

**A taxonomic revision of the genus
Henckelia Spreng. (Gesneriaceae) in
Thailand and surrounding countries**

Thesis submitted for the Degree of Doctor of Philosophy

August 2020

Sukontip Sirimongkol

Department of Botany
School of Natural Sciences
Trinity College Dublin, The University of Dublin

Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work except where duly indicated and clearly acknowledged in the text.

I agree to deposit this thesis in the University's open access institutional repository or allow the Library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement.

Sukontip Sirimongkol

Sukontip Sirimongkol

Acknowledgements

I wish to thank the Royal Thai Government for a scholarship and also would like to acknowledge the support from the Forest Herbarium, the Department of National Parks Wildlife and Plant Conservation that enabled me to study for a PhD in Trinity College Dublin, Ireland. This funding would not have been granted without the help of Dr Somran Suddee, who requested this grant to support the Forest Herbarium (BKF) staff in their study abroad. Thank you. He also supported me in obtaining taxonomic knowledge and in fieldwork in Thailand. I am grateful to The Office of the Civil Service Commission and the Office of Educational Affairs in London for all their help.

I would like to express my sincere thanks to my supervisor Prof Dr John Parnell for super support and guidance of my work and life in Trinity College Dublin. From the beginning of my plan to study abroad, he was kind, supportive and helpful with all facilities and even contacted third parties. I got a warm welcome from the first day in Ireland. He drove me from the airport to the accommodation. My life in Ireland was not easy and there are many things and conditions that made my life rough and difficult. I can stand strong again and again due to his kind support and non-stop solution to my problems. He was patient in correcting my work and never gave up trying to help me improve when I was studying here. My co-supervisor, Prof Dr Trevor Hodgkinson assisted me with DNA extraction and molecular analyses. He also kindly proof-read my work and always welcomed me when I needed help. I would like to thank Dr Chalernpol Suwanphakdee, Dr Phinyarat Kongprakhon, Mrs Chantana Khensiri, Ms Jet Beekwilder, Ms Wanniga Moonsuk and Ms Erika Soldi for helping with molecular work, Prof Dr Fraser Mitchell and Ms Jacqueline Stone for kindly helping with pollen grainswork, Dr Somran Suddee, Dr Joeri Sergej Strijk and Ms Anne Dubéarnès while I am writing the thesis. I would like to thank Dr Sutee Duangjai, Ms Rumrada Meeboonya, Dr Witsanu Saisorn for kindly helping with my molecular data analysis, and also Dr Ponlawat Pattarakulpisutti, Mr Jarupund Pakawat and Dr Alina Premrov for helping with data analysis.

My research has progressed because I got more great support from my colleagues, including the BKF staff, Dr Kongkanda Chayamarit, Dr Rachun Pooma, Dr Voradol Chamchumroon, Ms Nannapat Pattarahirantracin, Mr. Manop Poopath, Mr Piyachart Trisarasri, Mr Preecha Karraket, Mr Thanongsak Jonganurak, Ms Sawita Yooprasert, Mr Sukid Rueangruea, Mrs Orathai Kerdkaew, Mr. Saran Jirakan and Dr Kanchana Pruesapan from the Bangkok Herbarium (BK) for preparation of specimens to loan to Ireland and Mr Phattaravee Prommanut for kindly checking some specimens from Thailand. I also thank Dr Pimwadee Pornponggrueng from Khon Kaen University and

Dr Komsan Rueangritsarakul from the Royal Forest Department for guidance about life in Dublin, and Dr Bhanubong Bongcheewin for guidance before travelling.

My field survey in Thailand, would not have been so smooth and successful without help from Mr. Piroos Boonchum, Mr Akkanitphong Sawatna, Ms Chanchanok Phongsasat, Mr. Phromwarong Khuenwang, Mr. Wattana Saengkam, Mr. Wuttipong Dongkhamfuu, Mr. Krissayam Kongsatree, Mr Chatchai Yotawut, Ms Nipaporn Paisarn, Mr. Ta Sarniyomphong, Mrs Sopa Boonchum, and Ms Wipaphon Baiya. A special thanks to Dr Voradol Chamchumroon, Mr Manop Poopath, Mr Saran Jirankan, Ms Sawita Yooprasert, Dr Rachun Pooma and Ms Nannaphat Pattarahirantracin who accompanied and helped during the field trips.

I would like to thank Dr David J. Middleton from Singapore Botanic Gardens for helpful advice on the Gesneriaceae, Prof Anton Weber from University of Vienna for kind delivery of the Gesneriaceae references, Prof Michael Möller from Royal Botanic Gardens Edinburgh and Dr Carmen Puglisi from Royal Botanic Gardens Kew for great support and advice about the Gesneriaceae work and Dr Avishek Bhattacharjee from Central National Herbarium, Botanical Survey of India for kindly sending images of type specimens. I thank Dr Pramote Triboun for giving guidance and suggestions, Mr Pakkapol Thaowetsuwan from Royal Botanic Garden Edinburgh for kind help with many references from the Edinburgh Herbarium, Mr Jaresak Saewai from Prince of Songkla University, Hatyai Campus for accompanying me while visiting the Muséum National d'Histoire Naturelle and Kew Herbarium, Prof Dr Peter Bruyns for accompanying me while visiting the Kew Herbarium and The Natural History Museum Herbarium and Peter Phillipson from Muséum National d'Histoire Naturelle for kind helping while visiting the Herbarium.

I am very grateful to the Aarhus University Herbarium (AAU), University of Aberdeen (ABD), The Natural History Museum (BM), The Forest Herbarium (BKF), Bangkok Herbarium (BK), Royal Botanic Gardens Edinburgh (E), Royal Botanic Gardens Kew (K), Kunming Institute of Botany (KUN), Naturalis (L), Queen Sirikit Botanic Gardens Herbarium (QBG) and Singapore Botanic Gardens (SING) for the loan of materials. Also, I would like to thank the staff of the Muséum National d'Histoire Naturelle (P), Royal Botanic Gardens Kew (K), The Natural History Museum (BM), Vietnam Academy of Science and Technology (VAST) (HN), VNU University of Science Herbarium (HNU) and Vietnam National Museum of Nature (VNMN). A special thanks to Dr Prachaya Srisanga from QBG for helping during my visit, and Dr Do Von Truong from VNMN for kindly driving and helping me while visiting herbaria in Hanoi, and Peter B. Phillipson (P), Lesley Walsingham (Kew), Elizabeth M. Woodgyner (Kew), Ranee Prakash (BM), Dr Jovita C. Yesilyurt (BM), Dr Tanawat Chaowasku (CMUB).

Thanks to my colleagues Dr Zhao Dongwei, Dr Ponlawat Pattarakulpisutti,

Mr Conor Owens, Dr Anindita Lahiri, Ms Anne Dubéarnès, Dr Anna Kaja Høyer, Dr Eileen Power, Dr Alwynn McGeever, Dr Aoife Delaney, Dr Hannah Hamilton, Ms Jean Williams, Ms Marine Valmier, Mrs Ruchita Ingle and Dr Ana Lopez for their great support. Special thanks to Ms Anne Dubéarnès and Mr Pisut Wisessing for correcting my work and Ms Jean Williams for kindly helping submitted my thesis. I thank the staffs of the Botany Department, TCD, including Prof Dr Fraser Mitchell, Dr Daniel Kelly, Prof Dr Jennifer McElwain, Prof Dr Michael Williams, Dr Stephen Waldren, Prof Dr Nick Gray, Prof Dr Jane Stout, Dr Paul Dowding, Prof Dr Michael Jones, Dr Matthew Saunders, Ms Frances Leogue, Ms Siobhán McNamee, Ms Diane Touzel, Ms Sophia Ni Sheoin, Ms Aisling O'Mahony, Dr Jesko Zimmerman, Ms Jacqueline Stone, Ms Patricia Coughlan, Dr Michelle Murray, Dr Brian Murphy and Ms Mandy Lockhart.

Special thanks to Mrs Marcella Campbell for the first warm home in Ireland and nice food in every meal when I stayed with her. She also helped me with the loan of herbarium specimens and life in Dublin. She gave me the strong advice "look forward to summer!" that made me able to fight through homesickness and cold.

Thanks to Assistant Prof Dr Nattapong Kitsuwon from The University of Electro-Communications, Tokyo, Japan for advice and helping me a lot in the first six months in Dublin, Mr Pisut Wisessing, Dr Borwornsak Rotjanapittayakul, Mr Thantap Pankhao and Dr Wipawee Paulsson for giving moral support when I lived in Ireland. Ms Aroonsri Sarikhan, Mr Padraic Morgan, Mr Brian Conry, Ms Sarah Maloudi and family, Mr John Jameson, Kai and Lee Jameson, Dr Bhanumas Chantarasuwan, Mrs Watchartinnawee Pestel and family, Mrs Warin Harrison for the great support and good hospitality while I live in Ireland. I very much appreciate it. Special thanks to my friends from Thailand for a great support.

Finally, I most gratefully acknowledge my parents, my sisters and my brother for all their support throughout the period of this study.

Summary

The Gesneriaceae is a flowering plant family in the order Lamiales. Its members consist of herbs, shrubs, epiphytes, lianes (e.g. *Asteranthera*), and rarely trees (e.g. *Sanango*, *Solenophora*). This family is distributed in the tropics and subtropics of the Old and New World with approximately 146 genera and ca. 3,400 species.

Henckelia was resurrected from *Didymocarpus* whilst *Didymocarpus* was remodelled (Weber and Burt, 1997). Their classification is based on cytological data and the phytogeography of Old World Gesneriaceae. The delimitation of *Henckelia* has subsequently been changed on the basis of molecular data (Wang et al., 2011; Weber et al., 2011). Results showed that only *Henckelia* Sect. *Henckelia* remained in *Henckelia* (Weber et al., 2011), while the remainder should be assigned to other genera (Middleton et al. 2013). The total number of species in *Henckelia* is 68.

This thesis focused on a taxonomic revision of *Henckelia* in Thailand and surrounding countries (Myanmar, Laos, Cambodia and Vietnam). Twenty-four species are recognized. Identification keys and full descriptions are provided (including illustrations and distribution maps). Five new species were discovered i.e. *Henckelia amplexifolia* Sirim., *H. campanuliflora* Sirim., *H. candida* Sirim., *H. dasycalyx* Sirim. & D.J. Middleton, and *H. nakianensis* Sirim., J. Parn. & Hodk.

The morphological investigation was extended to a numerical taxonomy study. Morphological data were analysed using non-metric multidimensional scaling (NMDS) with Jaccard as the distance measure. The morphometric analysis showed the similarity of the species and divided them into two groups. Group one consists of *Henckelia amplexifolia*, *H. campanuliflora*, *H. candida*, and *H. nakianensis* based on the single leaf, campanulate flower, reniform anthers and peltate or subpeltate stigma. Group two consists of *Henckelia adenocalyx*, *H. anachoreta*, *H. burttii*, *H. calva*, *H. ceratoscyphus*, *H. dasycalyx*, *H. fruticola*, *H. grandifolia*, *H. heterostigma*, *H. insignis*, *H. lacei*, *H. lachenensis*, *H. longipedicellata*, *H. longisepala*, *H. nakianensis*, *H. oblongifolia*, *H. peduncularis*, *H. pumila*, *H. rotundata*, *H. speciosa*, and *H. urticifolia* based on the presence of several leaves, infundibuliform flower, elliptic anthers and bilobed stigma. *Henckelia amplexifolia* is unique in having a stolon and bulbils and producing less fruit. Further study of these features is required.

A molecular phylogenetic study was carried out using DNA sequences obtained for four marker genes (nuclear ribosomal ITS, and the plastid *trnL-F*, *atpB-rbcL*, and *rps16*) for seven species namely *Henckelia amplexifolia*, *H. anachoreta*, *H.*

grandifolia, *H. nakianensis*, *H. pumila*, *H. rotundata* and *H. speciosa*. Only ITS was obtained for one of the species (*H. campanuliflora*). These sequences were combined with existing data from GenBank. A *Henckelia* clade containing groups A-F was well supported (1.00 PP). Thus, *Henckelia sensu* Weber et al. 2011 and Middleton et al. (2013) is shown to be monophyletic. We found that *Henckelia* was sister to a clade of other genera including *Allocheilos*, *Gyrocheilos*, *Didymocarpus*, *Liebigia* and *Cathayanth*e. This finding is congruent with Middleton and Möller (2012). *Codonoboea* is then sister to this *Henckelia* and *Allocheilos*, *Gyrocheilos*, *Didymocarpus*, *Liebigia* and *Cathayanth*e group. The relationships of *Henckelia* species are well resolved and supported and 6 groups can be defined (A to F). Within the core *Henckelia*, two large groups can be defined namely A and B. The largest, Clade B includes *H. dielsii*, *H. amplexifolia*, *H. nakianensis*, *H. grandifolia*, and *H. speciosa* (1.00 PP) with *H. anachoreta* as its sister taxon (1.00 PP). Clade A includes *H. bifolia* and *H. rotundata*. The A and B group are sister to *H. pumila* (C). A group of *Henckelia longisepala* and *H. urticifolia* (clade F) are the most outlying taxa of *Henckelia*, followed by *H. walkerae* (E) and *H. incana* and *H. flocossa* (D; Section *Henckelia*) that diverge successively from the next most basal nodes. Clades A, B, and C of *Henckelia* includes species from Thailand and surrounding countries and Clade F only from surrounding countries including India, Nepal, Bangladesh, Bhutan, Myanmar, China, Vietnam and Laos. Group F is the most outlying clade in terms of its phylogeny in *Henckelia*. The species it contains are geographically separated from Clades D and E which are geographically well separated from all the other groups. The trees separated the *Henckelia* species well and it is clear that the DNA regions will be valuable for future DNA barcoding work on the group especially nrITS and *trnL-F* which are particularly well represented for *Henckelia* species.

This thesis also contains the first study of pollen morphology in this genus. Seventeen pollen samples were investigated. Pollen of two species, *Henckelia anachoreta* and *H. pumila*, were collected from the field and fifteen species, *H. adenocalyx*, *H. bifolia*, *H. communis*, *H. dielsii*, *H. forrestii*, *H. fruticola*, *H. grandifolia*, *H. humboldtiana*, *H. incana*, *H. lachenensis*, *H. mishmiensis*, *H. oblongifolia*, *H. speciosa*, *H. urticifolia*, and *H. walkerae* were collected from herbarium samples. Pollen grain data and a master key are provided. *Henckelia* pollen grains are tricolpate and the shape ranges from subprolate to euprolate. The pollen grains are small to medium in size and the exine sculpturing is reticulate.

Contents

Acknowledgements.....	iii
Summary	vii
List of Tables	x
List of Figures	xi
Appendices	xiv
Chapter 1–General Introduction and Objectives.....	1
1.1 General introduction.....	1
1.2 Taxonomic history of botanical works relevant to <i>Henckelia</i>	13
1.3 Objectives of the study	19
1.4 Area of study.....	19
Chapter 2-General Morphology of <i>Henckelia</i>	20
2.1 General Introduction.....	20
2.2 Glossary.....	20
2.3 General morphology of <i>Henckelia</i>	21
Figure 2.11 Cluster dendrogram of <i>Henckelia</i> based on morphological data and Jaccard distance.Discussion	39
3.1 Introduction	41
3.2 Materials and methods	49
3.3 Results.....	54
Discussion.....	70
Chapter 4-Taxonomic treatment.....	75
4.1 Introduction	75
4.2 Material and methods.....	75
Systematic treatment	77
Chapter 5- Pollen morphology.....	158
5.1 Introduction	158
5.3 Results.....	166
Generic delimitation of <i>Henckelia</i>	177
Infrageneric relationships and classification of <i>Henckelia</i>	178
Morphometric / morphological analysis of <i>Henckelia</i>	178
Taxonomic revision of <i>Henckelia</i> for the Flora of Thailand and surrounding countries	179
Pollen morphology	181
Conclusions	182

List of Tables

Table 1.1 Genera of the Gesneriaceae recorded from the area of study.....	12
Table 1.2 <i>Henckelia</i> species recorded from Thailand and surrounding countries.....	18
Table 2.1 Showing results from flower side view and dissection characters.....	34
Table 2.2 List of morphological characters.....	36
Table 3.1 Marker regions used for published studies on the molecular study of the Gesneriaceae.....	44
Table 3.2 Details of voucher specimens.....	52
Table 5.1 Details of voucher specimens.....	164
Table 5.2 Flower characters and pollen size for the Pearson correlation.....	171
Table 5.3 Pearson product-moment correlation between flower characters and pollen characters.....	174
Table 5.4 Pearson product-moment correlation between flower characters and pollen characters from Thailand and surrounding countries.....	174

List of Figures

Figure 1.1 Diagrams of pair-flowered cymes.....	2
Figure 2.1 Morphological character images.....	23
Figure 2.2 Morphological character images (continued).....	24
Figure 2.3 Morphological character images (continued).....	25
Figure 2.4 Description of flower parts with image of dry flower and fresh flower.....	26
Figure 2.5 Side view of <i>Henckelia</i> flowers.....	27
Figure 2.6 Side view of <i>Henckelia</i> flowers (continued).....	28
Figure 2.7 Dissection of <i>Henckelia</i> flowers with calyx.....	29
Figure 2.8 Dissection of <i>Henckelia</i> flowers with calyx (continued).....	30
Figure 2.9 Dissection of <i>Henckelia</i> flowers with calyx (continued).....	31
Figure 2.10 Morphological data used in morphology analysis.....	37
Figure 2.11 Cluster dendrogram of <i>Henckelia</i> based on morphological data.....	41
Figure 3.1 Formal Gesneriaceae classification diagram based on sequences of several regions including <i>matK</i> , <i>ndhF</i> , ITS, or <i>trnL-F</i>	43
Figure 3.2 Simplified Maximum parsimony majority-rule consensus tree of <i>Henckelia</i> and allies based on combined <i>trnL-F</i> and nrITS sequences.....	47
Figure 3.3 Cladogram of the Bayesian majority-rule consensus tree of <i>Henckelia</i> and allies from combined <i>trnL-F</i> and ITS sequences, depicting the <i>Henckelia</i> clade.....	48
Figure 3.4 Strict consensus parsimony tree of <i>Henckelia</i> and allies based on nrITS sequences.....	49
Figure 3.5 Majority-rule consensus Bayesian inference tree base on the nrITS shown as a cladogram.....	56
Figure 3.6 Majority-rule Bayesian inference consensus tree based on the <i>trnL-F</i> genes region shown as a cladogram.....	58
Figure 3.7 Majority-rule consensus Bayesian inference tree based on the nrITS and <i>trnL-F</i> genes shown as a cladogram.....	60
Figure 3.8 Majority-rule consensus Bayesian inference tree base on the nrITS DNA shown as a cladogram.....	62
Figure 3.9 Majority-rule consensus Bayesian inference tree based on the <i>trnL-F</i> gene region shown as cladogram.....	64
Figure 3.10 Majority-rule consensus Bayesian inference tree based on the ITS and <i>trnL-F</i> genes shown as a cladogram.....	66
Figure 3.11 Majority-rule consensus Bayesian inference tree base on the combined	

chloroplast genes (<i>atpB-rbcL</i> , <i>rps16</i> and <i>trnL-F</i>) shown as a cladogram.....	68
Figure 3.12 Majority-rule consensus Bayesian inference tree based on the nrITS, <i>atpB-rbcL</i> , <i>rps16</i> and <i>trnL-F</i> shown as a cladogram.....	70
Figure 3.13 Cladogram of the majority-rule consensus Bayesian inference tree from the full analysis on combined <i>trnL-F</i> and ITS.....	72
Figure 3.14 Majority-rule consensus Bayesian inference tree of <i>Henckelia</i> and allies based on combined <i>trnL-F</i> and nrITS sequences.....	73
Figure 3.15 Map showing the geographical distribution of taxa in the clades.....	75
Figure 4.1 Survey area map of plant collections.....	77
Figure 4.2 The dendrogram of the identification key.....	82
Figure 4.3 Distribution map of <i>Henckelia adenocalyx</i>	84
Figure 4.4 Distribution map of <i>Henckelia amplexifolia</i>	86
Figure 4.5 Line drawing of <i>Henckelia amplexifolia</i>	87
Figure 4.6 Distribution map of <i>Henckelia anachoreta</i>	93
Figure 4.7 Line drawing of <i>Henckelia anachoreta</i>	94
Figure 4.8 Distribution map of <i>Henckelia burtii</i>	96
Figure 4.9 Distribution map of <i>Henckelia calva</i>	98
Figure 4.10 Distribution map of <i>Henckelia campanuliflora</i>	100
Figure 4.11 Line drawing of <i>Henckelia campanuliflora</i>	101
Figure 4.12 Distribution map of <i>Henckelia candida</i>	103
Figure 4.13 Line drawing of <i>Henckelia candida</i>	104
Figure 4.14 Distribution map of <i>Henckelia ceratoscyphus</i>	106
Figure 4.15 Line drawing of <i>Henckelia ceratoscyphus</i>	107
Figure 4.16 Distribution map of <i>Henckelia dasycalyx</i>	109
Figure 4.17 Distribution map of <i>Henckelia fruticola</i>	111
Figure 4.18 Distribution map of <i>H. grandifolia</i>	115
Figure 4.19 Line drawing of <i>Henckelia grandifolia</i>	116
Figure 4.20 Distribution map of <i>Henckelia heterostigma</i>	118
Figure 4.21 Distribution map of <i>Henckelia insignis</i>	120
Figure 4.22 Distribution map of <i>Henckelia lacei</i>	122
Figure 4.23 Distribution map of <i>Henckelia lachenensis</i>	125
Figure 4.24 Distribution map of <i>Henckelia longipedicellata</i>	127
Figure 4.25 Distribution map of <i>Henckelia longisepala</i>	129
Figure 4.26 Distribution map of <i>Henckelia nakianensis</i>	131
Figure 4.27 Line drawing of <i>Henckelia nakianensis</i>	132
Figure 4.28 Distribution map of <i>Henckelia oblongifolia</i>	135
Figure 4.29 Distribution map of <i>Henckelia peduncularis</i>	137

Figure 4.30 Distribution map of <i>Henckelia pumila</i>	143
Figure 4.31 Line drawing of <i>Henckelia pumila</i>	144
Figure 4.32 Distribution map of <i>Henckelia rotundata</i>	146
Figure 4.33 Line drawing of <i>Henckelia rotundata</i>	147
Figure 4.34 Distribution map of <i>Henckelia speciosa</i>	151
Figure 4.35 Line drawing of <i>Henckelia speciosa</i>	152
Figure 4.36 Distribution of <i>Henckelia urticifolia</i>	155
Figure 4.37 Line drawing of <i>Henckelia urticifolia</i>	156
Figure 5.1 Drawings of glossary words of pollen.....	162
Figure 5.2 Equatorial view of <i>Henckelia adenocalyx</i> and <i>H. anachoreta</i>	165
Figure 5.3 Equatorial view of <i>Henckelia bifolia</i> and <i>H. communis</i>	166
Figure 5.4 Pollen grains of <i>Henckelia communis</i>	166
Figure 5.5 Equatorial view of <i>Henckelia dielsii</i> ; <i>H. forrestii</i> ; and <i>H. fruticola</i>	167
Figure 5.6 Equatorial view of <i>Henckelia grandifolia</i>	167
Figure 5.7 Pollen grains of <i>Henckelia incana</i>	168
Figure 5.8 Equatorial view of <i>Henckelia incana</i> and <i>C. H. lachenensis</i>	168
Figure 5.9 Equatorial view of <i>Henckelia mishmiensis</i> ; C-D. <i>H. oblongifolia</i>	169
Figure 5.10 Equatorial view of <i>Henckelia pumila</i> and <i>H. speciosa</i>	169
Figure 5.11 <i>Henckelia urticifolia</i> pollen grains.....	170
Figure 5.12 <i>Henckelia walkerae</i> pollen grains.....	170
Figure 6.1 Map showing the area of section distribution.....	180

List of Appendices

Appendix 1.1 Publication (Sirimongkol et al., 2019).....	195
Appendix 2.1 Morphological data matrix of <i>Henckelia</i>	216
Appendix 3.1 DNA extraction.....	219
Appendix 3.2 DNA purification by JET Quick Spin Column technique per each sample	220
Appendix 3.3 Gel electrophoresis.....	220
Appendix 3.4 PCR amplification per each sample and sequencing.....	220
Appendix 3.5 PCR amplification programs used for each molecular marker and to clean the PCR product.....	221
Appendix 3.6 Cleaning of PCR products by Exosap protocol.....	221
Appendix 3.7 Checking DNA concentration by Nanodrop Lite machine.....	222
Appendix 3.8 List of PCR primers used in this study.....	222
Appendix 3.9 Preparing PCR and sequencing.....	222
Appendix 3.10 The bootstrap consensus tree shown as a cladogram obtained from comparative sequence analysis of the nrITS sequences data.....	223
Appendix 3.11 The bootstrap consensus tree shown as a cladogram obtained from comparative sequence analysis of the <i>trnL-F</i> sequences data.....	224
Appendix 3.12 The bootstrap consensus tree shown as a cladogram obtained from comparative sequence analysis of the nrITS and <i>trnL-F</i> sequences data.....	225
Appendix 3.13 The bootstrap consensus tree shown as a cladogram obtained from comparative sequence analysis of the nrITS sequences data.....	226
Appendix 3.14 The bootstrap consensus tree shown as a cladogram obtained from comparative sequence analysis of the <i>trnL-F</i> sequences data.....	227
Appendix 3.15 The bootstrap consensus tree shown as a cladogram obtained from comparative sequence analysis of the nrITS and <i>trnL-F</i> sequences data.....	228
Appendix 3.16 The bootstrap consensus tree shown as a cladogram obtained from comparative sequence analysis of the chloroplast genes (<i>atpB-rbcL</i> , <i>rps16</i> and <i>trnL-F</i>)	229
Appendix 3.17 The bootstrap consensus tree shown as a phylogram obtained from comparative sequence analysis of the the nrITS, <i>atpB-rbcL</i> , <i>rps16</i> and <i>trnL-F</i> sequences data.....	230
Appendix 5.1 Pollen size and class.....	231
Appendix 5.2 The pollen grains data of <i>Henckelia</i> from this study.....	232

Appendix 5.3 The pollen grains data from the previous study with *Henckelia* species233

Appendix .5.4 Pollen grains photographs from the previous study.....238

Chapter 1–General Introduction and Objectives

In recent years, the delineation of the Gesneriaceae has been significantly changed by molecular work especially in the genera *Chirita* and *Henckelia*. *Chirita* was separated from other genera i.e. *Henckelia*, *Damrongia*, *Microchirita*, *Liebigia* and *Primulina* (Weber et al. 2011). After that some *Henckelia* members were placed in other genera i.e. *Codonoboea*, *Didissandra*, *Didymocarpus*, *Lindernia* and *Loxocarpus* (Weber et al., 2011; Middleton et al., 2013; Middleton et al., 2015). Therefore, *Henckelia* requires a new classification and key using morphological characters and revision based on molecular work.

1.1 General introduction

The Gesneriaceae is a flowering plant family in the order Lamiales. The Lamiales comprises approximately 1140 genera and 23,600 species (Kubitzki, 2004). They are generally herbaceous (although some are important woody and climbing species), typically characterised by opposite leaves, zygomorphic flowers, up to 4 fertile stamens, a superior ovary composed of two fused carpels and 5 petals which have fused into a tube (Byng, 2014; Kubitzki, 2004; Utteridge & Bramley, 2014) (although variations to any of these occur).

Gesneriaceae members consist of herbs, sometimes epiphytes (*Aeschynanthus*), or shrubs, sometimes lianes (e.g. *Asteranthera*), and rarely trees (e.g. *Sanango*, *Solenophora*). It has approximately 146 genera and ca. 3,400 species (Mabberley, 2008; Weber et al., 2011 and 2013; Möller et al., 2016) distributed mainly in the tropics and subtropics of the Old and New World (Utteridge & Bramley, 2014).

The habits are perennial or rarely annual herbs, subshrubs or shrubs, rarely small tree (*Besleria*, *Negría*). They have a shallow fibrous root system and the tap root is not well developed (Burt, 1954). Many plants in the family grow on cliffs, rocks and some species grow on tree trunks or on shady banks. The stems are present or absent and when present may be erect, decumbent, creeping or pendulous.

The leaf arrangement can be opposite, whorled (whorls of three or four), near distichous, or spiral. In the case of opposite leaves, members of a pair are often of unequal size. A petiole is usually present but a stipule is absent. The lamina is usually simple, the shape ranging from linear through lanceolate, elliptic, ovate, and cordate to almost orbicular. The leaf margin can be entire, crenate, dentate or serrate. The indumentum of the stem and leaf usually consists of glandular and eglandular multicellular hairs.

The axillary inflorescence is of indeterminate thyrses made up of pair-flowered cymes (Burt, 1954; Weber, 2004). The specialized pair-flowered cyme is a characteristic type which can be found in this family and a few genera in Scrophulariaceae (Heywood et al., 2007). The terminal flower (T) of each cyme unit of each cyme unit is associated with an additional flower in frontal position (front-flower, F) (Kubitzki, 2004). The variation of the pair-flowered cyme showed as figure 1.1. The cyme is sometimes reduced to a solitary flower. The calyx is five-merous and the segments are free to the base or fused to a variable extent. The corolla is sympetalous. The corolla's shape is variable in both tube and limb. The very base of the tube is often gibbous and holds nectar. Some taxa have a sac or spur at the base of the corolla such as *Gasteranthus* or *Sinningia*. The corolla's limb is bilabiate with 2 upper and 3 lower lobes, though sometimes a regular limb can also be found.

The corolla lobes are usually rounded or less commonly pointed or fimbriate. The androecium consists of 4 stamens; rarely 5 stamens. Two stamens are fertile and the others are reduced to sterile staminodes or are absent altogether. The anthers are often connate or coherent, placed either side by side or face to face or apically. A style is well developed and the shape is straight or geniculate. The ovary position is superior, semi-inferior or inferior and 1 or 2-loculed. The fruit is a capsule or berry (Weber, 2004).

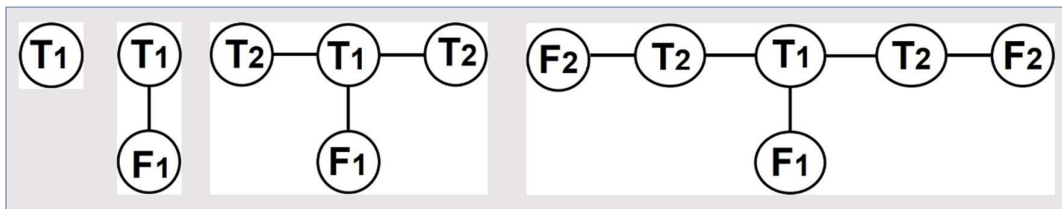


Figure 1.1 Diagrams of pair-flowered cymes arranged from single flower to several flowers. Modified from Kubitzki (2004).

In conclusion, the main morphological characters of Gesneriaceae are opposite leaves, zygomorphic flower, stamens 2(4), dehiscent or indehiscent fruit, numerous and minute seed with no appendage.

The history of Gesneriaceae classification is long. L.C.M. Richard and A.-L. de Jussieu first recognized this family in 1804. They considered grouping some genera to form a new family i.e. *Gesneria* L., *Besleria* L., *Columnea* L., *Achimense* Pers., *Gloxinia* L'Hér. and *Eriphia* P. Browne but they did not give it a name. In 1816, de Candolle used the name 'Gesneriées' without any description; the name being validated by his reference to de Jussieu's paper. De Jussieu also discussed placing other genera, including *Cyrtandra* in this group and was the first to recognize the Gesneriaceae as a

pan-tropical family (Weber et al., 2013).

Dumortier (1829) classified the Gesneriaceae in the Order Gesnerarieae. The important morphological characters of the family in the order Gesnerarieae that Dumortier used were the dehiscent fruit and the corolla with stamens. In terms of Gesneriaceae, Dumortier (1829) split them based on corolla lobe number which he thought more important than stamen number.

Many authors (De Candolle, 1838 and 1845; Bentham & Hooker, 1876; Clarke, 1885; Burt, 1962;) have split the Gesneriaceae into two subfamilies i.e. Gesnerioideae and Cyrtandroideae on the basis of flower and ovary, calyx tube, capsule and seed characters. Burt (1962) indicated that the “Old World” group have seedlings whose cotyledons become unequal in size soon after germination i.e. from subfamily Cyrtandroideae.

The Old World Gesneriaceae (tribe Cyrtandreae), were further split by Clarke (1885) into subtribes based on morphological data as outlined below:

Subtribe 1. Trichosporeae. Fruit a capsule long-linear, loculicidal 2-valved (dehiscing through the locules of a fruit rather than the septa), seed tip with very long hairs i.e. *Aeschynanthus*, *Lysionotus* and *Loxocarpus*.

Subtribe 2. Didymocarpeae. Fruit a capsule loculicidal 2-valved, seeds without hairs i.e. *Didymocarpus*, *Chirita*, *Didisandra*, *Platystemma*, *Championia*, *Boeica*, *Tetraphyllum*, *Trisepalum*, *Phyllobaea*, *Boea*, *Ornithoboea*, *Klugia*, *Rhychoglossum* and *Jerdonia*.

Subtribe 3. Leptoboea. Fruit a capsule septicidal 2-valved (dehiscing through the septa and between the locules), seeds without hairs i.e. *Leptoboea*.

Subtribe 4. Epithemeae. Fruit a capsule circumscissile (dehiscing along a transverse circular line), seeds without hairs i.e. *Epithema*.

Subtribe 5. Eucrytandreae. Fruit a berry, indehiscent, seeds without hairs i.e. *Monophyllaea*, *Cyrtandromoea*, *Stauranthera*, *Isanthera*, *Rhynchotechum* and *Cyrtandra*.

By contrast, the New World Gesneriaceae have seedlings with equal cotyledons i.e. from subfamily Gesnerioideae (Burt, 1962).

1. Subfamily Gesnerioideae

1.1. Tribe Coronanthereae

1.2. Tribe Mitrariaeae

2. Subfamily Cyrtandroideae

2.1 Tribe Cyrtandreae

2.2 Tribe Trichosporeae

2.3 Tribe Klugieae

2.4 Tribe Loxonieae

2.5 Tribe Didymocarpeae

Subsequently in 2004, Weber split the Gesneriaceae based on major systematic and geographical differences into 4 groups (informal level) i.e. Coronantheroid, Gesnerioid, Epithematoid and Didymocarpoid (Weber, 2004). The tribes were delineated in part as follows:

Group 1: Coronantheroid: nectary adnate to the ovary, distributed in E Australia, SW Pacific or temperate S America.

Group 2: Gesnerioid: nectary not adnate to the ovary, seedlings with equal cotyledons, the nectary often consists of separate glands if present; ovary often semi or completely inferior; ovary and fruit globose or ovoid, rarely elongate, fruit a dry or fleshy capsule or a berry, capsule never long-cylindrical and never twisted, distributed in the neotropics.

Group 3: Epithematoid: seedling with unequal cotyledons; nectary, if present, usually cup-shaped; ovary always superior, capsule cylindrical or less commonly globose, sometimes twisted; placenta triangular in cross section; ovary and fruit \pm globose, not twisted, style usually not well set off; seeds spirally striate-reticular; plant usually fleshy-succulent, sometimes with strongly asymmetrical leaves; distributed in the Old World.

Group 4: Didymocarpoid: placenta lamelliform in cross section, recurved to revolute; ovary and fruit usually elongate, rarely ovoid or globose, fruit valves straight or twisted; style usually not clearly defined; plant rarely fleshy-succulent; distributed in the Old World and Palaeotropics.

Weber et al. (2013, 2016) classified the Gesneriaceae based on molecular phylogenetic studies (the exemplar genera in brackets) as follows:

1. Subfamily Sanangoideae A. Weber, J.L. Clarke & Mich. Möller (*Sanango*)

2. Subfamily Gesnerioideae Burnett

2.1 Tribe 1: Titanotricheae Yamaz. ex W.T. Wang (*Titanotrichum*)

2.2 Tribe 2: Napeantheae Wiehler (*Napeanthus*)

2.3 Tribe 3: Beslerieae Bartl.

2.3.1 Subtribe 1: Besleriinae G. Don (*Besleria*, *Cremosperma*, *Gasteranthus*, *Reldia*)

2.3.2 Subtribe 2: Anetanthiniae A. Weber & J.L. Clarke (*Anetanthus*, *Cremospermopsis*, *Resia*, *Shuarua* and *Tylopsacas*)

2.4 Tribe 4: Coronanthereae Fritsch.

2.4.1 Subtribe 1: Coronantherinae Fritsch (*Coronanthera*, *Rhabdothamnus*)

- 2.4.2 Subtribe 2: Mitrariinae Hanst. (*Asteranthera*, *Fieldia*, *Mitraria* and *Sarmienta*)
- 2.4.3 Subtribe 3: Negriinae V.L. Woo, J.F. Smith & Garn. -Jones (*Depanthus*, *Lenbrassia* and *Negria*)
- 2.5 Tribe 5: Gesnerieae Dumort.
 - 2.5.1 Subtribe 1: Gesneriinae Oerst. (*Bellonia*, *Gesneria*, *Pheidonocarpa*, *Rhytidophyllum*)
 - 2.5.2 Subtribe 2: Gloxiniinae G. Don (*Achimenes*, *Amalophyllum*, *Chautemsia*, *Diastema*, *Eucodonia*, *Gloxinella*, *Gloxinia*, *Gloxiniopsis*, *Goyazia*, *Heppiella*, *Kohleria*, *Mandirolla*, *Monopyle*, *Moussonia*, *Niphaea*, *Nomopyle*, *Pearcea*, *Phinaea*, *Seemannia*, *Smithiantha* and *Solenophora*)
 - 2.5.3 Subtribe 3: Columneinae Hanst. (*Alloplectus*, *Alsobia*, *Christopheria*, *Chrysothemis*, *Cobananthus*, *Codonanthe*, *Codonanthopsis*, *Columnea*, *Corytoplectus*, *Crantzia*, *Cremersia*, *Drymonia*, *Episcia*, *Glossoloma*, *Lampadaria*, *Lembocarpus*, *Lesia*, *Nautilocalyx*, *Nematanthus*, *Neomortonia*, *Oestedina*, *Pachycaulos*, *Pagothrya*, *Paradrymonia*, *Rhoogeton* and *Rufodorsia*)
 - 2.5.4 Subtribe 4: Sphaerorrhizinae A. Weber & J.L. Clark (*Sphaerorrhiza*)
 - 2.5.5 Subtribe 5: Ligeriinae Hanst. (*Paliavana*, *Sinningia* and *Vanhouttea*)
- 3. Subfamily Didymocarpoideae Arn.
 - 3.1 Tribe 1: Epithemateae C.B. Clarke
 - 3.1.1 Subtribe 1: Loxotidinae G. Don (*Rhynchoglossum*)
 - 3.1.2 Subtribe 2: Monophyllaeinae A. Weber & Mich. Möller (*Monophylleae* and *Whytokia*)
 - 3.1.3 Subtribe 3: Loxoniinae A. DC. (*Gyrogyne*, *Loxonia* and *Stauranthera*)
 - 3.1.4 Subtribe 4: Epithematinae DC. ex Meisn. (*Epithema*)
 - 3.2 Tribe 2: Trichosporeae Nees
 - 3.2.1 Subtribe 1: Jerdoniinae A. Weber & Mich. Möller (*Jerdonia*)
 - 3.2.2 Subtribe 2: Corallodiscinae A. Weber & Mich. Möller (*Corallodiscus*)
 - 3.2.3 Subtribe 3: Tetrphyllinae A. Weber & Mich. Möller (*Tetrphyllum*)
 - 3.2.4 Subtribe 4: Leptoboestinae C.B. Clarke (*Beccarinda*, *Boeica*, *Championia*, *Leptoboeria*, *Platystemma*, *Rhynchotechum*)

- 3.2.5 Subtribe 5: Ramondinae DC. ex Meisn. (*Haberlea*, *Jancaea* and *Romonda*)
- 3.2.6 Subtribe 6: Litostigminae A. Weber & Mich. Möller (*Litostigma*)
- 3.2.7 Subtribe 7: Streptocarpinae Ivanina (*Acanthonema*, *Colpogyne*, *Hovanella*, *Linnaeopsis*, *Nodonema*, *Saintpaulia*, *Schizoboea*, *Streptocarpus* and *Trachystigma*)
- 3.2.8 Subtribe 8: Didissandrinae A. Weber & Mich. Möller (*Didissandra* and *Tribounia*)
- 3.2.9 Subtribe 9: Loxocarpinae A. DC. (*Damrongia*, *Dorcoceras*, *Emarhendia*, *Kaisupeea*, *Loxocarpus*, *Middletonia*, *Orchadocarpa*, *Ornithoboea*, *Paraboea*, *Rhobdothamnopsis*, *Senyumia*, *Somrania* and *Spelaeanthus*)
- 3.2.10 Subtribe 10: Didymocarpinae Nees (*Aeschynanthus*, *Agalmyla*, *Allocheilos*, *Allostigma*, *Anna*, *Briggsiopsis*, *Cathayanthe*, *Codonoboea*, *Conandron*, *Cyrtandra*, *Deinostigma*, *Didymocarpus*, *Didymostigma*, *Gyrocheilos*, *Hemiboea*, *Henckelia*, *Hexatheca*, *Liebigia*, *Loxostigma*, *Lysionotus*, *Metapetrocosmea*, *Microchirita*, *Oreocharis*, *Petrocodon*, *Petrocosmea*, *Primulina*, *Pseudochirita*, *Raphiocarpus*, *Ridleyandra* and *Sepikea*).

The Gesneriaceae in Thailand and surrounding countries

The following section reviews the accounts published for Thailand and the surrounding countries (Myanmar, Laos, Cambodia and Vietnam), for the Gesneriaceae (numbers of species are in brackets).

Thailand: Craib published Contributions to the Flora of Siam for the University of Aberdeen with the Gesneriaceae comprising 6 genera i.e. *Aeschynanthus* (3), *Lysionotus* (1), *Rhynchoglossum* (1), *Didymocarpus* (3), *Chirita* (3) and *Streptocarpus* (1) (Craib, 1912).

Later, Barnett (1962) published a species list of Thai Gesneriaceae containing about 26 genera and 123 species i.e. *Aeschynanthus* (15), *Boea* (15), *Boeica* (1), *Chirita* (24), *Chlamydoboea* (1), *Corallodiscus* (1), *Cyrtandra* (3), *Cyrtandromoea* (3), *Damrongia* (1), *Dichiloboea* (7), *Didisandra* (1), *Didymocarpus* (21), *Epithema* (3), *Leptoboea* (1), *Loxocarpus* (1), *Lysionotus* (1), *Monophyllea* (2), *Orchadocarpa* (1), *Oreocharis* (2), *Ornithoboea* (5), *Paraboea* (5), *Petrocosmea* (1), *Rhynchoglossum* (1), *Rhynchotechum* (3), *Stauranthera* (1), *Streptocarpus* (1) and *Tetraphyllum* (2).

After that, Burt (2001a) made a checklist of the family and made a number of changes in some genera. The first change was to move some genera to other genera or families i.e. *Chlamydoboea* moved to *Paraboea*, *Dichiloboea* moved to *Trisepalum*, *Loxocarpus* moved to *Henckelia*, whilst *Cyrtandromoea* was transferred to the family Scrophulariaceae. The second change was to state that *Didisandra* and *Orchadocarpa* did not occur in Thailand. Finally, Burt (2001b) recognized a number of new genera of Thai Gesneriaceae i.e. *Alineatus*, *Calcareaoboea*, *Henckelia*, *Ridleyandra* and *Tetraphyllum*. Burt (2001a) also published a new genus, *Kaisupeea* comprising three species i.e. *K. cyanea* B.L. Burt, *K. herbacea* (C.B. Clarke) B.L. Burt and *K. orthocarpa* B.L. Burt.

To sum up, a checklist of the Gesneriaceae from this time would recognise 25 genera and ca. 161 species (species numbers in brackets) i.e. *Aeschynanthus* (10), *Alineatus* (9), *Boea* (2), *Boeica* (5), *Calcareaoboea* (1), *Chirita* (25), *Corallodiscus* (1), *Cyrtandra* (5), *Damrongia* (1), *Didymocarpus* (17), *Epithema* (4), *Henckelia* (13), *Leptoboea* (1), *Lysionotus* (1), *Monophyllea* (2), *Oreocharis* (1), *Ornithoboea* (7), *Paraboea* (30), *Petrocosmea* (4), *Rhynchoglossum* (1), *Rhynchotechum* (3), *Ridleyandra* (3), *Stauranthera* (1), *Streptocarpus* (1), *Tetraphyllum* (2) and *Trisepalum* (11). Overall, Burt's work decreased the number of genera but increased the number of species.

In addition, the Gesneriaceae of Thailand have been subsequently revised as follows. In 2007, Middleton revised the genus *Aeschynanthus* which then comprised 20 species and provided descriptions and a key to species. Next, Middleton and Triboun (2010) described two new species of *Petrocosmea*, i.e. *Petrocosmea bicolor* D.J. Middleton & Triboun and *P. pubescens* D.J. Middleton & Triboun so the number of

Petrocosmea species rose to six. Later, Triboun and Middleton (2010) described a new endemic species of *Damrongia* - *Damrongia cyanantha* Triboun and recognized that this genus contained up to seven species (The Forest Herbarium, 2014).

After that, Middleton and Triboun (2012) described the new genus *Somrania* from Thailand. The genus then contained two new species i.e. *Somrania albiflora* D.J. Middleton and *S. lineata* D.J. Middleton & Triboun. One year later (2013b) they also found another new species i.e. *Somrania flavida* D.J. Middleton & Triboun making a total of three species. In 2013c, Middleton and Triboun found five new species of *Microchirita* i.e. *Microchirita purpurea* Middleton & Triboun, *M. karaketii* Middleton & Triboun, *M. suddeei* Middleton & Triboun, *M. albiflora* Middleton & Triboun & *M. woodii* Middleton & Triboun raising the number of species to about eighteen. Three years later Puglisi et al. (2016a) also published four new species of *Mirochirita* from Thailand i.e. *Microchirita hemrattii* C. Puglisi, *M. huppatatensis* C. Puglisi, *M. lilacina* C. Puglisi and *M. personata* C. Puglisi. This genus thus then contained 22 species.

Triboun (2013) also found a new species of *Paraboea* - *Paraboea middletonii* Triboun. In addition, he also described three new species of *Paraboea* with Middleton in 2015 i.e. *Paraboea chumphonensis* Triboun, *P. puglisiae* Triboun & D.J. Middleton and *P. romklaensis* D.J. Middleton & Triboun. Thus, the number of species in *Paraboea* increased to 76 species (The Forest Herbarium 2014, Triboun & Middleton 2015).

Nangngam (2013) also revised Thai *Didymocarpus* and found 18 species. She published five new species in 2014 i.e. *Didymocarpus brevicalyx* Nangngam & D.J. Middleton, *D. formosus* Nangngam & D.J. Middleton, *D. kasinii* Nangngam & D.J. Middleton, *D. pauciflorus* Nangngam & D.J. Middleton and *D. tribounii* Nangngam & D.J. Middleton making a total of twenty-three species of Thai *Didymocarpus*.

Pattharahirantricin (2014) revised *Rhynchoglossum* during treatment of Thai Gesneriaceae based on morphological characters: three species were recorded in total with one new species, *Rhynchoglossum saccatum* Patthar. At the same time, S.M Scott and Middleton (2014) also published 16 species of *Ornithoboea* and found 10 species to occur in Thailand i.e. *Ornithoboea arachnoidea* (Diels) Craib, *O. barbanthera* B.L. Burtt, *O. flexuosa* (Ridl.) B.L. Burtt, *O. lacei* Craib, *O. maxwellii* S.M. Scott, *O. occulta* B.L. Burtt, *O. parishii* C.B. Clarke, *O. pseudoflexuosa* B.L. Burtt, *O. puglisiae* S.M. Scott and *O. wildeana* Craib.

In addition, Middleton et al. (2015b) found one new species of *Petrocodon* i.e. *Petrocodon flavus* D.J. Middleton & Sangvir. Middleton et al. (2015a) also published a new genus, *Chayamaritia*, which has one species in Thailand, i.e. *Chayamaritia smitinandii* (B.L. Burtt) D.J. Middleton.

Puglisi et al. (2016b) described subtribe Loxocarpinae. She also submerged

Boea from Thailand into *Dorcoceras* i.e. *Boea geoffrayi* Pellegr. became *Dorcoceras geoffrayi* (Pellegr.) C. Puglisi and *Boea wallichii* R. Br. became *Dorcoceras wallichii* (R. Br.) C. Puglisi. Moreover, she also found a new genus '*Middletonia*' in Thailand that contain three species i.e. *Middletonia monticola* (Triboun & Middleton) C. Puglisi, *M. multiflora* (R. Br.) C. Puglisi and *M. multiflora* var. *caulescens* (Z.R. Xu & B.L. Burt) C. Puglisi. In 2018, Middleton et al. also published a new genus *Rachunia* with one species from Thailand, *Rachunia cymbiformis* D.J. Middleton & C. Puglisi.

Recently, Sukontip et al. (2019) discovered three new species of *Henckelia* from Northern Thailand i.e. *Henckelia amplexifolia* Sirim., *H. dasycalyx* Sirim. & D.J. Middleton, and *H. nakianensis* Sirim., J. Parn. & Hodk.

In summary, the family Gesneriaceae in Thailand currently contains 31 genera and 244 species in total according to the previous references and the Forest Herbarium (2014) (number of species in brackets) i.e. *Aeschynanthus* (20), *Boeica* (2), *Chayamaritia* (1), *Codonoboea* (11), *Corallodiscus* (1), *Cyrtandra* (6), *Damrongia* (7), *Didymocarpus* (23), *Dorcoceras* (5), *Epithema* (4), *Henckelia* (8), *Kaisupeea* (3), *Leptoboea* (2), *Loxocarpus* (1), *Lysionotus* (1), *Microchirita* (22), *Middletonia* (3), *Monophyllea* (2), *Oreocharis* (1), *Ornithoboea* (10), *Paraboea* (76), *Petrocodon* (1), *Petrocosmea* (6), *Rachunia* (1), *Rhynchoglossum* (3), *Rhynchotechum* (3), *Ridleyandra* (3), *Somrania* (3), *Stauranthera* (1), *Tetraphyllum* (2) and *Tribounia* (2).

Indo-China

The Gesneriaceae of Indo-China were revised by Pellegrin (1930). Twenty-five genera and 88 species were recognized (number of species in brackets) i.e. *Aeschynanthus* (12), *Anna* (1), *Boea* (14), *Boeica* (3), *Chlamydoeboea* (1), *Conandron* (1), *Damrongia* (2), *Didissandra* (5), *Didymocarpus* (10), *Epithema* (1), *Hemiboea* (3), *Henckelia* (5), *Liebigia* (1), *Loxostigma* (1), *Lysionotus* (4), *Microchirita* (5), *Oreocharis* (2), *Ornithoboea* (2), *Petrocosmea* (1), *Primulina* (6), *Rhynchoglossum* (1), *Rhynchotectum* (2), *Slackia* (1), *Stauranthera* (3) and *Streptocarpus* (1).

Moreover, accounts of the Gesneriaceae have also been published for Vietnam, Laos and Cambodia as follows:

Vietnam: Wang and Li (1992) described a new genus, *Deinostigma* that contained one species, *Deinostigma poilanei* (Pellegrin) W.T. Wang et Y. Li. Twenty years later Möller et al. (2016) discovered and described four new species i.e. *Deinostigma cycnostyla* (B.L. Burt) D.J. Middleton & A.J. Atkins, *D. eberhardtii* (Pellegr.) D.J. Middleton & A.J. Atkins, *D. minutihamata* (D. Wood) D.J. Middleton & A.J. Atkins and *D. tamiana* (B.L. Burt) D.J. Middleton & A.J. Atkins.

Pham (1993, 2003) also issued an illustrated Flora of Vietnam and found that

the Gesneriaceae contained 19 genera and 68 species (number of species in brackets) i.e. *Aeschynanthus* (9), *Anna* (1), *Boeica* (3), *Chirita* (20), *Didissandra* (5), *Didymocarpus* (3), *Episia* (1), *Epithema* (1), *Hemiboea* (2), *Loxostigma* (1), *Lysionotus* (3), *Oreocharis* (1), *Ornithoboea* (1), *Paraboea* (7), *Petrocosmea* (1), *Phylloboea* (1), *Rhynchotechum* (2), *Slackia* (1) and *Stauranthera* (3).

Additionally, Middleton and Ly (2008) published a new species of *Ornithoboea* from Vietnam i.e. *Ornithoboea emarginata* D.J. Middleton & N.S.Ly. Later Do et al. (2011) published a new record in *Loxostigma* i.e. *Loxostigma fimbrisepala* K.Y. Pan.

Middleton et al. (2014a) described *Billolivia*, a new genus from Vietnam with 5 new species i.e. *Billolivia longipetiolata* D.J. Middleton & Luu, *B. minutiflora* D.J. Middleton & H.J. Atkins, *B. poilanei* D.J. Middleton & H.J. Atkins, *B. vietnamensis* D.J. Middleton & Luu and *B. violacea* D.J. Middleton & H.J. Atkins. Middleton et al. (2014b), subsequently published another new species, *Billolivia moelleri* D.J. Middleton. In addition, Vŭ et al. (2015) described the new species, *Billolivia tichii* Luu, Q.D. Nguyen & N.L.Vu. Furthermore, Luu et al. (2015) described a new species, *Billolivia kyi* Luu & G. Tran. Recently, Dat et al. (2016) found a new species, *Billolivia cadamensis* from central Vietnam. In summary, this genus contains nine species.

Middleton (2015) found yet another new Gesneriaceae species, *Gyrocheilos orbiculatum* D.J. Middleton from Vietnam. Van et al. (2016) also published three new records from Vietnam i.e. *Hemiboea gracilis* Franch., *H. ovalifolia* (W.T. Wang) A. Weber & Mich. Möller and *Loxostigma glabrifolium* D. Fang & K.Y. Pan. Recently, Bui et al. (2020) published *Henckelia longisepala* (H.W. Li) D.J. Middleton & Mich. Möller, a new record from Vietnam.

In conclusion, the Gesneriaceae of Vietnam comprise 21 genera and 77 species.

Laos: Newman et al. (2007) found that the Gesneriaceae of Laos comprise 13 genera and 18 species (number of species in brackets) i.e. *Aeschynanthus* (6), *Baccarinda* (1), *Boea* (1), *Calcareoboea* (1), *Epithema* (1), *Loxostigma* (1), *Lysionotus* (1), *Henckelia* (1), *Microchirita* (1), *Ornithoboea* (1), *Paraboea* (1), *Rhynchoglossum* (1) and *Rhynchotechum* (1). In addition, *Chayamaritia banksiae* D.J. Middleton was recognized in Laos (Middleton et al., 2015).

Cambodia: Leti et al. (2013) recorded only one species of Gesneriaceae from Cambodia i.e. *Microchirita elphinstonia*. That terribly low number arises, almost certainly, due to a lack of botanical exploration and information.

Myanmar: Kress et al. (2003) published a checklist of the Burmese Gesneriaceae that contained 28 genera and 100 species (number of species in brackets) i.e. *Achimense* (2), *Aeschynanthus* (18), *Beccarinda* (1), *Boea* (1), *Boeica* (1), *Briggsia* (3), *Chirita* (2),

Corallodiscus (1), *Didymocarpus* (16), *Episcia* (2), *Epithema* (2), *Gloxinia* (1), *Henckelia* (15), *Kaisupeeae* (1), *Leptoboea* (1), *Loxostigma* (1), *Lysionotus* (5), *Ornithoboea* (2), *Paraboea* (9), *Petrocosmea* (2), *Phylloboea* (1), *Rhynchoglossum* (1), *Rhynchotechum* (4), *Saintpaulia* (1), *Stauranthera* (1), *Streptocarpus* (1) and *Trisepalum* (5).

Sirimongkol et al. (2019) (appendix 1.1) also published two new species of *Henckelia* from Myanmar i.e *Henckelia campanuliflora* Sirim. and *H. candida* Sirim. Both of them are from Shan state. So, the Gesneriaceae from Myanmar contained 28 genera and 102 species.

In conclusion, there are 52 genera of Gesneriaceae from Thailand and surrounding countries. The number of genera is shown in Table 1.1 (each genus with number of species in brackets).

The Gesneriaceae in this area are provided into 3 groups i.e. Indo-Burmese, Indo-Chinese and Malesian floristic element (Van Welzen et al., 2011).

Group 1: Indo-Burmese floristic element comprises of genus *Briggsia*, *Boeica*, *Corallodiscus*, *Dorcoceras*, *Henckelia*, *Kaisupeeae*, *Leptoboea*, *Loxostigma*, *Lysionotus*, *Petrocosmea*, *Phylloboea*, *Rachunia*, *Stauranthera*, *Tetraphyllum* and *Tribounia*.

Group 2: Indo-Chinese floristic element comprises of genus *Anna*, *Becarinda*, *Billoivia*, *Briggsia*, *Calcareaoboea*, *Chayamaritia*, *Damrongia*, *Gyrocheilos*, *Hemiboea*, *Henckelia*, *Lysionotus*, *Middletonia*, *Oreocharis*, *Paraboea*, *Petrocodon*, *Primulina*, *Rhynchoglossum*, *Rhynchotechum* and *Trisepalum*.

Group 3: Malaysian floristic element comprises of genus *Codonoboea*, *Cyrtandra*, *Damrongia*, *Didisandra*, *Loxocarpus*, *Monophyllea*, *Paraboea*, *Ridleyandra*, *Somrania* and *Trisepalum*.

Some genera also found widespread from India, China throughout Malaysia are *Aeschynanthus*, *Boea*, *Didymocarpus*, *Epithema*, *Microchirita*, *Ornithoboea* and *Stauranthera*.

The Gesneriaceae from the New World is *Episcia*, *Gloxinia* and *Streptocarpus*. They are ornamental.

Table 1.1 Genera of the Gesneriaceae recorded from the area of study (number of species in brackets).

Thailand	<i>Aeschynanthus</i> (20), <i>Boeica</i> (2), <i>Chayamaritia</i> (1), <i>Codonoboea</i> (11), <i>Corallodiscus</i> (1), <i>Cyrtandra</i> (6), <i>Damrongia</i> (7), <i>Didymocarpus</i> (23), <i>Dorcocheras</i> (5), <i>Epithema</i> (4), <i>Henckelia</i> (8), <i>Kaisupeea</i> (3), <i>Leptoboea</i> (2), <i>Loxocarpus</i> (1), <i>Lysionotus</i> (1), <i>Microchirita</i> (22), <i>Middletonia</i> (3), <i>Monophyllea</i> (2), <i>Oreocharis</i> (1), <i>Ornithoboea</i> (10), <i>Paraboea</i> (76), <i>Petrocodon</i> (1), <i>Petrocosmea</i> (6), <i>Rachunia</i> (1), <i>Rhynchoglossum</i> (3), <i>Rhynchotechum</i> (3), <i>Ridleyandra</i> (3), <i>Somrania</i> (3), <i>Stauranthera</i> (1), <i>Tetraphyllum</i> (2) and <i>Tribounia</i> (2)
Indo-China	<i>Aeschynanthus</i> (12), <i>Anna</i> (1), <i>Boea</i> (14), <i>Boeica</i> (3), <i>Chlamydoeboea</i> (1), <i>Conandron</i> (1), <i>Damrongia</i> (2), <i>Didissandra</i> (5), <i>Didymocarpus</i> (10), <i>Epithema</i> (1), <i>Hemiboea</i> (3), <i>Henckelia</i> (5), <i>Liebigia</i> (1), <i>Loxostigma</i> (1), <i>Lysionotus</i> (4), <i>Microchirita</i> (5), <i>Oreocharis</i> (2), <i>Ornithoboea</i> (2), <i>Petrocosmea</i> (1), <i>Primulina</i> (6), <i>Rhynchoglossum</i> (1), <i>Rhynchotectrum</i> (2), <i>Slackia</i> (1), <i>Stauranthera</i> (3) and <i>Streptocarpus</i> (1).
Vietnam	<i>Aeschynanthus</i> (9), <i>Anna</i> (1), <i>Billolivia</i> (9), <i>Boeica</i> (3), <i>Chirita</i> (20), <i>Didissandra</i> (5), <i>Didymocarpus</i> (3), <i>Episia</i> (1), <i>Epithema</i> (1), <i>Gyrocheilos</i> (1), <i>Hemiboea</i> (2), <i>Loxostigma</i> (1), <i>Lysionotus</i> (3), <i>Oreocharis</i> (1), <i>Ornithoboea</i> (1), <i>Paraboea</i> (7), <i>Petrocosmea</i> (1), <i>Phylloboea</i> (1), <i>Rhynchotechum</i> (2), <i>Slackia</i> (1) and <i>Stauranthera</i> (3).
Laos	<i>Aeschynanthus</i> (6), <i>Beccarinda</i> (1), <i>Boea</i> (1), <i>Calcareoboea</i> (1), <i>Dienostigma</i> (5), <i>Epithema</i> (1), <i>Loxostigma</i> (1), <i>Lysionotus</i> (1), <i>Henckelia</i> (1), <i>Microchirita</i> (1), <i>Ornithoboea</i> (1), <i>Paraboea</i> (1), <i>Rhynchoglossum</i> (1), <i>Rhynchotecchum</i> (1) and <i>Chayamaritia</i> (1)
Cambodia	<i>Microchirita</i> (1)
Myanmar	<i>Achimense</i> (2), <i>Aeschynanthus</i> (18), <i>Beccarinda</i> (1), <i>Boea</i> (1), <i>Boeica</i> (1), <i>Briggsia</i> (3), <i>Chirita</i> (2), <i>Corallodiscus</i> (1), <i>Didymocarpus</i> (16), <i>Episcia</i> (2), <i>Epithema</i> (2), <i>Gloxinia</i> (1), <i>Henckelia</i> (15), <i>Kaisupeea</i> (1), <i>Leptoboea</i> (1), <i>Loxostigma</i> (1), <i>Lysionotus</i> (5), <i>Ornithoboea</i> (2), <i>Paraboea</i> (9), <i>Petrocosmea</i> (2), <i>Phylloboea</i> (1), <i>Rhynchoglossum</i> (1), <i>Rhynchotechum</i> (4), <i>Saintpaulia</i> (1), <i>Stauranthera</i> (1), <i>Streptocarpus</i> (1) and <i>Trisepalum</i> (5)

1.2 Taxonomic history of botanical works relevant to *Henckelia*

Gesneriaceae was formally published by de Candolle (1816) as Gesneriées but without a description. The family name is based on the genus *Gesneria* which honours Conrad von Gesner. Gesner was a Swiss botanist and zoologist who lived from 1516-1615 (Brummit & Powell, 1992).

Henckelia was described in 1817 by Sprengel. He described the main characters of the genus, in particular of the flower. In the 19th Century the missionary and botanist J.P. Rottlera, who worked in India, sent specimens to M. Vahl, Prof of Botany at Copenhagen. Some of the plant specimens that Rottlera worked on were named by him as *Gratiola montana* Rottler ex D.C., but on receiving them Vahl realized that these specimens were distinct from *Gratiola* so he described them as *Roettlera* Vahl (1804) in honour of "Röttlera" and used the same specific epithet *Rottlera montana*. Later Vahl changed the epithet "*montana*" to "*incana*" because the leaves are covered with a dense white woolly indumentum. The authority for the genus can appear in the literature as Reöttlera (ö=oe) but elsewhere Rottlera's name has always appeared without the umlaut and it seems best to correct it to *Rottlera* (Burt, 1997).

However, Willdenow (1799) had used the name *Rottlera* Willd. for a member of the Euphorbiaceae before Vahl (1804) (*Rottlera* Willd. is now a synonym of *Trewia* L.). To resolve this, Sprengel decided to change the name *Rottlera* Vahl to *Henckelia* Spreng. in 1817 and transferred *Rottlera montana* to *Henckelia incana* (Vahl) Spreng. in 1824. The name *Henckelia* honours L.V.F. Henckel von Donnersmarck (1785-1861) who was a German administrator and amateur botanist. The type species of *Henckelia* is *Henckelia incana* (Vahl) Spreng. (Burt 1997, Sprengel 1824, Weber & Burt 1997).

The genus *Henckelia* was considered as a synonym of *Chirita* and *Didymocarpus* (Clarke, 1883). According to the classification of Gesneriaceae based on morphological characters by Clarke (1885), *Henckelia* was merged into 2 genera i.e. *Didymocarpus* and *Chirita*. *Didymocarpus* was placed within Section *Orthoboea* until 1997 when *Henckelia* was resurrected from *Didymocarpus* whilst *Didymocarpus* was remodelled (Weber & Burt, 1997). They also divided *Henckelia* into five sections.

The classification of the sections was based on morphological characteristics and the distribution of the type species of each section (Weber et al., 1997) i.e.

1. Sect. *Henckelia* A. Weber & B.L. Burt; Type: *Henckelia incana* (Vahl) Spreng.: The species in this group have alternate leaves, small flowers without a nectary, rather short fruit and occur in South India and Sri Lanka.

2. Sect. *Heteroboea* (Benth.) A. Weber & B.L. Burt; Type: *Henckelia crinita* (Jack) Spreng.: The species in this section have alternate leaves, usually tufted on top

of an unbranched stem, axillary single-flowered inflorescences, long fruits and are distributed from Sumatra to New Guinea.

3. Sect. *Loxocarpus* (R. Br.) A. Weber & B.L. Burt; Type *Henckelia browniana* A. Weber: The species in this section have alternate rosulate leaves, usually silver-hairy, pair-flowered cymes, short-tubed to flat-faced corolla, no nectary, a short fruit, usually with a basal hump and occur in West Malesia.

4. Sect. *Didymanthus* (C.B. Clarke) A. Weber & B.L. Burt; Type: *Henckelia serrata* (R. Br.) A. Weber & B.L. Burt: The species in this section have opposite leaves, pair-flowered cymes, the flower form is variable, a cylindrical or cup-shaped nectary is usually present; and are distributed in West Malesia.

5. Sect. *Glossadenia* A. Weber & B.L. Burt; Type *Henckelia flavobrunnea* (Ridl.) A. Weber: The species in this section have alternate distant or tufted leaves; flowers in much condensed cymes, nectary unilateral, forming a ventral blade and are distributed in West Malesia.

Weber et al. (2011) remodelled *Chirita* and placed some members of *Chirita* section *Chirita* into *Henckelia*. Middleton et al. (2013) also examined the name *Henckelia*. The impact of both papers radically changed the circumscription of *Henckelia* with 4 sections being moved to other genera and only Section *Henckelia* remaining. This *Henckelia sensu stricto* has subsequently increased in species number. Krishna and Lakshminarasimhan (2018) discovered *Henckelia pathakii* G. Krishna & Lakshmin from Arunachal Pradesh, India. Later, Sirimongkol et al. (2019) published 5 new species of *Henckelia*, *H. amplexifolia* Sirim., *H. campanuliflora* Sirim., *H. candida* Sirim., *H. dasycalyx* Sirim. & D.J. Middleton and *H. nakianensis* Sirim., J. Parn. & Hodk. Cai et al. (2019) published two new species from China, *Henckelia multinervia* Lei Cai & Z.L. Dao and *H. nanxiheensis* Lei Cai & Z.L. Dao and recently Borah et al. (2019) discovered one new species, *Henckelia collegii-sancti-thomasi* A. Joe & D. Borah & Taram & Sandhya from India.

The *Henckelia* sections that remain recognized are listed below.

1. *Henckelia* Sect. *Henckelia*

–*Didymocarpus* sect. *Orthoboea* Benth., Benth. & Hook. f., Gen. Pl. 2: 1022. 1876.

Type: *Henckelia incana* (Vahl) Spreng.

(leaves alternate, rosulate, flowers small, ventricose, without nectary, fruits rather short; S India, Sri Lanka)

Acaulescent or subacaulescent perennial herbs, scapigerous. Leaves alternate. Flowers small, ventricose, without nectar. Fruit rather short.

Distribution: South India, Sri Lanka.

2. *Henckelia* Sect. *Chirita*

–*Chirita* sect. *Chirita* Wood, Notes Roy Bot. Gard. Edinburgh **33**: 123-205.

–*Roettlera* sect. *Euchirita* (C.B. Clarke) K. Fritsch, Pflanzenfam 148, 1895.

–*Didymocarpus* sect. *Euchirita* (C.B. Clarke) Chun, Sunyatsenia 6: 294. 1946

Type: *Henckelia urticifolia* D. Don

Caulescent or caulescent perennial herbs, often slightly woody. Flowers one to many. Calyx usually tubular. Anthers fuse face to face.

Distribution: Western Himalayas, Eastern & Southern China to Southern India, Sri-Lanka, Myanmar, Laos, Vietnam and Thailand.

In total, *Henckelia* contains 68 species (Borah, 2019; Cai et al., 2019; Janeesha et al., 2015; Kiew, 2009; Krishna & Lakshminarasimhan, 2018; Manudev et al., 2012; Mendum, 2001; Middleton et al., 2013a; Ranasinghe et al., 2016; Sukontip et al., 2019; Weber et al., 2011). The presently recognized *Henckelia* species are listed below:

1. *Henckelia adenocalyx* (Chatterjee) D.J. Middleton & Mich. Möller
2. *H. amplexifolia* Sirim.
3. *H. anachoreta* (Hance) D.J. Middleton & Mich. Möller
4. *H. angusta* (C.B. Clarke) D.J. Middleton & Mich. Möller
5. *H. auriculata* (J.M. Li & S.X. Zhu) D.J. Middleton & Mich. Möller
6. *H. bifolia* (D. Don) A. Dietr.
7. *H. bracteata* Janeesha & Nampy
8. *H. briggsioides* (W.T. Wang) D.J. Middleton & Mich. Möller
9. *H. burttii* D.J. Middleton & Mich. Möller
10. *H. calva* (C.B. Clarke) D.J. Middleton & Mich. Möller
11. *H. collegii-sancti-thomasii* A. Joe & D. Borah & Taram & Sandhya
12. *H. campanuliflora* Sirim.
13. *H. candida* Sirim.
14. *H. ceratoscyphus* (B.L. Burtt) D.J. Middleton & Mich. Möller
15. *H. communis* (Gardner) D.J. Middleton & Mich. Möller
16. *H. dasycalyx* Sirim. & D.J. Middleton
17. *H. dibangensis* (B.L. Burtt, S.K. Srivast. & Mehrotra) D.J. Middleton & Mich. Möller
18. *H. dielsii* (Borza) D.J. Middleton & Mich. Möller
19. *H. dimidiata* (Wall. ex C.B. Clarke) D.J. Middleton & Mich. Möller
20. *H. fasciculiflora* (W.T. Wang) D.J. Middleton & Mich. Möller
21. *H. fischeri* (Gamble) A. Weber & B.L. Burtt

22. *H. floccosa* (Thwaites) A. Weber & B.L. Burt
23. *H. forrestii* (J. Anthony) D.J. Middleton & Mich. Möller
24. *H. fruticola* (H.W. Li) D.J. Middleton & Mich. Möller
25. *H. gambleana* (C.E.C. Fisch.) A. Weber & B.L. Burt
26. *H. grandifolia* A. Dietr.
27. *H. heterostigma* (B.L. Burt) D.J. Middleton & Mich. Möller
28. *H. hookeri* (C.B. Clarke) D.J. Middleton & Mich. Möller
29. *H. humboldtiana* (Gardner) A. Weber & B.L. Burt
30. *H. incana* (Vahl) Spreng.
31. *H. infundibuliformis* (W.T. Wang) D.J. Middleton & Mich. Möller
32. *H. innominata* (B.L. Burt) A. Weber & B.L. Burt
33. *H. insignis* (C.B. Clarke) D.J. Middleton & Mich. Möller
34. *H. lacei* (W.W. Sm.) D.J. Middleton & Mich. Möller
35. *H. lachenensis* (C.B. Clarke) D.J. Middleton & Mich. Möller
36. *H. longipedicellata* (B.L. Burt) D.J. Middleton & Mich. Möller
37. *H. longisepala* (H.W. Li) D.J. Middleton & Mich. Möller
38. *H. lyrata* (Wight) A. Weber & B.L. Burt
39. *H. macrostachya* (E. Barnes) A. Weber & B.L. Burt
40. *H. meeboldii* (W.W. Sm. & Ramaswami) A. Weber & B.L. Burt
41. *H. mishmiensis* (Debb. ex Biswas) D.J. Middleton & Mich. Möller
42. *H. missionis* (Wall. ex R. Br.) A. Weber & B.L. Burt
43. *H. monantha* (W.T. Wang) D.J. Middleton & Mich. Möller
44. *H. monophylla* (C.B. Clarke) D.J. Middleton & Mich. Möller
45. *H. moonii* (Gardner) D.J. Middleton & Mich. Möller
46. *H. multinervia* Lei Cai & Z.L. Dao
47. *H. nakianensis* Sirim., J. Parn. & Hodk.
48. *H. nanxiheensis* Lei Cai & Z.L. Dao
49. *H. oblongifolia* (Roxb.) D.J. Middleton & Mich. Möller
50. *H. ovalifolia* (Wight) A. Weber & B.L. Burt
51. *H. pathakii* G. Krishna & Lakshmin.
52. *H. peduncularis* (B.L. Burt) D.J. Middleton & Mich. Möller
53. *H. pradeepiana* Nampy, Manudev et A. Weber
54. *H. primulacea* (C.B. Clarke) D.J. Middleton & Mich. Möller
55. *H. puerensis* (Y.Y. Qian) D.J. Middleton & Mich. Möller
56. *H. pumila* (D. Don) A. Dietr.
57. *H. pycnantha* (W.T. Wang) D.J. Middleton & Mich. Möller
58. *H. repens* (Bedd.) A. Weber & B.L. Burt

59. *H. rotundata* (Barnett) D.J. Middleton & Mich. Möller
60. *H. shuii* (Z. Yu Li) D.J. Middleton & Mich. Möller
61. *H. sivagirensis* (Rajakumar, Selvak., S. Murug. & Chellap.) E.S.S. Kumar
62. *H. speciosa* (Kurz) D.J. Middleton & Mich. Möller
63. *H. tibetica* (Franch.) D.J. Middleton & Mich. Möller
64. *H. urticifolia* (Buch. - Ham. ex D. Don) A. Dietr
65. *H. walkerae* (Gardner) D.J. Middleton & Mich. Möller
66. *H. wightii* (C.B. Clarke) A. Weber & B.L. Burt
67. *H. wijesundarae* Ranasinghe & Mich. Möller
68. *H. zeylanica* (R. Br.) A. Weber & B.L. Burt

Almost all *Henckelia* literature so far published lacks of a key to species. The first preliminary classification of the genus *Henckelia* by Dietrich (1831) used stem characters for classification e.g. caulescent and acaulescent.

Henckelia from Thailand and surrounding countries (Myanmar, Laos, Cambodia and Vietnam) contained 24 species (Burt, 2011; Middleton et al., 2013; Pellegrin, 1930; Sirimongkol et al., 2019; Weber et al., 2011). They are shown in Table 1.2.

Table 1.2 *Henckelia* species recorded from Thailand and surrounding countries.

No	taxon	Distribution
1	<i>Henckelia adenocalyx</i>	India, Myanmar, China
2	<i>H. amplexifolia</i>	Northern Thailand
3	<i>H. anachoreta</i>	India, China, Myanmar, Thailand (Northern, North-Eastern, Eastern, Central and South-Eastern), Laos, Vietnam
4	<i>H. burtii</i>	India, Myanmar
5	<i>H. calva</i>	India, Bhutan and Myanmar
6	<i>H. campanuliflora</i>	Eastern Myanmar
7	<i>H. candida</i>	Eastern Myanmar
8	<i>H. ceratoscyphus</i>	Vietnam
9	<i>H. dasycalyx</i>	Northern Thailand
10	<i>H. fruticola</i>	China and Northern Vietnam
11	<i>H. grandifolia</i>	India, Bhutan, China, Myanmar and Northern Thailand
12	<i>H. heterostigma</i>	Myanmar
13	<i>H. insignis</i>	Myanmar
14	<i>H. lacei</i>	Myanmar
15	<i>H. lachenensis</i>	India, Bhutan, Myanmar and China
16	<i>H. longipedicellata</i>	Myanmar
17	<i>H. longisepala</i>	China and Laos
18	<i>H. nakianensis</i>	Northern Thailand
19	<i>H. oblongifolia</i>	India, Bangladesh, China and Myanmar
20	<i>H. peduncularis</i>	Northern Myanmar
21	<i>H. pumila</i>	India, Nepal, Bhutan, China, Myanmar, Northern Thailand and Vietnam
22	<i>H. rotundata</i>	Northern Thailand
23	<i>H. speciosa</i>	India, China, Myanmar, Northern Thailand, Laos, Vietnam
24	<i>H. urticifolia</i>	India, Nepal, Bhutan, China and Myanmar

1.3 Objectives of the study

The thesis specifically aimed:

1. To study the taxonomy and morphology of the genus *Henckelia* in Thailand and Indo-China including Myanmar.
2. To enumerate the existing species, establish synonyms, construct identification keys, and record ecological and distributional data for each.
3. To provide an identification key of *Henckelia* from Thailand and surrounding countries.
4. To perform phylogenetic analyses based on molecular DNA sequences (nuclear and chloroplast DNA) to evaluate the taxonomic results.
5. To update the molecular data of genus *Henckelia* from Thailand and surrounding countries for the purpose of DNA barcoding and taxon identification.
6. To clarify the genus concept of *Henckelia* from the relevant genera.
7. To study pollen morphology to determine any new taxonomically informative characters.

1.4 Area of study

The areas of study included Thailand, Laos, Vietnam, Cambodia and Myanmar. The Malay-Peninsula was excluded because some of the previous *Henckelia* species in this area have moved to *Codonoboea*, *Loxocarpus*, etc. (Middleton et al., 2013).

Chapter 2-General Morphology of *Henckelia*

2.1 General Introduction

Henckelia was described by Sprengel (1817) who noted that the main morphological characteristics of the flower were its five petals, the infundibuliform corolla tube with four stamens, two of which are fertile and the others sterile, and the fruit capsule (Sprengel, 1817). The new delineation of the genus based on molecular phylogenetic studies by Weber et al. (2011) gives the morphological characteristics of the genus as annual or perennial herbs, caulescent or acaulescent.

The simple leaves are usually opposite, alternate or whorled, sometimes forming a rosette at the base. The axillary inflorescence is a cyme with bracts. Bracts paired or whorled are free or united. The flowers are infundibuliform or campanulate with 5-merous. Calyx 5-lobed are fused into a tube or basally connate, the segment shapes vary from triangular to narrowly triangular. The corolla has a 3-lobed lower lip and a 2-lobed upper lip. The corolla tube is slightly pouched, rather constricted in the throat. There are 2 fertile stamens with geniculate, straight or curved filaments. The anthers are coherent (face to face). The pistil is stipitate with 2 or 1 carpel. The chiritoid stigma shapes are vary from deeply bilobed, shallowly bilobed or peltate. The straight capsular fruits are splitting along 2 valves or along the dorsal side, orthocarpic or plagiocarpic. Seeds are numerous, minute ellipsoid without appendaged.

2.2 Glossary

Anthers coherent – an anther attached to each other or anthers face to face.

Bract – a modified or specialized leaf in the inflorescence standing below partial peduncle, pedicels or flowers

Bulbil – a small bulb, capable of developing into a new plant.

Caducous calyx – calyx falling off soon after formation

Chiritoid stigma – a two-lipped stigma with the upper lip reduced and the lower lip enlarged or bilobed (Carmen, 2017) (Fig. 2.2F). In this thesis is represent the chiritoid stigma as bilobed, peltate or subpeltate.

Curved filament – filament continuously bending without angles

Cyme – a sympodial inflorescence in which the central flower opens first

Geniculate filament – filaments bent like a knee

Orthocarpic capsule – capsule held in line with pedicel

Pair-flowered cyme – the terminal flower (T) of each cyme unit of each cyme is

associate with an additional flower in frontal position (front-flower, F) (Kubitzki, 2004, page 73)

Persistent calyx – calyxes remain in place

Plagiocarpic capsule – capsule held roughly horizontally.

Rhizomatous – possessing an underground stem.

Rosette – a circle of tightly packed leaves.

Scape – inflorescence-stalk arising from ground.

Scapigerous – bearing a scape, herb with basal rosette and an inflorescence rising from.

Stoloniferous – bearing stolons.

2.3 General morphology of *Henckelia*

The morphological characters of this genus were studied based on field collections and dried herbarium specimens; they were:

1) Underground parts: fibrous root, tuber or storage root and sometimes with rhizome or prop roots (Fig. 2.1 A-D.).

2) Overground parts contain many characters as follows:

–Tuber (bulbil): most distinct in *Henckelia amplexifolia* Sirim. and present overground and underground (Fig. 2.1 E-F.).

–Stolon: several species have this character. In some species, new plants develop on the stolon e.g. *Henckelia burtii* D.J. Middleton & Mich. Möller or *H. lachenensis* (C.B. Clarke) D.J. Middleton & Mich. Möller but in others the stolon does not produce new plants (*H. amplexifolia* Sirim.) (Fig. 2.1 G-H).

–Habit: acaulescent or caulescent herb, rhizomatous, scapigerous, annual or perennial, herbaceous or woody, erect or creeping (Fig. 2.1 I-L.).

–Petiole: sessile or petiolate, glabrous or hairy with or without eglandular or glandular hairs.

–Leaves: opposite, alternate or whorled sometimes clustered at the stem apex, or reduced to just one or two leaves; lamina variable; apex acute, acuminate or round; base oblique, cuneate, cordate subcordate or round; margin entire, serrate or dentate; upper and lower surface glabrous or hairy with or without eglandular or glandular hairs; lateral veins pinnate.

–Petiole: terete glabrous or hairy with or without eglandular or glandular hairs.

–Inflorescence axillary, sometimes terminal, cyme, 1-several flowered.

–Bracts: paired (Fig. 2.2 A) or more than 2, free or connate at base; shape orbicular to linear, narrowly ovate or narrowly triangular; persistent or sometimes early

caducous.

–Calyx: tubular (Fig. 2.2 B) or 5-partite (calyx divided to the base) (Fig. 2.2 D); calyx lobes triangular, narrowly triangular (Fig. 2.2 B-C.), reflexed or not reflexed, sometimes strongly geniculate with a recurved tip (*H. ceratoscyphus* (C.B. Clarke) D.J. Middleton & Mich. Möller) (Fig. 2.2 E); caducous or persistent (*H. oblongifolia* (Roxb.) D.J. Middleton & Mich. Möller); with or without eglandular or glandular hairs (Fig. 2.3 G-I.).

–Corolla tube infundibuliform or campanulate, straight or curved; white, cream, yellow, pink to purple, often with yellow markings in throat; lobes bilabiate with 2-lobed, upper lip and 3-lobed lower lip (Fig. 2.2 F-H.).

–Stamens 2; inserted from the base of the corolla tube or near the middle or in the middle of the corolla tube; filaments geniculate or straight, with or without eglandular or glandular hairs; staminodes 2-3, stout or slender with or without eglandular or glandular hairs; anthers elliptic or orbicular, adaxial surface coherent, glabrous or hairy; disc a simple annular ring or 5-lobed, often very small (Fig. 2.2 I).

–Gynoecium linear or lanceolate, glabrous or hairy; ovary 2-loculed or 1-loculed; chiritoid stigma bilobed, subpeltate or peltate (Fig. 2.2 J-L.).

–Fruit: a capsule splitting into 2-valved or opening only along the dorsal side, plagiocarpic or orthocarpic, calyx persistent (Fig. 2.3 A-C.) or caducous (Fig. 2.3 D), Capsules splitting along 2 valves (Fig. 2.3 D).

–Seeds: several, minute, ellipsoid and rugose, no appendage (Fig. 2.3 E).



Figure 2.1 Morphological character images: A-B. Fibrous roots (A. *H. anachoreta* & B. *H. speciosa*); C. Rhizome (*H. ceratoscyphus*); D. Storage roots (*H. rotundata*); *H. amplexifolia*: E. Stolon with bulbil, F. Bulbil cross section, H. Stolon; G. Rosette leaves (*H. dielsii*). Photographs: Sukontip Sirimongkol.

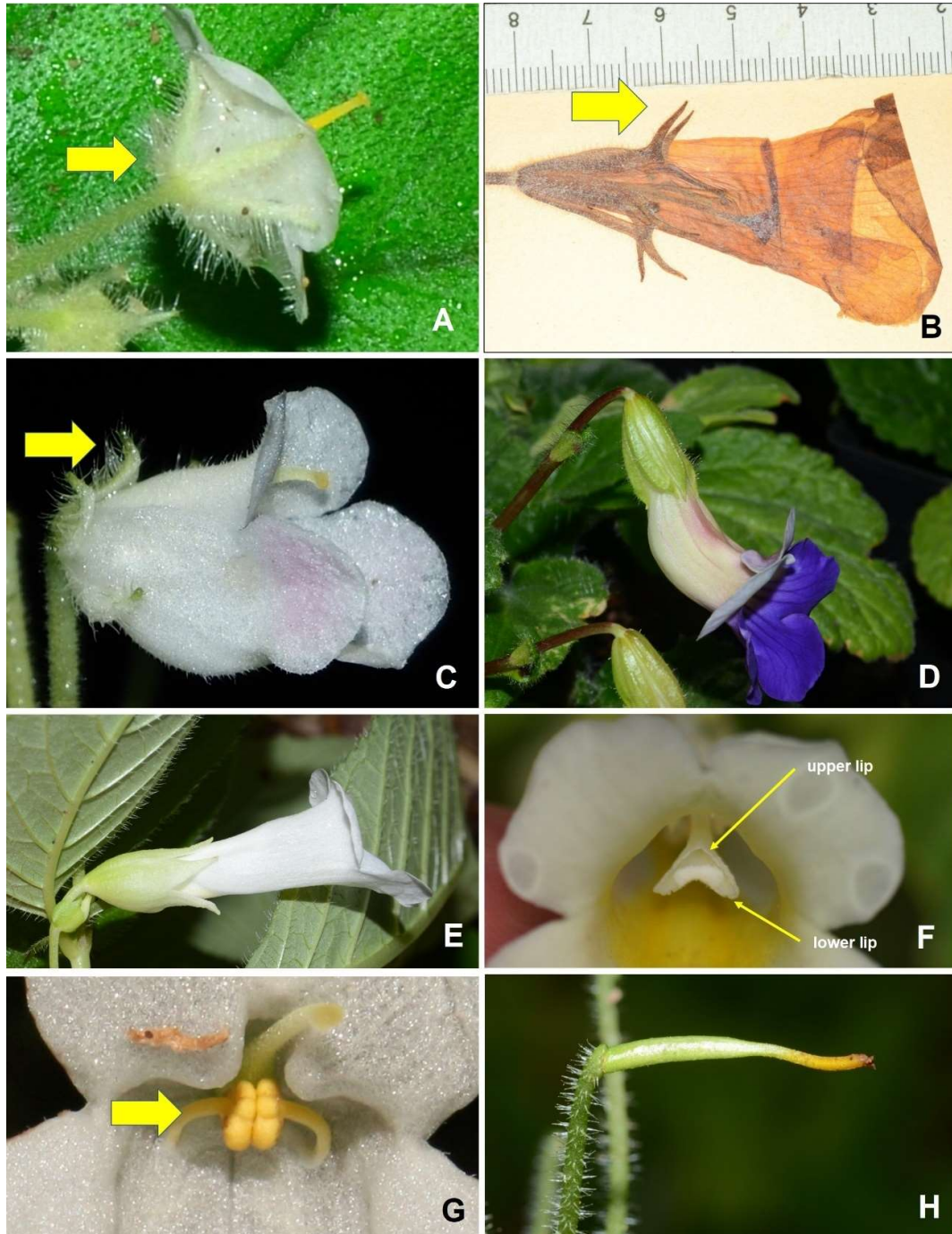


Figure 2.2 Morphological character images (continued): A. Calyx divided at base (*H. amplexifolia*); B. Strongly recurved calyx lobe tip (*H. ceratoscyphus*); C. Recurved calyx tip (*H. amplexifolia*); D. Infundibuliform corolla, slightly curved (*H. dielsii*); E. Infundibuliform corolla, straight corolla (*H. anachoreta*); F. Stigma chiritoid (deep bilobed) (*H. anachoreta*); G. Anthers coherent (*H. candida*); H. Capsule plagiocarpic (*H. amplexifolia*). Photographs: A-E. & H. by Sukontip Sirimongkol; F. by Joeri Strijk & G. by Michele Rodda.

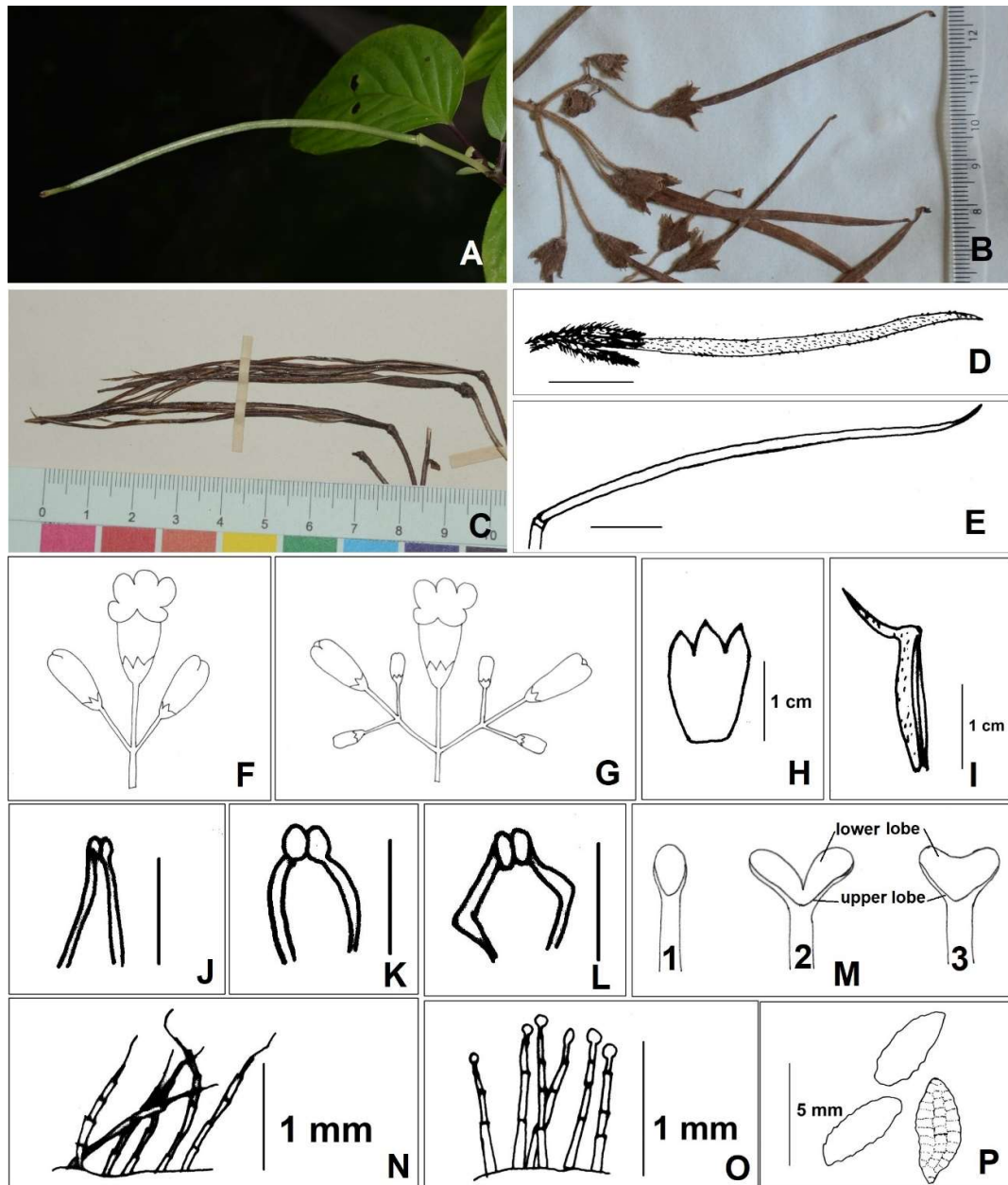


Figure 2.3 Morphological character images (continued): A. Capsule orthocarpic (*H. rotundata*); B. Calyx tube campanulate (*H. oblongifolia*); C. Capsule splitting (*H. speciosa*); D. Persistent calyx (*H. dasycalyx*); E. Caducous calyx (*H. grandifolia*); F. Single cyme; G. Compound cyme; H. Triangular calyx lobe; I. Strongly geniculate calyx lobe; J. Straight filament; K. curved filament; L. Geniculate filament; M. Chiritoid stigmas: M1. Peltate or subpeltate stigma, M2. Deep bilobed stigma, M3. Shallowly bilobed stigma; N. Multicellular eglandular hairs (*H. speciosa*); O. Multicellular glandular hairs (*H. forrestii*). P. Seeds (*H. grandifolia*); J. Simple cyme; K. Compound cyme. Photographs & drawings: Sukontip Sirimongkol.

2.4 Flower dissection drawing

The important part of *Henckelia* for identification is the flower. Every part is useful for identification to species. Starting from the calyx which may or may not be lobed or divided to the base. The corolla tube is infundibuliform and campanulate in shape. The position of stamens and staminodes also vary between each species.

In this section, I provide flower dissection drawings. The floral parts of dry and fresh flowers are described in figure 2.4, flowers dissections and a side view of the flowers of *Henckelia* from Thailand and surrounding countries are shown in the following figures (Fig. 2.5-2.9).

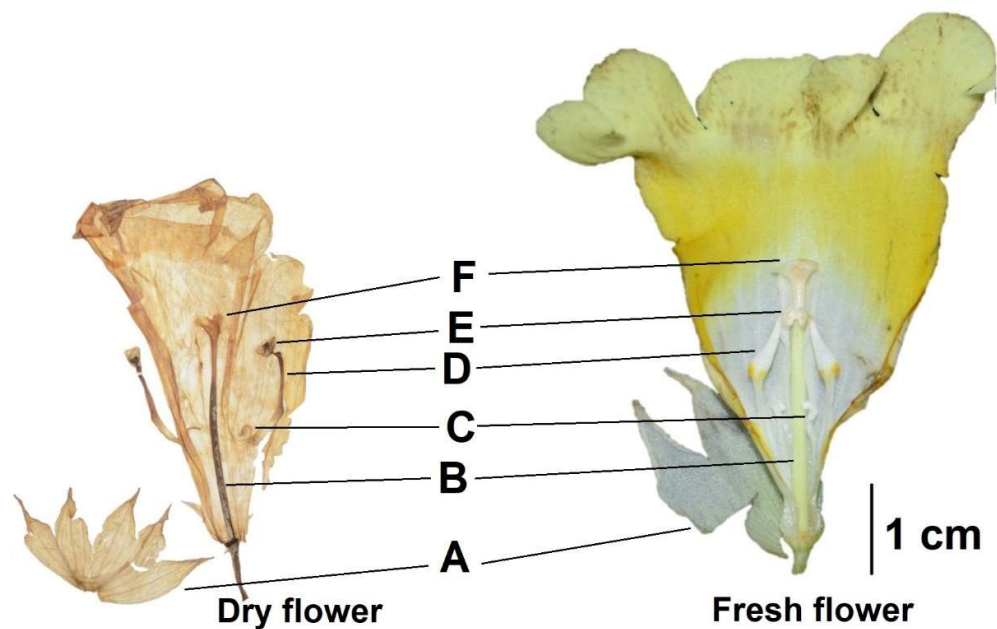


Figure 2.4 Description of flower parts with image of dry and fresh flower (*Henckelia anachoreta*): A. Calyx; B. Gynoecium; C. Staminode; D. Filament; E. Anther; F. Stigma.

Photographs: Sukontip Sirimongkol

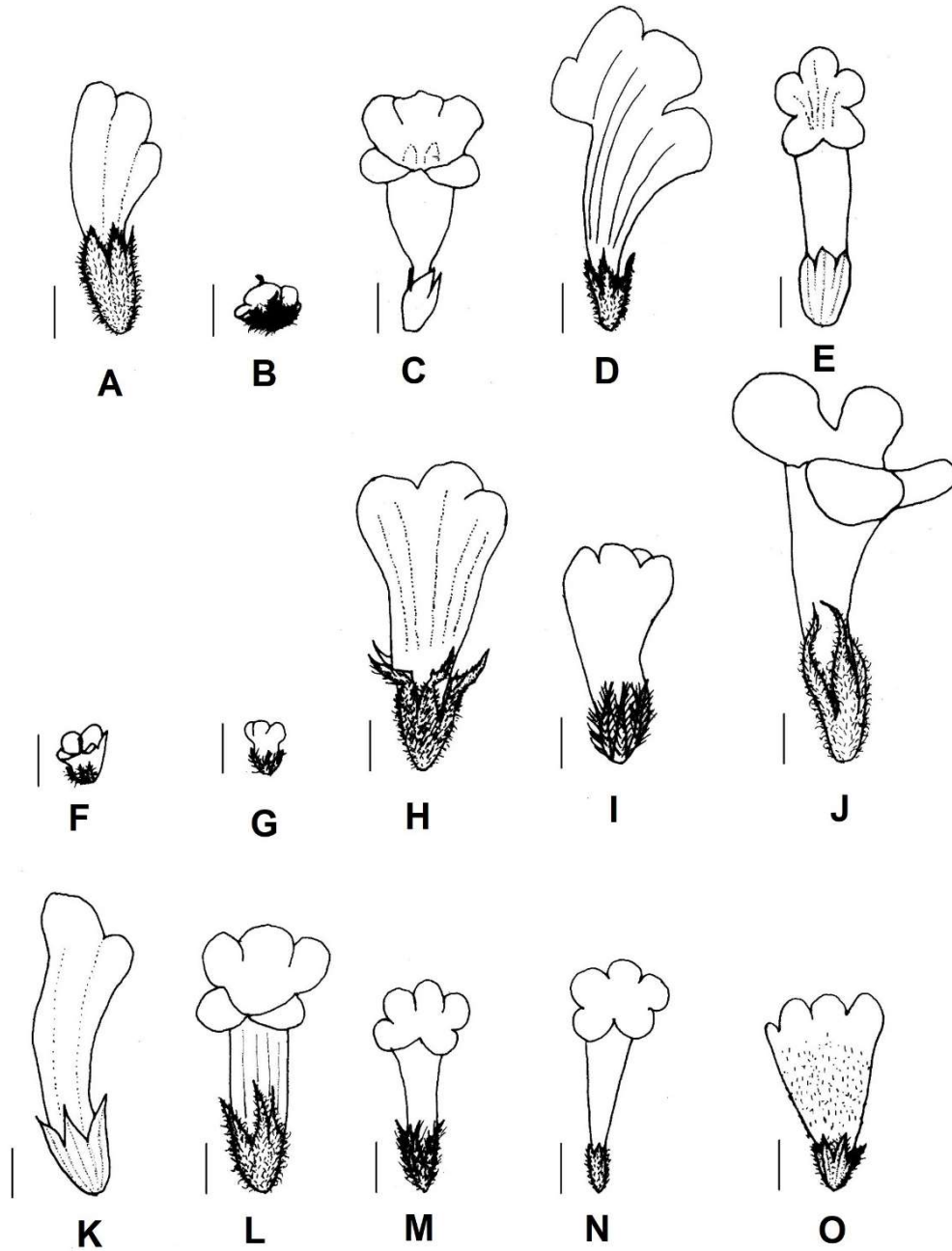


Figure 2.5 Side view of *Henckelia* flowers: A. *H. adenocalyx*; B. *H. amplexifolia*; C. *H. anachoreta*; D. *H. burtii*; E. *H. calva*; F. *H. campanuliflora*; G. *H. candida*; H. *H. ceratoscyphus*; I. *H. dasycalyx*; J. *H. fruticola*; K. *H. grandifolia*; L. *H. heterostigma*; M. *H. insignis*; N. *H. lacei*; O. *H. lachenensis*.

Drawings: Sukontip Sirimongkol. Scale bar = 1 cm.

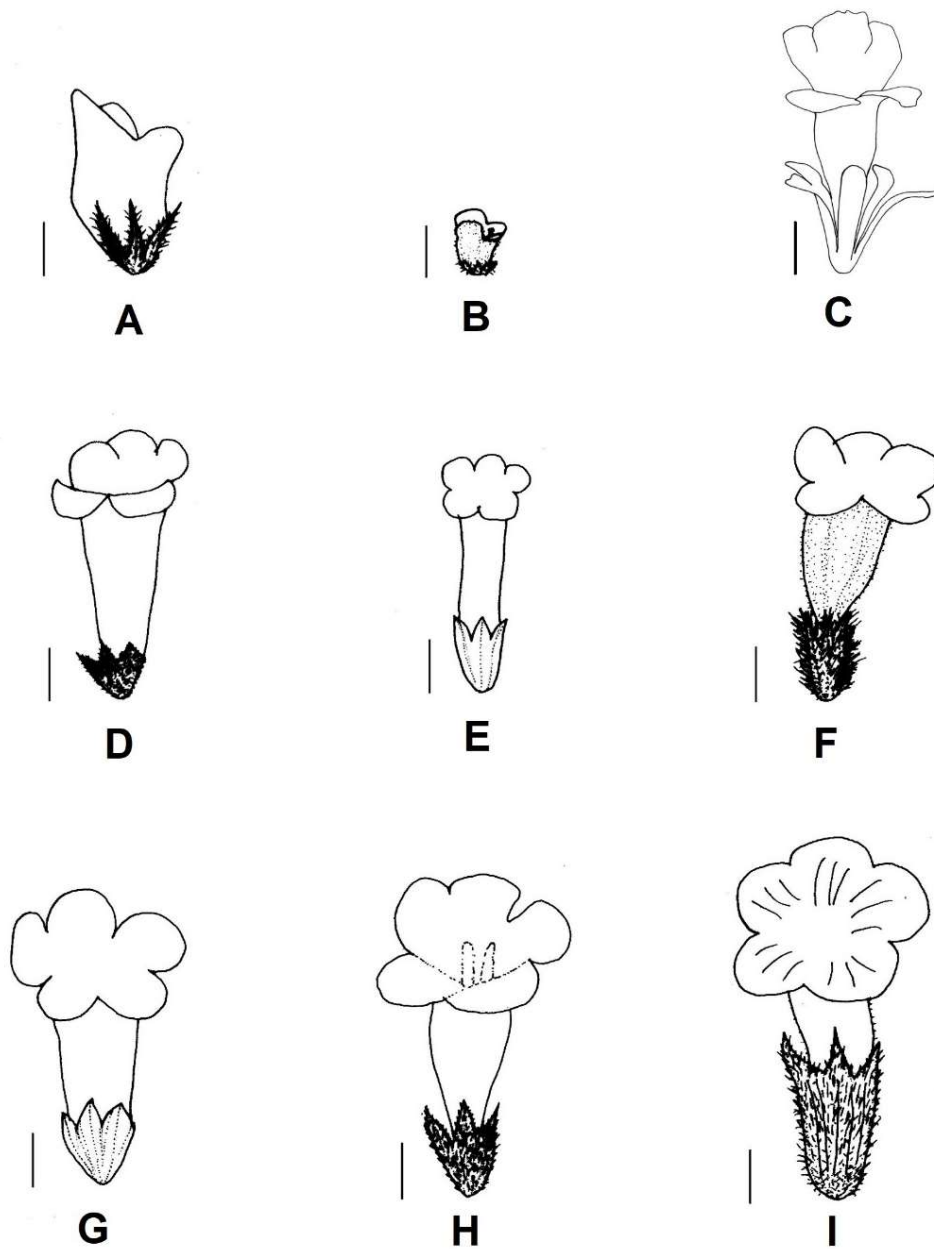


Figure 2.6 Side view of *Henckelia* flowers (continued): A. *H. longipedicellata*; B. *H. nakianensis*; C. *H. longisepala*; D. *H. oblongifolia*; E. *H. peduncularis*; F. *H. pumila*; G. *H. rotundata*; H. *H. speciosa*; I. *H. urticifolia*.

Drawings: Sukontip Sirimongkol. Scale bar = 1 cm.

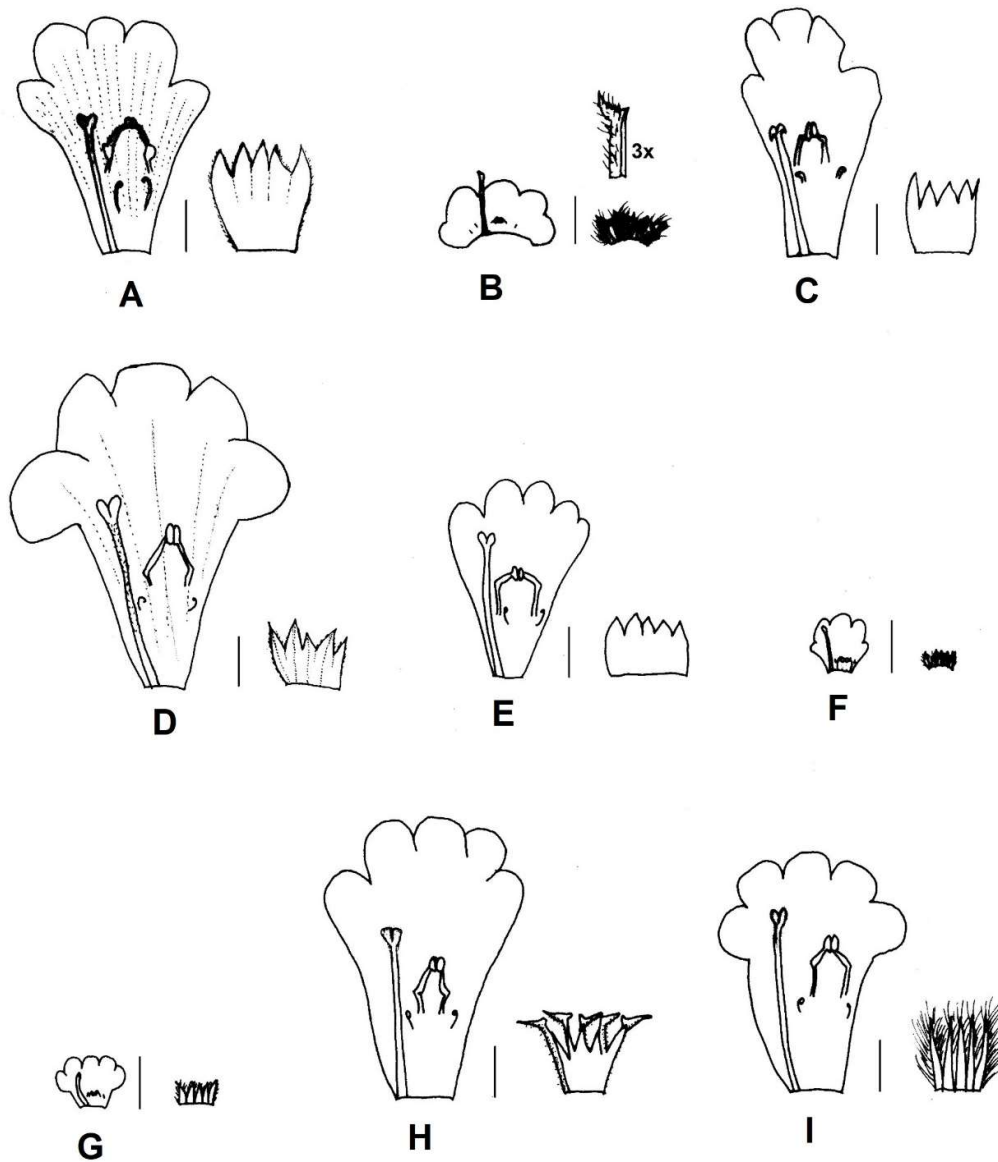


Figure 2.7 Dissection of *Henckelia* flowers with calyx: A. *H. adenocalyx*; B. *H. amplexifolia*; C. *H. anachoreta*; D. *H. burtii*; E. *H. calva*; F. *H. campanuliflora*; G. *H. candida*; H. *ceratoscyphus*; and I. *H. dasycalyx*.

Drawings: Sukontip Sirimongkol. Scale bar = 1 cm.

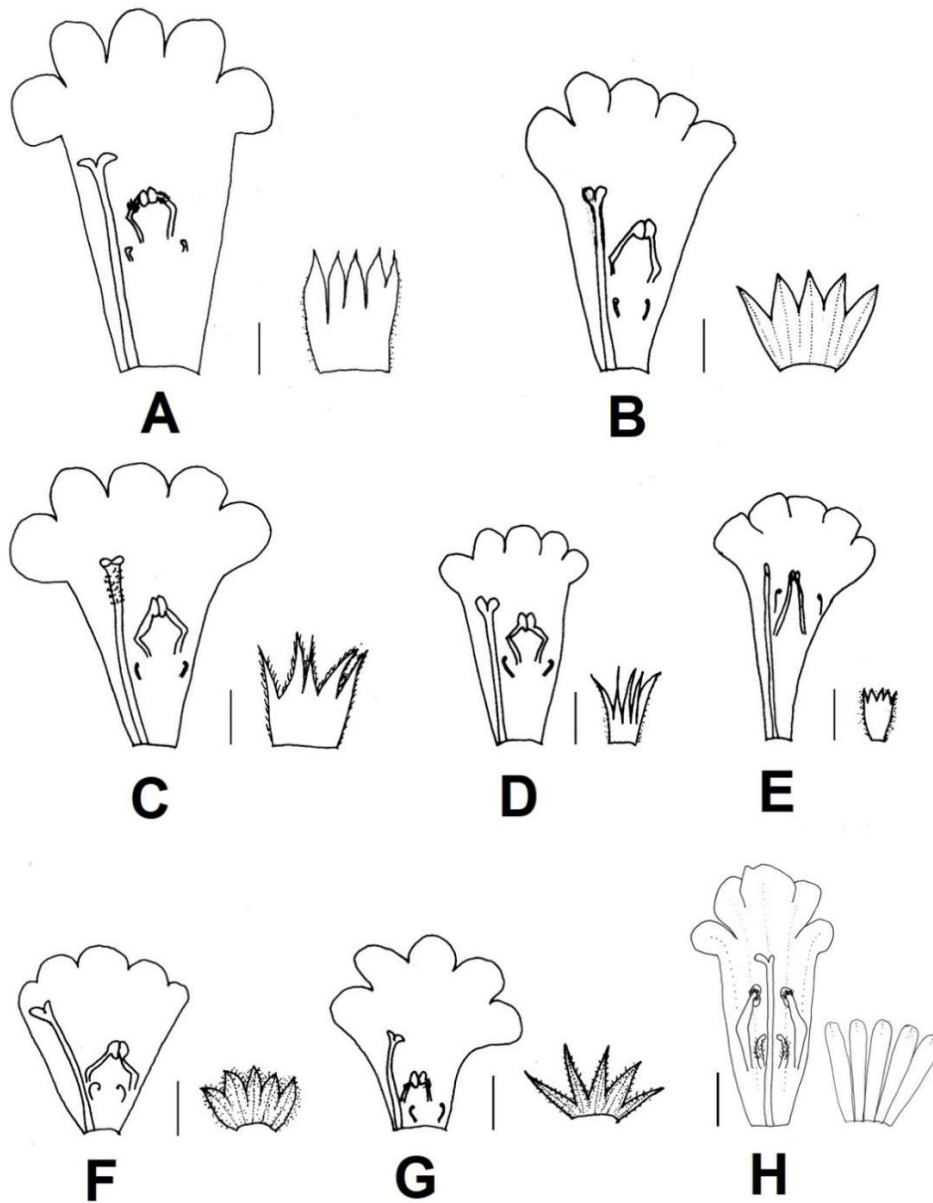


Figure 2.8 Dissection of *Henckelia* flowers with calyx continued: A. *H. fruticola*; B. *H. grandifolia*; C. *H. heterostigma*; D. *H. insignis*; E. *H. lacei*; F. *H. lachenensis*; G. *H. longipedicellata*.

Drawings: Sukontip Sirimongkol. Scale bar = 1 cm.

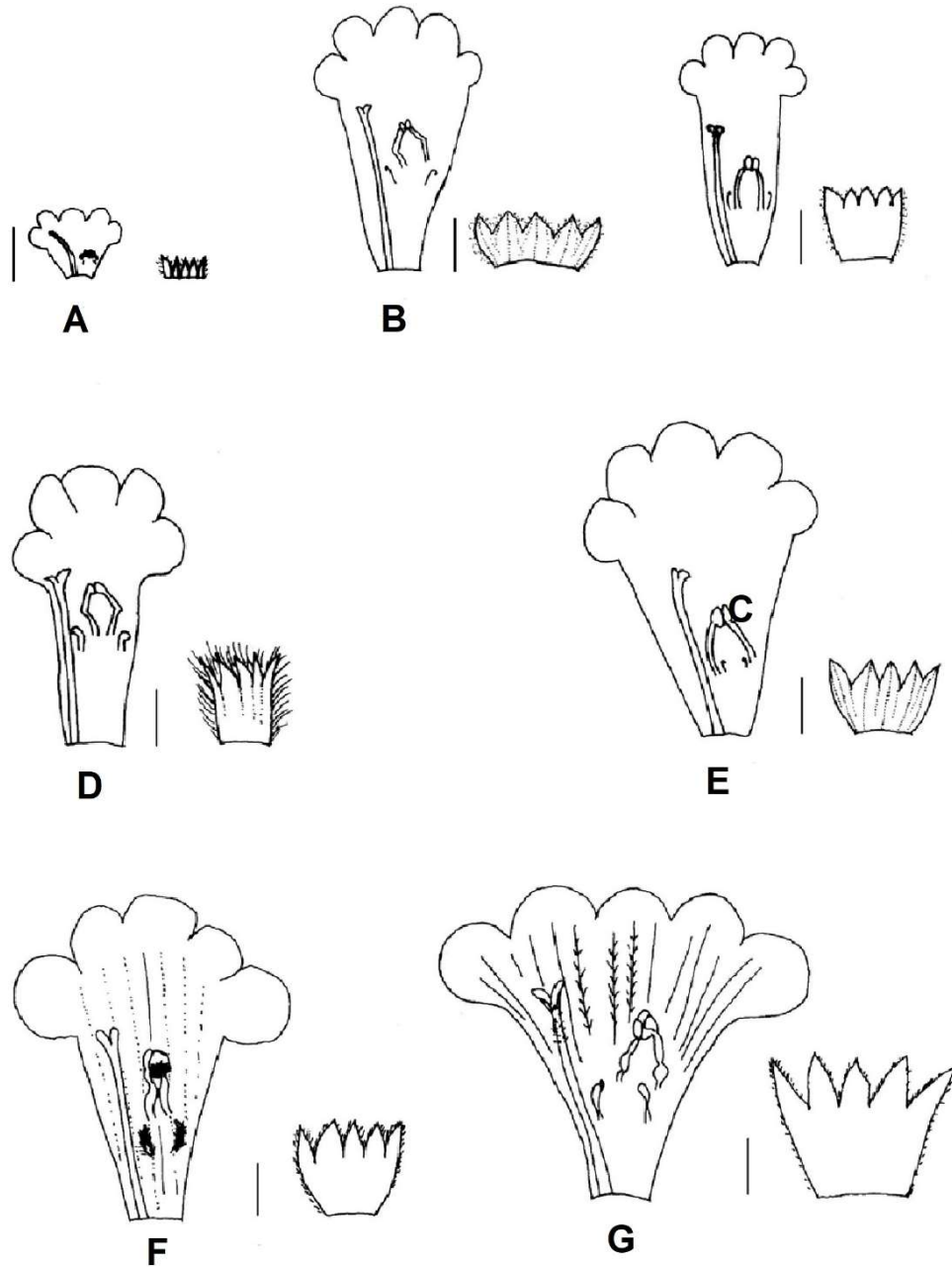


Figure 2.9 Dissection of *Henckelia* flowers with calyx (continued): A. *H. nakianensis*; B. *H. oblongifolia*; C. *H. peduncularis*; D. *H. pumila*; E. *H. rotundata*; F. *H. speciosa*; G. *H. urticifolia*.

Drawings: Sukontip Sirimongkol. Scale bar = 1 cm.

2.4.1. Results

Flowers were investigated from 24 species. Drawings show the outer and inner parts.

Outer part, on the side view drawing (Figs. 2.6 & 2.7), *Henckelia* flowers can be divided into three groups based on calyx divided and two groups based on corolla tube.

Inner part, flower dissection illustrations (Fig 8. 2.8, 2.9 and 2.10) show stamen and staminode position, and stigma shape and inner parts. The species can be divided based on position between stamens and staminodes into three groups.

The results shown as table 2.1.

Table 2.1 Showing results from flower side view and dissection characters.

Taxon\Character	Side view		Flower dissection		
	Calyx divided	Corolla tube	Staminode position	Staminode number	Stigma shape
<i>H. adenocalyx</i>	0	0	0	0	0
<i>H. amplexifolia</i>	2	1	1	1	2
<i>H. anachoreta</i>	0	0	0	0	0
<i>H. burtii</i>	0	0	0	0	0
<i>H. calva</i>	0	0	0	0	0
<i>H. campanuliflora</i>	2	1	0	1	2
<i>H. candida</i>	2	1	0	1	2
<i>H. ceratoscyphus</i>	0	0	0	0	0
<i>H. dasycalyx</i>	2	0	0	0	0
<i>H. fruticola</i>	0	0	0	0	A
<i>H. grandifolia</i>	0	0	0	0	A
<i>H. heterostigma</i>	0	0	0	0	1
<i>H. insignis</i>	0	0	0	0	0
<i>H. lacei</i>	0	0	2	0	2
<i>H. lachenensis</i>	0	0	0	0	0
<i>H. longipedicellata</i>	1	0	0	0	0
<i>H. longisepala</i>	2	0	1	0	0
<i>H. nakianensis</i>	2	1	0	0	2
<i>H. oblongifolia</i>	0	0	0	0	1
<i>H. peduncularis</i>	0	0	1	0	0
<i>H. pumila</i>	0	0	0	0	0
<i>H. rotundata</i>	0	0	1	0	0
<i>H. speciosa</i>	0	0	0	0	0
<i>H. urticifolia</i>	0	0	0	0	0

Remark. 1) Calyx divided: 0=divided less than half, 1=deeply divided & 2=divided to base

2) Corolla tube morphology: 0= infundibuliform & 1= campanulate

3) Staminode position: 0=lower than stamen, 1=same position as stamen & 2=upper than stamen

4) Staminode number: 0= 2 staminodes & 1= 3 staminodes

5) Stigma shape: A0=deeply bilobed, 1=shallowly bilobe & 2=subpeltate

2.5 Morphological data analysis

The previous classification studies have been based on morphological characters alone. The classification of the Gesneriaceae used morphological characters e.g. Smith & Systsma (1994) used morphological data to analyse the genus *Columnea*. Later, Smith (1996) investigated the cladistic relationship of tribes within Gesneriaceae based on morphological data. Thirty-four genera and 44 characters were used (Smith, 1996). Although recent molecular data sets have reduced the importance of morphological data they remain useful for the preliminary classifications and understanding of relationships.

Morphometric data used in this section comprised 49 characters in 24 species. The morphological characters were qualitative. Because of our specimens are suitable for collected the binary data than the quantitative data. The morphometric analysis on *Henckelia* was undertaken using the R-program to analyse the data using non-metric multidimensional scaling (NMDS) and cluster analysis. The morphological characters are described in Table 2.2. The data matrix for the selected characters is shown in Appendix 2.1.

2.5.1 Method

1. Binary morphological characters (49 characters) were obtained from *Henckelia* specimens in Thailand and surrounding countries (Table 2.2).
2. The morphological data were analysed using the vegan package version 2.5-2 (Oksanen et al., 2018) in R version 3.5.0 (R Core Team, 2018). A hierarchical, agglomerative method of cluster analysis was used for classification. Jaccard distance measure was used with the average linkage method because of the dataset is binary data (Robinowitz, 1975). The Jaccard dissimilarity matrix of *Henckelia* species was calculated using the vegdist function in the vegan package, and *Henckelia* species were sorted into groups using the hclust function in R version 3.5.0 (R Core Team, 2018).

Table 2.2 List of morphological characters.

Character	Code
Habit: annual (0), perennial (1)	C1
Habit: rhizomatous: not rhizomatous (0), rhizomatous (1)	C2
Stem: erect (0), creeping (1)	C3
Stem: acaulescent (0), caulescent (1)	C4
Stem: overground: without stolon (0), with stolon (1)	C5
Leaf: petiole: petiolate (0), sessile (1)	C6
Leaf: petiole covering: glabrous (0), hairy (1)	C7
Leaf: petiole with glandular hairs: absent (0), present (1)	C8
Leaf: petiole length ratio and leaf length (time): less than 1 time (0), more than 1 time (1)	C9
Leaf: one (0), several (1)	C10
Leaf arrangement: opposite (0), alternate or whorled (1)	C11
Leaf Cordate: absent (0), present (1)	C12
Leaf elliptic: absent (0), present (1)	C13
Leaf Lanceolate: absent (0), present (1)	C14
Leaf narrowly ovate or narrowly elliptic: absent (0), present (1)	C15
Leaf oblong: absent (0), present (1)	C16
Leaf Obovate: absent (0), present (1)	C17
Leaf Ovate: absent (0), present (1)	C18
Leaf-Orbicular: absent (0), present (1)	C19
Leaf margin: entire (0), serrate or dentate (1)	C20
Leaves clustered at the top of stem: not clustered (0), clustered (1)	C21
Bract connate: free (0), fuse (1)	C22
Bracts: paired (0), more than 1 (1)	C23
Inflorescence-hairs on pedicel: absent (0), present (1)	C24
Inflorescence-hairs on peduncle: absent (0), present (1)	C25
Inflorescence-ratio of inflorescence length with leaf length and petiole (time): less than 1 (0), more than 1 (1)	C26
Calyx lobes divided at base: not divided at base (0), divided at base (1)	C27
Calyx tube: tubular (0), basally connate (1)	C28
Calyx lobe tip: not reflex (0), reflex (1)	C29
Calyx lobe: triangular (0), deltate (1)	C30
Calyx lobes fimbriate: not fimbriate (0), fimbriate (1)	C31
Calyx outside texture: glabrous (0), hairy (1)	C32
Calyx attachment: caducous (0), persistent (1)	C33

Table 2.2 (continued).

Corolla tube: infundibuliform (0), campanulate (1)	C34
Stamens-filament attached from corolla tube (time): less than 1/3 of corolla tube (0), more than 1/3 of corolla tube (1)	C35
Stamens-glandular hairs on filament: absent (0), present (1)	C36
Stamens-hairs on filament: absent (0), present (1)	C37
Stamens-filament: geniculate (0), curved (1)	C38
Anthers shape: elliptic (0), reniform (1)	C39
Anthers texture: without beard (0), with beard (1)	C40
Staminode number: two (0), three (1)	C41
Staminode-hairs on staminode: absent (0), present (1)	C42
Gynoecium-glandular hairs on gynoecium: absent (0), present (1)	C43
Gynoecium-hairs on gynoecium: absent (0), present (1)	C44
Stigma: bilobed (0), subpeltate (1)	C45
Stigma deeply bilobed: absent (0), present (1)	C46
stigma shallowly bilobed: absent (0), present (1)	C47
Stigma subpeltate: absent (0), present (1)	C48
Capsule: orthocarpic, held in line with pedicel (0), plagiocarpic, held almost horizontally (1)	C49

Some morphological characters are illustrated in Fig. 2.10 and 2.11:

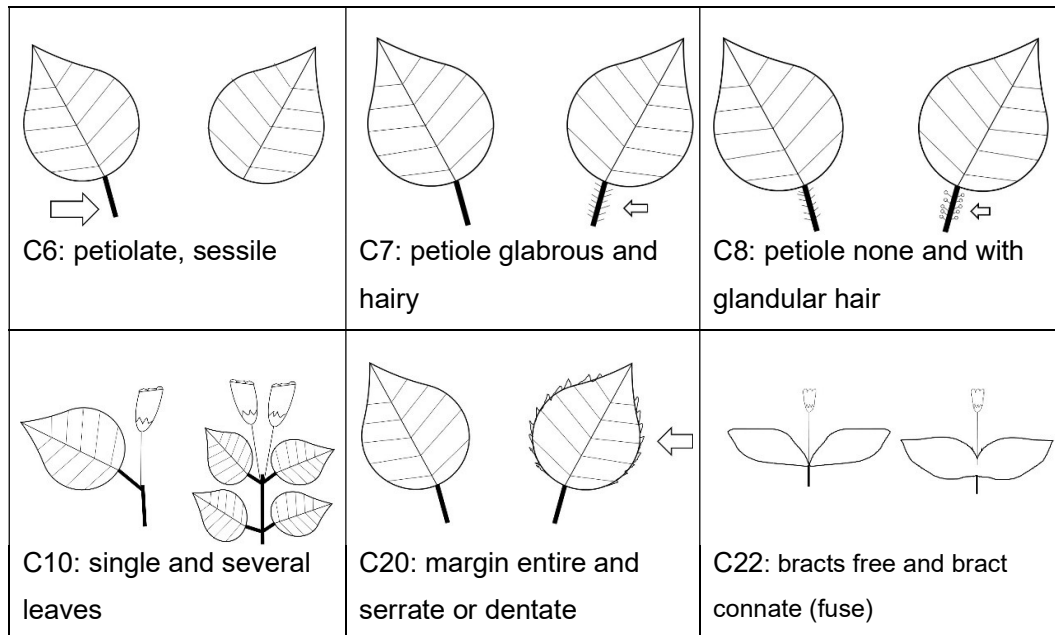


Figure 2.10 Morphological characters used in the morphology analysis.

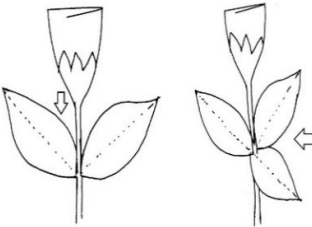
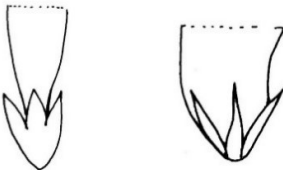
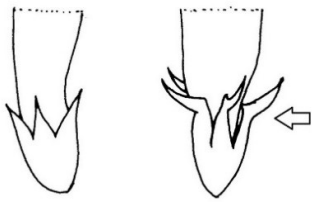
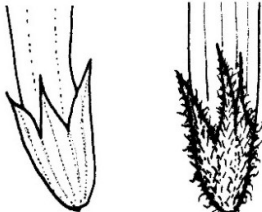

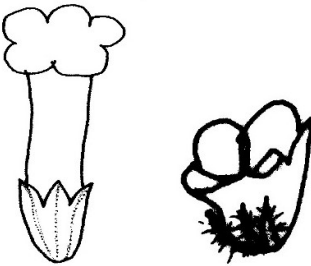
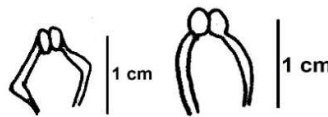

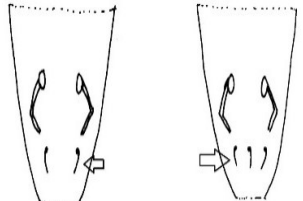
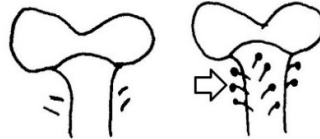
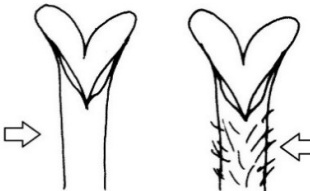
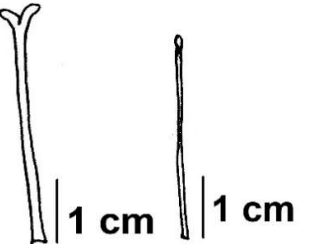
 <p>C23: bracts paired (2) and bracts more than 2</p>	 <p>C27: calyx not divided at base and divided at base</p>	 <p>C29: calyx lobes not reflex and reflex</p>
 <p>C32: calyx glabrous and hairy</p>	 <p>C33: calyx caducous and calyx persistent</p>	 <p>C34: corolla infundibuliform and campanulate</p>
 <p>C38: filaments geniculate and curved</p>	 <p>C39: anthers elliptic and reniform</p>	 <p>C41: staminodes 2 and 3</p>
 <p>C43: gynoecium no glandular hairs and with glandular hairs</p>	 <p>C44: gynoecium glabrous and hairy</p>	 <p>C48: stigma bilobed and subpeltate</p>

Figure 2.10 (continued).

2.5.2 Results

The cluster dendrogram shown as Fig. 2.12. It separated the species well and provides clearly linkage between individual species and allowed for analysis of the relationships of one of species to another.

This analysis showed that in group one, *Henckelia campanuliflora* and *H. candida* were most similar to each other and somewhat less similar to *Henckelia amplexifolia* and *H. nakianensis*. This reflects their joint possession of a single leaf, and peltate or subpeltate stigma: they differ due to the petiolate leaf in *H. campanuliflora* and sessile leaf in *H. candida* and peduncle with glandular hairs in *H. campanuliflora*.

Henckelia amplexifolia and *H. nakianensis* fall into this same group. They do so as they have a similar peltate or subpeltate stigma and reniform anthers: *H. amplexifolia* differs from the others as it may possess stolons.

In group two, *Henckelia heterostigma*, *H. peduncularis*, *H. lacei* and *H. oblongifolia* group together as they have a similar infundibuliform flower, hairy calyx and serrate leaves. The pattern of species relationships within this group can be understood by realizing that it reflects the facts that *H. heterostigma* has more than two bracts and a gynoeceum with glandular hairs and *H. peduncularis* has connate or fused bracts and *Henckelia lacei* and *H. oblongifolia* have a similar persistent calyx but differ due to the subpeltate stigma and curved filament in *H. lacei*.

Also, in group 2 *Henckelia dasycalyx*, *H. longipedicellata*, *H. urticifolia*, *H. anachoreta* and *H. pumila* form a group distinguished because they have a similar bilobed stigma, hairy petiole and hairy peduncle. The pattern of branching within the group arises due to the persistent calyx in *H. dasycalyx*; inflorescence length to leaf length ratio (including petiole) greater than one in *H. longipedicellata* and filament attached to corolla tube more than 1/3 times the length of the corolla tube length in *H. urticifolia*. *Henckelia anachoreta* and *H. pumila* are similar to each other in their caulescent and infundibuliform flower but differ in that the calyx hairs differ from the pedicel hairs in *H. pumila* but are similar in *H. anachoreta* and the gynoeceum is hairy in *H. anachoreta* but glabrous in *H. pumila*.

Finally, in group 2 *Henckelia insignis*, *H. adenocalyx*, *H. burtii*, *H. grandifolia*, *H. speciosa*, *H. fruticola*, *H. ceratoscyphus* and *H. lachenensis* group together as they have similar a petiole, pedicel and hairy filaments, and deeply bilobed stigma. The species split in this group because of various character states. The fimbriate calyx lobe in *H. insignis* splits it off early from the other species. Whilst *Henckelia adenocalyx*, *H. burtii*, and *H. speciosa* are similar in their rhizomatous habit but differ because the stem is creeping in *H. burtii* and is not creeping in *H. adenocalyx* and *H. speciosa*, the

bracts are fused or connate in *H. adenocalyx* but free in *H. burtii* and *H. speciosa*. *Henckelia speciosa* differs from *H. adenocalyx* and *H. burtii* as it has hairs on its anthers (beard).

Henckelia grandifolia and *H. speciosa* are similar in their rhizomatous habit but differ in relation to the hairs on anthers. *Henckelia fruticola*, *H. ceratoscyphus* and *H. lachenensis* have similar petiole, pedicel, calyx and hairy filament but differ in their bracts that are connate or fused in *H. fruticola*; calyx tip reflexed and leaf narrowly ovate or narrowly elliptic in *H. ceratoscyphus* and the creeping stem in *H. lachenensis*.

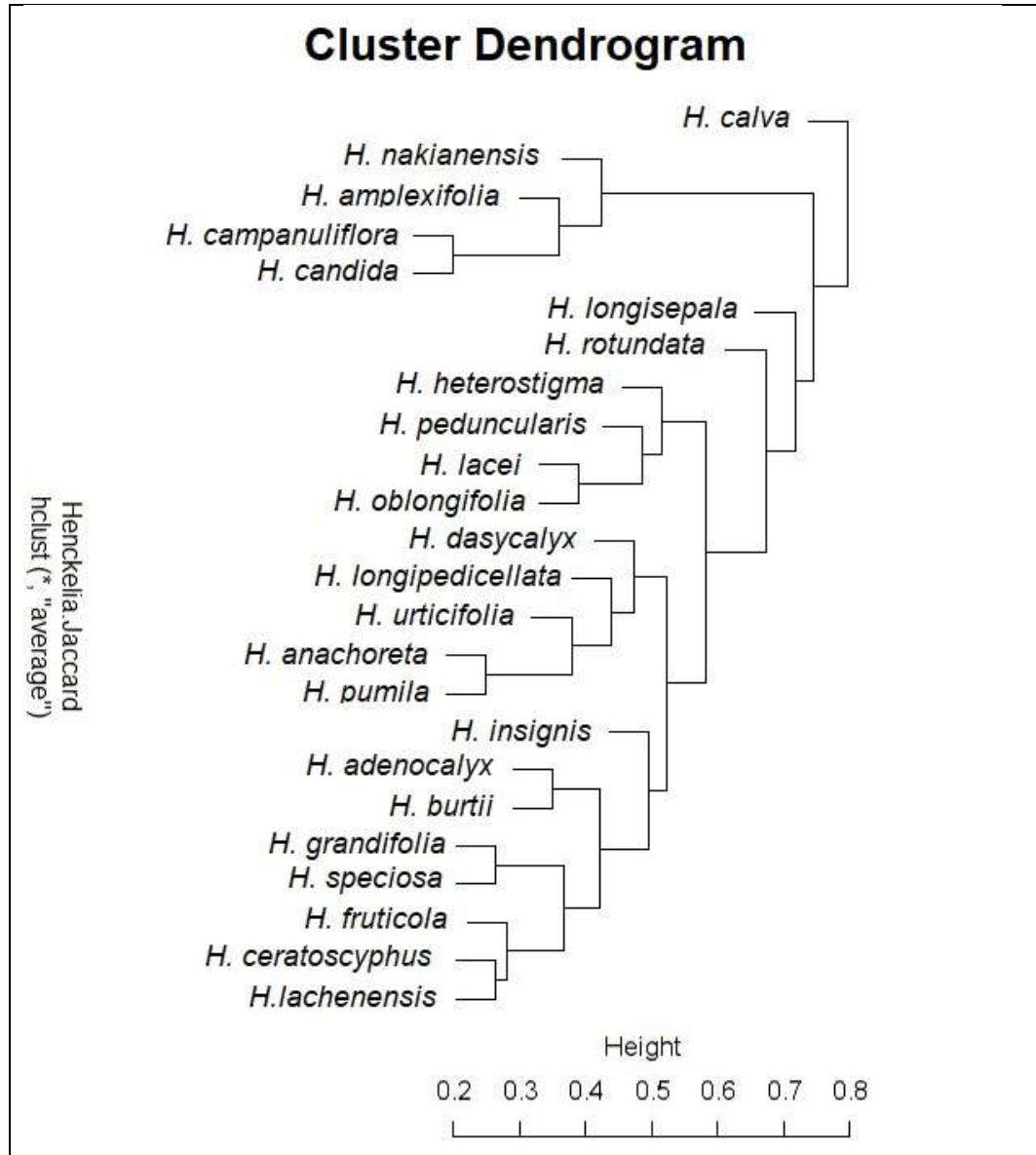


Figure 2.11 Cluster dendrogram of *Henckelia* based on morphological data and Jaccard distance.

Discussion

The morphology of *Henckelia* from Thailand, Indo-China including Myanmar was investigated for 24 species. Side views of the flower and dissection drawings were made for 23 species i.e. *Henckelia adenocalyx*, *H. amplexifolia*, *H. anachoreta*, *H. burttii*, *H. calva*, *H. campanuliflora*, *H. candida*, *H. ceratoscyphus*, *H. dasycalyx*, *H. fruticola*, *H. grandifolia*, *H. heterostigma*, *H. insignis*, *H. lacei*, *H. lachenensis*, *H. longipedicellata*, *Henckelia longisepala*, *H. nakianensis*, *H. oblongifolia*, *H. peduncularis*, *H. pumila*, *H. rotundata*, *H. speciosa* and *H. urticifolia*.

Side view drawings show an infundibuliform corolla tube in *Henckelia adenocalyx*, *H. anachoreta*, *H. burttii*, *H. calva*, *H. ceratoscyphus*, *H. dasycalyx*, *H. fruticola*, *H. grandifolia*, *H. heterostigma*, *H. insignis*, *H. lacei*, *H. lachenensis*, *H. longipedicellata*, *H. longisepala*, *H. oblongifolia*, *H. peduncularis*, *H. pumila*, *H. rotundata*, *H. speciosa* and *H. urticifolia*, with a campanulate corolla tube in *Henckelia amplexifolia*, *H. campanuliflora*, *H. candida*, and *H. nakianensis*.

The flower dissections show that stamens and staminode are positioned differently and the species can be divided into three groups with the staminodes lower than the stamens, the staminode at the same level as the stamens, and the staminode lying above than the stamens.

The morphometric analysis showed the similarity of the species and divided them into two groups. Group one consists of *Henckelia amplexifolia*, *H. campanuliflora*, *H. candida*, and *H. nakianensis* based on the single leaf, campanulate flower, reniform anthers and peltate or subpeltate stigma.

Group two consists of *Henckelia adenocalyx*, *H. anachoreta*, *H. burttii*, *H. calva*, *H. ceratoscyphus*, *H. dasycalyx*, *H. fruticola*, *H. grandifolia*, *H. heterostigma*, *H. insignis*, *H. lacei*, *H. lachenensis*, *H. longipedicellata*, *H. longisepala*, *H. nakianensis*, *H. oblongifolia*, *H. peduncularis*, *H. pumila*, *H. rotundata*, *H. speciosa*, and *H. urticifolia* based on the presence of several leaves, infundibuliform flower, elliptic anthers and bilobed stigma.

Henckelia amplexifolia is unique in having a stolon and bulbils and producing less fruit. Further study of these features is required.

Chapter 3-Molecular Phylogenetics of Henckelia and Allies

3.1 Introduction

Plant cells hold three genomes in the plastid (chloroplast), mitochondria and the nucleus. The majority of molecular systematic studies have used DNA sequences from the chloroplast and the nuclear genomes (Judd et al., 2002). The traditional way to classify plant families has been to use morphological data. The Lamiales are represented by a monophyletic group from molecular evidence proposed by APG II. Occasionally, families share some characters; such as the families in this order present opposite leaves, sympetalous and 5-merous flower, or zygomorphic lip flowers with dehiscent or indehiscent fruit, numerous and minute seeds (Byng, 2014; Utteridge & Bramley, 2014).

The general morphological characters of Gesneriaceae are simple and opposite leaves, although sometimes whorled or alternate, but usually with glandular or eglandular hairs. The inflorescence is a specialized “pair-flowered” cyme, with bisexual 5-merous flowers and a mostly superior ovary. They are different from the other families such as the Scrophulariaceae which have axillary placentation while the Gesneriaceae have T-shaped placentation. Rubiaceae are characterised by their stipules which are lacking in Gesneriaceae, while Solanaceae show alternate leaves and the Lamiaceae have seeds up to 4 nutlets or drupe with 1-5 stones (Gesneriaceae have numerous and minute seeds).

More recently, DNA sequence data have transformed the way that plants are classified. One of the earliest and most influential papers was by Chase et al. (1993) based on plastid *rbcL* sequences that assessed seed plant phylogeny. The work of the Angiosperm Phylogeny Group (APG IV, 2016) has subsequently expanded the early analyses and combined evidence from many sources to reclassify plants at the level of the family and above.

The Old World Gesneriaceae had previously been classified in the family Didymocarpaceae D. Don (1822) and Cyrtandraceae Jack (1825). Whilst, the New World Gesneriaceae was established by de Jussieu (1806). Later, Bentham and Hooker (1876) divided the family into subfamily Gesnereae (Gesnerioideae) and subfamily Cyrtandreae (Cyrtandroideae). The Gesnereae had an inferior ovary and a capsular fruit (Bentham & Hooker, 1876) compared to subfamily Cyrtandreae (Cyrtandroideae) that had a superior ovary and a fruit that was either capsular or a berry (Bentham & Hooker, 1876).

The two subfamilies can also be divided based on geography, namely the Old

World Cyrtandroideae and the New World Gesnerioideae (Fritsch, 1893).

Molecular studies have further assessed the classification of Gesneriaceae including work by the Angiosperm Phylogeny Group (Stevens, 2001; APG IV, 2016). The Gesneriaceae is considered to be monophyletic and has been divided into three subfamilies, Sanangoideae, Gesnerioideae and Didymocarpoideae (Stevens, 2001; Weber et al., 2013) and a number of tribes that are shown in Fig. 3.1.

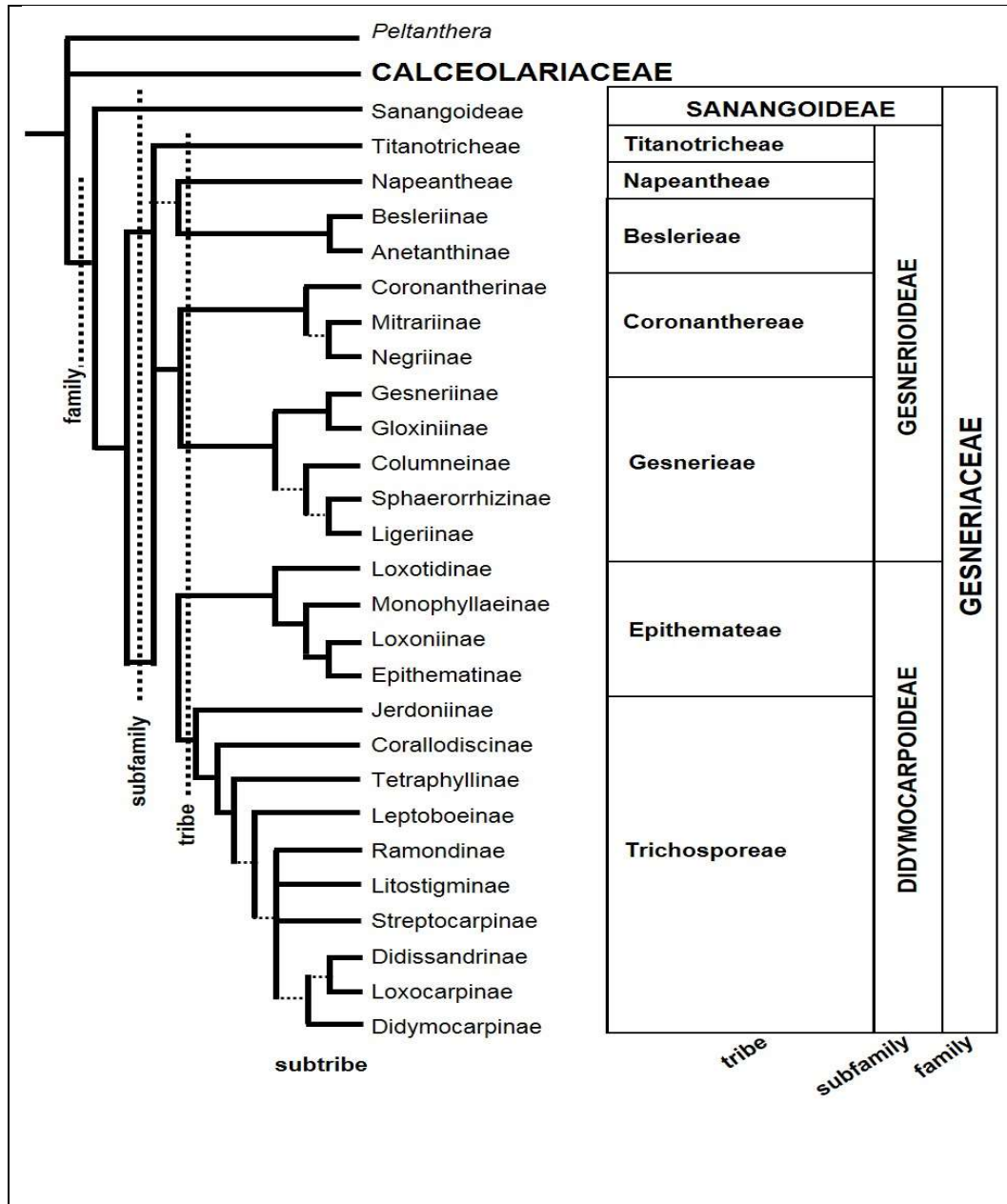


Figure 3.1 Formal Gesneriaceae classification and phylogenetic relationships. Modified from Weber et al. (2013) based on sequences of several DNA regions including *matK*, *ndhF*, nrITS and *trnL-F* (Möller & Clark, 2013).

Molecular studies on the Gesneriaceae have used nuclear and chloroplast DNA sequences (Table 3.1). Nuclear ribosomal ITS (nrITS) DNA has been used as a universal marker (Möller and Cronk, 1997a, 1997b and 2001; Denduangboripant and Cronk, 2000; Atkins et al., 2001; Clarke & Zimmer, 2003; Cronk et al., 2005). Some studies have used single sequences of chloroplast DNA such as *ndhF* (Smith et al. 1997; Smith and Carroll, 1997; Smith, 1997 and 2006). Other studies have used combined datasets including nrITS, *ndhF*, *trnL-F* (Clarke et al., 2006; Li et al., 2007; Wang et al., 2010; Möller et al., 2009; Perret et al., 2013; Zimmer et al. 2002), *atpB-rbcL* (Mayer et al., 2003; Wang et al., 2004; Möller et al., 2009; Perret et al., 2013), *rps16* (Perret et al., 2013), *trnH-psbA* (Clarke et al., 2006) and *ncpGS* (Table 3.1).

Table 3.1 Markers regions used for published studies on the molecular systematics of the Gesneriaceae.

Marker	References
ITS	Atkins et al. (2001)
ITS	Clarke & Zimmer (2003)
ITS	Denduangboripant & Cronk (2000)
ITS	Möller & Cronk (1997b)
ITS	Möller & Cronk (2001)
ITS	Möller & Cronk (1997a)
ITS and <i>ndhF</i>	Smith (2000a)
ITS and <i>trnL-trnF</i>	Zimmer et al., 2002
ITS, <i>trnH-psbA</i>	Clark et al. (2006)
ITS, <i>trnL-F</i>	Li et al. (2007)
ITS, <i>trnL-F</i>	Wang et al. (2010)
ITS, <i>trnL-F</i> , <i>atpB-rbcL</i>	Möller et al. (2009)
ITS, <i>trnL-F</i> , <i>trnE-T</i>	Zimmer et al. (2002)
ITS1-ITS2	Cronk et al. (2005)
<i>matK</i> , <i>rps16</i> and <i>trnL-F</i>	Perret et al. (2013)
<i>ndhF</i>	Smith & Carroll (1997)
<i>ndhF</i>	Smith (2000b)
<i>ndhF</i>	Smith et al. (1997)
<i>ndhF</i>	Smith, 1997

Table 3.1 (continued)

Marker	References
<i>rbcL-atpB</i> , <i>trnL-F</i>	Mayer et al. (2003)
<i>trnL-F</i> , <i>atpB-rbcL</i> , nr26S and the low-copy developmental gene <i>CYCLOIDEA</i> , <i>CYC</i>	Wang et al. (2004)
<i>trnT-L</i> , <i>trnL-F</i> , <i>trnS-trnG</i> , <i>atpB-rbcL</i> , <i>trnL</i> , <i>rps16</i> and <i>ncpGS</i>	Perret et al. (2003)

Intensive phylogenetic studies in the Gesneriaceae started with the subfamily Gesnerioideae. The Gesnerioideae was confirmed as a monophyletic based on forty-four morphological characters (Smith, 1996). Later, Smith et al. (1997) confirmed other monophyletic groups, in Cyrtandroideae and Gesnerioideae. The Gesneriaceae were also completely divided from the Scrophulariaceae and Acanthaceae (Smith et al., 1997). Later, Smith (2000a) used nrITS and *ndhF* regions to analyse the phylogeny of Episcieae (Gesnerioideae) and the results strongly supported the monophyly of this tribe (Smith, 2000a). In the same year, Smith (2000b) used *ndhF* to confirm the monophyly of Beslerieae and Napeantheae.

Zimmer et al. (2002) analysed nrITS and the chloroplast *trnL* intron, *trnL-trnF* spacer region and the *trnE-trnT* intergenic spacer region and confirmed the monophyly of Gesnerioideae including most of the New World members. Clarke and Zimmer (2003) also used nrITS sequences to demonstrate the polyphyly of *Alloplectus* (Gesnerioideae).

Perret et al. (2003) studied the systematics and evolution of tribe Sinningieae (Gesnerioideae) based on six plastid DNA regions (*trnT-L*, *trnL-F*, *trnS-trnG*, *atpB-rbcL*, *trnL*, *rp16*) and nuclear *ncpGS*. Their results strongly supported the monophyly of Sinningieae.

Wang et al. (2004) combined chloroplast DNA (*trnL-F*, *atpB-rbcL*), nuclear 26S ribosomal DNA and the low-copy developmental gene *CYCLOIDEA* in their phylogenetic reconstructions. The results strongly supported the placement of *Titanotrichum oldhamii* Soler. in the Gesneriaceae (Gesnerioideae) and not Scrophulariaceae.

Clark et al. (2006) used nrITS and *trnH-psbA* sequences and morphological data to clarify the phylogenetic relationships and generic boundaries in the Episcieae. The results showed that *Glossoloma* and *Crantzia* were segregated from *Alloplectus* and together with other genera they formed a strongly supported monophyletic group in Episcieae (Gesnerioideae) (Clarke et al., 2006). Wang et al. (2010) studied the origin and phylogenetic relationships of the Old World Gesneriaceae with

actinomorphic flowers using nrITS and *trnL-trnF* sequences. The results showed that the actinomorphic flowered genera were scattered within several zygomorphic clades and they hypothesized that evolution had occurred from zygomorphic to actinomorphic because of pollination strategies such as the position of nectar (Wang et al., 2010).

Möller and Cronk (1997a) studied the phylogeny and disjunct distribution of *Saintpaulia* (Cyrtandroideae) based on nrITS sequences and showed the paraphyly of *Streptocarpus* and the monophyly of *Saintpaulia* which was nested within *Streptocarpus*. In the same year, Möller and Cronk (1997b) used the nrITS region to study the phylogenetic relationships of *Saintpaulia* and the results also strongly supported the monophyly of the genus. However, Möller and Cronk (2001) later used nrITS to study *Streptocarpus* and found it was paraphyletic.

Denduangboripant and Cronk (2000) used the nrITS region to study the phylogeny of *Aeschynanthus* (Trichosporeae; Didymocarpoideae). They found that the data were difficult to interpret and that it indicated overlapping geographic species distributions. Classification based on morphological characters, especially the hair type on the seed, was presented. Atkins et al. (2001) also used nrITS marker sequences to clarify the biogeography and phylogeography of *Cyrtandra* (Cyrtandroideae) in the Sundaland region (Borneo and Peninsular Malaysia) and the Philippines. The results showed that the majority of *Cyrtandra* species on the island of Palawan are monophyletic and two species of Palawan *Cyrtandra* (*C. elatostemanoides* and *Cyrtandra* sp. C) fall within the Sundaland clade. Cronk et al. (2005) also used nrITS sequences to study the biogeography of *Cyrtandra* in the Pacific Ocean. The Pacific species (Polynesian and Micronesian) formed a highly supported monophyletic group. They inferred that *Cyrtandra* was able to disperse to the large Polynesian and Micronesian regions because of its fleshy fruit with almost white colour that aids dispersal by Columbiform birds (Cronk et al. 2005).

Mayer et al. (2003) analysed tribe Epithemateae (Didymocarpoideae) based on *rbcl-*atpB** and *trnL-F* intron spacer regions of chloroplast DNA. The results strongly support the monophyly of Epithemateae.

Li et al. (2007) undertook phylogenetic reconstruction of genus *Chiritopsis* and *Chirita* Sect. *Gibbosaccus* (Trichosporeae; Didymocarpoideae) based on nrITS and chloroplast *trnL-F* DNA sequences. The results demonstrated that section *Gibbosaccus* was paraphyletic and *Chiritopsis* was polyphyletic. This study changed the nomenclature of this section (Li et al., 2007).

Möller et al. (2009) conducted a preliminary phylogenetic reconstruction of didymocarpoid Gesneriaceae of the Old World taxa based on three molecular markers regions (nrITS, *trnL-F*, and *atpB-rbcL*). The results showed that some genera are not a

monophyletic i.e. *Briggia*, *Henckelia*, and *Chirita* (Möller et al., 2009). Weber et al. (2011) was remodelled *Chirita* and found it was placed close to other genera such as *Henckelia*, *Damrongia*, *Microchirita*, *Liebigia*, and *Primulina* (Figs. 3.2 and 3.3).

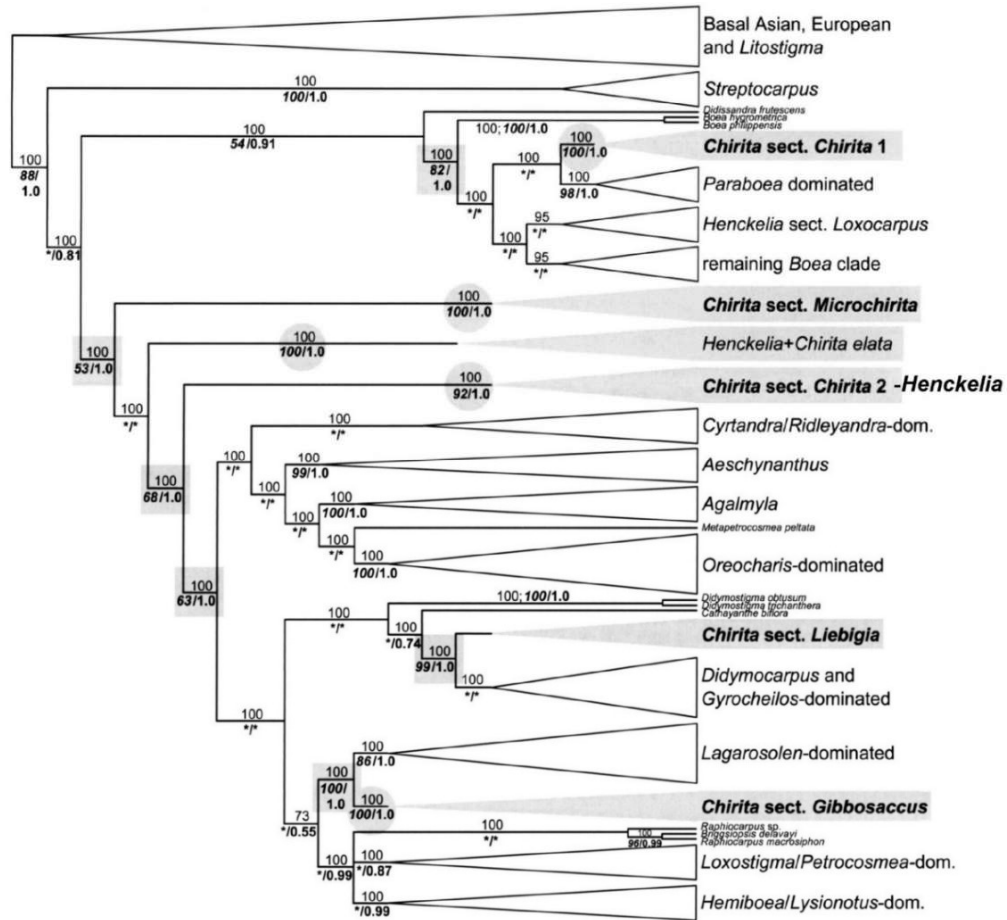


Figure 3.2 Simplified Maximum parsimony majority-rule consensus tree of *Henckelia* and allies based on combined *trnL-F* and nrITS sequences. The *Henckelia* clade is highlighted (Weber et al., 2011); Source Taxon 60(3), p 770. Values above the branches are majority-rule frequencies. Values below the branches are Bayesian posterior probabilities (PP) and bootstrap values (BS).

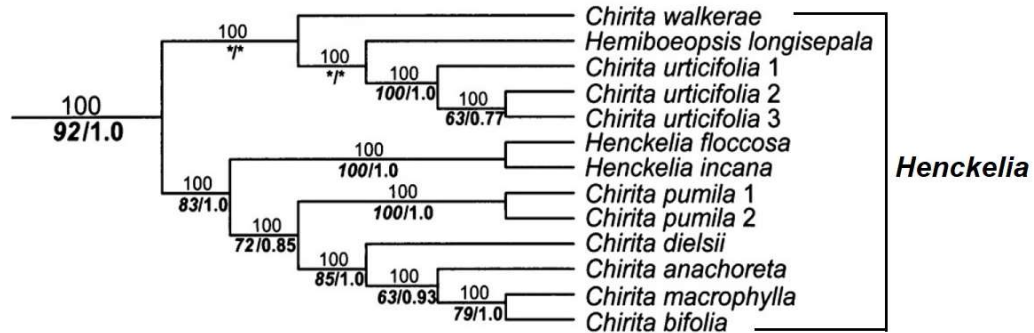


Figure 3.3 Cladogram of the Bayesian majority-rule consensus tree of *Henckelia* and allies from combined *trnL-F* and nrITS sequences, depicting the *Henckelia* clade (further explanation in Fig. 3.2) (Weber et al., 2011); Source Taxon 60(3), p 771.

Wang et al. (2011) continued work on *Chirita* using nrITS and chloroplast *trnL-F* to resolve the problem of polyphyly in *Chirita* and its allies. The analyses resulted in many changes to the nomenclature of *Chirita*. All taxa in *Chirita* sect. *Microchirita* form a clade and were supported with morphological characters i.e a monocarpic annual habit, unusual crested inflorescences with the peduncle fused to the petiole and anthers fused apically. Because of this, they placed this section into genus *Microchirita* (Wang et al., 2011). *Chirita* sect. *Chirita* also formed a separate clade from *Chirita* sect. *Gibbosaccus*. Therefore, Wang et al. (2011) placed *Chirita* sect *Gibbosaccus* into genus *Primulina* (Fig. 3.4). Later some *Henckelia* species were moved to other genera such as *Codonboea*, *Didissandra*, *Didymocarpus*, *Lindernia* and *Loxocarpus* by Middleton et al. (2013).

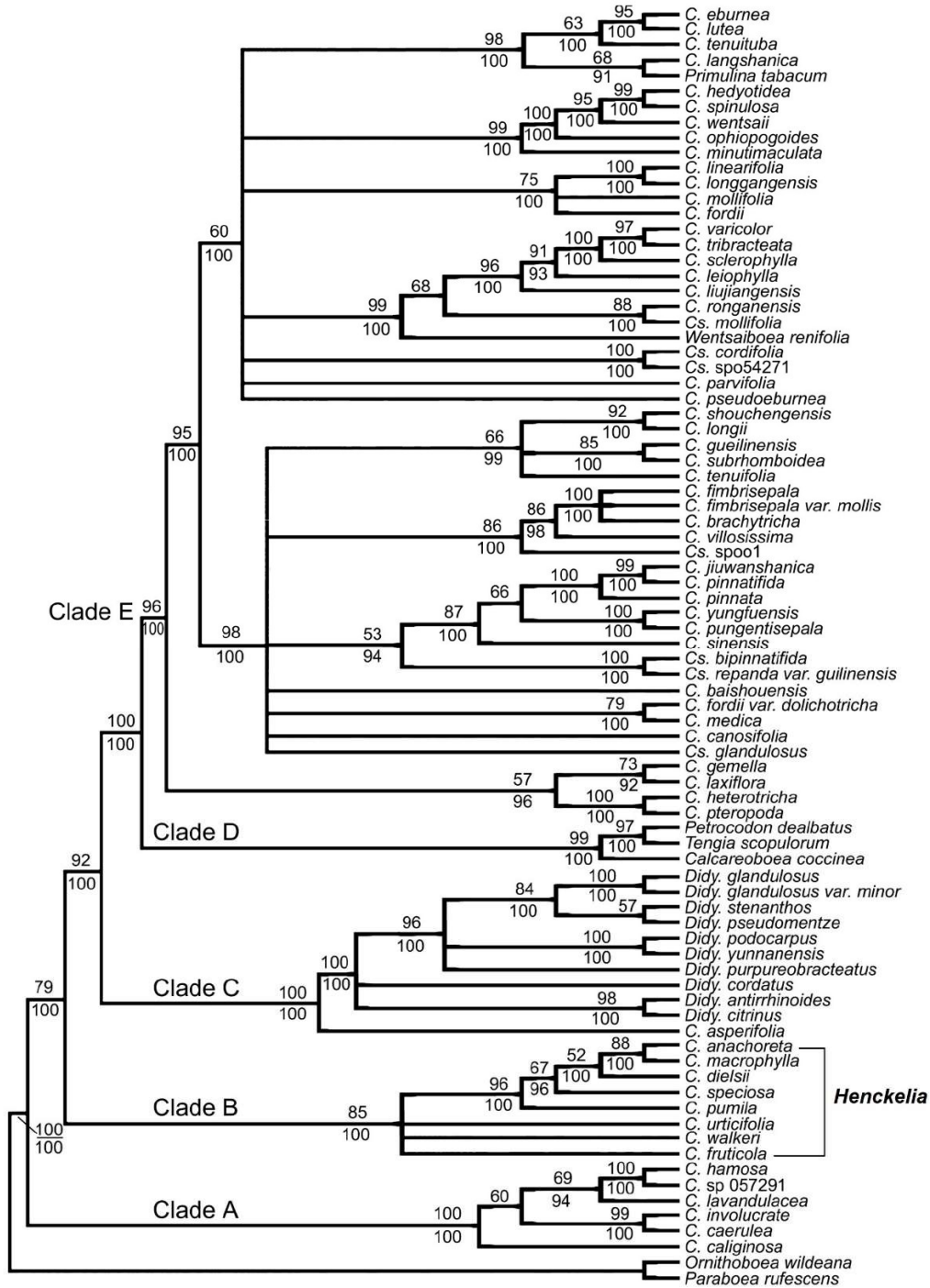


Figure 3.4 Strict consensus tree of *Henckelia* and allies based on nrITS sequences (Wang et al., 2011); Source Journal of Systematics and Evolution 49(1), p. 53. Number above branches are bootstrap values (BS) and below branches are posterior probabilities (PP). C.= *Chirita*; Cs. = *Chiritopsis* and Didy. = *Didymocarpus*.

Objectives

It is clear from the literature survey that many phylogenetic relationships within Gesneriaceae remain unresolved. Furthermore, *Henckelia* and related genera have been poorly sampled in previous studies.

The aim of this chapter was, therefore, to investigate the phylogenetic relationships of *Henckelia* and its allies with particular emphasis on the Thai species. The study used nrITS, and the following plastid gene regions: *trnL* and *trnF* (hereafter *trnL-F*), *atpB-rbcL* and *rps16*. These loci had been used before in studies of Gesneriaceae so we could combine the new data with existing sequences (from GenBank) to generate more comprehensive phylogenetic trees. DNA was sequenced using Sanger sequencing and data analysed using maximum parsimony and Bayesian approaches of single genes and combinations of genes.

3.2 Materials and methods

Plant materials were collected from the field and stored in silica gel using the Teabag Method to desiccate and preserve the material (Wilkie et al., 2013) (Table 3.2). Some sequences were downloaded from GenBank (<https://www.ncbi.nlm.nih.gov/genbank/>) (Table 3.2).

The data analysis comprises of 2 sets. The first set is the *Henckelia* current sequences with the other genera of Gesneriaceae. The results are shown as figure 3.5-3.7.

The second set is the sequences from this study. The ingroup taxa of this study were *Henckelia*. The outgroup comprised of three species, two species from Gesneriaceae, i.e. *Didymocarpus* sp. and *Microchirita* sp., and the other species from Scrophulariaceae i.e. *Verbascum bombyciferum*. They were all extracted from fresh material. The results are shown as figure 3.8-3.12.

DNA extraction was carried out using the modified hot 2xCTAB method (Doyle & Doyle, 1987; Hodkinson et al., 2007) and or the Qiagen DNeasy DNA Isolation Kit (Crawley, UK) following the manufacturer's protocol. Extraction of DNA is detailed in Appendix 3.1.

DNA purification of extracted DNA followed the manufacturer's protocol from the JETquick Spin Columns (ThermoFisher Scientific). The purification of DNA is detailed in Appendix 3.2. The quality of extraction was checked by agarose gel electrophoresis. A 1.2% agarose gel was used following Lee et al. (2012) to check the quality and quantity of extracted DNA or PCR product amplification. The protocol is detailed in Appendix 3.3.

PCR amplification of the nuclear ribosomal internal transcribed spacer (nrITS) followed Möller and Cronk (1997), intron and spacer region of *trnL* and *trnF* (*trnL-F*) followed Mayer et al. (2003), *atpB-rbcL* spacer followed Mayer et al. (2003), and *rps16* followed Oxelman et al. (1997) and Perret et al. (2013); the primers are listed in Appendix 3.8. The PCR conditions are detailed in Appendix 3.4 and 3.5. The PCR product was purified using the ExoSAP-IT™ product clean-up reagents. The protocol followed the manual from www.thermofisher.com (Appendix 3.6). Nanodrop Lite UV spectrophotometry was used to check total extracted DNA and PCR product quantity in combination with the gel method outlined above (Appendix 3.7). The cleaned PCR products were sent to the Source BioScience company for sequencing. The preparation of the PCR product for sequencing is outlined in Appendix 3.9.

Phylogenetic analyses

Raw data sequence files were edited using Mega 7 (Kumar et al., 2008 and 2016). Multiple sequences were then aligned using Muscle or Clustal in Mega7 and checked and corrected manually where necessary.

The DNA datasets were used to reconstruct phylogenetic trees using Bayesian inference (BI) and Maximum Parsimony (MP) analyses (Swofford, 2002) using MrBayes (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) and PAUP* (Swofford, 2002) respectively. The Nexus file (Maddison, 1997) for MrBayes and PAUP* was prepared using fastgap1.2 to convert files from Fasta format into Nexus format (following Borchsenius, 2009).

MrModeltest2 (Nylander, 2004) was run to select the model parameters for the Bayesian analyses. The parameters of Hierarchical Likelihood Ratio Tests (hLRT2) was GTR+G, akaike information CRITERION (AIC) was GTR+I+G. We selected GTR+I+G setting. The command to run MrBayes was therefore set to: Lset nst=6 rates=invgamma (=GTR+I+G), and the MCMC search run for 12 million generations sampled every 1000th generation and the first 25% of samples was discarded as burn-in. The resulting Newick Bayesian tree was viewed with PAUP*4.0 because it has a better tree graphics drawing option than MrBayes.

MP analysis was undertaken using PAUP*4.0 (Swofford, 2001) with heuristic search options including tree-bisection-reconnection (TBR), with 1000 replicates of random addition sequences (saving no more than 200 trees of a score greater than or equal to 1); max trees was automatically increased by 100 if reached. Internal support was evaluated with 1000 replicates of bootstrapping, with full heuristic search including simple sequence addition of taxa and TBR branch swapping.

Combined analyses were also undertaken for the nuclear and plastid gene

regions. Decision to combine was based on assessment of individual gene trees and their support values. Little incongruence was detected among them (especially supported incongruence) so the matrices could be combined (following Hodkinson et al., 2010). Final phylogenetic trees were displayed and labelled using the Microsoft Paint Program.

Table 3.2 Details of voucher specimens in this chapter.

Taxon	Marker	Voucher	Origin	Code
<i>Didymocarpus</i> sp.	atpB-rbcL, ITS, rps16, trnL-F	Poopath et al. 1730	Thailand	29
<i>Henckelia amplexifolia</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 701	Thailand	5
<i>H. amplexifolia</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 713	Thailand	49
<i>H. amplexifolia</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 714	Thailand	50
<i>H. anachoreta</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 698	Thailand	4
<i>H. anachoreta</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 660	Thailand	7
<i>H. anachoreta</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 673	Thailand	8
<i>H. anachoreta</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 665	Thailand	13
<i>H. anachoreta</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 710	Thailand	17
<i>H. anachoreta</i>	atpB-rbcL, ITS, rps16, trnL-F	Poopath et al. 1409	Thailand	44
<i>H. anachoreta</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 715	Thailand	51
<i>H. anachoreta</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 721	Thailand	54
<i>H. auriculata</i>	ITS (*FJ796197.1), trnL-F (*FJ796196.1)	Li Jia-Meivoucher="08816	China	A17 & B17
<i>H. bifolia</i>	ITS (*JF912549), trnL-F (*JF912522.1)	Bhaskar Adhikari L2B6, E"	Nepal	A18 & A18
<i>H. campanuliflora</i>	ITS	Puglisi	Myanmar	68
<i>H. dielsii</i>	ITS (*HQ632967.1), trnL-F (*HQ632871.1)	M.Moeller MMO 08-1211, E"	China	A19 & A19

Table 3.2 (continued)

Taxon	Marker	Voucher	Origin	Code
<i>H. dielsii</i>	ITS (*DQ872838.1), trnL-F (*DQ872818.1)	Li J.M.058132"	China	A20 & A20
<i>H. dielsii</i>	ITS (*KR336987.1), trnL-F (*KR476534.1)	HEAC: LJM118223"	China	A21 & A21
<i>H. floccosa</i>	ITS (*HQ632964.1), trnL-F (*FJ501486.1)	C.G. Jang G 157, WU"	Sri Lanka	A22 & A22
<i>H. glandifolia</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 707	Thailand	6
<i>H. grandifolia</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 720	Thailand	53
<i>H. grandifolia</i>	atpB-rbcL, ITS, rps16, trnL-F	Poopath et al. 1844	Thailand	61
<i>H. incana</i>	ITS (*HQ632965.1), trnL-F (*HQ632869.1)	S. Vogel SVG, E	India	A24 & A24
<i>H. longisepala</i>	ITS (*HQ632963.1), trnL-F (*HQ632868)	Y.M. Shui 73170, KUN"	China	A25 & A25
<i>H. nakianensis</i>	ITS, rps16, trnL-F	Pongamornkul et al. 5110	Thailand	56
<i>H. pumila</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 679	Thailand	1
<i>H. pumila</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 680	Thailand	9
<i>H. pumila</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 681	Thailand	10
<i>H. pumila</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 682	Thailand	14
<i>H. pumila</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 686	Thailand	15
<i>H. pumila</i>	atpB-rbcL, ITS, rps16, trnL-F	van de Bult 1358	Thailand	58
<i>H. rotundata</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 694	Thailand	3
<i>H. rotundata</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 697	Thailand	12
<i>H. rotundata</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 695	Thailand	16
<i>H. speciosa</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 693	Thailand	2

Table 3.2 (continue)

Taxon	Marker	Voucher	Origin	Code
<i>H. speciosa</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 696	Thailand	11
<i>H. speciosa</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 712	Thailand	48
<i>H. urticifolia</i>	ITS (*DQ872835.1), trnL-F (*DQ872821.1)	Li J.M.05851	China	A29 & A29
<i>H. urticifolia</i>	ITS (*JF912559.1), trnL-F (*JF912532.1)	NPSW 110, E"	Bhutan	A30 & A30
<i>H. urticifolia</i>	ITS (*FJ501328.1), trnL-F (*FJ501492.1)	EMAK 109 H 20.9.1991 (Edinburgh-Makalu Expedition 1991)"	Nepal	A31 & A31
<i>H. walkerae</i>	ITS (*FJ501326.1), trnL-F (*FJ501490.1)	Skog 7736 (US 590934), cult. Smithsonian 94-250"	Sri Lanka	A32 & B32
<i>Microchirita</i> sp.	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol et al. 677	Thailand	41
<i>Verbascum bombyciferum</i>	atpB-rbcL, ITS, rps16, trnL-F	Sirimongkol 725	Ireland	67

Remark: * The GenBank accession number.

3.3 Results

3.3.1 Phylogenetic analysis of *Henckelia* inferred from nrITS sequences.

The analysis of nrITS combined new data with the previous Gesneriaceae study of Middleton and Triboun (2012). The nrITS matrix comprised 123 sequences of Gesneriaceae and *Henckelia* from this study (table 3.2 and Middleton & Triboun, 2010). The nrITS sequences varied from 583-879 characters in length. The aligned matrix was 1006 base pairs (bp) long; after excluding ambiguous positions, 211 characters were constant, 112 characters are variable but parsimony-uninformative, and 683 characters were parsimony-informative.

The tree search conducted using MP resulted in 2400 trees of length 17192. The consistency index (CI)=0.10, homoplasy index (HI)=0.90, retention index (RI)=0.19, and rescaled consistency index (RC)=0.02. The bootstrap support (BS) percentages (≥ 50 % BS) are described as low (50-74 % BS), moderate (75-84 % BS), or high (85-100 % BS).

The bootstrap values are shown plotted on to the Bayesian tree in Fig. 3.5 with BS and posterior probabilities (PP); the parsimony bootstrap consensus tree is shown in Appendix 3.10.

The analysis of nrITS found *Henckelia* sensu Weber and Burt (1997) to be monophyletic (1.00 PP) i.e. *Henckelia incana* and *H. floccosa* except for *H. minima* and revealed a number of highly supported clades including *Henckelia dielsii* (100% BS; 1.00 PP), *H. rotundata* (100% BS; 1.00 PP), *Henckelia urticifolia* (100% BS; 1.00 PP), *H. pumila* (100% BS; 1.00 PP), *H. amplexifolia* (100% BS; 1.00 PP), *H. speciosa* (100% BS; 1.00 PP), *H. grandifolia* (100% BS; 0.99 PP), *H. pumila* (100% BS; 1.00 PP), *Tribounia* (100%BS; 0.98 PP), *Didymocarpus* (100%BS; 0.95 PP), *Gyrocheilos* (100%BS; 1.00 PP), *Microchirita* (100%BS; 0.96 PP), *Codonoboea* (99%BS; 0.99 PP), *Streptocarpus* (99%BS; 0.99 PP), *Ornithoboea* (98%BS; 0.99 PP), *Dorcoceras* (100%BS; 0.97 PP), *Damrongia* (100%BS; 1.00 PP), *Boeica* (100%BS; 1.00 PP) and *Rhynchotechum* (99%BS; 0.99 PP).

Inter-relationships of some of these *Henckelia* species are resolved (such as the grouping of *H. speciosa* with *H. grandifolia* (0.99 PP) and the grouping of *H. anachoreta* and *H. rotundata* (0.97 PP). The grouping of *Henckelia incana* with *H. floccosa* is also highly supported (100% BS; 0.84 PP).

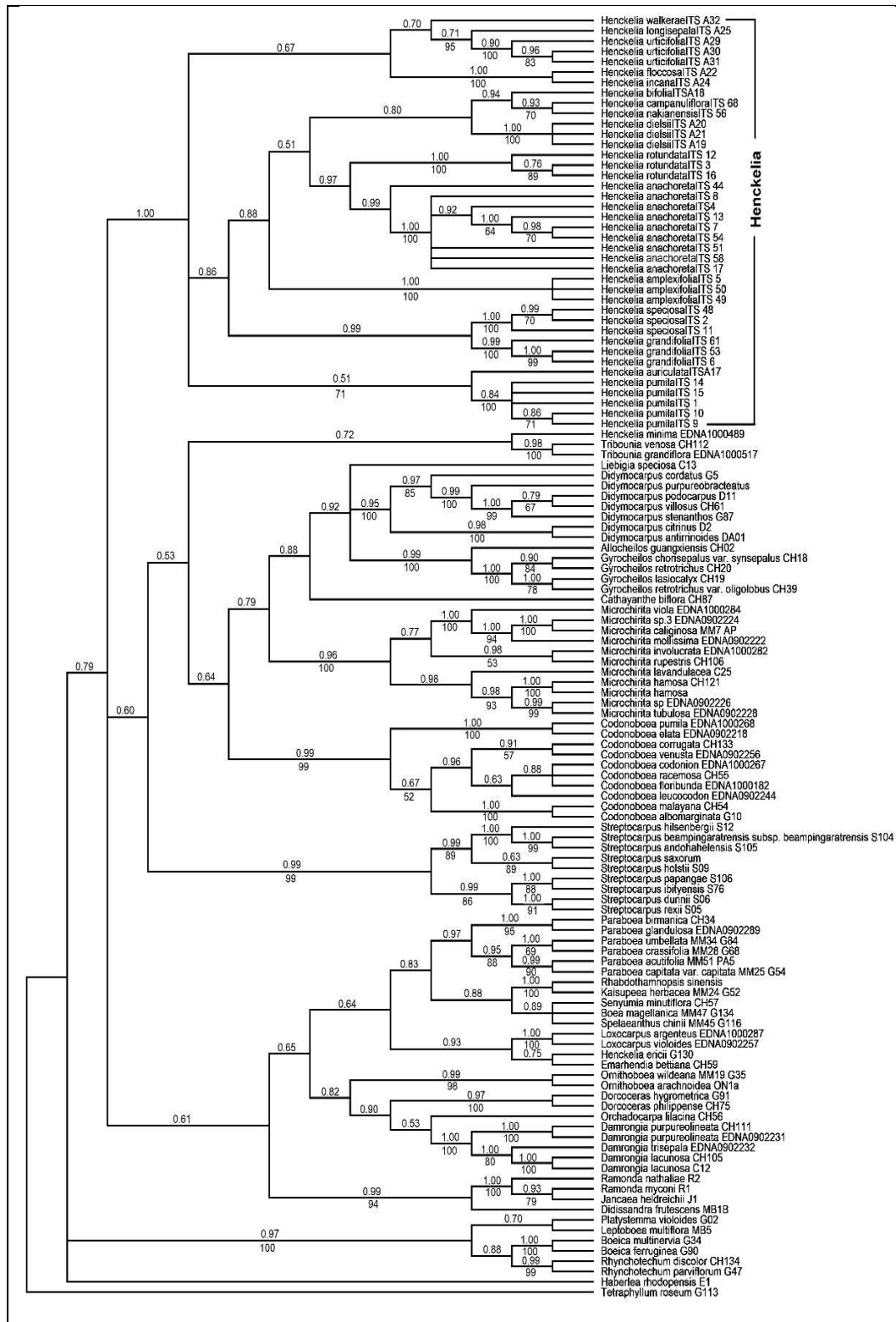


Figure 3.5 Majority-rule consensus Bayesian inference tree based on nrITS shown as a cladogram. Bayesian posterior probabilities (PP) ≥ 0.50 are shown above branches and bootstrap values (BS) from the MP analysis are shown below branches.

3.3.2 Phylogenetic analysis of *Henckelia* inferred from *trnL-F* sequences.

The analyses combined new data with the previous Gesneriaceae study of Middleton and Triboun (2010). The *trnL-F* matrix comprised 118 sequences of Gesneriaceae and *Henckelia* (table 3.2 and Middleton & Triboun, 2010). The *trnL-F* sequences varied in length from 761-970 bp. The aligned matrix was 1122 bp long; after excluding ambiguous positions, 641 characters were constant, 203 characters were variable but parsimony-uninformative, and 278 characters were parsimony-informative.

The tree search conducted using MP resulted in 199,400 trees of length 2069. CI=0.33, HI=0.67, RI=0.08, and RC=0.03. BS percentages (≥ 50 % BS) are described as low (50-74 % BS), moderate (75-84 % BS), and high (85-100 % BS).

The bootstrap values are shown plotted on to the Bayesian in Fig. 3.6. The parsimony bootstrap consensus tree is shown in appendix 3.11.

The *trnL-F* tree is highly congruent with the nrITS tree. Most *Henckelia* species group together in a clade but it is not strongly supported (0.72 PP). However, a number of genera including *Allocheilos*, *Gyrocheilos*, *Leibigia*, *Didymocarpus* and *Cathayanthe* form a well supported clade with *Henckelia* (1.00 PP). Genera allied to *Henckelia* are strongly supported as monophyletic including the *Rhynchotechum* (100% BS; 1.00 PP), *Streptocarpus* (100% BS; 1.00 PP), *Tribounia* (100% BS; 1.00 PP), *Dorcoceras* (100% BS; 1.00 PP), *Damrongia* (98% BS; 1.00 PP), *Loxocarpus* (98% BS; 1.00 PP), *Microchirita* (99% BS; 1.00 PP), *Gyrocheilos* (100% BS; 1.00 PP), *Henckelia urticifolia* (94% BS; 1.00 PP), *H. pumila* (96% BS; 1.00 PP), *H. amplexifolia* (99% BS; 1.00 PP), *H. grandifolia* (92% BS; 1.00 PP), *H. rotundata* (99% BS; 1.00 PP) and the group of *H. incana* and *H. floccosa* (100% BS; 1.00 PP).

Inter-relationships of *Henckelia* species were also consistent with the nrITS tree. Two *Henckelia* species did not group with the core *Henckelia* clade, namely *H. longisepala* and *H. walkerae*.

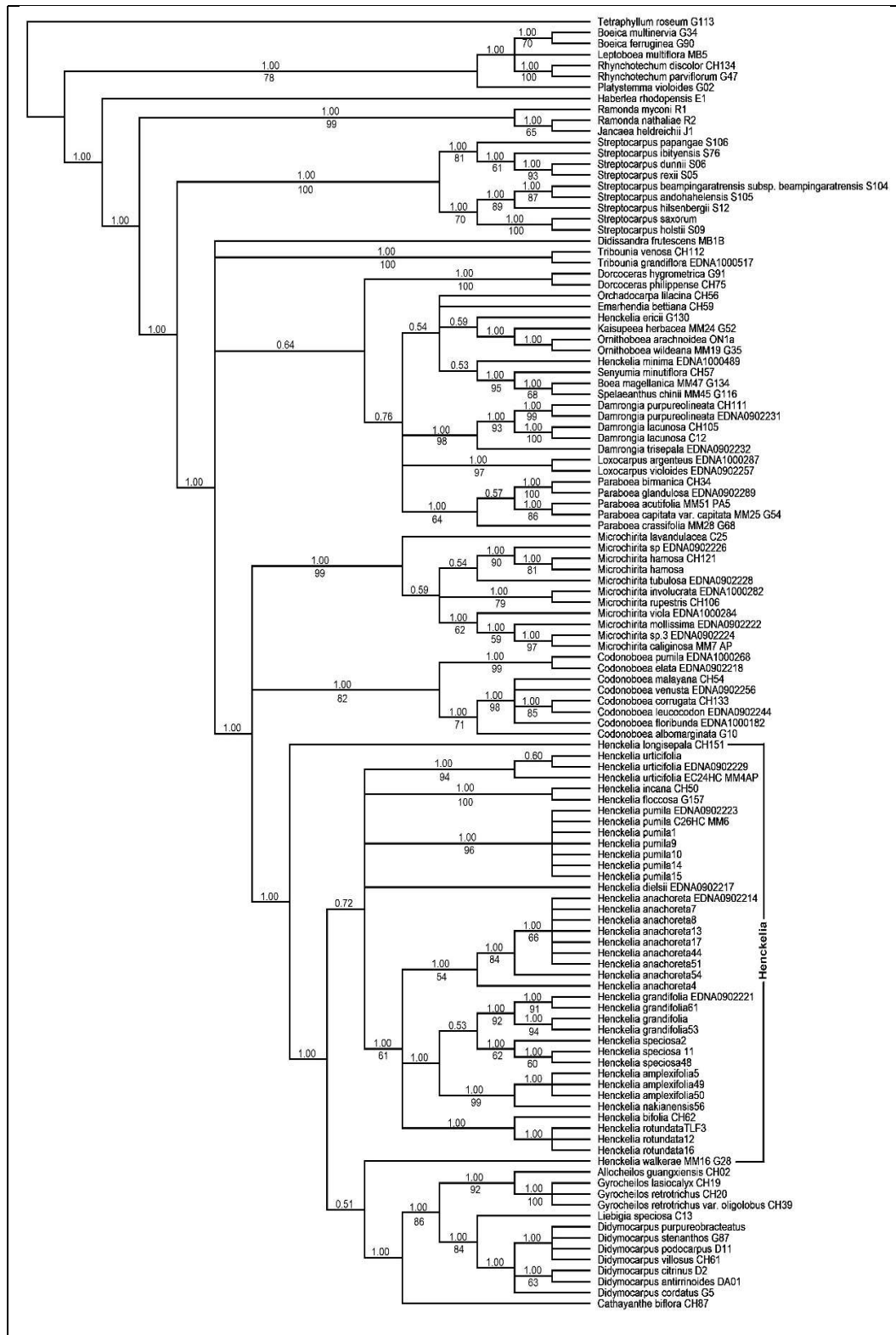


Figure 3.6 Majority-rule consensus Bayesian inference tree based on the *trnL-F* genes region shown as a cladogram. Bayesian posterior probabilities (PP) ≥ 0.50 are shown above branches and the MP bootstrap values (BS) are shown below branches.

3.3.3 Phylogenetic analysis of *Henckelia* inferred from combined nrITS and *trnL-F* sequences.

The analyses combined new data with the previous Gesneriaceae study of Middleton and Triboun (2010). The combined nrITS and *trnL-F* matrix comprised 236 sequences of Gesneriaceae and *Henckelia* (table 3.2 and Middleton & Triboun, 2010). The combined nrITS and *trnL-F* sequences varied from 583-970 bp in length. The aligned matrix was 2005 bp long; after excluding ambiguous positions, 861 characters were constant, 343 characters were variable but parsimony-uninformative, and 801 were characters are parsimony-informative.

The tree search conducted using MP resulted in 12001 trees of length 10444. CI=0.20, HI=0.81, RI=0.10, and RC=0.02. BS percentages ($\geq 50\%$ BS) are described as low (50-74 % BS), moderate (75-84 % BS), and high (85-100 % BS).

The bootstrap values are shown plotted on to the Bayesian tree in Fig. 3.7. The parsimony bootstrap consensus tree is shown in Appendix 3.12.

The combined data tree is well resolved and most groups receive high support values. Highly supported clades include *Boeica* (100% BS; 1.00 PP), *Rhynchotechum* (100% BS; 1.00 PP), *Streptocarpus* (100% BS; 1.00 PP), *Microchirita* (100% BS; 1.00 PP), *Henckelia urticifolia* (100% BS; 1.00 PP), *H. pumila* (100% BS; 1.00 PP), *H. amplexifolia* (100% BS; 1.00 PP), *H. grandifolia* (99% BS; 1.00 PP), *H. speciosa* (100% BS; 1.00 PP), *H. anachoreta* (99% BS; 1.00 PP) and *H. rotundata* (100% BS; 1.00 PP). *Henckelia incana* and *H. floccosa* are highly supported (100% BS; 1.00 PP) as a monophyletic group. As with the single gene analyses the genera allied to *Henckelia* are resolved in the same pattern. The genus *Henckelia* was found to be sister to a group of other genera including *Allocheilos*, *Cathayanthe*, *Didymocarpus*, *Gyrocheilos* and *Liebigia* (1.00 PP). The genus *Codonoboea* outlines this *Henckelia*, *Allocheilos*, *Cathayanthe*, *Didymocarpus*, *Gyrocheilos* and *Liebigia* group (1.00 PP). Within the core *Henckelia* clade two large groups can be defined: Clade 1) *H. dielsii*, *H. amplexifolia*, *H. nakianensis*, *H. grandifolia*, and *H. speciosa* (1.00 PP) with *H. anachoreta* as its sister taxon (1.00 PP); and Clade 2) *H. bifolia* and *H. rotundata*. Clade 1 and 2 are sister to each other. A group of *Henckelia longisepala* and *H. urticifolia* are the most outlying taxa of *Henckelia*, followed by *H. walkerae* and *H. pumila* that diverge successively from the next most basal nodes.

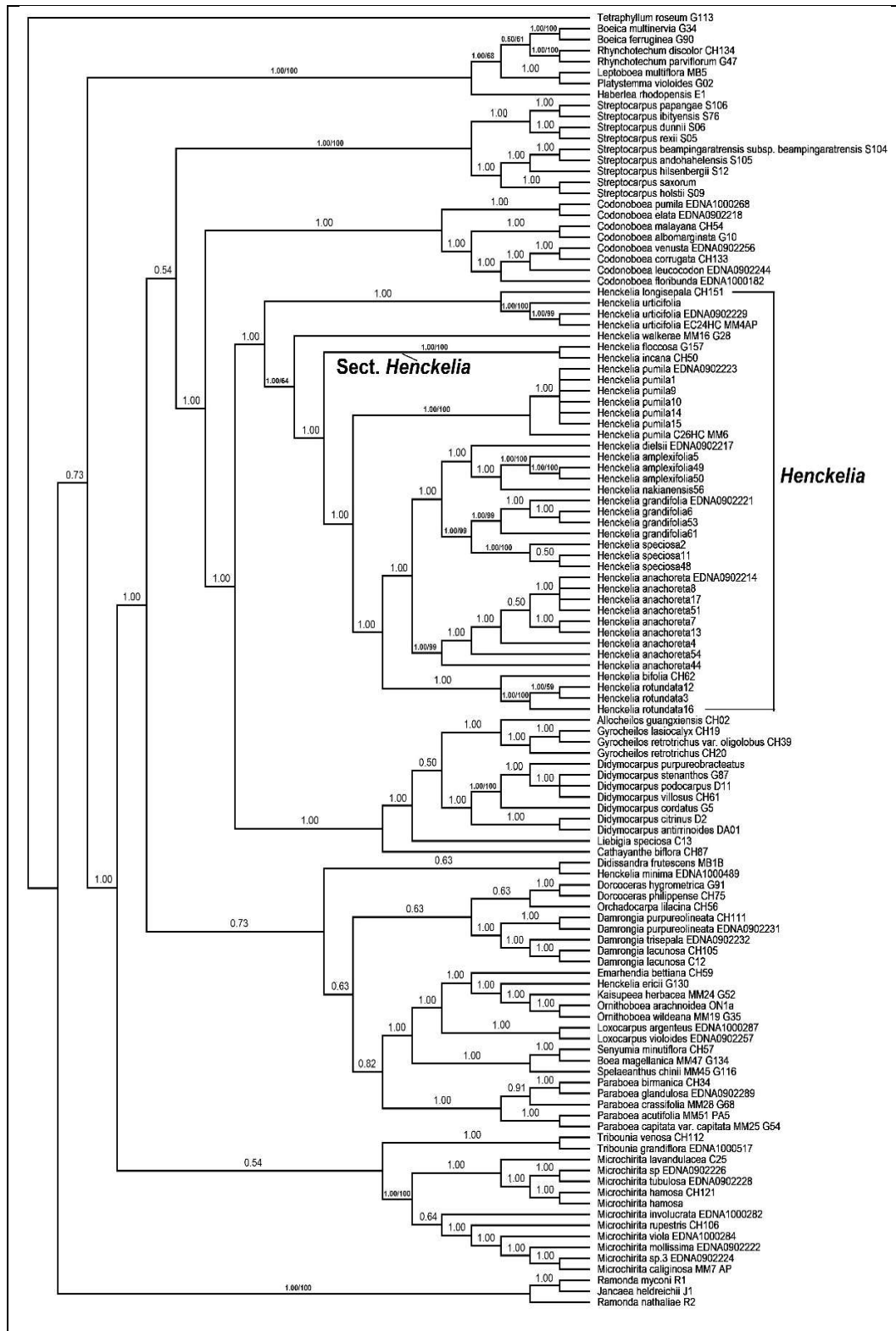


Figure 3.7 Majority-rule consensus Bayesian inference tree based on the nrITS and *trnL-F* genes shown as a cladogram. Bayesian posterior probabilities (PP) ≥ 0.50 are shown above branches and the MP bootstrap values (BS) are shown below branches.

3.3.4 Phylogenetic analysis of *Henckelia* inferred from nrITS sequences (reduced dataset).

A phylogenetic analysis of nrITS was also undertaken using a smaller dataset focused on *Henckelia*. The nrITS matrix comprised 40 sequences of *Henckelia* including 16 species (eight species from this study and eight species from GenBank) and with three species as out groups (table 3.2). The total of 43 nrITS sequences varied from 598-929 bp in length. The aligned matrix was 999 bp long; after excluding ambiguous positions, 512 characters were constant, 181 characters were variable but parsimony-uninformative, and 306 characters were parsimony-informative.

The tree search conducted using MP resulted in 2,041 trees of length 1,216. CI=0.58, HI=0.42, RI=0.70 and RC=0.41. BS percentages (≥ 50 % BS) are described as low (50-74 % BS), moderate (75-84 % BS), and high (85-100 % BS).

The bootstrap values are shown plotted on the Bayesian tree in Fig. 3.8. The parsimony bootstrap consensus tree is shown in Appendix 3.13.

Highly supported clades include the *Henckelia amplexifolia* group (100 % BS; 1.0 PP), *H. anachoreta* (86 % BS; 1.0 PP), *H. dielsii* (100 % BS; 1.00 PP), *H. grandifolia* (99 % BS; 1.00 PP), *H. rotundata* (100 % BS; 1.00), *H. pumila* (100 % BS; 1.00), and *H. speciosa* (100 % BS; 1.00 PP). *Henckelia floccosa* and *H. incana* are highly supported (100 % BS; 1.00 PP) as a monophyletic group. *Henckelia bifolia* is a sister group to *H. dielsii* with low support (52 % BS but a PP of 1.0). Inter-relationships among these clades are not clearly supported but are consistent with the large combined ITS and *trnL-F* tree (Fig. 3.7). There is support for Section *Henckelia* but there is no support for or against Section *Chirita*. The tree does however separate the species well.

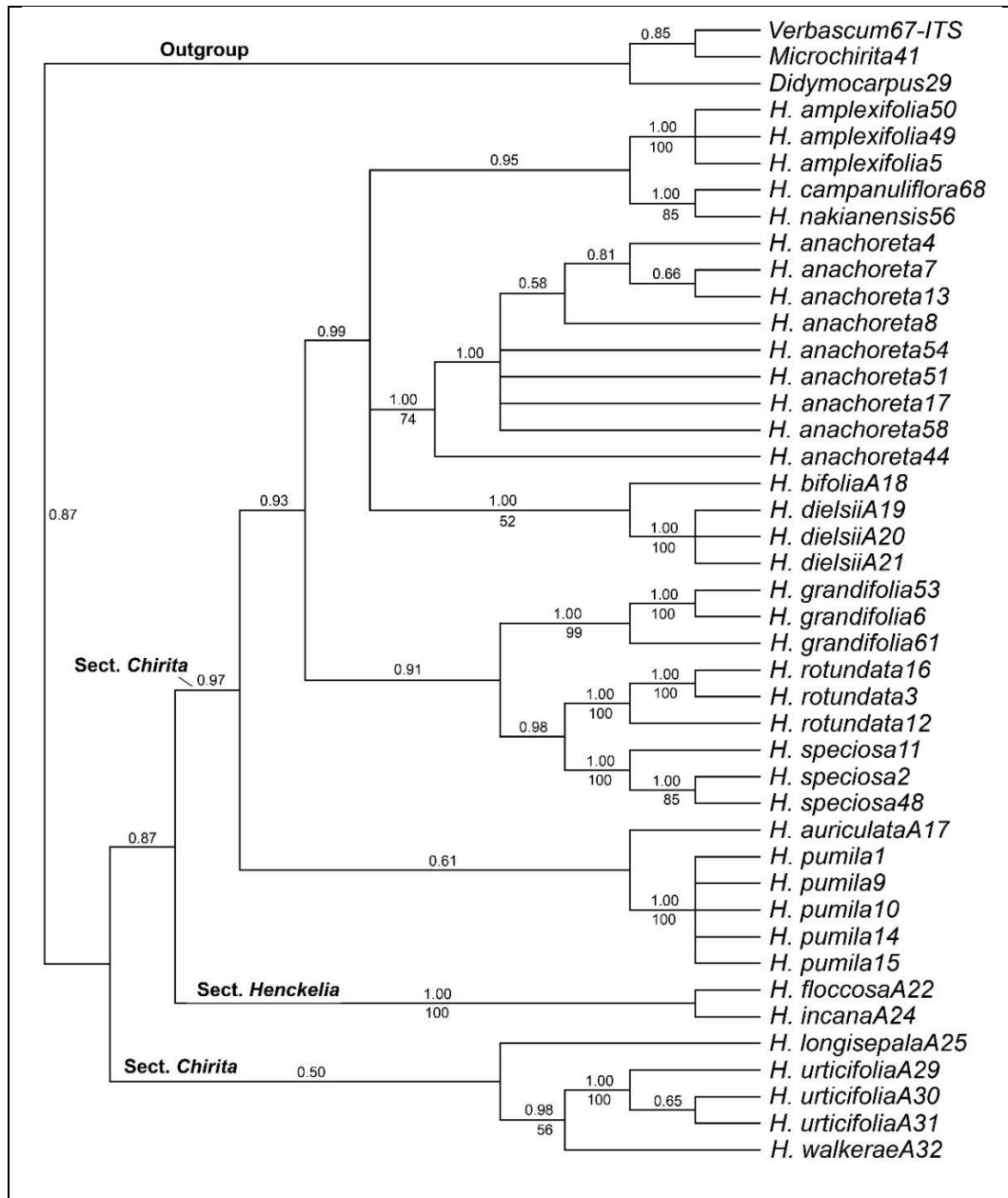


Figure 3.8 Majority-rule consensus Bayesian tree based on the nrITS DNA shown as a cladogram. Bayesian posterior probabilities (PP) ≥ 0.50 are shown above branches and the MP bootstrap values (BS) are shown below branches.

3.3.5 Phylogenetic analysis of *Henckelia* inferred from *trnL-F* sequences.

The *trnL-F* matrix comprised 39 sequences of *Henckelia* including 15 species (7 species from this study and 8 species from GenBank) and with three species as out groups (table 3.2). The total of 42 cpDNA *trnL-F* sequences varied from 774-908 bp in length. The aligned matrix was 932 bp long after excluding ambiguous positions, 739 characters were constant, 130 characters were variable but parsimony-uninformative, and 63 characters were parsimony-informative.

The tree search conducted using MP resulted in 12001 trees of length 263. CI=0.84, HI=0.16, RI=0.79, and RC=0.66. BS percentages (≥ 50 % BS) are described as low (50-74 % BS), moderate (75-84 % BS), and high (85-100 % BS).

The MP bootstrap values are shown plotted on the Bayesian tree in Fig. 3.9 with a bootstrap values (BS) and posterior probabilities (PP). The parsimony bootstrap consensus tree is show in Appendix. 3.14.

Highly supported clades include *Henckelia amplexifolia* group (94% BS; 1.00 PP), *H. grandifolia* (87% BS; 1.00 PP), *H. rotundata* (87% BS; 1.00 PP), *H. dielsii* (99% BS; 1.00 PP), and *H. urticifolia* (95% BS; 1.00 PP) and in Sect. *Henckelia*, the highly supported clades (100% BS; 1.00 PP) are *H. incana* and *H. floccosa*.

Inter-relationships among these clades are not clearly supported and they form a large polytomy. There is support for Section *Henckelia* (*H. floccosa* and *H. incana*) but there is no support for or against Section *Chirita* because of the lack of resolution and low supported values. The tree does however separate the species well.

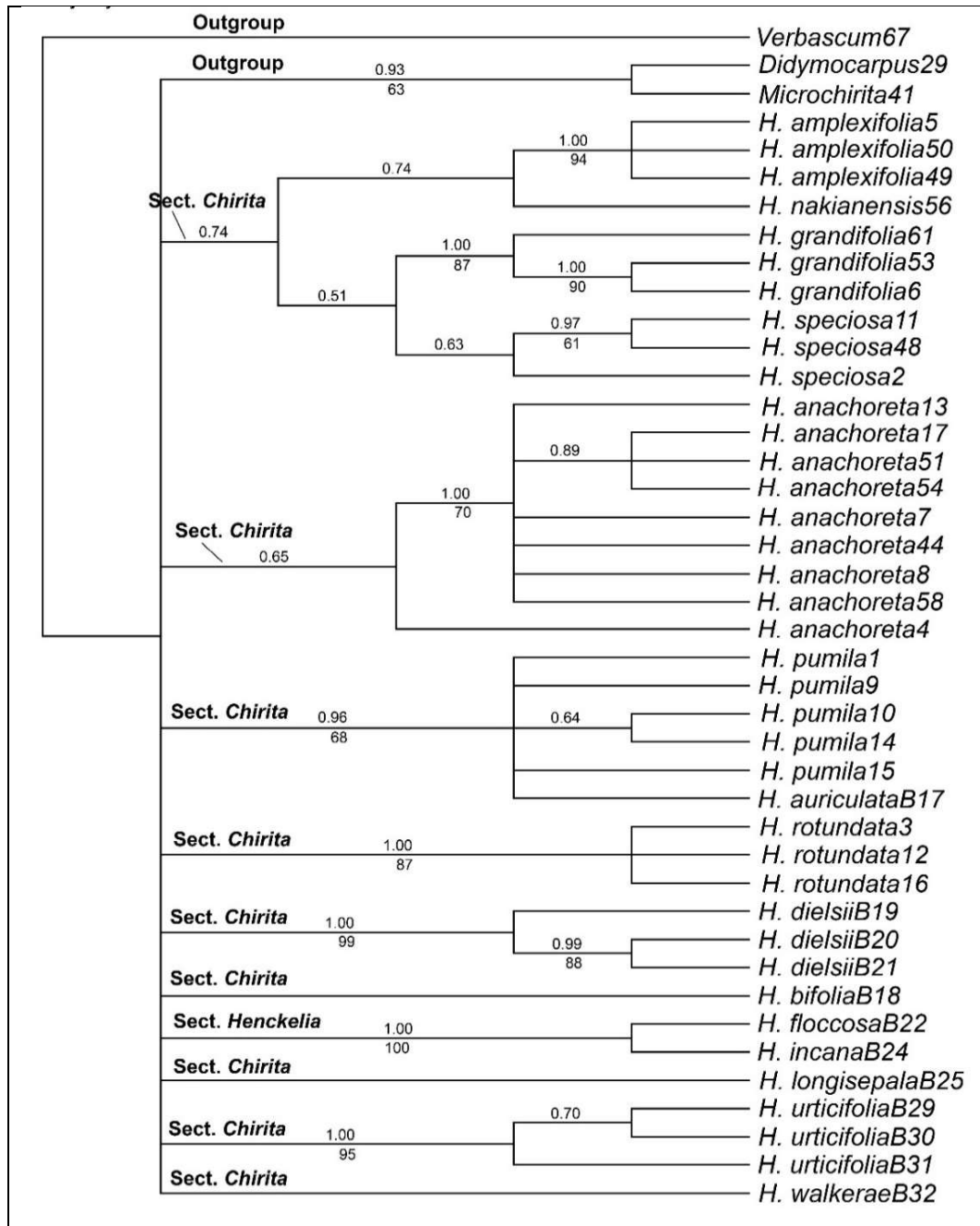


Figure 3.9 Majority-rule consensus Bayesian inference tree based on the *trnL-F* gene region shown as a cladogram. Bayesian posterior probabilities (PP) ≥ 0.50 are shown above branches and the MP bootstrap values (BS) are shown below branches.

3.3.6 Phylogenetic analysis of *Henckelia* inferred from combined nrITS and *trnL-F* sequences.

The nrITS and *trnL-F* matrix comprised 39 sequences of nrITS and 39 sequences of *trnL-F* of *Henckelia* including 15 species (7 species from this study and 8 species from GenBank) and with three species as the out group (table 3.2). The total of 78 nrITS and cpDNA *trnL-F* sequences varied from 598-929 bp in length. The aligned matrix was 1,914 bp long after excluding ambiguous positions, 1267 characters were constant after excluding ambiguous positions, 283 characters were variable, and 364 characters were parsimony-informative.

The tree search conducted using MP resulted in 12001 trees of length 1,411. CI=0.64, HI=0.36, RI=0.74, and RC=0.48. BS percentages (≥ 50 % BS) are described as low (50-74 % BS), moderate (75-84 % BS), and high (85-100 % BS).

The bootstraps values are shown plotted on to the Bayesian tree in Fig. 3.10. The bootstrap maximum parsimony consensus tree is shown in Appendix 3.15.

Highly supported clades include *Henckelia urticifolia* (100% BS; 1.00 PP), *H. pumila* (100% BS; 1.00 PP), *H. speciosa* (100% BS; 1.00 PP), *H. rotundata* (100% BS; 1.00 PP), *H. amplexifolia* (100% BS; 1.00 PP), and *H. grandifolia* (98% BS; 1.00 PP). *Henckelia incana* and *H. floccosa* are highly supported (100% BS; 1.00 PP) as a monophyletic group.

Inter-relationships among these clades are not clearly supported. There is support for Section *Henckelia* but there is no support or against Section *Chirita* because of the low resolution and support of the backbone of the tree. The tree does however separate the species well.

3.3.7 Phylogenetic analysis of *Henckelia* inferred from combined cpDNA (*trnL-F*, *atpB-rbcL*, and *rps16*) sequences.

The combined cpDNA (*trnL-F*, *atpB-rbcL*, and *rps16*) matrix comprised 116 sequences of *Henckelia* including 6 species (all from this study) and with three species as the out group (table 3.2). The total 29 cpDNA dataset varied from 678-940 characters in length. The aligned matrix was 2,747 base pairs after excluding ambiguous positions, 2,310 characters were constant, 292 characters were variable but parsimony-uninformative, and 145 characters were parsimony-informative.

The tree search conducted using MP resulted in 12001 trees of length 542. CI=0.89, HI=0.12, RI=0.86, and RC=0.76. BS percentages (≥ 50 % BS) are described as low (50-74 % BS), moderate (75-84 % BS), and high (85-100 % BS).

The bootstraps values are shown plotted on to the Bayesian tree in Fig. 3.11. The bootstrap consensus tree is shown in Appendix 3.16.

Highly supported clades include *Henckelia amplexifolia* (100% BS; 1.00 PP), *H. anachoreta* (100% BS; 1.00 PP), *H. rotundata* (100% BS; 1.00 PP), *H. grandifolia* (99% BS; 1.00 PP), *H. speciosa* (96% BS; 1.00PP), and *H. pumila* (100% BS; 1.00 PP). Inter-relationships among these clades are not clearly supported. There is high support for Section *Chirita* and the out group (100% BS; 1.00 PP).

Inter-relationships among these clades are not clearly supported. There is high support for Section *Chirita* and the out group (100% BS; 1.00 PP).

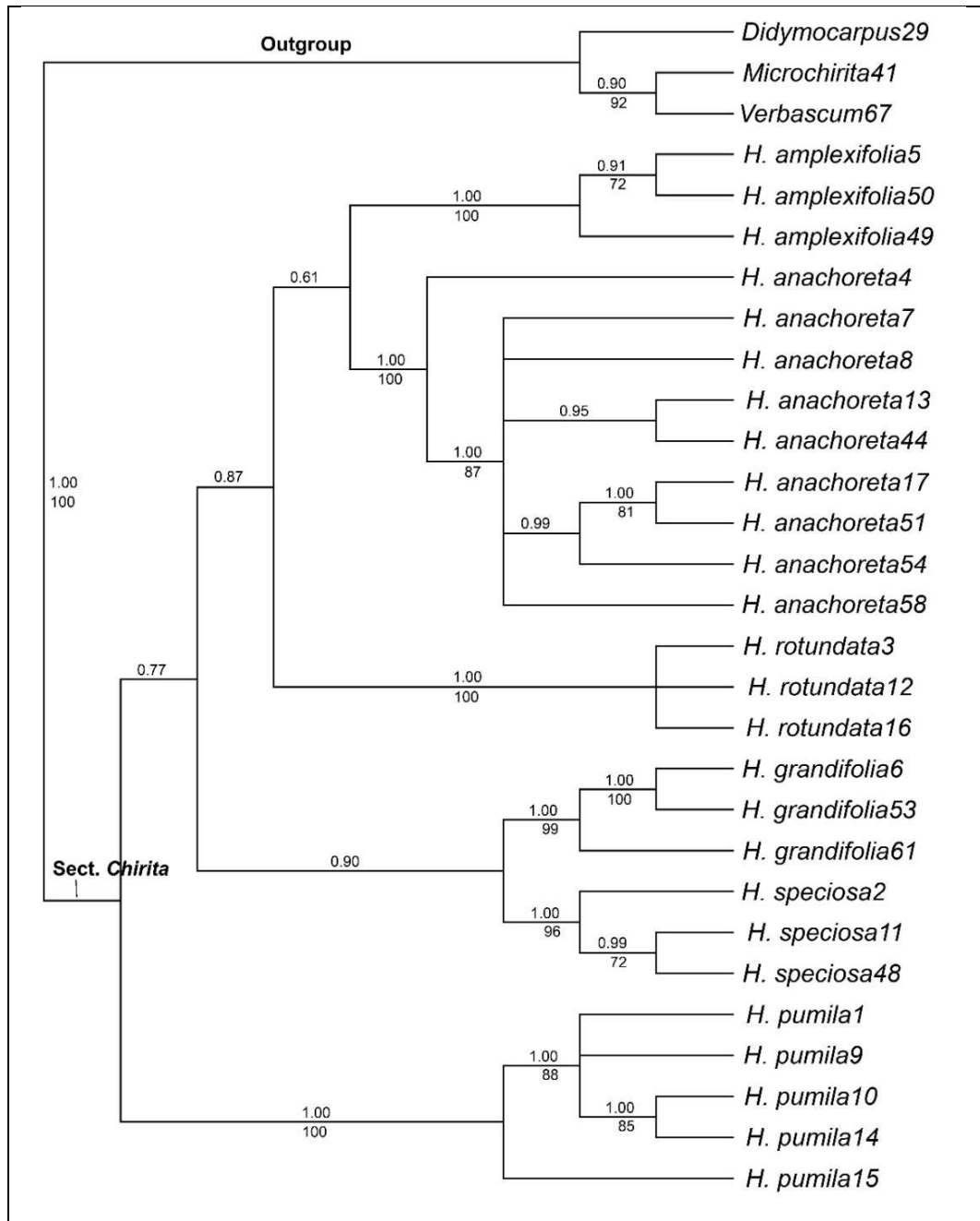


Figure 3.11 Majority-rule consensus Bayesian inference tree based on the combined chloroplast genes (*atpB-rbcL*, *rps16* and *trnL-F*) shown as a cladogram. Bayesian posterior probabilities (PP) ≥ 0.50 are shown above branches and the MP bootstrap values (BS) are shown below branches.

3.3.8 Phylogenetic analysis of *Henckelia* inferred from combined nrITS and cpDNA (*trnL-F*, *atpB-rbcL*, and *rps16*) sequences.

The combined nrITS and cpDNA (*trnL-F*, *atpB-rbcL*, and *rps16*) matrix comprised 28 sequences of each genes of *Henckelia* including 6 species (all from this study) and with three species as the out group (table 3.2). The total 112 nrITS and cpDNA dataset varied from 785-940 bp in length. The aligned matrix was 3,563 bp long after excluding ambiguous positions, 3,002 characters were constant, 377 characters were variable but parsimony-uninformative, and 184 characters were parsimony-informative.

The tree search conducted using MP resulted in 12,001 trees of length 542. CI=0.54, HI=0.46, RI=0.05, and RC=0.20. BS percentages (≥ 50 % BS) are described as low (50-74 % BS), moderate (75-84 % BS), and high (85-100 % BS).

The bootstraps values are shown plotted on to the Bayesian tree in Fig. 3.11. The parsimony bootstrap consensus tree is shown in Appendix 3.17.

Highly supported clades include *Henckelia amplexifolia* (100% BS; 1.00 PP), *H. anachoreta* (100% BS; 1.00 PP), *H. rotundata* (100% BS; 1.00 PP), *H. grandifolia* (99% BS; 1.00 PP), *H. speciosa* (98% BS; 1.00PP), and *H. pumila* (100% BS; 1.00 PP).

Inter-relationships among these clades are generally not clearly supported. There is support for Section *Chirita* (100% BS; 1.00 PP).

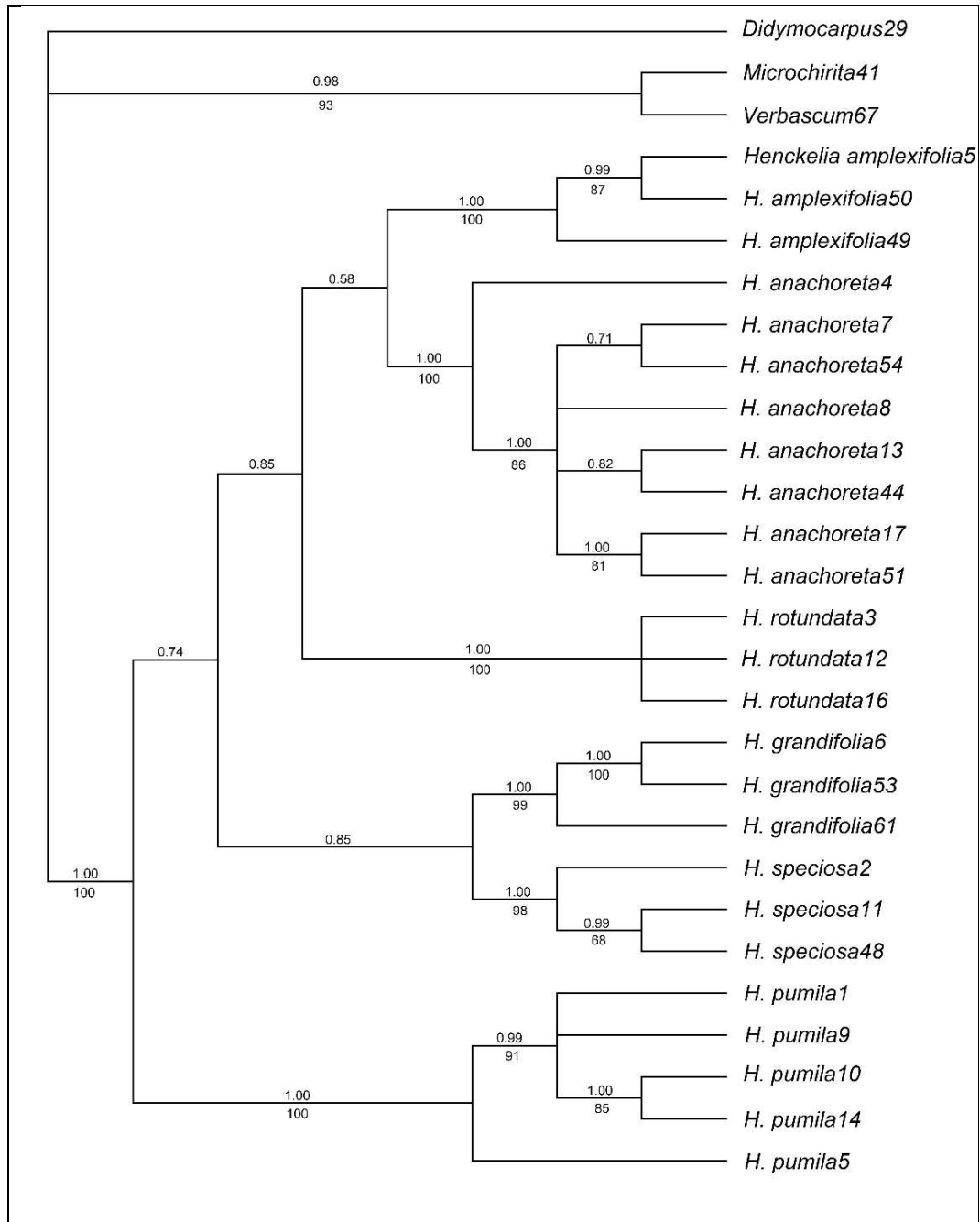


Figure 3.12 Majority-rule consensus Bayesian inference tree based on the nrITS, *atpB-rbcL*, *rps16* and *trnL-F* shown as a cladogram. Bayesian posterior probabilities (PP) ≥ 0.50 are shown above branches and bootstrap values (BS) are shown below branches.

Discussion

Henckelia was named by Sprengel (1817) but has a confused history linked with *Didymocarpus* (Jack, 1825; Sprengel, 1827; Wallich, 1829 and Dietrich, 1831) until *Didymocarpus* was remodelled (Weber and Burt, 1997). Weber and Burt (1997) separated *Henckelia* from *Didymocarpus* with morphological characters. *Henckelia* was distinguished from *Didymocarpus* by having an infundibuliform or campanulate corolla tube, bilobed or subpeltate stigma and plagiocarpic capsule. *Didymocarpus* has a salverform, infundibuliform or personate corolla, entirely capitate stigma and an orthocarpic capsule (Nangngam and Maxwell, 2013). *Henckelia* taxa have also been classified as *Chirita* (Wood, 1974) which was first described by Don (1822). *Chirita* has two fertile stamens like *Didymocarpus* but differs by having a bilobed stigma. Thus, phylogenetic studies are required to delimit *Henckelia* and assess its infrageneric classification.

The analyses of plastid and nuclear genes presented in this chapter are broadly congruent and have helped define major groups of taxa and their inter-relationships. It includes 10 taxa from Thailand and surrounding countries of which four taxa (*Henckelia amplexifolia*, *H. campanuliflora*, *H. nakianensis*, and *H. rotundata*) are sequenced for the first time here. The combined nrITS and *trnL-F* tree is the most comprehensive, well resolved and supported (Fig. 3.7). The part of it containing *Henckelia* and its closest allies is shown in Fig. 3.13. A *Henckelia* clade containing group A-F is well supported (1.0 PP). Thus, the genus *Henckelia* sensu Weber and Burt (2011) and Weber and Burt (1997) is monophyletic. The type species of the genus is *Henckelia incana* (group D) which embedded with this clade. It is sister to *H. floccosa* and both are part of *Henckelia* section *Henckelia* sensu Weber and Burt (1997).

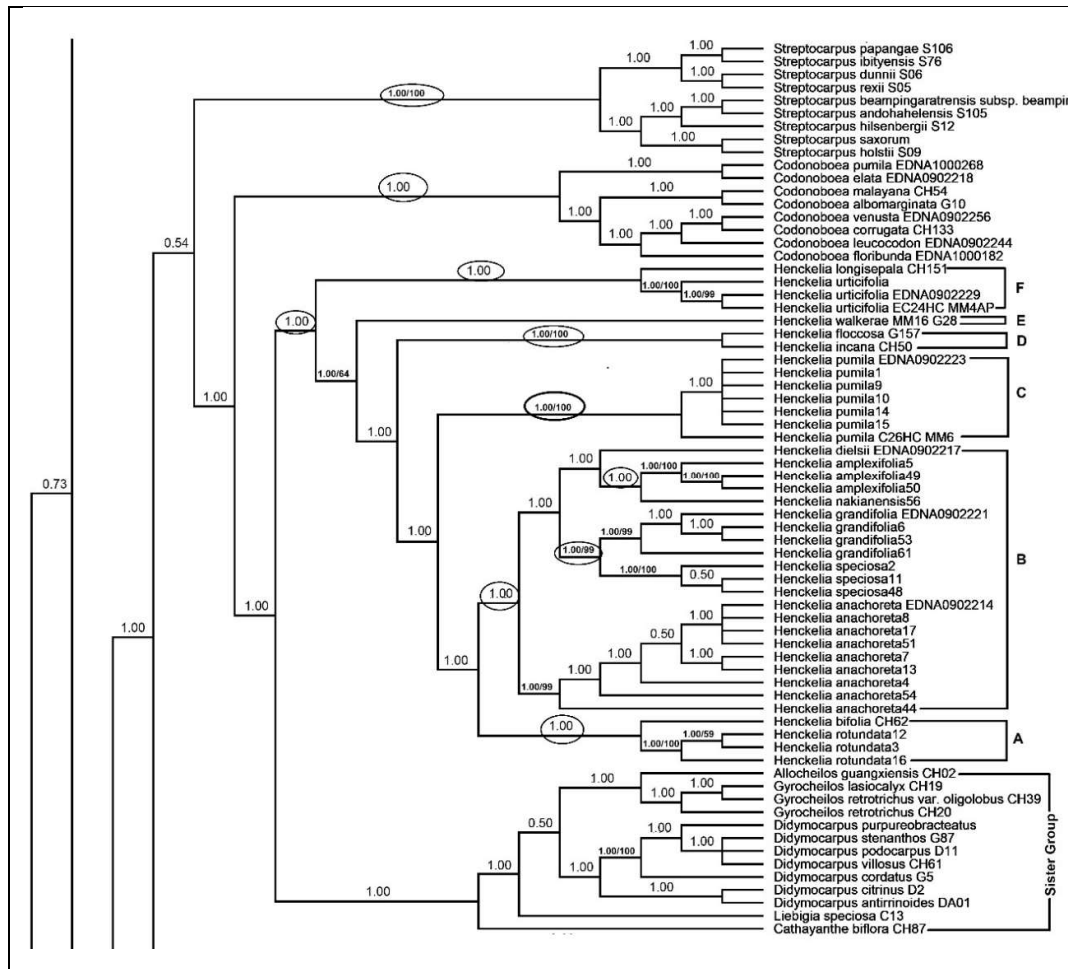


Figure 3.13 Cladogram of the majority-rule consensus Bayesian inference tree from the full analysis on combined *trnL-F* and ITS. For further explanation see Fig. 3.7.

Other phylogenetic studies have helped define *Henckelia* as supported here. For example, *Chirita* as defined at that time was not monophyletic because *Henckelia* sect *Henckelia* (represented by *H. humboldtiana*) formed a clade with *Chirita* sect. *Chirita* (*Chirita pumila*) (Weber and Burtt, 1997; Li and Wang, 2007 and Moller et al., 2009). Wang et al. (2011) separated *Chirita* into separate genera namely *Chirita*, *Microchirita*, *Liebigia*, *Primulina* and *Petrocodon* (Li and Wang, 2007: Fig. 1)

Weber et al. (2011) continued work and found that *Chirita*, *Henckelia* (from southern India) and *Hemiboeopsis* formed a monophyletic group (1.00 PP) (Weber et al., 2011: Fig. 1 and 4) (clade A: Fig. 3.13) (Fig. 3.2, 3.3 and 3.4). According to article 11 of the International Code of Nomenclature for Algae, Fungi, and Plants (Shenzhen Code) (Turland et al., 2018), the priority name of this clade is *Henckelia* because Sprengel described *Henckelia* in 1817 while *Chirita*, was described by Don in 1822 and

Hemiboeopsis, by Wang in 1984. Weber et al. (2011) also found that *Henckelia* from Southern Thailand and Malesia formed a monophyletic group namely *Codonoboaea* (Clade B: Fig. 3.14). *Codonoboaea* was also found to be monophyletic in the analyses present in this chapter (Fig. 3.14)

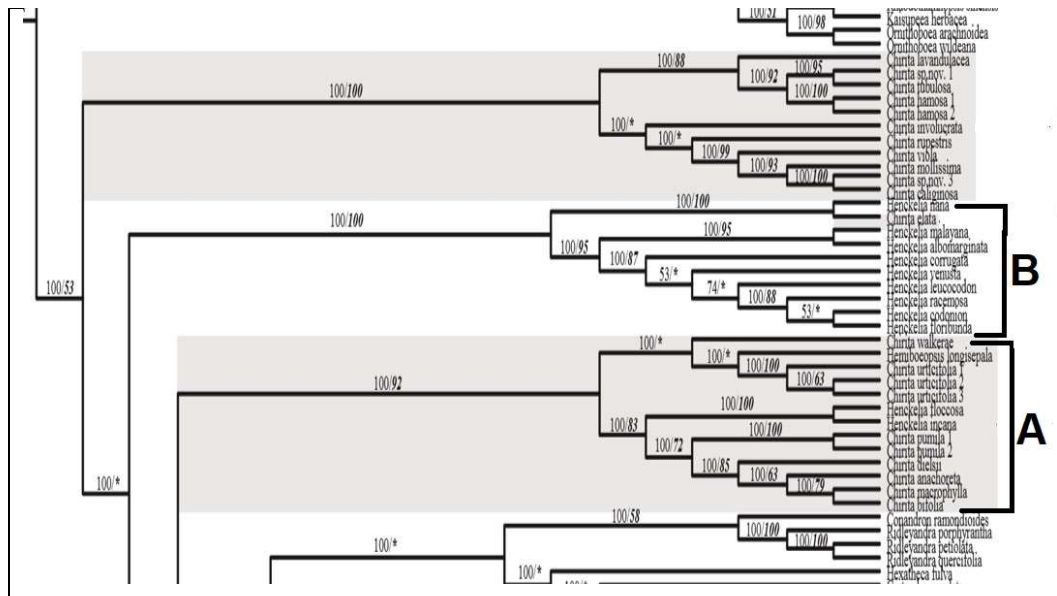


Figure 3.14 Majority-rule consensus Bayesian inference tree of *Henckelia* and allies based on combined *trnL-F* and nrITS sequences. The *Henckelia* clade is A, the *Codonoboaea* clade is B (Weber et al., 2011); Source Taxon 60(3), electronic supplement p S1. Values above branches before “/” are majority rule frequencies and bootstrap values (BS) after “/”.

We found that *Henckelia* was sister to a clade of other genera including *Allocheilos*, *Gyrocheilos*, *Didymocarpus*, *Liebigia* and *Cathayanthe*. This finding is congruent with Middleton (2012). *Codonoboaea* is then sister to this *Henckelia* *Allocheilos*, *Gyrocheilos*, *Didymocarpus*, *Liebigia* and *Cathayanthe* group. Thus, there is support for recognition for this set of genera and their inter-relationships. They are found in tribe Trichosporeae Nees and subtribe Didymocarpaceae (Weber et al., 2013).

The relationships of *Henckelia* species are well resolved and supported in the analyses presented here. Within the core *Henckelia*, two large groups can be defined namely A and B. The largest, Clade B includes *H. dielsii*, *H. amplexifolia*, *H. nakianensis*, *H. grandifolia*, and *H. speciosa* (1.00 PP) with *H. anachoreta* as its sister taxon (1.00 PP). Clade A includes *H. bifolia* and *H. rotundata*. The A and B group are sister to *H. pumila* (C).

A group of *Henckelia longisepala* and *H. urticifolia* (Clade F) are the most outlying taxa of *Henckelia*, followed by *H. walkerae* (E) and *H. incana* and *H. floccosa* (D: Section *Henckelia*) and *H. pumila* that diverge successively from the next most basal nodes (Fig. 3.14).

Clade A, B, and C, of *Henckelia* includes, all species from Thailand and surrounding countries, and clade F only from surrounding countries including India, Nepal, Bangladesh, Bhutan, Myanmar, China, Vietnam and Laos. Group F is the most outlying clade in terms of its phylogeny in *Henckelia*. Clade D occurs in India and Sri Lanka and Clade E in Sri Lanka (Fig. 3.15).

There is some correspondence of the clades with morphological characters i.e. Section *Henckelia* (Type *Henckelia incana* (Vahl) Spreng (Weber and Burtt, 1997)) is in clade D and is distinguished by having an acaulescent, scapigerous, calyx that is divided at the base and is persistent. It has a campanulate corolla tube and plagiocarpic capsule. All other clades (A, B, C, E, F) are in sect. *Chirita* (Type specimen is *Henckelia urticifolia* (Buch.-Ham ex D. Don) A. Dietr.) with caulescent, tubular calyx, infundibuliform or campanulate corolla tube, bilobed stigma with geniculate stamens, and caducous calyx. Thus, section *Chirita* sensu Wood (1974) is not monophyletic. There is also evidence from morphology for the mixing of these with sect. *Henckelia*. For example, *H. speciosa* and *H. glandifolia* have acaulescent habit, *H. amplexifolia* and *H. nakianensis* have campanulate corolla tube and subpeltate stigma and *H. nakianensis* has persistent calyx. There is little congruence with the Cluster analysis represented earlier (see 2.5.2 above), though there is some with for example, *H. nakiansis* and *H. amplexifolia* grouping together in both analyses. The lack of congruence may reflect the differences in the species analysed morphologically and molecularly.

The phylogeography, in figure 3.15 has shown that the clades A, B, C, and F are located in the Indo-Burmese and Indo-Chinese elements but the clades D and E are located in the Western Ghats and Sri Lanka. All elements are within the biodiversity hot spot (Myers et al., 2000).

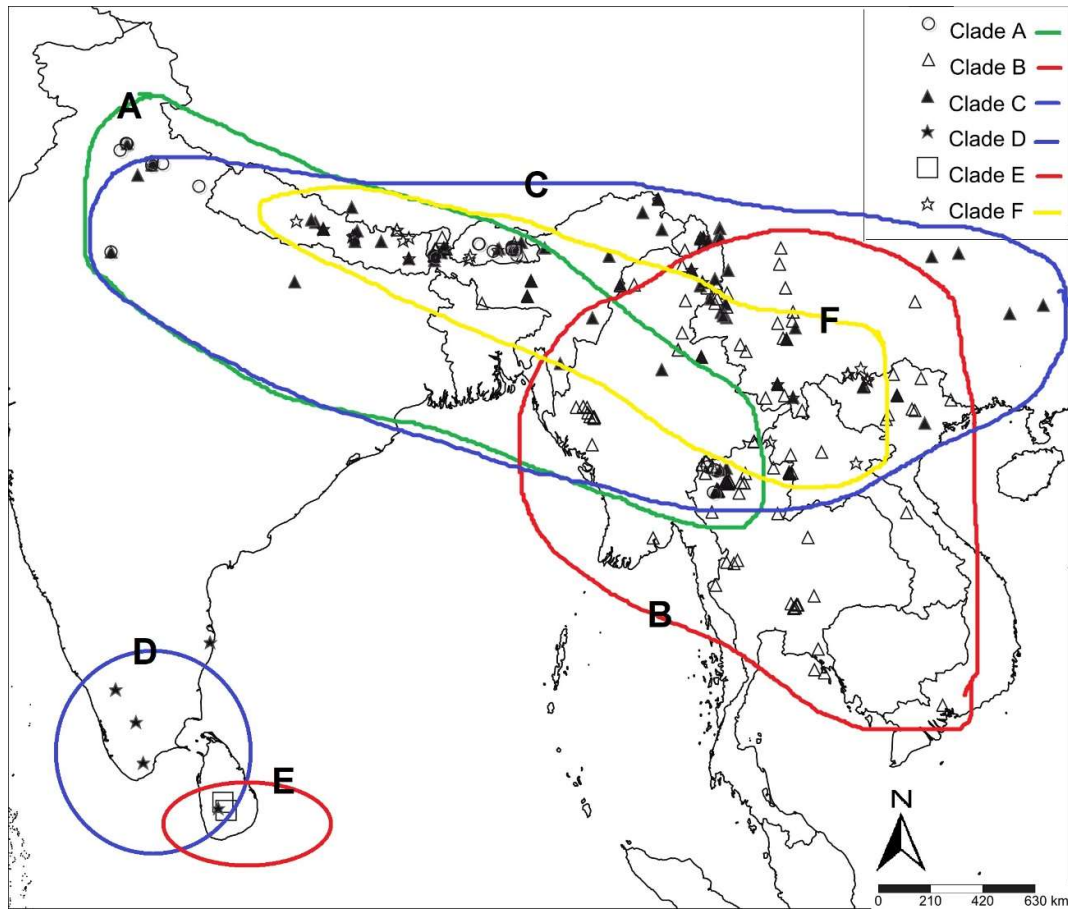


Figure 3.15 Map showing the geographical distribution of taxa in the clades A to F identified in the combined nrITS and *trnL-F* analysis. Base map from <https://www.simplemappr.net>

The current study focused on *Henckelia* from Thailand and surrounding countries (Myanmar, Laos, Vietnam and Cambodia). Although this study had low samples numbers, they are classified well. For example, *Henckelia* Sect. *Henckelia* is always monophyletic. Further work requires more samples and also more molecular markers before a new infrageneric classification at subgenus or sectional level can be produced but the clades recognized here (A to F) are candidate groups that deserve careful scrutiny as potential new infrageneric taxa.

Chapter 4-Taxonomic treatment

4.1 Introduction

The objective of this chapter was to provide a taxonomic revision of *Henckelia* from Thailand, Indo-China (Laos, Vietnam, and Cambodia), including Myanmar. Specimens were examined from various herbaria. Fresh material was collected by the author from Thailand. New fresh material from Myanmar was collected by the Makino Botanical Garden, Singapore Botanic Garden (SING) and Queen Sirikit Botanic Gardens (QBG). Vietnamese material was collected by the Vietnam National Museum of Nature Herbarium (VNMN) and Hanoi Herbarium (HN). It was not possible to collect fresh material from Cambodia and Laos, so only herbarium material was studied. The plant description follows The Thai Forest Bulletin (Botany) Journal and Flora of Thailand book format.

4.2 Material and methods

The descriptions are based on herbarium specimens and fresh materials. For the herbarium specimens, flowers were softened in water or 70% alcohol before measurements were taken. In the conservation assessments, the EOO (Extent of occurrence) and AOO (Area of occupancy) were derived in GeoCAT (Bachman et al. 2011), under default settings. Distribution maps were made using the basemap from <https://www.simplemappr.net>.

4.2.1 Herbarium collections and field study

In Thailand, the field surveys took place in 2016-2017 in the Northern, Central and South-Western regions of Thailand; 14 National Parks, one Ecotourism and Environmental Education Centre, one Royal Project, and two waterfalls were visited.

The northern region surveyed included eight national parks i.e. Doi Inthanon NP, Doi Pha Hom Pok NP, Doi Phu Kha NP, Doi Suthep-Pui NP, Huai Nam Dung NP, Mae Surin NP, Tham Pla- Namtok Pha Suea NP, and Tham Sakoen NP, one Royal Project i.e. Khun Win Royal Project and two waterfalls i.e. Mae Kam Pong Waterfall and Pam Bok waterfall.

In the central region, Khao Yai National Park was surveyed. This park occurs in four provinces i.e. Prachin Buri, Nakhon Nayok, Nakhon Ratchasima and Saraburi. Many other places were also surveyed i.e. Sarika waterfall, Nang Raung waterfall, Heaw Narok waterfall, Heaw Suwat waterfall, Dieo Dai cliff, Khao Khieo view point.

In addition, in the south-western region: five national parks i.e. Erawan NP, Khao Laem NP, Lam Klong Ngu NP, Sri Yok NP, and Srinakharin Dam NP were surveyed. All national parks located in Kanchanaburi Province. The field survey map is shown in Fig. 4.1.

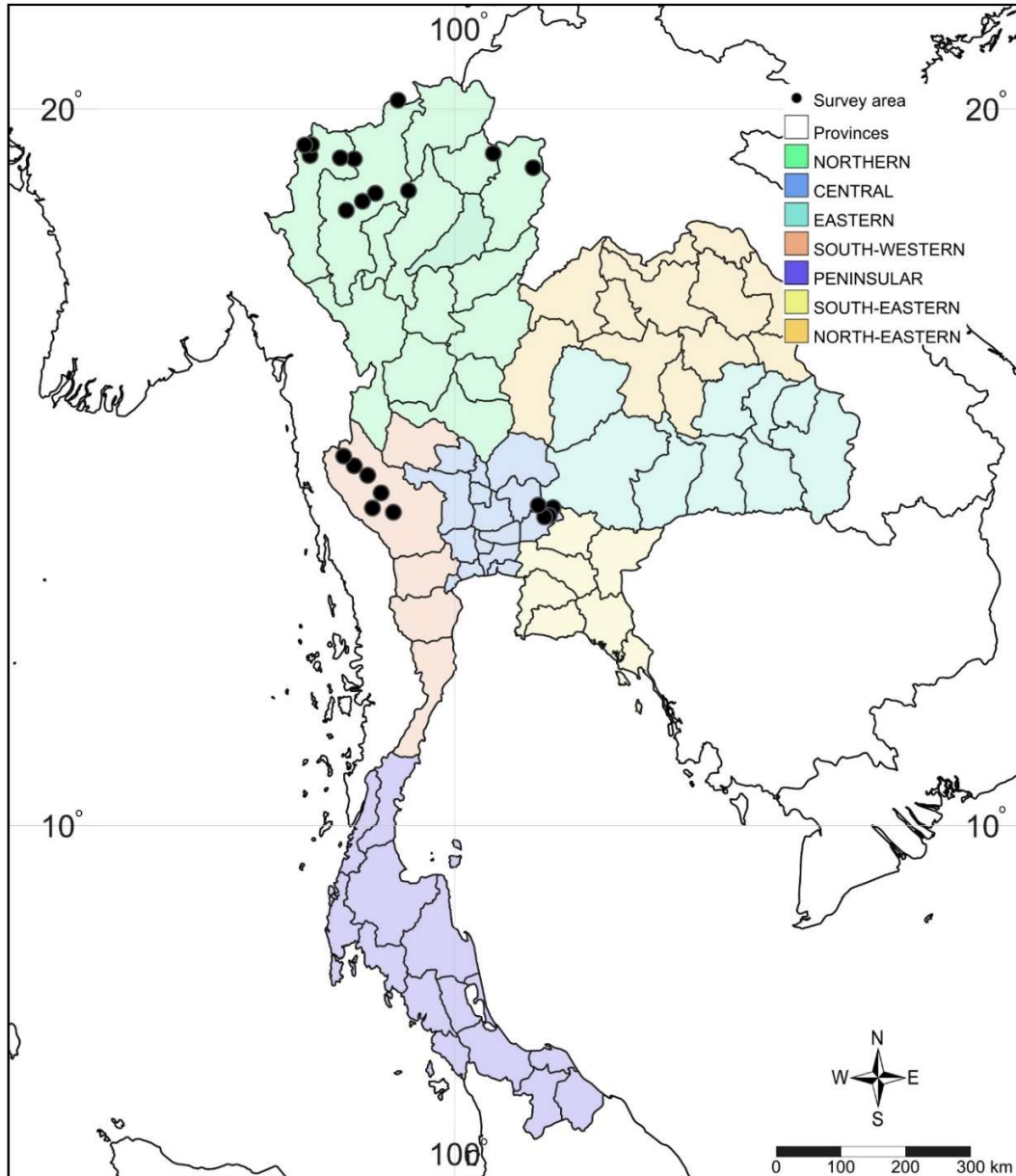


Figure 4.1 Survey area map of plant collections. Base map from <https://www.simplemappr.net>

4.2.2 Herbarium visits

Herbarium specimens were obtained on loan from ten herbaria, they comprised 389 specimens as follows:

Aarhus University Herbarium (AAU)

12 specimens

Aberdeen University Herbarium (ABD)	11 specimens
Bangkok Herbarium (BK)	29 specimens
The Forest Herbarium (BKF)	31 specimens
The Natural History Museum Herbarium (BM)	33 specimens
Royal Botanic Garden Edinburgh Herbarium (E)	128 specimens
Royal Botanic Gardens Kew Herbarium (K)	125 specimens
Kunming Institute of Botany Herbarium (KUN)	7 specimens
Naturalis Herbarium (L)	3 specimens
Singapore Botanic Gardens Herbarium (SING)	10 specimens

Herbarium visits also took place to BM, E, K and P in 2016-2018, and online surveys of specimens from other herbaria used the following resources:

Chinese Plant Catalogue: <http://www.cvh.ac.cn/en>

Geneva Herbarium: <http://www.ville-ge.ch/musinfo/bd/cjb/chg/index.php?lang=en>

Harvard University Herbarium:

<http://kiki.huh.harvard.edu/databases/specimeindex.html>

Jstor Global Plants online resource: <https://plants.jstor.org>

Naturalis: <http://bioportal.naturalis.nl>

New York Botanic Garden: <Http://nybg.org>

Royal Botanic Garden Edinburgh Herbarium:

<http://data.rbge.org.uk/Search/herbarium/>

Royal Botanic Garden Kew Herbarium: <http://apps.kew.org/herbcat/navigator.do>

Smithsonian Institution - National Museum of Natural History (USA):

<https://collections.nmnh.si.edu/search/botany/?ti=3>

Systematic treatment

***Henckelia* Spreng.**, Anleit. Kenntn Gew., ed.2, 2(1): 402. 1817.

–*Henckelia* sect. *Henckelia* Weber & Burtt, Beitr. Biol. Pflanzen 70: 334. 1998.

–*Didymocarpus* sect. *Orthoboea* Benth., Benth. & Hook. f., Gen. Pl. 2(2): 1022. 1876.

Tye: *Henckelia incana* (Vahl) Spreng.

Chirita Buch. -Ham. ex D. Don, Edinburgh Philos. J. 7: 83. 1822.

–*Chirita* sect. *Euchirita* C.B. Clarke, Monogr. Phan. 5(1): 111. 1883.

–*Didymocarpus* sect. *Euchirita* (C.B. Clarke) Chun, Sunyatsenia 6: 294. 1946.

–*Roettlera* sect. *Euchirita* (C.B. Clarke) Fritsch, Nat. Pflanzen. IV/3b: 1848. 1895.

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. (nom nud).

–*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 & Mich. Möller.).

–*Gonatostemon* Regel, Gartenflora 15: 353. 1866.

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

–*Ceratoscyphus* Chun, Sunyatsenia 6: 276. 1946.

Type: *Ceratoscyphus caerulea* Chun (= *Henckelia ceratoscyphus* (B.L. Burtt) D.J. Middleton & Mich. Möller.).

–*Hemiboeopsis* W.T. Wang, Acta Bot. Yunnan. 6: 397. 1984.

Type: *Hemiboeopsis longisepala* (H.W. Li) W.T. Wang (= *Henckelia longisepala* (H.W. Li) D.J. Middleton & Mich. Möller.).

Acaulescent or caulescent annual or perennial herbs; rhizomatous, stolons, stem short or distinct, glabrous or hairy; with tuber or without tuber. Indumentum multicellular hairs, glandular or eglandular hairs. Petiole: terete, opposite, alternate or whorled; glabrous or hairy.

Leaf sessile or petiolate; single or several; ovate, ovate-lanceolate, globose, cordate or subcordate; apex acute, acuminate or round; base oblique, cordate, subcordate or cuneate; margin serrate, dentate or entire; upper and lower surface glabrous or hairy.

Inflorescence axillary, sometimes terminal; cyme, 1-several flowered; white, cream, yellow, pink to purple color. Peduncle terete, glabrous, or with glandular or eglandular hairs. Bracts paired, free or connate. Pedicel terete with or without glandular or eglandular hairs. Calyx tubular or 5-partite, calyx lobes triangular or narrowly triangular, reflexed or not reflexed, sometimes horned; caducous or persistent. Corolla tube infundibuliform or campanulate, lobes round. Stamens 2, filaments inserted from the base to the middle on the corolla tube, geniculate or straight, glabrous or covering with eglandular or glandular hairs; anthers globose or elliptic, adaxial surface coherent; staminode 2 or 3, slender or stout, glabrous or hairy. Gynoecium glabrous or hairy; chiritoid stigma (deep bilobed, bilobed or peltate).

Fruit a capsule, orthocarpic or plagiocarpic, glabrous or hairy. Seeds numerous, minute, elliptic, rugose and without appendage.

KEY TO SPECIES OF *HENCKELIA* FROM THAILAND, AND SURROUNDING COUNTRIES

1. Mature plant developing one large leaf only
 2. Caulescent herb with petiolate leaf, persistent calyx, capsule held in line with pedicel (orthocarpic) 6. *H. campanuliflora*
 2. Caulescent herb with sessile leaf, caducous calyx, capsule held roughly horizontal with pedicel (plagiocarpic)
 3. Leaf usually less than 9 cm long, surface with dense hairs, calyx tip not reflexed, staminodes 2 7. *H. candida*
 3. Leaf 9 cm long or more, surface with sparse stout hairs, calyx tip reflexed, staminodes 3 18. *H. nakianensis*
1. Mature plant developing several leaves
 4. Plant with one large leaf and one small leaf 2. *H. amplexifolia*
 4. Plant with several large leaves
 5. Leaves alternate or whorled
 6. Plant creeping, with stolons; petiole more than 1-2 times the leaf length, corolla tube less than 4 cm long
 7. Corolla tube narrowly infundibuliform, more than 3.5 cm long, filaments inserted about 1 cm from base of corolla tube 4. *H. burtii*
 7. Corolla tube widely infundibuliform, less than 3.5 cm long, filaments inserted less than 1 cm from base of corolla tube 15. *H. lachenensis*
 6. Plant not creeping, without stolon, petiole less than the leaf length, corolla tube more than 4 cm long
 8. Calyx tip with a strongly geniculate recurved tip 8. *H. ceratoscyphus*
 8. Calyx tip without a strongly geniculate recurved tip
 9. Calyx glabrous or sparsely hairy 11. *H. grandifolia*
 9. Calyx densely hairy
 10. Petiole length less than twice the leaf length, leaf margin serrate, calyx covered with brown hairs, anther bearded 23. *H. speciosa*
 10. Petiole length more than twice the leaf length, leaf margin dentate or remotely dentate, calyx cover with white hairs, anther not bearded 10. *H. fruticola*
 5. Leaves opposite
 11. Plant with short stems, rhizomatous 13. *H. insignis*
 11. Plant not with short stems, not rhizomatous

- 12. Leaf margin entire
 - 13. Plant with storage root, leaf broadly ovate, upper and lower leaf surface glabrous or sparsely hairy 22. *H. rotundata*
 - 13. Plant without storage root, leaf narrowly ovate, whole plant glabrous 5. *H. calva*
- 12. Leaf margin serrate or dentate
 - 14. Calyx persistent
 - 15. Woody perennial herb, inflorescence lax, up to 12-flowered, calyx tube campanulate with short hairs 19. *H. oblongifolia*
 - 15. Woody annual herb, inflorescence dense, up to 9-flowered, calyx basally connate with long hairs 9. *H. dasycalyx*
 - 14. Calyx caducous
 - 16. Calyx glabrous,
 - 17. Leaves clustered at the top of stem, filament straight 14. *H. lacei*
 - 17. Leaves not clustered at the top of stem, filament geniculate 3. *H. anachoreta*
 - 16. Calyx hairy
 - 18. Calyx deeply divided or at base
 - 19. Leaves ovate, bracts elliptic 0.4 mm long, calyx lobes narrowly triangular 16. *H. longipedicellata*
 - 19. Leaves obovate-oblong, bracts suborbiculate 2 cm long, calyx lobes spatulate-linear 17. *H. longisepala*
 - 18. Calyx divided more or less than half way
 - 20. Bracts more than 2 12. *H. heterostigma*
 - 20. Bracts paired
 - 21. Bracts connate
 - 22. Corolla tube straight; filament glandular, strongly geniculate 1. *H. adenocalyx*
 - 22. Corolla tube curved; filament glabrous, curved 20. *H. peduncularis*
 - 21. Bracts free
 - 24. Corolla tube 4.5 cm long or less, staminode glabrous
 - 25. Calyx hairs similar to pedicel hair, 3. *H. anachoreta*
 - 25. Calyx hairs differ from pedicel hair,

almost pedicel glabrous 21. *H. pumila*

24. Corolla tube more than 4.5 cm long,
staminode hairy 24. *H. urticifolia*

The following diagram shows the pattern to the above key to species (Fig. 4.2A) and the diagram from the cluster analysis from chapter 2 (Fig. 4.2B)

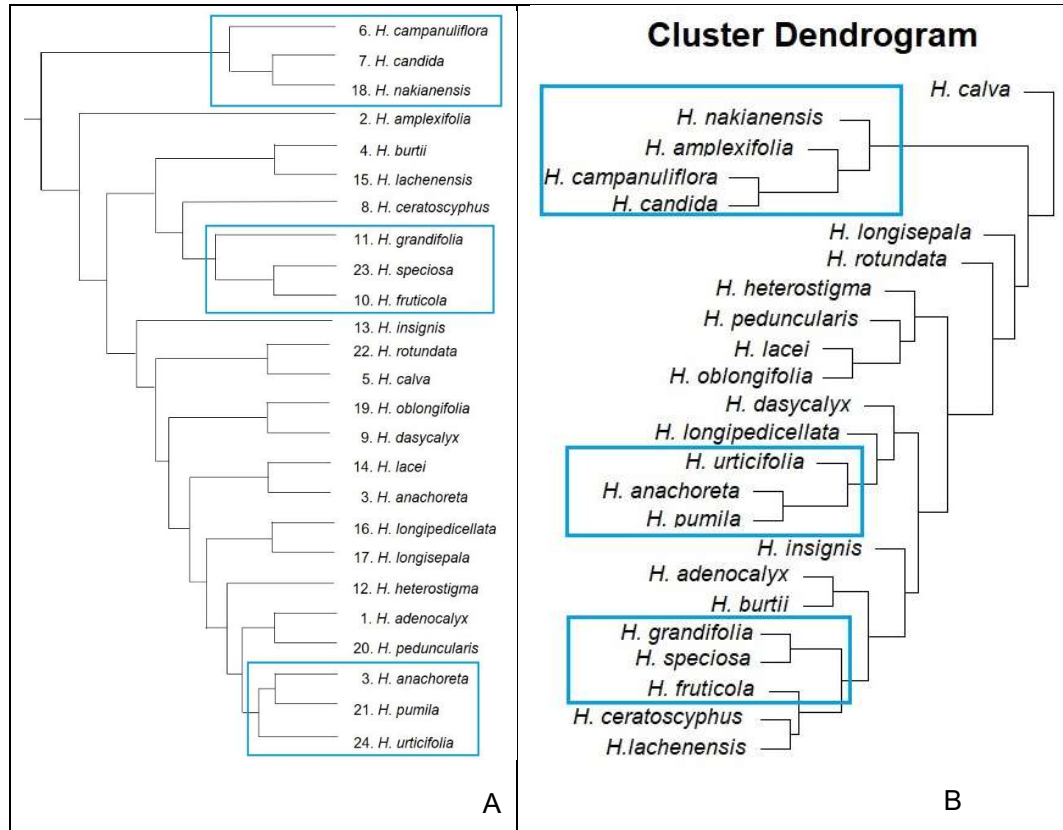


Figure 4.2 A branching diagram showing the pattern of the identification key & B. A branching diagram from cluster analysis.

Both identification diagrams have shown the similar group in 3 groups.

Group one comprises of characters as single leaf with campanulate flower i.e. *Henckelia candida*, *H. campanuliflora* and *H. nakianensis*.

Group two comprises of characters as several leaves, alternate or whorled with infundibuliform flower and corolla tube is longer than 4 cm i.e. *Henckelia grandifolia*, *H. fruticola* and *H. speciose*.

Group three comprises of characters as several leaves, opposite leaves, infundibuliform flower with bracts free i.e. *Henckelia anachoreta*, *H. pumila* and *H. urticifolia*.

4.3 Description

1. *Henckelia adenocalyx* (Chatterjee) D.J. Middleton & Mich. Möller, Taxon 60: 774. 2011.

≡ *Chirita adenocalyx* Chatterjee, Kew Bull. 1948: 63. 1948. TYPE: Burma, Upper Burma, Kachin Hills, 2,300 ft, Dec 1911, *S.M. Toppin 4251* (Holotype **K!** (K000858409)).

Caulescent annual herb, up to 30 cm tall; stem with multicellular hairs, up to 1.5 mm long. **Leaves** opposite: blade asymmetrically ovate or lanceolate, 7.5–11.7 by 4–6.9 cm, apex acute, base cuneate or suboblique, margin crenate (sometimes dentate), upper and lower surface densely hairy, lateral veins 7–10 on each side; petiole terete 0.8–4.5 cm long, densely hairy. **Inflorescence** axillary, simple or compound cymes, 1–7 flowered, violet with yellow stripes, yellow with red stripes, or cream, with red hairs; peduncle terete, 3–6.3 cm long, densely hairy; bracts paired, connate, ovate or elliptic, c. 0.9–1.25 by 0.5–1.4 cm, sparsely hairy, margin ciliate, apex acute; pedicel terete, 0.5–1.8 cm long, densely hairy. **Calyx** tubular, tube 0.6–1.4 cm long; lobes divided more or less than half way, narrowly triangular, 0.8–1.5 by 0.3–0.6 cm; densely hairy, caducous. **Corolla** purple; tube infundibuliform, 3.5–4 cm long, hairs glandular; lobes 0.5–1.1 by 0.7–1.5 cm. **Stamens** 2: filaments inserted about 1.6–2 cm from base of corolla tube, 1.1–1.4 cm long, hairs glandular, geniculate; anthers elliptic, adaxial surfaces coherent, 3.5–5 by 2–3.5 mm; staminodes 2, inserted 0.8–1.2 cm from base of corolla tube, c. 7 mm long, slender, long hairs on filaments. Gynoecium 2.6–4 cm long, hairy; stigma bilobed. **Fruit** orthocarpic (capsule held in line with pedicel), c. 4.5 cm long, densely hairy. Seeds not seen.

Distribution. India, Southern China and Myanmar (Fig. 4.3).

Ecology. On damp or wet shady banks, in sub-tropical forest, on granite rocks, alt. 600–2,400 m.

Provisional IUCN conservation assessment: Endangered EN B2ab(iii). This species has an EOO about 13,535 km² and AOO about 36 km². The EOO is <20, 000 km² within the threshold of Vulnerable but the AOO is ≤500 km² within the threshold of Endangered. However, populations can be described as 3 fragments in 3 countries. Some of them are outside a protected area and are threatened by a local construction project. An assessment of Endangered is more appropriate than Vulnerable.

Specimens examined. China: Yunnan, Gongshan Xian, Dulongjiang Xiang. W side of Gaoligong Shan, W of Gongshan, on trails from Qiqi to Bapo in the Dulong Jiang valley, 27°43'47" N, 98°23'29" E, 2,050 m, 17 July 2000, *Li 12906* (**E** (E00132439)). **India:** Assam, Theronliang, 27°05' N, 96°10' E, 600–900 m, 2 Nov. 1950, *Ward 20297* (**BM** (BM011025836)).

Myanmar: Kachin State: Frontier of Tibet and Myanmar, valley of the Nam Tamai, 27°42'27" N, 98°0'18" E, [1,524 m, 11 Sept. 1926, *Ward 7393* (**E** (E00096821), **K**); 914 m (*Ward 7320* (**K**), 8 Aug. 1937, *Ward 13553* (**BM** (BM011025840)), 27 Aug. 1938, *Kaulback 93* (**BM** (BM011025835), **E** (E00096818)), 15 Aug. 1938, *Kaulback s.n.* (**BM** (BM011025842)); Kachin Hills, 26 41'0"N, 97 14'0"E (610 m, 5 July 1930, *Ward 9025* (**BM** (BM011025837)), *Ward 9030* [**BM** (BM011025838)]). Sumprabum, Eastern approaches from Sumprabum to Kumon Range, Between Ning W'Krok and Kanang., 26°40' N, 97°20' E, 1,524 m, Mar. 1962, *Keenan et al. 3936* (**E** (E00627475), **K**). Mountain east of Fart Hertz, 27°23' N, 97°24' E, 914 m, 25 Aug. 1926, *Ward 7343* (**K**). N Myanmar, North Triangle (Hkinbum) (1,219 m, 14 Aug. 1953, *Ward 21266* (**BM** (BM011025839), **E** (E00627476)), 600–762 m, 3 Dec. 1953, *Ward 21669* (**BM** (BM011025841))). Kampti, Nama Uka Marao, *Toppin, S.M. 4521* (**K** (K000858409)). Nwai valley, 28 Mar. 1905 (*Ward 1914* (**E** (E00096817))), 0–2,400 m, 13 Sept. 1914, *Ward 1942* (**E** (E00096819)). *Keenan et al. 3051* (**K**).

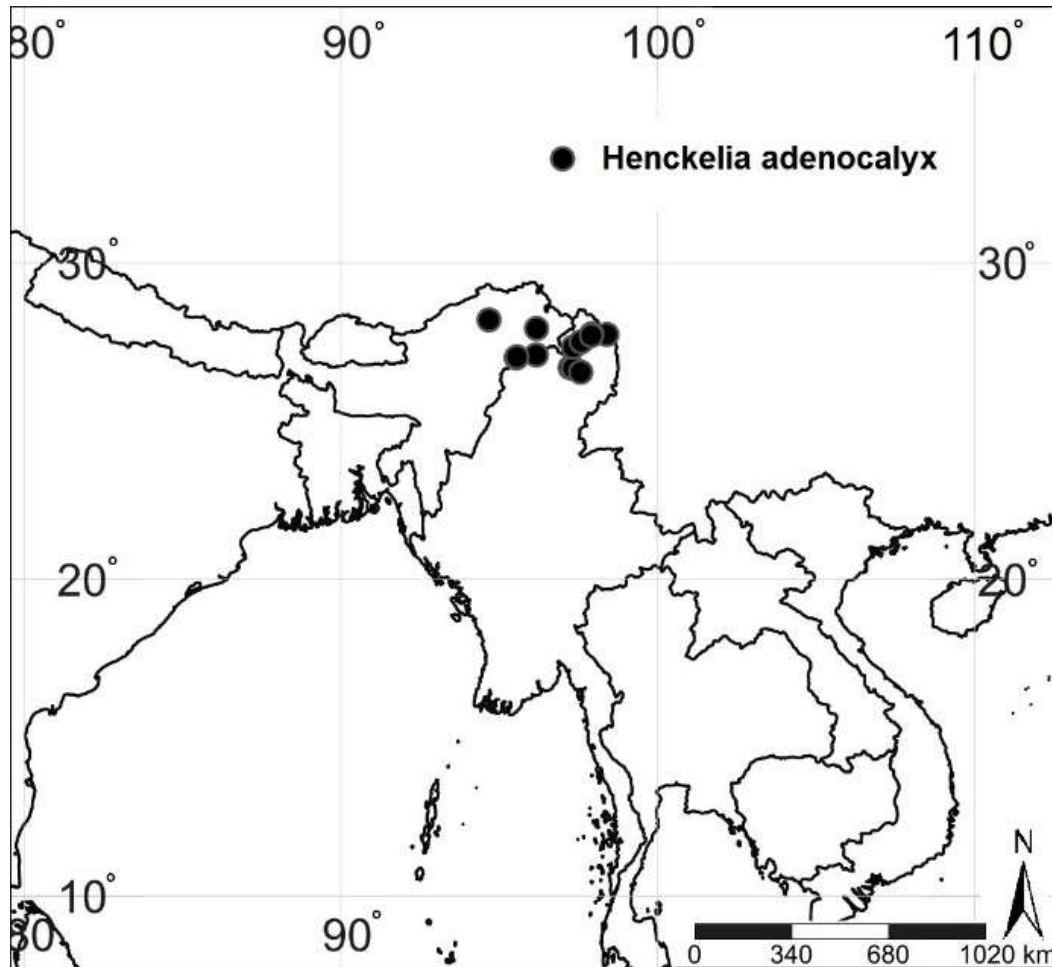


Figure 4.3 Distribution map of *Henckelia adenocalyx* (Chatterjee) D.J. Middleton & Mich. Möller. Base map from <https://www.simplemappr.net>

2. *Henckelia amplexifolia* Sirim., Thai Forest Bull., Bot. 47(1): 45–51. 2019.

TYPE: Thailand, Mae Hong Son, Muang Distr., Ban Nam Hu, road no 1–0003, km 20–21, 19°11'22.7"N, 98°04'12"E, alt. 800 m, fl. 12 July 2017, *Sukontip Sirimongkol, Ta Sapniyomphong and Chanthana Phongsasat* 714 (Holotype **BKF!**; Isotypes **E!**, **K!**, **L!**, **M!**, **P!**, **SING!**, **TCD!**).

Cauliscent annual herb, succulent, up to 15 cm tall; stem light green, 0.2–0.5 cm diam. with hispid white hairs; bulbils subglobose up to 1 cm diam., light brown; stolon white, terete, up to 0.1 cm diam. with lanceolate scale leaves up to 0.2 cm long. **Leaves** opposite: blade symmetrically cordate or orbicular, 6.2–16 by 6–14 cm, herbaceous, base cordate and sometimes amplexicaul, apex obtuse or rounded, margin serrate, upper surface hairy, lower surface glabrous, lateral veins 4–10 on each side, only the

first lateral vein ascending perpendicular to the midrib; usually two pairs of opposite leaves present, but some reduced such that there are only 1 or 2, rarely 3 or 4, full-sized leaves, sessile. **Inflorescence** terminal, single or compound cymes, rarely axillary, up to 2, 3–9-flowered, white; peduncles terete, light green, 2.3–5 by 0.2–0.3 cm, hairs glandular; bracts paired, free, sessile or linear, 0.5–7 by 1–15 mm, apex acute, hairy, margin ciliate. Pedicels terete, light green, 0.7–2 by 0.1 cm, with multicellular and glandular hairs. **Calyx** 5-partite; lobes basally connate, narrowly triangular, light green, 6–6.3 by 1–1.4 mm, apex with claw 2–3 mm long; with multicellular and glandular hairs, caducous. **Corolla** white; tube campanulate, 5 mm long, hairy outside, corolla lobes 5–6 by 7–8 mm. **Stamens** 2: filaments inserted 2 mm from base of corolla tube, 3 mm long (appressed 1.5 mm long), geniculate in the middle length of the filament, hairy on top; anthers reniform, yellow, adaxial surfaces coherent, c. 2.5 by 1.5 mm; staminodes 3, inserted 2 mm from base of corolla tube, c. 1 mm long, glabrous. Gynoecium 12–13 mm, ovary 1–1.5 mm across, hairs glandular; style terete, slender; stigma peltate, greenish. **Fruit** plagiocarpic (capsule held roughly horizontally), green, 1.2–2 by 0.1 cm. Seeds not seen (Fig. 4.5).

Distribution. Northern Thailand (Fig. 4.4).

Ecology. Mixed deciduous forest or bamboo forest, near a stream, moist area, conglomerate rocks, alt. 800–859 m, flowering in July–September, fruiting in September.

Provisional IUCN conservation assessment. Endangered EN B1ab(iii), B2ab(iii). This species is known from a small number of collections from along a short stretch of road in a National Park. However, the known populations could be subject to disturbance from any roadworks and, additionally, on at least one occasion one population appeared to have been heavily browsed.

Examined specimens. Thailand: NORTHERN: Mae Hong Son: Mueang, Mae Hong Son local road no. 1–0003 (between km. 19–20, Ban Nam Hu, 19°11'23.2" N, 98°03'56.3" E, 859 m, 12 July 2017, *Sirimongkol et al.* 713 (BKF, TCD), between km. 20–21, Ban Nam Hu, 19°11'22' N, 98°04'12' E, 800 m, 21 Sept. 2016, *Sirimongkol et al.* 701 (BKF, E, K, L, P, TCD), *ibidem*, 13 July 2017, *Sirimongkol et al.* 714 (BKF, E, K, P, SING, TCD)).

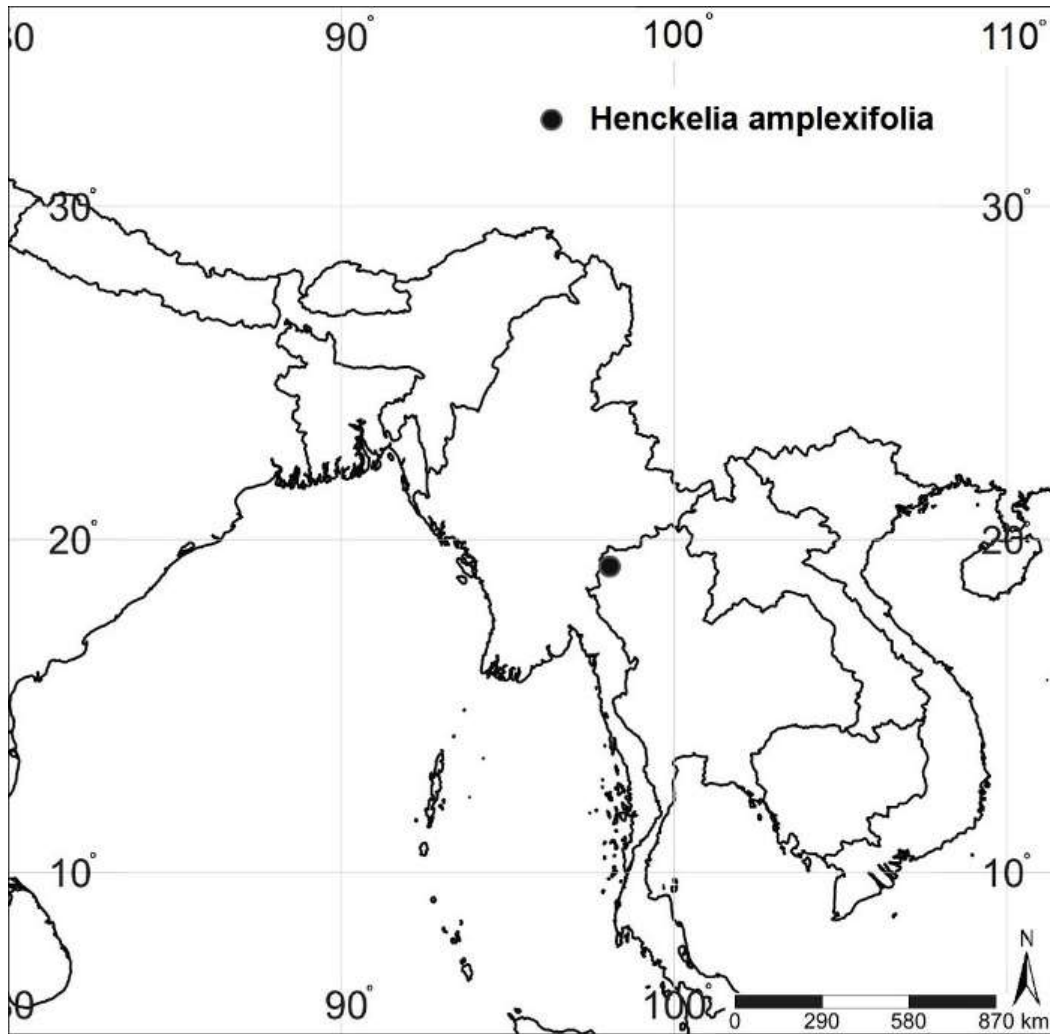


Figure 4.4 Distribution map of *Henckelia amplexifolia* Sirim.

Base map from <https://www.simplemappr.net>

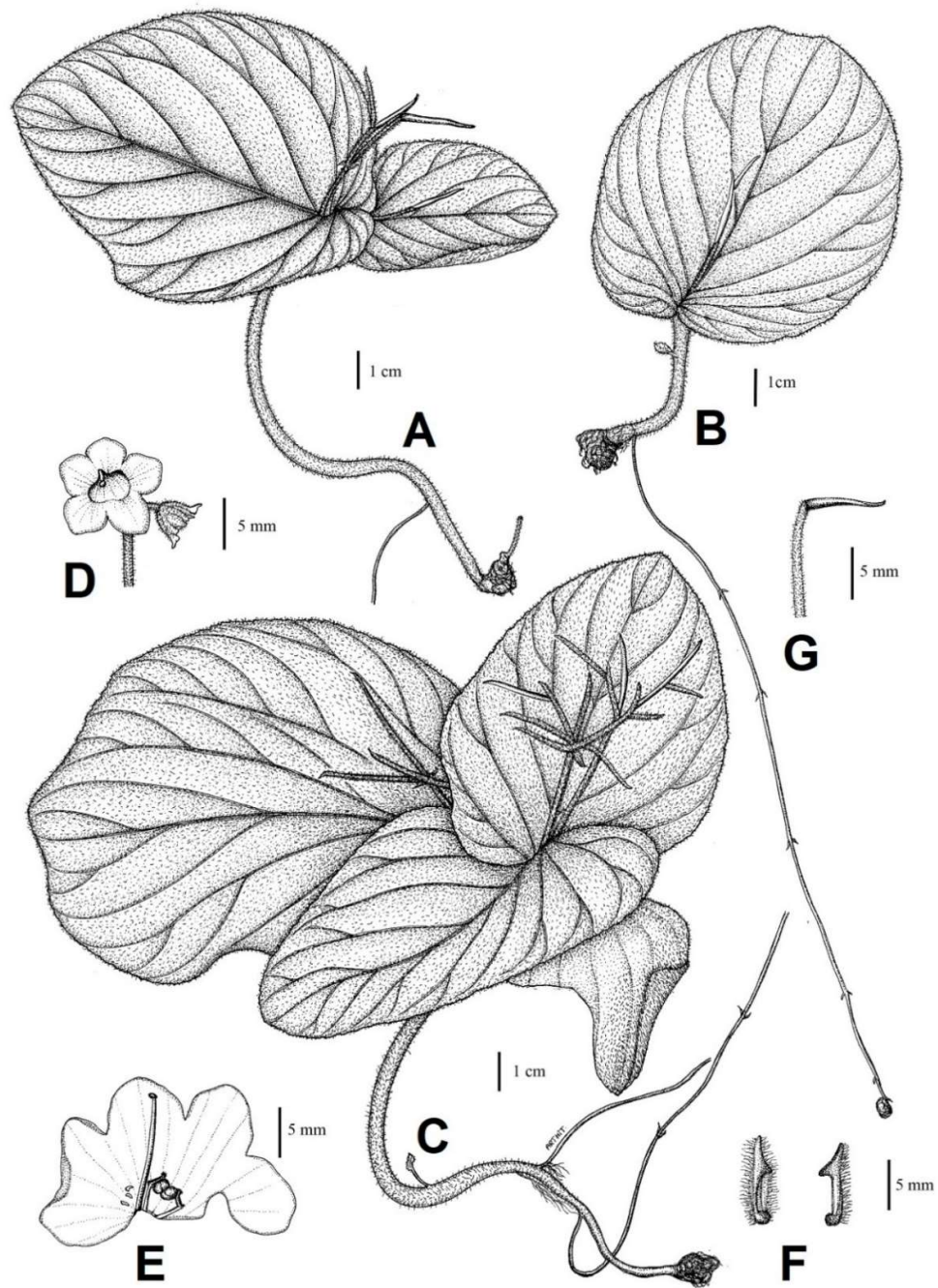


Figure 4.5 Line drawing of *Henckelia amplexifolia* Sirim.: A–C. Habit; D. Flower; E. Flower dissection; F. Calyx; G. Fruit. Drawings: Arthit Kamgannerd from *Sirimongkol et al.* 701 (BKF).

3. *Henckelia anachoreta* (Hance) D.J. Middleton & Mich. Möller, *Taxon* 60(3): 774. 2011; Middleton, Weber, Yao, Sontag & Möller, *Edinburgh J. Bot.* 70(3): 396. 2013. ≡ *Chirita anachoreta* Hance, *Ann. Sci. Nat., Bot. sér. 5*, 5: 234. 1866; Pellegrin, *Fl. Indo-Chine* 4(5): 538. 1930; Barnett, *Fl. Siam.* 3(3): 221–222. 1962; Wood, *Notes Roy. Bot. Gard. Edinburgh* 33: 164–165. 1974; Wang et al, *Fl. China* 18: 343. 1998. TYPE: China, 27 July 1864, G. *Theophilus Sampson 11377* (Holotype **BM!** (BM000041739); Isotype **GH!** (image)(HUH00015872), **K!** (K000858355)).
–*Didymocarpus anachoretus* (Hance) H. Lév., *Compt. Rend. Assoc. Franc.* 34: 427. 1906. 1905.
–*Chirita minuteserrulata* Hayata, *Icon. Pl. Formosan.* 5: 133. 1915. TYPE. Boho, Juli (FI) 1911, H. Inaba s.n.

Caulescent annual herb, non-rhizomatous, stem erect, (5) 10–40 (50) cm tall, glabrous, sparsely hairy or hairy. **Leaves** opposite: blade ovate, lanceolate or elliptic, 4.9–35.5 by 0.9–10.2 cm; apex acute, acuminate or rarely attenuate; base obliquely rounded on one side and acute on the other; margin serrate, shallowly or deeply serrate, rarely repand or undulate; upper surface and lower surface sparsely hairy to hairy except midrib and lateral veins on the upper surface glabrous, lateral veins 6–12 (14) on each side. Petiole terete, (0.5) 1–7.2 cm, glabrous to sparsely pubescent. **Inflorescence** axillary, simple or compound cymes, 1–8-flowered, white, creamy-white, yellow or purple with yellow markings; peduncle 3.1–8.3 cm, glabrous to sparsely pubescent; bracts paired, free, sessile, ovate or lanceolate, 3–13x1–8.5 mm, ciliate, margin entire or ciliate; pedicel 0.5–1.8 cm, glabrous to pubescent. **Calyx** tubular, tube 0.4–1.1 cm long; lobes divided more or less than half way, narrowly triangular, 0.4–1.2 cm long; outside glabrous or sparsely hairy, caducous. **Corolla** infundibuliform, 1.8–4.5 cm long, glabrous; lobes 0.8–1.2 by 0.8–2 cm. **Stamens** 2: filaments inserted 1–1.8 cm from base of corolla tube, 0.9–1.6 cm long, glabrous to puberulent on apex, geniculate; anthers elliptic, adaxial surface coherent, 2–3.2 mm long, glabrous; staminodes 2, inserted 1.5 cm from base of corolla tube, 5–7 mm long, glabrous. Gynoecium 1.5–2.9 cm, glabrous or sparsely hairy; stigma bilobed. **Fruit** orthocarpic, 3–15 cm long, glabrous or sparsely hairy. Seeds elliptic, c. 0.25 by 0.1 mm (Fig. 4.7).

Distribution. India (Sikkim, Assam), China, Myanmar, Vietnam, Laos and Thailand (Fig. 4.6).

Ecology. Moist or shaded area in primary or secondary evergreen forest, montane forest or near stream in deciduous forest, steep banks, on moist rocky crack or by

stream in open area, alt. 100–2,134 m.

Provisional IUCN conservation assessment: Least Concern LC. This species has an EOO about 3,474,533 km² and AOO about 244 km². The EOO is >20, 000 km² within the threshold of Least Concern but the AOO is ≤500 km² within the threshold of Endangered. However, this plant is common and widespread. An assessment of Least Concern is more appropriate than Endangered.

Specimens examined. India: Meghalaya, Khasi, *Hooker & Thompson s.n. (K)*, *Hooker & Thompson s.n. (K)*, *Hooker & Thompson s.n. (K)*, 10 Aug. 1950, *Pierre s.n. (K)*; Mowlong, khasia, 762 m, 24 Sep 1886, *Clarke 45073 (NMNS (NMNS00056633))*; Khasia, 914 m, 23 Oct. 1871, *Clarke 16521 (NMNS (NMNS00056632))*; Sikkim, Sikkim Himalaya., *Hooker s.n. (K)*; *Griffith s.n. (K)*; *Wallich 1832 (K (K000858408))*; *Wallich s.n. (K)*.

China: Chiayi Hsien, Chuchi Hsiang, Kuanyin Fall Forest Recreation Area, 4–500 m, 22 Sept. 1992, *Wen-Pen Leu 1618 (BM)*; Guangdong, Kwangtung, Tsing Wan Shan, Wong Chuk I and vicinity, Wung Tuen, 16 Aug. 1933, *Lau 2053 (NMNS (NMNS01343828))*; Guangdong: Guangzhou: Guangzhou City, Prope monastericum buddhisticum Filoitsz, secus fl. North River, Prov. Cantoniensis, 19 Sept. 1864, *Sampson 11377 (BM (BM000041739), GH (HUH00015872), K (K000858355))*; Guangzhou, Canton, Lo-fan shan, *Anonymous 477 (BM (BM000041646))*; Kouy-Tcheoe, 11 Oct. 1913, *Cavalerie s.n. (P (P03511089))*; Kwangtung-Tonkin Border, Kung Ping Shan and Vicinity, Faan Faan, Fang Cheng Distr., (25-30 Aug. 1936, *Tsang 26748 (K, P (P04079304))*), 10-18 Sept. 1936, *Tsang 26826 (K, P (P03511047))*); Lo Fau Shau-Ckiea, 1883, *Wallich 84 (K)*, Mengla County, Yunnan Prov., (Nangong Mountain, 800 m, 20 Sept. 2010, *Yin 1861 (QBG (QBG59116))*), Yunnan, South of Red River, 2,134 m, 10 Sept., *Henry 11275 (K, NMNS (NMNS00064565))*, 1,524 m, 7 Oct. 1901, *Henry 13438 (BM (BM000041563), E (E00087251), K, NMNS (NMNS00056623))*, *Henry 13438 (Henry 13521 (E (E00087250))*, 1,372 m, *Henry 12281A (K, NMNS (NMNS00064537))*, Xishuangbanna, on high way from Mengla to Mengyuan, Xiao-La high way, 950 m, 5 Nov. 2005, *Zhao 3171 (QBG (QBG43041))*); 183 m, *Anonymous 54 (K)*; *Wilson 2777 (K)*; Hong Kong, Lung Fu Shan, 9 Sept. 1994, *Ford 541 (K)*.

Myanmar: Chin State: Along footpath between Saw Loung Village and Saw Chaung River (Saw River), Kanpetlet Township., 21°10'22.3" -21°11'33.8"N, 94°02'41.6" -94°03'34.1" E, 750–1,375 m, 6 Sept. 2013, *Fujikawa et al. 095052 (MBK (MBK0255461))*; between Kanpetlet and Yelong Pan Village, 21°12.6' 38" N, 94°02.5'56"E, 1,445 m, 15

Sept. 2011, *Funakoshi et al.* 085363 (**SING**); track off road, approximately 2 miles along track from Matupi to Palawan town, on right to Chan Pyang Village, Matupi Township, Natma Taung NP., 21°34'22.47" N, 93°24'21.17" E, 1,676 m, 11 Mar 2014, *Srisanga et al.* 097836 (**SING**). Tnangkyi, 1,200 m, 15 Aug. 1919, *Ward* 3536A (**E** (E00096822)).

Thailand: NORTHERN. **Mae Hong Son:** Mueang (Tham Pla Pha Sue Waterfall NP, Pha Sue waterfall., 19°29'28.6" N 97°57'27.3", 367 m, 21 Oct. 2014, *Middleton et al.* 5805 (**BKF** (BKF189602), **SING** (SING0216587, SING0215160)), Route from Pha Sue waterfall to Pang Aung, Mae Hong Son road no. 4001, between km 7–8, 19°29'55" N, 97°57'98" E, 730 m, 20 Sept. 2016, *Sirimongkol et al.* 698 (**BKF, E, K, L, P, TCD**); Pang Ma Pha, (19 31'21"N, 98 14'46"E, 600 m, 5 Sept. 1999, *Suksathan* 1742–2 (**QBG** (QBG15659)), Ban Huay Nam Bong, Na Poo Pom subdistrict, near Nam Bong stream, 400 m, 29 Sept. 2003, *Palee* 627 (**CMUB** (CMUB22986))). **Chiang Mai:** Chom Thong, Doi Inthanon NP, Siriphum Waterfall., 18°32'52.4" N, 98°30'87.8" E, 13 Oct. 2011, *Norsaengsri et al.* 8207 (**BKF** (BKF189910), **QBG** (QBG54029)); Mae On, Huai Kaew Subdistrict, Mae Kam Pong Waterfall, (trail along waterfall., 18°51'43.2' N, 99°21'26.7" E, 1,100 m, 7 Sept. 2011, *Pooma et al.* 7775 (**BKF** (BKF184006)), *ibidem*, 8 Sept. 2011, *Pooma et al.* 7782 (**BKF** (BKF184010)), 1,200 m, 20 Sept. 2004, *Maxwell* 04–492 (**BKF** (BKF164736), **CMUB, L** (L3794635)), *ibidem*, 18°51'43.2' N, 99°21'26.7" E, 1,128 m, 20 July 2017, *Sirimongkol et al.* 715 (**BKF, TCD**)), Doi Lan, west side, pine ridge on the summit on the north slope above Mae Lie village, 1,175 m, 25 Oct. 2005, *Palee* 841 (**CMUB** (CMUB025807)); Mae Rim Mae Sa Botanical Garden, along Mae Sa Noi stream, 700 m, 2 Oct. 1990, *Maxwell* 90–1090 (**AAU**), Northern Botanic Garden, 18 54'11.88"N, 98 56'57.84"E, 8 Oct. 1990, *Chantaranothai et al.* 90/665 (**TCD**), Pong Yaeng, Huai Dee Mee, 800 m, 11 Sept. 1996, *Nanakorn et al.* 7445 (**QBG** (QBG7445)), Huai Mae Sa Noi, **QBG**, 854'11.88"N, 98 56'57.84"E, 3 Oct. 1996, *Nanakorn et al.* 7691 (**E** (E00679399), **QBG** (QBG7691)), Huai Waai., 18 55'12.36"N, 98 55'3.36"E, 8 Oct. 1997, *Nanakorn* 5-8-10-97 (**QBG** (QBG9705)), Mon Long, 700 m, 1 Sept. 1994, *Nanakorn et al.* 18998 (**QBG** (QBG18998))); Muang, Doi Suthep-Pui NP, Doi Suthep area (18 48'46"N, 98 53'37"E, 1,200 m, 12 Sept. 1958, *Sorensen et al.* 4937 (**ABD**), 900 m, 18 Sept. 1958, *Sorensen et al.* 5100 (**ABD**), *ibidem*, 18 Sept. 1958, *Sorensen et al.* 5101 (**ABD, BKF** (BKF28056)), east side, Ru-see cave, 1,075 m, 10 Oct. 1987, *Maxwell* 87-1160 (**BKF** (BKF93146)), middle elevation of Doi Suthep, 900–1,100 m, 20 Sept. 1967, *Shimizu et al.* T-10571 (**BKF** (BKF117765)), San Gou on way to Doi Pui, 18°48'03" N, 98°54'09" E, 1,030 m, 17 Sept. 2008, *Middleton et al.* 4480 (**E** (E00679396, E00629465), **SING** (SING0206191)), 1,250 m, 5 Sept. 1958, *Sorensen et al.* 4761 (**ABD, BKF** (BKF29295))); Wiang Papao, Mae Cha Dee Subdistr., Huai Nam Rin Village.,

19 11'0"N, 99 31'0"E, 1,200 m, 26 Sept. 1993, *C. Vial-Debas s.n.* (**CMUB** (CMUB04379)).

Chiang Rai: Doi Tung, 20 20'45"N, 99°50'4"E, 1,100 m, 4 Nov. 2004, *Palee 755* (**CMUB** (CMUB24424), **L** (L3794672)). **Payao:** Muang, Doi Luang NP, Jam Pa Thong water fall (19°13'04.2"N 99°44'15.9"E, 675 m, 24 Sept. 1997, *Petmitr 111* (**BKF** (BKF121859), **CMUB** (CMUB12797)), 600 m, 5 Nov. 2015, *Muangyen 345* [**QBG** (QBG88493)]). **Nan:** Bo Kleua, (Sapun waterfall., 19°12' N, 101°12' E, 820 m, 2 Sept. 200, Srisanga 1602 (**BKF** (BKF180735), **CMUB** (CMUB033641), **QBG** (QBG18049))), Khun Nan NP, 10 km from Bo Kluea to Chaloe Phra Kiat, 700 m, 7 Sept. 2008, *Phonsena 6224* (**BKF** (BKF163239)); Pua, Doi Phu Kha NP, (near Ban Bo Klua, 800 m, 19 Sept. 1992, *Santisuk 8615* (**BKF** (BKF105406)), in front of Nam Khwang watershed management station, on rock wall beside the road, 19°10'58' N, 101°04'28' E, 1,230 m, 4 Oct. 2016, *Sirimongkol et al. 710* (**BKF**, **K**, **TCD**); Song Kwai, Tham Sakoen NP (1,200 m, 2 Sept. 2006, *Pumicong 433* (**QBG** (QBG28552)), Than Thong waterfall, 19°23'12' N, 100°31'56' E, 762 m, 30 Aug. 2016, *Sirimongkol et al. 673* (**BKF**, **K**, **TCD**)). **Lamphun:** Doi Khun Tan NP [upper part of Tat Muey Falls, 600 m, 23 Oct. 1993, *Maxwell 93-1273* (**BKF** (BKF159198), **CMUB**), west side, above Yaw 3, 1,325 m, 2 Sept. 1994, *Maxwell 94-974* (**BKF** (BKF98662), **CMUB**), Tat Huay waterfall, 775 m, 30 Sept. 2001, *Palee 496* (**CMUB** (CMUB (19153), **L** (3794792))). **Lam Pang:** Jae Son NP [Ma Mawn village-Mae Sah Bau village., 18 50'11"N, 99°28'14" E, 975 m., 24 Aug. 1996, *Maxwell 96-1127* (**CMUB** (CMUB09402)), Pa Miang village, along Mae Ban stream, 1000 m, 27 Sept. 1995, *Maxwell 95-822* (**CMUB** (CMUB07103)), way to Mae Wan NP, ca. 500 m, 3 Sept. 2010, *Wathana 3523* (**QBG** (QBG50794)), Tad Rung, 519 m, 2 Sept. 2009, *Norsaengsri 6044* (**QBG** (QBG40824))). **Uttaradit:** Klong Tron NP, Klong Tron Falls, Nam Pie, North side of Pu Miang, 17 43'42"N, 100 41'4"E, 850 m, 14 Oct. 2005, *Maxwell 05-566* (**CMUB**, **L** (3794641)). **Kamphaeng Phet:** Mae Wong NP, Chong Yen Ranger Station, along trail about 400 m from station, 16°2'23"N 99 14'4"E, 1300, 29 Oct. 2002, *van de Bult 606* (**CMUB** (CMUB20972)). NORTH-EASTERN. **Loei:** Phu Kradueng NP, along trail from Samhaek (RS-5) to Langpae (RS-7), 550–1,280 m, 29 Aug. 1988, *Tagahashi T-63258* (**BKF** (BKF111721)). EASTERN. **Nakhon Ratchasima:** Pak Chong, Khao Yai NP (700 m, 21 Aug. 1968, *Smitinand 10457* (**BKF** (BKF39925)), near Orchid Waterfall (Pha Gluay Mai), 14°45' N, 102° E, 600 m, 22 Oct. 1969, *van Beusekom et al. 1833* (**AAU**, **BKF** (BKF152730), **E** (E00627682), **K**, **P** (P03511069)); Heaw Sai waterfall, 586 m, 17 Sept. 2002, *Charoenchai et al. 326* (**BK** (BK263298), **CMUB**), Heo Suwat Waterfall., 14°26'07" N, 101°24'49" E, 600 m, 20 Aug. 2012, *Middleton et al. 5655* (**E** (E00629433))). CENTRAL. **Sara Buri:** Sam Lan (9 Oct. 1973, *Maxwell 73-445* (**AAU**)); Kaeng Khoy (Jetkot Forest Plantation area, 250 m, 30 July 2000, *Wongprasert 007-3* (**BKF** (BKF129352)), Jet Kot Nuea,

Chet Kot Waterfall, 15°00'01" N, 101°24'88" E, 289 m, 25 Aug. 2016, *Sirimongkol et al.* 665 (**BKF**). **Nakhon Nayok**: Muang, Khao Yai NP, Nang Rong water fall (1960, *Chantraprasong 54* (**BK** (BK230724)), 100 m, 22 Oct. 1961, *Smitinand 6986* (**BKF** (BKF22138)), 16 Sept. 1972, *Maxwell 72-361* (**AAU**), ranger house area, end of the bridge cross the waterfall, 14°20'00" N, 101°19'15" E, 121 m, 23 Aug. 2016, *Sirimongkol et al.* 660 (**BKF, TCD**), *ibidem*, 14°19'59.7"N, 101°19'15.4" E, 121 m, 2 Sept. 2017, *Sirimongkol et al.* 721 (**BKF, K, TCD**)); near the Orchid Waterfall (Pha Kluay Mai) 650 m, 8 Oct. 1979, *Shimizu et al.* T-19640 (**BKF** (BKF78396)); near Pha Gluay Mai Station & Camp, along highway 2090 at km 46.5, 650 m, 9 Sept. 2002, *Maxwell 02-334* (**BKF** (BKF137815), **CMUB**); Sarika falls, trail to water fall (near summit, 7 Sept. 2006, *Pooma et al.* 6261 (**E** (E00679400)), 7 Sept. 2006, *Pooma et al.* 6261 (**BKF** (BKF167598)); Wang Ta Krai, 60 m, 4 Sept. 1977 (*Thephuttee 41* (**CMUB** (CMUB033587)), *Thephuttee et al.* 41 (**CMUB** (CMUB033587))). **SOUTH-EASTERN. Chanthaburi**: Khao Kitchakut, Khao Khitchakut NP, Khao Phra Bath (12°05' N, 102°01' E, 800 m, 17 Aug. 2008, *Phonsena 6182* (**BK** (BK265646), **BKF** (BKF163734)), road towards summit, 12°49'51" N, 102°09'03" E, 525 m, 27 Aug. 2012, *Middleton et al.* 5668 (**BKF** (BKF186859), **E** (E00629425))). **Trat**: Ko Chang, Lam Dan Kao, 11 58'44.4"N, 102°22'4.8"E, 400 m, 3 Oct. 1924, *Kerr 9302* [**ABD, BK** (BK23075, BK17753), **BM** (BM001010826), **K**]. Sept. 1910, *Kerr 1417* (**P** (P03511068), **TCD**).

Laos: Kammouan: Kaeng Meaung landing on Nakai Nam Theun, 17°45'47" N, 105°20'22" E, 538 m, 21 Oct. 2005, *Newman et al.* LAO-385 (**E** (E00219572)); Me Kong, *Thorel 2395* (**K, P** (P05311073, P05311070, P05311072)); Route from Laokay to Chapu, 600 m, July 1929, *Colani 5182* (**NMNS** (NMNS00056624)).

Vietnam: Saigon, 10 48'0"N, 106 39'0"E, 20 May 1921, *Poilane 1903* (**E** (E00627683), **P** (P03511071)); Tonkin, Sai Wong Mo Shan (Sai Vong Mo Leng) Lomg Ngong Village, Dam-ha, 21°0'0"N, 106°0'0" E, 9 Sept. 1940; Vi Xuyen, Lung Thao village, along Nap Ma stream, collection along path between Tam Ve village and Nap Ma village., 22°45'52" N, 104°51'52" E, 560 m, 7 Sept. 2000, *Harder et al.* 5302 [**E** (E00435284), **HN, SING** (SING204298)]; Bo Linh, Kim Anh, Vinh Phuc Prov., 21°18'N, 105°36'0"E, 21 Nov. 1959, *Anonymous s.n.* (**HNU**); Bo Linh, Kim Anh, Vinh Phuc Prov., *Anonymous s.n.* (**HNU**); Cao Bang Prov., Nguyen Binh Distr., municiplaity Nguyen Binh, 41 km to W from Cao Bang town, from Le A pass to Na Ni Mt. peak at 1400–1600 m, 22°37' N, 105°52' E, 1,400 m, 11-12 Nov. 1998, *Averyanov et al.* CBL083 (**HN**); Lang Son Prov., Loc Binh Distr., Mau Son, Way to Nui Cha., 21°50'42.6" N 106°56'37" E, 1063, 16 Sept. 2011, *Binh et al.* VMN-B1417 (**E!**, **P**, **PR!**, **SING!**, **VNMN**); Lao Cai Prov., Sa Pa Distr., San Sa Ho, Sin Chai., 22°20'44.3' N,

103°48'77"E, 1,377 m, 15 Sept. 2005, *Vu et al. HNK434 (K (K000612040))*; Suoi Khang Village, Suoi To, Phu Yen, Son La, 21°08'52.7" N 104°36'26.6" E, 7 Oct. 2008, *Du et al. HNK 2664 (SING (SING0215896))*; Tam Dao, Tam Dao District; approximately 100 km NNW of Hanoi, Tam Dao Forest Reserve. On trails from Tam Dao City to Tam Dao Mountain, trail side, 21°27'23" N, 105°38'39" E, 920 m, 8 Sept. 1993, *Harder et al. 1781 (SING (SING204297))*; Thai Ha Village, Muong Thai, Phu Yen, Son La., 21°18'58.7" N 104°41'10.8 E, 6 Oct. 2008, *Du et al. HNK 2587 (SING (SING0215895))*; Tonkin (22 July 1886, *Balansa 4286 [P (P03511075)]*), 14 Sept. 1886, *Balansa 4297 (P (P03511076, P03511077))*, 600 m, 21 Jan. 1930, *Petelot 5182 (P (P03511081))*, 1500 m, Aug. 1940, *Petelot 7225 (P (P03511083))*); *Anonymous 1642 (HNU)*; 17 Sept. 1980, *Anonymous P4283 (HNU)*; 8 Aug. 2009, *Anonymous VMN-B0000877 (VNMN)*; 17 Sept. 1980, *Anonymous s.n. (HNU)*; 600 m, July 1929, *Petelot 3182 [P (P03511079)]*, 1500 m, July 1927, *Petelot 5036 (P (P03511082, P03511078, P03511080))*, *Pluiong 6641 (HN)*; 9 July 1975, *TTH D2 25 616 (HNU)*.

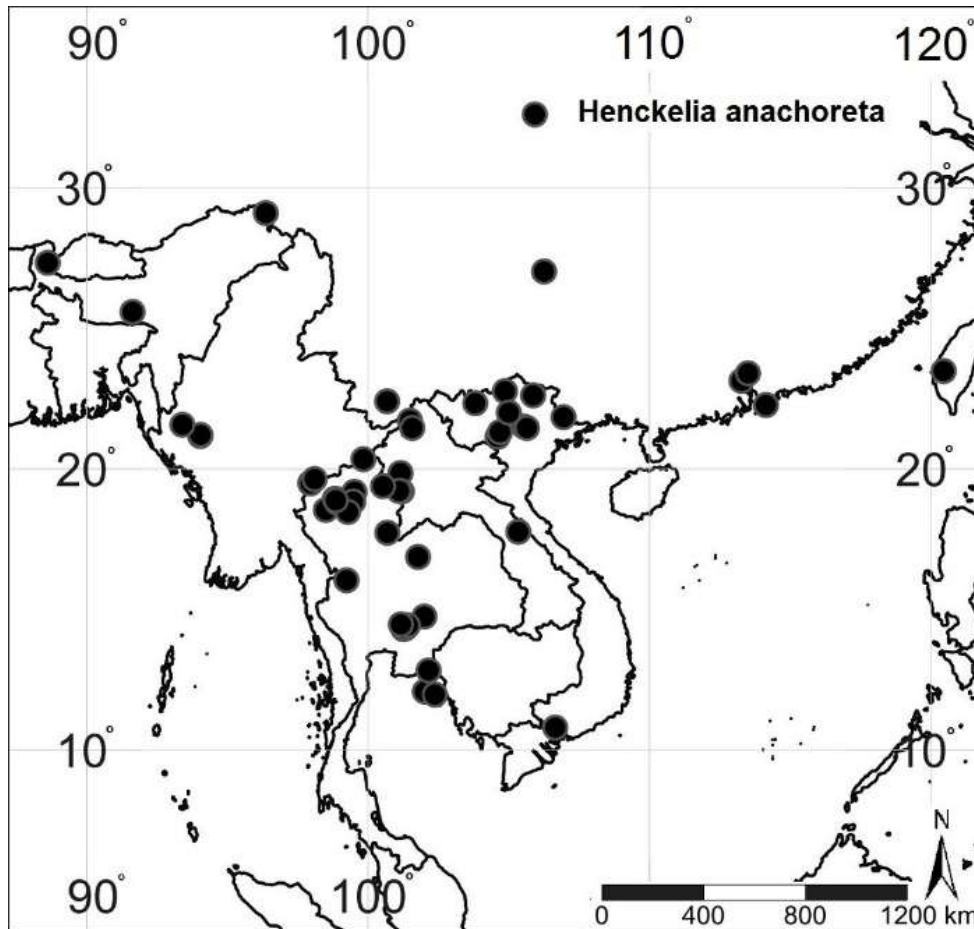


Figure 4.6 Distribution map of *Henckelia anachoreta* (Hance) D.J. Middleton & Mich. Möller. Base map from <https://www.simplemappr.net>

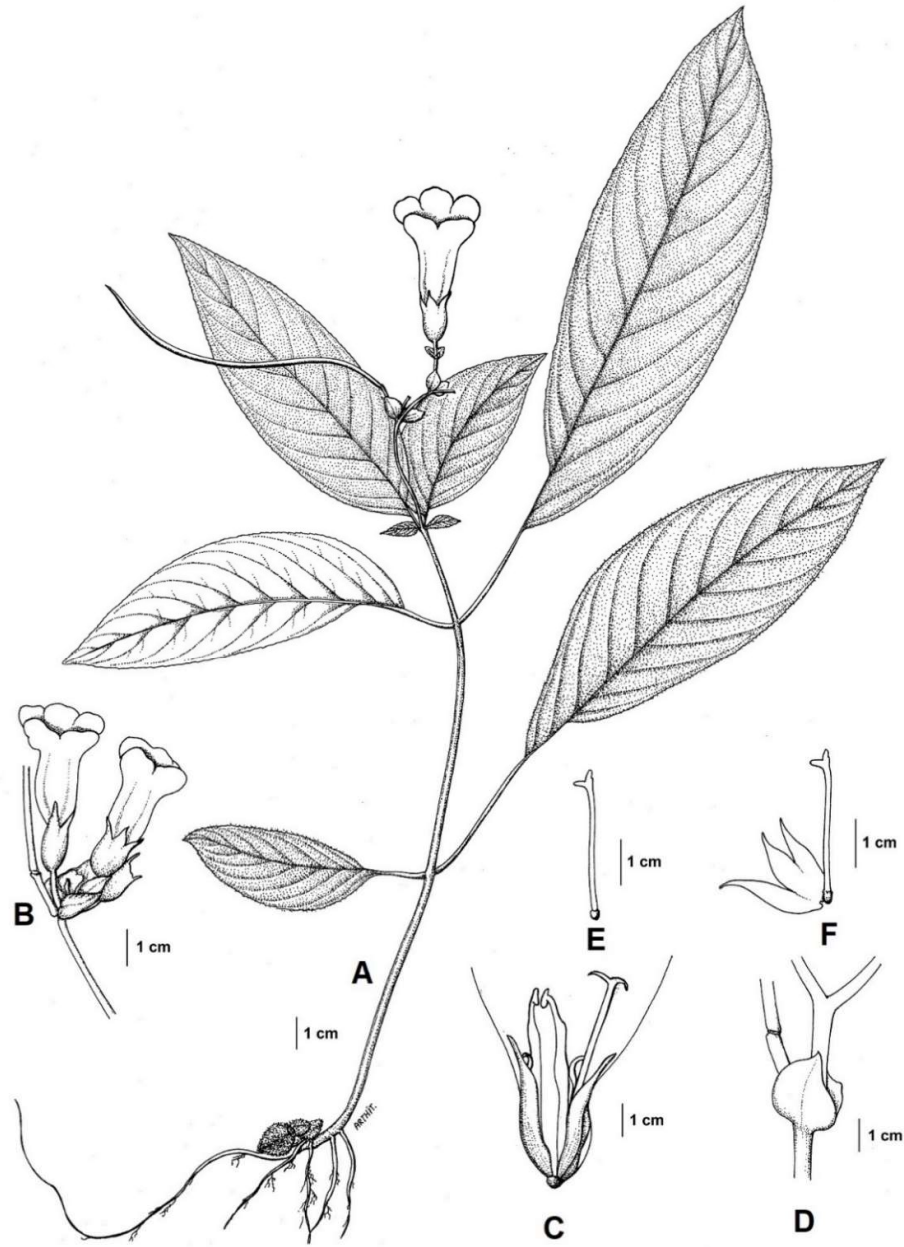


Figure 4.7 Line drawing of *Henckelia anachoreta* (Hance) D.J. Middleton & Mich. Möller: A. Habit; B. Inflorescence; C. Flower dissection; D. Bracts; E. Gynoecium; F. Gynoecium and calyx. Drawings: Arthit Kamgamnerd from *Sirimongkol et al.* 698

4. *Henckelia burtii* D.J. Middleton & Mich. Möller, *Taxon* 60(3): 775. 2011;
Middleton et al., *Edinburgh J. Bot.* 70(3): 396. 2013.
≡ *Chirita reptans* B.L. Burt & Panigrahi, *Notes Roy. Bot. Gard. Edinburgh* 26: 265.
1965. Wood, *Notes Roy. Bot. Gard. Edinburgh* 33: 157-158. 1974. TYPE. Myanmar,
Nytadi, head of Akhyang Valley, 27°15' N, 98°45' E, 1,800 m, 30 Apr. 1920, *Farrer*
1526 (Holotype. E! (E00155264)).

Creeping perennial herb, stem covered with brown hairs. **Leaves** alternate: blade ovate, 10–11 by 8–9.2 cm, apex acute, base cordate, margin serrate, upper surface sparsely shortly hairy, lower surface with mixed short and long hairs, secondary veins 5–6 on each side; petiole 10–17 cm long, hairy. **Inflorescence** several, solitary, flower violet with yellow streaks in the throat; peduncle 3–6 cm long, hairy. Bracts 2, free, lanceolate, 5 mm long, hairy. Pedicel 0.3–1 cm long, hairy. **Calyx** tubular, tube 1–1.3 cm long; lobes triangular, divided more or equally than half way, 5–8 mm long; hairy outside, caducous. **Corolla** tube infundibuliform, slightly curved, 4–6 cm long, sparsely short hairy; lobes 1–1.5 by 1–1.6 cm. **Stamens** 2: filaments inserted 1.5–2 cm from base of corolla tube, c. 1.5 cm long, glabrous, geniculate; anthers elliptic, 4–5 mm long, adaxial surfaces coherent. **Staminodes** 2, inserted 1.5 cm from base of corolla tube, c. 7 mm long, glabrous. **Gynoecium** 3.5 cm long, ovary c. 2 mm across, stigma deep bilobed. **Fruit** orthocarpic, young fruit 3.5 cm long.

Distribution. India, N Myanmar (Fig. 4.8).

Ecology. On moist soil slopes, damp rocky areas, deep shade in the forest or growing over rocks and tree trunks, alt. ca. 1,200 m.

Provisional IUCN conservation assessment. Endangered EN B1ab(iii) + B2ab(iii). This species has an EOO about 3,888 km² and AOO about 16 km². The EOO and AOO are within the threshold of Endangered. However, even though this species is found from 4 locations they are very close together and can be described as 2 fragments. One of them is beside the town and must be threatened by urban sprawl.

Specimens examined. India: Assam, Rotung, Dihang, 28°10' N, 95°10' E, 609–914 m, 11 Jan 1928, *Ward* 7832 (K).

Myanmar: Kachin State: Frontier of Tibet and Burma, Nam Tamai, 27°42'27" N, 98°0'18" E, 1,200 m, 5 May 1926, *Ward* 6689 (K); Myitkyina District, between Mali Hka and Sumprabum sub-district, 1,300 m, 10 Apr. 1953, *Tha Hla & Ehil Koko* 3792 (K)

(K000939233)). Langa Bum, 27°28' N, 97°42' E, 1,650 m, 19 Apr. 1926, Ward 6637 (K).

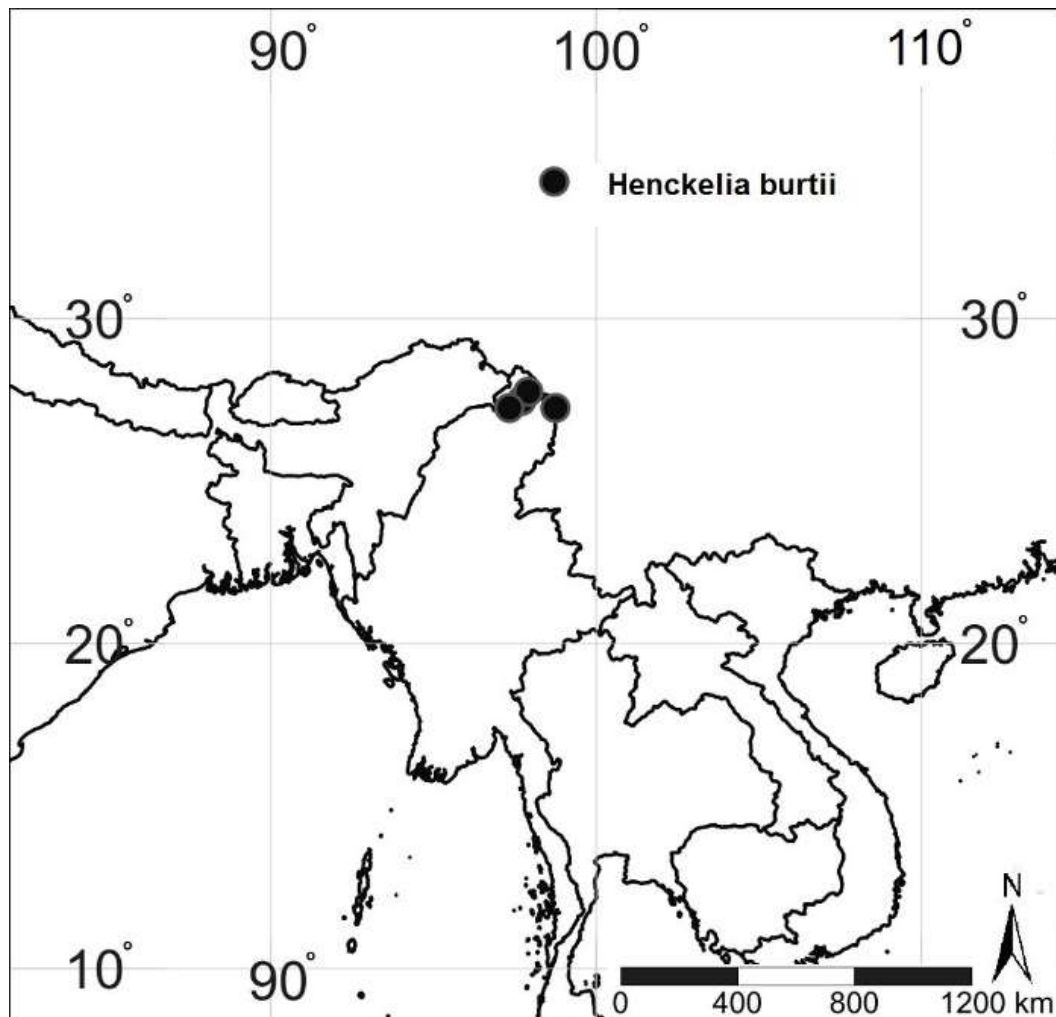


Figure 4.8 Distribution map of *Henckelia burtii* D.J. Middleton & Mich. Möller. Base map from <https://www.simplemappr.net>

5. *Henckelia calva* (C.B. Clarke) D.J. Middleton & Mich. Möller, *Taxon* 60(3): 775. 2011.

≡ *Chirita calva* C.B. Clarke, *Monogr. Phan. (A. DC. & C.DC.)* 5(1): 116. 1883 (Jul 1883); Hilliard, *Fl. Bhutan* 2(3): 1319. 2001. TYPE. India, Sikkim, 1,500 m, *Hook. f. s.n.* (Lectotype **K!** (K000858379)).

– *Roettlera calva* Kuntze-Revis. *Gen. Pl.* 2: 476. 1891.

– *Chirita glabra* C.B. Clarke (Illegitimate), *Commelyn. Cyrtandr. Bengal.* t. 73. 1874.

Caulescent annual herb, decumbent, stem 10–25 cm long, glabrous. **Leaves** opposite:

blade oblong-elliptic or oblong, 4–20 by 2–7.5 cm, apex acute or acuminate, base oblique; margin entire, ciliate; upper and lower surface glabrous, secondary veins 4–7 veins on each side; petiole 1–5 cm long, glabrous. **Inflorescence** axillary, 1–3 pairs, 1–4 flowered, purple-violet; peduncle 1–3, glabrous; bracts paired, free, lanceolate-acuminate or small scale, up to c.1 cm long; pedicel 0.8–2 cm long, glabrous. **Calyx** tubular, tube c. 0.7 cm long; lobes divided less than half way, triangular, c. 0.4–0.5 cm long; glabrous, caducous. **Corolla** narrowly infundibuliform, tube curved (slightly pouched), 3.5–6 cm long, glabrous. **Stamens** 2: filament inserted 1.2–1.5 cm from base of corolla tube, 0.7 cm long, minutely gland-dotted, curved; anthers elliptic, c. 2.2 by 1.5 mm long, glabrous, adaxial surfaces coherent; staminode 2, inserted 1 cm from base of corolla tube, 4 mm long, slender, glabrous. Gynoecium 2–2.5 cm long, stigma deeply bilobed. **Fruit** orthocarpic, young fruit 4.8 cm long, glabrous. Seeds not seen.

Distribution. India (Sikkim, Assam), Bhutan and North Myanmar (Fig. 4.9).

Ecology. Stream banks in the forest, alt. 1,006–1,891 m.

Provisional IUCN conservation assessment. Endangered EN B2ab(iii). This species has an EOO about 38,626 km² and AOO about 24 km². The EOO is within the threshold of Near Threatened but the AOO is within the threshold of Endangered. However, this species is only known from a small number of collections and some of them are near the town and must be threatened by local construction projects and human activities. An assessment of Endangered is more appropriate than Least Concern.

Specimens examined. Bhutan: Sankosh, above Daga Dzong towards Daga La, 27°05'49.2"N 89°52'18.5"E, 1,800 m, 7 Aug. 1989, *Wood 7047* (E (E00627481)).

India: Sikkim, West District, Rathong Chhu valley between Yoksam and Paha Khola, 27°23' N 88°13' E, 1940, 8 July 1992, *Long et al. 119* (E (E00627483)); Sikkim, *Hooker s.n.* (K); Lachen, 5 Aug. 1849, *Hooker s.n.* (K (Lectotype)); Darjeeling, Jakeras Road, Jigzags, 1,006 m, 18 Aug. 1875, *Gamble 3503C* (K); *ibidem*, 18 Aug. 1875, *Gamble s.n.* (K); Sikkim, Darjeeling, 1,891 m, Aug. 1882, *Gamble 10535* (BM (BM011025865), K).

Myanmar: Kachin state: Meng-Kyi, 26°16' N 98°19' E, 1219, 15 Aug. 1919, *Ward 3543* (E (E00627482)).

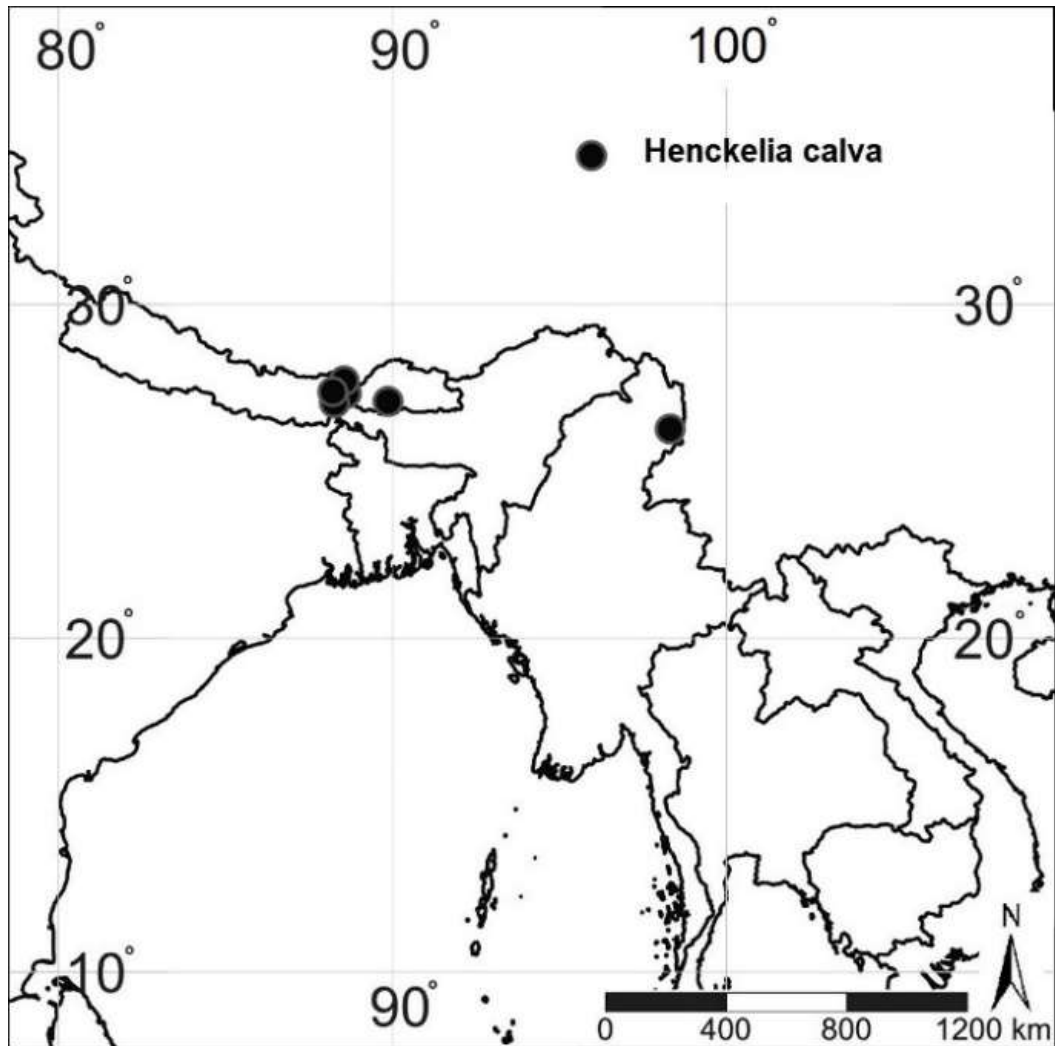


Figure 4.9 Distribution map of *Henckelia calva* (C.B. Clarke) D.J. Middleton & Mich. Möller. Base map from <https://www.simplemappr.net>

6. *Henckelia campanuliflora* Sirim., Thai Forest Bull., Bot. 47(1): 39-42. 2019.

TYPE: Myanmar, Shan State, Htan San Gu, Taunggyi Township, evergreen forest, limestone karst and colluvial slopes down to stream on dark brown loam, 20°49'14"N, 97°20'12.4"E, alt. 1,234 m, fl. 21 Sept. 2015, Y. Baba, K. Kertsawang, C. Kilgour, C. Puglisi, M. Rodda, P. Srisanga, Thant Shin & Phyu Phyu Hnin 103582 (Holotype, Isotype **SING!**).

Caulescent annual herb, up to 10 cm tall; stem 0.5–4.5 cm long, multicellular and glandular hairs, hairs 0.4–2.1 mm long. **Leaf** symmetrically orbicular (globose or rhombic), 4.2–7 by 4–6.5 cm, herbaceous, apex obtuse or acute, base cordate, margin

remotely dentate, upper and lower surface hairy, lateral veins 4–6 ascending on each side; only one leaf developing, petiole terete, 0.5–3.1 cm long, hairy. **Inflorescence** terminal, 3.8–6.2 cm long, inserted at the junction of the petiole and the stem, single or compound cymes, 2–9-flowered; peduncles terete, greenish or purplish, 3–3.7 cm long, hairy; bracts paired, free, sessile, lanceolate, 1.5–2 by 0.2–0.5 mm, apex acute, hairy, margin entire; pedicels terete, greenish or purplish, 3–3.7 cm long, with multicellular and glandular hairs. **Calyx** 5-partite; lobes basally connate, narrowly triangular, pinkish green, c. 3.4 by 0.6 mm; hairy, persistent. **Corolla** white, tube campanulate, c. 5 mm long, sparsely hairy; lobes c. 4 by 3 mm. **Stamens** 2: filaments inserted from base of corolla tube, c. 3 mm long, curved; anthers reniform, adaxial surfaces coherent, c. 1.2 by 1 mm; staminodes 3, inserted 2 mm from base of corolla tube, c. 0.4 mm long, glabrous. Gynoecium 9 mm, ovary c. 1 mm across, hairy; stigma peltate, dark purple, c. 0.4 by 0.2 mm. **Fruit** orthocarpic, green, 1–1.2 cm long by 2–2.1 mm wide, with eglandular hairs and few glandular hairs. Seeds prolate, brown, c. 0.3 by 0.2 mm (Fig. 4.11).

Distribution. Eastern Myanmar (Fig. 4.10).

Ecology. Edge of open semi-evergreen forest in the shade, evergreen forest or limestone karst and colluvial slopes above streams, dark brown loam, alt. 1,106–1,454 m, flowering and fruiting in September.

Provisional IUCN conservation assessment. Endangered (EN B1ab(iii), B2ab(iii)). The EOO and AOO are both well within the threshold of Critically Endangered but it is known from three populations which are fairly close together and could not be described as fragmented. At each site the species is fairly common. None of the populations are in a protected area and there has been extensive deforestation in the area, coupled with microclimate changes due to surrounding agricultural land.

Examined specimens. Myanmar. Shan State: Maopan Taung, Taunggyi Township, 20°48'47.51"N, 97°16'18.7"E, alt. 1,454 m, fr. 23 Sept 2015, *C. Kilgour, Thant Shin & Yo El* m–633 (**SING**). Lomkok mountain and pagoda, roadside, Taunggyi Township, 20°49'2.4" N, 97°13'26.6" E, alt. 1,106 m, fl. 20 Sept. 2015, *C. Puglisi, M. Rodda, S. Ruchisansakun, Thant Shin & Aung San* 103541 (**MBK** (MBK0272469, the left-hand side specimen)).

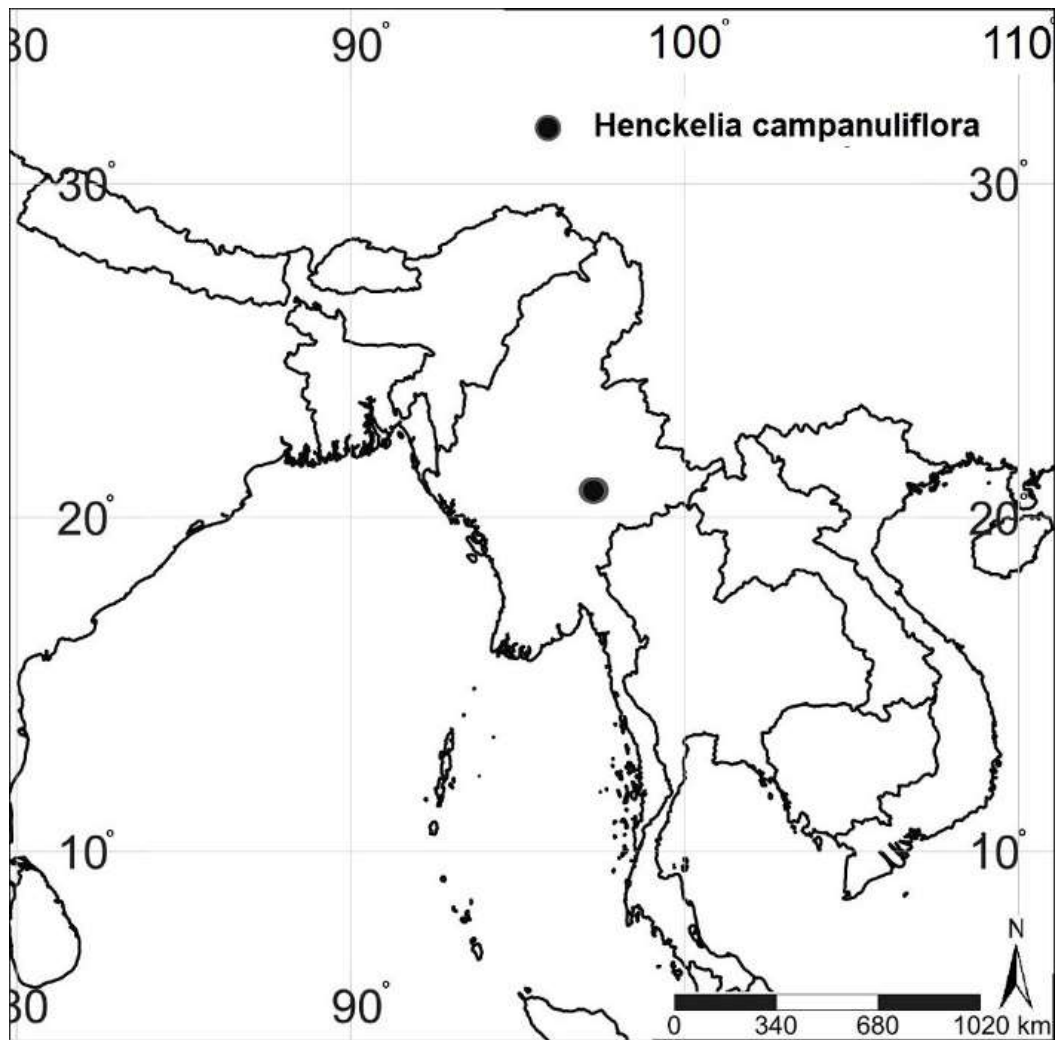


Figure 4.10 Distribution map of *Henckelia campanuliflora* Sirim. Base map from <https://www.simplemappr.net>

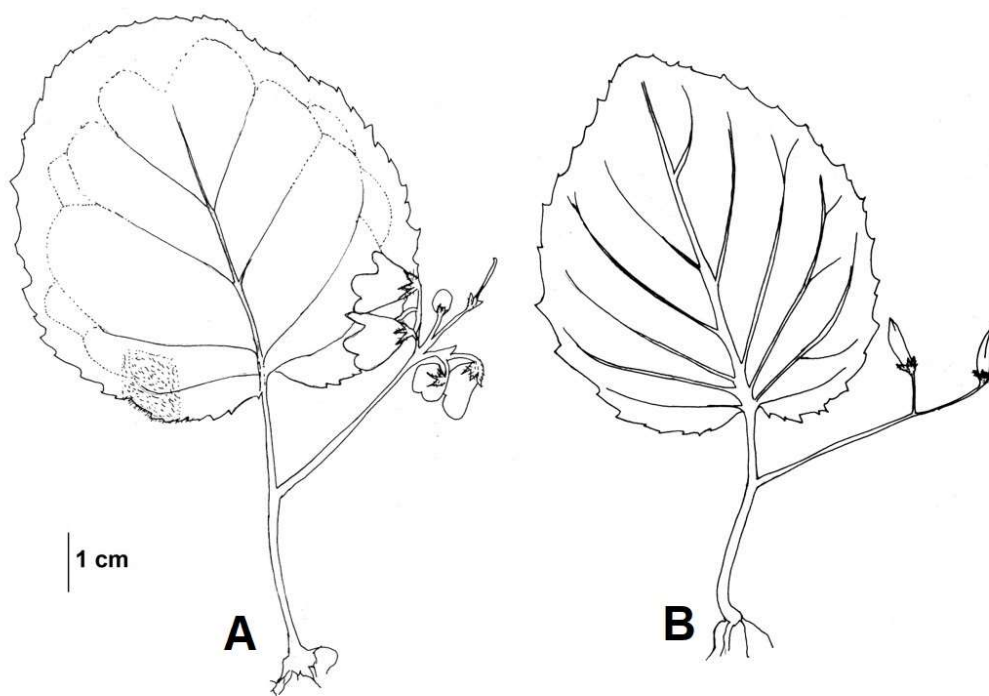


Figure 4.11 Line drawing of *Henckelia campanuliflora* Sirim.: A. Flowering stage; B. Fruiting stage. Drawings: Sukontip Sirimongkol, A. from *Baba et al.* 103582 (**SING**), B. from *Kilgour et al.* m-633 (**SING**).

7. *Henckelia candida* Sirim., Thai Forest Bull., Bot. 47(1): 42–45. 2019.

TYPE: Myanmar, Shan State, Kyauk Gu Taung, Paunglang Reserve Forest, Pinglong Township, 19°57'04.1"N, 96°38'40.3"E, alt. ca 395 m, fl. & fr. 12 Sept. 2015, Y. *Baba*, K. *Kertsawang*, C. *Kilgour*, C. *Pugnalsi*, M. *Rodda*, P. *Srisanga*, *Thant Shin* & *Phyu Phyu Hnin* 103111 (Holotype **SING!**).

Caulescent annual herb, 2.5–10 cm tall; stem with sparse multicellular and glandular hairs. **Leaves**: blade symmetrically orbicular, elliptic or ovate, 4.3–8.5 by 3.4–6 cm, apex acute, obtuse or rounded, base cordate, margin dentate, upper and lower surfaces densely hairy, lateral veins 4–6 on each side; only one leaf developing, sessile. **Inflorescence** terminal, inserted at the junction of the leaf blade and stem, single or compound cymes, 1–17-flowered; peduncles terete, green, 1.2–2.5 cm long, with multicellular hairs and few glandular hairs; bracts paired, free, sessile, elliptic, c. 5.5 by 2.5 mm, apex acute, sparsely hairy, margin ciliate; pedicels terete, green, 1.1–3 cm long, with multicellular hairs and few glandular hairs. **Calyx** 5-partite, basally connate, light green; lobes narrowly triangular, c. 5 by 1.1 mm, hairy, margin entire,

apex acuminate, not reflexed, caducous. **Corolla** campanulate, tube 5 mm long, sparsely hairy; corolla lobes c. 4 by 3 mm; pure white. **Stamens** 2: filaments inserted 2 mm from base of corolla tube, c. 1 mm long, curved; anthers reniform, c. 1 by 0.8 mm, yellow, adaxial surfaces coherent; staminodes 3, inserted 2 mm from base of corolla tube, c. 0.2 mm long, glabrous. Gynoecium 6.5 mm, ovary c. 1.3 mm across, hairy; style terete, slender; stigma peltate, greenish, c. 0.5 by 0.4 mm. **Fruit** plagiocarpic, elongate capsule, green, 1.2–1.8 cm long, sparsely hairy. Seeds prolate, brown, c. 0.6 by 0.2 mm (Fig. 4.13).

Distribution. Eastern Myanmar (Fig. 4.12).

Ecology. Damp gully with karst limestone boulders along the edge of a stream, shaded tall bamboo forest with dense layers of cryptogams and lithophytic plants or open semi-evergreen forest, on limestone, alt. 395–1,151 m, flowering & fruiting in September.

Provisional IUCN conservation assessment. Endangered (EN B2ab(iii)). This species is known from two populations, only one of which is in a protected area. The population that is not in a protected area is in a small patch of forest surrounded by agricultural land with a very high risk of disturbance.

Examined specimens. Myanmar. Shan State: Lomkok mountain and pagoda, roadside, Taunggyi Township, 20°49'2.4" N 97°13'26.6" E, alt. 1,106 m, fl. & fr. 20 Sept. 2015, *C. Puglisi, M. Rodda, S. Ruchisansakun, Thant Shin & Aung San 103541* (**MBK** (the two herbs on the right-hand side)).

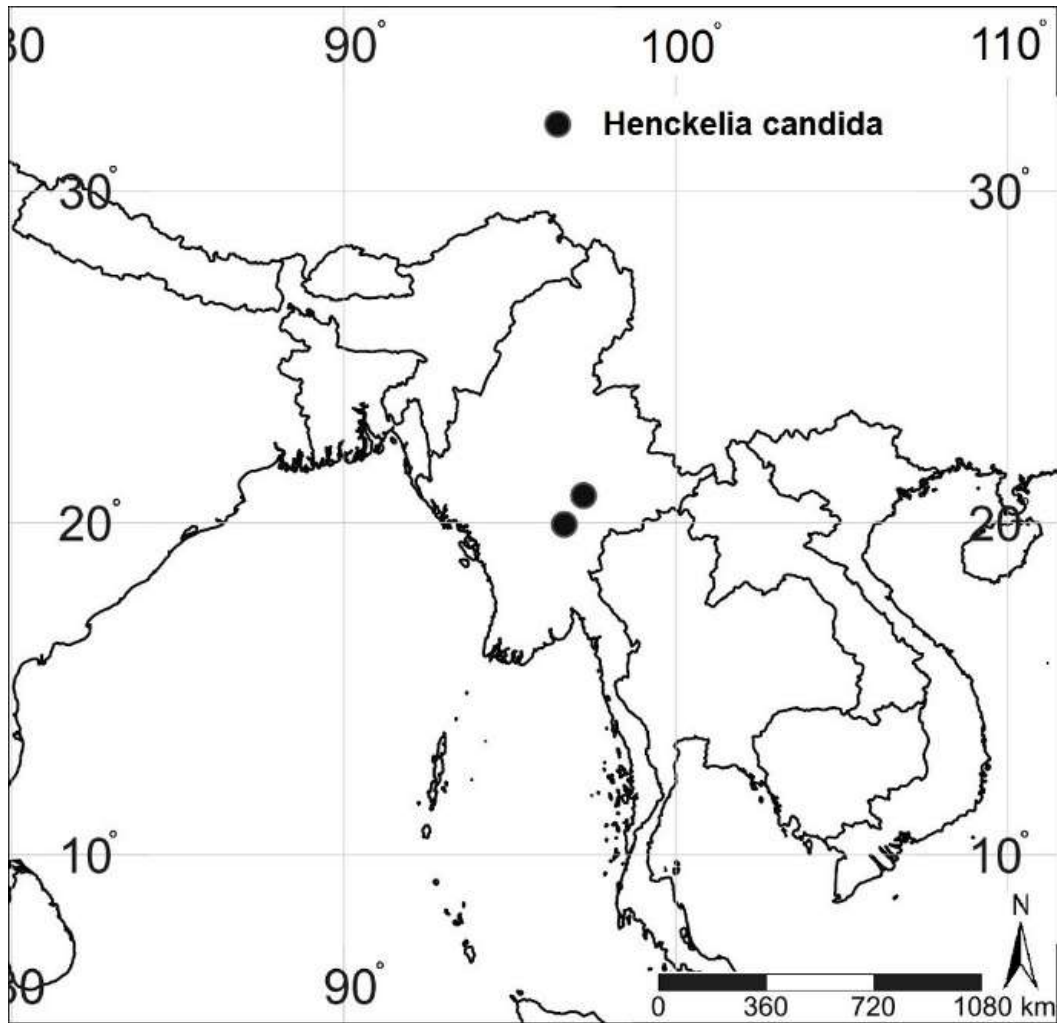


Figure 4.12 Distribution map of *Henckelia candida* Sirim. Base map from <https://www.simplemappr.net>

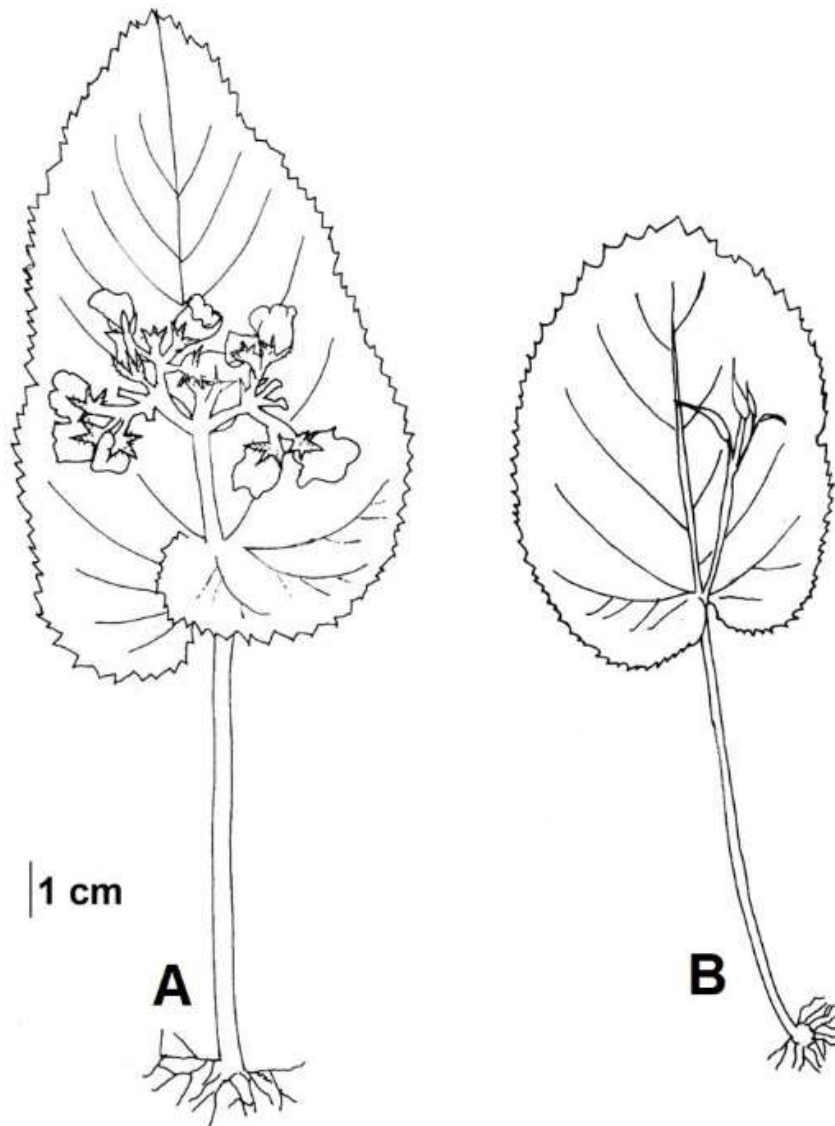


Figure 4.13 Line drawing of *Henckelia candida* Sirim.: A. Flowering stage; B. Fruiting stage. Drawings: Sukontip Sirimongkol from *Baba et al.* 103111 (SING).

8. *Henckelia ceratoscyphus* (B.L. Burt) D.J. Middleton & Mich. Möller, *Taxon* 60(3): 775. 2011.

≡ *Chirita ceratoscyphus* B.L. Burt, *Notes Roy. Bot. Gard. Edinburgh* 21: 212. 1954.

TYPE. Chen 4918 (not seen).

– *Ceratoscyphus caeruleus* Chun, non *Chirita caeruleus* R.Br. 1840.

– *Chirita corniculata* Pellegrin, *Bull. Soc. Bot. Fr.* 73: 420. 1926; Wang et al., *Fl. China* 18: 341. 1998, non *Henckelia corniculata* (Jack) Spreng. 1827; Pellegrin in Lecomte,

Fl. Gen. Indo-Chine 4: 535. 1930; Wood, Notes Royal Bot. Gard. Edinb 33: 158–159. 1974. TYPE: Viet Nam, Tonkin, Tuyen-Quang, Ban-Coc, Eberhardt 4004 (Holotype **P!** (P00602509), Isotype **P!** (P00602508)).

Acaulescent perennial herb, rhizomatous, up to 20 cm tall. **Leaves** whorled: blade narrowly ovate or narrowly elliptical, 8.5–21 by 2.2–6.2 cm, base oblique round to acute, apex acute or acuminate, margin serrate or crenate, upper surface hairy, lower surface with hairs on the lateral veins and midrib, lateral veins 8–13 on each side. Petiole 4–22 cm, sparsely hairy. **Inflorescence** axillary, 2–7, each 1–2-flowered; peduncle 1–7 cm long, hairy. Bracts paired, free, ovate, 4–13 by 1.4–7 mm, margin entire or serrate, sparsely hairy; pedicels 0.8–5 cm long, hairy. **Calyx** tubular, tube 0.8 cm long; lobes divided to half way, 0.7–0.8 cm long, with strongly geniculate recurved tip 0.5–1 cm long, caducous. **Corolla** white with yellow stripe in throat, infundibuliform, 6 cm long, glabrous; lobes violet, c. 1.3 by 2 cm. **Stamens** 2: filaments inserted c. 1.5–1.6 cm from base of corolla tube, 1 cm long, geniculate, sparsely hairy; anthers elliptic, 3 cm long, hairy on the lower surface, adaxial surface coherent; staminodes 2, inserted 1.2–1.4 cm from base of corolla tube, 0.5 cm long, sparsely hairy. Gynoecium 3–3.5 cm long, ovary 1 mm across, hairy; stigma deeply bilobed. **Fruit** orthocarpic, 6.5–7.6 cm long, glabrous. Seeds oblong, c. 0.5 by 0.15 mm (Fig. 4.15).

Distribution. Vietnam (Fig. 4.14).

Ecology. In shady and rocky places, alt. 400 m.

Provisional IUCN conservation assessment. Endangered EN B1ab(iii,v) + B2ab(iii,v).

This species has an EOO about 3,766 km² and AOO about 16 km². The EOO and AOO are within the threshold of Endangered. This plant has only a single sample per collection and none of them are in protected areas.

Specimens examined. Vietnam: Tonkin: 22°00'00.0"N 105°00'00.0"E, (Jan 1888, *Balansa* 4288 (**P** (P03511179, P03511178)), 21 July 1886, *Balansa* 4289 (**K, P** (P03511177, P03511176)), *Balansa* s.n. (**P** (P03511174)), 400 m, Feb 1838, *Petelot* 7248 (**P** (P03511175))); Tuyen-Quang, Ban-Coc, 21 49°0"N, 105°13'0"E, 7 Aug. 1917, *Pellegrin* 4004 (**P** (P00602508, P00602509)). *Anonymous VMN-CN373 (VNMN)*.

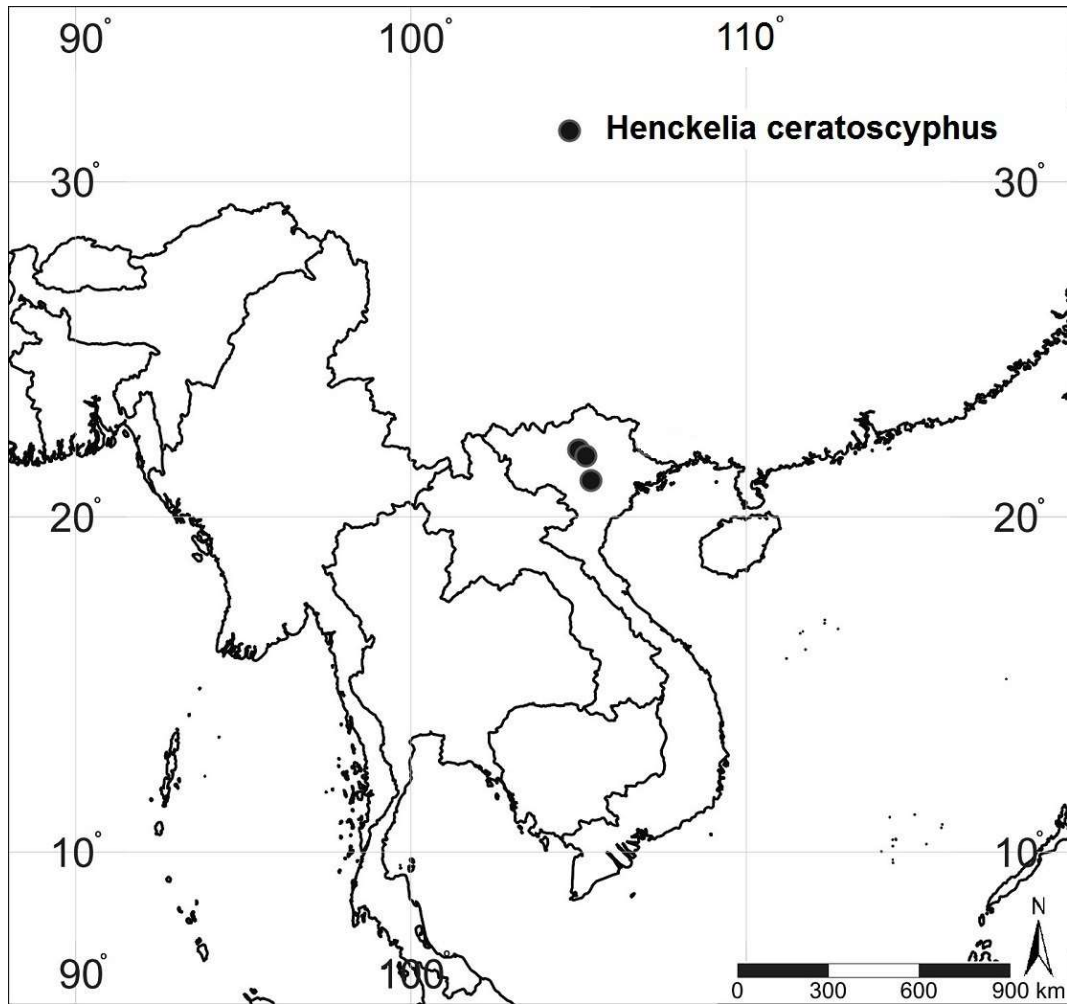


Figure 4.14 Distribution map of *Henckelia ceratoscyphus* (B.L. Burtt) D.J. Middleton & Mich. Möller. Base map from <https://www.simplemappr.net>.

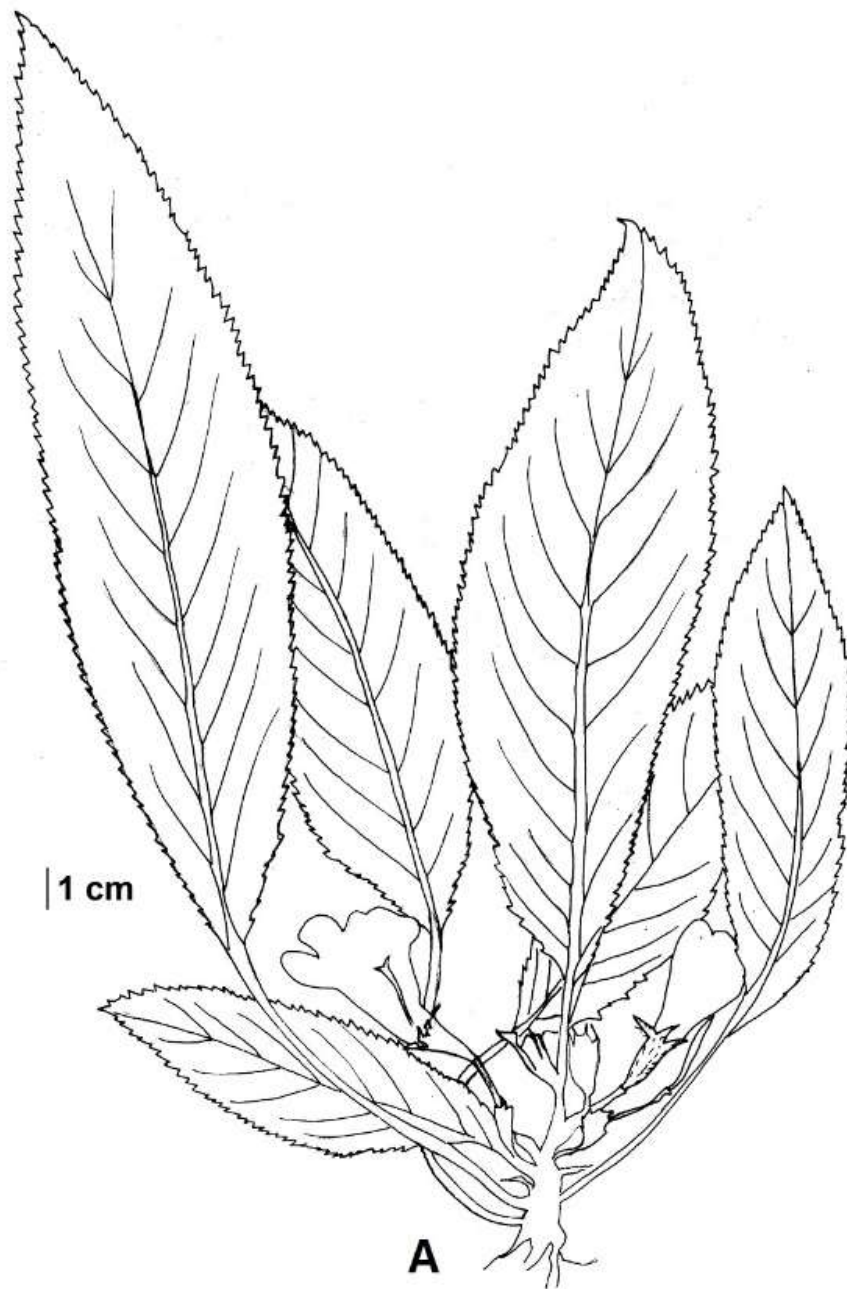


Figure 4.15 Line drawing of *Henckelia ceratoscyphus* (B.L. Burtt) D.J. Middleton & Mich. Möller: A. Habit. Drawing: Sukontip Sirimongkol from *Eberhardt* 4004 (P).

9. *Henckelia dasycalyx* Sirim. & D.J. Middleton, *Thai Forest Bull., Bot.* 47(1): 51–53. 2019.

TYPE: Thailand, Phitsanulok, Chat Trakan District, Namchung, Phumiang–Phuthong Wildlife Sanctuary, 17 July 2012, alt. 782 m, fl. & fr. 15 July 2012, *Romklao Botanical Garden* 0560/2555 (Holotype **QBG!** (QBG62273)).

Caulescent woody annual herb, 30 cm tall; stem with dense white multicellular hairs. **Leaves** opposite: blade symmetrically elliptic or ovate, each pair unequal in size, 11–14.4 by 5.2–9 cm, apex acute, base cuneate or sub-oblique, margin crenate (sometimes dentate), upper and lower surfaces densely hairy, lateral veins 8–9 on each side. Petiole terete, 3–8 cm long, densely hairy. **Inflorescence** axillary, c. 7, single or compound cymes, 1–9-flowered; peduncles terete, 3–6.3 cm long, densely hairy; bracts paired, free, sessile, elliptic, c. 5.5 by 2.5 mm, apex acute, sparsely hairy, margin ciliate; pedicels terete, 0.5–1.8 cm long, densely hairy. **Calyx** 5-partite; lobes narrowly triangular, basally connate, c. 11 by 1 mm; densely hairy persistent. **Corolla** purple; tube infundibuliform, c. 4 cm long, glandular hairs. **Stamens** 2: filaments inserted c 1.5–1.9 cm from base of corolla tube, c. 1 cm long; anthers elliptic, c. 2 mm long, adaxial surfaces coherent; staminodes 2, inserted c 1.5 cm from base of corolla tube, c. 3 mm long, glabrous. Gynoecium 3.6 cm; ovary 1 mm across, hairy; style terete, slender; stigma deeply bilobed. **Fruit** orthocarpic, 3.5–5.5 cm long, densely hairy, persistent calyx divided to the base. Seeds not seen.

Distribution. Northern Thailand, only known from the type locality (Fig. 4.16).

Ecology. In deciduous forest, alt. 782 m, flowering & fruiting in July

Provisional IUCN conservation assessment. Data Deficient (DD). This species is only known from the type collection that occurs within a protected area. The size of the population, the EOO and AOO are all unknown.

Specimens examined. Thailand: Phitsanulok, Chat Trakan District, Namchung, Phumiang–Phuthong Wildlife Sanctuary, 17 July 2012, alt. 782 m, fl. & fr. 15 July 2012, *Romklao Botanical Garden* 0560/2555 (**QBG!** (QBG62273)).

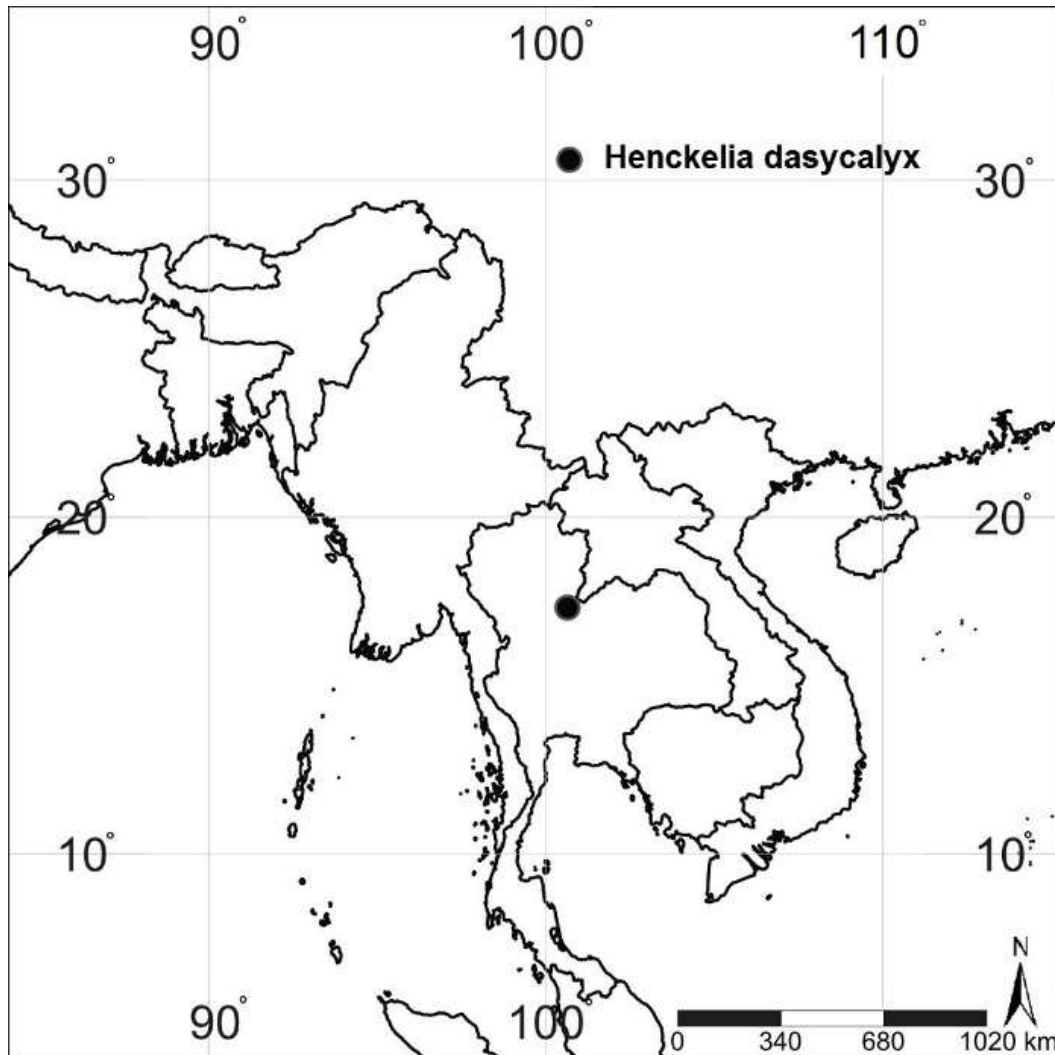


Figure 4.16 Distribution map of *Henckelia dasycalyx*. Base map from <https://www.simplemappr.net>

10. *Henckelia fruticola* (H.W. Li) D.J. Middleton & Mich. Möller, *Taxon* 60(3): 775. 2011.

≡ *Chirita fruticola* H.W. Li, *Bull. Bot. Res., Harbin* 3(2): 37. 1983; Wang et al., *Fl. China* 18: 341. 1989. TYPE: China, Yunnan, Jing Ping, 1,300 m, 22 May 1956, *Yunnan Complex. Exped 2489* (Holotype **KUN!** (image KUN1289687)).

Acaulescent perennial herb, rhizomatous, stem absent, simple, up to 30 cm tall, hairy. **Leaves** whorled: blade ovate, or cordate, 5.3–9.2 by 3.5–7 cm, apex acute, base cordate, upper surface hairy, lower surface glabrous, only midrib and secondary veins hairy, margin dentate or remotely dentate, lateral vein 6–8 on each side. Petiole terete,

5.5–26 cm long, sparsely hairy. **Inflorescence** single, axillary, 1–2-flowered, purple blue or violet; peduncle 5–7.5 cm, glabrous; bracts paired, connate on base, sessile, cordate, 5–18 mm, tomentose, margin entire, apex acute; pedicel 1.4 cm long, glabrous. **Calyx** tubular, tube c. 0.5 cm long; lobes divided more than half way, narrowly lanceolate, c. 1.8–2.2 cm long, apex acuminate; densely hairy, caducous. **Corolla** tube infundibuliform, c. 4.9–6 cm long, hairy. **Stamens** 2: filaments inserted c. 1.2–2.5 cm from base of corolla tube, c. 1 cm long, geniculate, hairy 1/3 times from the top of the filaments; anthers elliptic, 3 mm long, coherent by adaxial surface; staminodes 3, inserted 0.8–2.2 cm from base of corolla tube, 1–3 mm long, stout. Gynoecium 3.6–4 cm, stigma deeply bilobed. **Fruit** not seen.

Distribution. China (SE Yunnan, Jinping Xian) and Northern Vietnam (Fig. 4.17).

Ecology. Grows on limestone mountains, near streams, in wet places, primary evergreen forest on high mountains, or montane thickets, alt. 1,300–1,590 m.

Provisional IUCN conservation assessment. Endangered EN B1ab(iii,v)+B2ab(iii,v). This species has an EOO about 145 km² and AOO about 16 km². The EOO and AOO are within the threshold of Endangered. This population has two localities and can be divided into two fragments. None of them are in the protected area and within them there is only a single sample per collection.

Specimens examined. Vietnam: Lao Cai Province, Sapa District (beside road to Ban Khoang, 1,590 m, 3 May 1992, *Rushforth 2124* (E (E00627685)), San Sa Ho, Sin Chai, 22°20'44.3" N, 103°48'77" E, 1,377 m, 15 Sept. 2005, *Vu et al. HNK 445* (K (K000610677))).

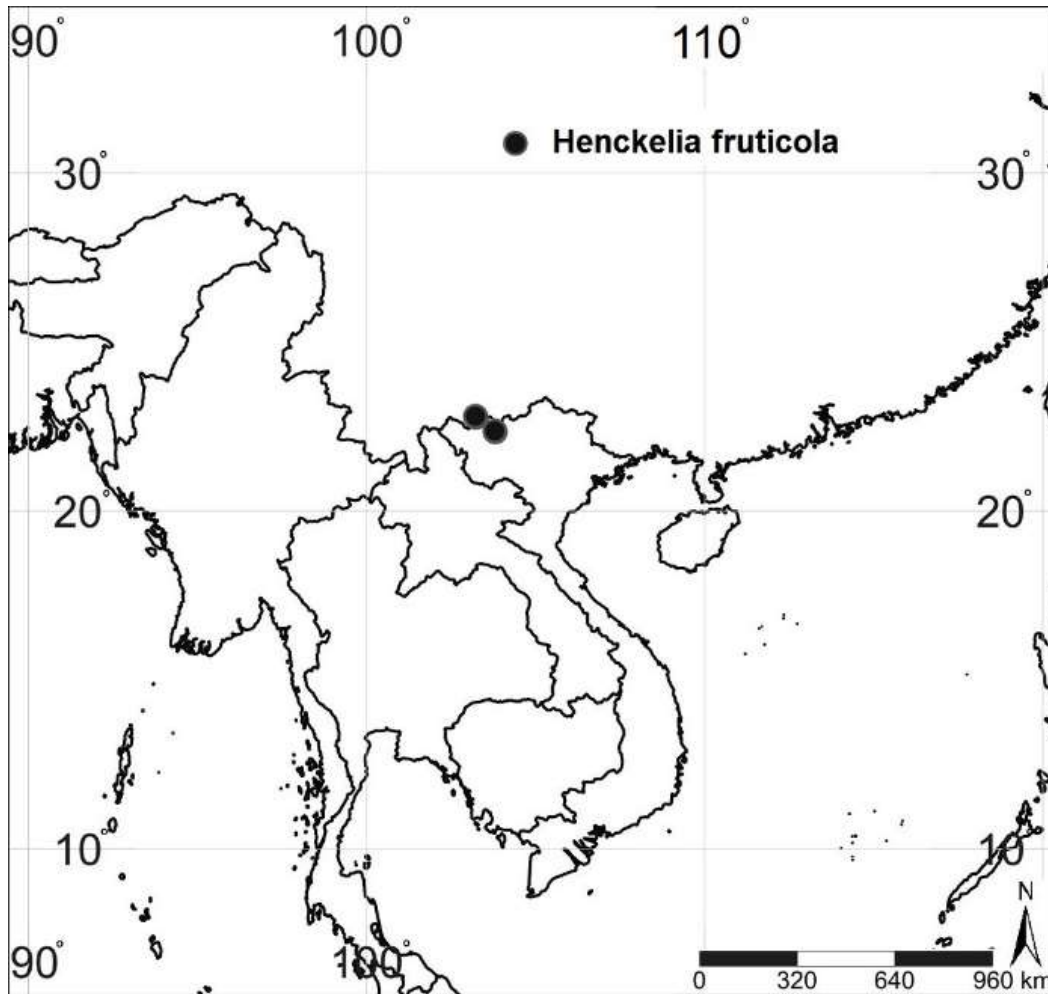


Figure 4.17 Distribution map of *Henckelia fruticola* (H.W. Li) D.J. Middleton & Mich. Möller. Base map from <https://www.simplemappr.net>

11. *Henckelia grandifolia* A. Dietr., Sp. Pl. ed. 6, 1:576. 1831; Weber et al, Taxon 60(3): 775. 2011; Middleton, Weber, Yao, Sontag & Möller, Edinburgh J. Bot. 70(3): 398. 2013.

–*Chirita macrophylla* Wall., Pl. Asiat. Rar. 1: 56, t 72. 1830, non *Henckelia macrophylla* (D. Don) Spreng.; Pellegrin, Fl. Indo-China 4(5): 537. 1930; C.B. Clarke Comm, et Cyrt. Beng. 1874. tab 71 et in DC. Monogr. Phaner. V: 116. 1883; Pellegrin, Fl. Indo-China 4(5): 537. 1930; Barnett, Fl. Siam. 3(3): 225. 1962; Hara, Enum. Fl. Pl. Nepal 3: 133. 1982; Wang et al., Fl. China 18: 342–343. 1989; Hilliard, Fl. Bhutan 2(3): 1318–1319. 2001. TYPE: Nepal, 1821, *Wallich 805* (Holotype. **BM!** (BM000571151); Isotype **G!** (image-G00133242), **K!** (K000858380, K000858382, K000858395), **M!** (image-M0185548), **NYBG!** (image-NYBG00063239), **PH!** (image-PH00000336)).

–*Chirita macrophylla* Wall. subsp. *tirapensis* Panigrahi, Bull. Bot. Soc. Bengal. 21 (1): 32. 1967.

–*Chirita macrophylla* Wall. var. *tirapensis* (Panigrahi) Deb & R.M. Dutta, J. Bombay Nat. Hist. Soc. 71(2): 271. 1974 (publ. 1975).

–*Calosacme macrophylla* Wall., Numer. List [Wallich] n. 805. 1829 (Nom nud).

Acaulescent or caulescent perennial herb, rhizomatous, stem short or erect, simple, 15–26 cm tall, hairy. **Leaves** opposite: blade asymmetrically ovate, or cordate, 10–17.2 by 7–11.5 cm wide, herbaceous; apex acute to acuminate; base cordate or oblique, rarely attenuate; base oblique about 0.2 cm distance; margin serrate, rarely repand; upper and lower surface sparsely hairy; especially on midrib and secondary veins, lateral veins 6–8 on each side. Petiole terete 7.3–22.5(30.6) cm, hirsute or strigose **Inflorescence** axillary, simple or compound cymes, 1–5 flowered, purple to blueish violet; peduncle 5.2–16.3 cm, glabrous or hairy; bracts paired, free, sessile, ovate or lanceolate, rarely rhomboid or linear, 0.5–1.1 cm long, sparsely hairy. Pedicel terete, 1.1–2 cm long, hairy or glabrous. **Calyx** tubular, tube 0.6–1.4 cm long; lobes divided equally or less than half way, narrowly triangular, 0.5–0.9 cm long; outside glabrous or sparsely hairy, caducous. **Corolla** infundibuliform, 4–6 cm long, glabrous to sparsely shortly hairy. **Stamens** 2: filaments inserted c. 1.5–1.8 cm from base of corolla tube, 1.3–1.7 cm long, hairy between filament and anther, geniculate; anthers elliptic, 2–3.7 mm long, adaxial surface coherent; staminodes 2, inserted 1 cm from base of corolla tube, 3 mm long. Gynoecium 2–3.6 cm long, sparsely hairy, stigma deeply bilobed. **Fruit** orthocarpic, 8.5–11 by 0.15–0.19 cm, hairs sparse, short. Seeds elliptic, c. 0.5 by 0.2 mm (Fig. 4.19).

Distribution. India, Bhutan, China, Myanmar and Northern Thailand (Fig. 4.18).

Ecology. In shady montane forest, on moist sandy banks and on rock, alt. 792–3,048 m.

Provisional IUCN conservation assessment. Least Concern. This species has an EOO about 1,370,814 km² and AOO about 140 km². The EOO is within the threshold of and the AOO is within the threshold of Endangered. However, this plant is common and widespread. An assessment of Least Concern is more appropriate than Endangered.

Specimens examined. India: East Himalaya, Sikkim (Figer hill, 792 m, 20656,

Chatterjee 274 (**AMD** (AMD73811)), *Griffith 3824* (**K, P** (P03884223)), *Griffith s.n.* (**U** (U1342361)), *Beddome s.n.* (**BM** (BM011025894)), *Hooker s.n.* (**K**), *Hooker s.n.* (**K**), *Hooker s.n.* (**K**), *Hooker s.n.* (**NMNS** (NMNS00064521)), *Hooker s.n.* (**P** (P03884222)), *Hooker s.n.* (**P** (P03884224)), *Hooker s.n.* (**U** (U1342362)), *Hooker s.n.* (**K** (K000858395)), 2,400 m, *Hooker s.n.* (**TCD**), *Hooker & Thompson s.n.* (**BM** (BM000040143)), Nor Lha, 1,219 m, 8 July 1862, *Anderson 980* (**P** (P03884220)), Yoksum to Bakhim forest trail. (AGSES = Alpine Garden Society's Expedition SIKKIM, 1983), 2,000 m, 10 June 1983, *Starling et al. AGSES 1* (**K**), 27°19'48"N, 88°37'12"E, 2,440 m, *Hooker s.n.* (**G** (G00492601))), Sikkim, Dajeeling (2100 m, *Gamble 3500A* (**K**), 3 July 1874, *C.K. 212* (**K**), 2,134 m, 30 Aug. 1869, *Clarke 8892C* (**BM** (BM011025898)), Lucknow, 1,981 m, July 1881, *Gamble 9504* (**K**), Rungbee, 20 June 1870, *Clarke 12104* (**K**), *ibidem*, 1,800 m, July 1870, *Clarke 12448* (**K**), *ibidem*, 20 June 1870, *Clarke 12104F* (**K**), *ibidem*, Rungbee, 1,829 m, July 1870, *Clarke 12448B* (**BM** (BM011025897))), Sikkim, Mangko, 1,676 m, 7 Oct. 1884, *Clarke 36340A* (**BM** (BM0000997762)); Nagaland, Fekwera, Naga hills, 1,524 m, 31 Aug. 1935, *Bor 6207* (**K**); Biwas s.n. (**NMNS** (NMNS00064517)); 2,286 m, 4 July 1923, *Cowan s.n.* (**K**); 2134 m, 2 July 1874, *Truetler 212* (**K**).

Bhutan: Chungkar, Diwangiri-Trashigung Road, 1,372 m, 24 June 1934, *Ludlow et al. 557* (**BM** (BM011025896)).

Nepal: 27°42'41"N, 85 18'31"E, *Wallich 885* (**SING** (SING123433)), March 1821, *Wallich 805* [Holotype. **BM!** (BM000571151); Isotype **G!** (image-G00133242), **K!** (K000858380, K000858382, K000858395), **M!** (image-M0185548), **NYBG!** (image-NYBG00063239), **PH!** (image-PH00000336)).

China: Yunnan: 3,048 m, June 1924, *Forrest 17649* (**BM** (BM000041515), **K**), *Forrest 18254* (**K**), *Forrest 18261* (**K**), *Forrest 30383* (**E** (E00627462)), Hill N.W. of Seugyueh, 25°30' N, 98°30' E, 2,400 m, 1 June 1925, *Forrest 26773* (**E** (E00087409), **K**, **NMNS** (NMNS00064518), **P** (P03884221)), Jinghong, Mt. Wuliang, Jingdong, 1°59'41.7"N 100°55'46.1"E, 27 July 2004, *Zhao 2009* (**SING** (SING42500)), Mekong-Salwin divide, 25°11'47.3"N 98°50'51.6"E, 2,400 m, 1 July 1919, *Forrest 18217* (**E** (E00087411)), Mianning, Poshang, 2,450 m, 4 Oct. 1938, *Yu 17862* (**E** (E00087408)), Shucli-Salwin divide, 3,000 m, 1 Aug. 1917, *Forrest 15886* (**BM** (BM000041509), **E** (E00087414), **K**), Sluneli-Salwin divide, 2,400 m, 1 Aug. 1919, *Forrest 18256* [**BM** (BM000041517), **E** (E00096807)], W. Yunnan, Shweli-Salwin divide, 3,000 m, 1 June 1924, *Forrest 24473* (**E** (E00096816), **K**), W. Yunnan, Shweli-Salwin divide, 2,700 m, Sept. 1924, *Forrest 25205* (**E** (E00087410), **K**), Tengchong Xian, NanKang, 24°49'42.7" N, 98°46'04.1"E, 2,172 m, 10 July 2007, *Wen et al. Tibet-MacArthur2013* (**NMNS** (NMNS00972224)).

Myanmar: Chin state: Natma Taung NP (along the roadside between 70 miles and 84 miles (Mindat-Matupi car road), Matupi Township, near the Natma Taung NP, 21°36' 0.7" N,

93°39' 31.3" E, 2,225 m, 22 July 2013, *Aung et al. 092624* (**SING** (SING0218671)); along the roadside between 5 and 6 miles from Kanpetlet to Natma Taung (Mt. Victoria), 21°12'0"N, 94°1'0"E, 2,100 m, 22 July 2011, *Man 087552* (**QBG** (QBG61747); **SING** (SING0218670)), in the vicinity of Kanpetlet, 21°12'0"N, 94°01' 00"E, 2 Aug. 2007, *Man 055305* (**SING** (SING0218668)); along the roadside between the entrance of Natma NP and 8 miles, Kampetlet Township, 20°12'89" N, 93°59'29.6" E, 2,400 m, 29 July 2013, *Aung et al. 092760* (**SING** (SING0218672)); along the walking trail between Hilong Village and the junction of 14 miles from Mindat, Mindat Township, 21°22'10.6" N 93°49'17.5", 1,740 m, 5 Aug. 2008, *Fujikawa et al. 053149* (**SING** (SING0218667)); 6 miles from entrance of Natma Taung NP, along unpaved road between Kanpetlet and 10 miles base camp of NP, 20°13'13" N, 93°58'41" E, 2,400 m, 21 Aug. 2013, *Fujikawa et al. 094116* (**QBG** (QBG76358); **SING** (SING0218673)); along foot path and unpaved new car road between Kanpetlet and Yelong Pan Village, Kanpetlet Township, 21°13' 07.9" N, 94°03'46.4" E, 1,260–1,435 m, 23 Aug. 2013, *Fujikawa et al. 094318* (**MBK** (MBK0254444)); 37 miles from Mindat, between Mindat and Matupi (Mindt-Matupi Road), Mindat Township, 21°33'58" N, 93°48'02" E, 2,450–2,545 m, 31 Aug. 2013, *Fujikawa et al. 094779* (**QBG** (QBG76043); **SING** (SING0218674)), in the pine forest, along the road to the entrance of the trail to the peak of Mt. Victoria, 21°12'18.5"–52.5" N 94°01'32.1"–00'36.0" E, 2,030–2,295 m, 27 June 2009, *Tanaka et al. 081292* (**SING** (SING0218669)); Esakan, Mt Victoria, 1,676 m, 4 Sept. 1956, *Ward 22660* (**BM** (BM011025895)); Moku-ji woods, 2,850 m, 28 July 1920, *Farrer 1760* (**E** (E00096831)). N.E. Myanmar (hills around Tyi-Zo-Ci, 2,400 m, Aug. 1925, *Forrest 27149* (**E** (E00096829), **K**, **NMNS** (NMNS00064520)), N Nakka-Salwin divide, 2,400 m, June 1931, *Forrest 29816* (**BM** (BM000041533), **E** (E00096830)); Sirhoi, 2,286 m, 16 June 1948, *Ward 17695* (**BM**, **NYBG** (NYBG02652108)); Upper Myanmar, Valley of New Chaung, 1,800 m, 24 Aug. 1914, *Ward 1898* (**E** (E00096832)).

Thailand: NORTHERN. **Mae Hong Son:** Pai, Lum Nam Pai WS, trails to Doi Chik Chong, 19°24'36.6" N, 98°19'15.9" E, 1,926 m, 15 Oct. 2018, *Tetsana et al. 1508* (**BKF**). **Chiang Mai:** Doi Inthanon NP (Pa Kampeng, east slope, Doi Augka Me klang Phat drainage, 18°33'56.1"N 98°32'13.7"E, 1,780 m, 2 Aug. 1927, *Garrett 412* (**ABD**, **E** (E00627687)), Pha Ngam (limestone cliffs), Mae Win, Mae Wang., 18°36'34.3"N 98°29'24.4"E, 1,850 m, 26 Aug. 2004, *Palee 704* (**CMU** (CMU24079), **L** (L3794596)), Pha Mon, 1,600 m, 29 Oct. 1962, *Smitinand et al. 7622* (**BKF** (BKF45743)), transect line 1,900, 18°32'37.27"N, 98°29'16.27" E, 1,900 m, 18 Nov 2012, *Tagane et al. T1318* (**BKF**195750)). **Nan:** Pua, Doi Phu Kha NP [19°13'N, 101°06' E, 1,870 m, 26 July 1999, *Srisanga 928* (**E** (E00161669, E00161670), **QBG** (QBG15213)), 19°10' N, 101°07' E, 1,800 m, 31 Aug.2000, *Srisanga 1535* (**QBG** (QBG17982)), 19°13' N, 101°06'

E, 1,700 m, 7 July 2001, *Srisanga 1986* (**QBG** (QBG20974)), Doi Dong Ya Waai, 1,400 m, 24 Sept. 1989, *Palsotypeoksantivatana Y-2495-89* [**BK** (BK062838)], trail from Lan Doo Dao to Doi Pha Tang, 1,700 m, 20 June 2008, *Pooma et al. 7102* (**BKF** (BKF172700), **E** (E00533232)), *ibidem*, 19°11'11" N, 101°05'52"E, 1,755 m, 4 Oct. 2016, *Sirimongkol et al. 707* (**BKF, E, K, P, TCD**), *ibidem*, 19°11'11"N, 101°05'52"E, 1,755 m, 31 Aug. 2016, *Sirimongkol et al. 720* (**BKF, TCD**)). **Tak**: Um Phang, Doi Hua Mut, 15 Aug. 1934, *Smith 659* (**NMNS** (NMNS00056602)). **Kamphaeng Phet**: Phang Sila Thong, Mae Wong NP, the route from Khlong 2nd camping area to Mo Ko Joo peak, 15°55'32.9"N, 99°06'01.03"E, 1,824 m, 17 Nov. 2017, *Poopath et al. 1844* (**BKF** (BKF194443)).

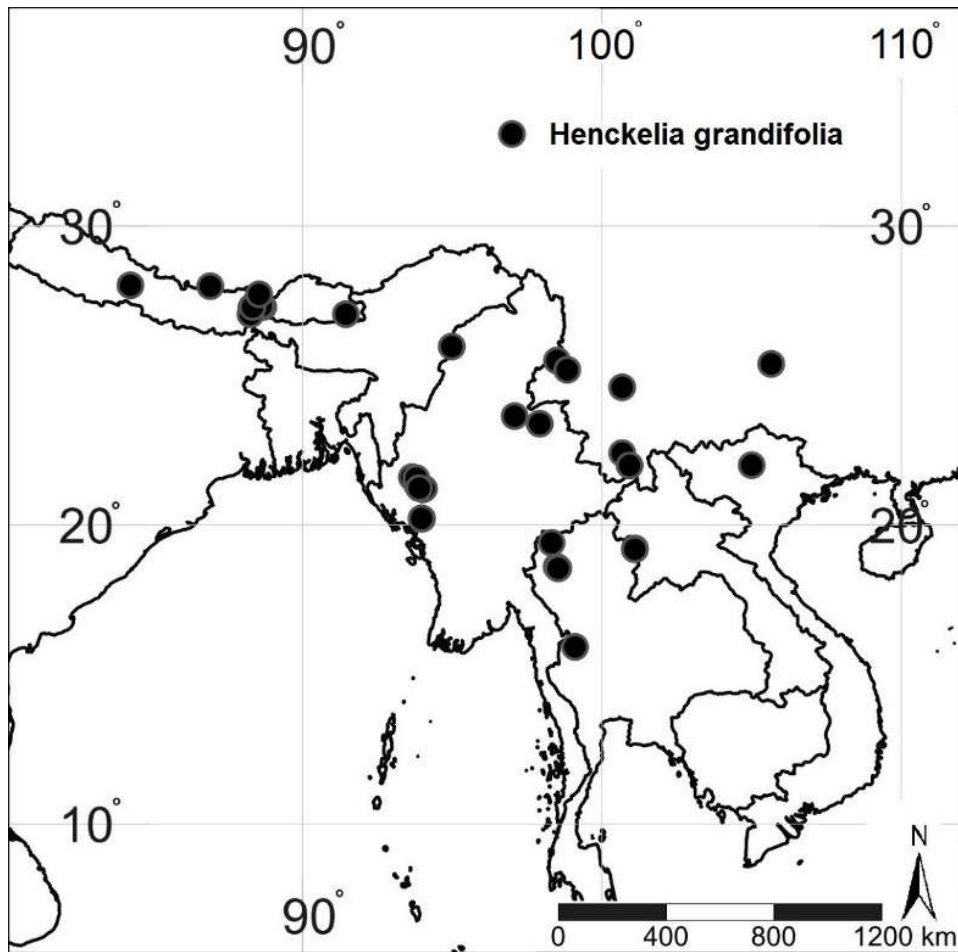


Figure 4.18 Distribution map of *Henckelia grandifolia* A. Dietr. Base map from <https://www.simplemappr.net>

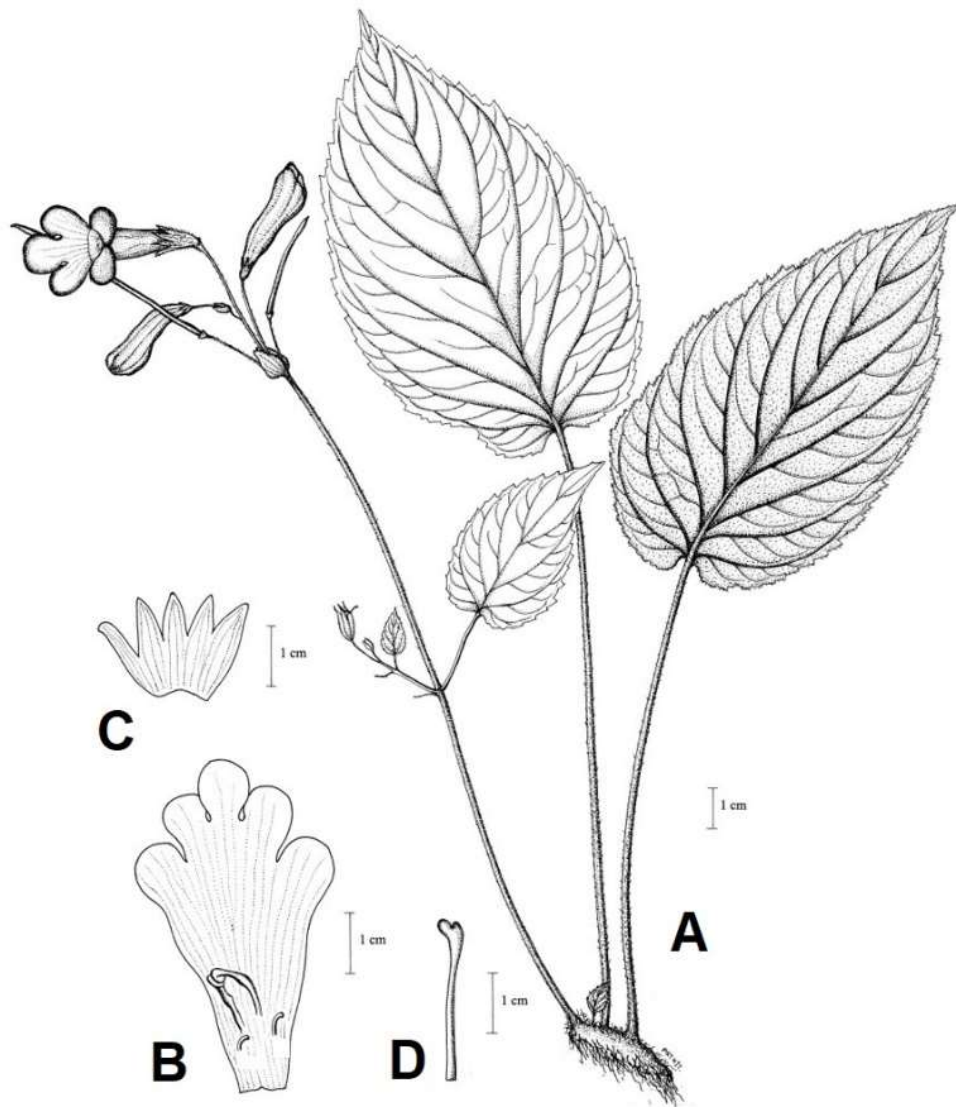


Figure 4.19 Line drawing of *Henckelia grandifolia* A. Dietr.: A. Habit; B. Flower dissection; C. Calyx; D. Gynoecium. Drawings: Arthit Kamgamnerd from *Pooma & Tamura 7102* (E).

12. *Henckelia heterostigma* (B.L. Burtt) D.J. Middleton & Mich. Möller, *Taxon* 60(3): 775. 2011.

≡ *Chirita heterostigma* B.L. Burtt, *Notes Roy. Bot. Gard. Edinburgh* 26: 262–263. 1965.
 Type: Myanmar, Nam Hat, 2,000–2,500 ft, 27 Apr. 1926, *Ward 6655* (Holotype. K! (K000858405)).

Cauliscent, rhizomatous, perennial herb, stem up to 60 cm tall, covered with red hairs.

Leaves opposite: blade elliptic, 10–17 by 3.5–8 cm, apex acuminate, base oblique-rounded, margin remotely dentate, upper surface sparsely hairy, lower surface hairy on veins, secondary veins 9–12 cm on each side. Petiole 0.5–1.5 cm long, hairy.

Inflorescence axillary, several on stem, solitary or pair flowered, pale violet; peduncle 0.5–1 cm long, hairy; bracts more than 2, free, several, up to 8 mm long, whorled; pedicels up to 8 mm long, hairy. **Calyx** tubular, tube 1.2 cm long; lobes divided more of half way, 1–1.3 cm long, hairy. **Corolla** infundibuliform, tube 5.5 cm long, lobes c. 1 by 1.2–1.5 cm. **Stamens** 2: filaments inserted 1.7 cm from base of corolla tube, 1–1.2 cm long, glabrous; anthers elliptic, 4 mm long, adaxial surfaces coherent; staminode 2, inserted 1.3 cm from base of corolla tube, 4 mm long, filaments hairy. Gynoecium 4.5 cm long, glandular hairs; stigma shallowly bilobed. **Fruit** not seen.

Distribution. Myanmar (Fig. 4.20).

Ecology. Growing on shady banks, alt. 900 m.

Provisional IUCN conservation assessment. Endangered EN B2ab(iii,v). This species has an EOO about 33,734 km² and AOO about 12 km². The EOO is within the threshold of Near Threatened and the AOO is within the threshold of Endangered. However, this species has only three collections each comprise single sample. None of them are in a protected area and must be threatened by urban development. An assessment of Endangered is more appropriate than Near Threatened.

Specimens examined. Myanmar: KACHIN STATE: Upper Myanmar, Nam Tamai Valley, 900 m, 9 Nov. 1922, *Ward 5528* (**E** (E00096824)); *Ward 6655* (**K** (K000858405)); Nmai Kha, 25°42'29.0"N 97°30'17.0"E, 3 May 1939, *Ward 484* (**NYBG** (NYBG02652115)).

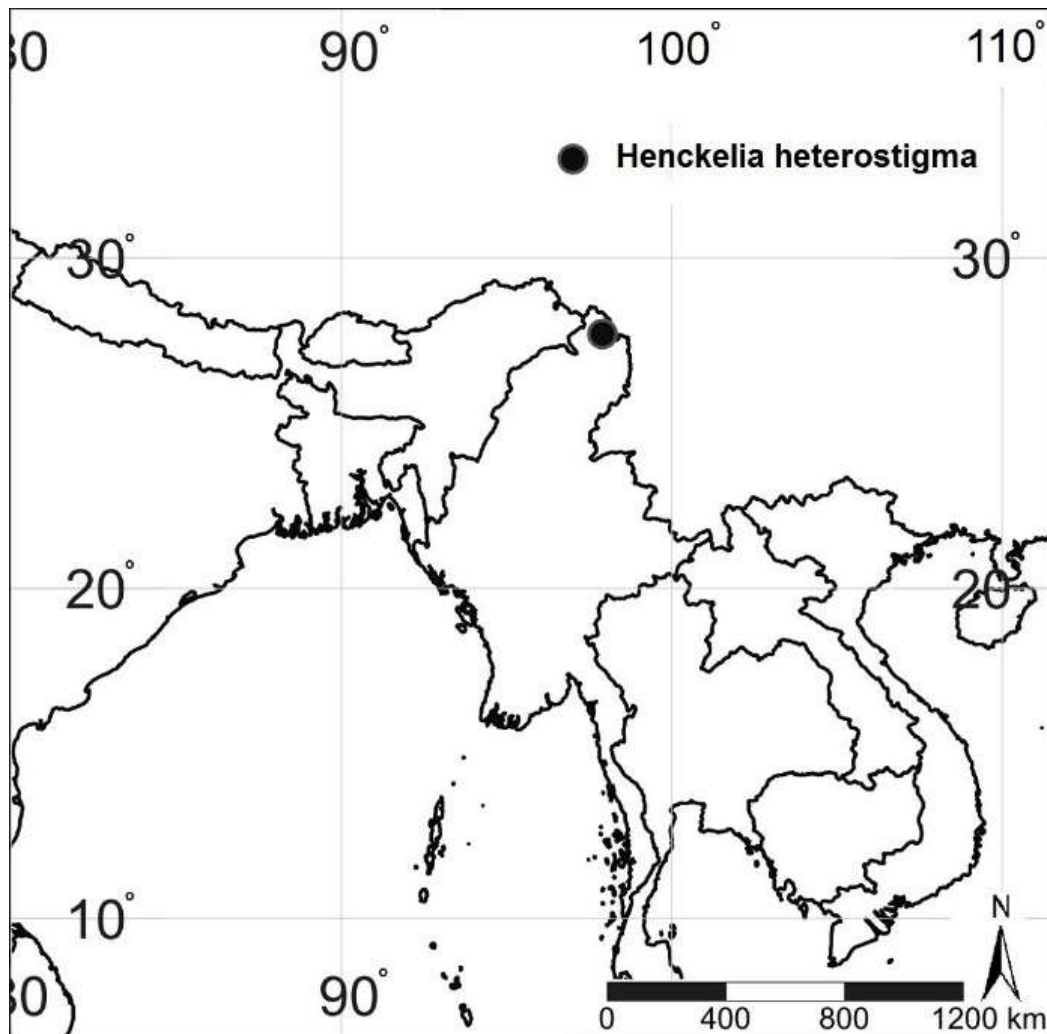


Figure 4.20 Distribution map of *Henckelia heterostigma* (B.L. Burtt) D.J. Middleton & Mich. Möller. Base map from <https://www.simplemappr.net>

13. *Henckelia insignis* (C.B. Clarke) D.J. Middleton & Mich. Möller, Taxon 60(3): 776. 2011.

≡ *Chirita insignis* C.B. Clarke in A. DC. Monogr. Phan. 5: 121. 1883; Wood, Notes Royal Bot. Gard. Edinb 33: 157. 1974. TYPE. Griffith 3831 (Holotype **K!** (K000858399), Isotype! **P** (P00602517)).

Acaulescent, shortly caulescent or rhizomatous perennial herb up to 15 cm. **Leaves** opposite: blade elliptic or ovate, 10.5–17 by 6.4–9 cm, base oblique or round, apex obtuse, margin serrate, upper and lower surface hairy, midrib and veins densely hairy on lower surface, lateral veins 12–15 on each side. Petiole 0.4–10 cm, sparsely hairy. **Inflorescence** 2–4, axillary, each 2-flowered, white with purple blotching. Peduncle 1–

1.5 cm, hairy; bracts paired, free, ovate to linear, 3–7 by 0.5–5 mm, hairy. Pedicel 0.5 cm, hairy. **Calyx** tubular, tube 6 mm long; lobes divided more or equal half way, narrowly triangular or frimbriate, c. 0.8–1.5 cm long, densely hairy. **Corolla** white, narrowly infundibuliform 3.4 cm long. **Stamens** 2: filaments inserted 1.6 cm from base of corolla tube, 1 cm long, geniculate, sparsely pubescent; anther elliptic, ca. 3 mm long, glabrous; staminode 2, inserted 1.3 cm from base of corolla tube, 5 mm long, sparsely hairy. Gynoecium 2.5–3 cm long, stigma deeply bilobed. **Fruit** not seen.

Distribution. Myanmar (Fig. 4.21).

Ecology. In mixed hill forest with *Podocarpus* and other, alt. 1,000–1,500 m.

Provisional IUCN conservation assessment. Data Deficient (DD). This species is only known from the type collection. The size of the population, the EOO and AOO are all unknown.

Specimens examined. Myanmar, Inter Assam et Ava; in convalle Hookong, *Griffith* 3831 (Holotype. **K!** (K000858399); Isotype. **P!** (P00602517)) Kachin State, Sumprabum, eastern approaches from Sumprabum to Kumon Range, Hills North-west of Kanang, 4°03'30" N, 115°00'90" E, 0–1,500 m, 1 Mar. 1962, *Keenan* 3863 (**E** (E00627556)).

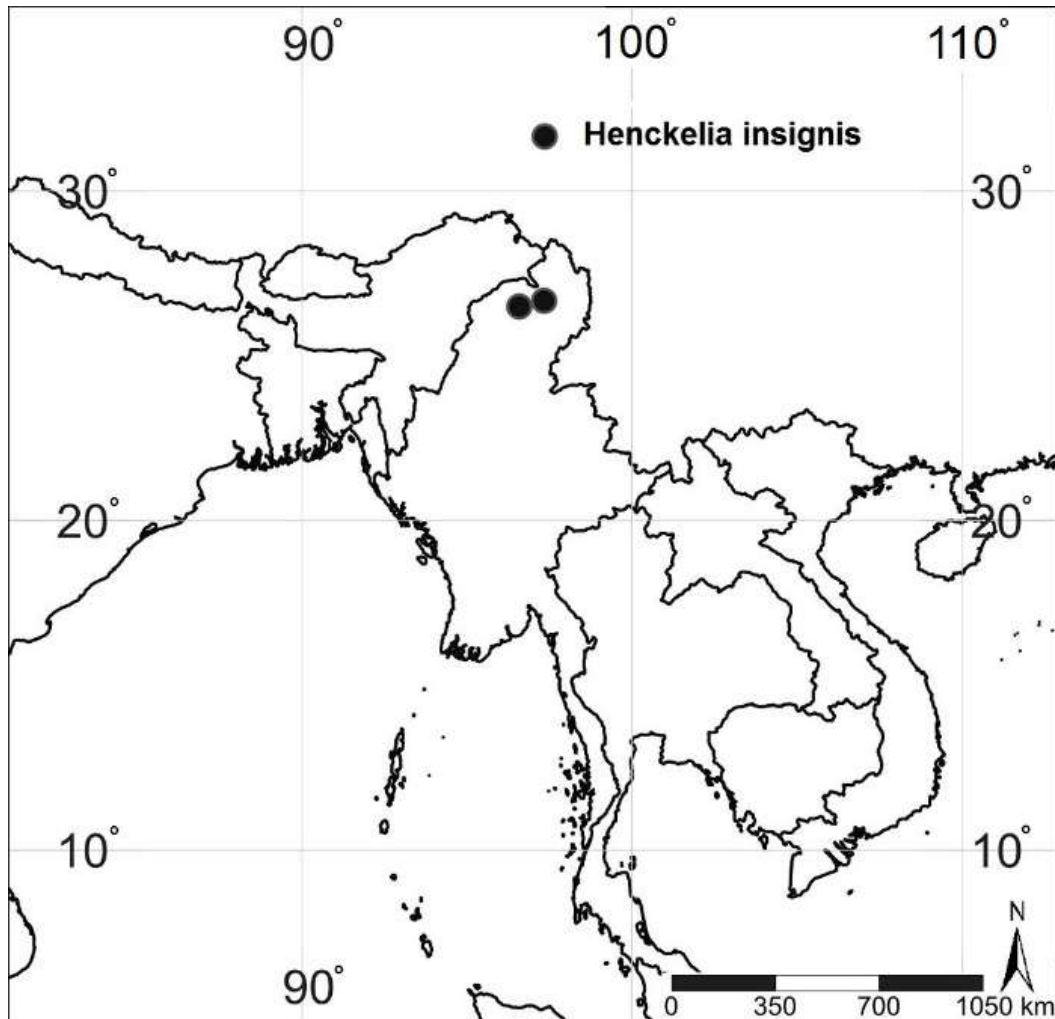


Figure 4.21 Distribution map of *Henckelia insignis* (C.B. Clarke) D.J. Middleton & Mich. Möller. Base map from <https://www.simplemappr.net>

14. *Henckelia lacei* (W.W. Sm.) D.J. Middleton & Mich. Möller, *Taxon* 60(3): 776. 2011.

≡ *Didymocarpus lacei* W.W.Sm., *Rec. Bot. Surv. India* 6: 41. 1913. TYPE: Myanmar, Shan State, Gokteik Gorge. 450 m, 2 Aug. 1908, Lace 4152 (Holotype. K! (K000858406); Isotype CAL! (image CAL0000019177), E! (E00096825, E00155258)).

– *Chirita lacei* (W.W.Sm.) B.L. Burtt, *Notes Roy. Bot. Gard. Edinburgh* 21: 187, in adnot. 1954.

Caulescent perennial herb, rhizomatous, up to 17 cm tall, stem hairy. **Leaves** opposite: blade elliptic, 2.5–10 by 1.7–3.2 cm, apex acute, base cuneate or obtuse, margin serrate, upper and lower surface hairy, lateral veins 4–8 on each side. Petiole

0.5–3.6 cm, hairy. **Inflorescence** axillary, flower solitary, purple; peduncle 0.5–1.6 cm, hairy; bract and pedicel not seen. **Calyx** tubular, tube 7–8 mm long: lobes divided less than half way, narrowly triangular c. 3.5 mm long, hairy, margin entire, apex acuminate, persistent. **Corolla** infundibuliform, c. 3.5 cm long, glabrous. **Stamens** 2: filaments inserted c. 2 cm from base of corolla tube, 1.2 cm long, glabrous; anthers elliptic, c. 1.5 mm long, adaxial surface coherent; staminodes 2, inserted 2.4 cm from base of corolla tube, c. 0.4 cm long, glabrous. Gynoecium 3.5 cm long, ovary 1 mm across, style terete; stigma peltate. **Fruit** orthocarpic, 2.9–5 cm long. Seed not seen.

Distribution. Myanmar (Fig. 4.22).

Ecology. On sandy rock, alt. 450–540 m.

Provisional IUCN conservation assessment. Data Deficient (DD). This species is only known from the type collection. The size of the population, the EOO and AOO are all unknown.

Specimens examined. Myanmar: Shan State: Gokteik Gorge, 450 m, 2 Aug. 1908, *Lace* 4152 (**CAL** (CAL0000019177), **E** (E00096825, E00155258), **K** (K000858406)), *ibidem*, 540 m, 8 Oct. 1911, *Lace* 5456 (**E** (E00627568, E00627569), **K**).

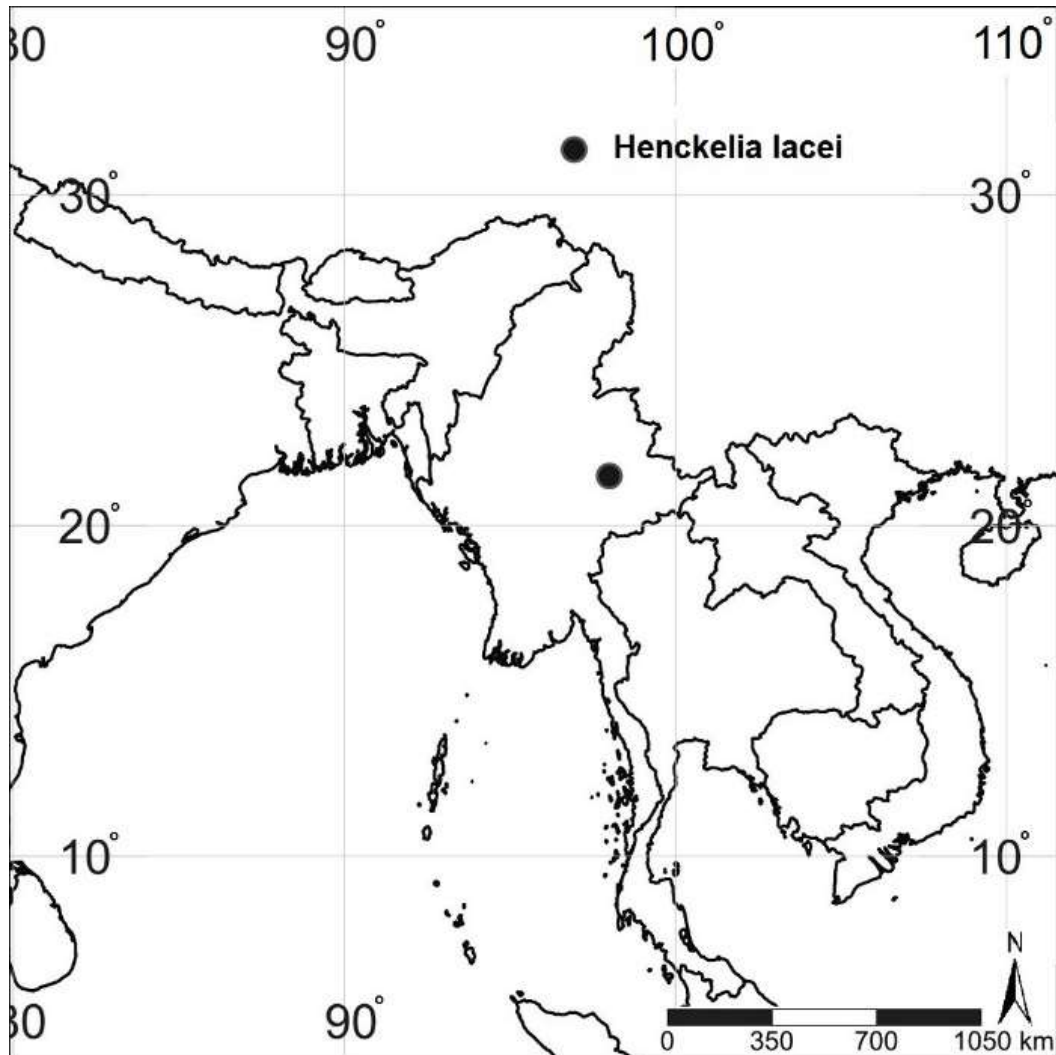


Figure 4.22 Distribution map of *Henckelia lacei* (W.W. Sm.) D.J. Middleton & Mich. Möller. Base map from <https://www.simplemappr.net>

15. *Henckelia lachenensis* (C.B. Clarke) D.J. Middleton & Mich. Möller, Taxon 60(3): 776. 2011.

≡ *Chirita lachenensis* C.B. Clarke in Candolle, Monogr. Phan. (A. DC. & C.DC.) 5(1): 118. 1883; Wang et al., Fl China 18: 341–342. 1998; Hilliard, Fl. Bhutan 2(3): 1317. 2001. TYPE: India, Sikkim, alt. 2,500 m, Hook. f. (Holotype. K! (K000858387)).

– *Chirita clarkei* Hook. f., Fl. Brit. India (J. D. Hooker) 4(11): 359. 1884. nom. illeg. (Status: nom. elleg. superfl. The protologue of *Chirita clarkei* includes the type collection of *C. lachenensis* C.B. Clarke (see Shenzhen Code Art. 9.6, 52.2(a))

– *Chirita umbricola* W.W. Sm. in Notes Roy. Bot. Gard. Edinburgh 10: 172. 1918. TYPE: Myanmar, Ridge of Naung-chaung, Nwai divide, 6,000–8,000 ft., July 1914,

Ward 1824 (Holotype **E!** (E00096828); Isotype **E!** (E00096827)).

–*Chirita stolonifera* C.E.C. Fisch. & Kaul in Kew Bull. Misc. Inform. 1940: 196. 1940.

TYPE: Delei Valley, 28°21'N 96°37'E, 6,000–7,000 ft, 16 July 1928, Ward 8462 (Holotype **K!**).

Acaulescent, rhizomatous, creeping, or slender stoloniferous perennial herb, aerial stem up to 10–30 cm long, with red-brown hairs. **Leaves** alternate: blade ovate or lanceolate 5.1–14 by 2.8–8.7 cm, apex acute, base oblique, margin serrate or dentate, upper and lower surfaces hairy, lateral veins 5–9 on each side. Petiole 3.5–18 cm long, hairy. **Inflorescence** axillary, 1–5 flowered, blue-violet; peduncle 2.5–19.2 cm long, hairy; bracts paired, free, lanceolate or ensiform, 3.5–7 mm, hairy; pedicels 0.3–3.2 cm, hairy. **Calyx** tubular, tube 0.2–0.8 cm long; lobes deeply divided of half way, narrowly triangular 0.6–1.4 by 0.3–0.4 cm, hairy, caducous. **Corolla** tube campanulate or wide infundibuliform, 1.8–3 cm long, hairy outside, lobes 0.7–0.9 by 0.8–1.2 cm. **Stamens** 2: filaments attached 1 cm from base of corolla tube, 0.5–0.6 cm long, geniculate, hairy after a base the middle; anthers subglobose, 2 mm long, hairy on the base, adaxial surfaces coherent; staminodes 2, inserted 0.6–0.7 cm from base of corolla tube, 3 mm long, glabrous. Gynoecium 1.7–2.3 cm long, stigma deeply bilobed. **Fruit** orthocarpic, 2.2–8.2 cm long. Seed not seen.

Distribution. India, Bhutan, Northern Myanmar and China (Yunnan) (Fig. 4.23).

Ecology. Growing on moist, shady banks and mossy rocks in dense mixed forest, facing slope among rocks or edges of cliffs, alt. 1,524–3,000 m.

Provisional IUCN conservation assessment. Endangered EN B2ab(iii). This species has an EOO about 428,598 km² and AOO about 64 km². The EOO is within the threshold of Least Concern and the AOO is within the threshold of Endangered. However, some of this species are outside the protected area and must be threatened by urban development. An assessment of Endangered is more appropriate than Near Threatened.

Specimens examined. Bhutan: Mangar, Salrug, 27°16'15.9"N 91°14'14.7"E, 1,981 m, 30 June 1969, *Lyon 15153* (**BM** (BM011025901)); Mangar, Salrug, 27°16'15.9"N 91°14'14.7"E, 2,134 m, 30 June 1969, *Lyon 15154* (**BM** (BM011025902)); Steep forested slopes near Namning, NW of Mongar, 27°19' N, 91°02' E, 2,500 m, 5 July 1979, *Grierson et al. 2503* (**K**); Tashiyangtse valley, 27°24'28.1"N 91°32'39.5"E, 2591

m, 2 Aug. 1933, *Ludlow et al.* 357 (**BM** (BM011025899)); Tobrang, Tashiyangtse Chu, E Bhutan, 27°32'15.5"N 91°25'36.1"E, 2,438 m, 5 July 1949, *Ludlow et al.* 20837 (**BM** (BM011025900)).

India: Sikkim, Lachen, 4 Aug. 1849, *Hooker s.n.* (**K** (K000858387)).

China: Yunnan (22°24'24.5"N 100°40'55.0"E, *Forrest* 11962 (**K**), Gongshan Xian, Dulongjiang Xiang. W side of Gaoligong Shan, W of Gongshan on the trail from Qiqi to Bapo in the Dulong Jiang valley., 27°43'38" N, 98°24'41" E, 2,400 m, 17 July 2000, *Li et al.* 12908 (**E** (E00132441)), Hills N.W. of Luggruh, 22°24'24.5"N 100°40'55.0"E, 0–2,400 m, 1 June 1931, *Forrest* 29681 (**E** (E00087405)), Shweli-Salwin divide, 2,700 m, 1 Nov. 1924, *Forrest* 25344 (**E** (E00096806), **K**), Upper Nuigkuong Valley, 2,100 m, 1 Nov. 1924, *Forrest* 25416 (**E** (E00087403), **K**)).

Myanmar: Chawchi Pass, 2,590 m, 29 June 1928, *Ward* 1661 (**E**); Deli Valley, 28°21' N, 96°37' E, 1,829–2,134 m, 17 July 1928, *Ward* 8462 (**K**). Kachin State, Upper Myanmar, Nam Tamai Valley, 27°42' N 97°54' E, 1,829 m, 19 Aug. 1926, *Ward* 7317 (**K**), Frontier of Tibet and Myanmar, Nam Tisang-Mali divide, 27°30' N, 97°50' E, 1,800 m, 24 Aug. 1926, *Ward* 7330 (**K**), Mali Kha, hills east of the Mali Kha, 27°30' N 97°30' N, 1,524 m, 31 July 1937, *Ward* 12853 (**BM** (BM011025903)); NE Upper Myanmar, 0–3,000 m, 1 Aug. 1924, *Forrest* 24838 (**K**); Ridge of Naung-chaung, Nwai divide, 1830–2,440 m, July 1914, 1,830–2,440 m, July 1914, *Ward* 1824 (Holotype. **E!** (E00096828); Isotype **E!** (E00096827)); *Forrest* 26511 (**NMNS** (NMNS0056646), **P** (P03884130)).

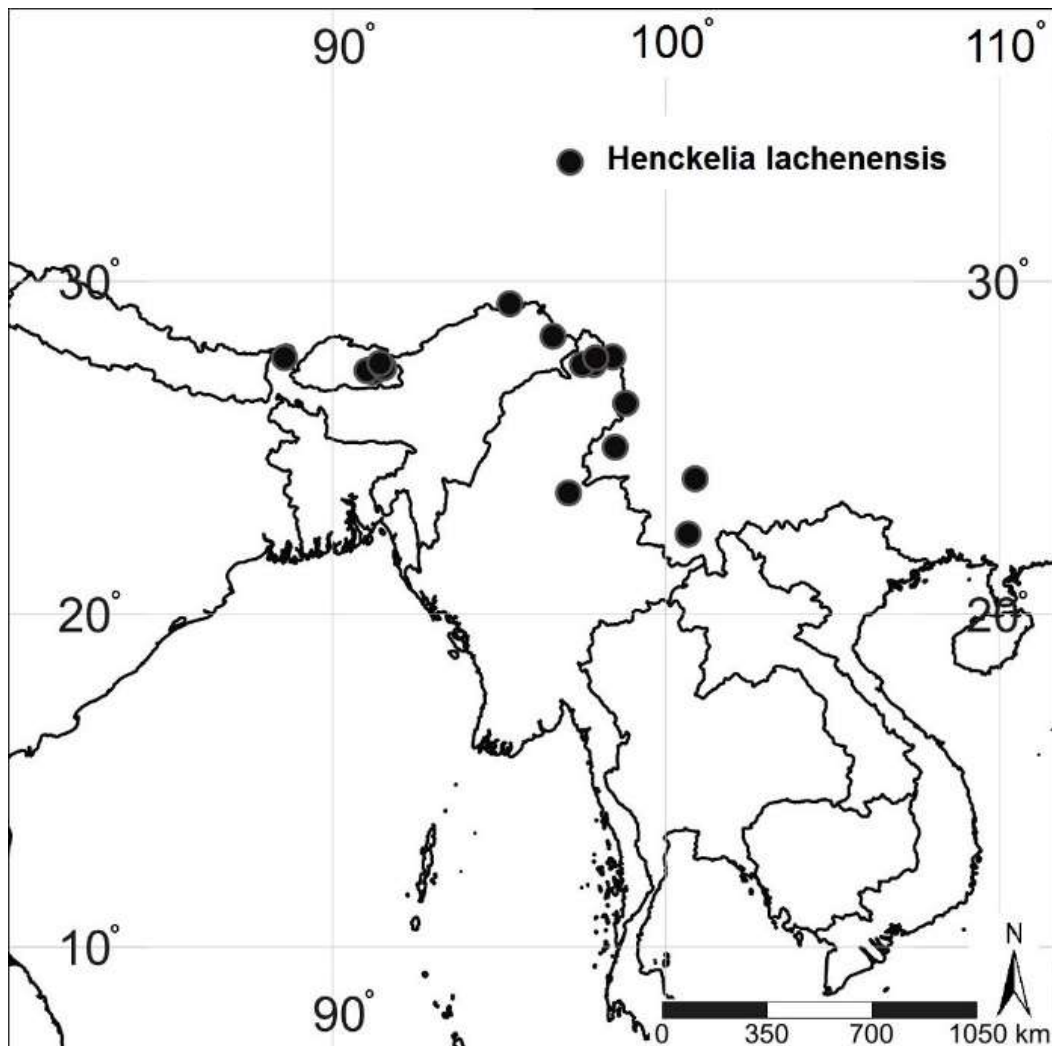


Figure 4.23 Distribution map of *Henckelia lachenensis* (W.W. Sm.) D.J. Middleton & Mich. Möller. Base map from <https://www.simplemappr.net>

16. *Henckelia longipedicellata* (B.L. Burtt) D.J. Middleton & Mich. Möller, Taxon 60(3): 776. 2011.

≡ *Chirita longipedicellata* B.L. Burtt, Notes Royal Bot Gard Edinb. 26: 264. 1965.

TYPE: *Ward 7241* (Holotype. **K!** (K000858404), Isotype. **E!** (E00155262)).

Cauliscent perennial herb, up to 27 cm tall, stem hairy. **Leaves** opposite: blade ovate, 3.5–6 by 2–3.5 cm, base cuneate, apex acute, margin serrate, upper and lower surface with eglandular hairs, lateral veins 5–7 on each side. Petiole 0.5–2.5 cm, with glandular and eglandular hairs. **Inflorescence** 2–4, axillary, flower solitary, violet; peduncle 3–7 cm, hairy; bracts paired, free, inserted below calyx, elliptic, 4 mm long;

pedicel absent. **Calyx** tubular, tube 3 mm long; lobes deeply divided, narrowly triangular, 9–10 mm long, sparsely glandular and eglandular hairs on both sides; caducous. **Corolla** infundibuliform, 3–3.5 cm long, straight pouched, sparsely hairy outside. **Stamens** 2: filaments inserted c. 5 mm from base of corolla tube, 6 mm long, curved, pubescent; anthers elliptic, 2–2.4 mm long, pubescent on lower surface, adaxial surfaces coherent; staminodes 2, inserted c. 1 mm from base of corolla tube, 1 mm long, glabrous. Gynoecium 1.8 cm long; stigma deeply bilobed. **Fruit** orthocarpic, young fruit 1.5 cm long.

Distribution. Myanmar (Fig. 4.24).

Ecology. Amongst high herbaceous undergrowth beneath alder copse, alt. 2,100 m.

Provisional IUCN conservation assessment. Data Deficient (DD). This species is only known from the type collection. The size of the population, and the EOO and AOO are all unknown.

Specimens examined. Myanmar: Shan State: Tibet - Myanmar Frontier, Valley of the Seinghku, 2,100 m, 5 Aug. 1926, *Ward 7241* (**E!** (E00155262), **K!** (K000858404)).

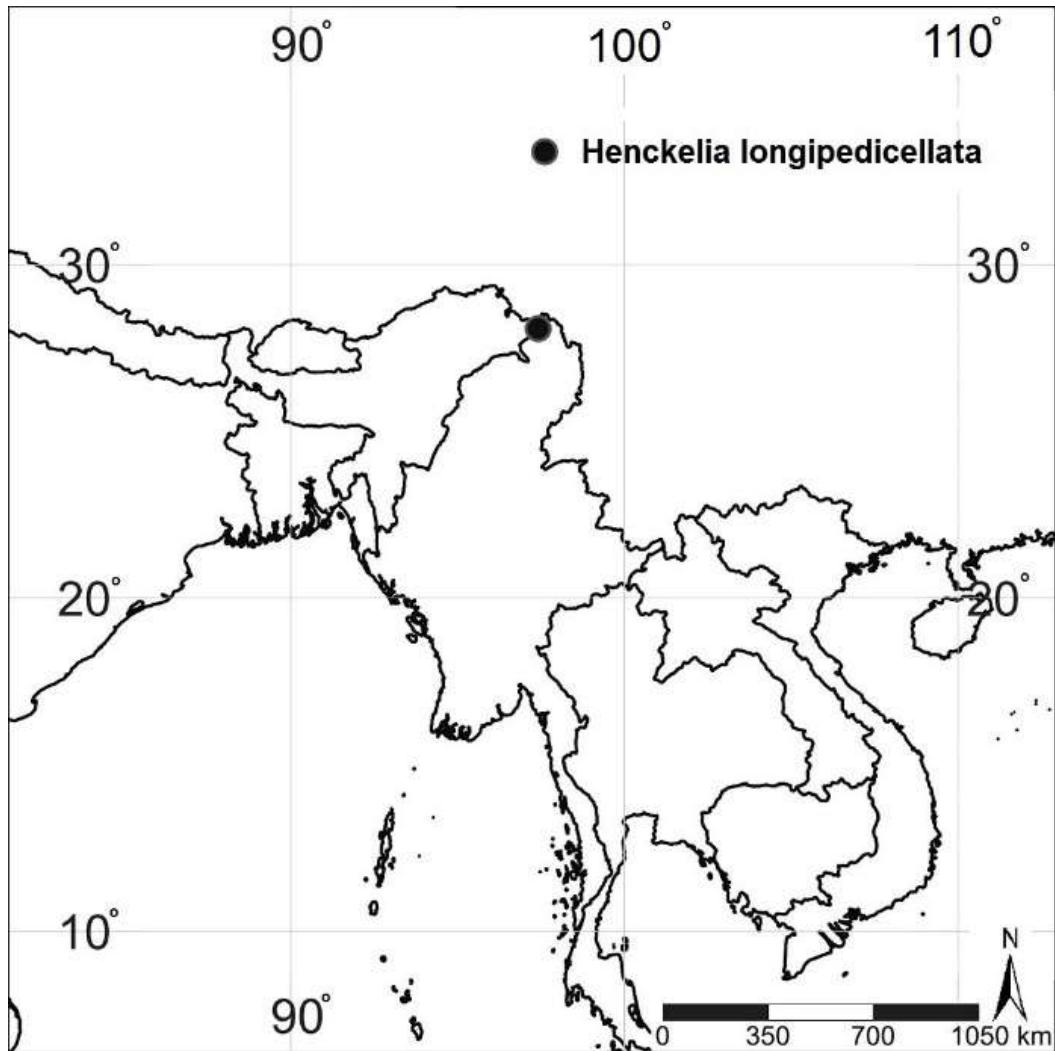


Figure 4.24 Distribution map of *Henckelia longipedicellata* (B.L. Burtt) D.J. Middleton & Mich. Möller. Base map from <https://www.simplemappr.net>

17. *Henckelia longisepala* (H. W. Li) D.J. Middleton & Mich. Möller, *Taxon* 60(3): 776. 2011; Bui, Nuraliev, Möller, Kuznetsov Kuznetsova, Middleton & Wen, *Rhedeia* 30(1): 176-186. 2020. Isolectotype: China, Yunnan, Hekou, Lengshui Gou, 250 m, 20 Apr 1953, K.H. Kai 464 (**KUN** (KUN1219162 digital image), PE (PE00030765 digital image)).

≡ *Lysionotus longisepalus* H.W. Li, *Bull. Bot. Res.*, Harbin 3(2): 1. 1983.

TYPE. K.H. Cai 464 (Holotype. **KUN** (KUN1219162 digital image), Isotype PE (PE00030765 digital image)).

– *Hemiboeopsis longisepala* (H.W. Li) W.T. Wang in *Acta Bot. Yunnan.* 6(4): 397. 1984.

Wang et al., *Fl. China* 18: 301–302. 1998.

Caulescent annual herb, up to 50 cm tall, stem appressed hairy. **Leaves** opposite: blade obovate-oblong, sometimes oblong, 11.5–23.6 by 2.8–6 cm, apex acuminate, base attenuate, upper and lower surface glabrous, margin entire, lateral veins 10–12 on each side; petiole terete, 1.5–6 cm long, sparsely hairy. **Inflorescence** several, axillary, 1–4-flowered, purple; peduncle 1.5–2 cm, hairy; bracts paired, free, suborbiculate to ovate-orbicular, 1.7–2 cm long. Pedicel terete, 3–4 mm long, glabrous. **Calyx** 5-partite; lobes basally connate, spatulate-linear, 17–20 by 2.5–3 mm, glabrous. **Corolla** purple, campanulate, tube 3.5–4.5 cm long, glabrous with yellow lines in throat. **Stamens 2**: filaments inserted 3 mm from base of corolla tube, 12 mm long, geniculate, sparsely hairy; anthers elliptic, 2 mm long, adaxial surfaces coherent; staminodes 2, inserted 3 mm from base of corolla tube, 5 mm long, sparsely hairy. Gynoecium 2.1–2.6 cm long, stigma deeply bilobed. **Fruit** orthocarpic, young fruit c. 7–8 cm long. Seeds not seen.

Distribution. China (SE Yunnan, Hekou Xian, Jinping Xian), Laos and Vietnam (Fig. 4.25).

Ecology. Shady montane valleys, alt. 200–1,300 m.

Provisional IUCN conservation assessment. Endangered EN B2ab(iii). This species has an EOO about 69,710 km² and AOO about 24 km². The EOO is within the threshold of Least Concern and the AOO is within the threshold of Endangered. However, none of them are in the protected area and must be threatened by urban development. An assessment of Endangered is more appropriate than Near Threatened.

Specimens examined. **China:** 800 m, 27 Apr. 1953, *Xu 284* (**KUN** (KUN0207862)); 120 m, 6 Apr. 1953, *Cai 84* (**KUN**); 8 Apr. 1953, *Cai 146* (**KUN** (KUN0207858)); 200 m, 8 May 1953, *Cai 990* (**KUN** (KUN0207859)). **Laos:** between Nadon and the Mine after km 12, Xieng Khouang, Apr. 1949, *Vidal 875B* (**P** (P03851426)); Pu Muten, Chiang Kwang, 1,300 m, 20 Apr. 1993, *Kerr 21170* (**K**).

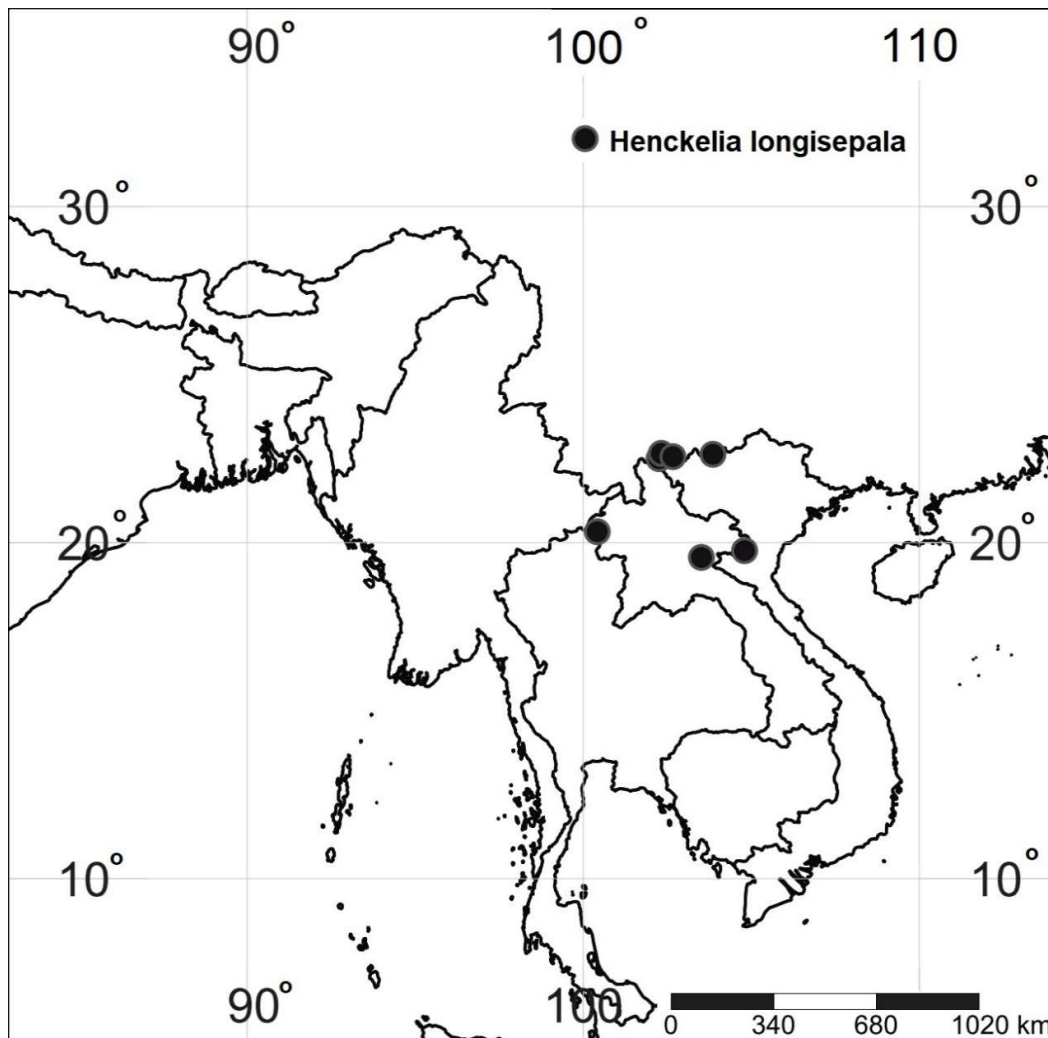


Figure 4.25 Distribution map of *Henckelia longisepala* (H. W. Li) D.J. Middleton & Mich. Möller. Base map from <https://www.simplemappr.net>

18. *Henckelia nakianensis* Sirim., J. Parn. & Hodk., Thai Forest Bull., Bot. 47(1): 45–47. 2019. TYPE: Thailand, Chiang Mai, Om Koi District, Tambon Na Kian, Ban Mae Kong, north of the village near stream, alt. 950 m, fl. & fr. 27 Aug. 2015, *W. Pongamornkul et al.* 5110 (Holotype **QBG!** (QBG8577), Isotype **BKF!**).

Cauliscent annual herb, up to 15 cm tall; stem glabrous. **Leaves**: blade symmetrically orbicular or cordate, 9–12 by 6–10 cm, herbaceous, apex acute, base cordate, margin remotely dentate, upper and lower surface nearly glabrous, but with sparsely stout hairs on upper surface, up to 0.7 mm long, lateral veins 4–6 on each side; only one leaf developing, sessile. **Inflorescence** terminal, inserted at the junction of the leaf

blade and stem, single or compound cymes, 2–10 flowered, white or purple; peduncles terete, 1.8–3.5 cm long, eglandular hairs; bracts paired, free, sessile, lanceolate, 5–17 by 0.1–6 mm, apex acute, hairy, margin dentate; pedicels terete, 1.2–3.5 cm long, multicellular and sparsely glandular hairs. **Calyx** 5-partite; lobe basally connate, triangular, c. 4.5 by 1.2–1.5 mm, apex acuminate, tip reflexed, hairy, margin entire, caducous. **Corolla** tube campanulate or wide infundibuliform, white-purplish, c. 7 mm long, glabrous; corolla lobes purple, glabrous, c. 5 by 3–4 mm. **Stamens** 2: filaments inserted 3 mm from base of corolla tube, c. 1.5 mm long, straight; anthers reniform, yellow, glabrous, c. 1.4 by 1 mm, adaxial surfaces coherent; staminodes 2, inserted 3 mm from base of corolla tube, c. 0.2 mm long, glabrous. Gynoecium 9.5 mm, ovary 1 mm across, hairy; style terete, slender; stigma peltate, yellow. **Fruit** a plagiocarpic, elongate capsule, green, 1–2.5 by 0.1–0.2 cm, sparsely hairy. Seeds not seen (Fig. 4.27).

Distribution. Northern Thailand, only known from the type locality (Fig. 4.26).

Ecology. Dry evergreen forest, a moist place near a stream, on the tree, c. 950 m, flowering and fruiting in August.

Provisional IUCN conservation assessment. Data Deficient (DD). This species is only known from the type collection from a rather remote and inaccessible site. The EOO and AOO are unknown.

Specimens examined. Thailand, Chiang Mai, Om Koi District, Tambon Na Kian, Ban Mae Kong, north of the village near stream, alt. 950 m, fl. & fr. 27 Aug. 2015, *W. Pongamornkul et al.* 5110 (**QBG** (QBG8577), **BKF**).

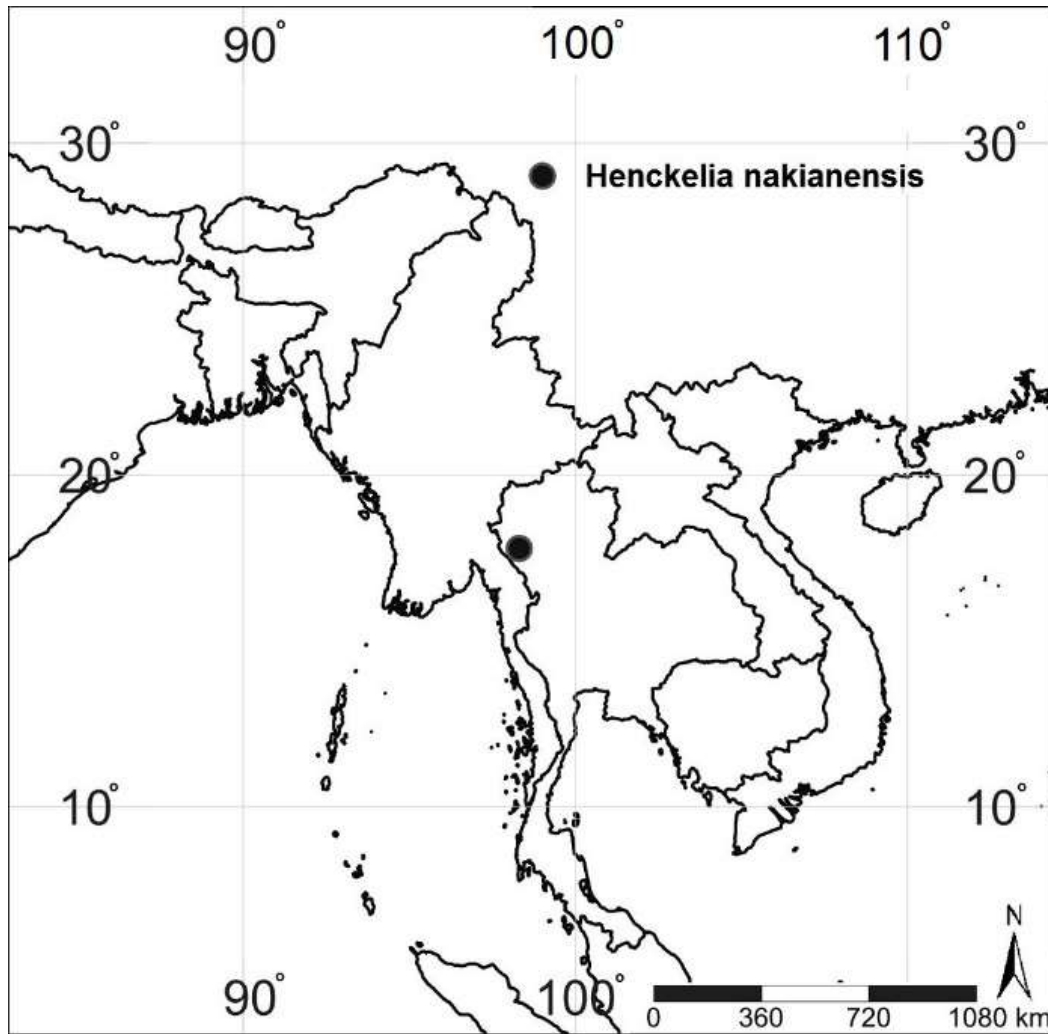


Figure 4.26 Distribution map of *Henckelia nakianensis* Sirim., J. Parn. & Hodk. Base map from <https://www.simplemappr.net>

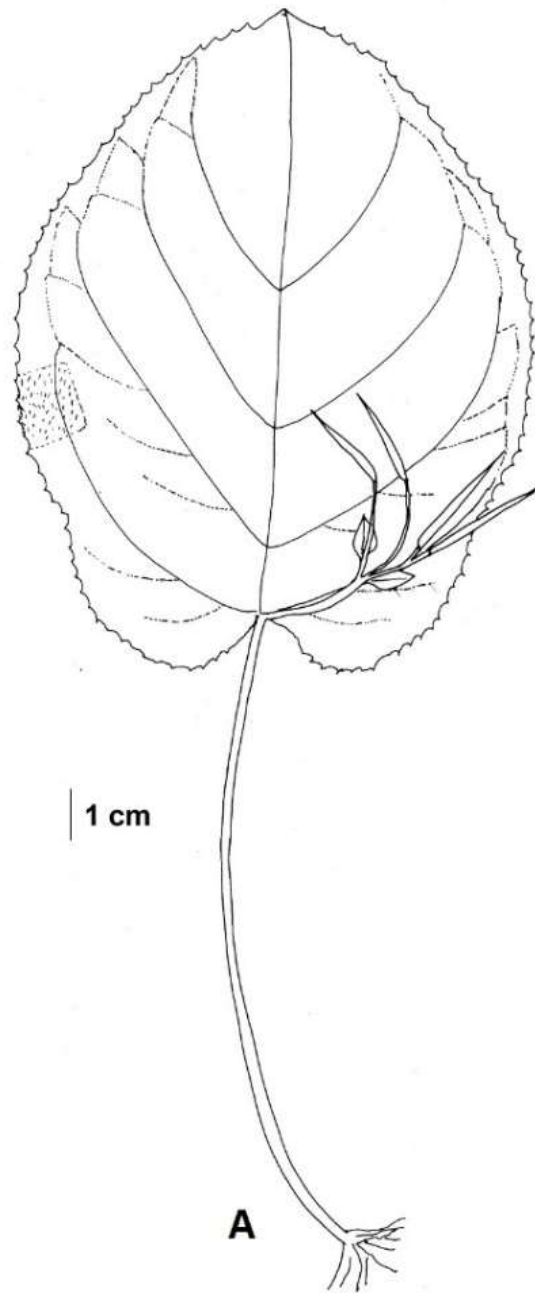


Figure 4.27 Line drawing of *Henckelia nakianensis* Sirim., J. Parn. & Hodk.: A. Habit.
Drawing: Sukontip Sirimongkol from *Pongamornkul et al.* 5110 (QBG).

19. Henckelia oblongifolia (Roxb.) D.J. Middleton & Mich. Möller, Taxon 60(3): 776. 2011.

≡ *Incarvillea oblongifolia* Roxb., Fl. Ind., ed. 2, 3: 113. 1832.

– *Chirita oblongifolia* (Roxb.) J. Sinclair in Bull. Bot. Soc. Bengal 9: 102. 1957. Wang et al., Fl. China 18: 342. 1998. TYPE: Chittagong Hills, 10 Nov. 1810, *sine col.* 238 (Lectotype **BM!** (BM000092133)).

– *Chirita oblongifolia* (Roxb.) B.L. Burtt, Notes Roy. Bot. Gard. Edinburgh 22: 307. 1958; Wood, Notes Roy. Bot. Gard. Edinburgh 33: 174–175. 1974; Wang et al., Fl. China 18: 113. 1989.

– *Chirita acuminata* Wall. ex R. Br., Cyrtandreae: 117. 1839, nom. nud.

– *Chirita acuminata* Steud., Nomencl. Bot., ed. 2, 1: 351. 1840, nom. nud.

Cauliscent herbaceous or woody perennial herb, stem up to 90 cm tall, covered with brown hairs. **Leaves** opposite: blade ovate to lanceolate, 6–26 by 2.5–12.5 cm, apex acuminate, base cuneate or oblique, margin remotely denticulate, upper surface shortly hairy, lower surface glabrous with black dot, hairy on veins, lateral veins 11–17 on each side; petiole 1–6 cm long, hairy. **Inflorescences** axillary, 2–4 pairs, up to 12-flowered, white or yellowish; peduncle terete, 0.5–3 cm long, hairy; bracts paired, free, narrowly lanceolate, up to 8 mm long. Pedicels 1–3 cm long, hairy. **Calyx** tube campanulate, tube 0.8–1 cm long; lobes divided less than half way, triangular, 0.3–0.6 by 0.4 cm long; caducous. Corolla infundibuliform, white, 3–4 cm long. **Stamens** 2; filaments inserted 1.8–2 cm from base of corolla tube, 0.7–1 cm long, glabrous; anthers elliptic, 1–2 mm long, adaxial surfaces coherent; staminodes 2, inserted 1.5 cm from base of corolla tube, 2 mm long, glabrous. Gynoecium 2.8 cm long, stigma shallowly bilobed. **Fruit** orthocarpic, 3–10 cm long.

Distribution. India, Bangladesh, China and Myanmar (Fig. 4.28).

Ecology. In shady forest, on rock or banks, alt. 220–1,950 m.

Provisional IUCN conservation assessment. Least Concern LC. This species has an EOO about 942,565 km² and AOO about 88 km². The EOO is within the threshold of Least Concern and the AOO is within the threshold of Endangered. However, this plant is common and widespread. An assessment of Least Concern is more appropriate than Endangered.

Specimens examined. India: Assam (*Griffith 1118* (**BM** (BM011025877)), Sept. 1882, *King s.n.* (**NMNS** (NMNS00064529), **P** (P03884215)), *Masters s.n.* (**P** (P03884206)), *Wallich s.n.* (**P** (P03884213)), *Wallich s.n.* (**U** (U1342090)), Henina, Naga hills, *Bor 6829* (**K**), Jfaflory, N. Cachar, 815 m, 17 Aug. 1908, *Craib 192* (**K**), Valley of the Bhareli, 914–1219 m, 26 Oct. 1935, *Ward 12482* (**BM** (BM011025881)); East Bengal, Nustwee, *Griffith 3829* (**G** (G00492577)); Dekho, 29 Mar 1895, *the Report on Economic Products to the Government of India 11044* (**NMNS** (NMNS00064533), **P** (P03884212)); Numklow, Khasia, 1067 m, 1 Nov. 1871, *Clarke 15772A* (**NMNS** (NMNS00064532)), *Clarke 15772B* (**BM** (BM011025874)); Meghalaya, Khasi (1,219 m, 1 Nov. 1872, *Clarke 19297B* (**BM** (BM011025870)), 1,524 m, *Griffith 3829* [**BM** (BM011025872), **P** (P03884208, P03884211)], *Griffith 19798* (**BM** (BM011025881)), *Griffith s.n.* (**K**), *Griffith s.n.* (**K**), 1 Oct. 1850, *Hooker & Thompson s.n.* (**K**), *Hooker & Thompson s.n.* (**K**), *Hooker & Thompson s.n.* (**K**), *Hooker & Thompson s.n.* (**NMNS** (NMNS00064528)), 305 m, *Hooker & Thompson s.n.* (**P** (P03884209)), 1,219 m, *Hooker & Thompson s.n.* (**P** (P03884210)), 610–1,219 m, *Hooker & Thompson s.n.* (**TCD**), 914–1,219 m, *Lobb s.n.* (**K**), *Lobb s.n.* (**K**), *Wallich s.n.* (**TCD**)); Meghalaya, Khasi hills (*Anonymous 3* (**K**), *Griffith s.n.* (**TCD**), *Griffith s.n.* (**TCD**), 1,219 m, *Hooker & Thompson s.n.* (**BM** (BM011025875)), Lemann, 1844, *Griffith 184* (**K**), *ibidem*, 1844, *Griffith s.n.* (**K**)); Shillong, 1,067 m, 14 Aug. 1886, *Clarke 44550D* (**BM** (BM011025876)), Sikkim, *Wallich s.n.* (**BM** (BM011025871)); Tuesenkan? Sushai hills, Assam, 1219 m, 23 Oct. 1927, *Parry 327* (**K**); *Booth s.n.* (**K**); *Griffith s.n.* (**K**); 1843, *Griffith 1843* (**P** (P03884214)); *Hooker s.n.* (**K**); 1859, *Hooker & Thompson s.n.* (**P** (P03884207)); *Lobb s.n.* (**BM** (BM011025878)); Nov. 1810, *Roxburgh 238* (**BM** (BM000092133)); *Wallich 802* (**BM** (BM011025873), **K**); 1,200 m, 1861, *Hooker & Thompson s.n.* (**G** (G00492368)); *Wallich s.n.* (**G** (G00492374)).

Bangladesh: Kasalong, Chitagong, 10 Jan 1869, *Clarke 8243* (**K**).

China: Yunnan: Kiukiang Valley, S of Kongpong, 1,200 m, Sept., *Yu 20458* (**E** (E00087416)).

Myanmar: Kachin State: Sumprabum, Eastern approaches from Sumprabum to Kumon Range. Ndum-Zup to Hpuginhku. Pathsides on western slope, 26°40' N, 97°20' E, 0–1,950 m, 30 Dec. 1961, *Keenan et al. 3092* (**E** (E00627595)); Sumprabum, Eastern approaches from Sumprabum to Kumon Range. Surrounds of Hpuginhku village, 26°40' N, 97°20' E, 1,500 m, 1 Feb. 1962, *Keenan et al. 3691* (**E** (E00626596), **K**); Sumprabum, 900–1,200 m, 18 Jan 1953, *Ward 20405* (**BM** (BM011025897), **SING** (0117817)); Numnea to Nammuu, Myitkyina District., 300 m, 1 Mar. 1910, *Lace 5187* (**E** (E00627598)); Upper Myanmar, Nam Tamai Valley 27°42' N 97°54' E (914–1,219 m,

12 Sept. 1926, *Ward 7401* (**K**), 1,200 m, 3 Sept. 1937, *Ward 13122* (**BM** (BM011025882), **E** (E00096834)), Nwai valley, 1,800 m, 9 Sept. 1914, *Ward 1931* (**E** (E00096833)). Sagaing Region: Chindwin, Upper Chindwin, Kodan Chaung near Yeson Camp, 21°28'26"N, 95°16'53"E, 240 m, 26 Nov. 1917, *Rogers 1023* (**E** (E00627593)); Hkawng Gaw, 25°58' N, 98°00' E, 10 Sept. 1939, *Kaulback 389* (**BM** (BM011025880)); Sagaing, Homalin Township, Basin of Chindwin River, Htamanthi WS, Nam Pa Gon area., 25°17'47", 95°27'64", 220 m, 25 Oct. 2014, *Aye et al. 48* (**NYBG** (NYBG02648378)).

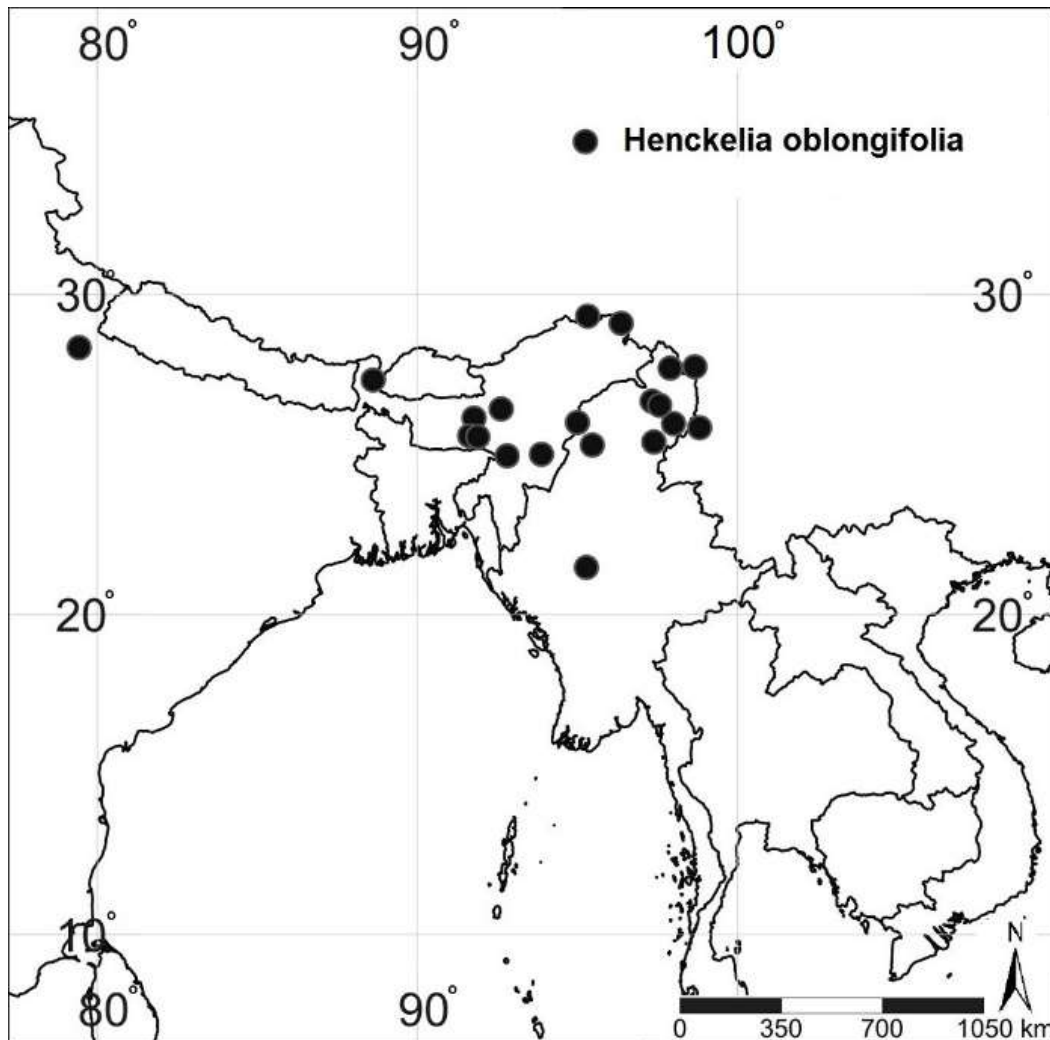


Figure 4.28 Distribution map of *Henckelia oblongifolia* (Roxb.) D.J. Middleton & Mich. Möller. Base map from <https://www.simplemappr.net>

20. *Henckelia peduncularis* (B.L. Burtt) D.J. Middleton & Mich. Möller, *Taxon* 60(3): 776. 2011.

≡ *Chirita peduncularis* B.L. Burtt in *Notes Roy. Bot. Gard. Edinburgh* 26: 265. 1965.
TYPE: Kachin State, Myanmar-Tibet frontier, Valley of the Nam Tamai., 4°03'30" N, 115°00'90" E, 1,200 m, 12 Sept. 1926, *Ward 7402* (Holotype. **K!** (K000858402); Isotype **E!**, **K!** (K000858403)).

Caulescent, long rhizomatous perennial herb, 30–40 cm tall. **Leaves** opposite: blade ovate, 5–12 by 4–7 cm, apex acute to acuminate, base wide cuneate, margin serrate, upper and lower surface sparsely stout hairy, secondary vein 6–8 on each side; petiole 2.5–4 cm long, hairy. **Inflorescence** 2–4 pairs, axillary, flowers solitary or pairs, white; peduncle slender, 2–2.3 cm long, with glandular hairs; bracts paired, connate, sub cordate, 1.5–1.7 by 0.5–1.6 cm, sparsely stout hair. Pedicel 0.8–1 cm, glabrous. **Calyx** tubular, tube 1.2 cm long; lobes divided less than half way, triangular, 3–4 mm long, sparsely hairy. **Corolla** infundibuliform, tube curved, slightly poached, 3.5–4 cm long, slightly hairy. **Stamens** 2: filaments inserted c. 1 cm from base of corolla tube, 1 cm long, curved, glabrous; anthers 3 mm long, glabrous, adaxial surfaces coherent; staminodes 2, inserted c. 1 cm from base of corolla tube, 2 mm long, glabrous. Gynoecium c. 2.5 cm long, stigma deeply bilobed. **Fruit** orthocarpic, young fruits 10–15 cm long.

Distribution. Northern Myanmar (figure 4.29).

Ecology. Growing in colonies in the shade or on the sheltered slope of the ridge, alt. 1,200–1,829 m.

Provisional IUCN conservation assessment. Data Deficient (DD). Only two collections from this species collected in the same location and same collector exist. The EOO and AOO are unknown.

Specimens examined. Myanmar: Kachin State: Myanmar-Tibet frontier, Valley of the Nam Tamai, 27°42'27" N, 98°0'18" E, 1,200 m, 12 Sept. 1926, *Ward 7402* (**E**, **K** (K000858402, K000858403)); 1,829 m, 5 Aug. 1937, *Ward 12935* (**BM** (BM011025873)).

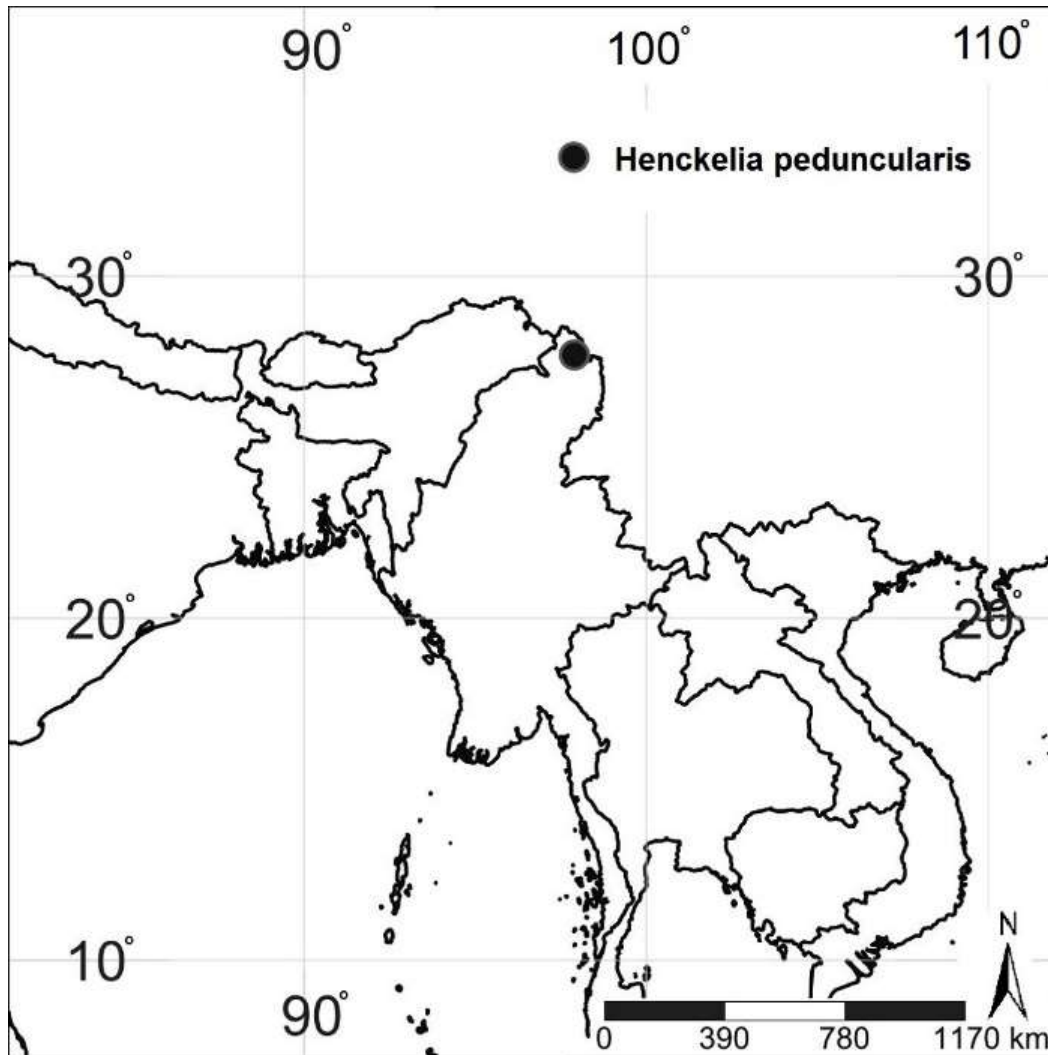


Figure 4.29 Distribution map of *Henckelia peduncularis* (B.L. Burtt) D.J. Middleton & Mich. Möller. Base map from <https://www.simplemappr.net>

21. *Henckelia pumila* (D. Don) A. Dietr., Sp. Pl., ed.6, 1: 574. 1831; Weber et al, Taxon 60(3): 774. 2011; Middleton, Weber, Yao, Sontag & Möller, Edinburgh J. Bot. 70(3): 401. 2013.

≡ *Chirita pumila* D. Don, Prodr Fl. Nepal. 90 (1825); Pellegrin, Fl. Indo-Chine 4(5): 537–538. 1930; Hara, Enum. Fl. Pl. Nepal 3: 134 (1982); Wang et al., Fl. China 18: 343–344. 1998. TYPE: Nathaniel Wallich, 801, Nepal (Holotype. **BM!** (BM000041656); Isotype. **G!** (G00133149 image), **K!** (K000858377, K000858388)).

–*Chirita diaphana* Royle, Ill. Bot. Himal. Mts. 294. 1836, nom. nud.

–*Chirita flava* Wall. ex R.Br., Cyrtandreae: 117. 1839, nom. superfl.

–*Calosacme flava* Steud., Nomencl. Bot. ed. 2, 1: 351. 1840, nom. nud.

–*Chirita edgeworthii* A.DC., Prodr 9: 269. 1845.

–*Chirita polyneura* Miq. var. *thomsonii* C.B. Clarke, Commelyn. Cyrtandr Bengal. t. 75. 1874.

–*Chirita sphagnicola* H. Lév. & Vaniot, Bull. Soc. Bot. France 53: 550. 1906.

TYPE: Jos Esquirol 171, Kouy-Tcheou, Ko-tchang-keou, Aug 1904 (Holotype **E!** (E00387545)).

Caulescent annual herb, stem short or erect, 6–19 cm tall, covered with multicellular eglandular hairs, pubescent to sparsely pilose. **Leaves** opposite: blade unequal or rarely nearly equal or equal in each pair, asymmetrically ovate to lanceolate, 4.9–10.6 by 2.3–5.2 cm, herbaceous; apex acute or rarely obtuse; base oblique; margin serrate or rarely sinuate or repand; upper and lower surfaces hairy or sparsely hairy, lateral veins 5–9 on each side; petiole terete 0.5–3.5 cm, glabrous or sparsely pilose. **Inflorescence** axillary, simple or compound cymes, 1–5 flowered, white to violet–blue; peduncle 0.95–5.2 (12.5) cm, glabrous or hispid; bracts paired, free, sessile, ovate or lanceolate, rarely spatulate or linear, 0.3–0.8 by 0.05–0.6 cm, villous or sometimes conspicuous on margin, margin entire, apex acute or obtuse, lateral veins. Pedicel 0.5–1.3 cm, glabrous, pubescent or pilose. **Calyx** tubular, tube 0.4–1.1 cm long; lobes more or less than half way, narrowly triangular, 0.5–1.05 cm long, outside pubescent or sparsely pilose, margin entire, apex acuminate or attenuate. **Corolla** infundibuliform, tube 3.1–4.5 cm long, white to violet-blue with yellow or purple marking, glabrous. **Stamens** 2: filaments inserted 2 cm from base of corolla tube, 1.1–1.9 cm long, geniculate; anthers elliptic, 1–2.5 mm, adaxial surfaces coherent; staminodes 2, inserted 1.8 mm from base of corolla tube, 2–4 mm long, glabrous. Gynoecium 2.4–3 cm, stigma deeply bilobed. **Fruit** orthocarpic, 6.7–11 by 0.11–0.15 cm, glabrous or sparsely puberulent. Seeds elliptic, 0.2–0.6 by 0.1–0.2 mm (Fig. 4.31).

Distribution. India, Nepal, Bhutan, China, Myanmar, Thailand and Vietnam (Fig. 4.30).

Ecology. Growing in shaded areas, on moist rock, in moist oak forest, in shady moist evergreen forest or on banks, alt. 610–3,048 m.

Provisional IUCN conservation assessment. Least Concern LC. This species has an EOO about 2,979,633 km² and AOO about 288 km². The EOO is within the threshold of Least Concern and the AOO is within the threshold of Endangered. However, this plant is common and widespread. An assessment of Least Concern is

more appropriate than Endangered.

Specimens examined. Nepal: Apies Gongaz, 1,350 m, 14 Sept. 1954, *Zimmermann* 1258 (**K**); Along path from Thak to Taprang, before Ghatti Khola, 1,500 m, 25 Aug. 1976, *Troth* 917 (**NMNS** (NMNS00064540)); Bagmati zone, between Dhaibunket and Ramche, east side of Trisuli River, 1,600 m, 15 Sept. 1966, *Nicolson* 2348 (**NMNS** (NMNS00064546)); E of Gonepani, NW of Porhara, 2,250 m, 20 July 1973, *Grey-Wilson et al. s.n.* (**K**); Khare, E of Lumle, 1,700 m, 17 July 1973, *Grey-Wilson et al.* 291 (**K**); Phulchoke, SW of Katmandu, 1,829 m, 27 Aug. 1965, *Schilling* 619 (**K**); Ramechap, Bhandar, Ramechap, Bhandar, 1,990 m, 20 July 1995, *Josni, C.M. J-1585614* (**BKF** (SN202431, SN202432)); Tamur river near Chirwa, 1,350 m, 3 Sept. 1989, *KEKE team: S. Crawford, C. Grey-Wilson, D. Long, R. McBeath, H. Noltie, M. Sinnott, M. Subedi, S. Zmarzty* *KEKE* 179 (**K**); *Anonymous s.n.* (**K** (K000858378)); 1821, *Wallich* 801 (**BM** (BM000041656), **G** (G00133149)); *Wallich* 1829 (**K** (K000858377)); 1819, *Wallich s.n.* (**BM** (BM000041744)); Nepal, 819, *Wallich s.n.* (**G** (G00133149)).

India: Arunachal Pradesh: Mishmi (*Griffith* 1044 (**K**), *Griffith* 3823 (**K, P** (P03884181), **U** (U1342368)), *Griffith* 19795 (**BM** (BM00001010974)), *Griffith s.n.* (**K**)); Assam (King s.n. {**U** (U1342367)}, Delei valley, 1,219 m, 30 July 1928, *Ward* 8499 (**K**), *ibidem* 1524 m, 18 July 1928, *Ward* 8468 (**K**); Dirang Dzong, 1,829 m, 15 Aug. 1938, *Ward* 14084 (**BM** (BM011025916)), *ibidem*, *Ward* 14093 (**BM** (BM011025938)), Henina, Naga Hills., 26 0'0"N, 95 0'0"E, 1,500 m, 26 Aug. 1935, *Bor* 5350 (**K**), near Rima, Lohit Valley, 28 0'0"N, 96 30'0"E, 1372 m, 2 July 1950, *Ward* 20064 [**BM** (BM011025917)]).

Himachal Pradesh: Shimla, 31 6'12"N, 77 10'20"E (1829 m, Aug. 1885, *Collett s.n.* (**K**), 1,676 m, 30 Aug. 1917, *Gamble* 4882A (**K**), 13 Aug. 1916, *Rich* 333 (**K**), Shimla waterfall, 31 6'12"N, 77°10'20"E, 1,463 m, *Gamble* 5094A (**K**)), Shimla, Aug. 1886, *Prain s.n.* (**NMNS** (NMNS00064549)); Hmifang, Lushai hills, 1,524 m, July 1926, *Mr & Parry* 31 (**K**); Khasia, Numklow, 25 35'0"N, 91°38'0"E (1,067 m, 9 Nov. 1871, *Clarke* 15258B (**BM** (BM011025920), 1,219 m, 26 Aug. 1885, *Clarke* 40195A (**BM** (BM011025921))); Kumoan, near Devi Dhurra, 30 19'48"N, 78 3'36"E, 1,524–1,829 m, 22 Aug. 1986, *Duthie* 5874 (**BM** (BM011025914)); Kurseong, 26°52'40"N 88°16'38"E, 1372 m, Aug. 1974, *Gamble* 3499A (**K**); Kusinoli Valley, Saharanpur, 1,219–1,524 m, 1 Sept. 1985, *Duthie* 4274 (**BM** (BM011025925)); Manipur, Ukhrul Distr., 5–1,829 m, 6 Aug. 1948, *Ward* 17904 (**BM** (BM011025927)); Meghalaya, Khasi (*Hooker s.n.* (**K**), *Hooker & Thompson* 2028 (**K**), 18 Oct. 1850, *Hooker & Thompson s.n.* (**K**), 19 Oct. 1850, *Hooker & Thompson s.n.* (**K**), Khasi hills, *Anonymous* 2 (**K**)); NW India, *Royle s.n.* (**K**); Mongpho, Sikkim, 30 Sept. 1882, *Clarke* 36030 (**NMNS** (NMNS00064536)), 1,676 m, 7 Oct, 1884, *Clarke* 36338 (**NMNS** (NMNS00064535)); Sikkim (1,524 m, 26 Aug. 1814, *C.K.* 649 (**K**), 1524 m, 24 Sept. 1884, *Clarke* 35814B (**K**), 1,829–2,134 m, 5 Aug. 1849,

Hooker s.n. (**K**), 1,219–1,829 m, *Hooker s.n.* (**K**), 1,219–1,829 m, *Hooker s.n.* (**P** (P03884180)), *Hooker s.n.* (TCD), 1,829 m, 5 Aug. 1849, *Hooker s.n.* (**K**); Sikkim, Darjeeling (1,800 m, 30 Aug. 1875, *Clarke 27292* (**L** (L2055073)), 914 m, 2 Aug. 1870, *Clarke 12313B* (**BM** (BM011025931)), 1,524 m, 11 Aug. 1875, *Clarke 26945* (**NMNS** (NMNS00064548))); 1,524 m, Sept. 1879, *Gamble 7069* (**K**), July 1881, *Gamble 9568* (**K**); Rishap, 914 m, 4 Sept. 1869, *Clarke 9005* (**K**), *ibidem*, 914 m, 27 Aug. 1870, *Clarke 12521C* (**K**); Rungbee, 1,524 m, 4 Sept. 1881, *Clarke 8558 B* (**K**), *ibidem*, 1,372 m, 20 Aug. 1869, *Clarke 8723G* (**BM** (BM011025932)), *ibidem*, 1,219 m, 1 Oct. 1884, *Clarke 36056* (**BM** (BM011025937))); Uttarakhand, Dehradun, Mussoorie (1524–1829 m, Aug. 1915, *Marten s.n.* (**BM** (BM011025911)), 2,000 m, 1 Aug. 1957, *Rau 3318* (**E** (E00630542)), Aug. 1898, *Duthie s.n.* (**P** (P03884179))); N. Garhwal, 2,000 m, 1 Sept. 1958, *Rau 8541* (**E** (E00630543)); Mussoorie, 1,829 m, 11 Aug. 193, *Stewart 15972* (**NMNS** (NMNS00064545)); Uttarakhand, Kumaon (1,433 m, *Anonymous s.n.* (**K**), 14 Aug. 1884, *Duthie 3276** (**K**), Oct. 1969, Pant, P.C. & Naithani 39547 (**K**), 1,524 m, 1843, *Strachey et al. 2* (**BM** (BM011025926), **K**, **P**)); 1,372 m, *Anonymous 317* (**K**); 31 Aug. 1885, *Barclay 109* (**BM** (BM011025912)); *Booth s.n.* (**K**); *Clarke s.n.* (**BM**); *Collett 245* (**K**); *Harris 115/2* (**BM** (BM011025913)); 6 Aug. 1850, *Hooker 2028* (**K**); *Hooker s.n.* (**K**); *Hooker s.n.* (**K**); *Royle 835* (**K**); *Royle s.n.* (**BM** (BM011025936)).

Bhutan: Deuchung, 27°18'58.3"N 90°37'12.4"E, 2,286 m, 6 Aug. 1949, *Ludlow et al. 21403* (**BM** (BM011025933)); Mongar, 27°22'16.4"N 91°05'08.1"E, 1,676 m, 24 July 1949, *Ludlow et al. 20939* (**BM** (BM011025934)); Tashiyangtse Kurted, 27°32'15.5"N 91°25'36.1"E, 1,829 m, 11 Aug. 1915, *Cooper et al. 4414* (**BM** (BM011025935)).

China: Tibet, Yigrong Valley, 3048 m, 3 Sept. 1935, *Ward 12127* (**BM** (BM000041576)). Guizho Prov, Zhenfeng Co, Lonchangzheng town, Shanhe village, 1,180–1,200 m, 29 July 1996, *Lua Yi-bo 117* (**K**); Huang tiao ha, 1916, *Cavalerie 4043* [**E** (E00135108), **P** (P03962951)]; Kouy-Tcheou (*Cavalerie 3120* [**E** (E00087424), **K**, **P** (P03884182)), Kotschang-keou, Aug. 1904, *Esquirol 171* (**E** (E00387545))); Menglun, S.E., 1,524 m, *Henry 11040* (**K**); Shweli valley, 2,100 m, 1 Sept. 1913, *Forrest 11990* (**BM** (BM000041495), **E** (E00087418), **K**); Yunnan (*Forrest 18294* (**K**), 1917–1019, *Forrest 18296* (**BM**), *Forrest 29935* (**E** (E00627466)), *Forrest 30387* (**E** (E00627468)), *Forrest s.n.* (**K**), 1,350 m, *Henry 12281* (**K**), 1,829 m, *Henry 13252* (**K**), 1,800 m, *Henry 11040A* (**K**, **NMNS** (NMNS00064539)), 1,829 m, *Henry 11040B* (**K**), *Henry 12281B* (**BM** (BM000041561), **K**), 1,524 m, *Henry 12281C* (**K**)); July, *Edgeworth 194* (**G** (G00133152)); Yunnan, Gaoligong Shan Region (Longling Xian, Zheng'an Zheng. Vicinity of Hupa village. E side of Gaoligong Shan along the new road from Baoshan to Tengchong via Nankang Yakou. NE facing 10–30' slope, 24°48'48" N, 98°49'58" E, 1,530 m, 23 Aug. 2003, *Li et al. 17646* (**E** (E00224715)), Longyang Qu, Mangkuan

Xiang. Baihualing Cun, Zotang area. E side of Gaoligong Shan, 25°18'18" N, 98°47'24" E, 1,590 m, 9 Sept. 2003, *Li et al. 18946* (**E** (E00224711)); Yunnan, Gongshan Xian, (Binzhongluo Xiang. Along the Niwa river gorge N of Bingzhongluo and Shimenguan (Stone Gate) on the W side of the Nu Jiang. E side of Gaoligong Shan. N facing 30–60 degrees slope, 28°02'33" N, 98°34'45" E, 1,750 m, 8 Oct. 2002, *Li et al. 17068* (**E** (E00224714)), Cikai Zheng. E side of Gaoligong Shan, W of Gongshan and E of Qiqi on the trail from Gongshan to the Dulong Jiang valley, 25°18'18" N, 98°47'24" E, 1,500 m, 20 July 2000, *Li 12976* (**E** (E00132438)); Yunnan, Salween, Tschamutong, 1,720 m, 14 Sept. 1916, *Handel-Mezzetti 1838* (**NMNS** (NMNS00064541)); Yunnan, Guilin, 25 16'0"N, 110 17'0"E, 1 Sept. 1912, *Cavalerie 3906* (**K**); Yunnan, Hills to the 12281 wheel-of-Fungyueh, 25° N, 98°38' E, 1,829 m, 1 July 1912, *Forrest 8537* (**E** (E00087419), **K**); Yunnan, Longling Xian, Zheng'an Zheng. Linggang area near Xiaohei Shan Provincial Nature Preserve. E side of Gaoligong Shan close to new road from Baoshan to Tengchong via Nankang Yakou. NE facing 10–30' slope, 1,900 m, 24 Aug. 2003, *Li et al. 17750* (**E** (E00224664)); Yunnan, Mid.W. Yunnan, 25°30' N, 98°15' E, 2,134 m, Aug. 1925, *Forrest 27180* (**K, P** (P038841683)); Yunnan, Nujiang Lisu Aut. Pref. (Fugong Co., West Bank of Salween across from village of Pi He, 26°31'38" N, 98°53'50" E, 1,165 m, 23 Oct. 1996, *Gaoligong Shan Expedition 1996 7938* (**E** (E00099072)); Gongshan Co., 2–3 km along track from Qi Qi towards Dong Shao Fang., 27°48'05" N, 98°34'07" E, 2,050–2,200 m, 20 Sept. 1997, *Gaoligong Shan Expedition 1997 9411* (**E** (E00099049)), *ibidem*, Shi Meng Guan (Stone Gate), 28°02'07" N, 98°36'08" E, 1,650 m, 11 Sept. 1997, *Gaoligong Shan Expedition 1997 8871* (**E** (E00099051)), *ibidem*, Track from Cu Lou Village towards Qi Qi Nature Reserve Station., 27°43' N, 98°37' E, 1,600 m, 13 Oct. 1996, *Gaoligong Shan Expedition 1996 7468* (**E** (E00099073)); Yunnan, Tengchong Xian (Sanyun Xiang. Qiaojie Cun in vicinity of Henghe village along a N-S facing ravine. W side of Gaoligong Shan on the old road from Baoshan to Tengchong via Dahaoping. W. facing 60 + degree slope., 24°59'19" N, 98°43'44" E, 1,960 m, 2 Aug. 2003, *Li et al. 18234* (**E** (E00224665)), Wuhe Xiang. Tenglan Cun, Laniba He. W side of Gaoligong Shan on the W side of a N-S ravine above the new road from Baoshan to Tengchong via Nankang Yakou, 24°52'57" N, 98°42'49" E, 1,630 m, 27 Aug. 2003, *Li et al. 18011* (**E** (E00222952)); Yunnan, Upper Kiukiang Valley, (Clulung) Letahca, 1,950 m, 31 July 1938, *Yu 19523* (**E** (E00627465)).

Myanmar: Above Wogok, Ruk Mines District, 1,350 m, 25 Oct. 1917, *Lace 6284* (**E** (E00627629), **K**); Dinghputyang, 25°58' N, 97°53' E, 610 m, 5 Aug. 1939, *Kaulback 316* (**BM** (BM011025919)); Mahtum, 26°06' N, 97°58' E, 1372 m, 20 Aug. 1939, *Kaulback 350* (**BM** (BM011025915)); Laktang, 1,500 m, 5 Aug. 1919, *Ward 3461* (**E**

(E00087427)); N.E. upper myanmar, Hills around Heawgaw, Sept. 1924, *Forrest 24896* (**BM** (BM011025923), **E** (E00096837), **K**); Nagkyi, 1,200 m, 15 Aug. 1919, *Ward 3537* (**E** (E00627628)); North Triangle (Htingnam), 1,219 m, 20 July 1953, *Ward 21182* (**BM** (BM011025922)); Tibet-Myanmar frontier. Valley of the Senighku, 2,100 m, 3 Aug. 1926, *Ward 7240* (**E** (E00627630), **K**); Upper Myanmar, Hills around Hlawgaw, 2,100 m, 1 Aug. 1924, *Forrest 24863* (**BM** (BM011025924), **E** (E00096836), **K**); Kachin State, Nam Tamai Valley, 27°42' N 97°54' E [1,219 m, 9 Aug. 1937, *Ward 12918* (**BM** (BM011025918)), 914 m, 16 Aug. 1938, *Kaulback 61* (**BM** (BM011025928)), 1,524 m, 9 Sept. 1938, *Kaulback 117* (**BM** (BM011025929)), 914 m, 15 Aug. 1938, *Kaulback s.n.* (**BM** (BM011025930))]; Kachin State, Putao District, buffer zone of Hkakaborazi NP (between Shingsankhu rest house and Golle village, 27°39'10.3"N, 97°53'39.6"E, 891 m, 9 Nov. 2015, *Armstrong et al. 1335* (**NYBG** (NYBG02649177)), between Tup Kwan rest house and Gushin village, 27°37'22.2"N, 98°10'44.6"E, 1,219 m, 26 Oct. 2015, *Armstrong et al. 1032* (**NYBG** (NYBG02648854)))).

Thailand: NORTHERN. Chiang Mai: Chom Thong, Doi Inthanon NP ((Siriphum Waterfall 18°32'52.4" N, 98°30'87.8" E, 1,100 m, 6 Aug. 2000, *Suksathan 2655* (**QBG** (QBG19407)), *ibidem*, 18°32.8' N, 98°30.8' E, 1,380 m, 19 Sept. 2008, *Middleton et al. 4511* (**BKF** (BKF167853), **E** (E00679398))), 18°32'47" N, 98°30'50" E, 1,326 m, 2 Sept. 2016, *Sirimongkol et al. 679* (**BKF**); Siriphum waterfall trail, 18°32'49" N, 98°30'54"E, 1,313 m, 2 Sept. 2016, *Sirimongkol et al. 680* (**BKF, TCD**); road to Doi Pha Tang, 18°31'03" N, 98°31'03" E, 1,300 m, 18 Sept. 2008, *Middleton et al. 4505* (**BKF** (BKF183053), **E** (E00679397)); road no 1284 to Khun Wang about 4 km from Royal Project, 18°34'02" N, 98°31'09" E, 1,466 m, 2 Sept. 2016, *Sirimongkol et al. 681* (**BKF, K, TCD**), *ibidem*, about 4.5 km from Royal Project, 18°34'12" N, 98°31'07" E, 1,459 m, 2 Sept. 2016, *Sirimongkol et al. 682* (**BKF, TCD**); Mae Pan Falls, 1,400 m, 1 Oct. 1979, *Santisuk s.n.* (**BKF** (BKF125354))); Chiang Dao, Doi Chiang Dao Wildlife Sanctuary, 1,200 m, 17 July 1955, *Bunchuai, K. 277* (**BKF** (BKF23021)); Mae Taeng, Huai Nam Dung NP, Doi Chang, 1,800 m, 23 Oct. 1979, *Santisuk s.n.* (**BKF** (BKF125352)); Muang, Doi Suthep-Pui NP, SW side, Doi Pui Meditation centre, above Doi Pui Mong village, 1,400 m, 25 June 2002, *Palee 521* (**CMUB** (CMUB21193)); Muang, Doi Suthep-Pui NP, Doi Pui trail to summit, beside the royal pavilion., 18°50'03" N, 98°53'18" E, 1,665 m, 3 Sept. 2016, *Sirimongkol et al. 686* (**BKF, TCD**). **Nan:** Pua, Doi Phu Kha NP, nature trails, 1,530 m, 18 Sept. 2003, *Phonsena et al. 3962* [**BKF** (BKF166036)]. *Kerr 2707* (**TCD**).

Vietnam: Sha-ba, Ban-si-phan, 1,800–1,900 m, 6 Aug. 1997, *Phengklay et al. 10472* (**BKF** (BKF143130)); road in to Khaya and the nursery, 1,700 m, Sept. 1927, *Petelot 5045* (**P** (P03884177)); Tonkin (1,600 m, July 1928, *Petelot 4996* (**P** (P03884176,

P03884184]], Between Chapa and the Lo qui Ho, 1,600 m, Aug. 1943, *Petelot 8473* (P (P03884185)), Sai Wong Mo Shan (Sai Wong Mo Leng) Long Ngong Village, Dam-ha, Tonkin, 18 July – 9 Sept. 1940, *Tsang 30398* (P (P03962952)), (Sai Wong Mo leng), Long Ngong village, Dam-ha, Tonkin, 18 Sept. 1940, *Tsang 30389* (E (E00627684), P (P03962950, P03962952), **SING** (SING0204299)); Tonkin, *Anonymous 4996* (NMNS (NMNS00064538)); *Anonymous HNK182* (HN); *Anonymous HNK378* (HN); 7 Aug. 1926, *Poilane 12884* (P (P03884186)); *Vu et al. HNK182A* (HN).

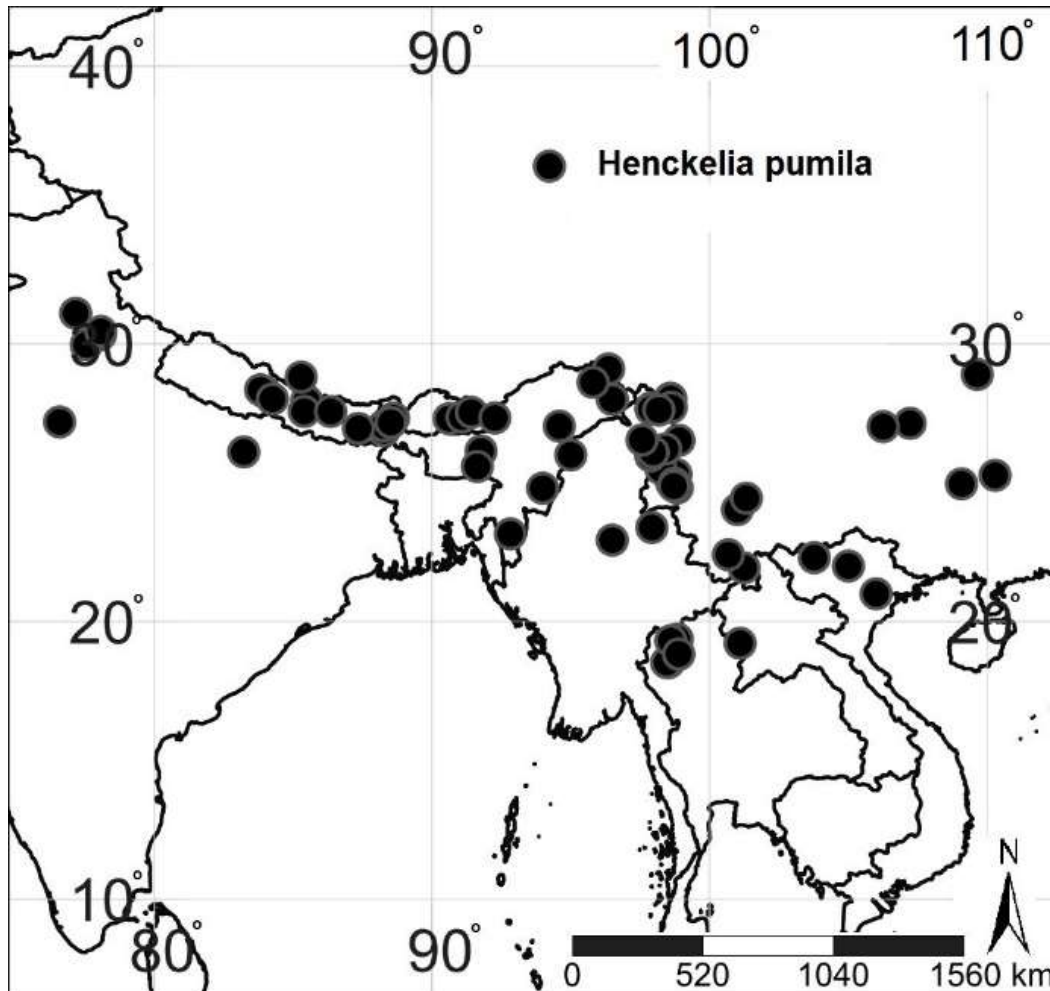


Figure 4.30 Distribution map of *Henckelia pumila* (D. Don) A. Dietr. Base map from <https://www.simplemappr.net>



Figure 4.31 Line drawing of *Henckelia pumila* (D. Don) A. Dietr.: A. Habit; B. Flower; C. Flower dissection. Drawings: Arthit Kamgamnerd from *Sirimongkol et al.* 681 (BKF)

22. *Henckelia rotundata* (Barnett) D.J. Middleton & Mich. Möller, *Taxon* 60(3): 7766 (2011); Middleton, Weber, Yao, Sontag & Möller, *Edinburgh J. Bot.* 70(3): 402. 2013.

≡ *Chirita rotundata* Barnett, *Nat. Hist. Bull. Siam. Soc.* 20: 17. 1961; *Kew Bull* 15(2): 254. 1961; Barnett, *Fl. Siam.* 3(3): 227. 1962. TYPE: A.F.G. Kerr, 6274, Thailand, Chiang Mai, Mae Cham, 11 July 1922 (Holotype. **K!** (K000545606); Isotype **ABD!**, **BK!**(BK257924), **BM!** (image BM000997772)).

Caulescent annual herb, stem slender, simple, up to 16 cm tall, glabrous. **Leaves** opposite: blade symmetrically or asymmetrically orbicular, ovate or obovate, 2.5–11 by 1.6–4.9 cm, herbaceous; obtuse or acute; base oblique or cuneate; margin entire or sparsely ciliate; upper surface glabrous or sparsely hairy; lower surface glabrous; lateral vein 4–5 on each side; petiole terete 0.5–5 cm, glabrous. **Inflorescence** axillary, solitary or cymes 1–4 flowered, pale purple with yellow marking, peduncle 0.5–1.7 cm long, glabrous; bracts paired, free, sessile, ovate, 5–10 mm long, glabrous and ciliate margin. Pedicel terete, 0.5–1.5 cm, glabrous. **Calyx** tubular, tube c. 0.9 cm long; lobes divided more or less than half way, triangular, c. 4 mm long, glabrous; caducous. **Corolla** infundibuliform, c. 3.6 cm long, glabrous. **Stamens** 2: filaments inserted c. 1.2 cm from base of corolla tube, c. 1.4 cm long, geniculate; anthers elliptic, c. 1.5 mm long, adaxial surface coherent; staminodes 2, inserted 1.2 cm from base of corolla tube, c. 5 mm long, curved, glabrous. Gynoecium 2.4 cm long, stigma deeply bilobed. **Fruit** orthocarpic, 7–11 by 0.15–2 cm, glabrous (Fig. 4.33).

Distribution. Northern Thailand (Fig. 4.32).

Ecology. On rocks near stream in deciduous forest, in moist deciduous forest with bamboo, alt. 700–1,075 m.

Provisional IUCN conservation assessment. Endangered EN B1ab(i,iii)+B2ab(i,iii). This species has an EOO about 1,760 km² and AOO about 16 km². The EOO and the AOO are within the threshold of Endangered. One location of this species is extremely threatened by land use. The site has been changed to agricultural land. The habitat is accessible by the local community and inaccessible due to its remote locality to the park authorities.

Specimens examined. Thailand: NORTHERN. Mae Hong Son: Pai (Huai Nam Dung NP, road no. 1095 from Pai to Pang Ma Pha, km. 80–81, route from Khun Mae Ya check point to Wat Chan tri-intersection, 19°15'49" N, 98°30'26" E, 860 m, 17 Sept. 2016, *Sirimongkol et al.* 694 (BKF, E, K, P, TCD), Pam Bok Waterfall, Pam Bok waterfall, beside minor small stream, 19°19'15" N, 98°24'19" E, 598 m, 17 Sept. 2016, *Sirimongkol et al.* 695 (BKF, TCD)), Pang Ma Pha, Ban Kued Sam Sib, Route from Ban Kued Sam Sib to Pang Koh, 10°25'27" N, 98°08'58" E, 1,075 m, 18 Sept. 2016, *Sirimongkol et al.* 697 (BKF, E, K, P, TCD).

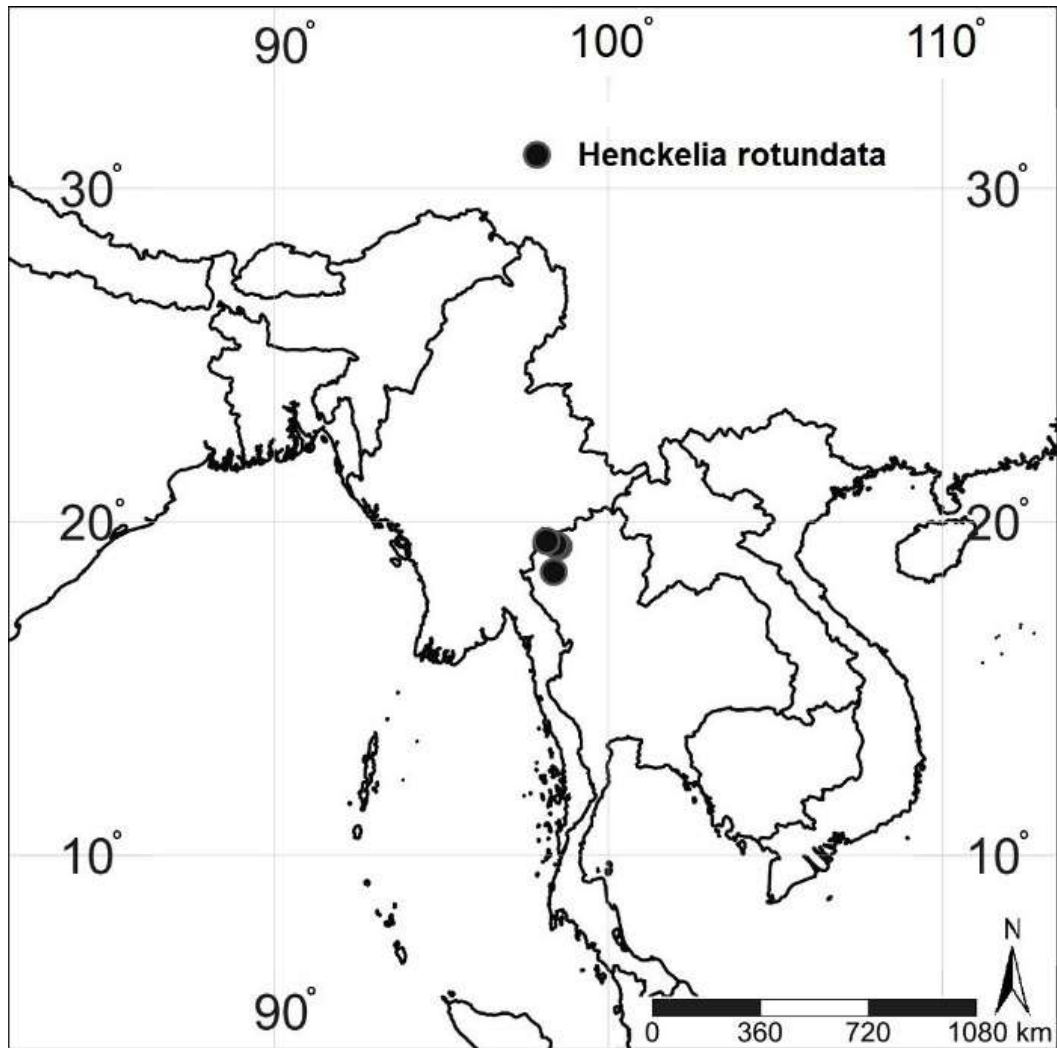


Figure 4.32 Distribution map of *Henckelia rotundata* (Barnett) D.J. Middleton & Mich. Möller. Base map from <https://www.simplemappr.net>

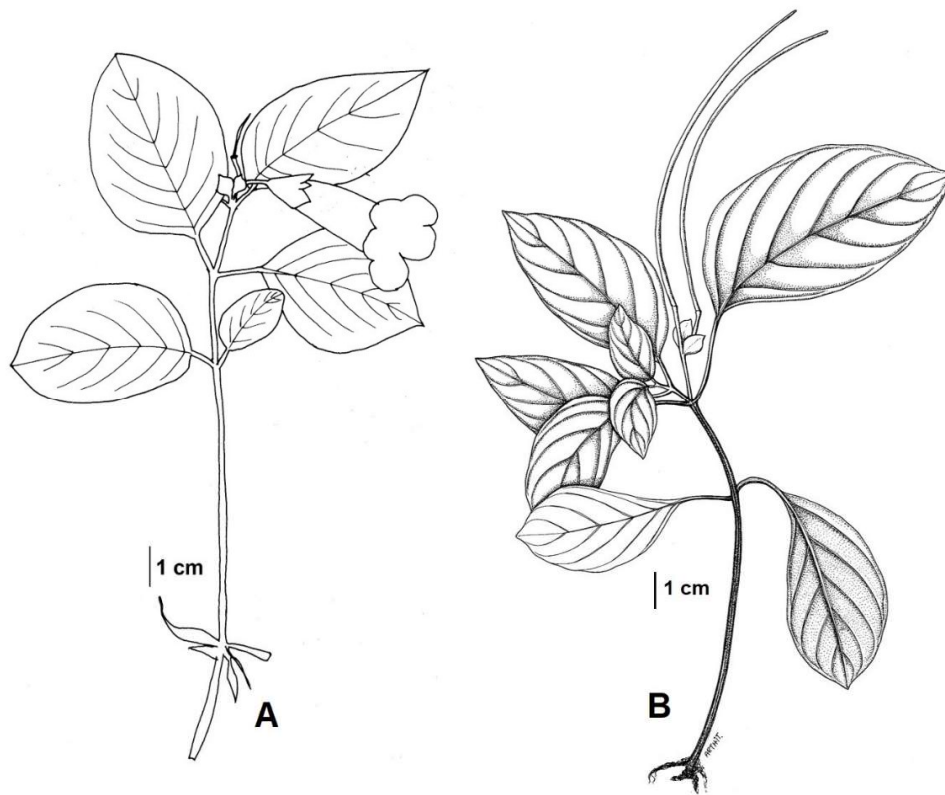


Figure 4.33 Line drawing of *Henckelia rotundata* (Barnett) D.J. Middleton & Mich. Möller: A. Flowering stage; B. Fruiting stage. Drawing: A. Sukontip Sirimongkol from Kerr 6274 (K); B. Arthit Kamgamnerd from Sirimongkol et al. 697 (BKF).

23. *Henckelia speciosa* (Kurz) D.J. Middleton & Mich. Möller, Taxon 60(3): 777.

2011; Middleton, Weber, Yao, Sontag & Möller, Edinburgh J. Bot. 70(3): 402. 2013.

≡ *Chirita speciosa* Kurz, J. Bot. 11: 195. 1873; Wang et al., Fl. China 18: 342. 1998.

TYPE: Yunnan Ponline, China, 5 March 1868, *Anderson s.n.* (Isolectotype **K**).

–*Chirita brevipes* C.B. Clarke, Candolle & Candolle, Monogr. Phan. 5(1): 120. 1883.

TYPE: Griffith, W. 3828, Meghalaya, East Bengal, Khasia, India, (Lectotype **K**);

Meghalaya, Mowpoot, Khasia, India, 14 Nov 1871, *Clarke, C.B. 14605* [Syntype **K**, **NMNS** (NMNS00064561)].

–*Chirita trailliana* Forrest & W.W. Sm. in Notes Roy. Bot. Gard. Edinburgh 9: 95. 1916.

TYPE: Forrest 7976, China, Yunnan, May 1912 (Lectotype **E!**, Isolectotype **K**), Forrest 8124, China, Yunnan, June 1912 (Syntype **E!**, Isosyntype **E!**).

Acaulescent perennial herb, rhizomatous, 15–20 cm tall, covered with brown hairs.

Leaves whorled: blade asymmetrically ovate, or cordate, 11.5–17.9 by 10–12.5 cm,

apex attenuate or acute, base subcordate or oblique; lower and upper surface hairy, especially on midrib and secondary veins, lateral veins 7–9 on each side; petiole terete 8–16.4 cm, hairy. **Inflorescence** several, axillary, simple or compound cymes, 1–7 flowered, purple with yellow marking or whitish-blue; peduncle 3–6 cm long, hairy; bracts paired, free, sessile, linear or lanceolate, 3–8 mm long, hairy. Pedicels terete, 1.3–1.4 cm long, hairy. **Calyx** tubular, 0.8–1.4 cm long; lobes divided more or less than half way, narrowly lanceolate, 0.4–1.8 cm long, outside hairy; caducous. **Corolla** infundibuliform, tube 4–6 cm long, hairy. **Stamens** 2: filaments inserted c. 1.8 cm from base of corolla tube, 1–1.7 cm long, the top of the filaments hairy; anthers elliptic, 2.5–3 mm long, lanate hairs on base, adaxial surface coherent; staminode 2, inserted 1.1 cm from base of corolla tube, 0.4–0.7 cm long, covered with long hairs. Gynoecium 3.1–4.5 cm, ovary 2 mm across, hairy, stigma deeply bilobed. **Fruit** orthocarpic, 5.6–9 cm long, hairy. Seeds elliptic, c. 0.3–0.5 by 0.1–0.3 mm (Fig. 4.35).

Distribution. India, Myanmar, China, Thailand, Laos and Vietnam (Fig. 4.34).

Ecology. In shaded and moist areas in primary evergreen forest, montane forest or stream valleys, alt. 300–3,000 m.

Provisional IUCN conservation assessment. Least Concern LC. This species has an EOO about 1,738,546 km² and AOO about 132 km². The EOO is within the threshold of Least Concern and the AOO is within the threshold of Endangered. However, this plant is common and widespread. An assessment of Least Concern is more appropriate than Endangered.

Specimens examined. India: Assam, (1,829 m, 26 July 1935, *Bor 4482* (K), Assam, *King s.n.* (U (U1342357))), Meghalaya, Khasi [914 m, 14 Nov. 1871, *Clarke 14605E* (BM (BM0000617794), K (K000858356)), 762 m, 16 Nov. 1871, *Clarke 14708D* (BM (BM0000997764)), *ibidem*, *Clarke 14714B* (BM (BM0000617793)), 1,219 m, 13 May 1886, *Clarke 43844A* (BM (BM0000997765)), *Griffith 3828* (K (K000858390), P (P03511053))); King 189 (P (P03511050)).

China: Kwangtung-Tonkin Border, Kung Ping Shan and Vicinity, Faan Faan, Fang Cheng Distr., 23°24'0"N, 113°30'0"E, 2,100 m, June 1925, *Forrest 26748* (BM (BM000041525), E (E00096811), K, NMNS (NMNS00064562), P (P03884132, P04079304)); Nujiang Lisu Autonomous Pref. Off provincial road 230 at Gang dang, Bai hua ling, Han long zhai (village) (at the end of Zoo3 county road). Circular road to Pu bu (waterfall), 25°30'82" N, 98°79' 49"E, 1,585 m, 22 July 2010, *Zhang et al.*

10CS2014 (K); Tibet & SW China, *Forrest 29834* (BM (BM000041534)); Yunnan, 22°24'24.5"N 100°40'55.0"E, (1868, *Anderson s.n.* (K (K000857507)), *Forrest 15813* (BM (BM000041508)), 1 Aug. 1917, *Forrest 15873* (E (E00096813), K), *Forrest 18428* (K), *Forrest 29918* (E (E00627469)), 1,524 m, *Henry 11789A* (K, NMNS (NMNS00064519)); Yunnan, Hill to the south of Seugyueh, 22°24'24.5"N 100°40'55.0"E, 2,100 m, June 1912, *Forrest 8124* [BM (BM000041487), E (E00135086, E00096812), K (K000858357)]; Yunnan, Hills N. W. of Lungywh, 25°20' N, 98°25' E, 2,100 m, 1 May 1931, *Forrest 29599* [BM (BM000041531), E (E00087433)]; Yunnan, Menghai, Mt. Bulang, Menghai Country, 12 Mar. 2011, *Li-Jianwu 386* [QBG (QBG59075)]; Yunnan, Salwin Valley, 25°20' N, 90°00' E, 1,200 m, 1 Apr. 1931, *Forrest 29391* [BM (BM000041530), E (E00087432)]; Yunnan, Shweli valley, 1,800 m, May 1912, *Forrest 7976* [E! (E00135140), K! (K000858358)]; Yunnan, Shweli-Salwin divide, 2,700 m [1 Oct. 1919, *Forrest 18365* [E (E00096814), P (P03884131)], 1 June 1924, *Forrest 24468* [E (E00096815), K]]; Yunnan, Szemer, 1,524 m, *Henry 11789* (K); Yunnan, Western Flauk of Hie Lali Rge, 3,000 m, 1 Sept. 1917, *Forrest 15539* [E (E00087431), K]; Yunnan, Xuelin to Zuodu, Xuelin, Lancang Co, Yunnan Prov., 23°95'37" N, 99°32'21" E, 1,945 m, 31 March 2010, *Liu et al. 2434* (K); 14 May 1935, *Smith 29599* (K).

Myanmar: Hills around Kan-Ruei, 2,700 m, 1924–1925, *Forrest 26522* (BM (BM011025885), K, NMNS (NMNS00064563), P (P03884155)); Kachin Hills, May 1898, *Mokim s.n.* (BM (BM011025887, 26513 K)); Kachin state, (Myitkyina (1,829 m, 9 May 1938, *Kermode 17349* (K), *ibidem*, near Pawte, 2,134 m, 4 May 1938, *Kermode 17285* (K); N W Yunnan and E Tibet, Kachin Hills, 600 m, 27 Mar. 1905, *Ward 203* (E (E00087435)); Khaiyang, 1,524–1,676 m, 30 Apr. 1948, *Ward 17359* (BM, NYBG (NYBG02652112)); Lapycka & Lweje, Bhamo District., 24 16'0"N, 97 14'0"E, 1,500 m, 7 Apr. 1912, *Lace 5758* (E (E00627602, E00627603), K, TCD); N Maikha-Salwin divide., 27°24' N, 97°31' E, 2,400 m, 1 June 1931, *Forrest 29736* (E (E00096839)); N. E. Upper Myanmar, Hills around Fyi Li, 25°58' N, 98°29' E, 2,438 m, Apr 1925, *Forrest 26513* (E (E00096840), K, NMNS (NMNS00064564), P (P03884143)); N.E. Upper Myanmar, Oct. 1925, *Forrest 27339* (E (E00096843), K); Ngawchang and Nmai valleys., 27°42' N 97°54' E, 1,200–1,500 m, 3 Apr. 1939, *Ward 479* (NYBG (NYBG02652109)); Ngawchang Valley, 1,200 m, 31 Mar. 1939, *Ward 471* (NYBG (NYBG02652110, NYBG02652113)); North Myanmar: North Triangle (Arahku), 1,219 m, 5 Apr. 1953, *Ward 20614* (BM (BM011025886), E (00627604)); Tamu-Chipwi New Road, 210 m, 25 Mar. 1938, *Kermode 16647* (K); Ti-ka-ho., 4°03'30" N, 115°00'90" E, 1,500 m, 24 Apr. 1921, *Ward 3771* (E (E00627605)); Upper Myanmar, Nwai Valley, 2,400 m, 10 May. 1914, *Ward 1534* (E (E00096844)); Upper Myanmar, Valley of Me Laping, 750 m, 1 Feb. 1914, *Forrest 12150* (E (E00096842)); 24 June 1915, *Forrest s.n.* (K).

Thailand: NORTHERN. **Mae Hong Son:** Pang Mapha, San Ban Dan Wildlife Sanctuary, border of Pang Ma Pha-Pai districts, along highway no 1095, km 123, 1425 m, 28 Sept. 2003, ((*Palee* 626 (**BKF** (BKF164119)), *Soe Myint Aye et al.* 626 (**CMUB** (CMU23015))); Pang Mapha, Kiew Lom, along highway 1095, km 123, Pai-Pang Ma Pha 1,475 m, 9 Nov. 2004, *Palee* 763 (**BKF** (BKF151048)); Road no.1095, Pai to Kiew Lom, km. 120, 19°26'35' N, 98°19'19' E, 1,416 m, 17 Sept. 2016, *Sirimongkol et al.* 696 (**BKF, E, K, L, P, TCD**). **Chiang Mai:** Muang, Doi Suthep-Pui NP, east side of Doi Pui, Chang Kian village area, near the Agricultural Station (site A), 1,475 m, 3 June. 1989, *Maxwell* 89-702 (**E** (E00627688)); Doi Pui, 18°50'03" N, 98°53'42" E, 1,400 m, 13 Aug. 2012, *Middleton et al.* 5585 (**BKF** (BKF186865)), road from Doi Pui Camp ground to Ban Khun Chang Khian, 18°50'03' N, 98°53'42' E, 1,408 m, 16 Sept. 2016, *Sirimongkol et al.* 693 (**BKF, E, K, TCD**), *ibidem*, 11 July 2017, *Sirimongkol et al.* 712 (**BKF, TCD**). Chiang Rai, Doi Tung, along the Wat Noi-Huai Nam Kun trail, 1 km east of Wat Noi, 1,180 m, 14 Aug. 2013, *van de Bult* 1331 (**CMUB** (CMUB21194)). SOUTH-WESTERN. **Kanchanaburi:** Kwai Noi River Basin Exp 1946, near Ka Tha Lai in Pan Paung River Valley, about 25 km east of Wanga, 300 m, 12 June 1946, *Hoed et al.* 933 (**BK** (BK263813), **P** (P03511097)). May 1910, *Kerr* 1164 (**P** (P03511052), **TCD**). **Laos:** 30 June 1892, *Henri d' Orleans s.n.* [**P** (P03511049, P03511051)]; 23 June 1892, *Henri d' Orleans s.n.* (**P** (P03884144, P03511048)). **Vietnam,** Tonkin, 400 m, 21 Apr 1926, *Poilane* 25821 (**P** (P03884141, P03884142)).

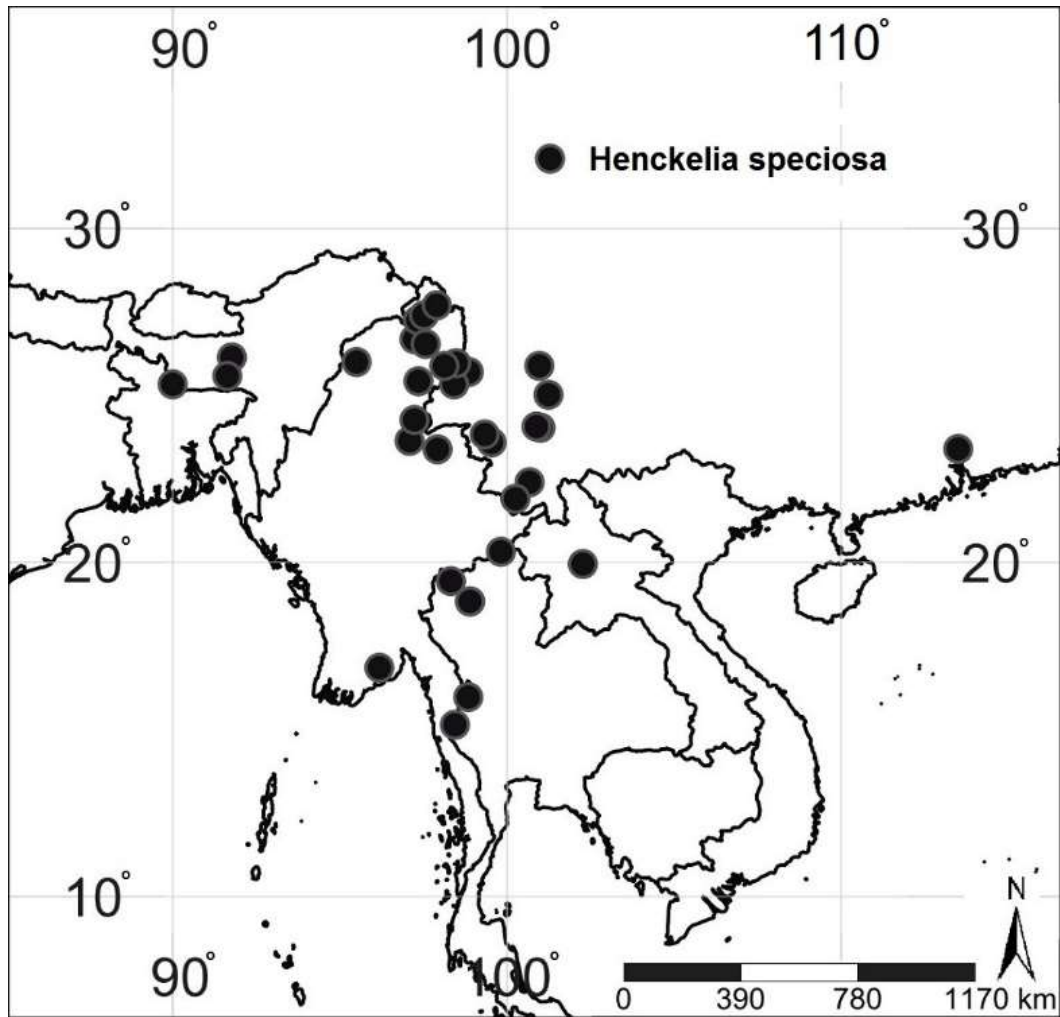


Figure 4.34 Distribution map of *Henckelia speciosa* (Kurz) D.J. Middleton & Mich. Möller. Base map from <https://www.simplemappr.net>

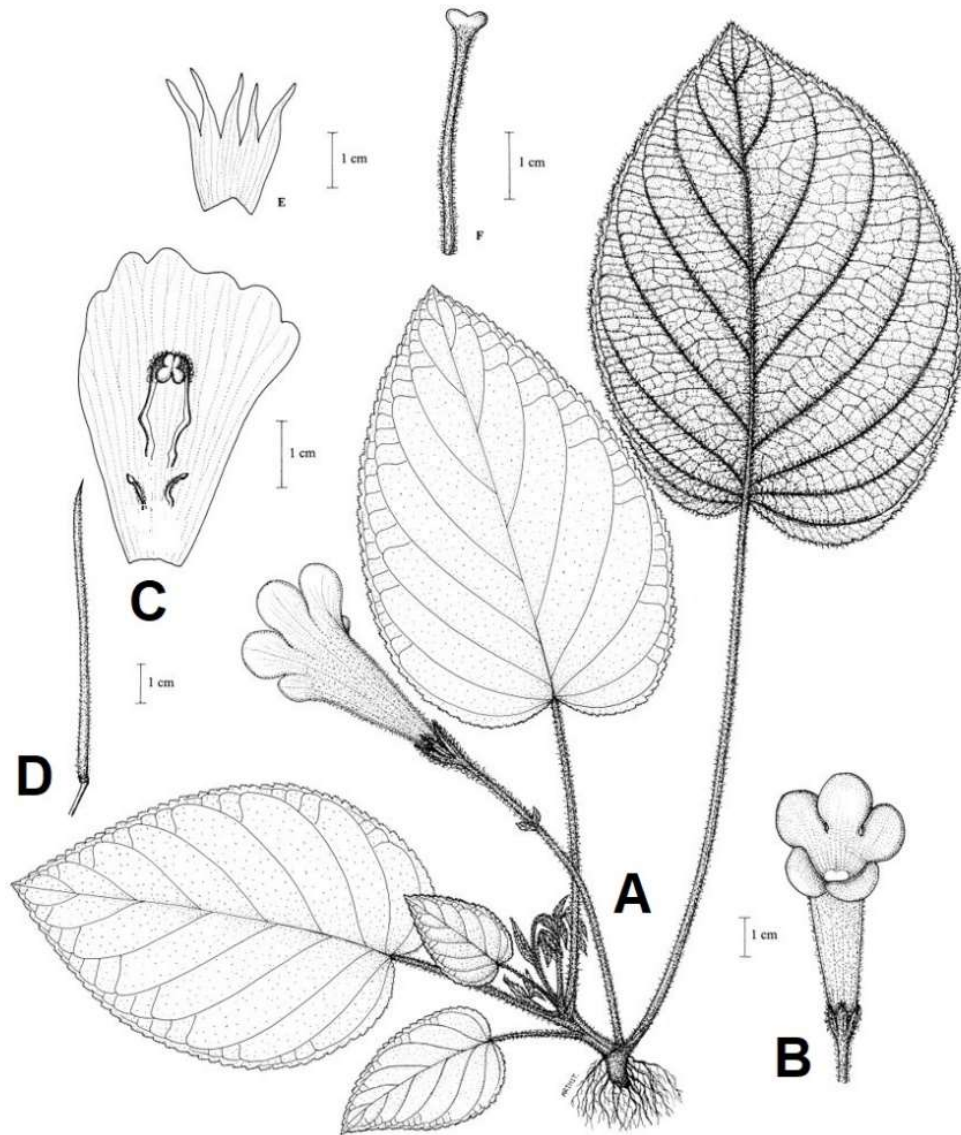


Figure 4.35 Line drawing of *Henckelia speciosa* (Kurz) D.J. Middleton & Mich. Möller: A. Habit; B. Flower; C. Flower dissection; D. Fruit. Drawings: Arthit Kamgamnerd.

24. *Henckelia urticifolia* (Buch. - Ham. ex D. Don) A. Dietr., Sp. Pl., ed. 6. 1: 574.

1831 (as "*urticaefolia*"); Weber & al., Taxon 60 (3): 777. 2011; Middleton et al., Edinburgh J. Bot. 70 (3): 403. 2013.

≡ *Chirita urticifolia* Buch. - Ham. ex D. Don, Prodr. Fl. Nepal. 90. 1825; Hara, Enum. Fl. Pl. Nepal 3: 134. 1982; Polunin & Stainton, Conc. fl. Himalaya 114. Pl 97. 1987; Wang et al., Fl. China 18: 343. 1989. Lectotype. Nepal, *Wallich list 800* (G!) (image-

G00133154), **K!** (K000858386), **M!** (image-M0185550), **P!** (P03884100, P03884101)).

–*Calosacme grandiflora* Wall., Numer. List 800. 1829.

–*Chirita grandiflora* Wall., Pl. Asiat. Rar. 1: 43. 1830.

–*Henckelia wallichiana* A. Dietr., Sp. Pl. (ed. 6) 1: 574. 1831.

–*Roettlera urticifolia* (Buch. - Ham. ex D. Don) Kuntze, Revis. Gen. Pl. 2: 477. 1891.

–*Didymocarpus urticifolius* (Buch. - Ham. ex D. Don) Wonisch, Sitzungsber. Kaiserl. Akad. Wiss., Math. - Naturwiss. Cl., Abt. 1 118: 454. 1909.

–*Gonatostemon boucheanum* Regel, Gartenflora 15: 353. 1866.

Caulescent perennial herb, decumbent, stem up to 50 cm tall, sparsely hairy. **Leaves** opposite: blade ovate, 3–13 by 1.5–8 cm, apex acuminate, base oblique or attenuate, margin serrate, lower and upper surface hairy, lateral veins 6–9 on each side; petiole 1–6 cm long, sparsely hairy to hairy. **Inflorescence** axillary, 1–2-flowered; peduncle 3–6 cm long, hairy; bracts paired, free, ovate or narrowly ovate, 4–15 mm long. Pedicel terete, 1–2.5 cm, glabrous or sparsely hairy. **Calyx** tubular, tube c. 1.5 cm long, hairy outside; lobes divided more or less than half way, narrowly triangular 1.2–2.5 by 0.3–0.3 cm long; caducous. **Corolla** infundibuliform, 4.8–5 cm long, pink with red streak or purple with yellow streak; lobes c. 1.2 by 1.5 cm. **Stamens** 2: filament inserted 2 cm from base of corolla tube with joint pod, c. 1.3 cm long; anthers c. 4.5 mm long, adaxial surface coherent; staminode 2, inserted 1.4 cm from base of corolla tube, 4.2–4.5 mm long. Gynoecium c. 4 cm long, stigma deeply bilobed. **Fruit** orthocarpic, 9.6–12.7 cm long. Seed not seen (Fig. 4.37).

Distribution. India, Nepal, Bhutan, China and Myanmar (Fig. 4.36).

Ecology. In moist shaded areas, shady banks by streams, or on wet mossy rocks, alt. 300–2,430 m.

Provisional IUCN conservation assessment. Least Concern LC. This species has an EOO about 507,613 km² and AOO about 92 km². The EOO is within the threshold of Least Concern and the AOO is within the threshold of Endangered. However, this plant is common and widespread. An assessment of Least Concern is more appropriate than Endangered.

Specimens examined. Nepal: along trail between Sundarijal and Pat-Bnjang leading

through cloud forest, heavily located with epiphytics, 2,450 m, 4 Sept. 1974, *Haas 2623* (**L** (L3805905)); Bagmati zone, between Bokajhunda and Dhunche, east side of Trisuli River, 1,900 m, 16 Sept. 1966, *Nicolson 2391* (**NMNS** (NMNS00064568)); Chaturaley, 1,524 m, 17 June 1914, *Lieut. Lal Dhoj 92* (**K**); in in Shivapuri, 1821, *Wallich 800* (**G** (G00133154), **NYBG** (NYBG00063247), **P** (P03884099, P03884100, P03884101)); Koshi Zone, Sankhuwasabha Distr, Simbung (1,740 m)-Deurali (2,030m)-Hatiya (1,540 m), 27°42'15" N, 87°21'30" E, 1,960 m, 16 Aug. 1998, Noshirio et al. 9830039 (**L** (L3794282)); N.E. Nepal. Tamur River between Chirwa and Hellok., 4°03'30" N, 115°00'90" E, 1,400 m, 4 Sept. 1989, *Keke; S. Crawfore, C. Grey-Wilson, D. Long, R. McBeath, H. Noltie, M. Sinnott, M. Subedi & S. Zmarzty 201* (**K**); Nanrding, 1600 m, 8 Sept. 1954, *Zimmermann 1110* (**K**); Sankhuwasabha, Bakang, above Sekidim, 2,800 m, 17 Aug. 1981, *Grey-Wilson et al. 4071* (**K**); Sheopuri, N of Katmandu, 2,210 m, 3 Sept. 1965, *Schilling 638* (**K**); *Delacour s.n.* (**P** (P03884129)); 1821, *Wallich s.n.* (**G** (G00133223)).

Bhutan: Kurn Chu Valley Kunley, 2,134 m, 27 Aug. 1915, Cooper et al. 4635 (**BM** (BM011025889)).

India: Himalaya, 1862, *Griffith 3822* (**G** (G00492420), **K**, **P** (P03884098), **U** (U1342372)); Sikkim (*Henry Haselfoot Haines 2739* (**K**), *Hooker s.n.* (**BM** (BM010760885)), 2,134 m, 15 Aug. 1849, *Hooker s.n.* (**K**), *Hooker s.n.* (**K**), *Hooker s.n.* (**K**), 2134 m, *Hooker s.n.* (**K**), *Hooker s.n.* (**P** (P03884102)), 300 m, *Hooker s.n.* (**P** (P03884103)), 2,134–2,438 m, *Hooker s.n.* (**TCD**), 2430 m, *Hooker s.n.* (**U** (U1342371)), 1 Oct. 1942, *Ludlow et al. 10111* (**BM** (BM011025891)), 27 19°48"N, 88 37'12"E, 1861, *Hooker s.n.* (**G** (G00492363))); Sikkim, Darjeeling (*Beddome 5812* (**BM** (BM010760886)), 13 Aug. 1869, *Clarke 8582A* (**BM** (BM011025890)), *Cowan s.n.* (**K**), 2,134 m, Oct. 1879, *Gamble 7217* (**K**), 2,134 m, Aug. 1874, *Gamble 3502A* (**K**)); Sikkim, Rangpo, 1,219 m, 1 Oct. 1884, *Clarke 36114* (**BM** (BM010760884)); 1829 m, 12 Aug., *Gamble s.n.* (**K**).

China: Yunnan, Menglun [1,500 m, *Henry 9161A* (**K**, **NMNS** (NMNS00064566)), S.E mt., 1,524 m 1901, *Henry 13521* (**K**), *ibidem*, *Henry 9161* (**E** (E00396451, E00527454, E00627464), **K**)).

Myanmar: Kachin State, Putao District. (Naungmung Township, buffer zone of Hkakaborazi NP, between Gatthu village and Hton Wan rest house., 27°28'36.2"N, 97°57'40.5' E, 587 m, 15 Oct. 2015, *Little et al. 1424* (**NYBG** (NYBG02649235)), between Hton Wan rest house and Khe Dam rest house, 27°30'51.6' N, 98°00'35.2' E, 1,135 m, 17 Oct. 2015, *Armstrong et al. 720* (**NYBG** (NYBG02648685))); between Hton Wan rest house and Khe Dam rest house, 27°30'51.6"N, 98°00'35.2", 1,135 m, 17 Sept. 2015, *Armstrong et al. 720* (**NYBG** (NYBG02648685))); Mahtum, 26°06' N 97°58' E, 1,372 m, 12 Aug. 1939, *Kaulback 333* (**BM** (BM0001191696)); North Myanmar, 914

m, 1 Dec. 1953, *Ward 21660* (**BM** (BM011025888)); Kachin State, Upper Myanmar, Nam Tamai Valley, 27°42' N 97°54' E, 1,219 m, 25 Nov. 1937, *Ward 13503* (**BM** (BM00001191697)).

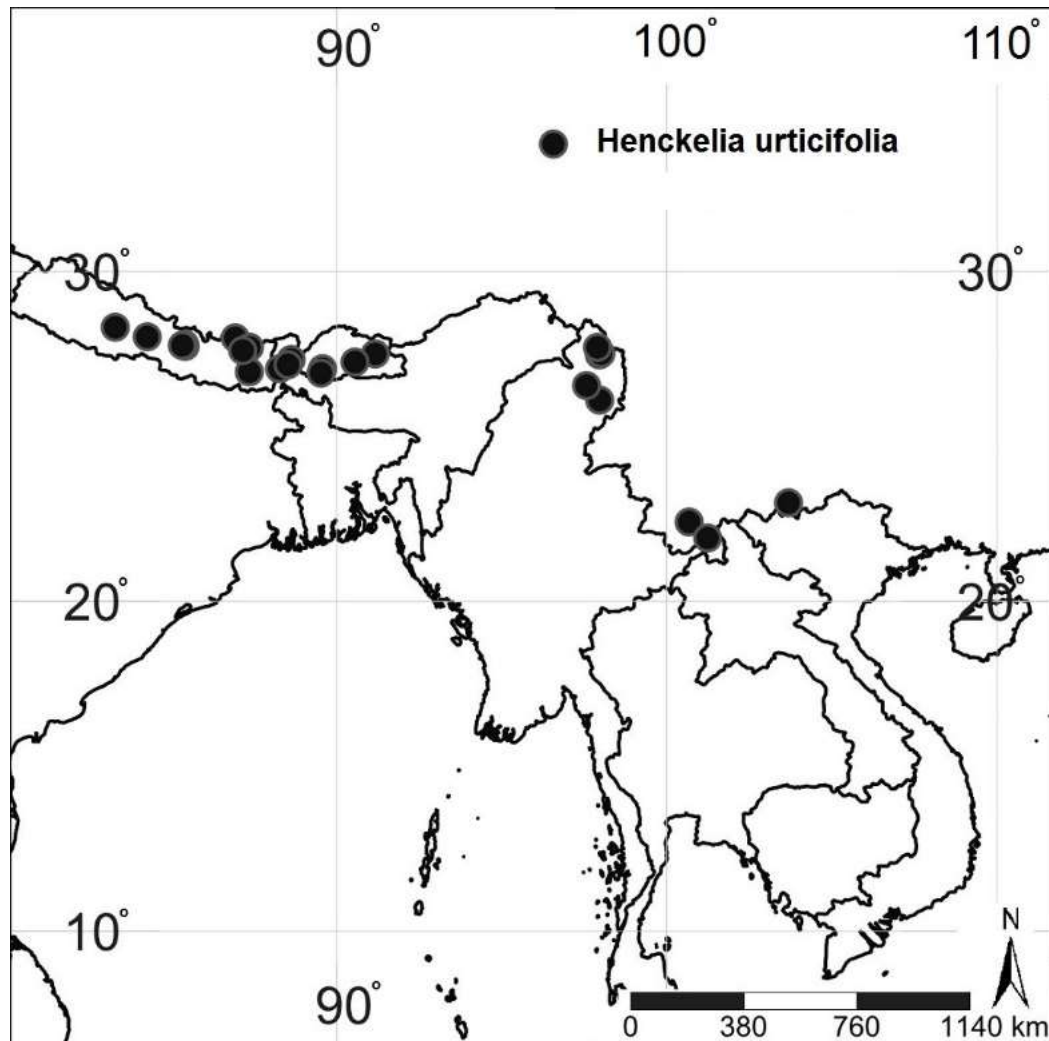


Figure 4.36 Distribution of *Henckelia urticifolia* (Buch. - Ham. ex D. Don) A. Dietr.
Base map from <https://www.simplemappr.net>

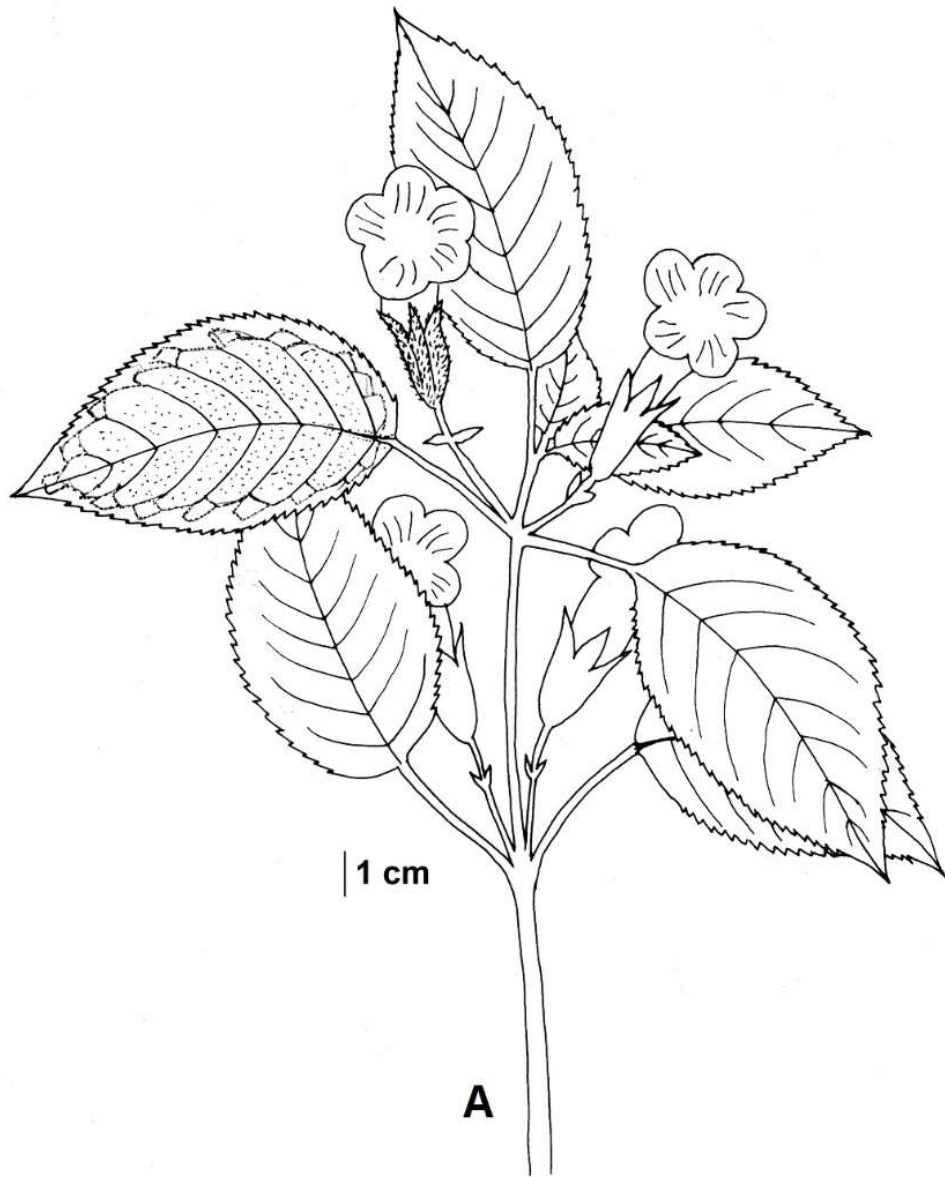


Figure 4.37 Line drawing of *Henckelia urticifolia* (Buch. - Ham. ex D. Don) A. Dietr.: A. Habit. Drawing: modified from Wall., Pl. Asiat. Rar. 1: 43.1830. P50.

Chapter 5- Pollen morphology

5.1 Introduction

Pollen morphology is part of palynological science and is the study of pollen grains and spores (Stuessy, 2014). Spores and pollen grains are similar but spores are the beginning of gametophytes, while pollen grains are mature microgametophytes (Judd et al. 1999). The early pollen studies have used light microscope (Stuessy, 2009). The utility of palynology in plant systematics depends in part on images produced Transmission Electron Microscope (TEM) and Scanning Electron Microscope (SEM) photographs (Stuessy, 2014). TEM provides information on pollen wall structure and SEM provides information on external structure (Moore et al., 1991).

The outer wall of pollen grains are so resistant to decay that they are preserved during fossilization (Stuessy, 1989). The pollen wall often contains a special compound, sporopollenin, which resists degradation by various chemicals, bacteria and fungi and contributes to the long persistence of pollen in sediments (Judd et al. 1999; Moore et al., 1991). Pollen grains are useful in palaeoecology, archaeology and the unravelling of angiosperm origin and phylogeny (Stuessy, 1989). Pollen grain data are useful at all levels of the taxonomic hierarchy (Stuessy, 1989). The most useful pollen characters are shape, sculpturing and aperture length (Fritze & Williams, 1988). The exine ornamentation patterns have been a help in the identification and delineation of taxa (Sivarajan, 1991).

Early studies of pollen grains in the Gesneriaceae were undertaken by Cranwell (1941) from New Zealand, on *Rhabdothamnus*. He described the pollen in this genus as simple, tricolpate, and the exine as thin and the sculpturing as granular. He indicated that this family is similar to some other families in pollen grain size and exine thickness e.g. Rutaceae (16 μm , exine very thin), Polygonaceae (26 μm , exine thick) and Sapotaceae (30 μm , exine thick) (Cranwell, 1941).

Later, Erdtman (1952) studied the pollen morphology of many Angiosperms. He investigated 20 species of the Gesneriaceae in *Streptocarpus*, *Columnea*, and *Coronanthera*. He described the pollen grain shape of *Streptocarpus grandis* N.E. Br as subprolate-prolate and suboblate-oblate spheroidal in *Streptocarpus rungwensis* Engl. He found that the minimum size (smallest grains) were in *Bellonia aspera* L. (14 by 11 μm) and the maximum pollen grains size were in *Columnea microphylla* Klotzsch & Hanst. ex Oerst. (51 by 34 μm). The sexine was found to be as thick as the nexine or thicker in *Boea hygrometrica* R.Br., *Columnea microphylla* Klotzsch & Hanst. ex Oerst., and *Coronanthera clarkeana* Schltr (Erdtman, 1952). In summary, the pollen

grains described were tricolporoidate to tricolpate, subprolate to prolate or suboblate to oblate in shape, with a polar diameter (P)/equatorial diameter (E) = 14–51/ 11–34 μm . Erdtman (1952) also mentions that pollen grains of this family are similar to these in the Bignoniaceae and Scrophulariaceae.

After Erdtman (1952), only individual genera or a few combined genera of the Gesneriaceae have been studied.

In the New World Gesneriaceae, Subfamily Gesneroideae, pollen morphology was studied by Fritze and Williams (1988). Five genera, i.e. *Columnea*, *Dalbergaria*, *Trichantha*, *Pentadenia*, and *Bucinellina* were intensively examined; all species belong to Tribe Columneinae. In *Columnea*, all species was found to have subprolate to spheroidal grains with a uniformly punctate exine. Meanwhile *Dalbergaria* have suboblate and reticulate sculpturing. *Trichantha*'s pollen grains are suboblate with reticulate exine. In *Pentadenia*, the pollen grains are suboblate to oblate and have reticulate sculpturing, whereas pollen grains in *Bucinellina* are oblate, with short oval apertures and reticulate or tectate-imperforate sculpturing. Fritze and Williams (1988) also mentioned that pollen grains of *Trichantha*, *Pentadenia*, and *Dalbergaria* can be used for identification only to sectional level. Later Kvist and Skog (1993), revised *Columnea* in Ecuador and merged four genera (*Dalbergaria*, *Trichantha*, *Pentadenia*, and *Bucinellina*) into *Columnea* based on morphology, chloroplast DNA, and the pollen study from Fritze and Williams (1988).

The pollen grains of New World Gesneriaceae were also studied by Fourny et al. (2010). Pollen grains of six Brazilian genera of two tribes in Subfamily Gesneroideae, were studied i.e. Tribe Beslerieae (*Besleria*) and Tribe Gesnerieae (*Codonanthe*, *Nematanthus*, *Paliavana*, *Sinningia* and *Vanhouttea*) (Fourny et al., 2010). The *Besleria* pollen grains were shown to be small (polar diameter 22.2–26.2 μm), prolate, tri-colporate and perforate. *Codonanthe* pollen grains were medium (polar diameter 27.5–32.5 μm), prolate-spheroidal, tri-colporate and with a micro reticulated surface. *Nematanthus* pollen grains were medium (polar diameter 26.2–43.7 μm), oblate-spheroidal or prolate spheroidal, tri-colpate and reticulate. The pollen grains of *Paliavana* were small to medium (polar diameter 25–30 μm), subtriangular, and heteroreticulate. *Sinningia* pollen grains were medium (polar diameter 26.2–37.5 μm), prolate, suboblate or subprolate, tri-colporate and reticulate. And *Vanhouttea* pollen grains were medium (polar diameter 27.5–37 μm), prolate spheroidal, tri-colporate and with cross-link sculpturing. Fourny et al. (2010) also provided a pollen identification key and found the opening and the sexine ornamentation useful for identification to taxa.

Kvist (1990) also described the pollen morphology of *Heppiella* and showed

that only small details distinguished the pollen of *Heppiella ulmifolia* Hanst. and *H. viscida* Fritsch as they showed only little variation between the species.

A study on European pollen and spores was published by Reille (1992). Two species of Gesneriaceae were studied. Both of them, *Ramonda nathaliae* Pinc & Petrov. and *Ramonda serbica* Pančić had tricolporate pollen.

Later, pollen grains of the Old World Gesneriaceae, Subfamily Cyrtandroideae, were studied by Luegmayer (1993a). One hundred and eight species in 18 genera were investigated by TEM and SEM i.e. *Aeschynanthus*, *Boea*, *Boeica*, *Chirita*, *Codonoboea*, *Cyrtandra*, *Didissandra*, *Didymocarpus*, *Epithema*, *Loxocarpus*, *Loxonia*, *Monophyllaea*, *Ornithoboea*, *Paraboea*, *Rhynchoglossum*, *Stauranthera*, *Streptocarpus*, and *Trisepalum*. In general, pollen grains were found to be tri-colpate or tri-colporate, spheroidal to oblate, with reticulate, perforate or regulate sculpturing and an equatorial diameter average range of 9–24 μm .

After that, Luegmayer (1993b) studied the pollen morphology of *Cyrtandra*. Forty-five Hawaiian *Cyrtandra*, four Malay Peninsular *Cyrtandra*, and 15 Bornean *Cyrtandra* were investigated. Luegmayer (1993b) found that their pollen grains were tricolporate and spheroidal with an average equatorial view ranging from 14–23 μm .

The pollen grains of South Pacific *Cyrtandra* was studied by Schlag-Edler and Kiehn (2001). Twenty-three species were investigated. Most of pollen grains were isopolar, spheroidal in equatorial view, with an equatorial diameter ranging from 9–16 μm , and reticulate. Schlag-Edler and Kiehn (2001) found the pollen grains of South Pacific *Cyrtandra* to be similar to the Hawaiian *Cyrtandra*.

Edwards (2003) studied the pollen morphology of *Streptocarpus* from South Africa. He described pollen of two species, *Streptocarpus kunhardtii* T.J. Edwards and *Streptocarpus hilburtianus* T.J. Edwards. Both species possessed tri-colpate grains but differed in size and shape. *Streptocarpus kunhardtii* pollen grains were prolate, scabrate and 17–20 by 9–10 μm , whilst *Streptocarpus hilburtianus* is spheroidal, scabrate and 10–12 by 10–12 μm .

Weber (2004) also investigated the pollen grains of Gesneriaceae and found them to be spheroidal, rarely prolate, suboblate or oblate and the sculpturing perforate or reticulate.

Pollen photographs form part of many new species publications in the Gesneriaceae, for example, Ramírez-Roa and Ibarra-Manríquez (1997) discovered a new species of *Solenophora tuxtliensis* Ram.-Roa and Ibarra-Manr. from Mexico. The pollen grain description was provided. The grains are tri-colpate, with a polar axis 26–34 μm , on equatorial axis 17–18 μm and micro-reticulate sculpturing.

Wei et al. (2013) also described pollen grains of *Anna rubidiflora* S.Z. He, F. Wen & Y.G. Wei as tri-colpate, prolate-spheroidal with a reticulate exine and diameter ranging from 14–15 µm.

In Thailand, Pattharahirantricin (2014) described the pollen morphology of *Rhynchoglossum* (*Rhynchoglossum mirabilis* Patthar. and *R. saccatum* Patthar.) as tri-colpate, with regulate sculpturing and a diameter between 18–19 µm.

According to the APG IV classification (Stevens, 2001 onwards), the Gesneriaceae comprise three subfamilies with 147 genera. But pollen morphology has only been investigated in 32 genera as follows:

1. Subfamily Sanangoideae - none
2. The New World Gesneriaceae, subfamily Gesneroideae
 - 2.1. Titanotracheae - none
 - 2.2. Napeantheae - none
 - 2.3. Beslerieae - *Besleria*
 - 2.4. Tribe Coronanthereae (*Coronanthera*, *Rhabdothamnus*)
 - 2.5. Tribe Gesnerieae (*Bellonia*, *Codonanthe*, *Columnea*, *Episia Heppiella*, *Nematanthus*, *Paliavana*, *Sinningia*, *Solonophora* and *Vanhouttea*)
3. The Old World Gesneriaceae, subfamily Didymocarpoideae
 - 3.1. Tribe Epithemateae (*Epithema*, *Monophylleae*, *Rhynchoglossum*, *Stauranthera*).
 - 3.2. Tribe Trichosporeae (*Aeschynanthus*, *Anna*, *Boea* (= *Dorcoceras*), *Boeica*, *Chirita*, (= *Microchirita*), *Codonoboaea*, *Cyrtandra*, *Didissandra*, *Didymocarpus*, *Loxocarpus*, *Ornithoboaea*, *Paraboaea*, *Ramonda*, *Rhynchoglossum*, *Streptocarpus*, *Trisepalum*)

The pollen grain data are shown in Appendix 6.3.

Henckelia belongs to Subfamily Didymocarpoideae, tribe Trichosporeae. The Trichosporeae comprise 44 genera. The pollen grains of this tribe had only 16 genera studied subject to the start of this investigation, and pollen grains of *Henckelia* had never been studied.

In some unrelated taxa it has shown that there is a relationship between pollen grain number, size and various floral characters and breeding system e.g. *Trichostema* (Spira, 1980), *Passiflora* (Garcia, Miguez and Gottsberger, 2014) and *Polygonum* (Crudon et al., 1985).

The aim of this study was to describe the pollen grains of *Henckelia*, to compare flower characters with pollen characters especially gynoecium and to accumulate data on the pollen grain morphology in the Gesneriaceae. A glossary is

provided as follows.

Glossary

Terminology follows Cranwell (1941) and Punt et al. (1994). Drawings that help to illustrate the use of terms in this list are given in Fig. 5.1.

Colpus or **Furrows** (pl. colpi, adj. colpate) – An elongated, aperture with a length/breadth ratio greater than 2

Equator – The dividing line between the distal and proximal faces of a pollen grain or spore

Equatorial axis – Often inappropriately used as a synonym of equatorial diameter

Equatorial diameter – A line, lying in the equatorial plane, perpendicular to the polar axis and passing through it.

Equatorial view – The view of a pollen grain or spore where the equatorial plane is directed towards the observer.

LO-analysis – A method for analysing patterns of sexine organization by means of light microscopy. Comment: This method is valuable for elucidating exine patterns. When focused at high level (H), raised sexine elements appear bright (Lux), whereas holes in the tectum are relatively dark (Obscuritas). At lower focus (L) holes become lighter and the sexine elements become darker. See also: LO-pattern, OL-pattern.

LO-pattern – A pattern of ornamentation that appears to show “bright islands” at high focus (H) and that become dark at low focus (L), observed when using LO-analysis. Comment: The reverse of OL-pattern.

Nexine – The inner, non-sculptured part of the exine which lies below the sexine. Antonym: sexine.

OL-pattern – A pattern of ornamentation that appears to show “dark islands” at high focus (H) and that become bright at low focus (L). Comment: The reverse of a LO-pattern.

Polar view – A view of a pollen grain or spore in which the polar axis is directed towards the observer

Prolate – Describing the shape of a pollen grain or spore in which the polar axis is larger than the equatorial diameter, $P/E=1.33-2$.

Sculpturing (adj. sculptured) – The surface relief, or topography, of a pollen grain or spore

Sexine – The outer, sculptured layer of the exine, which lies above the nexine.

Antonym: nexine.

Tricolpate, tricolporate, triporate (adj.) – Describing pollen grains with three ectocolpi, three compound apertures or three pores.

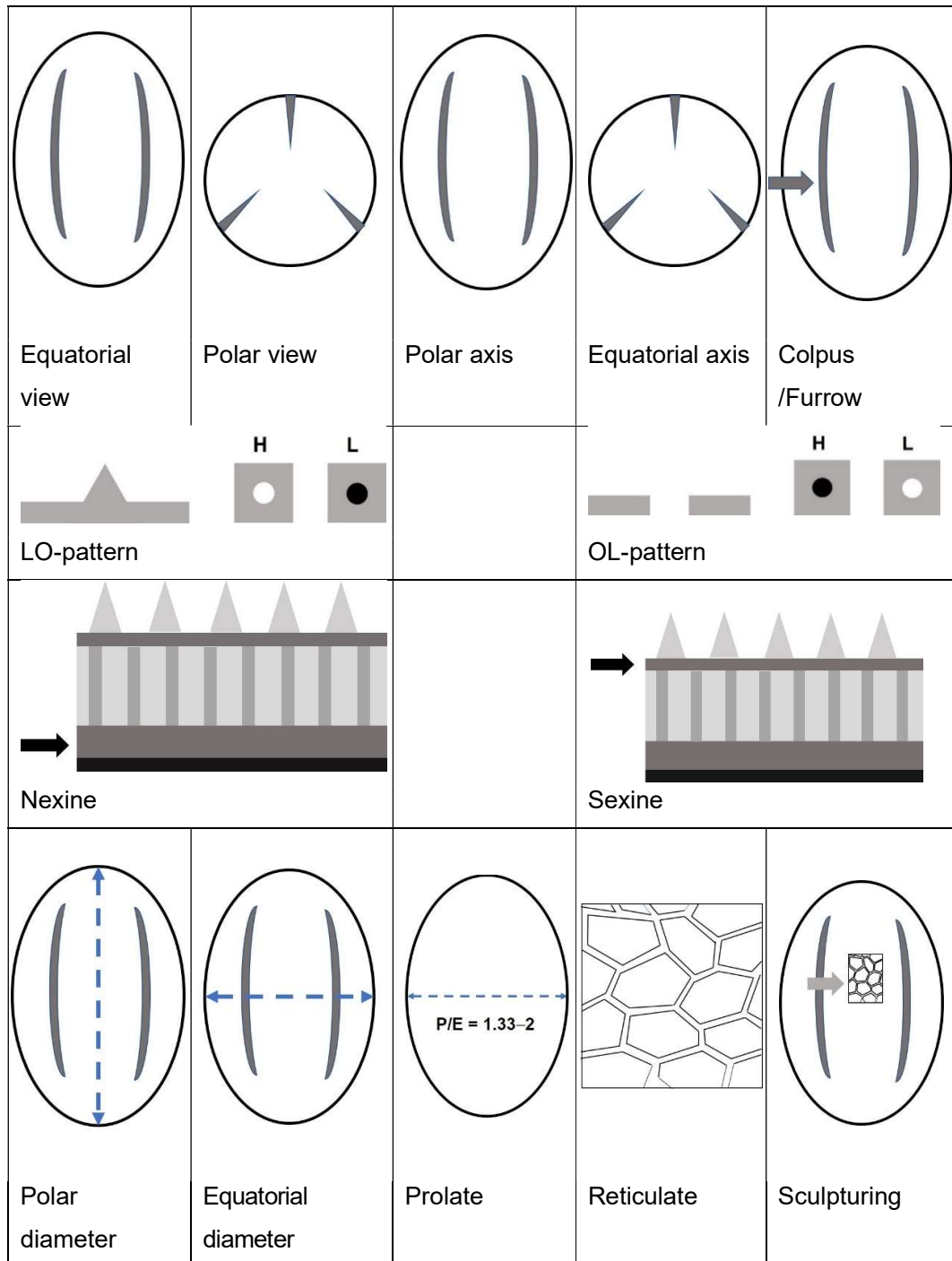


Figure 5.1 Drawings of glossary words of pollen. Modified from Punt et al., 2007.

5.2 Materials and methods

Preparation of pollen grains followed a modified method by Parnell (1991)

Step 1: Anthers were crushed in 50% acetone and filtered with the test sieves aperture 90 MIC to remove non pollen elements such as wall materials.

Step 2: The sample was centrifuged at 3,200 rpm for 3 minutes, the supernatant removed and then the pellet re-suspended in acetone in increasing concentrations from 60–100% being re-centrifuged and re-suspended of all concentration.

Step 3: Pollen pellets were dried overnight, applied to the slides and SEM stub.

Step 4: The slide samples were checked using a stereomicroscope at 100x magnification. The SEM stubs were coated in aluminum and were photographed with a SEM (KEW Herbarium).

All pollen samples were prepared for light microscope at 100x magnification. Ten pollen grains were measured per sample with the light microscope. The pollen voucher specimens showed as table 5.1.

Four species were preparing for SEM. Pollen grains for SEM were coated with platinum using a Quorum Q150T sputter coater, with a 90 seconds coating cycle and imaged with a Hitachi S-4700 (in the Kew Herbarium) field emission scanning electron microscope.

The pollen shape and size classes follow Walker and Doyle (1975) (Appendix 5.1).

A Pearson product-moment Correlation was calculated between some flower characters, i.e. corolla tube length, gynoecium length, filament length, and anther length with pollen size (polar diameter and equatorial diameter) (Table 5.2). The correlation was performed in the psych package version 1.8.4 (Revelle 2018) in R version 3.5.0 (R Core Team, 2018). As multiple tests were being undertaken the conservative Bonferroni correlation was applied (Legendre and Legendre 1998).

Table 5.1 Details of pollen material voucher specimens in this chapter.

Taxon	Vocher
<i>Henckelia adenocalyx</i>	Ward 7393 (K), Myanmar
<i>Henckelia anachoreta</i>	Sirimongkol et al. 721 (BKF), Thailand
<i>Henckelia bifolia</i>	Strachey & Winterbottom s.n. (TCD), India
<i>Henckelia communis</i>	Gardner s.n. (TCD), Sri Lanka
<i>Henckelia dielsii</i>	Yu 16288 (E), China
<i>Henckelia forrestii</i>	Forrest 11119, (E), China
<i>Henckelia fruticola</i>	Rushford 2141 (E), Vietnam
<i>Henckelia grandifolia</i>	Poore & Robbins 7622 (BKF), Thailand
<i>Henckelia humboldtiana</i>	Johnson s.n. (TCD), India
<i>Henckelia incana</i>	Bourne 5301 (K), India
<i>Henckelia lachenensis</i>	Forrest 29681 (E), China
<i>Henckelia mishmiensis</i>	Ward 7917 (K)
<i>Henckelia oblongifolia</i>	Griffith 3829 (K)
<i>Henckelia pumila</i>	Sirimongkol et al. 680 (BKF), Thailand
<i>Henckelia speciosa</i>	Lace 5758 (TCD), Myanmar
<i>Henckelia urticifolia</i>	Hooker s.n. (TCD), India
<i>Henckelia walkerae</i>	Thwaites 1789 (TCD), Sri Lanka

5.3 Results

Seventeen pollen samples were studied. Pollen grains were all radial symmetrical, tricolpate, with the longest axis occurring in *Henckelia grandifolia* (35 μm) and the shortest in *Henckelia incana* (13 μm), the polar diameter ranged between 13–36 μm and the equatorial diameter ranged between 10–23 (25) μm . All 17 species are described as follows with master key mixed with previous studied.

The pollen pictures in figure 6.2, 6.3, 6.5, 6.6, 6.7A, 6.8, 6.9, 6.10, 6.11 A, and 6.12A were analyzed from a light microscope and figure 6.4, 6.7 (B, C & D), 6.11 (B, C & D), and 6.12 (B, C & D) were analyzed from a SEM.

***Henckelia adenocalyx* (Chatterjee) D.J. Middleton & Mich. Möller**

The pollen grains are medium, euprolate; P by E = 26–30 by 15–20 μm , P/E = 27.6/16.7=1.65. The sculpturing is reticulate. Fig. 5.2.

***Henckelia anachoreta* (Hance) D.J. Middleton & Mich. Möller**

The pollen grains are small, subprolate; P by E = 20–26 by 14–18 μm , P/E = 24.1/16.2=1.49. The sculpturing is reticulate. Fig. 5.2.



Figure 5.2 Equatorial view: A-B. *Henckelia adenocalyx* (Chatterjee) D.J. Middleton & Mich. Möller. C-D. *H. anachoreta* (Hance) D.J. Middleton & Mich. Möller. A-B & D: none acetolysis. C: acetolysis. Scale bar=10 μm .

Photographs: Sukontip Sirimongkol.

***Henckelia bifolia* (D. Don) A. Dietr.**

The pollen grains are small to medium, euprolate; P by E = 20–25(27) by (12)14–18 μm , P/E = 23.3/15.1=1.54. The sculpturing is reticulate. Fig. 5.3.

***Henckelia communis* (Gardner) D.J. Middleton & Mich. Möller**

The pollen grains are small, euprolate; P by E = 19–22 by 11–13(15) μm , P/E = 20.6/12.2 = 1.69. The sculpturing is reticulate. Figs. 5.3 & 5.4.

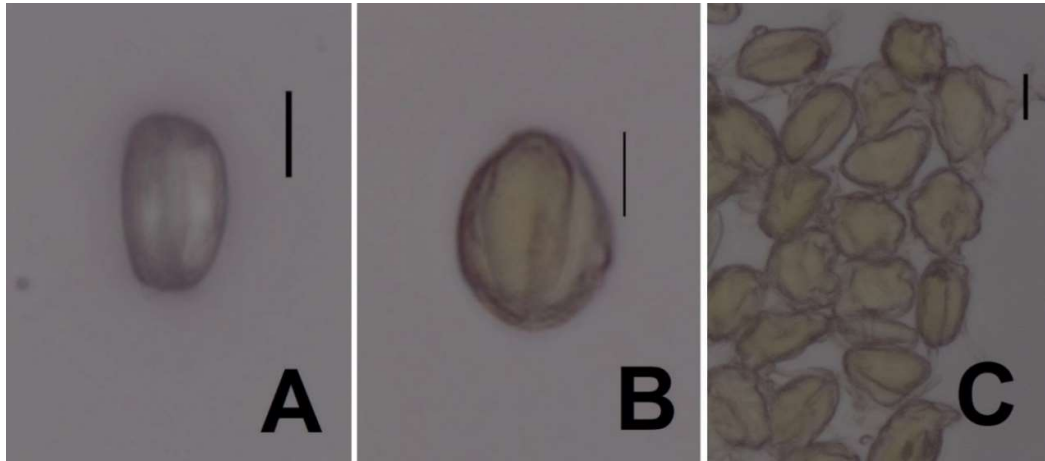


Figure 5.3 Equatorial view: A. *Henckelia bifolia* (D. Don) A. Dietr.; B-C. *H. communis*. A-C: no acetolysis. Scale bar=10 μm . Photographs: Sukontip Sirimongkol.

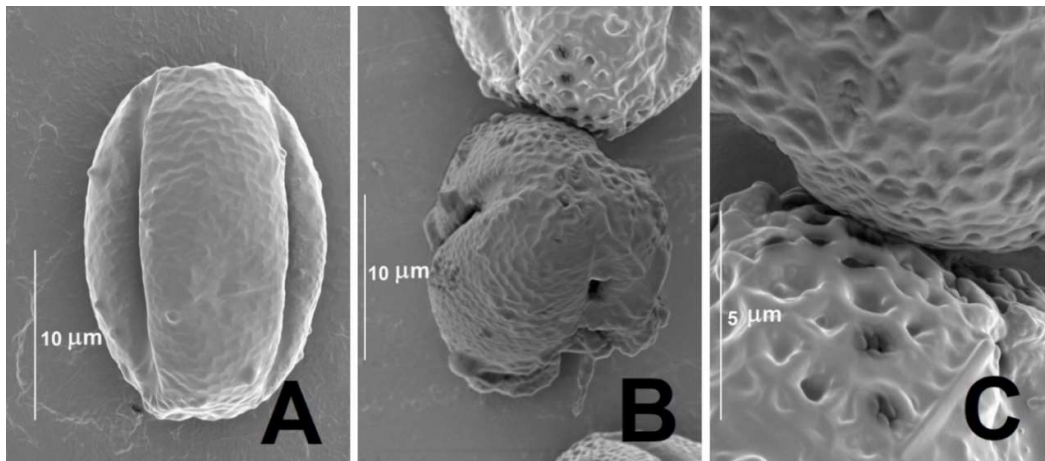


Figure 5.4 Pollen grains of *Henckelia communis* (Gardner) D.J. Middleton & Mich. Möller: A. Equatorial view; B. Polar view; C. Sculpturing. Photographs: Anne Dubéarnès.

***Henckelia dielsii* (Borza) D. J. Middleton & Mich. Möller**

The pollen grains are medium, subprolate; P by E = 24–30 by 20–24 μm , P/E = 27.4/22.4 = 1.22. The sculpturing is reticulate. Fig. 5.5.

***Henckelia forrestii* (J. Anthony) D.J. Middleton & Mich. Möller**

The pollen grains are medium, euprolate; P by E = 26–30 by 20–23 μm , P/E = 28.4/20.8 = 1.37. The sculpturing is reticulate. Fig. 5.5.

***Henckelia fruticola* (H.W. Li) D.J. Middleton & Mich. Möller**

The pollen grains are small to medium, subprolate; P by E = 23–27 by 20–21 μm , P/E = 24.7/20.3 = 1.23. The sculpturing is reticulate. Fig. 5.5.



Figure 5.5 Equatorial view: A. *Henckelia dielsii*; B. *H. forrestii*; C-D. *H. fruticola*. A-D.: none acetolysis. Scale bar=10 μm . Photographs: Sukontip Sirimongkol.

***Henckelia grandifolia* A. Dietr.**

The pollen grains are medium, euprolate; P by E = 30–35(36) by 20–23(25) μm , P/E = 33.5/21.1=1.59. The sculpturing is reticulate. Fig. 5.6.

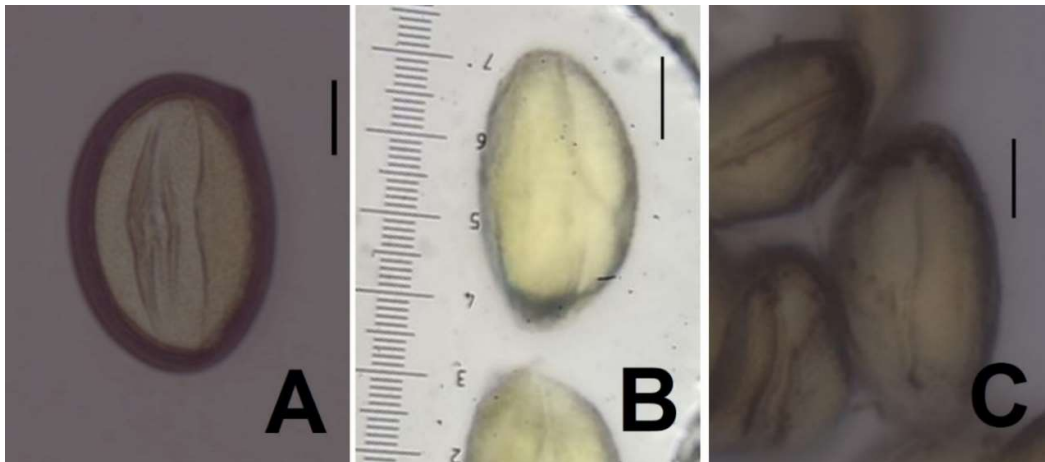


Figure 5.6 Equatorial view: A-C. *Henckelia grandifolia*. A.: acetolysis, B-C: none acetolysis. Scale bar=10 μm . Photographs: Sukontip Sirimongkol.

***Henckelia humboldtiana* (Gardner) A. Weber & B.L. Burt**

The pollen grains are small, euprolate; P by E = 19–22 by 10–13 μm , P/E = 20.7/11.3=1.83. The sculpturing is reticulate. Fig. 5.7.

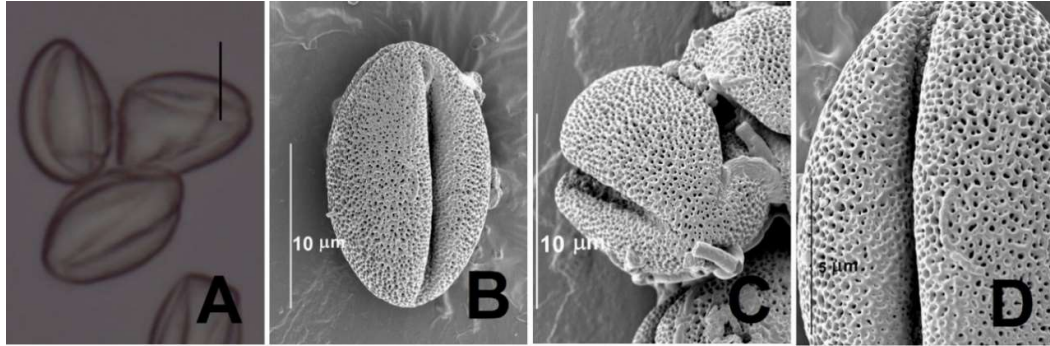


Figure 5.7 Pollen grains of *Henckelia incana*: A-B. Equatorial view; B. Polar view; C. Sculpturing. A: non acetolysis, B-D: acetolysis. Photographs: A: Sukontip Sirimongkol, B-D: Anne Dubéarnès.

***Henckelia incana* (Vahl) Spreng.**

The pollen grains are small, subprolate; P by E = 13–15 by 10–13 μm , P/E=14.2/10.7 = 1.33. The sculpturing is reticulate. Fig. 5.6.

***Henckelia lachenensis* (C.B. Clarke) D.J. Middleton & Mich. Möller**

The pollen grains are medium, euprolate; P by E = 26–30 by 13–18 μm , P/E= 27.6/15.3 = 1.8. The sculpturing is reticulate. Fig. 5.8.

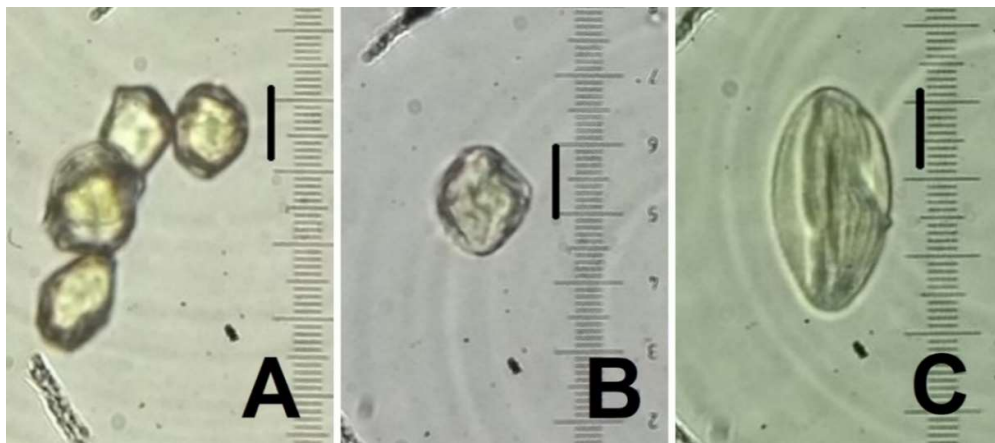


Figure 5.8 Equatorial view: A-B. *Henckelia incana*; C. *H. lachenensis*. A - C: none acetolysis. Scale bar=10 μm . Photographs: Sukontip Sirimongkol.

***Henckelia mishmiensis* (Debb. ex Biswas) D.J. Middleton & Mich. Möller**

The pollen grains are medium, euprolate; P by E = 25–27 by 16–20 μm ,
P/E = 26.2/18.3 = 1.43. The sculpturing is reticulate. Fig. 5.9.

***Henckelia oblongifolia* (Roxb.) D.J. Middleton & Mich. Möller**

The pollen grains are small, subprolate; P by E = 15–18 by 13–15 μm ,
P/E = 16.6/14.2 = 1.17. The sculpturing is reticulate. Fig. 5.9.



Figure 5.9 Equatorial view: A-B. *Henckelia mishmiensis*; C-D. *H. oblongifolia*. A-D: none acetolysis. Scale bar = 10 μm . Photographs: Sukontip Sirimongkol.

***Henckelia pumila* (D. Don) A. Dietr.**

The pollen grains are small, prolate spheroidal; P by E = 15–20 by (10)15–20 μm , P/E = 17.1/16 = 1.07. The sculpturing is reticulate. Fig. 5.10.

***Henckelia speciosa* (Kurz) D.J. Middleton & Mich. Möller.**

The pollen grains are medium, euprolate; P by E = 29–35 by 16–19 μm ,
P/E = 30.8/17.3 = 1.78. The sculpturing is reticulate. Fig. 5.10.



Figure 5.10 Equatorial view: A-B. *Henckelia pumila*; C-D. *H. speciosa*. A-B & D.: none acetolysis, C.: acetolysis. Scale bar = 10 μm . Photographs: Sukontip Sirimongkol.

***Henckelia urticifolia* (Buch. - Ham. ex D. Don) A. Dietr.**

The pollen grains are medium, euprolate; P by E = 30–34 by 19–21 μm ,
P/E = 31.7/19.9=1.59. The sculpturing is reticulate. Fig. 5.11.

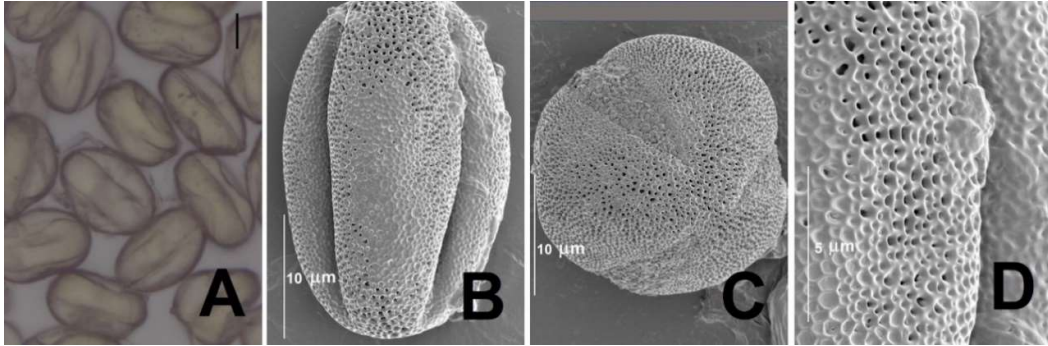


Figure 5.11 *H. urticifolia*. A-B. Equatorial view; C. Polar view; D. Sculpturing.

A: none acetolysis, B-D.: acetolysis. Scale bar=10 μm . Photographs: A: Sukontip Sirimongkol, B-D: Anne Dubéarnès.

***Henckelia walkerae* (Gardner) D.J. Middleton & Mich. Möller**

The pollen grains are small to medium, subprolate; P by E = 23–25 by 13–18 μm , P/E = 24.2/15.4=1.57. The sculpturing is reticulate. Figure 5.12.

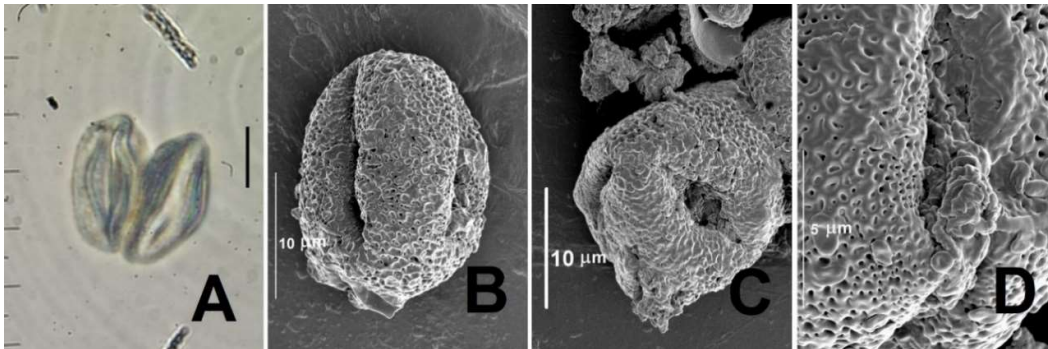


Figure 5.12 *H. walkerae*. A-B. Equatorial view; C. Polar view; D. Sculpturing.

A: none acetolysis, B-D.: acetolysis. Scale bar=10 μm . Photographs: A: Sukontip Sirimongkol, B-D: Anne Dubéarnès.

Table 5.2 Flower characters and pollen size for the Pearson correlation.

Taxon	Corolla tube Length (cm)	Gynoecium Length (cm)	Filament Length (cm)	Anther Length (cm)	Equatorial view Length (μm)	Polar view Length (μm)
<i>Henckelia adenocalyx</i>	4	3	1.3	0.4	27.6	16.7
<i>H. anachoreta</i>	4.5	2.5	0.8	0.32	24.1	16.2
<i>H. bifolia</i>	5	2.4	1.5	0.3	23.3	15.1
<i>H. communis</i>	4.5	1.8	1	0.1	20.6	12.2
<i>H. dielsii</i>	4.3	3.5	1.1	0.3	27.4	22.4
<i>H. forrestii</i>	2.5	1.9	1	0.2	28.4	20.8
<i>H. fruticola</i>	5.5	3.6	1	0.3	24.7	20.3
<i>H. grandifolia</i>	6	3	1.5	0.37	33.5	21.1
<i>H. humboldtiana</i>	1	0.45	0.15	0.15	20.7	11.3
<i>H. incana</i>	1	0.7	0.4	0.25	14.2	10.7
<i>H. lachenensis</i>	2.5	2	0.55	0.2	27.6	15.3
<i>H. mishmiensis</i>	5	3.6	1.5	0.4	26.2	18.3
<i>H. oblongifolia</i>	3.5	2.8	1	0.2	16.6	14.2
<i>H. pumila</i>	4.5	2.5	0.8	0.25	17.1	16
<i>H. speciosa</i>	6	3	1	0.3	30.8	17.3
<i>H. urticifolia</i>	6	3	1.4	0.35	31.7	19.9
<i>H. walkerae</i>	6	2.8	1.4	0.2	24.2	15.4

From the new data collected here I have made a master key to species based on pollen grain morphology as follows.

Master Key

1. Pollen grain shape euprolate
 2. Size small (19–22 μm) ***Henckelia communis, H. humboldtiana***
 2. Size medium (26–36 μm) ***Henckelia adenocalyx, H. forrestii, H. grandifolia, H. lachenensis, H. mishmiensis, H. speciosa, H. urticifolia***
 2. Size small to medium (20–27 μm) ***Henckelia anachoreta, H. bifolia, H. walkerae***
1. Pollen grain shape not as above
 3. Shape oblate- spheroidal *Codonanthe carnososa, Nematanthus crassifolius, N. fissus*
 3. Shape prolate *Streptocarpus kunhardtii, Besleria longimucronata, B. macahensis, B. melancholica, B. umbrosa, Sinningia brasiliensis*
 3. Shape prolate-spheroidal
 4. Size small (15–20 μm) ***Henckelia pumila***
 4. Size medium (28–41 μm) *Anna rubidiflora, Codonanthe devosiana, Nematanthus brasiliensis, N. fluminensis, Vanhouttea calcarata, V. lanata*
 3. Shape spheroidal *Aeschynanthus boschianus, A. parvifolius, Chirita caliginosa, Cyrtandra cordifolia, C. grandifolia, C. kaulantha, C. paludosa, C. pendula, C. pendula, C. platyphylla, C. procera, C. splendens, Didissandra morgani, Didymocarpus aff. floribundus, Ornithoboea arachnoidea, O. flexuosa, Rhychoglossum mirabilis, R. sacchatum, Stauranthera grandiflora, Streptocarpus gardenia, S. hiltburianus, S. orientalis, S. silvaticus, Rhabdothamnus.*
 3. Shape suboblate-oblate *Epithema membranaceum, Monophyllea horsfieldii, Streptocarpus grandis, S. rungwensis*

3. Shape subolate *Codonanthe gracilis*, *Sinningia douglasii*,
S. gigantifolia
3. Shape subprolate
5. Size small (13–18 μm) ***Henckelia incana*, *H. oblongifolia***
5. Size medium (24–37.2 μm) ***Henckelia dielsii*, *Nematanthus hirtellus*,**
Paliavana prasinata, *Sinningia bulbosa*,
S. guttata, *S. lateritia*
5. Size small to medium (23–27 μm) ***Henckelia fruticola***
3. Shape subprolate-prolate *Boea hygrometrica*, *Bellonia aspera*, *Coranthera*
clarkeana, *Columnea microphylla*

The Pearson product-moment correlation showed that there were positively significant correlation between corolla tube length with gynoecium length ($r = 0.81$), corolla tube length with filament length ($r = 0.8$), gynoecium length with filament length ($r = 0.76$), gynoecium length with polar view ($r = 0.75$) and equatorial view with polar view ($r = 0.75$) (Table 5.3).

When only nine species from Thailand, Indo-China including Myanmar were analysed (*Henckelia adenocalyx*, *H. anachoreta*, *H. fruticola*, *H. grandifolia*, *H. lachenensis*, *H. oblongifolia*, *H. pumila*, *H. speciosa* and *H. urticifolia*), the Pearson product-moment correlation showed that there were positively significant correlations between corolla tube length with gynoecium length ($r=0.70$), corolla tube length with polar view length ($r=0.81$), gynoecium length with polar view length ($r=0.67$) and equatorial view length and polar view length ($r=0.67$) (Table 5.4).

Table 5.3 Pearson product-moment correlation between flower characters and pollen characters. Pairs that are significantly different with the Bonferroni correction are in bold.

	Corolla tube length	Gynoecium length	Filament length	Anther length	Equatorial view length	Polar view length
Corolla tube length	1					
Gynoecium length	0.81	1				
Filament length	0.8	0.76	1			
Anther length	0.48	0.64	0.57	1		
Equatorial view length	0.53	0.52	0.53	0.52	1	
Polar view length	0.54	0.75	0.57	0.57	0.75	1

With 17 species from this study.

Table 5.4 Pearson product-moment correlation between flower characters and pollen characters from Thailand and surrounding countries. Pairs that are significantly different with the Bonferroni correction are in bold.

	Corolla tube length	Gynoecium length	Filament length	Anther length	Equatorial view length	Polar view length
Corolla tube length	1					
Gynoecium length	0.70	1				
Filament length	0.65	0.64	1			
Anther length	0.61	0.52	0.77	1		
Equatorial view length	0.52	0.22	0.53	0.63	1	
Polar view length	0.81	0.67	0.68	0.65	0.67	1

With 9 species from Thailand, Indo-China including Myanmar: *Henckelia adenocalyx*, *H. anachoreta*, *H. fruticola*, *H. grandifolia*, *H. lachenensis*, *H. oblongifolia*, *H. pumila*, *H. speciosa* and *H. urticifolia*.

5.4 Discussion

The genus *Henckelia* contains 64 species but pollen morphology was investigated in only 17 species. Nine of the twenty-four species of *Henckelia* from Thailand, Indo-China, including Myanmar i.e. *Henckelia adenocalyx*, *H. anachoreta*, *H. fruticola*, *H. grandifolia*, *H. lachenensis*, *H. oblongifolia*, *H. pumila*, *H. speciosa*, and *H. urticifolia* were studied. In figure 6.4, the pollen pictures were poorly acetolyzed even the sample preparation same as figure 6.7B, C & D. We assumed that the pollen coating was thicker than the other.

In this study, pollen grains were collected from herbarium specimens (E, K, TCD) and only two species were analysed using the fresh collections.

In general, the *Henckelia* pollen grains examined were tricolpate and the pollen shape ranged from subprolate to euprolate and the size ranged from small to medium (13–36 μm). The exine sculpturing was always reticulate.

The gynoecium length with pollen polar view length showed a positive significant correlation. When the gynoecium is long then the polar view is long too. The results confirm that the number of samples does not affect the positive correlation. Therefore, there appears to be a relationship between certain floral characters and pollen grain size. More pollen grain samples are required to confirm any relationship.

Future studies should also look at ovule number and other characters and may suggest that geography, topography, breeding system or pollinator may be relevant to any such relationship. For example, in *Trichostema* (Lamiaceae) it has been shown that outcrossing species have significantly larger flowers, larger nectar volumes and higher pollen to ovule ratios than do selfing species (Spira, 1980). And, according to Garcia et al. (2014) in *Passiflora* (Passifloraceae) species the pollen to ovule ratio of self-compatible species was lower than that for the self-incompatible ones. Therefore, although such relationships did not hold universally (Garcia et al., 2014) it would be of interest to investigate further if such a relationship holds in *Henckelia* as a high degree of self-compatibility might explain the overall pattern of morphological variation seen in the field.

Although only 17 *Henckelia* were investigated in this study, we can still use these characters to identify specimens to generic level (see master key). The most distinctive character is euprolate shape; only *Henckelia pumila* is prolate-spheroidal and *Henckelia dielsii*, *H. incana*, *H. fruticola* and *H. oblongifolia* are subprolate. In general pollen grains of *Henckelia* are tri-colpate with reticulate sculpturing. Further studies are required especially TEM and SEM photographs.

Chapter 6-General Discussion

Generic delimitation of *Henckelia*

The previous history of the taxonomy of *Henckelia* forms a very complex story as it intertwines with that of *Didymocarpus*. *Henckelia* was published by Sprengel (1817) and distinguished on the basis of having two fertile and two sterile stamens, and an infundibuliform corolla. After that *Didymocarpus* was described in a letter that Wallich sent to Francis Hamilton who passed it on for publication (Hamilton, 1819) as possessing an infundibuliform corolla and two fertile stamens. Nowadays, *Henckelia* is considered to differ from *Didymocarpus* by having an infundibuliform or campanulate corolla and peltate or subpeltate and bilobed stigma while *Didymocarpus* has a salverform, infundibuliform or personate corolla and an entirely capitate stigma (Nangngam and Maxwell, 2013). Furthermore, *Didymocarpus* always possesses a persistent calyx but *Henckelia* only sometimes does so.

Because they have such similar characteristics Jack (1825) transferred *Henckelia* to *Didymocarpus* whilst Sprengel (1827) transferred *Didymocarpus* to *Henckelia*. Wallich (1829) also listed *Didymocarpus* without referring to *Henckelia* but transferred the type species of *Henckelia* (*Rottlera incana* Vahl.) to *Didymocarpus rottlerianus* Wall. Until *Didymocarpus* was remodelled by Weber and Burt (1997) and *Chirita* was remodeled by Weber et al. (2011), both *Didymocarpus* and *Henckelia* were different.

In this study, I used both morphological and molecular data to clarify the status of *Henckelia* based on Weber and Burt (1997), Weber et al. (2011) and Middleton et al. (2013). My molecular and morphological data analysis supported the classification at species level and molecular data supported the separation of *Henckelia* from *Didymocarpus* and other genera. So, in this thesis, the concept of *Henckelia sensu* Weber et al. (2011) and Middleton et al. (2013) is supported using both morphological and molecular data. Thus, taxonomic stability in the generic delimitation of *Henckelia* looks like it has been achieved.

Henckelia was strongly separated from *Didymocarpus* and other genera on the basis of my molecular analysis. The molecular approach (see chapter 3) was used as an alternative tool to investigate the relationships between *Henckelia* members. Phylogenetic trees showed that *Henckelia incana* and *H. floccosa* (Section *Henckelia sensu* Weber and Burt, 1979) is always monophyletic (Fig. 3.5, 3.6, 3.7, 3.8, 3.9) but taxa belonging to *Henckelia* Section *Chirita* (Wood, 1974) are not monophyletic. The phylogenetic trees constructed supported the classification at species level (Fig. 3.5-3.12).

The section *Chirita* taxa included in this thesis are only a small subset of its species (*Henckelia amplexifolia*, *H. anachoreta*, *H. grandifolia*, *H. nakianensis*, *H. pumila*, *H. rotundata*, *H. speciosa*) but they did not form a monophyletic group.

Phylogenetic analysis using nrITS and plastid DNA found that *Henckelia* was sister to a clade of other genera including *Allocheilos*, *Gyrocheilos*, *Didymocarpus*, *Liebigia* and *Cathayanthe*. This finding is congruent with Middleton and Moller (2012). *Codonoboea* is then sister to this *Henckelia/Allocheilos*, *Gyrocheilos*, *Didymocarpus*, *Liebigia* and *Cathayanthe* group. This relationship to *Codonoboea* is a new finding from this thesis.

For future study more molecular data is needed to investigate the robustness and monophyly of section *Chirita* sensu Wood (1974).

Infrageneric relationships and classification of *Henckelia*

The relationships of *Henckelia* species are well resolved and supported in the phylogenetic analyses and six groups can be defined (A to F; Figure 3.13). Within the core *Henckelia*, two large groups can be defined namely A and B. The largest, Clade B includes *H. dielsii*, *H. amplexifolia*, *H. nakianensis*, *H. grandifolia*, and *H. speciosa* (1.00 PP) with *H. anachoreta* as its sister taxon (1.00 PP). Clade A includes *H. bifolia* and *H. rotundata*. The A and B group are sister to *H. pumila* (C). A group of *Henckelia longisepala/H. urticifolia* (clade F) are the most outlying taxa of *Henckelia*, followed by *H. walkerae* (E) and *H. incana* and *H. flocossa* (D; Section *Henckelia*) that diverge successively from the next most basal nodes. There is therefore support for *Henckelia* Section *Henckelia* (Weber and Burt, 1997) but no support for Section *Chirita* (Wood, 1974) as Section *Henckelia* is nested within it.

Clade A, B, and C of *Henckelia* includes species from Thailand and surrounding countries and Clade F from an inner subgroup of India, Nepal, Bangladesh, Bhutan, Myanmar, Thailand, China, Vietnam and Laos. Group F is the most outlying clade in *Henckelia*. These are geographically separated from Clade D. The trees separated the *Henckelia* species well and it is clear that the DNA regions will be valuable for future DNA barcoding work on the group especially nrITS and *trnL-F* which are particularly well represented for *Henckelia* species. Future phylogenetic work needs to focus on increasing the species sampling within *Henckelia* and to test if the groups (A-F) defined here remain robust. Once that is complete, a new infrageneric classification can be proposed. The phylogenetic work undertaken here will be submitted to the *Nordic Journal of Botany* or the *Journal of Plant Research*.

Morphometric / morphological analysis of *Henckelia*

In terms of morphology, *Henckelia* was confirmed to differ from *Didymocarpus* in having an infundibuliform or campanulate corolla, peltate or subpetate and bilobed

stigma and orthocarpic fruit while *Didymocarpus* has a salverform, infundibuliform or personate corolla, an entirely capitate stigma and plagiocarpic fruit (Nangngam and Maxwell, 2013). In *Didymocarpus*, the calyx is always persistent but in *Henckelia*, the persistent calyx only occurs in some species i.e. *Henckelia dasycalyx*, *H. floccosa*, *H. incana*, *H. lacei*. At specific level, *Henckelia* was clearly divided using non-metric multidimensional scaling (NMDS) (Figure 2.13) and separate morphological characters (Figure 4.2). The calyx, corolla tube, stigma and fruit of *Henckelia* are the most important characters for identification. For future study, more morphological data from more *Henckelia* species from an expanded area are required. *Henckelia* samples are difficult to collect and so more collaboration with local collectors is required. Equally, it would be good to expand the range of characters studied, for example, leaf anatomy. In the genus *Cyrtandra*, leaf sclereid patterns found in different species were used for taxonomic characters (Bokhari & Burt, 1970).

The morphological investigations in this thesis were extended to a numerical taxonomy study. Morphological data were analysed using NMDS with Jaccard as the distance measure. The morphometric analysis showed the similarity of the species and divided them into two groups. Group one consists of *Henckelia amplexifolia*, *H. campanuliflora*, *H. candida*, and *H. nakianensis* based on the single leaf, campanulate flower, reniform anthers and peltate or subpeltate stigma. Group two consists of *Henckelia adenocalyx*, *H. anachoreta*, *H. burtii*, *H. calva*, *H. ceratoscyphus*, *H. dasycalyx*, *H. fruticola*, *H. grandifolia*, *H. heterostigma*, *H. insignis*, *H. lacei*, *H. lachenensis*, *H. longipedicellata*, *H. longisepala*, *H. nakianensis*, *H. oblongifolia*, *H. peduncularis*, *H. pumila*, *H. rotundata*, *H. speciosa*, and *H. urticifolia* based on the presence of several leaves, infundibuliform flower, elliptic anthers and bilobed stigma. *Henckelia amplexifolia* is unique in having a stolon and bulbils and producing less fruit. The relationships of these groups to the ones found using molecular techniques requires further investigation as whilst there is some similarity there are also a number of differences. Further study of these features is required.

Taxonomic revision of *Henckelia* for the Flora of Thailand and surrounding countries

This thesis focused on a taxonomic revision of *Henckelia* in Thailand and surrounding countries (Myanmar, Laos, Cambodia and Vietnam). Twenty-four species are recognized. Identification keys and full descriptions are provided (including illustrations). Five new species were discovered i.e. *Henckelia amplexifolia* Sirim., *H. campanuliflora* Sirim., *H. candida* Sirim., *H. dasycalyx* Sirim. & D.J. Middleton, and *H. nakianensis* Sirim.,

J. Parn. & Hodk. The new species have been published and already cited three times at the time of writing (Google Scholar):

Sirimongkol, S., Parnell, J., Hodkinson, T., Middleton, D., & Puglisi, C. (2019). Five new species of *Henckelia* (Gesneriaceae) from Myanmar and Thailand. *Thai Forest Bulletin (Botany)*, 47(1), 38-54. <https://doi.org/10.20531/tfb.2019.47.1.08>.

Since starting the revision of *Henckelia* from Thailand I collected many new herbarium specimens (54 number in total) from a wide geographical range including for example, *Henckelia rotundata* specimens: a species that had last been collected nearly hundred years ago in 1922. The thesis has therefore provided work for publication in the *Flora of Thailand* and deposited specimens in BKF, TCD, K, E and P herbaria for future work. This taxonomic work is essential for global and regional assessments of biodiversity and for conservation work. I have shown that the conservation status of *Henckelia* varies:

–Endangered (EN) taxa are: *Henckelia adenocalyx*, *H. amplexifolia*, *H. burttii*, *H. calva*, *H. campanuliflora*, *H. candida*, *H. ceratoscyphus*, *H. fruticola*, *H. heterostigma*, *H. lachenensis*, *H. longisepala*, *H. rotundata*,

–Least Concern (LC) taxa are: *H. anachoreta*, *H. grandifolia*, *H. oblongifolia*, *H. pumila*, *H. speciosa*, *H. urticifolia* and

–Data Deficient (DD) taxa are: *Henckelia dasycalyx*, *H. insignis*, *H. lacei*, *H. longipedicellata*, *H. nakianensis*, *H. peduncularis*. Many are threatened and needing conservation. The treatment has also provided highly valuable biogeographic data on species which can be used in conservation and many aspects of biodiversity research. For example, the taxa that are Data Deficient (DD) may only be known from a single specimen. Future conservation studies may change the status. From herbaria data collection a map of *Henckelia* sections can be drawn (Figure 7.1) and Sect. *Henckelia* is clearly separated.

According to the phytogeography, *Henckelia* distributed in Indo-Burmese, Indo-Chinese and Western Ghats and Sri Lanka element. All elements are the biodiversity hot spot.

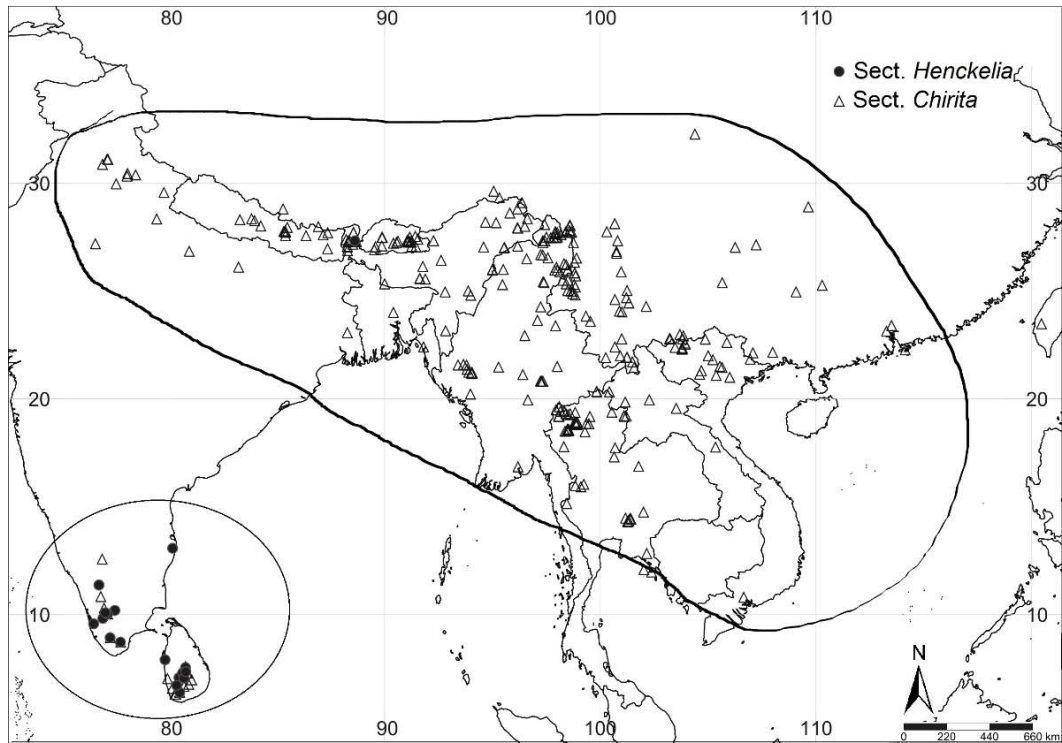


Figure 6.1 Map showing the distribution area of each *Henckelia* section. Base map from <https://www.simplemappr.net>

Pollen morphology

This study provides the first description of pollen morphology in *Henckelia*. Seventeen pollen samples were investigated. Pollen of two species, *Henckelia anachoreta* and *H. pumila*, were collected from the field and fifteen species, *H. adenocalyx*, *H. bifolia*, *H. communis*, *H. dielsii*, *H. forrestii*, *H. fruticosa*, *H. grandifolia*, *H. humboldtiana*, *H. incana*, *H. lachenensis*, *H. mishmiensis*, *H. oblongifolia*, *H. speciosa*, *H. urticifolia*, and *H. walkerae* were collected from herbarium samples. Pollen grain data and a master key are provided. The pollen grains of *Henckelia pumila* are similar in appearance to *Anna rubidiflora*, *Codonanthe devosiana*, *Nematanthus brasiliensis*, *N. fluminensis*, *Vanhouttea calcarata*, *V. lanata* in having a prolate-spheroidal pollen grain shape. Also, *Henckelia incana*, *H. oblongifolia*, *Henckelia dielsii*, *Henckelia fruticosa* are similar to *Nematanthus hirtellus*, *Paliavana prasinata*, *Sinningia bulbosa*, *S. guttata*, *S. lateritia* in having subprolate pollen grain shape. *Henckelia communis*, *H. humboldtiana*, *Henckelia adenocalyx*, *H. forrestii*, *H. grandifolia*, *H. lachenensis*, *H. mishmiensis*, *H. speciosa*, *H. urticifolia*, *Henckelia anachoreta*, *H. bifolia*, *H. walkerae* are distinguished by having euprolate pollen grains

shape. The morphology of *Henckelia* pollen grains were found to be generally tricolpate, with the shape ranging from subprolate to euprolate. The pollen grains are small to medium size and the exine sculpturing is reticulate. It will be interesting to see if, following the further data accumulation, these groupings of *Henckelia* are reflected in molecular or morphological analysis.

Future studies of pollen morphology require more pollen grains and possibly fresh material. Transmission Electron Microscope (TEM) and Scanning Electron Microscope (SEM) and light microscopy techniques could then be used to compare all the species. Further study of the interrelationships of pollen to ovule ratios and of their relationships to floral morphology may yield insights into the breeding biology of *Henckelia* and therefore into its evolution and morphological diversity.

Conclusions

This thesis has provided the required revision of *Henckelia* for the Flora of Thailand project (Middleton, 2003) and therefore the information required for the conservation and utilization of its taxa in Thailand. The work has provided phylogenetic evidence, from multiple gene regions, to support the current generic delimitation of *Henckelia* and has found infrageneric groupings (A-F) that can be investigated further to assess whether they each merit sectional or subgeneric status. The morphological data, including pollen morphology, has provided essential taxonomic data for the group. It is recommended that taxonomic study of *Henckelia* is expanded into the center of *Henckelia*'s distribution in India and China.

It is clear that *Henckelia* from Thailand is dramatically understudied as this thesis presents the first collection of *Henckelia rotundata* specimens for nearly one hundred years (the first collection was in 1922) and I discovered five new species i.e.

Henckelia amplexifolia, *H. campanuliflora*, *H. candida*, *H. dasycalyx* and *H. nakianensis* and I found *Henckelia amplexifolia*. More field collections are therefore required as it is quite possible that further new species await discovery. Although only 24 of the 68 species of *Henckelia* were dealt with in this study I am convinced that *Henckelia* is a distinct genus and this is supported by my morphological and molecular data. I have also shown that the genus can be split into section *Henckelia* and section *Chirita*.

References

- Atkins, H., Preston, J. and Q.C.B. Cronk. (2001). A molecular test of Huxley's line: *Cyrtandra* (Gesneriaceae) in Borneo and the Philippines. *Biological Journal of the Linnean Society* **72**: 143-159. Doi: 10.1006/bjpl.2000.0500.
- Bachman S., Moat J., Hill A.W., de la Torre J. and B. Scott. (2011). "Supporting Red List threat assessments with GeoCAT: geospatial conservation assessment tool." In: Smith, V. and L. Penev (Eds) e-Infrastructures for data publishing in biodiversity science. *ZooKeys* **150**: 117–126. (Version BETA)*.
- Barnett, E.C. (1954). Gesneriaceae. In: W.G. Craib & A.F.G. Kerr (eds.), *Florae Siamensis Enumeratio*: a list of the plants known from Siam, with records of their occurrence **3**: 196-238.
- Bentham, G. and J.D. Hooker. (1876). *Genera Plantarum*. **2**: 990-1025.
- Bernardo, J., and J. Ramón. (1998). An Introduction to Bayesian Reference Analysis: Inference on the Ratio of Multinomial Parameters. *Journal of the Royal Statistical Society. Series D (The Statistician)*, **47(1)**: 101-135. Retrieved from <http://www.jstor.org/stable/2988429>.
- Blummitt, R.K. and C.E. Powell. (1992). *Authors of plant names*. Royal Botanic Garden, Kew, UK.
- Bokhari, M.H. and B.L. Burtt. (1970). Studies in the Gesneriaceae of the Old World XXXII: foliar sclereids in *Cyrtandra*. *Notes from the Royal Botanic Garden Edinburgh* **30**: 11-21.
- Borah, D., Taram, M., Joe, A. and S. Vincent. (2019). *Henckelia collegii-sancti-thomasi*: a new species of *Henckelia* (Gesneriaceae) from Northeastern India. *Phytotaxa* **415(4)**: 248 (2019). 247-251. DOI: <http://dx.doi.org/10.11646/phytotaxa.415.4.10>.
- Borchsenius, F. (2009). FastGap 1.2. Department of Biosciences, Aarhus University, Denmark. Published online at http://www.aubot.dk/FastGap_home.htm.
- Bui, H.Q., Nuraliev, M.S., Moller, M., Kuznetsov, A.N., Kuznetsova, S.P., Middleton, D.J. & F. Wen. (2020). *Henckelia longisepala* (Gesneriaceae), a new record for Vietnam. *Rheedea* 30(1): 176-186.
- Burtt, B.L. (1954). Studies in the Gesneriaceae of the Old World II: Type and Lectotype of certain genera and group of lower rank. *Notes from the Royal Botanic Garden Edinburgh* **21**: 193-208.
- Burtt, B.L. (1962). Studies on the Gesneriaceae of the Old World, XXIV: Tentative keys to the tribes and genera in temperate South America. *Notes from the Royal Botanic Garden Edinburgh* **24**: 205-220.

- Burt, B.L. (1997). Taxonomic history of *Didymocarpus* and *Henckelia* (Gesneriaceae). *Beitrage zur Biologie der Pflanzen* **70**: 365-375.
- Burt, B.L. (2001a). Flora of Thailand: Annotated checklist of Gesneriaceae. *Thai Forest Bulletin (Botany)* **29**: 81-109.
- Burt, B.L. (2001b). *Kaisupeea*: a new genus of Gesneriaceae centred in Thailand. *Nordic Journal of Botany* **21**: 115-119.
- Byng, J.W. (2014). *The Flowering Plants Hand Book: A practical guide to families and genera of the world*. Plant Gateway Ltd., Hertford, UK.
- Cai, L., Liu, D.-T., Zhang, P. & Z.L. Dao. (2019). Two new species of *Henckelia* (Gesneriaceae) from Southeastern Yunnan, China. In: Cai J, Yu W-B, Zhang T, Li D-Z (Eds) Revealing of the plant diversity in China's biodiversity hotspots. *PhytoKeys* **130**: 151-160. <https://doi.org/10.3897/phytokeys.130.33988>.
- Candolle, A.P. 1816. *Essai sur les propriétés médicales des plantes, comparées avec leurs formes extérieures et leur classification naturelle* pp. 192. Crochard, Paris.
- Candolle, A.P. (1838). Gesneriaceae. *Prodromus Systematis Naturalis Regni Vegetabilis* **7**: 523-547.
- Candolle, A.P. (1845). Cyrtandraceae. *Prodromus Systematis Naturalis Regni Vegetabilis* **9**:259-286.
- Chase, M.W., Soltis, D.E., Olmstead, R.G., Morgan, D., Les, D.H., Mishler, B.D., Duvall, M.R., Price, R.A., Hills, H.G., Qiu, Y.-L., Kron, K.A., Rettig, J.H., Conti, E., Palmer, J.D., Manhart, J.R., Sytsma, K.J., Michaels, H.J., Kress, W.J., Karol, K.G., Clark, W.D., Hedren, Brandon S. Gaut, Robert K. Jansen, Ki-Joong Kim, Charles F. Wimpee, James F. Smith, M., Furnier, G.R., Strauss, S.H., Xiang, Q.-Y., Plunkett, G.M., Soltis, P.S., Swensen, S.M., Williams, S.E., Gadek, P.A., Quinn, C.J., Eguiarte, L.E., Golenberg, E., Learn, G.H., Graham, S.W., Barrett, S.C.H., Dayanandan, S., and V.A. Albert. (1993). Phylogenetics of Seed Plants:
An Analysis of Nucleotide Sequences from the Plastid Gene *RbcL*. *Annals of the Missouri Botanical Garden* **80(3)**: 528-548+550-580. doi:10.2307/2399846.
- Clarke, C.B. (1883). Cyrtandreae. *Monographiae Phanerogamarum* **5**: 1-303.
- Clarke, C.B. (1884). *Chirita monophylla* C.B. Clarke. In: J. D. Hooker (ed.), *Flora of British India* **4(11)**: 360.
- Clarke, C.B. (1885). Gesneriaceae. *Flora of British India* **IV**: 336-375.
- Clark, J.L. and E.A. Zimmer. (2003). A Preliminary Phylogeny of *Alloplectus* (Gesneriaceae): Implications for the evolution of flower resupination. *Systematic Botany* **28(2)**: 365-375.
- Clark, J., Herendeen, P., Skog, L., and E.A. Zimmer. (2006). Phylogenetic relationships

- and generic boundaries in the Episcieae (Gesneriaceae) inferred from nuclear, chloroplast, and morphological Data. *Taxon* **55(2)**: 313-336. doi: 10.2307/25065580.
- Craib, W.G. (1912). Gesneriaceae. *Contributions to the Flora of Siam: Dicotyledones* pp 148-150. Printed for the University of Aberdeen.
- Cranwell, L. (1942). New Zealand Pollen Studies: 1. Key to the pollen grains of families and genera in the native flora. *Records of the Auckland Institute and Museum* **2(6)**: 280-308. Retrieved from <http://www.jstor.org/stable/42905990>.
- Cronk, Q.C.B., Kiehn, M., Wagner, W.L., and J.F. Smith. (2005). Evolution of *Cyrtandra* (Gesneriaceae) in the Pacific Ocean: The Origin of a Supertramp. *American Journal of Botany* **92(6)**: 1017-1024. Retrieved from <https://www.jstor.org/stable/4126079>.
- Dat, N., Cuong, N., Long, V. and L. Truong. (2016). *Billolivia cadamensis* (Gesneriaceae), a new species from Central Vietnam. *Tap Chi Sinh Hoc* **38(4)**. doi:10.15625/0866-7160/v38n4.8837.
- De Candolle, A.P. 1816. de Candolle. (1816). *Gesneriées. Essai Sur Les Propriétés Médicales des Plantes*. Page 192.
- De Candolle, A.P. (1838). Gesneriaceae. *Prodromus Systematis Naturalis Regni Vegetabilis* **7(2)**: 523-547.
- De Candolle, A.P. (1845). Cyrtandraceae. *Prodromus Systematis Naturalis Regni Vegetabilis* **9**:259-286.
- De Jussieu, A.L. (1806). Third Memoir on the general characters of families of plants, derived from the seeds, as confirmed or corrected by the observation of Gaertner. In Charles, K. & J. Sims (eds.). *Annals of Botany* **2**: 558-568.
- Denduangboripant, J. and Q.C.B. Cronk. (2000). High Intraindividual Variation in Internal Transcribed Spacer Sequences in *Aeschynanthus* (Gesneriaceae): Implications for Phylogenetics. *Proceedings: Biological Sciences* **267(1451)**: 1407-1415. Retrieved from <http://www.jstor.org/stable/2665628>.
- Dietrich, A. (1831). *Henckelia*. *Species Plantarum* **6(2)**: 568-577.
- Do, X.T., Shu, L., Yi-Gang, W., Long-Fei, F. and W. Fang. (2016). New records and keys to species of *Hemiboea* and *Loxostigma* (Gesneriaceae) for the flora of Vietnam, *Taiwania* **61**: 369-374.
- Don, D. (1822). Descriptions of two new genera of Nepal plants. *Edinburgh Philosophical Journal* **7**: 82-87.

- Don, D. (1825). *Chirita*. Prodrromus Florae Nepalensis, London. P 89.
- Dumortier, B.C.J. (1829). Gesneriaceae. *Analyse des Familles de Plantes: avec l'indication des principaux genres qui s'y rattachent*. Tournay. p 30.
- Edwards, T.J. (2003). Two new species of *Streptocarpus* (Gesneriaceae) from South Africa. *Novon* **13(2)**: 185–188.
- Fourny, Ana Carolina da Silva, Mendonça, Cláudia Barbieri Ferreira, Lopes, Thereza Cristina Costa and Vania Gonçalves-Esteves. (2010). Palinologia de espécies de Gesneriaceae Rich. & Juss. ocorrentes no Estado do Rio de Janeiro. Brasil. *Acta Botanica Brasilica* **24(3)**: 812-824. <https://dx.doi.org/10.1590/S0102-33062010000300024>.
- Fritsch, K. (1893). Gesneriaceae. in Engler, H.G.A. & K. Prantl. *Die natürlichen Pflanzenfamilien IV* Theil, Ableitung 3B: 133-185.
- Fritze, K.J. and N.H. Williams. (1988). The Taxonomic Significance of Pollen Morphology in the *Columnnea* Alliance (Gesneriaceae: Gesnerioideae). *Annals of the Missouri Botanical Garden* **75(1)**: 168–191.
- García, A.T.A., Miguez, M.B. and G. Gottsberger. (2014). Pollen: ovule ratio and its relationship with other reproductive traits in some *Passiflora* species (Passifloraceae). *Anales del Jardín Botánico de Madrid* **71(2)**:1-8, e009. doi:<http://dx.doi.org/10.3989/ajbm.2360>.
- Jack, W. (1820). Didymocarpus. *Description of Malayan plant 2. Malayan Miscellanies* **1(5)**. Bencoolen page 1-6.
- Jack, W. (1825). On Cyrtandra, a new Natural order of plants. *Transactions of the Linnean Society of London* **14**: 23-44.
- Janeesha, A.P. & S. Nampy. (2015). *Henckelia bracteata*, a new species from S Western Ghats, India, and lectotypification of *Didymocarpus humboldtianus* (*H. humboldtiana*). *Willdenowia* **45**: 53-59.
- Judd, W.S., Campbell, C.S., Kellogg, E.A., Stevens, P.F. and M.J. Donoghue. (2002). *Plant Systematics: a phylogenetic approach 2nd edition*. Sinauer Associates, Inc. Sunderland, Massachusetts, U.S.A.
- Heywood, V.H., Brummitt, R.K., Culham, A. and O. Seberg. (2007). *Flowering Plants Families of the World*. Royal Botanic Gardens, Kew, UK.
- Hodkinson, T. R., Ni Chonghaile, G., Sungkaew, S., Chase, M.W., Salamin, N. and C.M.A. Stapleton (2010) Phylogenetic analyses of plastid and nuclear DNA sequences indicate a rapid late Miocene radiation of the temperate bamboo tribe Arundinarieae (Poaceae, Bambusoideae), *Plant Ecology & Diversity*, 3:2, 109-120, DOI: 10.1080/17550874.2010.521524.

- Hodkinson, T.R., Waldren, S., Parnell, J.A., Kelleher, C.T., Salamin, K. and N. Salamin. (2007). DNA banking for plant breeding, biotechnology and biodiversity evaluation. *Journal of Plant Research*, **120(1)**: 17-29.
- Hodkinson, T.R., Waldren, S., Parnell, J.A.N., Kelleher, C.T., Salamin, K. and N. Salamin. (2007). DNA banking for plant breeding, biotechnology and biodiversity evaluation. *Journal of Plant Research* **120**: 17-29.
- Horikoshi, H. and Yuan Tang (2016). ggfortify: Data Visualization Tools for Statistical Analysis Results. <https://CRAN.R-project.org/package=ggfortify>
- Huelsenbeck, J. P. and F. Ronquist. (2001). MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**:754-755.
- IUCN. (2012). *IUCN Red List Categories and Criteria: Version 3.1. Second edition*. Gland, Switzerland and Cambridge, UK: IUCN. iv + 32pp.
- Kiew, R. (2009). Three new species of Gesneriaceae from Kelantan, Malaysia. *The Gardens' Bulletin (Singapore)* **61**: 73-79.
- Kress, W.J., De Filippis, R.A., Farr, E. and Daw Yin Yin Kyi. (2003). *Contributions from the United States National Herbarium* **45**: 1-590: A Checklist of the Trees, Shrubs, Herbs, and Climber of Myanmar pp. 261-264. Department of Systematic Biology Botany, National Museum of Natural History, Washington, DC.
- Krishna, G. and P. Lakshminarasimhan. (2018). A new species of *Henckelia* (Gesneriaceae) from Arunachal Pradesh, India. *Taiwania* **63(4)**: 397-401.
- Kumar, S., Nei, M., Dudley, J. and K. Tamura. (2008). MEGA: A Biologist-Centric Software for Evolutionary Analysis of DNA and Protein Sequences. *Briefings in Bioinformatics* **9**: 299-306.
- Kumar, S., Stecher, G. and K. Tamura. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**: 1870-1874.
- Kvist, L.P. 1990. Revision of *Heppiella* (Gesneriaceae). *Systematic Botany* **15(4)**: 720–735.
- Kvist, Lars P. and L. E. Skog. (1993). The genus *Columnnea* (Gesneriaceae) in Ecuador. *Allertonia* **6 (5)**: 327-400. <http://www.jstor.org/stable/23188176>.
- Legendre, P. and L. Legendre. *Numerical ecology*. Elsevier, Amsterdam.
- Leti, M. Hul, S., Fouché, J., Cheng, S.K. and B. David. (2013). *Flore photographique du Cambodge* pp 295. Editions Private, Toulouse, France.
- Li, J. and Y. Wang. (2007). Phylogenetic Reconstruction among Species of *Chiritopsis* and *Chirita* Sect. *Gibbosaccus* (Gesneriaceae) Based on nrDNA ITS and cpDNA *trnL-F* Sequences. *Systematic Botany* **32(4)**: 888-898. Retrieved from <http://www.jstor.org/stable/25064302>.

- Luegmayr, E. (1993). Pollen characters of Old World Gesneriaceae (Cyrtandroideae) with special reference to SE Asian taxa. *Grana* **32**: 221-232.
- Luegmayr, E. (1993a). Pollen Characters of Old World Gesneriaceae (Cyrtandroideae), *Grana* **32**:4-5, 221-232, DOI: 10.1080/00173139309429985.
- Luegmayr, E. (1993b). Pollen of Hawaiian *Cyrtandra* (Gesneriaceae) including notes on Southeast Asian taxa. *Blumea* **38**: 25–38.
- Luu, T.H., Pham, N.H., Tran, G., Ngo, D.T.T., Dinh, L.N. and M.T. Ton. (2015). *Billolivia kyi* (Gesneriaceae), a New Species from Vietnam. *Annales Botanici Fennici* **52**: 362–364.
- Mabberley, D.J. (2008). *Mabberley's Plant-book: a portable dictionary of plants, their classification and uses*. 3rd ed. Cambridge University Press, Cambridge.
- Maddison, D.R., Swofford, D.L. and W.P. Maddison. (1997). NEXUS: an extensible file format for systematic information. *Systematic Biology* **46(4)**: 590-621.
- Manudev, K.M., Weber, A. and S. Nampy. (2012). *Henckelia pradeepiana*, a new species of Gesneriaceae from the southern Western Ghats, India. *Rheedea* **22**: 119-123.
- Mayer, V., Möller, M., Perret, M. and A. Weber. (2003). Phylogenetic position and generic differentiation of Epithemateae (Gesneriaceae) inferred from plastid DNA sequencing data. *American Journal of Botany* **90(2)**: 321-329.
- Mendum, M. (2001). Three new Gesneriaceae from Palawan, Philippines. *Edinburgh Journal of Botany* **58**: 435-441.
- Middleton, D.J. (2003). Progress on the Flora of Thailand. *Telopea* **10(1)**: 33-42.
- Middleton, D.J. (2007). A revision of *Aeschynanthus* (Gesneriaceae) in Thailand. *Edinburgh Journal of Botany* **64**: 363-429.
- Middleton, D.J. and N.S. Ly. (2008). A new species of *Ornithoboea* (Gesneriaceae) from Vietnam. *Edinburgh Journal of Botany* **65**: 353–357.
- Middleton, D.J. (2015). A new species of *Gyrocheilos* (Gesneriaceae) from Vietnam. *Edinburgh Journal of Botany* **72**: 235-238.
- Middleton, D.J. and P. Triboun. (2010). Two new species of *Petrocosmea* (Gesneriaceae) from Thailand. *Thai Forest Bulletin (Botany)* **38**: 42-47.
- Middleton, D.J. and P. Triboun. (2013b). A new species of *Somrania* (Gesneriaceae) from Thailand. *The Gardens' Bulletin Singapore* **65**: 181-184.
- Middleton, D.J. and P. Triboun. (2013c). New species of *Microchirita* (Gesneriaceae) from Thailand. *Thai Forest Bulletin (Botany)* **41**: 13-22.
- Middleton, D.J, Atkins, H., Luu, H.T., Nishii, K. and M. Möler. (2014a). *Billolivia*, a new genus of Gesneriaceae from Vietnam with five new species. *Phytotaxa* **161**: 241-269.

- Middleton, D.J., Kanae Nishii, K., Puglisi, C., Forrest, L.L and M. Möller. (2015a). *Chayamaritia* (Gesneriaceae: Didymocarpoideae), a new genus from Southeast Asia. *Plant Systematics and Evolution* **301**: 1947–1966.
- Middleton, D.J., Khew, G.S., Poopath, M., Möller, M. and C. Puglisi. (2018). *Rachunia cymbiformis*, a new genus and species of Gesneriaceae from Thailand. *Nordic Journal of Botany* **36(11)**-e0199: 4.2018.
- Middleton, D.J., Leong-Škorničková, J. and Q.B. Nguÿen. (2014b). A new species of *Billolivia* (Gesneriaceae) from Vietnam. *The Garden's Bulletin Singapore* **66**: 189-194.
- Middleton, D.J., Sangvirotjanapat, S. and W. La-ongsri. (2015b). A new species of *Petrocodon* (Gesneriaceae) from Thailand. *Thai Forest Bulletin (Botany)* **43**: 15-17.
- Middleton, D.J., Weber, A., Yao, T.L., Sontag, S. and M. Möller. (2013a). The current status of the species hitherto assigned to *Henckelia* (Gesneriaceae). *Edinburgh Journal of Botany* **70**: 385-404.
- Möller, M. and J. Clarke. (2013). The state of molecular studies in the family Gesneriaceae: a review. *Selbyana* **31(2)**: 95-125.
- Möller, M. and Q.C.B., Cronk. (1997). Origin and relationships of *Saintpaulia* (Gesneriaceae) based on ribosomal DNA internal transcribed spacer (ITS) sequences. *American Journal of Botany* **84(8)**: 956-965.
- Möller, M., Nishii, K., Atkins, A.J., Kong, H.H., Kang, M., Wei, Y.G., Wen, F., Hong, X. and D.J. Middleton. (2016). An expansion of the genus *Deinostigma* (Gesneriaceae). *The Gardens' Bulletin Singapore* **68**: 145-172.
- Möller, M., Wei, Y.-G., Wen, F., Clarke, J.L., and A. Weber. (2016). You win some you lose some: updated generic delineations and classification for the family in China. *Guihaia* **36**: 44-60.
- Möller, M., Pfosser, M., Jang, C.-G, Mayer, V, Clark, A., Hollingsworth, M.L., Barfuss, M.H.J., Wang, Y.-Z., Kiehn, M. and A. Weber. (2009). A Preliminary Phylogeny of the 'Didymocarpoid Gesneriaceae' Based on Three Molecular Data Sets: Incongruence with Available Tribal Classifications. *American Journal of Botany* **96(5)**: 989-1010. Retrieved from <http://www.jstor.org/stable/27733429>.
- Möller, M., and Q.C.B. Cronk. (1997a). Phylogeny and Disjunct Distribution: Evolution of *Saintpaulia* (Gesneriaceae). *Biological Sciences* **264(1389)**: 1827 -1836. Retrieved from <http://www.jstor.org/stable/51120>.
- Möller, M., and Q.C.B. Cronk. (1997b). Origin and relationship of *Saintpaulia* (Gesneriaceae) based on ribosomal DNA internal transcribed spacer (ITS)

- sequences. *American Journal of Botany* **84(8)**: 956-965.
- Möller, M., and Q.C.B. Cronk. (2001). Phylogenetic studies in *Streptocarpus* (Gesneriaceae): reconstruction of Biogeographic history and distribution patterns. *Systematics and Geography of Plants* **71(2)**: 545-555.
- Moore, P.D., Webb, J.A. & M.E. Collinson. 1991. *Pollen analysis: second edition*. Blackwell Scientific Publications, Oxford. 216 pp.
- Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. & J. Kent. *Nature* **403**: 853-858.
- Nangngam, P and D.J. Middleton. (2014). Five new species of *Didymocarpus* (Gesneriaceae) from Thailand. *Thai Forest Bulletin (Botany)* **42**: 35-42.
- Nangngam, P and J.F. Maxwell. (2013). *Didymocarpus* (Gesneriaceae) in Thailand. *The Gardens' Bulletin Singapore* **65**: 185-225.
- Newman, M., Ketphanh, S., Svengsuksa, B., Thomas, P., Sengdala, K., Lamxay, V. and K. Armstong. (2007). *A checklist of the Vascular Plants of Lao PDR* pp. 146-147. Royal Botanic Garden Edinburgh, Scotland.
- Nylander, J.A.A. (2004). *MrModeltest v2*. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Oksanen, K., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlenn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E. and Wagner, H. (2018) *vegan: Community Ecology Package* version 2.5-2. <https://CRAN.R-project.org/package=vegan>.
- Oxelman, B., Liden, M. and D. Berglund. (1997). Chloroplast *rps16* intron phylogeny of the tribe Sileneae (Caryophyllaceae). *Plant Systematics and Evolution* **206**: 393-410.
- Parnell, J. (1991). Pollen Morphology of *Jouibarba* Opiz and *Sempervivum* L. (Crassulaceae). *Kew Bulletin* **46(4)**: 733–738. doi:10.2307/4110418.
- Pattarahirantricin, N. (2014). The genus *Rhynchoglossum* Blume (Gesneriaceae) in Thailand. *Thai Forest Bulletin (Botany)* **42**: 24–34.
- Pavlopoulos, G.A., Soldatos, T.G., Barbosa-Silva, A. and R. Schneider. (2010). A reference guide for tree analysis and visualization. *BioData Mining* **3**: 1-28. doi:10.1186/1756-0381. <http://www.biodatamining.org/content/3/1/1>.
- Pellegrin, F. (1930). Gesneriaceae. *Flore de L'Indo-China* **4**: 487-565.
- Perret, M., Chautems, A., Araujo, A.O. and N. Salamin. (2013). Temporal and spatial origin of Gesneriaceae in the New World inferred from plastid DNA sequences. *Botanical Journal of the Linnean Society* **171**: 61-79.
- Perret, M., Chautems, A., Spichiger, R., Geoffrey Kite, and V. Savolainen. (2003).

- Systematics and Evolution of Tribe Sinningieae (Gesneriaceae): Evidence from Phylogenetic Analyses of Six Plastid DNA Regions and Nuclear ncpGS. *American Journal of Botany* **90**(3): 445-460. Retrieved from <http://www.jstor.org/stable/4124163>.
- Pham, H.H. (1993). Gesneriaceae. *An Illustrated Flora of Vietnam* **3**: 2-25.
- Pham, H.H. (2003). Gesneriaceae. *An Illustrated Flora of Vietnam* **3**: 12-29.
- Puglisi, C. and D.J. Middleton. (2017). A revision of *Microchirita* (Gesneriaceae) in Thailand. *The Gardens' Bulletin Singapore* **69** (2): 211-284.
- Puglisi, C., Middleton D.J. and S. Suddee. (2016a). Four new species of *Microchirita* (Gesneriaceae) from Thailand. *Kew Bulletin* **71**: 2. DOI 10.1007/S12225-016-9614-0.
- Puglisi, C., Yao, T.L., Milne, R., Möller, M. and D.J. Middleton. (2016b). Generic recircumscription in the *Loxocarpinae* (Gesneriaceae), as inferred by phylogenetic and morphological data. *Taxon* **65**: 277-292.
- Punt, W., Hoen, P.P., Blackmore, S., Nilsson, S. and A. Le Thomas. (2007). Glossary of pollen and spore terminology. *Review of Palaeobotany and Palynology* **143**: 1-81. <http://www.sciencedirect.com>.
- Rajakumar, T.J.S., Selvakumari, R., Murugesan, S. & Chellaperumal, N. 2009. *Didymocarpus sivagirensis*, a new species of Gesneriaceae from Tirunelveli. *Indian Journal of Forestry* **32**(3): 481–483.
- Rambaut A, Drummond AJ, Xie D, Baele G and MA. Suchard. (2018) Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*. syy032. doi:10.1093/sysbio/syy032.
- Ramírez-Roa, A. and G. Ibarra-Manríquez. (1997). A new species of *Solenophora* (Gesneriaceae) from Southeast Veracruz, Mexico. *Novon* **7**(3): 281-284.
- Ranasinghe, S., Milne, R., Jayasekara, Rubasinghe, S. and M. Möller. (2016). *Henckelia wijesundarae* (Gesneriaceae), a new endemic species from Sri Lanka, and lectotypification of *Chirita walkerae* and *C. walkerae* var. *parviflora*. *Willdenowia* **46**: 213-224.
- R Core Team. (2018). *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing <http://www.R-project.org>.
- Reille, M. (1992). *Pollen et spores d'Europe et d'Afrique du Nord*. Louis-Jean, France. 520 pp.
- Revelle, W. (2018). psych: Procedures for Personality and Psychological Research Version = 1.8.4., Northwestern University, Evanston, Illinois, USA, <https://CRAN.R-project.org/package=psych> Version = 1.8.4.
- Robinson, G.B. (1975). An introduction to Nonmetric Multidimensional Scaling. *American Journal of Political Science* **19**(2): 343-390.

- Ronquist, F. and J. P. Huelsenbeck. (2003). MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**:1572-1574.
- Royle, J.F. (1839). *Chirita bifolia*. Illustrations of the Botany and other branches of the Natural History of the Himalayan Mountains and of the Flora of Cashmere, Vol 2: 70.
- Scott, S.M. and D.J. Middleton. (2014). A revision of *Ornithoboea* (Gesneriaceae). *The Gardens' Bulletin Singapore* **66**: 73-119.
- Schlag-Edler, B. & M. Kiehn. (2001). Palynology of South Pacific *Cyrtandra* (Gesneriaceae) with notes on some Hawaiian taxa. *Grana* **40**: 192-196. DOI: 10.1080/001731301317223123.
- Sirimongkol, S., Parnell, J., Hodkinson, T., Middleton, D., & Puglisi, C. (2019). Five new species of *Henckelia* (Gesneriaceae) from Myanmar and Thailand. *Thai Forest Bulletin (Botany)* **47(1)**: 38-54. <https://doi.org/10.20531/tfb.2019.47.1.08>
- Sivarajan, V.V. (1991). *Introduction to the principles of plant taxonomy. Second edition*. In Robson, N.K.B. (ed.). Oxford & IBH Publishing Co. Put. Ltd., New Delhi, India. pp 149-156. (299 pp).
- Smith, J.F. (1996). Tribal relationships within Gesneriaceae: a cladistic of morphological data. *Systematic Botany* **21(4)**: 497-513.
- Smith, J. F. (2000a). Phylogenetic resolution within the tribe Episcieae (Gesneriaceae): congruence of ITS and NDHF sequences from parsimony and maximum-likelihood analyses. *American Journal of Botany* **87**: 883-897.
- Smith, J. F. (2000b). A phylogenetic analysis of Tribes Beslerieae and Nepeantheae (Gesneriaceae) and Evolution of fruit types: Parsimony and Maximum Likelihood Analyses of *ndhF* Sequences. *Systematic Botany* **25(1)**: 72-81.
- Smith, J., and C. Carroll. (1997). A Cladistic Analysis of the Tribe Episcieae (Gesneriaceae) Based on *ndhF* Sequences: Origin of Morphological Characters. *Systematic Botany* **22(4)**: 713-725. doi:10.2307/2419437.
- Smith, J., Draper, S., Hileman, L., and D. Baum. (2004). A Phylogenetic Analysis within Tribes Gloxinieae and Gesnerieae (Gesnerioideae: Gesneriaceae). *Systematic Botany* **29(4)**: 947-958. Retrieved from <http://www.jstor.org/stable/25064023>.
- Smith, J. and K.J. Systma. (1994). Molecules and morphology: congruence of data in *Columnea* (Gesneriaceae). *Plant systematics and evolution* **193**: 37-52.
- Smith, J., Wolfram, J., Brown, K., Carroll, C., and D. Denton. (1997). Tribal Relationships in the Gesneriaceae: Evidence from DNA Sequences of the Chloroplast Gene *ndhF*. *Annals of the Missouri Botanical Garden* **84(1)**: 50-66. doi:10.2307/2399953
- Spira, T.P. (1980). Floral parameters, breeding system and pollinator type in *Trichostema*

- (Labiatae). *American Journal of Botany* **67(3)**: 278-284.
- Sprengel, C. (1817). *Henckelia*. *Anleitung zur Kenntniss der Gewachse*, Zweite, 2nd ed.: 402.
- Sprengel, C. (1824). *Henckelia incana*. *Systema vegetabilium*, **1**: 38.
- Stevens, P. F. (2001 onwards). Angiosperm Phylogeny Website. Version 14, July 2017 [and more or less continuously updated since]." will do. <http://www.mobot.org/MOBOT/research/APweb/>.
- Stuessy, T.F. (1989). *Plant taxonomy: the systematic evaluation of comparative data*. Columbia University Press. USA. 514 pp.
- Stuessy, T.F. (2009). *taxonomy: the systematic evaluation of comparative data* 2nd ed. Columbia University Press. USA. 539 pp.
- Stuessy, T.F., Crawford, D.J., Soltis, D.E. & P.S. Soltis. (2014). *Plant taxonomy*. Koeltz Scientific Books, Germany. 425 pp.
- Sukumaran, E. & Kumar, S. (2014). A new combination in *Henckelia* (Gesneriaceae). *Polish Botanical Journal* 59(1): 149. DOI: 10.2478/pbj-2014-0002.
- Swofford, D.L. (2002). PAUP phylogenetic analysis using parsimony, version 4.0b10. Sinauer Associates, Sunderland, MA.
- Swofford, D.L. (2003). PAUP*: Phylogenetic analysis using parsimony (*and other methods), ver. 4.0b.10. Sinauer Associates, Sunderland, Massachusetts.
- The Forest Herbarium. (2014). *Thai Plant Names Tem Smitinand revised edition 2014*. The Forest Herbarium, Department of National Parks, Wildlife and Plant Conservation, Bangkok.
- Triboun, P. (2013). *Paraboea middletonii* (Gesneriaceae) from Thailand. *Thai Forest Bulletin (Botany)* **41**: 45-47.
- Triboun, P. and D.J. Middleton. (2010). A new species of *Damrongia* (Gesneriaceae) from Thailand. *Thai Forest Bulletin (Botany)* **38**: 108-110.
- Triboun, P. and D.J. Middleton. (2015). Three new species of *Paraboea* (Gesneriaceae) from Thailand. *Thai Forest Bulletin (Botany)* **43**: 18-23.
- Turland, N.J., Wiersema, J.H., Barrie, F.R., Greuter, W., Hawksworth, D.L., Herendeen, P.S., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T.W., McNeill, J., Monro, A.M., Prado, J., Price, M.J. and G.F. Smith. (2018). *International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adapted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017*. Regnum Vegetabile 159. Glashütten: Koeltz Botanical Books. <https://doi.org/10.12705/Code.2018>.
- Utteridge, T. and G. Bramley. (2014). *The Kew tropical plant families identification handbook*. Royal Botanic Gardens, Kew, UK.

- Van Welzen, P. C., Madern, A., Raes, N., Parnell, J. A. N., Simpson, D. A., Byrne, C., Curtis, T., Macklin, J., Trias-Blasi, A., Prajaksood, A., Bygrave, P., Dransfield, S., Kirkup, D. W., Moat, J., Wilkin, P., Couch, C., Boyce, P. C., Chayamarit, K., Chantaranothai, P., Esser, H. J., Jebb, M. H. P., Larsen, K., Larsen, S. S., Nielsen, I., Meade, C., Middleton, D. J., Pendry, C. A., Muasya, A. M., Pattharahirantricin, N., Pooma, R., Suddee, S., Staples, G. W., Sungkaew, S. & Teerawatananon, A. (2011). Land Use, Climate Change and Biodiversity Modeling: Perspectives and Applications. The current and future status of floristic provinces in Thailand. (pp.). IGI Global.
- Vahl, M. (1804). *Röttlera. Enumeratio Plantarum* **1**: 87-88.
- Vũ, N.L., Pham, H.N., Nguyễn, T.V. and H.T. Luu. (2015). *Billolivia tichii* (Gesneriaceae), a new species from Vietnam. *Phytotaxa* **219**: 190-194.
- Wang, W.T. and Z.Y. Li. (1992). Genus novum Gesneriacearum e Vietnam. *Acta Phytotaxonomica Sinica* **30**: 356–361.
- Wang, C., Möller, M., and Q. Cronk. (2004). Phylogenetic Position of *Titanotrichum oldhamii* (Gesneriaceae) Inferred from Four Different Gene Regions. *Systematic Botany* **29(2)**: 407-418. Retrieved from <http://www.jstor.org/stable/25063972>.
- Wang, Y., Liang, R., Wang, B., Li, J., Qiu, Z., Li, Z., Z.Y. A. Weber. (2010). Origin and phylogenetic relationships of the Old World Gesneriaceae with actinomorphic flowers inferred from ITS and *trnL-trnF* sequences. *Taxon* **59(4)**: 1044-1052. Retrieved from <http://www.jstor.org/stable/20773975>.
- Wang, Y.-Z., Mao, R.-B., Liu, Y., Li, J.-M., Dong, Y., Li, Z.-Y. and J.F. Smith. (2011). Phylogenetic reconstruction of *Chirita* and allies (Gesneriaceae) with taxonomic treatments. *Journal of Systematics and Evolution* **49(1)**: 50-64. doi: 10.1111/j.17596831.2010.00113.x.
- Walker, J., & Doyle, J. (1975). The Bases of Angiosperm Phylogeny: Palynology. *Annals of the Missouri Botanical Garden* **62(3)**: 664–723. doi:10.2307/2395271.
- Weber, A. (2004). Gesneriaceae. In K. Kubitzki and J.W. Kadereit (eds.), *The Families and Genera of Vascular Plants* **7**:63-158.
- Weber, A. and B.L. Burt. (1997). Remodelling of *Didymocarpus* and associated genera (Gesneriaceae). *Beitrage zur Biologie der Pflanzen* **70**: 293-363.
- Weber, A., Clarke, J.L. and M. Möller. (2013). A New Formal Classification of Gesneriaceae. *Selbyana* **31**: 68-94.
- Weber, A., Middleton, D.J., Forrest, A., Kiew, R., Lim, C.L., Rafidah, A.R., Sontag, S., Triboun, P., Wei, Y.-G., Yao, T.L. and M. Möller. (2011). Molecular systematics and remodelling of *Chirita* and associated genera (Gesneriaceae). *Taxon* **60**: 767- 790.

- Weber, A., Middleton, D.J., Forrest, A., Kiew, R., Lim, C.L., Rafidah, A.R., Sontag, S., Triboun, P., Wei, Y.-G., Yao, T.L. and M. Möller. (2011). Molecular systematics and remodelling of *Chirita* and associated genera (Gesneriaceae). *Taxon* **60**: 767-790.
- Wei, Y., Wen, F., Zhao, B. and S. He. (2013). *Anna rubidiflora* (Gesneriaceae), a new species from Guizhou, the southern part of China. *Plant Ecology and Evolution* **146(2)**: 203–211.
- Wilkie, P., Poulsen, A.D., Harris, D. and L.L. Forrest. (2013). The collection and storage of plant material for DNA extraction: The Teabag Method. *The Garden's Bulletin Singapore* **65**: 231-234.
- Wood, D. (1974). A revision of *Chirita* (Gesneriaceae). *Notes from the Royal Botanic Garden Edinburgh* **33**: 123-205.
- Zimmer, E., Roalson, E., Skog, L., John K. Boggan, Z.Y. A. Idnurm. (2002). Phylogenetic Relationships in the Gesnerioideae (Gesneriaceae) Based on nrDNA ITS and cpDNA *trnL-F* and *trnE-T* Spacer Region Sequences. *American Journal of Botany* **89(2)**: 296-311. Retrieved from <http://www.jstor.org/stable/4131303>.

Appendices

Appendix 1.1 Publication (Sirimongkol et al., 2019)

INTRODUCTION

This work has been published in Thai Forest Bulletin (Botany) Vol. 47(1), 2019.

A previously indicated the genus *Henckelia* was described by Sprengel (1817) but was subsequently mostly included in synonymy of *Didymocarpus* Wall. until resurrected and greatly expanded by Weber & Burtt (1998). *Henckelia* was then extensively remodelled by the removal of all Malesian species and the inclusion of many *Chirita* species by Weber *et al.* (2011). A clarification of the consequent confusion of names and the current status of each was provided by Middleton *et al.* (2013).

The genus is now morphologically fairly diverse and formerly includes two species which develop only a single leaf: *Henckelia monophylla* (C.B. Clarke) D.J. Middleton & Mich. Möller from central Arunachal Pradesh, India (Clarke, 1884; Wood, 1974), and *H. pradeepiana* Nampy, Manudev & A. Weber, from the southern Western Ghats, India (Manudev *et al.*, 2012). Recent field surveys and the examination of herbarium materials have revealed additional new species of *Henckelia* which develop only one large leaf. Three are similar to *H. monophylla* and are described here as *H. campanuliflora* Sirim., *H. candida* Sirim. and *H. nakianensis* Sirim., J. Parn. & Hodk. A fourth new species, *H. amplexifolia* Sirim., is most similar to *H. bifolia* (D. Don) A. Dietr. (Royle, 1839; Wood, 1974). A caulescent plant from Northern Thailand was also found to be a new species and is here described as *H. dasycalyx* Sirim. & D.J. Middleton. This taxon is closely related to *H. oblongifolia* (Roxb.) D.J. Middleton & Mich. Möller.

These new taxa increase the number of known *Henckelia* species to 64 (Janeesha *et al.*, 2015; Manudev *et al.*, 2012; Middleton *et al.*, 2013; Rajakumar *et al.*, 2009; Ranasinghe *et al.*, 2016; Sukumaran & Kumar, 2014; Weber *et al.*, 2011). The genus is found in Sri Lanka, southern and north-eastern India, Nepal, Bhutan, southern China, northern Laos, northern Vietnam and northern Thailand (Weber *et al.*, 2011).

MATERIALS AND METHODS

The descriptions of new species are based on herbarium specimens and fresh material. For the herbarium specimens, flowers were softened in water or 70% alcohol

before measurements were taken. The conservation assessments were made following the IUCN criteria (IUCN, 2012).

KEY TO THE SPECIES OF *HENCKELIA* WITH ONLY ONE OR TWO LEAVES

1. Mature plant developing one large leaf only
 2. Acaulescent herb, tuber present, capsule ovoid to subglobose *H. pradeepiana*
 2. Caulescent herb, tuber absent, capsule elongate
 3. Herb with long rhizome, scale-like leaves developed *H. monophylla*
 3. Herb without long rhizome, scale-like leaves incompletely developed
 4. Leaves petiolate, persistent calyx, capsule held in line with pedicel (orthocarpic) 1. *H. campanuliflora*
 4. Leaves sessile, caducous calyx, capsule held ± horizontal to the pedicel (plagiocarpic)
 5. Leaf less than 7 cm long, surface densely covered in fine hairs, calyx tip not reflexed, staminodes 2 2. *H. candida*
 5. Leaf more than 7 cm long, surface with sparse stout hairs, calyx tip reflexed, staminodes 3. *H. nakianensis*
 1. Mature plant developing one large leaf and one small leaf, rarely with some individuals in a population with 1, 3 or 4 leaves.
 7. Perennial herb lacking stolons and tubers, flower infundibuliform, 1–2 flowered, corolla tube more than 3 cm long, capsule orthocarpic *H. bifolia*
 7. Perennial herb with stolons and tubers, flower campanulate, more than 2-flowered, corolla tube less than 1 cm long, capsule plagiocarpic 4. *H. amplexifolia*

1. *Henckelia campanuliflora* Sirim. sp. nov. Type: Myanmar, Shan State, Htan San Gu, Taunggyi Township, evergreen forest, limestone karst and colluvial slopes down to stream on dark brown loam, 20°49'14"N, 97°20'12.4"E, alt. 1,234 m, fl., 21 Sept. 2015, Y. Baba, K. Kertsawang, C. Kilgour, C. Puglisi, M. Rodda, P. Srisanga, Thant Shin & Phyu Phyu Hnin 103582 (holotype SING!). Fig. 1.

Epilithic annual herb, up to 10 cm tall; stem 0.5–4.5 cm long, with dense multicellular and glandular hairs, 0.4–2.1 mm long. *Leaf* symmetrically orbicular (globose or rhombic), 4.2–7 by 4–6.5 cm, herbaceous, apex obtuse or acute, base cordate, margin remotely dentate, upper and lower surfaces hairy, lateral veins 4–6 ascending on each side of midrib; only one leaf developing. *Petiole* terete, 0.5–3.1 cm long, hairy. *Inflorescence* terminal, 3.8–6.2 cm long, inserted at the junction of the petiole and the

stem, single or compound cymes, 2–9-flowered; peduncles terete, greenish or purplish, 3–3.7 cm long, hairy; bracts 2, free, sessile, lanceolate, 1.5–2 by 0.2–0.5 mm, apex acute, hairy, margin entire; pedicels terete, greenish or purplish, 3–3.7 cm long, with a dense indumentum of multicellular hairs, sometimes glandular, sometimes simple. *Calyx* 5-partite, lobes narrowly triangular, basally connate, pinkish green, ca 3.4 by ca 0.6 mm, hairy, margin entire, apex acuminate, not reflexed, persistent. *Corolla* white, tube campanulate, ca 5 mm long, sparsely hairy; lobes 4 by 3 mm, glabrous. *Stamens* 2: filaments inserted at the base of the corolla tube, 3 mm long, curved; anthers reniform, adaxial surfaces coherent, ca 1.2 by ca 1 mm; staminodes 3, inserted 2 mm from the base of the corolla tube, 0.4 mm long, glabrous. *Gynoecium* 9 mm long; ovary 1 mm across, hairy; stigma subpeltate, dark purple, ca 0.4 by ca 0.2 mm. *Infructescence* 4.5–5 cm long, $\frac{3}{4}$ –1 time the length of the subtending leaf. *Fruit* held in line with the pedicel (orthocarpic), capsule elongate, green, 1–1.2 cm long by 2–2.1 mm wide, with eglandular hairs and few glandular hairs. Seeds prolate, brown, ca 0.3 by ca 0.2 mm (Fig 2).

Myanmar. **Shan State**: Maopan Taung, Taunggyi Township, 20°48'47.51"N, 97°16'18.7"E, alt. 1,454 m, fr., 23 Sept. 2015, *Kilgour et al. m-633 (SING)*. Lomkok mountain and pagoda, roadside, Taunggyi Township, 20°49'2.4" N, 97°13'26.6" E, alt. 1,106 m, fl., 20 Sept. 2015, *Puglisi et al. 103541 [MBK (MBK0272469), the left-hand side herb]*.

Distribution. — Only known from Shan State (Fig. 10).

Ecology. — Edge of open semi-evergreen forest in the shade, evergreen forest or limestone karst and colluvial slopes above streams, dark brown loam, alt. 1,106–1,454 m, flowering and fruiting in September.

Etymology. — The name of this species refers to the corolla shape.

Provisional conservation assessment. — Endangered (EN B1ab(iii), B2ab(iii)). The EOO and AOO are both well within the threshold of Critically Endangered but it is known from three populations which are fairly close together and could not be described as fragmented. At each site the species is fairly common. None of the populations are in a protected area and there has been extensive deforestation in the area, coupled with microclimate changes due to surrounding agricultural land.

Notes. — This species is similar to *Henckelia monophylla*, *H. pradeepiana*, *H. candida* Sirim., and *H. nakianensis* Sirim., J. Parn. & Hodk. in having only one large leaf but differs from *H. monophylla* in lacking a long rhizome character (*vs* present), scale-like leaf incompletely developed (*vs* fully developed), inflorescences mostly more than 2-flowered (*vs* strictly 2-flowered), broadly campanulate corolla tube, 5 mm long

(vs infundibuliform, 50–60 mm long). It is similar to *H. pradeepiana* in the petiolate leaf, campanulate flower, reniform anthers and persistent calyx, but differs in absence of a tuber (vs present), being caulescent (vs acaulescent), the hairy upper leaf surface (vs glabrous), the inflorescence $\frac{1}{2}$ – $\frac{3}{4}$ times the leaf length (vs 1–1 $\frac{1}{2}$ times), the elongate capsule (vs ovoid to subglobose). This species is also similar to *H. candida* and *H. nakianensis* but differs in the petiolate leaf and persistent calyx (Table 1).



Figure 1. Holotype of *H. campanuliflora* Sirim. Photograph: Derek Liew.

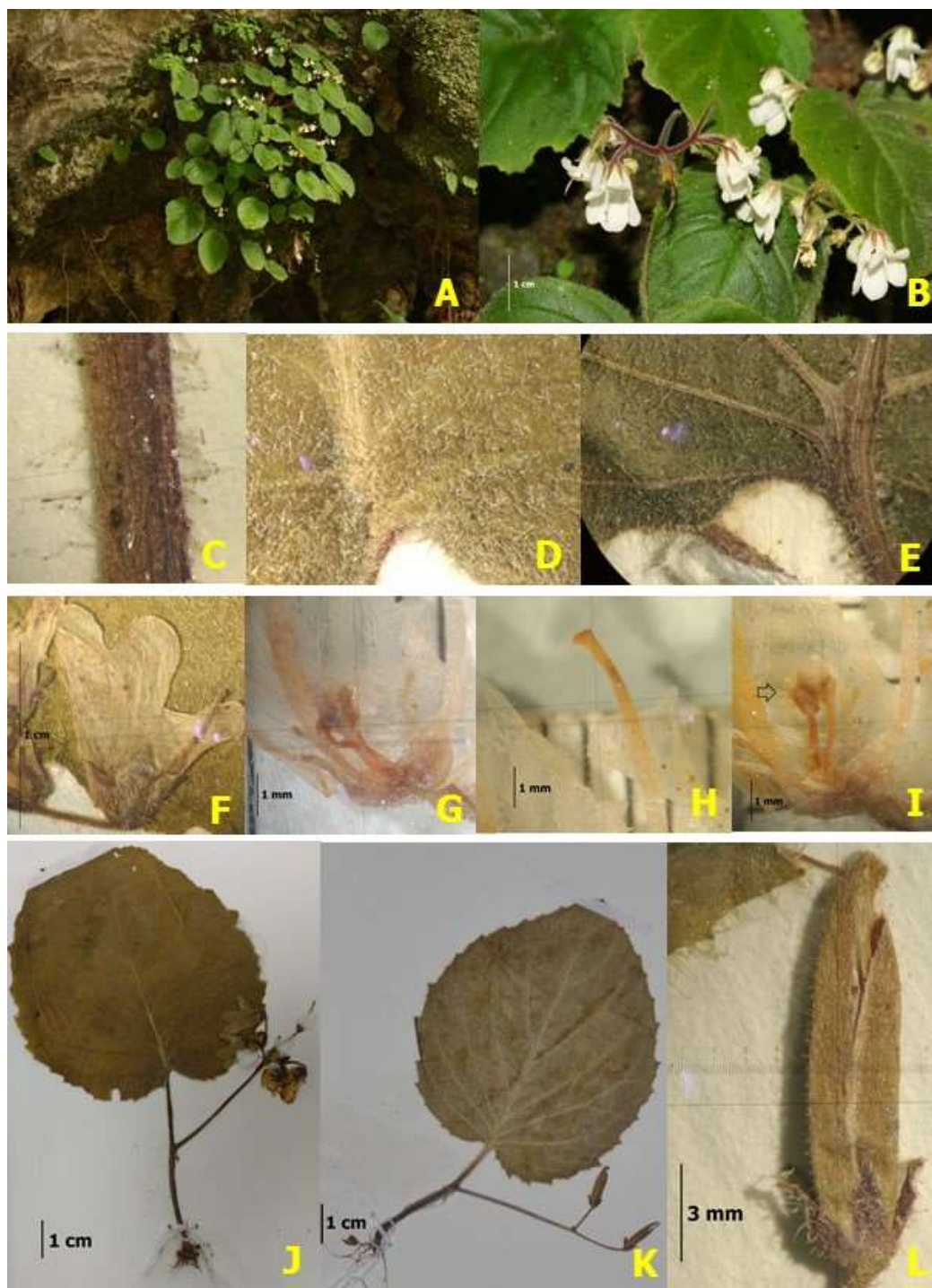


Figure 2. *H. campanuliflora* Sirim.: A. Habitat; B. Inflorescences; C. Glandular hair on pedicels; D. Upper leaf surface; E. Lower leaf surface; F. Flower; G. Ovary; H. Gynoecium, I. Anthers; J.–K. Habit; L. Fruit. Photographs: A.–B. by Michele Rodda; C.–L. by Sukontip Sirimongkol.

2. *Henckelia candida* Sirim. sp. nov. Type: Myanmar, Shan State, Kyauk Gu Taung, Paunglang Reserve Forest, Pinglong Township, 19°57'04.1"N, 96°38'40.3" E, alt. 395 m, fl. & fr., 12 Sept. 2015, Y. Baba, K. Kertsawang, C. Kilgour, C. Puglisi, M. Rodda, P. Srisanga, Thant Shin & Phyu Phyu Hnin 103111 (holotype SING!). Fig. 3.

Epilithic annual herb, 2.5–10 cm tall; stem sparsely multicellular and glandular hairs. *Leaf* symmetrically orbicular, elliptic or ovate, 4.3–8.5 by 3.4–6 cm, apex acute, obtuse or rounded, base cordate, margin dentate, upper and lower surfaces densely hairy, lateral veins 4–6 on each side of midrib; only one leaf developing, sessile. *Inflorescence* terminal, inserted at the junction of the leaf blade and stem, single or compound cymes, 1–17-flowered; peduncles terete, green, 1.2–2.5 cm long, with multicellular hairs and few glandular hairs; bracts 2, free, sessile, elliptic, 5.5 by 2.5 mm, apex acute, sparsely hairy, margin ciliate; pedicels terete, green, 1.1–3 cm long, with multicellular hairs and few glandular hairs. *Calyx* 5-partite, lobes narrowly triangular, basally connate, light green, 5 by 1.1 mm, hairy, margin entire, apex acuminate, not reflexed, caducous. *Corolla* white; tube campanulate, 5 mm long, sparsely hairy, corolla lobes 4 by 3 mm, glabrous. *Stamens* 2: filaments inserted 2 mm from the base of the corolla tube, 1 mm long, curved; anthers reniform, yellow, adaxial surfaces coherent, ca 1 by ca 0.8 mm; staminodes 3, inserted 2 mm from the base of the corolla tube, 0.2 mm long, glabrous. *Gynoecium* ca 6.5 mm; ovary 1.3 mm across, hairy; style terete, slender; stigma subpeltate, greenish, ca 0.5 by ca 0.4 mm. *Fruit* plagiocarpic, capsule elongate, green, 1.2–1.8 cm long, sparsely hairy. *Seeds* prolate, brown, 0.6 by 0.2 mm (Fig 4).

Myanmar. — Shan State: Lomkok mountain and pagoda, roadside, Taunggyi Township, 20°49'2.4" N 97°13'26.6" E, alt. 1,106 m, fl. & fr. 20 Sept. 2015, *Puglisi et al.* 103541 [MBK (MBK0272469), the two herbs on the right-hand side].

Distribution. — Only known from Shan State (Fig. 10).

Ecology. — Damp gully with karst limestone boulders along the edge of stream, shaded tall bamboo forest with dense layers of cryptogams and lithophytic plants or open semi-evergreen forest, on limestone, alt. 395–1,106 m, flowering & fruiting in September.

Etymology. — The name of this species refers to the white flower colour.

Provisional conservation assessment. — Endangered (EN B2ab(iii)). This species is known from two populations, only one of which is in a protected area. Another population is in a small patch of forest surrounded by agricultural land with very high risk of disturbance.

Notes. — This species is similar to *Henckelia monophylla*, *H. pradeepiana* and

H. nakianensis but differs from *H. monophylla* in lacking a long rhizome (vs present), having inflorescences mostly more than 2-flowered (vs always 2-flowered), the campanulate corolla tube ca 5 mm long (vs infundibuliform, 50–60 mm long), reniform anthers (vs elliptic), and horizontally held fruit (plagiocarpic) (vs in line with the pedicel). It is also similar to *H. pradeepiana* in the campanulate flower and reniform anthers but differs in absence of a tuber (vs present), being caulescent (vs acaulescent), the leaf being sessile (vs petiolate), upper leaf surface densely hairy (vs glabrous), inflorescence $\frac{1}{2}$ – $\frac{3}{4}$ times the subtending leaf length (vs 1–1 $\frac{1}{2}$ times the leaf length), calyx caducous (vs persistent), capsule plagiocarpic (vs orthocarpic) and capsule elongated (vs ovoid to subglobose). This species is also similar to *H. nakianensis* in having an inflorescence that is about $\frac{1}{2}$ times the subtending leaf length but differs, particularly, in the calyx that lacks of a strongly reflexed tip and in having 3 staminodes (vs 2) (Table 1).

Two doubtful specimens, *Srisanga et al.* 103884 (SING) and *Srisanga et al.* 103882 [MBK (MBK0272475)], are similar to this species in having one large sessile leaf. However, both specimens are in fruiting stage and differ slightly from *H. candida* in their indumentum and plant size, making them hard to confirm their identities. Further field study is necessary to assess such variation.



Figure 3. Holotype of *H. candida* Sirim. Photograph: Derek Liew.

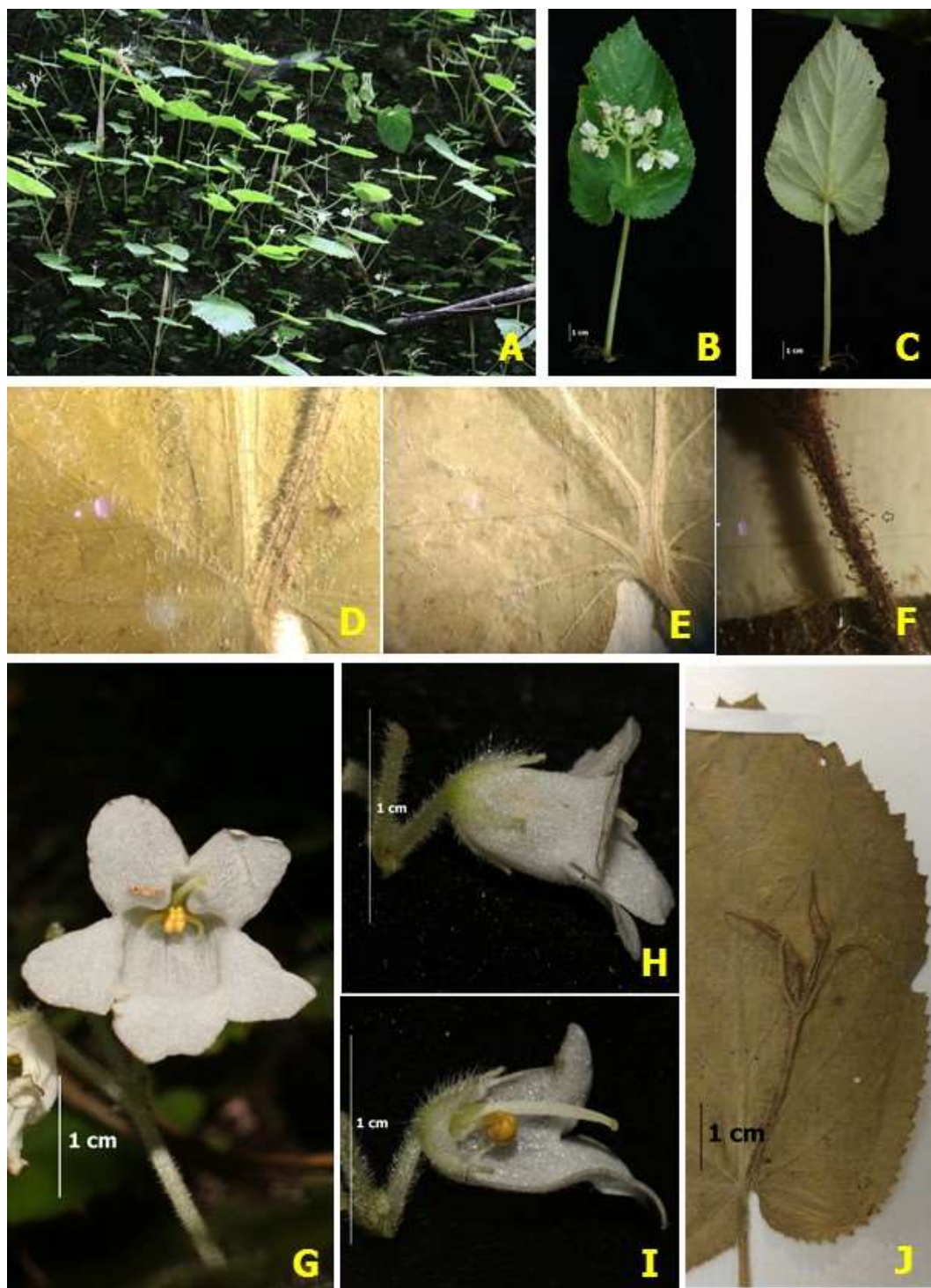


Figure 4. *Henckelia candida* Sirim.: A. Habitat; B.–C. Habit; D. Upper leaf surface; E. Lower leaf surface; F. Glandular hairs on pedicels; G.–I. Flowers; J. Fruits. Photographs: A.– C. & G.–I. by Michele Rodda; D.–F. & J. by Sukontip Sirimongkol.

3. *Henckelia nakianensis* Sirim., J. Parn. & Hodk., sp. nov. TYPE: Thailand, Chiang Mai, Om Koi District, Tambon Na Kian, Ban Mae Kong, north of the village near stream, alt. 950 m, fl. & fr. 27 Aug. 2015, *Wittaya Pongamornkul et al. 5110* [holotype QBG! (QBG85771), isotype BKF!]. Fig. 5.

Epiphytic herb, up to 15 cm tall; stem glabrous. *Leaf* symmetrically orbicular or cordate, 9–12 by 6–10 cm, herbaceous, apex acute, base cordate, margin remotely dentate, upper and lower surfaces nearly glabrous, but with sparse stout hairs on upper surface, up to 0.7 mm long, lateral veins 4–6 on each side of midrib; only one leaf developing, sessile. *Inflorescence* terminal, inserted at the junction of the leaf blade and stem, single or compound cymes, 2–10 flowered; peduncles terete, 1.8–3.5 cm long, with eglandular hairs; bracts 2, free, sessile, lanceolate, 5–17 by 0.1–6 mm, apex acute, hairy, margin dentate; pedicels terete, 1.2–3.5 cm long, multicellular and sparsely glandular hairs. *Calyx* 5-partite, lobes triangular, basally connate, 4.5 by 1.2–1.5 mm, apex acuminate, tip reflexed, hairy, margin entire, caducous. *Corolla* white-purplish, tube campanulate, 7 mm long, glabrous, corolla lobes purple, glabrous, 5 by 3–4 mm. *Stamens* 2: filaments inserted 3 mm from the base of the corolla tube, 1.5 mm long, straight; anthers reniform, adaxial surfaces coherent, yellow, glabrous, ca 1.4 by ca 1 mm; staminodes 2, inserted 3 mm from the base of the corolla tube, 0.2 mm long, glabrous. *Gynoecium* ca 9.5 mm, ovary 1 mm across, hairy; style terete, slender; stigma subpeltate, yellow. *Fruit* a plagiocarpic, elongate capsule, green, 1–2.5 by 0.1–0.2 cm, sparsely hairy. Seeds not seen (Fig. 6).

Distribution. — Only known from the type locality (Fig. 10).

Ecology. — On trees in dry evergreen forest, moist places near stream, ca 950 m alt., flowering & fruiting in August.

Vernacular name. — Dao noi.

Etymology. — The name of this species refers to the type locality.

Provisional conservation assessment. — Data Deficient (DD). This species is only known from the type collection from a rather remote and inaccessible site. The EOO and AOO are unknown.

Notes. — This species is similar to *Henckelia monophylla* and *H. pradeepiana* in having only one leaf, but differs from *H. monophylla* in lacking a long rhizome (*vs* present), in having the inflorescences being mostly more than 2-flowered (*vs* always 2-flowered), campanulate corolla tubes, ca 7 mm long (*vs* infundibuliform, 50–60 mm long), and the subpeltate stigma (*vs* bilobed). It differs from *H. pradeepiana* in being caulescent (*vs* acaulescent), lacking a tuber (present), having sessile leaf (*vs* petiolate), inflorescence shorter than the leaf length (*vs* longer than), caducous calyx

(vs persistent), plagiocarpic capsule (vs orthocarpic) and elongate (vs ovoid or subglobose). This species is also similar to *H. candida* in its inflorescence that is shorter than the subtending leaf but differs in the leaf length being more than 7 cm long (vs less than 7 cm long), reflexed calyx tip (vs not reflexed) and 3 staminodes (vs 2) (Table 1).

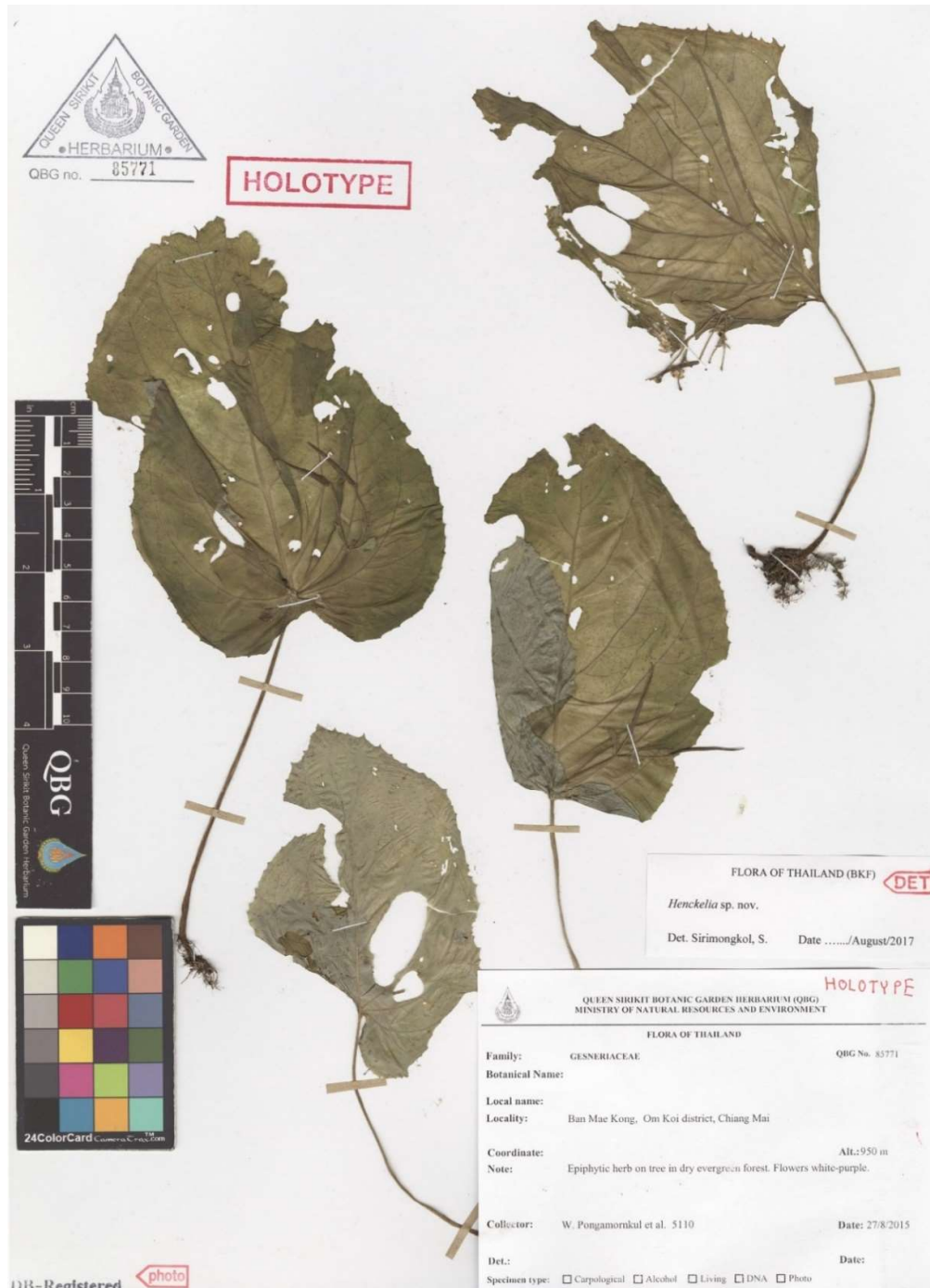


Figure 5. Holotype of *H. nakianensis* Sirim. Photograph: Susee Daoh.

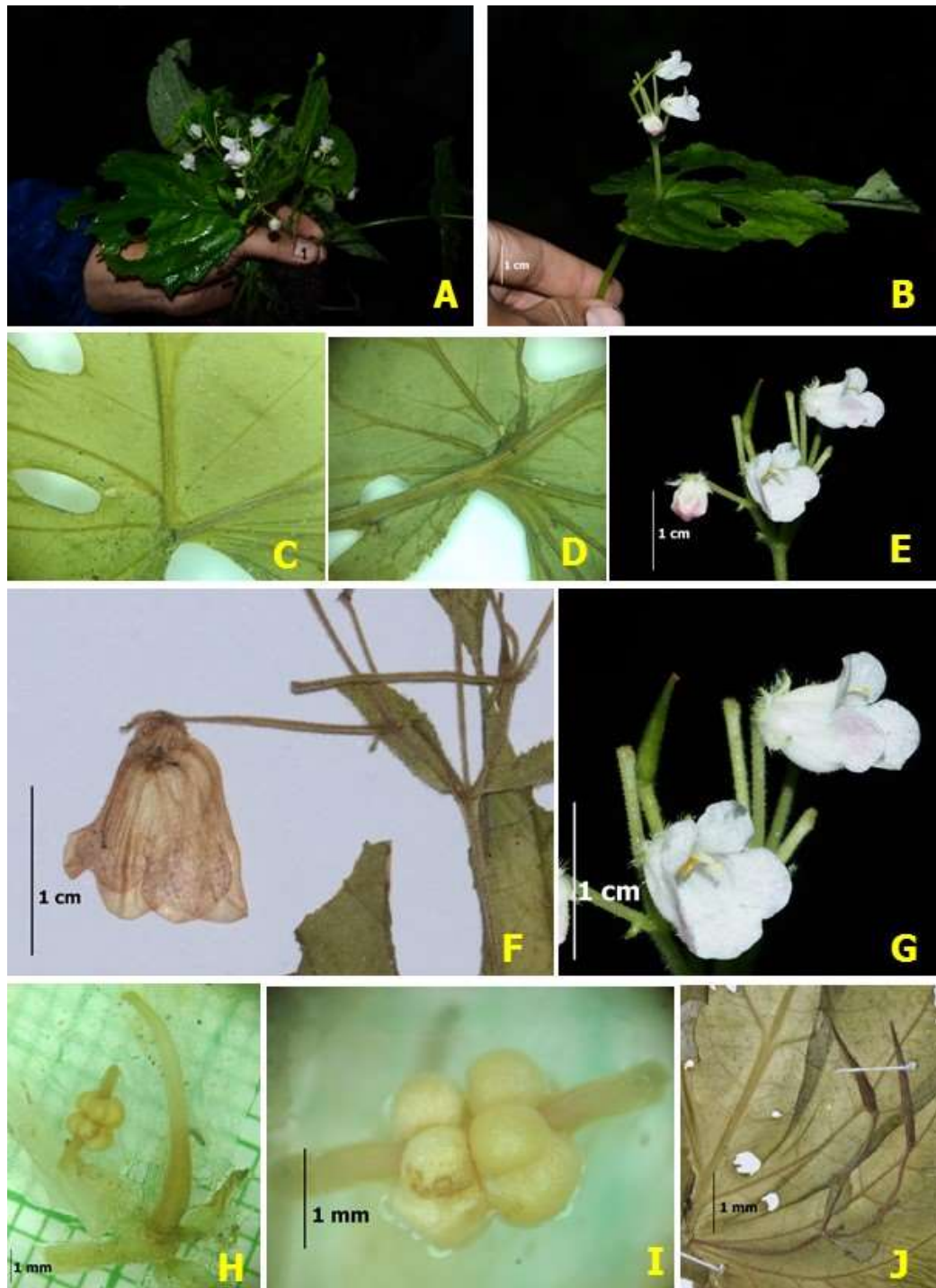


Figure 6. *Henckelia nakianensis* Sirim., J. Parn. & Hodk.: A.–B. Habit; C. Upper leaf surface; D. Lower leaf surface; E. Inflorescence; F.–G. Flowers; H. Gynoecium; I. Anthers; J. Fruits. Photographs: A.–B., E. & G. by Wittaya Pongamornkul; C.–D., F. & H.–J. by Sukontip Sirimongkol.

Table 1. Morphological differences between five *Henckelia* species; *H. monophylla*, *H. pradeepiana*, *H. campanuliflora*, *H. candida* and *H. nakianensis*. Data for *H. monophylla* and *H. pradeepiana* are adapted from Wood (1974) and Manudev *et al.* (2012), respectively.

Character	<i>H. monophylla</i>	<i>H. pradeepiana</i>	<i>H. campanuliflora</i>	<i>H. candida</i>	<i>H. nakianensis</i>
Rhizome	present	absent	absent	absent	absent
Tuber	absent	present	absent	absent	absent
Habit	perennial	perennial	annual	annual	annual
Stem	caulescent	acaulescent	caulescent	Caulescent	caulescent
Petiole	sessile	petiolate	petiolate	sessile	sessile
Leaf shape	ovate	broadly ovate, oblong-ovate, elliptic	orbicular	elliptic or ovate	remotely dentate
Leaf margin	serrate	serrulate	remotely dentate	dentate	remotely dentate
Upper leaf surface	glabrous	glabrous	hairy	densely hairy	sparsely stout hairs
Inflorescence	2-flowered	1–20-flowered	2–9-flowered	1–17-flowered	2–10-flowered
Calyx	caducous, tip not reflexed	persistent, tip not reflexed	persistent, tip not reflexed	caducous, tip not reflexed	caducous, tip reflexed
Corolla tube	infundibuliform	campanulate	campanulate	campanulate	campanulate
Tube length	50–60 mm	4-5 mm	ca 5 mm	ca 5 mm	ca 7 mm
Filament attachment	base of corolla tube	base of corolla tube	base of corolla tube	middle of corolla tube	middle of corolla tube
Filament length	ca 13 mm, geniculate	ca 4 mm, curved	ca 3 mm, curved	ca 1 mm, curved	ca 1.5 mm, curved
Anthers	elliptic	reniform	reniform	reniform	reniform

Table 1 (continued).

Character	<i>H. monophylla</i>	<i>H. pradeepiana</i>	<i>H. campanuliflora</i>	<i>H. candida</i>	<i>H. nakianensis</i>
Stamino-nodes	present, 2, 3 mm long, hairy	absent	present, 3, 0.4 mm long, glabrous	present, 3, 0.2 mm long, glabrous	present, 2, 0.2 mm long, glabrous
Infructescence length	about half of the leaf length	1–1½ times the length of subtending leaf	½–¾ times the length of subtending leaf	½–¾ times the length of subtending leaf	1/3–½ times the length of subtending leaf
Fruit (long)	Ca 90 mm, elongate, orthocarpic	6–8 mm, ovoid or subglobose, orthocarpic	10–12 mm, elongate, orthocarpic	12–18 mm, elongate, plagiocarpic	10–25 mm, elongate, plagiocarpic

4. *Henckelia amplexifolia* Sirim., sp. nov. Type: Thailand, Mae Hong Son, Muang District, Ban Nam Hu, road no 1–0003, km 20–21, 19°11'22.7"N, 98°04'12"E, alt. 800 m, fl., 12 July 2017, *Sukontip Sirimongkol, Ta Sapniyomphong & Chanthana Phongsasat 714* (holotype **BKF!** (BKF194734); isotypes **E!**, **K!**, **L!**, **M!**, **P!**, **SING!**, **TCD!**). Figs. 7-8.

Epilithic or terrestrial, succulent annual herb, up to 15 cm tall; stem light green, 0.2–0.5 cm diam. with hispid white hairs; bulbils subglobose up to 1 cm diam., light brown; stolon white, terete, up to 0.1 cm diam. with lanceolate scale leaves up to 0.2 cm long. *Leaves* symmetrically cordate or orbicular, 6.2–16 by 6–14 cm, herbaceous, base cordate and sometimes amplexicaul, apex obtuse or rounded, margin serrate, upper surface hairy, lower surface glabrous, lateral veins 4–10 on each side of midrib, only the first lateral vein ascending perpendicular to the midrib; usually two pairs of opposite leaves present, but some reduced such that there are only 1 or 2, rarely 3 or 4, full-sized leaves, sessile. *Inflorescence* terminal, single or compound cymes, rarely axillary, up to 2 inflorescences, 3–9-flowered; peduncles terete, light green, 2.3–5 by 0.2–0.3 cm, glandular hairs; bracts 2, free, sessile or linear, 0.5–7 by 1–15 mm, apex acute, hairy, margin ciliate. Pedicels terete, light green, 0.7–2 by 0.1 cm, with

multicellular and glandular hairs. *Calyx* 5-partite, lobes triangular, basally connate, light green, 6–6.3 by 1–1.4 mm, with multicellular and glandular hairs, margin entire, apex acute with acumen 2–3 mm long, caducous. *Corolla* white; tube campanulate, ca 5 mm long, hairy outside, corolla lobes 5–6 by 7–8 mm. *Stamens* 2: filaments inserted 2 mm from the base of the corolla tube, 3 mm long (appressed part 1.5 mm long), geniculate in the middle of the filament, hairy at the top; anthers reniform, yellow, adaxial surfaces coherent, 2.5 by 1.5 mm; staminodes 3, inserted 2 mm from the base of the corolla tube, 1 mm long, glabrous. *Gynoecium* ca 12–13 mm; ovary 1–1.5 mm across, with glandular hairs; style terete, slender; stigma subpeltate, greenish. *Fruit* plagiocarpic, green, 1.2–2 by 0.1 cm. Seeds not seen (Fig. 8).

Thailand. —NORTHERN: Mae Hong Son: Muang District, Ban Nam Hu, road no 1–0003, km 20–21, 19°11'22.7"N, 98°04'12"E, alt. 800 m, fr. 21 Sept. 2016, *Sirimongkol et al. 701* (BKF, E, K, P, TCD); *ibidem*, km 19–20, 19°11'23.2"N, 98°03'56.3"E, alt. 859 m, sterile, 12 July 2017, *Sirimongkol et al. 713* (BKF, TCD); Muang District, Mae Surin National Park, Road to Doi Pui, 19°11'22"N, 98°04'11"E, alt. 842 m, fl. & fr. absent, 21 Oct. 2014, *Middleton et al. 5813* [BKF, E (E00726602), SING].

Distribution. — Only known from Nam Tok Mae Surin, a National Park of Thanon Thong Chai Mountain Range (Fig. 10).

Ecology. — Mixed deciduous forest on limestone or bamboo forest, near streams, moist areas over conglomerate rocks or on muddy rocky bank, alt. 800–859 m, flowering in July, fruiting in September.

Vernacular name. — Dao pradap.

Etymology. — The name of this species refers to its amplexicaul leaf base.

Provisional conservation assessment. — Endangered (EN B1ab(iii), B2ab(iii)). This species is known from a small number of collections along a short stretch road in the National Park. However, this known population could be easily disturbed by any roadworks.

Notes. — This new species is similar to *H. bifolia* in having one large and one small leaves but differs in its distinct stolon (*vs* absent), the campanulate flowers (*vs* infundibuliform), the calyx apex with an acumen (*vs* without acumen), filaments inserted in the middle of the corolla tube (*vs* filament inserted near the base of the corolla tube), the apex of the filament with short hairs (*vs* with long hairs), glabrous staminodes (*vs* sparsely hairy), and the white flowers (*vs* purple) (Table 2); the most distinctive character is the stolon with bulbils. Field observations showed extremely high flower predation by insects such that fruit set was very low.



Figure 7. Holotype of *H. amplexifolia* Sirim. Photograph: Torsakul Nawail.



Figure 8. *Henckelia amplexifolia* Sirim.: A.–B. Habitat; C.–E. Habit; F.–G. Stolons; H.–I. Bulbils; J.–L. Inflorescences; M. Flower dissection; N. Gynoecium; O. Stamines; P. Fruit. Photographs: A.–P. by Sukontip Sirimongkol.

Table 2. Morphological comparison between *Henckelia bifolia* (from Wood, 1974) and *H. amplexifolia*.

Characters	<i>H. bifolia</i>	<i>H. amplexifolia</i>
Stolon	absent	present
Inflorescence	1, 1–2 (rarely 3)-flowered,	1–2, 3–9-flowered,
Calyx tip	without claw	with claw, 2–3 mm long
Corolla tube	infundibuliform, purple	campanulate, white
Tube length	30–50 mm	ca 5 mm
Filament inserted	nearly at the corolla tube base,	middle of the corolla tube
Filament length	10–12 mm, long hairs at the top	1.5 mm, short hairs at the top
Staminode	ca 3 mm long, hairy	ca 1 mm long, glabrous
Fruit	young fruit 2.7 cm long	mature fruit 1.2–2 cm long

5. *Henckelia dasycalyx* Sirim. & D.J. Middleton, sp. nov. Type: Thailand, Phitsanulok, Chat Trakan District, Namchuang, Phumiang–Phuthong Wildlife Sanctuary, 17 July 2012, alt. 782 m, fl. & fr., 15 July 2012, *Romklao Botanical Garden 0560/2555*, leg. Navin s.n. [holotype **QBG!** (QBG62273)]. Fig. 9

Terrestrial annual herb, 30 cm tall; stem with dense white multicellular hairs. *Leaves* opposite, symmetrically elliptic or ovate, each pair unequal in size, 11–14.4 by 5.2–9 cm, apex acute, base cuneate or sub-oblique, margin crenate (sometimes dentate), upper and lower surfaces densely hairy, lateral veins 8–9 on each side of midrib. *Petiole* terete, 3–8 cm long, densely hairy. *Inflorescences* axillary, 7 on a plant, single or compound cymes, 1–9-flowered; peduncles terete, 3–6.3 cm long, densely hairy; bracts 2, free, sessile, elliptic, 5.5 by 2.5 mm, apex acute, sparsely hairy, margin ciliate; pedicels terete, 0.5–1.8 cm long, densely hairy. *Calyx* 5-partite, lobes narrowly triangular, basally connate, ca 11 by 1 mm, densely hairy, margin entire, apex acuminate, persistent. *Corolla* purple; tube infundibuliform, 40 mm long, with glandular hairs. *Stamens* 2: filaments inserted at 1.5–1.9 cm from the base of the corolla tube, 1.5 cm long, geniculate; anthers elliptic, adaxial surfaces coherent, 2 mm long; staminodes 2, inserted 1.5 cm from the base of the corolla tube, 3 mm long, glabrous. *Gynoecium* ca 3.6 cm long; ovary 1 mm across, hairy; style terete, slender; stigma bilobed. *Fruit* orthocarpic, fusiform, 3.5–5.5 cm long, densely hairy. Seeds not seen (Fig. 9).

Distribution. — Only known from the type locality (Fig. 10).

Ecology. — In deciduous forest, flowering & fruiting in July

Vernacular name. —Muang tra kan.

Etymology. — The name of this species refers to its hairy calyx.

Provisional conservation assessment. — Data Deficient (DD). This species is only known from the type collection which is located within a protected area. The size of the population, the EOO and AOO are all unknown.

Notes. — This species is similar to *H. oblongifolia* in the distinct axillary inflorescence with 2–4 pairs, and the persistent calyx but differs in the annual habit (vs perennial), the compact flower arrangement (vs loosely arranged), calyx tube basally connate (vs campanulate), the lanceolate calyx lobes (vs triangular), the persistent calyx covered with long hairs (vs short hairs), and slender fusiform fruit (vs oblong) (Table 3).

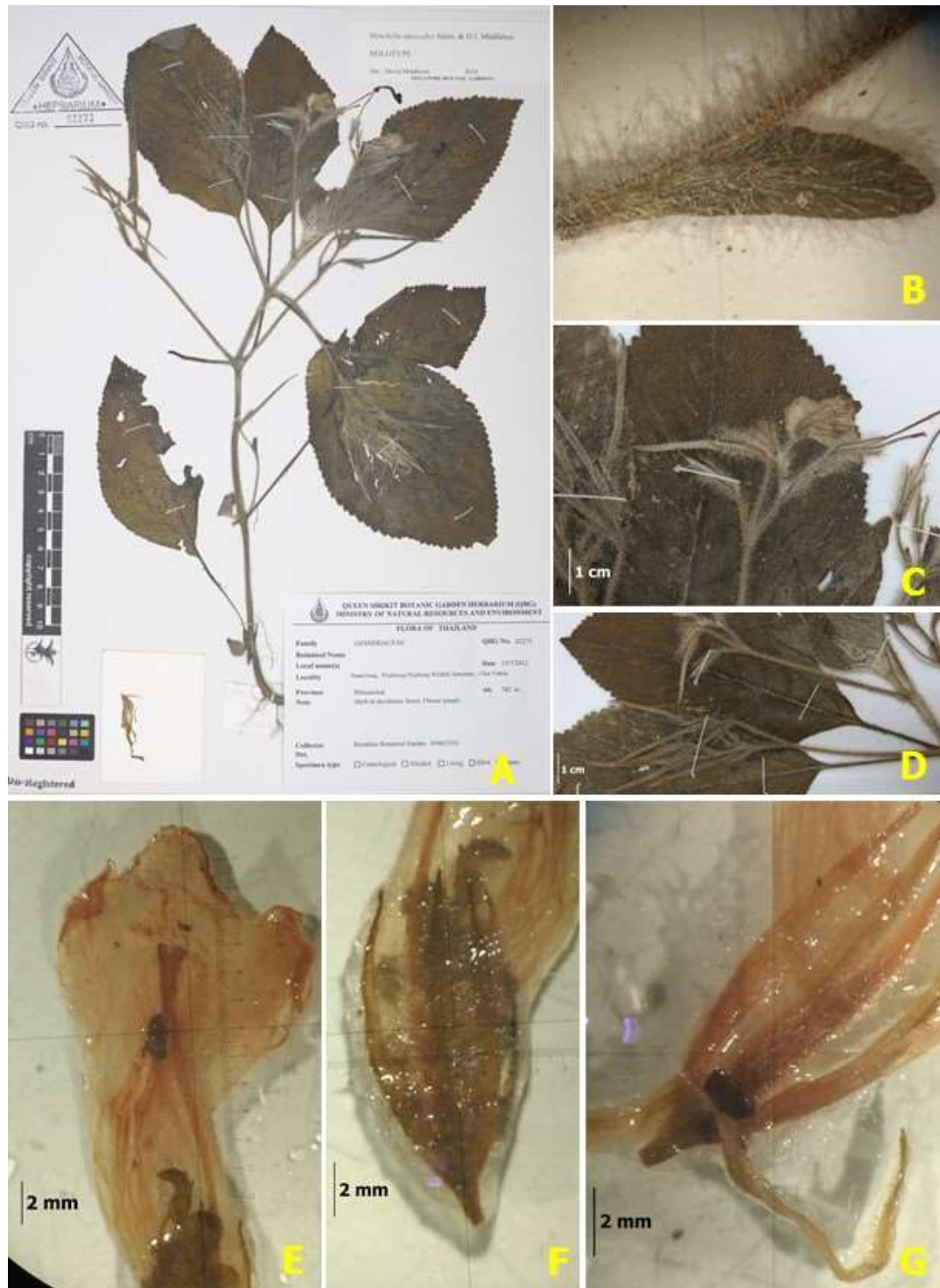


Figure 9. *Henckelia dasycalyx* Sirim. & D.J. Middleton.: A. Habit; B. Hairs on bract; C.–D. Infructescences; E. Flower; F.–G. Calyx. Photographs: A. by Derek Liew.; B–G. by Sukontip Sirimongkol.

Table 3. Morphological comparison between *Henckelia oblongifolia* (from Wood, 1974) and *H. dasycalyx*.

Characters	<i>H. oblongifolia</i>	<i>H. dasycalyx</i>
Habit	perennial herb, up to 90 cm tall.	annual herb, up to 30 cm tall.
Leaf	ovate, base strongly oblique, leaf covered with small brown glands.	elliptic or ovate, base weakly oblique, leaf without brown glands.
Inflorescence	loose, up to 12-flowered.	dense, up to 9-flowered.
Calyx	tube campanulate, lobes triangular, hairs shorter than calyx lobe	basally connate, lobes lanceolate, hairs equal or longer than calyx lobe.
Fruit	up to 9 cm long, oblong	up to 5.5 cm long, fusiform

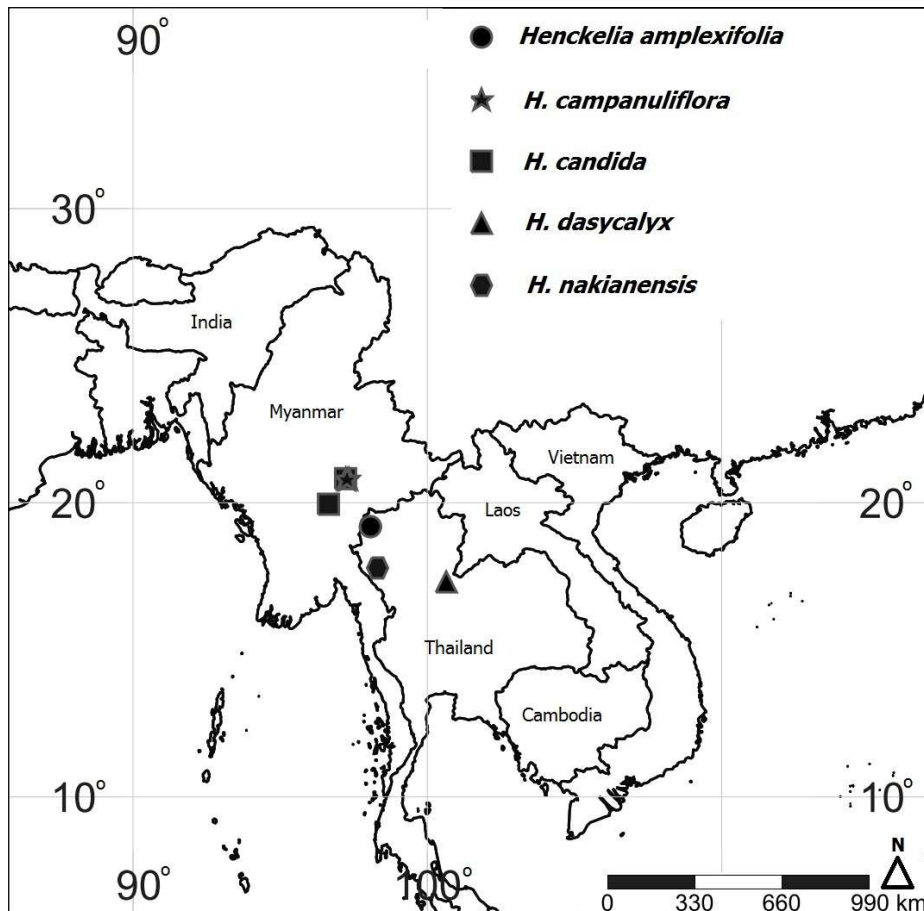


Figure 5.10. Distribution of the new species of *Henckelia* from Myanmar and Thailand.

Base map from <https://www.simplemappr.net>

Appendix 2.1 Morphological data matrix of *Henckelia* (characters C1-C49).

Taxon/Character	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
<i>H. adenocalyx</i>	1	0	0	1	0	0	1	0	0	1
<i>H. amplexifolia</i>	0	0	0	1	1	1	0	0	0	1
<i>H. anachoreta</i>	0	0	0	1	0	0	1	1	0	1
<i>H. burtii</i>	1	0	1	1	0	0	1	1	1	1
<i>H. calva</i>	0	0	0	1	0	0	0	0	0	1
<i>H. campanulifolia</i>	0	0	0	1	0	0	1	0	0	0
<i>H. candida</i>	0	0	0	1	0	1	0	0	0	0
<i>H. ceratoscyphus</i>	1	1	0	0	0	0	1	1	0	1
<i>H. dasycalyx</i>	0	0	0	1	0	0	1	1	0	1
<i>H. fruiticola</i>	1	1	0	0	0	0	1	1	1	1
<i>H. grandifolia</i>	1	1	0	1	0	0	1	1	1	1
<i>H. heterostigma</i>	1	1	0	1	0	0	1	1	0	1
<i>H. insignis</i>	1	0	0	0	0	0	1	1	0	1
<i>H. lacei</i>	1	1	0	1	0	0	1	0	0	1
<i>H. lachenensis</i>	1	1	1	0	0	0	1	1	1	1
<i>H. longipedicellata</i>	1	0	0	1	0	0	1	0	0	1
<i>H. longisepala</i>	0	0	0	1	0	0	1	0	0	1
<i>H. nakianensis</i>	0	0	0	1	0	1	0	0	0	0
<i>H. oblongifolia</i>	1	0	0	1	0	0	1	0	0	1
<i>H. peduncularis</i>	1	1	0	1	0	0	1	0	0	1
<i>H. pumila</i>	0	0	0	1	0	0	1	0	0	1
<i>H. rotundata</i>	1	1	0	1	0	0	1	0	0	1
<i>H. speciosa</i>	1	1	0	0	0	0	1	1	1	1
<i>H. urticifolia</i>	1	0	0	1	0	0	1	0	0	1
Taxon/Character	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20
<i>H. adenocalyx</i>	0	0	0	1	0	0	0	1	0	1
<i>H. amplexifolia</i>	0	0	0	1	0	0	0	1	1	1
<i>H. anachoreta</i>	0	1	0	0	0	0	0	1	0	1
<i>H. burtii</i>	1	0	0	0	1	0	0	1	0	1
<i>H. calva</i>	0	0	1	0	0	0	0	0	0	0
<i>H. campanulifolia</i>	0	0	0	0	0	0	0	1	1	1
<i>H. candida</i>	0	1	0	0	0	0	0	1	1	1
<i>H. ceratoscyphus</i>	1	0	1	1	0	0	0	1	0	1
<i>H. dasycalyx</i>	0	0	0	0	0	0	1	1	0	1
<i>H. fruiticola</i>	1	0	0	1	0	0	0	1	0	1
<i>H. grandifolia</i>	1	0	0	0	0	0	0	0	0	1
<i>H. heterostigma</i>	0	0	0	0	0	1	0	0	0	1
<i>H. insignis</i>	0	0	0	0	0	1	0	0	0	1
<i>H. lacei</i>	0	0	0	0	0	0	0	1	0	1
<i>H. lachenensis</i>	1	0	0	1	0	0	0	1	0	1

Appendix 2.1 (continued)

Taxon/Character	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20
<i>H. longipedicellata</i>	0	0	0	0	0	0	0	1	0	1
<i>H. longisepala</i>	0	0	0	0	0	0	0	1	0	0
<i>H. nakianensis</i>	0	0	1	0	0	0	0	1	1	1
<i>H. oblongifolia</i>	0	0	1	0	0	0	0	1	0	1
<i>H. peduncularis</i>	0	0	1	0	0	0	0	0	0	1
<i>H. pumila</i>	0	1	0	0	0	0	0	1	0	1
<i>H. rotundata</i>	0	0	0	0	0	0	0	1	1	0
<i>H. speciosa</i>	1	1	0	0	0	0	0	0	0	1
<i>H. urticifolia</i>	0	1	0	0	0	0	0	0	0	1
Taxon/Character	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30
<i>H. adenocalyx</i>	0	1	0	1	1	0	0	0	0	0
<i>H. amplexifolia</i>	0	0	0	1	1	0	1	1	1	0
<i>H. anachoreta</i>	0	0	0	1	1	0	0	0	0	0
<i>H. burtii</i>	0	0	0	1	1	0	0	0	0	0
<i>H. calva</i>	0	0	0	0	0	0	0	0	0	0
<i>H. campanulifolia</i>	0	0	0	1	1	0	1	1	0	0
<i>H. candida</i>	0	0	0	1	1	0	1	1	0	0
<i>H. ceratoscyphus</i>	0	0	0	1	1	0	0	0	1	0
<i>H. dasycalyx</i>	0	0	0	1	1	0	1	1	0	0
<i>H. fruticola</i>	0	1	0	1	0	1	0	0	0	0
<i>H. grandifolia</i>	0	0	0	1	1	1	0	0	0	0
<i>H. heterostigma</i>	0	0	1	1	1	0	0	0	0	0
<i>H. insignis</i>	0	0	0	1	1	0	0	0	0	0
<i>H. lacei</i>	1	0	0	1	1	0	0	0	0	0
<i>H. lachenensis</i>	0	0	0	1	1	0	0	0	0	0
<i>H. longipedicellata</i>	0	0	0	0	1	1	0	0	0	0
<i>H. longisepala</i>	0	0	0	0	1	0	1	0	0	1
<i>H. nakianensis</i>	0	0	0	1	1	0	1	1	1	0
<i>H. oblongifolia</i>	0	0	0	1	1	0	0	0	0	0
<i>H. peduncularis</i>	0	1	0	0	1	0	0	0	0	0
<i>H. pumila</i>	0	0	0	1	1	0	0	0	0	0
<i>H. rotundata</i>	0	0	0	0	0	0	0	0	0	0
<i>H. speciosa</i>	0	0	0	1	1	1	0	0	0	0
<i>H. urticifolia</i>	0	0	0	1	1	0	0	0	0	0
Taxon/Character	C31	C32	C33	C34	C35	C36	C37	C38	C39	C40
<i>H. adenocalyx</i>	0	1	0	0	0	1	1	0	0	0
<i>H. amplexifolia</i>	0	1	0	1	0	0	1	0	1	0
<i>H. anachoreta</i>	0	0	0	0	0	0	0	0	0	0
<i>H. burtii</i>	0	1	0	0	0	1	1	0	0	0
<i>H. calva</i>	0	1	0	0	0	1	0	1	0	0
<i>H. campanulifolia</i>	0	1	0	1	0	0	0	1	1	0
<i>H. candida</i>	0	1	0	1	0	0	0	1	1	0

Appendix 2.1 (continued)

Taxon/Character	C31	C32	C33	C34	C35	C36	C37	C38	C39	C40
<i>H. ceratoscyphus</i>	0	1	0	0	0	0	1	0	0	0
<i>H. dasycalyx</i>	0	1	1	0	0	0	0	0	0	0
<i>H. fruiticola</i>	0	1	0	0	0	0	1	0	0	0
<i>H. grandifolia</i>	0	0	0	0	0	0	1	0	0	0
<i>H. heterostigma</i>	0	1	0	0	1	0	0	0	0	0
<i>H. insignis</i>	1	1	0	0	0	0	1	0	0	0
<i>H. lacei</i>	0	1	1	0	1	0	0	1	0	0
<i>H. lachenensis</i>	0	1	0	0	0	0	1	0	0	0
<i>H. longipedicellata</i>	0	1	0	0	0	0	1	0	0	0
<i>H. longisepala</i>	0	0	0	0	0	0	1	0	0	0
<i>H. nakianensis</i>	0	1	0	0	0	0	0	0	1	0
<i>H. oblongifolia</i>	0	1	1	0	1	0	0	0	0	0
<i>H. peduncularis</i>	0	1	0	0	1	0	0	0	0	0
<i>H. pumila</i>	0	1	0	0	0	0	0	0	0	0
<i>H. rotundata</i>	0	0	0	0	0	0	0	0	0	0
<i>H. speciosa</i>	0	1	0	0	0	0	1	0	0	1
<i>H. urticifolia</i>	0	1	0	0	1	0	1	0	0	0
Taxon/Character	C41	C42	C43	C44	C45	C46	C47	C48	C49	
<i>H. adenocalyx</i>	0	0	0	1	0	1	0	0	0	
<i>H. amplexifolia</i>	1	0	1	1	1	0	0	1	1	
<i>H. anachoreta</i>	0	0	0	1	0	1	0	0	0	
<i>H. burtii</i>	0	0	0	1	0	1	0	0	0	
<i>H. calva</i>	0	0	0	0	0	1	0	0	0	
<i>H. campanulifolia</i>	1	0	0	1	1	0	0	1	0	
<i>H. candida</i>	1	0	0	1	1	0	0	1	1	
<i>H. ceratoscyphus</i>	0	0	0	1	0	1	0	0	0	
<i>H. dasycalyx</i>	0	0	0	1	0	1	0	0	0	
<i>H. fruiticola</i>	0	0	0	1	0	1	0	0	0	
<i>H. grandifolia</i>	0	0	0	1	0	1	0	0	0	
<i>H. heterostigma</i>	0	0	1	1	0	0	1	0	0	
<i>H. insignis</i>	0	0	0	0	0	1	0	0	0	
<i>H. lacei</i>	0	0	0	0	0	0	0	1	0	
<i>H. lachenensis</i>	0	0	0	0	0	1	0	0	0	
<i>H. longipedicellata</i>	0	0	0	0	0	1	0	0	0	
<i>H. longisepala</i>	0	0	0	0	0	0	1	0	0	
<i>H. nakianensis</i>	0	0	0	1	0	0	0	1	1	
<i>H. oblongifolia</i>	0	0	0	1	0	0	1	0	0	
<i>H. peduncularis</i>	0	0	0	0	0	1	0	0	0	
<i>H. pumila</i>	0	0	0	0	0	1	0	0	0	
<i>H. rotundata</i>	0	0	0	0	0	1	0	0	0	
<i>H. speciosa</i>	0	1	0	1	0	1	0	0	0	
<i>H. urticifolia</i>	0	0	0	1	0	1	0	0	0	

Appendix 3.1 DNA extraction.

The total genomic DNA was extracted by a modified hot 2xCTAB method (Doyle & Doyle, 1987; Hodkinson et al., 2007) as follows per each sample.

- 1) Preheat a water bath to 65°C with mortar and pestle and incubate for about 20 min.
- 2) Label a 12 ml polypropylene centrifuge tube and prepare the dry leaf material about 0.01-0.1 g.
- 3) Add a PVP 0.11 g, 5 ml of 2xCTAB extraction buffer (CTAB 20 g, 1M Tris pH 8 100 ml, 0.25M EDTA 80 ml, 5M NaCl 280 ml, make up to 1 liter with ultrapure water) and 20 µl mercapto-ethanol in a labelled tube and incubated in water bath about 10 min.
- 4) Grind leaf material in chilled mortar and pestle in a fume hood and add a small amount of extraction buffer and swirl to mix until the leaf grinds well.
- 5) Add the remaining extraction buffer, swirl to mix and pour back to the same label tube and cover with a lid.
- 6) Transfer the tube to the water bath at 65°C and incubate for at least 10 min with occasional swirling.
- 7) Add CI (24:1 Chloroform: isoamyl alcohol) 5 ml, shake well in horizontal mixer 250 rpm/ 30 mins then centrifuge 4,000 rpm/10 mins. In this step the material will separate in 3 layers i.e. the bottom is CI, the middle is plant material and the upper part is DNA.
- 8) Remove aqueous phase with a transfer pipette into a label 50 ml conical-base tube with an equal volume of cold isopropanol and gently mixed.
- 9) Place the tube into the -20°C freezer overnight or longer to further precipitate DNA.
- 10) The sample from the freezer was centrifuged at 2000 rpm/ 5 mins to pelletise the DNA.
- 11) Remove the supernatant and add 1.5 ml of 70% ethanol alcohol to wash the DNA.
- 12) Gently mix the sample and centrifuge at 2000 rpm/ 3 mins then remove the supernatant.
- 13) Gently place the tube upside down on a tissue paper to let the wash buffer away then place in the fume hood to let the pellet dry. The dried pellet was resuspended in 0.5 ml of TE buffer [10 mM Tris-Hydrochloric acid (pH 8.0) and 1 mM EDTA-Na₂] and transfer to 1.5 ml centrifuge tube. Store the sample into the -20°C freezer until required.
- 14) The quality of DNA was estimated by electrophoresis on 1.2 % agarose gel with DNA ladder (Thermo #SM0333, 100-10000 bp) in 1xTBE buffer (89 mM Tris, 89 mM boric acid and 2 mM EDTA-Na₂). The visualization used a fluorescent dye (GelRed).
- 15) The quantity and quality of DNA also can estimated from the Nanodrop lite UV spectrophotometer.

Appendix 3.2 DNA purification by JET Quick Spin Column technique per each sample.

- 1) Add DNA 100 μl and H1 400 μl into a JET quick spin column tubes set then centrifuge the tube at 12,000 rpm/ 1 min and remove liquid.
- 3) Add H2 500 μl into the same tube then centrifuge at 12,000 rpm/ 1 min and remove liquid.
- 4) Recentrifuge at 12,000 rpm/ 1 mins and replace the lower tube to 1.5 ml centrifuge tube.
- 5) Add a warm TE 50 μl and centrifuge at 12,000 rpm/ 2 mins. The pure DNA with TE buffer remains in the 1.5 ml centrifuge tube.

Appendix 3.3 Gel electrophoresis.

1. Preparation of the Gel

- Weigh out the agarose 1.2 g into an Erlenmeyer flask.
- Add in 10 percent of TE buffer and mixed well (TE=40 mM Tris-acetate, 1 mM EDTA, 45 mM Tris-borate, 1 mM EDTA).
- Melt agarose buffer with container flask by heating in the microwave at 30 seconds and swirl. Repeat it until the agarose gel has completely dissolved.
- Add 5 ml of 0.5 $\mu\text{g/ml}$ GelRed into 80 ml of agarose gel in the flask and mixed well. Then put in the gel tray with combs and taped both sides of the gel tray. This process was working in the fume hood.
- Let the gel set and cool down in the fume hood. It takes approximately 30 minutes.
- Remove the combs and place the gel tray into the gel box.

2. Setting up of Gel Apparatus and Separation of DNA Fragments.

- Mixed well 2 ml of loading dye and 3-5 ml of DNA sample (2-3 ml for PCR product).
- Load the mixer in the gel. Loading the 5 ml of gel ladder into the first line of gel tray and continue with the mixer.
- Turn on the power supply of gel box about 120 volts.
- Running gel was approximately 45 mins the turnoff.

3. Observing Separated DNA fragments

- Remove the gel tray from the gel box and drained.
- Remove the gel from the gel tray and expose the gel to UV light machine (transilluminator).
- Check DNA quality using the 1Kbp gel ladder to compare.

Appendix 3.4 PCR amplification per each sample and sequencing.

- 1) PCR was carried out with Biomix buffer, 12.50 μl , water 11.00 μl , Forward primer 0.25 μl , Reverse primer 0.25 μl and total DNA 1.00 μl , in a total 25 μl per reaction

- 2) Each component was added to a 200 μ l PCR tube on ice.
- 3) Sample was mixed well and placed in a thermal cycler (Veriti 96 Well, Applied Biosystems, #9902) with preheated lid and using specific program listed below (Table 3.1 and 3.2).

Appendix 3.5 PCR amplification programs used for each molecular marker and to clean the PCR product.

Region or Process	PCR Program
ITS	1.30 min. at 95°C followed by 30 cycles of 45 sec. denaturation (95°C), 1 min. annealing (54°C), and 1 min. extension (72°C). Final extended by 7 min. at 72°C. Then the temperature was kept at 4°C. Store the component at -20°C.
<i>trnL-trnF</i>	1.30 min. at 94°C followed by 30 cycles of 30 sec. denaturation (94°C), 1 min. annealing (56°C), and 1 min. extension (72°C). Final extended by 7 min. at 72°C. Then the temperature was keep at 4°C. Store the component at -20°C.
<i>atpB-rbcL</i>	3 min. at 94°C followed by 32 cycles of 45 sec. denaturation (94°C), 45 sec. annealing (52°C), and 2 min. extension (72°C). Final extended by 7 min. at 72°C. Then the temperature was keep at 4°C. Store the component at -20°C.
<i>Rps16</i>	1 min. at 95°C followed by 35 cycles of 45 sec. denaturation (95°C), 45 sec. annealing (64°C), and 2 min. extension (72°C). Final extended by 7 min. at 72°C. Then the temperature was kept at 4°C. Stored the component at -20°C.
ExoSap	30 min. at 37°C followed by 20 mins. at 82°C for cleaning PCR product. After that the temperature was kept at 4°C. Stored the component at -20°C.

Appendix 3.6 The cleaning of PCR by Exosap protocol.

- 1) PCR product (5 μ l) was mixed with the master mix [Exonuclease (20 unit/ μ l) 0.3 μ l, alkaline phosphate (1 unit/ μ l) 2.0 μ l, water 2.7 μ l] into a labelled PCR tube.
- 2) Place the sample into a thermo cycler (Veriti 96 well, Applied Biosystems, #9902) with preheated lid and using a specific program listed below (Table 3.1).
- 4) After that, check DNA concentration by Nanodrop lite machine then sequencing

process.

Appendix 3.7 Checking DNA concentration by Nanodrop Lite machine.

- 1) Turn on the Nanodrop Lite machine. The screen will show Home, then select dsDNA.
- 2) Measure blank 2 times by 1 μ l of autoclaved water and clean stage every time after use.
- 3) Drop 1 μ l of PCR product on stage then close and measure.
- 4) The screen will show the DNA concentration in ng/ μ l.
- 5) Then clean the stage and turn off the machine.

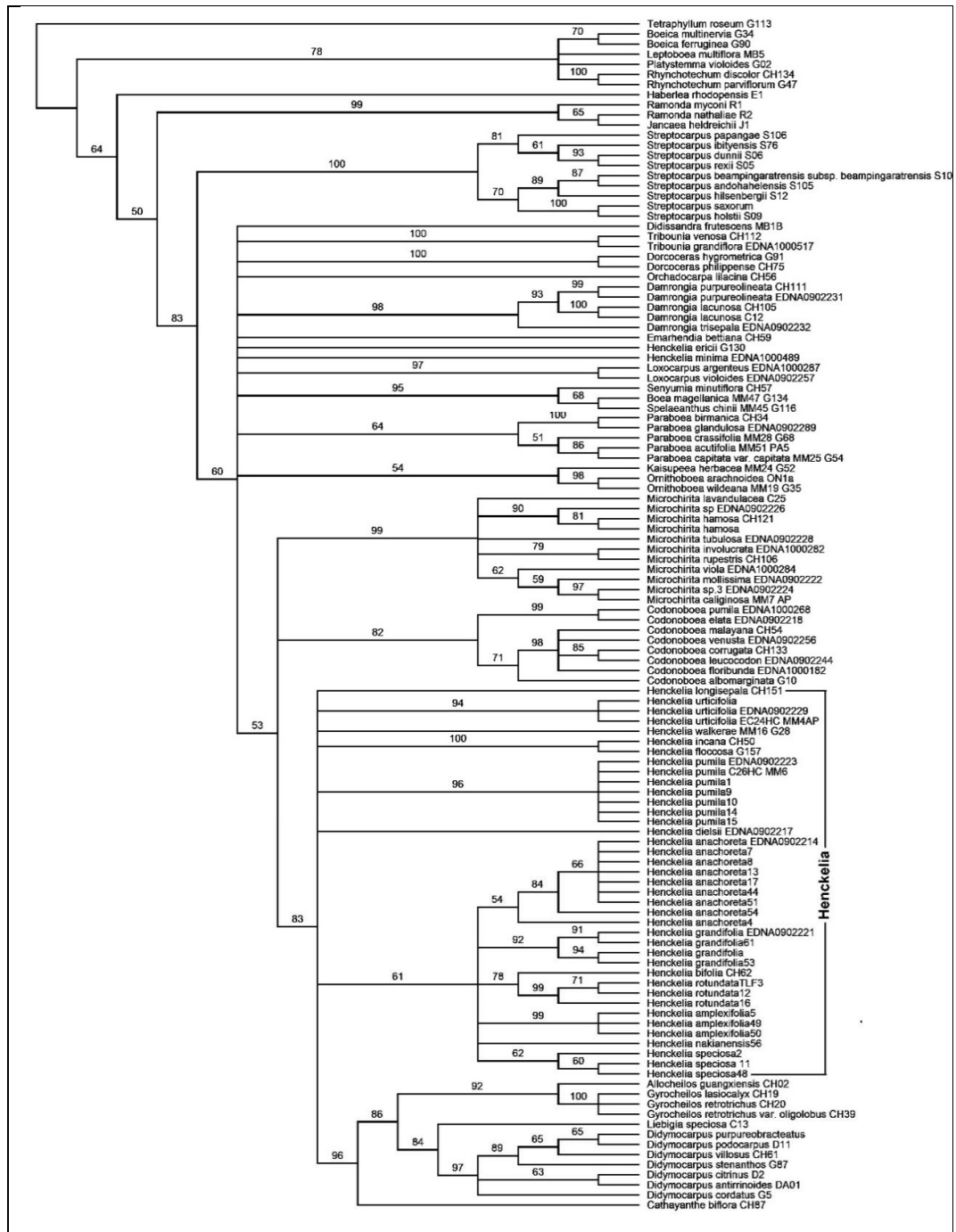
Appendix 3.8 List of PCR primers used in this study.

Type	Regions	Primer sequences (5' to 3')
Nuclear	ITS	ITS_AB101=5'-ACG AAT TCA TGG TCC GGT GAA GTG TTC-3' ITS_AB102=5'-TAG AAT TCC CCG GTT CGC TCG CCG TTA-3'
Plastid	<i>trnL-trnF</i>	<i>trnL-C</i> =5'-CGA AAT CGG TAG ACG CTA CG-3' <i>trnL-D</i> =5'-ATT TGA ACT GGT GAC ACG AG-3'
Plastid	<i>atpB-rbcL</i>	<i>atpB-rbcL</i> _2R=5'-GAA GTA GTA GGA TTG ATT CTC -3' <i>atpB-rbcL</i> _1R=5'-GTT TCT GTT TGT GGT GAC AT -3'
Plastid	<i>rps16</i>	<i>rps_16F</i> = 5'-GTG GTA GAA AGC AAC GTG CGA CTT-3' <i>rps_2R</i> = 5'-TCG GGA TCG AAC ATC AAT TGC AAC-3'

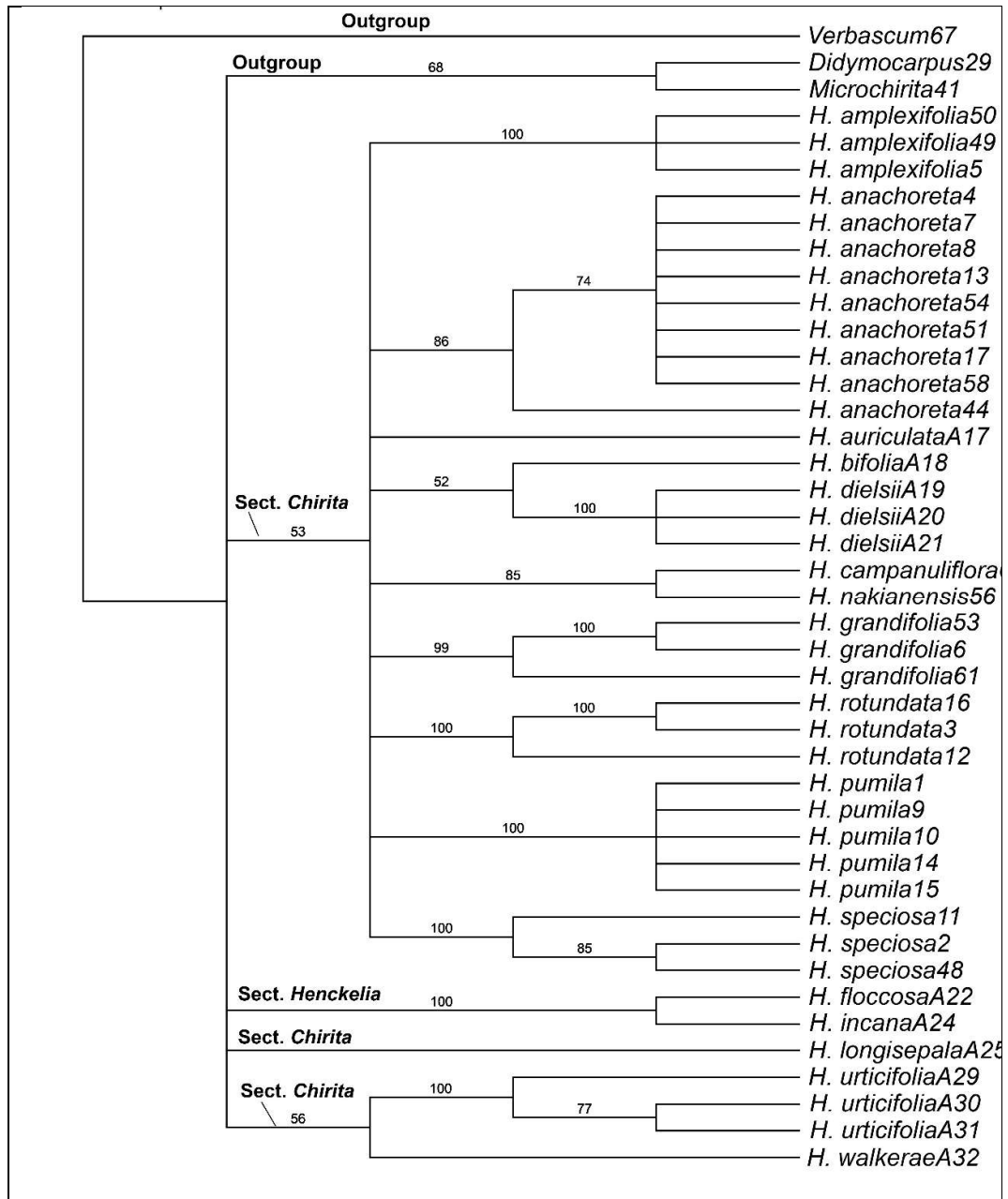
Appendix 3.9 Preparing PCR and sequencing

We sent the PCR product to the Source BioScience company for sequencing. The PCR product needed to have been checked that there is a clear band on the PCR with a gel. Then clean with Exosap and check the concentrate with the Nanodrop Lite machine before doing the dilution for the sequencing company. The samples are load as follow steps. the

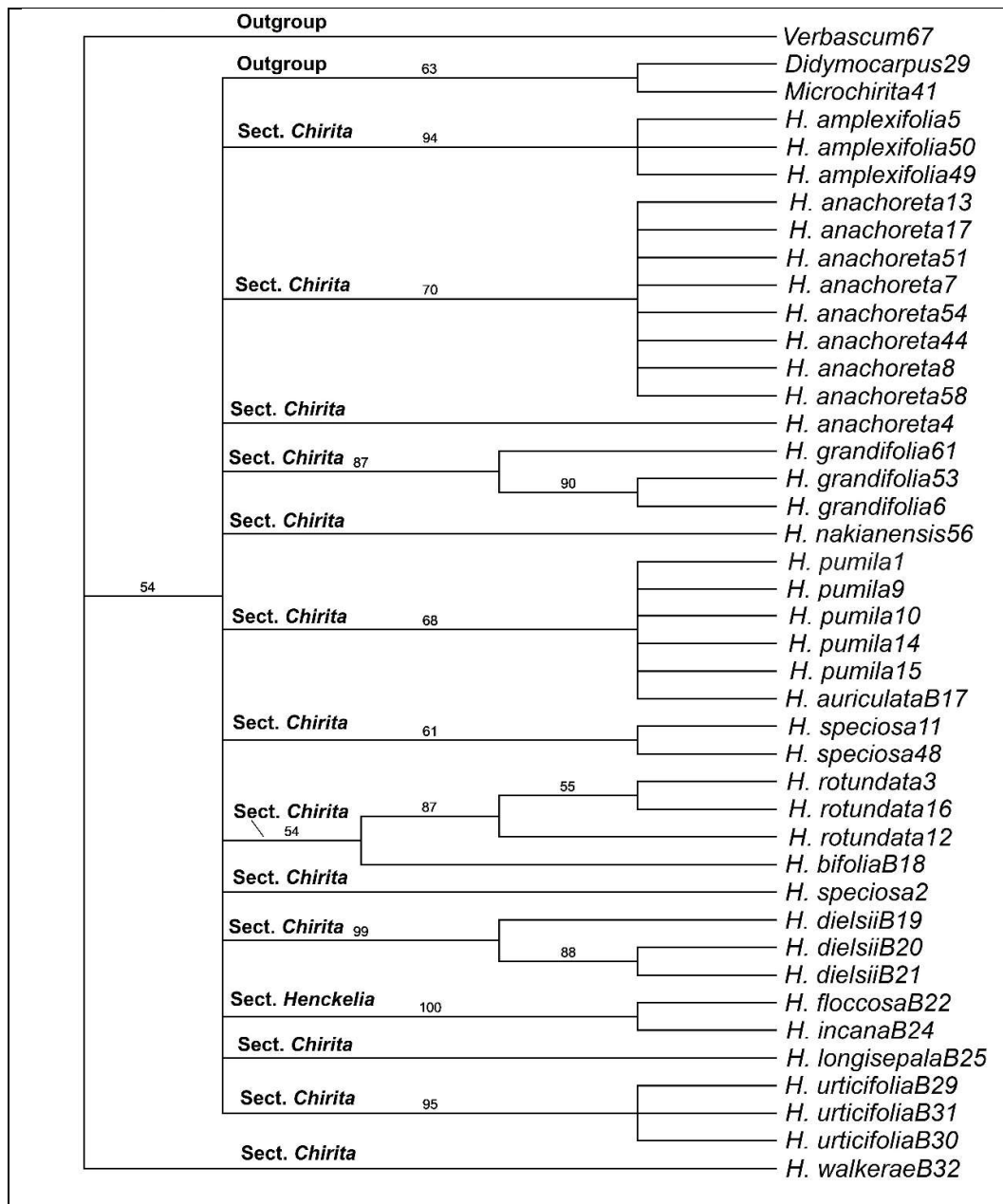
- 1) Load clean PCR product onto the plate in the 1-8 order (vertical column).
- 2) The PCR product at 10 ng/ μ l in a total of 10 μ l per each sample.
- 3) Seal plate with the sealing film.
- 4) Prepare primer at 3.2 pmol/ μ l in total 5 μ l per reaction into a tube and seal with



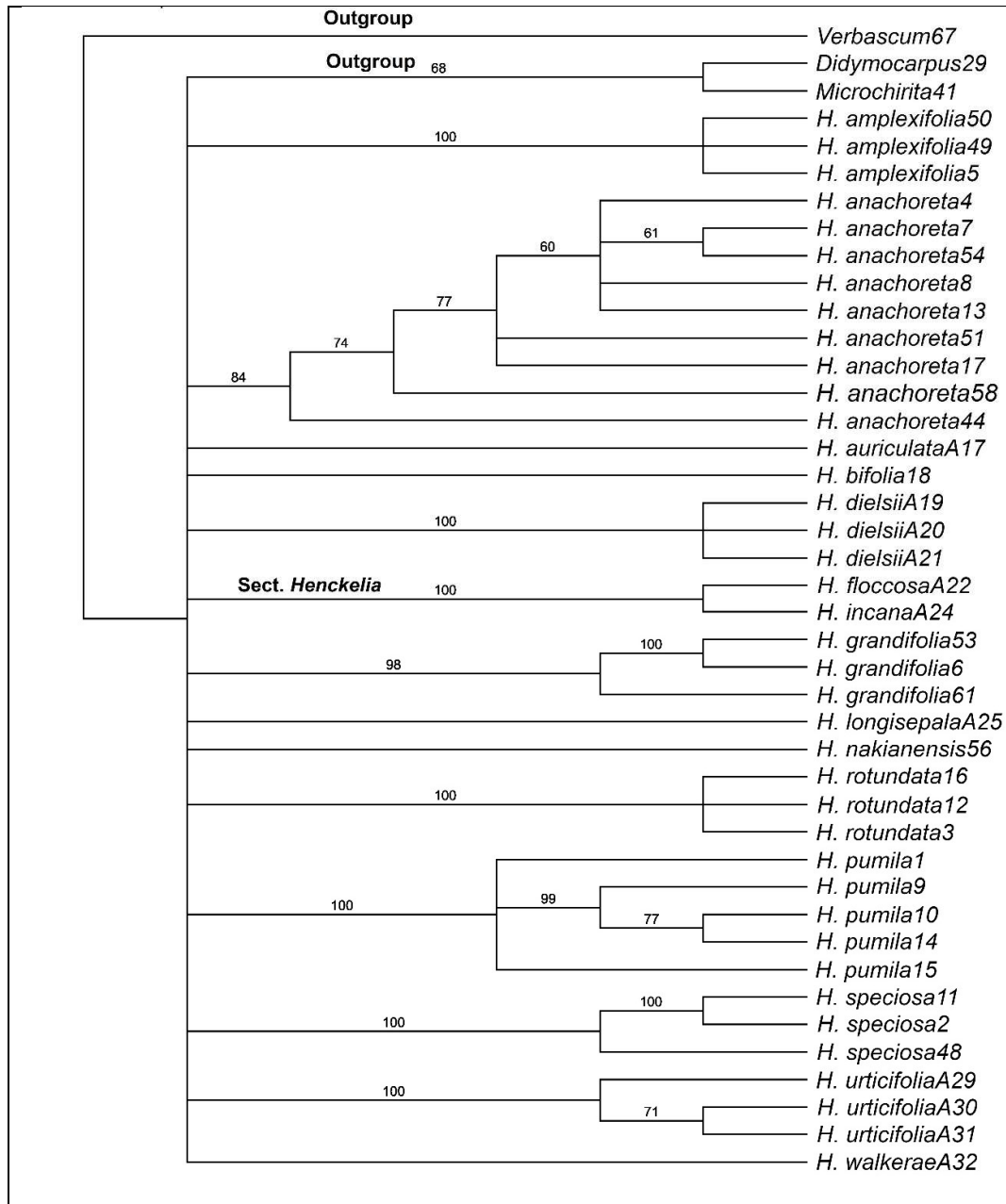
Appendix 3.11 The bootstrap consensus tree shown as a cladogram obtained from comparative sequence analysis of the *trnL-trnF* data. Values above branches represent the percentage of bootstrap supporting each branch.



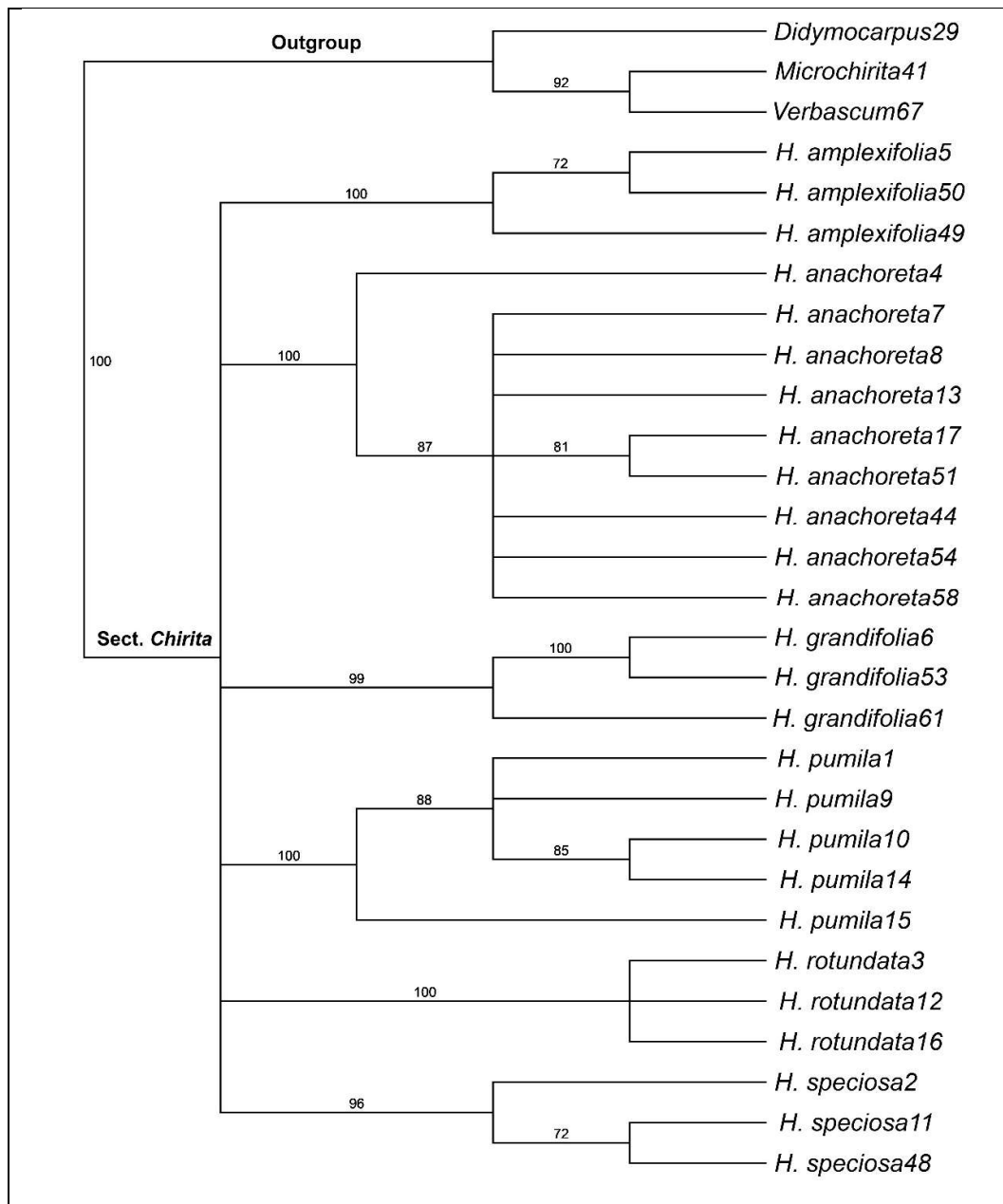
Appendix 3.13 The bootstrap consensus tree shown as a cladogram obtained from comparative sequence analysis of the ITS data. Values above branches represent the percentage of bootstrap supporting each branch.



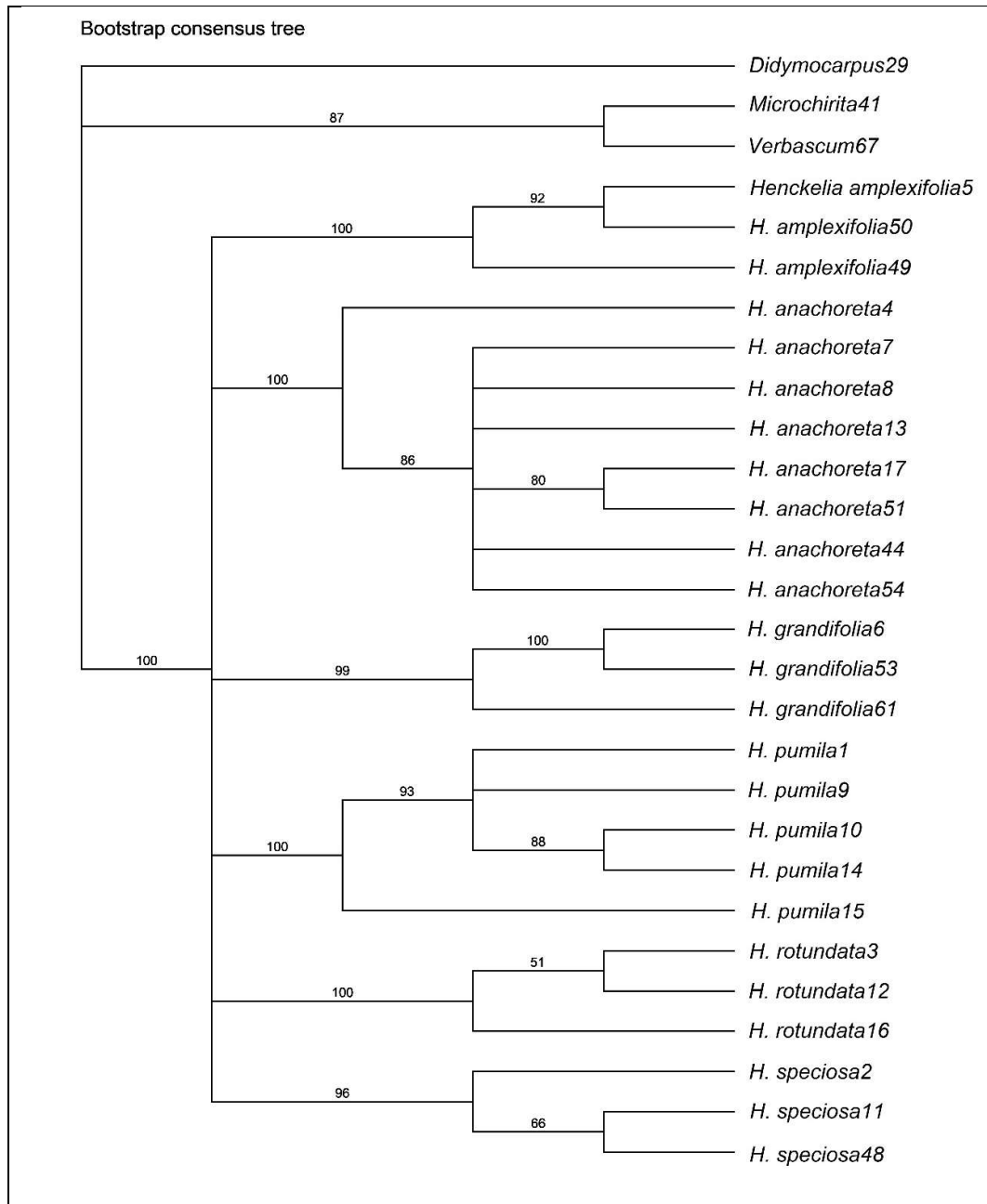
Appendix 3.14 The bootstrap consensus tree shown as a cladogram obtained from comparative sequence analysis of the *trnL-F* data. Values above branches represent the percentage of bootstrap support for each branch.



Appendix 3.15 The bootstrap consensus tree shown as a cladogram obtained from comparative sequence analysis of the ITS and *trnL-F* data. Values above branches represent the percentage of bootstrap supporting each branch.



Appendix 3.16 The bootstrap consensus tree shown as a cladogram obtained from comparative sequence analysis of the chloroplast gene regions (*atpB-rbcL*, *rps16* and *trnL-F*). Values above branches represent the percentage of bootstrap supporting each branch.



Appendix 3.17 The bootstrap consensus tree shown as a cladogram obtained from comparative sequence analysis of the ITS, *atpB-rbcL*, *rps16* and *trnL-F* data. Values above branches represent the percentage of bootstrap supporting each branch.

Appendix 5.1 Pollen size and class from Walker & Doyle (1975).

Designation	P/E ratio	Class	Size (μm)
Prolate		Minute grains	< 10
Perprolate	≥ 2.00	Small grains	10-24
Euprolate	1.34-1.99	Medium-sized	25-49
Subprolate	1.15-1.33	grains	50-99
Prolate spheroidal	1.01-1.14	Large grains	100-199
		Very large grains	≥ 200
Spherical	1.00	Gigantic grains	
Oblate			
Oblate spheroidal	0.88-0.99		
Suboblate	0.76-0.87		
Euoblate	0.51-0.75		
Perolate	≤ 0.50		

Appendix 5.2 The pollen grain data of *Henckelia* from this study.

Taxon	Shape	Polar diameter (P) (µm)	P mean (µm)	Equatorial diameter (E) (µm)	E mean (µm)	P/E	Size	Sculpturing	Vocher
<i>Henckelia adenocalyx</i>	euprolate	26-30	27.6	15-20	16.7	1.65	medium	reticulate	Ward 7393 (K), Myanmar
<i>H. anachoreta</i>	euprolate	20-26	24.1	14-18	16.2	1.49	small to medium	reticulate	Sirimongkol et al. 721 (BKF), Nakhon Nayok
<i>H. bifolia</i>	euprolate	20-25(27)	23.3	(12)14-18	15.1	1.54	small to medium	reticulate	Strachey & Winterbottom s.n. (TCD), India
<i>H. communis</i>	euprolate	19-22	20.6	11-13(15)	12.2	1.69	small	reticulate	Gardner s.n. (TCD), Sri Lanka
<i>H. dielsii</i>	subprolate	24-30	27.4	20-24	22.4	1.22	medium	reticulate	Yu 16288 (E), China
<i>H. forrestii</i>	euprolate	26-30	28.4	20-23	20.8	1.37	medium	reticulate	Forrest 11119 (E), China
<i>H. fruticola</i>	subprolate	23-27	24.7	20-21	20.3	1.23	small to medium	reticulate	Rushford 2141 (E), Vietnam
<i>H. grandifolia</i>	euprolate	30-35(36)	33.5	20-23(25)	21.1	1.59	medium	reticulate	Poore & Robbins 7622 (BKF), Chaing Mai, Thailand
<i>H. humboldtiana</i>	euprolate	19-22	20.7	10-13	11.3	1.83	small	reticulate	Johnson s.n. (TCD), India
<i>H. incana</i>	subprolate	13-15	14.2	10-13	10.7	1.33	small	reticulate	Bourne 5301 (K), India
<i>H. lachenensis</i>	euprolate	26-30	27.6	13-18	15.3	1.8	medium	reticulate	Forrest 29681 (E), China
<i>H. mishmiensis</i>	euprolate	25-27	26.2	16-20	18.3	1.43	medium	reticulate	Ward 7917 (K), India
<i>H. oblongifolia</i>	subprolate	15-18	16.6	13-15	14.2	1.17	small	reticulate	Griffith 3829 (K), India
<i>H. pumila</i>	prolate spheroidal	15-20	17.1	(10)15-20	16	1.07	small	reticulate	Sirimongkol et al. 680 (BKF), Chiang Mai, Thailand
<i>H. speciosa</i>	euprolate	29-35	30.8	16-19	17.3	1.78	medium	reticulate	Lace 5758 (TCD), Myanmar
<i>H. urticifolia</i>	euprolate	30-34	31.7	19-21	19.9	1.59	medium	reticulate	Hooker s.n. (TCD), India
<i>H. walkerae</i>	euprolate	23-25	24.2	13-18	15.4	1.57	small to medium	reticulate	Thwaites 1789 (TCD), Sri Lanka

Appendix 5.3 The pollen grain data from the previous study with *Henckelia* species.

Subfamily	Tribe	Taxon	P axis (µm)	E axis (µm)	Type	Sculturing	Shape	Size	Source
Didymocarpoideae	Epithemateae	<i>Epithema membranaceum</i>	17	21	tri-colpate	rugulate	suboblate-oblate	small	Luegmayr, 1993a
Didymocarpoideae	Epithemateae	<i>Monophyllea horsfieldii</i>	13.2	13	tri-colporate	reticulate	suboblate-oblate	small	Luegmayr, 1993a
Didymocarpoideae	Epithemateae	<i>Rhychoglossum mirabilis</i>	-	18	tri-colpate	rugulate	spheroidal	small	Pattharahirantricin, 2014
Didymocarpoideae	Epithemateae	<i>Rhychoglossum sacchatum</i>	-	19	tri-colpate	rugulate	spheroidal	small	Pattharahirantricin, 2014
Didymocarpoideae	Trichosporeae	<i>Aeschynanthus boschianus</i>	20.5	17.4	tri-colpate	reticulate	spheroidal	small	Luegmayr, 1993a
Didymocarpoideae	Trichosporeae	<i>Aeschynanthus parvifolius</i>	-	16.8	tri-colporate	reticulate	spheroidal	small	Luegmayr, 1993a
Didymocarpoideae	Trichosporeae	<i>Anna rubidiflora</i>	28	16	tri-colporate	reticulate	prolate-spheroidal	medium	Wei et al., 2013
Didymocarpoideae	Trichosporeae	<i>Boea hygrometrica</i>	-	-	tri-colpate	granulate	subprolate-prolate	-	Erdtman, 1952
Didymocarpoideae	Trichosporeae	<i>Chirita caliginosa</i>	19	22	tri-colpate	reticulate	spheroidal	small	Luegmayr, 1993a
Didymocarpoideae	Trichosporeae	<i>Cyrtandra cordifolia</i>	-	17	tri-colporooidate	microreticulate	spheroidal	small	Luegmayr, 1993b
Didymocarpoideae	Trichosporeae	<i>Cyrtandra grandifolia</i>	19	18	tri-colporooidate	microreticulate	spheroidal	small	Luegmayr, 1993b
Didymocarpoideae	Trichosporeae	<i>Cyrtandra kaulantha</i>	-	21.4	tri-colporooidate	microreticulate	spheroidal	small	Luegmayr, 1993b
Didymocarpoideae	Trichosporeae	<i>Cyrtandra paludosa</i>	17.5	19.6	tri-colporooidate	microreticulate	spheroidal	small	Luegmayr, 1993b
Didymocarpoideae	Trichosporeae	<i>Cyrtandra pendula</i>	20	-	tri-colpate	reticulate	spheroidal	small	Luegmayr, 1993a
Didymocarpoideae	Trichosporeae	<i>Cyrtandra pendula</i>	-	17.5	tri-colporooidate	microreticulate	spheroidal	small	Luegmayr, 1993b
Didymocarpoideae	Trichosporeae	<i>Cyrtandra platyphylla</i>	17.4	20	tri-colporooidate	microreticulate	spheroidal	small	Luegmayr, 1993b
Didymocarpoideae	Trichosporeae	<i>Cyrtandra procera</i>	17	19	tri-colporooidate	microreticulate	spheroidal	small	Luegmayr, 1993b
Didymocarpoideae	Trichosporeae	<i>Cyrtandra splendens</i>	-	11	tri-colporooidate	microreticulate	spheroidal	small	Luegmayr, 1993b
Didymocarpoideae	Trichosporeae	<i>Didissandra morganii</i>	16.25	12.5	tri-colpate	reticulate	spheroidal	small	Luegmayr, 1993a

Appendix 5.3 (continued)

Subfamily	Tribe	Taxon	P axis (μm)	E axis (μm)	Type	Sculturing	Shape	Size	Source
Didymocarpoideae	Trichosporeae	<i>Didymocarpus aff. floribundus</i>	16	17.3	tri-colporate	reticulate/ perfolate	spheroidal	small	Luegmayr, 1993a
Didymocarpoideae	Trichosporeae	<i>Henckelia adenocalyx</i>	27.6	16.7	tri-colpate	reticulate	euprolate	medium	This study
Didymocarpoideae	Trichosporeae	<i>Henckelia anachoreta</i>	24.1	16.2	tri-colpate	reticulate	euprolate	small to medium	This study
Didymocarpoideae	Trichosporeae	<i>Henckelia bifolia</i>	23.3	15.1	tri-colpate	reticulate	euprolate	small to medium	This study
Didymocarpoideae	Trichosporeae	<i>Henckelia communis</i>	20.6	12.2	tri-colpate	reticulate	euprolate	small	This study
Didymocarpoideae	Trichosporeae	<i>Henckelia dielsii</i>	27.4	22.4	tri-colpate	reticulate	subprolate	medium	This study
Didymocarpoideae	Trichosporeae	<i>Henckelia forrestii</i>	28.4	20.8	tri-colpate	reticulate	euprolate	medium	This study
Didymocarpoideae	Trichosporeae	<i>Henckelia fruticola</i>	24.7	20.3	tri-colpate	reticulate	subprolate	small to medium	This study
Didymocarpoideae	Trichosporeae	<i>Henckelia grandifolia</i>	33.5	21.1	tri-colpate	reticulate	euprolate	medium	This study
Didymocarpoideae	Trichosporeae	<i>Henckelia humboldtiana</i>	20.7	11.3	tri-colpate	reticulate	euprolate	small	This study
Didymocarpoideae	Trichosporeae	<i>Henckelia incana</i>	14.2	10.7	tri-colpate	reticulate	subprolate	small	This study
Didymocarpoideae	Trichosporeae	<i>Henckelia lachenensis</i>	27.6	15.3	tri-colpate	reticulate	euprolate	medium	This study
Didymocarpoideae	Trichosporeae	<i>Henckelia mishmiensis</i>	26.2	18.3	tri-colpate	reticulate	euprolate	medium	This study
Didymocarpoideae	Trichosporeae	<i>Henckelia oblongifolia</i>	16.6	14.2	tri-colpate	reticulate	subprolate	small	This study
Didymocarpoideae	Trichosporeae	<i>Henckelia pumila</i>	17.1	16	tri-colpate	reticulate	Prolate spheroidal	small	This study
Didymocarpoideae	Trichosporeae	<i>Henckelia speciosa</i>	30.8	17.3	tri-colpate	reticulate	euprolate	medium	This study
Didymocarpoideae	Trichosporeae	<i>Henckelia urticifolia</i>	31.7	19.9	tri-colpate	reticulate	euprolate	medium	This study
Didymocarpoideae	Trichosporeae	<i>Henckelia walkerae</i>	24.2	15.4	tri-colpate	reticulate	euprolate	small to medium	This study
Didymocarpoideae	Trichosporeae	<i>Ornithoboea arachnoidea</i>	17	17.7	tri-colpate	perfolate	spheroidal	small	Luegmayr, 1993a
Didymocarpoideae	Trichosporeae	<i>Ornithoboea flexuosa</i>	-	10.5	tri-colpate	perfolate	spheroidal	small	Luegmayr, 1993a
Didymocarpoideae	Trichosporeae	<i>Ramonda</i>	-	-	tri-colporate	-	-	-	Reille, 1992

Appendix 5.3 (continued)

Subfamily	Tribe	Taxon	P axis (μm)	E axis (μm)	Type	Sculturing	Shape	Size	Source
Didymocarpoideae	Trichosporeae	<i>Stauranthera grandiflora</i>	15.5	17.6	tri-colpate	reticulate	spheroidal	small	Luegmayr, 1993a
Didymocarpoideae	Trichosporeae	<i>Streptocarpus gardenii</i>	-	17.6	tri-colpate	reticulate/ perfoliate	spheroidal	small	Luegmayr, 1993a
Didymocarpoideae	Trichosporeae	<i>Streptocarpus grandis</i>	-	-	tri-colpate	granulate	suboblate- oblate	-	Erdtman, 1952
Didymocarpoideae	Trichosporeae	<i>Streptocarpus hilturianus</i>	11	11	tri-colpate	scabrate	spheroidal	small	Edwards, 2003
Didymocarpoideae	Trichosporeae	<i>Streptocarpus kunhardtii</i>	17	9	tri-colpate	reticulate	prolate	small	Edwards, 2003
Didymocarpoideae	Trichosporeae	<i>Streptocarpus orientalis</i>	14.2	14	tri-colpate	reticulate	spheroidal	small	Luegmayr, 1993a
Didymocarpoideae	Trichosporeae	<i>Streptocarpus rungwensis</i>	-	-	tri-colpate	granulate	suboblate-oblate		Erdtman, 1952
Didymocarpoideae	Trichosporeae	<i>Streptocarpus silvaticus</i>	12.5	-	tri-colpate	reticulate/ perfoliate	spheroidal	small	Luegmayr, 1993a
Gesnerioideae	Beslerieae	<i>Besleria longimucronata</i>	22.0	14.9	tri-colpate	perforate	prolate	small	Fourny et al., 2010
Gesnerioideae	Beslerieae	<i>Besleria macahensis</i>	25.6	18.7	tri-colpate	perforate	prolate	medium	Fourny et al., 2010
Gesnerioideae	Beslerieae	<i>Besleria melancholica</i>	22.4	16.2	tri-colpate	perforate	prolate	medium	Fourny et al., 2010
Gesnerioideae	Beslerieae	<i>Besleria umbrosa</i>	24.6	17.1	tri-colporate	perforate	prolate	medium	Fourny et al., 2010
Gesnerioideae	Coronanthereae	<i>Coranthera clarkeana</i>	-	-	tri-colpate	granulate	subprolate -prolate	-	Erdtman, 1952
Gesnerioideae	Coronanthereae	<i>Rhabdothamnus.</i>	20	-	tri-colpate	granular	spheroidal	small	Cranwell, 1941
Gesnerioideae	Gesnerieae	<i>Bellonia aspera</i>	14	11	tri-colpate	granulate	subprolate -prolate	small	Erdtman, 1952
Gesnerioideae	Gesnerieae	<i>Codonanthe carnosa</i>	31.7	35.6	tri-colporate	reticulate	oblate- spheroidal	medium	Fourny et al., 2010

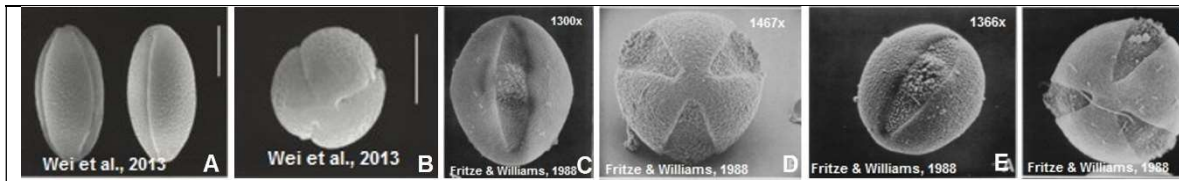
Appendix 5.3 (continued)

Subfamily	Tribe	Taxon	P axis (μm)	E axis (μm)	Type	Sculturing	Shape	Size	Source
Gesnerioideae	Gesnerieae	<i>Codonanthe devosiana</i>	30.7	28.0	tri-colporate	reticulate	prolate-spheroidal	medium	Fourny et al., 2010
Gesnerioideae	Gesnerieae	<i>Codonanthe gracilis</i>	29.5	34.1	tri-colporate	reticulate	subolate	medium	Fourny et al., 2010
Gesnerioideae	Gesnerieae	<i>Columnnea microphylla</i>	51	34	tri-colpate	granulate	subprolate-prolate	medium	Erdtman, 1952
Gesnerioideae	Gesnerieae	<i>Heppiella</i>	-	-	tri-colpate	reticulate	-	-	Kvist, 1990
Gesnerioideae	Gesnerieae	<i>Nematanthus brasiliensis</i>	29.0	27.0	tri-colporate	reticulate	prolate-spheroidal	medium	Fourny et al., 2010
Gesnerioideae	Gesnerieae	<i>Nematanthus crassifolius</i>	41.6	44.5	tri-colporate	reticulate	oblate-spheroidal	medium	Fourny et al., 2010
Gesnerioideae	Gesnerieae	<i>Nematanthus fissus</i>	37.3	41.1	tri-colporate	reticulate	oblate-spheroidal	medium	Fourny et al., 2010
Gesnerioideae	Gesnerieae	<i>Nematanthus fluminensis</i>	41.0	38.7	tri-colpate	reticulate	prolate-spheroidal	medium	Fourny et al., 2010
Gesnerioideae	Gesnerieae	<i>Nematanthus hirtellus</i>	37.2	32.8	tri-colpate	reticulate	subprolate	medium	Fourny et al., 2010
Gesnerioideae	Gesnerieae	<i>Paliavana prasinata</i>	26.4	22.0	tri-colporate	reticulate	subprolate	medium	Fourny et al., 2010
Gesnerioideae	Gesnerieae	<i>Sinningia brasiliensis</i>	33.0	28.9	tri-colpate	reticulate	prolate	medium	Fourny et al., 2010
Gesnerioideae	Gesnerieae	<i>Sinningia bulbosa</i>	30.3	22.8	tri-colpate	reticulate	subprolate	medium	Fourny et al., 2010
Gesnerioideae	Gesnerieae	<i>Sinningia douglasii</i>	36.5	24.2	tri-colpate	reticulate	subolate	medium	Fourny et al., 2010

Appendix 5.3 (continued).

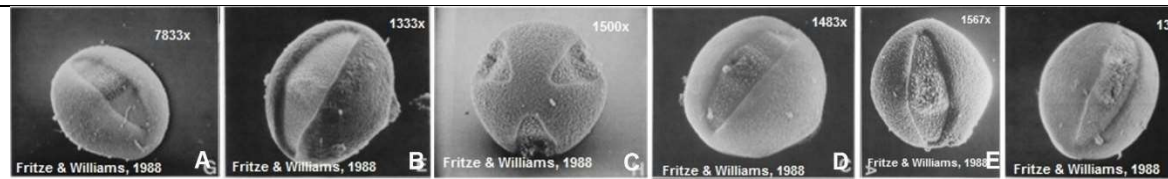
Subfamily	Tribe	Taxon	P axis (μm)	E axis (μm)	Type	Sculturing	Shape	Size	Source
Gesnerioideae	Gesnerieae	<i>Sinningia gigantifolia</i>	26.9	32.1	tri-colpate	reticulate	subolate	medium	Fourny et al., 2010
Gesnerioideae	Gesnerieae	<i>Sinningia guttata</i>	31.7	24.2	tri-colpate	reticulate	subprolate	medium	Fourny et al., 2010
Gesnerioideae	Gesnerieae	<i>Sinningia lateritia</i>	33.3	27.9	tri-colpate	perforate	subprolate	medium	Fourny et al., 2010
Gesnerioideae	Gesnerieae	<i>Vanhouttea calcarata</i>	31.2	28.2	tri-colpate	reticulate	prolate- spheroidal	medium	Fourny et al., 2010
Gesnerioideae	Gesnerieae	<i>Vanhouttea lanata</i>	29.1	26.0	tri-colpate	reticulate	prolate- spheroidal	medium	Fourny et al., 2010

Appendix 5.4 Pollen grains photographs from the previous study.



Polar axis: A. *Anna rubidiflora*, C. *Columnnea arguta*, E. *C. billbergiana*.

Equatorial axis: B. *Anna rubidiflora*, D. *Columnnea bilabiate*, F. *C. cobana*.



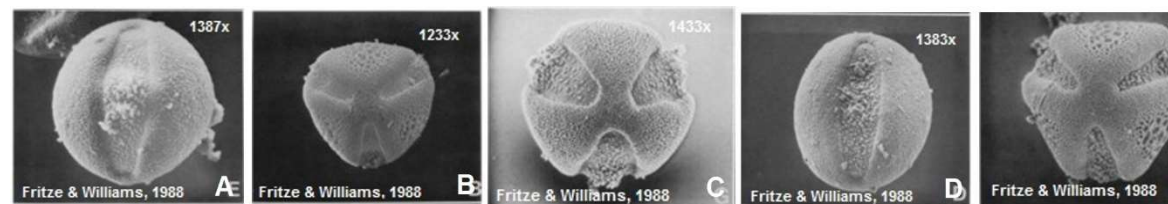
Polar axis: A. *Columnnea dodsonii*, B. *C. dressleri*, D. *C. flaccida*, E. *C. gallicauda*,

F. *C. guatemalensis*. Equatorial axis: C. *Columnnea erythrophaea*



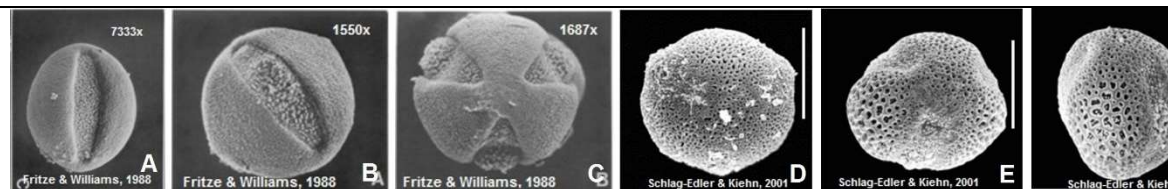
Equatorial axis: A. *Columnnea hirsutissima*, B. *C. kienastiana*, E. *C. purpusii*.

Polar axis: C. *C. linearis*, D. *C. oerstediana*.



Polar axis: A. *Columnnea querceti*, D. *C. rubicaulis*.

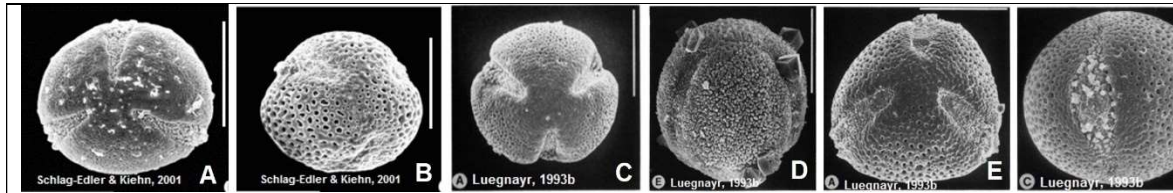
Equatorial axis: B. *Columnnea repens*, C. *C. rubra*, E. *C. rutilans*.



Polar axis: A-B. *Columnnea schiedeana*, F. *Cyrtandra feaniana*.

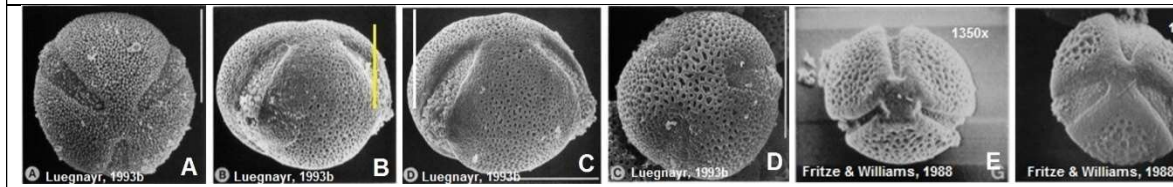
Equatorial axis: C. *Columnnea zebranella*, D. *Cyrtandra compressa*, E. *C. feaniana*.

Appendix 5.4 (continued)



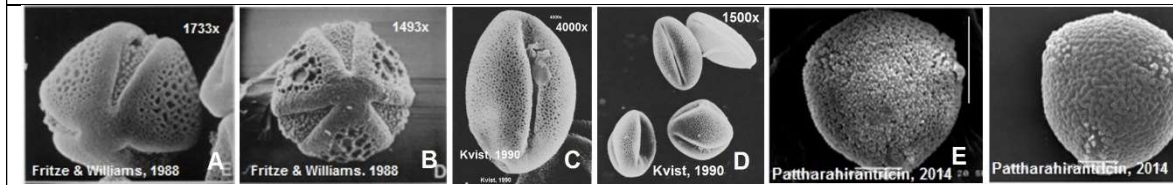
Equatorial axis: A. *Cyrtandra kamooloensis*, B. *C. richii*, C. *C. cordifolia*, E. *C. kaulantha*.

Polar axis: D. *Cyrtandra grandiflora*, F. *C. paludosa*.



Equatorial axis: A. *Cyrtandra pendula*, D. *Cyrtandra splendens*, E. *Dalbergia*

asteroloma, F. *D. polyantha*. Polar axis: B. *Cyrtandra platyphylla*, C. *C. procera*.



Equatorial axis: A. *Dalbergia puyana*, B. *D. silvarum*, E. *Rhynchoglossum mirabilis*, F. *R. saccatum*. Polar axis: C-D. *Heppiella ulmifolia*.



Polar axis: A-B. *Solonophora tuxtensis*, F. *Streptocarpus kunhardii*.

Equatorial axis: C-D. *Solonophora tuxtensis*, E. *S. hillmartianus*.