

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Phylogenetic affinities, species delimitation  
and adaptive radiation of New Zealand  
*Ranunculus*

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy  
in Plant Biology

at Massey University, Palmerston North  
New Zealand

Carlos Adolfo Lehnebach

March 2008

## ABSTRACT

*Ranunculus* is the largest genus in the Ranunculaceae family and comprises c. 600 species. Its distribution is almost worldwide and the largest number of species occurs in temperate zones of North and South America, Europe, Asia, Australia, New Zealand, and in the alpine regions of New Guinea. In New Zealand the genus *Ranunculus* contains about 41 species and is found both in lowland and alpine environments. This thesis reports a phylogenetic analysis of lowland and alpine New Zealand *Ranunculus*, an assessment of morphological variation and species boundaries among complex alpine species and examines evidence suggesting adaptive radiation of the alpine *Ranunculus* lineage.

Phylogenetic analysis suggests that New Zealand species of *Ranunculus* are not a monophyletic group. For some New Zealand species the closest affinities inferred from the analysis of nrDNA and cpDNA sequences are to species from other land masses such as Australia, the Northern Hemisphere, southern South America and islands in the southern Oceans. Contrary to Fisher's hypothesis (1965), the Andean South American *Ranunculus* in the section *Trollianthoideae* are not closely related to the New Zealand alpine group. The *Trollianthoideae* section was not monophyletic and the Peruvian-Ecuadorian species in it form a lineage sister to European alpine species. Instead, aquatic and sub-aquatic species from the Euro-Mediterranean region and southern South America and the Kerguelen Island were inferred as the closest relatives to the New Zealand alpine *Ranunculus*; albeit this relationship was weakly supported. Findings from this study suggest that colonisation of *Ranunculus* into the Southern Hemisphere has been a dynamic process and several long distance dispersal events and different colonisation routes have been used. Dispersal from New Zealand to Australia and vice versa, has also been inferred. Bird transportation and oceanic currents are speculated as being the most likely vectors for long dispersal for this group.

Morphological variability at the species level is a feature of several species of *Ranunculus* worldwide. In New Zealand, the alpine species *R. insignis* and *R. enysii* are characterised by extensive morphological variability across their distribution range. Currently, these two species include a number of geographically restricted forms that in earlier taxonomic treatments were considered as separate species. Analysis of qualitative and quantitative morphological characters using parametric and non-parametric statistical tests and multivariate analysis, habitat characterisation using environmental variables from the GIS database LENZ and molecular analyses of nrDNA and cpDNA sequences have provided a

framework for interpreting and understanding the nature of this phenotypic variation. An argument based on morphological, genetic and ecological support for the reinstatement of the species *R. insignis*, *R. lobulatus* and *R. monroi* is presented here. The last two species may correspond to lineages of recent origin. Hybridisation and introgression between *R. insignis* and *R. lobulatus* are suggested as being responsible for intermediate phenotypes found in areas where their distribution overlaps. Morphological variability in *R. enysii* is inferred to have had a complex origin. The species has a disjunct distribution and events of hybridisation and/or introgression with *R. monroi* and *R. gracilipes* seems to have occurred in some of the northern and southern populations, respectively. These hybrid lineages may have swamped out pure lineages of *R. enysii* and eliminated the ancestral phenotype. Studies including assessment of gene flow using microsatellites, phenotypic stability under common garden condition and pollination experiments will be necessary to further test these hypotheses. Contrary to the latter two species, *R. lyallii* is morphologically uniform across its distribution range but genetically diverse (11 haplotypes, one of them shared with *R. buechananii*). Morphological stability in this species is probably explained by morphological stasis and habitat specialisation.

The alpine *Ranunculus* group is outstanding in the New Zealand flora in terms of its great phenotypic and ecological diversity of its members. These two features plus the monophyletic nature of the group and its recent origin have suggested to previous researchers that the radiation of this group has been adaptive. Phylogenetic analysis of 20 *taxa* in this group using nrDNA and cpDNA sequences has shown that the group includes four lineages and that genetic diversity between the species forming each lineage is low. This confirms findings from earlier studies by Lockhart *et al.* (2001). Cluster Analysis, multidimensional scaling analysis and histological and scanning microscopy observations of morphological and anatomical vegetative and reproductive characters were used to quantify the extent of morphological diversity in the group. Habitat diversity of this group was characterised using 16 environmental variables available from the GIS database LENZ and analysed using Canonical variates analysis. Although four habitat types were identified, there was no correlation between habitat and phenotype as predicted for an adaptive radiation. A number of alternative explanations for this lack of correspondence are discussed. The conclusion drawn from this study was that available data layers and resolution of LENZ limit the use of GIS databases for testing hypotheses of adaptation in the New Zealand Alps.

## **ACKNOWLEDGMENTS**

I would like to thank my supervisors Peter Lockhart and Phil Garnock-Jones for their guidance and support during the time I conducted this study. The valuable advice and constant support from Trish McLenachan and Mark Large during these years are also greatly appreciated. The statistical advice and guidance from Simon Jolly while doing the morphological analyses are also very much appreciated as well as the comments and suggestions on earlier versions of this work by Heidi Meudt. E-mail discussions with Cecilia Ezcurra, Elvira Hörandl, Magaly Rivero were a great contribution to the development of this study (thanks!) as were the numerous “corridor-discussion” with Steve Trewick and Mary Morgan-Richards. I am also very grateful by the fantastic training on histological techniques by Barbara Ambrose and Charlotte.

I must thank the many people who have collected samples for me here in New Zealand and overseas (P. Garnock-Jones, H. Meudt, K. Ford, P. Novis, M. Steel, J. Goldberg, C. Voelckel, D. Havell, O. Morrone, A. Cano, A. Robertson, U. Jenzen, F. Ehrendorfer, N. Ritter, P. Sklenar, E. Cameron, V. McGlynn, C. Oegle., P. Heenan, Jennifer Tate), my field companions Jaime, Paola, Becky and my Mum! and the curator of numerous institutions (VALD, CONC, S, SI, WELT, AK, CHR, OTA, MPN, W, ILLS, USM) who kindly facilitated loans and material for morphological and genetic analysis. I would also like to thanks the great assistance from the AWC staff (Joy, Susan, Karen, Lorraine, Olga) and IMBS (Ann, Cynthia, Paul, Doug).

The financial support from the Allan Wilson Centre, IMBS Massey University, Sir Allan Stewart Scholarship and the New Zealand Postgraduate Study Abroad Award is greatly appreciated. This project would have not been possible without their support.

I thank my Savage family in Palmerston North: Viv, Tony, Becky, Rae, Steve and new additions (Craig and the new town crier Ellah) for their love and friendship during all these years and my family in Chile for their love and constant encouragement. Also a big thanks to my friends Carolina, Jean Luc, Pat, Dorothee, Yvone, Juana, Julia, Claudia, Alastair, Masha, Isabel, Andrea, Barbara, Anna, Arne, Pom, Russel, Diane, Wayne and little Findley, and all those that have been there during this time! And last but not least, a great thanks to Jaime who has been all the ups and downs of my “PhD journey” and patiently (or perhaps eagerly?) waits for it to end.

## TABLE OF CONTENTS

### CHAPTER I

#### **The genus *Ranunculus*; an overview**

---

INTRODUCTION	2
The genus <i>Ranunculus</i> L.	2
Taxonomy and phylogenetic relationships within <i>Ranunculus</i>	4
Pollination, breeding systems and seed dispersal	6
Hybrid speciation in <i>Ranunculus</i>	9
The New Zealand <i>Ranunculus</i>	10

### CHAPTER II

#### **Phylogenetic affinities and Biogeographic patterns of *Ranunculus* in the Southern Hemisphere**

---

INTRODUCTION	12
METHODS	15
Taxon sampling	15
DNA isolation, amplification and sequencing	15
Data analyses	16
RESULTS	17
Super-Network analyses of the ITS and matK trees	27
DISCUSSION	31
Phylogenetic affinities of New Zealand <i>Ranunculus</i>	31
Phylogenetic affinities of South American <i>Ranunculus</i>	34
Southern Hemisphere <i>Ranunculus</i> , regional patterns of dispersal and dispersal vectors	38

### CHAPTER III

#### **Morphological variation and species delimitation in complex *Ranunculus* taxa of the New Zealand Alps**

---

INTRODUCTION	44
METHODS	47
Study case 1: <i>Ranunculus insignis</i> s.l.	47
Study case 2: <i>Ranunculus enysii</i> s.l.	49

Study case 3: <i>Ranunculus lyallii</i>	50
Morphological variation	52
Phenetic analysis	52
Molecular analysis	58
DNA isolation, amplification and sequencing	58
Data analysis	59
Habitat characterisation and geographic distance	59
Matrix correlations	60
<b>RESULTS</b>	61
Morphological analysis of <i>R. insignis s.l.</i>	61
<i>Character variation</i>	63
<i>Discriminant analysis of intermediate individuals</i>	65
Morphological analysis of <i>R. enysii s.l.</i>	67
Morphological analysis of <i>R. lyallii</i>	70
Molecular analysis <i>R. insignis s.l.</i>	72
Molecular analysis <i>R. enysii s.l.</i>	72
Molecular analysis <i>R. lyallii</i>	74
Habitat characterisation	77
<i>Habitats R. insignis s.l.</i>	77
<i>Habitats R. enysii s.l.</i>	78
<i>Habitats R. lyallii</i>	80
Matrix correlations	81
<b>DISCUSSION</b>	82
Species delimitation and patterns of morphological variation in <i>R. insignis s.l.</i>	82
Patterns of morphological variation and species delimitation in <i>R. enysii s.l.</i>	85
Patterns of morphological variation and species delimitation in <i>R. lyallii</i>	88
Concluding remarks	89
Taxonomic implications	91
<b>CHAPTER IV</b>	
<b>Phenotypic and habitat diversification: does the New Zealand Alpine</b>	
<b><u><i>Ranunculus</i> group show features of an adaptive radiation?</u></b>	
<b>INTRODUCTION</b>	93
<b>METHODS</b>	96

Phylogenetic affinities	96
Morphological characterisation	96
Habitat types	97
RESULTS	99
Molecular analysis	99
Phenotypic diversity	103
Habitats distinguished by LENZ	116
Phenotypes are not explained by LENZ habitats	116
DISCUSSION	118
i) Evidence of adaptation in the New Zealand alpine <i>Ranunculus</i> radiation	118
ii) Characters of adaptive significance	120
iii) The limitation of LENZ in describing the habitats occupy by this radiation	120
Vegetative and reproductive characters	121
Summary	122
<b>CHAPTER V</b>	
<b><u>Concluding remarks</u></b>	<b>124</b>
<b>REFERENCES</b>	<b>127</b>
<b>APPENDIX</b>	<b>146</b>

## LIST OF FIGURES

<b>Figure 1:</b> Plant habit and detail of the reproductive structures of <i>Ranunculus</i>	3
<b>Figure 2:</b> Phylogram of Bayesian inference analysis, posterior probability values $\geq 80$ indicated on above the branches.	7
<b>Figure 3A:</b> Phylogenetic affinities of <i>Ranunculus</i> species and allied genera based on Bayesian analysis of nrDNA ITS sequences.	18
<b>Figure 3B:</b> Phylogenetic affinities of <i>Ranunculus</i> species and allied genera based on Bayesian analysis of nrDNA ITS sequences.	20
<b>Figure 3C:</b> Phylogenetic affinities of <i>Ranunculus</i> species and allied genera based on Bayesian analysis of nrDNA ITS sequences.	22
<b>Figure 3D:</b> Phylogenetic affinities of <i>Ranunculus</i> species and allied genera based on Bayesian analysis of nrDNA ITS sequences.	25
<b>Figure 3E:</b> Phylogenetic affinities of <i>Ranunculus</i> species and allied genera based on Bayesian analysis of nrDNA ITS sequences.	26
<b>Figure 4A:</b> Phylogenetic affinities of <i>Ranunculus</i> species and allied genera based on Bayesian analysis of cpDNA <i>matK</i> sequences.	28
<b>Figure 4B:</b> Phylogenetic affinities of <i>Ranunculus</i> species and allied genera based on Bayesian analysis of cpDNA <i>matK</i> sequences.	29
<b>Figure 5:</b> Super-network of the combined ITS and <i>matK</i> Bayesian phylograms	30
<b>Figure 6:</b> Distribution of <i>R. insignis</i>	48
<b>Figure 7:</b> Distribution of <i>R. enysii</i>	50

<b>Figure 8:</b> Distribution of <i>R. lyallii</i>	51
<b>Figure 9:</b> Geographic distribution of the intermediate specimens with respect to the forms “ <i>insignis</i> ” and “ <i>lobulatus</i> ” in the South Island of New Zealand.	57
<b>Figure 10:</b> Cluster analysis of the 124 individuals of <i>R. insignis s.l.</i> based on morphological characters.	62
<b>Figure 11:</b> Multidimensional scaling ordination in three dimensions of the 124 specimens of <i>R. insignis s.l.</i> based in morphological characters.	63
<b>Figure 12:</b> Frequency of four qualitative characters with the greatest degree of discontinuity between the three forms of <i>R. insignis s.l.</i>	64
<b>Figure 13:</b> Discriminant function analysis of the intermediate individuals and putative parental species.	66
<b>Figure 14:</b> Cluster analysis of the 83 specimens of <i>R. enysii s.l.</i> based on morphological characters.	68
<b>Figure 15:</b> Multidimensional scaling ordination in three dimensions of the 83 specimens of <i>R. enysii s.l.</i> based in morphological characters.	69
<b>Figure 16:</b> Multidimensional scaling ordination in three dimensions of <i>R. enysii s.l.</i> based in morphological characters.	69
<b>Figure 17:</b> Cluster analysis of the 41 specimens of <i>R. lyalii.</i> based on morphological characters.	70
<b>Figure 18:</b> Multidimensional scaling ordination in three dimensions of the 41 specimens of <i>R. lyalii</i> based in morphological characters.	71

<b>Figure 19:</b> Maximum parsimony trees based on nrITS (A) and cpJ <sub>SA</sub> (B) sequences of <i>R. insignis s.l.</i>	73
<b>Figure 20:</b> Maximum parsimony trees based on nrITS (A) and cpJ <sub>SA</sub> (B) sequences of <i>R. enysii s.l.</i>	75
<b>Figure 21:</b> Maximum parsimony trees based on nrITS (A) and haplotype network (B) based on cpJ <sub>sa</sub> sequences of <i>R. lyallii</i> , haplotypes distribution (C) and position of the polymorphic sites (D).	76
<b>Figure 22:</b> Loadings scores for each site on the first two PCA axes for the three forms of <i>R. insignis s.l.</i> studied.	78
<b>Figure 23:</b> Loadings scores for each site on the first two PCA axes for the five forms of <i>R. enysii s.l.</i> studied.	79
<b>Figure 24:</b> Loadings scores for each site on the first two PCA axes for <i>R. lyallii</i> .	80
<b>Figure 25:</b> Variation in nectary gland morphology and size in the three forms of <i>R. insignis s.l.</i>	83
<b>Figure 26:</b> MT tree showing phylogenetic affinities of the New Zealand alpine <i>Ranunculus</i> based on nrDNA sequences (ITS).	101
<b>Figure 27:</b> MT tree showing phylogenetic affinities of the New Zealand alpine <i>Ranunculus</i> based on cpDNA sequences (J <sub>SA</sub> ).	102
<b>Figure 28:</b> Multidimensional scaling ordination in three dimensions showing the morphological diversity of 20 <i>taxa</i> of alpine <i>Ranunculus</i> of New Zealand based on morphological and anatomical characters.	103
<b>Figure 29:</b> Multidimensional scaling ordination in three dimensions showing the morphological diversity of 20 <i>taxa</i> of alpine <i>Ranunculus</i> of New Zealand based on vegetative (A) and reproductive characters (B).	104

<b>Figure 30:</b> Cluster analysis of 20 <i>taxa</i> of alpine <i>Ranunculus</i> based on 19 morphological characters.	105
<b>Figure 31:</b> Diversity in plant habit observed within the New Zealand alpine <i>Ranunculus</i> .	109
<b>Figure 32:</b> SEM micrographs showing the diversity of nectary gland shape and size of New Zealand alpine <i>Ranunculus</i> .	110
<b>Figure 33A:</b> Achene SEM micrographs of New Zealand alpine <i>Ranunculus</i> and detail of the carpel wall cells.	111
<b>Figure 33B:</b> Achene SEM micrographs of New Zealand alpine <i>Ranunculus</i> and detail of the carpel wall cells.	112
<b>Figure 33C:</b> Achene SEM micrographs of New Zealand alpine <i>Ranunculus</i> and detail of the carpel wall cells.	113
<b>Figure 33D:</b> Achene SEM micrographs of New Zealand alpine <i>Ranunculus</i> and detail of the carpel wall cells.	114
<b>Figure 33E:</b> Achene SEM micrographs of New Zealand alpine <i>Ranunculus</i> and detail of the carpel wall cells.	115
<b>Figure 34:</b> Canonical variates analysis of 16 environmental variables obtained from LENZ indicating the habitats occupied by 20 <i>taxa</i> of the New Zealand alpine <i>Ranunculus</i> .	117
<b>Figure 35:</b> Habitats occupy by a selected group of alpine <i>Ranunculus</i> in the South Island.	119

## LIST OF TABLES

<b>Table 1:</b> Subdivision of the genus <i>Ranunculus s.l.</i> following Tamura (1995).	5
<b>Table 2:</b> Classification of New Zealand <i>Ranunculus</i> at the section level according to Fisher (1965), Garnock-Jones (1988) and Tamura (1995).	13
<b>Table 3:</b> Qualitative and quantitative morphological characters studied in 151 specimens of <i>R. insignis s.l.</i>	53
<b>Table 4:</b> Qualitative and quantitative morphological characters studied in 83 specimens of <i>R. enysii s.l.</i>	54
<b>Table 5:</b> Qualitative and quantitative morphological characters studied in 41 specimens of <i>R. lyallii.</i>	55
<b>Table 6:</b> List of variables obtained from the environmental layer IV of the LENZ database.	60
<b>Table 7:</b> K-W test and significance of each character for <i>R. insignis s.l.</i>	64
<b>Table 8:</b> Classification results of the DFA for the three groups; forms insignis and lobulatus and intermediate specimens.	65
<b>Table 9:</b> Standardised canonical discriminant function coefficients for the groups made up by insignis, lobulatus and intermediate specimens.	66
<b>Table 10:</b> Results of the Mantel tests for habitat, geographic distance and morphology for the three study cases: <i>R. insignis s.l.</i> , <i>R. enysii s.l.</i> and <i>R. lyallii.</i>	81
<b>Table 11:</b> Characters and character states recorded from 20 <i>taxa</i> of alpine <i>Ranunculus</i> of New Zealand.	98

<b>Table 12:</b> Morphological and anatomical character and character-states recorded from 20 <i>taxa</i> of alpine <i>Ranunculus</i> of New Zealand.	108
---	-----

## LIST OF APPENDICES

<b>Appendix 1:</b> Articles published during the production of this thesis	146
<b>Appendix 2:</b> Collection details of <i>Ranunculus</i> species sequenced in Chapter II and not published before (ITS and <i>matK</i> ) included in CD attached	203
<b>Appendix 3:</b> Sequences of the primers used for amplification of the Internal Transcribed Spacer (ITS) and the chloroplast regions <i>J<sub>SA</sub></i> and <i>matK-trnK</i> .	203
<b>Appendix 4:</b> Eigen values, percentage of variance explained by the first five components and loading scores for the environmental data of <i>R. isignis s.l.</i>	204
<b>Appendix 5:</b> Eigen values, percentage of variance explained by the first five components and loading scores for the environmental data of <i>R. enysii s.l.</i>	204
<b>Appendix 6:</b> Eigen values, percentage of variance explained by the first five components and loading scores for the environmental data of <i>R. lyalii s.l.</i>	204
<b>Appendix 7:</b> Multidimensional scaling ordination in three dimensions showing the morphological diversity of 20 <i>taxa</i> of alpine <i>Ranunculus</i> of New Zealand based on vegetative (A) and reproductive characters (B).	205