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Molecular Species Delimitation, Taxonomy and Biogeography of Sri Lankan Gesneriaceae

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Declaration

I hereby declare that the work contained in this thesis is my own unless otherwise acknowledged and cited. This thesis has not in whole or in part been previously presented for any degree

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24th January 2017.

Abstract

The plant family Gesneriaceae is represented in Sri Lanka by six genera: *Aeschynanthus*, *Epithema*, *Championia*, *Henckelia*, *Rhynchoglossum* and *Rhynchotechum*, with 13 species (plus one subspecies/variety) of which ten are endemic including the monotypic genus *Championia*, according to the last revision in 1981. They are exclusively distributed in undisturbed habitats, and some have high ornamental value. The species are morphologically diverse, but face a problem of taxonomic delineation, which is further complicated by the presence of putative hybrids.

Sri Lanka and Indian Peninsula, represent the Deccan plate of the ancient Gondwanan supercontinent. The presence of a relict flora may indicate the significance of the geological history of the Deccan plate for the evolution of angiosperms. The high degree of endemism here, along with their affinities to the global angiosperm flora paints a complex picture, but its biogeographic history is still unclear. The pantropical family Gesneriaceae distributed in Sri Lanka and South India is therefore an appropriate study group in this context. Besides, the family itself has a complex but largely unresolved biogeographical history especially concerning the origin and diversification of Old World Gesneriaceae.

Modern approaches for the taxonomic studies were applied, integrating morphological and molecular data. Multiple samples were collected for each species across their geographical distribution. Nuclear ITS and chloroplast *trn*L-F sequences for the taxa from Sri Lanka were used to generate regional genus phylogenies of all six genera, using maximum parsimony. The rate of evolution of the nuclear ITS region versus chloroplast *trn*L-F was varied greatly across the six genera studied. Molecular delimitations were mostly congruent with the classical taxonomy.

Over 65 taxonomic characters were studied in detail to recognize synapomorphies for clades and taxa. A complete taxonomic revision of Gesneriaceae in Sri Lanka, including lectotypification, was conducted based on both, the molecular and morphological data. This resulted in the recognition of 14 species in the six genera, including one newly described species *H. wijesundarae* Ranasinghe and Mich.Möller. *Henckelia communis* and *H. angusta* were not supported molecularly as two separate entities but are recognized as two species because of consistent

morphological differences between them. *Henckelia humboldtiana* is proposed to represent a species complex due to its highly variable and inconsistent molecular and morphological diversity and overlap with *H. incana* and *H. floccosa*; more research is needed here. National conservation assessments were conducted, and all 14 species were recognized as threatened.

Biogeographic affinities of Sri Lankan Gesneriaceae were elucidated, generating a dated phylogeny using an existing matrix of four plastid gene regions; *trn*L-F, *mat*K, *rps*16 and *ndh*F, amended by sequences generated in this study. The final combined matrix included 175 taxa including newly generated sequences for the 13 Sri Lankan taxa. Phylogenetic trees were generated using parsimony, maximum likelihood and Bayesian inference. Molecular dating was carried out using BEAST and ancestral area reconstruction using BioGeoBears. These analyses indicated that the six genera of Gesneriaceae arrived in Sri Lanka separately and sometimes different time periods. One lineage dated back to the early diversification of the subfamily Didymocarpoideae (generally regarded as the Old World Gesneriaceae), which occurred around the KT boundary, before the Deccan plate was connected to Asia.

Lay Summary

Sri Lanka harbours one of the most diverse floras in tropical Asia with a high species diversity and high levels of endemism. Similar to other tropical forests around the world those in Sri Lanka are also at risk of degradation and destruction and accompanying species extinction. It is therefore significant that Sri Lanka has been recognized as an important component of the world's biodiversity and has thus been recognized among one of the 34 global biodiversity hotspots, a spotlight which might lead to recognition of improved protection. The Sri Lankan flora was first catalogued and described dating back as far as the time of Linnaeus, and infrequently since, the last being the Revision of the Flora of Ceylon from 1968–2000. However, there are still more taxa being discovered and added in field studies. On the other hand, the country's flora has not been studied in the light of molecular methods to understand important aspects of their pattern of speciation and the origin and evolution of lineages, also with view to biogeographic affinities to the world flora.

The present study focused on the plant family Gesneriaceae in Sri Lanka as a case study. Here I present more insight into plant speciation and evolution of 13 Gesneriaceae taxa in six genera based on molecular and morphological data. The biogeographic affinities of these Sri Lankan taxa to other Gesneriaceae flora in the world were also studied.

The results show correspondence and also discrepancy between molecular and morphological data for recognizing species: one new species has been recognized on the basis of congruent molecular and morphological data, while in another case morphological evolution outstripped molecular evolution. The analysis of the assemblage of the Gesneriaceae flora on the Deccan Plate (i.e. Sri Lanka + South India) revealed at least six–seven events where Gesneriaceae lineages were introduced to the island over time, one dating back around the collision of the plate with Eurasia, others are more recent up to the perhaps as recent as the last glacial maximum.

This is the first detailed study on Sri Lankan plants to recognize "species" on the combination of molecular and morphological data and the inclusion of multiple samples per species. The study also assembled the most updated DNA sequence matrix of the Old World lineage of family Gesneriaceae and included all Sri Lankan

Gesneriaceae species. The study shed new light on our knowledge on the biodiversity and biogeography of the Sri Lankan and South Asian flora, and can be regarded as a blue print for future studies in this study area.

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Table of Contents

Declaration ..... **i**

Abstract ..... **ii**

Lay Summary ..... **iv**

Acknowledgements ..... **vi**

Chapter 1 Introduction **1**

1.1 Overview *1*

1.1.1 Floristic history of Sri Lanka 1

1.1.2 Geography and Climate in Sri Lanka 2

Geography 2

Climate 3

1.2 Background to the study *5*

1.2.1 Classification of the family Gesneriaceae 5

1.2.2 Gesneriaceae flora in Sri Lanka 8

1.2.3 Current knowledge of the Biogeographic history of family Gesneriaceae 9

1.3 Species concept for delimitation study *10*

1.3.1 Different aspects of speciation 11

1.4 Morphological and molecular data used in species delimitation. *11*

1.4.1 Morphological data 11

1.4.2 Molecular data 13

Problems of molecular species delimitation 13

1.5 Objectives *16*

Chapter 2 Species Delimitation of Sri Lankan Gesneriaceae **17**

2.1 Introduction *17*

2.2 Species delimitation approach in family Gesneriaceae *17*

2.2.1 Species delimitation concept applied to Sri Lankan Gesneriaceae 17

2.2.2 Genetic markers used for species delimitation in family Gesneriaceae 18

2.2.3 Sampling strategies in molecular species delimitation 18

2.3 Methodology *19*

2.3.1 Field Collections 19

2.3.2 Outgroup Taxa 19

Rhynchoglossum 20

Epithema....20
Championia....20
Rynchotechum....20
Henckelia....20
Aeschynanthus....21
2.3.3 Ingroup Taxa.... 21
Rhynchoglossum....21
Epithema....21
Championia....21
Rhynchotechum....21
Henckelia....21
Aeschynanthus....22
2.3.4 DNA Extraction, PCR and Sequencing.... 22
2.3.5 Calculating Genetic distance.... 25
2.3.6 Phylogenetic Analysis.... 26
2.4 Results.... 27
2.4.1 Molecular species delimitation of genera of Gesneriaceae in Sri Lanka.... 27
Rhynchoglossum....27
Epithema....31
Championia....35
Rhynchotechum....37
Henckelia....38
Aeschynanthus....42
2.5 Discussion.... 44
2.5.1 Molecular species delimitation of Gesneriaceae genera in Sri Lanka.... 44
Rhynchoglossum....44
Epithema....44
Championia....45
Rhynchotechum....45
Henckelia....46
Aeschynanthus....48
2.5.2 Comparison of the genetic distance data (ITS vs. trnL-F) across the species and genera. 48
2.5.3 General Discussion.... 51
2.6 Conclusions.... 52

Chapter 3 Taxonomic status and revision of Sri Lankan Gesneriaceae.....53

3.1 Introduction 53

3.2 Methodology 55

3.3 Revision of Sri Lankan Gesneriaceae 61

Rhynchoglossum Blume 61

Epithema Blume 72

Championia Gardn. 78

Rhynchotechum Blume 83

Henckelia Spreng. 88

Aeschynanthus Jack 130

Aeschynanthus ceylanicus 131

3.4 Conclusions 135

Chapter 4 Biogeographic affinities of the Gesneriaceae flora of Sri Lanka 142

4.1 Overview of historical biogeography 142

4.1.1 Current knowledge of the biogeographical history of the family Gesneriaceae 143

4.1.2 Biogeographic history of subfamily Didymocarpoideae 146

4.1.3 Geological and climatic history of Sri Lanka 147

4.1.4 Biogeographic affinities of the Sri Lankan Flora 153

4.1.5 Biogeographic history of family Gesneriaceae in Sri Lanka and South India . 155

4.2 Materials and Methods 156

4.2.1 Materials 156

4.2.2 DNA extraction, PCR and sequencing 157

4.2.3 Phylogenetic Analysis 158

4.2.4 Dating Analysis 160

4.2.5 Ancestral Area Reconstruction 161

4.3 Results 161

4.3.1 Phylogenetic Analysis 161

4.3.2 Dating Analysis 167

4.3.3 Ancestral Area Reconstruction 171

4.4 Discussion 172

4.4.1 Taxon Sampling 172

4.4.2 Phylogenetic Analysis 173

4.4.3 Biogeographic history of family Gesneriaceae 176
Sampling strategies of dated phylogenetic studies in family Gesneriaceae 176
Origin of family Gesneriaceae 177
Origin of Old World Gesneriaceae 178
Subfamily Didymocarpoideae 178
Tribe Trichosporeae 178
Tribe Epithemateae 180
4.4.4 Biogeographic affinities of Sri Lankan Gesneriaceae 180
Championia 180
Rhynchoglossum 180
Epithema 181
Rhynchotechum 181
Henckelia 181
Aeschynanthus 182
4.5 General Discussion 183
4.6 Conclusions 186

Chapter 5 Final Discussion and Conclusions **..... 188**

References 191

Appendices 207

Appendix 1 Details of DNA samples used in the molecular species delimitation in Chapter 2 .. 207-215

Appendix 2 Details of DNA samples used in the phylogeny and Biogeography of family Gesneriaceae 215-222

Appendix 3 Comparison of stem and crown age estimates for family Gesneriaceae from different studies 222-224

Appendix 4 Cladogram of Bayesian inference analysis of the family Gesneriaceae 225

Appendix 5 50% Majority Rule Consensus Tree of Maximum Parsimony Analysis of the family Gesneriaceae 226

Appendix 6 Maximum clade credibility tree results for family Gesneriaceae 227

Appendix 7 Results of the BioGeoBears analysis with DEC Model 228–229

Appendix 8 Results of the BioGeoBears analysis with DEC+J Model 230–231

Chapter 1 Introduction

1.1 Overview

1.1.1 Floristic history of Sri Lanka

The Sri Lankan angiosperm flora was celebrated in the work of several eminent botanists in the 17th to 18th century. First, Linnaeus (1747) recorded several new plant species in his *Flora Zeylanica* based on enumerations of a collection made by Paul Hermann, a Dutch botanist and medical officer in Sri Lanka (then Ceylon) in the late 17th century. Therefore, many Sri Lankan specimens of Hermann's collection became type specimens in an epochal book of Linnaeus, *Species Plantarum*, published in 1753. Among others, *Enumeratio Plantarum Zeylaniae* by G.H.K. Thwates in 1858, five volumes of the *Hand Book to The Flora of Ceylon* by Henry Trimen and Sir Joseph Dalton Hooker during 1893–1900, the sixth supplementary volume made by A.H.G. Alston, are remarkable contributions to the history of Sri Lankan botany (Gunatilleke and Gunatilleke, 1990). The *Flora of Ceylon* was later revised by F.R. Fosberg and M.D. Dassanayake for angiosperms in 14 volumes published during 1980–2000. Therein, a total of 3771 angiosperm species in 1363 genera were listed of which 1000 taxa including subspecies and varieties were recognized as endemic. These numbers also included naturalized species. The most updated information (Wijesundara *et al.*, 2012), excluding naturalized species revealed a total of 3154 native angiosperm species in 186 families recorded as native to the island. Although there are no endemic families, there are 15 genera endemic to Sri Lanka. It is also recognized that 894 (28%) of the angiosperm taxa are endemic to the island.

However, the current knowledge of the biodiversity in the country is largely based on outdated field expedition data (Pethiyagoda, 2005). There were no island-wide expeditions carried out since the work of a century or more ago. It is also accepted that the dry part is poorly botanized in comparison to the wet southwest and central part of the country (D.S.A. Wijesundara and G.A.D. Perera, 2005-2013, pers. comm.).

On the other hand, Sri Lanka is recognized, together with the Western Ghats of India, as one of the 34 biodiversity hotspots in the world due to the remarkably high

biodiversity and present threats from human activities (Myers *et al.*, 2000). The close biotic affinities between Sri Lanka and South India have been recognized and explained by the close geographical connection in the historic past (Ashton and Gunatilleke, 1987; Pethiyagoda, 2005). However, there is also evidence for a distinct biotic component uniquely assembled on the island that may be overlooked in the context of biogeographic structure, if Western Ghats and Sri Lanka are treated as a single hotspot unit (Bossuyt *et al.*, 2004). Several studies usin molecular data on animal groups (Miththapala *et al.*, 1995; Fernando *et al.*, 2000; Meegaskumbura *et al.*, 2002; Bossuyt *et al.*, 2004) using molecular data recognized a unique assembly of taxa on the island with insular speciation, despite its close proximity to the Indian subcontinent (Pethiyagoda, 2005). Ashton and Gunatilleke (1987) also recognized the unique assembly of the angiosperm flora on the island, especially in the southwest lowland forests. Pethiyagoda (2005) indicated that this biotic isolation from mainland India is perhaps due to the aridification of the land bridge between the two countries during a relatively dry glacial maximum. However, this phenomenon has not been studied so far for the angiosperm flora. Moreover, the presence of a relict flora with Gondwanan affinity has been indicated by phylogenetic studies of the families Dipterocarpaceae (Dayanandan *et al.*, 1999), and Crypteroniaceae (Conti *et al.*, 2002). Further details of the biotic affinities of Sri Lankan angiosperm flora are discussed in Chapter 4.

1.1.2 Geography and Climate in Sri Lanka

Geography

Sri Lanka is a continental island in the Indian Ocean (Figure 1.1), located in the southeastern tip of the Indian Peninsula between latitudes 5^0 55'– 9^0 50' N and longitudes 79^0 40'–81^0 53' E (Ashton *et al.*, 1997). It is considered a medium sized island with about 65,500 square kilometres of land cover. Sri Lanka and India are separated by the shallow Palk Strait but share the same continental plate (Ashton and Gunatilleke, 1987). They also shared the same tectonic plate of southern Gondwanan origin which is collectively termed the "Deccan Plate" (Ashton and Gunatilleke, 1987).

The diverse topography of the country resulted from volcanism, uplift and erosion events during the drifting of Sri Lanka from the southern to the northern hemisphere. The island has four peneplains recognized, each defined by its altitude range

(Katupotha, 2013). The first peneplain is between 0-30m with no land-based biota, leaving three peneplains for the island land-based biota. The first peneplain,

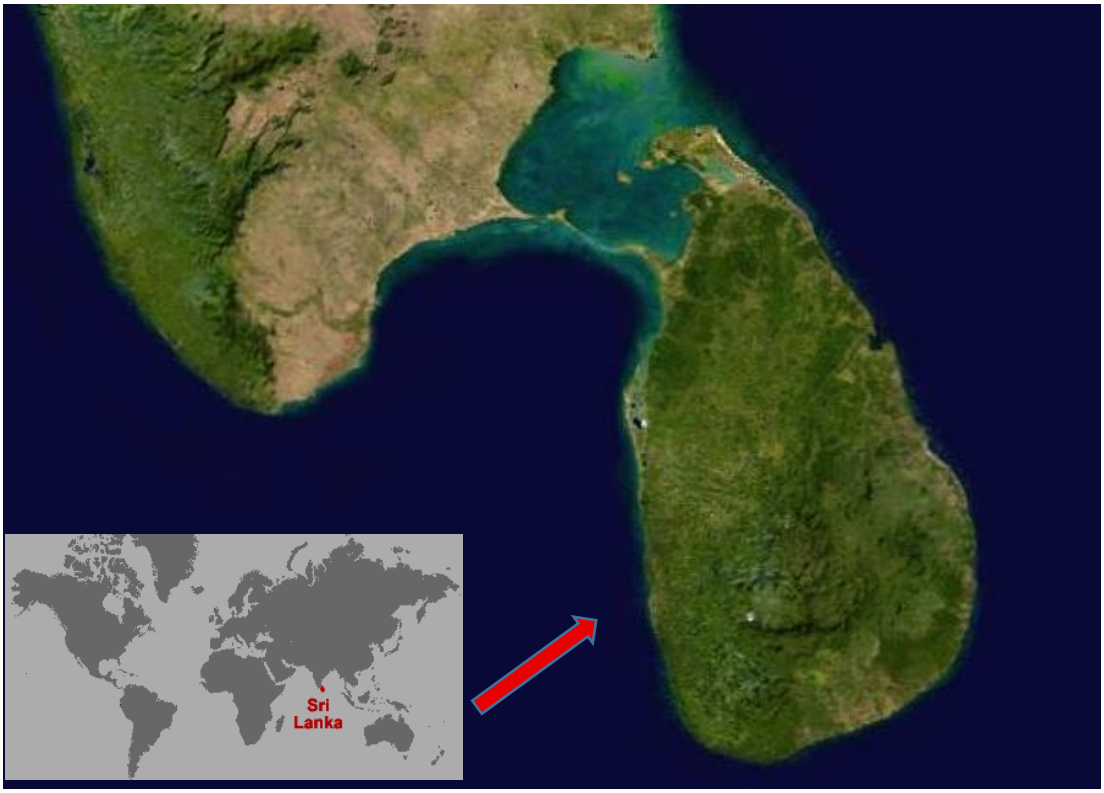


Figure 1.1 Sri Lanka, a continental island in the Southeast of the Indian subcontinent in the Indian Ocean

"lowland peneplain" encompasses about 2/3 of the island's landmass and reaches between 30-100 m in altitude above sea level. The distribution of lowland peneplain is wider in northern and eastern parts of the island and also characterized by many eroded remnant isolated hills and rock outcrops, occasionally as high as 600 m. The second peneplain, from about 100–500 m, is clearly recognizable on the northern and eastern parts, but is insignificant on the southern part of the country. The third peneplain, above 500-2524 m is characterized by mountain chains and deep valleys. Among them is the most prominent, roughly anchor-shaped central highland, which bears Sri Lanka's highest mountain, Pidurutalagala (2,524 m). There are few plateaux distinguishable in this peneplain such as the Horton plains (2,100 m) and the Hatton plateau (1,300 m).

Climate

Sri Lanka's near-equator position provides for a tropical climate with an average temperature range of 28–30 °C, and mean temperature variation from 16°C in the

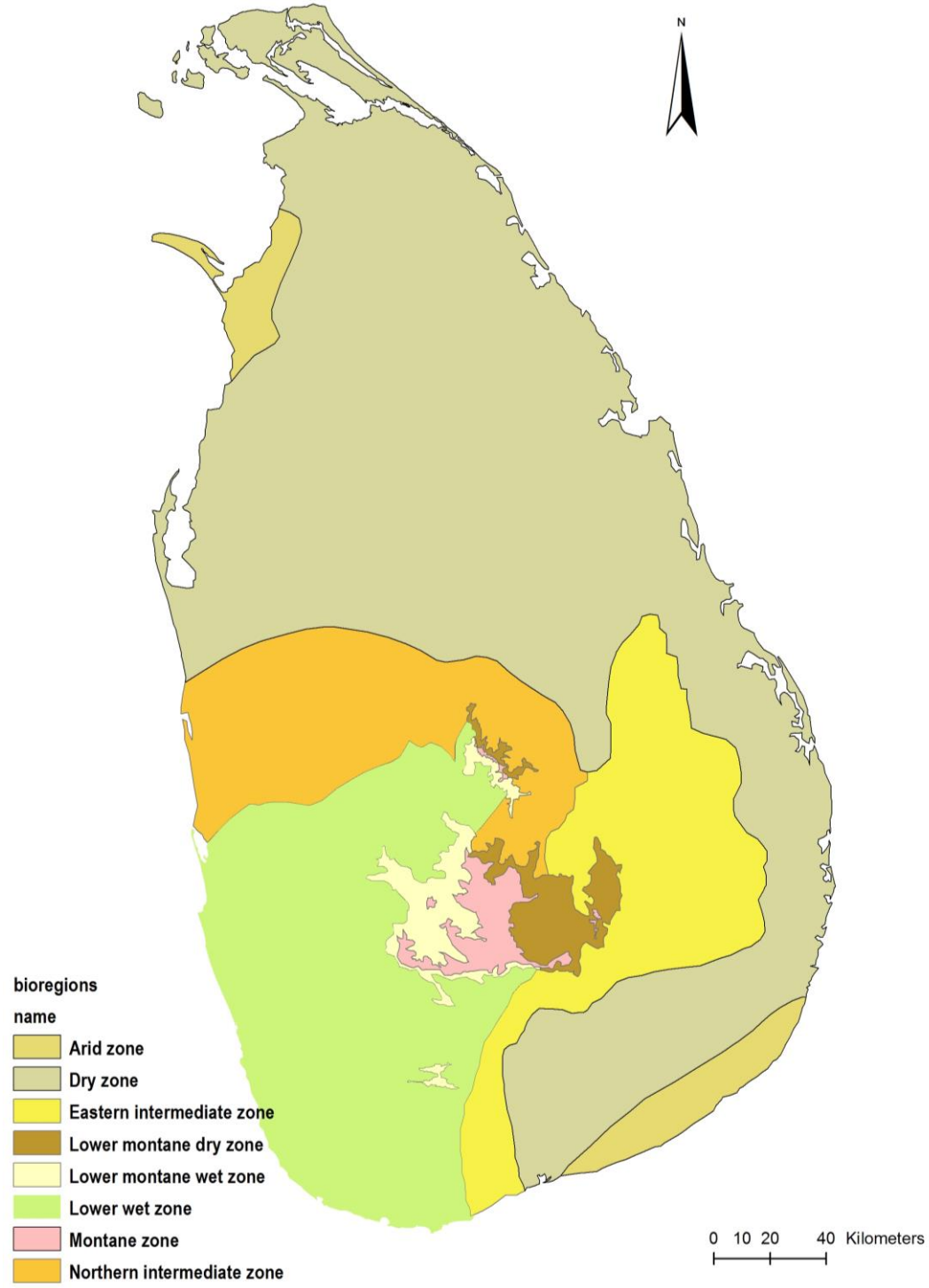


Figure 1.2 Bioclimatic Map of Sri Lanka.

central highland to 32°C (up to 38°C) for Trincomalee on the north-eastern coast. Temperature also plays an important role in the highland regions, for every 100 m

increase in elevation, the mean temperature falls by 0.5°C. During the months from December to March ground frosts occurs on the plateaus. However, in most parts of the country there is no significant temperature variation (Somasekaran, 1988).

According to Burt and Weerasinghe (2014), the mean annual rainfall varies from less than 1000 mm on the southeastern coast to over 4500 mm on the western slopes of the island. The variation of rainfall is mainly affected by monsoonal winds, which occur during two seasons of the year. From mid-May to September the southwestern monsoon brings moist air from the Indian Ocean and provides rainfall to the southwestern part and the Central Highland slopes. During December to February, the north-eastern monsoon brings moist air from the Bay of Bengal and causes rainfall across the whole country. The distinct inter-monsoonal periods receive conventional rains and cyclones. However, the two main monsoonal seasons resulted in the division of the country into two major climatic regions; the wet zone which receives rain from both monsoons, and the dry zone which receives rain from only one. The area between wet and dry zone is called intermediate zone. In addition, two small areas at the extreme northwest and southeast of the country have a very dry climate and are arid zones. The combinations of the varied climate, especially the rainfall influenced by the monsoon periods, and the diverse topography have created a variety of ecosystems that harbour a wide range of species. Wijesinghe *et al*. (1993) developed a map with bioclimatic zones of Sri Lanka based on climate, vegetation and faunal distribution (Figure 1.2). According to this map, mainly there are five main different climatic zones in Sri Lanka, namely the arid zone, dry zone, intermediate zone, wet zone and montane zone.

1.2 Background to the study

The present study is focused on plant family Gesneriaceae of Sri Lanka. According to the last revision of the flora, a total of thirteen Gesneriaceae taxa from six genera were recorded in Sri Lanka (Theobald and Grupe, 1981), of which ten taxa were considered endemic to the island.

1.2.1 Classification of the family Gesneriaceae

Gesneriaceae, the African violet family, is of tropical and subtropical distribution, and includes around 147 genera, and over 3400 species (Weber *et al.*, 2011, 2013; Möller *et al*., 2016). The family has been revised several times (Burtt and Wiehler,

1995; Weber, 2004), including the latest being an informal classification that recognized four major groups in the family: (1) coronantheroids, (2) gesnerioids, (3) epithematoids and (4) didymocarpoids (Weber, 2004). However, the first formal classification that was based on molecular phylogenetic studies was established recently and identified three subfamilies: Sanangoideae, Gesnerioideae (including the former coronantheroids and gesnerioids) and Didymocarpoideae (formerly Cyrtandroideae, including the former epithematoids and didymocarpoids) (Weber *et al*., 2013) (Figure 1.3).

According to this classification, subfamily Sanangoideae is represented by a single genus *Sanango* with a single species, recorded from the New World. It is sister to the other two subfamilies (Gesnerioideae + Didymocarpoideae). Subfamily Gesnerioideae with around 75 genera and over 1200 species, is almost exclusively distributed in the New World, but includes one species from the Old World: *Titanotrichum oldhamii* in the monospecific tribe Titanotricheae, with a distribution in the paleotropics (Southeast China, Taiwan, and South Japan). The third subfamily Didymocarpoideae with around 70 genera and around 2200 species, is almost exclusively Old World, with a distribution covering Asia, Malesia, Africa, Madagascar and Europe; however it includes one species which is recorded from Central America, *Rhynchoglossum azureum*. Because of these near-exclusive distribution patterns, the subfamily Didymocarpoideae has been traditionally recognized as the Old World Gesneriaceae and conversely subfamily Gesnerioideae has been termed the New World Gesneriaceae (Weber, 2004).

Weber et al.'s (2013) new classification recognized two lineages (tribes) in subfamily Didymocarpoideae, tribe Epithemateae (formerly epithematoids) and tribe Trichosporeae (formerly didymocarpoids) (Figure 1.3). The tribe Epithemateae was identified as a distinct clade as sister to tribe Trichosporeae and phylogenetic relationships within Epithemateae are well resolved (Mayer *et al.*, 2003; Möller *et al.*, 2009). The tribe includes four subtribes with seven genera and over 77 species. Trichosporeae was identified as the largest tribe (with 10 subtribes, with around 85 genera and over 2000 species). The tribe is phylogenetically divided into distinct grades and clades, but not all their relationships are fully resolved (Figure 1.3 from Weber *et al*., 2013). Four of the subtribes are monotypic or small and appear in basal

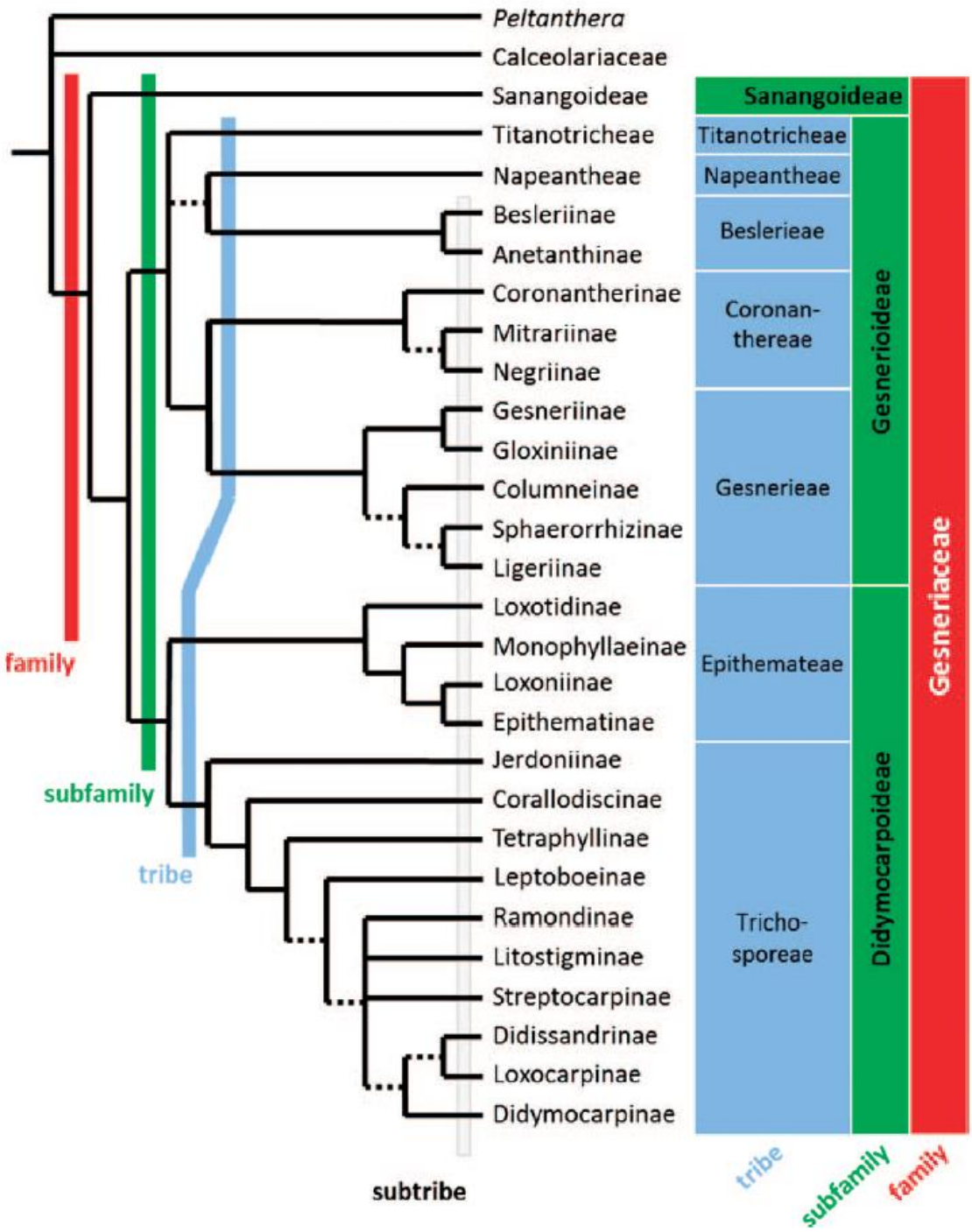


Figure 1.3 Diagrammatic representation of the formal classification of family Gesneriaceae (Weber *et al.*, 2013); Source Selbyana, 2013(31:2), p.75.

grades in the summary phylogeny. The subtribes Ramondinae (2-3 genera with five species), Litostigminae (one genus with 2 species) and Streptocarpinae (one genus with 176 species, Nishii *et al.*, 2015) appear on a polytomy, and the subtribes Didissandrinae (two genera with 10 species), Loxocarpinae (14 genera with over 200

species, Möller *et al.*, 2016) and Didymocarpinae (32 genera with 1660-1830 species, Möller *et al.*, 2016) (Weber *et al*., 2013).

Subtribe Didymocarpinae, has been identified as most difficult and complicated to resolve in the family phylogeny, difficulties of placing genera (*Allostigma* W.T.Wang, *Cathayanthe* Chun, *Conandron* Siebold and Zucc. and *Metapetrocosmea* W.T. Wang), and the existence of many mono-generic clades and several polyphyletic genera (Möller *et al*., 2009, 2011; Möller and Clark, 2013).

1.2.2 Gesneriaceae flora in Sri Lanka

In the most updated classification of family Gesneriaceae, Sri Lankan genera and species are distributed in both tribes, Epithemateae and Trichosporeae of subfamily Didymocarpoideae (Weber *et al.*, 2013). A summary of all the Sri Lankan taxa in the updated classification is given in Table 1.1.

A number of morphologically variable taxa were already encountered during initial field studies carried out in Sri Lanka especially in *H. humboldtiana* and *H. communis*. Therefore, it is necessary to conduct detailed studies of the species delineation and taxonomy of family Gesneriaceae in Sri Lanka.

According to Möller *et al*. (2009, 2011) and Weber *et al*. (2011), the Sri Lankan taxa are poorly represented in molecular phylogenetic studies of family Gesneriaceae. There are only two species from Sri Lanka (*Henckelia walkerae*, *H. floccosa*) included in these most recent phylogenies of the family. In species-level phylogenetic studies of the genus *Aeschynanthus* based on nuclear ITS (Internal transcribed spacer) only *A. ceylanicus* of Sri Lanka was included (Denduangboripant and Cronk, 2000, 2001).

Table 1.1 Summary of Sri Lankan Gesneriaceae taxa based on the updated Gesneriaceae classification (Weber et al., 2013). * genus placed in the subtribe based on morphological data.

Tribe	Sub Tribe	Genus	Species
Epithemateae	Loxotidinae	*Rhynchoglossum*	1. *Rhynchoglossum gardneri* W.L.Theob. and Grupe 2.*Rhynchoglossum notonianum* (Wall.) B.L.Burtt
	Epithematinae	*Epithema*	3. *Epithema ceylanicum* Gardner
Trichosporeae	Leptoboeinae	*Championia**	4. *Championia reticulata* Gardner

	Rhynchotechum	5. *Rhynchotechum permolle* (Nees) B.L.Burtt
Didymocarpinae	*Henckelia*	6. *Henckelia moonii* (Gardner) D.J.Middleton and Mich.Möller
		7. Henckelia walkerae (Gardner) D.J.Middleton and Mich.Möller
		8. *Henckelia communis* (Gardner) D.J.Middleton and Mich.Möller
		9. *Henckelia angusta* (C.B.Clarke) D.J.Middleton and Mich.Möller
		10. *Henckelia zeylanica* (R. Br.) A. Weber and B.L.Burtt
		11. *Henckelia floccosa* (Thwaites) A.Weber and B.L. Burtt
		12. *Henckelia humboldtiana* (Gardner) A.Weber and B.L.Burtt
	Aeschynanthus	13.*Aeschynanthus ceylanicus* Gardner

Considering the distribution of Gesneriaceae in Sri Lanka, most of the taxa have a rather restricted distribution, mostly in localities in undisturbed moist montane and lowland forests of the central and southwestern parts of the island. Only two species, *Henckelia humboldtiana* and *H. communis* show quite widespread distributions in both dry and wet climatic areas, as well as at low and high altitudes. The remaining *Henckelia* species are confined to wet forests at both low and high altitudes.

1.2.3 Current knowledge of the Biogeographic history of family Gesneriaceae

This section provides a brief overview of the current knowledge of the biogeography of family Gesneriaceae, with more details presented in Chapter 04.

The biogeographic history of the Gesneriaceae family has received relatively little attention since Burtt (1998) proposed a vicariance hypothesis for the origin of the family, and dispersal events throughout the tectonic history related to the Gondwana break up. Successive researchers focussed on the family's biogeographic history using different foci: Perret *et al*. (2013) focussed on the origin of New World

Gesneriaceae, while Petrova *et al*. (2015) focused on the origin of the European paleoendemic genus *Haberlea.* Most recently, Roalson and Roberts (2016) dated the phylogeny of family Gesneriaceae to understand th**e** diversification rates across the different clades. The former two studied only a part of the family in detail, whereas Roalson and Roberts (2016) used a 26 gene region molecular data matrix for the whole family but with missing data in the analysis. Therefore, these studies do not represent an even sampling across the distribution of family, nor do they offer comparable data sets to obtain a stable phylogenetic hypothesis of the family. However, the unpublished work of Luna-Castro (2016) combined comprehensive sampling from both New World and Old Word with data from four plastid DNA regions to study the global biogeography of Gesneriaceae, making it the most complete phylogenetic work to date. Despite this, it only included two species from South India and none from Sri Lanka, leaving the biogeographic affinities and role of the Sri Lankan Gesneriaceae unexamined. Therefore, incorporation of Sri Lankan taxa into Luna-Castro's phylogeny provides an effective means of determining the relationships of these taxa, and hence the biogeographic significance of Sri Lanka within this family.

Detailed studies of Gesneriaceae in Sri Lanka may provide more insights into the family itself. It may also reveal significant aspects of angiosperm diversification and evolution in Sri Lanka of regional and global importance. Therefore, looking at the picture of pantropically distributed family Gesneriaceae in Sri Lanka may clarify the different routes of floral assembly in the island.

1.3 Species concept for delimitation study

Species concepts or definitions are central to the recognition of species. There exist many species definitions, the oldest from ancient Greek authors, especially Plato, others from modern day scholars (Stuessy *et al.*, 2014). However, there are controversies over the use of different species concepts among contemporary scientists. The biological species concept (BSC) proposed by Ernst Mayr in 1942 was a turning point in this debate; it defined species as "a group of interbreeding natural populations that are reproductively isolated from other such groups" (Coyne and Orr, 2004).

1.3.1 Different aspects of speciation

Speciation, the process of origin of new and distinct species, is often linked with the species concepts (Stuessy *et al.*, 2014). There are four main evolutionary aspects recognized in the speciation process; mutation, natural selection, gene flow (migration) and genetic drift (de Quiroz, 2007). Mutations are changes in DNA sequences that may result in alterations in genes. However, most mutations are neutral (e.g. synonymous, or in noncoding regions), and have no effect on gene action (Page and Holmes, 1998). On the other hand, natural selection is a key process of evolution which alters gene frequency because alleles that confer greater fitness are passed on more frequently, leading to changes in allele frequency and eventually to adaptation (Page and Holmes, 1998). Further to that, genetic drift can be regarded as a change in frequency of an allele in a population due to random sampling at each generation. This may cause certain alleles to disappear subsequently reducing the genetic variation in a population (Page and Holmes, 1998). All three processes act to cause populations to gradually diverge from one another through progressive genetic change. However if there is migration of individuals or propagules between them, then this causes gene flow, which has a homogenising effect by increasing the proportion of alleles shared between those populations. Therefore, permanent cessation of gene flow between two populations is the starting point for speciation (de Queiroz, 2007).

1.4 Morphological and molecular data used in species delimitation.

In the process of speciation, diverging lineages or populations acquire and fix one to many different character states to form discrete entities that we recognize as species. Species may be recognized using two main diagnosable types of characters, such as those from morphology and molecular origin (Duminil and Michele, 2009).

1.4.1 Morphological data

Morphological data have usually been the primary source for identifying species in the field, especially in plant material collections for systematic studies. In fact, morphological data have traditionally been used in species delimitation, where species recognition is based on one or more qualitative or quantitative morphological

characteristics not overlapping with other closely related species (Wiens, 2007; Duminil and Michele, 2009). However, intraspecific variation, that is variation

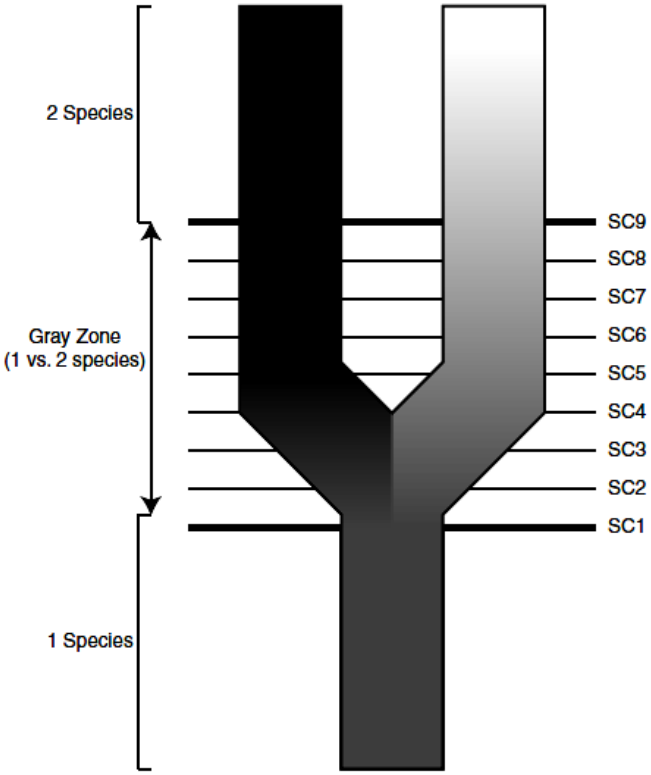


Figure 1.4 Diagram showing a single lineage (species) splitting to form two lineages (species). The gradation in the gray zone represents daughter lineages diverging through time, the horizontal lines labeled SC 1 to 9 represent the times at which they acquire different properties. Alternative species concepts come to conflict within the grey area where they acquire different properties as their species criteria (extracted from de Queiroz, 2007: 882).

that exists between different individuals in a species due to genetic variation or environmental influences, may complicate the problem of species delimitation. On the other hand, cryptic species are those that are morphologically very similar due to convergence, and/or genetic divergence without morphological divergence, and may be grouped into the same species despite the fact that they represent separately functioning entities (Duminil and Michele, 2009). Therefore, species delimitation entirely based on morphological taxonomy perhaps leads to incorrect estimations of biodiversity. Because of these problems, morphology is recognized as a complex and non-neutral marker even though its use is a simple practice (Page and Holmes, 1998). Also, species discriminated on morphological data often do not reflect their true

evolutionary relationships (Wiens, 2007; Figure 1.4). Therefore, it is problematic to use morphological data alone in phylogenetic species concepts.

1.4.2 Molecular data

With the development of molecular techniques and recognition of certain drawbacks of morphological data on species delimitation, DNA sequence data have become a popular and straightforward source of information to define species and understand relationships between species. The phylogenetic species concept based on molecular (DNA) characters has been widely employed to delimit species (Molina *et al*., 2004). Application of molecular data in the phylogenetic species concept enables us to select appropriate morphological characters for phylogenetic species (Mallet, 1995).

The relative ease of obtaining numerous characters from different loci, the level of resolution given by DNA data and the ability to distinguish divergence events even at the population level saw the rise of DNA data as a suitable method to delimit species, and even to determine the ages of species (Naciri and Linder, 2015). In a logical progression, the emergence of molecular data has brought species delimitation to an interesting crossroads where integration of diverse data types, commonly DNA sequence data and morphological data, are used to establish a more stable taxonomy (Fujita *et al.*, 2012).

Problems of molecular species delimitation

Developing a phylogeny at species level using DNA sequence data is often challenged by the most practical and common problem, which is the lack of clear phylogenetic signal, particularly considering closely related species (Naciri and Linder, 2015).

There are certain issues associated with the use of nuclear ITS data. A common problem is paralogy, where the gene locus is duplicated and occupies more than one position on non-homologous chromosomes in the genome and their divergent evolution can cause problems in PCR amplification and cloning is required (Denduangboripant *et al.*, 2007; Möller *et al*., 2008; Zhang *et al*., 2015). Cloned ITS copies, however, can lead to conflicting phylogenies due to the amplification of ancient copies or pseudogenes (e.g. Buckler *et al*., 1997; Xiao *et al*., 2010; Xiao and Möller, 2015). Nuclear ITS marker can also give incongruent signals between species due to concerted evolution (van Houten *et al*., 1993; Koch *et al*., 2003) where paralogous genes within one species are more closely related to each other than to

members of the same gene family in another species, despite the gene duplication event preceding the speciation event (Okuyama *et al.*, 2005). Several other issues encountered when using nuclear ITS data also recognized; characters are not necessarily independent from each other due to the secondary structure of ITS, which is sometime used to guide alignments, high levels of homoplasy due to the high rate of evolution when aligning distantly related taxa, and possible contamination with the use of universal primers (Alvarez and Wendel, 2003).

On the other hand, certain drawbacks exist with the use of plastid data. It is assumed that organelle genomes of angiosperms are maternally inherited; however, several studies have shown the existence of paternal and bi-parental inheritance in angiosperm taxa (Small *et al.*, 2004).

In plants which undergo hybridization, the use of chloroplast markers may fail to recognize true ancestry thus resulting in conflicting signals in the phylogeny. This can lead to sharing of a chloroplast haplotype among a set of closely related species even when a clear signal is shown in the ITS phylogeny, morphology or ploidy level, and/or the occurrence of distantly related haplotypes within the same species (e.g. Naciri and Linder, 2015). The former indicates the unequal rate of evolution of morphological and molecular markers with the cpDNA being slow, and both can result from hybridization events between closely related taxa (Hughes *et al*., 2005).

On the other hand, intra-population and intra-species polymorphism due to incomplete lineage sorting or hybridization events can cause non-monophyletic species and affect species resolution (e.g. Hughes *et al.*, 2005; Eckert and Carstens, 2008; Blanco-Pastor, *et al*., 2012; Smith *et al.*, 2015).

The problem is not only encountered in taxonomically complex groups (TCG) but also in less complex ones (Naciri and Linder, 2015). Here, the authors also recognized three key processes which consider under the present study as which can eventually blur the phylogenetic signal and obscure species delimitation of closely related species. These problems are as follows. (i) Intergenomic transfers (NuPt, NuMt) are the transfer of genetic material from chloroplast and mitochondrial to the nuclear genome of the same species. (ii) Hybridization, i.e. interbreeding between individuals of different species which, if hybrids are fertile, allows interspecific

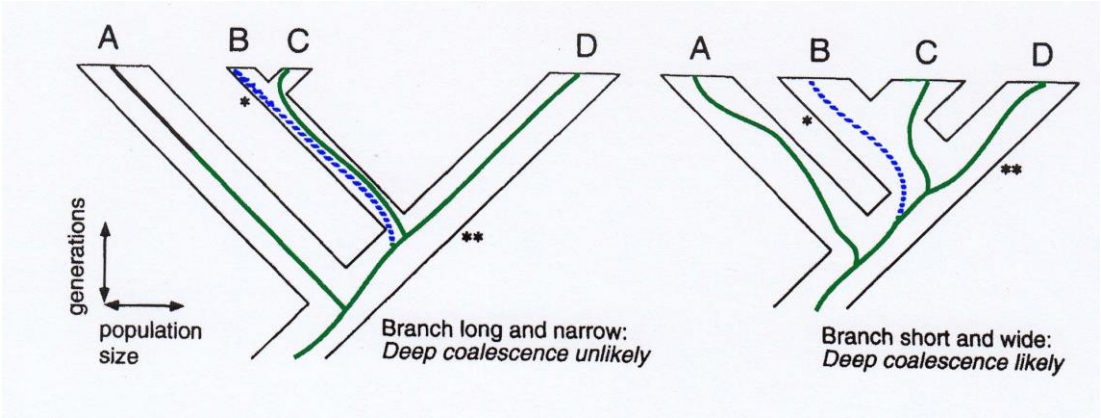


Figure 1.5 (a) lineage sorting (b) Incomplete lineage sorting; when the dashed and solid gene copies existed in the ancestral species shown in (**) neither were lost from the population by the time of the speciation event (*) then by chance only the dashed gene form might survive and be sampled in species B and only the solid form might be found in species C. Because the solid form was sampled from species D, the resulting gene tree would show the gene copies C and D (from species C and D, respectively) as most closely related (modified from Maddison, 1997: Fig. 4, pp 526).

transfer of genetic material resulting in reticulated relationships among closely related species (Doyle, 1992). (iii) Incomplete lineage sorting (Deep coalescence) refers to failure of gene copies to coalesce and homogenise, within the duration of the species (Figure 1.5). The coalescence time of genes and the speciation time is considered different to each other in this scenario. Incomplete lineage sorting occurs when a genetic polymorphism that predates a speciation event is inherited by one or both daughter species, leading eventually to the sharing of alleles between populations or species that might not be each other's' closest relatives. Incomplete lineage sorting is apparent when the different tree topologies of gene trees and species trees are encountered (Maddison, 1997) and can cause serious difficulties in recently diverged populations or species (Maddison and Knowles, 2006).

Therefore, it is necessary to take appropriate measures to overcome the potential problems discussed above in taxonomic delimitation when using both morphological and molecular data.

Therefore, considering all issues emphasized above, an intergrated and detailed study of the Sri Lankan Gesneriaceae is needed to resolve both local and global questions about the family. This study will have the specific objectives given below.

1.5 Objectives

1. Species delimitation of Sri Lankan Gesneriaceae taxa. This will be achieved by establishing regional phylogenies with population-level samples for all available species of six genera recorded in Sri Lanka (*Aeschynanthus*, *Championia*, *Epithema*, *Henckelia*, *Rhynchoglossum* and *Rhynchotechum*), using chloroplast *trn*L-F and nuclear ribosomal ITS (internal transcribed spacer) sequence data corroborated by morphological character differences.
2. Taxonomic revision of all Sri Lankan Gesneriaceae taxa with respect to the species identified in the molecular phylogeny, with integration of micro- and macro-morphological data.
3. Unravelling the biogeographical history of Gesneriaceae in Sri Lanka, by reconstructing a family-wide phylogeny of Gesneriaceae based on four chloroplast DNA regions with special focus on the Old World Gesneriaceae and including all Sri Lankan species, generating a dated phylogenetic tree and ancestral area reconstruction.

Chapter 2 Species Delimitation of Sri Lankan Gesneriaceae

2.1 Introduction

The intra specific diversity of Sri Lankan Gesneriaceae necessitated detail studies on species delimitation. The morphological taxonomy of the species is also complicated by the presence of putative hybrids. Hybridization events in family Gesneriaceae in Sri Lanka were also noted between *H. angusta* and *H. communis* by Theobald and Grupe (1972). Furter to that in the family Gesneriaceae, hybridization events have been recorded for example among species of *Oreocharis* from China (Puglisi *et al.*, 2011), and among *Streptocarpus* species in Africa (Hilliard and Burtt, 1971). The present study therefore, attempted to integrate both morphological and molecular data for understanding species limits and boundaries in the Sri Lankan Gesneriaceae flora.

Here we used the phylogenetic species concept considering the Hennig's concept of monophyly (de Queiroz and Donoghue, 1988) which is basically using molecular characters to define species as clades, corroborated by consistent morphological character differences to differentiate and describe species. In the phylogenetic species concept, a species is the smallest (exclusive) monophyletic group of common ancestry (Coyne and Orr, 2004).

2.2 Species delimitation approach in family Gesneriaceae

2.2.1 Species delimitation concept applied to Sri Lankan Gesneriaceae

As explained above, there are certain issues of species identification of Sri Lankan Gesneriaceae taxa especially in the genus *Henckelia*. Therefore, the present chapter is concerned with the delineation of taxonomic boundaries of the Gesneriaceae taxa from Sri Lanka. With an understanding of the different approaches and potential problems associated with taxonomic delimitation, especially at a lower taxonomic level among closely related taxa. The main focus here was to test the existing morphology-based taxonomic units (species) in the light of molecular phylogenetic hypotheses (trees), and make adjustments where necessary. Therefore, the phylogenetic species concept is tested initially with molecular data and then with

morphological characters. Species are then differentiated and described using morphological characteristics.

2.2.2 Genetic markers used for species delimitation in family Gesneriaceae

In previous phylogenetic studies on Old World Gesneriaceae, three genetic markers were widely used, the plastid *trn*L-F intron-spacer (*trn*L-F), the *atp*B-*rbc*L spacer (*atp*B-*rbc*L) and the ITS region of 18S-26S (Möller *et al.*, 2009, 2011; Weber *et al.*, 2011).

According to Appendix 2 of Möller and Clark (2013), ITS and *trn*L-F are the most extensively used gene regions for acquiring sequence data for different taxonomic levels in the entire family. Palee *et al*. (2006) stated that the nuclear ITS spacer sequences evolve five times faster than the 5.8S ribosomal gene which may need to be considered when using molecular evolutionary models. The *trn*L-F intron-spacer region was previously found suitable to resolve higher taxonomic level issues in Gesneriaceae such as generic relationships as well as infra- and intra-tribal relationships (Möller *et al.*, 1999, 2009, 2011; Mayer *et al.*, 2003) but can also discriminate at species and even population level as discussed in Chapter 1. The chloroplast *trn*L-F spacer regions from Gesneriaceae show twice the rate of evolution compared to the chloroplast *trn*L intron (Möller and Clark, 2013). On the other hand, the ITS spacer region is five times faster than the *trn*L-F region in Gesneriaceae (Möller *et al.*, 2009).

2.2.3 Sampling strategies in molecular species delimitation

There are examples from Gesneriaceae where molecular sequence data unambiguously establish new genera and species, demonstrating their relationships and allowing their description, for example the genera *Chautemsia* (Araújo *et al.*, 2010), *Shuaria* (Clark *et al.*, 2010), two *Litostigma* species, *L. coriaceifolium*Y.G. Wei, F. Wen and Mich. Möller and *L. crystallinum* Y.M. Shui and W.H. Chen (Wei *et al.*, 2010) and two *Chayamaritia* species, *C. smitinandii* (B.L. Burtt) D.J. Middleton and *C. banksiae* D.J. Middleton (Middleton *et al.*, 2015).

Usually the placement of new species or genera in Gesneriaceae, and in many other angiosperm plant families, is based on the use of a single accession. This can be misleading because from this approach, the genetic depth of the new species or genus

cannot be judged, and nor is it clear whether a continuum of variation might connect it to other existing taxa. It is therefore recommended that multiple individuals per species are included to give more insight into the species' history and relationships (Pennington and Lavin, 2016). The approach of using several individuals per species is recognized as a very effective approach to low taxonomic level studies (Duminil and Michele, 2009; Zhang *et al*., 2015; Pennington and Lavin, 2016; Ranasinghe *et al.*, 2016).

An example from Gesneriaceae involved the use of multiple individuals in *Haberlea* from Bulgaria to understand species delineation (Petrova *et al.*, 2014). Although it is not common, some studies used multiple samples for every taxon/species in taxonomic and phylogenetic studies in angiosperms, for example in genus *Ipomoea*, subgenus *Quamoclit* of family Convolvulaceae (Miller *et al*., 2004), a delimitation study of Asian Yews (*Taxus*) along the Hindu Kush-Himalaya and adjacent regions (Poudel *et al.*, 2012).

2.3 Methodology

2.3.1 Field Collections

Field sampling was conducted in Sri Lanka between May-August 2013, October-December 2014 and June-July 2015 with an attempt to collect all Gesneriaceae taxa at the population level from all of the six genera recorded in the country. The population sampling used only one leaf per plant from up to five individuals (where reasonable, i.e. considering population size) from each population and from 3-5 populations per taxon to represent their geographical distribution and possible intraspecific variation. Especially in the genus *Henckelia*, different populations were intentionally selected to reflect their observed population-level variation and to test species boundaries of the genus in Sri Lanka. Access to South Indian material was not possible in the duration of the project, though was highly desirable and necessary for some species. Location data of the field collections of Sri Lankan Gesneriaceae taxa are shown in bio-region maps in Chapter 3 (Figures 3.16–3.20).

2.3.2 Outgroup Taxa

The selection of outgroup taxa in the matrices was based on the recent phylogenetic studies (Mayer *et al.*, 2003; Möller *et al.*, 2009, 2011; Weber *et al.*, 2011) and the

family phylogeny prepared in Figure 4.9 in Chapter 4. Appendix 1 summarizes the details of outgroup and ingroup taxa used for the delimitation studies in this chapter.

Rhynchoglossum

According to the phylogeny of tribe Epithemateae, *Whytockia* and *Monophyllaea* are two sister group genera to *Rhynchoglossum* (Mayer *et al.*, 2003). For the genus *Rhynchoglossum* the ITS matrix used *M. horsfieldii*.

The *trn*L-F matrix used four *Monophyllaea* species, M. *elongata, M. hirticalyx, M. glauca* and *M. horsfieldii*. and further three *Whytockia* species, *W. purpurascens, W. sasakii* and *W. tsiangiana*.

Epithema

According to Mayer *et al*. (2003) and the phylogeny produced in Chapter 4, both *Stauranthera grandiflora* and *Loxonia hirsuta* are sister to the genus *Epithema*. However, ITS sequence data were available only for *S. grandiflora* which is used as the only outgroup taxon in the ITS matrix while both *S. grandiflora* and *L. hirsuta* were used in the *trn*L-F matrix.

The ITS sequences available for other Epithemateae genera, such as *Whytockia* and *Rhynchoglossum*, are too divergent and unalignable with *Epithema*. Already between *Epithema* and *Stauranthera*, its phylogenetically closest genus (Mayer *et al*., 2003), the sequence divergence was 28%, near the limit of alignability.

Championia

ITS and *trn*L-F matrices for *Championia* used three outgroup taxa, *Jerdonia indica*, *Corallodiscus conchifolius* and *Corallodiscus lanuginosus* (see Figure 4.9, Chapter 4).

Rynchotechum

The ITS matrix of *Rhynchotechum* included with five outgroup taxa from the genus *Boeica*, i.e. *B. filiformis, B. stolonifera, B. porosa, B. multinervia* and *B. ferruginea*.

The *trn*L-F matrix included with only three outgroup taxa from the genus *Boeica*, i.e. *B. porosa, B. multinervia* and *B. ferruginea*.

Henckelia

For *Henckelia*, there were 14 outgroup taxa taken from previous studies (Möller *et al.*, 2009, 2011; Weber *et al.*, 2011) for both ITS and *trn*L-F matrices. These comprised four *Microchirita* species: *M. viola, M. mollissima, M. sericea, M.*

calliginosa and ten *Codonoboea species: C. nana, C. elata, C. malayana, C. albomarginata, C. venusta, C. corrugata, C. leucocodon, C. codonion, C. floribunda and C. racemosa.*

Aeschynanthus

Both ITS and *trn*L-F matrices for *Aeschynanthus* used two outgroup taxa, specifically *Billolivia violacea* and *Ridleyandra porphyrantha* from the closest sister group genera.

2.3.3 Ingroup Taxa

Rhynchoglossum

The ITS phylogeny of *Rhynchoglossum* contains three ingroup taxa, *Rhynchoglossum notonianum, R. gardneri* and *R. obliquum* with nine samples. Eleven samples of these ingroup taxa represented in the *trn*L-F phylogeny for *Rhynchoglossum.*

Epithema

Both ITS and *trn*L-F phylogenies of *Epithema* contain nine taxa with thirteen samples from *Epithema ceylanicum, E. tenue, E. membranacea, E. saxatile, E. benthamii* and *E. taiwanensis* var. *fasciculata, E. taiwanense* and *E. benthamii.*

Championia

For *Championia*, the ITS and *trn*L-F phylogenies included five and two samples of *C. reticulata*, respectively.

Rhynchotechum

The ITS matrix of *Rhynchotechum* comprised ten samples from seven *Rhynchotechum* species: *R. permolle, R. discolor, R. brevipedunculatum, R. formosanum, R. vestitum, R. parviflorum and R. ellipticum*. The *trn*L-F matrix comprised five samples from five *Rhynchotechum* species, *R. permolle*, *R. discolor*, *R. parviflorum*, *R. formosanum* and *R. ellipticum*.

Henckelia

Both ITS and *trn*L-F matrices of *Henckelia* comprised eight species from previous studies, *H. urticifolia, H pumila, H. dielsii*, *H anachoreta*, *H. macrophylla*, *H. bifolia*, *H. incana* and *H. floccosa*. There are seven species of *Henckelia, H. angusta, H. communis, H. humboldtiana, H. moonii*, *H. walkerae*, *H.* sp.nov. and *H. zeylanica*

used in the present study with hundred and fourteen samples for each ITS and *trn*L-F matrices.

Aeschynanthus

There were nine samples from six *Aeschynanthus* species, *A. ceylanicus*, *A. roseoflorus*, *A. buxifolius*, *A. bracteatus*, *A. lancilimbus* and *A. micranthus* used in both ITS and *trn*L-F phylogenies produced here. Details of ingroup taxa are also included in the Appendix 1.

2.3.4 DNA Extraction, PCR and Sequencing

Leaf material, immediately silica gel-dried in the field, was ground up using a Tissuelyser II Mill Grinder (Qiagen, Hombrechtikon, Switzerland) for DNA extraction. Total genomic DNA was extracted following three methods: initially, a modified CTAB (cetyl trimethylammonium bromide) method (Doyle and Doyle, 1987) and Qiagen extractor (Crowley, UK) were used and for samples with poor sequence quality a Plant DNeasy kit (Qiagen) was used (Crowley, UK). DNA extractions were tested on 1% agarose gel at 80v for 40 minutes to check the quality and quantity of the extracted products.

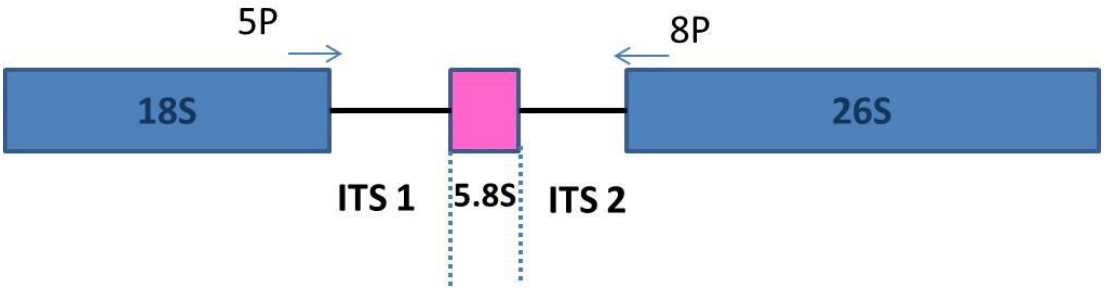


Figure 2.1 Map of the ribosomal DNA cluster of 18S, 5.8S and 26S showing the primer positions to amplify the complete ITS1, 5.8S and ITS2 region obtained in sequencing.

Sequences for ITS and *trn*L-F were generated. The complete ITS region contains two spacer regions (ITS1 located between 18S and 5.8S, and ITS2 located between 5.8S and 26S) and one ribosomal gene 5.8S rRNA with some portions of 18S and 26S gene regions where the primers are located (Figure 2.1). Both the ITS 1 and ITS 2 spacer regions are variable in length and variable in their nucleotide sequences among organisms. However, 5.8S is more conserved; it is about 164 base pairs (bp) in length (Yokota *et al.*, 1989). The complete ITS region was amplified using two primer pairs "ITS 5P" and "ITS 8P" for all the samples used in this study (Möller

and Cronk 1997), which are plant-specific primers modified from the fungal-specific primers of White et al. (1990).

The 25µl PCR amplification mix for ITS contained 2.5µl 2mM dNTPs, 2.5µl 10xNH_4 buffer, 1.25µl 25mM $MgCl_2$, 0.75µl each 10µM forward and reverse primer, 4µl TBT-PAR, 0.2µl Biotaq polymerase (Bioline), 1µl DNA template and 12.05µl dH_2O. TBT-PAR (Samarakoon *et al.*, 2013), a PCR additive, contains trehalose, bovine serum albumin (BSA) and polysorbate-20 (Tween-20) improving the successful PCR amplification of the ITS region for all the samples sequenced in the present study. The thermocycle used for PCR amplification of the ITS region was: 94˚C for 3 minutes, 30 cycles of 94˚C for 1 minute, 55˚C for 1 minute, 72˚C for 1.5 minutes, finishing with one cycle of 72˚C for 10 minutes, and terminated with 10˚C forever.

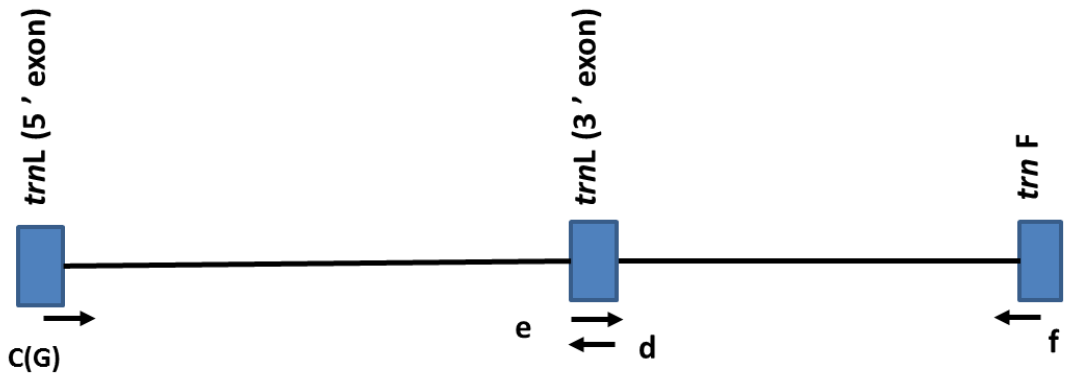


Figure 2.2 Map of the *trn*L-F region showing the positions and directions of extension of the primers used to amplify the two non-coding regions of plastid DNA located within trnL and between trnL and trnF.

The *trn*L-F region contains the plastid tRNA transferase gene *trn*L in which an approximately 550 bp long intron is inserted, and the tRNA transferase gene *trn*F separated by an approximately 550 bp long intergenic spacer from *trn*L (Fig. 2.2). Initially, the *trn*L-F region was amplified using primers "c" and "f" located in *trn*L and *trn*F respectively (Taberlet *et al.*, 1991). Although there was one clear band visible on the agarose gel from PCR products, the sequences generated created often messy electropherograms with multiple peaks of different heights. Different combinations of PCR mixtures and programs were tested, but it was very difficult to obtain usable sequences using the standard primers "c" and "f". Therefore, five more

primers were used: "a" is located in *trn*T ca. 550 bp upstream of *trn*L, "*ndh*JR" was located approximately 600 bp upstream of *trn*F (Shaw *et al.*, 2007), and internal primers "d" and "e" were in the *trn*L 3'exon (Taberlet *et al.*, 1991; Table 2.1). A new Gesneriaceae-specific forward primer "CG" was developed by Drs Michael Möller and Kanae Nishii at the Royal Botanic Garden Edinburgh and used instead of primer "c" for *trn*L-F.

The recipe for the PCR amplification mix for *trn*L-F contained 2.5µl 2mM dNTPs, 2.5µl 10xNH_4 buffer, 1.25µl 25mM $MgCl_2$, 0.75µl each 10µM forward and reverse primer, 4µl TBT-Par/5xCES, 0.2µl Biotaq polymerase (Bioline), 1µl DNA template and 12.05µl dH_2O to make up the total to 25µl. Either TBT-PAR (Samarakoon, *et al*., 2013) or 5xCES, a Combinatorial Enhancer Solution (Ralser *et al.*, 2006) which contains betaine, dithiothreitol, and dimethyl sulfoxide, were used as additives for improved PCR amplification of this region. The thermocycle used for the PCR amplification of the *trn*L-F region was: 94˚C for 4 minutes, followed by 30 cycles of 94˚C for 45 seconds, 55˚C for 45 seconds, 72˚C for 1.5 minutes, finishing with one cycle of 72˚C for 10 minutes, and terminated with 10˚C forever.

Both regions were amplified with a Biorad Tetrad DNA Engine PCR machine (Biorad, California, USA). All the primers used for PCR amplification and sequencing are listed in Table2.1.

Table 2.1 Sequences of the primers for amplification of nuclear ITS and plastid *trn*L-F regions.

No	Marker	Primer Name / direction	5' to 3' sequence	Reference
1	Nuclear ITS	5P forward	GGA AGG AGA AGT CGT AAC AAG G	Möller and Cronk 1997
2	Nuclear ITS	8P reverse	CAC GCT TCT CCA GAC TAC A	Möller and Cronk 1997
3	Plastid *trn*L-F	c forward	CGA AAT CGG TAG ACG CTA CG	Taberlet *et al*. 1991
4	Plastid *trn*L-F	CG forward	GTG AAG ACT TCT AAA TTC AGA GAA AC	Unpublished (Michael Möller and Kanae Nishii)
5	Plastid *trn*L-F	d reverse	GGG GAT AGA GGG ACT TGA AC	Taberlet *et al*. 1991
6	Plastid *trn*L-F	e forward	GGT TCA AGT CCC TCT ATC CC	Taberlet *et al*. 1991
7	Plastid *trn*L-F	f reverse	ATT TGA ACT GGT GAC ACG AG	Taberlet *et al*. 1991

The PCR products were tested on 1% agarose gels with 1Kb plus ladder (Thermo Fisher Scientific, Waltham, USA) for successful PCR amplification. The gels were run at 80V for 40 minutes. Samples which produced a clear single band were subsequently cleaned up using ExoSAP IT (USB Corporation, Ohio, USA). For this, a reaction mix of 5µl PCR product and 2µl ExoSAP IT was incubated at 37˚C for 15 minutes followed by heating at 80˚C for 15 mins in a thermocycler. For DNA sequencing, two reactions were prepared for each sample, one for the forward primer and the second for the reverse primer to obtain overlapping sequences for nucleotide confirmation. The 10µl sequencing reaction mix contained, 2µl 5x sequencing buffer, 0.5µl Bigdye mix (Applied Biosystems, Waltham, MA, USA), 0.32µl 10mM primer, 4.5µl dH_2O, 0.8-1.0µl template DNA. The cycle sequencing profile used was 95˚C for 30 seconds, and 25 cycles of 50˚C for 20 seconds, 60˚C for 4 minutes, and terminated by 4˚C forever.

There was a great difficulty of obtaining *trn*L-F sequences for two species, *Championia reticulata* and *Rhynchotechum permolle*. Irrespective of whether standard primers (‘c’ and ‘f’) were used, the regions amplified separately (‘c’ and ‘d’, ‘e’ and ‘f’), or further upstream located primers (‘a’) used in different combinations (‘a’ and ‘f’, ‘a’ and ‘d’) the electropherograms showed strongly polymorphic reads. Only the newly designed Gesneriaceae-specific primer ‘CG’ gave better results but not for all samples.

All sequences were edited using Sequencher version 5.1 (Gene Codes Corporation, 2012). These sequences were aligned using the Clustal W multiple alignment module in BioEdit version 7.1.11 (Hall, 1999) and the alignments manually adjusted in Mesquite version 2.75 (Maddison and Maddison, 2011).

2.3.5 Calculating Genetic distance

Genetic distances were calculated based on three criteria for the DNA sequence matrices, these were number of indels (insertions or deletions), nucleotide changes and uncorrected P-distance. The number of nucleotide changes and the P-distance among taxa was calculated using the DISTANCE MATRIX option in PAUP* beta version 4.0a146 for Microsoft Windows (Swofford, 2002). Indels were counted by eye for small matrices (both ITS and *trn*L-F) (i.e. *Aeschynanthus*, *Championia*,

Epithema, *Rhynchoglossum* and *Rhynchotechum*). For the determination of indels in *Henckelia* (both ITS and *trn*L-F) the programme SeqState (Müller, 2005) was used. These three measures of genetic distance data for Sri Lankan taxa (with multiple individuals) were calculated and presented here (Table 2.2 and 2.3) for within populations, between populations, within species and with respect to the closest sister group taxa/taxon found on the local phylogenetic trees (Figures 2.3 – 2.9).

2.3.6 Phylogenetic Analysis

Separately aligned matrices of ITS and *trn*L-F for each genus were analyzed by maximum parsimony (MP) implemented in PAUP* beta Version 4.0a146 for Microsoft Windows (Swofford, 2002). All characters were used unordered, with equal weight and gaps (indels) were treated as missing data. The 'Maxtrees' setting was 100, and automatically increased by 100, and no topological constraints were enforced. Different search strategies were implemented in PAUP*, due to different number of sequences assembled in the various matrices.

For *Championia* (ITS/*trn*L-F), *Epithema* ITS, *Rhynchoglossum* ITS, and *Rhynchotechum trn*L-F, parsimony trees were obtained with exhaustive searches. For *Epithema trn*L-F, *Rhynchoglossum trn*L-F, and *Rhynchotechum* ITS, parsimony trees were obtained with Branch-and-Bound searches with the ‘MulTrees’ option in effect. Statistical branch support was performed as bootstraps (BS; Felsenstein, 1985) with 10,000 heuristic bootstrap replicates of random stepwise addition with ‘TBR branch swapping’ algorithm and ‘MulTrees’ option in effect. For the analyses above, the setting for ‘Branches collapsed (creating polytomies) if minimum branch length is zero’ was chosen because the factory ‘maximum’ setting caused trees with identical topologies to be saved.

For both ITS and *trn*L-F matrices of *Henckelia*, parsimony trees were obtained in a two steps process. The first step was a heuristic search, performed via stepwise random addition sequence with 10,000 replicates, no branch swapping, ‘MulTrees’ and ‘Steepest Descent’ options not in effect, and no topological constraints in effect. In the second step, tree optimization of the trees saved in the first step was achieved with ‘TBR branch swapping’, ‘MulTrees’ and ‘Steepest Descent’ options in effect. Statistical branch support was evaluated using bootstraps (Felsentein, 1985) of

10,000 heuristic bootstrap replicates of random stepwise addition with 'TBR branch swapping' algorithm but no 'MulTrees' option in effect.

Due to a bug in PAUP, suboptimal trees are saved, so for all analyses where more than one tree was obtained, they were filtered to retain only the shortest trees.

The amount of parsimony signal for all analyses was given by the consistency index (CI) (Kluge and Farris, 1969), retention index (RI) and rescaled consistency index (RC) (Farris, 1989).

2.4 Results

2.4.1 Molecular species delimitation of genera of Gesneriaceae in Sri Lanka

Summary of characteristics of DNA matrices and genetic distances are iven in Table 2.2 and 2.3. The generic order followed the classification of the most updated family phylogeny of Weber *et al*. (2013).

Rhynchoglossum

According to Weber (2004) the genus *Rhynchoglossum* is distributed principally in the palaeotropics, except for *R. azureum* from the neotropics. On the other hand *R. notonianum* is recorded from South India and Sri Lanka while *R. gardneri* is endemic to Sri Lanka (Theobald and Grupe, 1981). *R. obliquum* is distributed from North and South India, China and South East Asia (Skog and Boggan, 2016 onw.).

The sequences of the ITS matrix of genus *Rhynchoglossum* are very variable with high number of nucleotide changes. Therefore, the ITS sequences of closest out group taxa (Weber *et al*., 2013) of the genus *Rhynchoglossum* are unalignable. Therefore, there was only one outgroup taxon, *Monophyllaea horsfieldii* in the ITS phylogeny of the genus (Figure 2.3a). The ITS matrix also contained three ingroup taxa, *Rhynchoglossum obliquum*, *R. notonianum* and *R. gardneri* of which the latter two Sri Lankan taxa were represented by seven samples from five populations.

Total aligned ITS matrix for *Rhynchoglossum* was 648 characters long of which 357 were constant. An additional 143 (22%) characters were variable and parsimony-informative, while another 148 variable characters were parsimony-uninformative. The phylogenetic analysis produced a single most parsimonious tree (Figure 2.5a) of 350 steps length and a consistency index (CI) = 0.9686, retention index (RI) = 0.9619 and rescaled consistency index (RC) = 0.9317. Three clades, *R. obliquum*, *R.*

Table 2.2 Genetic variation of Sri Lankan Gesneriaceae taxa within populations, between populations, between species of Sri Lanka (SL) and to sister species in local phylogenetic trees, for the ITS analysis.

Parameter	***Rhynchoglossum***	***Epithema***	***Championia***	***Rhynchotechum***	***Henckelia***	***Aeschynanthus***
No. of outgroup taxa (No. of samples)	1 (1)	1	3	5 (5)	14	2
No. of ingroup taxa (No. of populations from SL / No. of samples)	3 (5 / 7)	1 (2 / 3)	1 (3 / 5)	7 (3 / 3)	7 (29 / 114)	9 (3 / 4)
Total aligned matrix length (bp)	648	678	679	657	760	686
Within populations of species in SL						
Indels	0	0	0	NA	0-1	0
Nucleotide changes	0	0	0	NA	0-2	0
P-distance	0	0	0	NA	0.00-0.00309	0
Between populations of species in SL						
Indels	0	0	2	0	0-5	0
Nucleotide changes	0-1	0	20-21	0	0-30	0
P-distance	0-0.00165	0	0.03146-0.03298	0	0.00-0.04796	0
To sister group/s						
Indels	13*	NA	21-35	6	19-27**	8
Nucleotide changes	121-137*	NA	136-156	8-12	71-115**	35
P-distance	0.20055-0.22945*	NA	0.22734-0.25581	0.01256-0.01898	0.11932-0.1842**	0.05443
Between species in SL						
Indels	5	–	–	–	0-24	–
Nucleotide changes	45-46	–	–	–	0-111	–
P-distance	0.07509-0.07682	–	–	–	0.00-0.1764	–

Table 2.3 Genetic variation of Sri Lankan Gesneriaceae taxa within populations, between populations, between species of Sri Lanka (SL) and to sister species in local phylogenetic trees, for the *trn*L-F analysis.

Parameter	***Rhynchoglossum***	***Epithema***	***Championia***	***Rhynchotechum***	***Henckelia***	***Aeschynanthus***
No. of outgroup taxa (No. of samples)	7 (7)	2	3	3 (3)	14	2
No. of ingroup taxa (No. of populations from SL / No. of samples)	4 (6 / 7)	7 (3 / 4)	1 (2 / 2)	5 (5)	7 (29 / 114)	9 (3 / 4)
Total aligned matrix length (bp)	938	854	909	860	880	843
Within populations of species in SL						
Indels	0	0	NA	NA	0-1	0
Nucleotide changes	0	0	NA	NA	0-3	0
P-distance	0	0	NA	NA	0.00-0.00378	0
Between populations						
Indels	0	0	0	NA	0-2	0
Nucleotide changes	0	1-3	1	NA	0-4	0
P-distance	0	0.00130-0.00390	0.00124	NA	0.00-0.00503	0
To closest sister group						
Indels	4	2	10-16	1	0-6	4
Nucleotide changes	6-7	1-6	28-54	2-9	10-24	8-14
P-distance	0.02133-0.02379	0.00130-0.00781	0.03570-0.06736	0.00257-0.01144	0.01209-0.0346	0.00989-0.01725
Between species in SL						
Indels	4	–	–	–	0-5	–
Nucleotide changes	11	–	–	–	0-24	–
P-distance	0.01315	–	–	–	0.00-0.03048	–

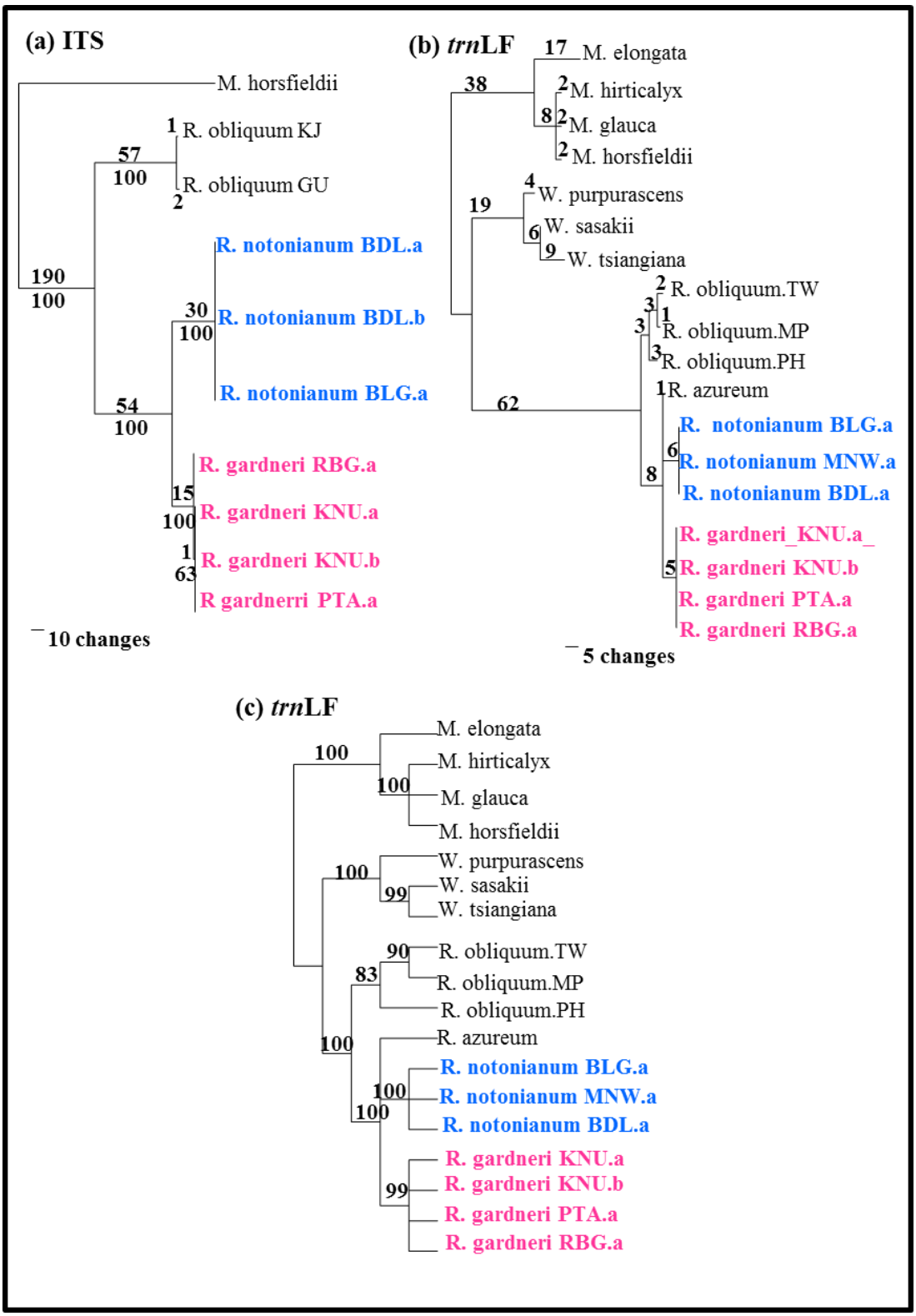


Figure 2.3 ***Rhynchoglossum***: (a) Phylogram of the single most parsimonious tree from the ITS MP analysis, (b) a single phylogram, and (c) the strict consensus tree of six most parsimonious trees based on the *trn*L-F MP analysis. Number of nucleotide changes above the branches of the phylograms, and bootstrap values below the branches of the phylogram and above the branches of the strict consensus tree. ***Rhynchoglossum*** (R), ***Monophyllaea*** (M) and ***Whytockia*** (W). Sri Lankan species are in coloured fonts.

notonianum and *R. gardneri* were highly supported (BS = 100%). A subclade within the *R. gardneri* clade was poorly supported (BS = 63%). The two Sri Lankan clades of *R. notonianum* and *R. gardneri* were sister with high support (BS = 100%). Here ITS showed no sequence variation within populations of *R. notonianum* and *R. gardneri*. One nucleotide change (P-distance = 0.00165) was found between populations of *R. gardneri*. The genetic P-distance between the two species was 0.07509-0.07682 and 5 indels. The genetic distance between two Sri Lankan species and *R. obliquum* was much higher (P-distance = 0.20055-0.22945, and 13 indels) (Table 2.2).

The *trn*L-F matrix for *Rhynchoglossum* contained seven outgroup taxa and four ingroup taxa, the three species examined for ITS, plus *R. azureum* with seven samples from six populations. There were 938 characters in the matrix of which 760 characters were constant. 143 (15.2%) variable characters were parsimony-informative, while 35 variable characters were parsimony-uninformative. The phylogenetic analysis produced six most parsimonious trees of 201 steps with a consistency index (CI) = 0.9801, retention index (RI) = 0.9932 and rescaled consistency index (RC) = 0.9734 (Figure 2.3b and c). Both, *R. notonianum* and *R. gardneri* samples from Sri Lanka, formed highly supported (BS = 100%) species-specific clades, and fell together with *R. azureum* on a polytomy. *Rhynchoglossum obliquum* clade also supported in the analysis (BS = 83%). *trn*L-F showed no genetic divergence within populations of *Rhynchoglossum* as well as between populations in Sri Lanka. However, there was a considerable genetic distance between the two species from Sri Lanka was P-distance = 0.01315 and 11 indels (Table 2.3). Furthermore, the genetic distance of two species from Sri Lanka to the closest sister taxon, *R. azureum* showed with P-distance = 0.00711-0.00838 and 4 indels and to *R. obliquum* P-distance = 0.02133-0.02379 and 6-7 indels.

Epithema

Epithema taiwanense and *E. taiwanense* var. *fasciculatum* which were synonymized under *E. ceylanicum* (Bransgrove and Middleton, 2015) were considered as different taxa in the phylogenetic *trn*L-F matrix here. Because the new concept of *E. ceylanicum* is based on a morphological revision it is more appropriate to test this under molecular aspects since molecular data give more direct insight to the

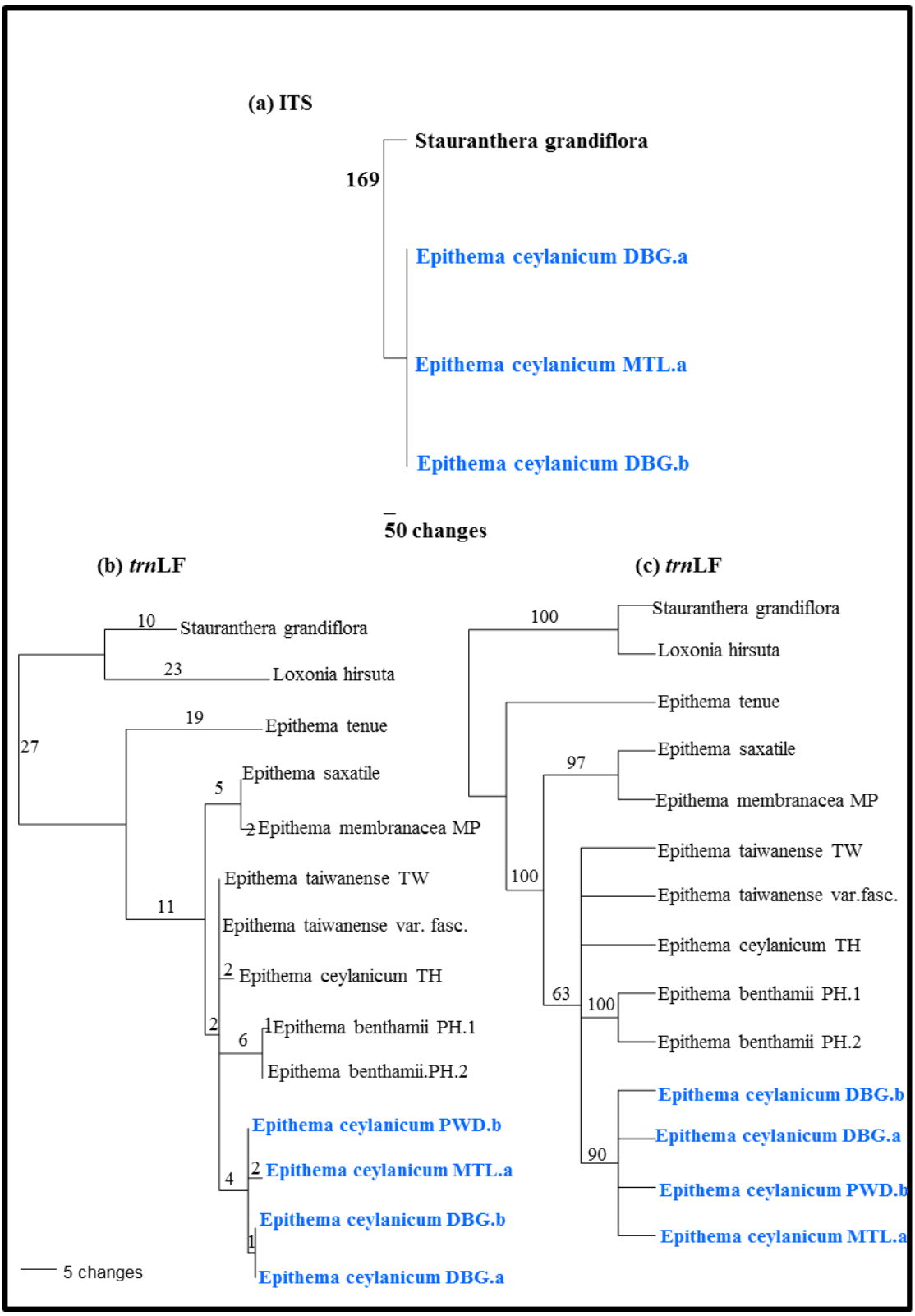


Figure 2.4 *Epithema*: (a) A phylogram of the single most parsimonious tree based on the ITS MP analysis, (b) a single phylogram and (c) the strict consensus tree of three most parsimonious trees from the *trn*L-F MP analysis. Number of nucleotide change on the phylogram and bootstrap values on the strict consensus tree are shown. Sri Lankan species are in coloured fonts.

phylogenetic relationships and speciation.

The ITS matrix of *Epithema* contained only one outgroup taxon, *Stauranthera grandiflora* due to the sequence alignment issues as discussed under the genus *Rhynchoglossum*. There is only one ingroup taxon *E. ceylanicum* with three individual samples from three populations (Figure 2.4a). ITS sequence data were not available for *Epithema* species other than *E. ceylanicum* from Sri Lanka. For ITS, the aligned matrix was 678 base pairs long that included 509 constant characters.

Because of the identical ingroup sequences and only one outgroup sample, no parsimony-informative characters were present, while 169 characters were variable. While no genetic variation was found between the populations of *Epithema*, the observed P-distance to the outgroup was 0.27892 and 12 indels (Table 2.2).

The *trn*L-F matrix for *Epithema*, contained two outgroup taxa, *Stauranthera grandiflora* and *Loxonia hirsuta*, and seven *Epithema* species with 12 samples (Figure 2.4b and c). Here, *Epithema ceylanicum* from Sri Lanka was included with four individual samples from three populations. The aligned matrix was 854 bp long of which 747 were constant. There were 51 (5.9%) variable parsimony-informative characters while 56 variable characters were parsimony-uninformative. The phylogenetic analysis produced three most parsimonious trees of 115 steps with a consistency index (CI) = 0.9652, retention index (RI) = 0.9481 and rescaled consistency index (RC) = 0.9151 (Table 2.3). The bootstrap values ranged from 57%-100% in the local matrix for *trn*L-F marker. A low support (BS = 62%) was found for the clade including *E. ceylanicum* in its wide sense as defined by Bransgrove and Middleton (2015) who united *E. taiwanense, E. taiwanense* var. *fasciculatum* with *E. ceylanicum*. The clade also included *E. benthamii*, a morphologically distinct taxon, though on a polytomy with *E. ceylanicum sens. lat*. However, all Sri Lankan samples of *E. ceylanicum* fell in a strongly supported (BS = 90 %) clade with respect to the rest of the *Epithema* species sampled here. Similar to the ITS results, there was no genetic difference within the population of *E. ceylanicum* in Sri Lanka, but between populations the genetic P-distance was 0.00130-0.00390 but with no indels. The genetic P-distance between the sister species of *E. ceylanicum* sens.str., was 0.00130-0.00781 and 2 indels (Table 2.3).

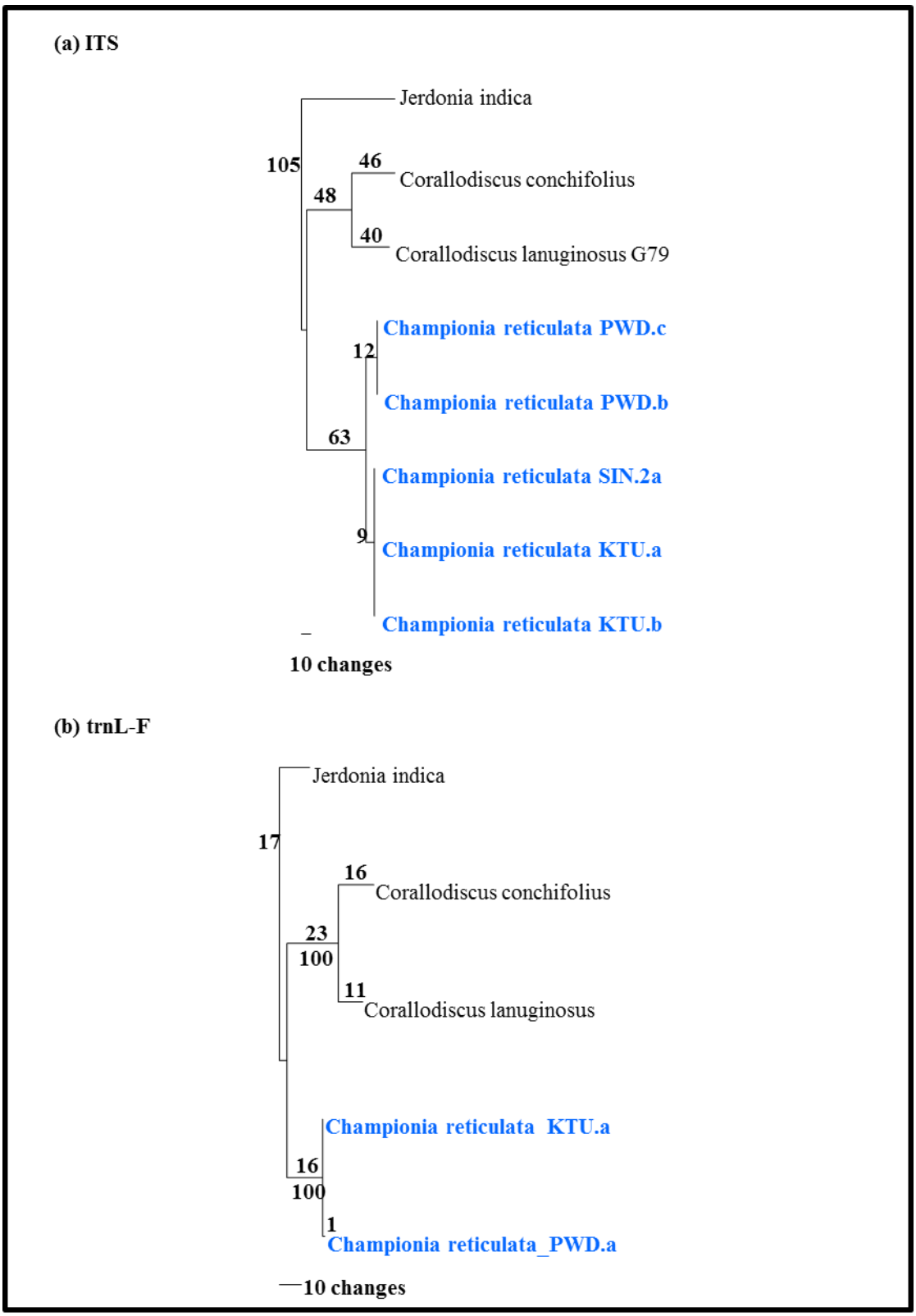


Figure 2.5 *Championia*: (a) A phylogram of the single most parsimonious tree based on the ITS MP analysis, (b) a phylogram of the single most parsimonious tree of the *trn*L-F MP analysis. Number of nucleotide changes above the branches and bootstrap value below the branches. Sri Lankan species in coloured fonts.

Championia

The ITS matrix contained three outgroup taxa and one ingroup taxon *C. reticulata* with five individuals from three populations (Figure 2.5a). The aligned ITS matrix was 679 bp long and included 433 constant characters. There were 122 (17.9%) parsimony-informative characters, while 126 characters were variable but parsimony-uninformative. The phylogenetic analysis for this ITS matrix produced a single most parsimonious tree of 323 steps with a consistency index (CI) = 0.9288, retention index (RI) = 0.8701 and rescaled consistency index (RC) = 0.8081 (Table 2.2). The bootstrap value of the sister clade relationships of *Corallodiscus* and *Championia* was 98%. A highly supported (BS = 100%) clade of the *C. reticulata* samples was recognized, comprising two highly supported subclades (BS = 100%). Two individuals from montane forests in Peak Wilderness (PWD) fell into one of these subclades, while the two individuals from Kalutara (KTU) and the one individual from Sinaharaja (SIN) representing lowland rainforest localities formed the second subclade. There was no genetic variation for ITS found to exist within the populations of two subclades of *C. reticulata.* However, the genetic distance between two subclades is considerable with P-distance = 0.03146-0.03298, nucleotide changes = 20–21, and two indels. Furthermore, there was a P-distance range of 0.22734-0.25581 and 21-35 indels observed between *C. reticulata* and *Corallodiscus* (*C. lanuginosus* + *C. conchifolius).*

The *trn*L-F matrix contained three outgroup taxa and one ingroup taxon *C. reticulata* with only two individuals from two populations (Figure 2.5b). This was due to the great difficulties in obtaining clean sequences as discussed under methods.

There were 909 included characters of which 827 were constant, 36 (3.9%) were parsimony-informative, and 46 were parsimony uninformative (Table 2.3). The phylogenetic analysis for *trn*L-F produced a single most parsimonious tree of 84 steps with a consistency index (CI) = 1.0000, retention index (RI) = 1.0000 and rescaled consistency index (RC) = 1.0000. The bootstrap values for both clades, *Corallodiscus* and *Championia*, were 100%. The genetic distance between two subclades of *Championia* for *trn*L-F shows in P-distance = 0.00124 and a single nucleotide change. The genetic distance to its closest sister group, *Corallodiscus* shows in P-distance = 0.03570-0.06736, nucleotide changes = 28–54 and 10-16 indels (Table 2.3).

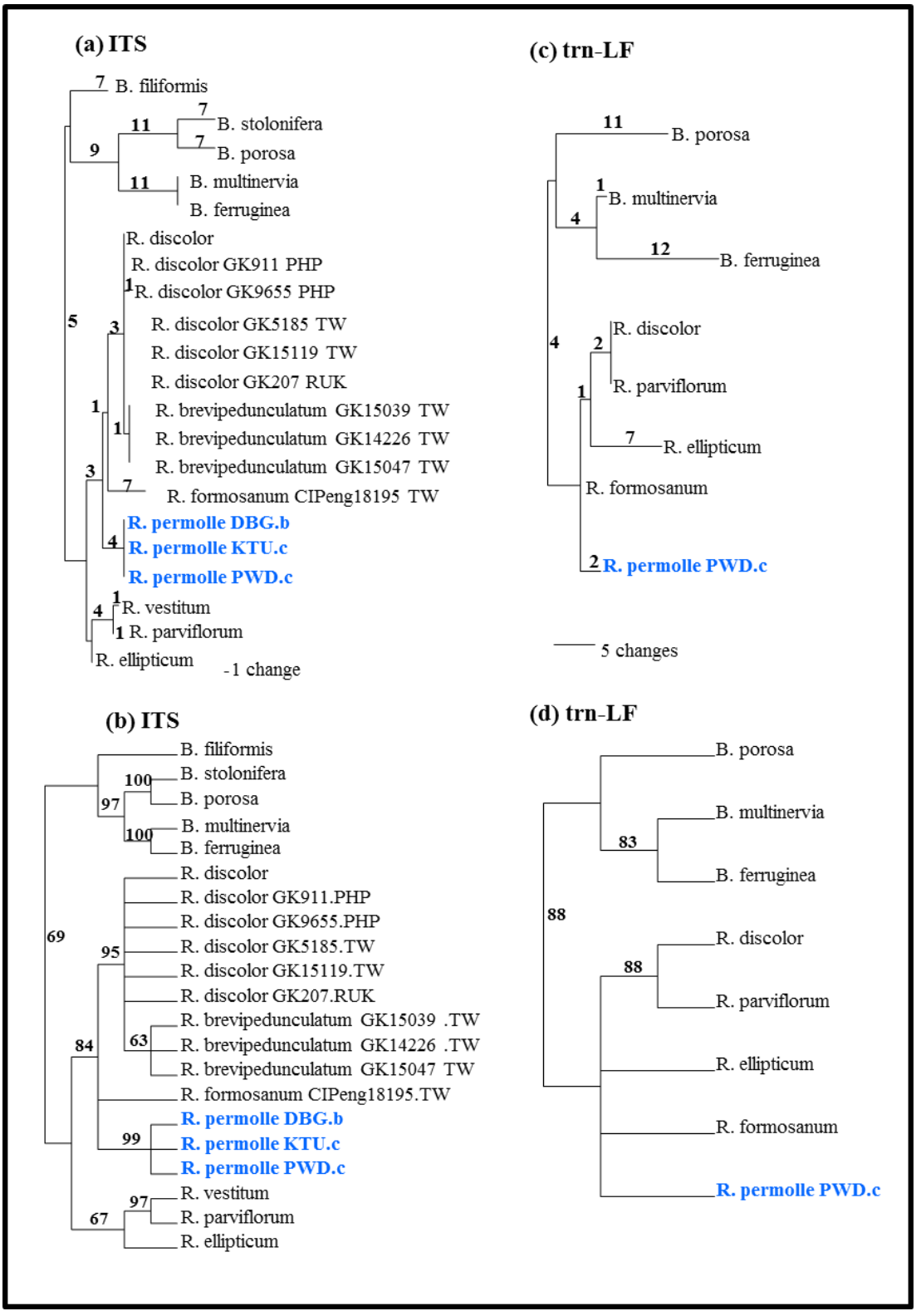


Figure 2.6 ***Rhynchotechum***: (a) A single phylogram and (b) the strict consensus tree of three most parsimonious trees based on the ITS MP analysis, (c) a single phylogram and (d) the strict consensus tree of three most parsimonious trees from the *trn*L-F MP analysis. Number of nucleotide changes on

phylograms and bootstrap values on the strict consensus trees are shown. Sri Lankan species in coloured fonts.

Rhynchotechum

The ITS matrix for *Rhynchotechum* contained five sequences from five taxa in the outgroup. The ingroup is comprising one sample each from three populations of *R. permolle* from Sri Lanka, plus six other *Rhynchotechum* spp. with 13 samples (Figure 2.6a and b). The aligned ITS matrix was 657 bp long and included 584 constant characters. Of the variable characters, 49 (7.5%) were parsimony-informative, while 24 were parsimony-uninformative. The phylogenetic analysis produced three most parsimonious trees of 83 steps length and a consistency index (CI) = 0.9398, retention index (RI) = 0.9587 and rescaled consistency index (RC) = 0.9009. The clade comprising Sri Lankan *R. permolle* is highly supported (BS = 99%). This clade is sister to *R. formosanum* and the clade comprising *R. discolor* + *R. brevipedunculatum*. The genetic P-distance within populations of *Rhynchotechum* was not available because only one sample per population could be examined. Also there was no genetic variation detected between the populations of *R. permolle*. The genetic distance to its closest sister group (*R. discolor* + *R. brevipedunculatum* + *R. formosanum*) is 0.01256-0.01898 with 6 indels (Table 2.2).

The *trn*L-F matrix for *Rhynchotechum* contained three sequences from three outgroup taxa and one sample each from five ingroup taxa (Figure 2.8c and d). The sequencing of *trn*L-F was hampered by issues discussed for *Championia* above. There were 860 bp included in the aligned matrix, of which 818 characters were constant, Furthermore, 10 (1.2%) variable characters were parsimony-informative, while 32 variable characters were parsimony-uninformative. The phylogenetic analysis produced three most parsimonious trees of 44 steps length with a consistency index (CI) = 0.9545, retention index (RI) = 0.8667 and rescaled consistency index (RC) = 0.8273. The *trn*L-F matrix had an only moderately supported backbone and clades. However, the single *R. permolle* sample from Sri Lanka fell in a polytomy with *R. formosanum*, *R. ellipticum* and a clade containing *R. discolor* and *R. parviflorum*.

Genetic variation data were not available within and between populations of *Rhynchotechum* in Sri Lanka due to difficulties in *trn*L-F sequencing similar to

Championia. The genetic distance of *R. permolle* to its sister taxa on the polytomy was P-distance = 0.00257-0.01144 and one indel (Table 2.3).

Henckelia

The ITS matrix of *Henckelia* contained 14 outgroup taxa and 124 samples of 15 ingroup taxa. The ingroup taxa from Sri Lanka included with 111 samples of 26 populations from eight taxa (seven species + one sp. nov.). And 13 samples from three populations of the putative hybrid. A single sample of *H. incana* from South India included as well (Figure 2.7a and 2.8a).

The aligned matrix of the ITS region was 760 bp long and included 406 constant characters (Table 2.2). Furthermore, 281 (36.9%) variable characters were parsimony- informative, while 73 variable characters were parsimony uninformative. The phylogenetic analysis produced 264 most parsimonious trees of 860 steps length and a consistency index (CI) = 0.6012, retention index (RI) = 0.9507 and rescaled consistency index (RC) = 0.5715.

The strict consensus tree and the one of 264 phylograms of ITS produced here are shown in Figure 2.7a and 2.10a respectively. All Sri Lankan *Henckelia* species plus the South Indian *H. incana* formed two highly supported clades (BS = 100%). One represented the **acaulescent clade (X)** that included *H. zeylanica, H. humboldtiana, H. floccosa* and *H. incana.* This clade was sister to a clade of five species with caulescent and acaulescent habits from China, Nepal and Thailand, but with low branch support (BS = 75%). The second Sri Lankan clade was a **caulescent clade (Y)** including *H. wijesundarae, H. walkerae, H. moonii, H. communis* and *H. angusta*. Further to that, samples of the Sri Lankan species *H. zeylanica* (BS = 100%) (clade "P"), *H. wijesundarae* (BS = 100%) (clade "S"), *H. walkerae* (BS = 98%) (clade "T") and *H. moonii* (BS = 100%) (clade "U") formed highly supported species-specific clades. On the other hand, *H. humboldtiana* formed two well supported clades, "Q" (BS = 100%) and "R" (BS = 93%). Clade "R" was sister to *H. incana* (BS = 96%) from South India. There were four populations distinct to *H. humboldtiana*-2 clade "Q": Knuckles (KNU), Peak Wilderness (PWD), Kegalle (KGL), and Rawanaella (RWE), and four populations distinct to clade "R": Pidirutalagala (PDG), Kikiliyamana (KIK), Ramboda (RMB), and Ritigala (RTG). However, individuals from population Moragahakanda (MGK) were shared between the two *H. humboltiana* clades. Two morphological types of *H. communis* (green leaf

type here termed, "*communis* green" and purple leaf type here termed, "*communis* purple"), *H. angusta* and the putative hybrid between *H. communis* and *H. angusta* formed a large highly supported clade (BS = 100%) with two polytomies, one containing most "*communis* green" samples, while the other included the remaining "*communis* green", "*communis* purple", the putative hybrid and *H. angusta*.

The genetic distance within populations of Sri Lankan *Henckelia* with P-distance = 0–0.00309 and 0–1 indels (Table 2.2). The genetic distances were higher between populations within species of *Henckelia* in Sri Lanka with P-distance = 0–04796 and 0–5 indels. Between species of *Henckelia* in Sri Lanka the P-distance was 0–0.1764 with 0–24 indels. The genetic variation between the species of Sri Lankan *Henckelia* and their sister species in the ITS matrix was P-distance = 0.11932–0.1842 and 71–115 indels excluding South Indian *H. incana*.

The *trn*L-F matrix of *Henckelia* contained the same ingroup and outgroups taxa as detailed in ITS data (Table 2.3). There were 880 (bp) characters in the *trn*L-F matrix of which 740 were constant. Furthermore, 83 (9.4%) variable characters were parsimony-informative while 57 variable characters were parsimony-uninformative. The phylogenetic analysis produced two most parsimonious trees of 164 steps length with a consistency index (CI) = 0.9146, retention index (RI) = 0.9895 and rescaled consistency index (RC) = 0.9050.

The strict consensus tree and the one of two phylograms of *trn*L-F produced here are shown in Figure 2.7b and 2.8b respectively. Similar to the ITS phylogeny all Sri Lankan *Henckelia* species plus the South Indian *H. incana* formed two well supported distinct clades, one the **caulescent clade** "X" (BS = 91%) containing *H. zeylanica*, *H. humboldtiana*, *H. floccosa*, and *H. incana*, and the **acaulescent clade "Y"** (BS = 94%) including *H.* sp.nov., *H. walkerae*, *H. moonii*, *H. communis*, *H. angusta* and a putative hybrid. However, only the Sri Lankan species *H. zeylanica* (BS = 96%) formed a well-supported clade in the *trn*L-F phylogeny. The *H. humboldtiana* samples (clades Q and R in the ITS phylogeny; Figure 2.9 a), and *H. floccosa* and *H. incana* formed a large polytomy.

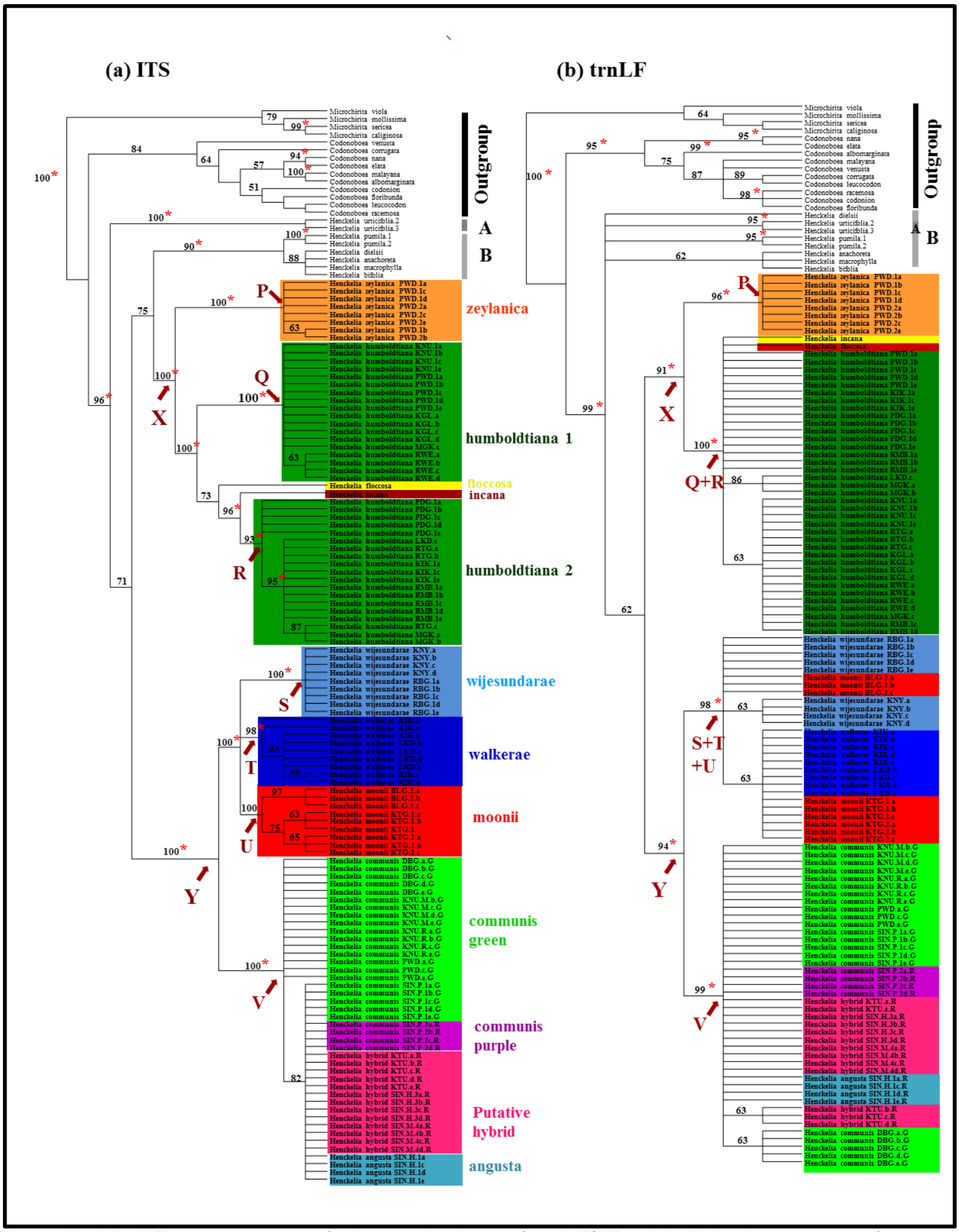


Figure 2.7 *Henckelia*: (a) The strict consensus tree of 264 most parsimonious trees from the ITS MP analysis, (b) the strict consensus tree of two most parsimonious trees from the *trn*L-F MP analysis. *H. zeylanica*; *H. humboldtiana* 1 and 2; *H. floccosa*; *H. incana*; *H. wijesundarae*; *H. walkerae*; *H. moonii*; *H. communis* "communis green", *H. communis* "communis purple"; Putative hybrid; H. *angusta*. Highly supported branches (BS = 90% - 100%) are shown with asterisk mark*.

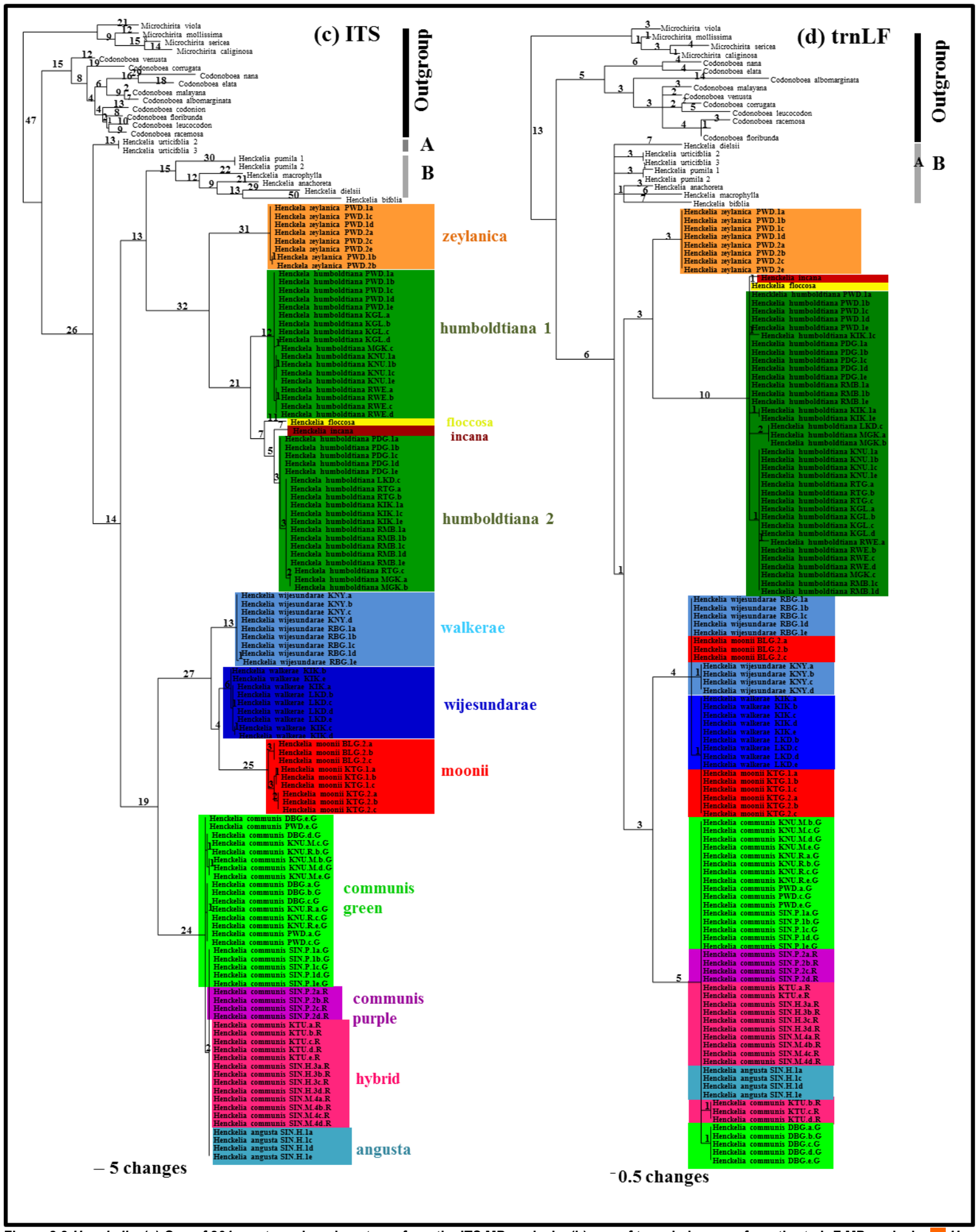


Figure 2.8 *Henckelia*: (a) One of 264 most parsimonious trees from the ITS MP analysis, (b) one of two phylograms from the *trn*L-F MP analysis. *H. zeylanica*; *H. humboldtiana* 1 and 2; *H. floccosa*; *H. incana*; *H.wijesundarae*; *H. walkerae*; *H. moonii*; *H. communis* "communis green"; *H. communis* "communis purple"; Putative hybrid; *H. angusta*. Number of nucleotide changes are shown above the branches.

However, this clade was well supported (BS = 100%). Similarly, *H. wijesundarae*, *H. walkerae* and *H. moonii* also formed a well-supported clade (BS = 98%), though with a large polytomy and poorly supported subclades. Similarly, the two morphological types of *H. communis* ("*communis* green" and "*communis* purple"), *H. angusta* and their putative hybrid also formed a clade (BS = 99%) with a large basal polytomy.

Similarly to the ITS matrix, the genetic variation was lower within (P-distance = 0-0.00378 and 0-1 indels) and between (P-distance = 0-0.00503 and 0-2 indels) populations than between species (P-distance = 0-0.03048 and 0-5 indels) of *Henckelia* in Sri Lanka. The genetic variation between species of Sri Lankan *Henckelia* and their sister species in the *trn*L-F matrix was lower than ITS with a P-distance between 0.01209-0.0346 and 10-24 indels excluding the South Indian *H. incana* (Table 2.4), which fell in the *H. humboldtiana* clade (P-distance to Sri Lankan samples = 0.00122-0.0283 and 1-3 indels) (Table 2.3).

Aeschynanthus

The aligned ITS matrix of *Aeschynanthus* was 686 characters long, of which 495 characters were constant. Furthermore, 72 (10.5%) variable characters were parsimony-informative while 119 variable characters were parsimony-uninformative. The phylogenetic analysis for ITS of *Aeschynanthus* produced one most parsimonious tree (Figure 2.9a and b) of 246 steps with a consistency index (CI) = 0.9024, retention index (RI) = 0.8095 and rescaled consistency index (RC) = 0.7305. Bootstrap values ranged from 62% –100%. All samples of *A. ceylanicus* from Sri Lanka formed a highly supported clade (BS = 100%). This *A. ceylanicus* clade is sister to a clade comprising two species, *A. lancilimbus* and *A. bracteatus*. There was no genetic variation observed within and between populations of *A. ceylanicus*. The genetic distance of *A. ceylanicus* to its closest sister clade with P-distance = 0.05443 and 8 indels (Table 2.2).

For the *Aeschynanthus trn*L-Fphylogeny, there were 843 characters in the aligned matrix of the *trn*L-F, where 802 characters were constant. Thirteen (1.5%) characters were variable and parsimony-informative, while 28 variable characters were parsimony-uninformative. The phylogenetic analysis produced one most parsimonious tree of 44 steps and a consistency index (CI) = 0.9773, retention index

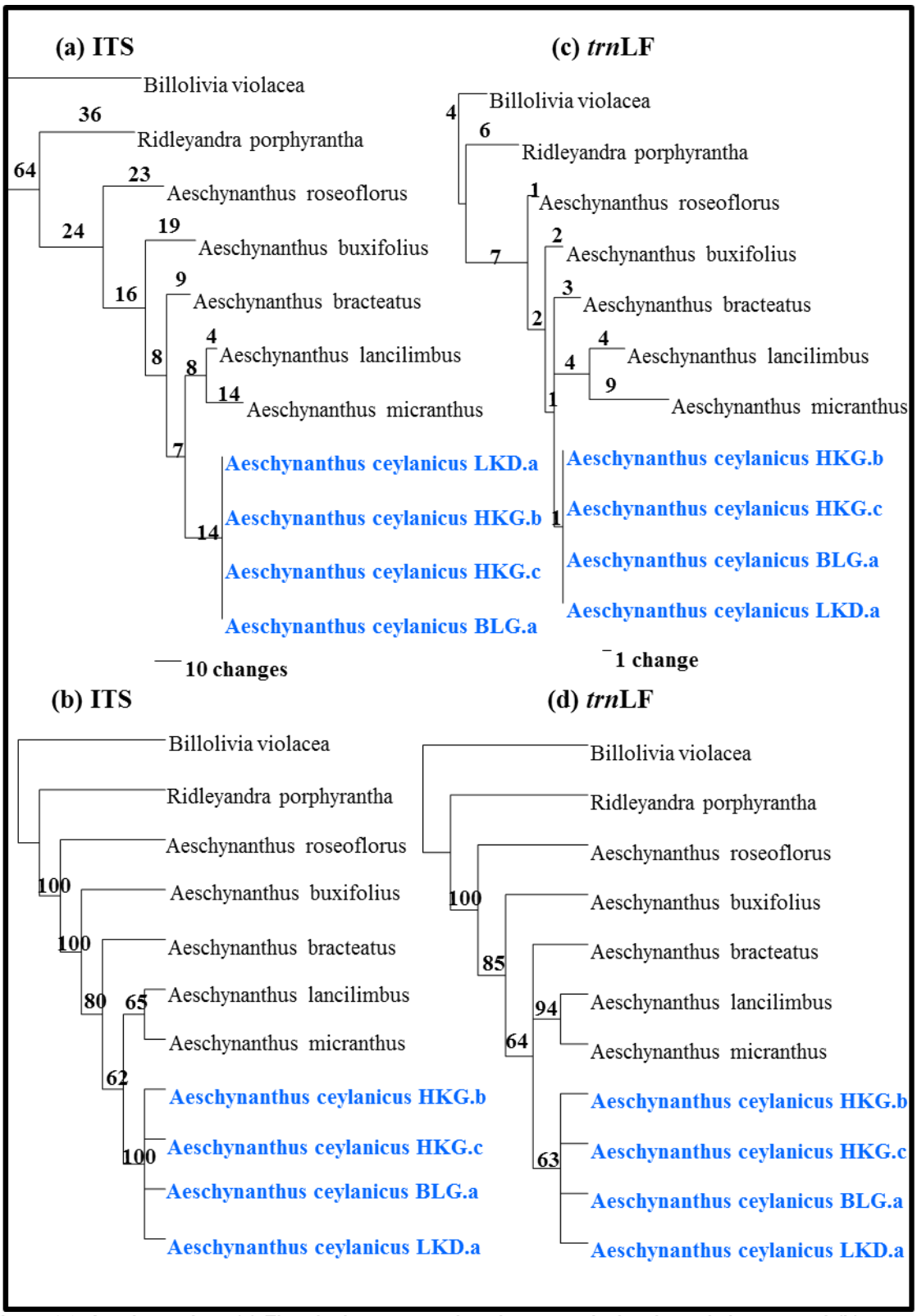


Figure 2.9 *Aeschynanthus*: (a) The single most parsimonious tree depicted as phylogram and (b) as cladogram based on the ITS MP analysis. (c) The single most parsimonious tree depicted as phylogram (c) and as cladogram (d) based on the *trn*L-F MP analysis. Sri Lankan species are showed in coloured fonts.

(RI) = 0.9474 and rescaled consistency index (RC) = 0.9258. The bootstrap values ranged from 63% - 100% (Figure 2.9c and d). A low branch support of 64% was found for the clade containing the Sri Lankan *A. ceylanicus* samples and *A. bracteatus*, *A. lancilimbus* and *A. micranthus*. All the samples of *A. ceylanicus* were monophyletic although the branch support was poor (BS = 63%) compared to the ITS phylogeny. Similarly to the ITS matrix, there was no genetic variation detected within and between populations of *A. ceylanicus* in Sri Lanka. The genetic distance between *A. ceylanicus* samples and its closest sister group clade (*A. bracteatus, A. micranthus* and *A. lancilimbus*) showed in P-distance range = 0.00989-0.01725 plus 4 indels (Table 2.3).

2.5 Discussion

2.5.1 Molecular species delimitation of Gesneriaceae genera in Sri Lanka

Rhynchoglossum

There are about 15 species described in the genus so far (Möller *et al*., 2016) of which only five are included here. Irrespective of the small number of species included here, some conclusions can be drawn. According to both ITS and *trn*L-F trees the multiple samples from each of the two Sri Lankan species, *R. notonianum* and *R. gardneri* formed highly supported (BS = 99% - 100%) clades, but did not show any intra-specific variation (Figure 2.3). The considerable genetic distance between *R. notonianum* and *R. obliquum* indicates that they are distinct species despite their overlapping geographical distribution in India. However, no material for *R. notonianum* from India could be included.

Epithema

For the ITS phylogeny only four sequences were available, three from *E. ceylanicum* and one *Stauranthera*. The *trn*L-F sequences evolve more slowly, i.e. show fewer nucleotide substitutions, and were readily alignable with the sister genera to *Epithema*. In this phylogeny *E. ceylanicum* samples from Sri Lanka formed a single well-supported clade, on a polytomy with *E. taiwanense* and *E. taiwanense* var. *fasciculatum* from Taiwan, *E. ceylanicum* from Thailand, and two *E. benthamii* samples from Thailand. This large clade contained taxa recently subsumed under *E. ceylanicum* with a broader distribution range, except *E. benthamii* which was kept as

a distinct taxon (Bransgrove and Middleton, 2015). Even though this clade included *E. benthamii* among *E. ceylanicum* sensu lato, it was poorly supported (BS = 66%) and thus uncertain whether the expanded *E. ceylanicum* does represent a natural lineage. To properly test the species definition of Bransgrove and Middleton (2015) inclusion of more samples from *E. ceylanicum* across its new distribution range is needed and perhaps more molecular markers are needed since *trn*L-F is too slowly evolving. Because of this uncertainty surrounding *E. ceylanicum* s.l. the taxonomic revision in Chapter three keeps the species description based only on Sri Lankan specimens.

Championia

This genus had never been included in molecular phylogenetic studies before, and the outgroup choice depended on the present biogeographic work (Chapter 4). Thus, *Jerdonia*, a monotypic genus from South India *Corallodiscus* from China were used (Figure 2.5). The samples of *Championia reticulata* fell in one clade in both ITS and *trn*L-F analyses (BS = 100%). Considering species delineation aspects, *Championia reticulata* formed two subclades in ITS and showed 1 nucleotide change between the ITS subclades in the *trn*L-F phylogeny. The two subclades (subclades I and II) differed in their distributions in montane forests in the central highlands, and lowland wet forests in the southwestern part of Sri Lanka. The high number of substitutions in ITS (20-21) suggests that these populations have been reproductively and geographically isolated for a considerable length of time.

Rhynchotechum

In the ITS trees, the individuals of *R. permolle* from Sri Lanka formed a highly supported clade (BS = 99%) (Figure 2.6a and b). There was an incongruence between the ITS and *trn*L-F tree concerning the position of *R. discolour* and *R. ellipticum*. It is difficult to comment on this observation since only seven out of 21 species in the genus (Möller *et al.*, 2016) were included here which does not reflect the geographical distribution range of the genus, and on the otherhand the different sampling between the ITS and *trn*L-F matrix. It is interesting to note that no variation in ITS was found across three populations of *R. permolle* and its distant position to its congeners suggest that it represent a good species. However, to fully confirm the species limits of *R. permolle*, more samples across its distribution range must be

included, since it is distributed also in India and possibly extends to Burma (Anderson and Middleton, 2013).

Henckelia

This phylogeny represents the largest for the genus to date. The most extensive previous work was published by Weber et al. (2011), to establish the new definition of *Henckelia*. Here, Weber included one species from India and two species from Sri Lanka, and seven further species now in *Henckelia*. To the latter were added the population samples from Sri Lanka. Single samples of two further species of *Henckelia* included in Weber *et al*.'s (2011) phylogeny were *H. incana* from South India and *H. flocossa* from Sri Lanka. *Henckelia floccosa* has only been collected twice from the type locality in 1845 and 1968 on the island. It was observed that the type locality was completely disturbed by habitat alteration and invasion of alien species, hence the species could not be found during the current study. Therefore, only seven *Henckelia* species from Sri Lanka are represented at the population level in the present phylogeny namely *H. zeylanica*, *H. humboldtiana*, *H. moonii*, *H. walkerae*, *H. communis*, *H. angusta* and the newly described *H. wijesundarae*.

A deep incongruence found in topology between the ITS and *trn*L-F tree concerned the position of the outgroup clade B, as sister to clade X (Sri Lanka + South India) in ITS and sister to clades X (Sri Lanka + South India) and Y (All Sri Lankan taxa) in *trn*L-F. These relationships were supported by 13 steps (BS = 75%) in ITS but only one step (BS = 62%) in *trn*L-F. The relationship of South Indian and Sri Lankan species of genus *Henckelia* to the rest of the species in the genus needs further studies in the future concerning the molecular based phylogenetic studies.

There were further incongruences observed between the two markers concerning *H. humboldtiana* and the species around *H. moonii*, *H. walkerae* and *H. wijesundarae*. Using different markers perhaps resolve the problem of this discrepancy in future.

ITS was entirely population specific, with the only exception being population MGK, that fell into two *H. humboldtiana* clades. In ITS phylogeny, the samples of *H. humboldtiana* were split into two clades by samples of *H. floccosa* and *H. incana*. The latter is the only sample of *Henckelia* included from South India, though this area includes 15 species (Möller *et al*., in press). The two *H. humboldtiana* clades were well supported and might represent distinct lineages (except for sample MGK.c that needs more investigations). *Henckelia humboldtiana* occurs also in South India.

Since only one of the 15 species of the purely Indian *Henckelia* species, *H. incana*, and only Sri Lankan samples of *H. humboldtiana* could be included in the present study, a taxonomic re-evaluation of this taxon has to be postponed until suitable material becomes available. However, the samples from the same population fell among different clades of *H. humboldtiana* in the *trn*L-F trees. There was no distinct geographical pattern discernible for these two *trn*L-F clades. There was also no evidence from the ITS sequencing that would have suggested that hybridisation might have occurred, such as polymorphic sequences or extensive polymorphic positions (cf. Puglisi *et al*., 2011). Such a scenario of conserved ITS signal and mixed cpDNA signal can be explained by the presence of populations with mixed cpDNA types due to incomplete lineage sorting (Naziri and Linder, 2015). In such case, it is necessary to fall back on using the ITS signal for species delineation.

The molecular markers used were unable to discriminate between the two accepted species, *H. angusta* and *H. communis*. Neither could the two distinct morphotypes of *H. communis*, *"*communis green*" and "*communis purple*"* be separated. *Henckelia angusta* was collected from a single locality in this study, despite several attempts to find the species from the few habitats where it was collected earlier. It was identical in ITS and *trn*L-F sequences data to a putative hybrid form bearing morphological characters from both *H. angusta* and *H. communis,* which is commonly distributed in lowland wet forests. All individuals of these three taxa formed a large polytomy/s in both phylogenetic trees. Therefore, the species boundaries of *H. communis* and *H. angusta* are difficult to interpret using nuclear ITS and chloroplast *trn*L-F data.

The individuals of *H. zeylanica* formed well supported clades with both markers which are supported by its morphological distinctness. Therefore, among all eight species recorded from Sri Lanka only one *H. zeylanica* provided a clear signal in delimitation perspective with these markers.

Their delimitation will be further studied using next generation sequencing data to overcome the problem of difficulties in taxonom and unresolved phylogeny (Leaché, 2010; Cariou *et al*., 2013; Eaton *et al*., 2013; McCormack *et al.*, 2013; Wagner *et al.*, 2013). This study will also give more insight into speciation and hybridization in the family Gesneriaceae in the future.

Aeschynanthus

The tree topologies constructed for the delineation of *A. ceylanicus* were similar for the ITS and *trn*LF data (2.11 b and d). In previous molecular phylogenetic analyses on *Aeschynanthus* two main clades were recognized, Clade I from Southeast Asia and Clade II from Malaysia (Denduangboripant *et al*., 2001). *Aeschynanthus roseoflorus* is the only sample included here from Clade II, and was sister the remaining samples from Clade I in the present study, which is congruent with the previous study. *Aeschynanthus ceylanicus* fell in a polytomy with two clades including *A. micranthus* (as *A. austroyunnanensis* in Denduangboripant *et al*., 2001) and nine other *Aeschynanthus* species in the phylogeny of Denduangboripant *et al*. (2001). In the present study the inclusion of multiple individuals from *A. ceylanicus* resulted in a highly supported clade (BS = 100%) in the ITS phylogeny but only poorly supported (BS = 63%) in the *trn*LF phylogeny.

The present phylogeny and divergence data on *A. ceylanicus* did not suggest the presence of molecularly diverging entities to be present in this species despite the inclusion of three populations sampled across the range of the species in Sri Lanka (Fig. 2.9).

However, *A. perrottetii* (a closely allied species to *A. ceylanicus*) from South India was not included here. *Aeschynanthus perrottetii* and *A. ceylanicus* show inconsistent overlaps in morphological characteristics (Clarke, 1883; Gardner, 1846). Therefore, there is a dispute over the discrimination of *A. perrottetii* from A. *ceylanicus* (Theobald and Grupe, 1981) which is further discussed in Chapter 3 on morphological aspects. Therefore, future work must include samples of this species to ascertain the species limits between the two species.

2.5.2 Comparison of the genetic distance data (ITS vs. *trn*L-F) across the species and genera.

This section compared genetic distances, indels (Figure 2.10a and b) and P-distances (Figure 2.10c and d) across the species and genera of Sri Lankan Gesneriaceae. This comparison may provide more insight into the behaviour of ITS and *trn*L-F markers across the six genera used from Sri Lanka at different taxonomic level. Figure 2.5a and b shows the genetic distance (indels) present in ITS and *trn*L-F data under each category of all six genera: within population; within species between populations; between species in Sri Lanka; and to their closest sister group. Considering both ITS

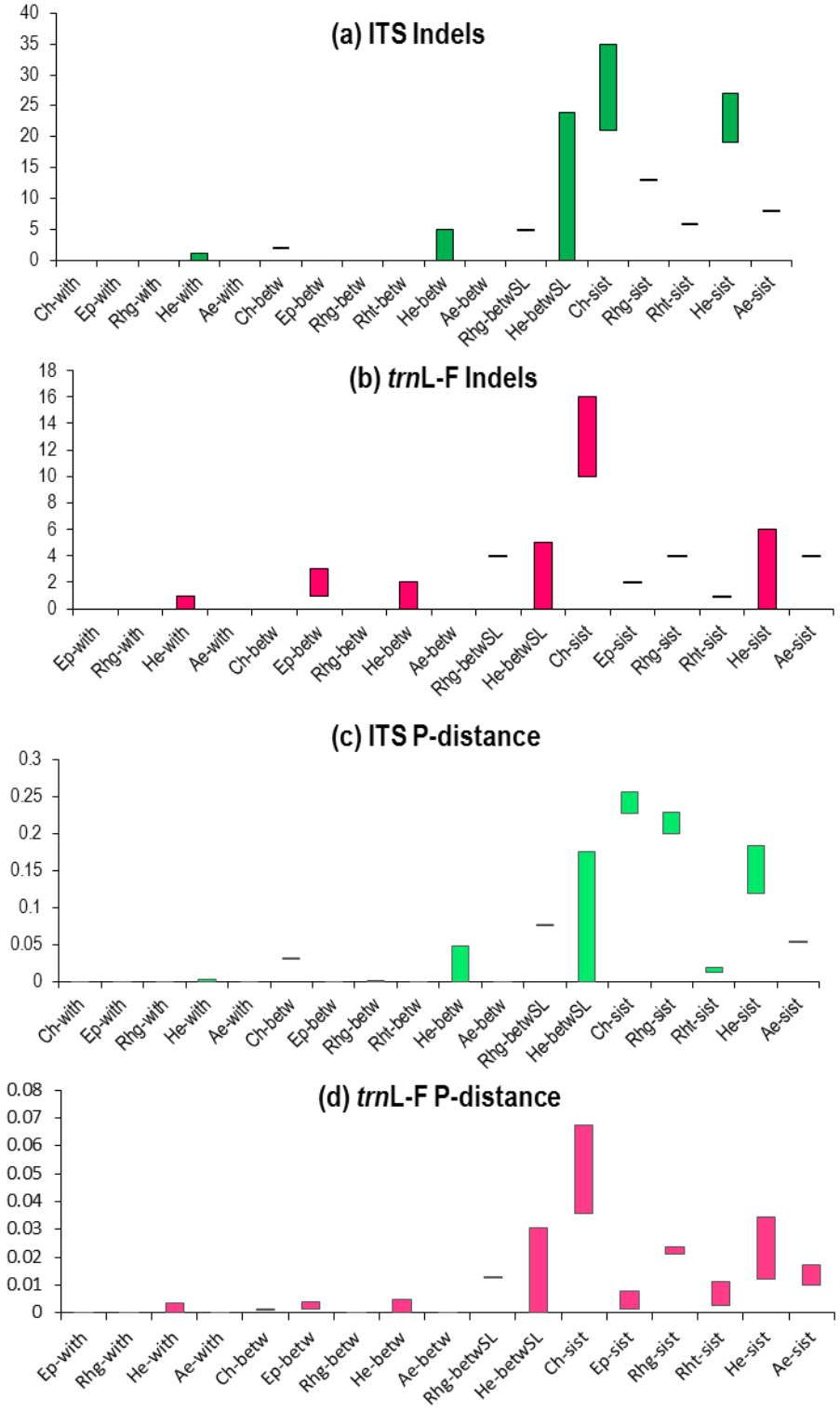


Figure 2.10 Graphical representation of indels observed in (a) ITS and (b) *trn*L-F ; P-distance observed in (c) ITS and (d) *trn*L-F under each category of within populations ('with'), between populations within species of Sri Lanka ('betw'), between species in Sri Lanka ('betwSL') and to the closest sister group ('sist').

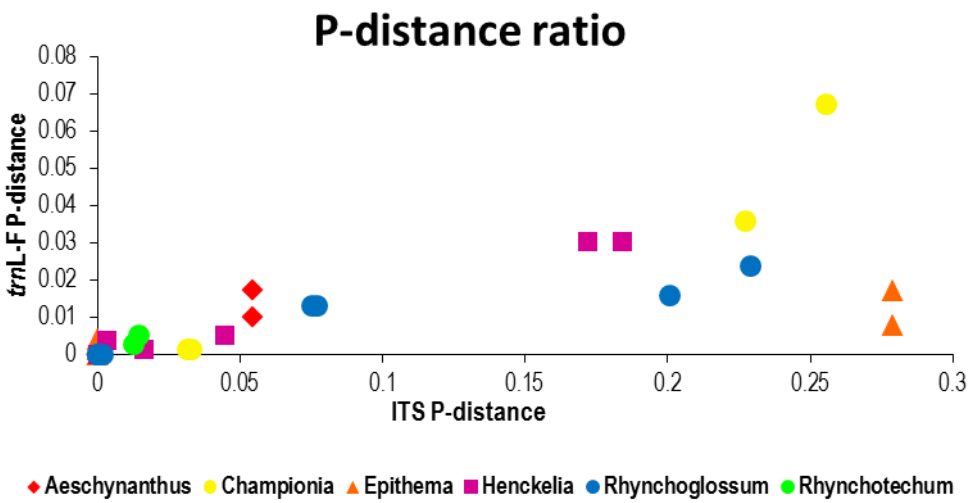


Figure 2.11 P-distance correlation between ITS and *trn*L-F for the six genera from Sri Lanka.

and *trn*L-F, within population distance (indels/P-distance) was observed only for the genus *Henckelia*. The genetic distance (indels/P-distance within species between populations were observed for two genera for ITS, *Championia* and *Henckelia*, and a further three genera for *trn*L-F, *Championia*, *Epithema* and *Henckelia*. The genetic distance (indels/P-distance) between the two *Rhynchoglossum* species, in Sri Lanka were lower compared to all eight *Henckelia* species in both ITS and *trn*L-F data. Considering the genetic distance to the closest sister group of each genus, *Championia* showed the highest genetic distance in both ITS and *trn*L-F. *Henckelia* also reflect a considerable genetic distance to the sister group compared to the other four genera. In these four genera, *Epithema* (no ITS data), *Rhynchoglossum*, *Rhynchotechum* and *Aeschynanthus* the genetic distance to the closest sister group was higher in *trn*L-F compared to ITS.

Figure 2.11 displays the P-distance correlation between ITS and *trn*L-F for the six genera from Sri Lanka. The P-distance ratio of *trn*L-F vs. ITS was different across the genera from Sri Lanka, for *Aeschynanthus* = 1:2–3; *Championia* = 1:2–4; *Rhynchoglossum* = 1:5–7; *Henckelia* 1:5–8. It was highest in *Epithema* = 1:13–27, and lowest in *Rhynchotechum* = 1:2. This may an indication of relative rate of evolution of nuclear ITS and Chloroplast *trn*L-F across these six genera. So that it is a deviation of the observation that ITS spacer region is five times faster than *trn*L-F

(Möller *et al*., 2009). According to present observations the lowest rate of evolution in *Rhynchotechum* where ITS spacer evolves 2 times faster than trnL-F while the highest rate of evolution is in *Epithema* where ITS space evolves 13-27 faster than trnL-F. Two genera, *Aeschynanthus* and *Championia* also show lower rate of evolution of ITS spacer where it is 2–3 times or 2–4 times faster than *trn*L-F. two genera, *Rhynchoglossum* and *Henckelia* have higher rate of ITS evolution vs. *trn*L-F evolution compared to *Aeschynanthus* and *Championia* while it is considerably lower than that of genus *Epithema*.

2.5.3 General Discussion

The study involved multiple samples per species across the geographical distribution of 13 Sri Lankan Gesneriaceae taxa (Theobald and Grupe, 1981) represented in six genera, two genera in tribe Epithemateae, *Rhynchoglossum* and *Epithema*, and a further four genera in tribe Trichosporeae, *Championia, Rhynchotechum, Henckelia* and *Aeschynanthus* (Weber *et al*., 2013), and provided detailed insights into the inter- and intra-species diversity and defined species boundaries.

The molecular delimitation of two species of the genus *Rhynchoglossum*, *R. notonianum* and *R. gardneri* was found to be two distinct monophyletic groups.

Molecular species delimitation of *Epithema ceylanicum sensu stricto,* supported the monophyly of the species within Sri Lanka. However, it did not support the monophyly of *Epithema ceylanicum* with the broader distribution in India (including Andaman Islands), Sri Lanka, Taiwan, Myanmar, Thailand, Cambodia, Vietnam, Philippines as suggested by Bransgrove and Middleton (2015).

Molecular delimitation studies in *Championia reticulata* revealed the presence of two distinct clades.

While the delimitation studies of *Rhynchotechum permolle* for samples included from Sri Lanka recognized its monophyly with respect to its congeneric species. The existing morphological taxonomy of three out of seven species in genus *Henckelia* recorded from Sri Lanka was supported by molecular delimitation studies: the morphological taxonomy of two species, i.e *H. moonii* and *H. walkerae*, was supported only by ITS, while *H. zeylanica* was supported by both ITS and *trn*L-F. The complex morphology observed in *H. humboldtiana* was not resolved through the molecular delimitation approach in this study. This was further complicated by the

lack of samples of this species from South India. The present molecular delimitation study could not detect any intraspecific variation in *H. communis* with the markers used in the present study, and did not resolve the existence of a putative hybrid between *H. communis* and *H. angusta.*

2.6 Conclusions

1. All the Sri Lankan Gesneriaceae species collected across their distribution to cover their geographical distribution which enabled us to find inter and intra species variation. All together there were 564 sequences from 268 samples generated for 14 taxa in six genera from Sri Lanka in the delimitation studies of the present chapter.

2. The molecular species delimitation studies based on both nuclear ITS and chloroplast *trn*L-F supported the morphological taxonomy of six Gesneriaceae species from Sri Lanka, i.e. *Rhynchoglossum gardneri R. notonianum, Epithema ceylanicum, Rhynchotechum permolle*, *H. zeylanica*, and *Aeschynanthus ceylanicus*. This shows the suitability of the approach hre to effectively delimit species. However, it also showed that while the nuclear ITS was a good candidate marker for species delimitation studies for the genus *Henckelia* the chloroplast *trn*L-F marker showed less success. Despite the observed incongruence between the two marker phylogenies, ITS supported the existing morphological taxonomy of *Henckelia walkerae* and *H. moonii*.

3. The species for which material from South India was not available, such as *Rhynchoglossum notonianum, Rhynchotechum permolle*, the species status supported by both ITS and *trn*L-F is tentative. Also, the inclusion of South Indian material of *Aeschynanthus perrottetii* is desirable to fully test the species limits of *A. ceylanicus.* For *Epithema ceylanicum*, while found to be a coherent species in Sri Lanka, the extended species concept needs further testing on an extended sample set.

4. A new species, *Henckelia wijesundarae* described from southwestern wet forests of Sri Lanka increased the species number to 14 species for the family Gesneriaceae on this island.

Chapter 3 Taxonomic status and revision of Sri Lankan Gesneriaceae

3.1 Introduction

Existing uncertainties over the morphological taxonomy of Gesneriaceae in Sri Lanka had been tested in the molecular studies here. The addition of molecular data provided more insight into species boundaries and the true patterns of diversity within Gesneriaceae in Sri Lanka. This recognized an apparent inter- and intra-species diversity. Nonetheless, morphological data still play a significant role in the description, identification and discrimination of taxa, especially in field botany. Therefore, in this chapter the taxonomy of Sri Lankan Gesneriaceae is revised, incorporating information from morphological data combined with the molecular results from Chapter 2.

As far as the taxonomic history of Gesneriaceae in Sri Lanka is concerned the collections made by Colonel George Warren Walker and his wife Anna Maria from 1830–1838 in Sri Lanka (then Ceylon) are most significant. These followed on from the earlier collections made by a Dutch phycisian, Paul Herman. The Walkers' collections became a valuable resource from which were described several new taxa from Sri Lanka (Noltie, 2013). These specimens were initially sent to Sir William Hooker (then at Glasgow University) and Robert Graham (then at RBGE), with a further set given to George Walker Arnott who worked in Glasgow for Hooker (Noltie, 2013). Dr. Robert Wight also made substantial collections in Sri Lanka accompanied by Colonel and Mrs. Walker during his short visit in 1836 while in service to the East India Company in Peninsular India. The Walker collections were mainly deposited in the herbaria K, E and G and some duplicates were sent to and deposited in other herbaria such as W, P and A (Thiers, 2016+; Noltie, 2013).

Part of the Walkers' 1830s collections were studied in the first detailed account of Gesneriaceae in Sri Lanka, i.e. Gardner (1846), *Contributions towards a Flora of Ceylon*; from these descriptions were made of several new species belonging to the tribe Cyrtandreae (now Trichosporeae). Gardner produced the account during his service as Superintendent and Chief Gardener of the Royal Botanic Garden, Ceylon (now Sri Lanka) from 1843–1849. George Gardner received some of Walker's collections from Sir William Hooker before he came to Sri Lanka to take up his duty

in the botanical garden. However, the first described species of Gesneriaceae from Sri Lanka is *Didymocarpus zeylanica* by Brown (1839) in his work on Cytandreae. George Gardner included fourteen species of Gesneriaceae (in tribe Cyrtandreae) with twelve species newly described, *Aeschynanthus ceylanicus*, *Didymocarpus longipetiolatus*, *Didymocarpus humboldtianus*, *Didymocarpus primulaefolius*, *Chirita moonii*, *Chirita walkerae* ["*walkeri*"], *Chirita communis*, *Isanthera floribunda*, *Championia reticulata*, *Klugia glabra*, *Klugia ceylanica* and *Epithema ceylanicum*, plus a detailed description for the already published species, *Klugia notoniana* (Wall.) A. DC.

George Henry Kendrick Thwaites became the next superintendent, succeeding George Gardner, and later appointed to the post of Director of the Sri Lankan Botanic Gardens. He continued Gardner's work and published *Enumeratio Plantarum Zeylaniae* in 1864. His account of tribe Cyrtandreae was based on those published accounts available at the time that included Sri Lankan Gesneriaceae taxa (Brown, 1839; De Candolle, 1845; Hooker, 1845, 1847; Gardner, 1846). Thwaites (1864) included 12 species and four varieties in his account including one new species, *Henckelia floccosa* recorded from the island. Clarke (1883) included Sri Lankan Gesneriaceae taxa already described by previous authors in his monograph on tribe Cyrtandreae in *Monographiae Phanerogamarum*.

The next comprehensive taxonomic treatment of the Sri Lankan Gesneriaceae was by Trimen (1895), *Flora of Ceylon*, the first such flora, published in 1893–1900 in five volumes, with the sixth volume later completed by Alston; this was one of the most comprehensive floras at that time (Wijesundara *et al.*, 2012). The flora basically followed the work by Clarke (1883). Trimen (1895) included 12 species and six varieties from six genera of Gesneriaceae from Sri Lanka, of which eight species were recorded as endemic. Much later, in 1968, a revision of the *Flora of Ceylon* was initiated with funding from the Smithsonian Institute, and later from the British Overseas Development project. The revision included 14 volumes in which the Gesneriaceae chapter appeared in volume three produced by Theobald and Grupe (1981). This work represents the most recent taxonomic account of Gesneriaceae in Sri Lanka. According to this revision, Sri Lanka harbours 13 Gesneriaceae species, ten of which were recognized as endemic.

This chapter aims to provide a comprehensive revision of the family Gesneriaceae in Sri Lanka. This revision includes an up to date set of taxonomic descriptions of Gesneriaceae taxa in Sri Lanka, based on morphological and molecular data.

3.2 Methodology

The present study performs a two-step approach to recognize taxa based on a combined molecular-morphological species concept, in which first the species are delimited using molecular data as detailed under Chapter 2 based on phylogenies, and then morphological data are used to define and recognized the taxonomic units. The phylogenetic groupings were used as guides to select/detect consistent morphological characteristics (synapomorphies) to then be considered in the taxonomic delimitation. Where phylogenetic signals were lacking or complex, a morphological taxonomy was applied.

Field collections were made as described in Chapter 2.3.1 and for this chapter included herbarium specimens and 'spirit' collections of flowers, fruits and seeds. For spirit collections the materials were fixed soon after collection in the field in FAA (50 ml of 100% ethanol, 35 ml of distilled water, 10 ml of glacial acetic acid and 5 ml of 40% formaldehyde). The preserved samples were kept in FAA solution for at least one week, and then transferred to 40% ethanol (for export purposes).

The herbarium material studied included, besides my own field-collected material, specimens received on loan from the following herbaria: BM, CAL, K, L, MO, US, W. In addition, specimens at PDA were studied during visits to Sri Lanka in 2014 and 2015. All specimens cited in this chapter were seen unless otherwise stated. As noted by Stafleu and Cowan (1986, p. 342) and Wheeler, (1983) the *CP* numbers of herbarium material from Sri Lanka ["Ceylon"] distributed by Thwaites were not standard collectors' numbers that were produced in a regular fashion for individual collections made of one taxon at one place at one time. Instead, as Wheeler (1983) also stated, the specimens distributed under one *CP* number may have been collected at various places and times by various collectors. He further specified that this numbered series was made up of specimens then housed in the herbarium at Peradeniya and which presumably were there when Thwaites arrived plus subsequent collections by Thwaites and others.

There is an inconsistency of terminologies used in the literature for several taxonomic characteristics for leaf shape, indumentum, etc. The terminology used for this study consistently followed *The Kew Plant Glossary* by Beentje (2012). All taxonomic names, author details and journal abbreviations follow the *International Plant Names Index* available at www.ipni.org. Authors of scientific names at genus and species level are abbreviated according to *Authors of Plant Names* by Brummitt and Powell and its online updates (http://www.ipni.org/ipni/authorsearchpage and http://kiki.huh.harvard.edu/databases/botanist_index.html). The bibliographic abbreviations followed *Botanico Periodicum Huntianum* (BPH) and *Taxonomic Literature*, 2nd edition (TL2) available online (http://kiki.huh.harvard.edu/databases/publication_index.html).

Basic characters such as plant height, branching pattern, stem, leaf size, shape and colour, flower shape, opening and colour, distribution of hairs, colour of indumentum etc., were noted in the field. Measurements for vegetative characters such as peduncle, pedicel, bracts and calyx were based on measurements taken in the field as well as from dried herbarium specimens. There were approximately 1–3 mm lower measurements noted in dry compared to fresh material and the 'dry' values were used for descriptions. Microscopic studies were conducted with a Zeiss Stemi 2000c dissection microscope equipped with a Zeiss Axiocam MRc 5 digital camera and Zeiss Axio Vision software version 3.0 with extended focus module (Carl Zeiss, Inc., Thornwood, NY 10594, USA). In particular, the reproductive structures, indumentum characters and seeds were observed under this microscope in detail. Basic seed morphological characters were considered in the present study. Additional seed characters were included defined in the detail studies of seed morphology in Gesneriaceae by Beaufort-Murphy (1983). All characters studied in this chapter for all taxa are listed in Table 3.1.

The comprehensive generic and species descriptions given here represent the diversity observed across the genera and species in Sri Lanka. The generic descriptions are presented here in taxonomic order (Weber *et al*., 2013), and species descriptions are in alpabatical order.

e generic descriptions particularly observed diversity in Sri Lankan esneriaceae specieNo field collections could be included for *Henckelia floccosa* in this study,

despite repeated attempts to locate populations of this species in the field; it might be extinct. Therefore the taxonomic description for *Henckelia floccosa* given here is based on herbarium specimens, the protologue description (Thwaites, 1864) and the revision in the *Flora of Ceylon* (Theobald and Grupe, 1981). The species descriptions focused on Sri Lankan specimens (*sensu stricto*: *s. s.*) for the non-endemic species *Rhynchglossum notonianum*, *Epithema ceylanicum*, *Rhynchotechum permolle*, *Henckelia humboldtiana* and *Aeschynanthus ceylanicus*.

Details of all the loan specimens, which number ca. 500, are entered into the PADME Database at the herbarium of the Royal Botanic Garden Edinburgh. Locality and distributional data from field studies and from herbarium sheets were taken to make interpretations on the ecology, habitat and distribution of the taxa. GIS data for mapping the distributions were taken in the field from material collected for this study, and from all herbarium material examined. Many recent herbarium specimens examined include GIS information, which was incorporated directly; however the majority of specimens lacked this, so GIS data were extracted using digital gazetteers of Sri Lanka based on location information on the herbarium sheets. Distribution maps were prepared using ARC GIS version 10.2 (ESRI, 2013). Local Red Listing Assessments followed guidelines and criteria in IUCN (2012). Area of Occupancy (AOO) and Extent of Occurrence (EOO) were calculated using GEOCAT tool available online at http://www.kew.org/science-conservation/research-data/science-directory/projects/geocat-%E2%80%93-geospatial-conservation (Bachman *et al.*, 2011).

Table 3.1 Details of characters used for taxonomic observations.

No	Main Character	Specific Characters	Character states/types
1	**Habit**	1a. Woodiness	Herbs, shrubs, semi-shrubs/suffruticose
		1b. Lifespan	Annual, perennial, monocarpic
		1c. Growth form	Caulescent, acaulescent, epiphyte
		1d. Height	[cm]
2	**Stem**	1a. Stem type	Erect, short rhizomatous, creeping rhizomatous
		1b. Branching pattern	Unbranched, branched (irregular, dichotomous)
		1c. Stem diameter	[cm]

		1d. Leaf scars	Conspicuous, inconspicuous
		1e. Indumentum I	Glabrous, pubescent, villous, floccose
		1f. Indumentum II	Glandular, eglandular
		1g. Indumentum III	Uniseriate, multiseriate
		1h. Indumentum IV	Sessile, stalked
3	**Leaves**	3a. Leaf arrangement	Opposite, whorled, alternate
		3b. Leaf petiole	Petiolate, sessile
		3c. Petiole: length	[cm]
		3d. Indumentum on petiole	Glabrous, pubescent, villous, floccose
		3e. Blade colour (upper surface and lower surface)	Green, pale green, yellow-rusty, rusty, purple
		3f. Shape	Orbicular, ovate, obovate, oblong, lanceolate, elliptic
		3g. Size	Length [cm], width [cm]
		3h. Texture	Thin, membranous, thick (fleshy, leathery), subcoriaceous
		3i. Base I	Symmetrical, unequal
		3j. Base II	Cuneate, attenuate, obtuse, rounded, cordate, oblique
		3k. Margin:	Entire, dentate, crenate, bi-crenate, serrate
		3l. Apex	Acute, attenuate, acuminate, rounded, obtuse
		3m. Lateral vein pairs	Number.
		3n. Tertiary veins	Inconspicuous, reticulate;
		3o. Upper surface	Smooth, wrinckled/bullate, glandular;
		3p. Indumentum upper/lower I	Glabrous, subglabrous, pubescent, sericeous, villous, wooly, velvet, floccose;
		3q. Indumentum upper/lower II	Eglandular, glandular;
		3r. Indumentum upper/lower III	Uniseriate, multiseriate
		3s. Indumentum upper/lower IV	Sessile, stalked
4	**Inflorescence**	4a. Position	Terminal, axillary
		4b. length	[cm]
		4c. Type	Simple dichasium (pair flowered cyme), compound cyme, pseudo-racemose
		4d. Peduncle length	[cm]
		4e. Indumentum on peduncle	Glabrous, pubescent, sericeous, villous, floccose
		4f. Bract shape	Linear, lanceolate, triangular
		4g. Bract size	Length [cm/mm], width [cm/mm]

		4h. Indumentum on bract	Glabrous, pubescent, villous
		4i. Pedicel length	[cm/mm]
		4j. Indumentum on pedicel	Glabrous, pubescent, sericeous, villous, floccose
5	**Calyx**	5a. Lobes	Number
		5b. Lobes shape	Lanceolate, linear, oblong,
		5c. Lobes aggregation	Free, united halfway, united
		5d. Lobes size	[cm/mm]
		5e. Tube I	Wings present, wings absent
		5f. Tube II	Wings equal, wings unequal
		5g. Attachment	Persistent, caducous
		5h. Indumentum I	White, yellow rusty
		5i. Indumentum II	Pubescent, sericeous, villous, ciliate
		5j. Indumentum III	Eglandular, glandular
		5k. Indumentum IV	Sessile, stalked
6	**Corolla**	6a. Symmetry	Actinomorphic, zygomorphic
		6b. Shape	Rotate, bilabiate, campanulate, infundibular
		6c. Lobes: number	4, 5
		6d. Lobes colour	White, purple
		6e. Lobes length, width	[cm/mm], [cm/mm]
		6f. lobes shape	Lanceolate, orbicular, round
		6g. tube colour	White, pale purple
		6h. tube length	[cm/mm]
		6i. Corolla indumentum I	Glabrous, pubescent, villous
		6j. Corolla indumentum II	Glandular, eglandular
		6k. Corolla indumentum III	Sessile, stalked
		6j. Two longitudinal ridges	Present, absent
		6k. Broad yellow line;	Present, absent
7	**Stamens**	7a. Number	2, 4
		7b. Arrangement:	Didynamous, equal
		7c. Filament length	[cm]
		7d. Filament shape;	Filiform,
		7d. Filament attachment;	Basifixed, dorsifixed
		7d. Indumentum on filaments:	Glabrous, pubescent
		7e. anthers:	Fused, free
		7d. Indumentum on anthers	Glabrous, pubescent
		7f. Staminodes	2,3
8	**Disk**	8a. Disk I	Absent, inconspicuous, present
		8b. Disk II	Lobes present, lobes absent
		8c. Disk III	Cupular, crenate
9	**Pistil**	9a. Ovary shape	Conical, globose, ovoid, linear
		9b.Ovary size;	[cm]

		9c. Ovary indumentum I	Glabrous, pubescent
		9d. Ovary indumentum II	Glandular, eglandular
		9e. Ovary indumentum III	Sessile, stalked
		9f. Ovary indumentum IV	Straight, hooked
		9g. Placentation	Axile; parietal
		9h. Style length	[cm/mm]
		9i. Style indumentum	Glabrous, glandular or eglandular pubescent
		9j. Lower lip of stigma	Subcapitate, spathulate, campanulate, truncate
10	**Fruit**	10a.Type	Berry, capsule, circumscissile (operculate capsule)
		10b. Shape	Ovoid, linear, ovate, obovate
		10c. Indumentum	Glabrous, pubescent
		10d. Seeds shape	Ovate, oblong, elliptic, sickle-shaped
		10e. Seeds size	[mm]
		10f. Seed appendages	Present, absent
		10g. Testa colour	Brown, orange
		10h. Testa ornamentation	Reticulate, sub-reticulate, hexagonal, plygonate

Key to the Genera

1.a Inflorescence a unilateral raceme, or a thyrse, congested and subcapitiate raceme with a large, solitary, leafy bract **2**

1.b Inflorescence a simple or a compound dichasium, sometimes reduced to 1–2 axillary flowers **3**

2.a Inflorescence of unilateral racemes; bracts small, linear; leaves pseudoalternate, strongly asymmetrical; fruit a globose or ovoid to elongate capsule dehiscing loculicidally; fertile stamens 4 ..... **1. *Rhynchoglossum***

2.b Inflorescence a thyrse, reduced to a single, much condensed pair-flowered cyme; bract cucullate; leaves opposite and equal, except for large basal leafy petiolate macrocotyledon and solitary leaf above it; fertile stamens 2, posterior ……...**2. *Epithema***

3.a Corolla 4-merous, actinomorphic flowers with regular symmetry .....

……..**3.*Championia***

3.b Corolla 5-merous, zygomorphic flowers with bilateral symmetry **4**

4.a Leaves alternate, fruit indehiscent, ovoid; pericarp fleshy .... **4. *Rhynchotechum***

4.b Leaves opposite-decussate or whorled; fruit dehiscent, a linear capsule; pericarp not fleshy..... **5**

5.a Fertile stamens 4, stamens didynamous; epiphytic or subshrubs climbing on tree trunks and rocks; inflorescence 1–2 flowered in axils of terminal leaves; seeds with hair-like appendage at each end ..... **6. *Aeschynanthus***

5.b Fertile stamens 2, anterior; caulescent or acaulescent perennial herbs, sometimes creeping rhizomatous; inflorescence 1-many flowered, axillary; seeds without hair-like appendages.....**5. *Henckelia***

3.3 Revision of Sri Lankan Gesneriaceae

Rhynchoglossum Blume

Bijdr. Fl. Ned. Ind. 14: 741 (1826) [’Rhinchoglossum’]; A.DC., Prodr. 9: 274 (1845); Benth. in Benth. and Hook. f., Gen. Pl. 2: 1019 (1876); C.B. Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 161 (1883); Hook. f., Fl. Brit. Ind. 4: 367 (1885); Boerl., Handl. Fl. Ned. Ind. 2: 571 (1891); Fritsch in Engl. and Prantl, Nat. Pflanzenfam. 4(3B): 156 (1894); B.L. Burtt, Notes Roy. Bot. Gard. Edinburgh 24: 168 (1962); A. Weber in Kubitzki and Kadereit, Fam. Gen. Vasc. Pl. 7: 128 (2004). – Type: *Rhynchoglossum obliquum* Blume.

Antonia R. Br. in Wall., Pl. Asiat. Rar. 3: 65 (1832), auct. non Pohl. – Type: *Antonia obliqua* (Wall.) R. Br.

Klugia Schltdl., Linnaea 8: 248 (1833); A.DC., Prodr. 9: 274 (1845); in Benth. and Hook. f., Gen. Pl. 2: 1019 (1876); C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 158 (1883); Hook. f., Fl. Brit. Ind. 4: 366 (1885); Fritsch in Engl. and Prantl, Nat. Pflanzenfam. 4(3B): 155 (1894). – Type: *Klugia azurea* Schltdl.

Loxotis R. Br. ex Benth., Scroph. Ind. 57 (1835); R. Br. in Benn., Pl. Jav. Rar. 102 (1838); Miq., Fl. Ned. Ind. 2: 731 (1856). – Type: *Loxotis obliqua* (Wall.) R. Br. ex Benth.

Glossanthus J.G. Klein ex Benth., Scroph. Ind. 57 (1835). – Type: *Glossanthus malabaricus* Klein ex Benth.

Habit erect to creeping fleshy-succulent herbs. **Stem** perennial or annual, rhizomatous or not rhizomatous. **Leaves** pseudoalternate, exstipulate, petiolate, petioles glabrous or villous above; leaf blade variable in size, lamina membranous or subcoriaceous, ovate to lanceolate, green above and pale green beneath, margin entire or shallowly dentate or serrate with hydathodes, somewhat sinuate, apex acuminate, base strongly unequal, upper surface puberulous with eglandular,

multiseriate hairs and lower surface sessile or short-stalked glandular hairs or glabrous. **Inflorescences** a unilateral racemes with two rows of flowers, terminal on the main branch or on lateral branches, small linear bracts, sometimes absent; peduncles villous along one side; bracts linear-filiform, margin entire. **Calyx** lanceolate, 5-lobed, connate, lobes shorter or longer than the tube, 5-angled, angles at the union of calyx lobes, margin ciliate. **Corolla** strongly zygomorphic, bilabiate, tube cylindrical, constriction at the throat, upper lip short with 2 united lobes, white or pale blue, upright or reclined, lower lip broader and longer with 3 united lobes, lower lip deep rich purplish-blue, sometimes slightly paler, with a yellow spot at base, glabrous. **Disk** cupular. **Stamens** 4; filament flat or terete, glabrous, adnate to corolla tube on adaxial side, all 4 weakly connate; anthers glabrous, c. 1 mm broad, basifixed, coherent in pairs, thecae nearly parallel or divaricate, dehiscing longicidally. **Pistil** ovary globose or ovoid, 1–loculed, placentas 2, parietal; stigma 1, terminal. **Fruit** a capsule, stalked, globose or ovoid to elongate, smaller (fully enclosed) or larger (half enclosed) than calyx remnant, dehiscing loculicidally to base; 2–valvate, straight, not twisted; Seeds minute, cuneate, unappendaged.

Note: *Rhynchoglossum* is a widespread genus which has approximately 10 to 12 species with a distribution in Sri Lanka, India and South China to New Guinea, and also one (to three?) species in Central America (Skog and Boggan, 2016). According to the last revision of the flora in Sri Lanka (Theobald and Grupe 1981), there are two species of *Rhynchoglossum* recorded, *R. notonianum* and *R. gardneri*.

Key to the species

1.a Wings of the calyx all equal in size, lobes narrowly lanceolate-acuminate, longer than the tube; lower lip of corolla less than 14 mm long.....**1. *R. gardneri***

1.b Wings on the calyx not all equal in size, free upper part of calyx lobes lanceolate, shorter than the tube; lower lip of corolla usually greater than 16 mm long ..... **2. *R. notonianum***

1. *Rhynchoglossum gardneri* W.L.Theob. and Grupe, Ceylon J. Sci., Biol. Sci. 10: 70 (1972); W.L.Theob. and Grupe in Dassan. and Fosberg, Revis. Handb. Fl. Ceylon 3: 101–102, Fig. 6(a, b) (1981). Type: Sri Lanka, Ratnapura district, in

the stream at highway marker 65/21 along highway A-17 from Rakwana to Deniyaya, c. 1000 m, 25 July 1968, *Theobald, W.L. and Grupe, G.A. 2360* (lectotype PDA, designated here). (Figures 3.1 and 3.16, Map, pp. 88).

Klugia ceylanica Gardner [Calcutta J. Nat. Hist. 6: 490 (1846); Thwaites, Enum. Pl. Zeyl. 208 (1864); C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 160 (1883); Trimen, Handb. Fl. Ceylon 3: 278 (1895). Type: Sri Lanka, *Gardner, G. 603* (lectotype K [K00080841], designated here).

Klugia notoniana auct. non (Wall.) A.DC.: Hook., Bot. Mag. 77: pl. 4620. (1851).

[*Glossanthus zeylanicus* R. Br. On Cyrtandreae 121 (1839) nom. nud.]

[*Klugia zeylanica* A.DC., Prodr. 9: 276 (1845) nom. nud.]

Herb 20–60 cm tall. **Stem** irregularly branched, villous line down one side of the stem; indumentum multiseriate, eglandular branched hairs. **Leaves** petiole 0.3–3.5 cm long; leaf blade length 4–12 cm, width 2.6–6.5 cm. **Inflorescences** peduncles 2–5 mm long; bracts 1–3 mm long; pedicels 3–5 mm long. **Calyx** 6–8 mm long, lobes longer than the tube, tube 2 mm, all angles equal and slightly winged. **Corolla** upper lip 4–5 mm high, c. 3 mm broad at base; lower lip 10–12 mm long, 10–16 mm broad, tube white, c. 10 mm long, 3–4 mm broad, glabrous outer surface, slightly pubescent inner surface. **Stamens** one pair c. 5 mm long, other c. 1 mm shorter. **Pistil** ovary c. 2 mm long, c. 2 mm wide, glabrous; style glabrous; 7–8 mm long. **Fruit** membranous, c. 8 mm long, 4–5 mm broad; seeds minute, orange-brown, broadly elliptic or obovate, testa with short polygonate cells and straight cell orientation.

Distribution. Species endemic to Sri Lanka. Found in wet forests at an elevation from (350–)500–1000 m.

Habitat and Ecology. Habitats similar to *R. notonianum*, on wet soil banks in shade near streams.

Chromosome count. *n = 10* (Ratter and Prentice, 1967)

Phenology. Flowering observed in June and November.

Note. As stated by Burtt (1962), the reason that *Klugia ceylanica* is not the earliest epithet and therefore the correct name under a combination in *Rhynchoglossum* is due to the existence of *Rhynchoglossum zeylanicum* Hook. already in the genus. He stated this as "*Klugia notoniana* and *K. ceylanica* (usually written *K. zeylanica*) have been kept as distinct species by most authors. The question needs further study and I am at present unwilling to make a new name for *K. ceylanica* in *Rhynchoglossum*, more especially as the epithet *ceylanica* (*zeylanica*) is preoccupied in that genus."
Rhynchoglossum zeylanicum was described by Hooker (1845) who noted that he received the plant from Sri Lanka by George Gardner. The illustration (Tab. 4198) and the description of Hooker (1845) however, go with *R. obliquum* that was described by Blume in 1826. Clarke (1883) and Burtt (1962) both treated *Rhynchoglossum zeylanicum* Hook. as a synonym of *R. obliquum* Blume. *Rhynchglossum obliquum* has never been recorded in Sri Lanka except for this note of Hooker (1845). The present study could not find any existing populations of *R. obliquum* in Sri Lanka as well. Skog and Boggan (2016 onw.) also stated under *R. obliquum* that, "This name, its circumscription, its synonyms, and their respective types needs intensive study. Numerous records probably due to misidentification". Thus, further studies on *R. obliquum* is required to confirm its states and the distribution.

Lectotypification of *Rhynchoglossum gardneri*. This species was published by Theobald and Grupe (1972) based on their studies and field work on the family Gesneriaceae for their revision for the *Flora of Ceylon* (Theobald and Grupe, 1981). In their original publication, they did not mention any specimen or location data for the species. Later in the revision of the *Flora of Ceylon* volume III, they cited several specimens including their collections (*Theobald and Grupe: 2315, 2360, 2370*). They also mentioned the distribution of the species as "moist lowlands of the southern Ratnapura District north into the Kandy District and to elevations about 1100 m". The specimen *Theobald and Grupe* 2360 closely tallies with this description and is here selected as the lectotype. They also noted duplicates under the same collection

Figure 3.1 *Rhynchoglossum gardneri* (a) habitat; (b) Seeds X 40, scale bar 200µm); (c) inflorescence with flowers showing equally winged calyx.

number at A, E, K, PDA, UC, and US. The possibility of the existence of isolectotypes in these herbaria must be further checked and verified except at E where no specimen of this number could be found.

Lectotypification of *Klugia zeylanica.* This species was originally described by Gardner (1846). It was then synonymized under *Rhynchoglossum gardneri* by Theobald and Grupe (1981). There are two specimens collected by Gardner mounted on the same sheet in K but individually numbered and barcoded as *Gardner 603* [K000858041] and *Gardner 604* [K000858042]. *Gardner 603* [K000858041] is the more complete specimen and is selected as the lectotype. The specimen collection year on the sheet is 1847 but Gardner's original description of the species was published in 1846. Probably the processing time between Gardner's collection and the receipt of specimens by KEW is the cause of this discrepancy.

Provisional IUCN Conservation Assessment. Extent of occurrence (EOO) was calculated as 3129 km^2 and area of occupancy (AOO) as 64 km^2. Number of locations is less than 5 (Figure 3.16). This species is very habitat specific and sensitive to habitat alterations because the plants are always found in shaded near streams on wet soil banks. Moreover, it has a very fragmented distribution, and always forms very small populations outside conservation areas, especially forest edges orin the buffer zone close to human settlements/villages. This species is therefore very vulnerable due to habitat loss and climatic changes. The present conservation status of *Rhynchoglossum gardneri* can be assessed as Endangered (EN) under both EOO and AOO under criteria B1 ab (i,ii,iii).+ B2ab(i,ii,iii).

Additional Specimens Examined. KANDY DISTRICT: Gannoruwa hill, Peradeniya, *Meijer 1701* (K, MO, US); Hantane, *s. coll., s.n.* part of *CP 1786* (PDA); Kandy, *Alston s.n.* (PDA); Raxawa, *s.coll.*, part of *CP 1786* (PDA); Peradeniya, *Amarathunga 281* (PDA); *Ranasinghe 1070* (E, PDA); Galagedara, *Amaratunga 950* (PDA); Nitre cave, Knuckles conservation area, *Jayasuriya 8753* (PDA); above Murutenna near Lakshapana Falls, *Grieson 1050* (PDA); Paranapattiya, *Ranasinghe*

874 (E, PDA). **KEGALLE DISTRICT:** on the north side of Highway A-l west of Kadugannawa, just below tunnel, *and Grupe 2370* (PDA). **MATALE DISTRICT:** Wiltshire Forest, *Ranasinghe 895* (E, PDA). **RATNAPURA DISTRICT:** on rocks in stream at highway marker 65/21 along Highway A-17 from Rakwana to Deniyaya, *Theobald and Grupe 2360* (PDA); Carney, Adam's Peak sanctuary south side, *Hepper, Maxwell and Jayasuriya 4529* (PDA); trail to Gongala above Longford division of Heyes group, *Jayasuriya, Balasubramanium and Greller 2909* (MO). **LOCALITY UNKNOWN:** *Gardner 603* (K); *Gardner 604* (K); *s.coll. CP 1786* (PDA, W); *Macrae 443* (K).

2. *Rhynchoglossum notonianum* (Wall.) B.L.Burtt, Notes R. Bot. Gard. Edinburgh 24: 170 (1962); W.L.Theob. and Grupe in Dassan. and Fosberg, Revis. Handb. Fl. Ceylon 3: 99–101, Fig. 6.c (1981). – *Wulfenia notoniana* Wall., Tent. Fl. Nepal. 46 (1826). – *Klugia notoniana* (Wall.) A.DC., Prodr. 9: 276 (1845); Gardner, Calcutta J. Nat. Hist. 6: 487 (1846); Thwaites, Enum. Pl. Zeyl. 208 (1864); C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 159–160 (1883); Trimen, Handb. Fl. Ceylon 3: 277 (1895). – *Glossanthus notoniana* (Wall.) R.Br. ["*notonianus"*], Cyrtandreae 121 (1839). Type: India, Nilghiry, *Norton, P.J. s.n.* (lectotype K [K001109983], designated here). (Figures 3.2 and 3.16, Map, pp.88).

Glossanthus malabaricus Klein ex Benth., Scroph. Ind. 57 (1835). Type: India, Travancore, *s.coll. Cat. No. 6394.A* (lectotype K [K001123774], designated here).

Klugia glabra Gardner, Calcutta J. Nat. Hist. 6: 489 (1846). – *Klugia notoniana* var. *glabra* (Gardner) C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 159 (1883).Type: Sri Lanka, Matelle (Matale) East, 06–1863, 'herb. Thwaites', *s.coll. CP 3369*. (neotype K [K000858046], designated here).

Rhynchoglossum scabrum Dalzell in Hook., Bot. Mag. 2: 140 (1850). – *Klugia scabra* (Dalzell) Dalzell in Dalzell and Gibson, Bombay Fl. 134 (1861). – *Klugia notoniana* var. *scabra* (Dalzell) C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 160 (1883). Type: India (Maharashtra), Bombay, *Dalzell, N.A. s.n.* (lectotype K [K000858044], designated here).

Herb caulescent, occasionally reduced to a single leaf. **Stem** irregularly branched, 60–80 cm tall, villous line down one side of the stem; indumentum multiseriate, eglandular branched hairs. **Leaves** petiole 0.5–4 cm long; **Inflorescences** peduncles 1–4 cm long; bracts 2–5 mm long; pedicels 3–5 mm long. **Calyx** 8–11 mm long, tube 2–4 mm long, upper angle with a rounded wing-like crest on its lower half; crest 3–5 mm high, other angles slightly or equally crested, shorter than the tube. **Corolla** upper lip 3–4 mm high, 3–4 mm broad at base; lower lip 16–26 mm long, 15–20 mm broad, tube white, 7–10 mm long, 3–4 mm broad. **Stamens** one pair 6–7 mm long, other c. 1 mm shorter. **Disk** cupular. **Pistil** ovary c. 2 mm long, c. 2 mm wide; style glabrous; c. 8 mm long. **Fruit** membranous, 8–10 mm long, 5–6 mm broad; seeds minute, orange-brown, ovate to oblong, testa with long polygonate cells and weakly spiral cell orientation.

Distribution. Recorded from both Sri Lanka and South India. Sri Lankan specimens were collected from montane and submontane forests between 1100 m to 2400 m elevation range. *Rhynchoglossum notonianum* is well separated from *R. gardneri* in geography as well as in elevation (see below the details under *R. gardneri*). However, these two species were recorded in the same area in Dolosbage (between Kandy and Kegalle districts). This area is an ecotone between two climatic zones and elevation generally between 600–1200 m. Hence, the two species are separated by altitude with l ittle overlap, with *R. gardneri* recorded from lower elevations while *R. notonianum* occurs at higher elevations.

Habitat and Ecology. Found adjacent to stream beds, waterfalls or open stream areas in rock crevices and on soil banks. The fleshy stem is used as a vegetable in rural areas.

Phenology. Most populations in Kandy and Matale were observed to flower in June and July, but one population in Peradeniya (Kandy district) was seen flowering in both June and December.

Chromosome count. *n = 10* (Eberle, 1956).

Vernacular Name. Diya-nilla (S). This is the only Sri Lankan Gesneriaceae for which a vernacular name could be found.

Lectotypification of *Wulfenia notoniana*. *Rhynchoglossum notonianum* was originally named as *Wulfenia notonia* (Wallich, 1826). Wallich named this species when he received the specimen from P.J. Norton who gathered it from a swamp near Nilghirry. But he did not specify any specimen information in his description. However, there is one specimen available at K with the collector's name as 'B. Norton'. This specimen has pencil written notes on it by Wallich, but it is difficult to read the words fully. However, it has *W. notoniana* and p. 46 (the same page number of Wallich's publication) written on the labels. N0. 409 on the original label of the sheet tallies with Wallich's Catalogue entry 409 for *W. notoniana*. That confirms the recognition of the specimen as the type of *W. notoniana*. Therefore, this specimen K001109983 is selected as the lectotype specimen. It is also suggested that the collector's name in the database at K to be changed to P.J. Norton.

Letotypification for *Glossanthus malabaricus*. *G. malabaricus* was validly published by Bentham (1835) based on Wallich's herbarium collections. Bentham noted Wallich's catalogue number as 6394 in the description of *G. malabaricus*. Also in the Wallich catalogue, available online (http://wallich.rbge.info/node/16820), *G. malabaricus* is listed under number 6394. There are two specimens at K with this number, 6394A and 6394B. The specimen 6394A (K001123774) is the better specimen to select as the lectotype because it contains clear flower and fruit characters to matc the protologue description.

Neolectotypification of *Klugia glabra*. This was first described by Gardner (1846). Later, this was recognized as a variety of *K. notoniana* by Clarke (1883). There are no specimens available at PDA or any other herbaria collected by Gardner under this name, *K. glabra*. The specimen K000858046, available at K, has two labels, one with CP 3369, the second label has *K. notoniana* var. *glabra* written on it by C.B. Clarke.

Therefore it is confirmed that Clarke proposed the variety "*glabra*" based on this specimen. In the absence of any original material from Gardner, the specimen at K, K000858046, is here proposed as a neotype for *K. glabra*.

Lectotypification of *Rhynchoglossum scabrum*. *Rhynchoglossum scabrum* was first described by Dalzell (1850). There are two specimens collected by Dalzell at K but only one specimen (K000858044) has *R. scabrum* and *Klugia notoniana* var. *scabra* written on it. The second specimen (K000858045) collected by Dalzell has only *K. notoniana* var. *scabra* written on it by Clarke. Only the first specimen is complete, with clear inflorescence and leaf characters. Therefore, the specimen K000858044 is designated as the lectotype specimen**.**

Provisional IUCN Conservation Assessment. The **e**xtent of occurrence (EOO) is 2973 km^2 and the area of occupancy (AOO) 88 km^2. Number of locations is ≤ 5 (Figure 3.16). This species is very habitat specific and sensitive to habitat changes of reduced water supply because they are always found near streams and on wet soil banks. Furthermore, they are always very fragmented in distribution, and in small populations of less than 20–25 individuals. This species is found in conserved areas as well in forested areas clos tohuman settlemens/villages. This species is therefore very vulnerable to human activites, forest clearing, illegal settlements etc. Therefore, the present conservation status of *Rhynchoglossum notonianum* could be assessed as Endangered (EN) under both EOO and AOO under criteria B1ab(i,ii,iii) +2ab(i,ii,iii).

Additional Specimens Examined. BADULLA DISTRICT: Namunukula, Passara, *Ranasinghe 1054* (PDA); Passara, *Balakrishnan NBK626* (MO, PDA, US); Ohiya to Bambarakanda Falls, *Theobald and Krahulik 2842* (US). **KANDY DISTRICT:** At the base of Kabaragala Mountain behind Raxawa Tea Estate, east of Dolosbage, *Theobald and Grupe 2356* (US); below the Laxapana Falls, *Burtt and Townsend 119* (K [two sheets], MO); Nawanagala, *Waas 1024* (K, MO). **NUWARA ELIYA DISTRICT:** Mandaram Nuwara, *Ranasinghe and Wijewickrama 1080* (PDA); Boragas, beside fort MacDonald road. ±6km from main road, *Cramer 3511* (PDA, US); about 2 miles N of Horton Plains towards Agrapatana, in forest, *Gould 13577*

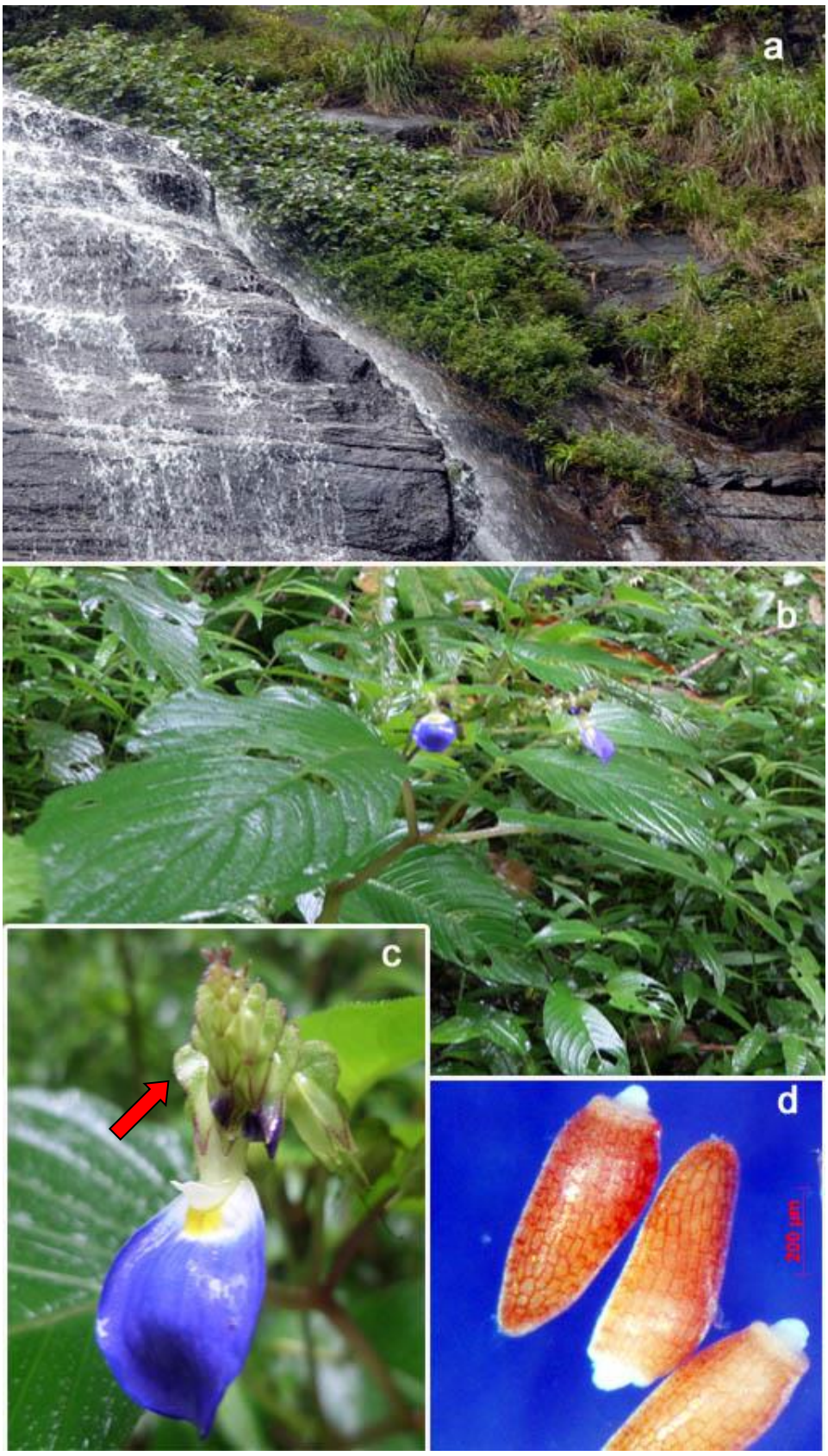


Figure 3.2 ***Rhynchoglossum notonianum*** **(a) habitat of wet rock outcrops adjascent to waterfalls; (b) a flowering branch; (c) inflorescence with open flower showing unequal dorsal wing of the calyx (arrow); (d) seeds X40.**

(US); Horton Plains, *Grey-Wilson and Silva 3068* (K, US); Rambodde, *Jayasuriya and Cramer 767* (K, MO, PDA, US); Horton Plains along road to Diyagama, *Jayasuriya and Robyns 80* (US); Great Western Range, above Lindula, *Kostermans 24611* (K, US [02 sheets]), road from Diyagama Tea Estate to Horton Plains, *Sohmer and Sumithraarachchi 9998* (K, MO, US); along Farr Inn-Diyagama road, Horton Plains, *Sumithraarachchi, Moldenke, Moldenke and Jayasuriya DBS58* (PDA, US); from Horton plains to Agarapatana, *Theobald and Grupe 2309* (US); Adams Peak, north of Pinnawala, south of divide, *Maxwell and Jayasuriya 909* (MO); Horton Plains, Agarapatana road, *Nowicke and Jayasuriya 266* (MO, PDA, US); Route A-4 en route to Belihul Oya, near waterfall at sharp bend in road, *Read 2259* (K, US). **RATNAPURA DISTRICT:** Near Galagama falls, *Bremer and Bremer 975* (PDA, K, US); Adams Peak, Bogawanthalawa-Boralanda road, *Comanor 1066* (K, MO, US); Maratenne, Bogawanthalawa road, *Waas 446* (K, MO). **LOCALITY UNKNOWN:** *s.coll. s.n. CP 1787* (W); *s.coll. s.n. CP 3369* (W).

Epithema Blume

Bijdr. 737 (1826); C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 176 (1883); C.B.Clarke in Hook.f., Fl. Brit. Ind. 4: 369 (1884); King and Gamble, J. Asiat. Soc. Bengal, Pt. 2, Nat. Hist. 74(2): 783 (1909); Ridl., Fl. Malay Penins. 2: 539 (1923); Gamble, Fl. Madras 2: 991 (1924); Pellegr., Fl. Indo-Chine 4: 558 (1930); Kanjilal et al., Fl. Assam 3: 399 (1939); Barnett, Fl. Siam. 3(3): 205 (1962); Backer and Bakh.f., Fl. Java 2: 527 (1965); W.L.Theob. and Grupe in Dassan. and Fosberg, Revis. Handb. Fl. Ceylon 3: 102 (1981); Pham-Hoàng Hô, Cayco Vietnam ed. 3, 3(1): 24 (1993); W.T.Wang et al., Fl. China 18: 400 (1998); B.L.Burtt, Thai For. Bull. (Bot.) 29: 93 (2001); Hilliard in Grierson and D.G.Long, Fl. Bhutan 2(3): 1328 (2001); A.Weber in Kubitzki, Fam. Gen. Vasc. Pl. 7: 129 (2004). Type: *Epithema saxatile* Blume.

Aikinia R.Br. in Wall., Pl. Asiat. Rar. 3: 65 (1832). Type: *Aikinia brunonis* Wall. (=*Epithema brunonis* (Wall.) Decne.).

[*Carpocalymna* Zipp., Alg. Konst- Lett.-Bode 1: 297 (1829), nom. nud.]

Habit caulescent or acaulescent herbs, annual, occasionally only one leaf developing. **Stem** sparingly branched, round, fleshy, subglabrous, sparsely pubescent or sparsely strigose, cluster of adventitious roots near the base. **Leaves** opposite or alternate, exstipulate, one of the lowermost leaf pair is larger and distinct while the second leaf is abortive, occasionally a single leaf per plant, lowest leaf petiolate and solitary, upper leaves petiolate or sessile; blades variable in size, lamina thinly to thickly membranous, usually ovate to cordate, more rarely elliptic to orbicular, upper surface pale to black-green, sometimes variegated, lower surface light to mid or olive-green or purplish, margin entire to crenate, serrate or (bi-)dentate, venation pinnate, apex rounded to acute, base mostly cordate or sub-auriculate to obtuse, inserted evenly or unevenly on petiole, upper surface with sparse straight and/or hooked hairs, sub-glabrous to strigose, hairs sparse to medium density, sometimes with hooked and straight hairs on veins. **Inflorescences** a thyrse, peduncles usually originating from the leaf axils, occasionally from the petiole and/or the midrib of the blade; singular bract subtending each inflorescence, greenish, cucullate, enclosing the entire inflorescence, margin entire to dentate; peduncle and pedicel densely pubescent with fine minute hairs and more sparsely with larger strigose hairs. **Calyx** cylindrical to campanulate, persistent, consisting of a tube and 5 lobes, lobes lanceolate with acute tips, green with an embedded gland towards the apex of each lobe. **Corolla** zygomorphic**,** tube tube cylindrical to narrowly fluted, occasionally slightly constricted at the apex; usually white, lobes 05, pale pink to blue or purple, lower 3 lobes larger, rounded, upper 2 lobes smaller, suborbicular, slightly fused next to each other, with a distinct H-shaped dark purple blotch across the two lobes, minute straight hairs scattered sparsely; corolla tube white, glabrous, commonly with darker markings on either lip; lobes with margins entire to fimbriate. **Disk** apparently absent or one- to three-lobed, margin entire to undulate. **Stamens** 4, filiform, glabrous; fertile stamens two; anthers sub-reniform, glabrous; staminodes 2, third staminode absent, staminodes curved and swollen at tip. **Pistil:** ovary cylindrical to spherical, unilocular, parietal placentation; glabrous to densely pubescent, hairs predominantly hooked and shorter than the hairs on rest of the flower, stigma bi-lobed, papillate, glabrous, lower part persistent. **Fruit** (sub-) cylindrical to (sub-) spherical circumscissile; operculum dehiscing at maturity, indumentum as ovary;

surrounded by persistent calyx. Seed numerous, ovoid, attached to the free apices of the placentae by long, whitish fine cords; testa brown, spirally arranged long lines with a sub-reticulate pattern.

Note: The majority of species are lithophytic, found on limestone and, to a lesser extent, on granite and sandstone. Bransgrove and Middleton (2015) recognised 20 species of *Epithema* from central tropical Africa, India, Sri Lanka, Nepal, Southern China and through Southeast Asia and Malesia to the Solomon Islands. The only representative of the genus recorded from Sri Lanka is *Epithema ceylanicum*, described by Gardner (1846). Recently, the circumscription of this species has been expanded to include several species, and the distribution range has consequently expanded from being endemic to Sri Lanka (Gardner, 1846) to being much more widespread in India, Indochina, Taiwan and to the Philippines (Bransgrove and Middleton, 2015).

Epithema ceylanicum Gardner, Calcutta J. Nat. Hist 6: 492 (1846); Wight, Icon. Pl. Ind. Orient. 4: pl. 1354 (1848); Walp., Ann. Bot. Syst. 3: 99 (1852). – *Epithema carnosum* var. *ceylanicum* (Gardner) C.B.Clarke in A.DC. and C.DC. Monogr. Phan. 5(1): 178 (1883). Type: Sri Lanka, *Gardner, G. 606* (lectotype K [K000438690], designated by Bransgrove and Middleton, 2015). (Figures. 3.3 and 3.16, Map, pp. 88).

Epithema carnosum var. *hispidum* C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 178 (1883). – *Epithema dentatum* subsp. *hispidum* (C.B.Clarke) Hilliard and B.L.Burtt, Edinburgh J. Bot. 54: 112 (1997). Type: India, Tamil Nadu, Western Ghats, Courtallum, August 1835, *Wight, R. 2350* (lectotype K [K001089591], designated by Bransgrove and Middleton, 2015); isolectotypes K (K001089589], [K001089590].

Epithema carnosum var. *dentatum* C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 177 (1883). – *Epithema dentatum* (C.B.Clarke) Hilliard and B.L.Burtt, Edinburgh J. Bot. 54: 111 (1997). Type: Burma, Mon, Moulmein, Farm Cave Rocks, *Parish, C.S.P. 63* (lectotype K, designated by Hilliard and Burtt, 1997).

Epithema brunonis var. *fasciculatum* C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 180 (1883). – *Epithema taiwanense* var. *fasciculatum* (C.B.Clarke)

Z.Yu Li and M.T.Kao, Fl. Taiwan ed. 2, 4: 697 (1998). Type: Philippines, Tayabas, 1841, *Cuming, H. 823* (lectotype K [K000438698], designated by Bransgrove and Middleton, 2015; isolectotypes BM, G-DC, K, L, P).

Herb height up to c. 30–40 cm, **Stem** c. 0.5–1.5 cm wide. **Leaves** length c. 30 cm, width c. 20 cm, petioles of lower leaves 2–12 cm long, petioles of upper leaves 0–2 cm long, lateral veins 6-10 pairs. **Inflorescences** peduncles c. 2–13 cm long, 1–4 arising at the axis or along the basal part of mid rib of the leaf; bracts c. 0.5–1.0 cm long, c. 0.5–1 cm wide,; pedicel c. 2–3 mm long. **Calyx** lobes c. 3.0–3.5 mm long, c. 0.6–0.8 mm wide. **Corolla** c. 6–7 mm long, lower 3 lobes larger, rounded, c. 3 mm broad, c. 3 mm wide; upper 2 lobes c. 2 mm long, c. 1 mm wide, corolla tube c. 3–3.5 mm long. **Stamens:** filaments c. 1.5–2 mm long. **Pistil** 1.0 mm long, 1.0 mm wide; stigma c. 5–6 mm long. **Fruit** 1.5–2 long, 1.5–2 mm wide; operculum c. 0.5 mm long; seeds 0.4 mm long, 0.1–0.2 mm wide.

Distribution. The latest revision by Bransgrove and Middleton (2015) suggested a more widespread distribution from India (including Andaman Islands), Sri Lanka, Taiwan, Myanmar, Thailand, Cambodia, Vietnam, Philippines. In Sri Lanka known from submontane forests in Kandy, Dolosbage and Kegalle.

Habitat and Ecology. In dense shade at mid elevations and submontane forest habitats between c. 500–1100 m. Usually found in crevices and shallow litter layer on wet rocks, or occasionally on wet soil banks. Short-lived plants.

Phenology. Flowering was observed during fieldwork for this project from July to September, but according to Theobald and Grupe (1981), flowering probably occurs throughout the year.

Note. *Epithema ceylanicum* (“zeylanicum”) Gardner was synonymized under *Epithema carnosum* by Theobald and Grupe (1981) in the last flora revision of Ceylon. Later, Bransgrove and Middleton (2015) revised *E. ceylanicum* and *E. carnosum* as separate species, now including the previously recognized taxa

Figure 3.3 *Epithema ceylanicum* (a) habitat on wet rock surfaces; (b) habit; (c) close-up of inflorescence.

Epithema carnosum var. hispidum, E. carnosum var. dentatum, E. brunonis var. fasciculatum and E. taiwanense. They proposed that the presence of hooked hairs on the operculum of *E. ceylanicum* as the primary characteristic to distinguish it from its closest relative *E. carnosum*.

Provisional IUCN Conservation Assessment. Bransgrove and Middleton (2015) suggested Least Concern (LC), considering the widespread distribution of the species. Considering *Epithema ceylanicum* in Sri Lanka alone, the extent of occurrence (EOO) was calculated as 4158 km^2 and area of occupancy (AOO) as 60 km^2. Number of loations is 6 (Figure 3.16). This species is recorded from very specific habitats, where moisture and shade are abundant. Also the populations are not very widespread and found as small populations restricted to their particular habitat. This species found in small fragmented forests, conserved forests as well as forest edges close to human settlements/villages. During the field expeditions it was also noted that the habitats of this species gradually invaded by an invasive species *Clusia rosea*. Therefore the present conservation category of *E. ceylanicum* in Sri Lanka can be assessed as Vulnerable (VU) with criteria B1ab (i,ii,iii) +B2ab(i,ii,iii).

Additional Specimens Examined. KANDY DISTRICT: Hantane, *s.coll.,* part of *CP 2844* (PDA); Raxawa, *Thwaites s.n.,* part of *CP 2844* (PDA); Kadugannawa, *Trimen s.n.* (PDA); Galamuduna Estate, Dolosbage (5 miles from Dolosbage to Bulathkohupitiya road), *Ranasinghe 867* (E, PDA); Seven Virgin Hills (Peak Wilderness Sanctuary), *Ranasinghe 1035* (PDA, E). **KEGALLE DISTRICT:** Northside of Hwy. A-l west of Kadugannawa, just below sharp bend in road on downgrade and rock tunnel, *Theobald and Grupe 2368* (PDA, E), *Theobald and Grupe 2404* (PDA). **MATALE DISTRICT:** Wiltshire forest, *Wijewickrama and Ranasinghe 896* (PDA, E) **RATNAPURA DISTRICT:** Katussagala Hill, *R.B. and A.J. Faden 76/494* (PDA). **LOCALITY UNKNOWN:** *Mrs. Walker s.n.* (K); *Gardner 606* (K); *Macrae 144, 244* (BM). *Davidse 8505* (L, MO); *Ranasinghe and Wijewickrama 1073* (E, PDA). **NUWARA ELIYA DISTRICT:** Hakgala, *Cramer 3449* (L).

Championia Gardn.

Calcutta J. Nat. Hist. 6: 485 (1846); Theobald and Grupe, in Dassanyake, Rev. Handb. Fl. Ceylon 3: 96-98 (1981) – Type: *Championia reticulata* Gardner.

Habit caulescent herb with strong woody base, perennial. **Stem** occasionally branched, young stem pubescent, mature stem glabrous. **Leaves** opposite, petiolate, petioles glabrous or pubescent, leaf blade upper surface dark green, lower surface pale green, oblong-oblanceolate, very variable in size and shape, texture slightly thick and leathery, base rarely obtuse, margin entire, apex acute, lateral veins conspicuous on lower surface, upper and lower surfaces pubescent, hairs becoming less dense with age, eglandular hairs crowded on midrib, occasionally glandular hairs present. **Inflorescences** a compound cyme, sometimes reduced to a single flower or pair-flowered cyme, short peduncles arising in axils of the upper leaves; peduncles sparsely pubescent, bracts 2, linear, glabrous or pubescent, pedicel glabrous or pubescent. **Calyx** 5-lobed, lobes lanceolate or rarely more linear, divided to the base, sparsely pubescent, persistent. **Corolla** distinctly 4-lobed, rotate shape, regular, corolla lobes white, lanceolate, obtuse apex, glabrous; tube short, white, glabrous. **Disk** inconspicuous. **Stamens** 4, equal, inserted on corolla tube; filaments shorter than lobes, glabrous; anthers basifixed, distally aggregated, ovate-oblong, 2 lager and 2 smaller locules. **Pistil** ovary oblong-conical, pubescent, sessile glandular hairs and eglandular hairs present, unilocular, 2 bifid parietal placentae, ovules numerous; style short, glabrous; stigma subcapitate. **Fruit** oblong-ovate capsule, pointed, loculicidal dehiscence, 2-valved, each valve splitting into 2 halves; seeds minute, numerous, broadly elliptic, unappendaged; testa brown, reticulate.

Note: *Championia* is a monotypic genus only found in Sri Lanka. Gardner (1846) described the genus and only species *Championia reticulata*. The genus is mentioned in different taxonomic treatments, and included in the family Gesneriaceae by Thwaites (1864), Clarke (1883), Trimen (1895) and Theobald and Grupe (1981). It is unusual with its actinomorphic flowers, which are only occasionally found in other genera of the family (e.g. the Old World *Bournea* Oliv. and *Thamnocharis* (H.Lév.) W.T.Wang, both now included in *Oreocharis* Benth. (Möller *et al*., 2011); *Tengia* W.Y.Chun (now in *Petrocodon* Hance), *Ramonda* Rich., *Conandron* Sieb. and Zucc.,

the New World *Bellonia* [Plum. ex] L., *Depanthus* S.Moore, and some species of *Napeanthus* Gardner)

Championia reticulata Gardner, Calcutta J. Nat. Hist. 6: 485 (1846); Walp., Ann. Bot. Syst. 3: 95 (1852); Thwaites, Enum. Pl. Zeyl. 208 (1864); C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 133 (1883); Trimen, Handb. Fl. Ceylon 3: 277 (1895); W.L.Theob. and Grupe in Dassan. and Fosberg, Revis. Handb. Fl. Ceylon 3: 96–97, Fig. 5 (1981). TYPE: Sri Lanka, *Mrs. Walker s.n*. (lectotype K [K000899489], designated here). (Figures 3.4 and 3.17, Map, pp. 89).

Herb height up to c. 30 cm. **Stem** c. 5 mm wide. **Leaves** length 5–15 cm, width 1.5–4 cm, petiole 1–3.5 cm long, lateral veins 8–12 pairs. **Inflorescences** peduncle 4–12 cm long; bracts 0.4–0.5 mm long, 2–3 mm wide; pedicel 5–20 mm long. **Calyx** lobes c. 0.5 mm long, 0.2–0.3 mm wide. **Corolla** 5.5 mm–8.5 mm long; lobes 5–8 mm long, 3–4 mm wide; tube c. 0.5 mm long. **Stamens** filaments 2–3 mm long. **Pistil** ovary 2.5–3 mm long, c. 1 mm wide; style 1–2 mm long. **Fruit** c. 10 mm long, 2–3 mm wide; seeds c. 0.4–0.5 long, 0.4–0.5 mm wide.

Distribution. Lowland wet forests to submontane forests of the foothills of Adam's Peak, found at elevations between 50–1000 m.

Habitat and Ecology. Under dense or semi-shady forests, on wet soil banks adjacent to stream beds in undisturbed forests. Possibly self-pollinating according to Theobald and Grupe (1981).

Phenology. In the present study flowering was observed from July to September, and fruiting in late September to November. According to Theobald and Grupe (1981) flowering takes place in February to March, and also July to August, and probably throughout the year at irregular intervals.

Lectotypification of *Championia reticulata*. In the protologue of *Championia reticulata* there is no type specimen proposed by Gardner (1846). There is one sheet

CP 358 at PDA with three specimens mounted on it and in pencil annotated with "Saffragam Adam's Peak, Gardner, March 1853, south of the Island, August September 1863 (1865?)"**.** However, this specimen is a doubtful one for the collection made by Gardner according to Theobald and Grupe (1981) since Gardner's service to the Garden at Peradeniya in Sri Lanka was from 1843–1849. On the other hand, Gardner mentioned the habitat in the original description as "Saffragam[1], Moon. Adam's Peak. Mrs. General Walker"**.** This indicates that Mrs. Walker's collections were studied by Gardner for his original description of *Championia reticulata*. Four herbarium sheets of specimens of this taxon collected by Mrs. Walker were found in different herbaria, two at K [K000899489 and K000899490], one at P [P03552821] and one at W [Acc. No. W0050320, not barcoded]. Only the two sheets at K were labeled as collected by "Mrs. Walker"; the sheets in P and W have only "Walker" as collector. Therefore, there is no evidence on the sheets at P and W to prove that the specimens were collected by General Walker or his wife (Mrs. Walker). None of the herbarium sheets noted here state the locality as "Adam's Peak". On the whole it seems more appropriate and accurate to select one sheet at K as lectotype. K000899489 includes the more complete specimens and all on the sheet appear to come from the same gathering. Therefore, K000899489 is selected as the lectotype of *Championia reticulata*.

Note. Taxonomic delineation studies based on molecular data in Chapter 2 (section 2.6.2, genus Championia and Figure 2.7) recognized two different clades in the ITS phylogeny with 100% bootstrap support as well as 21 nucleotide changes, but a only one nucleotide difference in the *trn*LF phylogeny. However, based on the present morphological studies, no character discriminates the two ITS clades. Thus, the *status quo* with one species in *Championia* is retained.

Provisional IUCN Conservation Assessment. An extent of occurrence (EOO) of 2778 km^2 and area of occupancy (AOO) of 80 km^2 were calculated for *Championia reticulata*. Number of locations ≤ 10 (Figure 3.17). This species is found in larger

[1] Saffragam" in Moon's Catalogue means "four Korales" (four Administrative divisions) in the British colonial period.

Figure 3.4 *Championia reticulata* (a) habitat in shade on forest floor; (b) habit; (c) flowers showing four corolla lobes.

populations, with about 100 individuals in wet forests of Sri Lanka. This pattern of occurrence is quite distinct from other Gesneriaceae members in Sri Lanka, all of which have few individuals per population and quite fragmented distributions. This was also found for *C. reticulata* in habitats on eroded wet soil banks similar to those of *Rhynchotechum permolle*. Probably, *C. reticulata* can tolerate more harsher climatic conditions compared to other members of the family perhaps due to the possession of woody stem bases and thick, leathery leaves. However, their natural habitat in lowland wet forests is under threat due to habitat alteration and fragmentation. These areas have a high threat of species extinction due to various kinds of human activities. This is mainly duty illegalencroachment of forest habitats for settlements and culivations of tea, *Cinnamomum*, *Cardamomum* etc.,. Therefore, a continuing decline of these populations can be projected for the EOO, AOO and quality of habitat in the future. Thus, the conservation status of *C. reticulata* is assessed as Endangered (EN) under both EOO and AOO with subcriteria B1ab(i,ii,iii) +2ab(i,ii,iii).

Additional Specimens Examined. KANDY DISTRICT. Nawalapitiya, *Kostermans 24590A* (K); Seven Virgin Mountains from Laxapana end, Peak Wilderness Sanctuary, *Ranasinghe 1033* (PDA); **GALLE DISTRICT:** Kottawa Forest Reserve, *Faden and Faden 76/521* (PDA, US); along Forest Department logging road, 2–3 miles east of turnoff, 3 miles north of Udugama on road to Hiniduma, *Theobald and Grupe 2379* (USA); along Forest Department logging road, 5.6 miles east of turnoff, 3 miles north of Udugama on road to Hiniduma, *Theobald and Grupe 2383* (PDA, US); Windsor Forest east of Dolosbage or road to St. Helen's Tea Estate, *Theobald and Grupe 2402* (PDA, US); Kanneliya Forest, Hiniduma, *Kostermans 24733* (K, US); Gilimale Forest Reserve, *Meijer 597* (US); **KALUTARA DISTRICT:** Abandoned timber camp, Nalella Ela [Ela = stream], Delgoda, *Jayasuriya and Balasubramaniam 4269* (PDA); near logging site, Morapitiya, *Cramer 4181* (E, PDA, K, US); Runakanda PR, *Jayasuriya and Balasubramaniam 5239* (PDA); behind the Buddhist monastery, Athwelthota, Morapitiya Forest Reserve, *Ranasinghe 1053* (PDA). **RATNAPURA DISTRICT:**

Dotalugaha, *Cramer 4706* (E, PDA, US); way to Adavi Kanda [kanda = mountain], Erathna, *de Silva 122* (PDA); near Waddagala Virgin Forest, Sinharaja Forest Reserve, *Hepper, Maxwell and Fernando 4539* (US); south of Weddagala, Sinharaja Forest Reserve, *Hepper, Maxwell and Fernando 963* (K, US); along Halmandiya Dola (dola = stream); Sinharaja Forest Reserve, *Jayasuriya and Balasubramaniam 3889* (PDA); Kalawana Forest Range, Karawita PR, *Jayasuriya and Balasubramaniam 5123* (PDA); transect 125B, near stream, Pitakele, approach Kudawa, *Jayasuriya and Wijesinghe 7948* (PDA); Titta Weralu Kotha, *s.coll. s.n.* (PDA); near stream in Sinharaja forest, ca 4 miles south of Atweltota, along forest department logging road, Atweltota, *Theobald and Krahulik 2806* (PDA); Erathna, *Trimen s.n.* (PDA); Hopewell Forest, *Waas 1602* (PDA); Warukandeniya, *Waas 1987* (E, PDA [two sheets], MO); Weddagala, Sinharaja Forest Reserve, *Weerasooriya and Jayasekera CER2080* (K); at the middle part of Mulawella New trail from Maguruwalla, Sinharaja Forest, *Ranasinghe 1017* (PDA); Pitakelle area, Sinharaja Forest, *Ranasinghe 1010* (PDA); on the way to Nawada Tree trail, Sinharaja Forest Reserve, Kudawa, *Ranasinghe 1061* (PDA). **LOCALITY UNKNOWN:** *s.coll. s.n. CP 358* (P [two sheets], PDA,W [three sheets]); *s.coll. s.n.* (K); *Mrs Walker s.n.* (K); *Gardner s.n.* part of *CP 358* (PDA).

Rhynchotechum Blume

Bijdr. Fl. Ned. Ind. 775 (1826); G.Don, Gen. Hist. 663 (1838); Endl., Gen. Pl. 719 (1839); R.Br. in Benn., Pl. Jav. Rar. 122 (1840); DC., Prodr. 9: 285 (1845); Miq., Fl. Ned. Ind. 2: 749 (1858); Benth. in Benth. and Hook.f., Gen. Pl. 2: 1016 (1876); C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 194 (1883); C.B.Clarke in Hook.f., Fl. Brit. India 4: 372 (1884); Fritsch in Engl. and Prantl, Nat. Pflanzenfam. 4(3b): 159 (1894); B.L.Burtt, Notes Roy. Bot. Gard. Edinburgh 24: 36 (1962). Type: *Rhynchotechum parviflorum* Blume.

Isanthera Nees, Trans. Linn. Soc. London 17: 82 (1834); Endl., Gen. Pl. 668 (1839); DC., Prodr. 9: 279 (1845); Benth. in Benth. and Hook.f., Gen. Pl. 2: 1016 (1876); C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 191 (1883); C.B.Clarke in Hook.f., Fl. Brit. India 4: 372 (1884); Fritsch in Engl. and Prantl, Nat.Pflanzenfam. 4(3b): 159 (1894). Type: *Isanthera permollis* Nees.

Corysanthera Wall. ex Endl., Gen. Pl. 719 (1839); L.E.Skog, Baileya 20: 1 (1976). Type: *Corysanthera elliptica* Wall. ex D.Dietr.

[*Cheilosandra* Griff. ex Lindl., Veg. Kingd. 672 (1846) nom. nud. pro syn.; Benth. In Benth. and Hook.f., Gen. Pl. 2: 1025 (1876).]

Chiliandra Griff., Not. Pl. Asiat. 4: 150 (1854); Benth. in Benth. and Hook.f., Gen. Pl. 2: 1025 (1876). Type: *Chiliandra obovata* Griff.

Habit subshrubs erect or decumbent. **Stem** unbranched, occasionally irregularly branched, woody at base, leaf scars conspicuous, glabrous. **Leaves** alternate (in Sri Lanka), closely aggregated towards the apex, petiolate. Young leaves and stem apices often densely hairy, the hairs becoming less dense with age. **Inflorescences** compound cymes, pedunculate from the axils of leaves or leaf scars or with the peduncle reduced and the inflorescence branches appearing fascicled; bracts linear to triangular at branch points and below some pedicels. **Calyx** 5-lobed, divided to near the base, persistent and surrounding the fruit. **Corolla** short tubular and two-lipped, the upper lip of two lobes, the lower of three; the upper lip typically smaller and often with some colouration towards the base. **Disk** small, surrounding the ovary at the base. **Stamens** four fertile plus one staminode, attached near the base of the corolla tube, the filaments twisted, the anthers globose with pollen sacs confluent and opening by a longitudinal slit with a valve-like dehiscence. **Pistil:** ovary of two carpels, unilocular; placentation parietal, the placentae nearly touching to make the ovary bilocular; style single, persistent in fruit, though maybe broken off. **Fruit** fleshy and indehiscent, green when immature becoming white at maturity, rarely brown; seeds numerous, very small, irregular ellipsoid, dimpled or grooved.

Note: According to the most recent revision of *Rhynchotechum* by Anderson and Middleton (2013) there are 16 species distributed from India to China, north to the Ryukyus in Japan, south through the Philippines and the Malay Peninsula to Sumatra and east to Papua New Guinea. One species *Rhynchotechum permolle* (Nees) B.L.Burtt is recorded from Sri Lanka and India and perhaps also from Burma (Theobald and Grupe, 1981).

Rhynchotechum permolle (Nees) B.L.Burtt, Notes Roy. Bot. Gard. Edinburgh 24: 39 (1962); Ramamoorthy in C.J.Saldanha and Nicolson, Fl. Hassan Dist. 531 (1976); W.L.Theob. and Grupe in Dassan. and Fosberg, Revis. Handb. Fl. Ceylon 3: 105, Fig. 8 (1981). – *Isanthera permollis* Nees, Trans. Linn. Soc. London 17: 82 (1834); Walp., Repert. Bot. Syst. 3: 124 (1844); DC., Prodr. 9: 280 (1845); Wight, Icon. Pl. Ind. Orient. 4: pl. 1355 (1848); Walp., Ann. Bot. Syst. 3(1): 99 (1852); Miq., Fl. Ned. Ind. 2: 749 (1858); C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 192 (1883); C.B.Clarke in Hook.f., Fl. Brit. India 4: 372 (1884). Type: Unknown locality [probably India], *Heyne 9073* (lectotype K [K000858004], designated by Anderson and Middleton, 2013); isolectotypes GH, K). (Figures 3.5 and 3.17, Map, pp. 89).

[*Cyrtandra lanuginosa* R.Br. in Wall., Numer. List 7131 (1832) nom. nud.]

Isanthera floribunda Gardner, Calcutta J. Nat. Hist. 6: 483 (1846); Walp., Ann. Bot. Syst. 3(1): 99 (1852). Type: Ceylon [Sri Lanka], *Gardner 605* (lectotype K [K000831996], designated by Anderson and Middleton, 2013; isolectotypes BR, K x 2).

Isanthera permollis Nees var. *paucinervia* C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 192 (1883). Type: Lower Burma, Mergui, *Griffith s.n.* (holotype K [K000735006]).

Subshrub 10–60 cm tall. **Stem** 5–6 mm wide. **Leaves** petiole 1–3 cm long, densely pubescent with rusty brown hairs; blade obovate to narrowly obovate or oblong to elliptic, upper surface dark green to green, lower surface pale green and yellow-rusty, length 7–25 cm, width 3–9 cm, 2–3 times as long as wide, apex acuminate or caudate to acute, margin denticulate, the teeth up to 1 mm long, base narrowly cuneate; lateral vein pairs 12–14; upper surface finely pubescent, hairs more dense on the midvein; lower surface woolly to rarely subglabrous, more dense on the rusty-brown veins. **Inflorescences** axillary, length 1–5 cm, 2–4 times-branched; peduncle 1–1.8 cm; first branch 0.5–2 cm long; second branch 0.5–1 cm long or absent; axes yellow rusty sericeous/villous to densely so; bracts linear to triangular, c. 0.5–1 cm long; pedicels 0.2–1 cm long, yellow-rusty, sericeous/villous. **Calyx** 5-lobed, lobes triangular, apices caudate, rarely toothed, c. 0.5 cm long, 0.2–0.3 cm wide, densely

yellow-rusty sericeous. **Corolla** zygomorphic, white, outer glabrous; upper lip 2-lobed, lobes c. 0.2–0.4 cm long, c. 0.2–0.3 cm wide, apices rounded to obtuse; lower lip 3-lobed; lobes 0.3–0.5 cm wide, 0.2–0.4 cm long, apices rounded to obtuse; tube 1–1.5 mm long, white. **Stamens** four, filaments minute, c. 0.2 cm long; anthers glabrous to slightly pubescent with glandular hairs; staminode minute. **Pistil** globose-rounded; ovary finely pubescent; 0.2 cm long; stigma lower lip truncate. **Fruit** ovoid-globose, white berry, c. 1 cm long, c. 1 cm wide, puberulent, short-stalked glandular hairs on outer wall; seeds 0.3–0.35 mm long, ovate, testa brown, with short hexagonal cells.

Distribution. India, Sri Lanka and possibly Burma (Anderson and Middleton, 2013).

Habitat and Ecology. Growing in evergreen and monsoon forests, sometimes dry or disturbed, often near streams (Theobald and Grupe, 1981), on moist clay or sandy soils, sometimes on steep slopes, at 250–1200 m.

Phenology. Flowering and fruiting June to November, probably year round (Theobald and Grupe, 1981).

Note. Usually the Sri Lankan specimens have 12(–14) pairs of lateral veins. This agrees with observations by Anderson and Middleton (2015) that *R. permolle* in Sri Lanka have different veins numbers than those in India (9–20 pairs).

Provisional IUCN Conservation Assessment. According to Anderson and Middleton (2013) this species can be categorized as Least Concern (LC) because of its common and widespread distribution. Considering *Rhynchotechum permolle* in Sri Lanka alone, the extent of occurrence (EOO) was calculated as 5174 km^2 and area of occupancy (AOO) with 84 km^2. Number of locations found to be ≤10 (Figure 3.17). This species is quite widespread in the lowland wetzone and submontane forests of Sri Lanka. However, as mentioned under the conservation assessment of *Championia reticulata,* lowland wet forests are under threat of habitat destruction

Figure 3.5 *Rhynchotechum permolle* (a) habitat on forest floor; (b) branch; (c) axillary inflorescence.

and fragmentation. Fieldwork in the submontane zone for this study also found land clearance for **u**rbanisation or agricultural land conversion to threaten the species. Therefore a continuing decline of these populations can be projected under EOO, AOO and quality of habitat. Therefore the present situation of *R. permolle* can be assessed as Vulnerable (VU) with both EOO and AOO under criteria B1ab(i,ii,iii) +B2ab(i,ii,iii).

Additional Specimens Examined: KANDY DISTRICT: Galamuduna Estate, Dolosbage, 5 miles from Dolosbage to Bulathkohupitiya road, *Ranasinghe 865* (E, PDA). **MATALE DISTRICT:** Rangala, Sept. 1888, *s.coll. s.n.* (PDA). **NUWARA ELIYA DISTRICT:** Seven Virgin Hills, Peak Wilderness Sanctuary, *Ranasinghe 1033* (E, PDA). **RATNAPURA DISTRICT:** Mulawalla, Sinharaja Forest Reserve, *Ranasinghe 1018* (E, PDA); Pitakelle, Sinharaja Forest Reserve, Ranasinghe 1009 (E, PDA). **LOCALITY UNKNOWN:** *s.coll. s.n. CP 1670* (PDA).

Henckelia Spreng.

Anleit. Kenntn. Gew., ed. 2, 2(1): 402 (1817). – *Henckelia* sect. *Henckelia* Weber and Burtt in Beitr. Biol. Pflanzen 70: 334 (1998 [‘1997’]). – *Didymocarpus* sect. *Orthoboea* Benth. in Benth. and Hook.f., Gen. Pl. 2(2): 1022 (1876). Type: *Henckelia incana* (Vahl) Spreng.

Chirita Buch.-Ham. ex D.Don, Edinburgh Philos. J. 7: 83 (1822). – *Chirita* sect. *Euchirita* C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 111 (1883). – *Didymocarpus* sect. *Euchirita* (C.B.Clarke) Chun in Sunyatsenia 6: 294 (1946). – *Roettlera* sect. *Euchirita* (C.B.Clarke) Fritsch in Engler and Prantl, Nat. Pflanzen. IV/3b: 148 (1895). Type (lectotype designated by Burtt, 1954): *Chirita urticifolia* Buch.-Ham. ex D.Don (= *Henckelia urticifolia* (Buch.-Ham. ex D.Don.) A.Dietr.).

Calosacme Wall., Numer. List: 800–806 (1829). All species names established in *Calosacme* by Wallich (1829) are nomina nuda.

Babactes DC. ex Meisn., Pl. Vasc. Gen. 1: tab. diag. 302, Comm. 211 (1840). Type: *Babactes oblongifolia* (Roxb.) DC. (= *Henckelia oblongifolia* (Roxb.) D.J.Middleton and Mich.Möller).

Gonatostemon Regel, Gartenflora 15: 353 (1866). Type: *Gonatostemon boucheanum* Regel (= *Henckelia urticifolia* (Buch.-Ham. ex D.Don.) A.Dietr.).

Ceratoscyphus Chun in Sunyatsenia 6: 276 (1946). Type: *Ceratoscyphus caerulea* Chun (= *Henckelia ceratoscyphus* (B.L.Burtt) D.J.Middleton and Mich.Möller).

Hemiboeopsis W.T.Wang in Acta Bot. Yunnan. 6: 397 (1984). Type: *Hemiboeopsis longisepala* (H.W.Li) W.T.Wang (= *Henckelia longisepala* (H.W.Li) D.J.Middleton and Mich.Möller).

Habit perennial or annual herbs, sometimes woody at base; caulescent with conspicuous or inconspicuous internodes, occasionally a creeping habit. **Leaves** opposite, alternate or whorled, possibly clustered at the stem apex or bearing only one or two leaves, petiolate, variable lamina shape, entire or pinnately lobed. **Inflorescences** predominantly axillary but sometimes pseudo-terminal with the reduction of the vegetative shoot; flowers 1–15; bracts paired, whorled, free or united at base, orbicular to linear, narrowly ovate or narrowly triangular, caducous when young. **Calyx** 5-lobed, triangular or narrowly triangular in shape, lobes free or basally united to form a tube, deeply divided on dorsal side especially when the calyx lobes are united. **Corolla** infundibuliform or rarely tubular, bilabiate, upper lip formed by 2 lobes and the lower lip formed by 3 united lobes, variable in colour with characteristic yellow marking present or absent in throat; tube slightly pouched, rather constricted in the throat. Stamens 2 fertile; filaments geniculate or straight; anthers fused face to face, glabrous or pubescent. **Disk** a simple annular ring or 5-lobed, often very small. **Pistil:** ovary shortly stipitate or not; stigma often chiritoid (lack of development of the upper lobe and the often bifid development of the lower lobe), the upper lobe of the stigma is reduced and the lower lobe is expanded or rarely almost truncate, bifid or not. **Fruit** a capsule, splitting along 2 valves or along the dorsal side, stipitate or not, plagiocarpic or not, calyx persistent or caduceus; seeds numerous, minute, ellipsoid, unappendaged.

Note: After its recent restructuring, *Henckelia* Spreng. has approximately 56 species with a distribution in Northeast and South India and Sri Lanka, the Himalayas and continental SE Asia, excluding Peninsular Thailand and Malaysia (Möller *et al*., 2011; Weber *et al*., 2011). The Sri Lankan species had previously been assigned to

two genera: *Chirita* Buch-Ham. ex D.Don (Woods, 1974) with four species and *Didymocarpus* Wall. (Wallich, 1828-1849) with three species. When the genus *Henckelia* was re-established by Weber and Burtt (1998), the three species of *Didymocarpus* occurring in Sri Lanka were moved into *Henckelia*. Later, based on molecular phylogenetic data (Möller *et al*., 2011; Weber *et al*., 2011), the four species of *Chirita* were also moved into *Henckelia*. Six out of these seven species are endemic to Sri Lanka: *H. angusta*, *H. communis*, *H. moonii*, *H. walkerae*, *H. floccosa* and *H. zeylanica*. The only species not endemic to Sri Lanka is *H. humboldtiana*, which occurs in both Sri Lanka and South India. All species have a rather restricted distribution in Sri Lanka, with their localities in undisturbed moist montane and lowland forests of the central and southwestern parts of the island. Based on molecular-morphological work in the present study, an additional species, *H. wijesundarae*, is recognized, bringing the species number to eight.

Key to the species

1.a Acaulescent herbs or creeping rhizomatous herbs **2**
1.b Caulescent herbs or shrubs with branched or unbranched stem **4**

2.a Rhizome erect, short; leaves in basal rosette, usually rugose; corolla tube without prominent dark veins; capsules pubescent **3**
2.b Rhizome creeping, elongate; leaves scattered along rhizome, not rugose; petiole un-winged; corolla tube with prominent dark veins; capsules glabrous…….....**8. *H. zeylanica***

3.a Peduncles and calyx floccose, corolla tube distinctly inflated and constricted at throat .....**6. *H. flocossa***
3.b Peduncles and calyx villous or pubescent, corolla tube not distinctly inflated and constricted at throat .....**7. *H. humboldtiana***

4.a Caulescent shrubs of rock cliffs; flowers solitary, sometimes 2 in leaf axils, 1–3- or 3–4-flowered in upper leaf axils..... **5**
4.b Caulescent herbs of stream beds or moist areas; flowers in paniculate compound dichasium **7**

5.a Calyx free to nearly base; corolla 6 – 7 cm long; flowers solitary or rarely 2 in upper leaf axils.....**3. *H. moonii***
5.b Calyx 5-parted, lobes distinct about half way; corolla up to 6 cm long; **6**

6.a Leaf upper surface sericeous with densely appressed short straight hairs; calyx lobes free to half of the length of the calyx, regularly spaced, calyx more than 2 cm long..... **4. *H. walkerae***

6.b Leaf upper surface pubescent with fine silvery and purple hairs throughout; calyx lobes divided to 1/3 to less than half of calyx length, apparent 2+2+1 arrangement of calyx lobes with the single calyx lobe on the adaxial side…….....**5. *H. wijesundarae***

7.a Leaves ovate-lanceolate or oblong-lanceolate to elliptical; corolla tube angular……**1. *H. angusta***

7.b Leaves broadly ovate; corolla tube not angular ..... **2. *H. communis***

1. ***Henckelia angusta*** (C.B.Clarke) D.J.Middleton and Mich.Möller, Taxon 60: 774 (2011). – *Chirita zeylanica* var. *angusta* C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 113 (1883); Trimen, Handb. Fl. Ceylon 3: 276 (1895). – *Chirita angusta* (C.B.Clarke) W.L.Theob. and Grupe in Ceylon J. Sci., Biol. Sci. 10(1): 70 (1972); W.L.Theob. and Grupe in Dassan. and Fosberg, Revis. Handb. Fl. Ceylon 3: 95–96, Fig. 4.aandb (1981). Type: Sri Lanka: *Thwaites s.n., CP* 3437 (lectotype BM [BM000997766], designated here). (Figures 3.6 and 3.18, Map, pp.90).

Herb caulescent, suffruticose, 10–30 cm tall. **Stem**s unbranched or few-branched, mature stem erect, perhaps creeping on the rock surface, 4–5 mm in diam., glabrous; young stem green, cylindric, 2–4 mm in diam., silky-pubescent with densely appressed silvery white hairs; leaf scars present but not prominent on mature stems. ***Leaves*** opposite decussate; petiole light green, cylindrical, usually 0.5–2 cm, densely pubescent with fine silvery white hairs throughout; leaf blade pale green on lower surface, bright green on upper surface, narrowly ovate to lanceolate, length 5–14 cm, width 0.8–3 cm, fleshy (thin when dried), base tapering, cuneate to attenuate, slightly unequal, margin entire, apex acute to attenuate pinnately veined, lateral veins in 5–6 pairs, rarely oblique, conspicuous on lower surface, densely pubescent on upper surface, less pubescent on lower surface, hairs aggregated on midrib and lateral veins. ***Inflorescences*** predominantly a paniculate, compound cyme; peduncles usually 10–12 cm long, glabrous; bracts ovate to lanceolate, 2–6 mm long, 2–4 mm wide, caducous; pedicels 0.5–1 cm long, glabrous. ***Calyx*** 5-lobed; calyx lobes

divided to near base, lobes regularly placed, narrowly ovate-lanceolate to lanceolate, 2.5–3 cm long and 2–3 mm wide, glabrous on both surfaces. ***Corolla*** 4–5 cm long, 5-lobed, lobes pale-bright purple, suborbicular, lower lip with 3 lobes 5–6 mm long, 6–9 mm wide; upper lip with 2 lobes slightly smaller than those of lower lip, corolla tube angular, white with purple tinge towards the lobes, c. 2.5 cm long, 1–1.5 cm wide at the throat, 2 longitudinal ridges along the floor of the tube and lower lip, a broad yellow line along the ridges inside the tube, glandular pubescent on the outer surface and less so on the inner surface. ***Stamens*** filaments white, geniculate, swollen at bend, twisted in lower half, glabrous to sparsely pubescent, lower part 4–4.5 mm long, upper part 5–6 mm long; anthers yellow, coherent, c. 2 mm long and c. 1 mm wide, tuft of hairs on back; staminodes 3, 2 lateral ones hooked, twisted near base, pubescent and with a tuft of hairs near swollen apex, c. 4 mm long, median one inconspicuous. ***Disk*** lobes crenate. ***Pistil*** linear; ovary linear, c. 1 cm long, glandular pubescent with short-stalked glands; style glandular-pubescent, c. 1 cm long; stigma upper lobe inconspicuous, lower lobe expanded laterally into rhomboidal to elliptical flattened plate, 2 mm long, 4 mm wide. ***Fruit*** a linear capsule, glabrous, 5–8 cm long; seeds minute, numerous, oblong to elliptic, ≤ 0.5 mm long, testa brown.

Distribution. Species is endemic to Sri Lanka. Distributed in forested areas of the low country from Galle District to the foothills above Ratnapura.

Habitat and Ecology. Found in habitats similar to those of *H. communis*, with which its distribution overlaps at lower elevations. *Henckelia angusta* is usually found on rocks and boulders along or in small streams in undisturbed forests, usually in dense shade.

Phenology. Flowering in March and August; probably irregularly throughout the year. Fruiting is from September to October.

Note. *Henckelia angusta* is morphologically distinct enough from *H. communis* to be recognized as a separate species, as proposed by Theobald and Grupe (1972) (Table

3.1), despite the lack of molecular differentiation between the two species (Chapter 2). The two taxa are likely to have diverged very recently.

Lectotypification of *Chirita zeylanica* var. *angusta.* Thwaites (1864) recognized a variety in *Chirita zeylanica*, var. β, an endemic of Sri Lanka, in his *Enumeratio Plantarum Zeylaniae,* citing specimen *CP 3437*. He described the location of the species as at the edge of a rivulet in the Singherajah (Sinharaja) forest between Galle and Ratnapoora. Later, Clarke (1883) named this variety as *Chirita zeylanica* var. *angusta*, and cited several specimens of the same number *CP 3437* at various herbaria as "Ceylon; inter Galle et Ratnapoora (Thwaites C.P., n. 3437 in hh. Kew, Mus. Brit., DC, Berol., Paris)". The present study found five herbarium sheets of *CP 3437* at different herbaria, one at BM [BM000997766], two at K (one sheet [K000858374], the second sheet with [K000838575] + [K000838576]) and two at PDA. None of these specimens contain the locality data noted by Thwaites. However, all three sheets at BM and K have pencil-written annotations made by Clarke. Also, the sheets at K have putative hybrid specimens along with proper *H. angusta* specimens on the same sheets. Therefore, the specimen CP 3437 at BM [BM000997766], which has three specimens with *Chirita zeylanica* var. *angusta* characters, is selected as the lectotype.

Provisional IUCN Conservation Assessment. The present study found only a single population of *Henckelia angusta*, in the Sinharaja Forest Reserve. Although this species was collected from other 5–6 locations in the past, from this reserve and from one location at Hiniduma in Galle, none of these populations exist today. Most of these previous habitats have been turned into tea cultivations or have been otherwise destroyed. On the other hand, a putative hybrid was observed in the few habitats where *H. angusta* was recorded previously. This is interesting in two aspects; firstly, that they may exist further as yet undiscovered populations, and secondly, that the habitat disruptions may have brought the two species *H. angusta* and *H. communis* in closer contact allowing hybridization to occur. The oldest specimen of this putative hybrid was recorded from the same area in Sinharaja forest by Hepper, Maxwell and Fernando in 1972. This has been observed in other

Figure 3.6 *Henckelia angusta* (a) habitat on rocks in a stream; (b) a flowering branch; (c) putative hybrid between *H. angusta* and *H. communis*.

Gesneriaceae before (Hilliard and Burtt, 1971). The number of mature individuals observed in the population in 2013, 2014 and 2015 was less than 250. The population is existing in a protected area, a forest reserve which is also recognized as a world heritage site. Therefore, any threat from human influence is likely to be negligible. However, no conservation measures on this particular population have been applied so far. Given the reduction in number of mature individuals in recent times, *H. angusta* should be categorized as endangered (EN) under criterion D.

Additional Specimens Examined. **GALLE DISTRICT:** along road in the rivulet, Hiniduma, *Kostermans 25004* (PDA, US). **MATARA DISTRICT:** Morawaka Korale, *Trimen s.n.* (PDA). **RATNAPURA DISTRICT:** Halmandiya Dola, near the research station, Sinharaja Forest Reserve, *Ranasinghe*, *1014* (PDA); 9 miles SW into forest from Weddagala, Sinharaja Forest Reserve, *Bremer and Bremer 892* (K, PDA, US); Waddagala, Sinharaja Forest Reserve, *Hepper, Maxwell and Fernando 4541* (PDA, US); south of Waddagala, Sinharaja Forest Reserve, *Hepper, Maxwell and Fernando 965* (Kx02, PDA, US); Kehelwatupola near Kudawe, Weddagala, *Hoogland 11447* (E, Lx02, PDA, US); Warukandeniya, Sinharaja Forest Reserve, *Waas 1977* (K, MO, PDA, US); 4 miles into Sinharaja forest on track Panapola-Deniyaya, Sinharaja Forest Reserve, *Wambeek 2789* (US); under shade along rock banks of Thundola Ela, Sinharaja Forest Reserve, *Cramer and Weerasooriya 6735* (K); forest of SW Ceylon, on wet rock in dark shady rivulet, Sinharaja Forest Reserve, *Kostermans 26695* (L); along Halmandiya Dola, Sinharaja forest, *Jayasuriya and Balasubramaniam 3909* (PDA); Halmandiya Dola, near research station, Sinharaja forest, *Jayasuriya, Gunatilleka and Gunatilleka 3628* (PDA). Sinharaja forest, Kukul Korale, *Thwaites s.n.*, *CP 3437* (K, PDA).

2. *Henckelia communis* (Gardner) D.J.Middleton and Mich.Möller *sensu stricto*, Taxon 60: 775 (2011). – *Chirita communis* Gardner, Calcutta J. Nat. Hist. 4: 481 (1846). – *Roettlera communis* (Gardner) Kuntze, Rev. Gen. Pl. 2: 476 (1891). Type: Sri Lanka, *Gardner 602* (lectotype K [K000858753], designated here). (Figs 3.7 and 3.18, Map, pp 90).

Chirita zeylanica Hook., Bot. Mag. 71: pl. 4182 (1845); Thwaites, Enum. Pl. Zeyl. 208 (1864); C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 112 (1883); Trimen, Handb. Fl. Ceylon 3: 276 (1895); W.L.Theob. and Grupe in Dassan. and Fosberg, Revis. Handb. Fl. Ceylon 3: 93–95, Fig. 4.c (1981). Type: Sri Lanka, (lectotype, Hook. Bot. Mag. 71: t. 4182 (1845), designated here).

[*Chirita vulgaris* Morren Belgique Hort. 3: 238, pl. 36. (1853) *nom. nud*.]

Herb caulescent, suffruticose, c. 30–60 cm tall. **Stem** branched or unbranched, mature stem cylindrical, 5–6 mm in diam., sparsely pubescent or glabrous, young stem cylindrical, 3–4 mm in diam., silky-pubescent; leaf scars present but not prominent on mature stems. **Leaves** opposite decussate; petiole light green or purple, cylindrical, usually 4–6 cm or extended up to 10 cm, densely pubescent with fine silvery hairs throughout; blade broadly ovate to lanceolate, length 8–15 cm, width 4–8 cm, fleshy (thin when dried), bright green upper surface, pale green lower surface, or maroon to purple upper and lower surfaces, base rounded to obtuse sometimes cuneate, slightly unequal, margin entire, apex attenuate to acuminate, lateral veins in 8–10 pairs, sometimes oblique, conspicuous on lower surface, less pubescent on lower surface and silky to pubescent on upper surface. **Inflorescences** axillary, predominantly a paniculate, compound dichasium; peduncles usually 8–12 cm or up to 18 cm long, glabrous to sparsely pubescent; bracts ovate to lanceolate, caducous, 2–6 mm long, 2–4 mm wide; pedicels 0.5–1 cm long. **Calyx** 5-lobed; lobes almost free but united at the base for 2–3 mm; lobes regularly placed, lanceolate, 1–1.5 cm long, 4–6 mm wide, glabrous, apex bluntly acute. **Corolla** 4–5 cm long, 5-lobed, 3–4 cm long, pale purple, suborbicular, lower lip 3 lobed, c. 1 cm long, 0.8–1 cm wide, upper lip 2-lobed, slightly shorter than those of lower lip, c. 0.8 cm long, c. 1.2 cm wide; corolla tube white with a purple tinge, 2.5–3 cm long, 1–1.2 cm wide at throat, absence of angular tube, 2 longitudinal ridges along floor of tube and lower lip, two broad yellow lines along the ridges, glandular pubescent on outside of tube and lobes, inner glabrous except for the distribution of hairs towards the adaxial side. **Stamens** filaments white, geniculate, swollen at bend, twisted in lower half, lower arm 4–6 mm long and glabrous, upper arm 5–6 mm long with scattered glandular pubescent hairs; anthers yellow, coherent, 1.8–2 mm long and 0.8–1 m wide, tuft of

hairs on back; staminodes 3, 2 longer, 6–6.5 mm long, twisted near base, pubescent and with a tuft of hairs near swollen apex, third smaller. **Pistil** linear, c. 1 cm long, ovary glabrous or minutely glandular pubescent; style c. 1 cm long, glandular-pubescent; stigma chiritoid, lower lobe broadly expanded laterally into rhomboidal or elliptical flattened plate, 4–5 mm long, 2–3 mm wide. **Disk** lobes crenate**. Fruit** a capsule, long-linear, glabrous, 5–10 cm long, 3–4 mm wide; seeds numerous, oblong-elliptic, c. 0.5 mm long, testa brown.

Distribution. Endemic to Sri Lanka. This is the second-most widespread species of Gesneriaceae in Sri Lanka after *Henckelia humboldtiana*, distributed from lowland wet forests to upper montane forests at elevations between c. 150–1500 m.

Habitat and Ecology. The species is usually found in rocky areas along streambeds and wet forest floors of undisturbed forests, usually in dense shade.

Phenology. Flowering in June-August, and perhaps irregularly throughout the year. Fruiting in late August to October.

Lectotypification of *Henckelia communis***. This species was originally described by Gardner (1864) as *Chirita communis* and later revised under genus *Henckelia* (Weber** *et al*., 2011). Looking for the original material at the time of Gardner's description there are two sheets with the same number *Gardner 602* as mentioned by Theobald and Grupe (1981), one at K and the second at BM. However, the sheet in BM could not be located. There are two specimens of *C. communis* mounted on the sheet *Gardner 602* which is available at K. These two specimens are bearing flowers, fruits and other vegetative characters that match with the original description made by Gardner. Therefore, the herbarium sheet at K, K000858373, is selected as the lectotype for *Henckelia communis*.

Lectotypification of *Chirita zeylanica*. Hooker (1845) described this species. However, the same taxon was described by Gardner (1846) as *Chirita communis*

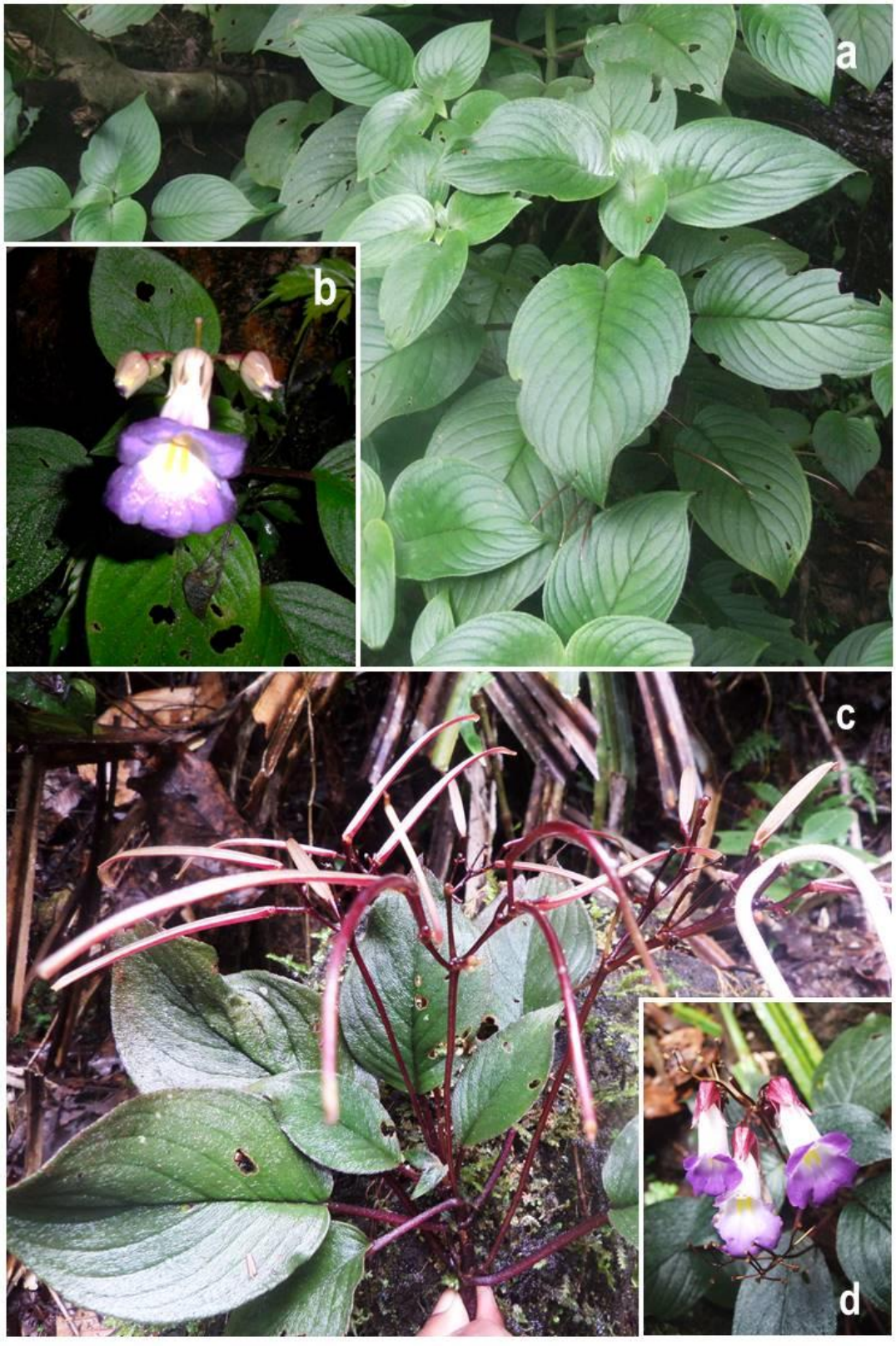


Figure 3.7 *Henckelia communis*, "communis green" (a) habit; (b) flowering branch; "communis purple" (c) a branch with capsules; (d) inflorescence with open flowers.

without mentioning Hooker's work. Later Theobald and Grupe (1981) used Hooker's name in their revision based on priority given for the species described. In the restructuring work for *Henckelia* Weber *et al*. (2011) recognized this species as *Henckelia communis* (Gardner) D.J. Middleton and Mich. Möller and *C. zeylanica* is recognized as heterotypic synonym. It was not possible to use *zeylanica* here as the name is *Henckleia zeylanica* already occupied in the genus to accommodate *Didymocarpus zeylanicus* R. Br.. None of these taxonomic treatments designated a type specimen for *C. zeylanica*. It is common with Hooker's names described from living material that a specimen is deposited in K (pers. comm. D.J. Middleton). But no specimen was found at K with the name *C. zeylanica* that could be traced back to Hooker's work. It is recommended to lectotypify the name on the illustration if the plant material clearly mentioned in the protologue is now lost (Art. 9.12; McNeill *et al.*, 2012). Therefore, the illustration, t. 4182 (Hooker, 1845) is selected as the lectotype specimen.

Note. Two morphologically distinct types of *Henckelia communis* are recognized in the present study, morphotype 1 with green leaves, "communis green" and morphotype 2 with maroon-purple leaves, "communis purple". Moreover, *H. communis* has close morphological affinities to *H. angusta.* Making the taxonomy even more complex is the presence of a putative hybrid between the two species. Because of the lack of conclusive molecular data obtained in Chapter 2, this revision considers the two morphotypes of *H. communis* to be one distinct species, and *H. angusta* to be another. See also Figure 3.6, 3.7 and Table 3.2. The distribution of "communis green", "communis purple", putative hybrid and *H. angusta* given in Figure 3.18.

Table 3.2 Diagnostic features to differentiate *Henckelia communis, Henckelia angusta* and their putative hybrid.

Character	***Henckelia communis* morphotype 1**	***Henckelia communis* morphotype 2**	***Henckelia angusta***	**Putative hybrid**
Plant height	c. 30 cm or up to c. 60 cm	c. 30 cm or up to c. 60 cm	c. 10–30 cm	5–10 cm

Leaf shape	broadly ovate to lanceolate	broadly ovate to lanceolate	narrowly ovate-lanceolate	ovate-lanceolate
Leaf size (L × W ratio)	8–15 cm long, 4–8 cm wide (1.9-2)	8–15 cm long, 4–8 cm wide (1.9–2)	5–-14 cm long, 0.8–3 cm wide (4.7–6.3)	6–12 cm long 2.5–4 cm wide (2.4–3)
Leaf colour	green	maroon to purple	green	purple to green
Corolla tube	straight	straight	angular	angular
Lateral veins	7–8 pairs	7–8 pairs	5–6 pairs	5–6 pairs

Provisional IUCN Conservation Assessment. Although this species is very widespread in its distribution, most of the populations exist in fragmented forest patches. They are also found in sensitive habitats along streams or on the forest floor in shade. Their habitat specificity may increases their vulnerability to habitat destruction in the face global warming. Therefore, a continuous decline of these populations can be projected under extent of occurrence (EOO), area of occupancy (AOO) and quality of habitat. The EOO was calculated as 6096 km^2 and AOO as 160 km^2. Considering all these factors, the conservation status of *Henckelia communis* can be assessed as Vulnerable (VU) under criterion B1ab(i,ii,iii) and Endangered (EN) under criterion B2ab(i,ii,iii).

Additional specimens examined. BADULLA DISTRICT: Palugama, *Cramer 3818* (K, US); Thalpitigala on road Badulla-Passara, *Kostermans 24441* (L). **GALLE DISTRICT:** Bambarawana, *Cramer 5219* (US); Hiniduma, *Kostermans 27687* (K, L.02). **KANDY DISTRICT:** at base road ascent to the falls, Hunnasgiriya, *Cramer 4501* (L, US); 3 miles from Kothmale on road to Nawalapitiya, *Grupe 199* (US), *Theobald and Grupe 2311* (US); Kalugammana, *Silva 38* (W); near summit of Hantane Mt. 02, *Theobald and Grupe 2314* (US); 28 miles east of Raugalla on road to Looloowatte, *Theobald and Grupe 2316* (US); Sentry Box East Mt. behind Raxawa Tea Estate, east of Dolosbage, *Theobald and Grupe 2344* (US); forest patch in Galamuduna Estate (5 miles from Dolosbage to Bulathkohupitiya road), *Ranasinghe 864* (PDA). **MATALE DISTRICT:** Corbet's Gap, near highway marker 29, on road from Rangala to Looloowatte, *Grupe 241*

(US); Midlands, Matale, *Jayasuriya and Bandaranayake 1764* (L, US); Cardamom Plantation near milepost 32, Knuckles, *Ranasinghe 1047* (PDA). **KALUTARA DISTRICT:** East of Kalugala Forest, *Waas 1551* (K, L, MO). **NUWARA ELIYA DISTRICT:** Thangamalai Forest Reserve, *Cramer 4198* (L, US); *Jayasuriya and Maxwell 768* (US); between Thunmodara and Medahinna on the trail to Adam's Peak, *Jayasuriya and Gunatilleke 3188* (MO); Seven Virgin Hills, Peak Wilderness Sanctuary, *Ranasinghe 1033* (PDA). **RATNAPURA DISTRICT:** Thundola Ela, Sinharaja Forest Reserve, *Cramer and Weerasooriya 6735* (K); Sinharaja Forest Reserve, *Kostermans 26697* (L); Sinharaja Forest Reserve, south of Weddagala, *Hepper, Maxwell and Fernando 964* (US); Dotalugaha, *Cramer 4709* (US); trail from Meriyakota to Waleboda, south of Peak Wilderness, *Jayasuriya, Balasubramaniam and Greller 2839* (MO); Rassagalle, Balangoda, *Kostermans 23580* (L); *Kostermans 23602A* (L, US); near Bambarakotuwa Oya, south of Gayirenagama, *Meijer 865* (MO); on north side of road beyond Rasaggalla, Doyawela Mukalana, *Read 2205* (US); Nalella Ela, Pitakele in Sinharaja Forest Reserve, *Ranasinghe 1059* (PDA); Gloxing Falls, Helakanda, Balangoda, *Ranasinghe 10*68 (PDA); Massena Forest Reserve, Balangoda, *Ranasinghe and Wijewickrama 1078* (PDA); Mulawella, Sinharaja Forest Reserve, Kudawa, *Ranasinghe 1*057 (PDA); Wawulkele, Pitakelle, Sinharaja Forest Reserve, *Ranasinghe 1058* (PDA). **LOCALITY UNKNOWN:** *Hooker 827* (W); *Hugel 3229* (W); *s.coll. s.n.* (L); *s.coll. 4360* (W); s.*coll. s.n. CP 1788* (W.02); *s.coll. 660* (L); *s.coll. 602* (L).

Specimens Examined for the Hybrid. GALLE DISTRICT: Forest behind Duwili Ella, Kosmulla, Neluwa, *Ranasinghe 1082* (E, PDA). **KALUTARA DISTRICT:** Denihena, *Waas 1876* (L, MO); Morapitiya Forest Reserve, Athwelthota, *Ranasinghe 1052* (E, PDA). **RATNAPURA DISTRICT:** South of Weddagala, Sinharaja Forest Reserve, *Hepper, Maxwell and Fernando 964* (W, MO); Halmandiya Dola (=stream), Sinharaja Forest Reserve, *Ranasinghe 1015* (E, PDA).

3. ***Henckelia moonii*** (Gardner) D.J.Middleton and Mich.Möller, Taxon 60: 776 (2011). – *Chirita moonii* Gardner, Calcutta J. Nat. Hist. 6: 479 (1846); Thwaites,

Enum. Pl. Zeyl.: 207 (1864); C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 112 (1883); Trimen, Handb. Fl. Ceylon 3: 275 (1895). – *Roettlera moonii* (Gardner) Kuntze, Revis. Gen. Pl. 2: 476 (1891). Type: Sri Lanka, *Gardner, G. s.n.*, part of *CP 1789* (lectotype PDA, designated here). (Figures 3.8 and 3.19, Map, pp.91).

[*Martynia lanceolata* Moon, Cat. 45 (1824), nom. nud**.]**

Herb caulescent, suffruticose, 60–150 cm tall. *Stem* irregularly branched, mature stem woody, cylindrical, 0.5–1.2 cm in diameter, glabrous; young stem green, cylindrical, 0.3–0.5 cm in diameter, densely pubescent; leaf scars prominent on mature stems. **Leaves** predominantly 3-whorled at nodes, rarely decussate in some individuals; petiole light green, cylindrical, 1–4 cm long, densely pubescent with fine silver or maroon coloured hairs throughout; leaf blade upper surface green, lower surface pale green, ovate to lanceolate, length 6–15 cm, width 2–4 cm, fleshy (thin when dried), base cuneate to obtuse, slightly unequal, margin entire to shallowly **Inflorescences** flowers solitary, or rarely 2 in upper leaf axils; peduncles 2–5 cm long, pubescent; bracts small, linear, 1–2 mm long, c. 0.5 mm wide. **Calyx** 5-lobed, 2.5–3 cm long, calyx lobes free to nearly base, 2+2+1 arrangement of the lobes absent, lanceolate, keeled, glandular pubescent, apex acuminate. **Corolla** 6–7 cm long; corolla lobes 5, pale purple, suborbicular, lower lip with three lobes c. 3 cm long and c. 3 cm wide, 2 lobes of upper lip slightly smaller than those of lower lip; corolla tube white with a purple tinge, 3–4 cm long, 1–2 cm wide at throat, 2 longitudinal yellow ridges along floor of the tube extending onto lower lip, outer surface of corolla tube glandular pubescent, inside less so. **Stamens** filaments white, geniculate, swollen at the bend, twisted in lower half, upper arm 6–7 mm long and lower arm 5– mm long, glandular pubescent on upper arm; anthers coherent, c. 5 mm long with a crenulate with inconspicuous hydathodes, apex acute to attenuate, pinnately veined, lateral veins in 8–10 pairs, sometimes oblique, conspicuous on lower surface, indumentum sericeous on upper surface and densely sericeous on lower surface. 6 tuft of hairs on back; staminodes 3, 2 hooked with a tuft of hairs on their back, twisted near base, median one inconspicuous. **Disk** 5 crenate lobes. **Pistil** linear, 1–1.5 cm long; ovary glabrous or glandular pubescent; style 1–1.5 cm long,

glandular pubescent; stigma chiritoid, lower lip white, expanded. **Fruit** linear capsule, 10–16 cm long, outer surface glabrous, loculicidal dehiscence along both sutures; seeds oblong or sickle-shaped, >5 mm long, testa brown.

Distribution. Species endemic to Sri Lanka, recorded from lowland and submontane forests, at altitudes of c. 350–1400 m.

Habitat and Ecology. Growing on rock outcrops usually exposed to full sunlight.
tuft of hairs on back; staminodes 3, 2 hooked with a tuft of hairs on their back, twisted near base, median one inconspicuous. ***Disk*** 5 crenate lobes. ***Pistil*** linear, 1–1.5 cm long; ovary glabrous or glandular pubescent; style 1–1.5 cm long, glandular pubescent; stigma chiritoid, lower lip white, expanded. ***Fruit*** linear capsule, 10–16 cm long, outer surface glabrous, loculicidal dehiscence along both sutures; seeds oblong or sickle-shaped, >5 mm long, testa brown.

Distribution. Species endemic to Sri Lanka, recorded from lowland and submontane forests, at altitudes of c. 350–1400 m.

Habitat and Ecology. Growing on rock outcrops usually exposed to full sunlight.

Chromosome Count. *2n = 18* (Kiehn and Lorence, 1996).

Lectotypification of *Chirita moonii.* This species was first described by Gardner (1846). However, none of the specimens specified in the protologue regarding the original description. There is a *C. moonii* specimen deposited at PDA with pencil annotation “Hantanei” and “Gardner” on the sheet. Also the number *CP1789* is written on it. None of the other *CP 1789* specimens of *C. moonii* found at other herbaria, PDA, K, or W, are indicated as being Gardner’s collections. Therefore, the *CP 1789* specimen at PDA collected by Gardner is selected as the lectotype specimen.

Figure 3.8 *Henckelia moonii* (a) habitat on very steep wet rock outcrops; (b) close-up of flower; (c) branch with mature capsules.

Phenology. According to field observations and specimens studied in herbaria, flowering time is from July to September and fruiting time from August to November.

Provisional IUCN Conservation Assessment. The extent of occurrence (EOO) is 2782 km^2 and area of occupancy (AOO) 48 km^2. Number of locations is ≤10 (Figure 3.19). This species is not widespread in its distribution, and very fragmented and isolated in small populations throughout its distribution. It is usually found at the edge of the forests on rocks exposed to full sunshine and is vulnerable to habitat clearance and alterations. Especially this species was found distributed in the tea cultivated areas or steep soil banks along roadsides where massive road construction work carried out .Therefore, a continuous decline of these populations in the future can be projected for EOO, AOO and quality of habitat. Therefore the present situation of *Henckelia moonii* is best assessed as Endangered (EN) under criteria B1ab(i,ii,iii) + B2ab(i,ii, iii).

Additional Specimens Examined. KANDY DISTRICT: Near summit of Hantane Mt. No. 1, Kandy, *Theobald and Grupe 2338* (PDA, US); *Rangala and Alston 462* (PDA); Doluwa Kande, *s. coll. s.n.* (PDA); tea estate just off road from highway marker 24/11 on road from Rangala to Looloowatta, *Grupe 254* (PDA); on a rock face in jungle area just off-road highway maker 23/5 on road from Rangala to Loolowatte, *Grupe 219* (PDA). **KEGALLE DISTRICT:** Above railroad tracks just west of Kadugannawa, *Theobald and Grupe 2372* (US); *Theobald and Grupe 2391* (PDA, US); above railroad track 1/2 mile *Wilson 3047a* (K); Dehena Ella, Wevelwatte, Balangoda, *Ranasinghe 1067* (PDA); Massena Forest Reserve, Balangoda, *Ranasinghe and Wijewickrama 1077* (PDA). **LOCALITY UNKNOWN:** *Mrs. Walker 42* (PDA); *Walker s.n.* (E); *s. coll. s.n., CP 1789* (PDA); two specimens, *Thwaites s.n., CP 1789* (W). of Kadugannawa west between tunnel 10 and 11, *Grupe 201* (PDA); Wewiyatalawa, approach from Halgolla Estate, Yatiyantota, *Jayasuriya and Wijesinghe 8526* (PDA); Hettikande, off Lellopitiya, *Jayasuriya and Wijesinghe 7561* (PDA); Wewiyatalawa, approach from Halgolla Estate, Yatiyantota, *Ranasinghe 1048* (PDA). **RATNAPURA DISTRICT:** Medakanda, Balangoda, *Balakrishnan NBK 304* (K, MO, US); Katussagala Hill west end, *Faden and Faden 76/473* (K, PDA); tea plantation bordering the Heramitigala forest, Balangoda, *Grey-Wilson and Grey-Wilson 3047a*

4. *Henckelia walkerae* (Gardner) D.J.Middleton and Mich.Möller, Taxon 60: 777 (2011). – *Chirita walkerae* ["*walkeri*"] Gardner, Calcutta J. Nat. Hist. 6: 480 (1846. – *Roettlera walkerae* ["*walkeri*"] (Gardner) Kuntze, Revis. Gen. Pl. 2: 477 (1891). Type: Sri Lanka ["Ceylon"], *Mrs. General Walker 191* (lectotype K top left-hand specimen on sheet [K000858368], designated by Ranasinghe *et al.*, 2016). (Figures 3.9 and 3.19, Map, pp. 91).

Herb caulescent, suffruticose, 30–90 cm tall. **Stem** irregularly or dichotomously branched; mature stem cylindrical, c. 1 cm in diameter, glabrous; *leaf scars* present on mature stems; *young stem* fleshy, green, 0.3–0.5 cm in diameter, cylindrical and pubescent, fine silvery brown hairs scattered all over. **Leaves** predominantly whorled in threes at a node, rarely decussate; petioles fleshy, light green and cylindrical, about 1–2 cm long and pubescent with fine silvery hairs throughout; blade ovate to lanceolate, length 6–12 cm and width 2–5 cm, bright light green on upper surface, greenish white on lower surface, base obtuse or tapering gradually, slightly unequal, apex attenuate, leaf margin slightly serrated with conspicuous hydathodes, indumentum pubescent on lower surface, sericeous with densely appressed short straight hairs on upper surface, lateral veins 8–10(–14) pairs, usually oblique, conspicuous on lower surface. **Inflorescences** axillary, 1–2 flowered, a simple dichasium (pair-flowered cymes) or rarely a compound cyme; peduncles pubescent, 5–7 cm long; bracts linear, caducous, c. 5 mm long, c. 2 mm wide; pedicels 1–2 cm long. **Calyx** 5-lobed; tube c. 1 cm long; lobes divided to 1/2 of the calyx length, lobes regularly placed, lanceolate, keeled, densely pubescent with eglandular hairs on the outer surface, apex acuminate, 1–1.5 cm long, 2–3 mm broad. **Corolla** up to 6 cm long, 5-lobed, lobes deep purple, lower lip with 3 lobes, 7–8 mm long, 9–14 mm broad, upper lip with 2 slightly smaller lobes shorter than lower lip, round in shape,; tube 3–4 cm long, 1–1.5 cm wide at throat, white or diffuse purple-tinged, with a broad yellow line in bottom of throat, 2 longitudinal ridges along the floor of the tube and lower lip, inside glabrous except glandular pubescent basal part of tube, outside with glandular hairs especially in proximal part. **Stamens** filaments white, geniculate, swollen at bend, twisted in lower half, glabrous to sparsely pubescent, lower part 4–5 mm long, upper part 5–6 mm long; anthers yellow, coherent, c. 2 mm,

with tuft of hairs on back; staminodes 3, lateral pair hooked, twisted near base, pubescent and with a tuft of hairs near swollen apex, median one inconspicuous. **Disk** crenate, lobes 5. **Pistil** linear, c. 3 cm long; ovary glandular pubescent with short-stalked glandular hairs; style green-white, glandular pubescent; stigma chiritoid, the upper lip of stigma inconspicuous; lower lip of stigma cuneate to truncated, c. 2 mm long, c. 2 mm wide. **Fruit** a linear capsule, glabrous, 8–13 cm long, 2–3 mm broad; seeds minute, numerous, nearly ovate, more than 0.5 mm long, testa brown.

Figure 3.9 *Henckelia walkerae* (A) habitat and habit; (B) flower front view; (C) flowering branch. Reproduced from Rranasinghe *et al.*, 2016, Figure 4, pp. 222.

Distribution. Endemic species to Sri Lanka, currently known from Kandy and Nuwara Eliya districts above c. 1200 m elevations.

Habitat and Ecology. Growing in crevices and top of vertical wet rock outcrops in submontane and montane forests partially or fully exposed to the sun. In the present study, specimens of *H. wijesundarae* and *H. communis* misidentified as *Henckelia walkerae* were identified. This allowed a more narrow definition of the habitat of *Henckelia walkerae*, which is confined to montane and submontane forests.

Phenology. Flowering specimens were collected in July to September; fruiting time is September to November.

Lectotypification of *Henckelia walkerae.* This species was first described from Ceylon (= Sri Lanka) as "*Chirita walkeri*" by Gardner (1846), correctly *Chirita walkerae* under Art. 60 Note 4 and Rec. 60.C.1 of the ICN (McNeill *et al.*, 2012), based on Mrs. Walker's collections. However, Gardner (1846) did not cite any specimen number or any specific location of Mrs. Walker's collections in Sri Lanka in his description. Until now no lectotype has been designated. Gardner (1846) stated that he described the species based on part of Mrs. Walker's collections which he received from Prof. William Hooker then at Glasgow University. Gardner took them to Ceylon when he accepted his appointment as Director of the Botanic Gardens at Peradeniya. However, none of the specimens of *H. walkerae* from Mrs. Walker's collections are present at the herbarium at Peradeniya (PDA). The collections of Mrs. Walker annotated as *Chirita walkerae* are available at the herbaria in Kew (K), Edinburgh (E) and Paris (P). Study of these specimens confirmed that there is no specific location cited on those herbarium sheets, except for the then country name "Ceylon". There are three sheets at K, *Walker 191* [K000858368], *Walker 1722* [K000858369] and *Walker 177* [not barcoded]. On sheet *Walker 191* there are three specimens mounted, and only those in the top left and at the bottom left are *H. walkerae*, whereas the one at the top right belongs to *H. communis*. The other sheets contain only *H. walkerae* specimens. Another three specimens of collection number *Walker 1722* are at E, mounted on two sheets [E00155265 and E00155266]; these

were confirmed as *H. walkerae*. Only one specimen from Mrs. Walker's collection is present at P [P03884698] but it does not have a collection number. The two specimens mounted on this sheet match in morphology *H. wijesundarae*. Therefore, only specimens at K and E match the description of *H. walkerae* made by Gardner (1846). The top left and bottom left specimens on the sheet of *Walker 191* at K [K000858368] are complete for the description of *H. walkerae* including flowers and fruits. However, careful observations suggested that it is very unlikely these specimens are from a single gathering. Therefore, the top left specimen of *Walker 191* is selected as the lectotype specimen for *H. walkerae*.

Provisional IUCN Conservation Assessment. The extent of occurrence (EOO) was calculated with 595 km^2 and area of occupancy (AOO) with 24 km^2. Number of locations ≤5 (Figure 3.19). This species is also not widespread in its distribution, very fragmented and isolated in small populations throughout its range. Usually, it is found in specific habitats in forests on very steep rock outcrops, where water seepage is present. They are thus more vulnerable to habitat destruction and climatic changes that would affect the water supply. There are unpredicted climatic changes especially long dry spells experience in recent past may affect the water seepageof these rock outcrops.A continuing decline of these populations in the future can be projected for EOO, AOO and quality of habitat. Therefore, the present situation of *Henckelia walkerae* can be assessed as Endangered (EN) under criteria B1ab(i,ii,iii) + 2ab(i,ii,iii). This represents an upgrade from the MOE (2012) conservation status, Vulnerable (VU). This was partly due to a narrower distribution range due to the inclusion of previously misidentified specimens of other species.

Additional Specimens Examined. KANDY DISTRICT: Forest behind James Taylor's bungalow, Loolkondara Tea Estate, Deltota, *Ranasinghe 1051* (PDA); Kotagala Hill, just outside Hatton, *Burtt and Townsend 79* (E two sheets, K, MO, US); at base of Kabaragala Mt. behind Raxawa Tea Estate, east of Dolosbage, *Grupe 137* (PDA, US); at base of Kabaragala Mt. behind Raxawa Tea Estate, Dolosbage, *Theobald and Grupe 2357* (PDA); Raxawa, *Thwaites s.n.*, part of *CP 2843* (PDA). **NUWARA ELIYA DISTRICT:** Kikiliyamana Forest Reserve from north end of

Pundaluoya Tea Estate, *Ranasinghe 961* (PDA); Fishing Hut, Moray Estate Maskeliya, *Clayton and Jayasekera 6127* (W); Batulu Oya near fishing hut, Moray Tea Estate, *Fosberg 58135* (E, K, L, MO, US); RBG-Hakgala cutting originally brought from Horton plains, *s.coll. s.n.* (PDA); Pidurutalagala, *Thwaites CP 2843* in part (PDA). **LOCALITY UNKNOWN.** *Gardner s.n. CP 2843* (W); *Thwaites s.n. CP 2843* (P, W); *Walker 177* (K); *Walker 1722* (E.02 sheets, K), *Walker s.n.* (P).

5. *Henckelia wijesundarae* Ranasinghe and Mich.Möller, Willdenowia, 46 (2): 213-224 (2016). Type: Sri Lanka, Southern Province, Galle District, Hiniduma, forest behind Nugagala Monastery, 208 m, 26th July 2013, *Ranasinghe, S. and Wijewickrama, T. 31* (holotype PDA; isotypes E, K). (Figures 3.10, 3.11 and 3.19, Map, pp. 91).

Chirita walkerae Gardner ("walkeri") var. "β", Thwaites, Enum. Pl. Zeyl: 207 (1864). – *Chirita walkerae* var. *parviflora* C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 112 (1883). – *Chirita walkerae* ("walkeri") subsp. *parviflora* (C.B.Clarke) W.L.Theob. and Grupe, Ceylon J. Sci., Biol. Sci. 10(1): 70 (1972). Type: Sri Lanka ["Ceylon"], *Mrs. General Walker CP 542* (lectotype K, designated by Ranasinghe *et al*., 2016).

Herb caulescent, suffruticose, 30–90 cm tall. ***Stem*** dichotomously branched, mature stem cylindrical, 0.7–0.8 cm in diameter, brownish pink hairs all over the stem; leaf scars present on mature stems; young stem fleshy, about 0.5 cm in diameter, cylindrical and pubescent, fine silvery hairs scattered all over. **Leaves** predominantly whorled in threes at a node, rarely decussate; petioles fleshy, light green and cylindrical, about 2–4 cm long and pubescent with fine silvery and purple hairs throughout; blade ovate to lanceolate; colour bright light green on upper surface and greenish white on lower surface, length about 6–8 cm and width 3.5–4.5 cm; fleshy texture (thin when dried), apex acuminate, base obtuse and slightly unequal; margin shallowly crenulate with inconspicuous hydathodes; indumentum silky to pubescent on lower surfaces and more velvety on upper surface, pinnately veined; lateral veins 8–10 pairs, sometimes oblique, conspicuous on lower surface. **Inflorescences** axillary, a compound cyme, 3–4 flowers open simultaneously most of the time;

peduncles pubescent, 3.5–4.5 cm long, bracts oblong-linear, caducous, 2–3 mm long, 0.5–1 mm wide, pedicels 0.5–1.0 cm long. **Calyx** 5-lobed, tube 0.5–1 cm long, calyx lobes divided to 1/3 to less than half of calyx length, apparent 2+2+1 arrangement of calyx lobes with the single calyx lobe on the adaxial side; lobes lanceolate, keeled, hairs present on outer surface aggregated in a sharp groove along the mid-axis, apex acuminate, glandular pubescent, 0.5–1 cm long, c. 0.2 cm wide. **Corolla** 3–4 cm long; lobes 5, white, lower lip with 3 lobes 0.7–1 cm long, c. 1 cm wide, upper lip with 2 lobes slightly smaller than of lower lip, round in shape, 2 longitudinal ridges along the floor of the tube and lower lip, throat somewhat angular in outline; corolla tube white with pink tinge, c. 3 cm long, c. 1 cm wide at throat, sometimes with a broad yellow line in throat, inside glabrous, outside with glandular hairs especially in the proximal part of the tube. **Stamens** filaments white, geniculate, swollen at bend, twisted in lower half, glabrous to sparsely pubescent, lower part 4–5 mm long, upper part 5–6 mm long, toothed at apex; anthers yellow, coherent, c. 2 mm, with tuft of hairs on back, staminodes 3, lateral 2 hooked, twisted near base, pubescent and with a tuft of hairs near swollen apex, median inconspicuous. **Disk** of 5 crenate lobes. ***Pistil*** linear; ovary glandular-hairy; style green-white, glandular pubescent, 2.5–3.5 cm long; stigma chiritoid, the upper lip of stigma inconspicuous; lower lip of stigma greenish white, spatulate, 2–3 mm long and 1.5–2 mm wide. **Fruit** a capsule glabrous, linear, 6–7 cm long, 1–2 mm broad, dehiscence along both sutures; seeds minute, numerous, ovate.

Distribution. Endemic species of Sri Lanka. Currently known only from Hiniduma, Galle, in the SW of the country, at altitudes of 200–300 m.

Phenology. Flowering specimens were collected in July to September, and fruiting time is September to November.

Habitat and Ecology. Growing in the shade, on wet rock outcrops in lowland rain forests.

Etymology. The specific epithet "*wijesundarae*" is given in honour of the great botanist, scientist and former Director General of the Botanic Gardens in Sri Lanka, Dr. Siril Wijesundara.

Lectotypification of *Chirita walkerae* var. *parviflora*. Thwaites (1864) recognized two varieties under *Chirita walkerae*, "var. α" and "var. β". Later, Clarke (1883) described "var β" as *C. walkerae* var. *parviflora* citing as its basis "Thwaites C. P. n. 542 in hh. Kew, DC, Mus. Brit., Berol.", a treatment that was followed by Trimen (1895) in the Handbook to the Flora of Ceylon. During revisionary work on the Flora of Ceylon for the family Gesneriaceae (Theobald, 1972) elevated *C. walkerae* var. *parviflora* to subspecific rank as *C. walkerae* subsp. *parviflora* (C.B.Clarke) W.L.Theob. and Grupe. This was based on the same material examined by Thwaites (1864) and Clarke (1883) because no other material determined as this taxon had been collected since. However, none of these accounts cited a particular specimen of Thwaites' *CP 542* collections as the type specimen for *C. walkerae* var. *parviflora*. We have located seven herbarium sheets in different herbaria bearing the number *CP 542*. Two sheets at W with the herbarium accession numbers 162356 and 65460, originally annotated as *C. walkerae* var. β under *CP 542*, had in 2014 been annotated as isotypes of *C. walkerae* by J. Walter (annotation numbers W-1889-0162356 and W-1889-0065460 respectively). Although these two specimens do not bear flowers there are other important characters, such as leaf pubescence, lateral vein number, fruit length and leaf margin characteristics that are comparable with other *CP 542* specimens. Hence, these specimens are better referred to *C. walkerae* var. *parviflora*, i.e. *H. wijesundarae*, not to typical *H. walkerae* ("*Chirita walkerae*") and therefore this re-annotation as isotype is not in accord with the present lectotypification of *H. walkerae*. There were two specimens of *CP 542* observed from P: one sheet with *no 542* [P03884094] annotated as *C. walkerae* var β and a second sheet *CP 542* [P03884093] annotated as *C. walkerae* var. *parviflora*. There are two further herbarium sheets of *CP 542* at PDA with the same annotation. There is only one sheet at K, mounted with two specimens annotated as *C. walkerae* var. β. Our present investigation suggests that all specimens numbered *CP 542* represent *C. walkerae* var. *parviflora*. The herbarium sheet of *CP 542* at K was noted by Clarke (1883) in

Figure 3.10 ***Henckelia wijesundarae*** **– (A) habit; (B) corolla front view; (C) corolla longitudinally dissected; (D) two inflorescences; (E) two fertile stamens with toothed filaments and coherent anthers and two lateral staminodes; (F) leaf abaxial (left) and adaxial (right) surfaces; (G) calyx showing lobes divided less than ½ length of calyx. Reproduced from Ranasinghe** ***et al.*****, 2016, Figure 4, pp. 219.**

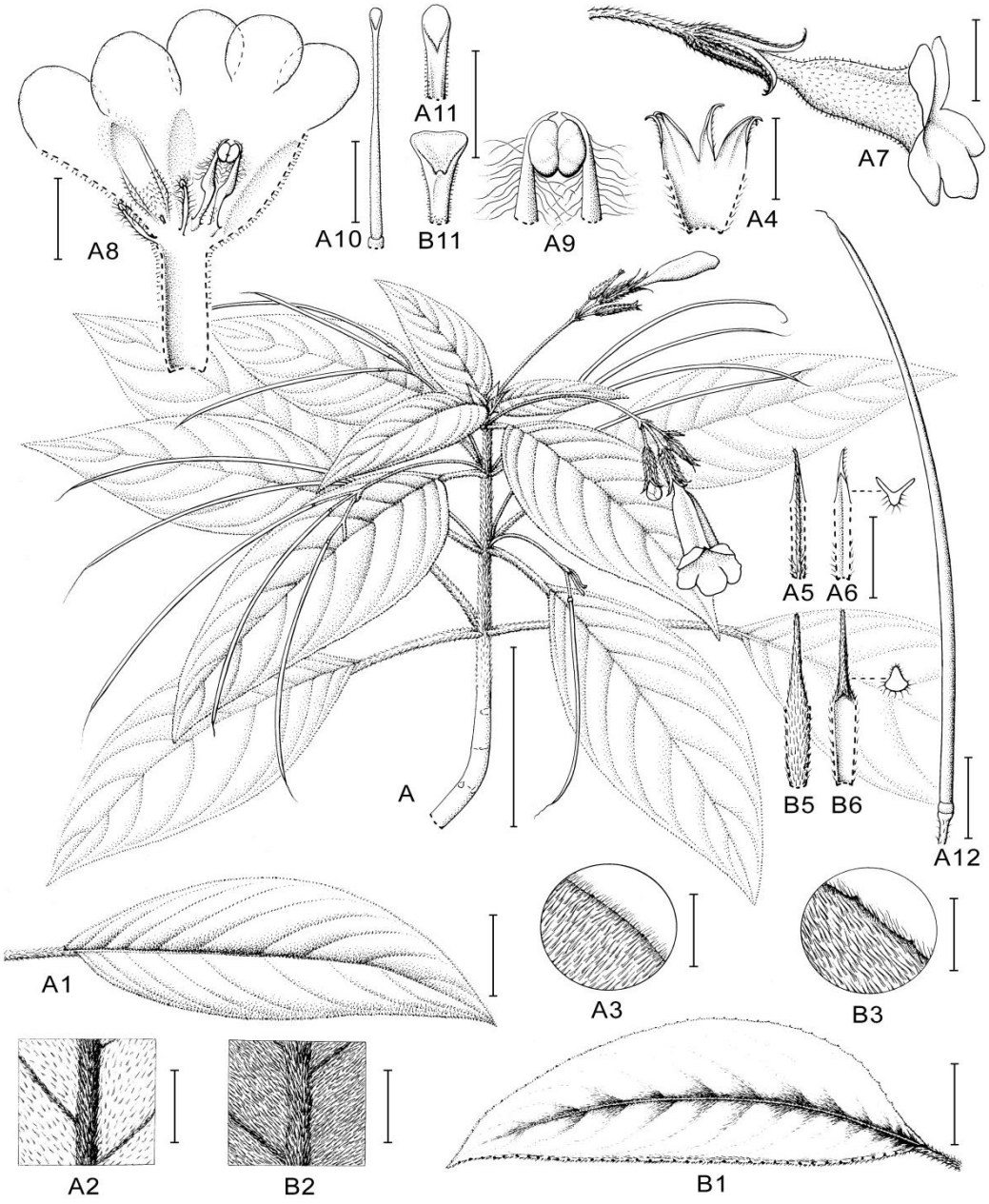


Figure 3.11 ***Henckelia wijesundarae*** **(A) and H. walkerae (B). – A: habit; A1 and B1: adaxial leaf surface; A2 and B2: abaxial leaf surface; A3 and B3: leaf margin; A4: calyx cut open showing 2+2+1 arrangement of lobes; A5 and B5: calyx lobe abaxial surface; A6 and B6: calyx lobe adaxial surface and cross-section; A7: flower lateral view; A8: flower longitudinal section; A9: coherent anther pair showing toothed filaments; A10: pistil; A11 and B11: stigma; A12: fruit. – Scale bars: A = 4 cm; A1, B1, A4, A5, B5, A6, B6, A7, A8, A10, A12 = 1 cm; A2, B2, A3, B3, A9, A11, B11 = 5 mm. – Drawn from** ***Ranasinghe and Wijewickrama 31*** **by Claire Banks. E Reproduced from Rranasinghe** ***et al.*****, 2016, Figure 3, pp. 218.**

his original description on *C. walkerae* var. *parviflora*. The two specimens mounted on this sheet appear to be from a single gathering. Therefore, we have chosen *CP 542* at K to lectotypify *C. walkerae* var. *parviflora.*

Provisional IUCN Conservation Assessment. *Henckelia wijesundarae* is at present known to exist in only two small subpopulations in Hiniduma, occurring in isolated forest patches in Southwest Sri Lanka which were discovered in 2008 and 2013 respectively. Three other collections from the same area during the last 50 years, discovered during the preparation of this study, suggest that the species was more widespread in the past: *Bernardi 1547* from Kalubovitiyangala mountain, Hiniduma, Nerugalkanda Forest, Udalamatta, collected in 1966 and 1971 respectively. Older collections before 1850 suggest that the species was present in Kalutara further northwest of Galle. This is now heavily urbanized. During extensive fieldwork carried out from 2013–2015 we did not find any further populations of *H. wijesundarae* in the Southwest lowland wet forests of Sri Lanka other than these two populations. The number of mature plants was less than 100 in each population and the extent of occurrence (EOO) has been calculated at 61.434 km^2 and an area of occupancy, AOO 12.0 km. The populations do not occur inside protected areas and their distribution is severely fragmented by the destruction of the surrounding habitat due to human settlements and tea plantations. A continuing decline in the future is likely due to continuing habitat loss and forest degradation. The situation of the new species is thus assessed as Critically Endangered (CR) under EOO criteria B1ab(i,iii).

Additional Specimens Examined. Galle district: Beaten path along border of Nerugalkanda Forest, Udalamatta, *Cramer 3390* (L); Udalamatta, *Cramer 2300* (E). circa Hiniduma ad cacumen montis Kalubovitiyangala ad pagum Dewalagama, *Bernardi 15479* (MO, PDA). Kalutara district, Rayigam Korale, *Thwaites s.n*. part of *CP 542* (PDA); Pasdun Korale, *Thwaites s.n*., part of *CP 542* (PDA). Locality unknown, *Thwaites s.n.*, *CP 542*, (two sheets P, two sheets W, K), *Mrs. Walker s.n.* (P).

6. *Henckelia floccosa* (Thwaites) A.Weber and B.L.Burtt in Beitr. Biol. Pflanzen 70: 344 (1998 [‘1997’]); Vitek *et al.*, Ann. Naturhist. Mus. Wien 495 (2000); Weber *et al.*, Taxon 60: 775 (2011). – *Didymocarpus floccosa* Thwaites, Enum. Pl. Zeyl.: 207 (1860) [“floccosa”]; Trimen, Handb. Fl. Ceylon 3: 274 (1895); W.L.Theob. and Grupe in Dassan. and Fosberg, Revis. Handb. Fl. Ceylon 3: 86–88, Fig. 2.b (1981). – *Roettlera floccosa* (Thwaites) Kuntze, Revis. Gen. Pl. 2: 476 (1891). Type: Sri Lanka, Raxawa, July 1855, *Thwaites, G.H.K. CP 3368* (lectotype PDA [not barcoded], designated here; isolectotypes BM [BM001135089], CAL [not barcoded]), K [K000858181], P [P04060475] and [P04060476]. (Figure 3.20, Map, pp. 92).

Herb perennial. ***Stem*** short, erect rhizome with indistinct internodes and adventitious roots. **Leaves** basal; petioles variable in length, narrowly winged and densely floccose; leaf blade rhomboid to ovate to orbicular, length 7–15 cm, width 4–10 cm, apex obtuse to acute, base gradually tapering and decurrent on petiole; leaf margin shallowly serrulate-crenulate; surface usually rugose, venation not conspicuous; indumentum on both upper and lower surfaces densely floccose with long, wooly tufts of hairs. **Inflorescences** pair flowered or a compound cyme, 2–3 times divided, 9–22 cm long; bracts oblong, densely floccose, 4–6 mm long, c. 1 mm wide; peduncles and pedicels slender, floccose, 0.5–1 cm long. **Calyx** 5-lobed, very deeply divided, lobes green, oblong, 3–5 mm long, c. 1 mm wide, densely floccose. **Corolla** 1.5–2.0 cm long, 5-lobed, lobes pale purple, sparsely pubescent outside and glabrous inside; lower three lobes c. 8 mm long, c. 8 mm broad, upper 2–6 mm long, 8 mm broad; tube white, expanded and then narrowly constricted at the throat 5–7 mm long on dorsal side, 1.0–1.5 cm long on ventral side yellow spot inside the tube; pubescent outside and towards back inside, glabrous in front, glandular-pubescent. **Stamens**, filaments white, glabrous, c. 3.5 mm long; anthers coherent, with violet spots, glabrous, c. 2 mm broad; 3 staminodes glabrous, two lateral ones c. 1.8 mm long, slightly swollen and tapering at tip, median one small, indistinct. **Disk** minute. **Pistil** oblong; ovary densely pubescent; style glabrous, stigma lower lobe capitate. **Fruit** a capsule, linear, cylindrical, straight or slightly curved, tapering at tip, 1.2–3 cm long, 1.8–2 mm broad at base, pubescent, usually dehiscing along dorsal side. Seeds minute, numerous, testa brown.

Distribution. Endemic species to Sri Lanka. This species has so far been collected twice from the Dolosbage area in Kandy district at elevations of about 650 m.

Habitat and Ecology. According to Theobald and Grupe (1981), the species grows on wet rock surfaces and rock crevices with abundant underground water seepage. The authors also mentioned that the area was very open and next to a tea plantation.

Phenology. According to Theobald and Grupe (1981), flowering is from July to September.

Lectotypification of *Didymocarpus floccosus.* *Henckelia floccosa* was originally described as *Didymocarpus floccosus* Thwaites (1846). Vitek *et al*. (2000) mentioned the *CP 3368* specimen with citation of three herbaria: BM, K and W, but without selecting any of these as a type. According to Theobald and Grupe (1981), this species had only been collected once on the island before their collection in 1968. This first collection was made by Thwaites (1846) and he mentioned *CP 3368* in the protologue in *Enumeratio Plantarum Zeylaniae* but no herbarium is mentioned there. He also described the location as "Dolosbage in the Central Province, at an elevation of 2000 feet". There are six sheets with *CP 3368* in different herbaria, one sheet at each of CAL, BM, K, PDA and two sheets at P. These specimens can be considered as parts of one collection from a single gathering from Dolosbage, the type locality for *D. floccosus* by Thwaites (1846). The sheet at PDA is selected as the lectotype while the other five sheets mentioned the above are treated as isolectotypes.

Provisional IUCN Conservation Assessment. This species had only been collected twice on the island of Sri Lanka, by Thwaites in 1846 and by Theobald and Grupe in 1968. Thwaites' type locality was the Raxawa Estate, Dolosbage. Theobald and Grupe also made their collection to the east of Dolosbage at an elevation equal to that of Thwaites. Extensive surveys in Dolosbage four times in 2013, 2014 and 2015 could not find any signs of the species; however, *Henckelia communis* populations were found in a few isolated forest patches among the tea cultivations of the type

locality area during field expeditions in 2013, 2014 and 2015. These surveys revealed that more than 90% of the previously forested area is entirely covered by tea plantations or represent abandoned cultivated land now dominated by one of the noxious weeds in the central highlands of Sri Lanka, *Clusia rosea* (Clusiaceae). This plant has already taken over a massive, previously forested area, following the clearance of lower areas for tea cultivation. Since *Henckelia floccosa* has not been collected for nearly 50 years, and the only known sites have been apparently destroyed, the conservation status Critically Endangered possibly extinct (CRp) is proposed.

Additional Specimens Examined. KANDY DISTRICT: Raxawa, Dolosbage, *Thwaites s.n., CP 3368* (CAL, BM, K, P [02 sheets], PDA). **KEGALLE DISTRICT:** Just east of waterfalls, c. 3 miles east of Dolosbage, *Theobald and Grupe 2403* (K, PDA, US).

7. *Henckelia humboldtiana* (Gardner) A.Weber and B.L.Burtt, Beitr. Biol. Pflanzen 70: 346 (1998 [‘1997’]); Vitek *et al*., Ann. Naturhist. Mus. Wien 499,512 (2000); Weber *et al.*, Taxon 60: 775 (2011). – *Didymocarpus humboldtianus* Gardner, Calcutta J. Nat. Hist. 6: 477 (1846) [“*humboldtiana*”]; Thwaites, Enum. Pl. Zeyl. 207 (1860); Trimen, Handb. Fl. Ceylon 3: 273 (1895); W.L.Theob. and Grupe in Dassan. and Fosberg, Revis. Handb. Fl. Ceylon 3: 84–86, Fig. 2.a (1981). – *Roettlera humboldtiana* (Gardner) Kuntze, Revis. Gen. Pl. 2: 476 (1891). Type: Sri Lanka [“Ceylon”], Rambodde, *Gardner, G. 600* (lectotype K [K000858189], designated by Janeesha and Nampy, 2015; isolectotype K [K000858188]). (Figures 3.12–3.14, 3.20, Map, pp. 92).

Didymocarpus primulifolius [“*primulaefolia*”] Gardner, Calcutta J. Nat. Hist. 6: 478 (1846), nom. illeg., non *D. primulifolius* Don (1825). – *Didymocarpus humboldtianus* var. *primulifolius* [“*primulaefolia*”] Thwaites, Enum. Pl. Zeyl. 207 (1860); Hook. Bot. Mag. 86: pl. 5161 (1860). Type: Sri Lanka *[“Ceylon”], Hantane, Gardner, G. 601 (lectotype K [K000858191]*, designated here*; isolectotypes BM [*BM000997738], K [K000858192]).

Didymocarpus humboldtianus var. *recedens* C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 103 (1883); Vitek *et al*., Ann. Naturhist. Mus. Wien 512 (2000). Type: Sri Lanka, Wallekelly Hill, 5000 ft., *Beckett, T.W.N. 364* (holotype K [K00858190]).

Herbs perennial. **Stems** very short, erect rhizome with indistinct internodes and adventitious roots. **Leaves** whorled 2, 3 or 4 at a node; petiole very variable in length, narrowly winged and sometimes lyrate, finely pubescent with brown or white hairs; leaf shape broadly ovate to orbicular, very variable in size, 3–20 cm long including the petiole, 3–10 cm broad, base rounded or very occasionally obtuse rarely acute or truncated, margin shallowly to coarsely serrulate-crenulate, apex mostly rounded, surface rugose or weakly so, venation conspicuous, indumentum on upper surface densely pubescent with eglandular hairs appearing white or with some populations sparsely pubescent or becoming weekly so with age, lower surface densely villous or pubescent with usually brown or rarely white eglandular hairs, sometimes accumulated on midrib and lateral veins, sessile glandular hairs also present and apparent when eglandular hairs are few in number. **Inflorescences** compound cyme, sometimes more branched and paniculate, 3–6 times divided; axis green or purple, both eglandular and glandular hairs or only eglandular hairs, 6–25 cm long; bracts linear-oblong, densely pubescent or villous, 3–8 mm long, c. 1 mm broad; pedicels of both flower pairs unequal in length, 3–20 mm long. **Calyx** 5-lobed, very deeply divided; lobes green or purple, linear-oblong, 2–4 mm long, apex obtuse, villous or pubescent. **Corolla** 1–2 cm long, variable in size and colour, 5-lobed, lobes pure white to pale purple or dark purple, rounded, lower three lobes 3.5–10 mm long, 5–12 mm broad, upper 2 lobes 2–6 mm long, 3.5–8 mm broad; tube not distinctly inflated and constricted at throat, 5–7 mm long on dorsal side, 10–15 mm long on ventral side, pure white to pale purple with a broad yellow spot within, pubescent outside and towards back inside, glabrous in front, glandular-pubescent. **Stamens** filaments white, glabrous or pubescent with eglandular multicellular hairs, sometimes twisted, 3–4 mm long; anthers yellow, coherent, glabrous, c. 2 mm broad; 3 staminodes, glabrous, two c. 2 mm long, slightly swollen and tapering at tip, median one small, indistinct. **Disk** minute. **Pistil** linear to oblong; ovary glandular

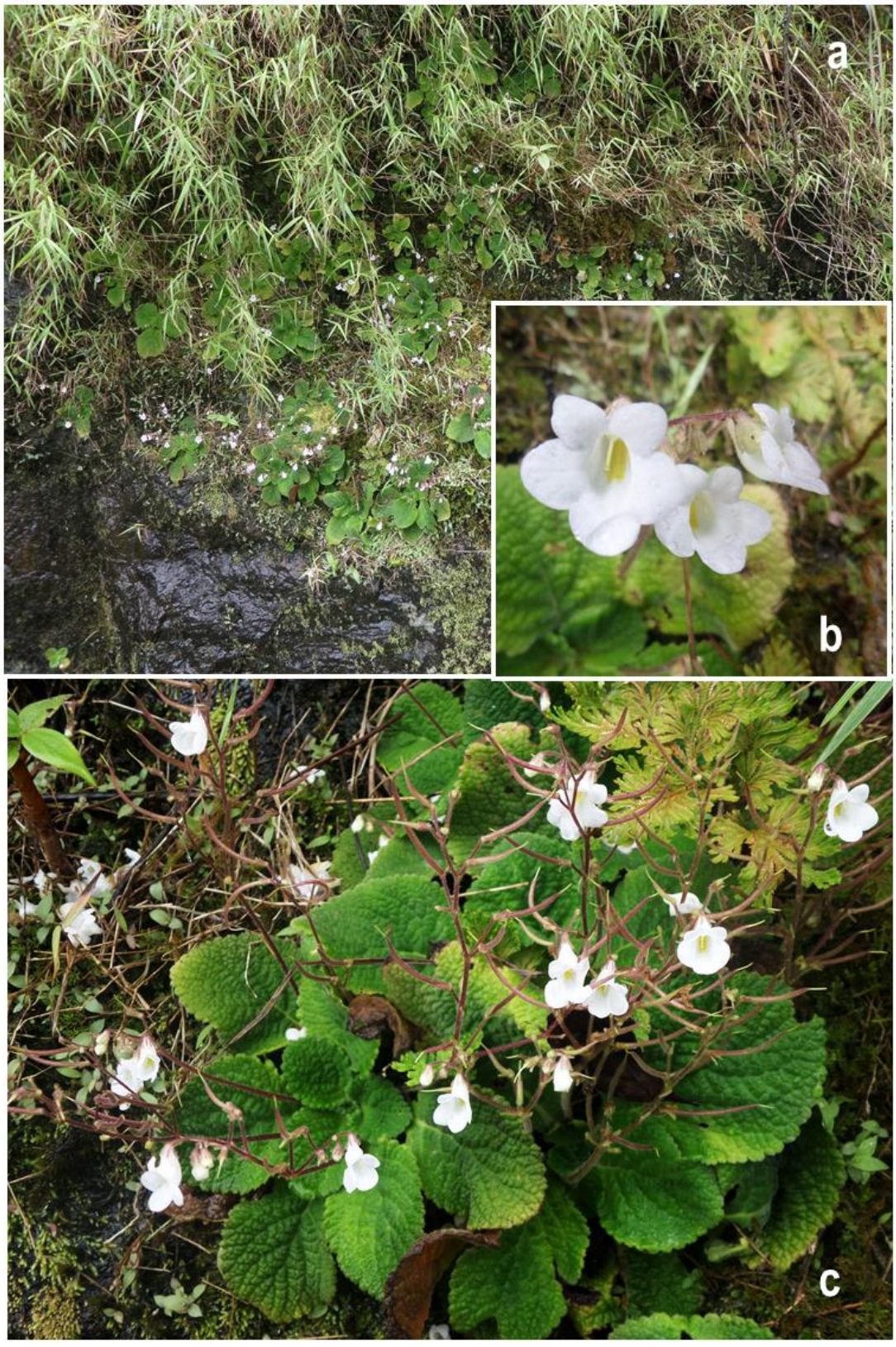


Figure 3.12 ***Henckelia humboldtiana*** **white flowers at all flowering stages and rugose leaves from Knuckles, Sri Lanka(a) habitat on wet rock surface; (b) flower (c) mature plants with compound cymes.**

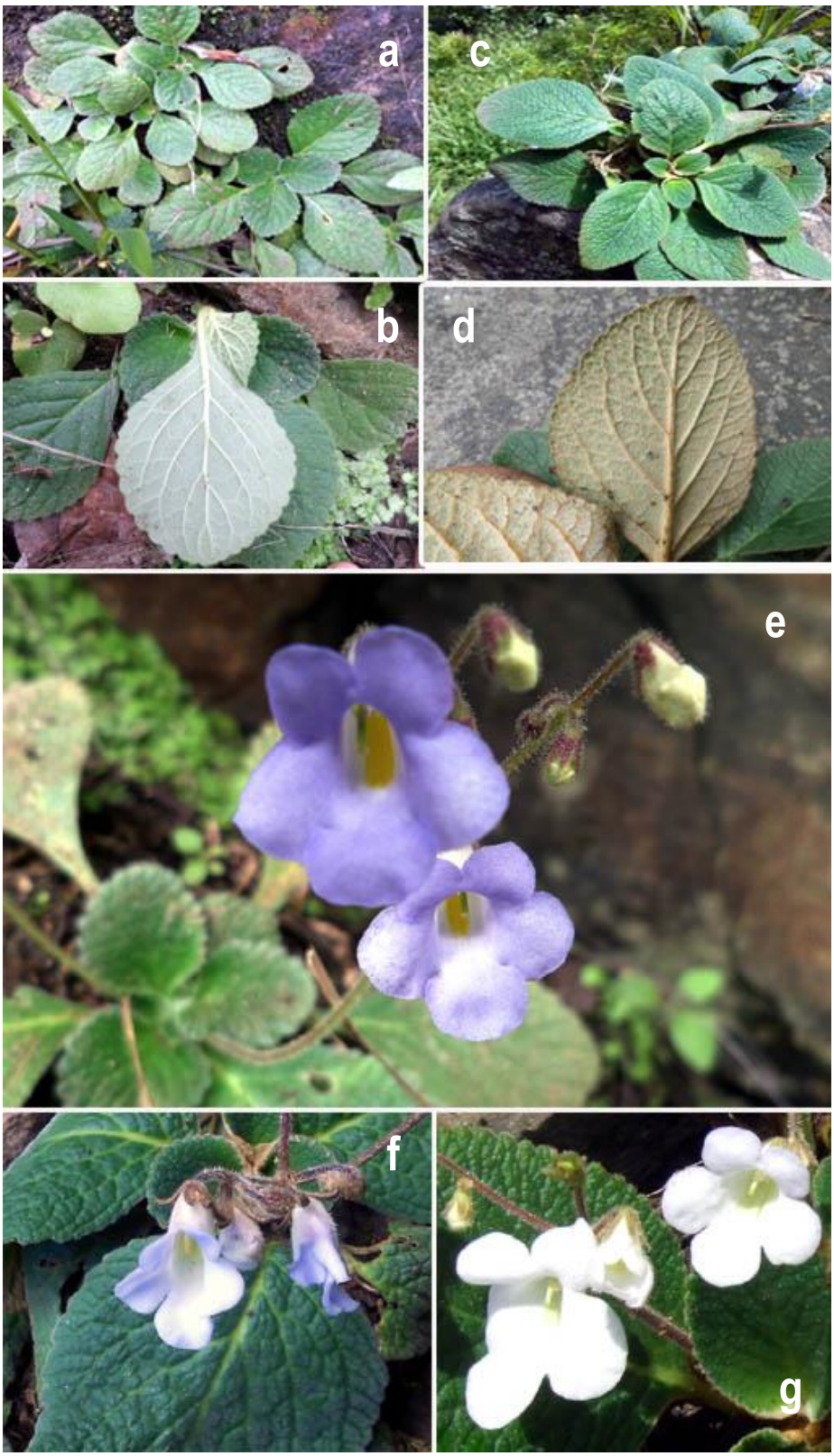


Figure 3.13 *Henckelia humboldtiana* intra-specific diversity. Intermediate zone populations, (a), (b) and (e). a: habit, (b): lower surface of leaf; (e): inflorescence with purple flowers. Upper montane zone populations, (c), (d), (f) and (g). (c): habit; (d): lower surface of leaf; f: pale purple flowers with narrow corolla tube; (g): corolla lobes more reflexed and turning white with maturity of flower.

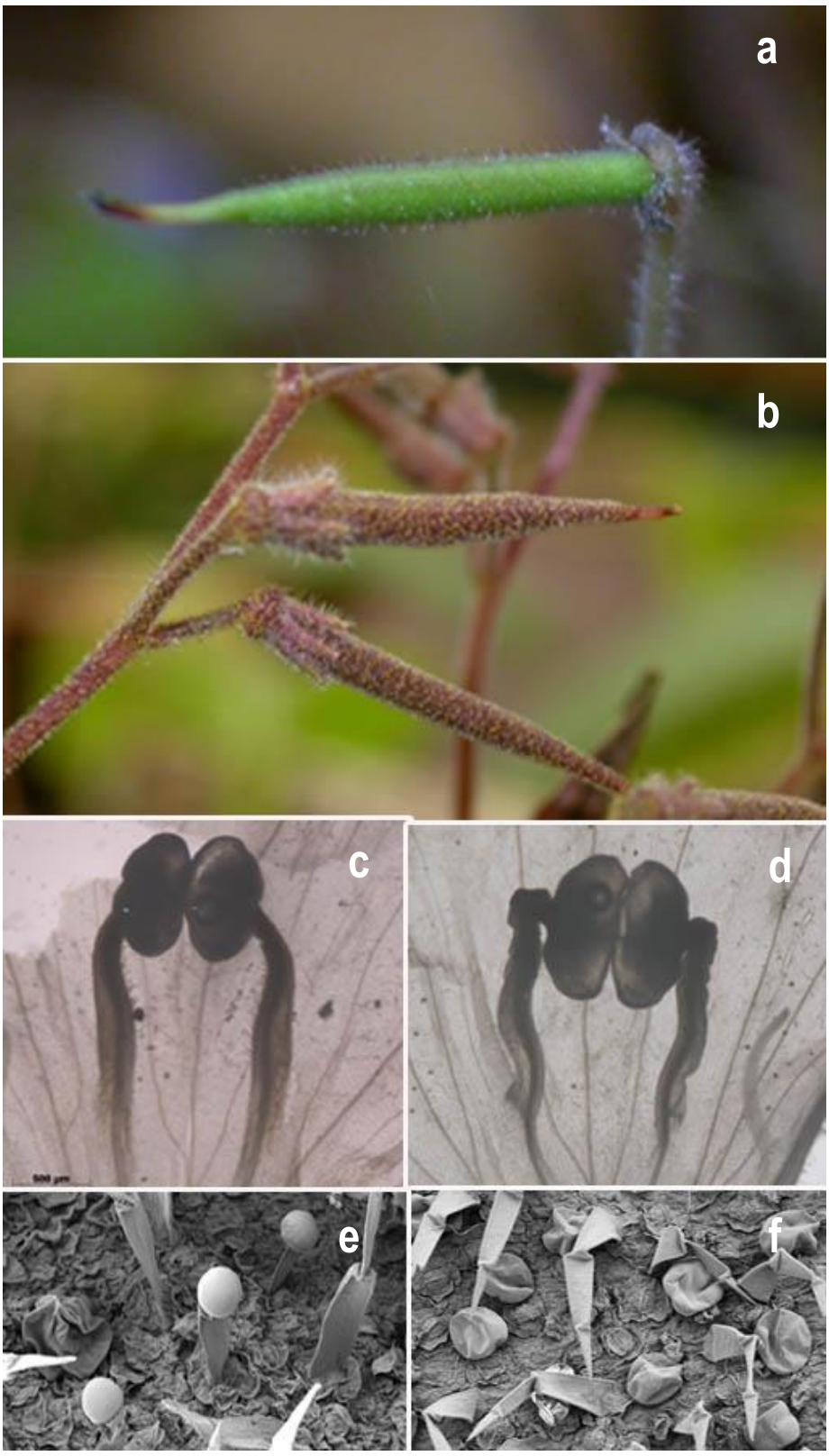


Figure 3.14 Diversity of glands and filaments in *Henckelia humboldtiana*. (a) colorless stalked glands on fruits; (b) yellow sessile glands on fruits; dissecting microscope view for (c) straight, glandular filaments, and (d) twisted, glabrous filaments; SEM images for e: rigid, stalked glands on lower surface of the leaf X 801; f: sessile glands on lower surface of the leaf X577.

pubescent with multicellular eglandular hairs and glandular hairs with multicellular stalks or short stalks; style glabrous, short; stigma lower lip sub-capitate. **Fruit** a linear capsule, straight or slightly curved, tapering at the tip, 1.5–2.5 cm long, c. 1.5 mm broad at base, glandular-pubescent, usually dehiscing along one side; seeds minute, numerous, testa brown.

Distribution. This is the most widespread member of the family in Sri Lanka. It is found in the dry and intermediate zones as well as in montane and submontane forests up to c. 200–2100 m elevation.

Habitat and Ecology. Found on wet rocks where there is abundant water seepage, or in rock crevices, in shade or exposed to the sun. However, in the dry zone this species is usually found in wet forest patches on isolated hills, and in the intermediate zone, it occurs on wet rock outcrops near waterfalls or in savanna grasslands.

Phenology. Flowering late July to September, and fruiting from September to December.

Note. This is the most variable species of all recorded Gesneriaceae species in Sri Lanka. None of the macro- or micromorphological characters are consistent to any identified clade or subclade in the molecular species delimitation in Chapter 2. In fact, there are a few populations possessing unique morphological characteristics. For example populations in submontane forests of Knuckles mountain range possess very wrinkled leaf surfaces, rigid, white stalked glands and rusty brown pubescence on leaf lower surface and pure white flower corolla (Figure 3.12). In contrast, the populations of the upper montane zone, in Pidurutalagala, possess less wrinkled leaves with brown villous-floccose hairs on the lower surface, and bright yellow pigmented glands present on the corolla, ovary and fruit. The populations of the dry intermediate zone in Badulla, Rawana Ella differ from all the other *H. humboldtiana* populations collected, in their smaller more round leaves with distinctly less pubescence on them, and the hairs that are silver white rather than rusty brown.

However, these populations do not form well-supported clades or subclades in the *Henckelia* phylogeny (Figs. 2.8 and 2.9) produced in Chapter 2. The species also occurs in India and shows great morphological variation there as well. In fact, a new species, *Henckelia bracteata* Janeesha and Namphy, was described recently from South India that is very similar to *H. humboldtiana* (Janeesha and Nampy, 2015). However, careful observations of Sri Lankan material in the present study found that this new species, apparently endemic to South India, is closely allied to populations *H. humboldtiana* of the upper montane zone in Sri Lanka. The molecular studies in this study also suggested a close affinity of Sri Lankan samples of *Henckelia humboldtiana* with South Indian *Henckelia incana*, the type species of the genus. Therefore, considering the wide distribution range of *H. humboldtiana* from Sri Lanka to South India and great morphological variation, a combined revision of the species occurring across the Palk Strait and Gulf of Mannar in South India and Sri Lanka is imperative to fully resolve the taxonomy of the species of *Henckelia* occurring on both sides, and their closest relatives.

Lectotypification of *Didymocarpus primulifolius* ["*primulaefolia*"] Gardner. This was described by Gardner (1846) without citing any specimen number but including locality data as "on shady rocks on the Hantane range, near Kandy". The name was recognized under *H. humboldtiana* as a heterotypic synonym by Vitek et al. (2000). They also mentioned *Gardner 601* specimens at K and BM as type specimens. A lectotype specimen could be selected since no single specimen was cited from a specific herbarium in Vitek et al. (2000). There are two herbarium sheets at K (K000858191 and K000858192) labelled with number 601 under var. *primulifolia*. The sheet K000858191 has no collector name but the location, "Hantane" written on it. The second sheet, K000858192, has no locality information but the name Gardner written on it. At the BM herbarium, specimens of *Gardner 601* (BM000617482) are mounted together with those of *CP 1785* (BM000997738) (see also the note by Vitek *et al*., 2000, on page 499). The specimen of *Gardner 601* at BM also has a pencil written label which contains "Hantane" and "*Gardner 601*". It is very likely that Gardner collected and studied all specimens of *Gardner 601* at BM and K. Therefore, the best specimen reflecting the characters described by Gardner for *D.*

primulifolius is the one in K, K000858191, and is here selected as the lectotype. The specimens at K (K000858192) and BM (BM000997738) are therefore isolectotypes.

Provisional IUCN Conservation Assessment. The extent of occurrence (EOO) of *Henckelia humboldtiana* in Sri Lanka is calculated as 10,472 km^2 and the area of occupancy (AOO) as 132 km^2. Although this species is very widespread in its distribution, it is also found in very sensitive habitats usually on wet rock outcrops and crevices of rocks with abundant underground water seepage. Their habitat specificity suggests that the plants are vulnerable to habitat destruction and the effect of plant invasion for example *Clusia rosea* and *Panicum maximum*. A continuing decline of these populations is possible. Therefore the National Conservation category of *Henckelia humboldtiana* can be assessed as Vulnerable (VU) under the EOO criteria with B2ab(ii,iii).

Additional Specimens Examined. **ANURADHAPURA DISTRICT:** Unakanda Hill, Ritigala, Anuradhapura, *Ranasinghe and Wijewickrama 1071* (E). **BADULLA DISTRICT:** road Haputale-Diyatalawa, *de Silva s.n.* (PDA); Rawana Ella, *Ranasinghe 1069* (E, PDA); Ooma Oya, *s.coll. s.n.* (PDA). **KANDY DISTRICT:** near summit of Hantane Mt. No. 2, Kandy, *Theobald and Grupe 2313* (PDA, US); below summit of Hantane Mt. No. 1, Kandy, *Theobald and Grupe 2334* (BM, NY, PDA, UC, US); rock cliff on summit of Hantane Mt. No. 1, Kandy, *Theobald and Grupe 2337* (E, PDA, US). *Theobald and Grupe 2395* (A, E, PDA, RSA, UC, US); Hantane, *Gardner s.n.,* part of *CP 1785* (PDA), *Gardner 601* (BM, K); Hantane, *Thwaites s.n.,* part of *CP 1785* (BM, K, PDA); Kandy, *Moon 698* (BM); base of Kabaragala Mt. behind Raxawa Tea Estates east of Dolosbage, *Theobald and Grupe 2358* (PDA, UC, US); Tamaravelly area east of Craighall Tea Estate, 8 miles South of Gampola, *Theobald and Grupe 2399* (PDA, US); Hunnasgiriya, *s.coll. s.n.* (PDA); Corbet's Gap, *Simpson 9443* (BM); near Madugoda on the Urugala road, *Simpson 8786* (BM); forest behind Loolcondera Tea Estate, Deltota, *Ranasinghe 1050* (E, PDA). **MATALE DISTRICT:** Sigiriya, *Gardner s.n.,* part of *CP 1784* (PDA); Matale, *Thwaites s.n.,* part of *CP 1784* (BM, PDA); Reverston, Knuckles Conservation, *Ranasinghe 1046* (E, PDA). **NUWARA ELIYA DISTRICT:** forest

behind Hakgala Botanic Gardens, *Theobald and Grupe 2302* (US), *Theobald and Grupe 2397* (A, E, K, LE, PDA, UC, US); south boundary, Hakgala, *de Silva s.n;* (PDA); Rambodde, 4500 ft, *Gardner 600* (BM, K), part of *CP 1784* (PDA); on rock surface of Ramboda old tunnel, *Ranasinghe 863* (PDA); Kikiliyamana Forest Reserve, *Ranasinghe 959* (E, PDA); Pidurutalagala, from the entrance from Lover's slip waterfall, *Ranasinghe 1021* (E, PDA). **DISTRICT UNKNOWN:** Condegala jungle, *Alston 1726* (K, PDA). **LOCALITY UNKNOWN:** *Macrae 245* (BM); *Col. Walker 184* (K); *Mrs. Walker 1291* (E); *Mackenzie s.n.,* (K); *Thomson s.n;* (K).

8. *Henckelia zeylanica* (R.Br.) A.Weber and B.L.Burtt, Beitr. Biol. Pflanzen 70: 359 (1998 [‘1997’]); Vitek et al., Ann. Naturhist. Mus. Wien 504,527 (2000); Weber *et al.*, Taxon 60: 778 (2011). – *Didymocarpus zeylanicus* R.Br. [“*zeylanica*”], Cyrtandreae 119 (1839); Thwaites, Enum. Pl. Zeyl. 207 (1864); C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 104 (1883); Trimen, Handb. Fl. Ceylon 3: 274 (1895); W.L.Theob. and Grupe in Dassan. and Fosberg, Revis. Handb. Fl. Ceylon 3: 88–89, Fig. 2.c (1981). – *Roettlera zeylanica* (R.Br.) Kuntze, Revis. Gen. Pl. 2: 477 (1891). Type: Sri Lanka, Ambagamuwa, Adam’s Peak, *Gardner s.n., CP 352* (Neotype PDA, designated here). (Figure 3.20, Map, pp. 92)
Didymocarpus longipetiolata Gardner, Calcutta J. Nat. Hist. 6: 475 (1846); Vitek *et al*., Ann. Naturhist. Mus. Wien 504,527 (2000). Type: Sri Lanka (“Ceylon”), *Walker 1720* (lectotype E [E00627485], designated here).

Herb perennial. **Stem** a prostrate, branched and creeping rhizome with adventitious roots. **Leaves** scattered alternating along the rhizome with long petioles; petioles 3–20 cm long, densely pubescent with appressed eglandular hairs; blade broadly ovate to orbicular, length 4–8.5 cm, width 3.5–7.5 cm, base cordate to rounded, leaf margin serrulate-crenulate, apex acute to obtuse, surface smooth, not rugose, midrib and lateral veins conspicuous on fresh leaves, lateral veins 5–7 pairs, indumentum upper surface densely and finely pubescent with eglandular hairs very often appressed to the leaf, lower surface less pubescent with eglandular hairs scattered and accumulated on the midrib and lateral veins. **Inflorescences** pair-flowered cyme or a compound cyme, 2–3 times divided; peduncle green, 7–15 cm long, pubescent;

bracts lanceolate, pubescent, 2–4 mm long, c. 1 mm wide; pedicels sub-filiform, 1–2 cm long. **Calyx** 5-lobed, very deeply divided, lobes green, linear-lanceolate, apex obtuse, pubescent, 3–5 mm long, c. 1 mm wide. **Corolla** 1–1.5 cm long, 5-lobed, lobes white, rounded, thinly pubescent on back, glabrous in front, lower 3 lobes 6–9 mm long, 6–9 mm wide, upper 2 slightly smaller; tube slightly gibbous at base, slightly constricted at throat, c. 11 mm long dorsally, c. 13 mm long ventrally, white to pale purple with prominent darker veins, corolla outside pubescent and glabrous inside. **Stamens** filaments white, glabrous; anthers coherent, yellow, glabrous, c. 2.5 mm long; staminodes 3, glabrous, two c. 1.2 mm long, slightly swollen at tip, other small, indistinct. **Pistil** oblong, glabrous; style glabrous, linear; stigma chiritoid, lower lobe flattened, c. 2 mm long, 1.5 mm broad. **Fruit** a linear capsule, cylindrical, straight or slightly curved, tapering at the tip, 2–3 cm long, 1–1.5 mm broad at base, glabrous, usually dehiscing along the dorsal side; Seeds. numerous, minute, testa brown.

Distribution. Endemic species to Sri Lanka. Confined to montane zone elevations above c. 1000 m.

Habitat and Ecology. In the present study no population was found in the Dolosbage area in Kandy district due to habitat loss (further discussed below under conservation status). Two populations were found at Peak Wilderness Sanctuary (Adam's Peak); one population grew on wet rock faces and in rock crevices, and the second one in the shade on the forest floor in leaf litter.

Phenology. Trimen's (1895) observation on flowering from December to March could be accurate as I could not find any flowering or fruiting material during my field work from July to August in 2013 or October to November in 2014.

Note. *Didymocarpus longipetiolatus* was validly published by Gardner (1846) as a species distinct from *D. zeylanica* Wall. (now *H. zeylanica*). This was later synonymized under *D. zeylanica* from Sri Lanka in subsequent taxonomic treatments (Thwaites, 1864; Clarke, 1883; Trimen, 1895; Theobald and Grupe, 1981; Weber

and Burtt, 1998). Also Vitek *et a*l. (2000) included this under *H. zeylanica* as a heterotypic synonym. The present study based on both morphological and molecular data also confirms that these two names refer to a single taxon, *H. zeylanica*.

Lectotypification of *Didymocarpus zeylanica.* This species was first described by Robert Brown in 1839 in his work on Cyrtandreae, but he did not mention any specimens or collector in this description. No specimen has been traced back to Robert Brown's original description in previous taxonomic treatments on this species or in the present study. In the absence of any original material, it is recommended to propose a neotype specimen (McNeill *et al.*, 2012). Thwaites (1864) suggested three *CP* numbered specimens, *CP 352*, *CP 395* and *CP 1783* for *D. zeylanica*. Clarke (1883) also mentioned the collector and the specimens for *D. zeylanica* as "(Domina Walker, in hh. Kew, Delessert); alt. 1600–1800 metr., prope Adam's Peak (Thwaites C. P., mi. 395, 352, 1783 in h. Kew, n. 352 in hh. Mus. Brit., DC, Boissier, Berol.)". Vitek, et al. (2000) proposed a type materia for this as "Type: Ceylon, coll.? [?]. - "Ceylon, descr. A", s. coll. [BM] could be type material". I could not find this specimen during my visit to the Natural History Museum in London. Checking for a specimen to be proposed as a neotype, I found *CP 352* at PDA bearing four specimens mounted on it. Therefore, specimen *CP 352* at PDA was selected as the neotype specimen.

Lectotypification of *Didymocarpus longipetiolata.* Gardner (1846) did not cite any specimen in his original description but he mentioned the locality and collector's information as "Adam's Peak, found by Mrs. General Walker". There are two herbarium sheets collected by Mrs. Walker available, each at K and E identified as collected by Mrs. Walker. The one at E, E00627485, has all the characters and "D. zeylanicus R.Br.", "Ceylon", "Walker No 1720" is written in pencil on it. But there are no annotations of D. longipetiolata. The second herbarium sheet at K was found during my visit to the herbarium at K, but this was not a barcoded herbarium sheet. This sheet contains three specimens of D. humboldtiana (now H. humboldtiana) with the name, Didymocarpus humboldtiana written on it along with the name of Gardner. One specimen at the top right hand of the same sheet identified as D. zeylanica (now

H. zeylanica). There is also a label with a pencil-written "No 24", "Ceylon" and "Mrs. Walker" on the same sheet. Of these two more complete herbarium sheet at E [**E00627485**] is proposed here as the lectotype.

Provisional IUCN Conservation Assessment. The extent of occurrence (EOO) is 89 km^2 and area of occupancy (AOO) is 24 km^2. This species is confined to the montane zone and very rare in distribution points. Only two small populations were found in the Peak Wilderness Sanctuary. Previous habitats in Dolosbage, Kandy district where this species was recorded in the late 1960's, were found to be invaded by *Clusia rosea* (Clusiaceae) and *Panicum maximum* (Poaceae). Therefore, it is very unlikely that it will be found in this area again, due to habitat loss. This species is very specific in habitat requirements and its distribution is, therefore, more vulnerable to climatic fluctuations. Hence, a continuing decline of the few remaining populations can be projected for EOO, AOO and quality of habitat. Therefore, the present situation of *Henckelia zeylanica* can be assessed as Critically Endangered (CR) with EOO criteria as B1ab(i,ii,iii).

Additional Specimens Examined. NUWARA ELIYA DISTRICT: near Carolina Tea Factory, 1 mile south of the turnoff to Watawala on road to Hatton, *Theobald and Grupe 2359* (PDA, US); near Carolina Tea Factory, *Tunnard s.n.* (PDA); Gartmore, Rosamaliya,
de Silva s.n. (PDA); Malgama, Maskeliya, *Trimen s.n.* (PDA); Adam's Peak, *Gardner s.n.*, part of *CP 352* (PDA); Adam's Peak Wilderness adjoining Moray Estate, *Jayasuriya, Kostermans and Balakrishnan 220* (PDA); *Ranasinghe 1041 and 1042* (E, PDA). **KANDY DISTRICT:** Ambagamuwa, *Gardner s.n.*, part of *CP 352* (PDA). **KEGALLE DISTRICT:** Windsor Forest east of Dolosbage on road to St. Helen's Tea Estate, *Theobald and Grupe 2401* (PDA, US), Windsor Forest west of Dolosbage on road to St. Helens Tea Estate, *Grupe 161* (PDA, US). **LOCALITY UNKNOWN:** *Thwaites s.n.*, part of *CP 352* (K, PDA), *Walker 1720* (E), *s.coll. s.n.* part of *CP 352* (CAL).

Aeschynanthus Jack

Trans. Linn. Soc. London 14: 42 (1823); C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 18 (1883); Ridley, Fl. Malay Penins. 2: 496 (1923); Wang, Fl. Reipubl. Popularis Sin. 69: 498 (1990); Middleton, Edinburgh J. Botany 64: 368 (2007); Middleton, Edinburgh J. Botany 66: 393 (2009). Type: *Aeschynanthus volubilis* Jack.

Trichosporum D.Don, Edinburgh Philos. J. 7: 82 (1822), nom. rej; Blume, Bijdr. Fl. Ned. Ind. (1826). Type: *Trichosporum parviflorum* D.Don (= *Aeschynanthus parviflorus* (D.Don) Spreng.), lectotype designated by Middleton (2007).

Rheitrophyllum Hassk., Flora 25 (2): beibl. 56 (1842). Type: *Rheitrophyllum subverticillatum* Hassk. (= *Aeschynanthus angustifolius* (Blume) Steud.).

Oxychlamys Schltr., Bot. Jahrb. Syst. 58: 286 (1923). Type: *Oxychlamys pullei* Schltr. (= *Aeschynanthus oxychlamys* Mendum)

Euthamnus Schltr., Bot. Jahrb. Syst. 58: 284 (1923). Type: *Euthamnus papuanus* Schltr. (= *Aeschynanthus papuanus* (Schltr.) B.L.Burtt)

Micraeschynanthus Ridl., Fl. Malay Penin. 5: 324 (1925). Type: *Micraeschynanthus dischidioides* Ridl. (= *Aeschynanthus dischidioides* (Ridl.) D.J.Middleton)

Habit epiphytic herbs or subshrubs. **Stem** erect, arching or pendulous, these sometimes rooting along their lengths when in contact with a suitable substrate. **Leaves** opposite or verticillate, pedicellate; blades coriaceous to distinctly fleshy, more rarely herbaceous, simple, margins entire to weakly crenate or weakly dentate, sometimes somewhat undulate, venation pinnate but more often than not obscure. **Inflorescences** axillary few-flowered cymes, or flowers solitary in the axils of leaves, or a pseudoterminal cluster. Flowers strongly protandrous. **Calyx** of 5 sepals, these free or variously fused into a tube for part or most of length. **Corolla** zygomorphic, tubular, widening towards lobes, distinctly inflated at the base, glabrous to variously pubescent outside and inside; with 5 lobes, these consisting of a 2-lobed upper lip, 2 lateral lobes and a lower lobe; very variable in colour but most frequently red, orange, yellow or green (or combination of these) and then often with other darker or lighter patterning. **Stamens** 4, in 2 pairs, attached to the inside of the corolla tube, included or exserted from corolla tube when mature; vestigial staminode present; anthers of each pair attached by their apices (occasionally all 4

attached together outside this region). **Disk** present, annular to dentate. **Pistil** consisting of a sterile stipe at the base, the fertile ovary section, the style and the peltate stigma; ovules many, anatropous. **Fruit** a long narrow capsule, opens loculicidally; seeds many, tiny, with short to long appendages at both ends.

Note: *Aeschynanthus* is one of the larger genera of the family Gesneriaceae, with approximately 160 species distributed from Sri Lanka and India through southern China and Southeast Asia to New Guinea and the Solomon Islands (Weber, 2004; Middleton, 2016). There is only one representative in Sri Lanka, *Aeschynanthus ceylanicus* Gardn., the only epiphytic plant among the Gesneriaceae from Sri Lanka..

Aeschynanthus ceylanicus Gardner, Calcutta J. Nat. Hist. 6: 474 (1846); Wight, Icon Pl. Ind. Orient. t. 1347 (1848); Walp., Ann. Bot. Syst. 3: 95 (1852); Thwaites, Enum. Pl. Zeyl.: 206 (1864); C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 26 (1883); Trimen, Handb. Fl. Ceylon 3: 272 (1895). – *Trichosporum ceylanicum* (Gardner) Kuntze, Revis. Gen. Pl. 2: 478 (1891). Type: Sri Lanka: *Gardner, G. 599* (lectotype K [K000096752], designated here). (Figures 3.15 and 3.17, Map, pp. 89).

[*Aeschynanthus wilsonii* Walker ex C.B.Clarke in A.DC. and C.DC., Monogr. Phan. 5(1): 26 (1883) ("Wilsoni"), nom. nud.]

Epiphyte, pendulous. **Stem** branched, green, glabrous with adventitious roots at the thickened nodes. **Leaves** opposite; petiole short, 2–3 mm long, green, glabrous; blade upper surface green and lower surface pale green, lanceolate or oblanceolate, length 4–10 cm, width 1–2.3 cm, usually more than 2 times as long as wide, thick, fleshy and rather coriaceous, margin entire, tapering at both ends, apex acute or sometimes attenuate, base cuneate or attenuate, both surfaces glabrous, usually 4 pairs of lateral veins, indistinct on fresh leaves, quite distinct on dried leaves. **Inflorescences** pseudo-terminal, umbel with 1–2 flowers; peduncle very short, indistinct; pedicels 1–2 cm long, glabrous, strongly protandrous flowers. **Calyx** 5-lobed, lanceolate or narrowly triangular, 6–8 mm long and 1–2 mm broad, calyx segments deeply divided and free, obtuse at the tip, outer surface glabrous, glandular hairs towards the tip of the margin, inner surface with short-stalked or nearly sessile glandular hairs, slightly

Figure 3.15 *Aeschynanthus ceylanicus* a: hanging down from tree brances; b and c: flowering branch; d: close-up of a flower.

spreading. **Corolla** zygomorphic, bilabiate, 5-lobed, upper 2 lobes 3–4 mm long, 3 mm wide, yellowish green with reddish- purple flecks and irregular stripes in front, dark maroon-red at the base, lower 3 lobes more spreading than upper lobes, 5–6 mm long, c. 5 mm wide, yellowish green with maroon colour blotch towards the base of the lobes and reddish- purple flecks and irregular stripes towards the tip, glandular pubescent on both surfaces and clearly distinct along margins; tube 3–3.5 cm long, outer and inner glandular pubescent, tube scarlet red, narrow at base, curved. **Stamens** epipetalous, filaments long, exserted and didynamous, anthers coherent in 2 pairs, one staminode inserted in the corolla tube; filaments pink-purple, glandular pubescent, anterior pair c. 2.5 cm long, lower 1.8–2 cm long; anthers c. 2.5 mm long, c. 1.5 mm broad, reddish-purple towards filament, yellowish-green above; staminode c. 8 mm long. **Disk** 5-lobed (crenate), c. 1 mm high. **Pistil** linear, c. 2 cm long; ovary glabrous, lower part a narrow, tapering stipe; style 5–15 mm long depending on the flowering stage, glabrous; stigma pale red, c. 2 mm across. **Fruit** a capsule, 10–20 cm long, 2–4 mm wide, glabrous; seeds 1.5–2.4 mm long, 0.4–0.5 mm broad, brown, testa papillose; appendages of one long hair at each end, papillose, c. 1 cm long.

Distribution. Sri Lanka and South India. In Sri Lanka, it is found in montane and submontane forests at elevations above c. 1100 m.

Habitat and ecology. Epiphytic, climbing and hanging from tree trunks and branches also growing on wet rock surfaces.

Phenology. Flowering observed from July to August, fruiting from September to October. According to Theobald and Grupe (1981) flowering in March, August to October, and probably throughout the year. The flower is strongly protandrous.

Chromosome count. *2n = 32* (Rashid *et al*., 2001).

Lectotypification of *Aeschynanthus ceylanicus*. There were only three specimens found at K belonging to number *599* collected by Gardner annotated with *Aeschynanthus ceylanicus* on the sheets. No further specimens of this collection

number are recorded from any other herbaria. Therefore, the herbarium sheet K00096752 with a complete specimen was selected as the lectotype.

Note. *Aeschynanthus ceylanicus* has close morphological affinities with *A. perrottetii* from South India (Theobald and Grupe, 1981; Skog and Boggan, 2007onw.). There are few characters to distinguish *A. ceylanicus* (Gardner, 1846) and *A. perrottetii* (De Candolle, 1845) based on the protologues. *Aeschynanthus ceylanicus* has broader and longer leaves, a cuneate leaf base, umbels with not more than two flowers, a glandular pubescent outer surface of the corolla tube and longer (c. 25 cm) fruits. *Aeschynanthus perottettii* has narrow, shorter leaves, an obtuse leaf base, umbels with 3–5 flowers, a glabrous outer surface of the corolla tube and about 8 cm long fruits. However, it is difficult to say whether these main differences are consistent because Clarke (1883) noted, for example, the corolla tube as glandular pubescent or subglabrous in *A. perrottetii*. This may be due to his view of *A. perrottetii sensu lato* from the Indian Peninsula to include two varieties, var. *malabarica* and var. *planiculmis*. Unfortunately, no material from India was available for molecular studies here. Future studies on *A. ceylanicus* and *A. perrottetii* are needed, particularly on material from India to understand whether taxonomic boundaries exist between these taxa, and among their varieties.

There is another variety of *A. ceylanicus* described by Clarke (1883) as *A. ceylanicus* var. *pinguis* from Sri Lanka and India. Clarke (1883) cited the specimen, *Mrs. Walker 28* at K, for this variety. Careful observations showed that this specimen has smaller leaves and flowers. However, it is otherwise not distinct enough to be recognized as a variety. Therefore, I agree with Theobald and Grupe (1981) where this variety perhaps can be regarded as a smaller form of *A. ceylanicus*.

Provisional IUCN Conservation Assessment. The extent of occurrence (EOO) for this species was calculated as 933 km^2 and the area of occupancy (AOO) as 32 km^2. This species was common in the past according to Gardner (1846) and Trimen (1895). Today, however, this is no longer the case in montane forests. Since most populations of *Aeschynanthus ceylanicus* were collected within protected areas there is a less adverse impact from anthropogenic activities and thus the decline of the

species may have different reasons, such as perhaps effects of climate change. A continuing decline of these populations can be projected under both EOO and AOO and quality of habitat in the future. Therefore, the present conservation status for *A. ceylanicus* is assessed as Endangered (EN) with both EOO and AOO as B1ab(i,ii,iii) +2ab(i,ii,iii).

Additional Specimens Examined. KANDY DISTRICT: just SW of Corbet's Gap junction, *Davidse 8505* (L, MO); Ambagamuwa, *Gardner* part of *CP 1782* (PDA); forest behind Loolkanduratea Estate, *Ranasinghe 1073* (PDA, E). **NUWARA ELIYA DISTRICT:** Adam's Peak, *Gardner* part of *CP 1782* (PDA) near Hakgala, Monte Galaha, *Bernardi 15826* (PDA); foot of Hakgala Peak above the Garden, *Cramer 3449* (PDA, L); just behind Hakgala Botanic Garden, *Davidse and Sumithraarachchi 7974* (K, L, MO, PDA); above the Hakgala Botanic Garden, *Nooteboom 3229* (PDA, L); Hakgala trail from Garden to the 1st summit of the Hakgala Mountains, *Sohmer and Sumithraarachchi 10134* (MO, PDA); *Sohmer and Sumithraarachchi 10136* (PDA); Hakgala, *Waas 94* (K, PDA); scrambling on rocks in forest area behind Hakgala Botanic Garden, *Theobald and Grupe 2396* (PDA); Kandapola, transect 141H, *Jayasuriya 8280* (PDA); middle part of Nuwara Eliya, *Attanayake 2* (PDA); *Jayasuriya 5493* (PDA); Hakgala Garden, trail to Hakgala Park, *Sohmer, Jayasuriya and Eliezer 8514* (PDA); *Ranasinghe 1063* (PDA, E); Rambodde, *Gardner 599* (K); Tammetiya Kele, NW of Pidurutalagala Range, *Jayasuriya, Balasubramaniam and Wijesundara 3016* (MO, PDA); *Jayasuriya 970* (PDA); *Tirvengadum and Cramer 50* (PDA). **RATNAPURA DISTRICT:** Massena Forest Reserve, Balangoda, *Ranasinghe 1075*. **LOCALITY UNKNOWN:** Thwaites, part of *CP 1782* (PDA); *s.coll.* part of *CP 1782* (PDA); de Silva *s.n.* (PDA); *Gardner 599* (K [three sheets]).

3.4 Conclusions

The present taxonomic revision of the family Gesneriaceae in Sri Lanka is based on extensive plant expeditions across the entire distribution range of the family in Sri lanka covering 12 administrative districts out of 24 such as Anuradhapura, Badulla, Balangoda, Galle, Kandy, Kegalle, Kurunegala,Matale, Matara, Nuwara Eliya and

Rathnapura. In total, 38 field expeditions were conducted totalling 76 fieldwork days which resulted in the collection of 152 specimens, of 84 populations in 14 species.

The present study represents a comprehensive taxonomic revision of the family Gesneriaceae considering all 14 species occurring in Sri Lanka, and included revised descriptions for six genera and 13 species and the description of one new species, *Henckelia wijesundarae*.

The taxonomic revision of the present study confirmed the existing taxonomy of eight Gesneriaceae species present in Sri Lanka, i.e. *Rhynchoglossum gardneri R. notonianum, Epithema ceylanicum, Rhynchotechum permolle, Henckelia moonii, H. walkerae, H. zeylanica*, and *Aeschynanthus ceylanicus*.

The two subclades displayed in the molecular phylogeny of *Championia reticulata* are not reflected in morphological characterization. Therefore, a single species in the existing morphological taxonomy is accepted. One new species, *Henckelia wijesundarae* Ranasinghe and Mich.Möller has been described using both molecular and morphological data and comprehensive sampling at population level.

The present taxonomic studies confirmed that the intra-specific variation observed in *Henckelia humboldtiana* is not consistently distributed. Hence, this study proposes it to form a species complex with *H. incana* from South India and *H. floccosa* from Sri Lanka. This complex requires more detailed studies in the future, especially with the inclusion of South Indian material.

Since the existence of the putative hybrid between *H.communis* and *H. angusta* could not be confirmed in the present study, the two species are treated here as distinct species. 17 lectotypifications were carried out for the 13 taxa treated here.

The highest threatened category for each species was selected based on Red Listing assessments conducted here. All 13 Sri Lankan Gesneriaceae taxa are recognized as threatened to varying degrees: critically endangered, endangered or vulnerable. *Henckelia floccosa* is Critically Endangered and Possibly Extinct (CRPE); *H. walkerae* and *H. wijesundarae* are Critically Endangered (CR); *A. ceylanicus, C. reticulata, H. angusta, H. humboldtiana, H. moonii, H. walkerae, H. zeylanic, R. notonianum* and *R. gardneri*, are endangered (EN); *R. permolle, E. ceylanicum* and *H. communis* are proposed ot be categorised Vulnerable (VU).

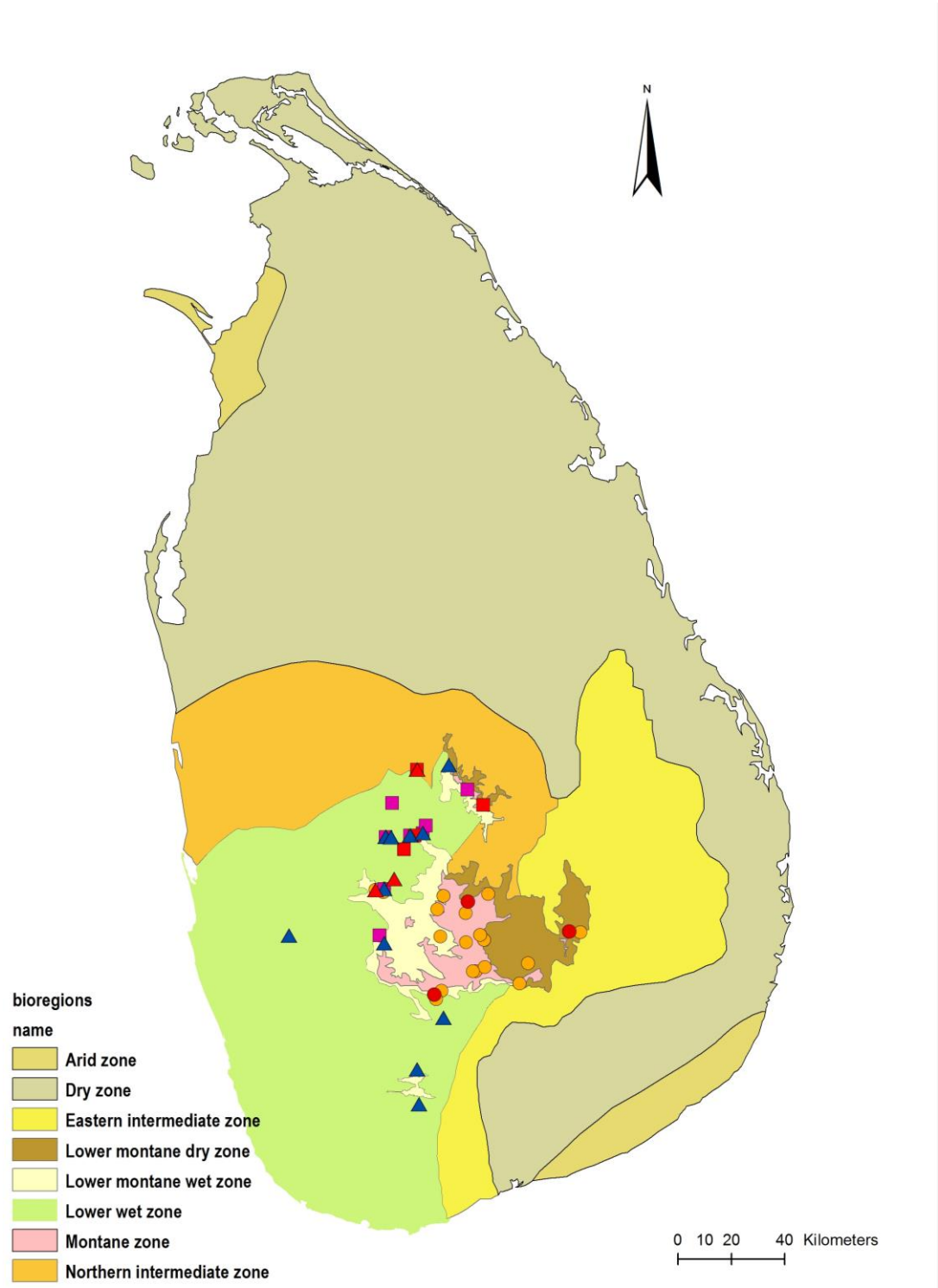


Figure 3.16 Distribution of *Rhynchoglossum gardneri* ■ ,*R. notonianum* ● , and *Epithema ceylanicum* ▲ in the bioclimatic map of Sri Lanka. Symbols coloured in red indicates collection localities of the present study.

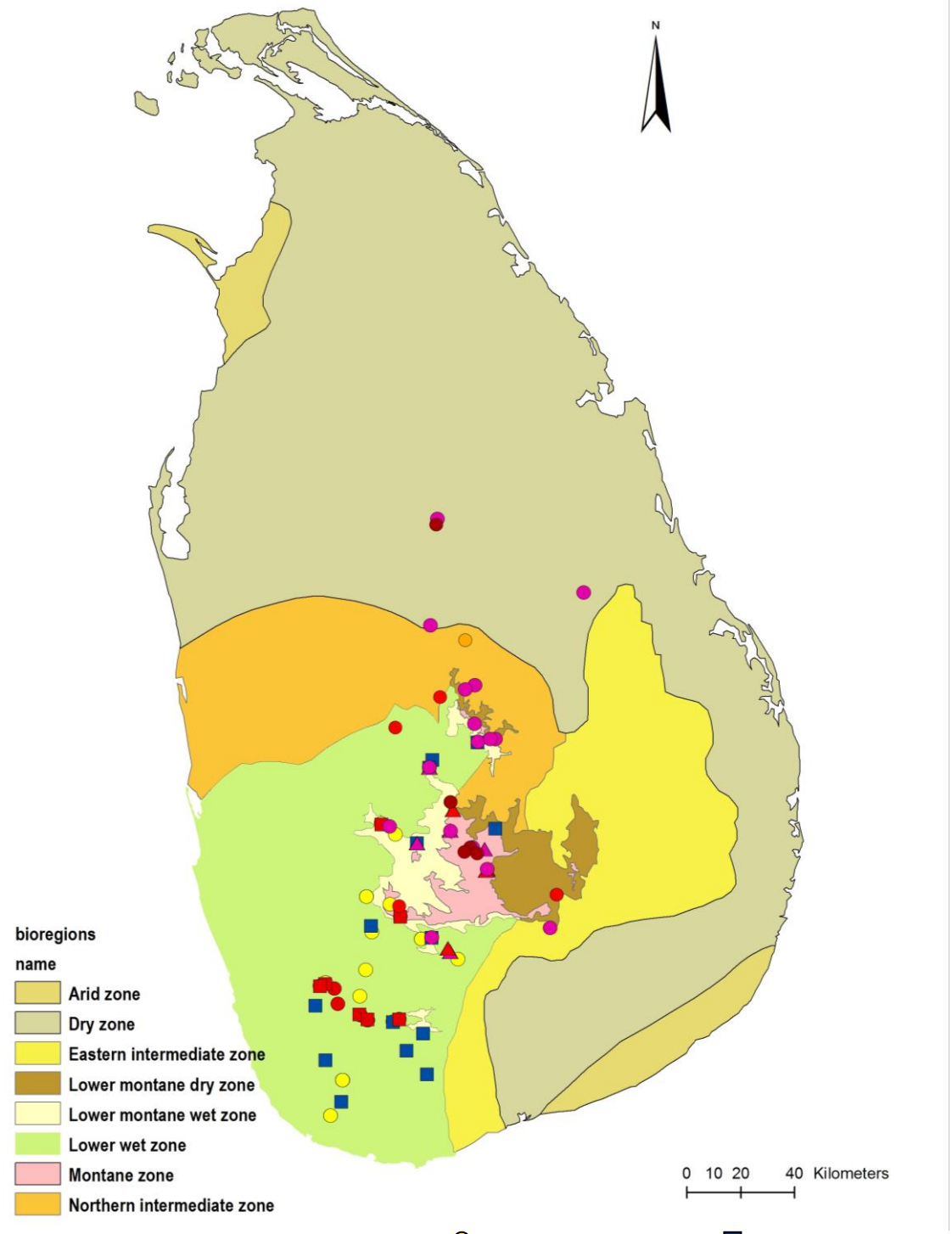


Figure 3.17 Distribution of *Championia reticulata* ○, *Rhynchotechum permolle* ■, and *Aeschynanthus ceylanicus* △ in the bioclimatic map of Sri Lanka. Symbols coloured in red indicates collection localities of the present study.

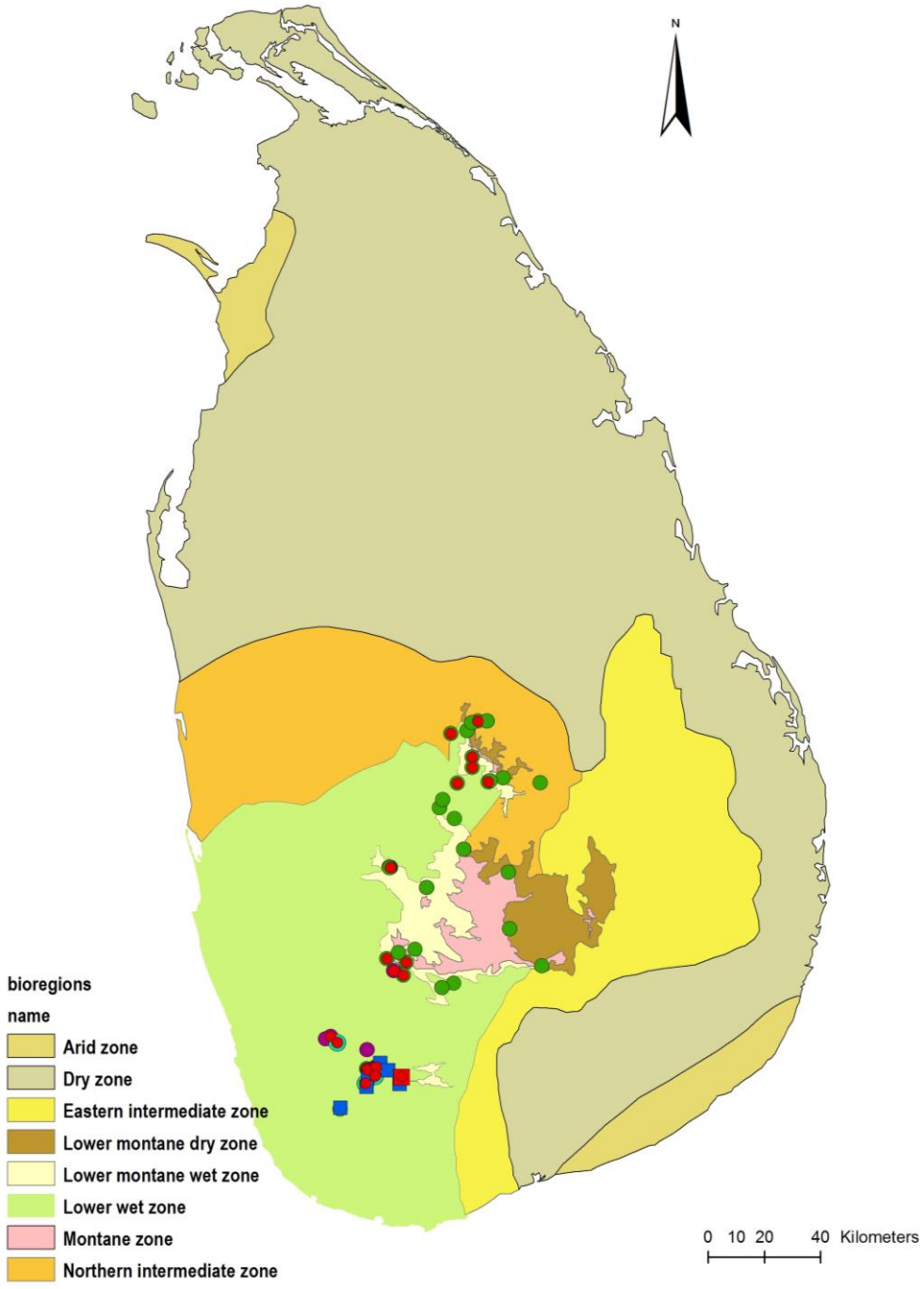


Figure 3.18 Distribution of *Henckelia communis* "communis green", ● , and *Henckelia angusta* ■ in the bioclimatic map of Sri Lanka. Symbols coloured in red indicates the collection localities of the present study. *H. communis*, "communis purple" ● and the putative hybrid ● Symbols coloured in red indicates collection localities of the present study.

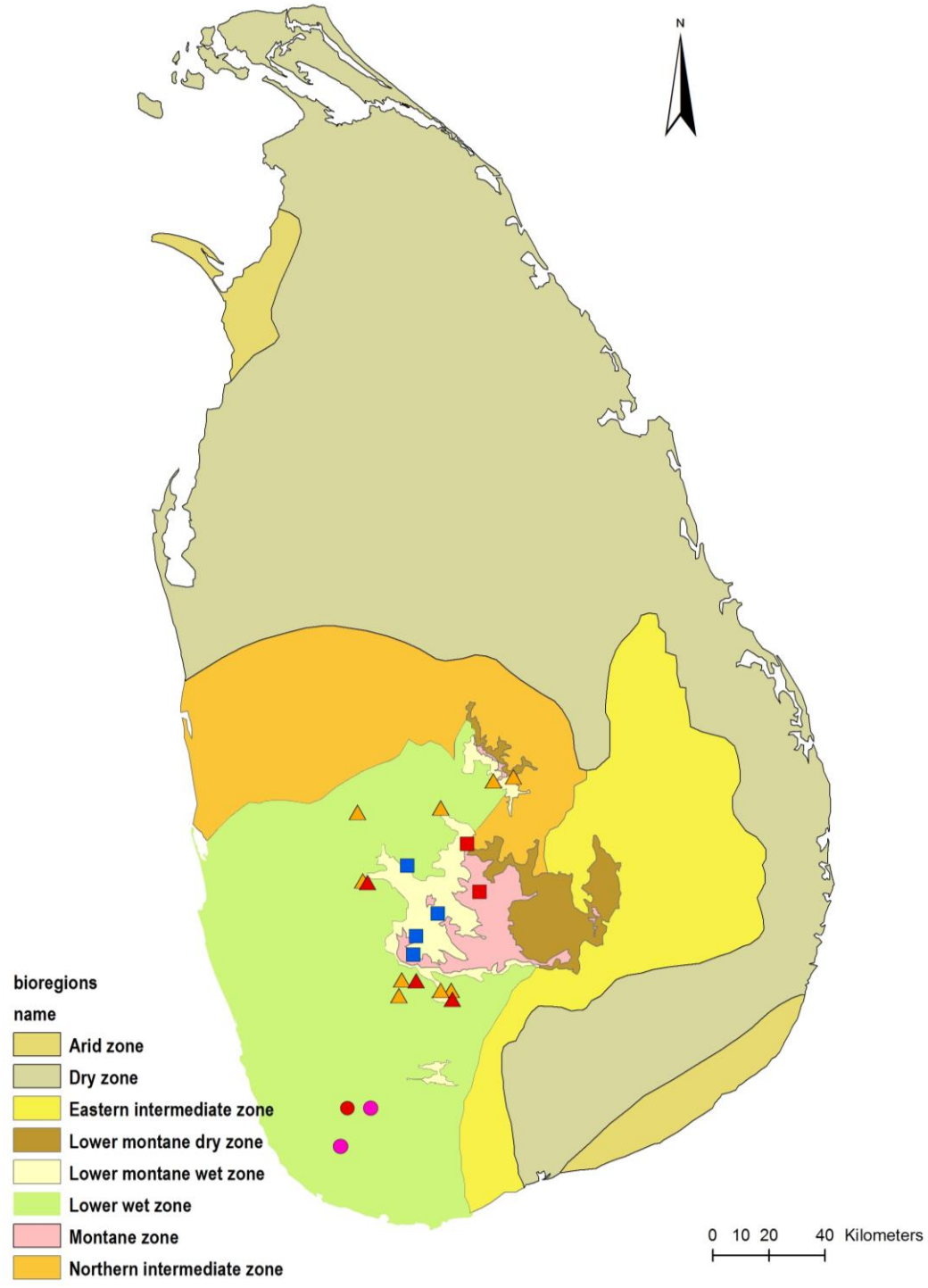


Figure 3.19 Distribution of *Henckelia moonii* △, *H. walkerae* □, and *H. wijesundarae* ○ in the bioclimatic map of Sri Lanka. Symbols coloured in red indicates collection localities of the present study.

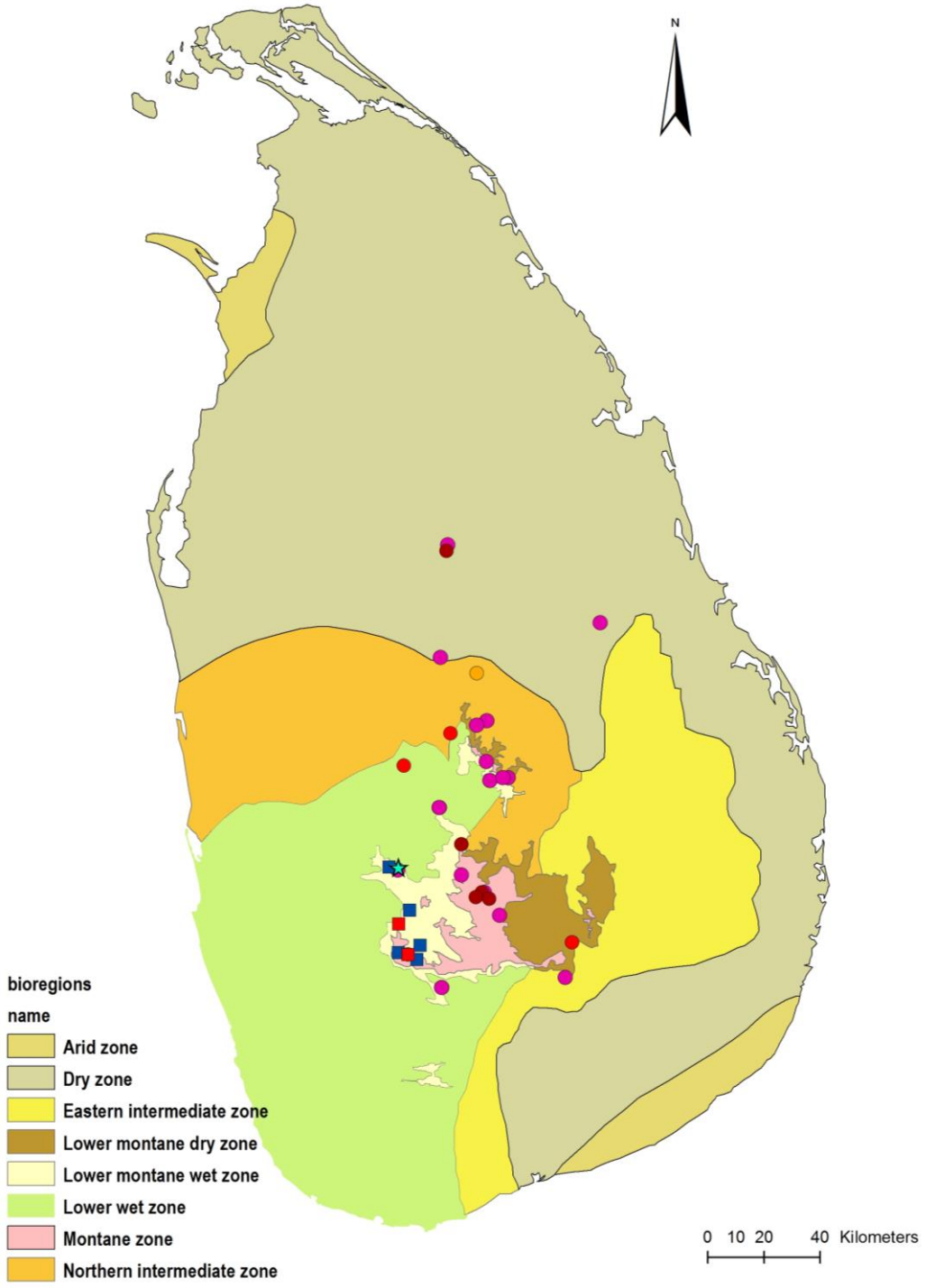


Figure 3.20 Distribution of *Henckelia humboldtiana* clade I ○, Clade II and Moragahakanda population found in both clades I and II ;, *H. floccosa* ☆ , and *H. zeylanica* ■ in the bioclimatic map of Sri Lanka. Symbols coloured in red indicates collection localities of the present study.

Chapter 4 Biogeographic affinities of the Gesneriaceae flora of Sri Lanka

4.1 Overview of historical biogeography

If the positions of continents and the climate had been static over the earth's history then it would be rather difficult to explain endemisms, provincialisms and disjunctions (Lomolino *et al*., 2010). It is apparent that organisms evolved under different geographical and climatic conditions that shaped the present day distribution of biodiversity on earth (Sanmartin, 2012). Understanding the origin and distribution of life on earth with respect to past geographical and climatic events is therefore very significant for the interpretations of the assembly of biodiversity on present day earth and its future directions.

The present day disjunct distributions of biota have been explained through plate tectonics, rare dispersal events and global climatic changes during glaciation and inter-glaciation periods based on the distribution of angiosperms and fossil evidence (Raven and Axelrod, 1974). These forces had a tremendous influence on the present day distribution patterns of various groups of flowering plants. Therefore, with the emergence of the plate tectonic theory in the early 1970's, vicariance was considered as a fundamental reason for disjunct plant distributions (Raven and Axelrod, 1974) and the importance of long-distance dispersal was then considered to be low (de Queiroz, 2005).

Several studies combining fossil evidence with dated phylogenies revealed that the disjunct distributions of flowering plants have occurred after the breakup of the Gondwanan supercontinent (Pennington *et al*., 2006; Olmstead, 2013; Armstrong *et al.*, 2014; Richardson *et al.*, 2016). The exact time at which angiosperms first appeared on land is still debated and the evidence from the oldest fossil pollen of angiosperms from China dated to ~125 Mya in the early Cretaceous (Sun *et al.*, 2002). In fact, several studies that included dated phylogenies showed that contintental disjunctions are too young to be explained by vicariance (Givnish *et al.*, 2004; Sytsma *et al.*, 2004; Pennington *et al*., 2006). There are three mechanisms proposed through which transoceanic long-distance dispersal can occur; rafting aided

by water currents, wind (Renner, 2004; Schaefer *et al.*, 2009; Lomolino *et al.*, 2010) and dispersal by birds (Viana *et al.*, 2015). Furthermore, several additional factors are significant for the distribution of plants and other organisms on earth especially at local and regional scale. These can be categorized as ecological (climate and geology) and biological (speciation and extinction) (Lomolino *et al.*, 2010).

Based on phylogenetic reconstruction, plate tectonics, climatic history and fossil records it is possible to formulate several possible explanations and hypotheses for the origin and disjunct distribution of plants on earth. Moreover, the importance of phylogenetic relationships and dated molecular phylogenies is recognized for understanding the biogeographic history of organisms on earth (Lomolino *et al.*, 2010).

4.1.1 Current knowledge of the biogeographical history of the family Gesneriaceae

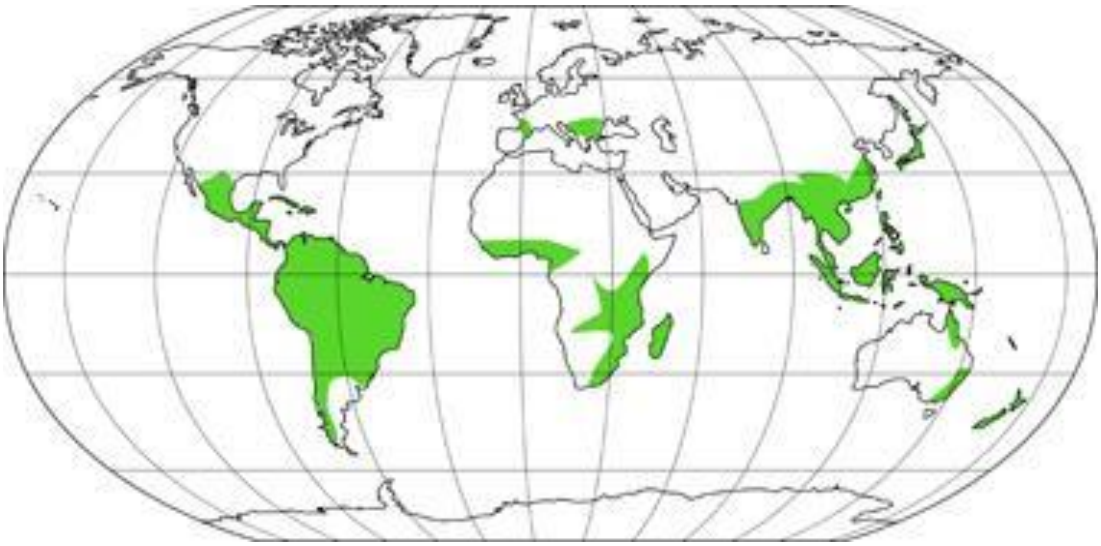


Figure 4.1 Present day global distribution of family Gesneriaceae. (Source:http://www.thecompositaehut.com/www_tch/webcurso_spv/familias_pv/gesneriaceae.html [Accessed on 9 November 2016].)

The pantropical distribution of Gesneriaceae across several biomes in tropical and sub-tropical areas on earth makes the family a good candidate to study and understand angiosperm evolution and biogeography (Figure 4.1; Weber, 2004). The

plants were recognized as megathermal angiosperms and the origin of the family was initially discussed by Raven and Axelrod (1974) in the context of angiosperm evolution. They suggested Gesneriaceae to be of ancient Paleogene origin (lower Tertiary about 65 million years ago, Mya) when South America and Africa were in close proximity to each other. Based on this view, Burtt (1998) hypothesized a Gondwanan origin for the family and subsequent diversification was attributed to the movement of tectonic plates by considering indirect evidence on the distribution of characters such as habit, floral morphology and seed dispersal.

Table 4.1 Details of primary and secondary calibration points used in dated molecular phylogenies of family Gesneriaceae

References from family Gesneriaceae	Fossil/node	Node age (Mya)	Prior distribution	Mean/SD (offset) of lognormal distribution	Original reference
Primary Calibration Points					
Perret et al., 2013; Petrova et al., 2015	Fossil fruits of *Fraxinus*	37	lognormal	2.5/0.5 (35.0)	In Call and Dilcher (1992)
Perret et al., 2013; Petrova et al., 2015	Fossil fruits of *Paulownia*	16–23	lognormal	1.5/0.5 (15.0)	In Butzmann and Fischer (1997); Fischer and Butzmann (2006); Manchester *et al.* (2009)
Perret et al., 2013; Petrova et al., 2015	Fossil seed of *Byblis*	37.2–48.6	lognormal	2.0/0.5 (35.0)	In Conran and Christophel (2004)
Perret et al., 2013; Petrova et al., 2015	Fossil fruits and seeds of Bignoniaceae	49.5	lognormal	1.5/0.5 (45.0)	In Wesley and Hopkins (1994); Pigg and Wehr (2002)
Roalson and Roberts 2016	*Acanthus rugatus*	28.8	—	—	In Reid and Chandler 1926
Roalson and Roberts 2016	*Ajuginucula smithii*	28.4	—	—	In Reid and Chandler 1926
Roalson and Roberts 2016	*Fraxinus wilcoxiana*	44.3	—	—	In Call and Dilcher 1992

Roalson and Roberts 2016	*Paulownia inopinata*	16	–	–	In Butzmann and Fischer 1997; Fischer and Butzmann 2006; Manchester et al. 2009
Roalson and Roberts 2016	*Cantisolanum daturoides*	44.3	–	–	In Collinson et al. 1993
Roalson and Roberts 2016	Unnamed (Bignoniaceae)	49.4	–	–	In Wehr and Hopkins 1994; Pigg and Wehr 2002
Roalson and Roberts 2016	Unnamed (Bignoniaceae)	35	–	–	In Manchester 1999
Secondary calibration points					
Perret et al., 2013; Petrova et al., 2015	Gesneriaceae/*Peltanthera* split	71	lognormal	2.5/0.5 (70.0)	In Bremer *et al*. (2004)
Perret et al., 2013; Petrova et al., 2015	Lamiales stem age	104–106	lognormal	2.5/1.0 (95.0)	In Bremer *et al*. (2004); Janssens *et al*. (2009)
Roalson and Roberts 2016	Lamiales	106.9			Janssens et al. 2009
Roalson and Roberts 2016	Coffea	112.8			Janssens et al. 2009

The first substantial dated phylogeny of the family Gesneriaceae included 303 accessions using 202 Gesneriaceae species and used three chloroplast markers, *mat*K, *rps*16 and *trn*L-F (Perret *et al*., 2013). This study used four primary calibration points based on fossils of Lamiales and the two secondary calibration points as detailed in the Table 4.1. They included a majority of samples from the New World, but only four from the Old World Gesneriaceae. According to Perret *et al*. (2013), the origin of Gesneriaceae is likely to be in South America, specifically in the temperate Andes and Amazonian rainforest in the late Paleocene period with an estimated stem age of 57.5 million years ago (Mya) (excluding *Sanango*).

In a later work, the Gesneriaceae family was sampled more equally between two major subfamilies, Gesnerioideae of predominantly New World and

Didymocarpoideae of predominantly Old World Gesneriaceae (Petrova *et al.*, 2015) using principally the same calibration points as Perret *et al*. (2013). Petrova *et al*. (2015) used 25 samples from subfamily Gesnerioideae and 56 samples from subfamily Didymocarpoideae, and analysed sequences from two chloroplast markers, *atp*B-*rbc*L and *trn*L-F. Their stem age estimates for the family Gesneriaceae excluding *Sanango* was 71.88 Mya and this was significantly older than that of Perret *et al*. (2013). Including *Sanango* increased the stem age to 77.79 Mya in the late Cretaceous (Petrova *et al*., 2015, Supplemental material fig. B7). The different age estimates of these two studies were attributed by the authors to the different sampling strategies for Old World and New World Gesneriaceae (Petrova *et al*., 2015).

Roalson and Roberts (2016) generated a dated phylogeny for Gesneriaceae that included all data available on GenBank for the family and included 768 species with an alignment matrix of 26 gene regions. They used 12 calibration points based on fossil and geologic data obtained from the literature (Table 4.1). Despite the very large gaps in their combined matrix, their crown age estimates of family Gesneriaceae were very similar to Petrova *et al*. (2015): excluding *Sanango* the stem age was 73.07 Mya, including *Sanango* it was 76.03 Mya. Luna-Castro (2016, unpublished) using a four chloroplast gene matrix (*mat*K, *ndh*F, *trn*L-F, *rps*16) and 13 calibration points, obtained values intermediate between those of Petrova *et al*. (2015) and Roalson and Roberts (2016) and Perret *et al*. (2013). A comparison of the ages of stems and crowns from Luna-Castro (2016), unpublished, Roalson and Roberts (2016), Petrova *et al*. (2015), Perret *et al*. (2013) given in Appendix 3.

4.1.2 Biogeographic history of subfamily Didymocarpoideae

The biogeographic history of subfamily Didymocarpoideae to which all the Sri Lankan and South Indian taxa belong, is a focus of the present chapter. The divergence time between the Old World (OW) Didymocarpoideae and the New World (NW) Gesnerioideae clades is dated at 44.7 Mya (Perret *et al*., 2013). The divergence time estimate for the split between the OW and NW clades is 65.52 Mya stem age and 71.88 Mya crown age (Petrova *et al.*, 2015). The divergence time estimates for this split from Luna-Castro (2016) and Roalson and Roberts (2016),

were 57.7 Mya. According to the ancestral area reconstruction studies by Luna-Castro (2016), the initial diversification of Didymocarpoideae was suggested to be 57.7 Mya and c. 67 Mya respectively. These different age estimates can be attributed to the discrepancies in sampling strategies as discussed in the previous section. In any case the age estimates are not congruent with a role of Gondwanan vicariance for the pantropical distribution of Gesneriaceae as suggested by Burtt 1998). Analysis of Perret et al. also suggested the dispersal of early lineages of Gesneriaceae to the Palaeotropics and Australasia during the Eocene and Oligocene, most likely through long-distance dispersals across the southern hemisphere. Thus, Perret *et al*. (2013) analysis favoured the alternative interplate dispersal hypothesis (Morley, 2003; Sanmartín and Ronquist, 2004; Renner, 2005) involving dispersal from South America to Australasia to explain the origin of the transoceanic distribution of Gesneriaceae.

The Didymocarpoideae lineage was established in eastern Asia/ South East Asia at 70 Mya following a dispersal event from Andean South America where the family was originated (Roalson and Roberts, 2016). Phylogenies of Gesneriaceae recognize the placement of *Jerdonia indica* as the most basal lineage of tribe Trichosporeae (Möller *et al.,* 2009; Weber *et al.,* 2011). In the most comprehensive phylogeny of the family *Jerdonia indica* falls on the most basal branch of tribe Trichosporeae (Möller *et al.,* 2009). The dated phylogenies of the family also recognized the basal lineage of *J. indica* in Trichosporeae (Petrova *et al*., 2015; Luna-Castro, 2016). Although this gives an indication of the importance of the Indian subcontinent for the biogeographic history of OW Gesneriaceae the lack of voucher specimen or any locality data for the collection of *J. indica* from South India so far delayed this elucidation.

4.1.3 Geological and climatic history of Sri Lanka

The diverse settings of topography and varied climate in Sri Lanka created different habitats and ecosystems for a range of biota, which subsequently resulted in a rich biodiversity of the island (Gunawardene *et al.,* 2007). The present topography of Sri Lanka was shaped by the past geological history and also has a tremendous influence on the present day biodiversity (Ashton and Gunatilleke, 1987; Katupotha, 2013).

The supercontinent Pangea began to break up in the early to mid-Jurassic at about 180-200 Mya. In the breakup of Pangaea, the southern land mass, Gondwanaland comprising present day Africa, Madagascar, Arabia, India, Australia, Tasmania, New Guinea, New Zealand, New Caledonia and Antarctica, separated from Laurasia (Lomolino *et al.*, 2010). The Gondwanaland started drifting further south after the split and Gondwana itself also broke apart. The breakup of Gondwanaland began also in the early Jurassic (about 180 Mya) (Figure 4.2) and continued during the late Cretaceous and throughout the Paleocene (100 to 58 Mya) (Figure 4.3, 4.4). The breakup resulted in four main landmasses (South America, Africa, Madagascar-India-Sri Lanka, and Antarctica-Australia-New Zealand).

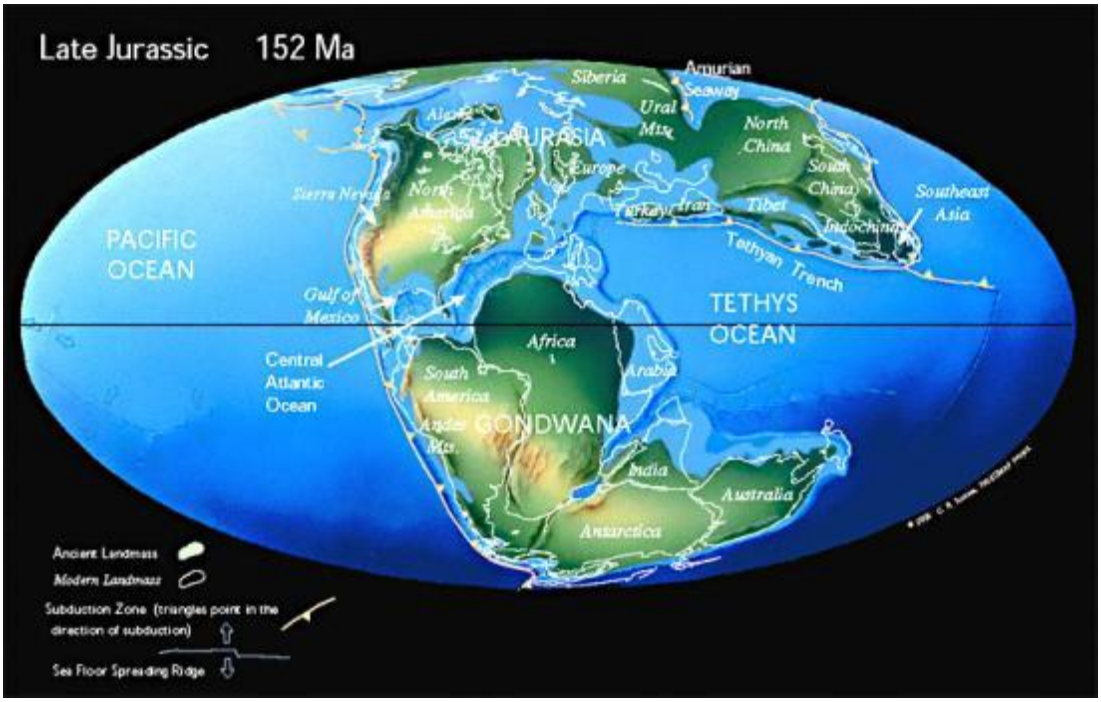


Figure 4.2 The supercontinent of Pangea began to break apart in the Middle Jurassic. In the Late Jurassic the Central Atlantic Ocean was a narrow ocean separating Africa from eastern North America. Eastern Gondwana had begun to separate from Western Gondwana. Source: http://www.scotese.com/late1.htm [Accessed 12 November 2016].

The Deccan Plate, present day Sri Lanka and India, separated from the southern part of the Gondwanan supercontinent in the Lower Cretaceous about 130 Mya (Lomolino *et al.*, 2010). During the Late Cretaceous, about 94 Mya, the global climate was warmer than today's climate and no ice existed at the poles (Scotese, 2016; Figure 4.4).

Subsequently the still joined Deccan and Madagascar-Seychelles plates moved rapidly northwards until they separated, in the Late Cretaceous or early Paleocene, 80 Mya and 65 Mya respectively (Ashton and Gunatilleke, 1987; Lomolino *et al.*, 2010). After the split, the Deccan Plate drifted North in isolation from neighbouring continents for about 20 million years until it collided with the Southern Laurasian coastline in the Eocene epoch of the early Tertiary period about 45 Mya (Ashton and Gunatilleke, 1987). Sri Lanka was still connected to India for another 20 million years somewhere around the Miocene (about 25 Mya) after the collision of the Deccan plate with the Eurasian landmass.

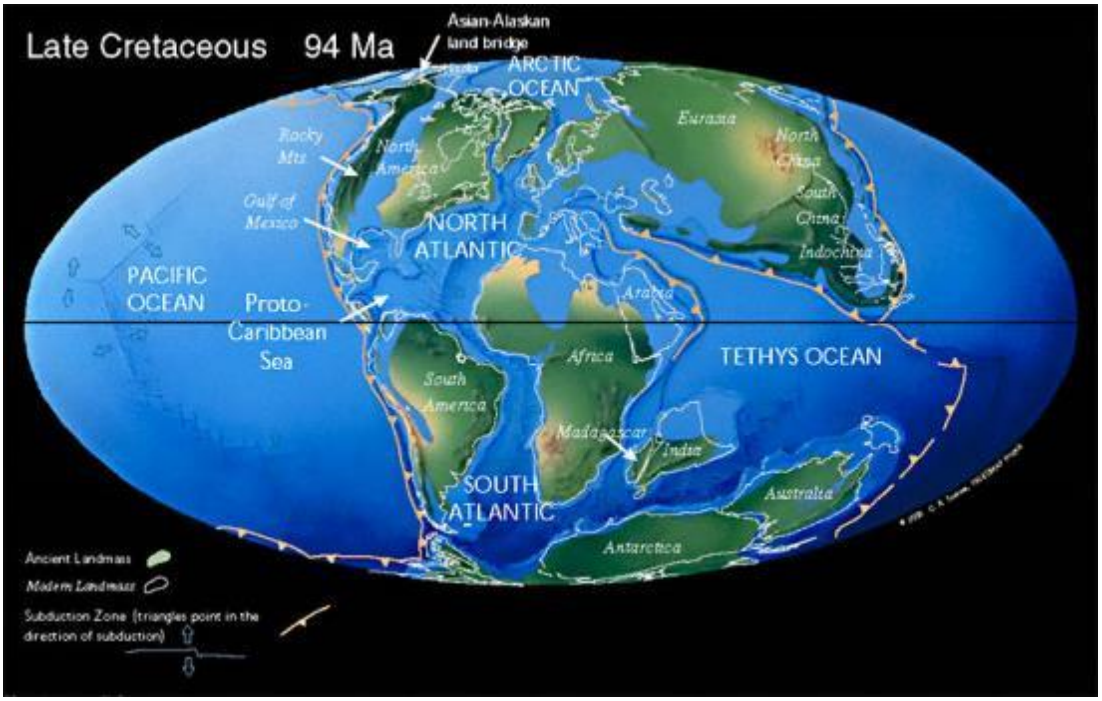


Figure 4.3 During the Cretaceous the South Atlantic Ocean was opened. India separated from Madagascar and drifted northward on a collision course with Eurasia. North America was connected to Europe, and Australia was still joined to Antarctica. Source: http://www.scotese.com/cretaceo.htm [Accessed 12 November 2016].

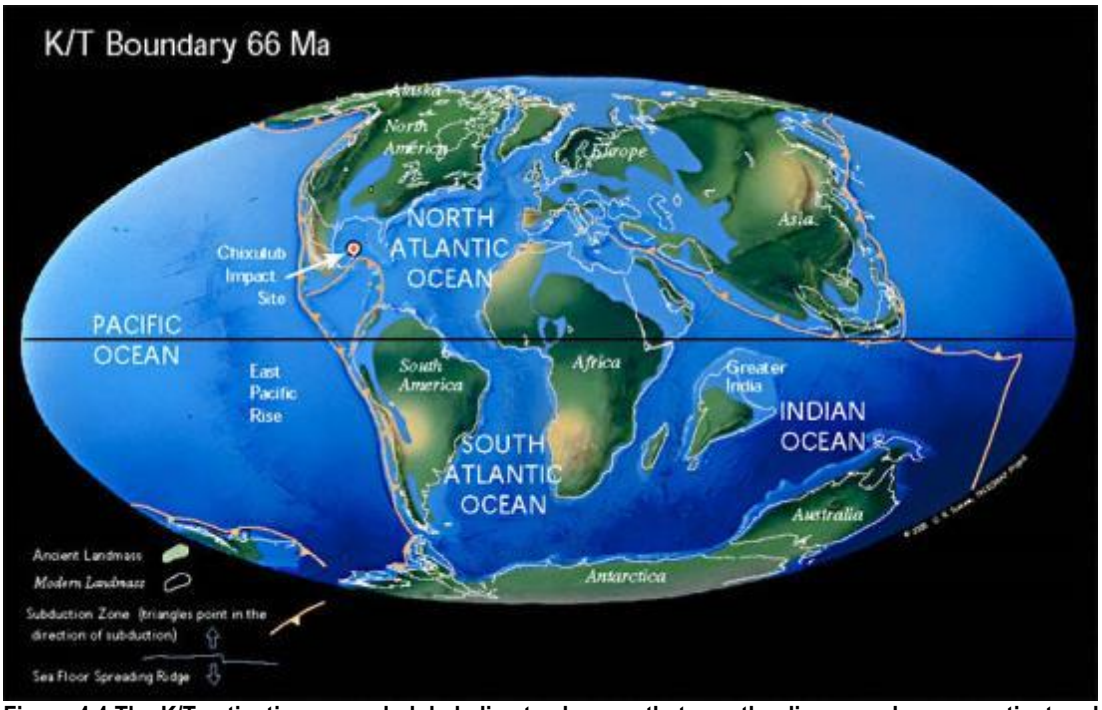


Figure 4.4 The K/T extinction caused global climate changes that saw the dinosaurs become extinct and many other forms of life. By the Late Cretaceous the oceans had widened, and India approached the southern margin of Asia. Source: http://www.scotese.com/K/t.htm [Accessed on 12 November 2016].

The first severance of the land connection between India and Sri Lanka was reported to have occurred in the Miocene to Pliocene (13–25 Mya) as evidenced by the nature of the marine deposits in the northern and north-western parts of Sri Lanka as well as Quilon on the Indian Peninsula (Majumder, 1984). Then, Sri Lanka was separated from the Indian mainland by the submersion of the land area between the two countries (Ashton *et al.*, 1997).

During the Pleistocene glaciations (0.5 – 2 Mya) in the Quaternary period, Sri Lanka was intermittently connected to Peninsular India (Rohling *et al.*, 1998). These repeated connections between India and Sri Lanka can be explained by sea level fluctuations resulting from glacial and interglacial events during the Pleistocene epoch. These land bridge connections between Sri Lanka and the Indian Peninsula in the Pleistocene epoch are also supported by the Siwalik (Himalayan hill range) fauna being present in Sri Lanka (Majumder, 1984). Sri Lanka reached its present position at 5º52´N-9º 55´N, 79º 30´E-81º 55´E by the Lower to Middle Pleistocene (1.806-0.781 Mya) (Katupotha, 2013). Then, Sri Lanka became a continental island due to

the sea level rise and submersion of the land area between the two countries at about 10,000 years ago (Vaz, 2000).

After the separation from Gondwana, the Deccan Plate (Sri Lanka + Peninsular India) was subjected to rising elevations (Ashton and Gunatilleke, 1987) during its northward movement. The occurrence of these uplifts in Sri Lanka was discussed in Katupotha (2013) and references cited therein. They stated that during the period from Lower to Upper Jurassic, Sri Lanka was subjected to at least four major upliftments in the Jurassic, Miocene, Pliocene and Pleistocene times. These uplifts resulted in the geological formation of the central mountain range and other mountains in Sri Lanka.

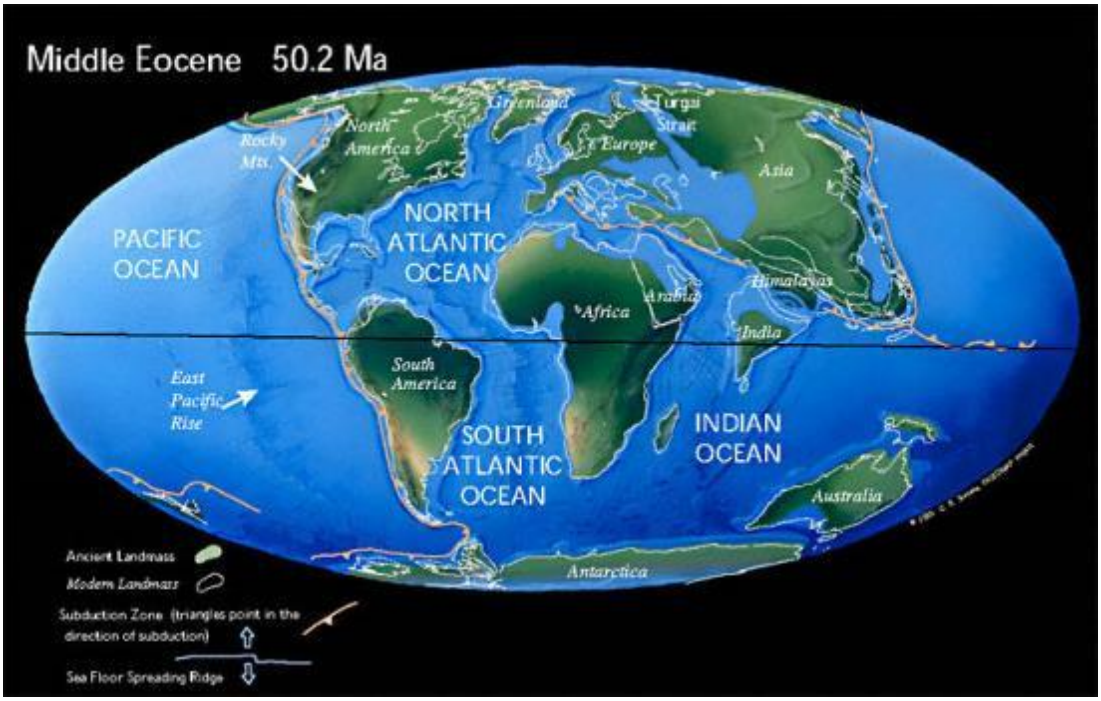


Figure 4.5 20 million years ago, Antarctica was covered by ice and the northern continents were cooling rapidly Source:http://www.scotese.com/newpage9.htm [Accessed on 12 November 2016].

There were several events contributing to extinctions of the original biota on the rafting Deccan Plate (Naggs and Raheem, 2005). The climate at the beginning of the formation of the plate, when it was part of Gondwana, was cold temperate which rapidly changed to a tropical climate during the northward rafting (Ashton and Gunatilleke, 1987). This influenced the replacement of the original temperate biota.

During the northward rafting of the Deccan Plate there were also massive volcanic eruptions and lava deposits known as the Deccan Trap, overlaying the Indian-Seychelles Plate. These volcanic eruptions dated back to 65 Mya, and may have played a part in the well-characterized mass extinction event around the late Cretaceous and Tertiary periods known as the K/T boundary extinction (Wignall, 2001; Courtillot and Renne, 2003).

Over most of the period of northward rafting Sri Lanka had an aseasonal humid climate (Ashton and Gunatilleke, 1987), which created favourable refugia for many taxa that were exposed to the extinction-level events on the Deccan plate reported above. Therefore, the present-day Sri Lankan biota, especially those in the southwest may harbour significant relict biogeographic elements of regional and global significance (e.g. Azuma *et al.*, 2008).

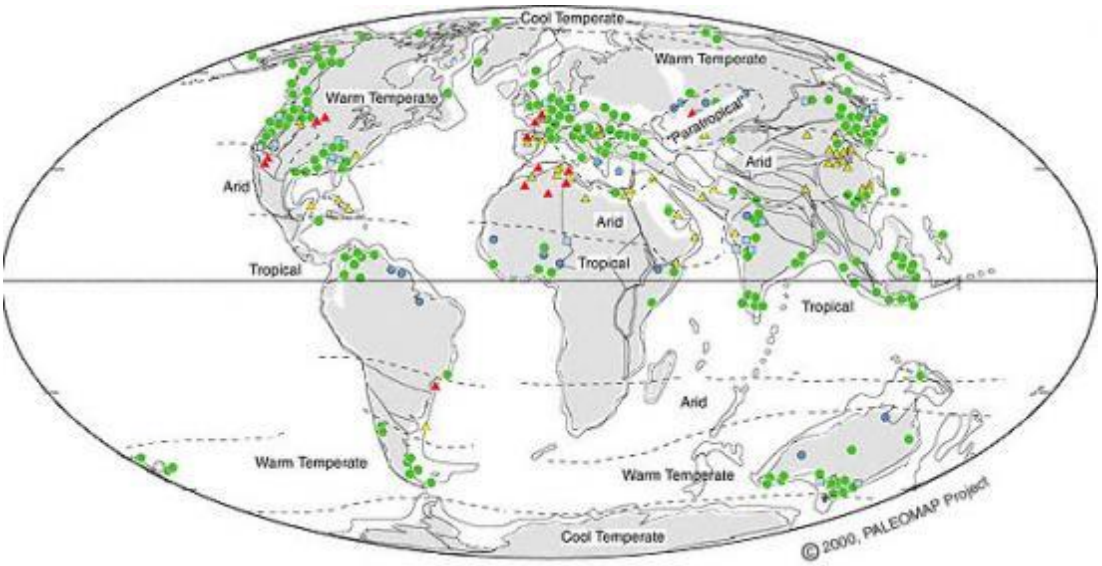


Figure 4.6 Global climate during the Late Eocene was warmer than today. Ice had just begun to form at the South Pole. India was covered by tropical rainforests, and warm temperate forests covered much of Australia. warm tropical, warm tropical or cool temperate, dry arid calcrete, dry arid evaporate, warm temperate. Source: http://www.scotese.com/lateeoc1.htm [Accessed on 20 December 2016].

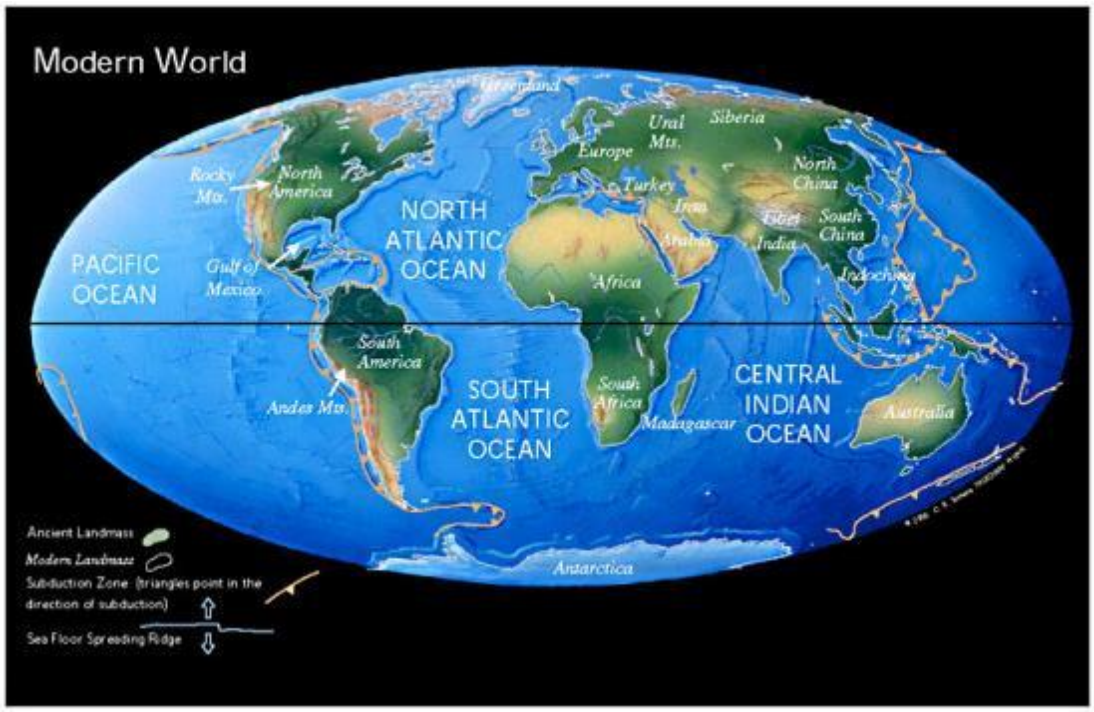


Figure 4.7 Modern world resultant from plate tectonic and climate historic events. Source: http://www.scotese.com/modern.htm [Accessed on 12 November 2016].

4.1.4 Biogeographic affinities of the Sri Lankan Flora

The composition of the Sri Lankan biodiversity is greatly affected by Sri Lanka being part of the Deccan plate. The Deccan Plate which has received much attention in the context of angiosperm evolution and diversification (Morley, 2003; Sanmartín and Ronquist, 2004; Raven and Axelrod, 1974). One of the significances of the plate is as vector for rain forest plants from Africa to Asia, involving biotic migration through the Deccan plate (referred to as the 'Out-of-India hypothesis'), and was recognized as a significant path for plant migration (Conti *et al.*, 2002).

There are very close affinities in the flora and fauna of Sri Lanka with South India, the southern part of the Peninsular India (Ashton and Gunatilleke, 1987; Bossuyt *et al.*, 2004). The close affinities between these areas lead to their collective recognition as one of four oriental biogeographical regions, the 'Ceylonese region' that included the Sri Lankan island and part of South India (e.g. Wallace, 1876; Blanford, 1888).

Diverse biogeographic elements were recognized in Sri Lankan biodiversity (Abeywickrama, 1956) which originated from a variety of sources, reaching back in time as far as ancient Gondwana. According to Raven and Axelrod (1974) for

example, subfamily Hortonioideae of family Monimiaceae which is restricted to Sri Lanka may have derived from West Gondwanaland stock, and survived during the northward movement (= "Noah's ark" distribution (Mckenna, 1973; Renner *et al.*, 2010). Trimen (1885) and Abeywickrama (1956) recognized six floristic elements in the angiosperm flora in Sri Lanka: “Sri Lankan endemics”, “Indo-Sri Lankan”, “Himalayan”, “Malayan”, “African”, and “Pantropical and Cosmopolitan” components. Despite the strong affinities to the Indian Peninsular the high endemism is a striking feature of the Sri Lankan angiosperm flora (Trimen, 1885; Gunatilleke and Gunatilleke, 1990). In total, 894 angiosperm species, i.e. about 28% of the entire angiosperm flora, represent the endemic component that diversified in Sri Lanka (Wijesundara *et al.*, 2012). Some plant species, such as those belonging to the Indo-Sri Lankan element, may have a shared origin after their northward drift in isolation on the Deccan Plate. Although the period of isolation was seemingly not long enough for the evolution of new families, evolution continued within families and genera: Gunatilleke and Gunatilleke (1990) reported that about 30% of all non-endemic plant species in Sri Lanka are only shared with South India. For example some orchid genera, such as *Seidenfadeniella*, *Cottonia*, *Taprobania*, and *Srihookera* (Sathish Kumar and Manilal, 1994) are recognized as South Indian elements that are also represented in the Sri Lankan flora. Contact with Laurasia in the mid-Tertiary, however allowed prolonged mixing of the Deccan flora of Gondwanan origin with the tropical flora that occupied southern coastal Laurasia (Ashton and Gunatilleke, 1987). The “Himalayan”, “Malayan”, “African” and “Pantropical and Cosmopolitan” floristic elements may have come into existence or migrated into India after the collision of the Deccan Plate with Laurasia (Gunatilleke and Gunatilleke, 1990). *Rhododendron* (Ericaceae) and *Berberis* (Berberidaceae) originated in the Himalayan region and have distribution points in montane forests of Sri Lanka, which is indicative of affinities to North India and Asia (Ashton and Gunatilleke, 1987). One species in Sri Lanka, *Nepenthes distillatoria* (Nepenthaceae), has affinities to the Malayan region, and the arrival of such Malayan species is considered through long distance dispersal (Ashton and Gunatilleke, 1987). *Rhipsalis baccifera* in family Cactaceae were thought to have initially diversified in Africa and Madagascar and its

presence in Sri Lanka an example of an African/Madagascan link (Ashton and Gunatilleke, 1987). There are several pantropical species present in Sri Lanka, for example *Tephrosia purpurea* in family Fabaceae, *Murdannia nudiflora* in family Commelinaceae and *Spiranthes sinensis* in Orchidaceae (Ashton and Gunatilleke, 1987).

However, there are little compelling evidences to link the biotic elements in Sri Lanka to different biogeographical-historic events of the Sri Lankan flora. For example, presence of Gondwanan elements is supported by a dated phylogeny of the family Crypteroniaceae with the Sri Lankan endemic species, *Axinandra zeylanica* Thw. (Conti *et al.*, 2002), and in a phylogeny of the family Dipterocarpaceae (Dayanandan *et al.*, 1999). A molecular dated study (Bossuyt *et al.*, 2004) on two invertebrate and four vertebrate groups suggested that the Sri Lankan fauna is derived from an evolutionarily diverse stock from the Indian mainland. According to their study the presence of a high number of endemics also confirmed an overall limited biotic interchange that has left both areas with unique biodiversity elements. Apparently the recognition of different floristic elements in Sri Lankan as well as on the entire Deccan Plate has been studied only sporadically and here, based on traditional taxonomic and phylogenetic methods (Ashton and Gunatilleke, 1987).

The present study is an opportunity to study historic biodiversity aspects with a comprehensive representation of the Sri Lankan biota using dated phylogenies in a global setting to unravel more precisely the biogeographic history of floristic elements in Sri Lanka.

4.1.5 Biogeographic history of family Gesneriaceae in Sri Lanka and South India

There exist few publications with a biogeographic element directly relevant to the Gesneriaceae in Sri Lanka. In a phylogenetic analysis of tribe Epithemateae, using the *rbc*L/*atp*B-spacer and *trn*L-F intron-spacer regions of chloroplast DNA, Mayer et al. (2003) recognized that the Sri Lankan/South Indian species, *Rhynchoglossum notonianum*, was closely related to the Neotropical *R. azureum* and both sister to *R. obliquum* which is widely distributed from India, Southern China, and Malay Archipelago.

The monotypic genus, *Jerdonia indica*, from South India resided on the most basal lineage of tribe Trichosporeae (Möller *et al*., 2009). This was confirmed in dated phylogenies of Gesneriaceae (Petrova *et al.*, 2015; Luna-Castro, 2016; Roalson and Roberts, 2016), which might indicate the importance of the Indian subcontinent for the origin and diversification of the majority of Old World Gesneriaceae, subfamily Didymocarpoideae (c. 65 genera with ca 2000 species) (Weber *et al*., 2013).

Thus far, there have been too few species included from Sri Lanka and India to understand the importance of the Indian subcontinent in the biogeographic history of the family Gesneriaceae. Therefore, the aims of this chapter were to elucidate the phylogenetic origins, affinities and ages of Sri Lankan and South Indian Gesneriaceae. The tree topology generated from the phylogenetic analysis will be used as framework for the dated BEAST tree and to infer the biogeographic movements of Gesneriaceae in ancestral area reconstructions in BioGeoBEARS (Matzke, 2013). BEAST is a standard software for the analyses of molecular sequences to infer time calibrated phylogenies (Drummond and Rambaut, 2007) that was also used in the previous dated phylogenetic analysis of family Gesneriaceae (Perret *et. al*. 2013; Petrova *et al*., 2015; Roalson and Roberts, 2016). This study used BioGeoBEARS (BioGeography with Bayesian Evolutionary Analysis in R Scripts; Matzke, 2013; http://cran.r-project.org/web/packages/BioGeoBEARS/index.html) to reconstruct the ancestral area distribution of Gesneriaceae with special focus on Old World Gesneriaceae and the Sri Lankan and Indian species therein. BioGeoBEARS is becoming a popular analytical method in ancestral area reconstruction in the recent times (Fonseca and Lohmann, 2015; Massana, *et al*., 2015; Roalson and Roberts, 2016).

4.2 Materials and Methods

4.2.1 Materials

The samples included in this chapter had been chosen for each to represent a species and the species to represent the distribution range of the genera (Weber *et al*., 2013). Each species was assigned between one and three of seven geographical sub regions recognized in this study, especially considering the global geographical distribution

of Gesneriaceae. These regions were: Central and South America (A), Africa and Madagascar (B), Malay Archipelago (C), Central and East Asia (D), South India and Sri Lanka (E), Australia and Pacific islands (F) and Europe (G).

The matrix used in the present chapter covers 170 Gesneriaceae species with four chloroplast markers. In this work thirteen species of Sri Lankan Gesneriaceae and another 14 samples of other Old World species were newly generated and added to the matrix of Luna-Castro (2016). Since the focus of this study is the Old World Gesneriaceae, the new World samples were reduced and the final matrices included 24 samples of subfamily Gesnerioideae, and 130 samples from subfamily Didymocarpoideae (Appendix 2). Appendix 2 gives the details of all the sequences used in the present study.

Based on previous phylogenetic work, Calceolariaceae is sister group to the family Gesneriaceae (Olmstead *et al.*, 2001; Oxelman *et al.*, 2005; Wortley *et al.*, 2005; Schäferhoff *et al.*, 2010). Because of the uncertain position of *Peltanthera* as sister to Gesneriaceae or sister to Gesneriaceae + Calceolariaceae in various studies (reviewed in Möller *et al*., 2013) this genus was omitted from the analysis here. Five outgroup taxa, *Jovellana violacea, J. sinclairii, Porodittia triandra, Calceolaria falklandica* and *Calceolaria* sp. from family Calceolariaceae were used as outgroup samples.

4.2.2 DNA extraction, PCR and sequencing

DNA extractions followed the methods as described in Chapter 02. The four chloroplast regions used for this part of the present study followed Luna-Castro (2016). These comprised two coding genes, *ndh*F (Olmstead and Reeves, 1995) and *mat*K + 32 *trnK* intron (Sang *et al*., 1997; Perret *et al*., 2013), and the *rps*16 intron (Oxelman *et al*., 1997) and the *trn*L-F intron / intergenic spacer region (Taberlet *et al.*, 1991).

The amplification of *ndh*F was performed in two reactions using the primer pair 11F–989R for the first section, and primer pair 916F–1813R for the second. Similarly, *mat*K amplifications also used two primer pairs, 206F–946R and 917F–1734R. The *rps*16 intron was amplified using a single primer pair 27F–983R. Details of all primers are given in Table 4.1. The amplification reactions were carried

out in 20 µl reaction mixture containing 2 µl 2mM dNTPs, 2 µl 10x NH_4 buffer, 1 µl 25mM $MgCl_2$, 1 µl each 10 µM forward and reverse primer, 4 µl TBT-Par, 0.4 µl BSA (Bovine Serum Albumin), 0.2 µl Biotaq polymerase (Bioline), 1 µl DNA template and 7.4 µl dH_2O. The thermocycle used for the PCR amplifications was: 94˚C for 3 minutes, 30× [94 ˚C for 1 minute, 55 ˚C for 1 minute, 72 ˚C for 1.5 minutes, 72˚C for 10 minutes], 10 ˚C forever. All regions were amplified in a Tetrad DNA Engine PCR machine (Biorad, Hertfordshire, United Kingdom). PCR amplification of *trn*L-F intergenic spacer region followed the protocol, programme and the primers used under Chapter 2 in this study.

Purification of PCR reaction mixtures and Big Dye reaction were as described in Chapter 2 using the respective PCR primers.

4.2.3 Phylogenetic Analysis

An ILD (Incongruence Length Difference; *Farris* et al., 1995; Mickevich and Farris, 1981) test was performed to measure any incongruence between the four data matrices of *mat*K, *ndh*F, *trn*LF and *rps*16, more to test for technical data coherence (absence of mixed-up or misidentified data or samples) than for evidence of recombination, since cpDNA is maternally inherited in Gesneriaceae (Möller *et al*., 2004; Puglisi *et al.*, 2011). The ILD test was carried out as partition homogeneity test with 100 replicates (where p-values < 0.05 indicate significant incongruence) in PAUP* version 4.0a150 (Swofford, 2002). The ILD suggested that there was no incongruence between the four data matrices (*p*-value = 0.08) and the matrices were analysed combined.

Three different approaches to phylogenetic analysis were performed on the concatenated matrix of all four chloroplast markers. These were Maximum Parsimony (MP) using PAUP* version 4.0a150 (Swofford, 2002), Maximum Likelihood (ML) using RAxML (Stamatakis *et al*., 2008; Stamatakis, 2014) through the CIPRUS gateway (www.phylo.org; Miller *et al*., 2010), and Bayesian Inference (BI: Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). For the BI analysis, the aligned matrices of *mat*K and *ndh*F were further partitioned into, 1st, 2nd and 3rd codon position, and for *mat*K additionally in the exon and intron region. MrModeltest version 2.3 (Nylander, 2004) was performed for the nine individual

matrices (*mat*K 1st, *mat*K 2nd, *mat*K 3rd, *mat*K intron, *ndh*F 1st, *ndh*F 2nd, *ndh*F 3rd, *trn*L-F and *rps*16) using PAUP version 4.0a150 and models selected under the AKAIKE information criterion (AIC; Akaike, 1974). Two nucleotide substitution models were selected, the GTR+I+G model for *ndh*F 1st, *ndh*F 2nd, *ndh*F 3rd, *trn*L-F and *rps*16, and GTR for *mat*K 1st, *mat*K 2nd, *mat*K 3rd and *mat*K intron.

Before the analysis PAUP* settings were adjusted to Branches collapsed (creating polytomies) if minimum branch length is zero. All characters were treated as unordered, with equal weight, and gaps treated as missing characters. Initial starting trees were obtained with PAUPRat (Sikes and Lewis, 2000), implemented with default settings with PAUP*. This method is based on the Parsimony Rachet (Nixon, 1999), a very effective starting tree searching strategy covering a broad tree space for rapid parsimony analysis especially useful for large DNA matrices. The 200 iterations conducted through PAUPRat generated 201 trees which were optimised with TBR branch swapping algorithm with steepest descent and MulTrees options in effect. Branch support was obtained in PAUP* through 10000 bootstrap replicates with random addition and TBR on, but MulTrees off (Möller *et al.*, 2009).

The ML phylogeny was generated using RAxML-HPC2 on XSEDE available in CIPRUS with default settings and implementing the MrModeltest partition models as described above, except the running time of the analysis was set to 24 hours with rapid bootstrapping with 1000 iterations and print branch lengths (-K) option in effect.

BI analyses were conducted based on the models selected from MrModeltest as described above. Two independent runs of four Markov Chain Monte Carlo (MCMC) were applied and run for one million generations, sampling every thousand generation and the burn-in set to 25% of the sampled trees after plotting the likelihoods versus generations of an initial run in Excel. Marginal densities were examined in Tracer v6.1 (Rambaut *et al.*, 2014) to check the convergence of both runs. A majority rule consensus tree with posterior probabilities was generated on the sampled trees excluding the 25% burn-in.

4.2.4 Dating Analysis

The ML phylogenetic tree that showed the most resolved tree topology generated in RAxML was used to constrain certain nodes to resolve polytomies in the dating analysis in BEAST version 1.8.3 (Bayesian Evolutionary Analysis by Sampling Trees; Drummond and Rambaut, 2007; Suchard and Rambaut, 2009). Input xml files for analysis in BEAST were created in BEAUti version 1.8.3 (Drummond and Rambaut, 2007; Drummond *et al.*, 2012). The same twoodels selected for the nine partitions in MrModeltest for the BI analysis above were applied here as well. Eight constraints were included for selected taxa based on the ML tree topology to obtain a fully resolved tree: **group 1** (3 *Agalmyra* spp.+ 3 *Deinostigma* spp. + *Metapetrocosmea peltata*); **group 2** (group 1 + *Conandron ramondioides* + *Hemiboea fangii* + *H. longgangensis* + *Oreocharis acaulis* + *O. henryana*); **group 3** (*Drymonia dodsonii* + *Rhoogeton cyclophyllus* + *Pheidonocarpa corymbosa* + *Rhytidophyllum exsertum* + *Gloxinia perennis* + *Phinaea pulchella*); **group 4** (group 3 + *Paliavana tenuiflora* + *Sinningia schiffneri*); **group 5** (*Henckelia floccosa* + *H. incana*); **group 6** (*Ornithoboea barbanthera*, *O. flexuosa*); **group 7** (group 2 + *Allocheilos cortusiflorus* + *Didymostigma obtusum* + *D. trichanthera* + *Petrocodon dealbatus* + *P. niveolanosus* + *P. dryas* + *P. gemella* + *P. lutea*); **group 8** (all Gesneriaceae taxa + *Sanango*; (Weber *et al*., 2013). Clock and tree models were linked. An uncorrelated relaxed clock model was selected with log normal distribution in order to co-estimate phylogeny and divergence times in a "relaxed phylogenetics approach" (Drummond *et al.*, 2006). The tree prior used the Yule process, a neutral model for speciation with a constant birth death rate (Gernhard, 2008). Because of the absence of fossils in Gesneriaceae, three secondary calibration points were selected based on Roalson and Roberts (2016) and a normal prior distribution set (Table 4.1).

Table 4.2 Details of calibration points used in the present study.

Calibration point	Node	Node Age (Mya)	Prior distribution	Mean/SD of lognormal distribution
1	Gesneriaceae (+ *Sanango*) crown	73.07	Normal	73.07/4.0

2	Core Gesneriaceae crown	69.66	Normal	69.66/3.5
3	Subfamily Didymocarpoideae	67.41	Normal	67.41/3.5

Twenty-four individual Markov Chain Monte Carlo (MCMC) chains each of 10 million generations were run. Each MCMC chain was sampled every thousand generation and the first 20% removed as burn-in. The individual and combined analyses were evaluated in Tracer version 1.6 (Drummond and Rambaut, 2007) to check for convergence of the runs, and an effective sampling size (ESS) >200 for all estimated parameters in BEAST for the combined analyses was reached.

All tree files of the MCMC runs were combined after the burn-in trees were removed using LogCombiner version 1.8.3. The maximum clade credibility (MCC) tree was calculated using TreeAnnotator version 1.8.3 with the tree samples produced in BEAST. This tree was visualized and edited in Fig Tree version 1.4.2.

4.2.5 Ancestral Area Reconstruction

Maximum of three areas allowed in the BioGeoBEARS analysis of all seven geographical regions defined here

These two models were run either as unconstrained (all areas equally probable and no limitation on dispersal direction) or constrained (accounting for area connectivity and dispersal probabilities between regions). The Likelihood Ratio Test was used to select the best fit model to generate the output of the biogeographical events and most probable areas of ancestral distribution (Matzke, 2013).

4.3 Results

4.3.1 Phylogenetic Analysis

The combined matrix of all four chloroplast markers contained 6271 characters for 175 species. There were 3013 constant characters of which 2297 (36.6%) were parsimony informative and 961 variable but parsimony uninformative. The maximum parsimony analysis produced 768 trees of 9667 steps with a consistency index (CI) = 0.5058, retention index (RI) = 0.7896 and rescaled consistency index (RC) = 0.3994. The resulting trees from the three analyses, MP, ML and BI were compared for branch support values and tree topologies. Branch support values,

bootstrap (BS) of MP and ML analysis and posterior probability (PP) of BI analysis were also compared in Figures 4.8 and 4.9. According to previous studies the BI PP values and MP bootstrap (MP BS) values are not directly comparable and PP values are usually higher than MP bootstrap values (e.g. Alfaro *et al*., 2003). Further to that, Alfaro *et al*. (2013) also recognized that ML bootstrap values (ML BS) are comparable to BI PP values in their study. The plots of the branch support values showed in Figure 4.8(a–c) indicates realatively overestimate of BI PP and ML BS support values. It was noted here that BI PP = 1.0 or ML BS = 100% applied to a range of MP BS from 50%–100%. Apparently the values of MP BS, BI PP and ML BS are not comparable to each other in this study. In the present study, the branch support values were categorized as highly supported MP BS ≥ 95% , well supported (MP BS = 80%–94%), moderately supported (MP BS = 70%) and poorly supported MP BS ≥ 50%.

The branch support values of the three analyses, MP, ML and BI, were compared. Bootstrap values of the MP and ML analysis and the posterior probability (PP) of the BI analysis were plotted (Figure 4.9). According to previous studies the BI PP values and MP bootstrap (MP BS) values are not directly comparable and PP values are usually higher than MP bootstrap values (e.g. Alfaro *et al*., 2003). Further to that, (Alfaro *et al*., 2003) also recognized that ML bootstrap values (ML BS) are comparable to BI PP values in their study. In the present study the plots of the branch support values (Figure 4.8 a-c) indicated the high overestimation of the BI PP values compared to the MP BS values, where MP BS support values down to almost 50% had BI PP values of over 0.98(Figure 4.8a). The ML BS support values were also higher than the MP BS values where values down to 50% MP BS values had up to 78% ML BS values (Figure 4.8b). Comparing BI PP and ML BS values indicated the former were consistently higher and low values of 50% ML BS had corresponding BI PP values of 0.7 (Figure 4.8c).

Thus, apparently the values of MP BS, BI PP and ML BS are not comparable to each other in this study, and BI PP were continuously highest, MP BS lowest, and ML BS in between.

The ML tree produced the most resolved tree compared to the majority rule consensus tree of the MP analysis and the majority rule consensus tree generated in the BI analysis. Branch support values of the three analyses are presented on the ML tree (Figure 4.9) which shows the subfamily Gesnerioideae as collapsed clade since the focus is on the Sri Lankan and Indian members of subfamily Didymocarpoideae.
In the phylogeny presented here the family Gesneriaceae including *Sanango* formed a highly supported (MP BS = 100%, BI PP= 1.0, ML BS = 100%) clade. Subfamily Sanangoideae represents the first diverging lineage in the family and is sister to a clade comprising subfamily Gesnerioideae + subfamily Didymocarpoideae. Details of the BI and MP analyses of subfamily Gesnerioideae are provided in Appendices 4 and 5 respectively. Subfamily Didymocarpoideae (MP BS = 88%, PP = 1.0, ML BS = 100%) is divided into two clades, one representing tribe Epithemateae (MP BS = 98%, BI PP = 1.0, ML BS = 100%) and one tribe Trichosporeae (MP BS = 85%, PP = 1.0, ML BS = 100%) (Figure 4.9). Tribe Epithemateae is further divided into four subclades representing the four subtribes, Loxotidinae (MP BS = 100%, BI PP = 1.0, ML BS = 100%), Monophyllaeinae (MP BS = 100%, BI PP = 1.0, ML BS = 100%), Loxoniinae (MP BS = 90%, PP = 1.0, ML BS = 94%) and Epithematinae (MP BS = 75%, PP = 0.99, ML BS = 96%). Loxoniinae was sister to Epithematinae (MP BS = 74%, PP = 0.99, ML BS = 96%), which together in turn were sister to Monophyllaeinae (MP BS = 81%, PP = 0.99, ML BS = 96%), and these sister to Loxotidinae (MP BS = 98%, PP = 1.0, ML BS = 100%). = 85%, PP = 1.0, ML BS = 100%). *Championia reticulata* from Sri Lanka represented the second diverging lineage (MP BS = 93%, PP = 1.0, ML BS = 100%). Subtribes Corallodiscinae (MP BS = 89%, BI PP = 1.0, ML BS = 100%), Tetraphyllinae (MP BS = 60%, BI PP = 1.0, ML BS = 100%) and Litostigminae (MP BS = 88%, BI PP = 1.0, ML BS = 100%) formed the third, fourth and fifth diverging lineages respectively. Two further subtribes, Ramondinae (MP BS = 94%, BI PP = 1.0, ML BS = 100%) and Leptoboeinae (MP BS = 92%, BI PP = 1.0, ML BS = 100%) were sister clades (BI PP = 1.0, ML BS = 90%) and formed the next diverging lineage though with little support (BI PP = 0.62). The majority of nodes in the backbone of the phylogeny beyond this lineage were not supported with MP BS. Subtribes Didissandrinae

represented with the genus *Didissandra* (MP BS = 52%, BI PP = 0.98, ML BS = 79%) and *Tribounia* (MP BS = 100%, BI PP = 1.0, ML BS =

Considering tribe Trichosporeae, subtribe Jerdoniinae, represented by the only species *Jerdonia indica* from South India, fell on the first diverging lineage (MP BS 100%) was not monophyletic. *Didissandra* and Streptocarpinae (MP BS = 100%, BI PP = 1.0, ML BS = 100%) formed the next grades though with little or no support. The following backbone split (BI PP= 0.92, ML BS = 70%) represent subtribe Loxocarpinae (BI PP= 1.0, ML BS = 100%) plus *Tribounia* as sister (BI PP= 1.0, ML BS = 97%), to which subtribe Didymocarpinae (BI PP= 1.0, ML BS = 100%) was sister. Of the 14 subtribes of Weber et al. (2013) for subfamily Didymocarpoideae, 12 were monophyletic. The two exceptions were subtribe Championiinae, resurrected in the present study which fell separate from the rest of the subtribes in the basallineages, and subtribe Didissandrinae as described above. Except for *Ramonda*, and *Petrocodon*, all genera were monophyletic.

Considering the Sri Lankan and South Indian taxa in the phylogeny, *Rhynchoglossum notonianum* and *Rhynchoglossum gardneri* formed a clade (BI PP = 0.71, ML BS = 52%) as sister to the New World *R. azureum* (MP BS = 98%, BI PP = 1.0, ML BS = 100%) in subtribe Loxotidinae. *Epithema ceylanicum* from Sri Lanka fell as sister (MP BS = 94%, BI PP = 1.0, ML BS = 99%) to the species pair *E. membranaceum* + *E. saxatile* (MP BS = 96%, BI PP = 1.0, ML BS = 100%) in subtribe Epithematinae of tribe Epithemateae. *Rhynchotechum permolle* was sister (MP BS = 99%, BI PP = 1.0, ML BS = 100%) to the species pair *R. parviflorum* + *R. discolour* (MP BS = 58%, BI PP = 1.0, ML BS = 82%) in subtribe Leptoboeinae of tribe Trichosporeae.

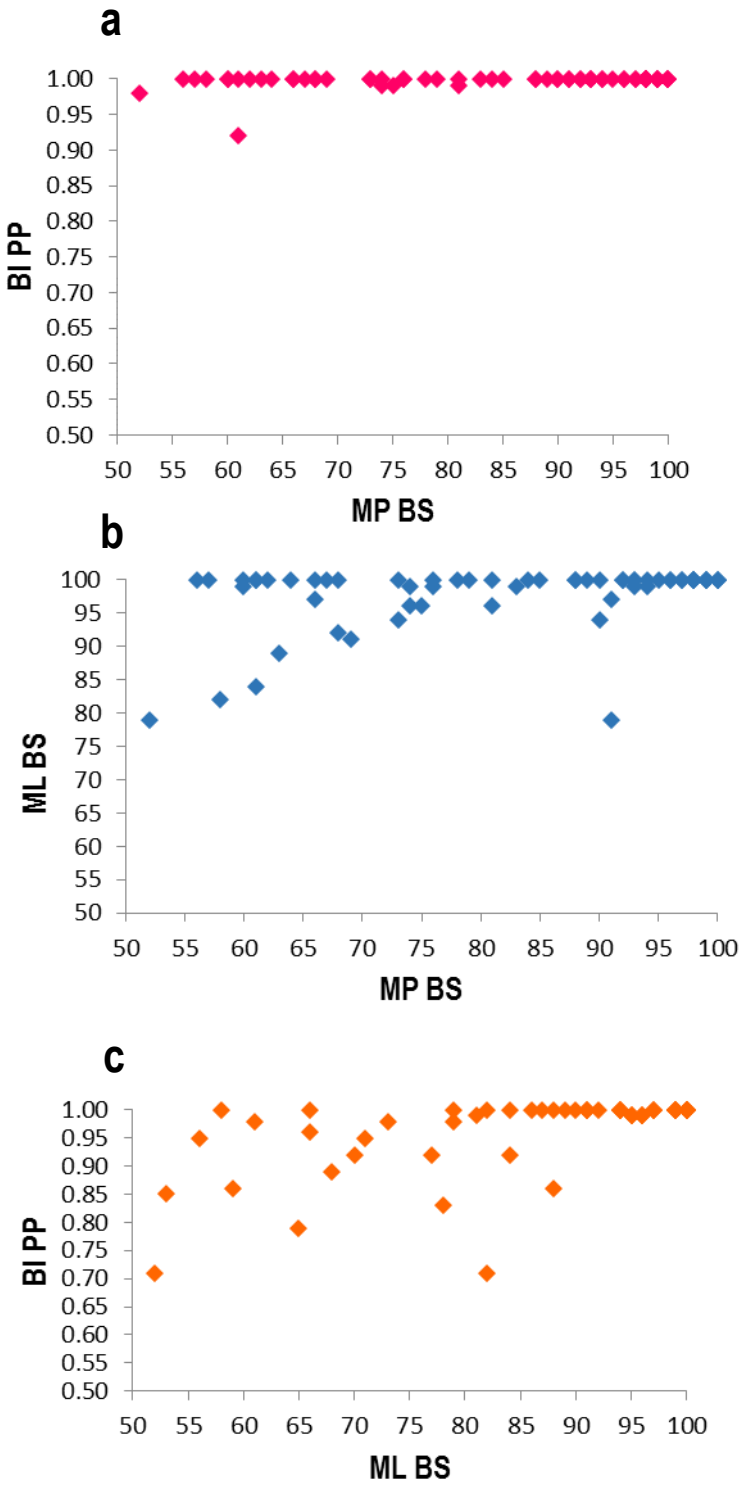


Figure 4.8 The branch support values, Bootstrap (BS) and Posterior probability (PI) of the three analyses, MP, ML and BI plotted; a. BI PP vs. MP BS; b. ML BS vs. MP BS; c. BI PP vs. ML BS.

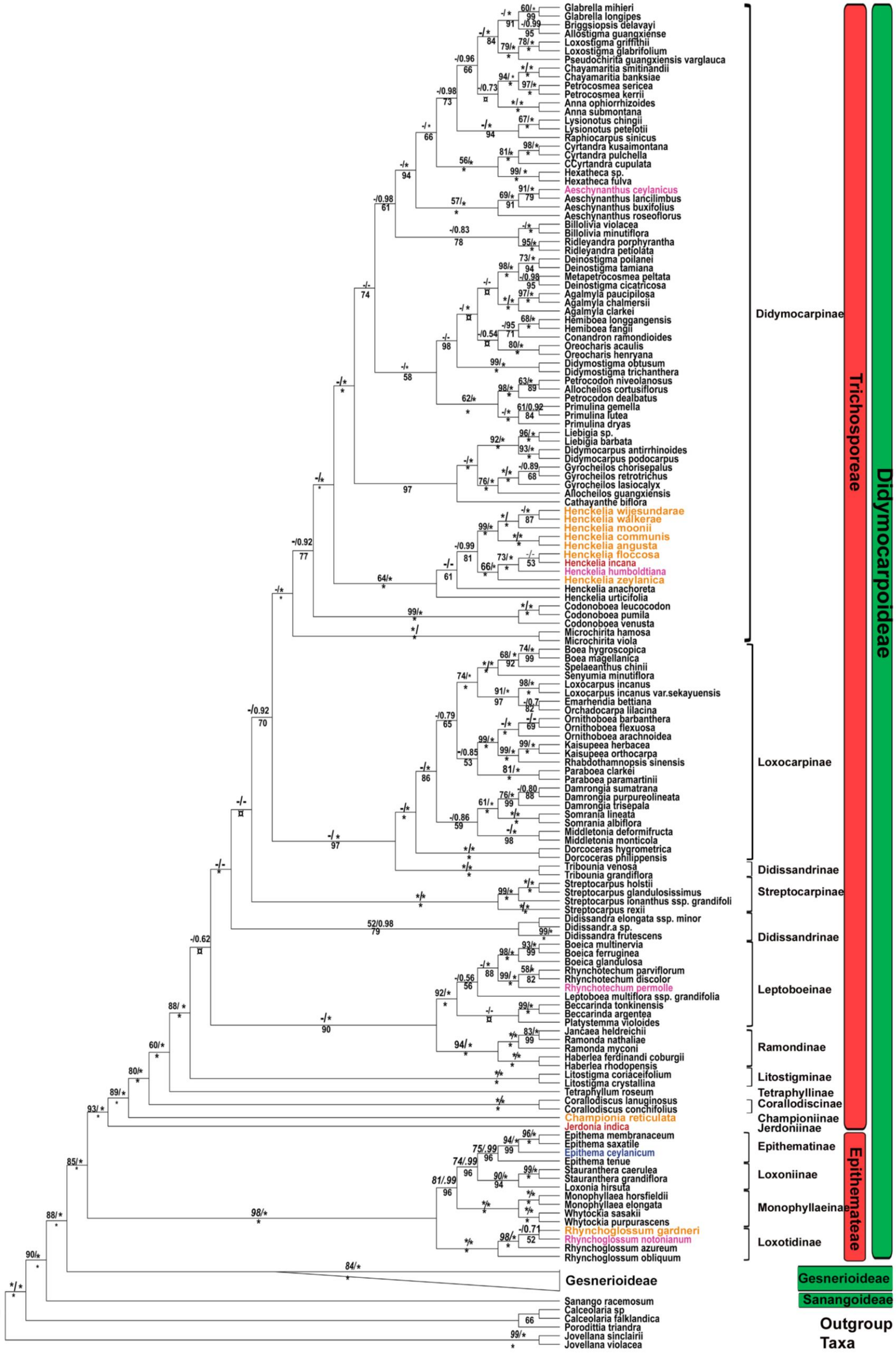

Glabrella mihieri
Glabrella longipes
Briggsiopsis delavayi
Allostigma guangxiense
Loxostigma griffithii
Loxostigma glabrifolium
Pseudochirita guangxiensis varglauca
Chayamaritia smitinandii
Chayamaritia banksiae
Petrocosmea sericea
Petrocosmea kerrii
Anna ophiorrhizoides
Anna submontana
Lysionotus chingii
Lysionotus petelotii
Raphiocarpus sinicus
Cyrtandra kusaimontana
Cyrtandra pulchella
CCyrtandra cupulata
Hexatheca sp.
Hexatheca fulva
Aeschynanthus ceylanicus
Aeschynanthus lancilimbus
Aeschynanthus buxifolius
Aeschynanthus roseoflorus
Billolivia violacea
Billolivia minutiflora
Ridleyandra porphyrantha
Ridleyandra petiolata
Deinostigma poilanei
Deinostigma tamiana
Metapetrocosmea peltata
Deinostigma cicatricosa
Agalmyla paucipilosa
Agalmyla chalmersii
Agalmyla clarkei
Hemiboea longgangensis
Hemiboea fangii
Conandron ramondioides
Oreocharis acaulis
Oreocharis henryana
Didymostigma obtusum
Didymostigma trichanthera
Petrocodon niveolanosus
Allocheilos cortusiflorus
Petrocodon dealbatus
Primulina gemella
Primulina lutea
Primulina dryas
Liebigia sp.
Liebigia barbata
Didymocarpus antirrhinoides
Didymocarpus podocarpus
Gyrocheilos chorisepalus
Gyrocheilos retrotrichus
Gyrocheilos lasiocalyx
Allocheilos guangxiensis
Cathayanthe biflora
Henckelia wijesundarae
Henckelia walkerae
Henckelia moonii
Henckelia communis
Henckelia angusta
Henckelia floccosa
Henckelia incana
Henckelia humboldtiana
Henckelia zeylanica
Henckelia anachoreta
Henckelia urticifolia
Codonoboea leucocodon
Codonoboea pumila
Codonoboea venusta
Microchirita hamosa
Microchirita viola
Boea hygroscopica
Boea magellanica
Spelaeanthus chinii
Senyumia minutiflora
Loxocarpus incanus
Loxocarpus incanus var.sekayuensis
Emarhendia bettiana
Orchadocarpa lilacina
Ornithoboea barbanthera
Ornithoboea flexuosa
Ornithoboea arachnoidea
Kaisupeea herbacea
Kaisupeea orthocarpa
Rhabdothamnopsis sinensis
Paraboea clarkei
Paraboea paramartinii
Damrongia sumatrana
Damrongia purpureolineata
Damrongia trisepala
Somrania lineata
Somrania albiflora
Middletonia deformifructa
Middletonia monticola
Dorcoceras hygrometrica
Dorcoceras philippensis
Tribounia venosa
Tribounia grandiflora
Streptocarpus holstii
Streptocarpus glandulosissimus
Streptocarpus ionanthus ssp. grandifoli
Streptocarpus rexii
Didissandra elongata ssp. minor
Didissandr.a sp.
Didissandra frutescens
Boeica multinervia
Boeica ferruginea
Boeica glandulosa
Rhynchotechum parviflorum
Rhynchotechum discolor
Rhynchotechum permolle
Leptoboea multiflora ssp. grandifolia
Beccarinda tonkinensis
Beccarinda argentea
Platystemma violoides
Jancaea heldreichii
Ramonda nathaliae
Ramonda myconi
Haberlea ferdinandi coburgii
Haberlea rhodopensis
Litostigma coriaceifolium
Litostigma crystallina
Tetraphyllum roseum
Corallodiscus lanuginosus
Corallodiscus conchifolius
Championia reticulata
Jerdonia indica
Epithema membranaceum
Epithema saxatile
Epithema ceylanicum
Epithema tenue
Stauranthera caerulea
Stauranthera grandiflora
Loxonia hirsuta
Monophyllaea horsfieldii
Monophyllaea elongata
Whytockia sasakii
Whytockia purpurascens
Rhynchoglossum gardneri
Rhynchoglossum notonianum
Rhynchoglossum azureum
Rhynchoglossum obliquum
Gesnerioideae
Sanango racemosum
Calceolaria sp
Calceolaria falklandica
Porodittia triandra
Jovellana sinclairii
Jovellana violacea
Didymocarpinae
Didissandrinae
Streptocarpinae
Didissandrinae
Leptoboeinae
Ramondinae
Litostigminae
Tetraphyllinae
Corallodiscinae
Championiinae
Jerdoniinae
Epithematinae
Loxoniinae
Monophyllaeinae
Loxotidinae
Loxocarpinae
Trichosporeae
Didymocarpoideae
Epithemateae
Gesnerioideae
Sanangoideae
Outgroup Taxa

Figure 4.9 ML tree with branch support values displayed on the branches from three analyses, MP, BI and ML. Numbers above the branches represent MP bootstrap (MP BS) / BI posterior probability (BI PP) values. Number below the branches represent ML bootstrap (ML BS) values. Support values = 100% from MP and ML analyses are indicated by asterisks (*), hyphens (–) indicate absence of branches in particular analyses, (¤) symbol indicates branches with less than 50% support from any of the three analyses. Taxa grouped in brackets represent subtribes, red boxes indicate tribes and green boxes and text indicate subfamilies. Species endemic to Sri Lanka , endemic to South India , common between Sri Lanka and South India , Sri Lanka, S. India and Asia .

Henckelia (MP BS = 64%, BI PP= 1.0, ML BS = 100%) fell on the third basal lineage in subtribe Didymocarpinae where the Sri Lankan + South Indian taxa were monophyletic (BI PP = 0.99, ML BS = 81%). Within this clade *Henckelia* formed two clades, one (MP BS = 66%, BI PP= 1.0, ML BS = 100%) included the two Sri Lankan endemic species *H. zeylanica* and *H. floccosa*, the Sri Lankan and South Indian *H. humboldtiana*, and the South Indian endemic *H. incana*. In the second clade (MP BS = 99%, BI PP= 1.0, ML BS = 100%) fell the remaining Sri Lankan endemics *H. communis*, *H. angusta*, *H. moonii*, *H. walkerae and H. wijesundarae*. All the Sri Lankan and South Indian *Henckelia* species are sister to the central and Southeast Asian *H. anachoreta*, although with low support (ML BS = 61%).

Aeschynanthus ceylanicus from Sri Lanka fell as sister to *A. lancilimbus* (MP BS = 91%, BI PP = 1.0, ML BS = 79%). The genus *Aeschynanthus* itself formed a well-supported derived clade in the BI and ML analyses (BI PP= 1.0, ML BS = 94%) within subtribe Didymocarpinae.

4.3.2 Dating Analysis

Mean ages with 95% highest posterior density (HPD) confidence intervals for the lineages and clades (except for the reduced Gesnerioideae) of family Gesneriaceae are illustrated in the maximum clade credibility (MCC) tree of the BEAST analysis

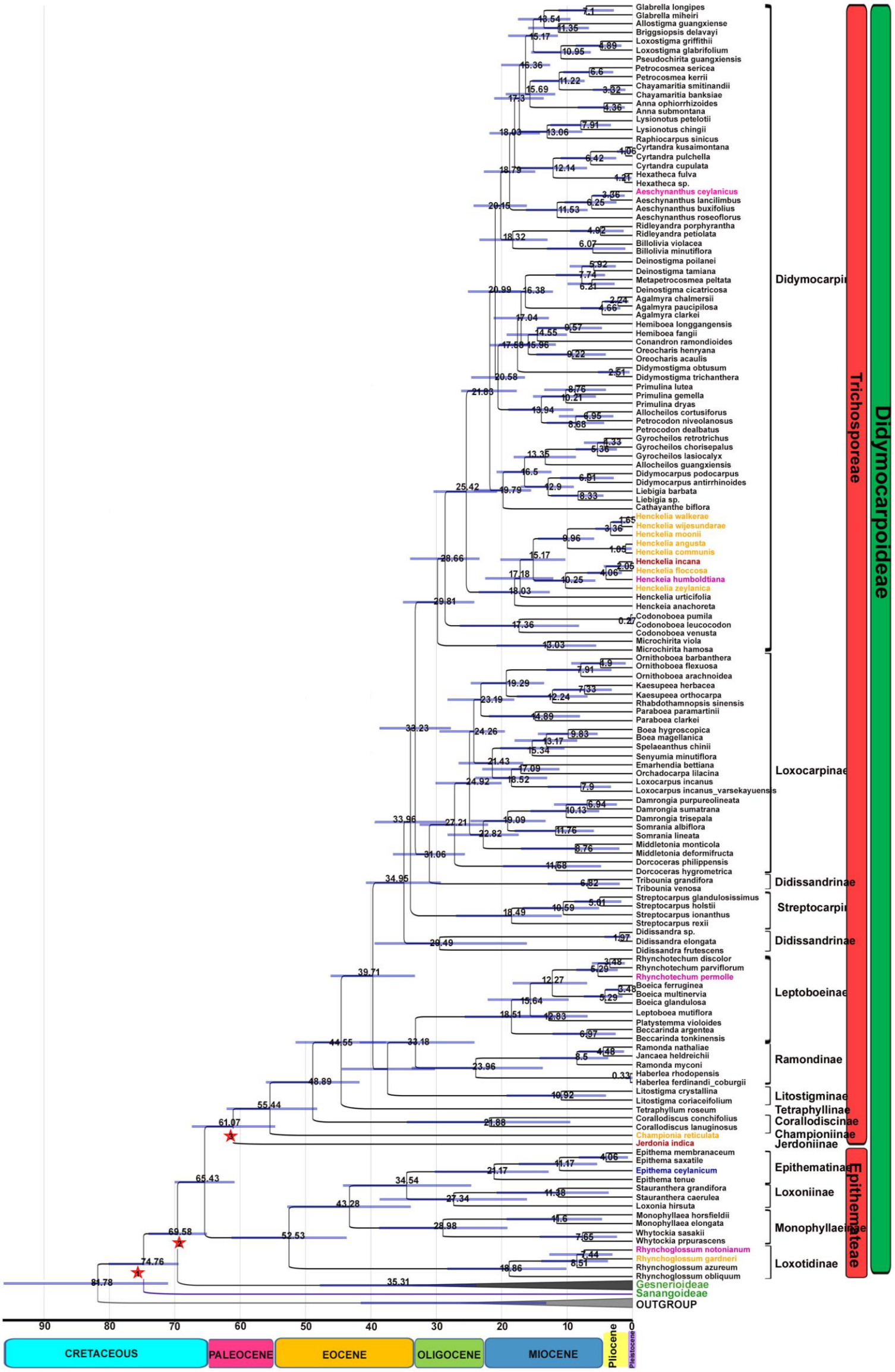

Glabrella longipes
Glabrella miheiri
Allostigma guangxiense
Briggsiopsis delavayi
Loxostigma griffithii
Loxostigma glabrifolium
Pseudochirita guangxiensis
Petrocosmea sericea
Petrocosmea kerrii
Chayamaritia smitinandii
Chayamaritia banksiae
Anna ophiorrhizoides
Anna submontana
Lysionotus petelotii
Lysionotus chingii
Raphiocarpus sinicus
Cyrtandra kusaimontana
Cyrtandra pulchella
Cyrtandra cupulata
Hexatheca fulva
Hexatheca sp.
Aeschynanthus ceylanicus
Aeschynanthus lancilimbus
Aeschynanthus buxifolius
Aeschynanthus roseoflorus
Ridleyandra porphyrantha
Ridleyandra petiolata
Billolivia violacea
Billolivia minutiflora
Deinostigma poilanei
Deinostigma tamiana
Metapetrocosmea peltata
Deinostigma cicatricosa
Agalmyra chalmersii
Agalmyra paucipilosa
Agalmyra clarkei
Hemiboea longgangensis
Hemiboea fangii
Conandron ramondioides
Oreocharis henryana
Oreocharis acaulis
Didymostigma obtusum
Didymostigma trichanthera
Primulina lutea
Primulina gemella
Primulina dryas
Allocheilos cortusiforus
Petrocodon niveolanosus
Petrocodon dealbatus
Gyrocheilos retrotrichus
Gyrocheilos chorisepalus
Gyrocheilos lasiocalyx
Allocheilos guangxiensis
Didymocarpus podocarpus
Didymocarpus antirrhinoides
Liebigia barbata
Liebigia sp.
Cathayanthe biflora
Henckelia walkerae
Henckelia wijesundarae
Henckelia moonii
Henckelia angusta
Henckelia communis
Henckelia incana
Henckelia floccosa
Henckeia humboldtiana
Henckelia zeylanica
Henckelia urticifolia
Henckeia anachoreta
Codonoboea pumila
Codonoboea leucocodon
Codonoboea venusta
Microchirita viola
Microchirita hamosa
Ornithoboea barbanthera
Ornithoboea flexuosa
Ornithoboea arachnoidea
Kaesupeea herbacea
Kaesupeea orthocarpa
Rhabdothamnopsis sinensis
Paraboea paramartinii
Paraboea clarkei
Boea hygroscopica
Boea magellanica
Spelaeanthus chinii
Senyumia minutiflora
Emarhendia bettiana
Orchadocarpa lilacina
Loxocarpus incanus
Loxocarpus incanus_varsekayuensis
Damrongia purpureolineata
Damrongia sumatrana
Damrongia trisepala
Somrania albiflora
Somrania lineata
Middletonia monticola
Middletonia deformifructa
Dorcoceras philippensis
Dorcoceras hygrometrica
Tribounia grandifora
Tribounia venosa
Streptocarpus glandulosissimus
Streptocarpus holstii
Streptocarpus ionanthus
Streptocarpus rexii
Didissandra sp.
Didissandra elongata
Didissandra frutescens
Rhynchotechum discolor
Rhynchotechum parviflorum
Rhynchotechum permolle
Boeica ferruginea
Boeica multinervia
Boeica glandulosa
Leptoboea mutiflora
Platystemma violoides
Beccarinda argentea
Beccarinda tonkinensis
Ramonda nathaliae
Jancaea heldreichii
Ramonda myconi
Haberlea rhodopensis
Haberlea ferdinandi_coburgii
Litostigma crystallina
Litostigma coriaceifolium
Tetraphyllum roseum
Corallodiscus conchifolius
Corallodiscus lanuginosus
Championia reticulata
Jerdonia indica
Epithema membranaceum
Epithema saxatile
Epithema ceylanicum
Epithema tenue
Stauranthera grandifora
Stauranthera caerulea
Loxonia hirsuta
Monophyllaea horsfieldii
Monophyllaea elongata
Whytockia sasakii
Whytockia prpurascens
Rhynchoglossum notonianum
Rhynchoglossum gardneri
Rhynchoglossum azureum
Rhynchoglossum obliquum
Gesnerioideae
Sanangoideae
OUTGROUP
Didymocarpi
Loxocarpinae
Didissandrinae
Streptocarpi
Didissandrinae
Leptoboeinae
Ramondinae
Litostigminae
Tetraphyllinae
Corallodiscinae
Championiinae
Jerdoniinae
Epithematinae
Loxoniinae
Monophyllaei
Loxotidinae
Trichosporeae
Didymocarpoideae
Epithemateae
90
80
70
60
50
40
30
20
10
0
CRETACEOUS
PALEOCENE
EOCENE
OLIGOCENE
MIOCENE
Pliocene
Pleistocene

Figure 4.10 Chronogram of Gesneriaceae with mean age estimates and 95% highest posterior density (HPD) bars based on BEAST analyses using secondary calibration points listed in Table 4.1. Geological time scale is shown at the bottom with the scale bar in millions of years ago (Mya). Red Star ( ) indicates the three secondary calibration points used in the BEAST analysis. Details of mean age estimates and 95% HPD bars for complete tree of family Gesneriaceae are shown in Appendix 3. Species endemic to Sri Lanka , endemic to South India , common between Sri Lanka and South India , Sri Lanka, S. India and Asia .

(Figure 4.10). The corresponding details for subfamily Gesnerioideae are included in the complete tree presented in Appendix 6. According to the dating analysis, the stem age of family Gesneriaceae including subfamily Sanangoideae was estimated with 81.78 Mya (HPD: 71.05-96.16) and the crown age with 74.76 Mya (HPD: 69.43-80.09 Mya). The crown age of the core Gesneriaceae (excluding *Sanango*) was 69.58 Mya (HPD: 65.09-74 Mya), for subfamily Gesnerioideae 35.31 Mya (HPD: 23.9-47.78 Mya) and for subfamily Didymocarpoideae much older with 65.43 Mya (HPD: 60.87-70 Mya). Tribe Epithemateae of subfamily Didymocarpoideae was estimated with a stem age of 65.43 Mya (HPD: 60.87-70 Mya) and crown age of 52.53 Mya (HPD: 43.74-61.31 Mya). The crown age of tribe Trichosporeae of subfamily Didymocarpoideae was 61.07 Mya (HPD: 54.66-67.36). Crown and stem ages of all subtribes are summarised in Appendix 6.

Considering the Gesneriaceae from Sri Lanka and South India (Table 4.2), *Rhynchoglossum* had a stem age of 52.53 (HPD: 33.93-52.79 Mya) and crown age of 18.86 Mya (HPD: 10.11-28.32 Mya), while the stem age of the genus in Sri Lanka plus South India was estimated to be 8.51 Mya (HPD: 3.76-13.91 Mya) with a divergence time between *R. notonianum* and *R. gardneri* of 7.44 Mya (HPD: 3.07-12.69 Mya). The stem age of *Epithema* was 34.54 Mya (HPD: 24.69-44.25 Mya), and crown age of 21.17 Mya (HPD: 12.84-30.21 Mya), with the age of *E. ceylanicum* being estimated with 11.17 Mya (HPD: 5.42-17.37 Mya). *Jerdonia indica* from South India represented the oldest genus of all Old World Gesneriaceae with an age of 61.07 Mya (HPD: 54.66-67.36 Mya). *Championia* was estimated as the oldest genus (and species) from Sri Lanka with an age of 55.44 Mya (HPD: 48.23-62.05 Mya). *Rhynchotechum permolle* was much younger with an age estimate of 5.29 Mya (HPD: 2.28-8.57 Mya), and a genus stem age of 12.27 Mya (HPD: 6.96-18.31 Mya). The genus *Henckelia* was estimated with a stem age of 25.42 (20.74-30.41)

and crown age of 18.03 Mya (HPD: 12.66–23.51 Mya). The age of *Henckelia* in Sri Lanka+South India was 17.18 Mya for the stem age (HPD: 12.12–22.5 Mya) and 15.17 Mya for the crown age (HPD: 10.28–20.19 Mya), with the oldest being *H. zeylanica* with an age of 10.25 Mya (HPD: 5.69–15.06 Mya), and youngest being *H. angusta* and *H. communis* with a divergence time of 1.05 Mya (HPD: 0.15–2.37 Mya). The stem age of genus *Aeschynanthus* was estimated with 18.79 Mya (HPD: 16.18–24.28 Mya) and crown age of 11.53 (HPD: 6.88–16.28Mya). *Aeschynanthus ceylanicus* in a derived clade with *A. lancilimbus* in the genus was much younger with 3.36 Mya (HPD: 1.21–6.02 Mya).

Table 4.3 Mean age values with 95% HPD range for crown and stem age estimates of Sri Lankan and South Indian Gesneriaceae species and their representative genera.

Tribe (Subtribe)	***Genus*** *Species*	**Crown Age (95% HPD) Mya**	**Stem Age (95% HPD) Mya**
Epithemateae (Loxotidinae)	***Rhynchoglossum***	18.86 (10.11–28.32)	52.53 (33.93–52.79)
	R. notonianum+gardneri	(species age in Sri Lanka)	(genus age in Sri Lanka)
		7.44 (3.07–12.69)	8.51(3.76–13.91)
Epithemateae (Epithematinae)	***Epithema***	21.17 (12.84–30.11)	34.54 (24.69–44.25)
	E. ceylanicum	n.a.	11.17 (5.42–17.37)
Trichosporeae (Jerdoniinae)	***Jerdonia***	n.a.	61.07 (54.66–67.36)
	J. indica	n.a.	61.07 (54.66–67.36)
Trichosporeae (‘Championiinae’)	***Championia***	n.a.	55.44 (48.23–62.05)
	C. reticulata	n.a	55.44 (48.23–62.05)
Trichosporeae (Leptoboeinae)	*Rhynchotechum*	5.29 (2.28–8.57)	12.27 (6.96–18.31)
	R. permolle	n.a.	5.29 (2.28–8.57)
Trichosporeae (Didymocarpinae)	***Henckelia***	18.03 (12.66–23.51)	25.42 (20.74–.41)
H. zeylanica	*H. zeylanica*	n.a.	10.25 (5.69–15.06)
H. humboldtiana	*H. floccosa+incana*	2.05 (0.12–4.37)	4.06 (1.64–6.9)
H. floccosa			
H. incana	*H. humboldtiana+floccosa+ incana*	4.06 (1.64–6.9)	10.25(5.69–15.06)
H. communis			
H. angusta	*H. angusta+communis*	1.05 (0.15–2.37)	9.96 (5.88–14.43)
H. moonii	*H. moonii*	n.a.	3.36 (1.44–5.66)
H. walkerae	*H. walkerae+wijesundarae*	1.65 (0.61–3.05)	
H. wijesundarae			3.36 (1.44–5.66)
Trichosporeae	***Aeschynanthus***	11.53 (6.88–16.28)	18.79 (14.89–22.71)
	A. ceylanicus	n.a.	3.36 (1.21–6.02)

4.3.3 Ancestral Area Reconstruction

Considering the two cladogenesis models, DEC and DEC + J, that were used in BioGeoBEARS, the DEC + J model with a log likelihood value of –215.15 was favoured over the DEC model that had a log likelihood value of –236.79 in both constrained and unconstrained analyses. Therefore, it is apparent that founder effects allowed for in the "+J" model significantly contributed to speciation and diversification (Matzke, 2014) of family Gesneriaceae. Results of the ancestral area reconstruction based on both DEC and DEC + J models in BioGeoBEARS illustrated in Appendices 7 and 8. Here the percent probability values of the ancestral area reconstruction analysis are given in pie charts mapped on the maximum clade credibility tree from the BEAST analysis (Figure 4.10). The analysis based on the DEC+J model suggested an origin of family Gesneriaceae (subfamily Sanangoideae) in the late Cretaceous in South America. According to the present study the probability of this ancestral area reconstruction is with 36% quite low. An origin of the subfamily Gesnerioideae in South America was supported with a 76% probability. Subfamily Didymocarpoideae has the highest probability of 42% for its ancestral area as South India plus Sri Lanka.

The ancestral area for tribe Epithemateae was uncertain with six different areas suggested, of which C + D, Malay Archipelago plus Central and East Asia, had the highest probability value of 24%. The ancestral areas of the subtribes of tribe Epithemateae also supported with low probability values except subtribe Loxoniinae. For subtribe Loxotidinae it was C + E with a probability of 46%, for Monophyllaeinae, C + D, i.e. the Malay Archipelago plus Central and East Asia, with a probability of 47%, for Loxoniinae C, the Malay Archipelago, with a probability of 100% and for Epithematinae also Malay Archipelago with probability of 27%. The ancestral area reconstructed for tribe Trichosporeae was E, South India plus Sri Lanka, supported by an 84% probability. Considering the subtribes recognized in this tribe, the most basal two, Jerdoniinae and Championiinae have an origin in area E with a probability of 84% and 77% respectively. The ancestral area for the subtribes Corallodiscinae, Tetraphyllinae and Litostigminae was D, Central and East Asia, with high probabilities of 100%, 99% and 100% respectively.

The ancestral area for subtribe Ramondinae was Europe (100%), for Leptoboeinae area D, Central and East Asia (99.4%), for *Didissandra* it is area C, the Malay Archipelago (100%), and for Streptocarpinae it is area B, Africa plus Madagascar (100%). The ancestral area for both subtribes Loxocarpinae+*Tribounia* and Didymocarpinae was D, Central and East Asia, with high probability support of 96% and 94% respectively.

Considering the focus of the present chapter, the affinity of Sri Lankan and South Indian Gesneriaceae, it is important to understand the ancestral areas of the genera that have wider distributions beyond South India and Sri Lanka, i.e. distributed there and in other areas. The genus *Rhynchoglossum* has an origin in C + E (Malay Archipelago + [South India plus Sri Lanka]) with a probability of 46%. However, the origin of the clade comprising the three species *R. notonianum*, *R. gardneri* and the Central American *R. azureum* is most likely in area E, South India plus Sri Lanka (78%).

The origin of the genus *Epithema* is suggested as area C, the Malay Archipelago, with the highest probability support of 27%, but with other areas close to it, D with 21% and B+C+D with 18%. The subclade which contains the Sri Lankan species *E. ceylanicum*, *E. membranaceum* and *E. saxatile* has ancestral area C + D (Malay Archipelago plus Central and East Asia) with a probability of 61%. The clade containing genus *Rhynchotechum* in subtribe Leptoboeinae has 100% probability for an origin in Central and East Asia (area D). There is a 98% probability for the origin of genus *Henckelia* also in Central and East Asia and a 100% likelihood for Sri Lanka and South India as ancestral state for the species occurring there. Similarly, the genus *Aeschynanthus* was also supported as originating in area D, Central and East Asia, with only with 87% probability, and a 93% probability for this area to be ancestral for *A. ceylanicus* and *A. lancilimbus*.

4.4 Discussion

4.4.1 Taxon Sampling

All fourteen Sri Lankan Gesneriaceae taxa were included in the unpublished matrix of Luna-Castro (2016) to determine their placement in the phylogeny, followed by

dating and ancestral area reconstruction to understand the biogeographic affinities of the Sri Lankan and South Indian Gesneriaceae taxa.

It was attempted to include all currently recognised genera of Old World Gesneriaceae and with species to cover all geographic areas of the genera. The genera *Championia*, *Chayamaritia*, *Deinostigma*, *Raphiocarpus* were added to the matrix of Luna-Castro (2016) since they were missing there. Molecular data were not available for the Chinese genus *Gyrogyne* which is likely extinct (Wang, 2003). An additional species of *Glabrella* was included here, since only one was included by Luna-Castro (2016).

For *Boeica*, *Damrongia*, *Epithema*, *Ornithoboea* and *Rhynchotechum*, additional samples were included to cover geographic areas of genus's distributions that were missing in Luna-Castro (2016). Additional samples for *Aeschynanthus* and *Henckelia* were incorporated to better place the Sri Lankan species. For stability and biogeographic reasons (see 4.4.3), additional samples of *Agalmyla*, *Cyrtandra*, *Gyrocheilos*, *Ornithoboea*, *Primulina* and *Streptocarpus*, were added.

However, covering all geographic areas was not possible in all cases (only for 6 out of 66 genera) due to the lack of samples, particularly from Southeast Asia. For example, *Beccarinda*, *Boeica* and *Middletonia* occur also in the Malay Archipelago but no sample for this area could be included. For *Codonoboea*, Central and East Asia could not be covered, *Microchirita* was not included from South India and *Aeschynanthus* not from the Solomon Islands (area F). However, none of these cases, except perhaps *Microchirita*, affected the main focus of the present study, the biogeographic affinities of Sri Lankan + South Indian species. The analysis here provided a first glance of their biogeographic affinities in the family Gesneriaceae (see 4.4.3).

Some potential errors of Luna-Castro (2016) were corrected here, such as replacing the misidentified *Codonoboea elata*, *Rhynchoglossum gardneri* samples.

4.4.2 Phylogenetic Analysis

The phylogeny produced in the present study (Figure 4.9) was congruent with almost all subfamilies, tribes and subtribes recognized in the latest updated classification of

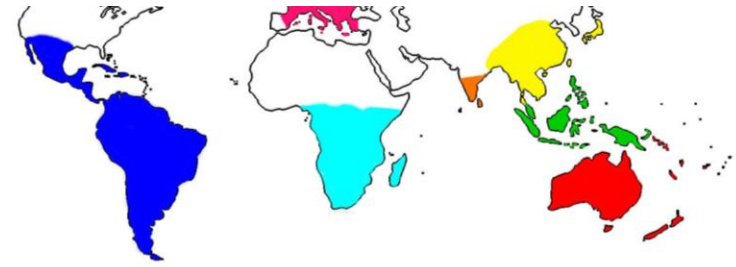
family Gesneriaceae (Weber *et al*., 2013), except for very few discrepancies in subfamily Didymocarpinae as explained below.

The genus *Championia* was placed in various positions in previous morphology-based classifications such as in subtribe Didymocarpeae of tribe Cyrtandreae by Bentham (1876); subtribe Championiinae of tribe Championieae by Fritsch (1893–1894); tribe Didymocarpeae of subfamily Cyrtandroideae by Burtt (1963), Ivanina (1965, 1967) and Burtt and Wiehler (1995).

This genus was tentatively placed in subtribe Leptoboeinae in tribe Trichosporeae along with five other genera, *Leptoboea*, *Boeica*, *Rhynchotechum*, *Tetraphyllum* and *Beccarinda* by Weber et al. (2013). The synapomorphy considered in Weber *et al.* (2013) to include *Championia* in Leptoboeinae was the presence of tetrandrous flowers.

However, the molecular analyses here palced the genus *Championia* on an early branching lineage near the monotypic genus *Jerdonia* from South India. It is proposed here to resurrect the subtribe 'Championiinae' to assign subtribe level to this lineage.

Subtribe Didissandrinae, established by Weber *et al*. (2013) that included two genera *Didissandra* and *Tribounia*, is also not monophyletic in the phylogeny presented here (Figure 4.9). The genus *Didissandra* was placed in a basal clade while *Tribounia* is sister to subtribe Loxocarpinae in the present study which is also suggested by Luna-Castro (2016). To expel *Tribounia* from Didissandrinae, however, may be premature since its phylogenetic position is not supported in the MP analysis. Also, apparent synapomorphies between it and genera of Loxocarpinae are lacking. Detailed carpological studies as suggested by Puglisi *et al.* (2016) and morphological analysis as suggested by Luna-Castro (2016) are necessary to understand the relationships here better.



A South and Central America
B Africa and Madagascar
C Malay Archipelago
D Central and East Asia
E South India and Sri Lanka
F Australia and Pacific Islands
G Europe
A + D
A + E
C + D
C + E
D + E
A + D + E
B + D + E
Other combinations

Figure 4.11 Maximum-likelihood reconstruction of geographical ranges (ancestral areas) inferred from the DEC + J model in BioGeoBEARS plotted on the maximum clade credibility chronogram from BEAST. The highest relative probability value for ancestral areas marked on the pie charts. The seven regions coded in the ancestral area reconstruction are given codes A–F with some significant area combinations denoted in different colours and showed in the world map and the legend. Scale bar reflect time in millions of years ago (Mya), with different time periods and respective epochs shown below the scale bar. Time calibration from 0 to 2 Mya: Quaternary period (present to Pleistocene epoch); from 13±1 to 63±2 Mya: Tertiary period (Pliocene, Miocene, Oligocene, Eocene and Paleocene); from 63±2 to 90 Mya: early to mid-Cretaceous period. Species endemic to Sri Lanka , endemic to South India , common between Sri Lanka and South India , Sri Lanka, S. India and Asia .

The majority of genera with two or more samples included were monophyletic in the phylogeny with few exceptions. One such exception was the genus *Ramonda* of subtribe Ramondinae, with three species of which two were included here, *R. myconi* and *R. nathaliae*. *Ramonda* was not monophyletic, because the genus *Jancaea* nested within it. This is perhaps correct since other datasets and analyses have shown this as well (e.g. Möller *et al*., 1999, 2009, 2011; Petrova *et al*., 2015), and therefore *Jancaea* should be returned to *Ramonda* as suggested by Weber *et al*. (2013).

Both recognized species of the genus *Allocheilos* of subtribe Didymocarpinae were included, but they fell in different places: *A. guangxiensis* as sister to *Gyrocheilos*, and *A. cortusiflorus* nested within *Petrocodon*. Since only two out of 29 species described in *Petrocodon* (Möller *et al*., 2016) were included here, this situation needs further study.

Finally, the recently expanded genus *Deinostigma*, previously monotypic with *D. poilanei*, now including seven species altogether (Möller *et al.*, 2016) was not monophyletic here. This might reflect a sampling artefact since it is monophyletic with five samples were included in the original work (Möller *et al.*, 2016), and only three species were included in the present analysis. Furthermore, in Möller *et al*. (2016) 132 samples of subtribe Didymocarpinae were included while in the present study there are only 74 samples.

4.4.3 Biogeographic history of family Gesneriaceae

Sampling strategies of dated phylogenetic studies in family Gesneriaceae

According to my results, the family Gesneriaceae diverged from its sister group 81.78 Mya (HPD: 71.05–96.16 Mya) in the late Cretaceous period. This age estimate is similar to Petrova *et al*. (2015), and Roalson and Roberts (2016), but higher than Perret

et al. (2013) and Luna-Castro (2016) (Appendix 3). The relatively older ages obtained in the present study compared to Luna-Castro (2016) were perhaps caused by reducing the New World samples here. It is also noted that Perret *et al*. (2013) obtained relatively younger age estimates for all the clades recognized here, which might have been the effect of an unbalanced sampling favouring subfamily Gesnerioideae, with only three samples from subfamily Didymocarpoideae. According to Linder *et al*. (2005) and Milne (2009), age estimates in dating analyses are very sensitive to under-sampling. However, Milne (2009) recognized only small to negligible effects from changes to the sampling density in neighbouring clades. Overall, the estimates here are in line with two other studies using different sampling and analysis approaches (Petrova *et al*., 2015; Roalson and Roberts, 2016), and the results will be discussed in the light of these comparable age ranges.

Origin of family Gesneriaceae

Perret *et al*. (2013) proposed an origin of family Gesneriaceae in South America with probable areas of origin in temperate Andes and the Amazonian rainforest. The inference of this place of origin in Perret *et al*. (2013) relied mainly on the placement of the NW taxa Calceolariaceae, *Sanango* and *Peltanthera* at the base of the Gesneriaceae. Analysis by Luna-Castro (2016), and Roalson and Roberts (2016) supported this recognition of origin of the family. However, in my analysis the origin of the family in South America was supported by a low probability value of 35%, while areas A+D+E had 21.5% and A+E 15.4% probability support values (Figure 4.11).

All previous work included the genus *Peltanthera* which was placed outside of family Gesneriaceae, sister to subfamily Sanangoideae in studies by (Wang *et al*., 2004; Perret *et al.*, 2013). *Peltanthera* is a monotypic genus with a single species, *P. floribunda*, distributed in northern South and Central America (Skog and Boggan, 2016 onw.). Due to its uncertain phylogenetic position within Lamiales and unusual characters of flowers and inflorescences, Weber et al. (2013) did not place *Peltanthera* within family Gesneriaceae. The origin of family Gesneriaceae in the South America with probable areas of origin in the temperate Andes and Amazonian rainforest by Perret *et al*. (2013) is thus greatly affected by the inclusion of the genus *Peltanthera*.

Origin of Old World Gesneriaceae

Subfamily Didymocarpoideae

The split between Old World Didymocarpoideae and New World Gesnerioideae fell near the late Cretaceous (~69.58 Mya). The age estimate of Old World Gesneriaceae (Crown age 65.43 Mya and stem age 69.58) is much older than the age estimate of New World Gesneriaceae (Crown age 35.31 Mya and stem age 69.58). A traditional vicariance hypothesis was initially proposed by Burtt (1998) to explain the distribution of palaeotropical Gesneriaceae. However, based on the young age of the disjunction predating the Gondwana break-up in the early Jurassic, Perret *et al*. (2013) favoured an alternative hypothesis for the distribution of palaeotropic Gesneriaceae. He proposed an inter-plate dispersal from South America to Australasia through Antarctica in the late Paleocene to mid Eocene. Luna-Castro (2016) hypothesized an initial diversification of Didymocarpoideae in three possible regions, the Indian subcontinent, East Asia or South America.

Tribe Trichosporeae

In the present study estimate of the age of the oldest Old World lineage, tribe Trichosporeae was 61.07 Mya for the crown age and 65.43 for the stem age with an 84% probability for its origin on the Deccan Plate (E: South India + Sri Lanka). Considering the time period between 60─65 Mya at about 65.5 Mya, mass extinction occurred where the majority of existing organisms including dinosaurs and many angiosperms went extinct at the boundary between Cretaceous and Tertiary (KT boundary; Figure 4.4). During this time Deccan plate was isolated between the landmasses of Africa, Asia and Australia. The large area of bare land following the volcanic eruptions may have encouraged the arrival and establishment of taxa from land masses nearby. These must have been pioneer plants that were able to get established on relatively marginal soilless niches, that later established forests and prepared the ground for forest-dwelling plants such as Gesneriaceae.

According to the present study based on most updated dated phylogeny of Roalson and Roberts (2016), it can be hypothesized that an ancient lineage of Gesneriaceae travelled through Antarctica to Australia and an ancestor of tribe Trichosporeae further expanded its distribution to the Deccan Plate through a transoceanic long-distance dispersal event.

Considering the biogeographic affinities of family Gesneriaceae in Australasian plate, eight genera in the present phylogeny include species from Australia and Pacific Islands (Appendix 5 and 6, species area coloured in red). Six species are represented in subfamily Gesnerioideae in tribe Coronanthereae: *Coronanthera* sp., *Rhabdothamnus solandri*, *Fieldia australis*, *Negria rhabdothamnoides*, *Depanthus* sp. and *Lenbrassia australiana* while two species are from subfamily Didymocarpoideae, in tribe Loxocarpinae: *Boea hygroscopica* and in tribe Didymocarpinae: *Cyrtandra pulchella*. The occurrence of the New World taxa in area F (Australia and Pacific islands) appears to be the result of relatively recent long-distance dispersal events 30 Mya – 18 Mya (Appendix 6─8) aided perhaps by the evolution of fleshy fruits (Woo *et al.*, 2011).

Likewise, there are no ancient lineages found amongst the Didymocarpoideae lineage in Australia and the Pacific Islands according to the dated phylogeny of family Gesneriaceae (Figure 4.11 and Appendix 6). Their absence here may be attributed to a complete species extinction with the onset of aridification that started in the mid Miocene (Martin, 2006). An alternative route for Gesnericaeae to the Old World would be from South America to Africa and from there to the Deccan Plate.

On the other hand it can be also hypothesized that the African clades, subtribe Streptocarpinae and *Epithema tenue* date back to the Oligocene and are thus too young. Furthermore, there is no evidence from BioGeoBEARS for such a scenario.

Therefore the most likely hypothesis from the present study is a modification of Perret *et al*. (2013), where the ancestral lineages of Old World Gesneriaceae dispersed from South America and migrated through Antarctica and eventually reached Australasia, followed by at least a single long-distance event to the Deccan plate. Under this modified Australasia hypothesis, the ancestors of the Epithemateae lineage could have remained extant in Australasia and entering Southeast Asia. An out-of-India into Eurasia scenario after the collision of the Deccan plate with Eurasia could account for the distribution of the Trichosporeae lineage since most of the major lineages of this tribe have their ancestral areas in Central Asia with high probability support (Figure 4.11).

Tribe Epithemateae

The geographic origin of tribe Epithemateae in the early Eocene is blurred by a collection of six probable combinations of areas. Migration through Australasia to Southeast Asia into Central plus East Asia is possible but needs more work to include members of this subtribe from the extremes of its distribution range, e.g. *E. rennellense* from the Solomon Islands (Rennell Island), or *Rhynchoglossum papuae* from Papua New Guinea, Bismarck Archipelago, or *Monophyllaea* and *Stauranthera* from New Guinea.

It appears that since the collision of India with Eurasia, four migration events occurred within the Trichosporeae lineage, from Central + East Asia to Africa (subtribe Streptocarpinae), to Europe (subtribe Ramondinae), and twice to the Malay Archipelago (subtribe Didissandrinae and *Cyrtandra*), apart from those involving taxa from South India and Sri Lanka.

4.4.4 Biogeographic affinities of Sri Lankan Gesneriaceae

Championia

Championia reticulata is the oldest member of the Sri Lankan Gesneriaceae flora originating about 55.44 Mya around the Paleocene / Eocene boundary. The lineage representing *C. reticulata* is in a derived grade to *Jerdonia indica* of South India. The two species are evidence for the view that Sri Lanka and India still retain a relict flora (Azuma *et al.*, 2008). Further, the deep origin of both, the *Jerdonia* and *Championia* lineage, in the context of historical biogeography of the family Gesneriaceae is further evidence that South India / Sri Lanka harbour a Gesneriaceae flora since the Paleocene, prior to the collision of the Deccan Plate with Eurasia. It is therefore recognized that these are key lineages to explain the history of the tribe Trichosporeae or even subfamily Didymocarpoideae.

Rhynchoglossum

On the contrary, in tribe Epithemateae *Rhynchoglossum gardneri* from Sri Lanka and *R. notonianum* from both South India and Sri Lanka (7.44 Mya crown age and 8.55 Mya stem age) are much younger and originate in the late Miocene, and have likely arrived

in Sri Lanka from the Malay Archipelago by long-distance dispersal. The means of this dispersal are unclear. Their fruits are not fleshy and their seeds small but not dust-like as in orchids and suitable for long-distance travel (Beaufort-Murphy, 1983). However, the Malay Archipelago is part of the ring of fire where frequent earthquakes and volcanic eruptions occur (Rosenberg, 2017), and these might have played a part in dispersing their propagules.

Epithema

Although the ancestral area of the genus *Epithema* is blurred in ancestral area reconstruction, *Epithema* may have diversified in the Malay Archipelago, Central or South East Asia in Oligocene (31.17–34.54 Mya), then migrated to or arrived via long distance dispersal in Sri Lanka and South India in the Miocene (11.17–21.17 Mya) before *Rhynhoglossum*. An oversea dispersal can be supported by the presence of *E. ceylanicum* distributed on the Andaman Islands of India (Bransgrove and Middleton, 2015), an island off the west coast of Sumatra that can act as a potential stepping stone for plants to arrive directly in Sri Lanka (Whittaker and Fernández-Palacios, 2007).

Rhynchotechum

Rhynchotechum permolle is part of a basal lineage of genus *Rhynchotechum* in subtribe Leptoboeinae. However, only three out of 21 recognised species have been included here (Möller *et al*., 2016), and the results must therefore be treated with some caution. The centre of diversity of the genus is in Central and Southeast Asia with an estimated late Miocene (5.29–12.27 Mya) origin. Therefore it is reasonable to conclude that *R. permolle* originated in Central and East Asia then migrated to Sri Lanka and South India in the late Miocene (5.29 Mya).

Henckelia

It is clearly evident that *Henckelia* has been present in Sri Lanka and South India since the mid Miocene (Figure 4.11), having migrated there after an origin in Central and East Asia. There are two distinct resolved clades: one clade consists of all caulescent species endemic to Sri Lanka: *H. walkerae, H. wijesundarae, H. moonii, H. communis* and *H. angusta*, while the second clade consists of all acaulescent species, including two endemic to Sri Lanka (*H. zeylanica* and *H. floccosa*); one shared between Sri Lanka and

South India (*Henckelia humboldtiana*); and one endemic to South India (*H. incana*). There are no caulescent *Henckelia* in South India recorded at present, the nearest only from Northeast India. The *Henckelia* clade arrived on the Deccan plate at ~15–18 Mya, much later in early Miocene but the origin of the clades representing existing taxa is dated ~10 Mya in late Miocene. After the caulescent lineage arrived in Sri Lanka, in the late Miocene, it further diversified and speciated in-situ especially in the Pliocene (Figure 4.10). *Henckelia zeylanica* a species with a prostrate, branched and creeping rhizomatous stem, is on a basal lineage in the acaulescent group in the dated phylogeny here. This might indicate a gradual reduction in caulescence leading to 'acaulescent' rosette plants (Nishii *et al.*, 2017). However, understanding the origin and affinities of the acaulescent group of *Henckelia* requires assemblage of more samples representing the 15 *Henckelia* species from South India and across the genus. Moreover, this acaulescent clade, especially *H. floccosa*, *H. humboldtina* and *H. incana*, are interesting to study since the data presented here indicate that exchanges between South India and Sri Lanka have occurred perhaps frequently, and at least *H. humboldtiana* is known to be present in both areas.

Aeschynanthus

The most comprehensive study of the genus *Aeschynanthus*, a nuclear ITS phylogeny by (Denduangboripant *et al*., 2001), placed *Aeschynanthus ceylanicus* in a derived position, and suggested the origin of the genus between Indo-China (South East Asia including Malay Peninsula) and the Philippines. They suggested an ancient vicariance event at the time of origin of the genus based on geographical difference between the two major clades recognized in the ITS phylogeny. In this study basal species in the clade I were from Indo-China and Taiwan while basal species of clade II were from Philippine and New Guinea. The present dated phylogeny with ancestral area analysis suggested, the ancestral area of the genus is to be in Central and East Asia with an origin in the late Miocene. Therefore, the ancestral area of the genus *Aeschynanthus* in Denduangboripant (2001) and the present study are nearly comparable. The present study agree with Denduangboripant (2001) for the origin of the genus in Miocene with the present estimation at 11.53–18.79 Mya.

Diversification of the clade comprising *A. ceylanicus* and *A. lancilimbus*, happened somewhat later in the late Pliocene (3.36 Mya) with an ancestral area in Central and East Asia. *Aeschynanthus ceylanicus* is distributed in South India and Sri Lanka while *A. lancilimbus* is from China (Yunnan) (Skog and Boggan, 2007 onw.). Therefore, it can be hypothesized with reasonable confidence that the lineage of *A. ceylanicus* originated in Central Asia and then migrated through India to South India and Sri Lanka also in Pliocene (3.36 Mya) probably the same time of the arrival of *H. humboldtiana* and *H. floccosa*.

According to the present study it can be hypothesized that there are at least six or seven events in the geological history during which the six genera of Gesneriaceae arrived in Sri Lanka.

4.5 General Discussion

Considering the biogeographic history of Sri Lankan Gesneriaceae, the finding of the presence of an ancient basal lineage of Deccan origin, i.e. *Championia* in subfamily Didymocarpoideae (Old World Gesneriaceae) in Sri Lanka with its age dated to the Eocene, about 55.44 Mya, is the most significant outcome. *Championia* may have arrived in Sri Lanka though a long distance dispersal event from Australasia.

The phylogenetic trees further suggested that the monotypic genus *Championia* is better placed in a separate subtribe Championiinae. The present study proposed to reinstate subtribe Championiinae, that was previously established by Fritsch (1894) and currently synonymized under Leptoboeinae by Weber *et. al*. (2013).

Considering the other five genera, the presence of *Epithema* and *Rhynchoglossum* in Sri Lanka was inferred to have been the result of long distance dispersal events from the Malay Archipelago. The genus *Epithema*, dispersed into Sri Lanka in the mid Miocene (11–21 Mya) while the genus *Rhynchoglossum* probably dispersed to Sri Lanka in a younger period, in the late Miocene (7–8 Mya). The confidence intervals for the timing of these two genera may suggest the arrival in a single event, perhaps via rafting, since the seeds of these plants have no specialized mechanisms for long distance dispersals. It is notable in the Gesneriaceae phylogeny presented here that the neotropical *R. azureum*

was closely related to the Sri Lankan/South Indian species which was also indicated in earlier work (Mayer *et al*., 2003).

Three genera, *Henckelia, Rhynchotechum* and *Aeschynanthus* appear to have migrated into the Indian Peninsular from Central and East Asia and have entered Sri Lanka at different occasions. The genus *Henckelia*, seems to be present in Sri Lanka since the mid Miocene (10– (15–18 Mya), more likely via overland migration from Central and East Asia and dispersed into Sri Lanka through the Indian Peninsula. Further diversification of the genus continued in the Pliocene (2.6–5.3 Mya) and Pleistocene (2.6 Mya–11,000 years) in Sri Lanka while some exchange events were also suggested between the Indian Peninsula and Sri Lanka during these periods.

The genus, *Rhynchotechum* enterd on Sri Lanka possibly at the Miocene–Pliocene boundary (5.29 Mya) also may have dispersed from Central and East Asia through Indian Peninsula. The genus arriving most recently on Sri Lanka is the genus *Aeschynanthus* dated to the Pliocene (3.36 Mya) which may also have entered in Sri Lanka from Central and East Asia through Indian Peninsula.

Sri Lanka remained separated from Indian Peninsula since the Cretaceous period and only connected sporadically from the late Pliocene onwards (Katupotha, 2013; Majumder, 1984; Scotese, 2016). During the Miocene to Pliocene epochs where the most of Gesneriaceae flora indicated to be appeared on the island Sri Lanka had severed the connection to the Indian mainland. This is due to a sea level rise, as the world's climate was warmer than today. There was a global greenhouse condition in the mid Miocene (Sayyed, 2014) called "Miocene Climatic Optimum". Therefore the genera of Gesneriaceae, especially *Henckelia* and *Rhynchotechum* possibly arrived on the Indian Peninsula then migrated to Sri Lanka during the Miocene.

Thus, the routes of dispersal or migration of these genera, *Henckelia, Rhynchotechum and Aeschynanthu*s in Sri Lanka indicated from the present study.

However, the mode of dispersal is unclear especially without evidence for a land connection between Sri Lanka and India (Majumder, 1984; Rohling *et al.*, 1998). *Rhynchotechum permolle* may have dispersed by birds since it bears a small fleshy berry. Seed dispersal mechanism of *Henckelia* is suggested by water over short distances. The seed dispersal mechanism of *H. humboldtina*, *H. floccosa* and *H.*

zeylanica described with the rain splash capsules of the plagiocarpic fruits.This is a special mechanism for dispersal of seeds through rain water (Weber and Burtt, 1998).

Aeschynanthus with its recent arrival can be oversea or perhaps through the land connection during Pleistocene glaciations when the renewal of the land connection between Indian Peninsula and Sri Lanka. *Aeschynanthus ceylanicus* has appendaged seeds that can account for wind dispersal as shown in the case of *Lysionotus* (Kokubugata *et al*., 2011). According to Denduangboripant *et al.* (2001) the wide distribution of the genus *Aeschynanthus* can be attributed to the presence of these appendages.

Katupotha (2013) suggested that from ~ 20 to 5 Mya Sri Lanka had a humid climate, with limestone facies in the North and Northwest while arenaceous (a geological term used to describe a condition with sand or sand-like particles) facies in the Southeast and a global position at 4–8°N and 77E–79°E. This indicates that during the Miocene the greater part of the island was covered with humid forests.

Although Gesneriaceae are recorded from limestone habitats elsewhere, so far no Gesneriaceae plants have been recorded from the northern limestone part of the island. However, this part of the country had not been botanised since 16th century, the beginning of the botanical history in Sri Lanka, and even now is omitted due to consequences of the country's civil war over several decades. Possibly, therefore, species there await discovery. However, perhaps a Gesneriaceae flora once existed in the past in the northern limestone vegetation and might have become extinct due to later climatic changes that led to greater aridity in this part of the country (Katupotha, 1994). On the other hand, the Gesneriaceae flora is distributed in the Central and Southwest wet forests of Sri Lanka at present, which might indicate that these wet forests act as refugia for lineages that were once more widely distributed across the island, when wet forest covered more of its area.

The above scenarios are made on the basis of the included samples. While the sampling in Sri Lanka is complete, only two of around 22 species in six genera that are known from South India (Möller *et al*., in press) could be included here. Thus, for a full and complete understanding of the evolution of Gesneriaceae in Sri Lanka, the addition of

more South India samples is required. Nevertheless, some important conclusions can be drawn.

4.6 Conclusions

1. The dated phylogeny of family Gesneriaceae present here included with all 13 Gesneriaceae species from Sri Lanka, except *Henckelia floccosa*, and two species from South India. Therefore, this is the first detailed study of family Gesneriaceae focusing the biogeographic history of the Decan plate, Sri Lanka and South India. 26 sequences for *trn*L-F, 52 sequences for matK, 24 sequences for *rps*16 and 46 sequences for *ndh*F were newly generated for the 13 Gesneriaceae species from Sri Lanka. Additionally, 36 *mat*K, 12 *rps*16, 32 *ndh*F and 12 *trn*L-F additional sequences were generated for another 14 Gesneriaceae species from the Old World.
2. According to the updated phylogeny presented here the origin of family Gesneriaceae was dated to a stem age of 81.78 Mya (74.76 Mya crown age) in the late Cretaceous period.
3. The study further estimated an origin of the Old Word Gesneriaceae lineage (subfamily Didymocarpoideae) 69.58 Mya at the K/T boundary. According to the most parsimonious hypothesis, an in-and-out of the Deccan Plate can be suggested for tribe Trichosporeae. In this scenario *Championia* and *Jerdonia* may have arrived as ancestors of Trichosporeae, via a long-distance dispersal event to the Deccan Plate from Australasia in the Paleocene. After the collision of the Deccan Plate with Eurasia, the Trichosporeae further diversified and expanded to areas such as Africa, Europe, and Asia in the early Eocene. The presence of an ancient basal lineage of Deccan origin, i.e. *Championia*, in subfamily Didymocarpoideae (Old World Gesneriaceae) in Sri Lanka with an age of 55.44 Mya is a significant outcome of this study.
4. Three genera, *Rhynchotechum*, *Henckelia* and *Aeschynanthus* were recognized with an origin in Central and East Asia and may have arrived in Sri Lanka through South India while two genera, *Rhynchoglossum* and *Epithema* likely arriving from the Malay Archipelago by long-distance dispersal events. However, there are at least

five events in the geological history where six Gesneriaceae genera arrived on the island spanning from the Eocene through to the Pleistocene.

Chapter 5 Final Discussion and Conclusions

The present study focused on members of the plant family Gesneriaceae from Sri Lanka and had three objectives, firstly to delineate species using a molecular approach, secondly to revise the taxonomy of all the Sri Lankan Gesneriaceae and thirdly, to investigate their phylogenetic affiliations and biogeographical history using a family-wide phylogeny and generating a dated phylogenetic tree and ancestral area reconstruction.

Overall, the morphological – molecular taxonomic delimitation approach was found to be an effective method to delineate species to propose a stable and evolutionary meaningful classification. Especially the integration of morphological data with different molecular markers proved very effective. In this aspect, the inclusion of multiple individuals per population and taxon was eseential to detect taxon boundaries and to show the intra-specific diversity and gave more insight into the |Sri Lankan Gesneriaceae taxa. For example the study of intra-specific diversity in *Henckelia walkerae* enabled the identification of a new species, *H. wijesundarae* (Ranasinghe *et al*., 2016). On the other hand, the complexity of morphology was reflected in the molecular delimitation attempt in the species complex of two Sri Lankan species *H. humboldtiana*, *H. floccosa* and with South Indian *H. incana*. The presence of unresolved clades of species indicated the existence of recently diverging lineages, for example *H. angusta* and *H. communis*. The application of the integrated method here also leads us to distinguish two closely related species as distinct taxa such as *Rhynchoglossum notonianum* and *R. gardneri*. These two species were recognized as two species based on a difference in only one calyx segment and thus there was a certain dispute over species boundaries of these taxa (Michael Möller, pers. com.).

The integrated molecular-morphological approach and the complete taxonomic revision made it possible to place all 14 Sri Lankan Gesneriaceae species directly in the family phylogeny. Therefore, it enables us to get a whole picture of the assembly of Gesneriaceae family in Sri Lanka. Overall, long-distance dispersals and overland migration through the Indian Peninsular have shaped the presence of Gesneriaceae in Sri Lanka. It is quite remarkable that the six genera arrived in Sri Lanka in several waves and their origin goes back to the establishment of subfamily Didymocarpoideae.

The ancestor of Epithemateae lineage seem to have been resident in Australasia in the Paleocene and the ancestor of tribe Trichosporeae appears to have dispersed onto the Deccan Plate in the Eocene and diversified there eventually leaving behind *Championia* in Sri Lanka. The lineage then appeared to have escaped to Central and East Asia upon collision of the Indian Plate with Eurasia and diversified into the many genera extant there today. Therefore, the present findings of assembly of the family Gesnericeae could establish important hypotheses on historical pathways through which the family is distributed globally.

The presence of very old lineages/species, for example *Championia reticulata* with an age estimate of 55.44 MYA, further confirms that Sri Lanka contains unique historical biotic components despite the close land connections between Sri Lanka and the Indian Peninsula (Ashton *et al.*, 1997; Bossuyt *et al.*, 2004) over a long geological history since their assembly in Southern Gondwanaland about 180 Mya (ref.).

The present study also suggests some important aspects for future work. The presence of a species complex around *H. humboldtiana* with the Sri Lankan endemic *H. floccosa* and the South Indian endemic *H. incana* must be further tested with the inclusion of more samples of these taxa from both Sri Lanka and South India in the future. Understanding of the morphological diversity of *H. communis* and its unresolved phylogeny with *H. angusta* and the putative hybrid would be an interesting study in the future with the application of modern methodologies, such as single nucleotide polymorphism (SNP) using next generation sequencing methods such as Restriction site Associated DNA sequencing (RADseq) or Hybrid baits capture approaches that generates hundreds of markers across the entire genome.

Taxon sampling outside Sri Lanka remains far from complete, with the lack of South Indian material a particular issue. There are about 24 Gesneriacaee species recorded from South India of which three, *Henckelia humboldtiana*, *Rhynchoglossum notonianum* and *E. ceylanicum* are shared between Sri Lanka and South India (Möller *et al*., 2016 in press; Skog and Boggan, 2016 onw.), and other needs to be tested thoroughly, such as *Aeschynanthus perrottetii*. Presently, the family phylogeny of Gesneriaceae includes all 14 species of Sri Lankan Gesneriaceae but only two out of ca.

24 species from South India, *H. incana* and *Jerdonia indica*. It is hoped that further sampling will continue to clarify the biogeographic history of this family.

Molecular delimitation studies in *Championia reticulata* revealed the presence of two distinct clades. However, there was no discriminating character found in the morphological studies that would warrant splitting the species *C. reticulata*. It was interesting to note that while the morphology was much conserved, the rate of molecular evolution was relatively high. Altough Theobald and Grupe (1981) sugested the species as self pollinated it has an actinomorphic flower which are thought of to be more suited for generalist pollinators and are thus under less functional constraints for shape diversification (e.g. Caruso, 2006). Whether the lack of morphological evolution in *C. reticulata* can be, at least in part, linked to the flower actinomorphy would be interesting to test.

Considering the complex molecular phylogeny recognized here, *H. humboldtiana* is proposed as a species complex with Sri Lankan endemic *H. floccosa* and South Indian endemic *H. incana*. Inclusion of samples of *H. humboldtiana* and further 17 *Henckelia* species from South India is very crucial for understanding of a clear picture of the taxonomy of the aculescent group of *Henckelia*. *Henckelia communis* and *H. angusta* could not be separated by the molecular markers applied here. The occurrence of a putative hybrid may hamper progress here.

Finally, the present study upholds the necessity of understanding the underlying history of a species and to sample the entire range of genetic and morphological diversity, to effectively define its boundaries, which is important for downstream applications from taxonomy, such as conservation and sustainable utilization. From this point of view, the combined morphological-molecular approach sampling at the population level proved highly effective and successful. This study also provided new insights into the biogeographic history of the family Gesneriaceae, in Sri Lanka and beyond, and in particular pointed to the role of the Deccan Plate in generating diversity and as a global plant distribution route.

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Appendices

Appendix 1 Details of DNA samples used in the molecular species delimitation in Chapter 02.

No	Tribe	Subtribe	Taxon	EDNA No. if applicable	Voucher number	Origin	*trn*L-F	ITS
1	Epithemateae	Epithematinae	*Epithema ceylanicum*	EDNA14-0034714	Ranasinghe S. SR 867a	Sri Lanka	This study	This study
2	Epithemateae	Epithematinae	*Epithema ceylanicum*	EDNA14-0035535	Ranasinghe S. SR1035b	Sri Lanka	This study	–
3	Epithemateae	Epithematinae	*Epithema ceylanicum*	EDNA14-0035536	Ranasinghe S. SR897a	Sri Lanka	This study	This study
4	Epithemateae	Epithematinae	*Epithema ceylanicum*	EDNA14-0035537	Ranasinghe S. SR867b	Sri Lanka	This study	This study
5	Epithemateae	Epithematinae	*Epithema ceylanicum*	EDNA16-0044917	Middleton et al., 4579.	Thailand	This study	–
6	Epithemateae	Epithematinae	*Epithema benthamii*	EDNA16-0044918	Argent et al., 29028-EP7	Philipine	This study	–
7	Epithemateae	Epithematinae	*Epithema benthamii*	–	CW15RBGE19972563	Philipine	AY423135	–
8	Epithemateae	Epithematinae	Epithema membranaceum	EDNA16-0044915	P. Wilkie et al., FR152899	Malay peninsula	This study	–
9	Epithemateae	Epithematinae	Epithema taiwanense	–	G103Wang.et.al	Taiwan	AJ492276.	–
10	Epithemateae	Epithematinae	Epithema taiwanense var. fasciculatum	EDNA16_0044919		Taiwan	This study	–
11	Epithemateae	Epithematinae	*Epithema saxatile*	EDNA13-0031196	Weber, A. and Anthonysamy 870521-3/2	cult. HBV	AJ492275	–
12	Epithemateae	Epithematinae	*Epithema tenue*	EDNA13-0031203	D. Harris DTH 5815	Cameroon	AJ492277	–
13	Epithemateae	Monophyllaeinae	*Monophyllaea elongata*	EDNA13-0031197	Weber, A. and Anthonysamy 870518-1/1		AJ492279	–
14	Epithemateae	Monophyllaeinae	*Monophyllaea glauca*	NA	G88, Vogel and Weber 790106-1/1 (WU)	Borneo, Sarawak, Bkt. Mentawa	AJ492280	–
15	Epithemateae	Monophyllaeinae	*Monophyllaea horsfieldii*	NA	RS/Y1	Malaysia	AJ492269	–
16	Epithemateae	Monophyllaeinae	*Monophyllaea hirticalyx*	NA	G66, Vogel and Weber 790801	Malay peninsula	AJ492281	–
17	Epithemateae	Monophyllaeinae	*Whytockia purpurascens*	EDNA13-0031187	Möller, M. MMO 01-87	China	FJ501428	–
18	Epithemateae	Monophyllaeinae	*Whytockia sasakii*	EDNA10-00778	Möller, M. s.n.	Taiwan	AY423134	–
19	Epithemateae	Monophyllaeinae	*Whytockia tsiangiana*	–	Nr. 200	China	AJ492289	–

20	Epithemateae	Monophyllaeinae	*Rhynchoglossum azureum*	–	Huber and Weissenhofer 722	Costa Rica	AJ492282	–
21	Epithemateae	Loxoniinae	*Stauranthera grandiflora*	EDNA13-0031193	Weber, A. 870602-1/1; HNBT04	–	AJ492287	KJ475410
22	Epithemateae	Loxotidinae	*Rhynchoglossum obliquum*	–	Tao,J. and Kang,M, HNBT05	China	–	KJ475424
23	Epithemateae	Loxotidinae	*Rhynchoglossum obliquum*	–	Liang, R. H., GX-NP-02	China	–	GU350652
24	Epithemateae	Loxotidinae	*Rhynchoglossum obliquum*	–	G102.	Taiwan	TW.AJ492286	–
25	Epithemateae	Loxotidinae	*Rhynchoglossum obliquum*	–	G16,Weber870510.1/3	Malay peninsula	AJ492284	–
26	Epithemateae	Loxotidinae	*Rhynchoglossum obliquum*	–	G97	Philipine	AJ492285	–
27	Epithemateae	Loxotidinae	*Rhynchochlossum notonianum*	EDNA15-0040446	Ranasinghe, S., SR 1080a	Sri Lanka	This study	This study
28	Epithemateae	Loxotidinae	*Rhynchochlossum notonianum*	EDNA15-0038224	Ranasinghe, S. SR 1051a	Sri Lanka	This study	This study
29	Epithemateae	Loxotidinae	*Rhynchochlossum notonianum*	EDNA15-0038225	Ranasinghe, S. SR 1051b	Sri Lanka	–	This study
30	Epithemateae	Loxotidinae	*Rhynchochlossum notonianum*	EDNA15-0040445	Ranasinghe, S. SR 1076a	Sri Lanka	This study	This study
31	Epithemateae	Loxotidinae	*Rhynchochlossum notonianum*	–	G19.HBM.	cult. HB München, rec. Dec. 1997	AJ492283	–
32	Epithemateae	Loxotidinae	*Rhynchoglossum gardneri*	EDNA13-0034719	Ranasinghe, S. SR 905a	Sri Lanka	This study	This study
33	Epithemateae	Loxotidinae	*Rhynchoglossum gardneri*	EDNA14-0034720	Ranasinghe, S. SR 905b	Sri Lanka	This study	This study
34	Epithemateae	Loxotidinae	*Rhynchoglossum gardneri*	EDNA13-0034718	Ranasinghe, S. SR 874a	Sri Lanka	This study	This study
35	Epithemateae	Loxotidinae	*Rhynchoglossum gardneri*	EDNA14-0034717	Ranasinghe, S. SR 875a	Sri Lanka	This study	This study
36	Trichosporeae	Championiinae	*Championia reticulata*	EDNA13-0034520	Ranasinghe, S. SR 1033a	Sri Lanka	This study	–
37	Trichosporeae	Championiinae	*Championia reticulata*	EDNA14-0035173	Ranasinghe, S. SR1010b	Sri Lanka	–	This study
38	Trichosporeae	Championiinae	*Championia reticulata*	EDNA14-0035174	Ranasinghe, S. SR1033b	Sri Lanka	–	This study
39	Trichosporeae	Championiinae	*Championia reticulata*	EDNA14-0035175	Ranasinghe, S. SR1018a	Sri Lanka	–	This study
40	Trichosporeae	Championiinae	*Championia reticulata*	EDNA14-0035538	Ranasinghe, S. SR 1027c	Sri Lanka	This study	This study
41	Trichosporeae	Championiinae	*Championia reticulata*	EDNA14-0035539	Ranasinghe, S. SR 1033c	Sri Lanka	–	This study
42	Trichosporeae	Corallodiscinae	*Corallodiscus conchifolius*	EDNA09-00441; –	Wang, H. 105; CH137chd	China; –	FJ501433	Unpublished
43	Trichosporeae	Corallodiscinae	*Corallodiscus lanuginosus*	EDNA09-00449; –	Möller, M. MMO 01-138; G79	China	FJ501432	FJ501432
44	Trichosporeae	Jerdoniinae	*Jerdonia indica*	EDNA09-00456	G155 Jang	India	FJ501429	Unpublished
45	Trichosporeae	Didymocarpinae	*Aeschynanthus bracteatus*	EDNA13-0034449	G37, Wang 991113	China	FJ501501	AF349203/AF349284

46	Trichosporeae	Didymocarpinae	*Aeschynanthus buxifolius*	–	HMS1	China	KM232642	KJ475429
47	Trichosporeae	Didymocarpinae	*Aeschynanthus ceylanicus*	EDNA13-0034451	Ranasinghe S., SR861b	Sri Lanka: Hakgala	This study	This study
48	Trichosporeae	Didymocarpinae	*Aeschynanthus ceylanicus*	EDNA13-0034452	Ranasinghe S., SR861c	Sri Lanka: Hakgala	This study	This study
49	Trichosporeae	Didymocarpinae	*Aeschynanthus ceylanicus*	EDNA15-0040443	Ranasinghe S., SR1075a	Sri Lanka: Balangoda	This study	This study
50	Trichosporeae	Didymocarpinae	*Aeschynanthus ceylanicus*	EDNA15-0040444	Ranasinghe S., SR1073a	Sri Lanka: Loolkandura	This study	This study
51	Trichosporeae	Didymocarpinae	Aeschynanthus lancilimbus	–	G106, Y.Z.Wang S-10868, PE	China	FJ501499	HQ632992
52	Trichosporeae	Didymocarpinae	*Aeschynanthus micranthus*	–	G67, M.Moeller 01-79	–	FJ5015003	Unpublished
53	Trichosporeae	Didymocarpinae	*Aeschynanthus roseoflorus*	EDNA09-00403	Argent 87/14	Indonesia	HQ632896	HQ632993
54	Trichosporeae	Didymocarpinae	Billolivia violacea	EDNA0000052; –	SVFDE201b, KN179; CH78	–	KU985111 (Unpublished)	KU985115 (Unpublished)
55	Trichosporeae	Didymocarpinae	*Ridleyandra porphyrantha*	EDNA09-00452	Weber, A. 870420-2/4; G111chkd	Malaysia	FJ501520	HQ633031
56	Trichosporeae	Leptoboeinae	Boeica filiformis	–	MB7	–	–	Unpublished
57	Trichosporeae	Leptoboeinae	Boeica stolonifera	–	CH33	–	–	Unpublished
58	Trichosporeae	Leptoboeinae	Boeica porosa	–	G92, Gu 99-705	China	FJ501441	Unpublished
59	Trichosporeae	Leptoboeinae	Boeica multinervia	–	Y.Z.Wang 015, PE, G34	China, Yunnan, Yingjiang	HQ632951	HQ632951
60	Trichosporeae	Leptoboeinae	Boeica ferruginea	–	G90, M.Möller MMO 01-182B ex Zhang Chang Qin 200012	China, SE Yunnan	FJ501440	Wei et al., 2010
61	Trichosporeae	Leptoboeinae	Rhynchotechum discolor	–	CH134	China	–	Unpublished
62	Trichosporeae	Leptoboeinae	Rhynchotechum vestitum	–	CH30	China	–	Unpublished
63	Trichosporeae	Leptoboeinae	Rhynchotechum parviflorum	–	G 47; M. Mendum, G. Argent and Hendrian 00148	Central Sulawesi, Mt. Sojol	FJ501437	Wei.et.al.201
64	Trichosporeae	Leptoboeinae	Rhynchotechum discolor	–	RBGE-PNH Expedition 1997/SM8, cult. RBGE 19972562; GK911	Philippines	FJ501436	Unpublished
65	Trichosporeae	Leptoboeinae	Rhynchotechum discolor	–	; GK9655	Philippines	–	Unpublished
66	Trichosporeae	Leptoboeinae	Rhynchotechum discolor	–	; GK5185	Taiwan	–	Unpublished
67	Trichosporeae	Leptoboeinae	Rhynchotechum discolor	–	; GK15119	Taiwan	–	Unpublished
68	Trichosporeae	Leptoboeinae	Rhynchotechum discolor	–	; GK207	RyuKyu	–	Unpublished
69	Trichosporeae	Leptoboeinae	Rhynchotechum brevipedunculatum	–	; GK15039	Taiwan	–	Unpublished

70	Trichosporeae	Leptoboeinae	Rhynchotechum brevipedunculatum	–	; GK14226	Taiwan	–	Unpublished
71	Trichosporeae	Leptoboeinae	Rhynchotechum brevipedunculatum	–	; GK15047	Taiwan	–	Unpublished
72	Trichosporeae	Leptoboeinae	Rhynchotechum formosanum	–	HNQZ01; CIPeng18195	Taiwan	–	Unpublished
73	Trichosporeae	Leptoboeinae	Rhynchotechum ellipticum	–	isolate LS01; CH29	China	KM232661	Wei.et.al.2010
74	Trichosporeae	Leptoboeinae	Rhynchotechum permolle	EDNA14-0035177		Sri Lanka	NA	This study
75	Trichosporeae	Leptoboeinae	Rhynchotechum permolle	EDNA14-0035182		Sri Lanka	NA	This study
76	Trichosporeae	Leptoboeinae	Rhynchotechum permolle	EDNA14-0035181	Ranasinghe S., SR1033c	Sri Lanka	This study	This study
77	Trichosporeae	Didymocarpinae	Microchirita viola	EDNA10-00284	A.R.Rafidah, FRI-64388, KEP	Malaysia	JF912533	JF912560
78	Trichosporeae	Didymocarpinae	Microchirita mollissima	EDNA09-02222	D.Middleton et al. 4361, E	Thailand	JF912528	JF912555
79	Trichosporeae	Didymocarpinae	Microchirita sericea	EDNA09-02224	A.R.Rafidah, FRI-64328, KEP	Malaysia	JF912521	JF912548
80	Trichosporeae	Didymocarpinae	Microchirita caliginosa	–	cult. HBV GS-96-02 ex HB Muenchen-Nymphenburg; Kiehn and Pfosser 2000-1	Malaysia	FJ501325	FJ501325
81	Trichosporeae	Didymocarpinae	Codonoboea nana 00268	EDNA10-00268	T.L.Yao, FRI-55963. KE	Malaysia	JF912543	JF912570
82	Trichosporeae	Didymocarpinae	Codonoboea elata	EDNA09-02218	A.R.Rafidah, FRI-64321, KEP	Malaysia	JF912523	JF912550
83	Trichosporeae	Didymocarpinae	Codonoboea malayana	EDNA08-00888	CH54Q	Malaysia	JF912541	JF912568
84	Trichosporeae	Didymocarpinae	Codonoboea albomarginata	EDNA09-00445	"WU";G10, A.Weber 840805-1/12, WU	Malaysia	AJ492297	HQ632961
85	Trichosporeae	Didymocarpinae	Codonoboea venusta	EDNA09–02256	R.Kiew, RK-5430, KEP	Malaysia	JF912545	JF912572
86	Trichosporeae	Didymocarpinae	Codonoboea corrugata	–	RBGE-PNH Expedition 1998, DNA no. D12	Philippines	FJ501484	HQ632962
87	Trichosporeae	Didymocarpinae	Codonoboea leucocodon	EDNA09–02244	C.L.Lim, FRI-64821, KEP	Malaysia	JF912540	JF912567
88	Trichosporeae	Didymocarpinae	Codonoboea racemosa	EDNA08-00889	P.S.Smith, SMTSU-110/110, E	Indonesia	JF912544	JF912571
89	Trichosporeae	Didymocarpinae	Codonoboea codonion	EDNA10-00267	C.L.Lim, FRI-65040, KEP	Malaysia	JF912538	JF912565
90	Trichosporeae	Didymocarpinae	Codonoboea floribunda	EDNA10-00182	C.L.Lim, FRI-64971, KEP	Malaysia	JF912539	JF912566
91	Trichosporeae	Didymocarpinae	Henckelia urticifolia	EDNA09-02229	NPSW 110, E	Bhutan	JF912532	JF912559
92	Trichosporeae	Didymocarpinae	Henckelia urticifolia	–	EMAK 109 H 20.9.1991	Nepal	FJ501492	FJ501328
93	Trichosporeae	Didymocarpinae	Henckelia pumila	EDNA09-02223	D.Middleton et al. 4505, E	Thailand	JF912529	JF912556

94	Trichosporeae	Didymocarpinae	Henckelia pumila	–	cult. RBGE 19962271, Gaoligong Shan Expedition 1996	China	FJ501491	FJ501327
95	Trichosporeae	Didymocarpinae	Henckelia dielsii	EDNA09-02217	M.Moeller MMO 08-1211, E	China	HQ632871	HQ632967
96	Trichosporeae	Didymocarpinae	Henckelia anachoreta	EDNA09-02214	D.J.Middleton et al 4480, E	Thailand	HQ632870	HQ632966
97	Trichosporeae	Didymocarpinae	Henckelia macrophylla	EDNA09-02221	M.Moeller, MMO 08-1222, E	China	JF912527	JF912554
98	Trichosporeae	Didymocarpinae	Henckelia bifolia	EDNA08-00896	Bhaskar Adhikari L2B6, E	Nepal	JF912522	JF912549
99	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034439	Ranasinghe, S. SR DBG 866.a		This study	This study
100	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034440	Ranasinghe, S. SR DBG 866.b	Sri Lanka	This study	This study
101	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034441	Ranasinghe, S. SR DBG 866.c	Sri Lanka	This study	This study
102	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034442	Ranasinghe, S. SR DBG 866.d	Sri Lanka	This study	This study
103	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034443	Ranasinghe, S. SR DBG 866.e	Sri Lanka	This study	This study
104	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034432	Ranasinghe, S. SR KNU.M 879.b	Sri Lanka	This study	This study
105	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034433	Ranasinghe, S. SR KNU.M 879.c	Sri Lanka	This study	This study
106	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034434	Ranasinghe, S. SR KNU.M 879.d	Sri Lanka	This study	This study
107	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034435	Ranasinghe, S. SR KNU.M 879.e	Sri Lanka	This study	This study
108	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034444	Ranasinghe, S. SR KNU.R 901.a	Sri Lanka	This study	This study
109	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034445	Ranasinghe, S. SR KNU.R 901.b	Sri Lanka	This study	This study
110	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034446	Ranasinghe, S. SR KNU.R 901.c	Sri Lanka	This study	This study
111	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA14-0034448	Ranasinghe, S. SR KNU.R 901.e	Sri Lanka	This study	This study
112	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034290	Ranasinghe, S. SR PWD 1033.a	Sri Lanka	This study	This study
113	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034437	Ranasinghe, S. SR PWD 1033.c	Sri Lanka	This study	This study
114	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA14-0034438	Ranasinghe, S. SR PWD 1033.e	Sri Lanka	This study	This study
115	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034241	Ranasinghe, S. SR SIN.P1- 1012.a	Sri Lanka	This study	This study
116	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034242	Ranasinghe, S. SR SIN.P1- 1012.b	Sri Lanka	This study	This study
117	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034243	Ranasinghe, S. SR SIN.P1- 1012.c	Sri Lanka	This study	This study
118	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034244	Ranasinghe, S. SR SIN.P1- 1012.d	Sri Lanka	This study	This study
119	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034245	Ranasinghe, S. SR SIN.P1- 1012.e	Sri Lanka	This study	This study

120	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034494	Ranasinghe, S. SR SIN.P2- 1011.a	Sri Lanka	This study	This study
121	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034495	Ranasinghe, S. SR SIN.P2- 1011.b	Sri Lanka	This study	This study
122	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034496	Ranasinghe, S. SR SIN.P2- 1011.c	Sri Lanka	This study	This study
123	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034497	Ranasinghe, S. SR SIN.P2- 1011.d	Sri Lanka	This study	This study
124	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034289	Ranasinghe, S. SR KTU 1026.a	Sri Lanka	This study	This study
125	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034490	Ranasinghe, S. SR KTU 1026.b	Sri Lanka	This study	This study
126	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034491	Ranasinghe, S. SR KTU 1026.c	Sri Lanka	This study	This study
127	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034492	Ranasinghe, S. SR KTU 1026.d	Sri Lanka	This study	This study
128	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034493	Ranasinghe, S. SR KTU 1026.e	Sri Lanka	This study	This study
129	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034498	Ranasinghe, S. SR Sin.H-3 1015.a	Sri Lanka	This study	This study
130	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034499	Ranasinghe, S. SR Sin.H-3 1015.b	Sri Lanka	This study	This study
131	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034500	Ranasinghe, S. SR Sin.H-3 1015.c	Sri Lanka	This study	This study
132	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034501	Ranasinghe, S. SR Sin.H-3 1015.d	Sri Lanka	This study	This study
133	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034502	Ranasinghe, S. SR Sin.M 1016.a	Sri Lanka	This study	This study
134	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034503	Ranasinghe, S. SR Sin.M 1016.b	Sri Lanka	This study	This study
135	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13	Ranasinghe, S. SR Sin.M 1016.c	Sri Lanka	This study	This study
136	Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13	Ranasinghe, S. SR Sin.M 1016.d	Sri Lanka	This study	This study
137	Trichosporeae	Didymocarpinae	*Henckelia angusta*	EDNA13-0034288	Ranasinghe, S. SR Sin.H-1 1014.a	Sri Lanka	This study	This study
138	Trichosporeae	Didymocarpinae	*Henckelia angusta*	EDNA13-0034454	Ranasinghe, S. SR Sin.H-1 1014.c	Sri Lanka	This study	This study
139	Trichosporeae	Didymocarpinae	*Henckelia angusta*	EDNA13-0034455	Ranasinghe, S. SR Sin.H-1 1014.d	Sri Lanka	This study	This study
140	Trichosporeae	Didymocarpinae	*Henckelia angusta*	EDNA13-0034456	Ranasinghe, S. SR Sin.H-1 1014.e	Sri Lanka	This study	This study
141	Trichosporeae	Didymocarpinae	Henckelia sp.nov.	EDNA13-0034293	Ranasinghe, S. SR KNY 922.a	Sri Lanka	This study	This study
142	Trichosporeae	Didymocarpinae	Henckelia sp.nov.	EDNA13-0034343	Ranasinghe, S. SR KNY 922.b	Sri Lanka	This study	This study
143	Trichosporeae	Didymocarpinae	Henckelia sp.nov.	EDNA13-0034344	Ranasinghe, S. SR KNY 922.c	Sri Lanka	This study	This study
144	Trichosporeae	Didymocarpinae	Henckelia sp.nov.	EDNA14-0034511	Ranasinghe, S. SR KNY 922.d	Sri Lanka	This study	This study
145	Trichosporeae	Didymocarpinae	Henckelia sp.nov.	EDNA13-0034345	Ranasinghe, S. SR RBG 1044.a	Sri Lanka	This study	This study

146	Trichosporeae	Didymocarpinae	Henckelia sp.nov.	EDNA13-0034346	Ranasinghe, S. SR RBG 1044.b	Sri Lanka	This study	This study
147	Trichosporeae	Didymocarpinae	Henckelia sp.nov.	EDNA13-0034513	Ranasinghe, S. SR RBG 1044.c	Sri Lanka	This study	This study
148	Trichosporeae	Didymocarpinae	Henckelia sp.nov.	EDNA13-0034514	Ranasinghe, S. SR RBG 1044.d	Sri Lanka	This study	This study
149	Trichosporeae	Didymocarpinae	Henckelia sp.nov.	EDNA13-0034515	Ranasinghe, S. SR RBG 1044.e	Sri Lanka	This study	This study
150	Trichosporeae	Didymocarpinae	*Henckelia walkerae*	EDNA13-0034292	Ranasinghe, S. SR KIK 961.a	Sri Lanka	This study	This study
151	Trichosporeae	Didymocarpinae	*Henckelia walkerae*	EDNA13-0034481	Ranasinghe, S. SR KIK 961.b	Sri Lanka	This study	This study
152	Trichosporeae	Didymocarpinae	*Henckelia walkerae*	EDNA13-0034482	Ranasinghe, S. SR KIK 961.c	Sri Lanka	This study	This study
153	Trichosporeae	Didymocarpinae	*Henckelia walkerae*	EDNA13-0034483	Ranasinghe, S. SR KIK 961.d	Sri Lanka	This study	This study
154	Trichosporeae	Didymocarpinae	*Henckelia walkerae*	EDNA13-0034484	Ranasinghe, S. SR KIK 961.e	Sri Lanka	This study	This study
155	Trichosporeae	Didymocarpinae	*Henckelia walkerae*	EDNA13-0034486	Ranasinghe, S. SR RBG.1045.b	Sri Lanka	This study	This study
156	Trichosporeae	Didymocarpinae	*Henckelia walkerae*	EDNA13-0034487	Ranasinghe, S. SR RBG.1045.c	Sri Lanka	This study	This study
157	Trichosporeae	Didymocarpinae	*Henckelia walkerae*	EDNA13-0034488	Ranasinghe, S. SR RBG.1045.d	Sri Lanka	This study	This study
158	Trichosporeae	Didymocarpinae	*Henckelia walkerae*	EDNA13-0034489	Ranasinghe, S. SR RBG.1045.e	Sri Lanka	This study	This study
159	Trichosporeae	Didymocarpinae	*Henckelia moonii*	EDNA15-0038220	Ranasinghe, S. SR BLG.1062.a	Sri Lanka	This study	This study
160	Trichosporeae	Didymocarpinae	*Henckelia moonii*	EDNA15-0038221	Ranasinghe, S. SR BLG.1062.b	Sri Lanka	This study	This study
161	Trichosporeae	Didymocarpinae	*Henckelia moonii*	EDNA15-0038222	Ranasinghe, S. SR BLG.1062.c	Sri Lanka	This study	This study
162	Trichosporeae	Didymocarpinae	*Henckelia moonii*	EDNA15-0038214	Ranasinghe, S. SR KTG.1048.a	Sri Lanka	This study	This study
163	Trichosporeae	Didymocarpinae	*Henckelia moonii*	EDNA15-0038215	Ranasinghe, S. SR KTG.1048.b	Sri Lanka	This study	This study
164	Trichosporeae	Didymocarpinae	*Henckelia moonii*	EDNA15-0038216	Ranasinghe, S. SR KTG.1048.c	Sri Lanka	This study	This study
165	Trichosporeae	Didymocarpinae	*Henckelia moonii*	EDNA15-0038217	Ranasinghe, S. SR KTG.1049.a	Sri Lanka	This study	This study
166	Trichosporeae	Didymocarpinae	*Henckelia moonii*	EDNA15-0038218	Ranasinghe, S. SR KTG.1049.b	Sri Lanka	This study	This study
167	Trichosporeae	Didymocarpinae	*Henckelia moonii*	EDNA15-0038219	Ranasinghe, S. SR KTG.1049.c	Sri Lanka	This study	This study
168	Trichosporeae	Didymocarpinae	Henckelia incana	EDNA08–00884	S.Vogel SVG, E	South India	HQ632869	HQ632965
169	Trichosporeae	Didymocarpinae	*Henckelia floccosa*	EDNA09-00457	G157 Jang	Sri Lanka	FJ501486	HQ632964
170	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034476	Ranasinghe, S. SR KNU-1 883.a	Sri Lanka	This study	This study
171	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034477	Ranasinghe, S. SR KNU-1 883.b	Sri Lanka	This study	This study

172	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034478	Ranasinghe, S. SR KNU-1 883.c	Sri Lanka	This study	This study
173	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034480	Ranasinghe, S. SR KNU-1 883.e	Sri Lanka	This study	This study
174	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034287	Ranasinghe, S. SR PWD-1 1031.a	Sri Lanka	This study	This study
175	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034472	Ranasinghe, S. SR PWD-1 1031.b	Sri Lanka	This study	This study
176	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034473	Ranasinghe, S. SR PWD-1 1031.c	Sri Lanka	This study	This study
177	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034474	Ranasinghe, S. SR PWD-1 1031.d	Sri Lanka	This study	This study
178	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034475	Ranasinghe, S. SR PWD-1 1031.e	Sri Lanka	This study	This study
179	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA15-0040508	Ranasinghe, S. SR LKD 1072.c	Sri Lanka	This study	This study
180	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA15-0040510	Ranasinghe, S. SR RTG 1071.a	Sri Lanka	This study	This study
181	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA15-0040511	Ranasinghe, S. SR RTG 1071.b	Sri Lanka	This study	This study
182	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA15-0040512	Ranasinghe, S. SR RTG 1071.c	Sri Lanka	This study	This study
183	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA15-0040522	Ranasinghe, S. SR MGK 1083.a	Sri Lanka	This study	This study
184	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA15-0040523	Ranasinghe, S. SR MGK 1083.b	Sri Lanka	This study	This study
185	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA15-0040514	Ranasinghe, S. SR KGL 1079.a	Sri Lanka	This study	This study
186	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA15-0040515	Ranasinghe, S. SR KGL 1079.b	Sri Lanka	This study	This study
187	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA15-0040516	Ranasinghe, S. SR KGL 1079.c	Sri Lanka	This study	This study
188	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA15-0040517	Ranasinghe, S. SR KGL 1079.d	Sri Lanka	This study	This study
189	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA15-0040518	Ranasinghe, S. SR RWE 1074.a	Sri Lanka	This study	This study
190	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA15-0040519	Ranasinghe, S. SR RWE 1074.b	Sri Lanka	This study	This study
191	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA15-0040520	Ranasinghe, S. SR RWE 1074.c	Sri Lanka	This study	This study
192	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA15-0040521	Ranasinghe, S. SR RWE 1074.d	Sri Lanka	This study	This study
193	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA15-0040524	Ranasinghe, S. SR MGK 1083.c	Sri Lanka	This study	This study
194	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034462	Ranasinghe, S. SR KIK 959.a	Sri Lanka	This study	This study
195	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034464	Ranasinghe, S. SR KIK 959.c	Sri Lanka	This study	This study
196	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034466	Ranasinghe, S. SR KIK 959.e	Sri Lanka	This study	This study
197	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034457	Ranasinghe, S. SR PDG 1021.a	Sri Lanka	This study	This study

198	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034458	Ranasinghe, S. SR PDG 1021.b	Sri Lanka	This study	This study
199	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034459	Ranasinghe, S. SR PDG 1021.c	Sri Lanka	This study	This study
200	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034460	Ranasinghe, S. SR PDG 1021.d	Sri Lanka	This study	This study
201	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034461	Ranasinghe, S. SR PDG 1021.e	Sri Lanka	This study	This study
202	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034467	Ranasinghe, S. SR RMB 863.a	Sri Lanka	This study	This study
203	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA14-0034468	Ranasinghe, S. SR RMB 863.b	Sri Lanka	This study	This study
204	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA14-0034469	Ranasinghe, S. SR RMB 863.c	Sri Lanka	This study	This study
205	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA14-0034470	Ranasinghe, S. SR RMB 863.d	Sri Lanka	This study	This study
206	Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034471	Ranasinghe, S. SR RMB 863.e	Sri Lanka	This study	This study
207	Trichosporeae	Didymocarpinae	*Henckelia zeylanica*	EDNA13-0034341	Ranasinghe, S. SR PWD 1041.a	Sri Lanka	This study	This study
208	Trichosporeae	Didymocarpinae	*Henckelia zeylanica*	EDNA14-0034342	Ranasinghe, S. SR PWD 1041.b	Sri Lanka	This study	This study
209	Trichosporeae	Didymocarpinae	*Henckelia zeylanica*	EDNA14-0034506	Ranasinghe, S. SR PWD 1041.c	Sri Lanka	This study	This study
210	Trichosporeae	Didymocarpinae	*Henckelia zeylanica*	EDNA14-0034507	Ranasinghe, S. SR PWD 1041.d	Sri Lanka	This study	This study
211	Trichosporeae	Didymocarpinae	*Henckelia zeylanica*	EDNA13-0034286	Ranasinghe, S. SR PWD 1042.b	Sri Lanka	This study	This study
212	Trichosporeae	Didymocarpinae	*Henckelia zeylanica*	EDNA14-0034339	Ranasinghe, S. SR PWD 1042.a	Sri Lanka	This study	This study
213	Trichosporeae	Didymocarpinae	*Henckelia zeylanica*	EDNA13-0034340	Ranasinghe, S. SR PWD 1042.c	Sri Lanka	This study	This study
214	Trichosporeae	Didymocarpinae	*Henckelia zeylanica*	EDNA13-0034510	Ranasinghe, S. SR PWD 1042.e	Sri Lanka	This study	This study

Appendix 2 Details of DNA samples used in the phylogeny and Biogeography of family Gesneriaceae including samples from Luna-Castro (2016) unpublished matrix and data generated for the present study.

Tribe	**Subtribe**	**Taxon**	**EDNA No. if applicable**	**Voucher number**	**Origin**	***mat*K**	***ndh*F**	***trn*L-F**	***rps*16**
Epithemateae	Epithematinae	*Epithema ceylanicum*	EDNA14-0034714	Ranasinghe S. SR 867a	Sri Lanka, Dolosbage	This study	This study	This study	–
Epithemateae	Epithematinae	*Epithema saxatilettia*	EDNA13-0031196	Weber, A. and Anthonysamy 870521-3/2		Luna Castro, 2016	Luna Castro, 2016	AJ492275	Luna Castro, 2016
Epithemateae	Epithematinae	*Epithema tenue*	EDNA16-0044914	D. Harris DTH 5815	Cameroon	This study	This study	This study	This study
Epithemateae	Loxoniinae	*Loxonia hirsuta*	EDNA13-0031195	Weber, A. 870602-1/5		Luna Castro,	Luna Castro,	AJ492278	Luna Castro,

						2016	2016		2016
Epithemateae	Loxoniinae	*Stauranthera caerulea*	EDNA13-0033734	Möller, M. MMO G-20		Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016
Epithemateae	Loxoniinae	*Stauranthera grandiflora*	EDNA13-0031193	Weber, A. 870602-1/1			Luna Castro, 2016	AJ492287	Luna Castro, 2016
Epithemateae	Loxotidinae	*Rhynchoglossum azureum*	EDNA13-0031198	Huber and Weissenhofer 722	Costa Rica	Luna Castro, 2016		AJ492282	Luna Castro, 2016
Epithemateae	Loxotidinae	*Rhynchoglossum gardneri*	EDNA14-0034717	Ranasinghe, S. SR 875a	Sri Lanka: Peradeniya	This study	This study	This study	This study
Epithemateae	Loxotidinae	*Rhynchoglossum notonianum*	EDNA15-0038224	Ranasinghe, S. SR 1051a	Sri Lanka: Namunukula	This study	This study	This study	This study
Epithemateae	Loxotidinae	*Rhynchoglossum obliquum*	EDNA13-0031194	Weber, A. 870510-11/3		Luna Castro, 2016	Luna Castro, 2016	AJ492284	Luna Castro, 2016
Epithemateae	Monophyllaeinae	*Monophyllaea elongata*	EDNA13-0031197	Weber, A. and Anthonysamy 870518-1/1		Luna Castro, 2016	Luna Castro, 2016	AJ492279	Luna Castro, 2016
Epithemateae	Monophyllaeinae	*Monophyllaea horsfieldii*							AJ492269
Epithemateae	Monophyllaeinae	*Whytockia purpurascens*	EDNA13-0031187	Möller, M. MMO 01-87	China	Luna Castro, 2016	Luna Castro, 2016	FJ501428	Luna Castro, 2016
Epithemateae	Monophyllaeinae	*Whytockia sasakii*	EDNA10-00778	Möller, M. s.n.	Taiwan	Luna Castro, 2016	Luna Castro, 2016	AY423134	Luna Castro, 2016
Trichosporeae	Championiinae	*Championia reticulata*	EDNA14-0035538	Ranasinghe, S. SR 1027c	Sri Lanka: Kalutara	This study	This study	This study	This study
Trichosporeae	Corallodiscinae	*Corallodiscus conchifolius*	EDNA09-00441	Wang, H. 105	China	Luna Castro, 2016	Luna Castro, 2016	FJ501433	Luna Castro, 2016
Trichosporeae	Corallodiscinae	*Corallodiscus lanuginosus*	EDNA09-00449	Möller, M. MMO 01-138	China		Luna Castro, 2016	FJ501432	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Aeschynanthus ceylanicus*	EDNA13-0034452	Ranasinghe S., SR861c	Sri Lanka: Hakgala	This study	This study	This study	This study
Trichosporeae	Didymocarpinae	*Aeschynanthus lancilimbus*	EDNA09-01249	Wang, Y.Z. S-10868	China	Luna Castro, 2016	Luna Castro, 2016	FJ501499	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Aeschynanthus roseoflorus*	EDNA09-00403	Argent 87/14	Indonesia	Luna Castro, 2016	Luna Castro, 2016	HQ632896	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Agalmyla clarkei*	EDNA13-0032525	RBGE and Philippine National Herb. Exped. (1997) IS26	Philippines	Luna Castro, 2016	Luna Castro, 2016	FJ501540	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Agalmyla paucipilosa*	EDNA09-00428	Smith and Galloway 261	Indonesia		Luna Castro, 2016	HQ632893	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Allocheilos cortusiflorus*	EDNA10-02658	Zhou, P. 2010-075	China	Luna Castro, 2016	Luna Castro, 2016	unpublished	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Allocheilos guangxiensis*	EDNA08-00301	Wei, Y.G. WYG 06-02	China	This study	This study	HQ632897.1	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Allostigma guangxiense*	EDNA08-00302	Möller, M. MMO 05-755	China	Luna Castro, 2016	Luna Castro, 2016	HQ632880	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Anna ophiorrhizoides*	EDNA09-00079	Möller, M. MMO 08-1280	China	Luna Castro, 2016	Luna Castro, 2016	HQ632937	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Anna submontana*	EDNA13-0031190	Möller, M. MMO 01-85	China	Luna Castro, 2016	Luna Castro, 2016	FJ501542	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Billolivia minutiflora*	EDNA13-0000053	Ly Ngoc Sam LY498	Vietnam			unpublished	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Billolivia violacea*	EDNA13-0000052	201	Vietnam	Luna Castro,	Luna Castro,	unpublished	Luna Castro,

						2016	2016		2016
Trichosporeae	Didymocarpinae	*Briggsiopsis delavayi*	EDNA13-0032534	Wen Fang 1	China		Luna Castro, 2016	HQ632879	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Cathayanthe biflora*	EDNA09-00083	Möller, M. MMO 08-1327	China	Luna Castro, 2016	Luna Castro, 2016	HQ632899	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Chayamaritia banksiae*	EDNA13-0032309	MMOG-174		This study	This study	–	This study
Trichosporeae	Didymocarpinae	*Chayamaritia smitinandii*	EDNA13-0032310	MMOG-173		This study	This study	–	This study
Trichosporeae	Didymocarpinae	*Codonoboea leucodon*	EDNA09-02244	Lim FRI 64821	Malaysia	Luna Castro, 2016	Luna Castro, 2016		Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Codonoboea pumila*	EDNA09-0002247	J. Tosh Lim		This study	This study	unpublished	This study
Trichosporeae	Didymocarpinae	*Codonoboea venusta*	EDNA09-0002256			This study	This study	JF912545	–
Trichosporeae	Didymocarpinae	*Conandron ramondioides*	EDNA13-0031192	Takeda Herbal Garden Kyoto s.n.	Japan	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Cyrtandra cupulata*	EDNA13-0034330	Möller, M. MMO G-238	Cultivated	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Cyrtandra kusaimontana*		PTBG.NTBG960873	–	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Cyrtandra pulchella*	EDNA13-0034255	C0029/CY67	Cultivated		Luna Castro, 2016	HQ632906	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Deinostigma cyrtocarpa*	EDNA13-0000047	MMO 06-908	–	This study	This study	–	This study
Trichosporeae	Didymocarpinae	*Deinostigma poilanei*	EDNA14-0035161	MMOG-289	–	This study	This study	–	This study
Trichosporeae	Didymocarpinae	*Deinostigma tamiana*	EDNA16-0044912	1997-3431	–	This study	This study	–	–
Trichosporeae	Didymocarpinae	*Didymocarpus antirrhinoides*	EDNA13-0030198	Jong, K. 9009	Malaysia	Luna Castro, 2016	Luna Castro, 2016	FJ501513	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Didymocarpus podocarpus*	EDNA13-0032529	NPSW 193	Nepal	Luna Castro, 2016	Luna Castro, 2016	FJ501514	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Didymostigma obtusum*	EDNA09-00085	Möller, M. MMO 08-1310	China	Luna Castro, 2016	Luna Castro, 2016	HQ632875	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Didymostigma trichanthera*	EDNA09-00086	Möller, M. MMO 08-1335	China	Luna Castro, 2016	Luna Castro, 2016	HQ632876	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Glabrella longipes*	EDNA13-0031188	MMO01-122		–	–	–	This study
Trichosporeae	Didymocarpinae	*Gyrocheilos chorisepalus var synsepalus*	EDNA08-00317	Wei, Y.G. 07-708	China	Luna Castro, 2016	Luna Castro, 2016	HQ632900	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Gyrocheilos lasiocalyx*	EDNA08-00318	Möller, M. MMO 06-881	China	Luna Castro, 2016	Luna Castro, 2016	HQ632901	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Gyrocheilos retrotrichus*	–	M.Moeller MMO 07-1136, E	China	KJ137893			
Trichosporeae	Didymocarpinae	*Hemiboea fangii*	EDNA09-00088	Möller, M. MMO 08-1284	China	Luna Castro, 2016	Luna Castro, 2016	HQ632882	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Hemiboea longgangensis*	EDNA08-00339	Wei, Y.G. Wei 07550	China	Luna Castro, 2016	Luna Castro, 2016	HQ632889	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Henckelia anachoreta*	EDNA09-02214	Zhang et al., 2015; D.J. Middleton et al 4480	China; Thailand	KJ137903	–	HQ632870	–

Trichosporeae	Didymocarpinae	*Henckelia angusta*	EDNA13-0034454	Ranasinghe, S. SR Sin.H-1 1014.c	Sri Lanka: Halmandiya	This study	This study	This study	This study
Trichosporeae	Didymocarpinae	*Henckelia communis*	EDNA13-0034289	Ranasinghe, S. SR KTU 1026.a	Sri Lanka: Kalutara	This study	This study	This study	This study
Trichosporeae	Didymocarpinae	*Henckelia floccosa*	EDNA09-00457	G157 Jang	Sri Lanka	–	–	FJ501486	–
Trichosporeae	Didymocarpinae	*Henckelia humboldtiana*	EDNA13-0034476	Ranasinghe, S. SR KNU-1 883.a	Sri Lanka: Corbet's Gap	This study	This study	This study	This study
Trichosporeae	Didymocarpinae	*Henckelia incana*	EDNA08-00884	Vogel, S. SVG	India	Luna Castro, 2016	Luna Castro, 2016	HQ632965	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Henckelia moonii*	EDNA15-0038214	Ranasinghe, S. SR KTG.1048.a	Sri lanka: Kitulgala	This study	This study	This study	This study
Trichosporeae	Didymocarpinae	*Henckelia urticifolia*	EDNA09-02229	NPSW 110	Bhutan	Luna Castro, 2016	Luna Castro, 2016	JF912532	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Henckelia walkerae*	EDNA13-0034292	Ranasinghe, S. SR KIK 961.a	Sri Lanka: Kikiliyamana	This study	This study	This study	This study
Trichosporeae	Didymocarpinae	*Henckelia wijesundarae*	EDNA13-0034344	Ranasinghe, S. SR KNY 922.c	Sri Lanka: Kanneliya	This study	This study	This study	This study
Trichosporeae	Didymocarpinae	*Henckelia zeylanica*	EDNA13-0034340	Ranasinghe, S. SR PWD 1042c	Sri Lanka: Peakwilderness	This study	This study	This study	This study
Trichosporeae	Didymocarpinae	*Hexatheca fulva*	EDNA10-00516	Sang, J. S 99358	Malaysia Borneo	Luna Castro, 2016	Luna Castro, 2016	HQ632969	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Hexatheca sp*	EDNA13-0034238	Puglisi, C. 11	Malaysia Borneo	Luna Castro, 2016	Luna Castro, 2016		Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Liebigia asperifolia*	EDNA13-0031179	Woods, P. 1071 (herb C6570)	Indonesia	Luna Castro, 2016	Luna Castro, 2016	FJ501538	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Liebigia sp.*	EDNA12-0027014	Puglisi, C., M. Hughes, D. Girmansyah, Roki 65	Sumatra	Luna Castro, 2016	Luna Castro, 2016	unpublished	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Loxostigma glabrifolium*	EDNA08-00320	Wei, Y.G. 709	China	Luna Castro, 2016	Luna Castro, 2016	HQ632910	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Loxostigma griffithii*	EDNA10-02823	Kew/Edinburgh Kanchenjunga Expedition (1989) 940	Nepal	Luna Castro, 2016	Luna Castro, 2016	FJ501508	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Lysionotus chingii*	EDNA13-0031181	Wang, Y.Z. S10669		Luna Castro, 2016	Luna Castro, 2016	FJ501498	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Lysionotus petelotii*	EDNA09-00408	Möller, M. MMO 01-100/4	China	This study	This study	FJ501496	This study
Trichosporeae	Didymocarpinae	*Metapetrocosmea peltata*	EDNA08-00304	Wei, Y.G. 07-702	China	Luna Castro, 2016	Luna Castro, 2016	HQ632872	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Microchirita hamosa*	EDNA09-00407	Möller, M. MMO 05-753A	China	Luna Castro, 2016	Luna Castro, 2016	JF912524	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Microchirita viola*	EDNA10-00284	Rafidah, A.R. FRI 64388	Malaysia	Luna Castro, 2016	Luna Castro, 2016	JF912533	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Oreocharis acaulis*	EDNA09-00092	Möller, M. MMO 08-1328	China	Luna Castro, 2016	Luna Castro, 2016	HQ632916	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Oreocharis henryana*	EDNA10-02656	Möller, M. MMO 10-1691	China	Luna Castro, 2016	Luna Castro, 2016	JF697586	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Petrocodon dealbatus*	EDNA13-0031183	Qingjian, X. J-042 (USA294382)	China	Luna Castro, 2016	Luna Castro, 2016	FJ501537	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Petrocodon niveolanosus*	EDNA10-02651	Möller, M. MMO 06-861	China	Luna Castro,	Luna Castro,	JF697588	Luna Castro,

						2016	2016		2016
Trichosporeae	Didymocarpinae	*Petrocosmea kerrii*	EDNA13-0032626	Möller, M. MMO 167	China	Luna Castro, 2016	Luna Castro, 2016	FJ501502	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Petrocosmea sericea*	EDNA09-00409	Gu 991104	China	Luna Castro, 2016	Luna Castro, 2016	FJ501503	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Primulina gemella*	EDNA13-0030199	Averyanov, L. s.n.	Vietnam	Luna Castro, 2016	Luna Castro, 2016	FJ501523	Luna Castro, 201ob
Trichosporeae	Didymocarpinae	*Pseudochirita guangxiensis var glauca*	EDNA08-00315	Möller, M. MMO 05-751	China	Luna Castro, 2016	Luna Castro, 2016	HQ632909	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Ridleyandra petiolata*	EDNA10-00590	Mohd. Hairul, M.A. FRI 60092	Malaysia	Luna Castro, 2016	Luna Castro, 2016	HQ632935	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Ridleyandra porphyrantha*	EDNA09-00452	Weber, A. 870420-2/4	Malaysia	Luna Castro, 2016	Luna Castro, 2016	FJ501520	Luna Castro, 2016
Trichosporeae	Didymocarpinae	*Raphiocarpus sinicus*	–	Q675	–	–	–	–	–
Trichosporeae	Jerdoniinae	*Jerdonia indica*	EDNA09-00456		India	–	–	FJ501429	–
Trichosporeae	Leptoboeinae	*Beccarinda argentea*	EDNA08-00899	Möller, M. MMO 01-58	China	Luna Castro, 2016	Luna Castro, 2016	Unpublished	Luna Castro, 2016
Trichosporeae	Leptoboeinae	*Beccarinda tonkinensis*	EDNA08-00278	Möller, M. MMO 07-1135		Luna Castro, 2016	Luna Castro, 2016	Unpublished	Luna Castro, 2016
Trichosporeae	Leptoboeinae	*Boeica glandulosa*	EDNA13-0034237	Middleton, D. 5569	Thailand	Luna Castro, 2016	Luna Castro, 2016		Luna Castro, 2016
Trichosporeae	Leptoboeinae	*Boeica multinervia*	EDNA09-00447	Wang, Y.Z. 15	China	Luna Castro, 2016	Luna Castro, 2016	HQ632861	Luna Castro, 2016
Trichosporeae	Leptoboeinae	*Leptoboea multiflora*	EDNA13-0031189	Maxwell, J.F. 94-834	Thailand			FJ501442.1	
Trichosporeae	Leptoboeinae	*Platystemma violoides*	EDNA09-00443	Projektteam 197-241	Nepal	Luna Castro, 2016		FJ501443	Luna Castro, 2016
Trichosporeae	Leptoboeinae	*Rhynchotechum discolor*	EDNA09-00438	RBGE-PNHE 1997 SM8	Philippines	Luna Castro, 2016	Luna Castro, 2016	FJ501436	Luna Castro, 2016
Trichosporeae	Leptoboeinae	*Rhynchotechum parviflorum*	EDNA09-00448	M.Mendum, G.Argent, Hendrian 148	Indonesia	Luna Castro, 2016	Luna Castro, 2016	FJ501437	Luna Castro, 2016
Trichosporeae	Leptoboeinae	*Rhynchotechum permolle*	EDNA14-0035181	Ranasinghe S., SR1033c	Sri Lanka: Peakwilderness	This study	This study	This study	This study
Trichosporeae	Litostigminae	*Litostigma coriaceifolium*	EDNA08-00325	Möller, M. MMO 07-1162	China	Luna Castro, 2016	Luna Castro, 2016	Unpublished	Luna Castro, 2016
Trichosporeae	Litostigminae	*Litostigma crystallina*	EDNA09-00405	Shu, Y.M.i 43865	China	Luna Castro, 2016	Luna Castro, 2016	Unpublished	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Boea hygroscopica*	EDNA09-00631	Tan, B., Coveny, R.G. and Brown, E.A. TCB 443	Australia	Luna Castro, 2016	Luna Castro, 2016	FJ501477	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Boea magellanica*	EDNA13-0031191	Lambinon 87/830	Papua New Guinea	Luna Castro, 2016	Luna Castro, 2016	FJ501478	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Damrongia purpureo-lineata*	EDNA09-02231	Middleton, D. et al. 4812	Thailand	Luna Castro, 2016	Luna Castro, 2016	JF912534	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Damrongia sumatrana*	–	–	–	–	–	–	–
Trichosporeae	Loxocarpinae	*Damrongia trisepala*	EDNA12-0028117	Middleton, D. et al. 5626	Thailand	Luna Castro, 2016	Luna Castro, 2016	unpublished	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Dorcoceras hygrometrica*	EDNA11-02037	Ping, Z. ZP 2010-022A	China	Luna Castro,	Luna Castro,	FJ501476	Luna Castro,

						2016	2016		2016
Trichosporeae	Loxocarpinae	*Dorcoceras philippinensis*	EDNA08-01209	Steve Scott 02-142	Indonesia	Luna Castro, 2016	Luna Castro, 2016	HQ632862	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Emarhendia bettiana*	EDNA08-00893	FRI 55716	Malaysia	Luna Castro, 2016	Luna Castro, 2016	HQ632864	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Kaisupeea herbacea*	EDNA11-00672	Middleton, D. et al. 4518	Malaysia	Luna Castro, 2016	Luna Castro, 2016	unpublished	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Kaisupeea orthocarpa*	EDNA11-00673	Middleton, D. et al. 4356	Thailand	Luna Castro, 2016	Luna Castro, 2016	unpublished	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Loxocarpus incanus*	EDNA12-0024116	Middleton, D. 5517	Thailand	Luna Castro, 2016	Luna Castro, 2016	unpublished	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Loxocarpus sekayuensis*	EDNA10-00188	Yao Tze Leong FRI 65445	Malaysia	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Middletonia deformifructa*	EDNA12-0028122	Middleton, D. et al. 5559	Thailand	Luna Castro, 2016	Luna Castro, 2016	unpublished	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Middletonia monticola*	EDNA09-01772	Middleton, D., Triboun, P., Chamchumroon, V., Saengrit, S. and Simma, A. 4363	Thailand		Luna Castro, 2016	unpublished	
Trichosporeae	Loxocarpinae	*Orchadocarpa lilacina*	EDNA08-00890	RK 5410	Malaysia	Luna Castro, 2016	Luna Castro, 2016	HQ632863	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Ornithoboea arachnoidea*	EDNA13-0030201	Cultivated 1997 2903	Thailand	Luna Castro, 2016	Luna Castro, 2016	FJ501461	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Ornithoboea barbanthera*	EDNA09-00110	Middleton, D. et al. 4257	Thailand	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Paraboea clarkei*	EDNA10-02521	Puglisi, C. 10	Malaysia	Luna Castro, 2016	Luna Castro, 2016	unpublished	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Paraboea paramartinii*	EDNA09-01792	Möller, M. MMO 06-852b	China	Luna Castro, 2016	Luna Castro, 2016	unpublished	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Rhabdothamnopsis sinensis*	EDNA09-00432	Möller, M. MMO 08-1059	China	Luna Castro, 2016	Luna Castro, 2016	AJ492302	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Senyumia minutiflora*	EDNA08-00891	Yao Tze Leong FRI 55722	Malaysia	Luna Castro, 2016	Luna Castro, 2016	HQ632865	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Somrania albiflora*	EDNA12-0028112	Williams, K. et al. 2123	Thailand	Luna Castro, 2016	Luna Castro, 2016	unpublished	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Somrania lineata*	EDNA11-00679	Middleton, D. et al. 5434	Thailand	Luna Castro, 2016	Luna Castro, 2016	HQ632866	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Spelaeanthus chinii*	EDNA08-00892	Yao Tze Leong FRI 60012	Malaysia	Luna Castro, 2016	Luna Castro, 2016	FJ501457	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Tribounia grandiflora*	EDNA10-00517	Middleton, D. 5205	Thailand	Luna Castro, 2016	Luna Castro, 2016	JX839281	Luna Castro, 2016
Trichosporeae	Loxocarpinae	*Tribounia venosa*	EDNA09-00108	Middleton, D. et al. 4589	Thailand	Luna Castro, 2016	Luna Castro, 2016	JX839282	Luna Castro, 2016
Trichosporeae	Ramondinae	*Haberlea ferdinandi-coburgii*	EDNA08-01822	Mladenov, P. 0909	Bulgaria		Luna Castro, 2016	unpublished	Luna Castro, 2016
Trichosporeae	Ramondinae	*Haberlea rhodopensis*	EDNA11-0022149	Ioannis Tsiripidis G107	Greece	Luna Castro, 2016	Luna Castro, 2016	AJ492296	Luna Castro, 2016

Trichosporeae	Ramondinae	*Jancaea heldreichii*	EDNA13-0031180	Cultivated 19771605	Greece	Luna Castro, 2016	Luna Castro, 2016	FJ501439	Luna Castro, 2016
Trichosporeae	Ramondinae	*Ramonda myconni*	EDNA12-0029742	Lausanne BotGard 19711477	Spain	Luna Castro, 2016	Luna Castro, 2016	AJ492301	Luna Castro, 2016
Trichosporeae	Ramondinae	*Ramonda nathaliae*	EDNA12-0029744	Cultivated 19784020		Luna Castro, 2016	Luna Castro, 2016	FJ501438	Luna Castro, 2016
Trichosporeae	Streptocarpinae	*Streptocarpus glandulosissimus*	EDNA13-0032523	Hilliard, O. s.n.	South Africa	Luna Castro, 2016	Luna Castro, 2016	unpublished	Luna Castro, 2016
Trichosporeae	Streptocarpinae	*Streptocarpus rexii*	EDNA13-0032524	Jong, K. s.n.	South Africa	Luna Castro, 2016	Luna Castro, 2016	AJ492305	Luna Castro, 2016
Trichosporeae	Tetraphyllinae	*Tetraphyllum roseum*	EDNA09-00453	Kurzweil, H.K. 798	Thailand	Luna Castro, 2016		FJ501434	Luna Castro, 2016
Beslerieae	Anetanthinae	*Anetanthus gracilis*	EDNA13-0032628	Clark, J.L. 9050	Ecuador	Luna Castro, 2016	Luna Castro, 2016	JX195724.1	Luna Castro, 2016
Beslerieae	Besleriinae	*Besleria lutea*	EDNA13-0033699	Clark, J.L. 10541	Cuba	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016
Coronanthereae	Coronantherinae	*Depanthus sp*		V. Savolainen 19211	Lord Howe Islands	JX195987	GQ383545	GQ383584	
Coronanthereae	Coronantherinae	*Rhabdothamnus solandri*	EDNA10-00775	Kealy, J. 1966 0192	New Zealand	Luna Castro, 2016	Luna Castro, 2016	FJ501426	Luna Castro, 2016
Coronanthereae	Coronantherinae	*Coronanthera clarkeana*	EDNA13-0031185	JG137	New Caledonia			GQ497192	
Coronanthereae	Mitrariinae	*Asteranthera ovata*	EDNA13-0032533	Möller, M. MMOG-170	Argentina and Chile	Luna Castro, 2016	Luna Castro, 2016	FJ501427	Luna Castro, 2016
Coronanthereae	Mitrariinae	*Fieldia australis*	EDNA10-03490	1969 6862	Australia	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016
Coronanthereae	Mitrariinae	*Sarmienta scandens*	EDNA13-0030204	Clark, J.L. 10652	Chile	Luna Castro, 2016	Luna Castro, 2016	AJ492309.1	Luna Castro, 2016
Coronanthereae	Negriinae	*Lenbrassia australiana*	EDNA13-0030202	Hind, P.D. 6654	Australia	Luna Castro, 2016	Luna Castro, 2016	AJ492308	Luna Castro, 2016
Coronanthereae	Negriinae	*Negria rhabdothamnoides*	EDNA13-0034326	Clark, J.L. 13740	Lord Howe Island	Luna Castro, 2016	Luna Castro, 2016	GQ383564.1	Luna Castro, 2016
Gesnerieae	Columneinae	*Drymonia dodsonii*	EDNA13-0033114	Clark, J.L. 6205	Ecuador	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016
Gesnerieae	Columneinae	*Rhoogeton cyclophyllus*	EDNA13-0034320	Clarke, H.D. 10350	Guyana	Luna Castro, 2016		Luna Castro, 2016	Luna Castro, 2016
Gesnerieae	Gesneriinae	*Pheidonocarpa corymbosa*	EDNA13-0033134	Clark, J.L. 10556	Cuba	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016
Gesnerieae	Gesneriinae	*Rhytidophyllum exsertum*	EDNA13-0033136	Clark, J.L. 10038	Cuba	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016
Gesnerieae	Gloxiniinae	*Gloxinia perennis*	EDNA13-0033141	Clark, J.L. 6855	Venezuela	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016
Gesnerieae	Gloxiniinae	*Phinaea pulchella*	EDNA13-0033723	Clark, J.L. 10583	Cuba	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016
Gesnerieae	Ligeriinae	*Paliavana tenuiflora*	EDNA13-0033735	s.n.	Brazil	Luna Castro, 2016	Luna Castro, 2016	AJ439807.1	Luna Castro, 2016
Gesnerieae	Ligeriinae	*Sinningia schiffneri*	EDNA10-00777	Mason, L.M. 476	Brazil	Luna Castro, 2016	Luna Castro, 2016	AJ439745.1	Luna Castro, 2016

Gesnerieae	Sphaerorrhizinae	*Sphaerorrhiza sarmentiana*	EDNA13-0033729	Clark, J.L. 8837	Brazil	Luna Castro, 2016	Luna Castro, 2016	GQ383577.1	Luna Castro, 2016
Gesnerieae	Sphaerorrhizinae	*Sphaerorrhiza burchellii*		A.O. Araujo and al. 535	Brazil	JX196123	GQ383575	GQ383624	
Napeantheae	Napeantheae	*Napeanthus bracteatus*	EDNA13-0033153	Clark, J.L. 8744	Panama	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016
Napeantheae	Napeantheae	*Napeanthus robustus*	EDNA13-0033714	Clark, J.L. 5651	Ecuador	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016	Luna Castro, 2016
Titanotricheae	Titanotricheae	*Titanotrichum oldhamii*	EDNA10-00776	Wang, C.N. 1265	China	Luna Castro, 2016	Luna Castro, 2016	AY423129	Luna Castro, 2016
Sanangoideae	Sanangoideae	*Sanango racemosum*	EDNA13-0033725	Clark, J.L. 8863	Ecuador	Luna Castro, 2016	Luna Castro, 2016	JX195799.1	Luna Castro, 2016

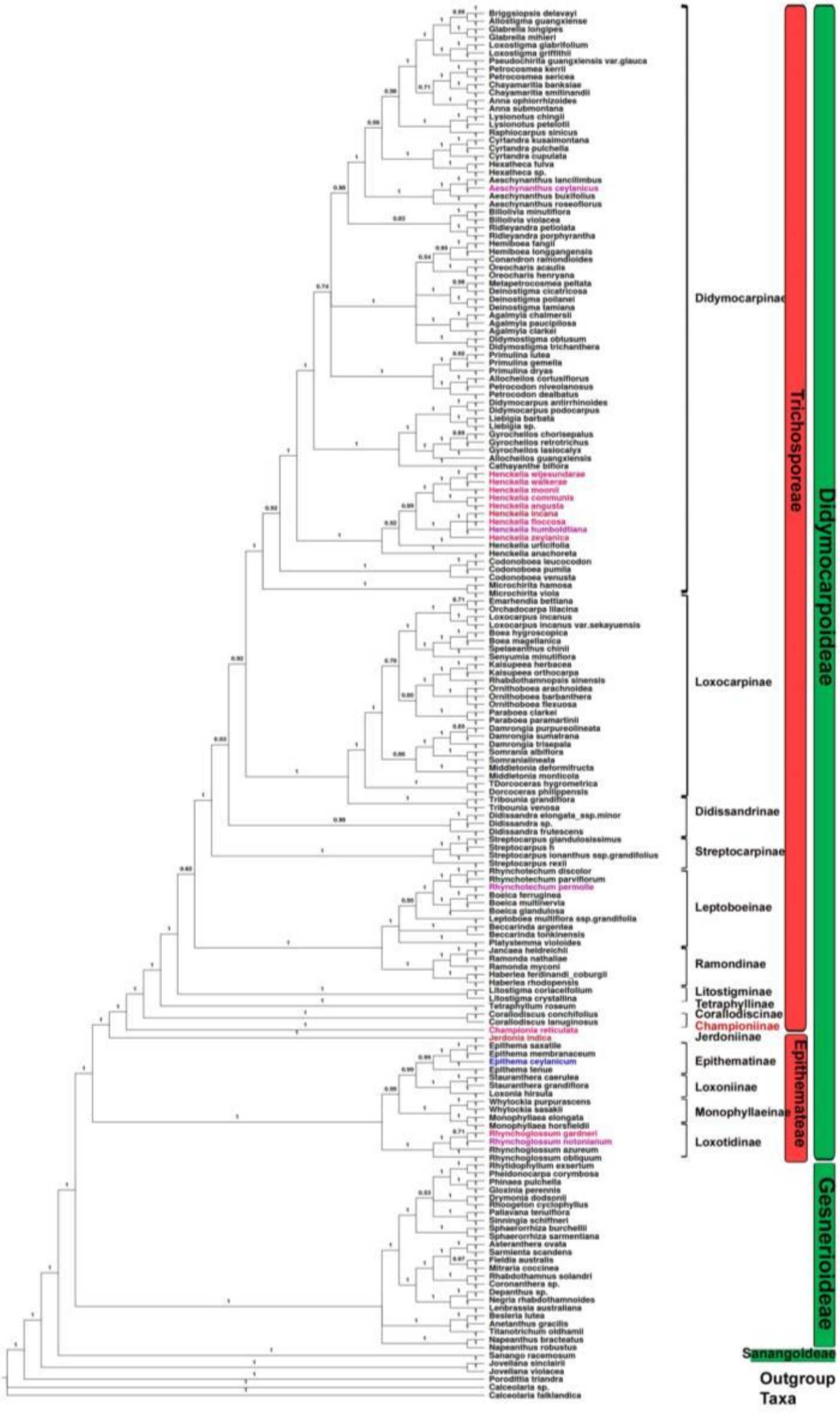
Appendix 3 Stem and crown age estimates for Gesneriaceae clades and outgroups. For comparison, the ages of stems and crowns from Luna-Castro (2016), unpublished, Roalson and Roberts (2016), Petrova et al. (2015), Perret et al. (2013), are provided. Estimation methods are indicated below reference names. Dates are indicated as Mean (Minimum, Maximum). Abbreviations: BEAST, Bayesian Evolutionary Analysis Sampling Trees; PL, penalized likelihood.

Clade	Present Study	Luna-Castro Unpublished 1	Luna-Castro Unpublished 2	Roalson and Roberts 2016	Petrova et al. 2015	Perret et al. 2013
Outgroup Taxa						
Lamiales stem	–	–	–	107.00 (107.00, 107.00)	109.30 (95.1, 181.7)	~97.00
Lamiales crown	–	–	–	101.62 (98.25, 104.00)	96.18 (77.1, 150.0)	~95.00
Calceolariaceae/ Gesneriaceae stem	81.78 (71.05, 96.16)	~62.50	–	79.35 (58.23, 85.62)	79.60 (71.3,110.8)	~74 (71, 80)
Calceolariaceae/ Gesneriaceae crown	26.53 (13.2, 41.54)	~26.00	–	82.31 (76.80, 86.74)	77.79 57.0,106.5)	~71 (68, 73)
Peltanthera	–	~62.5	–	81.90 (76.45, 87.10)	79.60 (71.3,110.8)	~64 (58-72)
Family Gesneriaceae						
Gesneriaceae (+Sanango) Stem	81.78(71.05-96.16)	64.0 (56.3-72.2)	63.6 (55.4-73.0)	76.03 (54.17, 82.14)	77.79 57.0,106.5)	~64 (58, 72)
Gesneriaceae(+Sanango) Crown	74.76 (69.43-80.09)	61.3 (53.5–69.3)	**–**	73.07 (51.93, 81.27)	71.88 (50.5,102.2)	57.50 (45.10, 68.10)
Core Gesneriaceae stem	74.76 (69.43–80.09)	61.3 (69.3–53.5)	61.1 (52.9–70.3)	73.07 (51.93, 81.27)	71.88	57.50 (45.10, 68.10)

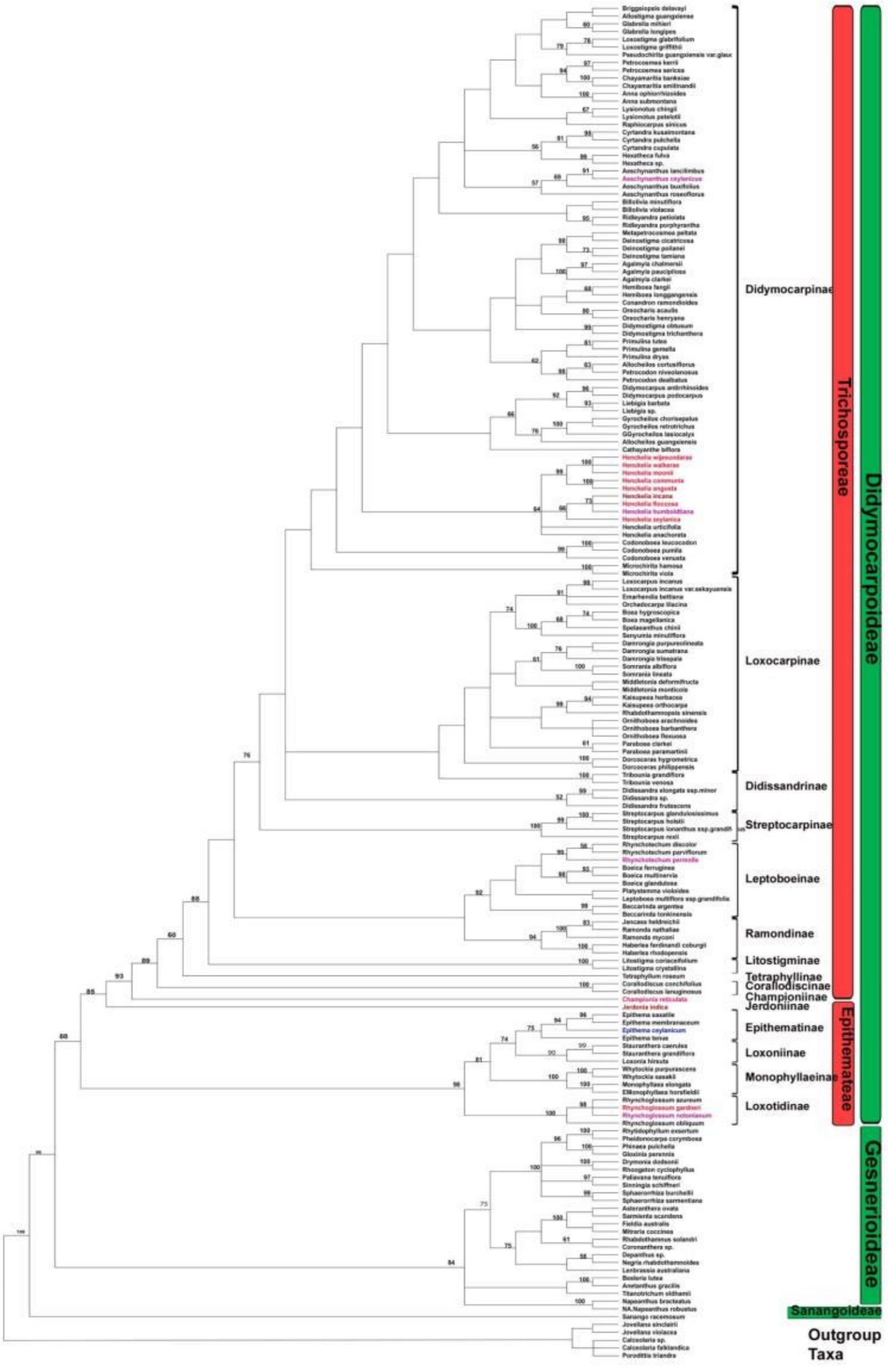
					(50.5,102.2)	
Core Gesneriaceae crown	69.58 (65.09–74.00)	57.7 (50.4–65.7)	57.6 (49.4–66.5)	69.66 (48.20, 77.06)	65.52 (57.0, 106.5)	44.70 (37.10, 60.50)
Subfamily Gesnerioideae						
Gesnerioideae stem	69.58 (65.09–74)	57.7 (50.4–65.7)	57.6 (49.4–66.5)	69.66 (48.20, 77.06)	65.52 (45.4, 94.4)	44.70 (37.10, 60.50)
Gesnerioideae crown	35.31(23.9–47.78)	41.8 (32.1–54.3)	42.4 (32.6–53.5)	46.85 (40.30, 66.20)	41.90 (18.0,78.6)	36.20 (32.30, 48.70)
Titanotricheae stem	na	37.2 (27–50.3)	37.7 (25.6–49.9)	41.71 (21.00, 69.22)	–	~22 (20, 45)
Napeantheae stem	34.66(23.73–47.69)	41.8 (32.1–54.3)	42.4 (32.6–53.5)	41.71 (21.00, 69.22)	35.88 (8.63, 78.47)	~22 (20, 45)
Napeantheae crown	6.2 (1.66–11.6)	6.3 (1.6–14.4)	6.2 (1.6–14.2)	9.99 (7.10, 12.65)	–	~5.5 (2, 15)
Beslerieae stem	31.32 (19.52–45.16)	37.3 (27.0–50.3)	37.7 (25.6–49.9)	43.92 (23.51, 55.25)	35.88 (8.63, 78.47)	~33 (27, 49)
Beslerieae crown)	16.9 (6.58–27.61)	21.8 (15.7–29.7)	–	22.62 (18.24, 28.83	15.96 (1.62, 58.76)	~14 (11, 28)
Anitanthinae stem	na	20.3 (14.2–27.8)	18.8 (12.4–26.3)	–	–	–
Anitanthinae crown	na	16.7 (10.5–24.0)	–	–	–	–
Coronanthereae stem	29.57(23.73–47.69)	36.2 (27.9–48.2)	37.0 (27.7–46.9)	41.73 (22.11, 66.31)	36.68 (10.71, 52.85)	34.30 (32.30, 48.70)
Coronanthereae crown	16.03 (10.08–22.45)	18.0 (11.7–25.8)	17.7 (11.2–25.0)	23.25 (19.33, 40.32)	23.18 (4.3, 37.27)	9.50 (7.60, 32.20)
Coronantherinae stem	14.49 (8.96–20.72)	15.7 (10.2–22.9)	15.4 (7.6–19.3)	–	–	–
Coronantherinae crown	10.56 (5.01–16.44)	13.1 (7.6–19.9)	12.9 (7.6–19.3)	–	–	–
Mitrariinae stem	14.49 (8.96–20.72)	15.7 (10.2–22.9)	15.4 (10.2–22.1)	–	–	–
Mitrariinae crown	8.05 (4.25–12.07)	8.5 (4.5–12.3)	8.3 (4.4–12.8)	–	–	–
Negriinae stem	16.03 (10.08–22.45)	18.0 (11.7–25.8)	17.7 (11.2–25.0)	–	–	–
Negriinae crown	11.95 (5.51–18.61)	12.7 (4.7–21.7)	12.7 (4.9–20.9)	–	–	–
Gesnerieae stem	29.57(19.89–40.5)	36.2 (27.9–48.5)	37.0 (46.9–27.7)	41.73 (22.11, 66.31)	36.68 (16.1, 73.02))	34.30 (29.20, 44.30)
Gesnerieae crown	19.13 (13.46–26.51)	26.7 (21.4–32.9)	26.8 (21.3–33.5)	27.31 (25.18, 29.86)	22.53 (2.68, 22.64)	31.70 (24.80, 36.90)
Gesneriinae stem	13.25 (8.24–18.67)	20.9 (16.2–26.2)	20.9 (16.4–26.4)	20.45 (18.79, 23.68)	16.68 (6.25,39.5)	26.10 (17.90, 29.90)
Gesneriinae crown	6.59 (2.48–11.33)	8.6 (4.6–13.7)	8.5 (4.3–13.6)	8.49 (6.64, 10.83)	5.01 (0.2, 31.2)	11.80 (3.50, 20.80)
Gloxiniinae stem	13.25 (8.24–18.67)	20.9 (16.3–26.2)	20.9 (16.4–26.4)	20.45 (18.79, 23.68)	16.68 (6.25,39.5)	26.10 (17.90, 29.90)
Gloxiniinae crown	7.97 (3.87–12.61)	16.9 (13.3–21.3)	16.9 (13.1–21.2)	16.46 (15.33, 17.52)	10.42 (2.99, 28.13)	21.70 (14.80, 25.00)
Sphaerorrhizinae stem	19.15(13.46–25.51)	26.7 (21.4–32.9)	26.8 (21.3–33.5)	25.65 (23.72, 29.13)	–	29.20 (22.80, 33.80)
Sphaerorrhizinae crown	5.75 (1.6–11.12)	6.4 (1.6–15.4)	6.4 (1.5–15.5)	10.22 (8.03, 12.78)	–	8.70 (2.20, 14.40)
Ligeriinae stem	18.27 (12.77–24.4)	25.3 (20.3–31.1)	25.3 (20.2–31.8)	25.65 (23.72, 29.13)	20.73 (9.74, 45.86)	31.70 (24.80, 36.90)
Ligeriinae crown	10.19(3.9–16.52)	14.7 (8.9–21.5)	14.7 (8.7–21.3)	15.17 (13.53, 17.47)	14.94 (3.02, 38.72)	31.70 (24.80, 36.90)
Columneinae stem	17.69 (12.18–23.7)	25.9 (20.9–31.8)	25.9 (20.7–32.3)	26.45 (24.68, 29.48)	22.53 (2.68, 22.64)	29.20 (23.30, 35.10)
Columneinae crown	12.29 (6.42–18.3)	20.9 (16.5–26.0)	20.9 (16.5–26.1)	22.40 (20.29, 25.70)	15.46 (0.62, 21.78)	28.60 (20.90, 31.20)
Subfamily Didymocarpoideae						
Didymocarpoideae stem	69.58 (65.09–74)	57.7 (50.4–65.7)	57.6 (49.4–66.5)	69.66 (48.20, 77.06)	65.52 (45.4, 94.5)	44.70 (37.10, 60.50)
Didymocarpoideae crown	65.43 (60.87–70)	54.9 (47.9–62.8)	–	67.41 (46.61, 75.03)	61.17 (41.6, 92.3)	~42 (28, 54)
Epithemateae stem	65.43 (60.87–70)	54.9 (47.9–62.8)	54.6 (46.8–63.3)	67.41 (46.61, 75.03)	61.17 (41.6, 92.3)	–
Epithemateae crown	52.53(43.74–61.31)	46.3 (37.9–55.1)	–	64.73 (48.49, 74.06)	51.63 (28.6, 81.6)	–
Loxotidinae stem	52.53 (33.93–52.79	46.3 (55.1–37.9)	46.1 (37.2–54.9)	–	–	–
Loxotidinae crown	18.86 (10.11–28.32)	15.7 (7.8–26.2)	15.6 (7.6–25.2)	–	–	–
Monophyllaeinae stem	43.28 (33.93–52.79)	39.0 (30.3–48.1)	38.6 (29.2–47.0)	–	–	–
Monophyllaeinae crown	28.98 (19.13–38.74)	27.6 (18.0–37.5)	–	–	–	–
Loxoniinae stem	34.54 (24.69–44.25)	30.8 (21.4–40.1)	30.4 (20.4–40.2)	–	–	–

Loxoniinae crown	27.34 (16.15–38.65)	24.1 (13.4–34.8)	–	–	–	–
Epithematinae stem	34.54 (24.69–44.25)	30.8 (21.4–40.1)	30.4 (20.4–40.2)	–	–	–
Epithematinae crown	21.17 (12.84–30.21)	15.9 (7.4–25.5)	16.0 (7.6–25.8)	–	–	–
Trichosporeae stem	65.43 (60.87–70.0)	54.9 (47.9–62.8)	54.6 (46.8–63.3)	67.41 (46.61, 75.03)	61.17 (41.6, 92.3)	~43 (28, 54)
Trichosporeae crown	61.07(54.66–67.3)	52.6 (45.5–60.3)	–	63.65 (43.62, 72.81)	55.90 (34.3, 89.7)	~23 (8, 32)
Jerdoniinae stem	61.07(54.66–67.3)	52.6 (45.5–60.3)	52.1 (44.5–60.7)	63.65 (43.62, 72.81)	55.90 (34.3, 89.7)	–
'Championiinae'	55.44 (48.23–62.05)	–	–	–	–	–
Corallodiscinae stem	48.89 (41.76–56.04)	46.8 (39.5–54.2)	46.6 (30.6–54.0)	54.08 (31.86, 62.72)	47.59 (28.6, 75.6)	–
Corallodiscinae crown	21.88 (9.53–34.48)	23.8 (10.4–37.6)	–	32.79 (16.89, 47.36)	22.15 (3.5, 60.0)	–
Tetraphyllinae stem	44.55 (37.72–51.5)	43.8 (37.1–51.3)	43.7 (36.8–50.6)	49.36 (29.38, 58.37)	41.74 (24.7, 68.3)	–
Litostigminae stem	37.45 (30.21–44.44)	–	–	–	–	–
Litostigminae crown	10.92 (4.07–19.24)	–	–	–	–	–
Ramondinae stem	33.18 (24.18–41.69)	34.1 (25.4–42.5)	34.0 (26.0–42.0)	41.5 (24.01, 50.34)	32.61 (10.1, 58.2)	–
Ramondinae crown	23.96 (13.74–33.74)	24.7 (15.8–35.3)	–	19.04 (9.25, 26.98)	25.45 (5.1, 55.3)	–
Leptoboeinae stem	33.18 (24.18–41.69)	34.1 (25.3–42.5)	34.0 (26.0–42.0)	31.13 (13.26, 41.86)	32.61 (10.1, 58.2)	–
Leptoboeinae crown	18.51 (11.77–25.64)	22.4 (14.8–31.2)	–	22.50 (9.23, 34.32	21.96 (6.4, 47.5)	–
Didissandrinae stem	34.95 (29.33–40.74)	–	–	–		
Didissandrinae crown	29.49 (16.17–39.42)	–	–	–		
Streptocarpinae stem	33.96 (28.43–39.47)	33.2 (27.0–40.3)	35.9 (30.5–42.1)	40.87 (23.31, 47.93)	33.78 (19.8, 55.6)	
Streptocarpinae crown	18.49 (10.84–26.96)	18.3 (9.2–27.9)	18.0 (9.9–27.7)	30.91 (17.86, 37.15)	17.80 (4.2, 42.9)	
Loxocarpinae stem	33.23 (27.79–38.68)	33.1 (27.8–39.0)	32.9 (27.4–38.8)	40.87 (23.31, 47.93)	31.91 (18.4, 51.6)	
Loxocarpinae crown	31.06 (25.66–36.6)	29.3 (24.2–35.0)	–	36.17 (19.50, 43.01)	25.17 (10.0, 49.8)	
Didymocarpinae stem	33.23 (27.79–38.68)	34.7 (29.4–40.8)	34.5 (29.0–40.1)	43.77 (25.30, 51.90)	30.27 (16.3, 50.9)	
Didymocarpinae crown	29.81 (24.21–35.08)	31.1 (26.0–37.1)	–	41.26 (21.96, 51.00)	31.91 (18.4, 51.6)	

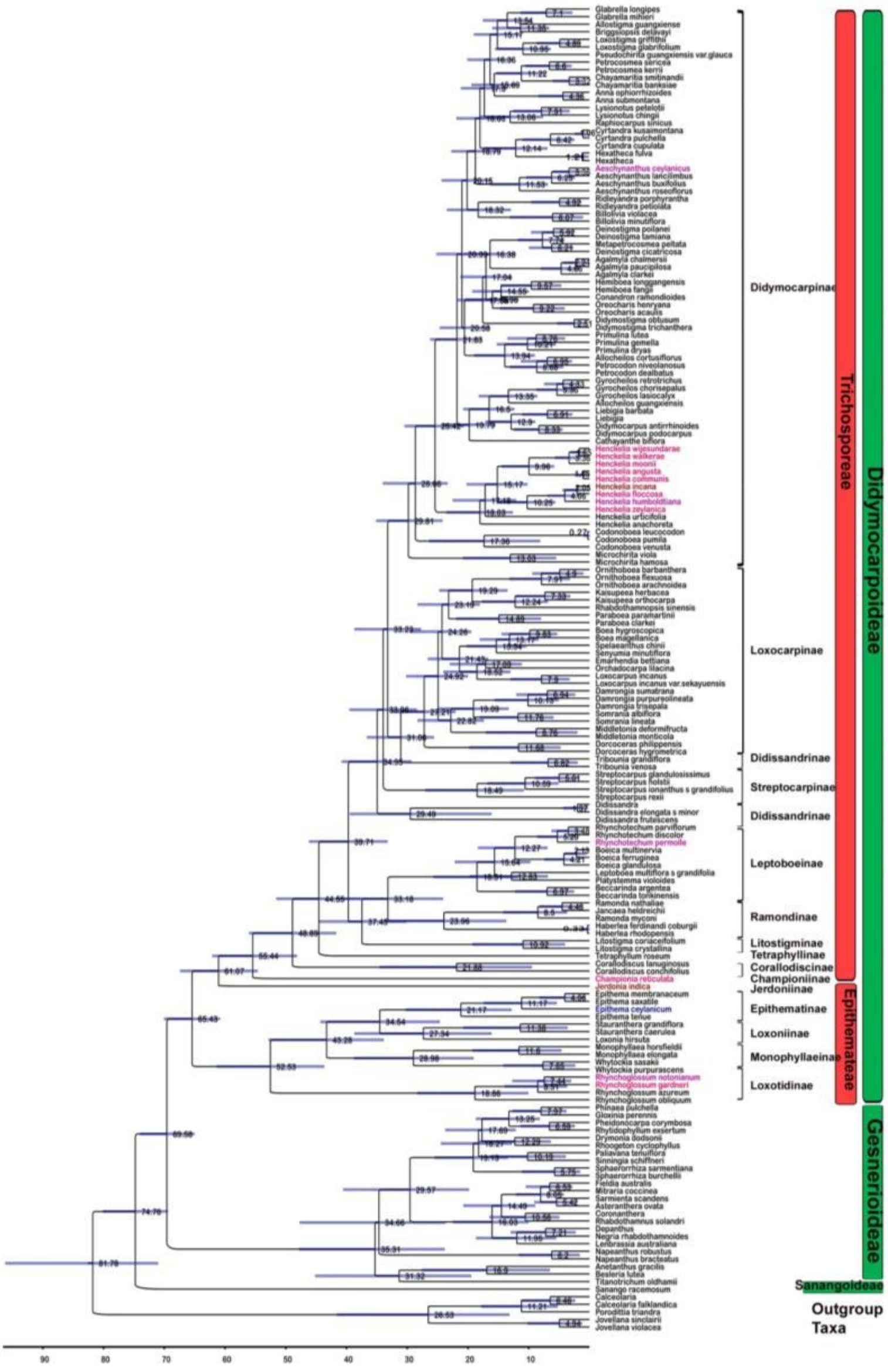
Appendix 4 Cladogram of Bayesian inference analysis with posterior probabilities shown on based on combined matrix of four chloroplast markers, *trn*L-F, *mat*K, *rps*16 and *ndh*F



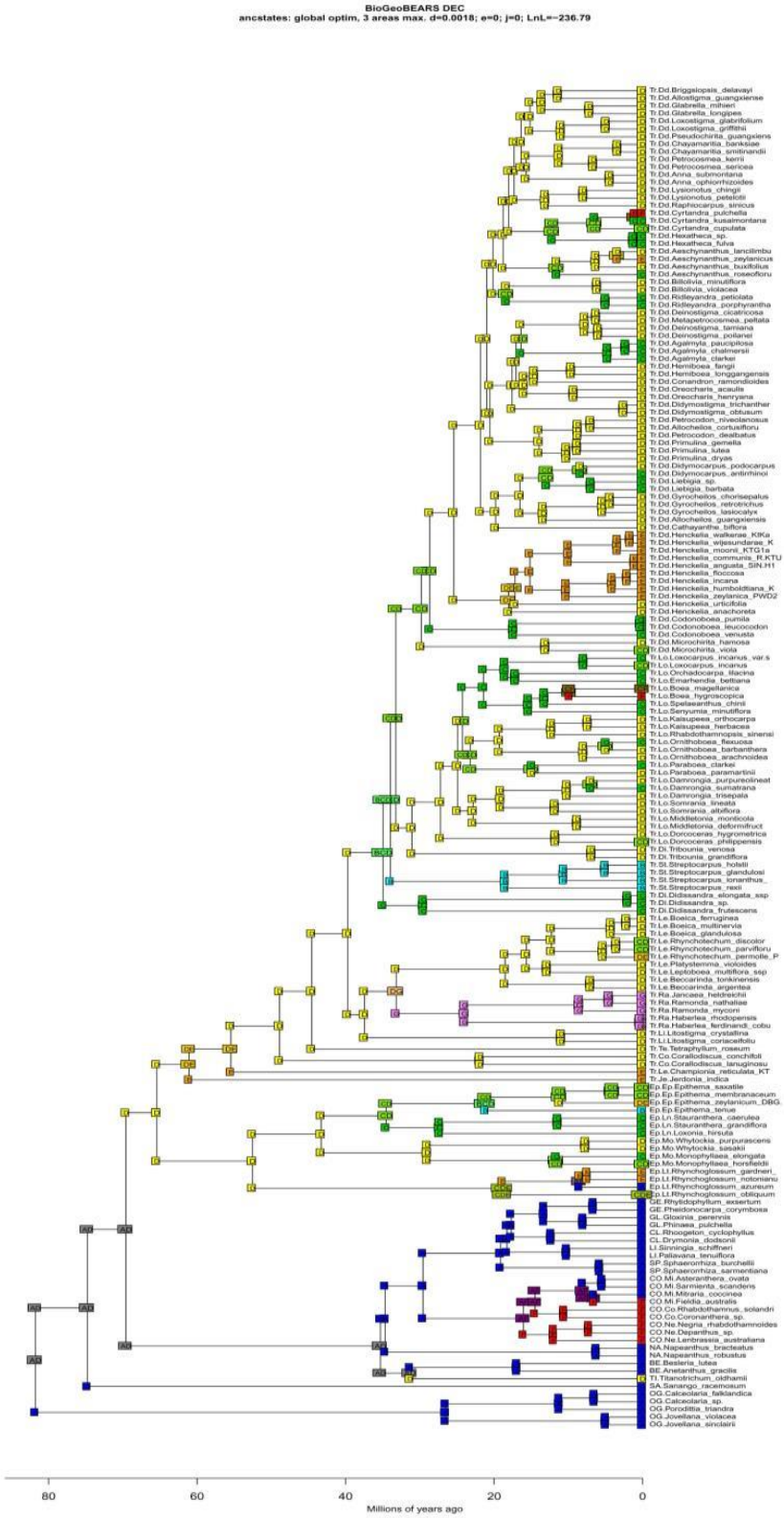
Appendix 5 50% Majority rule consensus tree of the maximum parsimony analysis with bootstrap shown on branches. Analysis based on combined matrix of four chloroplast markers, trnL-F, matK, rps16



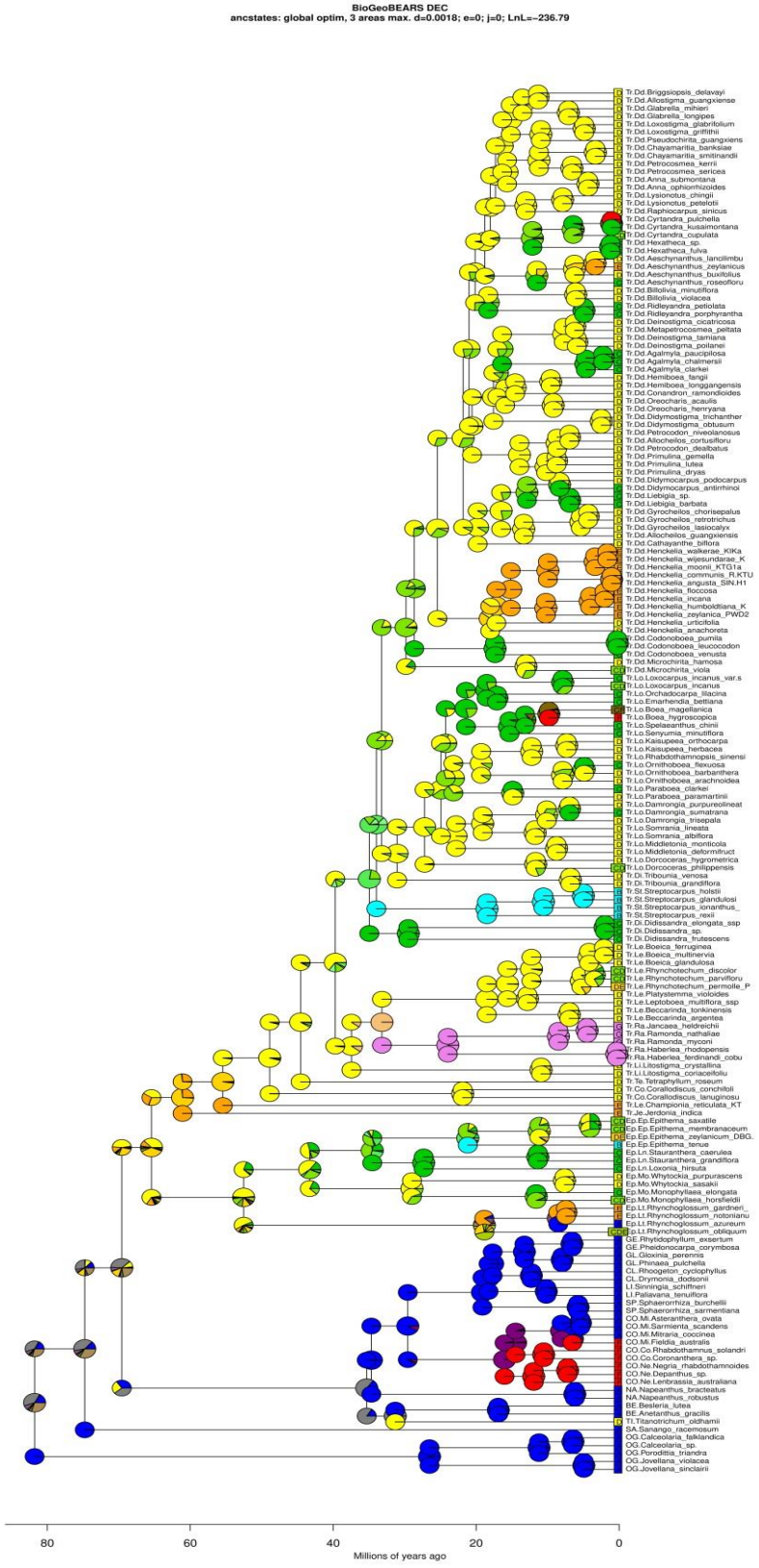
Appendix 6 Maximum clade credibility tree results for family Gesneriaceae with mean age with node bars for confidence intervals. Scale bar below shows age estimates of million of years ago (Mya)



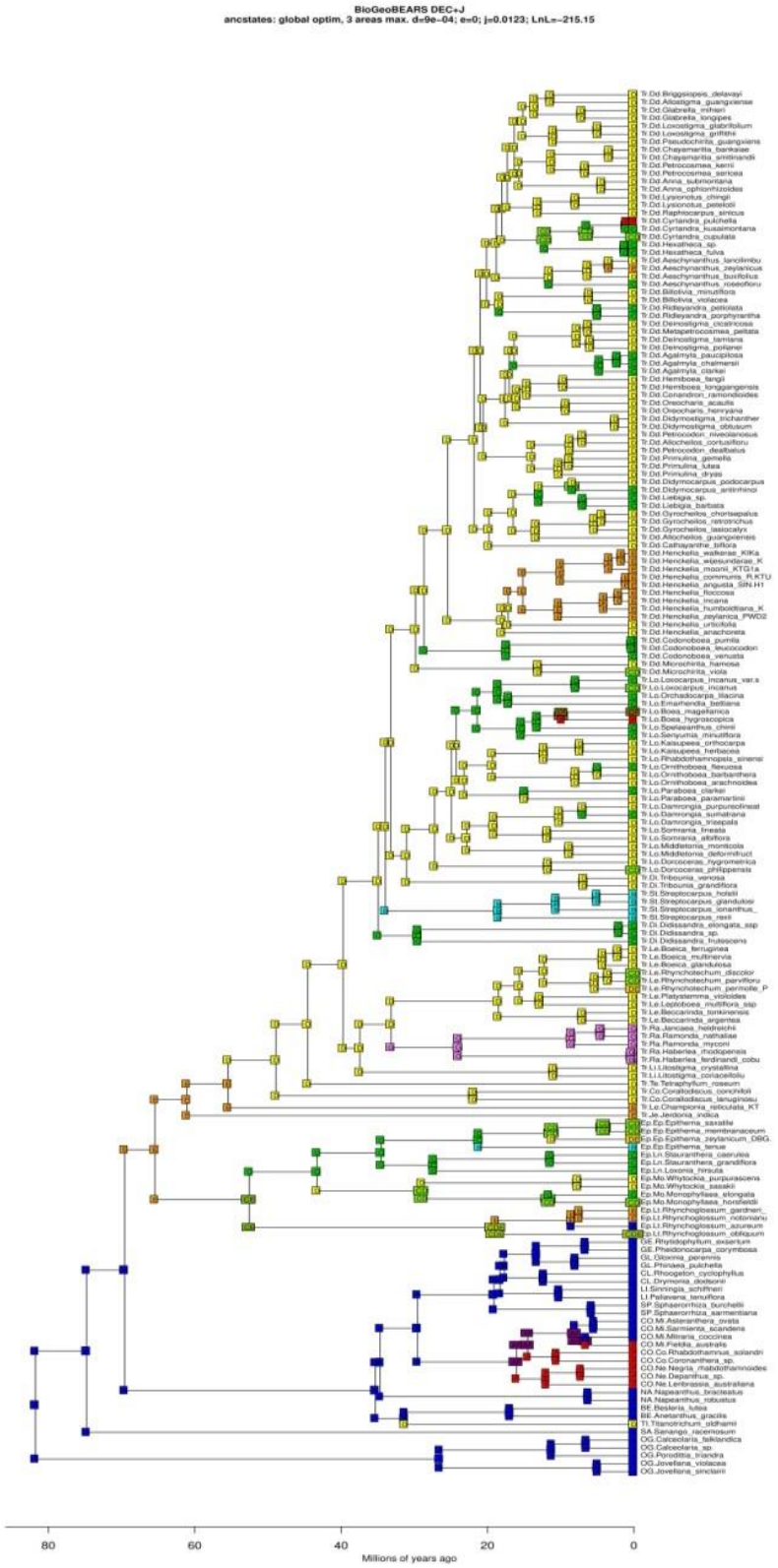
Appendix 7 Results of the BioGeoBears analysis with DEC Model



Appendix 6 BioGeoBears analysis results with DEC Model



Appendix 1 BioGeoBears analysis results with DEC+j Model



Appendix 2 BioGeoBears analysis results with DEC+j Model