Systematic studies on *Thysanotus* R.Br. (Asparagales: Laxmanniaceae)

Udani Megha Sirisena (B.Sc.Hons.)

School of Earth and Environmental Sciences

The University of Adelaide

A thesis submitted for the degree of Doctor of Philosophy of the University of Adelaide

January 2010

The University of Adelaide, SA 5005, Australia

Cover photo: *Thysanotus multiflorus* R.Br. from Western Australia (Photo- Udani M. Sirisena) This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by any other person, except where due reference is made in the text.

I give consent to this copy of my thesis when deposited in the University Library being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works contained within this thesis (as listed on page ix) resides with the copyright holder(s) of those works.

This study was funded by a faculty of sciences divisional scholarship (2006-2009), a Field Research Grant by the Biological Society of South Australia (2007/2008), a grant from the Nature Foundation SA (2007/2008), and a Conservation Biology Grant from the Nature Conservation Society of SA (2008/2009). Additional funds were provided by a Mark Mitchell Research Foundation Grant (2009) was awarded to Dr. John Conran.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Udani Megha Sirisena (January 2010)

DEDICATION

Dedicated to my loving husband and parents

v

My heartfelt gratitude is extended to all those who supported and encouraged me throughout this PhD study.

I am indebted to my supervisors Dr. John Conran and Dr. Terry Macfarlane for their valuable guidance, assistance and useful discussions. I thank Dr. John Conran for continuous valuable feedback on the work (experimental and writing) and help given in data analyses and preparation of manuscripts. Dr. Terry Macfarlane is gratefully thanked for assisting in field work, manuscript preparation and sharing his vast knowledge on *Thysanotus*.

Sincere thanks go to Ms. Helen Vonow and Mr. Graham Bell for their friendly and generous assistance at the State Herbarium of South Australia. Helen also provided brief but valuable discussions on ambiguities related to *Thysanotus* specimens. Mr. Daniel Duvall and his team at the seed conservation centre of South Australia are also thanked for collecting and providing me with *Thysanotus* specimens in South Australia. Many thanks to AD and PERTH herbaria directors: Prof. Andrew Lowe and Dr. Kevin Thiele, for the provision of facilities to undertake the research in those herbaria. The staff of the Adelaide School of Microscopy is thanked for the assistance and training provided for Scanning Electron Microscopy. Fellow postgraduate Mr. Mathew Donnon is also thanked for the training provided on molecular techniques. I specially thank Assoc. Prof. José Facelli and his lab group for valuable discussions provided during the presentation practices for conferences and various society meetings.

Fellow postgraduates whom I shared the office with are also thanked for their friendship, advice and discussions. Continuous wonderful friendship and support of my friends Cecilia Trujillo and Gael Campbell-Young are remembered with love and gratitude. My dearest friend Sue Gehrig is thanked for her friendship and help given in preparing the master document.

My parents Indrani and Sarath Sirisena are remembered for their endless love and support throughout this PhD. Last but not least, I thank my husband Dinesh Monaragala for his continuous love, support and understanding.

vii

ANTICIPATED PUBLICATIONS ARISING FROM THIS PH.D.

Sirisena UM, Conran JG, Macfarlane TD (20XX) Generic relationships within Laxmanniaceae inferred from non-coding chloroplast DNA and morphology. *Australian Systematic Botany*.

Sirisena UM, Conran JG, Macfarlane TD (20XX) Phylogenetic relationships and major lineages within the genus *Thysanotus* R.Br. (Laxmanniaceae) determined from combined morphological data and anatomical data. *Australian Systematic Botany*.

Sirisena UM, Conran JG, Macfarlane TD (20XX) Phylogeny of *Thysanotus* R.Br. inferred from non coding chloroplast DNA sequences, nuclear sequences and morphological data. *Plant Systematics and Evolution*.

Sirisena UM, Conran JG, Macfarlane TD (20XX) The systematic significance of seed coat morphology in *Thysanotus* R.Br. and related Laxmanniaceae. *Botanical Journal of the Linnean Society*.

Sirisena UM, Conran JG, Macfarlane TD (20XX) The systematic significance of aerial stem anatomy in *Thysanotus* R.Br. (Asparagales: Laxmanniaceae). *Botanical Journal of the Linnean Society*.

Sirisena UM, Macfarlane TD, Conran JG (2009) *Thysanotus unicupensis* (Laxmanniaceae), a new species discovered in Unicup Nature Reserve, south-west Western Australia. *Nuytsia* **19** (2): 259–263.

Sirisena UM, Macfarlane TD, Conran JG (20XX) *Thysanotus racemoides* (Asparagales: Laxmanniaceae), a new species from South Australia and western Victoria. *Telopea*.

ABSTRACT

Thysanotus R.Br. (Asparagales; Laxmanniaceae) is a genus native to Australia with c. 50 species distributed chiefly in Australia. To date, *Thysanotus* lacks proper and detailed systematic studies based on molecular and/or non-molecular data. Therefore, carrying out a detailed systematic study using molecular and/or non-molecular data seemed important. Furthermore, generic placement of *Murchisonia* Brittan has always been controversial and this placement required testing under a phylogenetic framework. The generic relationships within Laxmanniaceae/Lomandraceae are considered uncertain; therefore, a phylogenetic analysis using molecular and morphological data is necessary to properly understand the generic relationships of Laxmanniaceae.

Detailed studies on stem anatomy and morphology were carried out in order to understand the systematic significance and phylogenetic signal of these characters. The cp DNA (*trnL* intron and *trnL*–F intergenic spacer) and nuclear ITS2 gene regions were amplified and the results compared and combined with a morphological analysis. Phylogenetic analyses were carried out using *Arthropodium* R.Br and *Eustrephus* R.Br as outgroup taxa.

There was sufficient variation in general morphology, seed micromorphology and stem anatomy and were potentially useful in understanding phylogenetic relationships of Thysanotus. A number of synapomorphies based on general morphology and stem anatomy such as absence of pendent flowers and absence of irregular shaped epidermal cells were recognised. The molecular data and the combined data yielded highly resolved consensus trees and enabled us to recognise three main lineages within the genus, each representing life history adaptations. Murchisonia was consistently nested within Thysanotus in all analyses showing a need for the return of both species to Thysanotus. Insights to intraspecific variation were also discernable from morphological, molecular and the combined analyses in species such as T. patersonii and T. juncifolius. Two new Thysanotus species, T. unicupensis and T. racemoides are also described. Our data strongly support the current circumscription of Laxmanniaceae, but suggest that there are three main lineages within the family, rather than the two previously recognised subfamilies.

CONTENTS

CHAPTER 1: General introduction and literature review1-1

CHAPTER IV: Phylogeny of *Thysanotus* R.Br. inferred from non coding chloroplast DNA sequences, nuclear sequences and morphological data 4-1

CHAPTER V: The systematic significance of seed coat morphology in	
Thysanotus R.Br. and related Laxmanniaceae	

CHAPTER VI: The systematic significance of aerial stem anatomy in	
Thysanotus R.Br. (Asparagales: Laxmanniaceae)	

CHAPTER VII: *Thysanotus unicupensis* (Laxmanniaceae), a new species discovered in Unicup Nature Reserve, south-west Western Australia7-1

CHAPTER IX: General discussion	-	1	ĺ
--------------------------------	---	---	---