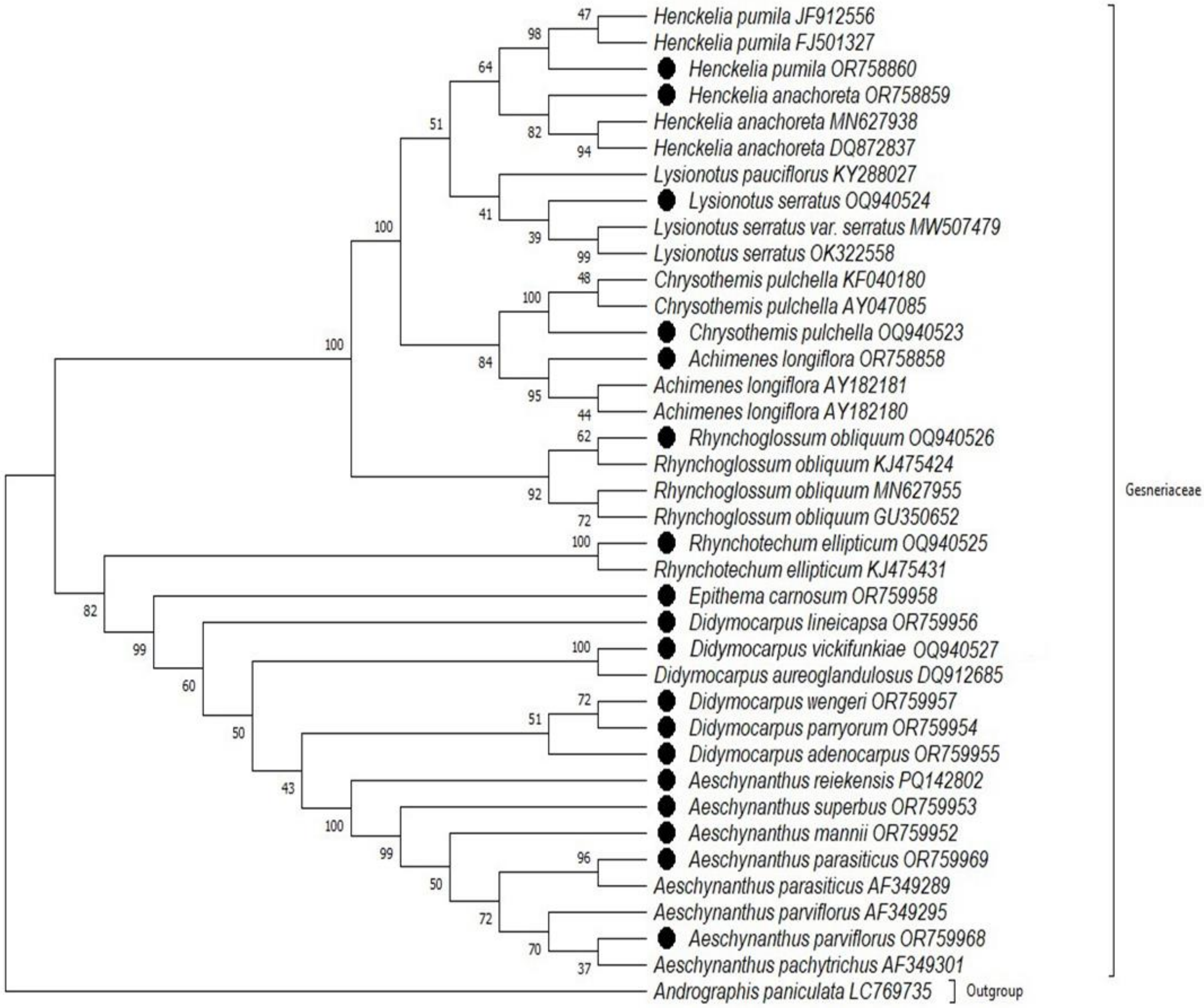

Figure 5.38: Maximum Likelihood (ML) Based Phylogenetic Tree analysis of ITS2 Sequences using MEGA X (Bootstrap value:1,000).

and *rbcL* sequence database and enhancing our understanding of the phylogenetic relationship within the Gesneriaceae family.

The DNA database submitted by the author to NCBI GenBank with their accession no. *Aeschynanthus mannii* (OR759953),*A. reiekensis* (PQ142802), *A. superbus* (OR759953), *Didymocarpus adenocarpus* (OR759955), *D. lineicapsa* (OR759956), *D. parryorum* (OR759954), *D. wengeri* (OR759957), and *D.vickifunkiae* (OQ940527) for ITS2 and *Aeschynanthus mannii* (PP479926), *A. parviflorus* (PP479928), *A. parasiticus* (PP479927), *A. reiekensis* (PP502407), *A. superbus* (PP479929), *Didymocarpus adenocarpus* (PP479933), *D. lineicapsa* (PP479932), *D. parryorum* (PP479934), *D. wengeri* (PP479931), *D. vickifunkiae* (PP479930), *Henckelia anachoreta* (PP502400), and *Rhynchotechum ellipticum* (PP502403) for *rbcL* respectively, is added to the global database for the first time that can improve the accuracy and reliability of phylogenetic information of the Gesneriaceae family.

5.5 ENDEMIC SPECIES

In the present study,a total of six taxa endemic to the state of Mizoram could be collected —five from the genus *Didymocarpus* namely *D. adenocarpus*, *D. lineicapsa*, *D. parryorum*, *D. wengeri* and *D. vickifunkiae* and one from the genus *Aeschynanthus* (i.e., *Aeschynanthus reiekensis*) newly discovered. Despite being widely distributed in the world constituting the eighth-largest genus in the Gesneriaceae, all the species of *Didymocarpus* species present and collected from Mizoram are narrow endemics with restricted ranges, occurring in small, isolated groups, and often only known from their type localities. This promotes its endemism within its original habitat and according to Prentice (1976) they are considered as the neo-endemic.

Gesneriads from Mizoram are known to flourish in a variety of habitat with altitudinal variation.The average altitudinal gradient in Mizoram ranges between 21°.58′ N to 24°.35′ N and 90°.15′ E to 93°.29′ E (Anonymous, 2001), indicating that these endemic plants are found in an average altitudinal gradient (Table 5.9) of the state.The hilly state of Mizoram, which is completely encased in a sedimentary basin, is home to mineral resources including limestone and according to Tseng (2019); Xu et al. (2017); Liu et al. (2021) this limestone karst has bred a high degree of species diversity and significant endemism. These limestones can be seen in locations like Muthi, Tuirial, Nisapui,Saipum, Tuirial River's bed, Kawrtethawveng, Lallen, West Phaileng, Kawnpui, Chawngte village, Phawngpui, Nisapui, Lungmuatram and Demdum area (Shiva Kumar & Verma, 2005).

In addition, the geographical position and climatic conditions of the state, with high rainfall and moderate temperatures might have contributed to the evolution of many endemic plant species of Gesneriaceae. The combination of heavy monsoon rains and relatively mild winters makes Mizoram a favourable environment for Gesneriaceae, many of which thrive in moist and shaded habitats, such as forest understories, stream banks, and limestone cliffs. The limited geographical distribution of Mizoram Gesneriaceae with the absence of existence for some species in their historical locations makes them high conservation priority.

Table 5.9: List of Endemic species of Gesneriaceae family from Mizoram

Sl.No	**SCIENTIFIC NAME**	**Location (GPS)**	**Altitude**
1.	*Aeschynanthus reiekensis* Lalhlupuii, S.D.Khomdram & S.D.Yumkham	23°40′58.2″N 92°36′22.0″E,	1391.70 m
2.	*Didymocarpus adenocarpus* C.E.C.Fisch.	23°68′35.1″N 92°60′78.7″E	1384 m
3.	*Didymocarpus lineicapsa* (C.E.C.Fisch.) B.L.Burtt	23°76′68.3″N 92°75′96.2″E	1090 m
4.	*Didymocarpus parryorum* C.E.C.Fisch.	22°83′84.0″N 92°81′67.4″E	1349 m
5.	*Didymocarpus wengeri* C.E.C.Fisch.	22°30′48.4″N 93°02′70.9″E	1426 m
6.	*Didymocarpus vickifunkiae* V.Gowda & N.S.Prasanna	22°83′84.0″N 92°81′67.4″E	1349 m

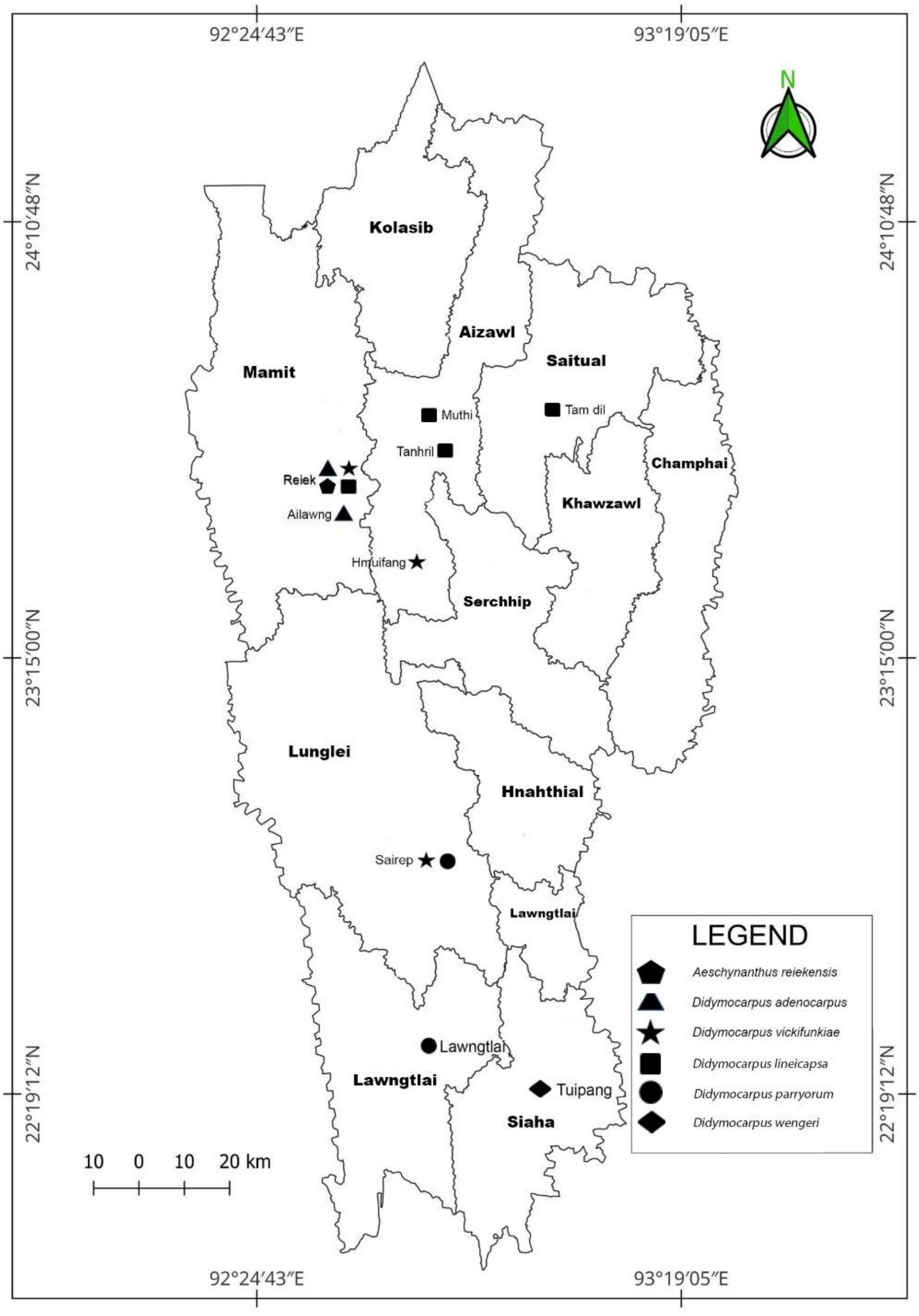

Figure 5.39: Distribution map of endemic taxa from Mizoram

5.6 RET

Mizoram is rich in biodiversity and is part of the Indo-Burma Biodiversity hotspot harbouring rich flora diversity and high species endemism. The species richness was reported high in the undisturbed and moderately disturbed stand. This could be attributed due to favourable climatic conditions like high and prolonged rainfall, moderate temperature, high relative humidity and status of soil. The conservation and management of Rare, Endangered and Threatened (RET) plant species have become an important issue in the present study area. The development of the economy, due to frequent human activities like tourism development, road construction extinct species like *Rhynchoglossum obliquum*, some species of *Didymocarpus* and *Henckelia* which grow along the shaded roadside were destroyed and the fragile ecological balance of these diversified microhabitat is easily destroyed which threaten the survival of gesneriad.Some of the species classified as RET offer diverse benefits, ranging from medicinal, ornamental and nutritional value to fulfilling various ecological roles.

The Gesneriaceae family members of Mizoram falls into six categories i.e., Least Concern, Not Threatened, Endangered, Critically Endangered, Not Evaluated and Vulnerable based on the IUCN Red List Categories and Criteria (IUCN 2022). As per IUCN Red List Categories and Criteria (IUCN, 2022) and standard research articles and online portal website, a total of 13 species belonging to 5 genera of the Gesneriaceae family are identified under the Rare, Endangered and Threatened (RET) taxa and 5 species are under NE category (Table 5.8). The species *Didymocarpus adenocarpus* and *D. vickifunkiae* are in endangered (EN) category because population count is limited to an area of about 15 km^2 and the population has 200 to 300 mature plants available during survey. Based on the fragmented population and rapidly degrading habitat, the species of *D. wengeri* should also be considered as critically endangered. Although provisionally assessed as CR by Lalhlupuii et al. (2023), *Aeschynanthus reiekensis* is also one of the critically endangered due to an area occupancy less than 10 km^2. Population of individual less than 1000 along with rapid forest degradation classified the *D. lineicapsa* under vulnerable (VU). The wide spread distribution and common in parts of its ranges categorized the species under least concern. Species under the family Gesneriaceae in Mizoram are at risk of extinction due to various factors such as habitat loss, overexploitation, pollution, and climate change. The conservation status of the unassessed plant needs to be taken into consideration for their habitat.

Table 5.8: Lists of taxa with RET status with botanical name, vernacular name, accession number, conservation status and references.

Sl. No	Botanical name	Vernacular name	MZUH Accession	Conservation status	References
1	*Achimenes longiflora* DC.	NA	MZUH129815	NE	NA
2	*Aeschynanthus mannii* Kurz ex C.B.Clarke	Hnahchhah	MZUH000036	NE	NA
3	*Aeschynanthus parasiticus* (Roxb.) Wall.	Hnahchhah	MZUH000037	LC	Middleton (2009)
4	*Aeschynanthus parviflorus* (D.Don) Spreng.	Hnahchhah	MZUH000038	LC	Middleton (2007)
5	*Aeschynanthus reiekensis* Lalhlupuii, S.D.Khomdram & S.D.Yumkham	Hnahchhah	MZUH000039	CR	Lalhlupuii et al. (2023)
6	*Aeschynanthus superbus* C.B. Clarke	Hnahchhah	MZUH000040	LC	Middleton (2009)
7	*Chrysothemis pulchella* (Donn ex Sims) Decne.	NA	MZUH129812	NE	NA
8	*Didymocarpus adenocarpus* C.E.C.Fisch.	Sekhupthur suak	MZUH000041	EN	Prasanna & Gowda (2020)
9	*Didymocarpus vickifunkiae* V.Gowda & N.S.Prasanna	Sekhupthur suak	MZUH000042	EN	Prasanna & Gowda (2021)
10	*Didymocarpus*	Sekhupthur	MZUH000043	VU	Prasanna &

	lineicapsa (C.E.C.Fisch.) B.L.Burtt	suak			Gowda (2020)
11	*Didymocarpus parryorum* C.E.C.Fisch.	Sekhupthur suak	MZUH000044	CR	Prasanna & Gowda (2020)
12	*Didymocarpus wengeri* C.E.C.Fisch.	Sekhupthur suak	MZUH000045	CR	Prasanna & Gowda (2020)
13	*Epithema carnosum* Benth.	Anthurte	MZUH000046	LC	Bransgrove & Middleton (2015)
14	*Henckelia anachoreta* (Hance) D.J.Middleton & Mich.Möller	NA	MZUH000047	LC	Sirimongkol (2020)
15	*Henckelia pumila* (D.Don) A.Dietr.	Ramthingsuak	MZUH000048	LC	Sirimongkol (2020)
16	*Lysionotus serratus* D.Don	NA	MZUH000049	NT	POWO
17	*Rhynchoglossum obliquum* Blume	NA	MZUH000050	NE	NA
18	*Rhynchotechum ellipticum* (Wall. ex D.Dietr.) A.DC.	Tiarrep	MZUH000051	NE	NA

Abbreviation: LC=Least Concern; NT=Not Threatened; EN=Endangered; NE=Not Evaluated; CR=Critically Endangered; VU=Vulnerable; NA=Not Available

5.7 Discussion

Mizoram flora including the taxa from the Gesneriaceae family is facing high degree of threat due to anthropogenic disturbances as a result of increasing human population and developmental activities. The exploitation of forest resources for foraging purpose by the local populations has resulted in depletion of the biodiversity of forest communities (Ramakrishnan, 2003) where *Rhynchotechum ellipticum* were collected for fodder and for local dishes. Land use pattern has been ranked as one of the most important drivers for terrestrial biodiversity change in the 21st century (Sala et al., 2000) with its more prominent role in tropical forests where jhum cultivation is promoted. Land degradation arising out of mining and quarrying activities is altering ecosystem structure due to excavation and overturning of top soil for dumping of mined overburden in adjoining areascreating unfavourable habitat conditions for Gesneriaceae plants. Sandstone quarrying, a prevalent practice in tropical hilly areas of Mizoram, effects on plant community characteristics (Singh et al., 2012). As many of the Gesneriaceae members are growing along the roadsides, open forest and even on the rocks, desctruction of the fragile ecosystem for their growth will affect many of the Gesneriaceae members, especially the ones which are endemic to the region and already placed under the RET category. So, a balanced approach that prioritize for conservation, sustainable development and community participation can safeguard the unique flora of the region while accommodating necessary development.

5.7 ETHNOBOTANY

5.7.1 Survey and data collection

Mizoram is one of the eight states in the northeastern region of India, a part of Indo-Burma biodiversity hotspot renowned for its rich ethnic diversity and traditional culture. Mizoram has international boundaries with Bangladesh to the west and Myanmar to the east. The ethnobotanical field survey was conducted during 2022–2023 to document ethnobotanical information related to Gesneriaceae flora of Mizoram.The areas coveredfor ethnobotanical survey include seven districts where the plants are commonly distributed i.e., Aizawl, Lunglei, Lawngtlai, Siaha, Serchhip, Champhai and Mamit covering 14 villages i.e., Muthi, Durtlang, Sihphir, Tuipang, Zobawk, Sairep, Thaltlang, Hmuifang, Murlen, Ngur, Vapar, Reiek, Ailawng and Dampa.

5.7.2 Collection and Identification

Following the methodology proposed by Martin (1995), a total of 60 respondents from indigenous Mizo tribes, aged between 40 to 90 years. Among the 60 key informants, 26 were female (30%) and 34 were male (70%). Ethnobotanical data were gathered through questionnaires, personal interviews, and open-ended group discussions with key informants. Prior Informed Consent (PIC) was obtained from all key informants. Plant identification was done through observation, dissection and description of plant detail characters which are further compared with relevant literature and online portal. For authentication of the identified specimens, herbarium specimens deposited at ASSAM (BSI, Shillong) and CAL (BSI, Kolkata) were consulted along with the digital herbarium databases from Royal Botanic Garden, Kew(K) and Royal Botanic Garden, Edinburgh(E).

5.7.3 Data analysis

The data were organized in Microsoft Excel, with columns detailing traditional uses, scientific and vernacular names, habits and usage modes. Statistical analyses were performed using GraphPad Prism software version 8.0.1. (244). Quantitative analysis and the creation of a chord diagram for usage modes were carried out withethnobotanyRpackage in R software version 0.1.8.2021.

Figure 5.40. Photograph of the informants and Questionnaires sample.

5.7.4 Quantitative Indices

5.7.4.1 Use Value (UV) as proposed by Sharma et al. (2012)

To determine the relative importance of the ethnobotanical plants, use value (UV) was used. UV is calculated by the formula:

$$\mathrm{UV} = \frac{\mathrm{U}}{\mathrm{n}}$$

Where, U represents the number of use reports cited by every respondent for a given species and n is the total number of respondents interviewed. A higher UV suggests greater importance of the species, whereas a lower UV indicates less reported use.

5.7.4.2 Importance Value (IVs) using Byg & Balslev (2001)

This index reflects the proportion of informants valuing the species as most important, with values ranging from 0 to 1.

Importance Value (IV_s) is calculated by the formula:

$$IV_s = \frac{n_{is}}{n}$$

Where, n_{is} is the number of informants considering a species most important, and n is the total number of informants.

5.7.4.3 Relative Frequency Citation (RFCs)

The relative frequency citation (RFC) is employed (Tardio & Pardo de Santayana, 2008) to assess how often a reference or mention is made by study participants who acted as informants. It is calculated using a specific formula

$$RFC = \frac{FC}{N}$$

Here, FC represents the number of informants who cited or mentioned the plant, and N stands for the total number of informants. Values close to 1 indicate that almost all informants referenced a specific medicinal plant for treating a particular illness, while lower values suggest that the plant's usage was mentioned by only a few or, at times, a single informant.

5.7.5 Results and Discussion

5.7.5.1 Socio-demographic characteristics of respondents.

The study documented the indigenous knowledge of the Mizo ethnic group regarding the traditional uses of Gesneriaceae family. It focuses on their uses as a medicinal plant, ornamentals, food and fodder. A total of 60 key informants were selected from the study area, with their age and gender details presented in Table 5.10. The ranges of age for the informants were from 40 to 90 years. Most of the informants reported acquiring their knowledge from relatives descended from their ancestors, while a minority gained knowledge through their own practical experiences. Additionally, there was a noticeable variation in age-related knowledge of medicinal plants within the study area.

Table 5.10: Demography of Informants in the study area

Parameters	**Category**	**Number**	**Frequency**
Sex	Male	34	56.7%
	Female	26	43.3%
Age	40-49	14	23.3%
	50-59	22	36.7%
	60-69	17	28.3%
	70-79	4	6.7%
	80-89	3	5%

5.7.5.2 Plant use diversity

Based on the uses of the ethnobotanically important members, the distribution is given in Figure 5.41. The distribution of plants in the studied area mostly focuses on their usage for ornamental purposes, while a lesser fraction is used for medicinal, food, and fodder purposes. Among the documented species, the majority, which amounts to 54% (7 species), fall under the category of ornamental. Conversely, the medicinal category consists of 23% (3 species), whilst the food category comprises 15% (2 species). The fodder group comprised of 8% (1 species).

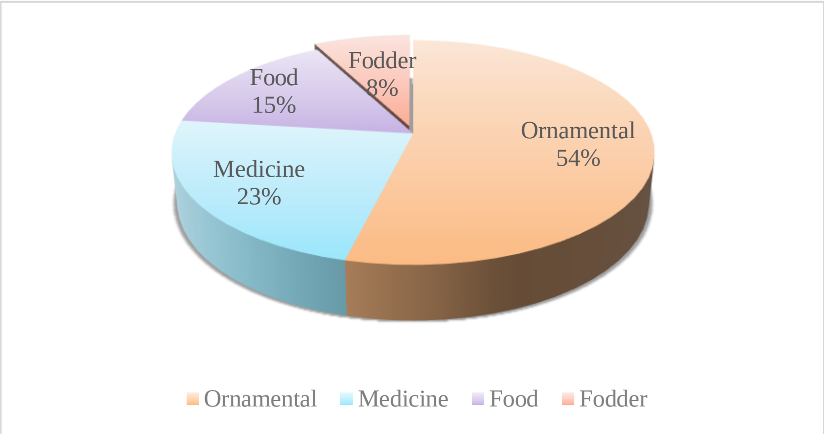

Figure 5.41: Plant Species by Use Category in percentage

5.7.5.3 Plant part used

The study demonstrates that *Achimenes longiflora*, *Aeschynanthus mannii*, *A. parasiticus, A. reiekensis, A. parviflorus* and *A. superbus* are utilised for decorative purposes by growing as ornamental plants, with *A. parviflorus* being esteemed for its foliage and *A. superbus* for its blossoms. Leaves of *Epithema carnosum* and roots and stems of *Rhynchotechum ellipticum* is employed for their medicinal applications.

A. parviflorus is utilised for its flower juice, whilst *R. ellipticum* is valued for its leaves and stem in relation to food consumption. *R. ellipticum* is used as fodder, while other species such as *E. carnosum* do not have any usage as fodder. This distribution emphasises the distinct purposes for which these species are utilised, demonstrating a predilection for decorative and some medical uses, while having limited applications in terms of food and animal feed.

5.7.5.4 Quantitative indices

Use value (UV) of all the reported species of Gesneriaceae ranges between 0.75 to 0.8 (Table 5.11). The plants with the highest UV indicate the species as economically most significant among the Gesneriaceae members. *Chrysothemis pulchella* and *Rhynchotechum ellipticum* have shown the highest use value (UV) of 0.75 among all the Gesneriaceae species reported, while *A. parasiticus* and *A. parviflorus* have shown the lowest use value (UV) of 0.8 within this family. Importance value (IVs) of all the Gesneriaceae members were found ranging between 0.05 - 0.25 (Table 5.11). *R. ellipticum* have the highest Importance Value (0.4), reflecting their significant ethnobotanical and cultural importance to the tribal communities. In contrast, *A.reiekensis* has the lowest Importance Value (0.05) which may be because it was discovered as novel taxa and so not much familiar to people of the locality. All the plants having used the mode shared the same Relative Frequency of Citation (RFC) value ranging from 0.01 to 0.53, showing that the usage or purpose of a medicinal plant species is mentioned by few of the informants. This comprehensive evaluation highlights the most valued species and their respective significance within the community.

Table 5.11: Ethnobotanical uses for Gesneriaceae members of Mizoram with botanical name, local name, habit, traditional uses/Mode, IVs, UV, RFC.

Botanical name	**Local name**	**Habit**	**Traditional Uses/ Mode**	**IVs**	**UV**	**RFC**
Achimenes longiflora DC.	NA	PH	Ornamental/O	0.25	0.9	0.33
Aeschynanthus mannii Kurz ex C.B.Clarke	Hnahchhah	EH	Ornamental /O	0.1	0.25	0.08
Aeschynanthus parasiticus (Roxb.) Wall.	Hnahchhah	EH	Ornamental /O	0.15	0.8	0.41
Aeschynanthus parviflorus (D.Don) Spreng.	Hnahchhah	EH	1. Ornamental 2.Leaves used for skin inflammation 3. Flowers juice/ O, M, F	0.3	0.8	0.43
Aeschynanthus reiekensis Lalhlupuii, S.D.Khomdram & S.D.Yumkham	Hnahchhah	EH	Ornamental / O	0.05	0.25	0.01
Aeschynanthus superbus C.B.Clarke	Hnahchhah	EH	Ornamental/ O	0.1	0.35	0.21
Chrysothemis pulchella (Donn ex Sims) Decne.	NA	PH	Ornamental/ O	0.15	0.75	0.41
Epithema carnosum Benth.	Anthurte	PH	Leaves used for treatment of haemorrhoids/ M	0.2	0.15	0.06
Rhynchotechum ellipticum (Wall. ex D.Dietr.) A.DC.	Tiarrep	SS	1.Roots used for treating kidney 2.Leaves serves as dishes 3.Leaves and stem used as fodder/ M, F, FD	0.4	0.75	0.53

Abbreviations: O = Ornamental; M = Medicine; F = Food; FD = Fodder; U = Unknown: Habit groups: Epiphytic herb = EH; SS = Subshrub; Perennial herb = PH; Lithophytic herb = L

5.7.5.5 R chord

The present ethnobotanical study of Gesneriaceae members has documented 9 species from 5 genera utilized by the indigenous Mizo tribe of Mizoram with detailing traditional uses, scientific and vernacular names, habit and usage modes (Table 5.11). An ethnobotanical field survey revealed that the Gesneriaceae family holdssignificant value, visually represented in the Figure 5.42, which was created using R software. The Indigenous Mizo tribe of Mizoram prioritizes the use of Gesneriaceae species, with the highest usage for ornamental purposes, particularly species from the genus *Aeschynanthus* and other species that include *A. longiflora* and *C. pulchella*. Medicinal applications are primarily associated with *A. parviflorus*, *E. carnosum* and *R. ellipticum.* Additionally, *A. parviflorus* and *R. ellipticum* species are used as food, while *R. ellipticum* is found to be the least employed for its use as a fodder plant fodder purpose.

5.7.5.6 Discussion

Though not extensively studied for its ethnobotanical uses, the Gesneriaceae taxa of Mizoram plays an important role in the traditional knowledge and practices of the local indigenous communities. The ethnobotanical documentation highlights that importance of the plants for their traditional uses and aesthetic values. The medicinal uses of some plants are exclusively reported for the local Mizo community

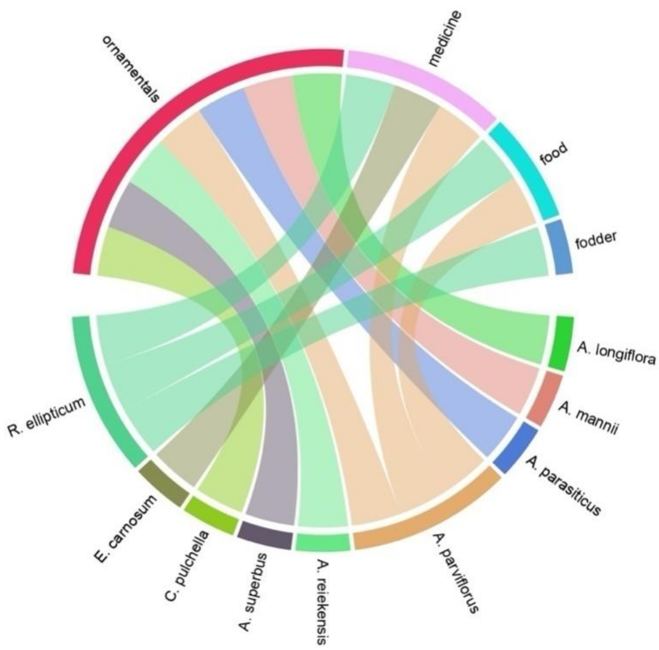

Figure 5.42: Ethnobotanical significance of Gesneriaceae family of Mizoram using R chord.

(Lalmuanpuii et al., 2021; Ralte et al., 2024), marking their first documentation in the uses of *R. ellipticum* roots, which are used to cure kidney problems and *E. carnosum* leaves, which are used to treat hemorrhoids.The ethnobotanical uses for the members of Gesneriaceae are limited largely due to the narrow endemic distribution of many of its members. This restricted range often confines the traditional knowledge about their uses to local communities underscoring the need for further documentation and study to preserve and underst and their cultural and medicinal significance. Most of the common uses reported for these Gesneriaceae members are found to be ornamental which may be due to their perennial nature, attractive flowers and their resurrection properties which are observed in most of the ornamental plants. So, preserving the traditional knowledge on the use of these Gesneriaceae members by the local communities will be a key for conservation of the plants while exploring the scientific research for modern applications in the field of horticulture and pharmaceutical industries.

Chapter 6

6. Conclusion

6.1 Species diversity and distribution

The present study successfully collected, identified and documented 18 species belonging to 9 genera of the Gesneriaceae family in Mizoram, India. The members of Gesneriaceae family in Mizoram is divided under three sub-family, three tribes and six sub-tribes. The 28% of the total species recorded are lithophytic, 33% are Geophytic, while 39% are reported as epiphytic distributed along different altitudinal gradient ranging from 23°66′56.0″ N 92°66′35.0″ E, 980m to 22°41′47.4″ N 92°02′96.8″ E, 1663m above the sea level in Mizoram. Identification and the relationship among the Gesneriaceae members were done based on the morpho-taxonomical, palynological, seed coat morphology and molecular studies.

6.2 Habitat

Habitat of Gesneriaceae members from Mizoram preferred a wide variation, reflecting their adaptability to diverse environmental conditions. These plants thrive in various ecosystems and they are high altitudinal and moisture loving plant and grows best in high humidity. The altitude and type of land preference differ in each genus. The genus *Aeschynanthus* are particularly abundant at mid-elevation to high elevation in the mountain forests preferably in undisturbed habitats and high humidity with proper sunlight where they grow as epiphytes. *Didymocarpus* genus are lithophytic plants which prefers rock crevices, overhanging rocks, shallow caves, rocky lodges and cliff. The genus *Henckelia* and *Epithema carnosum* are found close to streams banks, waterfalls and moist shaded environments. *Lysionotus serratus* are epiphytic sub shrub which prefer woodlock for their growth and adaptation. The two most widely distributed species among them are *Rhynchotechum ellipticum* and *Rhynchoglossum obliquum,* which grow in low to high altitudinal variation and have adaptable adaptations. Both *Achimenes longiflora* and *Chrysothemis pulchella* are ornamental plants that are often grown and have good growth potential.

6.3 Novelties in taxonomy

The discovery of a new species, *Aeschynanthus reiekensis*, shows the biodiversity richness and under exploration of the family in the region. The addition of *Henckelia anachoreta* and two naturalized invasive species, *Achimenes longiflora* and *Chrysothemis pulchella*, marks the significant contributions to the flora of the region.

6.4 Palynology and seed coat morphology

The detailed analysis of pollen characteristics and seed coat anatomy has been pivotal in constructing a comprehensive identification key for the Gesneriaceae family. Seed coat morphology, which examines the structure and texture of seeds, offers significant systematic implications, showing genus level consistency. The study of palynology and seed coat anatomy in Gesneriaceae confirms their role to advancing knowledge in plant systematics, ecology, and conservation.

6.5 Clustering analaysis of Palynology and Seed coat morphology

The significance of PCA analysis in both pollen and seed characterization lies in its ability to simplify complex datasets, identifying key variables that most effectively distinguish between species. In pollen, polar length contributed the highest in grouping the species marking as key character among the eight variables and in seed among the six variables, apical character made the highest contribution. This clustering method aids in identifying species-specific characteristics as well as a powerful tool for exploring the relationships between different traits and can help in understanding evolutionary patterns and ecological adaptations. Overall, PCA enhances the precision and efficiency of species grouping. identification, contributing to more accurate taxonomic and ecological studies.

6.6 Molecular studies

Creating the DNA barcode database using *rbcL* and ITS2 markers for the Gesneriaceae members of Mizoram in global repositories (GenBank) could provide a crucial step, particularly for establishment of the first DNA database for the endemic taxa that include all the collected species of *Didymocarpus* and some species from the *Aeschynanthus* aiding in precise identification. It will be a significant milestone that will reveal the hidden diversity among the members of the Gesneriaceae taxa from the region. The barcoding database will complement the morpho-taxonomic including the pollens and seed coats data for accurately identifying the members of Gesneriaceae. In addition to that, the database generated using the *rbcL* and ITS2 markers contribute to resolve the phylogenetic relationships among the Gesneriaceae members of the region offering insights into their evolutionary history and adaptive strategies among the members in the unique habitats available in Mizoram.

6.7 Ethnobotany

Ethnobotanical investigations have reported 9 species of Gesneriaceae used by the indigenous community of the state as food, medicine, fodder and ornamental purposes.The ethnomedicinal uses of *Rhynchotechum ellipticum* roots, which are used to cure kidney problems and *Epithema carnosum* leaves, which are used to treat hemorrhoids are reported for first time. This indicates the unique traditional knowledge of the region, which underscores the importance of preserving the ethnobotanical information. It highlights the potential for discovering novel bioactive compounds and unexplored medicinal properties that can contribute to scientific research and pharmacological development. Many of the Gesneriaceae members like all species of *Aeschynanthus,* though locally used as ornamental plants, possess significant horticultural development due to their aesthetic appeal. Their adaptability to subtropical conditions makes them ideal for cultivation in gardens and nurseries offering economic opportunities for local communities. Notably, *Chrysothemis pulchella* and *Achimenes longiflora* are widely cultivated ornamental plants with high economic value and have become naturalized in the region.

6.8 Endemic and RET species

Of the total 18 species reported in the present studies 6 (33%) species are endemic to the state while the remaining taxa are mostly endemic to broader region except for the naturalized taxa. Because of their limited range, sensitivity to habitat disturbances, small, isolated populations and tendency to be known only from its type localities, the *Didymocarpus* sp. and the newly discovered *Aeschynanthus reiekensis* are endemic to the state of Mizoram and serves as an indicator of pristine landscape. They are highly threatened from numerous threats, including habitat destruction due to shifting agriculture (jhum cultivation), deforestation, infrastructure development, and climate change. Given their narrow distribution and habitat specialization, they are particularly susceptible to extinction.

6.9 Conservation

Habitat preservation for the Members of Gesneriaceaespecies is crucial as they are highly sensitive to environmental changes. Conservation strategies should focus on the protection of fragile ecosystems and curbing destructive human activities in its habitat. Protecting their natural habitats and preventing further deforestation are essential as many taxa were found to be epiphytes and lithophytes. Co-ordinated conservation efforts, involving habitat protection, research, and community engagement, are crucial to safeguarding these species from extinction.

In conclusion, the present study on the members of Gesneriaceae in Mizoram highlight the unique and diverse flora of the region with many of the taxa are endemic or restricted to specific habitats. The overall systematic studies based on morphology, palynology, seed coat anatomy and molecular approaches have unraveled evolutionary relationships and diversification of lineage within the family. Exploring morphological diversity, micromorphological study on pollen and seeds along with the molecular data can highlighted the adaptive traits that have evolved in response to local environmental pressures. The discovery of novel taxa and new records underscores the rich biodiversity and the underexplored nature of Gesneriaceae family in Mizoram. Such findings will form a crucial foundation for documenting the biodiversity of the region and filling the gap at the global and regional database. Raising awareness by encouraging initiatives for habitat protection for conservation of the new taxa as well as the RET taxa and enabling exploration of the potential uses for their sustainable uses will reinforce the importance of detailed taxonomic studies, both for biodiversity and for ensuring their long-term preservation. The study on the ethnobotanical uses of Gesneriaceae members reflect the intricate relationship between these plants and local communities. So, the integrative approaches combining ethnobotany, conservation and further scientific research can ensure the sustainable management and protection of these valuable plant resources.

Appendices

Appendix 1

Questionnaires on Ethnobotanical Data Collection of Gesneriaceae family

Informants'detail

Name/Hming: ....

Gender : Male ☐ / Famale ☐

Age/kum : ....

Community / Hnam Hming: Mizo ☐ / Hnam dang ☐

Data on ethnobotanical importance of Gesneriaceae plants

Local name of plant /Thlai hming :………………………...

Habit of plant / Nihphung :………………………...

Mode of consumption / Chawhmeh a

Siam dan :………………………

Part used as food :………………………

Season of availability / a awm hun

1) Whole year / kumtluan in

2) From...........to……………..(Month)

Medicinal value (If any)/ Damdawi atan a hmanna:

………………………………………………………………………

Use report for other disease treatment (Natna danga hmanna):

.... here by give my full consent and willingly accepted to participate in this study and declare that the information provided by me during the course of interview was true and accurate to the best of my knowledge.

Plate 1: *Achimenes longiflora* DC., Prodr. A. Habit; B. Leaves; C. Habit zoom; D. Flower; E. Calyx; F. Pistil; G. Stamens.

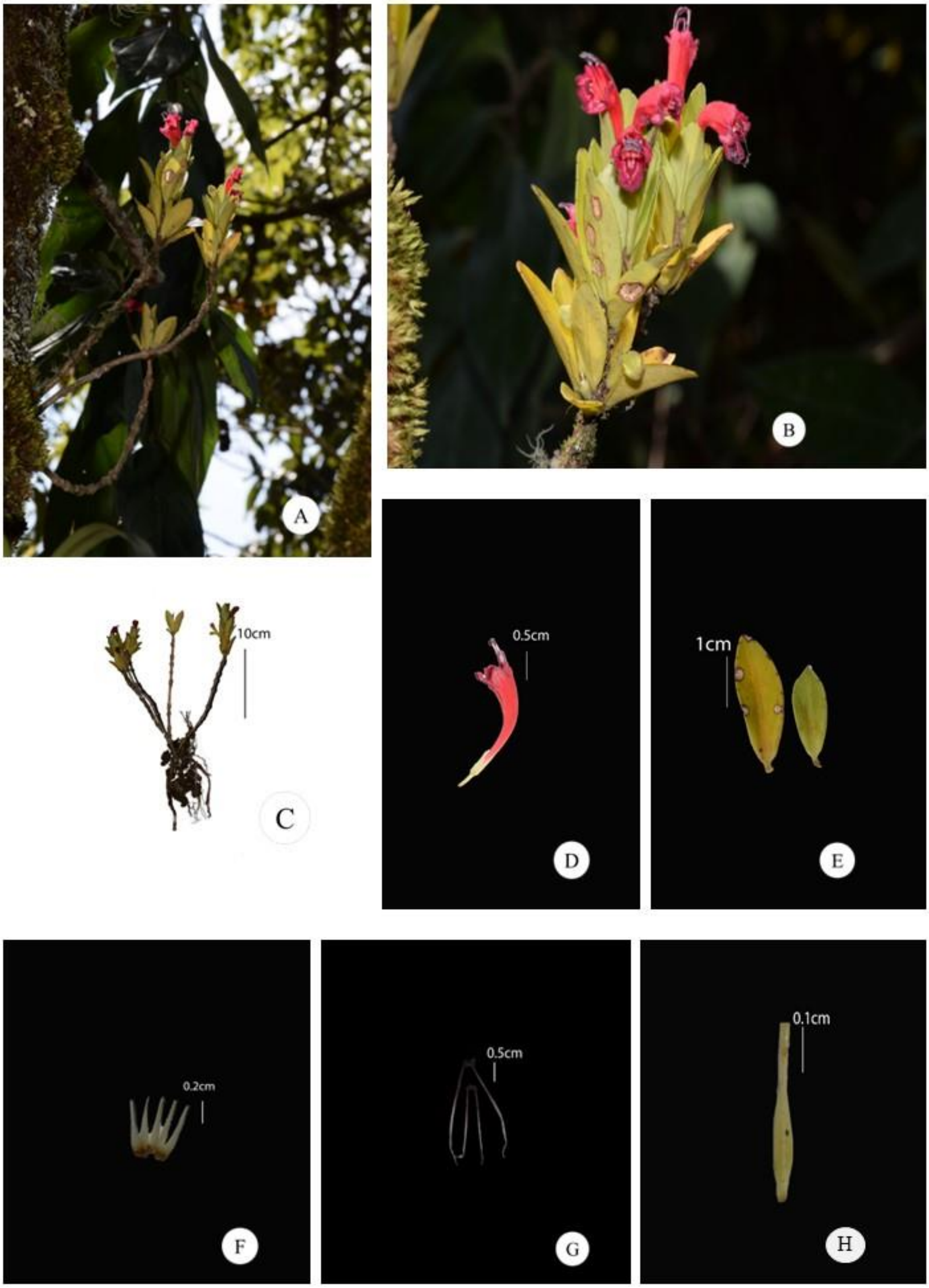

Plate 2: *Aeschynanthus mannii* Kurz ex C.B.Clarke A. Habit; B. Inflorescence; C. Whole plant; D. Flower; E. Leaves; F. Calyx; G. Stamens; H. Pistil.

Plate 3: *Aeschynanthus parasiticus* (Roxb.)

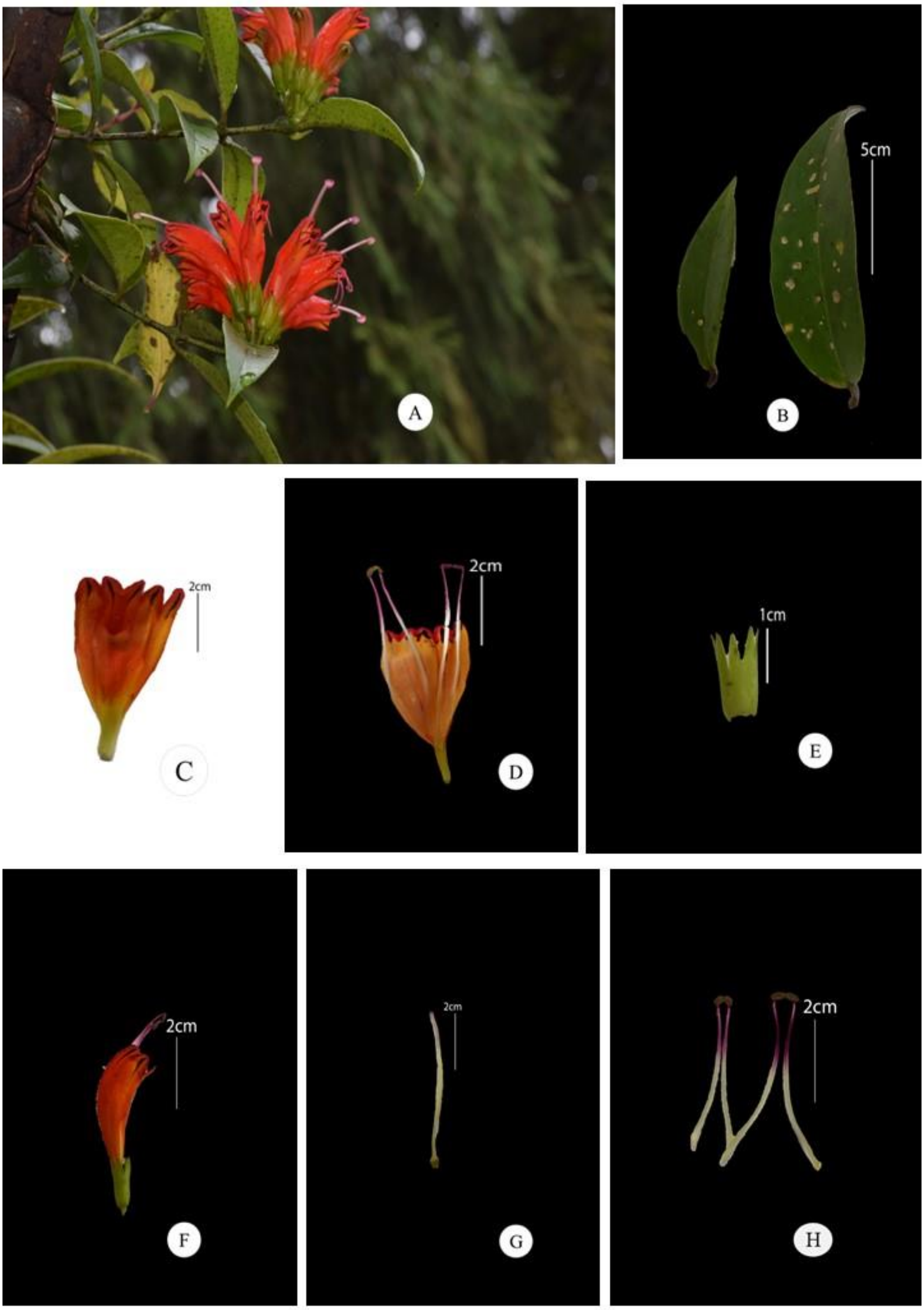

Plate 3 : Wall. A. Habit; B. Leaves; C. Dorsally opened corolla; D. Ventrally opened corolla; E. Calyx; F. Flower; G. Pistil; H. Stamens.

Plate 4: *Aeschynanthus parviflorus* (D.Don) Spreng. A. Habit with the inflorescence; B. Flower; C. Ventral flower; D. Dorsally opened corolla; E. Leaves; F. Ventrally opened corolla; G. Calyx; H. Stamens; I. Pistil.

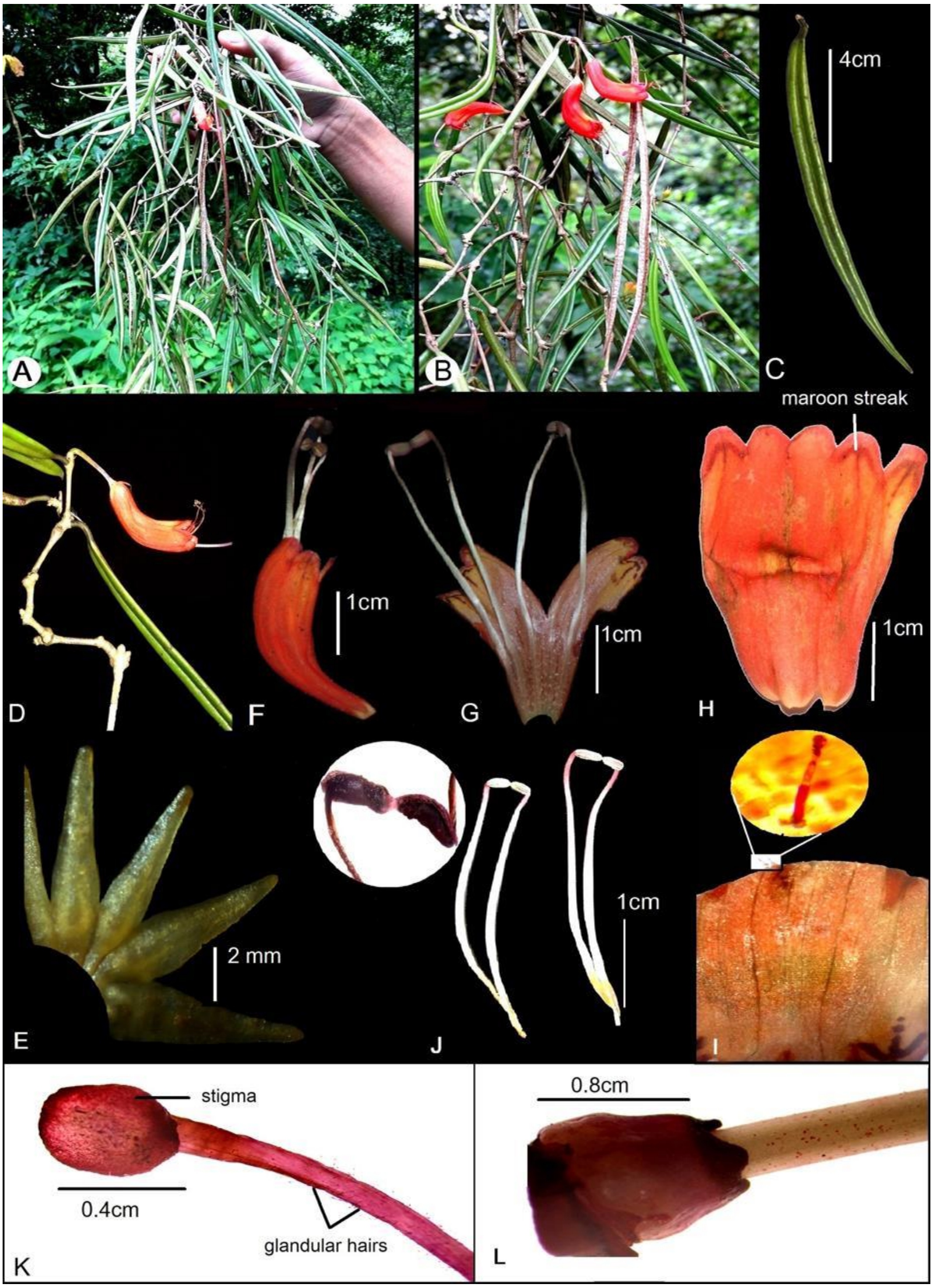

Plate 5: *Aeschynanthus reiekensis* M.Lalhlupuii, S.D.Khomdram & S.D.Yumkham A-B. Habit; C. Leaf; D. Flowering twig; E. Calyx; F.Mature Flower G. Fully opened flower; H. Opened corolla showing maroon streaks; I. Inner corolla base showing glandular hairs; J.Stamen pairs showing fused anthers; K. Part of pistil showingglandular hairs on style; L. Ovary.

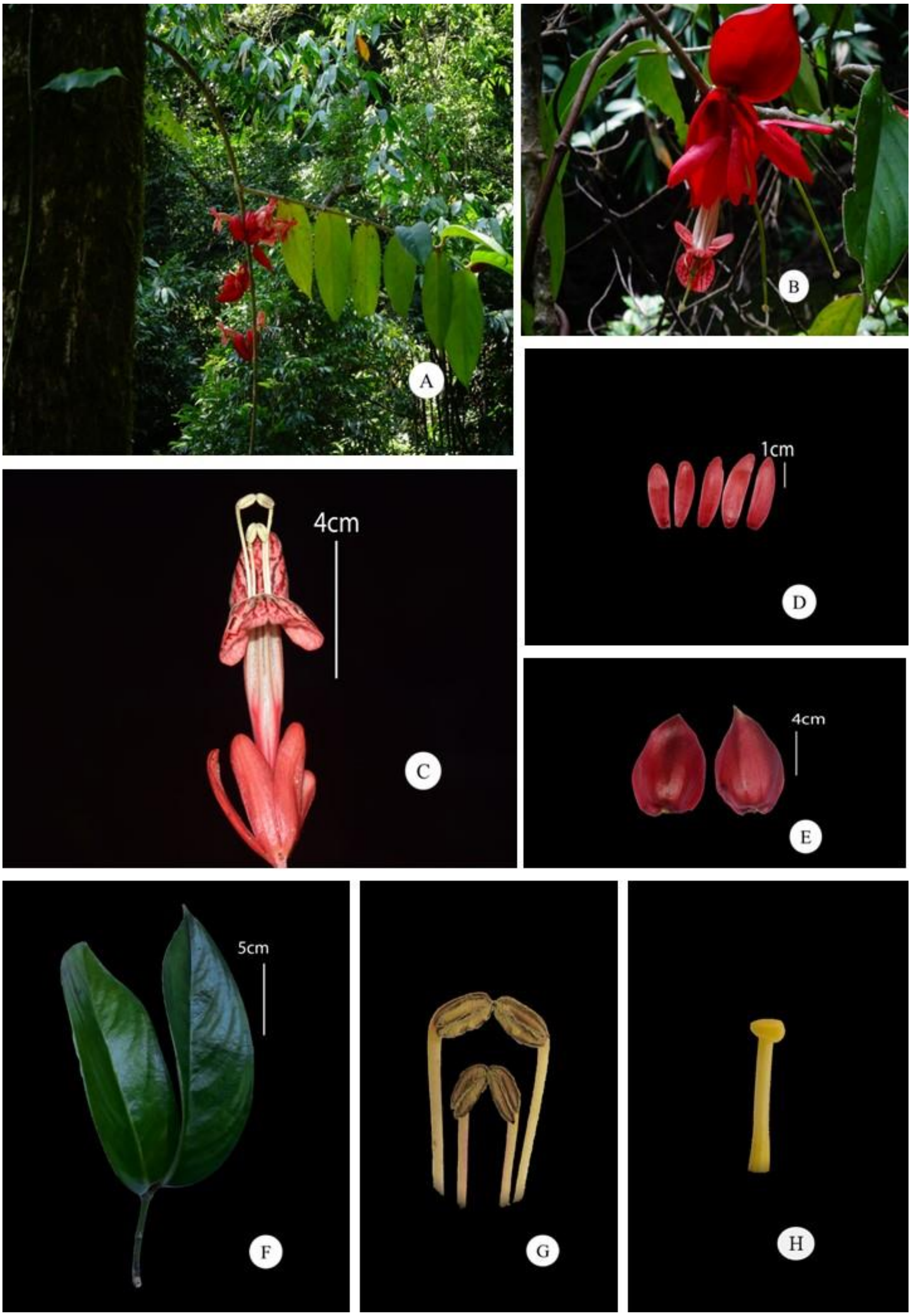

Plate 6: *Aeschynanthus superbus* C.B.Clarke A. Habit; B. Inflorescence; C. flower; D. Calyx; E. Bract; F. Leaves; G. Anther; H. Pistil.

Plate 7: *Chrysothemis pulchella* (Donn ex Sims) Decne. A. Habit; B. flower; C. Inflorescence; D. Bract; E. Corolla; F.Calyx; G. Leaves; H. Stamens; I. Pistil.

Plate 8: *Didymocarpus adenocarpus* C.E.C.Fisch. A. Habit; B.Whole plant; C. Leaves; D. Opened corolla; E. Flower; F.Calyx; G. Bract; H. Stamens; I. Pistil.

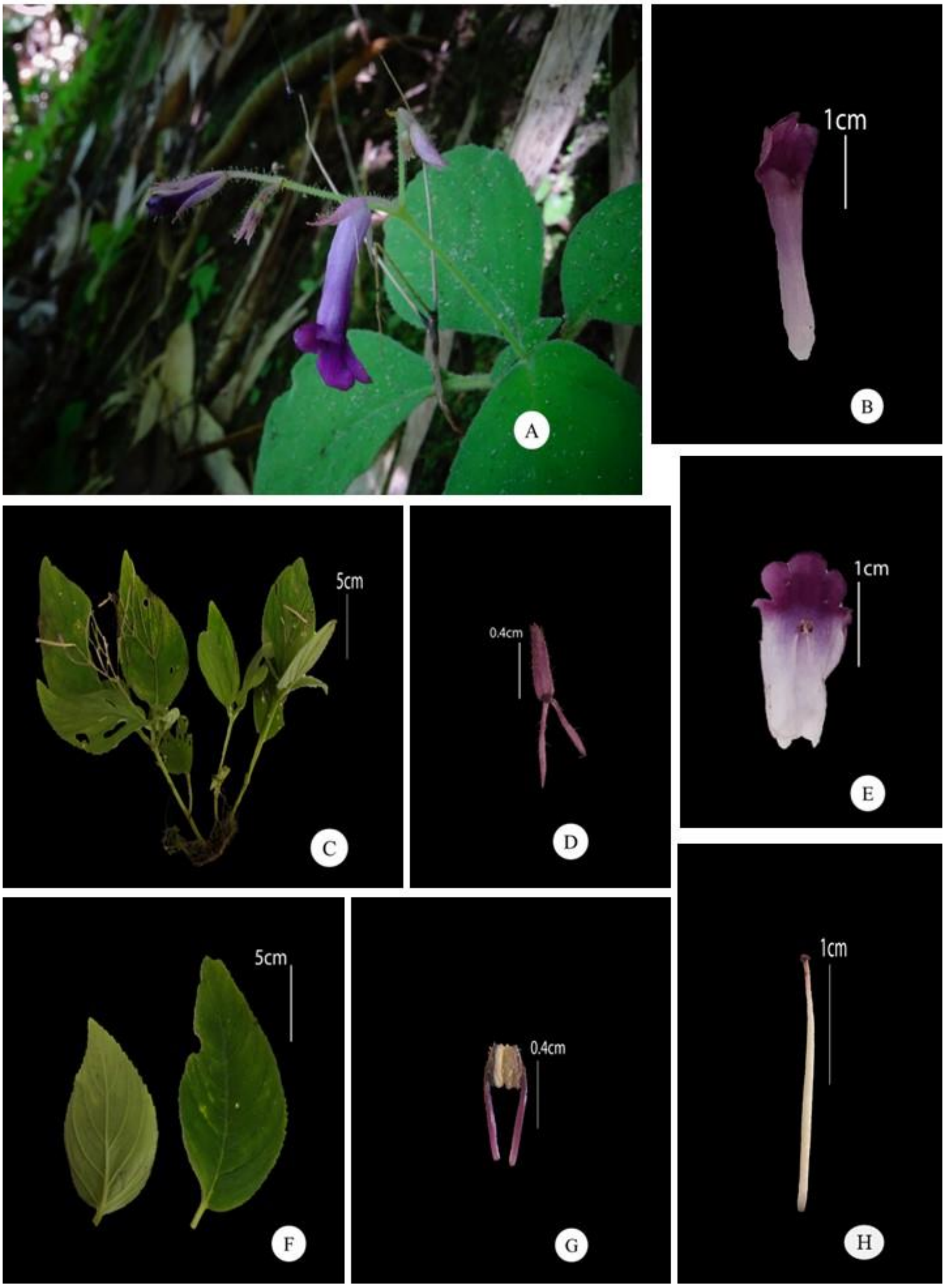

Plate 9: *Didymocarpus lineicapsa* (C.E.C.Fisch.) B.L. A. Habit; B. Corolla; C. Whole plant; D. Calyx; E. Opened corolla; F. Leaves; G. Stamens;H. Pistil.

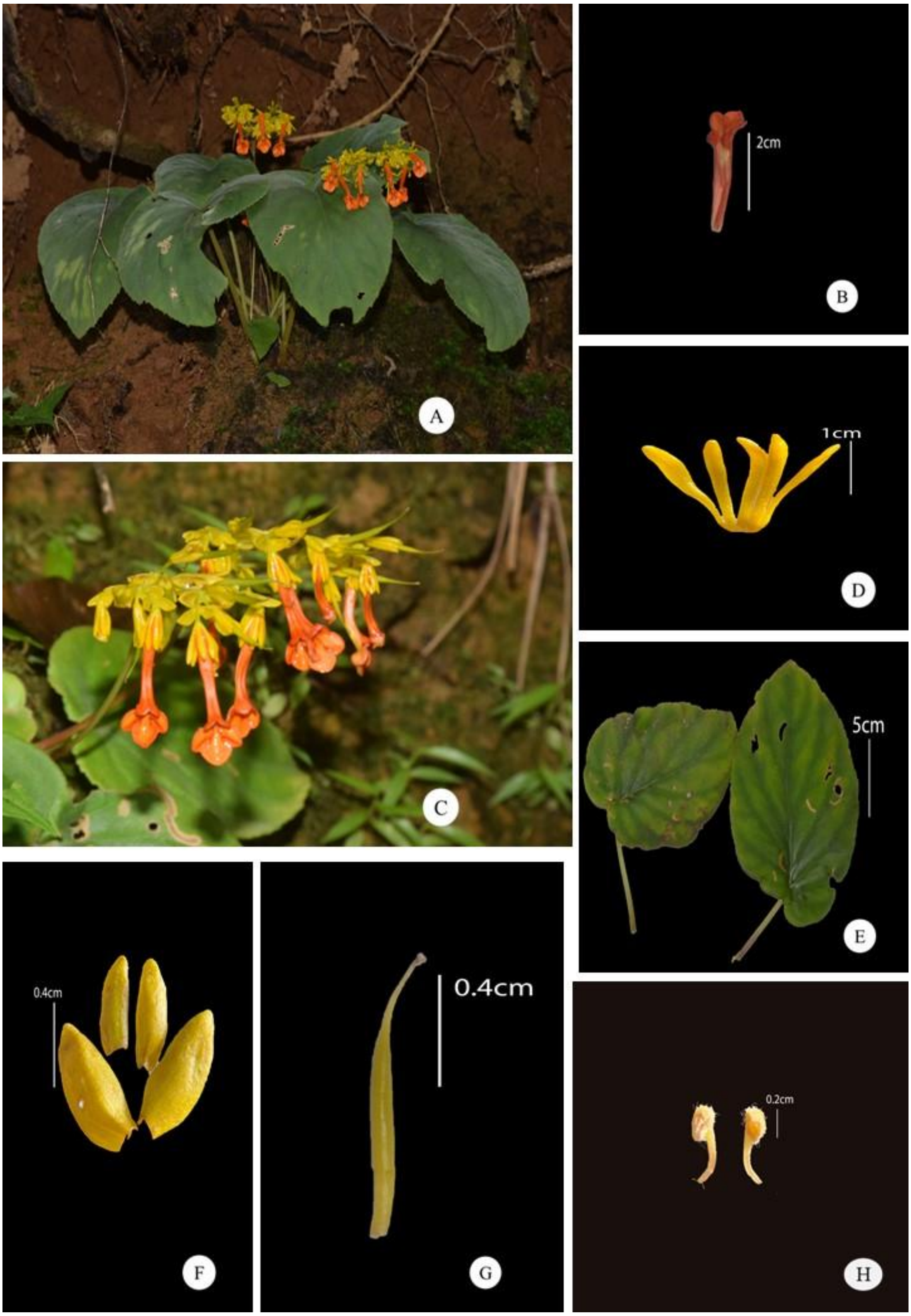

Plate 10. *Didymocarpus parryorum* C.E.C.Fisch. A. Habit; B. Opened corolla; C. Inflorescence; D. Calyx; E. Leaves; F. Bract; G. Pistil; H. Stamens.

Plate 11: *Didymocarpus vickifunkiae* V.Gowda & N.S.Prasanna A. Habit; B. Inflorescence; C. Whole plant; D. Leaves; E. Flower; F. Opened corolla; G. Bract; H. Calyx; I. Stamens; J. Pistil.

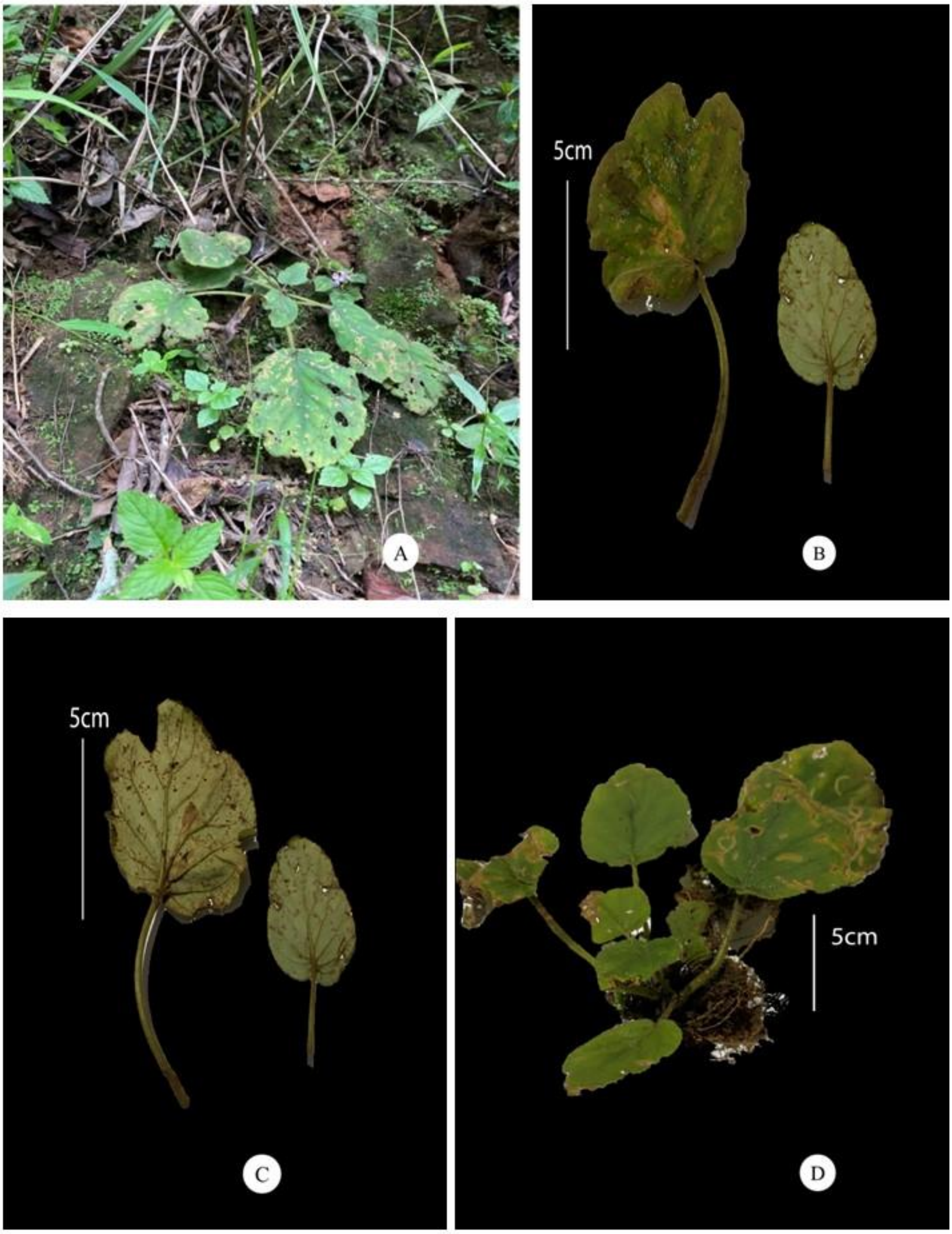

Plate 12: *Didymocarpus wengeri* C.E.C.Fisch. A. Habit; B. Adaxial leaves; C.Abaxial leaves; D. Whole plant.

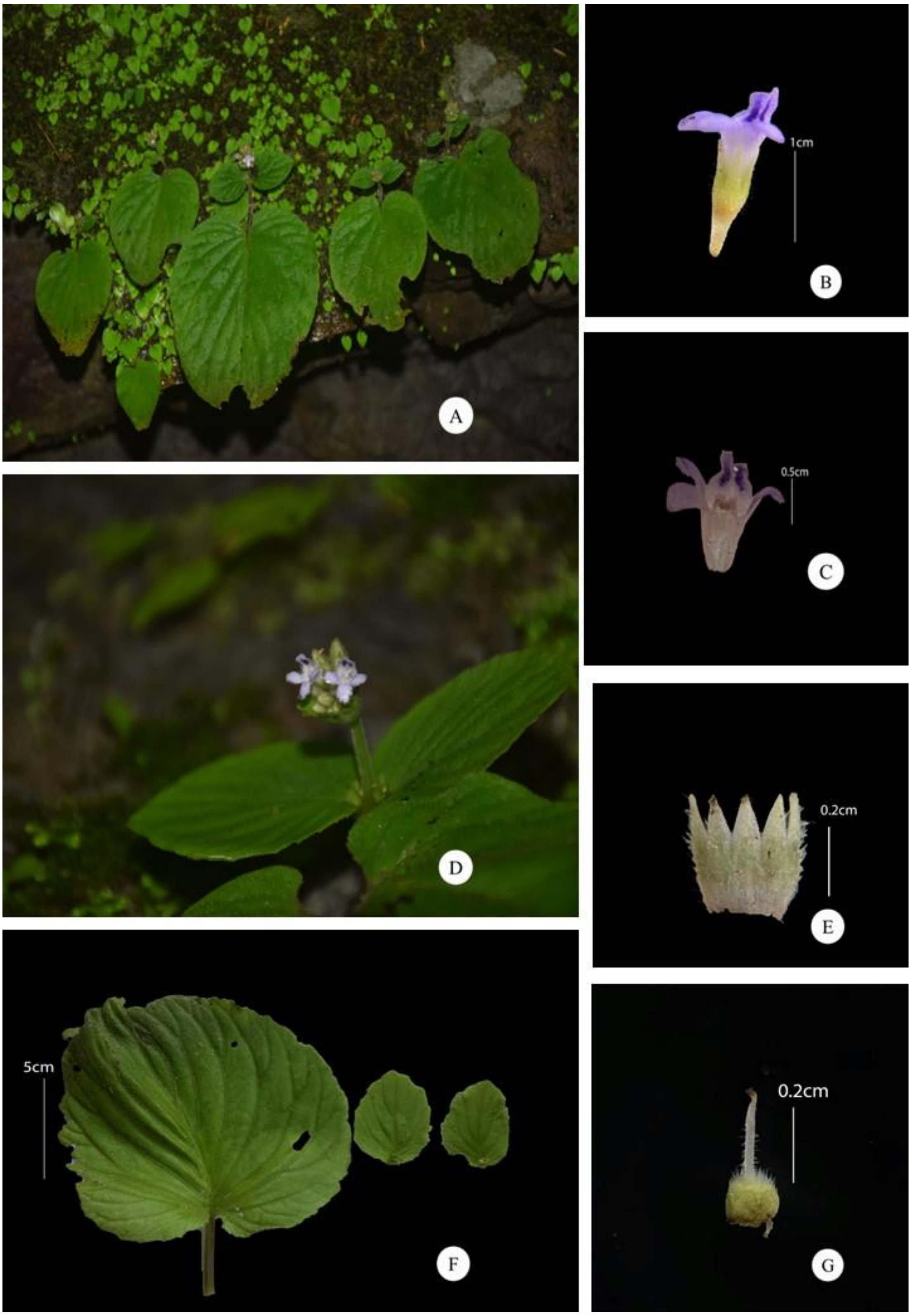

Plate 13: *Epithema carnosum* Benth. A. Habit; B. Flower; C. Opened corolla; D. Inflorescence; E. Calyx; F. Leaves; G. Pistil.

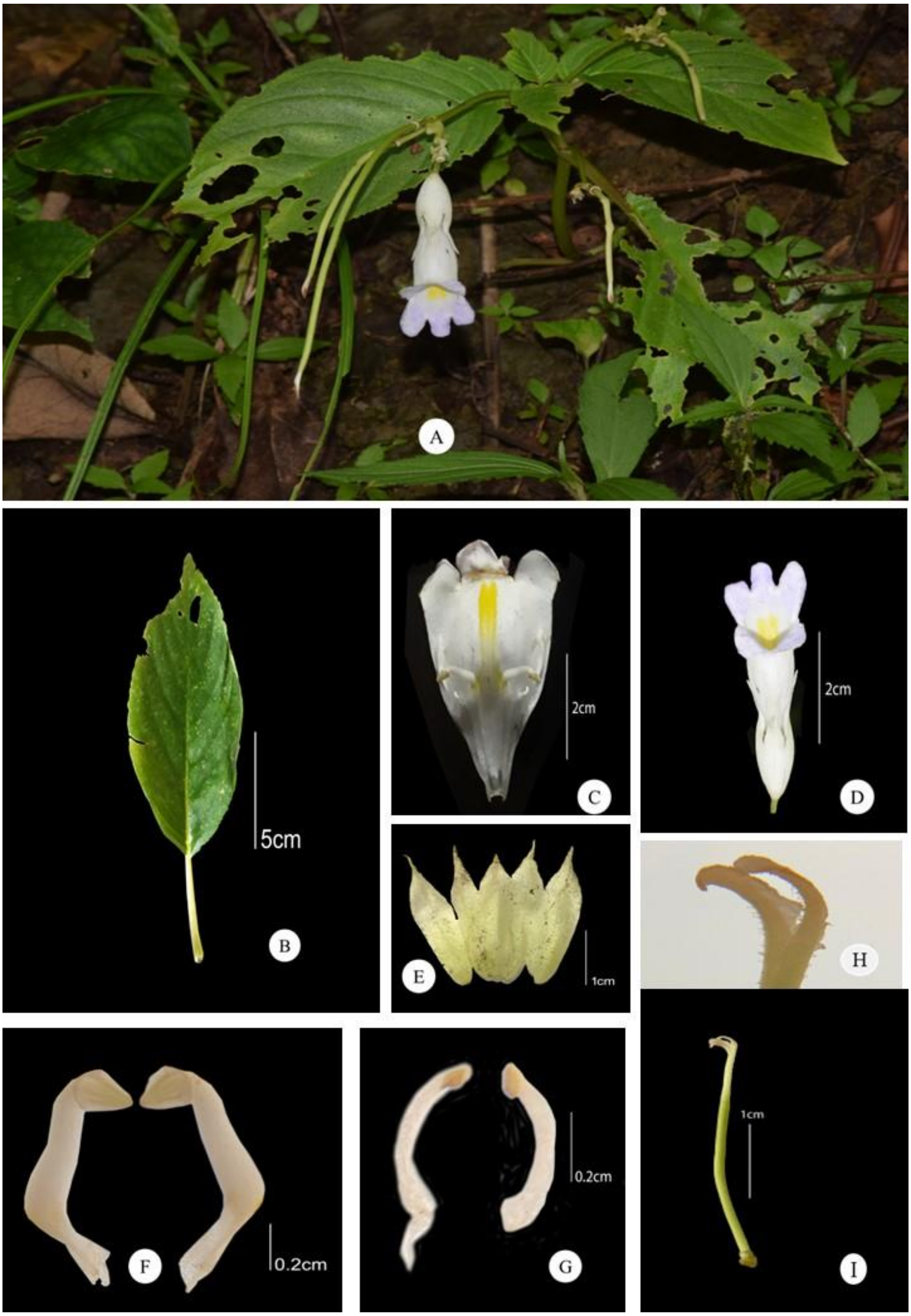

Plate 14: *Henckelia anachoreta* (Hance) D.J.Middleton & Mich.Möller A. Habit; B. Leaves; C. Opened corolla; D. Flower; E. Calyx; F. Stamens; G. Staminode; H. Pistil zoom ; I. Pistil.

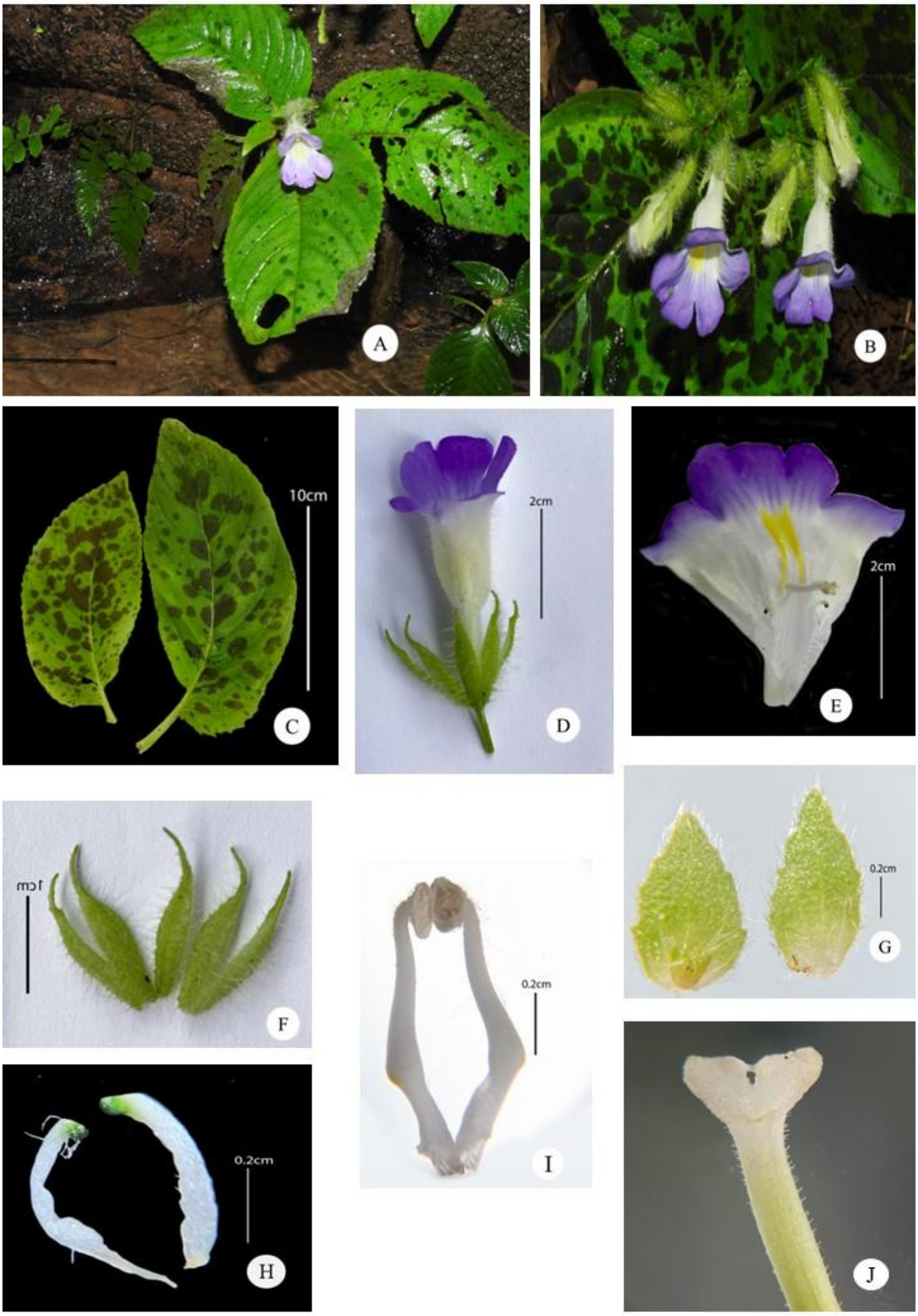

Plate 15: *Henckelia pumila* (D.Don) A.Dietr.A. Habit; B. Inflorescence; C. Leaves; D. Flower; E. Opened corolla; F. Calyx; G. Bract;H. Staminode; I. Stamens; J. Pistil zoom.

Plate 16: *Lysionotus serratus* D.Don A. Habit; B. Closed bud; C. Opened corolla; D. Leaves; E. Flower; F. Bract; G. Calyx; H. Stamens; I. Pistil.

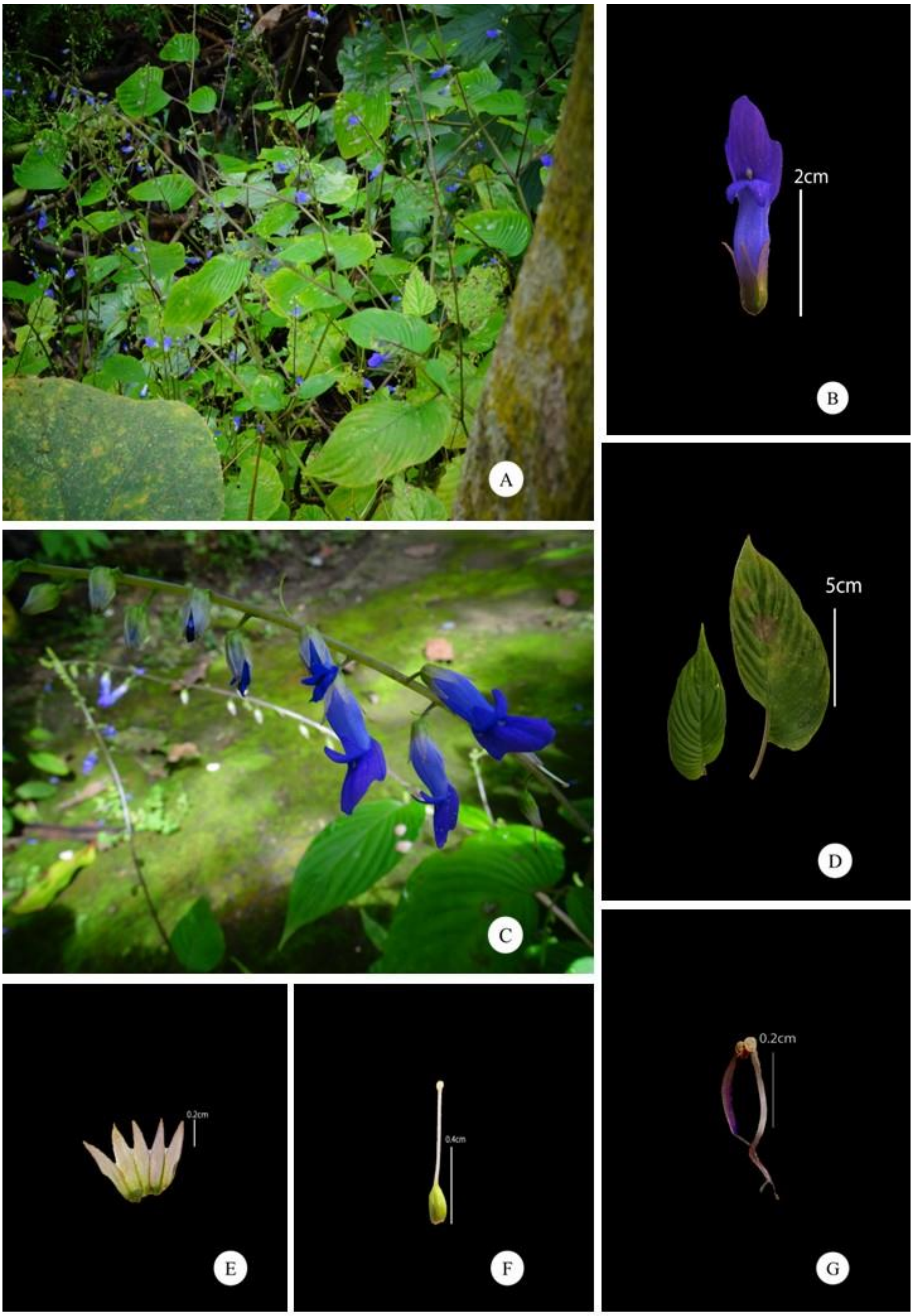

Plate 17: *Rhynchoglossum obliquum* Blume A. Habit; B. Flower; C. Inflorescence; D. Leaves; E. Calyx; F. Pistil; G. Stamens.

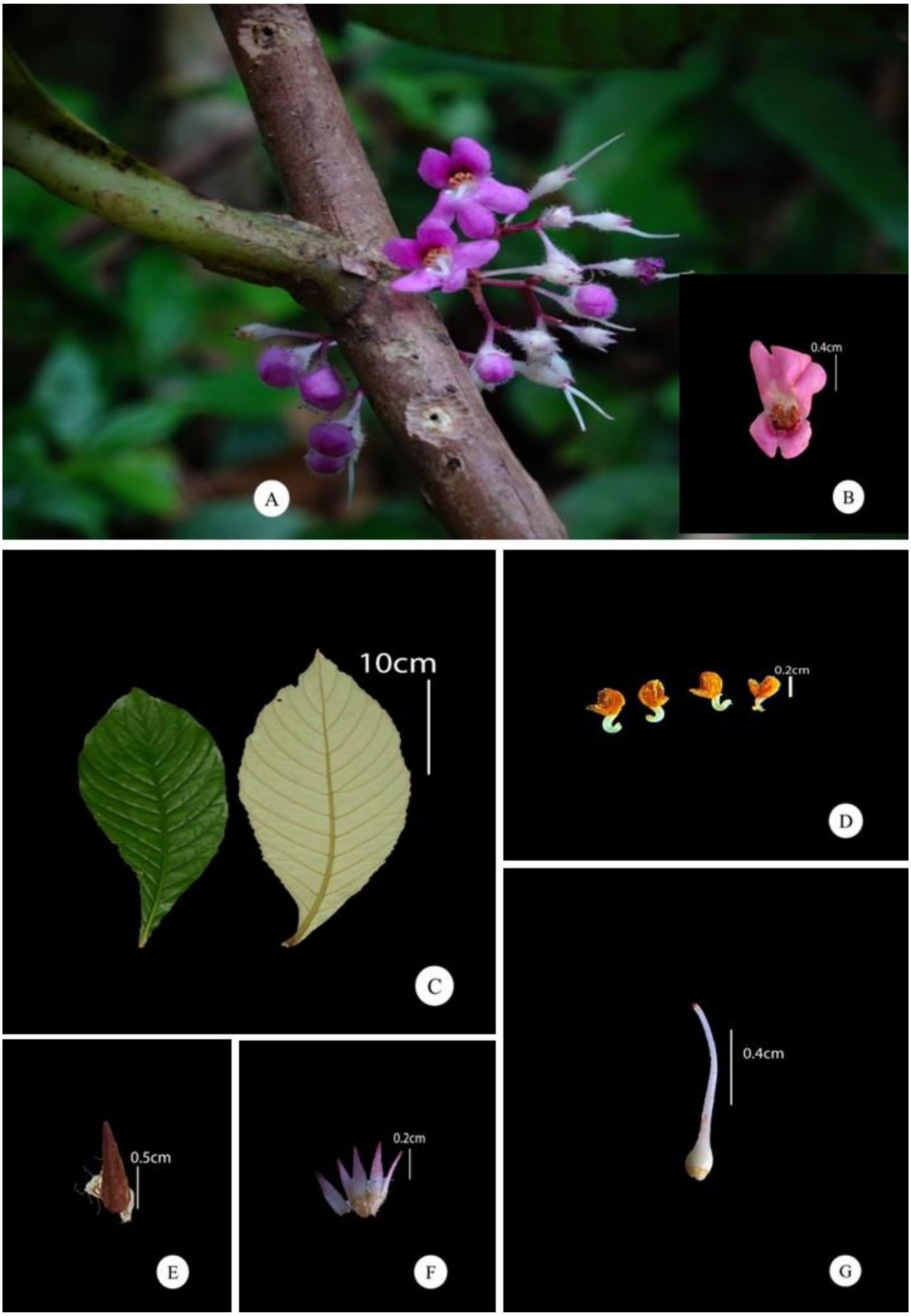

Plate 18: *Rhynchotechum ellipticum* (Wall. ex D.Dietr.) A.DC.A. A. Habit; B. Corolla; C. Leaves; D. Stamens; E. Bract; F. Calyx; G. Pistil.

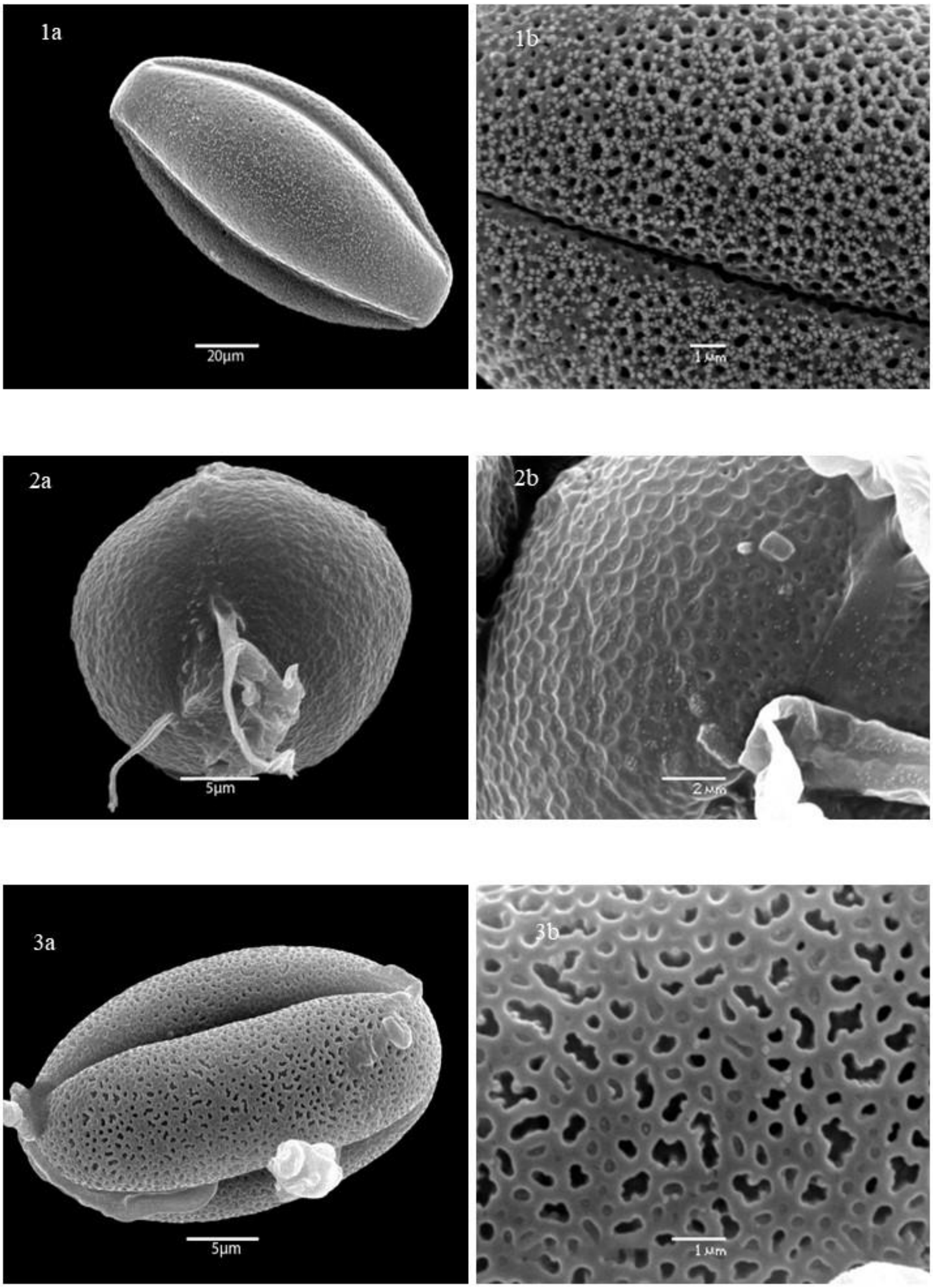

Plate 19: SEM microphotographs of pollen grains: 1. *Achimenes longiflora*, 2. *Aeschynanthus mannii*, 3. *Aeschynanthus parasiticus*; a-outline view, b- details of exine ornamentation.

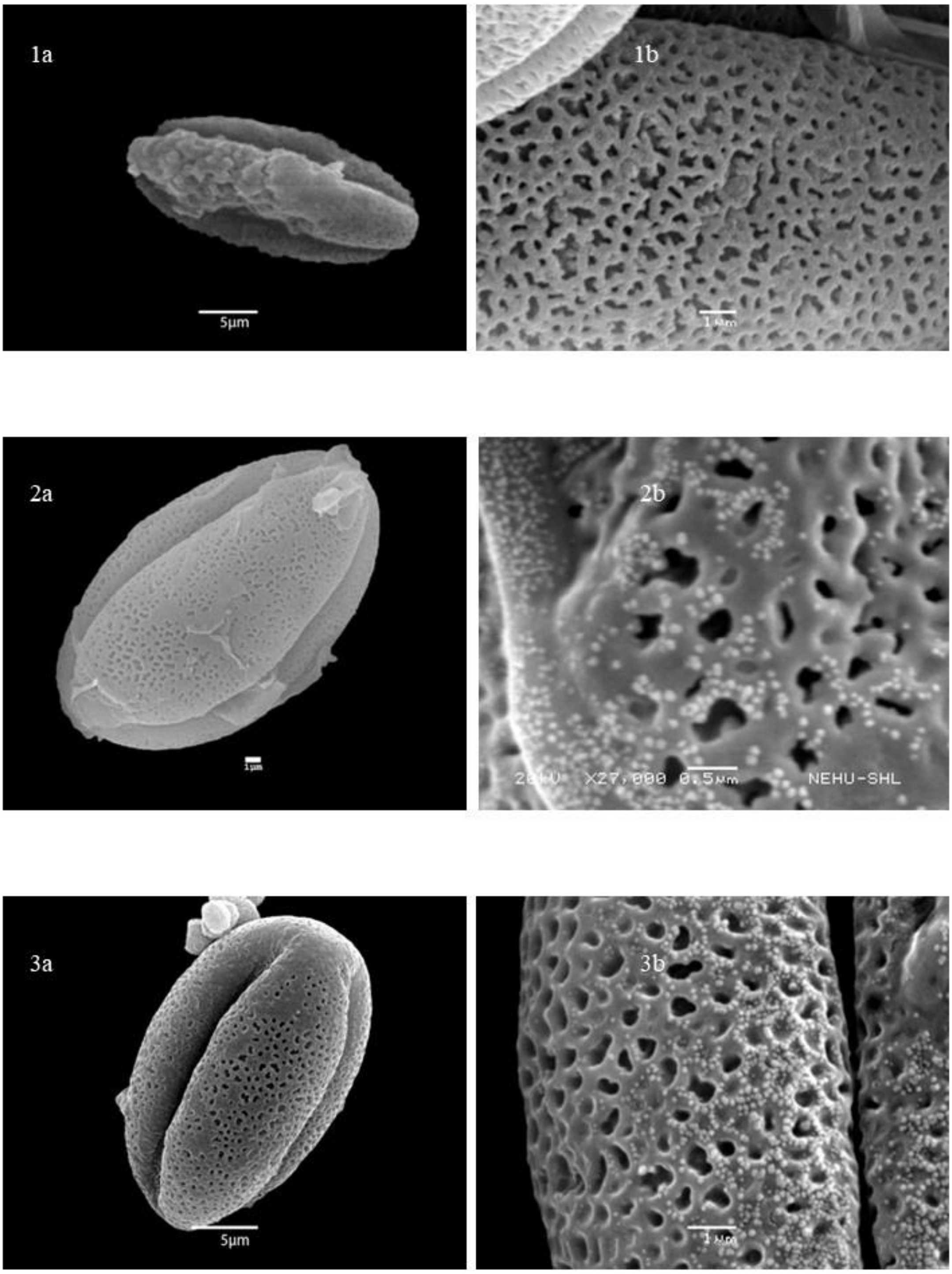

Plate 20: SEM microphotographs of pollen grains: 1. *Aeschynanthus parviflorus*, 2. *Aeschynanthus reiekensis*, 3. *Aeschynanthus superbus*; a-outline view, b-details of exine ornamentation.

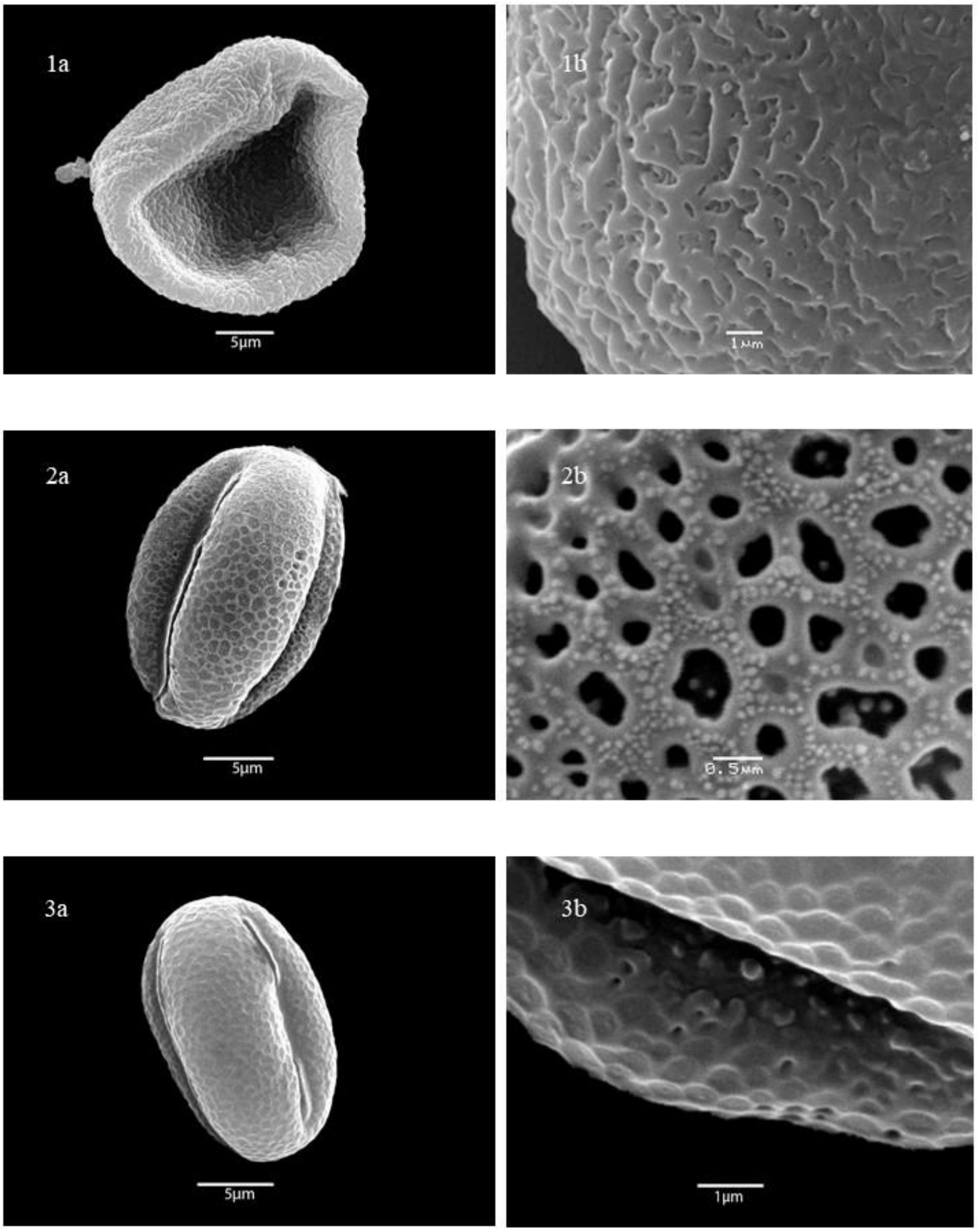

Plate 21: SEM microphotographs of pollen grains: 1. *Chrysothemis pulchella*, 2. *Didymocarpus adenocarpus*, 3. *Didymocarpus lineicapsa*; a-outline view, b-details of exine ornamentation.

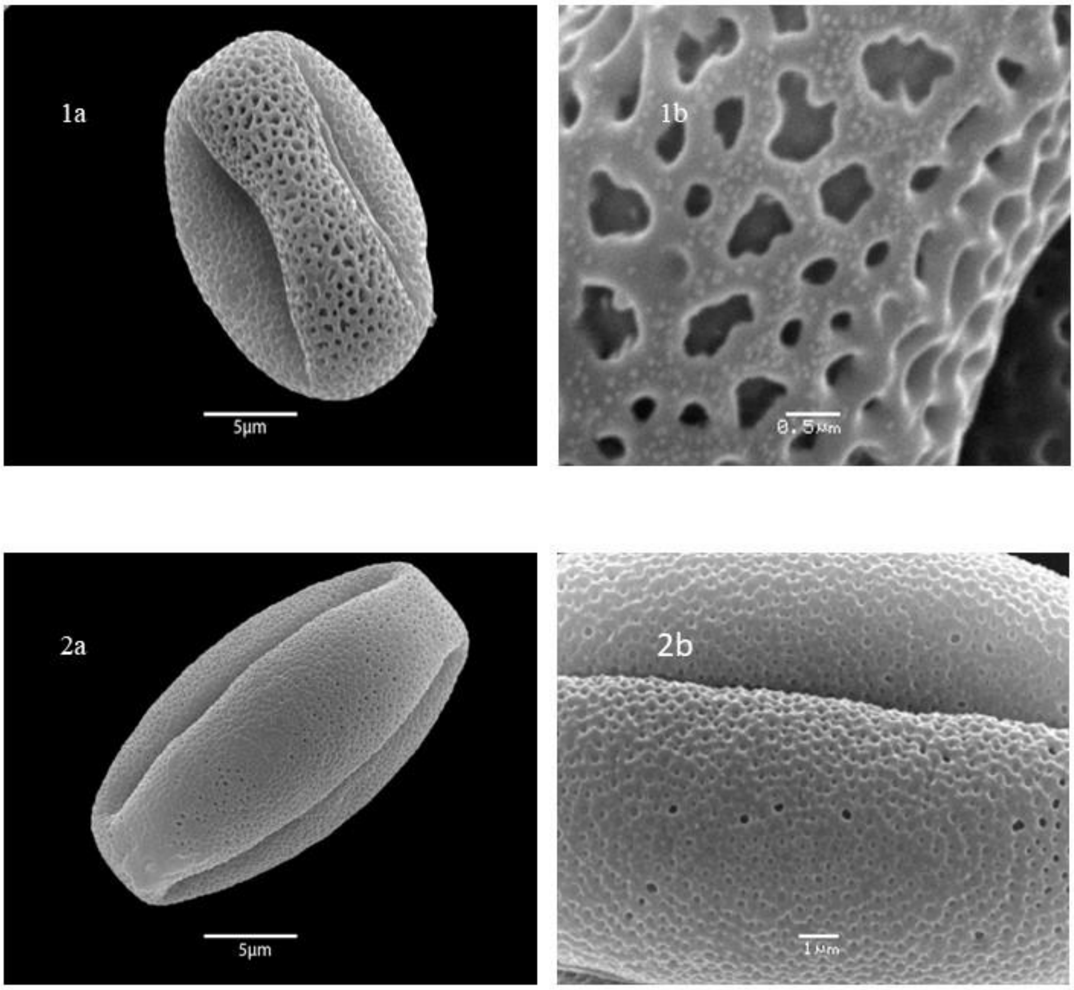

Plate 22: SEM microphotographs of pollen grains: 1. *Didymocarpus parryorum*, 2. *Didymocarpus vickifunkiae*; a-outline view, b-details of exine ornamentation.

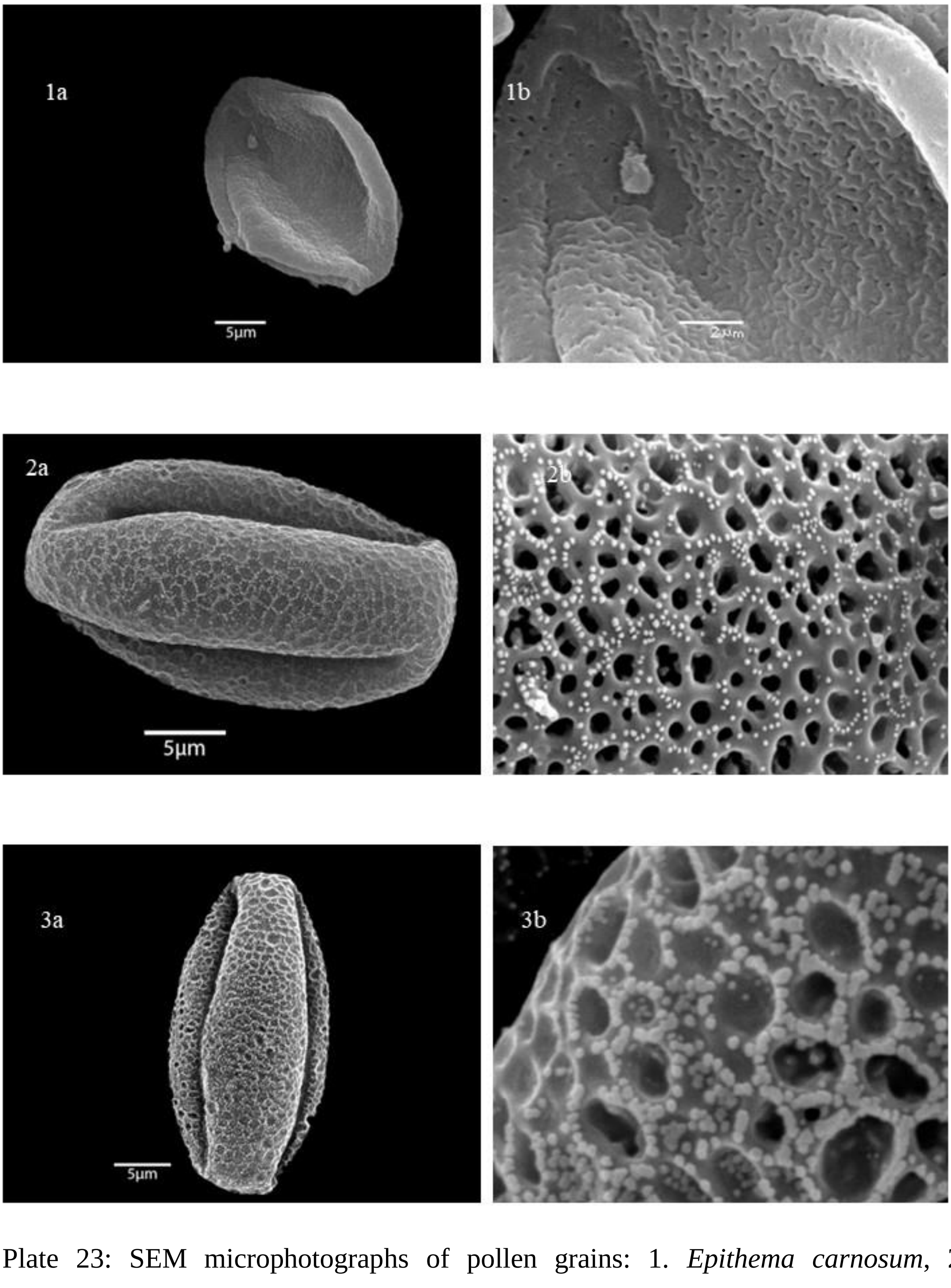

Plate 23: SEM microphotographs of pollen grains: 1. *Epithema carnosum*, 2. *Henckelia anachoreta*, 3. *Henckelia pumila*; a-outline view, b-details of exine ornamentation.

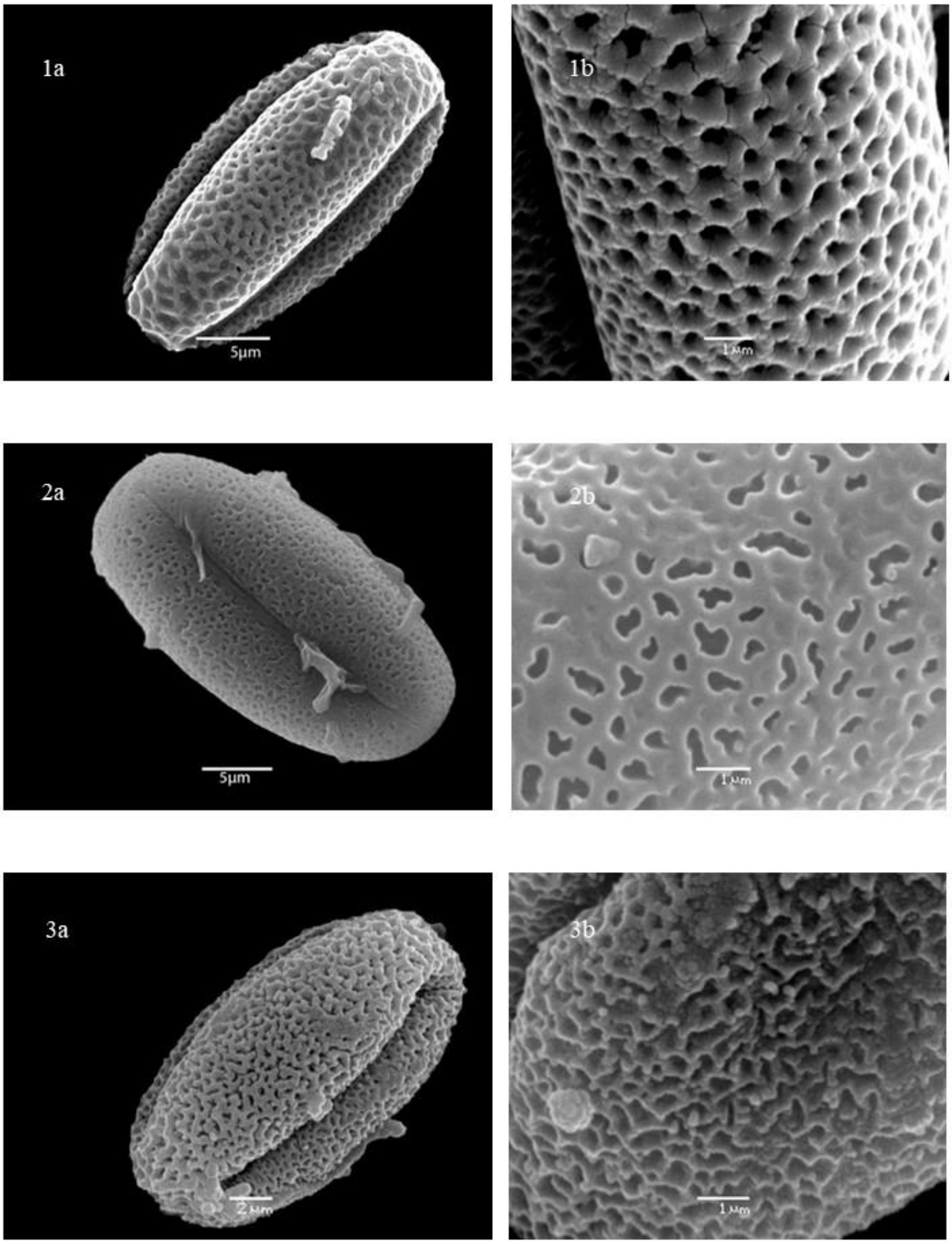

Plate 24: SEM microphotographs of pollen grains: 1. *Lysionotus serratus*, 2. *Rhynchoglossum obliquum*, 3. *Rhynchotechum ellipticum*; a-outline view, b-details of exine ornamentation.

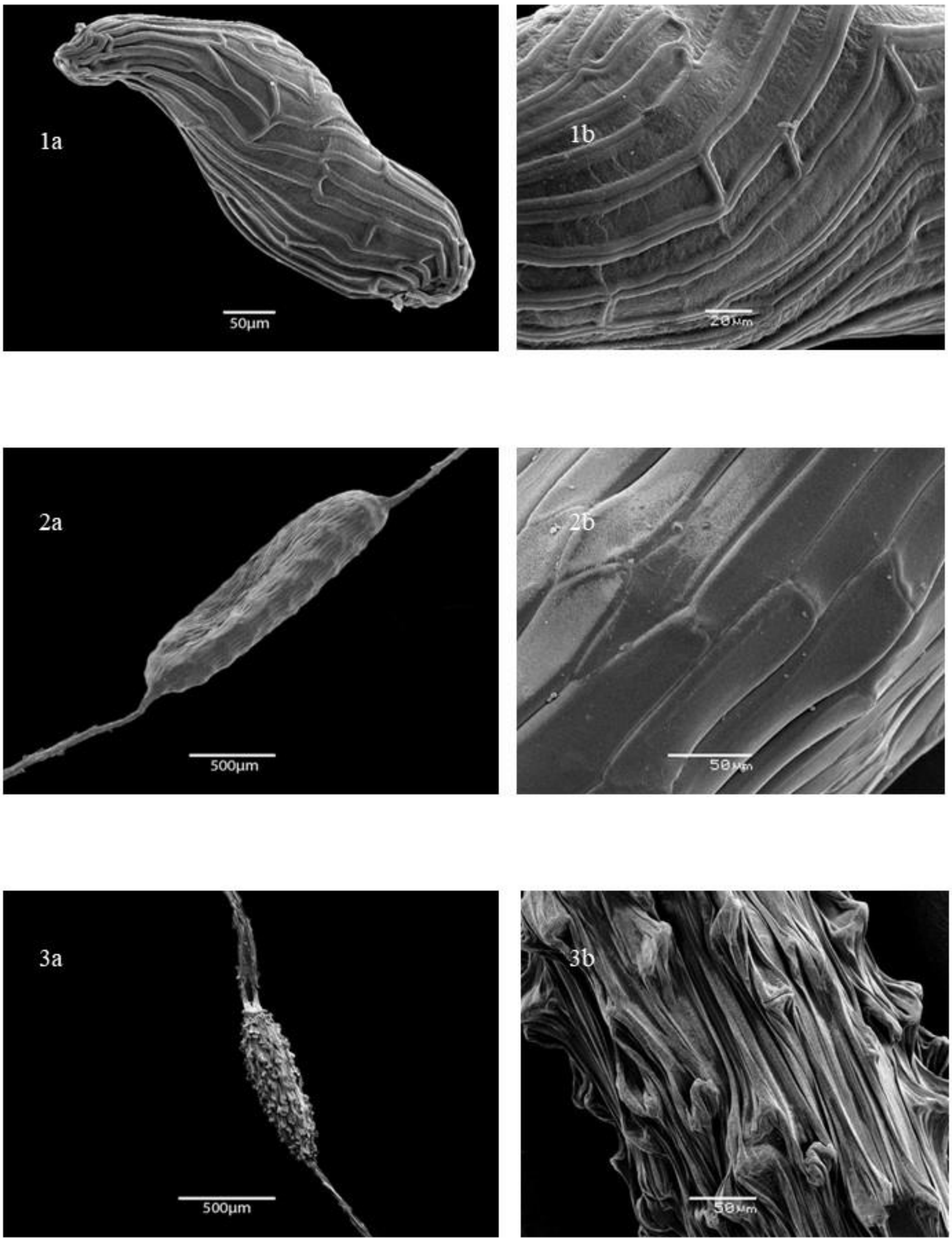

Plate 25: SEM microphotographs of seed coat: 1. *Achimenes longiflora*, 2. *Aeschynanthus mannii*, 3. *Aeschynanthus parasiticus*; a-outline view, b-details of cell character.

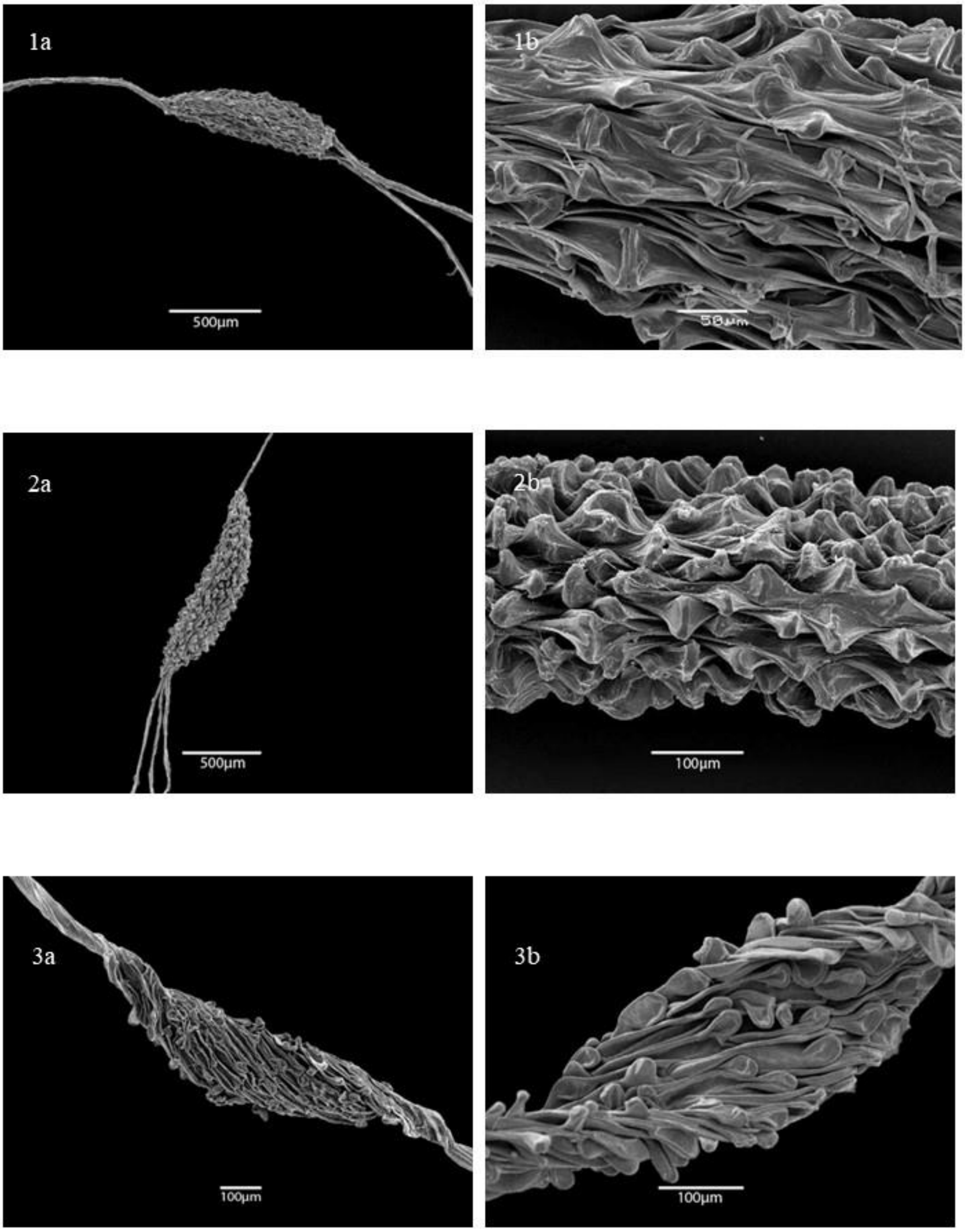

Plate 26: SEM microphotographs of seed coat: 1. *Aeschynanthus parviflorus*, 2. *Aeschynanthus reiekensis*, 3. *Aeschynanthus superbus*; a-outline view, b-details of cell character.

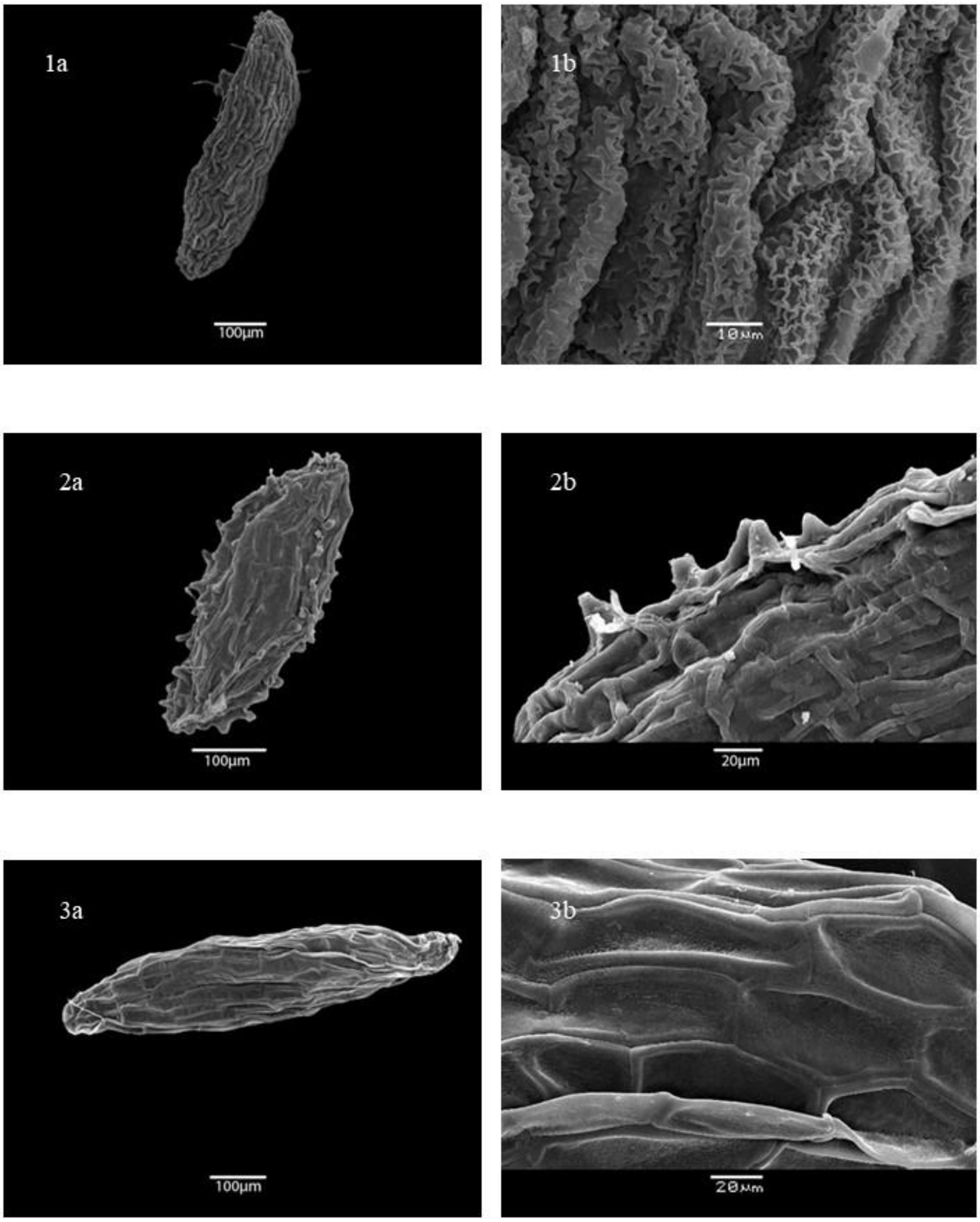

Plate 27: SEM microphotographs of seed coat: 1. *Chrysothemis pulchella*, 2. *Didymocarpus adenocarpus*, 3. *Didymocarpus lineicapsa*; a-outline view, b-details of cell character.

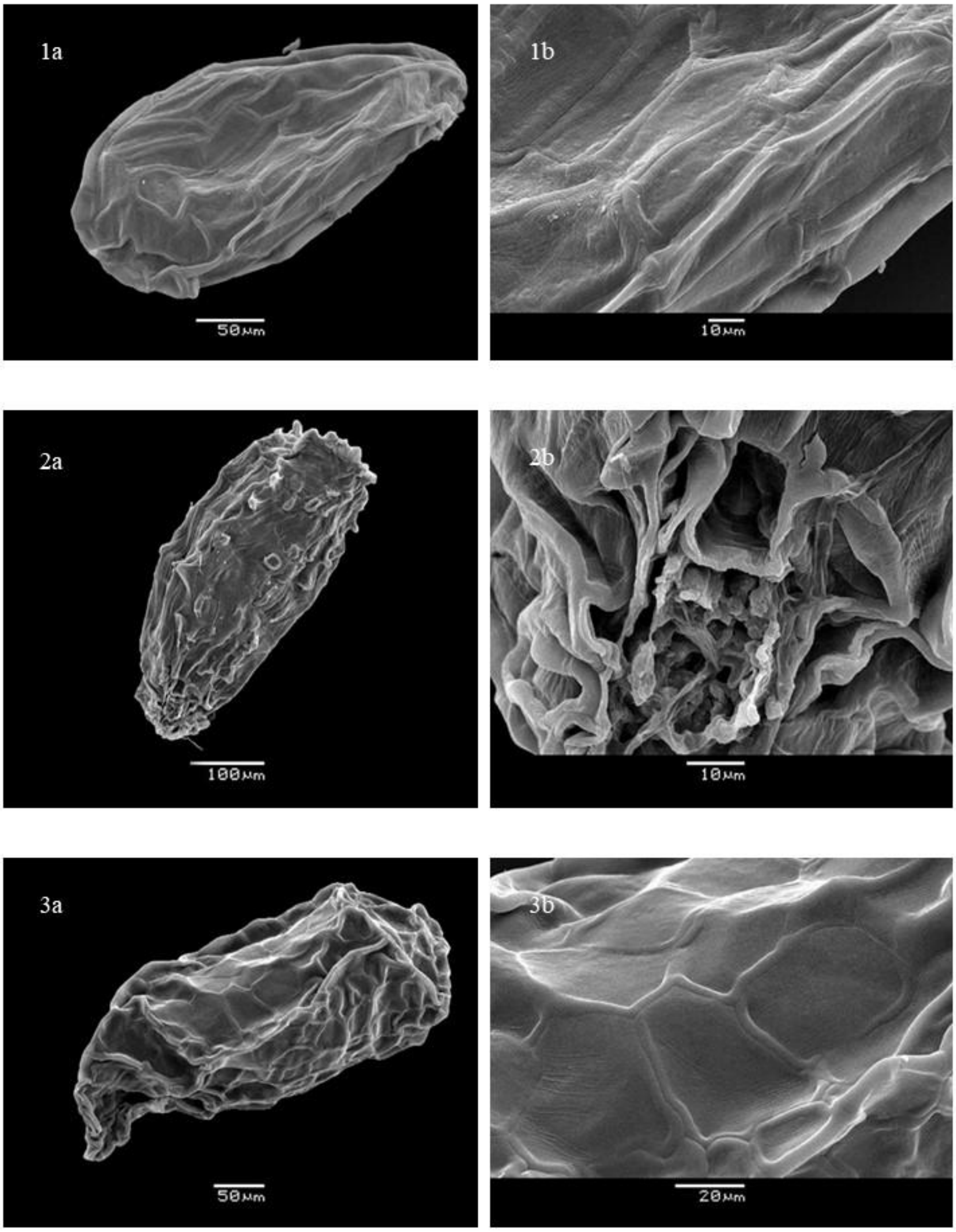

Plate 28: SEM microphotographs of seed coat: 1. *Didymocarpus parryorum*, 2. *Didymocarpus wengeri*, 3. *Didymocarpus vickifunkiae*; a-outline view, b-details of cell character.

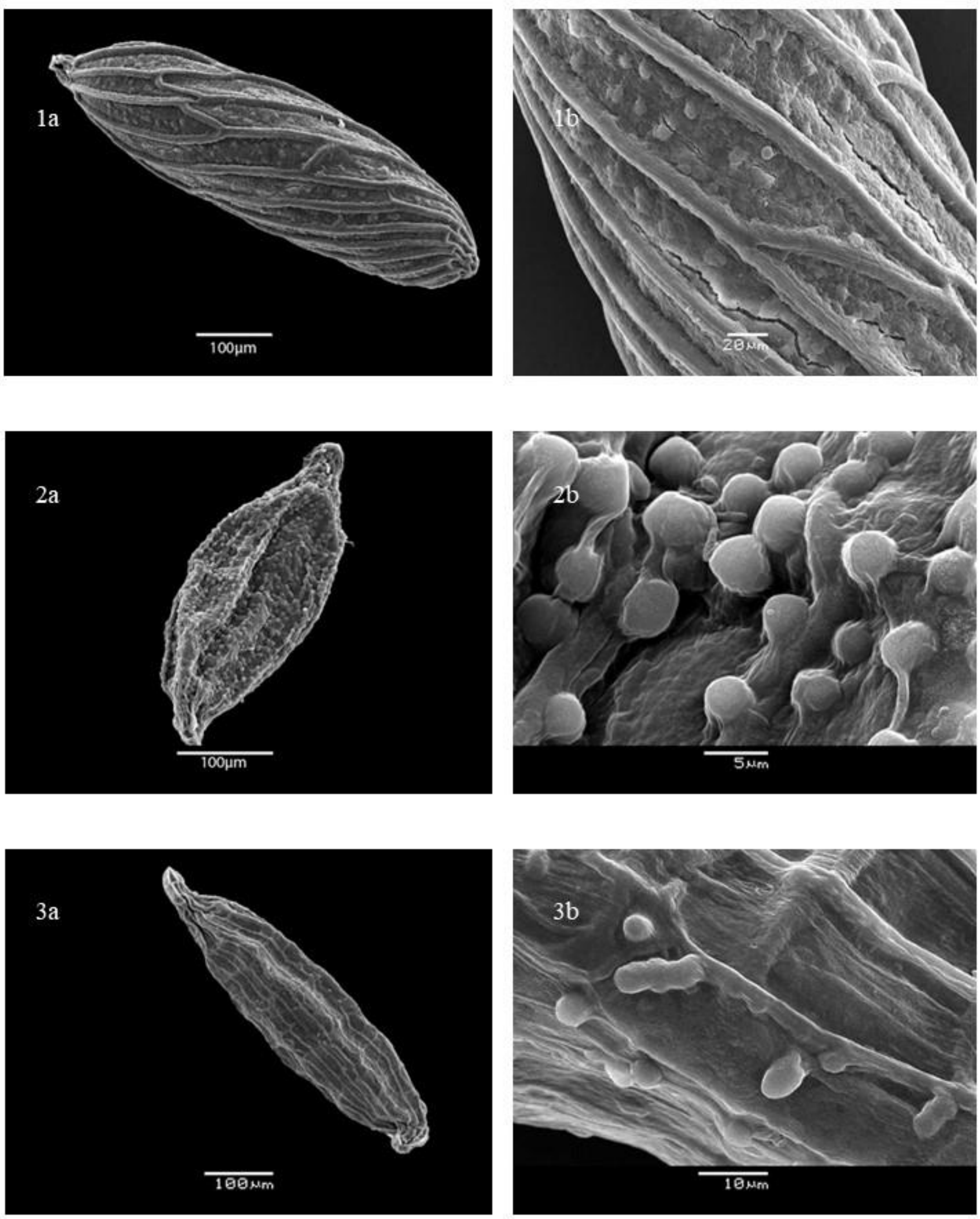

Plate 29: SEM microphotographs of seed coat: 1. *Epithema carnosum*, 2. *Henckelia anachoreta*, 3. *Henckelia pumila*; a-outline view, b-details of call character.

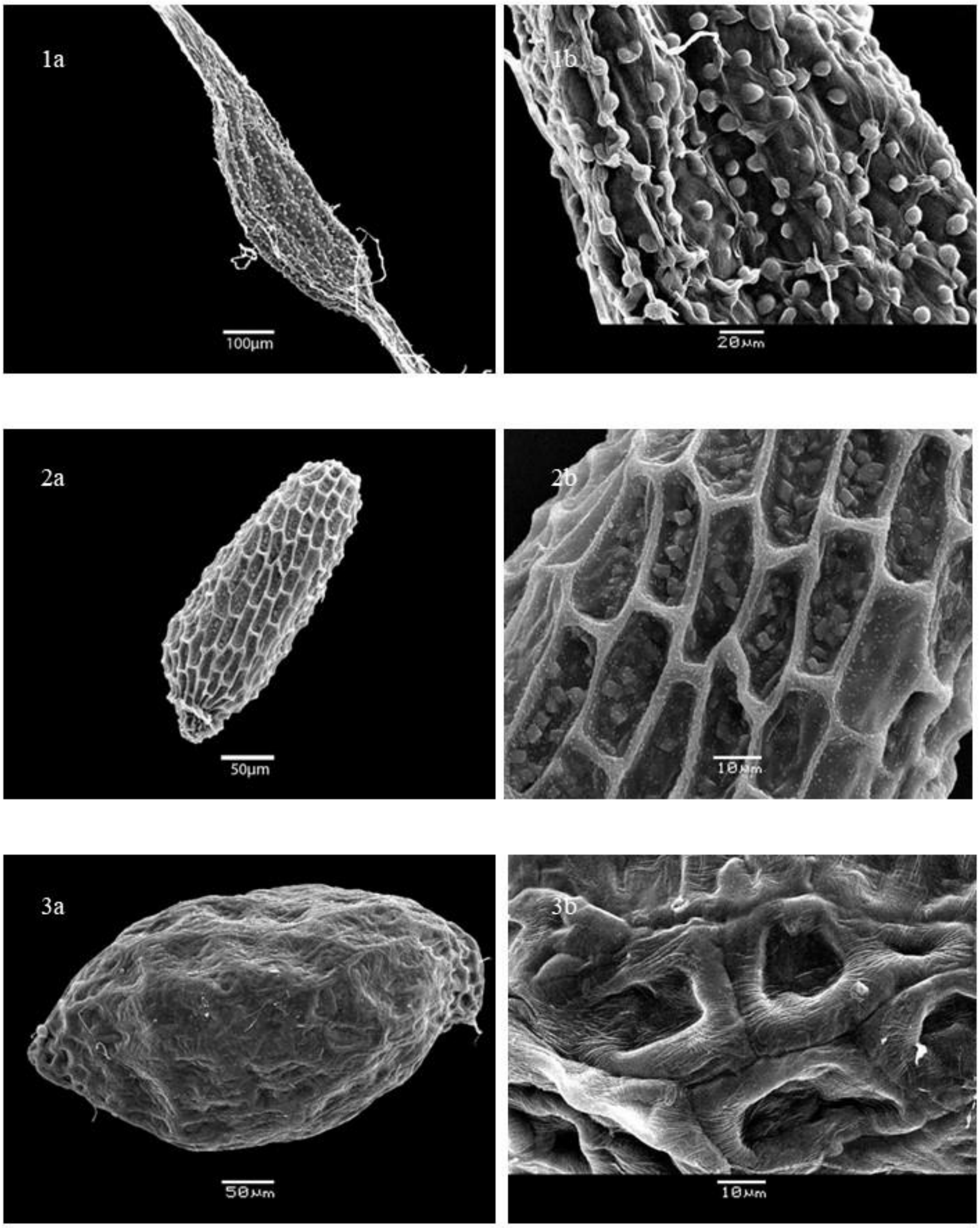

Plate 30. SEM microphotographs of seed coat: 1. *Lysionotus serratus*, 2. *Rhynchoglossum obliquum,* 3. *Rhynchotechum ellipticum*; a-outline view, b-details of cell character.

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List of Paper Publications

1. **Lalhlupuii, M**., Khomdram, S. D., Yumkham, S. D., Akhil, M. K., &Nampy, S. (2023). *Aeschynanthus reiekensis*, a new species of Gesneriaceae from Mizoram, Northeast India. *Taiwania*, *68*(1).

2. Tlanhlui, L., **Lalhlupuii, M.,**Yumkham, S. D., & Khomdram, S. D. (2023). Addition of four invasive alien plant species to state flora of Mizoram, India. *Journal of Threatened Taxa*, *15*(10), 24135-24139.

3. Tlanhlui, L., Khomdram, S. D .,Yumkham, S. D., **Lalhlupuii, M**., Laldinliana Khiangte & Sureshkumar Singh (2025). *Chamaegastrodia reiekensis*: A new holomycotrophic orchid from Mizoram, Northest India. *Journal of Plant Taxonomy and Geography, 80*(2): 229-238.

List of Presentations

1. Presented paper on **'Preliminary collection of *Aeschynanthus*Jack (Gesneriaceae) of Mizoram, NE India'**at the International Symposium on Plant Taxonomy and Ethnobotany, organised by Botanical Survey of India, Ministry of Enviroment, Forest & Climate Change, Kolkata from 13th–14th February, 2020

2. Presented paper on '**Pollen morphology and its systematic implication on *Didymocarpus* of Gesneriaceae family from Mizoram India'** in the National conference on Recent Advances in Plant Biology with special references to North-East India, organized by Department of Botany, School of Life Sciences, Mizoram University, Aizawl, Mizoram, India-796004 from 20th–21st April, 2023

3.Presented paper on **'A noteworthy collection and identification of interesting plants in Mizoram'** in the National conference on Emerging trends in Environmental Research (NACETER 2019), organised by Department of Enviromental Science, Pachhunga University College, Aizawl in collaboration with Enviroment, Forest & Climae Change Department, Government of Mizoram, during 31st October–2nd November. 2019.

Conferences/Seminars/Workshops Attended

1. Participated in the "Botanical Nomenclature Course" organised by Botanical Survey of India from 27th –31st January, 2020, in Shillong

2. Participated in One Day Awareness Programme cum Workshop on Invasive Alien Plants in Himalayas: Status, Ecological Impact and Management (Mizoram & Tripura Chapter), organized by Botanical Survey of India in collaboration with Department of Botany, Mizoram University, Aizawl on 26th April, 2019 under National Mission for Himalayan Studies.

3. Participated at the 'State Level Workshop cum Awareness Programme on Tinospora cordifolia', held at Administrative Conference Hall, Mizoram University on 19th November, 2019.

4. Participated in the 12thAnnual Convention of Association of Biotechnology and Pharmacy (ABAP) & International Conference on Biodiversity, Enviroment and Human Health: Innovations and Emerging Trends (BEHIET 2018) organuzed at the school of Life Sciences, Mizoram University, Aizawl, Mizoram 796004 during November 12 to 14, 2018.

5. Participated in the International Conference on Chemistry &Enviroment Sustainability (ICCES–2019) organise by department of Chemistry, Mizoram University during February 19–22, 2019.

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ABSTRACT

TAXONOMIC AND PALYNOLOGICAL STUDIES ON MEMBERS OF GESNERIACEAE FAMILY OF MIZORAM, INDIA

AN ABSTRACT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

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DEPARTMENT OF BOTANY

SCHOOL OF LIFE SCIENCES

DECEMBER, 2024

TAXONOMIC AND PALYNOLOGICAL STUDIES ON MEMBERS OF GESNERIACEAE FAMILY OF MIZORAM, INDIA

BY

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Submitted

In partial fulfillment of the requirement of the Degree of Doctor of Philosophy

in Botany of Mizoram University, Aizawl.

Abstract

The family Gesneriaceae Rich. & Juss. commonly referred to as gesneriads or the African violet family of the order Lamiales are known for its taxonomic complexity and remarkable global diversity comprising of 3,525 species across 147–151 genera. The members of the family plays a significant ecological and evolutionary role within the tropical and subtropical flora. The family has large representation from Northeastern States of India including Mizoram. Although some taxonomic work has been done on Gesneriaceae of Indian part of Eastern Himalayas and from other Northeastern States of India, most of the studies or reports from Mizoram was based on earlier collections which dates back 80-90 years ago. These historical studies lack updated information on the current distribution, diversity and characteristics of the family. The present study explores the comprehensive exploration of Gesneriaceae members in Mizoram. The research involves detailed examinations of their taxonomic characters, geographic distribution, micromorphological characters based on pollens of all the collected taxa from the family. Additionally, seed micromorphology along with molecular phylogenetic studies using the molecular markers (*rbcL* and ITS2) has been done to significantly gain a more comprehensive understanding of the plant family.

The thesis focused on the morphological taxonomy providing an important taxonomic information based on description, distribution map, photographic illustration and phenology of the species and the identification keys are provided at the genus to species level. The study of pollen grains along with seed coat anatomy provides critical morphological characteristics that aid in the identification and classification of many species. Very less work has been done on the palynological studies in context to Gesneriaceae taxa worldwide and in India. This study is being undertaken to address the significant gap in palynological data as well as seed micromorphological related to the distribution of Gesneriaceae family in Mizoram, India. Molecular studies on members of Gesneriaceae in Mizoram and from other parts of Northeast India are relatively sparse. This limits the understanding of genetic diversity, phylogenetic relationships and species delineation based on molecular data.

Molecular phylogenetic methods such as DNA sequencing of molecular markers (*rbcL* and ITS2) can help to clarify the taxonomic relationships between different taxa of Gesneriaceae of Mizoram which could significantly contribute to global database about them.

Extensive field surveys were conducted during 2018 to 2022 across Mizoram covering five protected areas and 31 villages under 11 districts. The main focused was on the flowering and fruiting season with the surveys targeted on diverse habitats, including places close to streams, waterfalls, rocky ledges, and moist, shaded environments. GPS coordinates were recorded for all collection sites, and high quality photographs of documented plant were taken for their morphology and habitat.

Species identifications was done using morphological observations, relevant literature and online databases such as POWO, Tropicos, WFO and the Gesneriad Reference Web. Herbarium specimens at ASSAM (BSI, Shillong) and CAL (BSI, Kolkata) and digital herbarium Kew (K) and Edinburgh (E) were consulted for authentication. Collected specimens were processed using standard herbarium methods and deposited at the Mizoram University Herbarium (MZUH).

For palynology and seed coat anatomy studies, fresh pollen and seed samples were dried, sputter-coated with gold and imaged using scanning electron microscope (JEOL JSM-6360) at Cyto-Gene Research and Development, Lucknow, and SAIF, NEHU, Shillong.

Hierarchical cluster analysis (UPGMA method with EUCLIDEAN distance) and principal component analysis (PCA) were performed using R software (V.4.1.2) to group species based on pollen characteristics and seed coat morphology. Results were visualized through dendrograms and PCA plots, highlighting species similarities and differences based on their groupings.

Molecular studies utilized fresh leaf samples for DNA extraction using the CTAB method. The two molecular marker regions ITS2 and *rbcL*were amplified, sequenced and submitted to GenBank, with accession number allotted. Phylogenetic tree was constructed using the ClustalW in MEGA X software.

The study documented 18 species from 9 genera (i.e., *Achimenes longiflora* DC., *Aeschynanthus mannii* Kurz ex C.B.Clarke, *Aeschynanthus parasiticus* (Roxb.) Wall., *Aeschynanthus parviflorus* (D.Don) Spreng., *Aeschynanthus reiekensis* Lalhlupuii, S.D.Khomdram & S.D.Yumkham, *Aeschynanthus superbus* C.B. Clarke, *Chrysothemis pulchella* (Donn ex Sims) Decne., *Didymocarpus adenocarpus* C.E.C.Fisch., *Didymocarpus aureoglandulosus* C.B.Clarke, *Didymocarpus lineicapsa* (C.E.C.Fisch.) B.L.Burtt, *Didymocarpus parryorum* C.E.C.Fisch., *Didymocarpus wengeri* C.E.C.Fisch., *Epithema carnosum* Benth., *Henckelia anachoreta* (Hance) D.J.Middleton & Mich.Möller, *Henckelia pumila* (D.Don) A.Dietr., *Lysionotus serratus* D.Don, *Rhynchoglossum obliqum* Blume and *Rhynchotechum ellipticum* (Wall. ex D.Dietr.) A.DC.) of the Gesneriaceae family in Mizoram, India, classified into three subfamilies, three tribes, and six sub-tribes. Habitat analysis revealed that 28% of species are lithophytic, 33% geophytic, and 39% epiphytic, distributed across altitudes ranging from 980 m to 1663 m.

Gesneriaceae species exhibit diverse habitat preferences thriving in high humidity and varying altitudinal gradients. *Aeschynanthus* species are abundant at mid- to high elevation forest area with high humidity and sunlight, growing as epiphytes. *Didymocarpus* species prefer rocky habitats like crevices, caves and cliffs. The *Henckelia* species and *Epithema carnosum* thrive near streams and waterfalls and while *Lysionotus serratus* grow as epiphytic subshrubs on tree trunks. *Rhynchotechum ellipticum* and *Rhynchoglossum obliquum* are widely distributed across attitudes showing broad adaptability. *Achimenes longiflora* and *Chrysothemis pulchella* are notable ornamental species with significant growth potential.

The discovery of a new species, *Aeschynanthus reiekensis*, highlights the rich biodiversity and under explored nature of the Gesneriaceae family in Mizoram. Additionally, the inclusion of *Henckelia anachoreta* and two naturalized invasive species, *Achimenes longiflora* and *Chrysothemis pulchella*, marks the significant contributions to the flora of the region and emphasizes its ecological importance.

Palynological studies involved detailed morphological characterization of pollen grains, focusing on size, shape, exine ornamentation, and apertural patterns. These features were examined to assess their taxonomic relevance and potential adaptations to pollination mechanisms in diverse ecological niches. Seed coat morphology, which examines the structure and texture of seeds, offers significant systematic implications, showing genus level consistency. The detailed analysis of pollen characteristics and seed coat anatomy has been pivotal in constructing a comprehensive identification key for the Gesneriaceae family. The study of palynology and seed coat morphology in Gesneriaceae confirms their role to advancing knowledge in plant systematics, ecology, and conservation.

The significance of PCA analysis in both pollen and seed characterization lies in its ability to simplify complex datasets, identifying key variables that most effectively distinguish between species. In pollen, polar length contributed the highest in grouping the species marking as a key character among the variables and in seed among the variables, apical character made the highest contribution. This clustering method aids in identifying species-specific characteristics as well as a powerful tool for exploring the relationships between different traits and can help in understanding evolutionary patterns and ecological adaptations. Overall, PCA enhances the precision and efficiency of species grouping, identification, contributing to more accurate taxonomic and ecological studies.

Creating the DNA barcode database using *rbcL* and ITS2 markers for the Gesneriaceae members of Mizoram in global repositories (GenBank) could provide a crucial step, particularly for establishment of the first DNA database for the endemic taxa that include all the collected species of *Didymocarpus* and some species from the *Aeschynanthus* aiding in precise identification. It will be a significant milestone that will reveal the hidden diversity among the members of the Gesneriaceae taxa from the region. The barcoding database will complement the morpho-taxonomic including the pollens and seed coats data for accurately identifying the members of Gesneriaceae. In addition to that, the database generated using the *rbcL* and ITS2 markers contribute to resolve the phylogenetic relationships among the Gesneriaceae members of the region offering insights into their evolutionary history and adaptive strategies among the members in the unique habitats available in Mizoram.

Among the reported 18 species of Gesneriaceae in Mizoram, 33% (6 species) are found endemic to the state, including all the *Didymocarpus* sp. and the newly discovered *Aeschynanthus reiekensis*. These species, indicators of pristine landscapes, face significant threats from habitat destruction caused by jhum cultivation, deforestation, infrastructure development, and climate change. Their narrow distribution, habitat specialization, and vulnerability to disturbances increase their extinction risk. A total of 13 species belonging to 5 genera have been identified under the Rare, Endangered and Threatened (RET) taxa. Additionally, 5 species fall under the Not Evaluated (NE) category, likely due to their limited distribution and lack of comprehensive research conducted on them.The threat status of the taxa highlights the limitation in population count of the taxa due to fragmented population caused by rapidly degrading habitat, forest degradation and insufficient data with less information available to determine their conservation status. So, conservation strategies must prioritize habitat preservation, protect of fragile ecosystems and curbing destructive human activities particularly for epiphytic and lithophytic species. Community engagement, research, and coordinated strategies are essential to safeguard these taxa.

Ethnobotanical investigations revealed the use of nine Gesneriaceae species used by the indigenous community of Mizoramfor food, medicine, fodder and ornamental purposes. Notably, the ethnomedicinal applications of *Rhynchotechum ellipticum* roots for treating kidney problems and *Epithema carnosum* leaves for hemorrhoids are documented for first time, reflecting the unique traditional knowledge of the region. This highlights the critical need to preserve ethnobotanical heritage and its potential for uncovering novel bioactive compounds and unexplored medicinal properties, contributing to scientific and pharmacological advancements.

The ornamental value of many Gesneriaceae species, particularly those in the species *Aeschynanthus*, though locally used as ornamental plants, possess significant horticultural development due to their aesthetic appeal. Their adaptability to tropical and subtropical conditions makes them ideal for cultivation in gardens and nurseries offering economic opportunities for local communities. Additionally, *Chrysothemis pulchella* and *Achimenes longiflora* are widely cultivated ornamental plants for their ornamental value, have become naturalized in the region, further emphasizing their horticultural and economic significance

In conclusion, the study highlights the taxonomic complexity and ecological significance of the family Gesneriaceae in Mizoram, emphasizing the integration of micromorphological, molecular and ethnobotanical approaches in addition to the morphotaxonomical approach. The additional data obtained by examining pollen and seed micromorphology alongside molecular data refines phylogenetic relationships, uncovers evolutionary adaptations and documents the rich diversity of this underexplored family. The findings reveal the unique and diverse flora of Mizoram, with many taxa being endemic or habitat specific. The discovery of novel species and new records underscores the untapped biodiversity of the region and fills critical gaps in global and regional databases. Morphological and molecular analyses provide insights into adaptive traits shaped by local environmental pressures, while ethnobotanical investigations reveal the intricate relationship between these plants and local communities. This research forms a foundational resource for biodiversity documentation, conservation strategies, and sustainable utilization of Gesneriaceae. Encouraging habitat protection, raising awareness and promoting sustainable management can ensure the long-term preservation of these valuable plant resources including newly discovered and RET taxa. Integrative approaches combining taxonomy, conservation, and community engagement are essential for protecting the botanical heritage of Mizoram.