Species delimitation and the population genetics of rare plants: A case study using the New Zealand native pygmy forget-me-not group (*Myosotis*; Boraginaceae)

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

Doctor of Philosophy
in
Plant Biology

at Massey University,
Manawatū,
New Zealand.

Jessica Mary Prebble

2016

Abstract

Myosotis L., the forget-me-nots, is a genus of about 100 species distributed in the Northern and Southern Hemispheres. There are two centres of diversity, Eurasia and New Zealand. The New Zealand species are a priority for taxonomic revision, as they comprise many threatened species and taxonomically indeterminate entities. This thesis includes a taxonomic revision of the native New Zealand *Myosotis pygmaea* subgroup, followed by an exploration of the genetic effects of rarity, and implications for conservation management.

Species delimitation follows the general lineage model, in which multiple lines of evidence are analysed to identify evolutionary lineages. The morphological data collected from herbarium specimens and live plants grown in a common garden were used to delineate the *M. pygmaea* group and identify several groups within it that nearly matched the current taxonomy. High levels of plasticity were also uncovered. Microsatellite loci were developed as polymorphic markers for the *M. pygmaea* group for species delimitation and conservation genetics. Over 500 individuals were genotyped, mostly focusing on the *M. pygmaea* group but including several outgroup species for comparison. Several genetic clusters were identified showing morphological or geographic patterns. Considering both the genetic and morphological data, as well as novel ecological niche modelling, there is evidence for three main lineages within the *M. pygmaea* group which are formally recognised as *M. antarctica*, *M. brevis* and *M. glauca*. *M. antarctica* is further subdivided into two subspecies based on allopatry and morphology, namely subsp. *antarctica* and subsp. *traillii* (formerly *M. drucei* + *M. antarctica* and *M. pygmaea*, respectively).

Using this new taxonomic framework to explore genetic variation relative to rarity shows very little difference among species. This is most likely due to the confounding effect of high levels of self-fertilization and low dispersal, which means that the majority of genetic variation within these species is partitioned between, rather than within populations. The implication for conservation is that each population is equally important in terms of their contribution to the genetic diversity of each species. This thesis represents a major increase in our knowledge of the evolution, systematics, taxonomy, rarity and conservation of New Zealand native forget-me-nots.

Acknowledgments

A large number of people and institutions have helped me to complete this thesis, thank you all. Most importantly my supervisors, Vaughan Symonds, Heidi Meudt and Jen Tate, thanks for your unwavering support and helpful input throughout all stages of the PhD process.

In the field: For collecting samples for me, or accompanying me in to the hills thanks to: Graeme Atkins, Lesley Bagnall, John Barkla, Jesse Bythell, Jan Clayton-Green, Liz and Chris Conner, Shannel Courtney, Peter de Lange, Charlie Devonish, Micheline Evans, Alex Fergus, Teresa Herleth, Rowan Hindmarsh-Walls, Cathy Jones, Carlos Lehnebach, Graeme Low, Heidi Meudt, Colin Ogle, Kay Pilkington, Mark, Meg and Sam Prebble, Brian Rance, Geoff Rogers, Neill and Barbara Simpson, Tony Silbury, Nick Singers, Mike Thorsen and Hugh Wilson. For helping with permits, location information, or assisting with access thanks to Castle Hill Station, Joy Comrie, Jacob Dexter, Paul Jansen, Graeme La Cock, Darren Peters, Viv McGlynn, Kiersten McKinley, New Zealand's Aluminium Smelter, Rodney Russ, Te iwi o Ngātiwai, Bev and Allan Potts, Ryonier Forests, Catherine Warren, Tama Wipaki and the Aorangi Awarua Trust and many additional Department of Conservation staff around the country. For help funding the fieldwork stage thanks to the Royal Society of New Zealand for a grant from the Hutton Fund, the Australasian Systematic Society for a Hansjörg Eichler Research Grant, Project Tongariro for a Memorial Award, and the Enderby Trust for a scholarship to join the Forgotten Islands of the South Pacific cruise with Heritage Expeditions.

In the lab: For helping me feel at home in the lab thanks to the LoST lab crew of Prashant Joshi, Rebecca Bloomer, Kay Pilkington, Cindy Skema, Tina Sehrish, Megan Van Ettan, Sofie Pearson and Mohamed Owis. For helping me run various analyses and connect with the Massey computer cluster thanks to Mac Campbell, Gillian Gibb, Elizabeth Daly, Mary Morgan-Richards and Steve Trewick. For sharing scripts to run Structure in parallel thanks to Kevin Emerson. For helping look after my plants in the common garden experiment thanks to Prashant Joshi, Rebecca Bloomer and Vaughan Symonds.

In the herbarium: Thanks to the Museum of New Zealand Te Papa Tongarewa for hosting me. To the Te Papa Botany team for day to day support, lunch time conversations and all of the birthday celebrations thanks to Pat Brownsey, Heidi Meudt, Carlos Lehnebach, Leon Perrie, Ant Kusabs, Julia Wilson-Davey, Peter Beveridge, Barbara Polly, and Phil Garnock-Jones. For facilitating the transfer of many herbarium specimens thanks to AK, CHR, OTA

and most of all Ant Kusabs at WELT and Jen Tate at MPN. For funding a course on morphometrics thanks to the Wellington Botanical Society for an Arnold and Ruth Dench NZ Botanical Award.

In general: For proof reading all of the chapters for me with enthusiasm thanks to Fiona Hodge. For funding thanks to Massey University for the Vice-Chancellor's Doctoral Scholarship, the Massey University Institute of Fundamental Science for post-scholarship funding, the Eastbourne Bays Community trust for the Eastbourne Freemason's Scholarship, Zonta Manawatū for their Scholarship for Women in Science and Technology – funded by Graduate Women Manawatū, and Universities New Zealand for the Claude McCarthy Fellowship.

At home: for being my home base at different times over the years (aka thanks for all of the cups of tea) thanks to Alex Fergus, Bronwyn Haines, Emmie Ellis, Ella Hayman, Dean Clarke, Anna Costley, Frances Moore, Rob Odlin, Amy Shears, Joe McCarter and Liz Willoughby-Martin. Most of all thanks to my parents, Mark Prebble and Lesley Bagnall who first took me tramping and gave me the names for plants; and my siblings, honorary siblings and niblings for the adventures, love and support along the way thanks to Joe, Priya, Nayan, Sam, Em, Meg, Amelia, Charlotte and Tim.

This thesis is dedicated to my brother Sam (1982–2014) who when I told him what I was researching said, "Is pressing flowers work now? Is that what they're teaching you in your so-called 'university,' hmm?"

Yes, it was work, but it was also fun, due to all of the people listed above who helped me along the way, but most of all due to my lovely family and friends. So for helping me all of the times, thanks, you're the best.

Table of contents

List of Figures

List of Tables

List of Appendices

Chapter 1 General Introduction

This thesis is concerned with questions regarding species delimitation and rarity, and how these concepts relate to conservation. Each chapter within this thesis contains its own introduction to the literature uniquely relevant to it; therefore this general introduction serves as a broader overview of species delimitation and rarity, and an explanation of the thesis structure itself. It begins with a brief discussion of what is a species and how species delimitation can be undertaken in an integrative way. This is followed by an introduction to some of the concepts of rarity, which is then related to the New Zealand situation and the study group of this thesis, the New Zealand native *Myosotis pygmaea* species group. Finally, the study group is introduced in more detail, along with the specific topics addressed in each chapter.

Species delimitation

What is a species?

The debate over how to define and delimit species is longstanding and ongoing (de Queiroz 2007). The species is one of the fundamental units of biology, and an understanding of species limits is essential, e.g., for the effective conservation management of endangered species. However, there is still a great deal of debate around how species should be defined, and once defined how they should be delimited. The sometimes heated discussion has yielded upwards of 20 species "concepts" (de Queiroz 2007) that when applied individually can result in different numbers of species, or even different species, being delimited. For example, the criterion of one or more fixed character state differences—the basis of one of the phylogenetic species concepts—commonly leads to the recognition of more species than the criterion of intrinsic reproductive barriers which is the basis of the biological species concept (Cracraft 1997).

Some of the influential species concepts include the biological species concept (Mayr 1942), the ecological species concept (van Valen 1976), and the phylogenetic species concept (e.g., Hennig 1966; Rosen 1979). The biological species concept states that species are groups of interbreeding populations that are reproductively isolated from other such groups (Mayr 1970). This is one of the most intuitive species concepts, but it becomes difficult to apply to species that are able to self-reproduce, and if applied rigidly would

lead to fewer species being recognised, particularly among plant species, where hybridisation is common (Rieseberg and Willis 2007). The ecological species concept takes into account the niche or adaptive zone that organisms inhabit, and when applied meticulously it has been used to name a cell line as a new species (van Valen and Maiorana 1991). There are several different phylogenetic species concepts, some of which rely on monophyly (e.g., Rosen 1979) while others focus on fixed character differences (e.g., Baum and Shaw 1995).

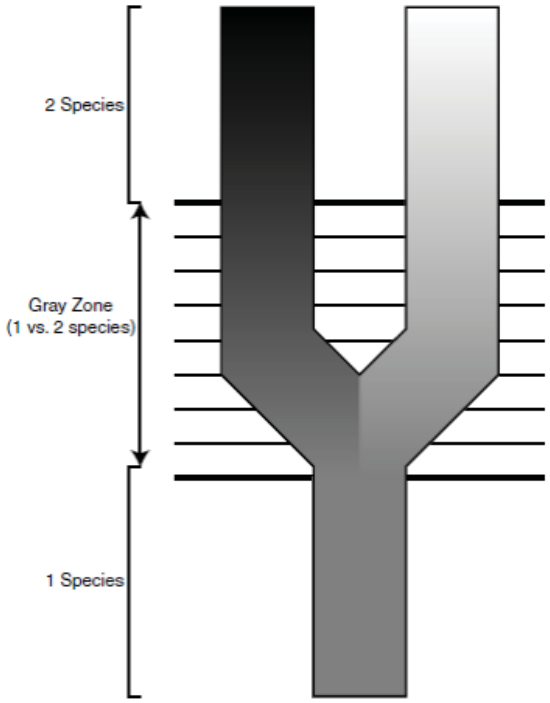


Figure 1.1 Speciation of two lineages through time, modified from de Queiroz (2007). Lines indicate where different properties might be acquired, such as morphological distinctiveness, ecological distinctiveness, reciprocal monophyly, reproductive incompatibility, etc. Properties are not necessarily acquired in the same rate or order during different speciation events.

With so many different existing concepts that have different strengths and weaknesses and different ways of defining and delimiting species, it has more recently been asked whether there are any common elements that could bring them together. This has led to the general lineage concept (also known as the unified species concept) of de Queiroz (2007). In the general lineage concept, species can be thought of as separately evolving metapopulation lineages, and what used to be considered "species concepts" can be seen as different methods that can be used to help decide at which point to make the cut-off between populations and species (Figure 1.1). As evolution and speciation are ongoing processes, it can be difficult to determine when this cut-off point should be drawn.

Therefore, the general lineage concept emphasises combining data from many different sources for the purpose of lineage discovery. Older and more divergent species can usually be easily recognised by considering any of the different species concepts and criteria. However, difficulties arise during the early stages of lineage divergence, when lineages may have diverged and formed new species, but may not have acquired all of the different properties on which species concepts are based (de Queiroz 2007). A pragmatic way to approach the question of species delimitation is to start with the null hypothesis that two individuals comprise the same species, and then aim to falsify this null hypothesis by one or more sources of evidence (the more the better). Therefore if one piece of evidence does not falsify the null hypothesis, it is not proof that there is only one species present, it simply indicates that that particular piece of evidence is not informative, yet others might be.

What are some of the different lines of evidence to delimit plant species?

Morphology Morphological characters are the most common characters used for plant species delimitation, and historically phenotypic similarity has been the main criterion used by taxonomists to group individuals into species. There is a strong correlation between phenetically defined groups based on morphological data and reproductively independent lineages (Rieseberg et al., 2006), which confirms that morphological data are worthwhile to use in species delimitation. Both quantitative and qualitative data can be used, with different analyses appropriate for different data types (Stuessy 2009).

Current taxonomy based on morphological data ideally uses a statistically rigorous approach, and a wide range of analyses can be used, from phylogenetic to phenetic, including clustering, ordination and statistical tests, to determine if groups are significantly different. Examples of analyses include phenetic techniques such as principle components analyses (PCA) [e.g., *Phormium* (Smissen and Heenan 2010)] and multidimensional scaling (MDS) [e.g. New Zealand *Plantago* (Meudt 2012)], mapping morphological data onto molecular phylogenies [e.g., *Lithospermum* (Cohen 2011) and *Zostera* (Les et al., 2002)], and geometric morphometrics (e.g., Viscosi and Cardini 2011).

Morphological variation is influenced by both environmental and genetic factors (and the interaction between them), and it is ideal to distinguish between the two so that taxonomic decisions can be based on characters that have a genetic basis. Due to their sessile lifestyle and modular construction, plants may be phenotypically plastic; that is, they have the capacity to produce new copies of organs (e.g., leaves) with different sizes and/or shapes, depending on the environmental stimulus (Price et al., 2003; Stuessy

2009). In Chapter 2 of this thesis I test species limits within the *Myosotis pygmaea* species group using statistical analyses of morphological characters measured from herbarium specimens. I also undertake a common garden experiment to determine which characters reliably distinguish between taxa of interest under common growth conditions to help determine the environmental vs. genetic basis of morphological variation in the *M. pygmaea* group.

Highly polymorphic markers and population genetics The use of highly polymorphic markers, such as nuclear microsatellites and amplified fragment length polymorphism (AFLP) data for species delimitation has become widespread in the last fifteen years (Koopman et al., 2008; Duminil and Michele 2009; Hausdorf and Hennig 2010). Microsatellite loci are tandem repeat DNA motifs of 1–6 bp in length. These loci occur at high frequency in all eukaryotes examined (Katti et al., 2001). Until recently, the use of microsatellite loci was near universal in population genetic studies, especially those studying animals, mostly due to their ease of use and power for population genetic analyses. Microsatellite loci are typically highly variable, even in organisms that otherwise display little DNA-sequence variation (Zwettler et al., 2002; Duminil 2012). Microsatellite markers for non-model organisms can now be fairly quickly and easily designed using next-generation sequencing data (Zalapa et al., 2012). Microsatellite loci have been used to test species limits (Edwards et al., 2009), explore the differences in genetic variation between pairs of rare and common congeners (e.g., Takahashi et al., 2011), and to investigate the differences in genetic variation between selfing and outcrossing plants (e.g., Mable and Adam 2007). Furthermore, as several independent loci are used, they should be able to provide a more accurate representation of the genome and limit the risks of incomplete lineage sorting, which can confound single-locus analyses.

The advances of next-generation sequencing, more accurately described as high-throughput sequencing, have vastly increased the amount of, and speed at which, DNA can be sequenced (Schuster 2008). This has resulted in multiple new methods relevant to generating data for species delimitation. New methods for developing markers include via whole genome skimming (e.g., Dodsworth 2015), sequencing transcriptomes (e.g., Mendoza et al., 2015) and Hyb-Seq (e.g., Folk et al., 2015). New methods for sequencing single nucleotide-polymorphisms (SNPs) from restriction based techniques such as restriction site associated DNA markers (RAD-Seq) and genotyping by sequencing (GBS) (e.g., Beck and Semple 2015) are also of relevance to species delimitation, and the ability to sequence multiple specimens in the same run is a significant break-through. Although these methods do not necessarily produce highly polymorphic markers, the large numbers

of markers able to be developed and sequenced, and the number of SNPs identified (often in the tens of thousands), means these methods are rivalling and even surpassing microsatellites in terms of generating informative data.

However, there are currently still benefits to using microsatellites rather than next generation sequencing data, in that as the marker of choice for the last ~20 years much is known about the properties of microsatellite loci, including how they evolve (Ellegren 2004), how to score them (e.g., software such as GeneMapper, Applied Biosystems) and how to analyse and interpret the results (e.g., Oliveira et al., 2006). Furthermore, microsatellites still have the advantage over SNPs in that each locus has the potential to display more alleles, and they are still the marker of choice for many studies involving fine-scale population structure, recent demographic events, and breeding or pedigree estimation (Oliveira et al., 2006; Zalapa et al., 2012). An additional benefit to using microsatellites vs. high-throughput techniques is that additional samples can be analysed efficiently as small numbers of samples can be genotyped at one time (Hodel et al., 2016). This can be particularly useful when studying rare, hard-to-find species, of which additional populations may be collected unexpectedly.

Under a population genetics framework, species boundaries can be identified using clustering methods that assign individuals to gene pools according to their genotype using analysis software such as Structure (Pritchard et al., 2000) and Instruct (Gao et al., 2007). Genetically differentiated gene-pools reflect barriers to gene flow, especially when found in sympatry (e.g., Duminil 2012). Studies have used microsatellite data as a tool for species delimitation in recent plant radiations [e.g., *Shoenoplectiella* (Cyperaceae) from South Korea (Kim et al., 2012), *Sphagnum* from Australia and New Zealand (Karlin et al., 2008), and *Conradina* (Lamiaceae) from Florida (Edwards et al., 2009)]. The relatively fast mutation rate of microsatellites (Jarne and Lagoda 1996) can allow the distinction of species in cases where DNA sequencing methods fail. For example, the population genetic analyses of microsatellite data showed that individuals and populations clustered together in accordance with the traditional morphological boundaries of six *Conradina* species, after species-level phylogenetic analyses based on multiple nuclear and plastid gene regions had failed to recover the monophyly of any of them (Edwards et al., 2006; Edwards et al., 2008; Edwards et al., 2009).

Population genetic studies of members of the Boraginaceae have been conducted using microsatellite data [*Echium* (Romeiras et al., 2007)], AFLP data [the New Zealand *Myosotis petiolata* complex (Meudt et al., 2013) and *Onosma* (Kolarčik et al., 2010)], and RAPD data

[*Borago* (Sales et al., 2008)]. No microsatellites have previously been developed for the genus *Myosotis*. In Chapter 3 of this thesis I report on microsatellite loci developed from next-generation sequencing data (e.g., Zalapa et al., 2012). Chapter 3 has already been published in the journal *Applications in Plant Sciences* (see Appendix 1). In Chapter 4 these microsatellites are used to explore the population genetic structure of the *Myosotis pygmaea* group and to help delimit species within it.

Ecological niche modelling Ecological and environmental variables can also be used to help delimit species. Botanists frequently record information such as the geology and substrate of the area, the habitat type (e.g. forest, tussock, and coastal turf), elevation and aspect and use this information to help inform taxonomic decisions. This can be done in more rigorous ways such as mapping the underlying geology with species distributions [e.g., *Pachycladon* (Heenan and Mitchell 2003)], or even by ecological and environmental modelling.

Ecological niche models use associations between environmental variables and known species' occurrence localities to define the abiotic conditions within which the species can survive (Elith et al., 2006). Although species distribution modelling is not yet commonly used to delimit species, it has been found to be useful when delimiting recently evolved species, and to delimit cryptic species (Raxworthy et al., 2007; Reeves and Richards 2011). Niche modelling can provide evidence for geographical isolation between populations, and therefore can suggest these populations are separately evolving lineages when gene flow is considered unlikely for the intervening unsuitable region (Raxworthy et al., 2007).

One of the challenges of using ecological niche modelling is getting good quality abiotic data to use in the model. Data for New Zealand is available in the form of WorldClim bioclimatic variables (http://www.worldclim.org/current)(Hijmans et al., 2005), which are available in a maximum of a 30 arc-second quadrat resolution (~1 km grid squares at the equator). There are additional layers developed for Land Environments New Zealand (LENZ) (https://lris.scinfo.org.nz/)(Leathwick et al., 2002), and these are available at a higher resolution (25 x 25 m^2 grid) . The LENZ layers have been used for ecological modelling, for example to reconstruct distributions of stick insects during the last glacial maximum (Buckley et al., 2010). Botanists have attempted to use the LENZ database to model ecological niches for *Ranunculus* (Lehnebach 2008) and for *Veronica* (Pufal 2010) but have found the abiotic data to be of limited use due to the relatively coarse scale at which it is available. Nevertheless, ecological niche modelling using LENZ may be useful for explicitly testing whether *Myosotis* species have distinctive ecological niches. This is

because as currently circumscribed they appear to have very restricted geographical ranges and/or occupy very specific habitats (Given 1981; Stanley et al., 1998; Rogers and Walker 2002). For example, the tag-named entity *M.* "Volcanic Plateau" is primarily considered a separate entity based on its differing ecology (Table 2.1). Chapter 5 of this thesis uses ecological niche modelling to compare between the habitat requirements of species and tag-named entities within the *M. pygmaea* group.

Importance of combining lines of evidence

The general lineage concept (de Queiroz 2007) emphasises the importance of combining lines of evidence for species delimitation. Evaluating multiple criteria not only increases our ability to detect recently separated lineages, but also can provide stronger support for lineage separation when they are in agreement (e.g., Ornelas-García et al., 2008; Reeves and Richards 2011). The process by which lineages are translated into taxonomic rank, i.e. how the decision is made as to whether a lineage is best recognised as a species or subspecies, is often not explicitly stated in taxonomic treatments, and can appear to be somewhat arbitrary. If there is good evidence that lineages are reproductively isolated, i.e. morphological or genetic data separates two lineages with sympatric ranges, then species rank is generally warranted (Steussy 2009). The rank of subspecies is probably more appropriate in situations where minor morphological or molecular differentiation is not obviously linked to reproductive isolation, i.e., because the lineages are allopatric (Stuessy 2009). This concept of subspecies was also identified in a meta-analyses exploring the use of subspecies in taxonomic treatments, which found that the rank of subspecies was most often used for lineages united by morphological and either evolutionary or ecogeographic data (Hamilton and Reichard 1992). Combining lines of evidence has loosely been termed "integrative taxonomy" by some, but Yeates et al., (2011) suggest it is better termed "iterative" taxonomy, and the term integrative be reserved for those studies that truly combine or concatenate multiple sources of data. One of the key requirements for true integration is usually that the data from different sources (e.g., morphological and molecular) be collected from the same individuals. In Chapters 4 and 5 of this thesis both iterative and integrative methods are explored, taking into account the morphological, molecular and ecological niche datasets generated to explore species delimitation of the *Myosotis pygmaea* group.

Rarity

The biology of rarity – what does it mean to be a rare plant?

The implications of a species being rare has been the topic of extensive discussion among biologists, particularly the relationship between rarity, extinction risk and conservation effort. Rare species are of interest to conservationists, as rare plants are generally considered to be more at risk of extinction than common species. However, there is a growing understanding that not all rare species have the same evolutionary history, the same causes of rarity or the same risk of extinction (Rabinowitz 1981). Species that are rare due to human-influenced factors such as habitat loss or the introduction of invasive species may require more conservation effort than those that are naturally uncommon e.g., the Naturally Uncommon category in the New Zealand Threat Classification System (NZTCS) (Townsend et al., 2008), Figure 1.2. Further research into the nature and causes of rarity, along with the implications of different kinds of rarity, is of great relevance to the field of conservation. The importance of rare plants to ecological functioning has recently been highlighted (Leitão et al., 2016), reinforcing the importance of rare plants as a key part of healthy ecosystems.

What is rarity?

The question of whether a plant species is or is not rare appears at first to be a simple matter; it is merely a question of how uncommon the plant species is (Given 1981). But what does uncommon mean? Rarity depends on the scale, space, and time considered. Plants can be rare in one country but common in another (Harper 1981). Furthermore, rare species can be geographically widespread but infrequent throughout their distribution, or be locally abundant in a very narrow geographic range. Abundance may vary in geological or evolutionary time in that relictual species that were once widespread and are now limited in distribution, or new species that are recent in origin and have yet to reach their potential to become geographically widespread (Stebbins 1980). In practice, definitions of biological species rarity tend to depend on the purpose for measuring the rarity, which makes it even more important for researchers to clearly communicate their measures of rarity (Reilly 2010).

Rabinowitz (1981) distinguished three aspects of the situation of a species: geographic range, habitat specificity, and local population size (Table 1.1). Stebbins (1980) emphasised that the nature and occurrence of rare species is not a simple problem that can be solved by applying one or few general principles. Each example of a rare species

must be considered with respect to the environment in which it grows, the population genetic structure and amount of genetic variability, and its reproductive system as well as the past history of the populations to understand its current distribution.

Table 1.1 The seven forms of rarity, adapted from Rabinowitz (1981), numbers as assigned by Reilly (2010). Numbers 1–4 correspond to rarer and more threatened species, 5–7 are less rare and less threatened, and 8 is common.

Geographic range	**Large**		**Small**	
Habitat specificity	Wide	Narrow	Wide	Narrow
Local population size: Large, dominant somewhere	8. Locally abundant over a large range in several habitats (Common)	7. Locally abundant over a large range in a specific habitat	5. Locally abundant in several habitats but restricted geographically	3. Locally abundant in a specific habitat but restricted geographically
Local population size: Small, non-dominant	6. Constantly sparse over a large range and in several habitats	4. Constantly sparse in a specific habitat but over a large range	2. Constantly sparse and geographically restricted in several habitats	1. Constantly sparse and geographically restricted in a specific habitat

In her PhD thesis, Reilly (2010) proposed a quantitative approach for defining rarity that included local abundance, geographic range size, habitat preference, frequency, and occupancy. Following Rabinowitz (1981), Reilly (2010) states that rarity is three-dimensional, consisting of abundance, range, and habitat volume. In Chapter 5 of this thesis the rarity types of the *M. pygmaea* group species are assessed following Rabinowitz (1981) and Reilly (2010).

Why are some plant species rare and some common?

It is possible to distinguish causes of rarity that relate to human influences such as habitat loss from those that relate to geological and evolutionary history ("natural" causes, here meaning any causes that do not relate to human intervention). It is widely accepted that a large number of species are rare due to human-induced factors, and the major drivers of contemporary biodiversity loss are considered to be human-influenced land use change such as habitat loss and/or fragmentation, and biotic exchange such as invasive introduced species (Sala et al., 2000; Didham et al., 2007). Teasing apart natural vs. human-influenced causes of rarity has important implications for conservation effort, as

people often place a higher priority on conserving species that are rare due to human causes (e.g., see the "Naturally Uncommon" category in the NZTCS; Townsend et al. 2008). However, understanding the causes of natural rarity also has important implications for conservation management as Naturally Uncommon species may easily become threatened if their habitat is threatened (de Lange et al., 2010).

Causes of natural rarity At times natural rarity has been correlated with "old" species nearing extinction (Darwin 1859; Fiedler 1986) or with newly formed species (Willis 1922), but more recently it is accepted that species of any age can be rare (Stebbins 1980; Schwartz 1993; Levin 2000). There is some indication that rare species are over-represented in large taxonomic groups (Schwartz and Simberloff 2001; Lozano and Schwartz 2005), possibly due to differential rates of speciation among families leading to both high species richness as well as numerous species with relatively small distributions (Schwartz and Simberloff 2001). Conversely, in lineages where extinction rates exceed speciation rates, the few species that persist may be those with larger distributions (Lozano and Schwartz 2005). Binks et al. (2015) argue that it may be possible to distinguish between declining vs. expanding scenarios using population genetic data as declining species are likely to show evidence of recent bottlenecks whereas expanding species will often exhibit star-shaped haplotype networks. On the other hand species that have persisted whilst rare may maintain levels of genetic diversity and be less affected by inbreeding. Other hypotheses that are sometimes used to explain natural rarity are discussed below in turn including poor competitive ability, various restrictions of the habitat the species occupies, life history traits such as dispersal method, and reduced genetic variation.

Poor competitive ability Common plants in pairs of rare/common congeners are often better competitors (Dawson et al., 2012) and poor competitive ability has been found to be linked to rarity (e.g., *Eriocaulon kornickianum*; Watson et al., 1994). However, in a study of the comparative competitive ability of rare and common prairie grasses, Rabinowitz (1980) found the paradoxical result that sparse species were nearly uniformly superior competitors to the common grasses. She concluded this was due to the sparse grasses growing best when sparse, and the common species growing best when common. Lloyd et al. (2002) studied the competitive ability of rare and common New Zealand *Chionochloa* and *Aceana* and found that some common species had better competitive ability than some rare species, but that the trend was not universal. Therefore, although rare species may have poor competitive ability in some cases, it should not be assumed that this is the cause of rarity in all cases.

Occupying a specific habitat There are two different ways that specific habitats can be limiting; either in space or in time. Numerous rare or endangered species occupy temporary types of vegetation that are soon succeeded by other vegetation in which they are unable to survive (Given 1981). This can be a difficult strategy to maintain and a number of rare native New Zealand seral species are outcompeted by invasive species with better competitive ability, including some of New Zealand's native threatened orchids such as *Thelymitra aemula* (Hatch 1995; Reid 1998). When habitat is limited by space, rather than time, many rare species occur on "ecological islands", such as living on a different substrate type (Stebbins 1980). Lloyd et al. (2002) also raise the idea that plants occupying a specific substrate type may be escaping competition, and in doing so, this tactic may be linked to poor competitive ability on the original substrate. There are many plants in New Zealand that are Naturally Uncommon due to their being restricted to a specific habitat type (de Lange et al., 2009), and several *Myosotis* species fit this category, such as *M. monroi* and *M. laeta*, which are confined to ultramafic rocks (de Lange et al., 2010).

Dispersal ability Differences in dispersal ability can sometimes explain the difference in ranges between rare and common plants (Stebbins 1980). There are differences in the dispersal mechanisms used by New Zealand plant species at a higher risk of extinction compared to those more common species (Thorsen et al., 2009). Threatened plants are more likely to use the following mechanisms: epizoochory (dispersal by attachment to animals), capsulivory (whereby a subfleshy capsule or peduncle could act as a nutritional reward for herbivorous species) and "foliage as fruit" (seeds being ingested and dispersed incidentally by herbivores). Seed dispersal mechanisms in New Zealand *Myosotis* are difficult to assess, as the hooked hairs often present on their calyces indicate dispersal by epizoochory, but in most species the calyx is attached firmly to the rachis (Thorsen et al., 2009). In prostrate species, such as the *Myosotis pygmaea* group, seeds are dispersed by water splash, wind, or possibly "foliage as fruit" dispersal (Thorsen et al., 2009); with evidence for the latter found in the presence of *Myosotis* seeds in moa coprolites (Wood et al., 2012).

Rare-common differences

There has been a great deal of research into whether there are overall trends of differences between rare and common species, and whether despite the different ways of being rare there are in fact common traits shared among rare plants. These studies can

generally be categorized into those investigating genetic trends, ecological trends, or biological trends as discussed below.

Genetic differences between rare and common species There is ongoing debate about whether rare species have lower levels of genetic variation than more common species, and if this is the case, whether it is a cause or a consequence of rarity (Stebbins 1980; Gitzendanner and Soltis 2000; Cole 2003). In a comparison of genetic variation as evidenced by isozymes between 34 pairs of rare and common congeners (not necessarily sister species), rare species did not, on average, have lower genetic diversity than widespread species, but there was a slight reduction of genetic diversity in rare species relative to their widespread congeners (Gitzendanner and Soltis 2000). This suggests it is of great importance to study related pairs of species when considering differences between rare and common species, as a significant amount of variation in genetic diversity can be linked to phylogenetic history. Cole (2003) also looked at the impact of different breeding systems on levels of genetic diversity and found that the combination of small population size and self-compatibility can be especially severe for reducing genetic variation. This is not surprising given that self-compatible plants generally have lower levels of genetic diversity, due to fixation of alleles (Lande 1995). Overall, Cole (2003) concluded that the reduced genetic variability in rare vs. common congeners indicates that genetic variation is frequently lost in rare species (but note he does not consider if it could be a cause of rarity rather than a result).

Biological and ecological differences between rare and common species Narrow-range species differ significantly from congeneric widespread species for a number of ecological and biological attributes (Lavergne et al., 2004). With respect to ecology, narrow-range species occur in habitats on steeper slopes, with higher rock cover and in lower and more open vegetation than their widespread congeners (Lavergne et al., 2004). This could potentially be tied into either the "ecological island" hypothesis (Stebbins 1980) whereby species are adapted to certain habitats, or the escaping competition hypothesis (Lloyd et al. 2002) whereby poor competitors are forced into marginal habitat. With respect to biological attributes, narrow-range species are significantly smaller than widespread species: even after their smaller stature is accounted for, narrow-range species still have fewer and smaller flowers. Narrow-range species also have flowers with less stigma-anther separation and lower pollen/ovule ratios and produce fewer seeds per plant than their widespread congeners. This would appear to suggest that narrow-range species are more likely to be self-compatible, a phenomenon that has also been noted for some groups within the New Zealand flora, e.g., the speedwell hebes (Garnock-Jones 1976). This is

directly in contrast to research on rarity in New Zealand *Myosotis* however, which found that narrow-range endemics are more likely to be outcrossing (Brandon 2001).

Is it bad to be a rare plant? Rarity and extinction risk

Darwin considered rarity to be the precursor to extinction, and thought that until the reasons for rarity were understood, extinction would not be understood either (Darwin 1859, Fiedler 1986). Rare plants are generally held to be at greater risk of extinction. If extinctions are due to the combined effects of predictable factors such as habitat loss, over exploitation, introduced species and pollution, and random or stochastic factors such as demographic, environmental, genetic and catastrophic factors (Shaffer 1981; Frankham 2005), then small populations will have a higher chance of going extinct. The influence of genetic factors in extinction events is an interesting one, and has at times been controversial (Frankham 2005). Three key ways in which genetic factors can influence extinction risk are 1. inbreeding depression, 2. loss of genetic diversity, and 3. deleterious mutation accumulation and meltdown.

Inbreeding depression occurs when usually outcrossing species either mate with closely related individuals, or become selfing, resulting in measurable losses in fitness such as reduced fecundity and reduced survival. Inbreeding has been shown to increase extinction risk in measurable ways in both laboratory experiments in mice and flies, and in field studies of butterflies and plants (Brook et al., 2002; Frankham 2005). Inbreeding depression due to self-fertilization is typically less in species that self naturally (23% reduction in mean fitness) relative to natural outcrossers (53% reduction), based on a meta-analysis (Husband and Schemske 1996). This is in part because as homozygosity increases through selfing, deleterious recessive mutations should be exposed to selection and "purged" from populations, thereby reducing the magnitude of inbreeding depression. Although some experiments have found higher inbreeding depression in predominantly outcrossing populations than predominantly selfing populations (Dart and Eckert 2013), the most comprehensive meta-analysis does not support the prediction that inbreeding depression consistently declines with increasing proportion of self-fertilized seeds (Winn et al., 2011). An important question that has received little experimental attention to date is how extinction risk for naturally inbreeding species is influenced by inbreeding depression (Frankham 2005).

Loss of genetic diversity in rare plants has been well documented (Cole 2003; Gitzendanner and Soltis 2000). Loss of genetic diversity in small populations is expected to increase extinction risk by decreasing the ability of small populations to evolve to cope with

environmental change, known as environmental potential (Frankham 2005). Reduced genetic diversity in the form of reduced heterozygosity is also implicated in reducing fitness in its own right (Reed and Frankham 2003), which could then contribute to extinction risk. Change in the structure of genetic diversity, rather than just the loss of diversity, can also be a feature of rare plants. Genetic drift can lead to the decrease of variation within populations due to loss of heterozygosity and fixation of alleles and the increase of differentiation among populations (Ellstrand and Elam 1993)

Deleterious mutation accumulation In large populations, the balance between mutation and natural selection maintains deleterious alleles at low frequencies. However, in small populations, deleterious mutations can become fixed as genetic drift plays a more important role. Fixed deleterious alleles can lead to inbreeding depression and an increased risk of extinction (Lande 1995). In large populations, chance variations in allele frequencies due to drift are generally small; in contrast, in small populations (e.g., < 100 individuals), large unpredictable changes in allele frequencies can often be attributed to drift (Ellstrand and Elam 1993).

Breeding system and rarity

It is important to understand the reproductive systems of rare plants, as species with different breeding systems are likely to have different population structures, to partition their genetic diversity in different ways, and to experience different consequences of rarity. Plants that are able to self-fertilize are able to colonise a new area from the germination of just one seed (Randle et al., 2009), are usually not dependent on pollinators, and will suffer far less from inbreeding depression (Frankham 2005). Their genetic variation will be structured with low variability within populations. On the other hand, plant species that usually outcross can be severely affected by inbreeding depression and may require pollinators to persist. Their genetic variation is more commonly structured with high heterozygosity within populations, and low differentiation between populations. For outcrossing populations that require pollination vectors, the pollinator and population density, as well as population size, may be more of an issue than for self-fertilizers. Note as well that seed dispersal can influence the amount of genetic variation structure within vs. between populations, with low levels of dispersal contributing to higher population differentiation (Loveless and Hamrick 1984).

The New Zealand situation

The New Zealand flora has a high proportion of rare endemic species; currently there are 2370 vascular plants known to be native to New Zealand, and 1932 (82%) are thought to be endemic (de Lange et al., 2010). Of these, 897 (38%) have a threat listing under the NZTCS, the majority being included in the At Risk - Naturally Uncommon category (542; 60%). When first conceptualised, this category represented a key difference between the IUCN Red List categories (IUCN 2012) and the NZTCS; it was created to more accurately reflect the nature of insular rarity within New Zealand (de Lange and Norton 1998). The Naturally Uncommon category is for species whose distribution is naturally confined to specific substrates, habitats, or geographic areas, and for which the distribution is not thought to be the result of past or recent human disturbance (Townsend et al., 2008). Categories of the NZTCS are shown in Figure 1.2.

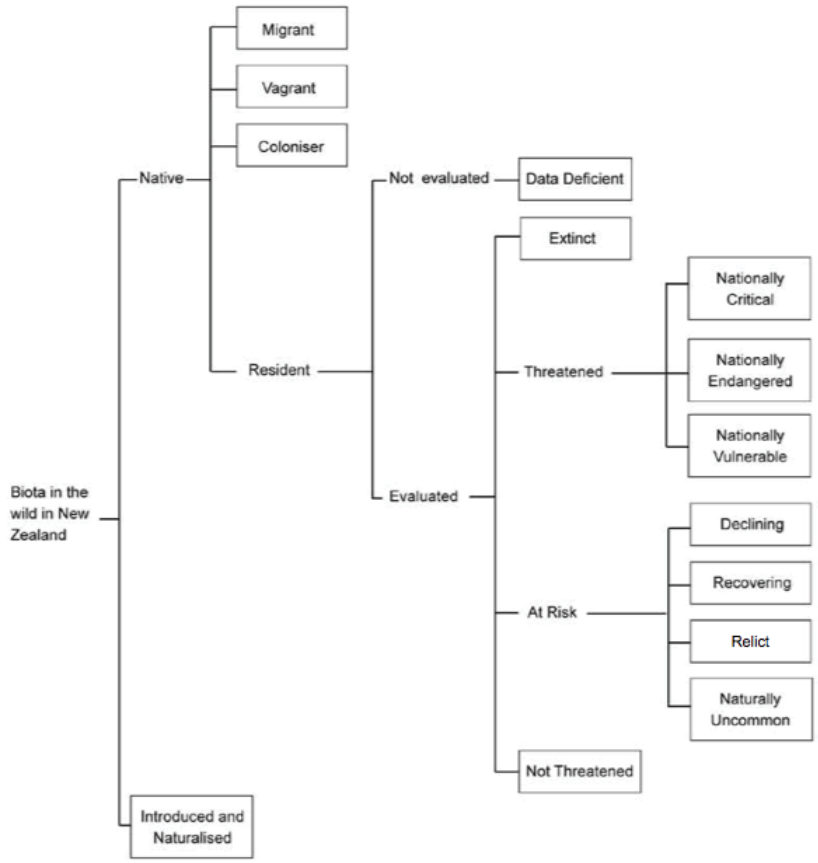


Figure 1.2 Flow chart showing the categories of the New Zealand Threat Classification System, reproduced from the manual of the NZTCS (Townsend et al., 2008).

Why New Zealand has so many Naturally Uncommon species is an area of much speculation (de Lange and Norton 1998). It is possibly linked to another well-documented feature of the New Zealand flora, that is, the large number of species radiations that exhibit extensive morphological and ecological differentiation but limited genetic

variation (Winkworth et al., 2005 and references therein). This is thought to be a result of relatively recent (2–5 mya) evolution, perhaps in response to environmental instability (including climate and geological) during the Pliocene and Pleistocene.

In New Zealand as elsewhere the destruction or alteration of habitats due to human-induced changes to the environment has been frequently implicated in plants becoming rare, particularly plants that were already restricted by their habitat requirements (i.e., were Naturally Uncommon already). In New Zealand, lowland plants are over-represented on the threatened plant list (Rogers and Walker 2002; de Lange et al., 2009), which is the area that has been most heavily modified since humans arrived (de Lange et al., 2010). A specific example is the greater bamboo rush (*Sporadanthus ferrugineus,* threat category Relict), a wetland plant from the north of the North Island, which is thought to have been widespread prior to human settlement but whose range has seriously declined as a result of wetland drainage and it is now only known from three bogs in the Waikato (de Lange et al., 1999; de Lange et al., 2010). In contrast, naturally uncommon plants from the least-modified environments, the alpine and subalpine, are infrequently listed as Threatened.

Few population genetic studies have been conducted on the New Zealand flora to date. Those based on microsatellite data have mostly been published in the last five years and include: *Sophora microphylla* (Van Etten et al., 2015), *Craspedia* (Breitwieser et al., 2015), *Fuscospora* (Smissen et al., 2014; Smissen et al., 2015) *Pseudopanax ferox* (Shepherd and Perrie 2011), *Phormium* (Scheele and Smissen 2010) and *Sphagnum* moss (Karlin et al., 2008). Those based on AFLP data include: the *Myosotis petiolata* group (Meudt et al., 2013), *Austroderia turbaria* (Houliston et al., 2012), *Olearia gardneri* (Barnaud and Houliston 2010), *Metrosideros excelsa* (Broadhurst et al., 2008), *Veronica speciosa* as *Hebe speciosa* (Armstrong and de Lange 2005), and *Metrosideros bartlettii* (Drummond et al., 2000). *Metrosideros excelsa* has also been studied based on allozyme data (Young et al., 2001). Microsatellite markers have recently been published for New Zealand native *Selliera* (Pilkington and Symonds 2016), *Clianthus* (Houliston et al., 2015), *Fuchsia* (Van Etten et al., 2013) and *Dactylanthus* (McLay et al., 2012) suggesting an increase in the number of population genetic studies is on the way. Due to this low number of studies, there are many outstanding questions regarding the differences in structuring of population genetic variation between rare and common species in general, and among Naturally Uncommon, Threatened (i.e., have gone through a human-influenced decline) and Not At Risk (i.e., common) plant species in particular. This thesis helps to address this gap by presenting a population genetic study of the *Myosotis pygmaea* species group in

Chapter 4, and investigating the differences in population genetic variation and structure between species with different threat classifications and rarity types in Chapter 5.

Rarity and New Zealand *Myosotis*

New Zealand *Myosotis* is an excellent study system for exploring how genetic variation is structured over different types of rarity, as there are examples of nearly every type of rarity exhibited (Brandon 2001). In addition, the genus includes selfing and outcrossing species, and plants native to all types of habitats (i.e., some more altered by humans than others). Furthermore, Brandon's (2001) unpublished PhD thesis entitled "Breeding systems and rarity in New Zealand *Myosotis"* laid the groundwork for further research on this genus.

Myosotis

The genus *Myosotis* L. (Boraginaceae) consists of approximately 100 species and is found in both the Northern and Southern Hemispheres (Mabberley 2008). The genus has two centres of diversity: western Eurasia, where approximately 60 species, subspecies and varieties occur (Al-Shehbaz 1991), and New Zealand where 41 species have been listed recently (Breitwieser et al., 2012; de Lange et al., 2013). It is likely that several species in New Zealand remain undescribed (Druce 1993; de Lange et al., 2013) and a taxonomic revision of the genus in New Zealand is a top priority (de Lange et al., 2009). Species delimitation within New Zealand *Myosotis* has predominantly been based on morphological grounds, but several entities that do not conform to current species descriptions have been identified, making species identification difficult.

Almost all of the native New Zealand *Myosotis* species are endemic, and many have very restricted geographical ranges and/or occupy very specific habitats (Given 1981; Stanley et al., 1998; Rogers et al., 2002). New Zealand *Myosotis* has 18 Threatened taxonomically determinate taxa (species, subspecies or varieties) and 16 At Risk (usually sub-category Naturally Uncommon) plus a further 10 Threatened or At Risk taxonomically indeterminate entities (de Lange et al., 2013). One *Myosotis* species and one variety have already gone extinct in New Zealand (de Lange et al., 2013). A range of different rarity types are exhibited by New Zealand *Myosotis* (Brandon 2001). Brandon (2001) found that breeding system (selfing vs. outcrossing) and floral structure (levels of herkogamy and the associated flower size) together accurately predict the range size of New Zealand *Myosotis*. In general species with wider distributions have little herkogamy, small flowers and are able to self-pollinate, whereas restricted species tend to show greater herkogamy, larger

flowers and require a pollinator to set seed. She found no evidence to suggest that dispersal ability (based on calyx properties, seed size and winged seed morphology) was correlated with current distribution. To date, there has been no research into the levels of genetic variation in rare vs. common *Myosotis*, nor on the implications that different levels of genetic variation may have for conservation management plans.

Phylogenetic analyses of nuclear and chloroplast sequences suggest *Myosotis* originated in the Northern Hemisphere (Winkworth et al., 2002; Meudt et al., 2015) and is nested within the tribe Cynoglosseae (Weigend et al., 2010; Nazaire and Hufford 2012). The first published study on the phylogeny of New Zealand *Myosotis* (Winkworth et al., 2002) analysed nuclear ITS and chloroplast 3' *mat*K DNA sequences from 34 individuals of *Myosotis*, including 8 from New Zealand and 26 from Europe and elsewhere. The Southern Hemisphere *Myosotis* was shown to be monophyletic. The most recently published phylogeny focusing on New Zealand *Myosotis* increased the sampling to 134 *Myosotis* samples, and number of loci analysed to four (*rps16–trnQ*, *atpI–atpH*, ITS and ETS), and also found that the Southern Hemisphere species form a clade (Meudt et al., 2015). However, multiple New Zealand *Myosotis* individuals were largely undifferentiated at several neutrally evolving DNA loci, and the New Zealand species were also largely unresolved by AFLP data (Meudt et al., 2015). This lack of differentiation, combined with molecular dating of the Southern Hemisphere clade based on the earliest known pollen fossil in New Zealand (~ 3 mya); suggest a relatively recent arrival and diversification of New Zealand *Myosotis* (Meudt et al., 2015). Despite the low levels of DNA sequence and AFLP divergence, the New Zealand species are morphologically diverse (see Figure 1 in Meudt et al., 2015). The habit of New Zealand *Myosotis* plants ranges from prostrate to erect, and some alpine species form cushions. All of the New Zealand *Myosotis* species have a basal rosette, with axillary lateral flowering branches that bear progressively more sessile cauline leaves (Moore 1961). Flower colour ranges from white to yellow to bronze, and is usually linked to breeding system and pollination method (Brandon 2001).

Due to the low levels of DNA sequence variation and lack of resolution with AFLP data, the lineages within the New Zealand clade are still unclear. However, there are several informal groups of morphologically and/or ecologically similar species that can be recognised within the genus that were identified by Robertson (1989) and included in Meudt et al., (2015). These are useful as starting hypotheses for identifying sister species pairs or groups within the genus. The *Myosotis pygmaea* group is one such group, and it is the main study system for this thesis. It is a subgroup of the bracteate-prostrate group (see Table 1 in Meudt et al., 2015).

The *Myosotis pygmaea* group consists of five named species and several tag-named entities, illustrated in Figure 2.1 and listed in Table 2.1. The first species described in the group was *Myosotis antarctica* (Hooker 1844), followed by *M. pygmaea* (Colenso 1883). Varieties of *M. pygmaea* were added over time – var. *glauca* (Simpson and Thomson 1942), var. *minutiflora* (Simpson and Thomson 1943) and var. *drucei* (Moore 1961); these varieties were recently elevated to species rank (de Lange et al., 2010) as *M. glauca*, *M. drucei* and *M. brevis,* respectively (the name *Myosotis minutiflora* was already in use). It appears likely that all members of the *M. pygmaea* complex usually self-fertilise, as their anthers are entirely included (Moore 1961). *Myosotis pygmaea, M. drucei* and *M. brevis* (as *M. pygmaea* var. *minutiflora*; note *M. glauca* was not studied) have a herkogamy distance of 0 mm, suggesting their pollination syndrome is autonomous (Robertson and Lloyd 1991; Brandon 2001). Plants in the *M. pygmaea* complex are found in a range of habitats from coastal sand dunes and turfs, to mountain turfs, damp scree and tarn edges (Moore 1961). Details of their taxonomic history, distribution, conservation status and distinguishing morphological characters are listed in Table 2.1. *Myosotis drucei* is Not at Risk and is considered the common entity in this group whereas *M. pygmaea* is considered to be Declining and historically was possibly common. *M. brevis* is Nationally Endangered (although it was probably never common), whereas *M. glauca* is Nationally Vulnerable and Declining from a population that was probably historically rare. Thus, a range of rarity types are available for testing hypotheses addressing the amount and structure of population genetic variation between different types of rarity in this group. A number of taxonomically indeterminate entities may also be associated with the *M. pygmaea* group (Table 2.1), which in conjunction with the threat levels of the named species makes a taxonomic revision of this group a high priority to inform their conservation management plans.

Thesis Structure

Overall this thesis addresses questions regarding the nature of rarity, population genetic variation, species delimitation and the conservation of plants. I have undertaken research that addresses some fundamental questions including: 1) How can population genetic information be used to inform species delimitation and conservation management of rare plants? And 2) How is population genetic variation structured in rare plants? Specifically are there differences in the population genetic structure or variation between Naturally Uncommon species and those that are thought to be rare due to human-influenced decline?

Using the *Myosotis pygmaea* group as the study system, Chapter 2 of this thesis uses morphological data from herbarium and common-garden grown plants to identify lineages within the *M. pygmaea* group. In Chapter 3, microsatellite markers are developed and tested across the *M. pygmaea* group as well as more widely in the *Myosotis* genus. In Chapter 4, over 500 individuals are genotyped using the newly described microsatellite markers to undertake a population-genetic study of the *M. pygmaea* group. Iterative and integrative taxonomy is performed, and in Chapter 5 a taxonomic revision based on those results, along with ecological niche modelling, is reported. The differences in population genetic variation and structure between species with different rarity types is also explored in Chapter 5. Chapter 6 is a general conclusion chapter, in which the significance of this thesis, along with possibilities for future work, are considered.

References

Al-Shehbaz IA (1991) The genera of Boraginaceae in the Southeastern United States. *Journal of the Arnold Arboretum, Supplementary Series* 1: 1–169.

Armstrong TT, de Lange PJ (2005) Conservation genetics of *Hebe speciosa* (Plantaginaceae) an endangered New Zealand shrub. *Botanical Journal of the Linnean Society* 149: 229-239.

Barnaud A, Houliston GJ (2010) Population genetics of the threatened tree daisy *Olearia gardneri* (Asteraceae), conservation of a critically endangered species. *Conservation Genetics* 11: 1515-1522.

Baum DA, Shaw KL (1995) *Genealogical Perspectives on the Species Problem*. In: Hoch PC, Stephenson AG, editors. *Experimental and Molecular Approaches to Plant Biosystematics*. St. Louis: Missouri Botanical Garden. p. 289–303.

Beck JB, Semple JC (2015) Next-generation sampling: Pairing genomics with herbarium specimens provides species-level signal in *Solidago* (Asteraceae). *Applications in Plant Sciences* 3: DOI: 10.3732/apps.1500014.

Binks RM, Millar MA, Byrne M (2015) Not all rare species are the same: Contrasting patterns of genetic diversity and population structure in two narrow-range endemic sedges. *Biological Journal of the Linnean Society* 114: 873-886.

Brandon AM (2001) *Breeding systems and rarity in New Zealand Myosotis*. PhD Thesis. Palmerston North: Massey University.

Breitwieser I, Brownsey P, Garnock-Jones PJ, Perrie L, Wilton A (2012) *New Zealand Inventory of Biodiversity. Phylum Tracheophyta, Vascular Plants*. In: Gordon D, editor. *Kingdoms Bacteria, Protozoa, Chromista, Plantae, Fungi*. Christchurch: Canterbury University Press. p. 411-459.

Breitwieser I, Ford KA, Smissen RD (2015) Characterisation of SSR markers for New Zealand *Craspedia* and their application in Kahurangi National Park. *New Zealand Journal of Botany* 53: 60-73.

Broadhurst LM, Young AG, Murray BG (2008) AFLPs reveal an absence of geographical genetic structure among remnant populations of pohutukawa (*Metrosideros excelsa*, Myrtaceae). *New Zealand Journal of Botany* 46: 13-21.

Brook B, Tonkyn D, O' Grady J, Frankham R (2002) Contribution of inbreeding to extinction risk in threatened species. *Conservation Ecology* 6: 1-16.

Buckley TR, Marske K, Attanayake D (2010) Phylogeography and ecological niche modelling of the New Zealand stick insect *Clitarchus hookeri* (White) support survival in multiple coastal refugia. *Journal of Biogeography* 37: 682-695.

Cohen J (2011) A phylogenetic analysis of morphological and molecular characters of *Lithospermum* L. (Boraginaceae) and related taxa: evolutionary relationships and character evolution. *Cladistics* 27: 559-580.

Cole CT (2003) Genetic variation in rare and common plants. *Annual Review of Ecology, Evolution, and Systematics* 34: 213-237.

Colenso W (1883) A further contribution towards making known the flora of New Zealand. *Transactions and Proceedings of the Royal Society of New Zealand* 16: 334.

Cracraft J (1997) *Species Concepts in Systematics and Conservation Biology – an Ornithological Viewpoint*. In: Claridge MF, Dawah HA, Wilson MR, editors. *Species: the Units of Biodiversity*. London: Chapman and Hall. p. 325–339.

Dart S, Eckert CG (2013) Experimental and genetic analyses reveal that inbreeding depression declines with increased self-fertilization among populations of a coastal dune plant. *Journal of Evolutionary Biology* 26: 587-599.

Darwin C (1859) *On the Origin of Species*. London: John Murray.

Dawson W, Fischer M, Kleunen M (2012) Common and rare plant species respond differently to fertilisation and competition, whether they are alien or native. *Ecology Letters* 15: 873-880.

de Lange P, Heenan P, Norton D, Rolfe J, Sawyer J (2010) *Threatened Plants of New Zealand*. Christchurch: Canterbury University Press.

de Lange P, Norton D (1998) Revisiting rarity: a botanical perspective on the meanings of rarity and the classification of New Zealand's uncommon plants. *Ecosystems, Entomology & Plants. Royal Society of New Zealand Miscellaneous series* 48: 145-160.

de Lange PJ, Heenan PB, Clarkson BD, Clarkson BR (1999) Taxonomy, ecology, and conservation of *Sporadanthus* (Restionaceae) in New Zealand. *New Zealand Journal of Botany* 37: 413-431.

de Lange PJ, Norton DA, Courtney SP, Heenan PB, Barkla JW, Carmeron EK, Hitchmough R, Townsend AJ (2009) Threatened and uncommon plants of New Zealand (2008 revision). *New Zealand Journal of Botany* 47: 61-69.

de Lange PJ, Rolfe JR, Champion PD, Courtney SP, Heenan PB, Barkla JW, Cameron EK, Norton DA, Hitchmough RA (2013) *Conservation Status of New Zealand Indigenous Vascular Plants, 2012*. Wellington: New Zealand Department of Conservation.

de Queiroz K (2007) Species concepts and species delimitation. *Systematic Biology* 56: 879-886.

Didham RK, Tylianakis JM, Gemmell NJ, Rand TA, Ewers RM (2007) Interactive effects of habitat modification and species invasion on native species decline. *Trends in Ecology & Evolution* 22: 489-496.

Dodsworth S (2015) Genome skimming for next-generation biodiversity analysis. *Trends in Plant Science* 20: 52 - 527.

Druce AP (1993) *Indigenous Higher Plants of New Zealand, Unpublished Checklist*. Lower Hutt: Landcare Research.

Drummond R, Keeling D, Richardson T, Gardner R, Wright S (2000) Genetic analysis and conservation of 31 surviving individuals of a rare New Zealand tree, *Metrosideros bartlettii* (Myrtaceae). *Molecular Ecology* 9: 1149-1157.

Duminil J (2012) Testing species delimitation in sympatric species complexes: The case of an African tropical tree, *Carapa* spp. (Meliaceae). *Molecular Phylogenetics and Evolution* 62: 275 - 285.

Duminil J, Michele MD (2009) Plant species delimitation: a comparison of morphological and molecular markers. *Plant Biosystems* 143: 528-542.

Edwards C, Judd W, Ionta G (2009) Using population genetic data as a tool to identify new species: *Conradina cygniflora* (Lamiaceae), a new, endangered species from Florida. *Systematic Botany* 34: 747-759.

Edwards CE, Lefkowitz D, Soltis DE, Soltis PS (2008) Phylogeny of *Conradina* and related southeastern scrub mints (Lamiaceae) based on gene sequences. *International Journal of Plant Sciences* 169: 579-594.

Edwards CE, Soltis DE, Soltis PS (2006) Molecular phylogeny of *Conradina* and other scrub mints (Lamiaceae) from the southeastern USA: evidence for hybridization in Pleistocene refugia? *Systematic Botany* 31: 193-207.

Elith J, Graham C, Anderson R, Dudík M, Ferrier S, Guisan A, Hijmans RJ, Huettmann F, Leathwick JR, Lehmann A, Li J, Lohmann LG, Loiselle BA, Manion G, Moritz C, Nakamura M, Nakazawa Y, Peterson AT, Phillips SJ, Richardson K, Scachetti-Pereira R, Schapire RE, Soberón J, Williams S, Wisz MS, Zimmermann NE (2006) Novel methods improve prediction of species' distributions from occurrence data. *Ecography* 29: 129-151.

Ellegren H (2004) Microsatellites: simples sequences with complex evolution. *Nature Reviews Genetics* 5: 435-445.

Ellstrand NC, Elam DR (1993) Population genetic consequences of small population-size – implications for plant conservation. *Annual Review of Ecology and Systematics* 24: 217-242.

Fiedler P (1986) Concepts of rarity in vascular plant species, with special reference to the genus *Calochortus* Pursh (Liliaceae). *Taxon* 35: 1-18.

Folk RA, Mandel JR, Freudenstein JV (2015) A protocol for targeted enrichment of intron-containing sequence markers for recent radiations: A phylogenomic example from *Heuchera* (Saxifragaceae). *Applications in Plant Sciences* 3: doi.org:10.3732/apps.1500039.

Frankham R (2005) Genetics and extinction. *Biological Conservation* 126: 131-140.

Gao H, Williamson S, Bustamante CD (2007) A Markov chain Monte Carlo approach for joint inference of population structure and inbreeding rates from multilocus genotype data. *Genetics* 176: 1635–1651

Garnock-Jones PJ (1976) Breeding systems and pollination in New Zealand *Parahebe* (Scrophulariaceae). *New Zealand Journal of Botany* 14: 291-298.

Gitzendanner MA, Soltis PS (2000) Patterns of genetic variation in rare and widespread plant congeners. *American Journal of Botany* 87: 783-792.

Given D (1981) *Rare and Endangered Plants of New Zealand*. Wellington: A.H. & A.W. Reed Ltd.

Hamilton W, Reichard SH (1992) Current practice in the use of subspecies, variety, and forma in the classification of wild plants. *Taxon* 41: 485-498.

Harper JL (1981) *The Meanings of Rarity*. In: Synge H, editor. *The Biological Aspects of Rare Plant Conservation*. New York: John Wiley & Sons. p. 189-203.

Hatch ED (1995) Plant succession and the problems of orchid conservation. *New Zealand Botanical Society* 41: 15.

Hausdorf B, Hennig C (2010) Species delimitation using dominant and codominant multilocus markers. *Systematic Biology* 59: 491-503.

Heenan P, Mitchell A (2003) Phylogeny, biogeography and adaptive radiation of *Pachycladon* (Brassicaceae) in the mountains of South Island, New Zealand. *Journal of Biogeography* 30: 1737-1749.

Hennig W (1966) *Phylogenetic Systematics, translated by D. Davis and R. Zangerl*. Urbana: University of Illinois Press.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965-1978.

Hodel RGJ, Segovia-Salcedo MC, Landis JB, Crowl AA, Miao Sun XL, Gitzendanner MA, Douglas NA, Germain-Aubrey CC, Chen S, Soltis DE, Soltis PS (2016) The report of my death was an exaggeration: A review for researchers using microsatellites in the 21st century. *Applications in Plant Sciences* 4: 1600025.

Hooker JD (1844) *The Botany of the Antarctic Voyage of H.M. Discovery Ships Erebus and Terror in the Years 1839-1843: Under the Command of Captain Sir James Clark Ross*. London: Reeve Brothers.

Houliston GJ, Dawson MI, Lange PJD, Heenan PB (2012) Using AFLP markers to inform population management of the endemic Chatham Island toetoe, *Austroderia turbaria* (Poaceae). *Pacific Conservation Biology* 18: 33-30.

Houliston GJ, Ramón-Laca A, Jain R, Mitchell CM, Goeke DF (2015) Simple sequence repeat markers for the endangered species *Clianthus puniceus* and *C. maximus* (Fabaceae). *Applications in Plant Sciences* 3: 1400102.

Husband B, Schemske D (1996) Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50: 54-70.

Jarne P, Lagoda P (1996) Microsatellites, from molecules to populations and back. *Trends in Ecology & Evolution* 11: 424-429.

Karlin EF, Boles SB, Shaw AJ (2008) Systematics of *Sphagnum* section *Sphagnum* in New Zealand: a microsatellite-based analysis. *New Zealand Journal of Botany* 46: 105-118.

Katti MV, Ranjekar PK, Gupta VS (2001) Differential distribution of simple sequence repeats in eukaryotic genome sequences. *Molecular Biology and Evolution* 18: 1161-1167.

Kim C, Jung J, Choi H-K (2012) Molecular identification of *Schoenoplectiella* species (Cyperaceae) by use of microsatellite markers. *Plant Systematics and Evolution* 298: 811-817.

Kolarčik V, Zozomová-Lihová J, Mártonfi P (2010) Systematics and evolutionary history of the *Asterotricha* group of the genus *Onosma* (Boraginaceae) in central and southern Europe inferred from AFLP and nrDNA ITS data. *Plant Systematics and Evolution* 290: 21-45.

Koopman WJM, Wissemann V, De Cock K, Van Huylenbroeck J, De Riek J, Sabatlno GJH, Visser D, Vosman B, Ritz CM, Maes B, Werlemark G, Nybom H, Debener T, Linde M, Smulders MJM (2008) AFLP markers as a tool to reconstruct complex relationships: A case study in *Rosa* (Rosaceae). *American Journal of Botany* 95: 353-366.

Lande R (1995) Mutation and conservation. *Conservation Biology* 9: 782-791.

Lavergne S, Thompson JD, Garnier E, Debussche M (2004) The biology and ecology of narrow endemic and widespread plants: a comparative study of trait variation in 20 congeneric pairs. *Oikos* 107: 505-518.

Leathwick J, Morgan F, Wilson G, Rutledge D, McLeod M, Johnston K (2002) *Land Environments of New Zealand: A Technical Guide*. Wellington, New Zealand: Ministry for the Environment

Lehnebach CA (2008) *Phylogenetic affinities, species delimitation and adaptive radiation of New Zealand Ranunculus*. PhD Thesis. Palmerston North: Massey University.

Leitão RP, Zuanon J, Villéger S, Williams SE, Baraloto C, Fortunel C, Mendonça FP, Mouillot D (2016) Rare species contribute disproportionately to the functional structure of species assemblages. *Proceedings of the Royal Society B-Biological Sciences* 283: DOI: 10.1098/rspb.2016.0084

Les D, Moody M, Jacobs S, Bayer R (2002) Systematics of seagrasses (Zosteraceae) in Australia and New Zealand. *Systematic Botany* 27: 468-484.

Levin D (2000) *The Origin, Expansion and Demise of Plant Species*. New York: Oxford University Press.

Lloyd KM, Lee WG, Wilson JB (2002) Competitive abilities of rare and common plants: comparisons using *Acaena* (Rosaceae) and *Chionochloa* (Poaceae) from New Zealand. *Conservation Biology* 16: 975-985.

Loveless MD, Hamrick JL (1984) Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* 15: 65-95.

Lozano FD, Schwartz MW (2005) Patterns of rarity and taxonomic group size in plants. *Biological Conservation* 126: 146-154.

Mabberley D (2008) *Mabberley's Plant Book. A portable dictionary of plants, their classifications and uses*. Seattle: University of Washington Botanic Gardens.

Mable BK, Adam A (2007) Patterns of genetic diversity in outcrossing and selfing populations of *Arabidopsis lyrata*. *Molecular Ecology* 16: 3565-3580.

Mayr E (1942) *Systematics and Origin of Species*. New York: Columbia University Press.

Mayr E (1970) *Populations, Species, and Evolution*. Cambridge, Massachusetts: Belknap Press of Harvard University Press.

McLay T, Tate J, Symonds V (2012) Microsatellite markers for the endangered root holoparasite *Dactylanthus taylorii* (Balanophoraceae) from 454 pyrosequencing. *American Journal of Botany* 99: e323-325.

Mendoza CG, Naumann J, Samain M-S, Goestghbeur P, Smet YD, Wanke S (2015) A genome-scale mining strategy for recovering novel rapidly-evolving nuclear single-copy genes for addressing shallow-scale phylogenetics in *Hydrangea*. *BMC Evolutionary Biology* 15: DOI: 10.1186/s12862-12015-10416-z.

Meudt HM (2012) A taxonomic revision of native New Zealand *Plantago* (Plantaginaceae). *New Zealand Journal of Botany* 50: 101-178.

Meudt HM, Prebble JM, Lehnebach CA (2015) Native New Zealand forget-me-nots (*Myosotis*, Boraginaceae) comprise a Pleistocene species radiation with very low genetic divergence. *Plant Systematics and Evolution* 301: 1455–1471.

Meudt HM, Prebble JM, Stanley RJ, Thorsen MJ (2013) Morphological and amplified fragment length polymorphism (AFLP) data show that New Zealand endemic *Myosotis petiolata* (Boraginaceae) comprises three rare and threatened species. *Australian Systematic Botany* 26: 210-232.

Moore LB (1961) *Boraginaceae*. In: Allan H, editor. *Flora of New Zealand. Vol. 1*. Wellington, New Zealand: PD Hasselberg, Government Printer. p. 806-833.

Nazaire M, Hufford L (2012) A broad phylogenetic analysis of Boraginaceae: Implications for the relationships of *Mertensia*. *Systematic Botany* 37: 758-783.

Oliveira E, Gomes Pádua J, Zucchi M, Vencovsky R, Carneiro Vieira M (2006) Origin, evolution and genome distribution of microsatellites. *Genetics and Molecular Biology* 29: 294-307.

Ornelas-García CP, Domínguez-Domínguez O, Doadrio I (2008) Evolutionary history of the fish genus *Astyanax* Baird & Girard (1854) (Actinopterygii, Characidae) in Mesoamerica reveals multiple morphological homoplasies. *BMC Evolutionary Biology* 8: 340.

Pilkington KM, Symonds VV (2016) Isolation and characterization of polymorphic microsatellite loci in *Selliera radicans* (Goodeniaceae). *Applications in Plant Sciences* 4: 1600012.

Price TD, Qvarnstrom A, Irwin DE (2003) The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society B: Biological Sciences* 270: 1433-1440.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.

Pufal G (2010) *The evolution and ecology of hygrochastic capsule dehiscence*. PhD Thesis. Wellington: Victoria University.

Rabinowitz D (1981) *Seven Forms of Rarity*. In: Synge H, editor. *The Biological Aspects of Rare Plants Conservation*. p. 205-217.

Randle AM, Slyder JB, Kalisz S (2009) Can differences in autonomous selfing ability explain differences in range size among sister-taxa pairs of *Collinsia* (Plantaginaceae)? An extension of Baker's Law. *New Phytologist* 183: 618-629.

Raxworthy C, Ingram C, Rabibisoa N, Pearson R (2007) Applications of ecological niche modeling for species delimitation: A review and empirical evaluation using day geckos (*Phelsuma*) from Madagascar. *Systematic Biology* 56: 907-923.

Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. *Conservation Biology* 17: 230-237.

Reeves PA, Richards CM (2011) Species delimitation under the general lineage concept: An empirical example using wild North American hops (Cannabaceae: *Humulus lupulus*). *Systematic Biology* 60: 45-59.

Reid VA (1998) *The Impacts of Weeds on Threatened Plants*. Wellington: Department of Conservation

Reilly LA (2010) *A quantitative approach for defining rarity*. PhD Thesis. Chapel Hill: University of North Carolina.

Rieseberg L, Willis J (2007) Plant speciation. *Science* 17: 910-914.

Rieseberg LH, Wood TE, Baack EJ (2006) The nature of plant species. *Nature* 440: 524-527.

Robertson A (1989) *Evolution and pollination of New Zealand Myosotis (Boraginaceae).* PhD Thesis. Christchurch: University of Canterbury.

Robertson AW, Lloyd DG (1991) Herkogamy, dichogamy and self-pollination in six species of *Myosotis* (Boraginaceae). *Evolutionary Trends in Plants* 5: 53-63.

Rogers G, Walker S (2002) Taxonomic and ecological profiles of rarity in the New Zealand vascular flora. *New Zealand Journal of Botany* 40: 73-93.

Rogers G, Walker S, Tubbs M, Henderson J (2002) Ecology and conservation status of three "spring annual" herbs in dryland ecosystems of New Zealand. *New Zealand Journal of Botany* 40: 649-669.

Romeiras MM, Cotrim HC, Duarte MC, Pais MS (2007) Genetic diversity of three endangered species of *Echium* L. (Boraginaceae) endemic to Cape Verde Islands. *Biodiversity and Conservation* 16: 547-566.

Rosen DE (1979) Fishes from the uplands and intermontane basins of Guatemala: Revisionary studies and comparative geography. *Bulletin of the American Museum of Natural History* 162: 267-376.

Sala OE, Chapin FS, Armesto JJ, Berlow E, Bloomfield J, Dirzo R, Huber-Sanwald E, Huenneke LF, Jackson RB, Kinzig A, Leemans R, Lodge DM, Mooney HA, Oesterheld M, Poff NL, Sykes MT, Walker BH, Walker M, Wall DH (2000) Biodiversity – Global biodiversity scenarios for the year 2100. *Science* 287: 1770-1774.

Sales E, Montaner C, Muniozguren JM, Carravedo M, Álvarez JM (2008) Genetic diversity in a collection of borage (*Borago officinalis*) germplasm. *Botany* 86: 603-609.

Scheele S, Smissen R (2010) Insights into the origin and identity of National New Zealand Flax Collection plants from simple sequence repeat (SSR) genotyping. *New Zealand Journal of Botany* 48: 41-54.

Schuster SC (2008) Next-generation sequencing transforms today's biology. *Nature Methods* 5: 16 - 18.

Schwartz MW (1993) The search for pattern among rare plants: Are primitive species more likely to be rare? *Biological Conservation* 64: 121-127.

Schwartz MW, Simberloff D (2001) Taxon size predicts rates of rarity in vascular plants. *Ecology Letters* 4: 464-469.

Shaffer ML (1981) Minimum population sizes for species conservation. *BioScience* 31: 131–134.

Shepherd LD, Perrie LR (2011) Microsatellite DNA analyses of a highly disjunct New Zealand tree reveal strong differentiation and imply a formerly more continuous distribution. *Molecular Ecology* 20: 1389-1400.

Simpson G, Thomson JS (1942) Notes on some New Zealand plants and descriptions of new species (No. 2). *Transactions and Proceedings of the Royal Society of New Zealand* 72: 26.

Simpson G, Thomson JS (1943) Notes on some New Zealand plants and descriptions of new species. *Transactions and Proceedings of the Royal Society of New Zealand* 73: 161.

Smissen R, Richardson S, Morse C, Heenan P (2014) Relationships, gene flow and species boundaries among New Zealand *Fuscospora* (Nothfagaceae: southern beech). *New Zealand Journal of Botany* 52: 389-406.

Smissen R, Roth M, Heenan P (2015) Absence of hybridisation between *Fuscospora* species at a site in Arthur's Pass National Park, New Zealand. *New Zealand Journal of Botany* 53: 168-174.

Smissen RD, Heenan PB (2010) A taxonomic appraisal of the Chatham Islands flax (*Phormium tenax*) using morphological and DNA fingerprint data. *Australian Systematic Botany* 23: 371-380.

Stanley R, Dickinson K, Mark A (1998) Demography of a rare endemic *Myosotis*: boom and bust in the high-alpine zone of southern New Zealand. *Arctic and alpine research*: 1-15.

Stebbins G (1980) Rarity of plant species: a synthetic viewpoint. *Rhodora* 82: 77-86.

Stuessy TF (2009) *Plant Taxonomy: The Systematic Evaluation of Comparative Data*. New York: Columba University Press.

Takahashi Y, Takahashi H, Maki M (2011) Comparison of genetic variation and differentiation using microsatellite markers among three rare threatened and one

widespread toad lily species of *Tricyrtis* section *Flavae* (Convallariaceae) in Japan. *Plant Species Biology* 26: 13-23.

Thorsen MJ, Dickinson KJM, Seddon PJ (2009) Seed dispersal systems in the New Zealand flora. *Perspectives in Plant Ecology, Evolution and Systematics* 11: 285-309.

Townsend AJ, de Lange PJ, Duffy CAJ, Miskelly CM, Molloy J, Norton DA (2008) *New Zealand Threat Classification System Manual*. Wellington: Science and Technical Publishing, Department of Conservation.

Van Etten MA, Robertson AW, Tate JA (2013) Microsatellite markers for the New Zealand endemic tree *Fuchsia excorticata* (Onagraceae). *Applications in Plant Sciences* 1: doi: 10.3732/apps.1300045.

Van Etten ML, Tate JA, Anderson SH, Kelly D, Ladley JJ, Merrett MF, Peterson PG, Robertson AW (2015) The compounding effects of high pollen limitation, selfing rates and inbreeding depression leave a New Zealand tree with few viable offspring. *Annals of Botany* 116: 833-843.

van Valen L (1976) Ecological species, multispecies, and oaks. *Taxon* 25: 233-239.

van Valen L, Maiorana C (1991) HeLa, a new microbial species. *Evolutionary Theory* 10: 71-74.

Viscosi V, Cardini A (2011) Leaf morphology, taxonomy and geometric morphometrics: A simplified protocol for beginners. *PLOS One* 6: e25630.

Watson L, Uno G, McCarty N, Kornkven A (1994) Conservation biology of a rare plant species, *Eriocaulon kornickianum* (Eriocaulaceae). *American Journal of Botany* 81: 980-986.

Weigend M, Gottschling M, Selvi F, Hilger H (2010) Fossil and extant western hemisphere Borageineae, and the polyphyly of "Trigonotideae" Riedl (Boraginaceae: Boraginoideae). *Systematic Botany* 35: 409-419.

Willis JC (1922) *Age and Area*. Cambridge, England: Cambridge University Press.

Winkworth RC, Grau J, Robertson AW, Lockhart P (2002) The origins and evolution of the genus *Myosotis* L. (Boraginaceae). *Molecular Phylogenetics and Evolution* 24: 180-193.

Winkworth RC, Wagstaff SJ, Glenny D (2005) Evolution of the New Zealand mountain flora: Origins, diversification and dispersal. *Organisms Diversity & Evolution* 5: 237-247.

Winn AA, Elle E, Kalisz S, Cheptou PO, Eckert CG, Goodwillie C, Johnston MO, Moeller DA, Ree RH, Sargent RD, Vallejo-Marin M (2011) Analysis of inbreeding depression in mixed-mating plants provides evidence for selective interference and stable mixed mating. *Evolution* 65: 3339-3359.

Wood JR, Wilmshurst JM, Worthy TH, Cooper A (2012) First coprolite evidence for the diet of *Anomalopteryx didiformis*, an extinct forest ratite from New Zealand. *New Zealand Journal of Ecology* 36: 164-170.

Yeates DK, Seago A, Nelson L, Cameron SL, Joseph L, Trueman JWH (2011) Integrative taxonomy, or iterative taxonomy? *Systematic Entomology* 36: 209-217.

Young AG, Schmidt-Adam G, Murray BG (2001) Genetic variation and structure of remnant stands of pohutukawa (*Metrosideros excelsa*, Myrtaceae). *New Zealand Journal of Botany* 39: 133-140.

Zalapa JE, Cuevas H, Zhu H, Steffan S, Senalik D, Zeldin E, McCown B, Harbut R, Simon P (2012) Using next-generation sequencing approaches to isolate simple sequence repeat (SSR) loci in the plant sciences. *American Journal of Botany* 99: 193-208.

Zwettler D, Vieira CP, Schlotterer C (2002) Polymorphic microsatellites in *Antirrhinum* (Scrophulariaceae), a genus with low levels of nuclear sequence variability. *Journal of Heredity* 93: 217-221.

Chapter 2 Delimiting the New Zealand native *Myosotis pygmaea* species group and identifying lineages within it using morphological data from herbarium specimens and living plants

Abstract

The New Zealand forget-me-not genus (*Myosotis,* Boraginaceae) contains many threatened species, as well as several taxonomically indeterminate entities, and is therefore a priority for taxonomic revision. For this study, morphological data were collected from herbarium specimens and live plants grown in a common garden environment to define and delimit the *Myosotis pygmaea* species group and identify morphological clusters within it. High levels of morphological plasticity were uncovered in the common garden experiment. In multidimensional scaling analyses of morphological data, four clusters were identified. Of the five named species that make up the *M. pygmaea* group three formed separate clusters (*M. pygmaea*, *M. glauca* and *M. brevis*), and the two others were indistinguishable from each other (*M. antarctica* and *M. drucei*). Within the *M. pygmaea* species group, no tag-named entities were shown to be morphologically distinct, but several tag-named entities part of the wider "bracteate-prostrate" group may warrant recognition at species rank with further study. This study represents the first important step towards a taxonomic revision of the *Myosotis pygmaea* species group, which is not yet attempted here. Further work to integrate the morphological results presented here with genetic data from microsatellites and ecological niche modelling are the subject of the following chapters.

Introduction

Plant taxonomy has essential applications in conservation (e.g. Cameron 2010), biosecurity (e.g. Pyšek et al., 2013), trade and agriculture (e.g. Zare et al., 2004), and ecological research (e.g. Knowlton and Jackson 1994), despite it being sometimes under-appreciated (Padial and de la Riva 2007). Taxonomy is relevant to conservation because the species is the major unit of conservation e.g., the RED list (IUCN 2001), and the New Zealand Threat Classification System (NZTCS: de Lange et al., 2013). In New Zealand, there are currently 150 threatened plants listed as "taxonomically indeterminate" (de Lange et al., 2013). Without the formal study of species limits, it is unclear whether these taxonomically indeterminate entities are separate species and therefore should be included on the threat list, or even if their conservation status needs to be considered given their taxonomic uncertainty. Thus, taxonomy is often the first important step towards conservation both globally, as well as specifically in New Zealand.

Delimitation of species following the general-lineage model of de Queiroz (2007) integrates multiple lines of evidence to detect separately evolving lineages. Morphology has historically been the primary data type used in species delimitation, and even when integrative or iterative taxonomic methods are employed (e.g. Yeates et al., 2011; López-Reyes et al., 2015), it is still the foundation for taxonomy and classification today (Steussy 2009). Over the last 50 years, methods to analyse morphological data in more statistically rigorous ways have been developed, allowing species hypotheses to be tested and species delimited using a more scientific approach e.g., Poaceae (Consaul et al., 2008), *Plantago* (Meudt 2012), *Myosotis* (Meudt et al. 2013), and *Caesalpinia* (Gagnon et al., 2015).

Morphology may even be considered more useful than genetic data in determining relatedness in situations such as rapid plant radiations in New Zealand where high morphological variation is often not underpinned by correspondingly high levels of genetic variation (Winkworth et al., 2005 and references within). However, there is a risk that the morphological variation present reflects the environment the plant grows in, and not its evolutionary history, due to either phenotypic plasticity or convergent local adaptation (Steussy 2009). Phenotypic plasticity can be tested by growing plants under controlled conditions in a common garden (e.g., Consaul et al., 2008) but local adaptation leading to homoplasious morphological characters is more difficult to detect using morphological data alone. Nevertheless, if genetic markers with sufficient variation can be assessed in conjunction with morphological data, such complicated patterns of adaptation and evolution can theoretically be disentangled (e.g., cichlid fish; Elmer et al., 2010).

Myosotis L., or the forget-me-nots, is a genus of about 100 species distributed in both the Northern and Southern Hemispheres (Mabberley 2008). There are two centres of diversity, Eurasia and New Zealand. New Zealand comprises the central point of a Southern Hemisphere species radiation, meaning plants from Australia and South America are nested within the otherwise New Zealand clade (Winkworth et al., 2002; Meudt et al., 2015). Of the more than 40 species native to New Zealand, two-thirds are listed as threatened to some degree and a number of taxonomically indeterminate entities have been identified, making a taxonomic revision of the genus a top priority (de Lange et al., 2013). Determining phylogenetic relationships and making taxonomic decisions in New Zealand forget-me-nots has proven challenging due to its relatively recent radiation in New Zealand of ~ 2 mya (Meudt et al., 2015), which has led to complicated patterns of incomplete lineage sorting and the hypothesis that speciation is ongoing (Meudt et al., 2013; Meudt et al., 2015). Based on morphology, informal species groups for the Southern Hemisphere representatives of the genus were proposed by Robertson (1989) and expanded upon by Meudt et al. (2015). Two major groupings are currently hypothesised, i.e., the "bracteate-prostrate" group (17 species, 16 native to New Zealand) and the "ebracteate-erect" group (29 species, 27 native to New Zealand), see Table 1 in Meudt et al. (2015). A number of subgroups within each major group were also identified. Meudt et al. (2015) attempted to delimit species groups within New Zealand *Myosotis* phylogenetically by using two chloroplast and two nuclear ribosomal markers but were hindered by a lack of resolution.

As the name suggests, the bracteate-prostrate group is characterised by plants with prostrate flowering stems on which each flower is usually associated with a leaf-like bract (also called a cauline leaf) (Robertson 1989, Meudt et al. 2015). Five of the 17 bracteate-prostrate species are considered part of the diminutive *Myosotis pygmaea* subgroup, which is the focus of the current study. Those five species are *M. antarctica*, *M. brevis*, *M. drucei*, *M. glauca* and *M. pygmaea* (see Table 2.1 for taxonomic history and distinguishing characters and Figure 2.1 for photographs). In addition to the described species, seven tag-named entities with a bracteate-prostrate growth form may be associated with the *M. pygmaea* subgroup, but have uncertain taxonomic status (Figure 2.1; Table 2.1).

As well as using morphology when attempting to identify major lineages within the genus (Moore 1961; Robertson 1989; Meudt et al., 2015), morphology has previously been used to aid species delimitation in New Zealand *Myosotis* (see recent examples: de Lange et al., 2010; Lehnebach 2012; Meudt et al., 2013). Morphological data such as herkogamy distances have also been used to inform breeding system studies (Brandon 2001) and it

appears likely that all members of the *M. pygmaea* species group usually self-fertilise, as their anthers are entirely included (Moore 1961). *Myosotis pygmaea, M. drucei* and *M. brevis* have a herkogamy distance of 0 mm, suggesting their pollination syndrome is autonomous (note *M. glauca* was not studied; Robertson and Lloyd 1991; Brandon 2001). Additionally, chromosome counts for several species of New Zealand *Myosotis* show that chromosome number is variable (e.g., 2*n* = 36, 40, 44, 46 and 48: de Lange and Murray 2002). Due to lack of data, it is not known how much intraspecific variation exists in the New Zealand species (but chromosome races have been found within the European *M. alpestris* group; Stepankova 1996). There have been three counts within the *Myosotis pygmaea* group undertaken: *M. drucei n* = 24 (Beuzenberg and Hair 1983), *M. pygmaea n* = $22_{II,}$ and *M.* aff. *drucei* from the Volcanic Plateau area *n* = 22_{II} (Murray and de Lange 2013). Additional counts could be helpful to assist with delimiting species within the group.

In her treatment of *Myosotis* in the *Flora of New Zealand* (1961), Moore's finishing remarks state that due to morphological polymorphism in the *M. pygmaea* group, "no real solution is possible without intensive study of living plants". To this end, two approaches were used to collect morphological data and analyse morphological variation within the *M. pygmaea* group. Firstly, morphological characters were measured from herbarium specimens from throughout the geographic ranges of all species in the group. Secondly, field-collected seeds from selected populations and species were grown in the common garden environment of a growth room.

The aims of this chapter are to:

1. Analyse the morphological variation from herbarium specimens to determine whether the *M. pygmaea* species group forms a morphologically identifiable subgroup of the bracteate-prostrate group.
2. Explore morphological variation, determine if variation seen in the field and on herbarium specimens of the *M. pygmaea* group species has a genetic and/or environmental basis (via a common garden experiment), and determine which characters are most useful to delimit species within the group.
3. Use these informative morphological characters to delimit morphological clusters within the *M. pygmaea* species group and compare these findings with previous and current species circumscriptions.

Materials and Methods

Morphological data from herbarium specimens

Sampling

Morphological measurements were taken from 144 bracteate-prostrate *Myosotis* individuals from specimens held at AK, CHR, K, OTA, UPS and WELT herbaria, including 80 plants of the five species in the *M. pygmaea* group (see Table 2.1) sampled from across their geographic ranges and including type material (*M. antarctica* [19], *M. brevis* [13], *M. drucei* [21], *M. glauca* [11], and *M. pygmaea* [16]). Measurements were taken from an additional 39 bracteate-prostrate tag-named plants (see Table 2.1) that may or may not be affiliated with the *M. pygmaea* group (*M.* "Volcanic Plateau" [9], *M.* "Tapuae-o-Uenuku" [4], *M.* aff. *tenericaulis* [6], *M.* aff. *glauca* [5], *M. glauca*? [1], *M.* "intermedia" [8], *M.* "Rock & Pillar" [4] and *M.* "non-pulvinaris" [2]). A further 25 additional plants representing the remainder of the 11 named species belonging to the bracteate-prostrate group were measured as outgroup specimens (*M. lyallii* [3], *M. elderi* [4], *M. matthewsii* [2], *M. spathulata* [2], *M. albiflora* [2], *M. tenericaulis* [3], *M. colensoi* [2], *M. glabrescens* [1], *M. pulvinaris* [2], *M. cheesemanii* [1], and *M. uniflora* [3]). Geographic location of the individuals included in this study, collection details and voucher information are presented in Appendix 2.

Data collection

Where possible, herbarium specimens were selected that contained both flowers and mature calyces containing nutlets, but 24 specimens were lacking one or the other. Those 24 specimens include the type of *M. pygmaea* (WELT SP004743; no flowers), five specimens that were included in order to ensure as much cross-over with the microsatellite dataset as possible (see Chapter 4), plus an additional 18 specimens that were included to best represent the geographic and morphological range of each species and tag-named entity.

Measurements were made with a dissecting microscope and Ultra-Cal IV digital callipers to the nearest hundredth of a millimetre (Fred V. Fowler Co., Inc., Newton, MA, USA) connected to a laptop computer. Values were automatically recorded in Excel via GageWedge software (Fred V. Fowler Co.). Characters measured or observed are listed in Table 2.2. All widths were measured at the widest point. Two rosette leaves (largest and smallest fully developed) were observed for leaf characters, and the individual average for each character was used; for all other characters a single observation or measurement per

individual was made. Trichome abundance was measured by counting the number of trichomes present in the field of view on the highest (×7) magnification, and then the density per mm^2 was calculated.

Characters chosen were those mentioned in species descriptions and keys of the *Flora of New Zealand* Boraginaceae treatment (Moore 1961) and de Lange et al. (2010). Additionally characters that appeared to be useful in separating species in preliminary analyses of the herbarium dataset (data not shown) were used, as were those that appeared to have a genetic basis. Whether or not characters had a genetic basis was determined by comparing the variation present in the herbarium specimens of field collected plants to the living plants grown in a common garden environment (see below).

Two morphological datasets were generated from the herbarium measurements: a "bracteate-prostrate" dataset, containing all 144 individuals and 52 characters (26 vegetative and 26 reproductive characters, of which 14 were binary or multistate and 38 quantitative); and a reduced subset "pygmaea group" dataset of 103 individuals and 26 characters (12 vegetative and 14 reproductive characters, of which 6 were binary or multistate and 20 quantitative), see Table 2.2.

The bracteate-prostrate dataset contained 5.4% missing data in total. Of the 144 individuals, 13 had missing data for 6 or more characters (> 10%), and of the 52 characters 11 had missing data from more than 14 individuals (> 10%). To determine if missing data affected the analysis, a reduced dataset containing 131 individuals and 46 characters (missing 1.6% data in total) was generated in such a way that no individual or character was missing more than 10% data (with the exception of the type specimen of *M. pygmaea* WELT SP004743 which was retained despite it lacking 10 characters [19%]).

Morphological data from common garden grown plants

Sampling and set-up

Seeds were collected over four field seasons (2010–2014) whilst collecting herbarium vouchers for morphological analyses and leaves into silica gel for genotyping (Chapters 3 & 4); details are listed in Table 2.3. Seed was not able to be collected from all populations visited, due either to the timing of the visit or the small number of plants present. Seeds were dried and stored at room temperature initially, then stored at 4°C.

Plants were grown under 16 hour days at 25°C in a physical containment level 2 (PC2) growth room at Massey University, Palmerston North. The soil was seedling mix (Daltons,

Matamata, New Zealand) that was initially autoclaved, and up to 10 seeds per pot were placed on top of the soil then watered-in with a fungicide (Terraclor 1.8g/L H_20). Growth room conditions were determined from a preliminary trial involving a total of 180 seeds in 36 pots (5 seeds per pot) from one population each of three species: *M. drucei* (WELT SP100445), *M. pygmaea* (WELT SP100462) and *M. brevis* (WELT SP093294). The trial investigated covering seeds with soil (not implemented as led to reduced germination), cold treatment of the seeds at 4°C (not implemented as it increased time to germination) and cold treatment of the plants in an attempt to promote flowering (not implemented as neither cold, dark treatment at 4°C for 6 weeks nor 1–4 week periods of -20°C triggered flowering).

A total of 964 seeds were sown in 159 pots, representing 38 populations from all five named species in the *M. pygmaea* group and several tag-named entities (Table 2.3). Until germination, the trays of pots were kept covered by clear plastic lids. The pots were sorted in a random block. The trays were checked and rotated every two days; this allowed for accurate recording of length of time to germination. After germination, checks were made weekly to record length of time until flowering. If more than one plant germinated in the same pot, the most central seedling was left in the original pot and all others were transplanted to separate pots. The majority of the seeds were planted on the same date, however seeds from the following six populations were sown or re-sown three to six months later due to either seedling die-off or new collections of seed from the field being made: *M. pygmaea* (WELT SP100477, WELT SP100462), *M. drucei* (WELT SP100445, WELT SP104519), *M. brevis* (WELT SP093294) and *M. glauca*? (WELT SP103892).

Data collection

Four weeks after each plant first flowered, morphological characters were measured on each live plant, a series of microscope photographs were taken, and the plant was then made into an herbarium specimen. Four additional colour characters that were generally not obtainable from herbarium specimens were evaluated on the living plants (i.e., colour of lamina, petiole, leaf midrib and corolla, Table 2.2). Sixteen characters included in the herbarium dataset were not measured on live plants, because they had already been shown to be non-variable within the *M. pygmaea* group (Table 2.2). Two data matrices were generated, both made up of 37 characters; the first of 26 individuals (those that flowered, “flowering”) is a subset of the second, made up of 39 individuals (all those that survived to adulthood including those that flowered, “all adults”). Of the 37 total characters, 16 were vegetative and 21 reproductive; further 7 were binary or multistate and 30 were quantitative. The “flowering” dataset contained only 0.2% or two missing

data points (for one individual that was lacking nutlets). The "adult" dataset contained 19% missing data, as the 13 plants that did not flower were missing data for 21 characters each. Characters were re-measured on five live plants once they had been made into herbarium specimens to assess degree of shrinking of dried pressed plants.

Data analyses for living and herbarium specimens

All morphological analyses were undertaken in R (RCoreTeam 2015) using RStudio (RStudio Team 2015). The following steps were applied to all four datasets ("bracteate-prostrate" and "pygmaea group" datasets from the herbarium specimens, as well as "flowering" and "all adults" datasets from the growth room). An integrated analysis, combining the herbarium "pygmaea group" data with the growth room "all adults" was also undertaken. MorphoTools functions (Koutecky 2015) were used to assess correlation between morphological characters using Pearson's coefficient (function ***cormat.p***), and when two non-independent characters were found to be highly correlated (> 0.85) one was excluded (e.g., calyx width at the base was correlated with calyx width at the tip, and so only calyx width at the tip was included). Cluster dendrograms based on WARD distances (function ***clust.ward***) were generated. Gower's coefficient was used to convert the data to a distance matrix using the ***daisy*** function of the package "cluster" (Maechler et al., 2015). Gower's coefficient was used because it is suitable for datasets that contain missing data and a mixture of qualitative and quantitative character types (Podani 1999; Steussy 2009).

The resulting distance matrix was analysed using non-metric multidimensional scaling (nMDS) performed with the ***metaMDS*** function in the "vegan" package (Oksanen et al., 2015). To decide how many dimensions to retain, stress values (as a scree plot) and Shepard diagrams were compared between analyses with one to six dimensions retained. The least number of dimensions was selected which had a "fair" stress score (less than 0.2; Wickelmaier 2003), was close to the "elbow" on the scree plot, and explained the most variation present.

The nMDS points were then used as input for Bayesian model-based clustering by the "mclust" package (Fraley and Raftery 2002; Fraley et al., 2012). The function ***Mclust*** identifies the probable number of clusters present using Bayesian information criteria (BIC), and assesses the classification uncertainty of each individual to its assigned cluster. Using default "mclust" settings, 14 models and K of 1–9 were assessed by the BIC.

Boxplots (function ***boxplot*** from the MorphoTools functions for quantitative characters) and stacked barcharts (function ***ggplot*** from package "ggplot2" (Wickham 2009) for qualitative characters) were also created to explore how characters separated along species and cluster (i.e. clusters identified in Figure 2.2A) lines and to investigate the differences between the herbarium specimens and the growth room plants to get an estimate of phenotypic plasticity. For characters of interest, whether the observed differences were significant was determined by ANOVA and paired t-tests (for groups with n > 3) with the Bonferroni correction applied using functions ***aov*** and ***pairwise-t-test*** from the basic statistics package in R.

Seven populations that were measured both as live plants in the growth room and as herbarium specimens were compared to get a direct measure of phenotypic plasticity. Plants from three of those populations flowered in the growth room (two of *M. brevis,* WELT SP090543 and WELT SP093294; one of *M. pygmaea,* WELT SP100462); four did not flower (two populations of *M. drucei,* WELT SP100445 and WELT SP100428; one *M. pygmaea* WELT SP100472; and one tag-named *M. glauca?* WELT SP103892).

Results

Herbarium morphological data

Within the *Myosotis* bracteate-prostrate group, three main clusters were identified by the "mclust" analyses of the nMDS points (with two dimensions retained). The dataset created to minimise missing data showed the same patterns reported below, and is not discussed further (data not shown). The three clusters correspond to a "pygmies" cluster (all representatives of the five *M. pygmaea* species plus three *M. elderi* and one *M. lyallii* specimen, although individuals from these latter two species were assigned with high uncertainty), a "cushions" cluster (*M. pulvinaris*, *M. colensoi*, *M. glabrescens*, *M. uniflora* and the remaining *M. lyallii* and *M. elderi* specimens) and a "creepers" cluster (*M. matthewsii*, *M. spathulata*, *M. albiflora* and *M. tenericaulis*) (Figure 2.2A). When tag-named entities are considered, it is apparent that *M.* "Volcanic Plateau", *M.* "intermedia", *M.* "Rock and Pillar", *M* aff. *glauca*, *M. glauca*? and *M.* "Tapuae-o-Uenuku" are closely affiliated with the *M. pygmaea* group, as all individuals were assigned to the "pygmies" cluster with low uncertainty. Half of the representatives of *M.* aff. *tenericaulis* were assigned to the "pygmies" cluster, and half to the "creepers" cluster, mostly with high uncertainty. In contrast, *M.* "non-pulvinaris" is clearly part of the "cushions" group and most likely closely affiliated to *M. pulvinaris*.

Of the two tag-named entities with uncertain affiliation, *M.* "Tapuae-o-Uenuku" clusters with *M. elderi* and *M. lyallii* with low uncertainty in "mclust" analyses from which the "cushions" and "creepers" were removed (Figure 2.2B; two dimensions retained). Additionally, both *M.* "Tapuae-o-Uenuku" and *M.* aff. *tenericaulis* have fixed morphological characters that separate them from the *M. pygmaea* group. For *M.* aff. *tenericaulis* this is most clearly illustrated by corolla diameter (Table 2.4, Figure 2.3A). Plants of *M.* "Tapuae-o-Uenuku" have bigger corollas but not significantly so (Table 2.4, Figure 2.4A), they also have distinctively long trichomes (Table 2.4, Figure 2.4B). Corolla diameter (Table 2.4 Figure 2.4A) also separates *M.* "Rock and Pillar" from the *M. pygmaea* group along with the presence of retrorse trichomes on the underside of the rosette leaves and hooked trichomes on the calyx.

The 103-individual "pygmaea group" dataset shows two major clusters in the "mclust" analysis (with three dimensions retained), corresponding to *M. pygmaea*, *M. glauca* and *M.* aff. *glauca* being mostly separated from *M. drucei*, *M. antarctica*, *M. brevis*, and *M.* "intermedia", and *M.* "Volcanic Plateau" specimens are assigned to both clusters with high uncertainty (Figure 2.3A). Although not significant in the "mclust" analyses, the nMDS plot shows *M. brevis* is somewhat separated from *M. drucei* and *M. antarctica* on the 2nd dimension, and *M. pygmaea* and *M. glauca* are separated from each other on the 3rd dimension (Figure 2.3B). Thus, only *Myosotis antarctica*, *M. drucei*, *M.* "Volcanic Plateau" and *M.* "intermedia" are unable to be distinguished from each other at all (Figure 2.3A) using multidimensional scaling.

Exploring the boxplots and stacked bar charts showed that the grouping of *M. pygmaea* and *M. glauca* in the nMDS is likely due to their both having appressed to patent trichomes on the rosette leaf margins, but they can be distinguished from each other by *M. pygmaea* having curved trichomes (Figure 2.6L), whereas *M. glauca* and *M.* aff. *glauca* have consistently straight trichomes (Figure 2.6A-D). Having flexuous trichomes that are either patent or erect along the rosette leaf margins unites the rest of the *M. pygmaea* species group (e.g., Figure 2.6E-J). When qualitative characters are excluded (i.e., Characters 1–20; Table 2.2) *M. glauca* and *M. pygmaea* can no longer be distinguished from the *M. antarctica* + *M. drucei* cluster, and only individuals of *M. brevis* form a separate cluster based on taxonomy (data not shown).

Although the ranges of each character are overlapping, *M. brevis* can be distinguished from the rest of the *M. pygmaea* group by having usually smaller calyces, flowers and nutlets (see Table 2.5 and Figures 2.4C, E). *Myosotis antarctica* and *M. drucei* differ from each

other only in the density of trichomes on their rosette leaves (Table 2.5). *Myosotis* "Volcanic Plateau" and *M. drucei* differ from each other only on the shape of their rosette leaves, calculated by the ratio of leaf length to width (Table 2.5). However, *M.* "Volcanic Plateau" and *M.* "intermedia" do not have significantly different rosette leaf length to width ratios (Table 2.5).

Common garden germination and growth, and morphological data

Germination and growth rates

Of the 964 seeds sown in the main growth room experiment a total of 306 (32%) seeds germinated; of those, 97 (32%) survived to adulthood, and of those, 33 (34%) flowered (Table 2.3). Certain populations were over-represented in the individuals that survived to adulthood and flowered, and all morphological characters were measured on only 26 of the 33 that flowered by measuring three (or in one case five) individuals per population. Those 26 that were measured represent four populations of *M. pygmaea,* four of *M. drucei* and two of *M. brevis* (Table 2.3). Seed from a further 64 individuals representing an additional seven populations germinated and survived to adulthood, but did not flower. The vegetative characters of 13 of those individuals (two or three representatives per population) were measured. Taking into account all sown seeds, including additional seeds that were germinated for the initial trial and attempts at chromosome counts, there is a clear negative trend between age of seed and germination rate (age of seed ranged from one to 92 months, average of 16 months; $p < 0.01$), and significant differences in length of time to germination between species as follows. In the initial trial, *M. drucei* seed took on average 29 days to germinate, *M. brevis* seed took on average 41 days to germinate, whereas *M. pygmaea* seed took on average 65 days [*M. drucei* ($n = 40$) vs. *M. pygmaea* ($n = 32$), and *M. brevis* ($n = 39$) vs. *M. pygmaea* were significantly different at $p < 0.01$; *M. brevis* vs. *M. drucei:* $p = 0.04$].

Morphological variation found

The "flowering" dataset of the 26 plants that flowered showed good separation among the three species represented based on the nMDS (with two dimensions retained), and this was corroborated by three clusters being found in the "mclust" analyses (Figure 2.5A). The "adults" dataset of 39 plants showed less-clear discrimination in nMDS plots (with two dimensions retained), and this is likely due to the large amount of missing data added to the dataset when plants lacking reproductive characters were included (Figure 2.5B). Exploring the boxplots and stacked bar charts revealed the characters that were

significantly different between plants of different species and thus were contributing to forming the clusters seen in Figures 2.5A and 2.5B. The eight quantitative characters for which plants of *M. brevis* are significantly shorter or smaller than plants of *M. drucei* and/or *M. pygmaea* are shown in Table 2.6 and three are illustrated in Figures 2.4C-D.

The one quantitative character for which plants of *M. drucei* are significantly larger than those of *M. pygmaea* is calyx length at fruiting (character number 44, Table 2.6). The most stable qualitative characters that differentiates the species is that plants of *M. pygmaea* have curved trichomes that are appressed to patent at the rosette leaf margins, whereas plants of *M. brevis* and *M. drucei* have flexuous trichomes that are patent to erect at rosette leaf margins, and the single *M. glauca* specimen had straight trichomes (trichome straightness illustrated in Figure 2.3F).

Measurements of live plants that were re-measured once they had been made into herbarium specimens showed that on average (n = 5) the leaves shrunk by 1.9 mm in length (13.1%) and 1.2 mm (19.6%) in width. Average calyx length at fruiting did not differ, but the average calyx width at the tips was 1.2 mm (19.9%) smaller once specimens had been dried and pressed, most likely due to the change in shape once the calyces were flattened. Additionally, corolla diameter shrunk by an average of 0.8 mm (34.3%) with drying.

Comparison of herbarium and common garden data

The nMDS and "mclust" analyses of the integrated "pygmaea group" and "all adults" datasets found three clusters, which correspond loosely to 1) all *M. pygmaea* individuals grown in the growth room, 2) *M. drucei* + *M. glauca?* individuals grown in the growth room, and 3) the remainder of the samples i.e., including all herbarium specimens + *M. brevis* grown in the growth room (See Appendix 3).

Species by species comparisons of the eight quantitative characters that were found to be useful in differentiating species in the growth room common garden environment, among all of the growth room plants and all of the herbarium *M. pygmaea* group plants, showed that seven of the eight quantitative characters had significantly higher values for plants grown in the growth room compared to the herbarium specimens for at least one of the three species (only nutlet length was not altered for any of the three species; Table 2.7, Figure 2.4E).

Despite their plasticity, three of those traits still showed significant differences among species in the herbarium data (Rosette leaf length [character 24]; Figure 2.4D, and characters floral lobe length [44] and length of calyx at fruiting [54]; Table 2.8). However, the one quantitative trait that separated *M. pygmaea* and *M. drucei* in the growth room data, calyx length at fruiting (44) (Table 2.6), was not significantly different between these two species in the herbarium data (Table 2.8). The main qualitative trait (whether trichomes were straight, curved or flexuous) that was found to separate species in the growth room was still a good character for differentiating between herbarium specimens (Figure 2.4F).

Plants from the seven populations for which both herbarium and growth room data were available all grew larger in the growth room compared to the field (see Figure 2.7); for example average rosette leaf length across all species increased from 6.4 mm (n = 7) to 19.3 mm (n = 18). For the three populations that flowered, average branch length increased from 15 mm (n = 3) to 72 mm (n = 11) and internode length from 1.4 mm (n = 3) to 8.9 mm (n = 11).

Discussion

Identifying lineages and delimiting species within New Zealand *Myosotis* is not straightforward due to the recent radiation of the genus (Meudt et al. 2013, Meudt et al. 2015), coupled with high levels of morphological plasticity (this study). Following the general-lineage model (de Queiroz 2007), morphological data from both living plants and herbarium specimens of the *Myosotis pygmaea* group have been analysed as a first step to both define this group and delimit species and subspecific taxa within it. Overall, the data show the *M. pygmaea* group can be defined based on morphology, and several morphological clusters within the group can be identified. Some of these morphological clusters align with the current taxonomy, but not all currently recognised species or tag-named entities are morphologically distinguishable and these are discussed below. As these morphological clusters will be further tested by and integrated with microsatellite and other data, a taxonomic revision is not included here; instead the findings are compared with the original descriptions, and previous and current species circumscriptions.

Comparing and integrating herbarium and growth room data

Historically, only a few studies contributing to New Zealand plant taxonomy have included common garden studies, instead often referring to a very small number of live plants [e.g.,

Wahlenbergia (Petterson 1997); though see the recent revision of *Kunzea* which considers 280 plants grown under common conditions (de Lange 2014)]. The low levels of seed germination and seedling survivorship in the current study highlight some of the reasons why results of common garden experiments are not more often published, i.e., the small samples sizes give low statistical power compared to the amount of effort required to generate the data. Those studies that do manage to gather morphological data in the field and in a common garden from the same individuals can therefore be considered all the more impressive (e.g., Consaul 2008). Despite the limited germination success, by growing *Myosotis* in a common garden it has been possible to identify which morphological characters have a stronger genetic vs. environmental basis, and therefore which characters will be most useful when delimiting and describing species. The large amount of vegetative plasticity seen, specifically the larger size attained by plants grown in the common garden compared to the field (Table 2.7 and Figure 2.7), is of particular interest, and will have to be taken into account when describing and delimiting species and generating keys.

Delimiting the *M. pygmaea* species group

The *M. pygmaea* group is recovered as one of three definable subsets of the bracteate-prostrate group (Figures 2.2A), and can be identified as being separate based on a suite of morphological characters. Individuals of the five described species (*M. antarctica*, *M. brevis*, *M. drucei*, *M. glauca* and *M. pygmaea*) are all unambiguously assigned to the "pygmies" subset, and are confirmed as forming the *M. pygmaea* group. Of the tag-named plants included in this study, *M.* "Volcanic Plateau", *M.* "intermedia" and *M.* aff. *glauca* are also assigned to the "pygmies" subset and morphologically belong to the *M. pygmaea* group (Figure 2.2A). Individuals of other species and tag-names of the bracteate-prostrate group formed the "cushions" and "creepers" subsets. Not unexpectedly, the two individuals of *M.* "non-pulvinaris" are unambiguously assigned to the "cushions" subset, together with individuals of *M. pulvinaris* and other cushion-forming species (Figure 2.2A). Individuals of three tag-named entities that are more ambiguous in how closely they affiliate to the *M. pygmaea* group are here all treated as being separate from it, i.e., *M.* aff. *tenericaulis*, *M.* "Tapuae-o-Uenuku" and *M.* "Rock & Pillar". Despite at least some of their representatives falling within the "pygmies" subset (Figure 2.2A), all have characters, for example larger corolla diameter, that separate them from the *M. pygmaea* group as treated here. Although individuals of *M.* aff. *tenericaulis* (n = 6) appear more similar morphologically to representatives of the *M. pygmaea* group than to individuals of *M. tenericaulis* itself (Figure 2.2A), corolla diameter reliably separates them from the *M.*

pygmaea group (Table 2.4; Figure 2.4A). Therefore it appears that morphologically *M.* aff. *tenericaulis* it is a distinct entity and species level recognition is most likely appropriate (pending genetic analyses and additional morphological studies). Based on further nMDS analyses with reduced sampling (Figure 2.2B), it is apparent that plants of *M.* "Tapuae-o-Uenuku" are more closely affiliated to individuals of *M. elderi* than to the "pygmies" and more detailed analyses of the morphological characters that separate these two entities is required before taxonomic decisions can be made. Representatives of the third tag-named entity of uncertain affiliation, *M.* "Rock and Pillar" appear somewhat differentiated from the *M. pygmaea* group in the nMDS (Figure 2.2B) despite falling within the "pygmies" cluster (Figure 2.2A). As well as the larger corolla diameter (Table 2.4; Figure 2.4A), the presence of retrorse trichomes on the underside of the rosette leaves, along with the presence of hooked trichomes on the calyx, and larger leaf size and internode size characters all indicate that plants of this entity are unified and differentiated from the *M. pygmaea* group. Additional comparisons of specimens of *M.* "Rock and Pillar" with other bracteate-prostrate species, including the "creepers" subset, is warranted.

This study confirms that the characters that define the *M. pygmaea* group include several that were used by Moore (1961) in her *Myosotis* treatment and key in the *Flora of New Zealand*, i.e., anther placement at least partly below the corolla scales, corolla diameter 1–4 mm, anthers < 1mm long, style < calyx length, calyx lobed about half way to the base, and petioles, internodes and pedicels generally "short". According to the analyses here, the *M. pygmaea* group can be further characterised by the following floral characters: corolla lobe length < 1.5 mm, anthers (sub) sessile (i.e., filament length of 0 or nearly 0), calyx length at flowering < 3.5 mm, pedicel length at fruiting < 2 mm, and corolla tube length < 3 mm. Additionally, members of the *M. pygmaea* group do not have adventitious roots. Individually none of these characters exclude all other New Zealand *Myosotis* species from the group, but taken together they do.

This suite of characters that unites the *M. pygmaea* group, particularly the small white flowers, are essentially those to which self-pollinating plants converge (Ornduff 1969). It is therefore unclear whether the *M. pygmaea* group represents an evolutionary lineage, or whether their morphological similarity is an artefact of a shared breeding syndrome (Robertson and Lloyd 1991; Brandon 2001).

Delimiting morphological clusters within the pygmy forget-me-nots

Taking into consideration the herbarium and common garden morphological analyses, four morphological clusters can be recognised within the *M. pygmaea* group. Three of the

four clusters correspond to a currently described species (*M. pygmaea*, *M. glauca* and *M. brevis*) but the fourth includes two named species (*M. antarctica* and *M. drucei*) as well as several tag-named entities. Each morphological cluster is discussed in more detail below. The first cluster discussed is *M. brevis*, as it has support from both herbarium and growth room datasets, and can be separated from the other groups by multiple quantitative characters. In contrast, the other three clusters are more similar to each other, and are mostly only separated by qualitative characters.

Myosotis brevis

Support for *M. brevis* is found in both the herbarium and growth room analyses; a cluster of individuals identified as *M. brevis* is evident (though with limited separation from *M. antarctica* and *M. drucei*) on the 2nd dimension of the nMDS of the "pygmaea group" dataset (Figure 2.3A), and all flowering individuals grown in the growth room identified as *M. brevis* form a cluster (N = 7; Figure 2.4A). In both datasets, this cluster is separated from the other *M. pygmaea* group samples by a mixture of quantitative and qualitative characters: all individuals identified as *M. brevis* have smaller floral characters including corolla and calyx size (Table 2.5), and despite all plants growing larger these size differences are maintained in the common garden (Table 2.6). The trichomes of *M. brevis* appear most similar to those of *M. drucei* and *M. antarctica* in that they are usually flexuous (Figures 2.4F and 2.6), and along with the smaller corolla, calyx and nutlet size of *M. brevis* (Tables 2.5 and 2.6), these characters differentiate *M. brevis* from *M. pygmaea* and *M. glauca.*

In the original description of *M. brevis* (as *M. pygmaea* var. *minutiflora*), it was described as differing from all other varieties and forms of the species "by its densely leafy stems and many minute flowers" (Simpson and Thomson 1943:161). In the key of Moore's (1961) treatment, *M. brevis* (as *M. pygmaea* var. *minutiflora*) was characterised by the corolla diameter being usually < 1 mm. Although the present study confirms *M. brevis* has smaller flowers than other pygmy forget-me-nots (e.g., corolla lobe length; Table 2.5), the corolla diameter was shown to be usually slightly larger than 1 mm (floral diameter from combined herbarium and growth room data: mean = 1.2 mm, range = 0.8–1.8 mm, n = 20). The data presented here confirms that *M. brevis* has a higher ratio of trichome length to leaf length (Table 2.5), as mentioned in the *Flora* ("leaf hairs being sometimes up to ½ leaf-width") and confirms the smaller nutlet size (average length of 1.1 mm for *M. brevis* vs. 1.4 mm for other pygmy forget-me-nots, $p < 0.01$, Table 2.5). Characters such as lateral branches being "infinite" and "short" mentioned in the *Flora* are shown to be plastic based on the common garden experiment (Table 2.7). It was also found that the calyx is generally

less than 2 mm long at flowering (and nearly up to 4 mm long at fruiting, Table 2.5) in contrast to 2–3 mm long as described by Moore (1961). The *M. brevis* plants grown in the growth room all had creamy-yellow petals, as did plants observed in the field.

M. brevis has an interesting distribution, being coastal in the North Island and inland in the South Island. Some New Zealand botanists suspect the North Island vs. South Island representatives may belong to separate entities, but this study has found no morphological evidence to support this (data not shown).

Myosotis drucei + M. antarctica

Support for a combined *M. drucei* + *M. antarctica* is found in the herbarium data analyses; essentially these two entities cannot be distinguished using multidimensional scaling of morphological data (Figure 2.3). As no *M. antarctica* individuals survived beyond germination in the growth room, their similarities or differences under common garden conditions could not be assessed. Although the name *M. antarctica* was originally applied to plants throughout New Zealand, based on the *Flora* and current usage, the name *M. antarctica* is reserved for plants from Campbell Island and southern Chile only (Moore 1961; Zuloaga et al., 2008). The couplet in the *Flora* key that separates *M. antarctica* from the rest of the *M. pygmaea* group reads: "Fls [Flowers] usually blue, hairs long, very fine and silky, crowded" (Moore 1961:809). In addition, a note at the end of the *M. drucei* (as *M. pygmaea* var. *drucei*) description states, "this var. approaches *M. antarctica* Hook.f. but hairs are less silky and less crowded, fls [flowers] rather smaller and cream coloured, nutlets more elongated" (Moore 1961:816). However, the data presented here show that of these characters, only trichome density is quantifiably different between individuals from Campbell Island and Chile (*M. antarctica*) versus those from mainland New Zealand (*M. drucei*). Although blue-flowered *M. antarctica* are present on Campbell Island, white-flowered individuals are also present in approximately equal abundance (J.M. Prebble, pers. obs.); the corolla colour of Chilean *M. antarctica* is described as "weiss oder blauweiss [white or blue white]" by Skottsberg (1916). The trichome lengths of *M. drucei* and *M. antarctica* are nearly identical (*M. antarctica* mean = 0.8 mm, range = 0.5–1.2 mm, n = 20; *M. drucei* mean = 0.9 mm, range = 0.5–1.6 mm, n = 21). As no satisfactory way of quantifying whether trichomes were "fine and silky" was found, this character was not assessed, however trichome density was quantified on the rosette leaf blade, and it was found that *M. antarctica* individuals do have a significantly higher number of trichomes relative to *M. drucei* individuals (20 per mm^2 vs. 7 per mm^2 $p < 0.01$; Table 2.5), although the ranges overlap considerably (7.4–33.3 vs. 1.7–19.1 per mm^2). *Myosotis drucei* does not have significantly smaller flowers than *M. antarctica* (mean *M. drucei* corolla diameter =

2.3 mm, range = 1.0–4.0 mm, n = 20; mean *M. antarctica* corolla diameter = 2.6 mm, range = 1.7–3.5 mm, n = 18) and the nutlets are not more elongated (mean *M. drucei* nutlet length to width ratio = 1.5 mm, range = 1.4–1.8 mm, n = 14; mean *M. antarctica* nutlet length to width ratio = 1.4 mm, range = 1.3–1.6, n = 12). Given therefore the only character that separates *M. antarctica* and *M. drucei* is rosette leaf trichome density, the possibility of the two entities comprising the same species must be considered. The type specimen of another published species, *M. ramificata* (Table 2.1) also clusters within the *M. drucei* + *M. antarctica* cluster (Figure 2.3), and was not able to be distinguished based on any morphological characters.

Several tag-named entities are also closely affiliated to *M. drucei* + *M. antarctica*, i.e., *M.* "Volcanic Plateau" and *M.* "intermedia" (Figure 2.3). Only a brief published description exists of *M.* "Volcanic Plateau" (Table 1.1) in which it is described as having white flowers (vs. cream for *M. drucei*), with narrower leaves than *M. drucei* (Murray and de Lange 2013). In addition it is considered morphologically distinguishable based on its "brown coloured leaf bases over the lower third of the leaf. It has strongly obovate leaves with wide gradually tapering leaf bases/petioles" (Geoff Rogers pers. comm., August 2012). The *M.* "Volcanic Plateau" individuals included in this study are shown to have a significantly higher length to width ratio of their rosette leaves, meaning they have more narrowly obovate leaves than individuals of *M. drucei* (Table 2.5). However, plants identified as *M.* "intermedia"—which is probably a shade form of *M. drucei* (see below)—also have a higher length to width ratio than *M. drucei* and cannot be significantly differentiated from *M.* "Volcanic Plateau" individuals (Table 2.5), or indeed from *M. drucei* individuals ($p = 0.342$). The cream vs. white petal character could not be assessed on *M.* "Volcanic Plateau" plants as none grew in the common garden, but as *M. drucei* plants had white (not cream) petals when grown in the growth room, this character does not appear to be a suitable one to differentiate the two entities. In addition several *M. drucei* plants grown in the growth room had dark coloured petioles and leaf bases, which means this character is not diagnostic for *M.* "Volcanic Plateau". Although most individuals identified as *M.* "Volcanic Plateau" grouped with *M. antarctica* + *M. drucei* (Figure 2.3), four grouped instead with *M. pygmaea* and *M. glauca* in the nMDS analysis (i.e., CHR 86263, CHR 244442, CHR 131697 and WELT SP100412; Figure 2.3). Two of these were plants that had been grown in cultivation (i.e., CHR 131697 and WELT SP100412), which may explain their unusual placement, and all were allocated with high uncertainty.

Morphological variation within *M. drucei* is quite high, which probably reflects the phenotypic plasticity of these plants. A number of herbarium specimens that have been

annotated with the tag name *M*. "intermedia" (Table 2.1), i.e., of intermediate morphology between *M. pygmaea* and *M. drucei* (e.g., WELT SP089911), fall within the range of morphological variation seen in *M. drucei*. It is considered these are most likely shade forms (e.g., WELT SP100498 growing under a rock overhang), as for example the rosette leaf length is more similar to that found on the plants grown in the common garden (Figure 2.4D).

A final entity included in this cluster (*M. glauca*?; Table 2.1) requires further collection and study to understand its morphology and affiliations, as it is currently only known from a single collection from Central Otago (WELT SP103892). It has the glaucous colour of *M. glauca*, but flexuous trichomes, a longer calyx and more erect flowering branches. Plants grown in the growth room did not flower.

The morphological analyses here suggest that *M. antarctica*, *M. drucei, M.* "Volcanic Plateau" and *M.* "intermedia" may be considered one morphologically variable, widespread species, found in the North and South Islands in mostly sub-alpine locations, as well as Campbell Island and southern Chile in coastal locations. *M. antarctica* is the first name published of the two, and so would have priority over *M. drucei*, however any taxonomic changes will await testing these taxonomic hypotheses (and assess which rank is most appropriate) with genetic and ecological niche modelling data.

Myosotis pygmaea

Support for *M. pygmaea* is found in both the herbarium and growth room analyses; a cluster of individuals identified as *M. pygmaea* is evident on the 3rd dimension of the nMDS of the herbarium "pygmaea group" dataset (Figure 2.3B), and all flowering individuals grown in the growth room identified as *M. pygmaea* also form a cluster (N = 14; Figure 2.5A). In both datasets, this cluster is separated from the samples of other pygmy forget-me-not species by qualitative trichome characters: individuals identified as *M. pygmaea* usually have curved trichomes on their rosette leaves (Figures 2.D-F; 2.6L), which are appressed to patent on the margins (Figure 2.6M).

The herbarium dataset identifies individuals of *M. glauca* (Figure 2.3A-B) as being most similar to *M. pygmaea*. *Myosotis glauca* and *M. pygmaea* share the character of appressed to patent trichomes on the rosette leaf margins, but they are otherwise distinguishable as *M. glauca* and *M.* aff. *glauca* have straight rather than curved trichomes (Figures 2.4F; 2.6A-D). The similarity between *M. glauca* and *M. pygmaea* is not recovered in growth room data (Figure 2.5B) probably because the additional character of leaf colour is taken

into account (*M. pygmaea* has green leaves, whereas *M. glauca* has blue-green leaves), along with reduced sampling of *M. glauca*.

In Moore's (1961) treatment, *M. pygmaea* is separated from *M. glauca* in the key by having more and longer trichomes on the calyx, and is separated from *M. drucei* by having strigose rather than flexuous trichomes, and a shorter calyx tube from which the nutlets protrude. The data collected for this study show that the lengths of trichomes on the calyx do not differ significantly between *M. glauca* and *M. pygmaea* (mean for both is 0.6 mm), although the trichomes on *M. glauca* (including on the calyx) are differentiated by being straight as already mentioned. To attempt to quantify the length of the calyx and whether this would lead to nutlets "protruding", the ratio of the length of the calyx lobes to the length of the calyx was calculated (Character 51, Table 2.2). The mean of *M. pygmaea* is slightly higher than that of *M. drucei* (0.5 vs. 0.4) indicating a greater degree of lobedness on average. However, this difference is not significant and the ranges greatly overlap (0.4–0.7 vs. 0.3–0.7), making this a poor character to differentiate *M. pygmaea* and *M. drucei*. However, in the growth room *M. pygmaea* individuals do have significantly shorter calyces at fruiting (Table 2.6), although that this pattern is not recovered in the herbarium dataset (Table 2.8). Calyx length was one of the few characters found not to shrink when growth room plants were re-measured as herbarium specimens. This difference in calyx length found between *M. pygmaea* and *M. drucei* in the growth room therefore potentially reflects genetic variation that is unrealized in field conditions. Mark (2012:256) suggests that *M. drucei* is "best distinguished from *M. pygmaea* by its cream- to lemon-coloured flowers". However both *M. drucei* and *M. pygmaea* plants grown in the common garden had white petals, occasionally tinged with blue or pink (data not shown).

There are two individuals identified as *M. pygmaea* that do not fall into the same cluster as the rest in the herbarium dataset (Figure 2.3A-B). One is placed with some uncertainty (CHR 245193, probably due to its having erect rather than appressed leaf trichomes), however the other has low uncertainty and is the type specimen of *M. pygmaea* (WELT SP004743). The type specimen lacks all floral characters, which could account for this placement. However it also lacks the curved trichomes that are appressed to patent at the leaf margins, which unite the rest of the individuals here identified as *M. pygmaea*. Given that curved trichomes are not a character exhibited by the type specimen, why have the specimens (e.g., in Appendix 2) been identified as *M. pygmaea*? The current usage of the name is demonstrated in Mark (2012: 256: a photograph identified as *M. pygmaea* is of a plant growing at the same location (inland Hawke's Bay) as WELT SP090631; this individual clusters with *M. pygmaea* specimens in Figure 2.3A. Specimens identified as *M.*

pygmaea are usually found in coastal locations, but inland populations from Kahurangi National Park (South Island, WELT SP100472), and inland Hawke's Bay (North Island, WELT SP090629), have been included in this study and are mentioned as belonging to *M. pygmaea* (as var. *pygmaea*) by Moore (1961). The taxonomic and nomenclatorial implications of the type specimen not sharing the key characters that appear to unite an entity will be discussed in a subsequent chapter once genetic and ecological niche modelling analyses have been undertaken. Note the type specimen of *M. antarctica* subsp. *traillii* clusters with the plants identified as *M. pygmaea* (Figure 2.3).

Myosotis glauca

Support for *M. glauca* is mostly from the herbarium dataset; a cluster of individuals identified as *M. glauca* or *M.* aff *glauca* is evident on the 3rd dimension of the nMDS of the herbarium "pygmaea group" dataset (Figure 2.3B). As only a single plant identified as *M. glauca* grew in the growth room, and this plant did not flower, limited conclusions can be made from the common garden study. Nevertheless, the cultivated plant did retain the straight trichomes that define *M. glauca* in the herbarium dataset, even if they became less appressed, and the blue-green colour remained pronounced (e.g., Figure 2.6A). Specimens of *M. glauca* were all collected from Central Otago, which fits the range identified by Moore (1961) in the *Flora*. One specimen previously identified as *M. glauca* collected from the Central Plateau region of the North Island (CHR 252337) does not share the stiff straight appressed trichomes that unite the rest of the individuals identified as *M. glauca*. In the nMDS analyses (Figure 2.3A) this individual clustered with *M. antarctica* subsp. *antarctica*, and is considered to belong to that entity. The tag name *M.* aff. *glauca* is given to specimens from the Pisa Range that key out to *M. glauca* using the *Flora* but lack that species' characteristic glaucous colouration (Table 2.1).

In the original description *M. glauca* (as *M. pygmaea* var. *glauca*) was described as being "everywhere dull glaucous green and dotted with long, stout, pointed, closely appressed, strigose hairs" (Simpson and Thomson 1942). In Moore's (1961) treatment, *M. glauca* (as *M. pygmaea* var. *glauca*) is again characterised by having stiff, short, sparsely distributed and appressed trichomes (the trichomes can become less appressed as they age), characters that separate it from other *M. pygmaea* group species and confirmed by the present study. Another character mentioned in the *Flora* is that internodes are longer than bracts. This was quantified by taking the ratio of the longest internode at fruiting to the length of the lowest cauline leaf (Character 47, Table 2.2). Both *M. glauca* and *M*. aff. *glauca* have a mean ratio of ~ 1 for this character (range: 0.5 to 2), indicating that their internodes are not always longer than their bracts.

Summary and Conclusions

This chapter has used morphological data from herbarium specimens and plants grown in a common garden to delimit the *Myosotis pygmaea* group within the bracteate-prostrate group of Southern Hemisphere *Myosotis*. It has identified characters that define the *M. pygmaea* group, and the species and tag-named entities that comprise it. All five species previously hypothesised to form the *M. pygmaea* group are confirmed as members (*M. antarctica*, *M. brevis*, *M. drucei*, *M. glauca* and *M. pygmaea*) as well as the following tag-named entities: *M.* "Volcanic Plateau", *M.* "intermedia" *M.* aff. *glauca* and *M. glauca*?. Whether the morphological similarities that unite the *M. pygmaea* group are due to them being each other's closest relatives, or whether they are instead united by sharing the same suite of traits adapted to self-pollination, remains unclear.

The common garden experiment revealed a high rate of morphological plasticity in the *Myosotis pygmaea* group species. Growing plants in the growth room also allowed the identification of characters that vary significantly between species clusters in both the common garden experiment and the herbarium dataset, which indicates they are more likely to have a genetic rather than environmental basis. Using these characters, only *M. brevis* can be separated based on quantitative morphological characters. Considering also qualitative characters, four morphological groups can be identified within the *M. pygmaea* group, three of which match currently described species, and one which comprises two described species and two tag-named entities. Individuals from the described species *M. pygmaea*, *M. glauca* and *M. brevis* are recovered as forming their own morphological groups and can be distinguished from one another primarily using trichome straightness (curved, straight and flexuous, respectively). By contrast, although rosette leaf trichome density differs between individuals of *M. drucei* vs. *M. antarctica,* these species form a single cohesive group in the nMDS analyses. *M. drucei* + *M. antarctica* is separated from *M. glauca* and *M. pygmaea* by having flexuous trichomes, and from *M. brevis* by the larger corolla and calyx size. For the tag-named entities considered part of the *M. pygmaea* group, *M.* aff. *glauca* is shown to be closely affiliated with *M. glauca*, *M*. "Volcanic Plateau" and *M.* "intermedia" are considered indistinguishable from *M. drucei* + *M. antarctica*, and *M. glauca*? (WELT SP103892) requires more collections before conclusions can be made.

Identifying these morphological groups is an important first step towards making a taxonomic revision, determining which entities deserve taxonomic recognition and at which rank, and hence determining the threat levels and conservation priorities in the *Myosotis pygmaea* species group. The high levels of morphological plasticity recovered in part explains the number of tag names that have been applied to this group (Table 2.1), as

individual plants are frequently seen in the field that do not match the species' descriptions in the current *Flora*. Other studies investigating polymorphic species using morphological data have shown that taxa previously recognized at the specific or infraspecific level show continuous variation and should be treated as one or few taxonomic entities, such as in *Picris hieracioides* L. (Slovák et al., 2012). Whether the few trichome and leaf colour characters that do appear to distinguish between the currently described species are sufficient to warrant species rank, as used by de Lange et al. (2010), or subspecies or perhaps varietal rank (as used by Moore 1961) is the next question. Before undertaking a taxonomic revision, the morphological groups identified here will be used as species hypotheses to be further tested and integrated with microsatellite markers (Chapters 3 & 4). Additional studies using ecological niche modelling (Chapter 5) will also contribute to understanding species limits and threat levels of this group of New Zealand forget-me-nots.

Figure 2.1 Photographs of *M. pygmaea* group species and potentially affiliated entities (see Table 2.1). **A.** *Myosotis glauca* WELT SP093284 **B.** *M.* "aff. *glauca*" WELT SP093282 **C.** *M. glauca*? WELT SP103892 **D.** *M. pygmaea* WELT SP090542 **E.** *M. brevis* WELT SP093294 (note green- vs brown-leaved individuals in photo **F.** *M. brevis* WELT SP090543 **G**. *M. drucei* WELT SP100445 **H.** *M. antarctica* WELT SP102777 **I.** *M.* "Volcanic Plateau" JMP12013 (no voucher) **J.** *M.* "intermedia" WELT SP093292 **K.** *M*. "Tapuae-o-Uenuku" WELT SP100449 **L.** *M*. "Rock and Pillar" WELT SP102784 **M.** *M.* aff. *tenericaulis* WELT SP091590. Photo C by Geoff Rogers, remainder by JMP. Voucher information in Appendix 2.

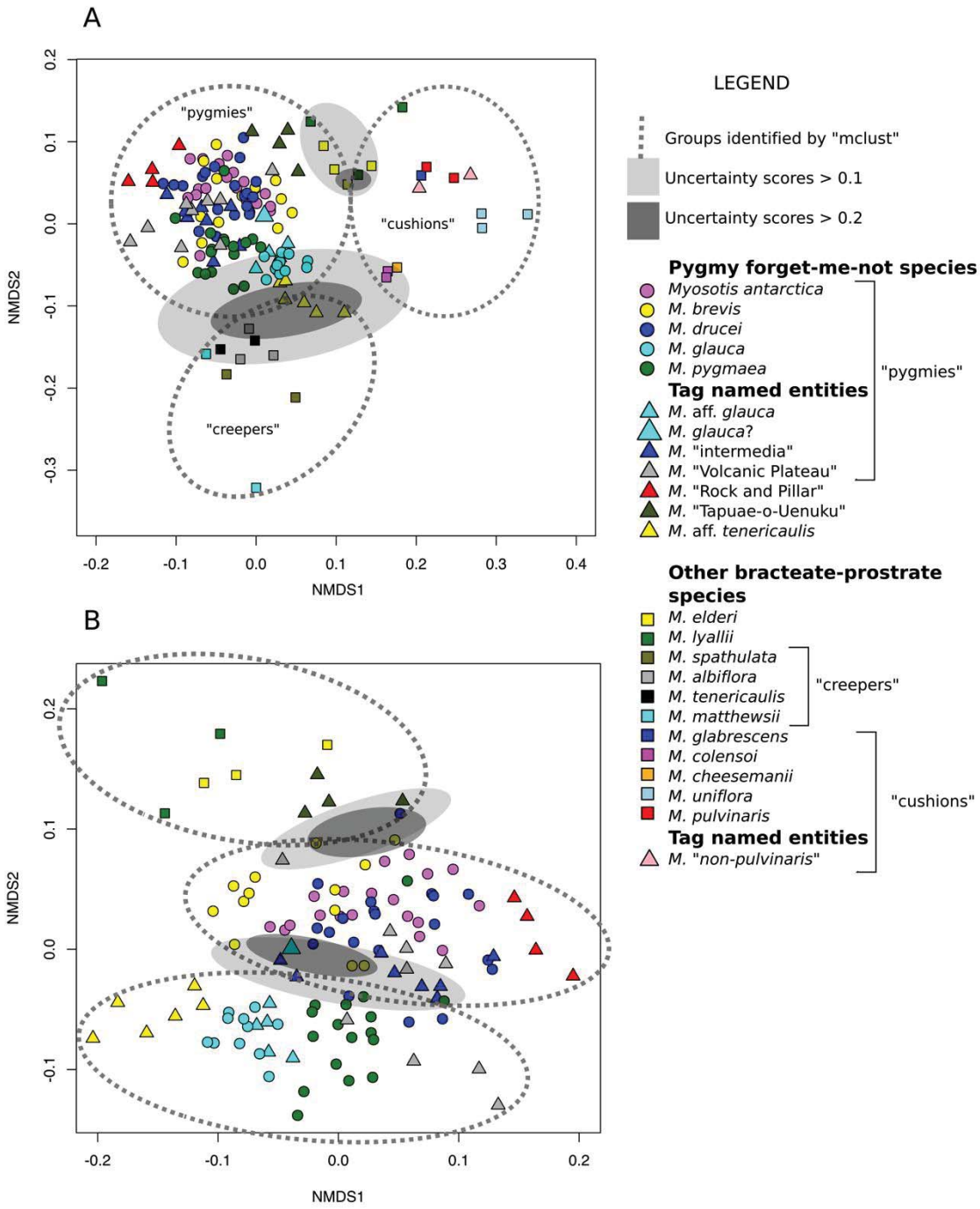


Figure 2.2 Non-metric multidimensional scaling (nMDS) plots of individuals of the *Myosotis pygmaea* species group and other bracteate-prostrate species, based on the herbarium specimen derived morphological datasets, converted to dissimilarity matrices with Gower's coefficient. Clusters and individual uncertainty calculated using "mclust" is shown. **A.** The "bracteate-prostrate" dataset comprising 144 individuals and 52 characters, showing both dimensions that were retained. **B**. Reduced subset of the "bracteate-prostrate" dataset comprising 113 individuals and 52 characters, including those identified as "pygmies" in Figure 2.2A, and those tag-named entities and species for which at least one individual was placed in the "pygmies" cluster, showing both dimensions that were retained.

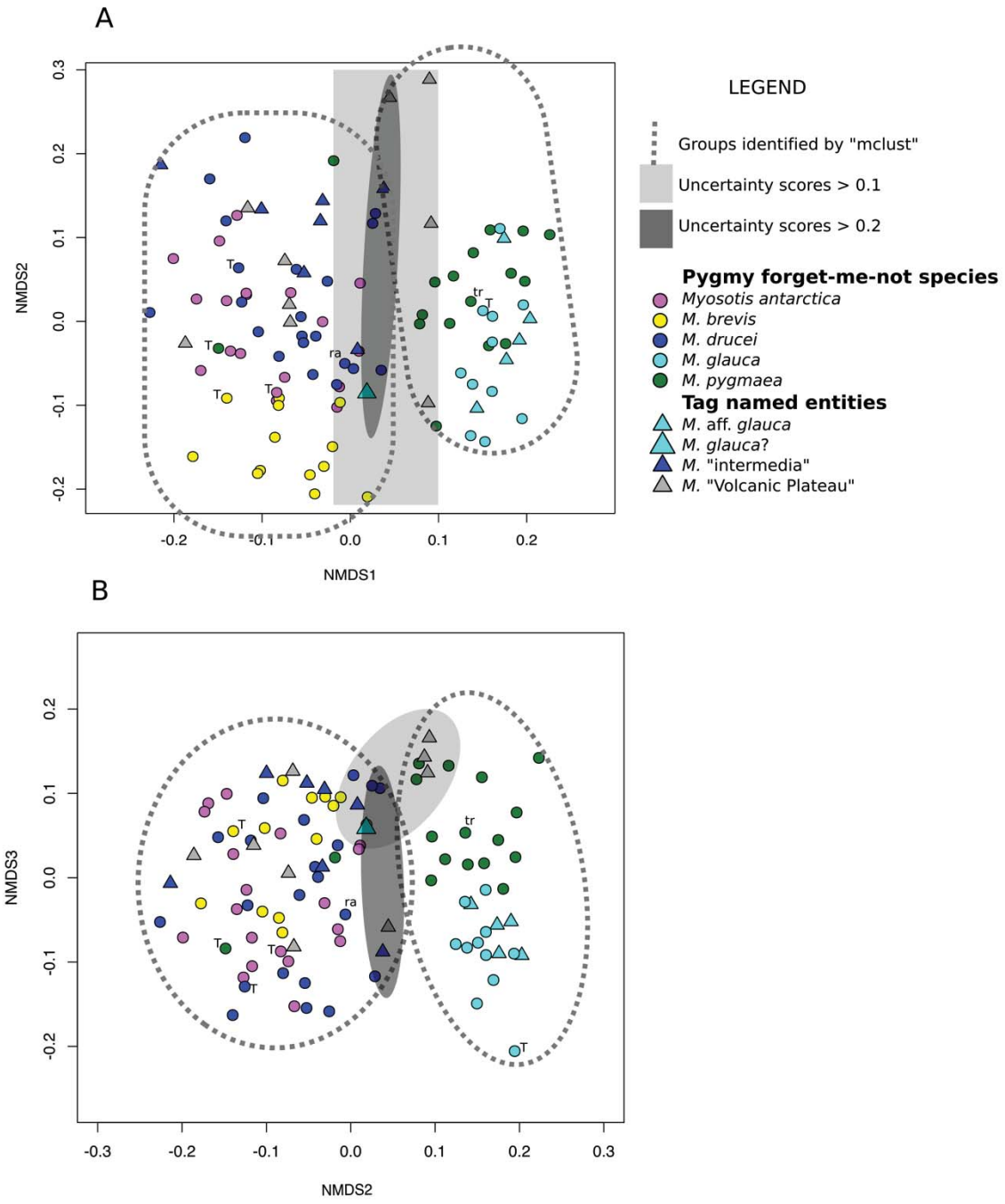


Figure 2.3 Non-metric multidimensional scaling (nMDS) plots of *Myosotis* individuals of the "pygmaea group" dataset comprising 103 individuals and 26 characters, based on the herbarium specimen derived morphological datasets, converted to dissimilarity matrices with Gower's coefficient. Three dimensions were retained. Clusters and individual uncertainty calculated using "mclust" is shown. T indicates type specimens of pygmy forget-me-not species, tr = type specimen of *M. antarctica* subsp. *traillii*, and ra = type specimen of *M. ramificata*. **A.** Dimensions 1 vs. 2 **B.** Dimensions 2 vs. 3.

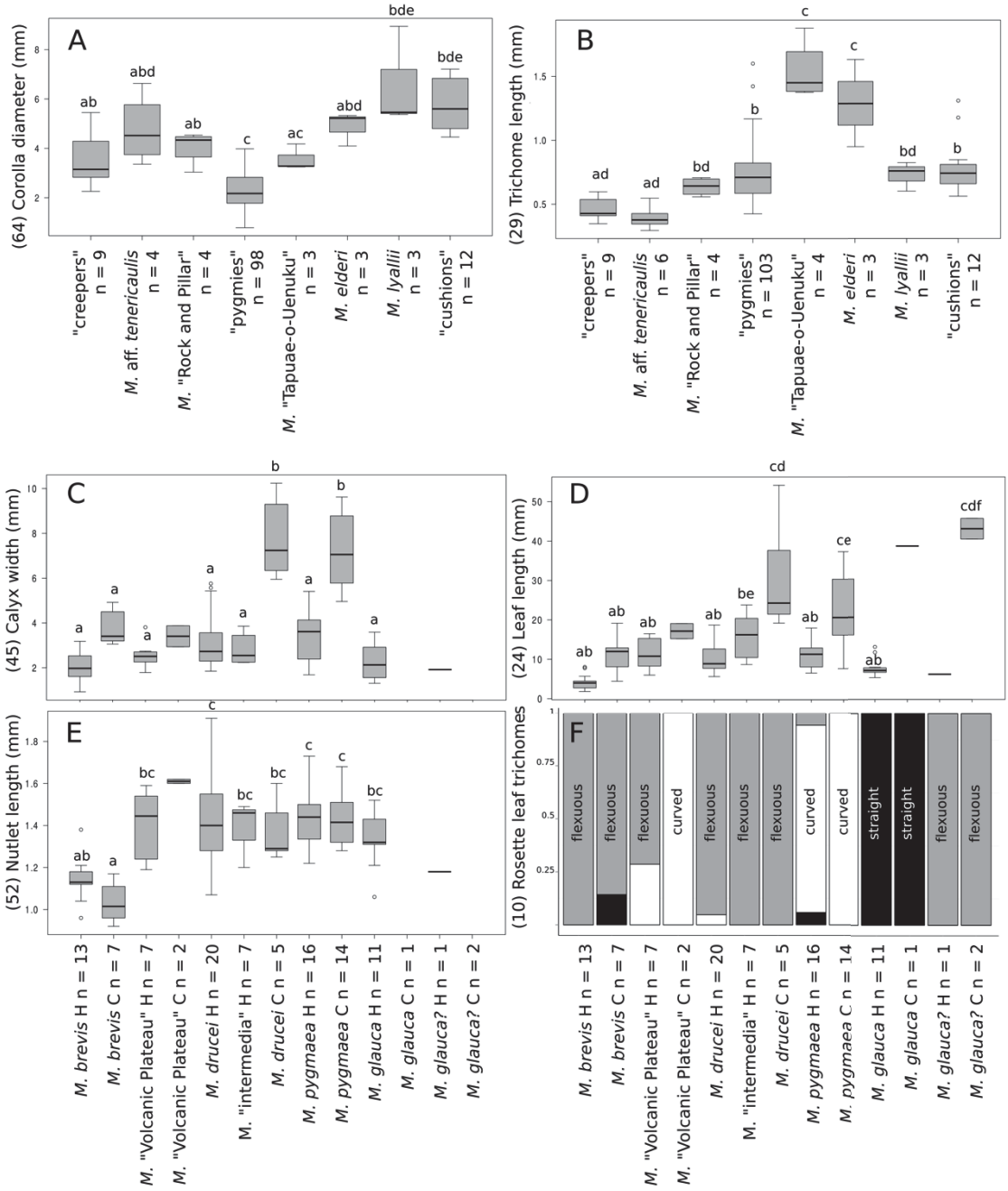


Figure 2.4 Box plots of selected morphological characters showing the *Myosotis pygmaea* group and affiliated species and tag-named entities. **A & B** are based on herbarium specimens, comparing groups as categorized in Figure 2.2A. **C–E** compare data from herbarium specimens (H) with cultivated specimens (C). Measurements of cultivated specimens were taken from live plants grown in the growth chamber with the exception of two specimens of *M.* "Volcanic Plateau", that are herbarium specimens of cultivated plants (WELT SP100412 and CHR 131697, see Appendix 2). Box plots that share the same lowercase letters are not significantly differentiated at p = 0.05, calculated for groups for which n ≥ 3. **F** is a stacked bar chart showing quantitative morphological character 10, which classifies rosette leaf trichomes as straight (black), flexuous (grey) or curved (white). Further information about each morphological character can be found in Table 2.2.

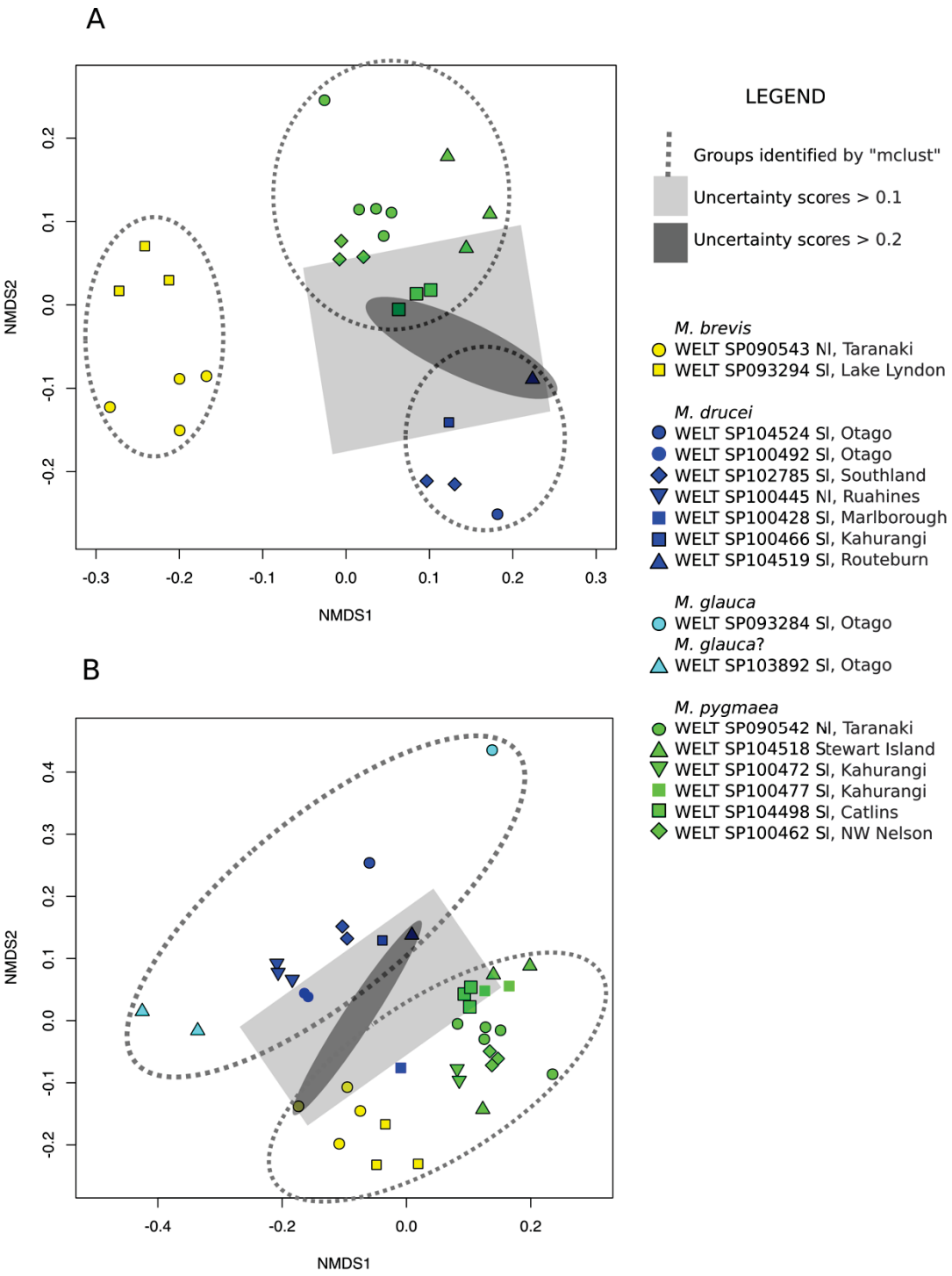


Figure 2.5 Non-metric multidimensional scaling (nMDS) plots based on the Gower's dissimilarity matrix of the morphological data measured on live cultivated plants from individuals of the *Myosotis pygmaea* species group grown in the common garden. Clusters and individual uncertainty calculated using "mclust" is shown. **A.** The "flowering" dataset of 26 individuals and 37 characters, showing both dimensions retained. **B.** The "all adults" dataset of 39 individuals and 36 characters, showing both dimensions retained. Voucher details in Appendix 2 and Table 2.3. NI = North Island, SI = South Island.

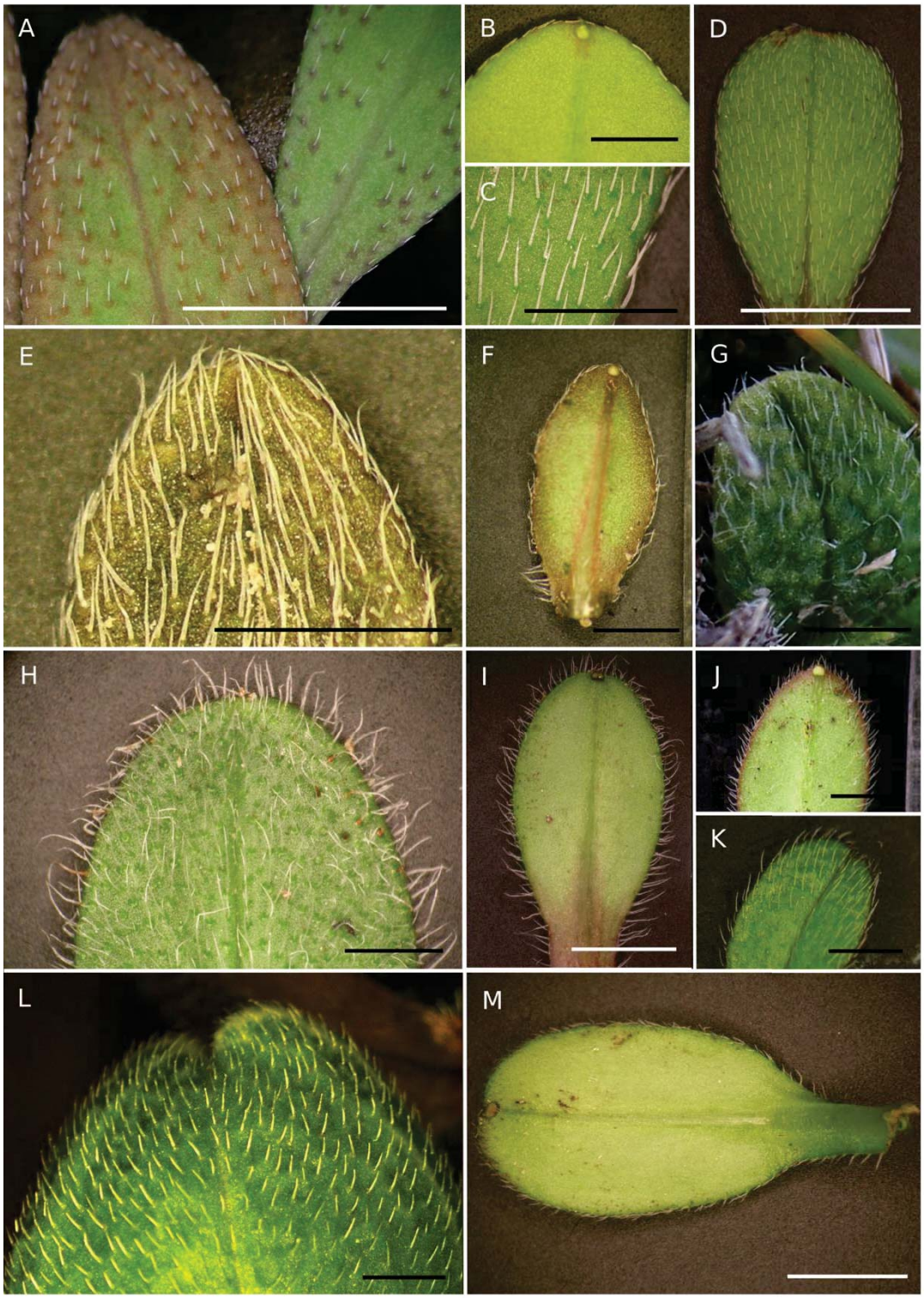


Figure 2.6 Photographs of rosette leaves of *Myosotis pygmaea* group plants illustrating trichome characters. White scale bars represent 5 mm, black scale bars represent 2 mm. **A-D** *M. glauca* showing straight trichomes that are appressed on the blade and at margins; **A, B** *M. glauca* WELT SP100497; **C, D** *M.* aff. *glauca* WELT SP092202. **E-G** *M. brevis* showing flexuous trichomes that are appressed to patent on the blade and at margins; **E, F** *M. brevis* WELT SP093294; **G** *M. brevis* WELT SP090543. **H-K** *M. drucei* and affiliated tag-named entities showing flexuous trichomes that are patent to erect on the blade and at margins. **H, I** *M. drucei* WELT SP092204; **J** *M.* "intermedia" WELT SP093292; **K** *M.* "Volcanic Plateau" WELT SP100412; **L-M** *M. pygmaea* showing curved trichomes that are patent on the blade and at margins; **L** *M. pygmaea* WELT SP104498; **M** *M. pygmaea* growing at Percy Scenic Reserve, Wellington (no voucher). B, F, I, J & M showing abaxial leaf surface, the remainder showing adaxial. Photos C, D, H, I & M taken by Carlos Lehnebach, remainder by JMP.

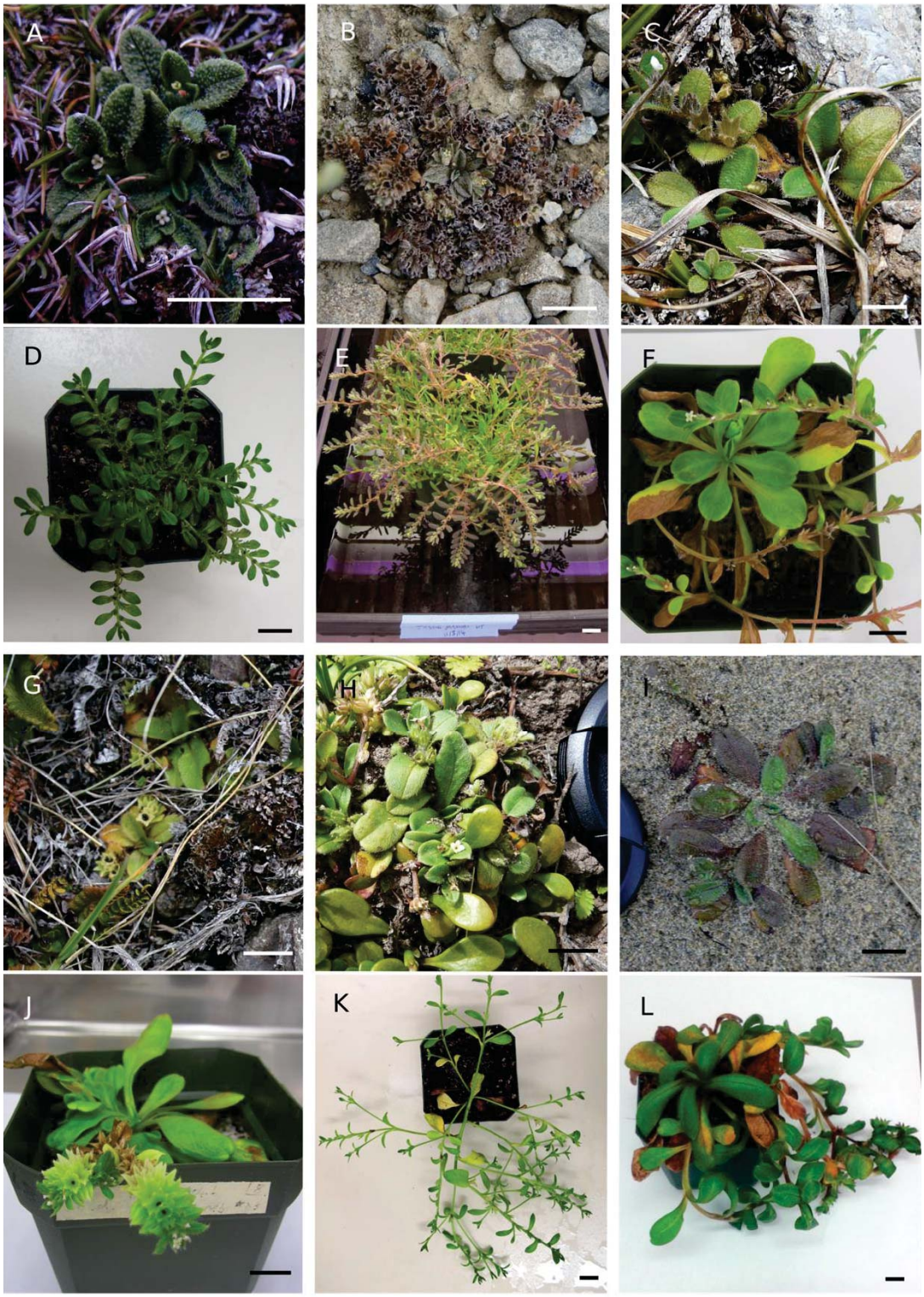


Figure 2.7 Photographs of *M. pygmaea* group plants in the field vs. their offspring growing in common garden conditions. All scale bars are 1 cm. **A&D** *M. brevis* North Island, coastal Taranaki, WELT SP090543. **B&E** *M. brevis*, South Island, Canterbury, Lake Lyndon, WELT SP093294. **C&F** *M. drucei*, north western South Island, Lake Peel, WELT SP100466. **G&J** *M. drucei*, South Island, Southland, Livingstone Mountains, WELT SP102785. **H&K** *M. pygmaea*, coastal north western South Island, WELT SP100462. **I&L** *M. pygmaea* South Island, Southland, the Catlins, Tahakopa Bay, WELT SP104498. Photos C&H by Mark Prebble, remainder by JMP.

Table 2.1 Taxonomic history, distribution, conservation status and important morphological characters of each published species in the *Myosotis pygmaea* species group, as well as other tag-named New Zealand *Myosotis* with a bracteate-prostrate growth form. NI = North Island, SI = South Island.

Species or tag name	Taxonomic description	As treated in Moore (1961)	Distribution from Moore (1961)	Conservation status (de Lange et al. 2013)	Distinguishing morphological characteristics from the literature	Summary of morphological evidence (this chapter)	Distinguishing morphological characters (this chapter)
***Myosotis antarctica* Hook.f.**	Hooker (1844)	*M. antarctica*	Campbell Island, and southern Chile[2]	Naturally Uncommon – Island Endemic and One Location	"Flowers usually blue; hairs long, very fine and silky, crowded" (Moore 1961:809)	Unable to distinguish *M. antarctica* from *M. drucei* (Figure 2.3A&B) apart from on average higher density of trichomes on rosette leaves (20 per mm^2 vs.7 per mm^2; Table 2.5)	Trichomes flexuous, patent to erect on blade and margins; corolla diameter 1.5–4 mm; corolla colour blue, white or cream; calyx 3–8 mm at fruiting; nutlets 1.2–1.9 mm long, 0.8–1.2 mm wide
***M. antarctica* subsp. *traillii* Kirk**	Kirk (1884)	*M. pygmaea s.l.*	Stewart Island: coastal	Not assessed	"The stiffish, rather sparse, +/- appressed hairs distinguish this form from *M. antarctica* […], but it does not seem to be varietally distinct from *M. pygmaea* Col." (Moore 1961:814)	The type specimen clusters with all specimens identified as *M. pygmaea* in this study (apart from the *M. pygmaea* type), Figures 2.3A&B	See *M. pygmaea*
***M. brevis* de Lange & Barkla**	As *M. pygmaea* var. *minutiflora*: Simpson and Thomson (1943); as *M. brevis*: de Lange et al., (2010)	*M. pygmaea* var. *minutiflora*	NI: coastal; SI: montane	Nationally Endangered – Data Poor and Extreme Fluctuations	Flowers cream coloured, corollas up to 1 mm diameter, hairs not strigose, small seeds, annual life-cycle (de Lange et al., 2010:437)	All specimens identified as *M. brevis* cluster together (Figures 2.3A and 2.5A&B). Some characters for which *M. brevis* is significantly smaller than other members of the *M. pygmaea* group are highlighted in Tables 2.5 and 2.6	Trichomes flexuous, patent to erect on blade and margins; corolla diameter 0.5–2 mm; corolla colour usually cream, calyx 2–4 mm at fruiting, nutlets 0.9–1.2 mm long, 0.5–0.8 mm wide
***M. drucei* (L.B.Moore) de Lange & Barkla**	As *M. pygmaea* var. *drucei*: Moore (1961); as *M. drucei*: de Lange et al. (2010)	*M. pygmaea* var. *drucei*	NI & SI: montane	Not Threatened (i.e. common)	"Hairs tapering to soft ± flexuous tip; nutlets quite immersed in long calyx" (Moore 1961:815) and "Approaches *M. antarctica* Hook.f. but hairs are less silky and less crowded, fls rather smaller and cream-coloured, nutlets more	*M. drucei* is indistinguishable from *M. antarctica* (Figure 2.3A&B), apart from on average lower density of trichomes on rosette leaves (7 per mm^2 vs. 20 per mm^2; Table 2.5). All specimens of *M. drucei* cluster together in the growth room data	See *M. antarctica*

Species or tag name	Taxonomic description	As treated in Moore (1961)	Distribution from Moore (1961)	Conservation status (de Lange et al. 2013)	Distinguishing morphological characteristics from the literature	Summary of morphological evidence (this chapter)	Distinguishing morphological characters (this chapter)
					elongated" (Moore 1961:816)	(Figure 2.5A)	
***M. glauca* (Simpson & Thomson) de Lange & Barkla**	As *M. pygmaea* var. *glauca*: Simpson and Thomson (1942); as *M. glauca*: de Lange et al. (2010)	*M. pygmaea* var. *glauca*	SI: Central Otago, montane	Nationally Vulnerable – Declining, Data Poor and Sparse	Flowers white; hairs appressed, stiff, regular, sparse, strigose; leaves glaucous and only few forming basal rosette; seed broadly ovoid (de Lange et al., 2010:438)	All specimens identified as *M. glauca* cluster together (Figure 2.3A&B)	Trichomes straight, appressed to patent on blade and at margins; corolla diameter 1.5–4 mm, corolla colour usually white, calyx 2.5–8 mm at fruiting, nutlets 1.2–1.5 mm long, 0.8–1.2 mm wide
***M. pygmaea* Col.**	Colenso (1883)	*M. pygmaea* var. *pygmaea*	NI & SI: coastal and inland	At Risk – Declining and Sparse	"Hairs strigose; calyx-tube short, nutlets protruding" (Moore, 1961:815)	Almost all specimens identified as *M. pygmaea* cluster together, with the notable exception of the type specimen which clusters with *M. antarctica* and *M. drucei* (Figure 2.3A&B; 2.5A)	Trichomes flexuous to curved, appressed to patent on blade and at margins (excluding the type specimen which has flexuous erect trichomes); corolla diameter 1.5–4 mm, corolla colour usually white, calyx 2.5–6 mm at fruiting, nutlets 1.2–1.9 mm long, 0.8–1.2 mm wide
***M. ramificata* Simpson**	Simpson (1952)	*M. pygmaea* s. l.	SI: Central Otago	Not assessed	"Simpson points out very accurately the differences between his sp. and *M. tenericaulis* but does not compare it to *M. pygmaea*" (Moore 1961:815)	The type specimen clusters with specimens identified as *M. antarctica* and *M. drucei* (Figure 2.3A&B)	See *M. antarctica*

Tag name	Example voucher specimen or publication of tag name	As treated in Moore (1961)	Distribution based on herbarium specimens	Conservation status[1] (de Lange et al., 2013)	Suspected distinguishing morphological characters	Summary of morphological evidence (this chapter)	Distinguishing morphological characters (this chapter)
M.* aff. *glauca	WELT SP089898	N/A	SI: Central Otago, Pisa	Not assessed	"Similar to *M. glauca* but visually quite different" Mike Thorsen,	Specimens cluster with *M. glauca* (Figure 2.3A&B). Trichomes similar	See *M. glauca*

Tag name	Example voucher specimen or publication of tag name	As treated in Moore (1961)	Distribution based on herbarium specimens	Conservation status[1] (de Lange et al., 2013)	Suspected distinguishing morphological characters	Summary of morphological evidence (this chapter)	Distinguishing morphological characters (this chapter)
			Range		note on specimen label	to *M. glauca,* but with leaves green rather than glaucous	
***M. glauca* ?**	WELT SP103892	N/A	SI: Central Otago	Not assessed	"Very erect, strikingly blue-mauve plants" Geoff Rogers, note on specimen label	Specimen clusters with *M. drucei* (Figures 2.3A&B; 2.5A&B). Leaf colour similar to *M. glauca* but with flexuous trichomes rather than straight, and somewhat erect habit. Additional collections and study are required	Additional collections and study are required.
***M.* "intermedia"**	WELT SP089916	N/A	SI: Central Otago lowlands	Not assessed	"Dark base to leaf, intermediate between *M. drucei* and *M. pygmaea*" Mike Thorsen, note on specimen label	Specimens cluster with *M. antarctica* + *M. drucei* (Figures 2.3A&B). No characters significantly differentiate *M.* "intermedia" specimens from *M. drucei*	See *M. antarctica*
***M.* "Rock and Pillar"**	WELT SP089903	N/A	SI: Central Otago, Rock and Pillar Range	Not assessed	"…Lower hairs dense, appressed, retrorse" Mike Thorsen, note on specimen label	All specimens form a cluster (Figure 2.2A) and can be separated from the pygmy forget-me-nots by their larger flower size (average corolla diameter 4.1 vs. 2.2, Table 2.4). Further comparison with other bracteate-prostrate species needed	Retrorse trichomes on the undersides of rosette leaves; hooked trichomes on the calyx; corolla diameter > 4 mm
***M.* "Volcanic Plateau"**	Druce (1993); de Lange et al. (2013) as *M.* aff. *pygmaea* "Volcanic Plateau" CHR 244566; Murray and de Lange (2013) AK 331000	N/A	NI: Volcanic Plateau	Naturally Uncommon – Extreme Fluctuations, Range Restricted and Sparse	"its leaves are narrower than usual for that species [*M. drucei*] and further, it grew under *Chionochloa pallens* Zotov subsp. *pallens* in an alpine flush" Murray and de Lange (2013:43)	Apart from a narrower length to width ratio of 3.1:1 for *M.* "Volcanic Plateau" vs. 2.0:1 for *M. drucei*, see Table 2.5), unable to distinguish this tag-named entity from *M. drucei*. It also does not have narrower leaves than *M.* "intermedia", Table 2.5.	See *M. antarctica*
***M.* "Tapuae-o-Uenuku"**	de Lange et al. (2013) as *M.* (b) "Mt Tapuae-o-Uenuku"; CHR 386966	N/A	SI: Marlborough, Mt Tapuae-o-Uenuku	Nationally Critical – Data Poor, One Location in New Zealand	"Cushion 5 cm diameter. Long hairs on upper surface and margins of leaf; few on lower surface; flowers 4–5mm diameter; anthers included" AP	All specimens identified as *M.* "Tapuae-o-Uenuku" cluster separately from other pygmy forget-me-nots (Figure 2.2B); further comparison with other bracteate-	Trichomes long (1.3–1.9 mm, Table 2.4); corolla diameter > 3.5mm

Tag name	Example voucher specimen or publication of tag name	As treated in Moore (1961)	Distribution based on herbarium specimens	Conservation status[1] (de Lange et al., 2013)	Suspected distinguishing morphological characters	Summary of morphological evidence (this chapter)	Distinguishing morphological characters (this chapter)
					Druce, comment on specimen label	prostrate species–particularly *M. elderi*–is needed	
M.* aff. *tenericaulis	de Lange et al. (2013) AK 7570	*M. tenericaulis*	SI: Central Otago and Southland	Naturally Uncommon – Range restricted and Sparse	"Specimens [of *M. tenericaulis*] from boggy places in the Garvie Mts are dwarfed and flower at c. 2 cm. tall; internodes and pedicels are short and lf-texture is less membr. than in larger plants" (Moore 1961; 814)	All specimens identified as *M.* aff. *tenericaulis* cluster together (Figure 2.2A&B); further comparison with other bracteate-prostrate species–particularly *M. tenericaulis*–is needed	Trichomes short, appressed, straight; corolla diameter > 4 mm
***M.* "non-pulvinaris"**	Robertson (1989); Druce (1993); de Lange et al. (2013) as *M.* aff. *pulvinaris* CHR 431563	N/A	SI: Fiordland and Otago	Data Deficient	OTA 043909 and CHR 272474 determined by A Robertson, no comments to explain in what way they are suspected to differ from *M. pulvinaris* on specimen labels	Specimens of *M.* "non-pulvinaris" cluster with those of *M. pulvinaris* and *M. glabrescens*, separate from the pygmy forget-me-nots (Figure 2.2A). Further comparison with other bracteate-prostrate species–particularly *M. pulvinaris*–is needed.	Cushion forming

1. "Only those informally recognised entities that are currently or have previously been believed to be Threatened or At Risk, and for which there is compelling evidence to suggest that they may be worthy of future formal taxonomic recognition were assessed" (de Lange *et al.* 2013:2).

2. Zuloaga et al. (2008)

Table 2.2 Description of morphological characters measured on *Myosotis* herbarium specimens and live plants grown in common garden conditions.

Character number	Character description	Qualitative (QUAL) or quantitative (QUANT)	Vegetative (V) or reproductive (R)	Used in the 144 individual herbarium "bracteate-prostrate" dataset	Used in the 103 individual herbarium "pygmaea in group" dataset	Used in the two growth room datasets
1	Cushion = 0, not cushion = 1	QUAL	V	Y		
2	Adventitious roots on branches absent = 0, present = 1	QUAL	V	Y		
3	Rosette leaf attachment petiolate = 0, indistinctly petiolate = 1, sheathing = 2	QUAL	V			Y
4	Rosette leaf apex acute = 0, obtuse = 1	QUAL	V	Y		
5	Rosette leaf apex not mucronate = 0, mucronate = 1	QUAL	V	Y		
6	Rosette leaf trichomes adaxial appressed = 0, patent = 1, erect = 2	QUAL	V	Y	Y	
7	Rosette leaf trichomes adaxial orientation relative to midrib parallel = 0, 45 degrees = 1	QUAL	V	Y		
8	Rosette leaf margin trichomes appressed = 0, patent = 1, erect = 2	QUAL	V	Y	Y	Y
9	Rosette leaf trichomes abaxial not retrorse = 0, retrorse = 1	QUAL	V	Y	Y	Y
10	Rosette leaf trichomes adaxial straight = 0, curved = 1, flexuous = 2	QUAL	V	Y	Y	Y
11	Uppermost cauline leaf apex obtuse = 0, acute = 1	QUAL	V	Y		
12	Retrorse trichomes on the calyx absent = 0, present = 1	QUAL	R	Y	Y	Y
13	Hooked trichomes on the calyx absent = 0, present = 1	QUAL	R	Y		
14	Density of trichomes inside the mature calyx is glabrous = 0, sparse = 1, dense = 2	QUAL	R	Y		
15	Corolla colour white = 0, white with pink lines = 1, creamy-yellow = 2[1]	QUAL	R			Y
16	Anthers entirely below scales = 0, at least partially below scales = 1, entirely above scales = 2	QUAL	R	Y	Y	
17	Rosette leaf blade colour bright green = 0, grey-green = 1, blue-grey = 2[1]	QUAL	V			Y
18	Rosette leaf petiole the same colour as the blade = 0, different colour = 1[1]	QUAL	V			Y
19	Rosette leaf midrib the same colour as the blade = 0, different colour = 1[1]	QUAL	V			Y
20	Rosette leaf trichomes abaxial glabrous = 0, solely on mid-rib = 1, all over = 2	QUAL	V			Y
21	Total number rosette leaves	QUANT	V		Y	Y
22	Rosette leaf petiole length (mm, average of 2)	QUANT	V	Y	Y	Y
23	Rosette leaf petiole width (mm, average of 2)	QUANT	V	Y		
24	Rosette leaf lamina length (mm, average of 2)	QUANT	V	Y	Y	Y
25	Ratio of rosette leaf length to widest point: length (average of 2)	QUANT	V	Y		Y

Character number	Character description	Qualitative (QUAL) or quantitative (QUANT)	Vegetative (V) or reproductive (R)	Used in the 144 individual herbarium "bracteate-prostrate" dataset	Used in the 103 individual herbarium "pygmaea in group" dataset	Used in the two growth room datasets
26	Ratio of rosette leaf length: width (average of 2)	QUANT	V	Y	Y	Y
27	Rosette leaf trichome count/mm2	QUANT	V	Y	Y	
28	Ratio of rosette leaf trichome length: rosette leaf length	QUANT	V	Y		Y
29	Trichome length rosette and cauline leaves (mm, average of 2 growth room, 4 herbarium)	QUANT	V	Y		Y
30	Lowest cauline leaf petiole length (mm)	QUANT	V	Y	Y	
31	Lowest cauline leaf petiole width (mm)	QUANT	V	Y		
32	Lowest cauline leaf length (mm)	QUANT	V	Y	Y	Y
33	Ratio of lowest cauline leaf length to widest point: length	QUANT	V	Y		Y
34	Ratio of lowest cauline leaf length: width	QUANT	V	Y	Y	Y
35	Uppermost cauline leaf length (mm)	QUANT	V	Y		Y
36	Ratio of uppermost cauline leaf length to widest point: length	QUANT	V	Y		Y
37	Ratio of uppermost cauline leaf length: width	QUANT	V	Y		Y
38	Total number flowers and fruits on longest branch including side branches	QUANT	R			Y
39	Length of longest branch (mm)	QUANT	R	Y	Y	Y
40	Width of longest branch (mm)	QUANT	R	Y		
41	Longest internode length on longest branch (mm)	QUANT	R			Y
42	Calyx length at flowering (mm)	QUANT	R	Y	Y	
43	Pedicel length at fruiting, longest one on longest branch (mm)	QUANT	R	Y	Y	Y
44	Calyx length at fruiting (mm) (mature fruits)	QUANT	R	Y	Y	Y
45	Calyx width at fruiting at tip of calyx lobes (mm) (mature fruits)	QUANT	R		Y	Y
46	Calyx trichome length average (mm) (whole calyx)	QUANT	R	Y		
47	Calyx lobe length at fruiting (mm)	QUANT	R			Y
48	Calyx lobe width at widest point at fruiting (mm)	QUANT	R	Y		
49	Ratio of largest internode at fruiting: lowest cauline leaf length	QUANT	R	Y	Y	Y
50	Ratio of calyx width at tip of lobes: width at base of lobes at fruiting	QUANT	R	Y		Y
51	Ratio of calyx lobe length: calyx length	QUANT	R	Y		Y
52	Nutlet length (mm)	QUANT	R	Y	Y	Y
53	Ratio of nutlet length: width	QUANT	R	Y	Y	Y

Character number	Character description	Qualitative (QUAL) or quantitative (QUANT)	Vegetative (V) or reproductive (R)	Used in the 144 individual herbarium "bracteate-prostrate" dataset	Used in the 103 individual herbarium "pygmaea in group" dataset	Used in the two growth room datasets
54	Corolla lobe length (mm)	QUANT	R	Y	Y	Y
55	Ratio of corolla lobe length: width	QUANT	R	Y		Y
56	Corolla tube length from base to scales (mm)	QUANT	R	Y	Y	
57	Corolla tube width at scales (mm)	QUANT	R	Y	Y	
58	Ratio of corolla tube width at scales: width at base	QUANT	R	Y		
59	Filament length (mm)	QUANT	R	Y		
60	Anther length (mm)	QUANT	R	Y	Y	
61	Ratio of style length: calyx length at flowering	QUANT	R	Y		
62	Ratio of style length: corolla tube length at flowering	QUANT	R	Y		
63	Ratio of style length: calyx length at fruiting	QUANT	R	Y		
64	Corolla diameter (mm) (lobe length × 2, plus width at scales)	QUANT	R			
	Total number of characters in each dataset			**52**	**26**	**37**

1. Assessments of corolla and leaf colours were made by eye

Table 2.3 Details of seed germination, survival to adulthood and flowering rates in the *Myosotis pygmaea* species group common garden experiment. Rows in bold are totals for each species or tag-named entity

Species	Voucher of original field collection of each population	Age of seed at planting (months)	No. seeds sewn	No. germinated	No. survived to adulthood	No. flowered	Days to germination	Days to flowering (since germination)	Notes	Vouchers of flowering plants measured, or no. vegetative plants measured
M. antarctica	WELT SP102775	9	20	3	0		39 to 41			
M. antarctica	**1 population**	**9**	**20**	**3**	**0**		**39 to 41**			
M. brevis	WELT SP090545	34	10	0						
M. brevis	WELT SP090549	34	15	1	0		72			
M. brevis	WELT SP093294[1]	3 to 31	120	33	9	6	6 to 87	43 to 49	3 attempts combined	WELT SP104515 WELT SP104997 WELT SP104506
M. brevis	WELT SP102760	13	27	0						
M. brevis	WELT SP102762	13	18	0						
M. brevis	WELT SP102763	13	36	0						
M. brevis	WELT SP090543[2]	9	33	18	15	6	70 to 126	33 to 92		WELT SP104511 WELT SP104495 WELT SP104496 WELT SP104555
M. brevis	WELT SP090550	34	12	0						
M. brevis ?	No voucher, seed collected by Graeme Atkins at Hicks Bay, East Cape, 2005	92 to 108	40	0					May be *M. pygmaea,* not *M. brevis*	
M. brevis	**9 populations**	**9 to 108**	**311**	**52**	**24**	**12**	**11 to 126**	**33 to 92**		**7 flowering**
M. drucei	WELT SP100465	20	12	10	1	1	15 to 35	191		WELT SP104500
M. drucei	WELT SP100428	19	37	3	1	0	38 to 70			1
M. drucei	WELT SP100440	20	11	0						
M. drucei	WELT SP093286	31	15	5	0		23 to 26			

Species	Voucher of original field collection of each population	Age of seed at planting (months)	No. seeds sewn	No. germinated	No. survived to adulthood	No. flowered	Days to germination	Days to flowering (since germination)	Notes	Vouchers of flowering plants measured, or no. vegetative plants measured
M. drucei	WELT SP091599	31	38	13	0		15 to 103			
M. drucei	WELT SP100492	19	35	32	8	0	15 to 79			2
M. drucei	AK 331000	?31	4	0						
M. drucei	WELT SP102785	7	27	8	8	2	7 to 103	97		WELT SP104502 WELT SP104503
M. drucei	WELT SP104524	6	6	3	1	1	70 to 79	144		WELT SP104526
M. drucei	WELT SP100445[1]	8 to 12	80	58	6	0	10 to 29		2 attempts combined	3
M. drucei	WELT SP100425	19	1	1	0		23			
M. drucei	WELT SP104519	3	40	7	7	1	15 to 37	79		Pending[3]
M. drucei	**11 populations**	**3 to 31**	**306**	**140**	**32**	**5**	**7 to 103**	**79 to 191**		**5 flowering, 6 vegetative**
M. glauca	WELT SP093284	31	11	1	1	0	44			1
M. glauca	WELT SP100497	19	4	3	0		11 to 15			
M. aff. *glauca*	WELT SP093282	31	10	0						
M. glauca ?	WELT SP103892	3	42	2	2	0			Including 2 self-sown from the original pots	2
M. glauca	**4 populations**	**3 to 31**	**67**	**6**	**3**	**0**	**11 to 44**			**3 vegetative**
M. pygmaea	WELT SP100472	20	26	14	5	0	23 to 126			2
M. pygmaea	WELT SP100477	20 to 24	60	24	3	0	10 to 29		2 attempts combined	2
M. pygmaea	WELT SP100462[1]	3 to 20	34	8	6	5	38 to 63	67 to 75	2 attempts combined	WELT SP104504 WELT SP104516 WELT SP104517
M. pygmaea	WELT SP100460	20	18	2	0		49 to 70			
M. pygmaea	No voucher, seed collected by JMP	12	40	31	15	8	15 to 70	60 to 183		WELT SP104498 WELT SP104508

Species	Voucher of original field collection of each population	Age of seed at planting (months)	No. seeds sewn	No. germinated	No. survived to adulthood	No. flowered	Days to germination	Days to flowering (since germination)	Notes	Vouchers of flowering plants measured, or no. vegetative plants measured
	from Tahakopa Bay, Southland, August 2012									WELT SP104509
M. pygmaea	WELT SP090542[2]	9	27	10	9	7	75 to 103	31 to 97		WELT SP104499 WELT SP104505 WELT SP104510 WELT SP104513 WELT SP104514
M. pygmaea	No voucher, seed from Otari-Wilton's Bush, ex Stewart Island	2 to 18	22	8	7	7	44 to 70	64	Including 5 seeds self sewn	WELT SP104518 WELT SP104501 WELT SP104512
M. pygmaea	WELT SP090629	33	22	6	0		35 to 72			
M. pygmaea ?	No voucher, seed collected by Alex Fergus from Martins Bay, Fiordland	3 to 4	1	0					May be *M. drucei* not *M. pygmaea*	
M. pygmaea	**9 populations**	**2 to 33**	**250**	**103**	**45**	**27**	**10 to 126**	**31 to 183**		**14 flowering, 4 vegetative**
M. "intermedia"	WELT SP100498	19	5	2	0		31 to 35			
M. "intermedia"	**1 population**	**19**	**5**	**2**	**0**		**31 to 35**			
M. "Volcanic Plateau"	No voucher, seed collected by Geoff Rogers from Kiwi Burn, Southland, December 2013	19	2	0						
M. "Volcanic	WELT SP100412	21	3	0						

Species	Voucher of original field collection of each population	Age of seed at planting (months)	No. seeds sewn	No. germinated	No. survived to adulthood	No. flowered	Days to germination	Days to flowering (since germination)	Notes	Vouchers of flowering plants measured, or no. vegetative plants measured
Plateau"										
***M.* "Volcanic Plateau"**	**2 populations**	**19 to 21**	**5**	**0**						
	TOTAL	3 to 108	964	306	104	38	11 to 126	31 to 191		26 flowering, 13 vegetative

1. Additional 60 seeds per population germinated as part of the initial trial
2. Seed collected two years after the original voucher specimen from the same population
3. Specimen still at Massey in growth room (as at June 2016), it was measured after transferring other specimens to WELT.

Table 2.4 Comparison of characters differentiating individuals of several *Myosotis* tag-named entities from those of the *M. pygmaea* species group based on herbarium specimen data. For explanation of character numbers see Table 2.2.

Character (Character No.)	Comparison (n = number of specimens measured)	Mean (Range)	p-value
Flower diameter (mm) (64)	*M.* aff. *tenericaulis* (n = 4) Pygmy group (n = 98)	4.8 (3.4–6.6) 2.2 (0.8–4.0)	0.0000018
Flower diameter (mm) (64)	*M.* "Rock and Pillar" (n = 4) Pygmy group (n = 98)	4.1 (4.0–5.0) 2.2 (0.8–4.0)	0.00165
Flower diameter (mm) (64)	*M.* "Tapuae-o-Uenuku" (n = 3) Pygmy group (n = 98)	3.5 (3.5–4.3) 2.2 (0.8–4.0)	0.261
Average trichome length (mm) (29)	*M.* "Tapuae-o-Uenuku" (n = 3) Pygmy group (n = 103)	1.5 (1.3–1.9) 0.7 (0.4–1.6)	3.0e-11

Table 2.5 Comparison of herbarium specimen data showing differences between individuals of selected species and some tag-named *Myosotis* entities within the *M. pygmaea* species group.

Character (Character No.)	Comparison (n = number of specimens measured)	Mean (Range)	p-value
Length of calyx at fruiting (mm) (44)	*M. brevis* (n = 13) Remainder of the *M. pygmaea* group (n = 90)	2.5 (1.7–3.7) 3.8 (2.1–6.3)	1.81e-05
Floral lobe length (mm) (54)	*M. brevis* (n = 13) Remainder of the *M. pygmaea* group (n = 90)	0.4 (0.2–0.7) 0.8 (0.3–1.4)	1.06e-06
Nutlet length (mm) (52)	*M. brevis* (n =13) Remainder of the *M. pygmaea* group (n = 90)	1.1 (1.0–1.4) 1.4 (1.1–1.9)	6.25e-05
Ratio of trichome length to rosette leaf length (28)	*M. brevis* (n = 13) Remainder of the *M. pygmaea* group (n = 90)	0.2 (0.1–0.4) 0.09 (0.0–0.3)	0.00200
Rosette leaf trichome density per mm^2 (27)	*M. antarctica* (n =19) *M. drucei* (n= 20)	20.4 (7.4–33.3) 7.4 (1.7–19.1)	5.5e-09
Ratio of rosette leaf length to width (26)	*M.* "Volcanic Plateau" (n = 9) *M. drucei* (n = 21)	3.1 (2.1–4.8) 2.0 (1.4–3.3)	8.8e-07
Ratio of rosette leaf length to width (26)	*M.* "Volcanic Plateau" (n = 9) *M.* "intermedia" (n = 7)	3.1 (2.1–4.8) 2.5 (1.7–3.4)	0.343

Table 2.6 Comparison of characters of growth room specimens of *Myosotis pygmaea* species group individuals; values given are mean (range). Measurements taken from live plants. See Table 2.2 for explanation of character numbers. (R) = reproductive, (V) = vegetative. P-values that are significant at p < 0.01 are denoted with an asterisk (*), including calyx length at fruiting between *M. drucei* and *M. pygmaea* (compare with Table 2.8).

Character (Character No.)	*M. brevis* n = 7	*M. drucei* n = 5 (R) or 11 (V)	*M. pygmaea* n = 14 (R) or 18 (V)	p-value		
				M. brevis vs. *M. drucei*	*M. brevis* vs. *M. pygmaea*	*M. drucei* vs. *M. pygmaea*
Rosette leaf petiole length (mm) (22)	4.9 (1.3–10.7)	11.9 (6.6–22.1)	8.9 (4.1–16.7)	0.0017*	0.0756	0.1336
Rosette leaf length (mm) (24)	11.1 (4.5–14.1)	29.7 (19.2–54.2)	21.9 (7.6–37.3)	0.00017*	0.01899	0.06140
Lowest cauline leaf length (mm) (32)	10.9 (6.6–18.1)	22.7 (15.0–27.2)	15.5 (5.8–31.2)	0.037	0.572	0.227
Branch length (mm) (39)	54.7 (8.0–106.8)	157 (87.8–240.0)	148.0 (95.3–222.1)	0.00327*	0.00079*	1
Calyx length at fruiting (mm) (44)	2.3 (1.8–3.2)	6.7 (5.1–8.0)	4.7 (2.6–5.8)	7.9e-08*	3.0e-05*	0.00089*
Calyx at fruiting width at tips (mm) (45)	3.8 (3.1–4.9)	7.8 (5.9–10.2)	7.2 (4.9–9.6)	0.00043*	0.00019*	1.00000
Nutlet length (mm) (52)	1.0 (0.9–1.2)	1.4 (1.3–1.6)	1.4 (1.3–1.7)	0.00033*	2.2e-06*	1
Floral lobe length (mm) (54)	0.5 (0.2–0.6)	1.3 (1.1–1.5)	1.3 (0.9–1.6)	2.3e-07 *	9.7e-09*	1

Table 2.7 Comparison of characters between growth room and herbarium plants of *Myosotis pygmaea* species group individuals. For explanation of character numbers see Table 2.2. (R) = reproductive, (V) = vegetative.

Character (Character No.)	Growth room *M. brevis* n = 7	Herbarium *M. brevis* n = 13	p-value	Growth room *M. drucei* n = 5 (R) or 11 (V)	Herbarium *M. drucei* n = 21	p-value	Growth room *M. pygmaea* n = 14 (R) or 18 (V)	Herbarium *M. pygmaea* n = 17	p-value
Branch length (mm) (39)	54.7 (8.0–106.8)	18.1 (3.6–48.0)	1	157.0 (87.8–240.0)	55.1 (17.8–224.0)	0.00113	148.0 (95.3–222.1)	68 (15.6–195.0)	0.00027
Rosette leaf petiole length (mm) (22)	4.9 (1.3–10.7)	2.3 (0.5–6.0)	1	11.9 (6.6–22.1)	5.7 (2.0–17.7)	0.00022	8.9 (4.1–16.7)	5.3 (2.1–14.7)	0.12664
Rosette leaf length (mm) (24)	11.1 (4.5–14.2)	4.1 (1.8–8.1)	0.32490	29.7 (19.2–54.2)	10.2 (5.6–18.7)	6.9e-14	22.0 (7.6–37.3)	10.9 (6.5–18.0)	2.4e-06
Lowest cauline leaf length (mm) (32)	10.9 (6.6–18.1)	4.0 (2.3–6.2)	0.01719	22.7 (15.0–27.2)	7.2 (4.0–12.4)	5.4e-10	15.5 (5.8–31.2)	6.1 (3.7–8.7)	1.6e-07
Floral lobe length (mm) (54)	0.5 (0.2–0.6)	0.4 (0.2–0.7)	1	1.3 (1.1–1.5)	0.8 (0.3–1.4)	0.00221	1.3 (0.9–1.6)	0.84 (0.4–1.3)	0.00103
Calyx at fruiting width at tips (45)	3.8 (3.1–4.9)	2.1 (1.0–3.2)	0.069	7.8 (5.9–10.2)	3.2 (1.9–5.8)	2.0e-10	7.2 (5.0–9.6)	3.4 (1.7–5.4)	1.4e-12
Calyx length at fruiting (44)	2.3 (1.8–3.2)	2.5 (1.7–3.7)	1	6.7 (5.1–8.1)	4.2 (2.8–6.2)	1.7e-06	4.7 (2.6–5.8)	3.8 (2.2–5.1)	0.15185
Nutlet length (52)	1.0 (0.9–1.2)	1.1 (1.0–1.4)	1	1.4 (1.3–1.6)	1.4 (1.0–1.4)	1	1.4 (1.1–1.9)	1.5 (1.2–1.7)	1

Table 2.8 Additional comparisons of herbarium data between individuals of the *Myosotis pygmaea* species group, highlighting three of the characters that found significant differences between species in the growth room data. For explanation of character numbers see Table 2.2. P-values that are significant at $p < 0.01$ are denoted with an asterisk (*), excluding calyx length at fruiting between *M. drucei* and *M. pygmaea* (compare with Table 2.8).

Character (Character No.)	***M. brevis* n = 13**	***M. drucei* n = 21**	***M. pygmaea* n = 17**	**p-value**		
				M. brevis* vs. *M. drucei	***M. brevis* vs. *M. pygmaea***	***M. drucei* vs. *M. pygmaea***
Rosette Leaf length (mm) (24)	4.1 (1.8–8.1)	10.2 (5.6–18.7)	10.9 (6.5–18.0)	0.08911	0.04681	1
Floral lobe length (mm) (54)	0.4 (0.2–0.7)	0.7 (0.3–1.4)	0.84 (0.4–1.3)	0.00237*	0.00020*	1
Length of calyx at fruiting (mm) (44)	2.5 (1.7–3.7)	4.2 (2.8–6.2)	3.8 (2.2–5.1)	1.4e-05*	0.00453*	1

References

Beuzenberg EJ, Hair JB (1983) Contributions to a chromosome atlas of the New Zealand flora—25. Miscellaneous species. *New Zealand Journal of Botany* 21: 13-20.

Brandon A (2001) *Breeding systems and rarity in New Zealand Myosotis*. PhD Thesis. Palmerston North: Massey University.

Cameron KM (2010) On the value of taxonomy, phylogeny, and systematics to orchid conservation: Implications for China's Yachang Orchid Reserve. *Botanical Review* 76: 165-173.

Colenso W (1883) A further contribution towards making known the flora of New Zealand. *Transactions and Proceedings of the Royal Society of New Zealand* 16: 334.

Consaul LL, Gillespie LJ, Waterway MJ (2008) Systematics of North American Arctic diploid *Puccinellia* (Poaceae): Morphology, DNA content, and AFLP markers. *Systematic Botany* 33: 251-261.

de Lange P, Heenan P, Norton D, Rolfe J, Sawyer J (2010) *Threatened Plants of New Zealand*. Christchurch: Canterbury University Press.

de Lange PJ (2014) A revision of the New Zealand *Kunzea ericoides* (Myrtaceae) complex. *PhytoKeys* 40: 1-185.

de Lange PJ, Murray BG (2002) Contributions to a chromosome atlas of the New Zealand flora—37. Miscellaneous families. *New Zealand Journal of Botany* 40: 1-23.

de Lange PJ, Rolfe JR, Champion PD, Courtney SP, Heenan PB, Barkla JW, Cameron EK, Norton DA, Hitchmough RA (2013) *Conservation Status of New Zealand Indigenous Vascular Plants, 2012*. Wellington: New Zealand Department of Conservation.

de Queiroz K (2007) Species concepts and species delimitation. *Systematic Biology* 56: 879-886.

Druce AP (1993) *Indigenous Higher Plants of New Zealand, Unpublished Checklist*. Lower Hutt: Landcare Research.

Elmer KR, Kusche H, Lehtonen TK, Meyer A (2010) Local variation and parallel evolution: morphological and genetic diversity across a species complex of neotropical crater lake cichlid fishes. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365: 1763-1782.

Fraley C, Raftery AE (2002) Model-based clustering, discriminant analysis and density estimation. *Journal of the American Statistical Association* 97: 611-631.

Fraley C, Raftery AE, Murphy TB, Scrucca L (2012) Mclust version 4 for R: Normal mixture modeling for model-based clustering, classification, and density estimation technical report no. 597, Department of Statistics, University of Washington.

Gagnon E, Hughes CE, Lewis GP, Bruneau A (2015) A new cryptic species in a new cryptic genus in the *Caesalpinia* group (Leguminosae) from the seasonally dry inter-Andean valleys of South America. *Taxon* 64: 468-490.

Hooker JD (1844) *The Botany of the Antarctic Voyage of H.M. Discovery Ships Erebus and Terror in the Years 1839-1843: Under the Command of Captain Sir James Clark Ross*. London: Reeve Brothers.

IUCN (2001) IUCN red list categories and criteria: version 3.1. IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, UK.

Kirk T (1884) Description of new plants collected on Stewart Island. *Transactions and Proceedings of the New Zealand Institute* 16: 373.

Knowlton N, Jackson JBC (1994) New taxonomy and niche partitioning on coral reefs: jack of all trades or master of some? *Trends in Ecology & Evolution* 9: 7-9.

Koutecky P (2015) MorphTools: a set of R functions for morphometric analysis. *Plant Systematics and Evolution* 301: 1115-1121.

Lehnebach C (2012) Two new species of forget-me-nots (*Myosotis*, Boraginaceae) from New Zealand. *PhytoKeys* 16: 53-64.

López-Reyes A, de la Rosa JP, Ortiz E, Gernandt DS (2015) Morphological, molecular, and ecological divergence in *Pinus douglasiana* and *P. maximinoi*. *Systematic Botany* 40: 658-670.

Mabberley D (2008) *Mabberley's Plant Book. A portable dictionary of plants, their classifications and uses*. Seattle: University of Washington Botanic Gardens.

Maechler M, Rousseeuw P, Struyf A, Hubert M, Hornik K (2015) Cluster: Cluster analysis basics and extensions. R package version 2.0.1.

Meudt HM (2012) A taxonomic revision of native New Zealand *Plantago* (Plantaginaceae). *New Zealand Journal of Botany* 50: 101-178.

Meudt HM, Prebble JM, Lehnebach CA (2015) Native New Zealand forget-me-nots (*Myosotis*, Boraginaceae) comprise a Pleistocene species radiation with very low genetic divergence. *Plant Systematics and Evolution* 301: 1455–1471.

Meudt HM, Prebble JM, Stanley RJ, Thorsen MJ (2013) Morphological and amplified fragment length polymorphism (AFLP) data show that New Zealand endemic *Myosotis petiolata* (Boraginaceae) comprises three rare and threatened species. *Australian Systematic Botany* 26: 210-232.

Moore LB (1961) *Boraginaceae*. In: Allan H, editor. *Flora of New Zealand. Vol. 1*. Wellington, New Zealand: PD Hasselberg, Government Printer. p. 806-833.

Murray BG, de Lange PJ (2013) Contributions to a chromosome atlas of the New Zealand flora—40. Miscellaneous counts for 36 families. *New Zealand Journal of Botany* 51: 31-60.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2015) vegan: Community Ecology Package. R package version 2.3-0. http://CRAN.R-project.org/package=vegan.

Ornduff R (1969) Reproductive biology in relation to systematics. *Taxon* 18: 121-133.

Padial JM, de la Riva I (2007) Taxonomy, the Cinderella of science, hidden by its evolutionary stepsister. *Zootaxa*: 1-2.

Petterson JA (1997) Revision of the genus *Wahlenbergia* (Campanulaceae) in New Zealand. *New Zealand Journal of Botany* 35: 9-54.

Podani J (1999) Extending Gower's general coefficient of similarity to ordinal characters. *Taxon* 48: 331-340.

Pyšek P, Hulme PE, Meyerson LA, Smith GF, Boatwright JS, Crouch NR, Figueiredo E, Foxcroft LC, Jarošik V, Richardson DM, Suda J, Wilson JRU (2013) Hitting the right target: taxonomic challenges for, and of, plant invasions. *AOB Plants* 5: 1-25.

RCoreTeam (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Robertson A (1989) *Evolution and pollination of New Zealand Myosotis (Boraginaceae).* PhD Thesis. Christchurch: University of Canterbury.

Robertson AW, Lloyd DG (1991) Herkogamy, dichogamy and self-pollination in six species of *Myosotis* (Boraginaceae). *Evolutionary Trends in Plants* 5: 53-63.

Simpson G (1952) Notes on some New Zealand plants and descriptions of new species (No. 5). *Transactions and Proceedings of the Royal Society of New Zealand* 79: 426.

Simpson G, Thomson JS (1942) Notes on some New Zealand plants and descriptions of new species (No. 2). *Transactions and Proceedings of the Royal Society of New Zealand* 72: 26.

Simpson G, Thomson JS (1943) Notes on some New Zealand plants and descriptions of new species. *Transactions and Proceedings of the Royal Society of New Zealand* 73: 161.

Skottsberg C (1916) *Botanische Ergebnisse der Schwedischen Expedition nach Patagonien und dem Feuerlande 1907–1909*. Stockholm, Sweden: Almqvist & Wiksells Boktryckeri-A. -B.

Slovák M, Kucera J, Marhold K, Zozomová-Lihová J (2012) The morphological and genetic variation in the polymorphic species *Picris hieracioides* (Compositae, Lactuceae) in Europe strongly contrasts with traditional taxonomical concepts. *Systematic Botany* 37: 258-278.

Stepankova J (1996) Karyological variation in the group of *Myosotis alpestris* (Boraginaceae). *Folio Geobotanica and Phytotaxonomica* 31: 251-262.

Steussy T (2009) *Plant Taxonomy: The Systematic Evaluation of Comparative Data*. New York: Columbia University Press.

Wickelmaier F (2003) An Introduction to MDS https://homepage.uni-tuebingen.de/florian.wickelmaier/pubs/Wickelmaier2003SQRU.pdf (accessed 5.11.15).

Wickham H (2009) ggplot2: elegant graphics for data analysis. Springer New York.

Winkworth R, Grau J, Robertson A, Lockhart P (2002) The origins and evolution of the genus *Myosotis* L. (Boraginaceae). *Molecular Phylogenetics and Evolution* 24: 180-193.

Winkworth RC, Wagstaff SJ, Glenny D (2005) Evolution of the New Zealand mountain flora: Origins, diversification and dispersal. *Organisms Diversity & Evolution* 5: 237-247.

Yeates DK, Seago A, Nelson L, Cameron SL, Joseph L, Trueman JWH (2011) Integrative taxonomy, or iterative taxonomy? *Systematic Entomology* 36: 209-217.

Zare R, Gams W, Schroers H-J (2004) The type species of *Verticillium* is not congeneric with the plant-pathogenic species placed in *Verticillium* and it is not the anamorph of *'Nectria' inventa*. *Mycological Resources* 108: 675-582.

Zuloaga F, Morrone O, Belgrano M, Marticorena C, Marchesi E (2008) Catálogo de las plantas vasculares del Cono Sur (Argentina, southern Brazil, Chile, Paraguay y Uruguay). *Monographs in Systematic Botany from the Missouri Botanical Garden* 3 volumes: 1-3486.

Chapter 3 Microsatellite markers for the New Zealand native *Myosotis pygmaea* species group (Boraginaceae) amplify across species

Note the published version of this chapter is included as Appendix 1.

Abstract

Premise of the study: Microsatellite loci were developed as polymorphic markers for the New Zealand native *Myosotis pygmaea* species group (Boraginaceae) for use in species delimitation and population and conservation genetic studies.

Methods and Results: Illumina MiSeq sequencing was performed on genomic DNA from seedlings of *M. drucei*. From trimmed paired-end sequences >400 bp, 484 microsatellite loci were identified. Twelve of 48 microsatellite loci tested were found to be polymorphic and consistently scorable when screened on 53 individuals from four populations representing the geographic range of *M. drucei*. They also amplify in all other species in the *M. pygmaea* species group, i.e., *M. antarctica*, *M. brevis*, *M. glauca*, and *M. pygmaea,* as well as 18 other *Myosotis* species.

Conclusions: These 12 polymorphic microsatellite markers establish an important resource for research and conservation of the *M. pygmaea* species group and potentially other Southern Hemisphere *Myosotis*.

Introduction

Forget-me-nots (*Myosotis* L., Boraginaceae) are found in both the Northern and Southern Hemispheres, with a center of diversity in New Zealand. The *M. pygmaea* species group (Meudt et al., 2015) comprises *M. antarctica* Hook.f., *M. brevis* de Lange & Barkla, *M. drucei* (L.B.Moore) de Lange & Barkla, *M. glauca* (G.Simpson & J.S.Thomson) de Lange & Barkla, and *M. pygmaea* Colenso, all native to New Zealand. Questions persist regarding the delimitation of these morphologically similar species (de Lange et al., 2010), four of which appear on the New Zealand threatened species list (de Lange et al., 2013). Indeed, of the 44 endemic New Zealand *Myosotis* taxa, 32 are considered threatened or at risk (de Lange et al., 2013). A priority in the conservation management of members of this genus is to both accurately delimit species and understand the levels and structure of genetic diversity present. Low genetic diversity in New Zealand *Myosotis*, as evidenced by previous studies (Meudt et al., 2013; Meudt et al., 2015), suggests that additional molecular markers are needed.

Here the development of 12 polymorphic microsatellite markers for the *M. pygmaea* species group are reported, which will be used in future studies of species delimitation and population genetic research. Additionally, the utility of these loci in 18 other *Myosotis* species are evaluated.

Methods and Results

Sibling individuals were selected from the type locality of *M. drucei* as the source DNA for marker development (WELT SP100445; Table 3.4). Genomic DNA was extracted from fresh young leaf tissue from 15 seedlings using a modified cetyltrimethylammonium bromide (CTAB) method (Shepherd and McLay 2011). To generate sufficient template for the requirements of Illumina MiSeq library preparation, extracted DNA was pooled and amplified using a REPLI-g kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. DNA was quantified using a Qubit 2.0 Fluorometer (Thermo-Fisher Scientific, Waltham, Massachusetts, USA), and a genomic library was prepared using the TruSeq Library Preparation Kit (Illumina, San Diego, California, USA) by the Massey Genome Service (Massey University, Palmerston North, New Zealand). The indexed library was pooled with three other libraries in equal concentration and sequenced using the paired-end 250-bp chemistry on a MiSeq (Illumina) by the Massey Genome Service. The resulting 2.7 million sequences were trimmed of low-quality results using a 0.01 quality cut-off in DynamicTrim in SolexaQA (Cox et al., 2010), which yielded 1,449,369 trimmed paired-end

sequences with an average length of 380 bp, ranging in size from 11–492 bp. Paired-end sequences were joined using the program FLASH (Magoc and Salzberg 2011).

The paired-end sequences were then imported into Geneious 6.1.5 (Biomatters, Auckland, New Zealand), where only sequences >400 bp were retained. Organellar sequences were removed by performing a local BLAST search of the *M. drucei* sequences against the phylogenetically closest relatives (Soltis et al., 2011) with the most complete mitochondrial and chloroplast sequences from GenBank. The chloroplast genomes used were: *Nicotiana undulata* Ruiz & Pav. NC_016068 (Solanaceae), *Olea europaea* L. subsp. *maroccana* (Greuter & Burdet) P. Vargas, J. Hess, Muñoz Garm. & Kadereit NC_015623 (Oleaceae), *Coffea arabica* L. NC_008535 (Rubiaceae), and *Arabidopsis thaliana* (L.) Heynh. NC_000932 (Brassicaceae). The mitochondrial genomes used were: *N. tabacum* L. NC_006581, *A. thaliana* NC_001284, and *Vigna radiata* (L.) R. Wilczek NC_015121 (Fabaceae). The remaining 397,224 sequences were split into four groups (due to computer memory constraints), and the first group of 99,999 sequences was searched for perfect di- to hexanucleotide microsatellite repeats with a minimum of seven uninterrupted repeat units using a search tool in Geneious (Phobos plugin; Mayer 2010), which identified 484 repeats. Sequences were removed from consideration if the paired-end sequences were found to be overlapping only in the repeat region, if regions near the microsatellite contained other microsatellite loci or single base pair repeats >4 bp, or if there were greater than 14 repeats. After removing unsuitable loci, primers were designed for 147 microsatellite regions using Primer3 within Geneious (Untergasser et al., 2012). The default settings were used except for: product size = 100–400 bp with a 50-bp buffer on both sides of the target region; primer size = 18 bp (minimum)–20 bp (optimal)–22 bp (maximum); melting temperature (T_m) = 47–55–60°C; 3′ GC content = 40–50–60%; maximum T_m difference = 10°C; GC clamp = 1; max poly N = 4. An M13 tag (CACGACGTTGTAAAAC- GAC) was added to the 5′-end of the forward primer for each locus, and a PIG-tail sequence; (GTTTCTT; Brownstein et al., 1996) was added to the 5′-end of each reverse primer.

For reasons of practicality, 48 primer pairs were chosen to trial a range of: uninterrupted number of repeats, types of microsatellites (e.g., di-, tri-, tetra-, penta-, and hexa-), and PCR product sizes. These 48 were initially trialed on seven individuals from five populations of four *M. pygmaea* group species (Table 3.4). Each locus was amplified individually in 10-μL PCR reactions that contained 1 μL of a 1:50 dilution of template DNA (5–50 ng), 0.02 μM forward primer, 0.45 μM reverse primer, 0.45 μM M13 primer (labeled with FAM, NED, or VIC), 1.5 mM $MgCl_2$, 1× buffer BD (Solis BioDyne, Tartu, Estonia), 250 μM of each dNTP,

and 1 unit FIREPol *Taq* polymerase (Solis BioDyne). PCRs were carried out with the following cycling program: an initial denaturation of 95°C for 3 min; 40 cycles of 95°C for 30 s, 53°C for 40 s, and 72°C for 1 min; and a final extension at 72°C for 10 min. A volume of 0.75 μL of each PCR product for three loci, each with a different fluorophore, was added to 9 μL of Hi-Di formamide (Applied Biosystems, Carlsbad, California, USA) premixed with a ROX-labeled CASS ladder (Symonds and Lloyd 2004) for subsequent fragment separation on an ABI 3730 Genetic Analyzer (Applied Biosystems) by the Massey Genome Service.

Alleles were visualized and scored using GeneMapper version 3.7 (Applied Biosystems). Of the 48 primer pairs tested, 25 were polymorphic, two were monomorphic, seven were unscorable, and 14 did not amplify. Twenty-four of the polymorphic loci were further tested using the above PCR conditions on 15 individuals from five *Myosotis* species. The 12 markers (Table 3.1) with the best amplification rates were selected for further investigation using four populations of *M. drucei* to demonstrate the utility of the markers in a population genetic framework. For these four populations, Table 3.2 shows the number of alleles, and observed (H_o) and expected (H_e) heterozygosities, which were determined using GenAlEx (Peakall and Smouse 2012). The average number of observed alleles per locus was 3.75, and average H_o was 0.059 (Table 3.2). H_o was typically lower than H_e, which matches the hypothesized mostly selfing nature of the *M. pygmaea* species group (Robertson and Lloyd 1991; Brandon 2001). The 12 markers amplified well across the other four species (one population each) in the *M. pygmaea* group (voucher information in Table 3.4) and were also trialed in an additional 18 species of *Myosotis*, 14 endemic to New Zealand, one from Australia, and three introduced to New Zealand from Europe. Amplification rates and polymorphism are reported in Table 3.3.

Conclusions

Twelve polymorphic microsatellite loci are described that will be useful for exploring species limits within the *M. pygmaea* species group, as well as determining the population genetic variation within and among other species of Southern Hemisphere *Myosotis*.

Table 3.1 Primer sequences and characteristics of 12 microsatellite loci developed in *Myosotis drucei.*

Locus	Primer sequences (5′–3′)	Fluorescent dye (pooling group)	Repeat motif	Allele size range (bp)[a]	T_a (°C)	GenBank accession no.
MYPY-4	F: TATGCTCGTACCGAAACAC R: AGTGCTTATGTTTGCCCTC	NED 2	$(TGT)_8$	248–255	53	KP861356
MYPY-10	F: GCGACATTGCAACTGATAC R: TACCTCATCGCTCAATACC	VIC 1	$(GAT)_{10}$	312– 45	53	KP861353
MYPY-14	F: AAGAACATTTTGCCACAGC R: TTAAATCATTGCACGTCCG	VIC 2	$(GAA)_7$	211–217	53	KP861350
MYPY-17	F: CCTCTCTCTATATGTCGCG R: GGATTACCTTGAGGCAGTG	VIC 3	$(ATA)_{12}$	273–311	53	KP861357
MYPY-20	F: GTTGAGAGAGCTCTACTGC R: GTACCCAGCATTAACCAGG	FAM 4	$(AT)_9$	228–236	53	KP861359
MYPY-26	F: ACTTGGAGAACGATTTGTCCG R: AACCGCCGCAAAATTCAAAC	NED 3	$(TC)_7$	374–477	53	KP861355
MYPY-28	F: TGACTCTGGACAATGATGAGAGAG R: CGGCTGTTTTAGAACCACCC	VIC 4	$(TA)_9$	341–357	53	KP861352
MYPY-29	F: GGTTCAGTGATAATGTTCGAGCC R: CACAGGAAGGATCAATGACTGC	FAM 2	$(AC)_9$	334–342	53	KP861351
MYPY-36	F: GTTGTGCTTGATGGTGACCC R: CCCATCCTTCTTCTCCACCC	NED 4	$(GAT)_{10}$	259–296	53	KP861360
MYPY-40	F: CTGCCTCATTATTCTCTGGG R: CACGACCATTCCATGTTAAC	FAM 1	$(AG)_7$	261	53	KP861358
MYPY-41	F: CTTCTTGACGCTTTTGCTAC R: TTCAGAATAGCAATTGTCGC	NED 1	$(TG)_8$	269–271	53	KP861354
MYPY-48	F: ATTCGACGTAGATCTTGTGC R: AAAGAAAACTGCAGAACGTG	FAM 3	$(GATGAA)_7$	251–275	53	KP861349

[a] Fragment size range based on 53 *Myosotis drucei* samples from four populations: WELT SP091599, WELT SP100445, WELT SP100440 and WELT SP100428, voucher information in Table 3.4.

Table 3.2 Summary statistics of microsatellite polymorphism determined by screening 53 *Myosotis drucei* samples from four populations; three from the South Island and one from the North Island of New Zealand[1].

	South Island									North Island			Total
	Coronet Peak *N*=13			Tapuae-o-Uenuku *N*=14			Mt Altimarlock *N*=11			Ruahine Ranges *N*=15			N=53
Locus	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A_T
MYPY-4	2	0.077	0.204	2	0.000	0.375	1	0.000	0.000	1	0.000	0.000	2
MYPY-10	3	0.000	0.462	3	0.000	0.500	2	0.091	0.351	1	0.000	0.000	7
MYPY-14	1	0.000	0.000	2	0.000	0.408	1	0.000	0.000	2	0.000	0.391	3
MYPY-17	2	0.077	0.074	1	0.000	0.000	1	0.000	0.000	1	0.000	0.000	4
MYPY-20	2	0.000	0.153	2	0.000	0.408	3	0.100	0.515	1	0.000	0.000	4
MYPY-26	2	0.000	0.142	2	0.000	0.408	1	0.000	0.000	3	0.000	0.561	5
MYPY-28	2	0.000	0.500	2	0.000	0.355	2	0.091	0.087	1	0.000	0.000	4
MYPY-29	2	0.000	0.165	3	0.667	0.667	2	1.000	0.500	2	0.600	0.420	4
MYPY-36	3	0.077	0.210	2	0.000	0.408	1	0.000	0.000	1	0.000	0.000	4
MYPY-40	2	0.000	0.165	1	0.000	0.000	1	0.000	0.000	1	0.000	0.000	2
MYPY-41	1	0.000	0.000	2	0.000	0.142	1	0.000	0.000	1	0.000	0.000	2
MYPY-48	2	0.000	0.473	2	0.000	0.408	1	0.000	0.000	2	0.000	0.337	4

Note: A = number of alleles; H_o = observed heterozygosity; H_e = expected heterozygosity; N = sample size for each population; A_T = total number of alleles.

[1.] South Island: Coronet Peak = WELT SP091599, Tapuae-o-Uenuku = WELT SP100440, Mt Altimarlock = WELT SP100428. North Island: Ruahine Ranges = WELT SP100445. See Table 3.4 for voucher information.

Table 3.3 Cross-amplification of 12 novel microsatellite loci in 22 *Myosotis* species [a].

Species name	Voucher number [b]	*N*	Location [c]	MYPY-4	MYPY-10	MYPY-14	MYPY-17	MYPY-20	MYPY-26	MYPY-28	MYPY-29	MYPY-36	MYPY-40	MYPY-41	MYPY-48
***Myosotis pygmaea* species group**															
M. antarctica	SP102775	12	CI	2	1	2	1	2	1	2	1	1	1	1	1
M. brevis	SP090361	25	NZ	1	1	1	1	2	2	1	1	1	1	1	1
M. glauca	SP093284	17	NZ	1	1	1	1	1	1	2	1	1	2	1	1
M. pygmaea	SP090540	13	NZ	1	1	1	1	1	1	2	1	1	1	1	1
Other New Zealand *Myosotis*															
M. arnoldii	SP100473	3	NZ	6	8	5	6	1	2	+	3	2	3	-	4
	SP100439	3													
M. cheesemanii	SP092210	1	NZ	+	+	+	+	-	-	-	+	+	+	-	-
M. colensoi	SP092419	1	NZ	+	-	-	+	-	-	-	+	+	+	-	-
M. forsteri	SP089691	1	NZ	2	1	2	2	-	2	-	2	3	1	1	1
	SP089928	1													
	SP092179	1													
M. glabrescens	SP089801	1	NZ	+	+	2	+	-	-	-	+	+	+	-	-
M. macrantha	SP100468	3	NZ	3	7	4	4	2	1	2	3	4	2	3	3
	SP100494	3													
M. pansa* subsp. *pansa	SP089670	2	NZ	2	1	2	2	-	-	1	1	-	1		-
	SP089674	1													
M. pansa* subsp. *praeceps	SP089685	2	NZ	2	1	3	-	-	-	-	1	-	2	-	-
	SP089686	1													
M. petiolata	SP089853	3	NZ	2	1	2	2	-	-	-	1	-	1	1	-
M. pottsiana	SP089687	2	NZ	1	2	1	2	-	1	1	1	-	2	1	-
	SP089689	1													
M. pulvinaris	SP092196	1	NZ	-	2	+	+	-	-	+	2	+	+	+	+

Species name	Voucher number [b]	*N*	Location [c]	MYPY-4	MYPY-10	MYPY-14	MYPY-17	MYPY-20	MYPY-26	MYPY-28	MYPY-29	MYPY-36	MYPY-40	MYPY-41	MYPY-48
***M.* "small white"**	SP090247 SP090251	1 1	NZ	2	1	1	2	-	1	-	1	3	1	1	-
M. spathulata	SP090628 SP092757	2 1	NZ	2	1	1	1	-	-	1	1	-	2	1	-
M. tenericaulis	SP092404	1	NZ	2	-	+	+	-	-	-	+	-	+	-	-
Other *Myosotis*															
M. arvensis	SP094173	1	Euro	-	-	+	+	-	-	-	-	-	-	-	-
M. australis	MPN44757	2	Aust	1	-	1	2	-	-	-	-	1	2	-	-
M. discolor	SP089930	1	Euro	-	-	-	+	-	-	+	+	-	+	-	-
M. laxa	SP090206	1	Euro	-	-	+	+	-	-	-	-	-	-	-	-

Note: *N* = number of individuals trialed from each population.

[a] Number of amplified alleles are indicated, + = amplified with unknown levels of polymorphism as only one allele in one individual amplified, — = no amplification.

[b] See Table 3.4 for voucher information.

[c] Aust = Australian native; CI = Campbell Island native; Euro = European native growing in New Zealand; NZ = New Zealand endemic.

Table 3.4 Voucher and location information for all *Myosotis* populations used in this study. One voucher was collected for each population used; all vouchers are deposited in WELT or MPN. An * indicates the five populations the makers were initially trialed on.

Species	Location	Voucher
***Myosotis pygmaea* species group**		
***Myosotis antarctica* Hook.f.**	New Zealand, Campbell Island, cliffs near Menhir	WELT SP102775
***Myosotis brevis* de Lange & Barkla**	New Zealand, Coastal Taranaki, Puketapu Rd end *	WELT SP090361
***Myosotis brevis* de Lange & Barkla**	New Zealand, Coastal Taranaki, Stent Rd	WELT SP090543
***Myosotis drucei* (L.B.Moore) de Lange & Barkla**	New Zealand, North Island, Ruahine Ranges, near Mt Maungamahue*	WELT SP100445
***Myosotis drucei* (L.B.Moore) de Lange & Barkla**	New Zealand, South Island, Marlborough, Tapuae-o-Uenuku	WELT SP100440
***Myosotis drucei* (L.B.Moore) de Lange & Barkla**	New Zealand, South Island, Central Otago, Coronet Peak	WELT SP091599
***Myosotis drucei* (L.B.Moore) de Lange & Barkla**	New Zealand, South Island, Marlborough, Mt Altimarlock *	WELT SP100428
***Myosotis glauca* (G.Simpson & J.S.Thomson) de Lange & Barkla**	New Zealand, South Island, Central Otago, Nevis Valley *	WELT SP093284
***Myosotis pygmaea* Colenso**	New Zealand, North Island, Coastal Taranaki, Opunake treatment ponds	WELT SP090540
***Myosotis pygmaea* Colenso**	New Zealand, South Island, Northwest Nelson, near Sandhill Creek river mouth *	WELT SP100460
Other New Zealand *Myosotis*		
***Myosotis arnoldii* L.B.Moore**	New Zealand, South Island, Marlborough, Mt Benmore	WELT SP100439
***Myosotis arnoldii* L.B.Moore**	New Zealand, South Island, Northwest Nelson, Hoary Head	WELT SP100473
***Myosotis cheesemanii* Petrie**	New Zealand, South Island, Central Otago, Pisa Range	WELT SP092210
***Myosotis colensoi* (Kirk) J.F.Macbr.**	New Zealand, cultivated, Origin: South Island, Canterbury, Castle Hill	WELT SP092419
***Myosotis forsteri* Lehm.**	New Zealand, North Island, Kaweka Ranges	WELT SP089928
***Myosotis forsteri* Lehm.**	New Zealand, North Island, Raukumara, Waioeka Conservation Area	WELT SP089691
***Myosotis forsteri* Lehm.**	New Zealand, South Island, Northwest Nelson, Kahurangi National Park	WELT SP092179
***Myosotis glabrescens* L.B.Moore**	New Zealand, South Island, Central Otago, Hector Mountains	WELT SP089801
***Myosotis macrantha* (Hook.f.) Benth. & Hook.f.**	New Zealand, South Island, Central Otago, Queenstown, Moke Creek	WELT SP100494

Species	Location	Voucher
***Myosotis macrantha* (Hook.f.) Benth. & Hook.f.**	New Zealand, South Island, Northwest Nelson, Lake Peel	WELT SP100468
Myosotis pansa* (L.B.Moore) Meudt et al. subsp. *pansa	New Zealand, North Island, Auckland Region, Anawhata stream	WELT SP089670
Myosotis pansa* (L.B.Moore) Meudt et al. subsp. *pansa	New Zealand, North Island, Auckland Region, Pararaha Valley	WELT SP089674
***Myosotis pansa* subsp. *praeceps* Meudt et al.**	New Zealand, North Island, Taranaki, Paraninihi/White Cliffs	WELT SP089686
***Myosotis pansa* subsp. *praeceps* Meudt et al.**	New Zealand, North Island, Waikato, Ngarupupu Point	WELT SP089685
***Myosotis petiolata* Hook.f.**	New Zealand, North Island, Hawkes Bay, Te Waka Range	WELT SP089853
***Myosotis pottsiana* (L.B.Moore) Meudt et al.**	New Zealand, North Island, Bay of Plenty, Ohutu Stream	WELT SP089689
***Myosotis pottsiana* (L.B.Moore) Meudt et al.**	New Zealand, North Island, Bay of Plenty, Waikokopu Stream	WELT SP089687
***Myosotis pulvinaris* Hook.f.**	New Zealand, South Island, Central Otago, Pisa Range	WELT SP092196
***Myosotis* "small white"**	New Zealand, South Island, Northwest Nelson, Kahurangi National Park	WELT SP090251
***Myosotis* "small white"**	New Zealand, South Island, Northwest Nelson, Kahurangi National Park	WELT SP090247
***Myosotis spathulata* G.Forst.**	New Zealand, North Island, Hawkes Bay	WELT SP090628
***Myosotis spathulata* var. *radicata* L.B.Moore**	New Zealand, cultivated, origin Kaweka Ranges, North Island	WELT SP092757
***Myosotis tenericaulis* Petrie**	New Zealand, South Island, Northwest Nelson, Kahurangi National Park	WELT SP092404
***Myosotis uniflora* aff.**	New Zealand, South Island, Central Otago, Pisa Flats	WELT SP089883
Other *Myosotis*		
***Myosotis arvensis* (L.) Hill**	New Zealand, North Island, Wellington, Karori	WELT SP094173
***Myosotis australis* R.Br.**	Australia, New South Wales, Barrington Tops National Park	MPN 44757
Myosotis discolor Pers.	New Zealand, South Island, Central Otago, Ranfurly Holiday Park	WELT SP089930
***Myosotis laxa* Lehm.**	New Zealand, South Island, Canterbury, Arthurs Pass	WELT SP090206

References

Brandon AM (2001) *Breeding systems and rarity in New Zealand Myosotis*. PhD Thesis. Palmerston North: Massey University.

Brownstein MJ, Carpten JD, Smith JR (1996) Modulation of non-templated nucleotide addition by taq DNA polymerase: Primer modifications that facilitate genotyping. *Biotechniques* 20: 1004-1010.

Cox MP, Peterson DA, Biggs PJ (2010) SolexaQA: At-a-glance quality assessment of Illumina second-generation sequencing data. *BMC Bioinformatics* 11: 485.

de Lange P, Heenan P, Norton D, Rolfe J, Sawyer J (2010) *Threatened Plants of New Zealand*. Christchurch: Canterbury University Press.

de Lange PJ, Rolfe JR, Champion PD, Courtney SP, Heenan PB, Barkla JW, Cameron EK, Norton DA, Hitchmough RA (2013) *Conservation Status of New Zealand Indigenous Vascular Plants, 2012*. Wellington: New Zealand Department of Conservation.

Magoc T, Salzberg S (2011) FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27: 2957-2963.

Mayer C. 2010 Phobos Version 3.3.11. http://www.ruhr-uni-bochum .de/spezzoo/cm/cm_phobos.htm [accessed 21 May 2015].

Meudt HM, Prebble JM, Lehnebach CA (2015) Native New Zealand forget-me-nots (*Myosotis*, Boraginaceae) comprise a Pleistocene species radiation with very low genetic divergence. *Plant Systematics and Evolution* 301: 1455–1471.

Meudt HM, Prebble JM, Stanley RJ, Thorsen MJ (2013) Morphological and amplified fragment length polymorphism (AFLP) data show that New Zealand endemic *Myosotis petiolata* (Boraginaceae) comprises three rare and threatened species. *Australian Systematic Botany* 26: 210-232.

Peakall R, Smouse PE (2012) GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28: 2537-2539.

Robertson AW, Lloyd DG (1991) Herkogamy, dichogamy and self-pollination in six species of *Myosotis* (Boraginaceae). *Evolutionary Trends in Plants* 5: 53-63.

Shepherd LD, McLay TGB (2011) Two micro-scale protocols for the isolation of DNA from polysaccharide-rich plant tissue. *Journal of Plant Research* 124: 311-314.

Soltis DE, Smith SA, Cellinese N, Wurdack KJ, Tank DC, Brockington SF, Refulio-Rodriguez NF, Walker JB, Moore MJ, Carlsward BS, Bell CD, Latvis M, Crawley S, Black C, Diouf D, Xi Z, Rushworth CA, Gitzendanner MA, Sytsma KJ, Qiu Y-L, Hilu KW, Davis CC, Sanderson MJ, Beaman RS, Olmstead RG, Judd WS, Donoghue MJ, Soltis PS (2011) Angiosperm phylogeny: 17 genes, 640 taxa. *American Journal of Botany* 98: 704–730.

Symonds VV, Lloyd AM (2004) A simple and inexpensive method for producing fluorescently labelled size standard. *Molecular Ecology Notes* 4: 768-771.

Untergasser A, Cutcutache I, Koressaar T, Ye J, Faircloth BC, Remm M, Rozen SG (2012) Primer3–new capabilities and interfaces. *Nucleic Acids Research* 40.

Chapter 4 How many pygmy forget-me-not species are there? Testing the morphology-based taxonomy of the New Zealand native *Myosotis pygmaea* species group with population genetic data

Abstract

Following the general lineage concept, data from molecular markers were analysed to identify lineages within the New Zealand native *Myosotis pygmaea* species group. Over 500 *Myosotis* individuals were genotyped, and the pattern of low within and high between population genetic variation is congruent with self-fertilization and low levels of seed dispersal. Within the *M. pygmaea* group, several genetic clusters can be identified, some of which match the current taxonomy and morphological variation previously identified (Chapter 2), whereas some other groupings represent geographic clustering. Integrative vs. iterative methods for combining molecular and morphological data are compared, and integrative techniques are shown to have higher power to distinguish clusters. Considering both the genetic and morphological data, there is evidence of three lineages within the *M. pygmaea* species group. These lineages correspond to: 1) *M. brevis*, 2) *M. glauca* and 3) the remainder of the *M. pygmaea* group (i.e. *M. antarctica, M. drucei, M. pygmaea,* and *M.* "Volcanic Plateau"). Within this third lineage, there is no evidence from the molecular data to separate *M. pygmaea* and *M. drucei,* despite one minor morphological character able to distinguish these two entities (Chapter 2). A formal taxonomic revision awaits the future incorporation of ecological niche modelling data.

Introduction

Taxonomy has important implications for conservation, ecology, and biosecurity (Knowlton and Jackson 1994; Zare et al., 2004; Cameron 2010; Pyšek et al., 2013). Modern best practice taxonomy and species delimitation are typically based on the general lineage concept of de Queiroz (2007). In the general lineage concept, species are defined as separately evolving metapopulation lineages. As evolution and speciation are ongoing processes, often it can be difficult to determine species boundaries. Therefore, the general lineage concept emphasises analysing data from many different sources for the purpose of lineage discovery. Evaluating multiple criteria not only increases our ability to detect recently separated lineages, but also can provide stronger support for lineage separation when they are in agreement (e.g. Ornelas-García et al., 2008; Reeves and Richards 2011; Meudt et al., 2013). Older and more diverged species usually can be easily recognised by considering any of the different species concepts and delimitation criteria. However, difficulties arise with relatively young species that are in the early stages of lineage divergence (such as species radiations or complexes) and may not have acquired all of the different properties on which species concepts are based (de Queiroz 2007).

Furthermore, delimiting species in a way that reflects the evolutionary history of the species lineages has important implications for conservation. Although species are the major unit of conservation, there is growing recognition that conserving genetic diversity is important in its own right and essential to the survival and maintenance of species (Moritz 2002). If the taxonomic classification system reflects evolutionary relationships, then conservation management decisions can be made based on this broader expectation to conserve genetic diversity. A cautionary tale is the case of the now extinct dusky seaside sparrow: a breeding program was set up based on an intra-specific taxonomy that did not accurately reflect the phylogeny of the species, which contributed to their extinction (Avise and Nelson 1989).

Under a population genetics framework, species boundaries can be identified using model-based clustering methods for assigning individuals to gene pools according to genotype data; e.g. Structure (Pritchard et al., 2000) and Instruct (Gao et al., 2007). Several studies have used microsatellite data as a tool for species delimitation in recent plant radiations (e.g., Edwards et al., 2009; Kim et al., 2012; Turini et al., 2014). In these studies, the current morphology-based taxonomy was tested using the program Structure. All three studies found that each species included in the structure analyses (up to eight species were included in one study) formed a separate cluster, and given the correlation

between morphological and molecular data this was used as justification for recognising these entities at species rank. The use of multiple data sets in this way uses an iterative taxonomic framework (Yeates et al., 2011) and uses complementarity among disciplines to improve rigor in delimiting lineages, and consequently species. To move from iterative to integrative taxonomy, the data from different methods (e.g. molecular and morphological) must be collected from the same individuals, but this sampling strategy is not always feasible. Programs such as integrated Bayesian phylogenetics and phylogeography (iBPP: Yang and Rannala 2010; Solis-Lemus et al., 2014) have been developed for integrating multi-locus sequence data with morphological data. A methodology for integrating other data types (e.g. genotypic, environmental and morphological) is described in Edwards and Knowles (2013), whereby Gaussian clustering is combined with multivariate approaches. The current study will assess the ability of this approach to find lineages within the New Zealand native *Myosotis pygmaea* group, by integrating molecular and morphological data and comparing the results to an iterative approach.

Myosotis is a genus of roughly 100 species distributed in both the Northern and Southern Hemispheres (Mabberley 2008). There are two centres of diversity, Eurasia and New Zealand, the latter of which is the central point of the Southern Hemisphere radiation (Winkworth et al., 2002; Meudt et al., 2015). Of the more than 40 species native to New Zealand, two-thirds are threatened at some level, and a number of taxonomically indeterminate entities have been identified, making a taxonomic revision of the genus a top priority (de Lange et al., 2009; de Lange et al., 2013).

Low levels of genetic differentiation at neutrally evolving markers (Winkworth et al., 2002; Meudt et al., 2015) suggest New Zealand *Myosotis* species are a recent species radiation and therefore species delimitation via a population genetic approach (e.g., Edwards et al., 2009) may be necessary. Although amplified fragment length polymorphisms (AFLPs) have proven useful for delimiting species within some North Island endemic species of *Myosotis* (i.e., the *M. petiolata* complex, Meudt et al., 2013), they were shown to be less effective at reconstructing the phylogeny of the South Island representatives and of the New Zealand species as a whole (Meudt et al., 2015).

The *Myosotis pygmaea* species group is made up of five native New Zealand *Myosotis* species (Chapter 2; Meudt et al., 2015): *M. antarctica*, *M. brevis*, *M. drucei*, *M. glauca* and *M. pygmaea* (Figure 2.1). All are endemic to New Zealand except *M. antarctica* which is also known from southern Chile (Zuloaga et al., 2008). The *M. pygmaea* species complex is a

subgroup of the bracteate-prostrate group of Southern Hemisphere *Myosotis* which comprises 12 other named species (Robertson 1989; Meudt et al., 2015). Several additional entities thought to be part of the *M. pygmaea* group or the bracteate-prostrate group have been given tag-names (Table 2.1). Based on herkogamy distances of 0 mm, it appears likely that members of the *M. pygmaea* complex typically self-fertilise (Moore 1961; Robertson and Lloyd 1991; Brandon 2001). A combination of small population size (common in the *M. pygmaea* group; see Table 4.4) and self-compatibility can be especially severe for reducing genetic variation (Lande 1995; Cole 2003) and must be taken into account when interpreting population genetics results.

In Chapter 2 of this thesis, an extensive morphological study of the *M. pygmaea* group was undertaken. In that chapter, the morphological characters that define the *M. pygmaea* group were for the first time clearly delimited, the composition (species and tag-named entities) of the *M. pygmaea* group was determined, and morphological clusters within the *M. pygmaea* group were identified. To test and integrate these morphological hypotheses with a molecular dataset, 12 microsatellite markers were developed (Chapter 3). Samples were collected as widely as possible across the geographic ranges of all species currently included in the *M. pygmaea* group, as well as several tag-named entities and representatives of the bracteate-prostrate group and additional New Zealand-based *Myosotis*, and over 500 individuals were genotyped.

The aims of this chapter are to:

1. Determine whether the *M. pygmaea* species group, as delimited in Chapter 2, forms an identifiable sub-group of the bracteate-prostrate group based on microsatellite genetic data.
2. Assess whether discrete genetic clusters within the *M. pygmaea* group occur, and if so whether these correspond to either the current taxonomy, morphological groups identified in Chapter 2, or neither (i.e. an iterative taxonomic approach)
3. Undertake an integrative taxonomic approach to lineage discovery in the *Myosotis pygmaea* group by co-analysing morphological data from Chapter 2 with microsatellite data, thereby assessing the effectiveness of the method of Edwards and Knowles (2013).

Methods

Sampling

Populations were sampled from across the known ranges of all five described species and several tag-named entities that may or may not be part of the *M. pygmaea* species group. Collection locations were identified based on herbarium records from WELT, AK, CHR and OTA, as well as advice from the New Zealand Department of Conservation staff. Samples were collected under permit number CA-31615-OTH, which contains restrictions for collecting threatened plants. An herbarium specimen, and leaves from 1–20 individuals from 58 locations around New Zealand were collected. The aim was to collect more than ten individuals per population as simulations have shown six is the minimum number required to successfully reconstruct clusters of mostly selfing species using cluster-based analyses of population structure (Fogelqvist et al., 2010). Details regarding each population such as their unique population code, location, as well as voucher information are listed in Appendix 2. Details regarding population size and number of individuals per population included in the microsatellite dataset are reported in Table 4.4.

DNA extraction and genotyping

DNA was extracted from silica-dried leaves using one of two modified CTAB methods (Doyle and Doyle 1990). Samples were genotyped using the 12 microsatellite loci described in Chapter 3 following polymerase chain reaction (PCR). The PCR conditions and genotyping specifications are listed in full in Chapter 3; the only difference was that for especially weakly amplifying markers the number of PCR cycles was in some instances increased from 35 to 40. Briefly, PCR products of three loci with differing flourophores were co-loaded for subsequent fragment sizing on an ABI 3730 Genetic Analyzer (Applied Biosystems) by Massey Genome Service at Massey University (Palmerston North, New Zealand). Alleles were visualized and scored using GeneMapper version 3.7 (Applied Biosystems). PCRs were repeated once, or occasionally twice.

Datasets

Eleven microsatellite data partitions were generated to explore questions at different scales. The largest dataset ("*Myosotis*-wide") comprised 586 samples and 12 markers, including 424 newly genotyped *M. pygmaea* group individuals and affiliated tag-named entities, combined with 120 *M. pygmaea* group samples and 42 representatives of additional *Myosotis* species already genotyped as part of the primer trials (Chapter 3). Only *M. pygmaea* group individuals that amplified for at least 9 of the 12 loci were

included (an additional 32 individuals were therefore excluded), whereas amplification rates for additional *Myosotis* species ranged from 1–12 loci (see Table 3.3). The other ten data partitions are subsets of the "*Myosotis*-wide" dataset, based either on morphological clusters (Chapter 2), or groupings identified in Structure analyses; details of the 11 datasets can be found in Table 4.1. The datasets were organised and coded in GenAlEx (Peakall and Smouse 2012), and exported to Structure (Pritchard et al., 2000) and R (RCoreTeam 2015) for analyses. All datasets were analysed using the methods outlined below.

Determining genetic structure and differentiation

Genetic structure was assessed using the program Structure (Pritchard et al., 2000). Default settings, including the admixture ancestry model as recommended by Francois & Durand (2010) and popflag set to 0, were used, with one exception: allele frequencies was set to "correlated" rather than "independent". Setting frequencies to "correlated" has been shown to improve clustering for closely related populations (Falush et al., 2003) and an initial trial yielded better likelihood scores with this setting (data not shown). Each Structure run had 150 000 iterations discarded as burnin, followed by 1 million iterations saved, following Gilbert et al.'s (2012) recommendations for reproducibility in population genetic studies. For each data partition, 10 runs of each K from 1 to n + 2 were run (where n is the number of populations included in each data partition). Structure runs were set up using the StrAuto python script (Chhatre 2012), modified to allow it to run in parallel (K. Emerson, pers. comm. 2015), and ran on ~30 cores on the Institute of Fundamental Sciences, Massey University computer cluster, thus significantly speeding up the analysis time.

Structure assumes that within populations loci will be at Hardy-Weinberg equilibrium, and linkage equilibrium. As the *M. pygmaea* group are most likely selfing (Robertson and Lloyd 1991; Brandon 2001) this assumption is probably not met. Therefore the data were also analysed using the programs Instruct (Gao et al., 2007) and Admixture (Alexander et al., 2009), which do not assume Hardy-Weinberg equilibrium. To run Admixture the dataset was first converted to binary format. Trials using the default settings showed that Instruct and Admixture gave nearly identical results to each other and to Structure (data not shown), and these methods are not discussed further.

Running the StrAuto Python script produces a zipped file ready to upload to Structure Harvester (Earl and vonHoldt 2012). Structure Harvester implements the Evanno method (Evanno et al., 2005), an "ad hoc" yet popular method for determining the optimal K value

for a given dataset. The outputs from Structure Harvester were downloaded and the run with the best likelihood for each value of K of interest was then processed using CLUMPP (Jakobsson and Rosenberg 2007) and DISTRUCT (Rosenberg 2004).

Each data set was also visualised as a NeighborNet network in SplitsTree (Huson 1998; Huson and Bryant 2006) and principal components analyses (PCoA) in R, and the number of clusters was assessed using "mclust" (Fraley and Raftery 2002; Fraley et al., 2012). The distance matrices that both the networks and the PCoAs are based on were calculated in two ways, firstly based on the proportion of shared alleles (POSA; Chakraborty and Jin 1993; Bowcock et al., 1994) using the program MSA (Dieringer and Schlotterer 2003). This calculation does not impute missing data and makes the minimum of assumptions. Secondly, following Ferrão et al., (2014), the dissimilarity matrix of Kosman and Leonard (2005; KL) was calculated in R using the ***dist.codom*** function from the "mmod" package (Winter 2012). This calculation is similar to POSA except the distance is calculated as a proportion of shared alleles at each locus. Distance matrices were calculated both based on individuals and populations. Again missing data is not imputed; the genetic pairwise distance is only calculated over loci for which there are data. PCoA were implemented in R using the ***pcoa*** function from the package "ape" (Paradis et al., 2004). The first three principal components were then used as input for Bayesian model-based clustering using the ***mclust*** function of the "mclust" package (Fraley and Raftery 2002; Fraley et al., 2012). "Mclust" identifies the number of clusters present using Gaussian clustering, and assesses the classification uncertainty of each individual to its assigned cluster. Slightly modifying the "mclust" default settings, 14 models and K of 1 to n+2 populations (rather than the default of 9 populations) were assessed using the Bayesian information criteria (BIC). The distance matrices were exported from R and MSA, converted into NEXUS files, and used to generate NeighborNet networks using SplitsTree (Husan and Bryant 2006).

Coding null alleles

A trial was undertaken using the "pygmy-plus" dataset in which null alleles were identified and coded in order to assess how this would influence the analyses. Software such as MicroChecker (Van Oosterhout et al., 2004) is able to correct raw genotypes and account for the presence of non-amplifying alleles based on deviations from Hardy-Weinberg equilibria. Given the deviations from Hardy-Weinberg observed in this dataset, an alternative method was chosen. Alleles were determined to be "null" if a population was missing data for more than 90% of individuals at a certain locus. Each null allele was then

coded with a unique allele number, as a homozygote. Coding null alleles was determined to not be useful for this dataset and was not implemented; details are given in the results.

Assessing population genetic variation

The genetic diversity of each population with more than five individuals was evaluated across all loci using the observed number of alleles (N_A), the effective number of alleles (N_E), expected heterozygosity (H_E), observed heterozygosity (H_O), observed heterozygosity of an individual relative to the expected heterozygosity of individuals in the population (F_{IS}), the percentage of polymorphic loci (%P) and the number of private alleles, all calculated in GenAlEx 6 (Peakall and Smouse 2006; Peakall and Smouse 2012). Each individual microsatellite locus was also assessed for the total number of alleles (A), the observed and expected heterozygosity (H_O and H_E) and observed heterozygosity of an individual relative to the expected heterozygosity of individuals across all populations (F_{IT}), and expected heterozygosity of individuals within a subpopulation relative to the total expected heterozygosity of individuals across all populations (F_{ST}). GenAlEx was also used to perform analysis of molecular variation (AMOVA; Excoffier et al., 1992). The significance of variance components was tested using 999 non-parametric permutations. Several AMOVA were run, including: 1) Comparing among all populations of the *M. pygmaea* group for which n > 5; 2) Comparing among all morpho-species of the *M. pygmaea* group as identified in Chapter 2; and 3) Comparing among individuals scored as the "*M. pygmaea* group", "other bracteate-prostrate", "ebracteate-erect" and "European". Mantel tests were undertaken at both the population and morphological species levels to test for isolation by distance using **mantel.rtest** function in the "adegenet" R package (Jombart 2008; Jombart and Ahmed 2011).

Integrating microsatellite and morphological data

True data integration can only be achieved when molecular and morphological data are collected from the same individual. In this thesis there are 31 *M. pygmaea* group populations for which both molecular and herbarium morphological data were collected; these are indicated in Appendix 2. For each population included in the molecular dataset, the morphological data were collected from one representative individual. Following Edwards and Knowles (2013), the first three dimensions of non-metric multidimensional scaling (nMDS) points calculated from distance matrices of the morphological (n = 31) and molecular datasets (31 populations; n = 326) were concatenated, and the number of clusters in the data was explored using "mclust" as described above (Fraley and Raftery 2002; Fraley et al., 2012). The nMDS were calculated in R using the ***metaMDS*** function in

the "vegan" package (Oksanen et al., 2015) as described in Chapter 2. The non-concatenated datasets were also analysed using "mclust" in order to determine the effect of concatenation. Additionally, following the methods outlined in Chapter 2, box plots, significance tests and stacked bar charts were generated to assess if there were any morphological characters that co-vary with the clusters identified at K = 3 of the 497-individual ("pygmy-only") microsatellite structure analyses; this was done using both the herbarium (n = 31) and growth room (n = 37) morphological datasets.

Results

Delimiting the *Myosotis pygmaea* group

There is a difference in microsatellite amplification rates between *Myosotis* species from the *M. pygmaea* group (92%; n = 532), other bracteate-prostrate species (85%, n = 55), ebracteate-erect *Myosotis* (56%, n = 31) and introduced European *Myosotis* (22%, n = 3) (Table 4.2). However, this may relate to the age of the extracted DNA at the time of genotyping, as older samples (extracted DNA stored -20°C for ~ 2 years) of bracteate-prostrate *Myosotis* had an average of 54% vs. 90% amplification for recent DNA extractions (DNA extracted within ~4 months of genotyping). The AMOVA and PCoA of the "*Myosotis*-wide" dataset should be interpreted with caution, as the low amplification rates of non-*M. pygmaea* group samples contribute a large amount of missing data (Table 4.2). An AMOVA shows these four *Myosotis* groupings are moderately differentiated (Hartl and Clark 1997) with an FST value of 0.15 (P = 0.001; 999 permutations). Given the low rates of amplification for the "ebracteate-erect" and "European *Myosotis*", a "bracteate-prostrate" dataset excluding those samples was generated. The PCoA of the "bracteate-prostrate" dataset by populations based on the KL distance matrix analysed with "mclust" recovered five groups in the data (Figure 4.1). One cluster contains almost all of the non-*M. pygmaea* group samples, including all of the bracteate-prostrate samples genotyped from older DNA extractions (i.e., those with more missing data), two populations of unclear affiliation extracted from new DNA represented by multiple samples (*M. lyallii* LY, *M. glauca?* CL), three populations of *M. pygmaea* group species represented by only one sample (RM, P1 & SI), and one population of the *M. pygmaea* group represented by multiple samples (HB). The remaining four groups are made up of *M. pygmaea* group samples and samples from three populations of tag-named entities that were not considered part of the *M. pygmaea* group based on morphological data (*M.* "Tapuae-o-Uenuku", *M.* "Rock and Pillar" and *M.* aff. *tenericaulis*). The networks of the pygmy-plus dataset show these three tag-named populations do not group with each other; instead

they are interspersed amongst the *M. pygmaea* group (Appendix 4). The deltaK plot of the Structure analyses of the pygmy-plus dataset had a small peak at k=4 (data not shown).

Lineages within the *M. pygmaea* group

When considering the "pygmy-only" dataset, no clusters based solely on traditional morphology, nor the morphological clusters identified in Chapter 2, are recovered in any of the analyses (Figures 4.2–4.4). However, three main clusters are evident: 1) "brevis-plus", 2) "pygmaea-reduced", and 3) "drucei-plus". In the Structure analyses of the optimal K = 3 (Evanno et al., 2005, see Appendix 5 for ΔK graph), the "brevis-plus" cluster comes the closest to forming a group based on traditional taxonomy and morphological similarity (Figures 4.2, 4.3), comprising all ten sampled populations of *M. brevis* plus additional populations identified as *M. pygmaea* (Hawke's Bay, H1–3), *M. drucei* (Lake Tennyson, LT), and *M. antarctica* (Campbell Island, HB, HW). Network and PCoA analyses also recover the "brevis-plus" grouping (Figure 4.4).

The "pygmaea-reduced" cluster comprises five geographically proximate *M. pygmaea* populations from coastal north-western South Island (PR, SC) and coastal Taranaki, North Island (MN, AN, OK) and is the most obviously differentiated group in the network and PCoA analyses (Figure 4.4). The "drucei-plus" cluster comprises the remaining samples; a mixture of individuals of *M. antarctica*, *M. drucei*. *M. glauca*, *M. pygmaea*, *M.* "intermedia", *M.* "Volcanic Plateau" and *M.* aff. *glauca*.

At K = 24 (which had the second highest peak in the ΔK graph, see Appendix 5) several populations group along geographic or taxonomic lines (Figure 4.3). For example two groups of geographically proximate *M. brevis* populations (North Island: TI, TO, NG and South Island: LL, BA, partial BE), three *M.* "Volcanic Plateau" populations (CP, T1, T2), two groups of geographically proximate *M. drucei* (central Otago: CO, C1, C2 and Mt Peel, Kahurangi National Park: L1&2), and the four *M. glauca* populations (M1, M2, N1, N2). The four *M. glauca* populations form their own cluster in all Structure runs of K values from 10 and higher, which is congruent with network results (Figures 4.3, 4.4).

Several populations each appear to comprise two different gene pools for most values of K; *M. antarctica* (HW), *M. brevis* (BE), *M. drucei* (TP and R2), and *M. pygmaea* (HH and OP) (Figure 4.3). Individuals from these populations also appear in different places in the networks (Figure 4.4).

Additional Structure runs undertaken after splitting the dataset into the clusters identified by Structure at K = 3 ("brevis-plus" n = 177, "pygmaea–reduced" n = 53, and "drucei-plus" n = 267), and by splitting the dataset into the clusters identified as morpho-clusters in Chapter 2, generally had peaks in the ΔK plots (following the Evanno method) at k = 2, and then smaller secondary peaks once the value of K came close to the number of populations included in each analyses (Table 4.1; Appendices 6-7).

A Mantel test of the "pygmy-only" 497-individual dataset by population vs. geographic location showed a low level of correlation that was not significant, indicating that isolation by distance is not the most significant pattern in the dataset. Conversely, Mantel tests of each of the four morphological clusters from Chapter 2 show higher correlation with mostly significant p-values (*M. brevis* 0.25, P = 0.03; *M. glauca* 0.5, p = 0.07 (not significant, possibly due to the small number of populations sampled); *M. pygmaea* 0.46, p = 0.005; *M. drucei* + *M. antarctica* 0.19, p = 0.02).

Comparison of two genetic distance calculations

The two distance matrix calculations trialled, POSA and KL, yielded similar but not identical results (compare Figure 4.4 with Appendix 5). The main relationships recovered by both were: 1. five *M. pygmaea* populations ("pygmaea–reduced" PR, SC, MN, AN, OK), 2. slight variations on the "brevis-plus" grouping, and, 3. all *M. glauca* s.s. populations. The main differences between the two networks were in the relationships among populations within the remainder of the samples – i.e. the "drucei-plus" group of the Structure analyses. Another difference between the networks is the greater degree of reticulation visible in the KL network, for example the "brevis-plus" grouping was separated by a larger split in the POSA network. In both networks, *M.* aff. *glauca* and *M. glauca?* were grouped with the "brevis-plus" cluster, which was not found in the Structure analyses of the "pygmy-only" dataset (Figure 4.3), but was found in the Structure analyses of the "pygmy-plus" dataset (Appendix 4).

Coding null alleles

Nine null alleles were identified and coded, six from marker MYPY-20, and one each from markers MYPY-4, 40 and 41. The populations for which null alleles were coded were: *M. brevis* CH and SP, *M. drucei* L1 and LT, *M. glauca* N2, *M.* "Volcanic Plateau" M1 and CP, *M. lyallii* LY and *M*. aff *tenericaulis* TE. There were only very slight differences between the POSA NeighborNet network reconstructed with the dataset with null alleles coded compared to that without null alleles coded. For example one individual of population CP

that clustered with OP, WA and TB without the null alleles coded, clustered with the rest of the individuals from CP once the allele had been coded. This one individual was missing 3 data points, so that most likely influenced its relationships in the first instance. The other changes were that populations with a null allele coded were shown as being more different to other populations once they had an additional data point uniting them, but relationships between populations were not altered (data not shown). Given the theoretical difficulties of coding null alleles, and the little benefit shown to this dataset, null alleles were not coded for the rest of the analyses.

Genetic variation

Across all populations included in the "pygmy-plus" dataset, a total of 138 alleles were observed with a mean of 11.5 alleles per locus. The number of alleles per locus ranged from five to 19 (Table 4.3). Two loci had higher than expected heterozygosity (MYPY-29 & MYPY-36), which corresponded to a negative F_{IS}.

In total, 47 private alleles were observed, representing 34% of the total alleles, with an average of 0.062 private alleles per population (Table 4.4). The four populations with the most private alleles were: *M.* "Tapuae-o-Uenuku" TU (4), *M. brevis* BE (4) and CH (3), and *M.* "Volcanic Plateau" T1&T2 (3); whereas 21 populations (42%) had no private alleles. The within-population mean number of alleles per locus (N_A) ranged from 1.0 (6 populations monomorphic at all loci) to 2.7 (average 1.4), but the number of effective alleles per locus (N_E) was lower (range 1.0–1.9, average 1.2)(Table 4.4). Polymorphic loci per population ranged from one to nine (10–92%), with an average of 30%. There is a trend of populations with smaller sample sizes having a lower number of polymorphic loci (Table 4.4). Observed heterozygosity (H_O) ranged from 0 (20 populations) to 0.45 (*M. pygmaea* H1–3) (average 0.06)(Table 4.4). The range of expected heterozygosity (H_E) was 0.00 (at 7 populations) to 0.44 (*M. brevis* BE) (average 0.11)(Table 4.4).

Integrating morphological and microsatellite data

The integrated "mclust" analyses of the 31 *M. pygmaea* group populations for which both molecular and morphological data were available recovered three clusters; one corresponding to all populations identified as *M. brevis*, a second to the *M. pygmaea* populations from coastal Taranaki and coastal north-western South Island, and the third making up the remainder of the samples (Figure 4.5A). When these morphological and molecular datasets were analysed separately only a single cluster was identified in each case (Figure 4.5B).

No morphological characters were found to differentiate the "pygmaea-reduced" group from the "drucei-plus" or the "brevis-plus" clusters in either the growth room or herbarium morphological datasets. A number of morphological characters were found to differentiate the "brevis-plus" cluster based on analyses of the growth-room morphological data (from Chapter 2), but this is most likely because the only two populations in the growth room data that represented this cluster were *M. brevis* populations (LL and ST), so the characters that united these populations were the same as those that unite the *M. brevis* morphological group (see Chapter 2). In contrast, the herbarium dataset (from Chapter 2) contained several non *M. brevis* populations that had been included in the microsatellite "brevis-plus" cluster, including *M. pygmaea* (H2), *M. drucei* (LT) and *M. antarctica* (HB and HW). There were no morphological characters found to significantly differentiate the "brevis-plus" cluster based on the herbarium morphological dataset.

Discussion

Testing species concepts with multiple lines of evidence is a powerful tool that enables lineage discovery and species delimitation based on evolutionary relationships, even in recent and ongoing species radiations (de Queiroz 2007; Yeates et al., 2011). In the current study, this has been achieved for the New Zealand native *Myosotis pygmaea* group and affiliated species by genotyping over 500 individuals at 12 microsatellite loci to test the morphological delimitation of the *M. pygmaea* group and lineages within it. Whereas in some cases correlation between molecular and morphological variation supports the current taxonomy (Moore 1961), in other cases entities are unable to be distinguished. There are some incongruences between the morphological and genetic markers, and these are hypothesized to relate to either incomplete lineage sorting, local adaptation, and/or hybridisation (Habel et al., 2015).

Iterative vs. integrative taxonomy

Modern best-practice taxonomy is undertaken in an integrative way; but it has been pointed out that the term "iterative taxonomy" perhaps better explains the process usually undertaken, whereby multiple datasets are analysed and interpreted in light of one another (Yeates et al., 2011). Integrative taxonomy, whereby multiple datasets are co-analysed, requires data to be collected from the same individuals, and also required advances in analysis techniques and software before it will be routinely implemented (Edwards and Knowles 2013). In this study, two datasets, morphological and molecular, are analysed following both iterative and integrative methods. Integration was

accomplished following the method outlined in Edwards and Knowles (2013), which is a flexible methodology allowing the concatenation of any types of data. Briefly, distance matrices of datasets with matching individuals are generated, these distance matrices are transformed via multidimensional scaling, and the outputs of different datasets are concatenated before Gaussian clustering is used to determine the number of distinct groups present. Both iterative and integrative methods yielded similar results in this study. However, the additional power of the integrated approach is highlighted, given similar results were found even with a much smaller dataset (31 populations and 326 individuals vs. 54 populations and 497 individuals in the molecular dataset, and 103 individuals in the morphological dataset). It is revealing that when the reduced molecular and morphological datasets are analysed separately, no clusters can be identified in either dataset, but when they are combined, three clusters that have biological relevance are identified (Figure 4.5). This approach therefore appears to be a useful method of integration, and particularly so when other constraints may limit researchers' ability to sample widely, for example when studying rare or threatened species such as the New Zealand *Myosotis* species studied here.

Delimiting the *M. pygmaea* group

Evidence from the microsatellite data corroborates the morphological delimitation of the *M. pygmaea* group from Chapter 2. Firstly, individuals identified as belonging to the *M. pygmaea* group based on morphology have a higher amplification rate of the nuclear microsatellites than other New Zealand *Myosotis* (Table 4.2). The decreasing amplification rate indicates that the primer binding sites are not conserved across less closely related species due to mutations (Selkoe and Toonen 2006). Secondly, most non-*M. pygmaea* group individuals can be excluded from the *M. pygmaea* group in the PCoA and "mclust" analyses (Figure 4.1). Although *M.* "Rock & Pillar", *M.* "Tapuae-o-Uenuku" and *M.* aff. *tenericaulis* are both morphologically (Chapter 2) and genetically (Figure 4.1, appendix 2) similar to the *M. pygmaea* group, comparisons with their geographically closest *M. pygmaea* group populations finds evidence of separate gene pools, even in cases of sympatry (e.g. *M.* "Tapuae-o-Uenuku TU vs. *M. drucei* TP Appendix 4). Although this is good evidence they are separate species from the taxa they were growing in sympatry with, it does not necessarily shed any light on their inclusion or otherwise in the *M. pygmaea* group. As only one population for each of these tag-named entities was included in the molecular analyses, it is not possible to conclude decisively whether they are most closely related to the *M. pygmaea* group, or to other bracteate-prostrate species, without further studies. Therefore the morphological definition of the *M. pygmaea* group (which

excludes these three entities based on flower size and other characters, see Chapter 2) still stands as the best evidence to define the pygmy forget-me-not group. In contrast, other tag-named entities (e.g. *M.* "Volcanic Plateau" and *M.* "intermedia") for which multiple populations were included in the genetic analyses, are unambiguously shown to be part of the *M. pygmaea* group based on both morphological and genetic data.

Comments on the *M. pygmaea* group as a whole

The low amount of heterozygosity and allelic-variation within populations provides the first genetic evidence that all *M. pygmaea* group species probably regularly self-fertilise (Nybom 2004). Additionally the large amount of between-population differentiation suggests low levels of gene flow and low levels of seed dispersal (Hamrick and Godt 1996). This leads to difficulties in reconstructing genetic lineages within the group, and particularly makes analysing microsatellite data in a taxonomic or systematics context difficult as their high mutation rate combined with low levels of gene flow can introduce homoplasy into the dataset (Selkoe and Toonen 2006). Nevertheless, some signal can be seen in the clustering of *M. brevis* and *M. glauca,* respectively, across their geographic ranges (Figures 4.3 & 4.4).

The pattern of within population homogeneity is so extreme that individuals from a single population are found in different clusters in only a handful of cases e.g. populations BE, HW, TP, HH and OP (Figures 4.3 & 4.4). Similarly, observed heterozygosity was usually much lower than expected, and heterozygosity was higher than expected only in a few populations (H1–3, BP, LT, M3, MR and N2; Table 4.4). In almost all cases, these populations are growing in sympatry, or at least nearby, to another *Myosotis* species, suggesting the molecular variation found may be due to gene flow via hybridization. In the cases of higher than expected heterozygosity, this could potentially be explained by the phenomenon termed "isolation-breaking", whereby previously isolated populations mix, or because heterozygotes are being favoured by selection (Hartl and Clark 1997). Alternatively it could be an artefact of the genotyping process; sequencing the microsatellites would be one way to assess whether the heterozygosities are homologous (e.g., Germain-Aubrey et al., 2016).

Interpreting the "pygmy-only" network in light of geographic location shows increasing complexity of relationships from north to south (Figure 4.4). Interestingly, a similar pattern was revealed when the *M. petiolata* complex was studied using AFLPs, in that those individuals from the North Island were more able to be easily distinguished, whereas the relationships between the South Island individuals was less clear (Meudt et

al., 2013), and the same pattern was noted when attempting to reconstruct the New Zealand *Myosotis* phylogeny (Meudt et al., 2015). In those earlier studies, the pattern may have been an artefact of sparser sampling across the South Island vs. the North Island (Meudt et al., 2013). However, the same cannot be said for this study, in which the majority of samples were collected from central Otago, which is where the largest number of *M. pygmaea* group species and populations are found.

More complicated patterns of genetic relationships in the South Island can be related to large-scale patterns of speciation and endemism in the New Zealand flora. Regions with high endemism have been identified in both the North and South Islands (McGlone et al., 2001), but recently it has been shown that the North Island endemics are on average older, more diverged species whereas the South Island endemics are more likely to be younger species (P. Heenan, Landcare Research, unpubl. data). Species radiations and complexes still evolving species boundaries are more likely to exhibit incomplete lineage sorting and hybridisation, hence leading to more complicated population genetic patterns (de Queiroz 2007).

Lineages within the *M. pygmaea* group

Of the four clusters within the *M. pygmaea* group identified using morphological data (Chapter 2), only *M. brevis* and *M. glauca* are also supported by the microsatellite data (Figure 4.3 & 4.4). The other two morphological clusters, *M. pygmaea* and *M. drucei* + *M. antarctica,* cannot be separated from each other using molecular data. Furthermore the populations of *M. glauca* only group above K = 10, by which point subdivisions within *M. brevis* are apparent (Figure 4.3). The results of the Structure analyses as regards species limits are therefore less clear in this scenario than has been found previously when analysing species limits using microsatellite data (e.g., Edwards et al., 2009; Kim et al., 2012; Turini et al., 2014). This is most likely due to the confounding effects of highly differentiated populations within the *M. pygmaea* group, a result of self-fertilization coupled with low levels of dispersal, as discussed above. The molecular support for or against each of the four morphological clusters is discussed in more detail below, in order of most supported to least supported based on the genetic and integrated data analyses.

M. brevis morphological cluster

The integrated dataset recovers all ten sampled populations of *M. brevis* from the North and South Islands as a cluster (Figure 4.5). These ten populations also cluster together when considering just the genetic data, although they are joined by populations of *M.*

drucei (LT), *M. pygmaea* (HB1–3), and *M. antarctica* (HB and HW) (Figures 4.3 & 4.4). The voucher specimen of *M. drucei* LT was placed ambiguously in the morphological nMDS analyses (Chapter 2) as being intermediate between *M. brevis* and *M. drucei* groups. Plants collected from the same location as this population had previously been identified as *M. brevis* (Rogers et al., 2002), and the sample included in the morphological analyses was identified as *M. drucei* based mostly on its relatively large nutlet size (1.4 × 1.0 mm vs. the range for *M. brevis* nutlets of 0.9–1.2 × 0.5–0.8 mm; Chapters 2, 5), but other characters that would help to identify which species this population belonged to, such as flowers, were missing. Additionally, the lakeshore habitat is reminiscent of that of the type locality of *M. brevis*, at Lake Lyndon, indicating *M. brevis* may be the more appropriate identification for this population. However, the integrated analyses did not assign this population to the *M. brevis* cluster (Figure 4.5). The other two populations that cluster with *M. brevis* genetically, *M. pygmaea* (H1–3) and *M. antarctica* (HB and HW), are morphologically separate from *M. brevis* judging by the nMDS plots in Chapter 2. Their clustering with *M. brevis* could be a case of incomplete lineage sorting (Habel et al., 2015), or long branch attraction effect (Kalinowski 2011; Kuhls et al., 2013). The *M. brevis* lineage is therefore supported by both iterative and integrative methods in this study.

Myosotis brevis is usually a spring annual species (though see Rogers et al. 2002), and peak flowering is earlier than that of other members of the *M. pygmaea* group (Sep–Nov vs. Dec–Jan; Chapter 5). This earlier flowering could contribute to the maintenance of a mostly separate gene pool from other members of the *M. pygmaea* group.

M. glauca morphological cluster

All of the *M. glauca* specimens included in the microsatellite dataset cluster together in Structure runs above K = 10 (Figure 4.3), and they form a group in network analyses (Figure 4.3, Appendices 4 & 5). Plants from two populations whose morphology differs subtly from *M. glauca* and from each other were also included: *M.* aff. *glauca* from the Pisa range (RM; n = 1), and *M. glauca*? from the Clutha River outwash (CL; n = 4). In the network based on the POSA genetic distance, these two populations are found near the *M. glauca* s.s. populations (Appendix 5), but they are not close in the network based on the KL distance (Figure 4.4). Given that only one individual of *M.* aff. *glauca* was included in the genetic study, little can be concluded regarding its relationships, however the morphological data does appear to unite this entity with *M. glauca* (Chapter 2). The population from the Clutha outwash (*M. glauca*? CL) is a little more complex given it is morphologically intermediate between *M. glauca* and *M. drucei,* and further collections and study are recommended.

In the integrated analyses, *M. glauca* specimens did not form a cluster, but this could be due to the limited sampling. The smallest group that would be theoretically able to be recovered using this type of analysis is the number of dimensions analysed plus one (Edwards and Knowles 2013). As six dimensions were retained in this analysis, and only two (*M. glauca* s.s.) or four (*M glauca* s.s. plus *M.* aff. *glauca* and *M. glauca*?) populations of *M. glauca* were included, it is therefore not surprising that *M. glauca* does not form a cluster in this integrated analysis. The *M. glauca* lineage is thus supported by iterative, but not integrative methods in this study.

M. pygmaea morphological cluster

The 11 *M. pygmaea* populations included in the microsatellite analyses do not form a cluster when analysed by Structure, networks or PCoA. The morphological characters that unite *M. pygmaea* were shown to remain stable when plants from six populations from around New Zealand (including two inland populations) were grown under common garden conditions (Chapter 2). This suggests the nature of the trichomes that most obviously separates *M. pygmaea* from *M. drucei* (curved, appressed to patent on the margins of the leaf blade vs. flexuous, patent to erect on the margins of the leaf blade) does have a genetic basis rather than being solely environmentally induced. However, in the Brassicaceae, branching trichomes have evolved independently several times (Beilstein et al., 2006). Therefore, it is unknown whether or not the mutations leading to the trichome variation observed in the pygmy forget-me-nots have arisen only once, and would therefore be considered sufficient evidence to unite *M. pygmaea* as a separate lineage. Not all of the plants identified as *M. pygmaea* cluster together in the integrated analyses (Figure 4.5A); this lineage is therefore supported by iterative methodologies based on the morphological differentiation, but not by integrated analyses.

The five populations of *M. pygmaea* from the coastal north-west of the South Island (PR, SC), and coastal Taranaki (MN, AN, OK) that cluster together genetically (Figures 4.1–5) are not differentiated from other individuals identified as *M. pygmaea* in the herbarium nor growth room morphological datasets (Chapter 2). The genetic differentiation of these populations could therefore indicate cryptic speciation has taken place or is in the process of occurring. Genetically differentiated populations of morphologically indistinguishable self-fertilizing Arctic *Draba nivalis* were found to have undergone cryptic speciation due to chromosome rearrangements (Gustafsson et al., 2014). A similar process may have occurred in this situation, or the genetic differentiation could be due to other processes not necessarily indicating speciation has occurred, such as genetic drift and/or isolation by distance.

M. drucei-plus morphological cluster

There are 23 populations included in this study that were grouped into the "*M. drucei*-plus" morphological cluster; these 23 populations are not shown to be united genetically in this study. In none of the analyses do the 15 populations identified as *M. drucei*, three populations identified as *M. antarctica*, three populations identified as *M.* "Volcanic Plateau", or two populations identified as *M.* "intermedia" form a cluster, either individually by entity or as a whole. Each of these species and tag names are discussed in turn below. The *M. drucei*-plus morphological cluster is also not recovered using integrative analyses (Figure 4.5A), like *M. pygmaea* this lineage is therefore supported by iterative methodologies based on the morphological differentiation, but not by integrated analyses.

The three populations of *M. antarctica* collected from Campbell Island do not cluster with each other in the molecular analyses. The only morphological character that separates *M. antarctica* from *M. drucei* is a higher density of trichomes (Moore 1961). However there is considerable overlap in trichome density between these two species (Chapter 2), and this character was shown to be highly plastic and likely a factor of how large the leaves grow, based on growth in a common garden (Chapter 2). The lack of morphological differentiation combined with the lack of support for a genetic monophyletic lineage on Campbell Island, already hinted at from DNA sequence data (Meudt et al. 2015), shows that there is no evidence for recongnising *M. antarctica* and *M. drucei* as separate species. Potential explanations for a lack of monophyly at this geographically isolated location include: 1. a single introduction combined with incomplete lineage sorting, 2. multiple introductions to Campbell Island followed by local adaption/hybridisation leading to a similar morphology, or 3. gene flow with the other *Myosotis* species present on Campbell Island, *M. capitata*.

The tag-name *M.* "Volcanic Plateau" has been given to plants morphologically affiliated to *M. drucei*, found growing in what was considered an unusual habitat for that species i.e. in runnels under tussocks (Table 2.1). Given the lack of morphological (Chapter 2) or genetic (e.g. Figures 4.3 & 4.4) differentiation, the populations this tag name refers to are better considered an expansion of the known habitat for *M. drucei* to occur in. The three populations from the Volcanic Plateau region do sometimes group together (e.g. Figure 4.3 K = 24, Appendix 5, but not Figure 4.4), but just as frequently they cluster with the geographically closest *M. drucei* population (MM; Structure K = 2–10 Figure 4.3). Given additional time, there is the possibility for ecological speciation (Rundle and Nosil 2005) between *M. drucei* populations growing in different habitats, but there is no genetic or

morphological evidence this has yet occurred. Whether these plants do in fact inhabit a separate niche is explored using ecological niche modelling in Chapter 5.

M. "intermedia" is a name written on herbarium specimen labels (M. Thorsen, pers. comm.), here considered to be most often applied to large, shade-form plants of *M. drucei.* There is no molecular evidence to unite the two populations suspected to fit this tag-name included in this dataset. As no morphological characters were found to unite them or separate them from *M. drucei* (Chapter 2), the plants this tag-name has been applied to should not be considered a separate entity from *M. drucei.*

Summary and conclusions

A dataset of over 500 *Myosotis pygmaea* group individuals and associated species based on nuclear microsatellite loci was generated to study a closely related, ongoing species radiation. It was found to be difficult to define the *M. pygmaea* group genetically, and the morphological description of Chapter 2 is still considered the best delimitation of the group. Low within population allelic variation and heterozygosity provides the first genetic support to the hypothesis that *M. pygmaea* entities most likely frequently self-fertilize. High between population differentiation points to low gene flow and hence low levels of seed dispersal.

Integrating two sources of data, morphological and molecular, is shown to be more powerful in terms of discovering lineages compared to analysing the same data separately. The method of integration employed here has the benefits of flexibility in terms of the data types that can be used. It is shown to be a useful method to employ until such time as more complex analyses are developed. The results of the iterative interpretation of these datasets is still considered valid though, and necessary here as only a subset of the data were able to be included in the integrative analyses due to sampling constraints.

By analysing data from two morphological datasets (herbarium and growth groom) and one molecular dataset based on nuclear microsatellites, it has been found that the *M. pygmaea* group can best be considered to be made up of three main lineages, corresponding to 1) *M. brevis*, 2) *M. glauca* and 3) *M. drucei* + *M. pygmaea* + *M. antarctica* combined. Within this third lineage, two sub-lineages, only supported by one minor morphological character but no molecular data, can be recognised. These correspond to *M. pygmaea* and *M. drucei* + *M. antarctica.*

A taxonomic revision is not undertaken here, as firstly these lineages will be further analysed with ecological niche modelling, and secondly the population genetic variation within and between these newly described lineages will be explored in the next chapter, along with the implications for their conservation. The taxonomic rank at which each lineage is best recognised will also be considered in the next chapter. This study adds considerably to our knowledge of the evolution of a large genus comprising many threatened species in New Zealand, as well as highlighting a useful data integration method that can be applied to any species delimitation question.

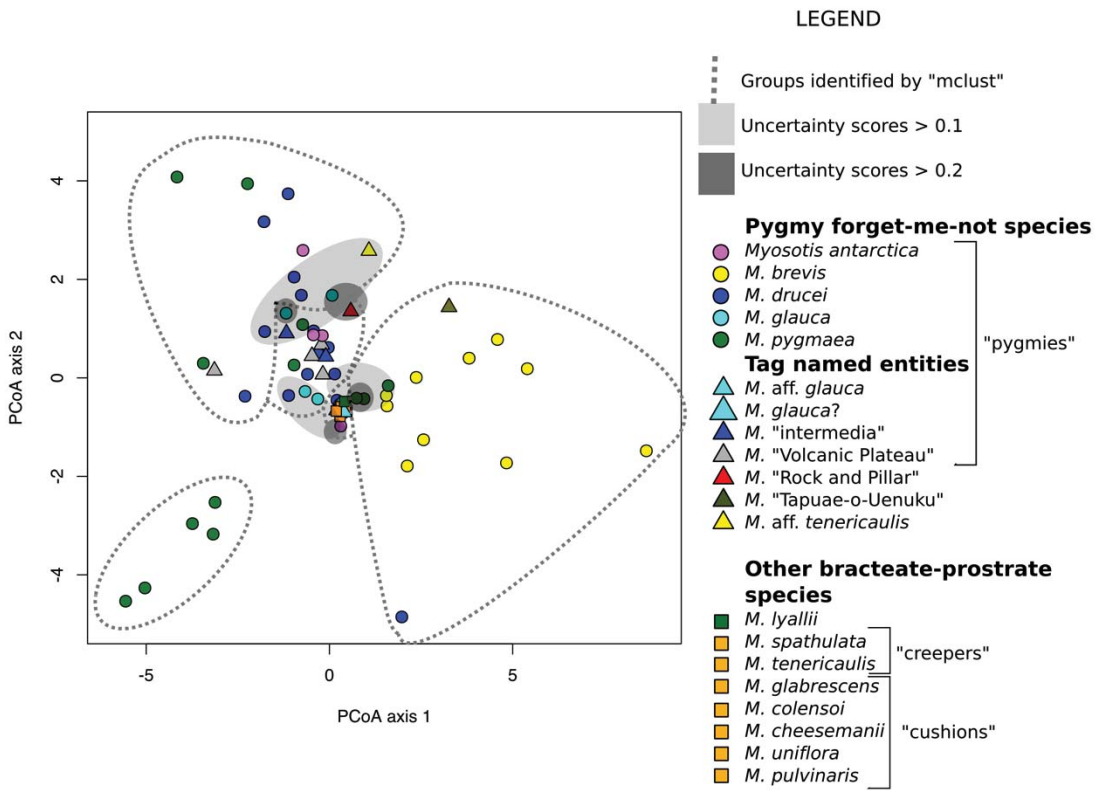


Figure 4.1 Principle coordinates analyses (PCoA) of the *Myosotis* "bracteate-prostrate" microsatellite dataset of 12 loci and 65 populations (552 individuals) showing the first two axes. The PCoA is based on the genetic distance of Kosman and Leonard (2005) calculated by population. Groups identified by "mclust" are indicated. The "pygmies", "creepers" and "cushions" groups in the legend are as identified by morphology in Chapter 2, see Figure 2.2A.

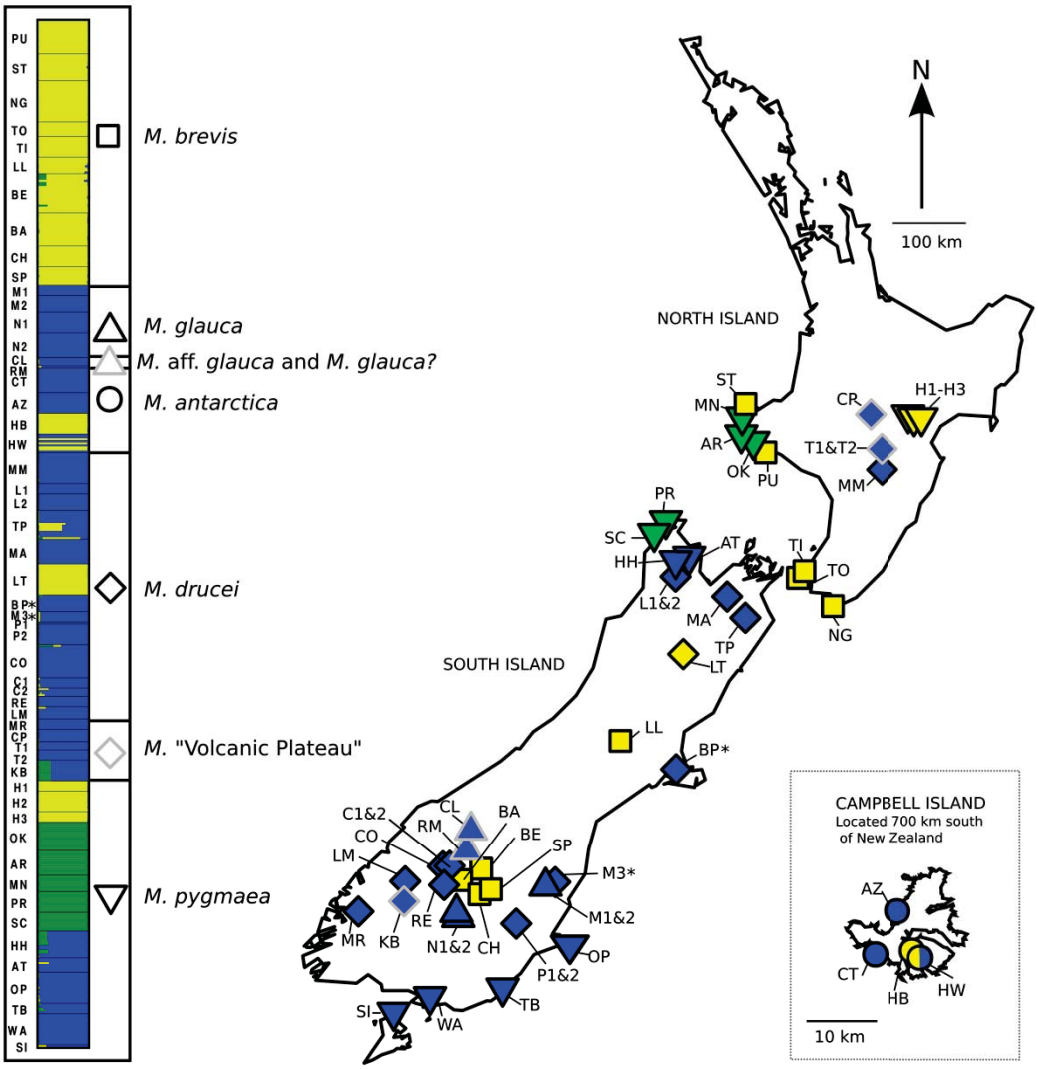


Figure 4.2 Map of New Zealand showing the locations of 54 *Myosotis* populations included in the "pygmy-only" microsatellite dataset of 497 individuals. Symbols on the map are coloured according to the group that population belongs to as assigned by Structure at K = 3. An explanation of the population codes can be found in Table 4.4, voucher details are in Appendix 2. An asterisk (*) indicates populations identified as *M.* "intermedia".

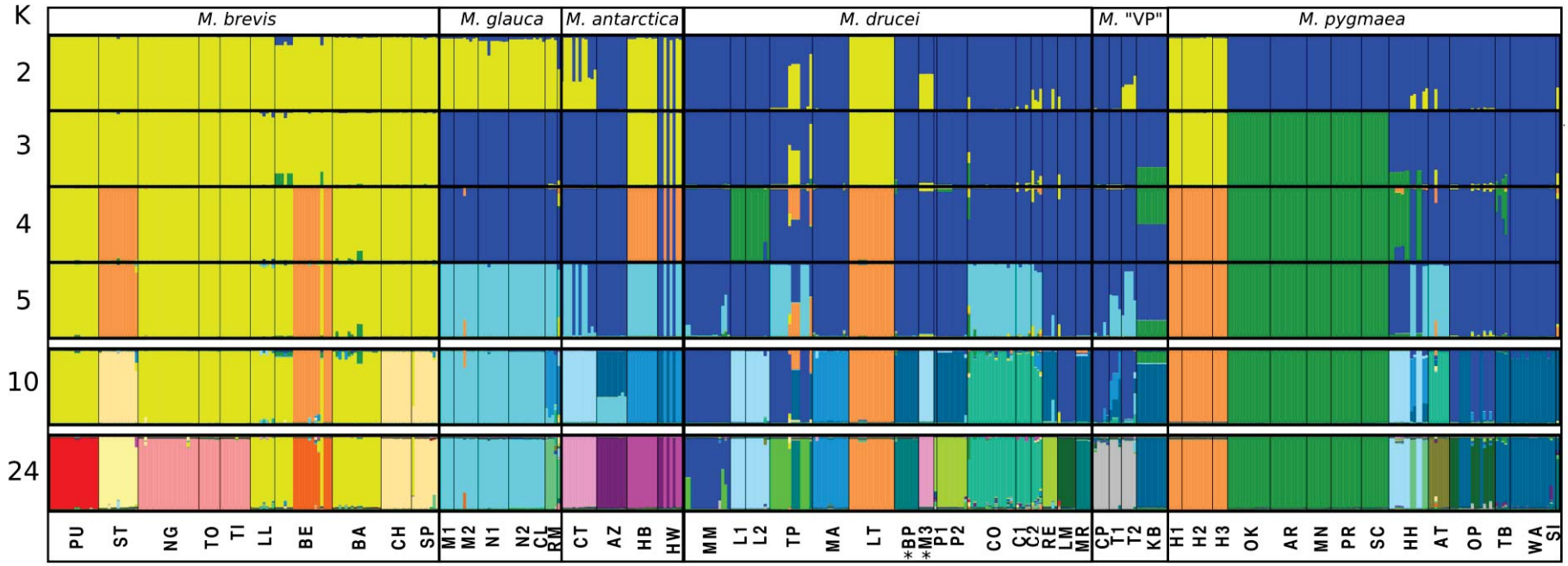


Figure 4.3 Structure runs for selected K values based on the "pygmy-only" *Myosotis* microsatellite dataset of 12 loci and 54 populations (497 individuals). Ten runs were undertaken for each K, the run with the best likelihood score is displayed here. Based on the Evanno method, K = 3 had the highest peak, closely followed by K = 24. An asterisk (*) indicates populations identified as *M.* "intermedia". *M.* "VP" = *M.* "Volcanic Plateau". An explanation of the population codes can be found in Table 4.4, and voucher information is in Appendix 2.

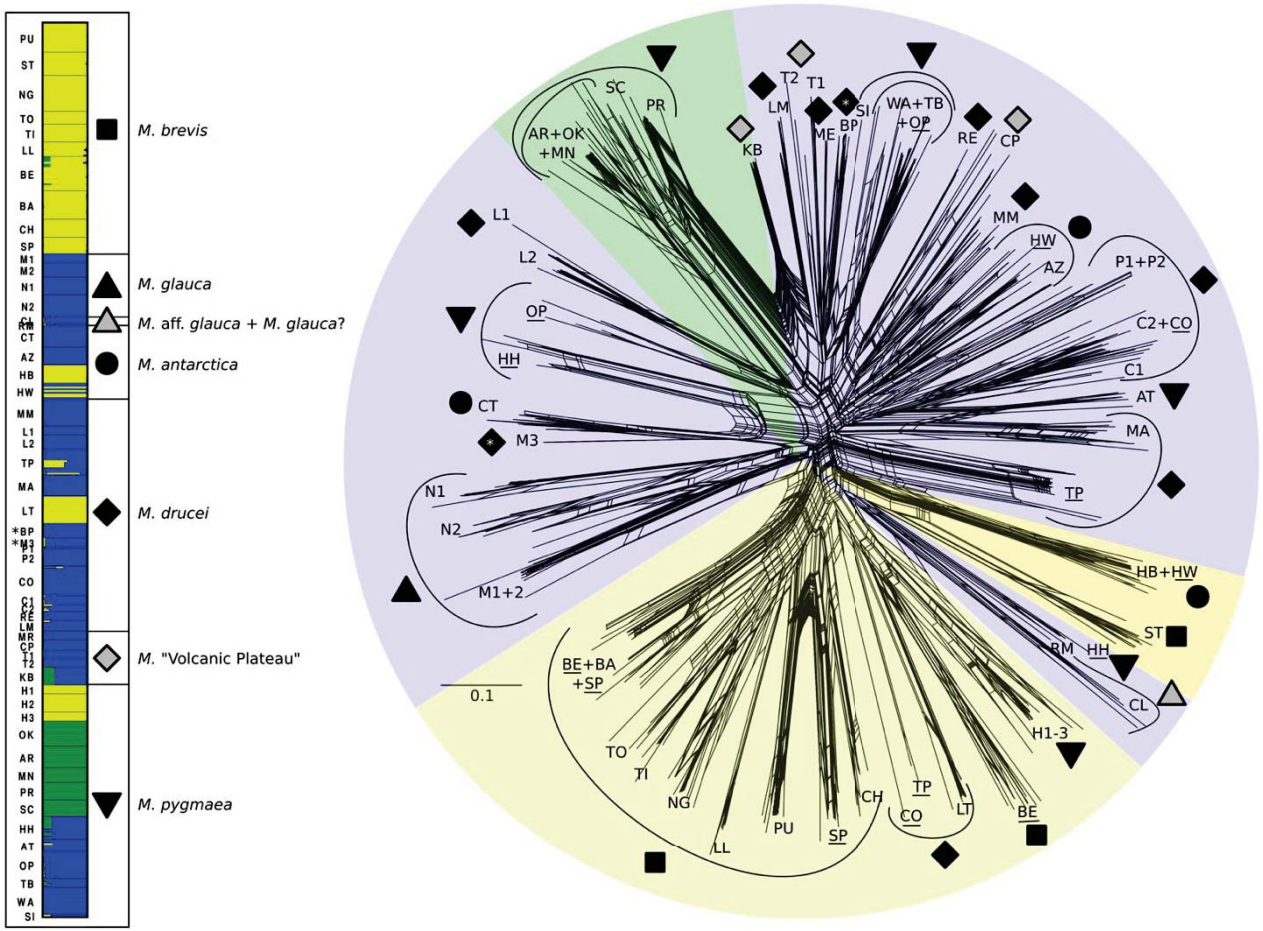


Figure 4.4 Structure plot at K = 3 and NeighborNet network of the "pygmy-only" microsatellite dataset based on the Kosman and Leonard (2005) distance matrix for 54 populations (497 individuals) of New Zealand *Myosotis*. The network is coloured by the Structure groups at K = 3. An explanation of population codes can be found in Table 4.4 and voucher information is in Appendix 2. An asterisk (*) indicates populations identified as *M.* "intermedia". Underlined population codes indicate populations that occur in multiple places on the network.

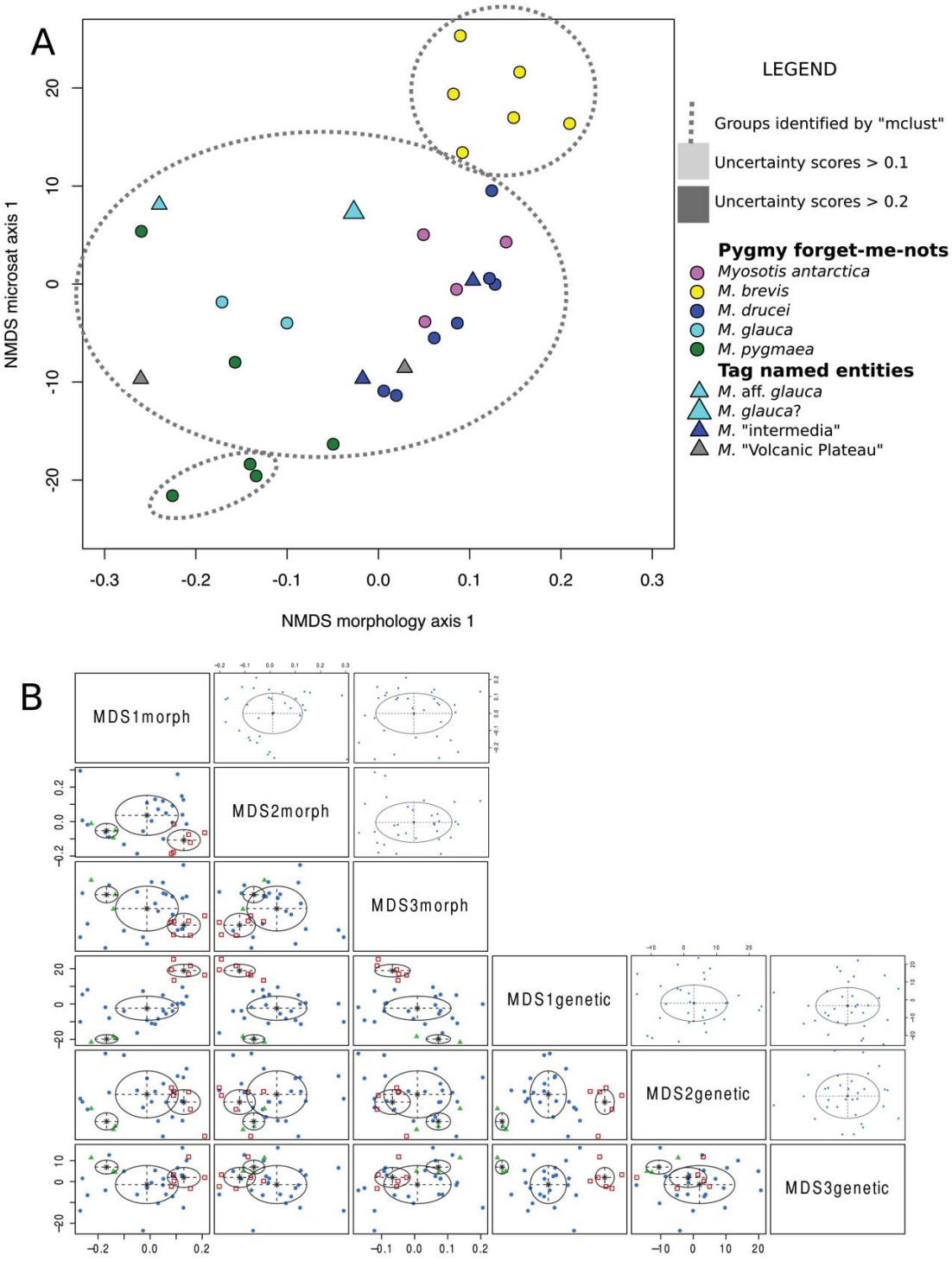


Figure 4.5 Integrated analyses of morphological and molecular data sets of 31 populations of *M. pygmaea* group forget-me-not individuals for which data from both data sets were available. **A.** Non-metric dimensional scaling (nMDS) plot of the first dimension of the morphological data vs. the first dimension of the molecular data. Points are colour coded by morphological species. **B.** Classification plots generated by "mclust". Bottom triangle showing three groups identified when morphological and molecular data integrated, top triangle showing no groups identified when analysing the morphological and molecular data separately.

Table 4.1 Details of the 11 *Myosotis* microsatellite data partitions including the dataset name and description, number of individuals and populations included in the dataset, the level of K identified by the Evanno method and the range in K values assessed via Structure, and the figure in this chapter in which each dataset is visualized. DeltaK plots are included in the relevant appendix.

Dataset name	Description of dataset	Number of individuals (number of locations/ populations)	K identified via the Evanno method (range of Ks assessed).	Figure
***Myosotis*-wide**	All individuals genotyped in this chapter and Chapter 3	586 (86)	N/A	N/A
bracteate-prostrate	All bracteate-prostrate specimens	552 (65)	N/A	Figure 4.1
pygmy-plus	All individuals potentially morphologically and genetically affiliated to the *M. pygmaea* group (Figures 2.2A and 4.1)	543 (58)	4 (1–60)	Appendix 4
pygmy-only	Only those individuals thought to be included in the *M. pygmaea* group (Chapter 2)	497 (54)	3 (1–56)	Figures 4.2–4, Appendix 5
***M. glauca* morph**	Plants identified as *M. glauca*; see figure 2.3B	36 (5)	4 (1–7)	Appendix 6A
***M. brevis* morph**	Plants identified as *M. brevis*; see figure 2.3A	128 (10)	10 (1–12)	Appendix 6B
***M. pygmaea* morph**	Plants identified as *M. pygmaea*; see figure 2.3B	129 (14)	8 (1–16)	Appendix 6C
***M. drucei* morph**	Plants identified as *M. drucei* and those unable to be distinguished from *M. drucei* i.e. including *M. antarctica* and *M.* "Volcanic Plateau"; see Figure 2.3A	200 (24)	7 & 15 (1–26)	Appendix 6D
***M. brevis*-plus Structure**	The yellow cluster in Figure 4.3	177 (16)	12 (1–18)	Appendix 7A
***M. drucei*-plus Structure**	The blue cluster in Figure 4.3	267 (34)	13 & 28 (1–36)	Appendix 7B
***M. pygmaea*-reduced Structure**	The green cluster in figure 4.3	53 (5)	2 (1–7)	Appendix 7C

Table 4.2 Percentage amplification across 12 microsatellite loci, by *Myosotis* morphological group (see Meudt et al., 2015 for explanation of the groups).

Locus Name (see Chapter 3)	*M. pygmaea* group (n = 532)	Bracteate-prostrate group excluding *M. pygmaea* subgroup (n = 55)	Ebracteate-erect group (n = 31)	European *Myosotis* (n = 3)
MYPY-4	91.7	90.9	87.1	0.0
MYPY-10	97.9	85.5	71.0	0.0
MYPY-14	96.8	92.7	96.8	66.7
MYPY-17	97.0	98.2	83.9	100.0
MYPY-20	80.6	27.3	16.1	0.0
MYPY-26	92.9	76.4	32.3	0.0
MYPY-28	85.7	87.3	16.1	33.3
MYPY-29	95.5	98.2	71.0	33.3
MYPY-36	90.6	92.7	48.4	0.0
MYPY-40	94.5	98.2	93.5	33.3
MYPY-41	89.5	85.5	32.3	0.0
MYPY-48	92.3	85.5	22.6	0.0
Total % amplification	**92.1**	**84.8**	**55.9**	**22.2**

Table 4.3 Frequency statistics by microsatellite locus for 12 markers. Calculated from 58 populations across the *Myosotis pygmaea* group and affiliated tag-named entities (the "pygmy-plus" dataset). Details for each locus include the number of alleles (A), the range in size of those alleles, F-statistics, as well as observed (H_O) and expected (H_E) heterozygosity.

Locus	A	Size range (bp)	F_{IS}	F_{IT}	F_{ST}	H_O (mean by pop)	H_E (mean by pop)
MYPY-4	15	233–264	0.532	0.922	0.833	0.070	0.151
MYPY-10	14	303–254	0.476	0.905	0.818	0.081	0.155
MYPY-14	14	184–235	0.617	0.958	0.889	0.036	0.095
MYPY-17	14	270–317	0.508	0.950	0.898	0.042	0.085
MYPY-20	19	220–266	0.969	0.996	0.865	0.003	0.112
MYPY-26	10	372–384	1.000	1.000	0.910	0.000	0.073
MYPY-28	9	341–362	0.979	0.998	0.909	0.001	0.068
MYPY-29	8	334–354	-0.585	0.474	0.668	0.365	0.230
MYPY-36	14	259–312	-0.444	0.759	0.833	0.178	0.123
MYPY-40	5	257–267	0.962	0.996	0.890	0.002	0.050
MYPY-41	6	260–277	0.609	0.960	0.898	0.021	0.055
MYPY-48	10	245–293	0.633	0.948	0.859	0.045	0.122

Table 4.4 Frequency statistics by population for *Myosotis pygmaea* group collections of n > 5. For each population, the following information is given: Population code, geographic location, number of individuals included in the microsatellite dataset (N), population size, size of area occupied by the population, percentage of polymorphic loci (%P), number of private alleles (which marker the private alleles occur in, see Chapter 3), number of alleles (N_A), number of effective alleles (N_E), observed (H_O) and expected heterozygosity (H_E) and F_{IS} (average observed heterozygosity of an individual relative to the expected heterozygosity of individuals in the population, ranges from -1 when all individuals are heterozygous, to +1 when all individuals are homozygous). NI = North Island, SI = South Island, pop = population.

Species	Pop Code	Location	N	Pop size	Size of area occupied by pop (m)	%P	Private alleles (marker)	N_A	N_E	H_O	H_E	F_{IS}
M. antarctica	CT	Campbell Island, cliff tops	12	~30	5 × 5	0.25	1(14)	1.25	1.24	0.00	0.12	1.00
M. antarctica	AZ	Campbell Island, Mt Azimuth	10	~60	50 × 5	0.08		1.08	1.08	0.08	0.04	-1.00
M. antarctica	HB & HW	Campbell Island, Mt Honey	19	~50 & 100s	200 × 20	0.92	1(4)	2.00	1.66	0.08	0.38	0.85
M. brevis	PU	NI, Coastal Taranaki, Puketapu Rd end	16	~100	30 × 5	0.17	2(14 & 20)	1.17	1.04	0.00	0.03	1.00
M. brevis	ST	NI, Coastal Taranaki, Stent Rd	13	400+	50 × 10	0.17	1(20)	1.25	1.07	0.00	0.05	1.00
M. brevis	NG	NI, Wairarapa, Kawakawa Rocks, near Ngawi	20	~150	30 × 10	0.25		1.25	1.05	0.00	0.04	1.00
M. brevis	TI	NI, Wellington South Coast, Te Ikaamaru Bay	7	~200	40 × 5	0.00	1(20)	1.00	1.00	0.00	0.00	
M. brevis	TO	NI, Wellington South Coast, Te Ohau Bay	10	80	120 × 40	0.08	1(20)	1.08	1.02	0.00	0.02	1.00
M. brevis	LL	SI, Canterbury, Lake Lyndon	8	20+	150 × 20	0.17	2(20 x 2)	1.25	1.14	0.00	0.08	1.00
M. brevis	BE	SI, Otago, Bendigo	19	~1000	50 × 50	0.83	4(10 x 2, 29,	2.75	1.99	0.00	0.44	1.00

Species	Pop Code	Location	N	Pop size	Size of area occupied by pop (m)	%P	Private alleles (marker)	N_A	N_E	H_O	H_E	F_{IS}
							36)					
M. brevis	BA	SI, Otago, Bannockburn	16	1000s	500 × 50	0.42	1 (17)	1.58	1.36	0.00	0.18	1.00
M. brevis	CH	SI, Otago, Chapman Rd Reserve	10	100s	150 × 20	0.08	3(41,10,36)	1.00	0.93	0.01	0.01	-0.07
M. brevis	SP	SI, Otago, Springvale Reserve	9	~30	50 × 20	0.67	1(36)	1.75	1.18	0.04	0.13	0.66
M. drucei	RR	NI, Ruahine Ranges, near Mt Maungamahue	15	~50	30 × 30	0.33		1.42	1.26	0.05	0.14	0.64
M. drucei	LT	SI, Marlborough, Lake Tennyson	15	~150	20 × 4	0.08	1(29)	1.08	0.98	0.05	0.04	-0.28
M. drucei	MA	SI, Marlborough, Mt Altimarlock	12	~60	400 × 20	0.33		1.42	1.21	0.10	0.12	0.13
M. drucei	TP	SI, Marlborough, Tapuae-o-Uenuku	14	~50	10 × 2	0.83	1(29)	2.00	1.65	0.06	0.34	0.90
M. drucei	L1&2	SI, NW Nelson, Ridge above Lake Peel	13	6 + 20	1 × 1 & 2 × 1	0.25		1.25	1.14	0.04	0.08	0.57
M. drucei	BP	SI, Canterbury, Banks Peninsula, Port Hills, Trig O	8	10	1 × 0.2	0.17		1.17	1.10	0.10	0.05	-0.54
M. drucei	CO	SI, Central Otago, Coronet Peak	16	30–50	100 × 10	0.83	1 (10)	2.17	1.34	0.03	0.21	0.78
M. drucei	M3	SI, Central Otago, Macraes flat	5	~10	0.4 × 0.2	0.08	1(36)	1.08	1.08	0.08	0.04	-1.00
M. drucei	C1 & 2	SI, Central Otago, Mt Hocken, Pisa Range	9	~15 & 22	20 × 4 & 300 × 2	0.67	1(10)	2.17	1.64	0.16	0.26	0.35
M. drucei	P1 & 2	SI, Central Otago, Rock and Pillar Range	11	12 & 20+	5 × 5 & 10 × 4	0.58		1.83	1.60	0.08	0.28	0.71

Species	Pop Code	Location	N	Pop size	Size of area occupied by pop (m)	%P	Private alleles (marker)	N_A	N_E	H_O	H_E	F_{IS}
M. drucei	RE	SI, Central Otago, The Remarkables ski area	5	40	5 × 2	0.08	1(36)	1.08	1.04	0.00	0.03	1.00
M. drucei	LM	SI, Southland, Livingstone Mts	6	10	10 × 5	0.08		1.08	1.05	0.00	0.03	1.00
M. drucei	MR	SI, Southland, Merrie Range, Tamatea Peak	5	Unknown	Unknown	0.08	1(14)	1.08	1.08	0.08	0.04	-1.00
M. glauca	N1	SI, Otago, School House Flat, Nevis Valley	10	~55	1.5 × 1.5	0.08		1.17	1.06	0.01	0.04	0.74
M. glauca	N2	SI, Otago, School House Flat, Nevis Valley	12	30–40	2 × 1.5	0.08		1.08	1.08	0.08	0.04	-1.00
M. glauca	M1 & 2	SI, Otago, Macraes flats, collected on different years	13	200 & Unknown	10 × 10	0.42	2(40,20)	1.42	1.06	0.01	0.05	0.79
M. glauca ?	CL	SI, Lake Wanaka, Clutha River	4	Unknown	Unknown	0.42	1(26)	1.42	1.30	0.11	0.17	0.36
M. pygmaea	H1–3	NI, Hawke's Bay, Hukanui Station	20	5 & ~15 & 5	5 × 5 & 100 × 100 & 5 × 5	0.58	2(14,48)	1.58	1.49	0.45	0.26	-0.66
M. pygmaea	OK	SI, Coastal Taranaki, Opunake treatment ponds	14	~50	2 × 1	0.08	1(14)	1.08	1.01	0.00	0.01	1.00
M. pygmaea	AR	NI, Coastal Taranaki, Arawhata Rd end	12	20	1 × 1.5	0.00		1.00	1.00	0.00	0.00	
M. pygmaea	MN	NI, Coastal Taranaki, Manihi Rd end	8	20	2 × 1	0.00		1.00	1.00	0.00	0.00	
M. pygmaea	HH	SI, NW Nelson, Hoary Head	13	~30	200 × 100	0.92		1.92	1.57	0.05	0.34	0.78
M. pygmaea	AT	SI, NW Nelson, ridge track to Mt Arthur	7	200	50 × 10	0.33		1.33	1.18	0.08	0.11	0.50
M.	PR	SI, NW Nelson, South of	10	100	7 × 3	0.00		1.00	1.00	0.00	0.00	

Species	Pop Code	Location	N	Pop size	Size of area occupied by pop (m)	%P	Private alleles (marker)	N_A	N_E	H_O	H_E	F_{IS}
pygmaea		Paturau River mouth										
M. pygmaea	SC	SI, NW Nelson, near Sandhill Ck river mouth	9	15	10 × 5	0.00		1.00	1.00	0.00	0.00	
M. pygmaea	OP	SI, Coastal Otago, The Chasm, Otago Peninsula	15	50+	10 × 5	0.58	1 (4)	1.67	1.57	0.06	0.28	0.85
M. pygmaea	TB	SI, Coastal Otago, the Catlins, Tahakopa Bay	5	35	200 × 10	0.17		1.17	1.13	0.00	0.07	1.00
M. pygmaea	WA	SI, Southland, Waituna, past Tiwai Point	15	~80	40 × 5	0.33	1(26)	1.33	1.13	0.00	0.08	1.00
***M.* "Volcanic Plateau"**	T1 & 2	NI, Cultivated in Dunedin ex. Ruahine Ranges, Makirkiri tarns mixed with some from new from the same location	9	Unknown & 6	10 × 2	0.75	3(17x2, 26)	2.17	1.72	0.07	0.34	0.83
***M.* "Volcanic Plateau"**	CP	NI, Central Plateau, Waipakihi River	6	Unknown	50 × 20	0.50		1.58	1.43	0.10	0.23	0.59
***M.* "Volcanic Plateau"**	KB	SI, Southland, Kiwi burn, near Mavora Lakes	10	Unknown	Unknown	0.00		1.00	1.00	0.00	0.00	

References

Alexander DH, Novembre J, Lange K (2009) Fast model-based estimation of ancestry in unrelated individuals. *Genome Research* 19: 1655-1664.

Avise JC, Nelson WS (1989) Molecular genetic relationships of the extinct dusky seaside sparrow. *Science* 243: 646-648.

Beilstein MA, Al-Shehbaz IA, Kellogg EA (2006) Brassicaceae phylogeny and trichome evolution. *American Journal of Botany* 93: 607-619.

Bowcock AM, Ruiz-Linares A, Tomfohrde J, Minch E, Kidd JR, Cavalli-Sforza LL (1994) High resolution of human evolutionary trees with polymorphic microsatellites. *Nature* 368: 455-457.

Brandon A (2001) *Breeding systems and rarity in New Zealand Myosotis*. PhD Thesis. Palmerston North: Massey University.

Cameron KM (2010) On the value of taxonomy, phylogeny, and systematics to orchid conservation: Implications for China's Yachang Orchid Reserve. *Botanical Review* 76: 165-173.

Chakraborty R, Jin L (1993) Determination of relatedness between individuals using DNA fingerprinting. *Human Biology* 65: 875-895.

Chhatre V (2012) StrAuto ver0.3.1: A Python utility to automate Structure analysis. Available at http://www.crypticlineage.net/pages/software.html.

Cole CT (2003) Genetic variation in rare and common plants. *Annual Review of Ecology, Evolution, and Systematics* 34: 213-237.

de Lange PJ, Norton DA, Courtney SP, Heenan PB, Barkla JW, Carmeron EK, Hitchmough R, Townsend AJ (2009) Threatened and uncommon plants of New Zealand (2008 revision). *New Zealand Journal of Botany* 47: 61-69.

de Lange PJ, Rolfe JR, Champion PD, Courtney SP, Heenan PB, Barkla JW, Cameron EK, Norton DA, Hitchmough RA (2013) *Conservation Status of New Zealand Indigenous Vascular Plants, 2012*. Wellington: New Zealand Department of Conservation.

de Queiroz K (2007) Species concepts and species delimitation. *Systematic Biology* 56: 879-886.

Dieringer D, Schlotterer C (2003) MICROSATELLITE ANALYSER (MSA): a platform independent analysis tool for large microsatellite data sets. *Molecular Ecology Notes* 3: 167-169.

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* 12: 13-15.

Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4: 359-361.

Edwards C, Judd W, Ionta G (2009) Using population genetic data as a tool to identify new species: *Conradina cygniflora* (Lamiaceae), a new, endangered species from Florida. *Systematic Botany* 34: 747-759.

Edwards DL, Knowles LL (2013) Species detection and individual assignment in species delimitation: Can integrative data increase efficacy? *Proceedings of the Royal Society B: Biological Sciences* 281:20132765.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology* 14: 2611-2620.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479-491.

Falush D, Stephens M, Pritchard J (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164: 1567-1587.

Ferrão LFV, Caixeta ET, Cruz CD, de Souza FF, Ferrão MAG, Maciel-Zambolim E, Zambolim L, Sakiyama NS (2014) The effects of encoding data in diversity studies and the applicability of the weighting index approach for data analysis from different molecular markers. *Plant Systematics and Evolution* 300: 1649-1661.

Fogelqvist J, Nittyvuopio A, Agren J, Savolainen O, Lascoux M (2010) Cryptic population genetic structure: the number of inferred clusters depends on sample size. *Molecular Ecology Resources* 10: 314-323.

Fraley C, Raftery AE (2002) Model-based clustering, discriminant analysis and density estimation. *Journal of the American Statistical Association* 97: 611-631.

Fraley C, Raftery AE, Murphy TB, Scrucca L (2012) Mclust version 4 for R: Normal mixture modeling for model-based clustering, classification, and density estimation technical report no. 597, Department of Statistics, University of Washington.

Francois O, Durand E (2010) Spatially explicit Bayesian clustering models in population genetics. *Molecular Ecology Resources* 10: 773-784.

Gao H, Williamson S, Bustamante CD (2007) A Markov chain Monte Carlo approach for joint inference of population structure and inbreeding rates from multilocus genotype data. *Genetics* 176: 1635–1651

Germain-Aubrey CC, Nelson C, Soltis DE, Soltis PS, Gitzendanner MA (2016) Are microsatellite frangment lengths useful for population-level studies? The case of *Polygala lewtonii* (Polygalaceae). *Applications in Plant Sciences* 4: 1500115.

Gilbert KJ, Andrew RL, Bock DG, Franklin MT, Kane NC, Moore J-S, Moyers BT, Renaut S, Rennison DJ, Ween T, Vines TH (2012) Recommendations for utilizing and reporting population genetic analyses: the reproducibility of genetic clustering using the program STRUCTURE. *Molecular Ecology* 21: 4925–4930.

Gustafsson ALS, Skrede I, Rowe HC, Gussarova G, Borgen L, Rieseberg LH, Brochmann C, Parisod C (2014) Genetics of cryptic speciation within an arctic mustard, *Draba nivalis*. *PLOS One* 9: e93834.

Habel JC, Zachos FE, Dapporto L, Rödder D, Radespiel U, Tellier AL, Schmitt T (2015) Population genetics revisited – towards a multidisciplinary research field. *Biological Journal of the Linnean Society* 115: 1-12.

Hamrick JL, Godt MJW (1996) Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions: Biological Sciences* 351: 1291-1298.

Hartl DL, Clark AG (1997) *Principles of Population Genetics*. Sunderland, Massachusetts: Sinauer Associates.

Huson D (1998) SplitsTree: analyzing and visualizing evolutionary data. *Bioinformatics* 14: 68-73.

Huson DH, Bryant D (2006) Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* 23: 254-267.

Jakobsson M, Rosenberg N (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23: 1801-1806.

Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24: 1403-1405.

Jombart T, Ahmed I (2011) adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. *Bioinformatics*: DOI: 10.1093/bioinformatics/btr1521.

Kalinowski S (2011) The computer program STRUCTURE does not reliably identify the main genetic clusters within species: simulations and implications for human population structure. *Heredity* 106: 625-632.

Karlin EF, Boles SB, Shaw AJ (2008) Systematics of *Sphagnum* section *Sphagnum* in New Zealand: a microsatellite-based analysis. *New Zealand Journal of Botany* 46: 105-118.

Kim C, Jung J, Choi H-K (2012) Molecular identification of *Schoenoplectiella* species (Cyperaceae) by use of microsatellite markers. *Plant Systematics and Evolution* 298: 811-817.

Knowlton N, Jackson JBC (1994) New taxonomy and niche partitioning on coral reefs: jack of all trades or master of some? *Trends in Ecology & Evolution* 9: 7-9.

Kosman E, Leonard KJ (2005) Similarity coefficients for molecular markers in studies of genetic relationships between individuals for haploid, diploid, and polyploid species. *Molecular Ecology* 14: 415-424.

Kuhls K, Cupolillo E, Silva SO, Schweynoch C, Boité MC, Mello MN, Mauricio I, Miles M, Wirth T, Schönian G (2013) Population structure and evidence for both clonality and recombination among Brazilian strains of the subgenus *Leishmania* (Viannia). *PLoS Neglected Tropical Diseases* 7: e249.

Lande R (1995) Mutation and conservation. *Conservation Biology* 9: 782-791.

Mabberley D (2008) *Mabberley's Plant Book. A Portable Dictionary of Plants, their Classifications and Uses*. Seattle: University of Washington Botanic Gardens.

McGlone MS, Duncan RP, Heenan PB (2001) Endemism, species selection and the origin and distribution of the vascular plant flora of New Zealand. *Journal of Biogeography* 28: 199-216.

Meudt HM, Prebble JM, Lehnebach CA (2015) Native New Zealand forget-me-nots (*Myosotis*, Boraginaceae) comprise a Pleistocene species radiation with very low genetic divergence. *Plant Systematics and Evolution* 301: 1455–1471.

Meudt HM, Prebble JM, Stanley RJ, Thorsen MJ (2013) Morphological and amplified fragment length polymorphism (AFLP) data show that New Zealand endemic *Myosotis petiolata* (Boraginaceae) comprises three rare and threatened species. *Australian Systematic Botany* 26: 210-232.

Moore LB (1961) *Boraginaceae*. In: Allan H, editor. *Flora of New Zealand. Vol. 1*. Wellington, New Zealand: PD Hasselberg, Government Printer. p. 806-833.

Moritz C (2002) Strategies to protect biological diversity and the evolutionary processes that sustain it. *Systematic Biology* 51: 238-254.

Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology* 13: 1143-1155.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2015) vegan: Community Ecology Package. R package version 2.3-0. http://CRAN.R-project.org/package=vegan.

Ornelas-García CP, Domínguez-Domínguez O, Doadrio I (2008) Evolutionary history of the fish genus *Astyanax* Baird & Girard (1854) (Actinopterygii, Characidae) in Mesoamerica reveals multiple morphological homoplasies. *BMC Evolutionary Biology* 8: 340.

Paradis E, Claude J, Strimmer K (2004) APE: analyses of phylogenetics and evolution in R language. V3.3. *Bioinformatics* 20: 289-290.

Peakall R, Smouse PE (2006) GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288-295.

Peakall R, Smouse PE (2012) GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28: 2537-2539.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155: 945-959.

Pyšek P, Hulme PE, Meyerson LA, Smith GF, Boatwright JS, Crouch NR, Figueiredo E, Foxcroft LC, Jarošik V, Richardson DM, Suda J, Wilson JRU (2013) Hitting the right target: taxonomic challenges for, and of, plant invasions. *AOB Plants* 5: 1-25.

RCoreTeam (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Reeves PA, Richards CM (2011) Species delimitation under the general lineage concept: An empirical example using wild North American hops (Cannabaceae: *Humulus lupulus*). *Systematic Biology* 60: 45-59.

Robertson A (1989) *Evolution and pollination of New Zealand Myosotis (Boraginaceae).* PhD Thesis. Christchurch: University of Canterbury.

Robertson AW, Lloyd DG (1991) Herkogamy, dichogamy and self-pollination in six species of *Myosotis* (Boraginaceae). *Evolutionary Trends in Plants* 5: 53-63.

Rogers G, Walker S, Tubbs M, Henderson J (2002) Ecology and conservation status of three "spring annual" herbs in dryland ecosystems of New Zealand. *New Zealand Journal of Botany* 40: 649-669.

Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes* 4: 137-138.

Rundle HD, Nosil P (2005) Ecological Speciation. *Ecology Letters* 8: 336-352.

Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. *Ecology Letters* 9: 615-629.

Solis-Lemus C, Knowles LL, Ane C (2014) Bayesian species delimitation combining multiple genes and traits in a unified framework. *Evolution* 69: 492-507.

Turini FG, Steinert C, Heubl G, Bringmann G, Lombe BK, Mudogo V, Meimberg H (2014) Microsatellites facilitate species delimitation in Congolese *Ancistrocladus* (Ancistrocladaceae), a genus with pharmacologically potent naphthylisoquinoline alkaloids. *Taxon* 63: 329-341.

Van Oosterhout C, Hutchinson W, Wills D, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4: 535-538.

Winkworth R, Grau J, Robertson A, Lockhart P (2002) The origins and evolution of the genus *Myosotis* L. (Boraginaceae). *Molecular Phylogenetics and Evolution* 24: 180-193.

Winter D (2012) MMOD: an R library for the calculation of population differentiation statistics. *Molecular Ecology Resources* 12: 1158-1160.

Yang Z, Rannala B (2010) Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences* 107: 9264-9269.

Yeates DK, Seago A, Nelson L, Cameron SL, Joseph L, Trueman JWH (2011) Integrative taxonomy, or iterative taxonomy? *Systematic Entomology* 36: 209-217.

Zare R, Gams W, Schroers H-J (2004) The type species of *Verticillium* is not congeneric with the plant-pathogenic species placed in *Verticillium* and it is not the anamorph of *'Nectria' inventa*. *Mycological Resources* 108: 675-582.

Zuloaga F, Morrone O, Belgrano M, Marticorena C, Marchesi E (2008) Catálogo de las plantas vasculares del Cono Sur (Argentina, southern Brazil, Chile, Paraguay y Uruguay). *Monographs in Systematic Botany from the Missouri Botanical Garden* 3 volumes: 1-3486.

Chapter 5 Taxonomic revision of the New Zealand native pygmy forget-me-nots (*Myosotis*; Boraginaceae) based on morphological, genetic and ecological niche modelling data

Abstract

A taxonomic classification system that reflects evolutionary lineages has important implications, including for species conservation. The general lineage model, whereby multiple lines of evidence are used to discover evolutionary lineages, is implemented here to delimit species in the New Zealand pygmy forget-me-not group (*Myosotis*; Boraginaceae). Pygmy forget-me-nots are part of a genus of threatened plants in need of taxonomic revision in New Zealand. Combining morphological, molecular, and ecological niche modelling data to undertake a taxonomic revision provides evidence for three species within the New Zealand native pygmy forget-me-not group: *Myosotis antarctica*, *M. brevis* and *M. glauca*. Two subspecies of *M. antarctica* are recognised: subsp. *antarctica* and subsp. *traillii.* These three species are described morphologically, their genetic variation and patterns of similarity and distinction are examined, and their ecological niches are modelled. Additionally the threat status, rarity type and implications for conservation are discussed.

Introduction

Using multiple lines of evidence to delimit species following the general lineage concept can result in a taxonomy that more accurately reflects the evolutionary history of a group (de Queiroz 2007), which can have important implications for conservation, biosecurity, trade and agriculture and ecological research (e.g., Knowlton and Jackson 1994; Zare et al., 2004; Cameron 2010; Pyšek et al., 2013). The general lineage concept is especially useful for groups that have gone through a rapid and recent species radiation, which is a common feature of the New Zealand flora, particularly in the alpine zone (Winkworth et al., 2005).

The New Zealand native species of *Myosotis* (Boraginaceae) are an example of a relatively recent and rapid species radiation in New Zealand (Winkworth et al., 2002; Meudt et al., 2015). The general lineage concept has been applied with success to species delimitation within New Zealand *Myosotis* in the past, for example the *M. petiolata* complex (Meudt et al., 2013). The main aim of this chapter is to undertake a taxonomic revision, after applying the general lineage concept, of the New Zealand native *Myosotis pygmaea* species group. The *M. pygmaea* species group (hereafter called the pygmy species group) is made up of small selfing herbaceous plants with petiolate obovate rosette leaves, decumbent branches with many flowers each associated with a cauline leaf. The white or cream corollas are up to 4 mm in diameter, with cylindric corolla tubes and included stamens (Chapter 2).

Lineages within the pygmy forget-me-not group have been identified using morphological (Chapter 2) and molecular data (Chapters 3 and 4) and in this chapter I also present data investigating the similarity and differences between ecological niches of the plants that make up this group. Ecological niche models estimate a species' niche across a geographical area by relating presence records of the species to environmental variables to generate predictions. These models estimate the probability that species occur in areas where they have not been observed, given the environmental variables (Elith et al., 2006). The field of niche modelling has grown rapidly in recent years and the methods have been used in a variety of applications; e.g. to test for ecological speciation (Joly et al., 2014) to compare lineage diversification with niche divergence (Wooten and Gibbs 2012), to assess evidence for glacial refugia (Buckley et al., 2010), to find previously unrecorded populations of threatened species (Bourg et al., 2005), and most relevant here, to aid in species delimitation (e.g., Raxworthy et al., 2007; Rissler and Apodaca 2007; Reeves and Richards 2011; Ahmadzadeh et al., 2013). There are many different methods for modelling

species niches such as bioclim, garp, domain and MaxEnt (Elith et al., 2006). The MaxEnt model is one of the most commonly executed, because it performs well with presence only data, and also because it is shown to be consistently highly performing relative to other methods (Elith et al., 2006). Additional advantages of the MaxEnt model are that it is frequently updated (e.g., Radosavljevic and Anderson 2014) and easy to implement due to a standalone graphical user interface (GUI) (Phillips et al., 2004; Phillips et al., 2006).

To use ecological niche modelling data in species delimitation, niches are modelled for each hypothesised species, and then measures of niche overlap are calculated (e.g., Warren et al., 2008). Hypotheses relating to whether niches are the same between two entities can be tested using pseudo-replicates of niche overlap, as implemented in programs such as ENMTools (Warren et al., 2010). However, whether niches are significantly different or not does not necessarily equate to two entities being different species. Godsoe (2010) suggests that niche modelling can only provide useful data for species delimitation in cases where the two entities' distributions are overlapping, and their niches are shown to differ.

Ecological niche data can also be used in an integrated taxonomic framework. In Chapter 4 of this thesis, morphological and molecular data were integrated for 31 populations of pygmy forget-me-nots for which both data types were available. The integration methods followed Edwards and Knowles (2013), and their technique can also be expanded to include ecological data. To do this, the environmental predictor values can be extracted for each population location, a distance matrix calculated, and the non-metric multidimensional scaling (nMDS) points are then concatenated with the nMDS point of the morphological and molecular data. Gaussian clustering methods are then used to identify clusters within the data.

In this chapter, the ecological niches of all pygmy forget-me-not species are modelled, for both the traditional taxonomic species and the groups identified in Chapter 4 (see Table 5.7). The niche overlap between each species pair is calculated, and whether their niches differ significantly is investigated. Apart from *M. antarctica* which grows on the Subantarctic Campbell Island and in Chile, the ranges overlap for the other pygmy forget-me-not species. This range overlap means that finding evidence of the lineages inhabiting different niches should contribute evidence as to whether they are separate species or not (Godsoe 2010). The niche of one tag-named entity, *M.* "Volcanic Plateau" is also modelled. This entity was originally identified as potentially ecologically differentiated because it is found in a unique habitat of "periodically scoured, shallowly incised flood channels or

runnels within red tussock covered valley floors" (G. Rogers pers. comm., August 2012). As *M.* "Volcanic Plateau" is considered to be a putatively different species based on its ecological niche, a niche modelling approach is therefore an appropriate method to test its distinctiveness, even though this tag-named entity was not recovered as a separate group in either morphological (Chapter 2) or genetic data analyses (Chapter 4).

In addition to a taxonomic revision and ecological niche modelling, this chapter assesses the conservation status of each of the pygmy forget-me-not species. Understanding patterns of genetic diversity in rare and threatened species, as evidenced by structure and variation within and among populations, is of fundamental importance to their conservation (Ellstrand and Elam 1993). It is therefore important to understand the genetic variation within and among the different pygmy forget-me-not group species, and to align their conservation status to best reflect this. Although none of the species here recognized are newly described, the new circumscription outlined here of the species present in the pygmy group requires a reassessment of the threat classification of each species.

Therefore, the aims of the present study are to:

1. Model the ecological niches of species and tag-named entities of the pygmy forget-me-not group, and to assess the utility of this data type to aiding species delimitation both as a stand alone dataset and by integrating ecological data with morphological and molecular data where possible;
2. Undertake a taxonomic revision of the pygmy species group having considered morphological, genetic and ecological niche modelling data under the general lineage model;
3. Assess the threat status of the newly circumscribed species; and
4. Compare the population genetic variation within and between each species with different threat levels and rarity types, and consider the implications for conservation.

Based on the morphological, genetic and ecological niche modelling data presented here, three lineages within the pygmy species group are recognized at the rank of species; they are *Myosotis brevis*, *M. glauca* and *M. antarctica*. Two lineages within *M. antarctica* are recognized at the rank of subspecies as *M. antarctica* subsp. *antarctica* and *M. antarctica* subsp. *traillii*, and these names will be used throughout this manuscript (see Table 5.7).

Methods

Ecological niche modelling

Latitude and longitude points for niche modelling were obtained from 290 herbarium specimens (Figure 5.1, Appendix 8) from AK, CHR, K, OTA, UPS, and WELT. Herbarium codes follow Index Herbariorum (Thiers 2016). There are over 700 specimens of the pygmy forget-me-not group housed across these herbaria, but to include a specimen in the niche modelling the identification of each specimen was assessed by JMP, and all latitude and longitude points were individually checked. Collections unable to be plotted precisely (i.e. to within 100 m) were not georeferenced, and only one specimen from each collection location (e.g. "Lake Lyndon") was included. The known geographic range of each species was well represented, including specimens from the only two known localities of *M. antarctica* from Chile (Punta Arenas and Puerto Altamirano; pers. obs. based on study of specimens from AK, BM, CHR, CONC, K, OTA, S, UPS, and WELT).

For the niche modelling, 33 environmental layers were considered (Table 5.1), including elevation, 19 WorldClim bioclimatic variables (http://www.worldclim.org/current) (Hijmans et al., 2005), and 13 layers developed for Land Environments New Zealand (LENZ) (https://lris.scinfo.org.nz/)(Leathwick et al., 2002). Raster layers from WorldClim are available in a maximum of a 30 arc-second quadrat resolution (~1 km grid squares at the equator). The LENZ layers are available at a higher resolution (25 × 25 m^2 grid), however the data in these layers has not been modelled for the New Zealand Subantarctic Islands, nor for Chile, and therefore does not encompass the entire geographic range of *M. antarctica*. In order to co-analyse the LENZ and WorldClim datasets, the resolution and projection of the LENZ layers was transformed to match that of the WorldClim data. This was undertaken in R (RCoreTeam 2015) using the function ***spatial_sync_raster*** from the package "spatial.tools" (Greenberg 2014). The raster files were then stacked using the ***stack*** function from the "raster" package (Hijmans 2015) and a Pearson's correlation (using function ***layerStats*** also from the "raster" package) showed that 16 of the 33 layers assessed were correlated with other layers (> 0.8) and should therefore be excluded (Table 5.1). An additional eight layers were shown to contribute little to the fit of the models after trials run in MaxEnt (Phillips et al., 2006). Three separate combinations of layers ("datasets") were collated to explore the data (Table 5.1). Firstly, four LENZ layers (soil, slope, temperature and balance) were used to model the niches of just the New Zealand specimens (excluding the Subantarctic Islands). Secondly, two LENZ layers (slope and soil) were transformed to match the resolution of the WorldClim layers, and

combined with seven WorldClim layers (Bio1, Bio2, Bio3, Bio8, Bio9, Bio12, Bio15). Due to the incorporation of the two LENZ layers, the New Zealand Subantarctic Islands were still excluded in this dataset. Thirdly, in order to assess New Zealand, the Subantarctic Islands and the region of southern Chile that comprises the range of *M. antarctica,* seven WorldClim bioclimatic data layers were downloaded for both "Tile 411" (New Zealand) and "Tile 43" (southern South America). The two tiles were joined into a single raster file for each environmental layer in R using the ***merge*** function from the "raster" package, with a blank raster the extent of the area between the two tiles included in the merge. These merged layers were cropped and masked to the world map extent for the relevant areas.

Ecological niche modelling was undertaken using presence-only data and the maximum entropy model as implemented by the program MaxEnt v.3.3.3 (Phillips et al., 2006). The "maximum iterations" threshold was set to 5000 and the "convergence threshold" was left at the default (0.00001); these two parameters determine the stopping point for the maximization algorithm. The "regularization multiplier", which controls the degree of over or under fitting of the model, was set to the default of 1. Duplicated localities were eliminated, meaning if multiple collections were from the same grid square, only one was randomly selected to be retained. Five independent runs of each niche model were combined to get the average. Model performance was evaluated by cross-validation using the area under the receiving operating characteristic curve (AUC). The AUC varies from 0.5 for a model that performs no better than random, to 1.0 for a model that always predicts presence versus absence. There are known issues with using the AUC for testing ecological niche models obtained from presence-only data, not least because AUC values are strongly affected by the extent of the background from which pseudo-absences are drawn (VanDerWal et al., 2009). Notably AUC values are usually higher for species with narrow ranges in comparison to the study area (Phillips 2010). This problem can be avoided by reducing the background points to a fixed area surrounding occurrence points (VanDerWal et al., 2009). The background region was delimited in three different ways, namely using: 1) the MaxEnt default of the whole region of interest, 2) the union area of circles of radius of 80 km around each occurrence (following Joly et al., 2014), and 3) the union area of circles of radius of 200 km (following VanDerWal et al., 2009). One thousand pseudo-absences were sampled at random within each background region to train the model.

Niche overlap for each pair of niches generated under the same conditions was calculated in ENMTools (Warren et al., 2010) using the "D" similarity statistic, developed by Schoener

(1968), and applied to environmental niche models by Warren et al. (2008). The D statistic describes the difference between two niche models in the predicted probability of presence across a study area, scaled from 0 (no overlap) to 1 (identical models). Two measures were calculated to test whether different species' niches were statistically differentiated using ENMTools (Warren et al., 2010). Both tests were run using 100 pseudo-replicates. Firstly, the niche identity test assesses whether the environmental niches of two species are indistinguishable by comparing the observed overlap. Niche identity is a stringent test for niche similarity and usually can only find two niches to be identical if the ranges of the two species being compared are also fully overlapping. Secondly, the background similarity test attempts to overcome this limitation and assesses whether the observed niche overlap can be attributed to the general environmental conditions that are available within the accessible area of one species. It is a two-tailed test, which is therefore calculated for each species separately. Due to computer memory constraints the background test was only run to compare between the niches of *M. antarctica* subsp. *antarctica* and *M.* "Volcanic Plateau". Background points used in this analysis were a random subsample of the background points from the union area of circles of radius of 80 km around each occurrence.

A principal components analysis (PCA) was undertaken on the environmental data extracted from each of the four (LENZ), seven (WorldClim) and nine (WorldClim + LENZ) layer datasets. PCA was undertaken in R using the ***prcomp*** function from the "Stats" package. The data was centred and scaled. The broken stick model was implemented using the "vegan" package (Oksanen et al., 2015) to assess how many principal components were relevant to retain to explain the variation in the data. The relevant PCs were then used as input for Bayesian model-based clustering using the "mclust" package (Fraley and Raftery 2002; Fraley et al., 2012). The function ***Mclust*** identifies the probable number of clusters present using Bayesian information criteria (BIC), and assesses the classification uncertainty of each individual to its assigned cluster. Using default "mclust" settings, 14 models and 1–9 clusters (K) were assessed by the BIC.

Integration of morphological, molecular and niche modelling data

The environmental data of the geographic locations of the 31 populations common to the morphological and molecular datasets were extracted. Data from both the seven WorldClim layers and the four LENZ layers (Table 5.1) were extracted; the four Subantarctic *M. antarctica* populations were excluded from the analyses that used the LENZ layers. Following Edwards and Knowles (2013), the distance matrices of both the

WorldClim and LENZ datasets were calculated using the Gower's matrix, and the nMDS points were concatenated to the nMDS points of the morphological and molecular datasets as described in Chapter 4. The number of clusters present in the dataset was explored using the "mclust" R package as described above. The non-concatenated datasets were also analysed using "mclust", as were pairs of datasets e.g. molecular and environmental datasets.

Taxonomic treatment

The taxonomic treatment was assembled in R using the function ***tableToDescription*** from the package "MonographaR", which facilitates writing parallel descriptions (Reginato 2016). Descriptions are based on morphological data measured or observed as detailed in Chapter 2 on herbarium specimens from WELT (n = 54), CHR (n=33), OTA (n = 5), AK (n=4), K (n = 3) and UPS (n = 2) (see Appendix 2). The description of *M. antarctica* is based on a total of 73 specimens, *M. brevis* on a total of 14 specimens and *M. glauca* on a total of 16 specimens. The description of *M. antarctica* is based on a higher number as it is made up of specimens originally identified as *M. drucei* (n = 21), *M. pygmaea* (n = 16), *M. antarctica* s.s. (i.e. from Campbell Island n = 15 and Chile n = 4), *M*. "Volcanic Plateau" (n = 9) and *M.* "intermedia" (n = 8). Measurements from an additional 39 plants grown in common garden conditions in a growth chamber were also taken into account (*M. antarctica* subsp. *antarctica* (as *M. drucei*) n = 11; *M. antarctica* subsp. *traillii* (as *M. pygmaea*) n = 18, *M. brevis* n = 7; *M. glauca* n = 1). Morphological terminology follows the latest Boraginaceae treatment (Weigend et al., 2016). Specifically, the appendages found between the corolla lobes are called "faucal scales" here (vs. "corolla scales" in Moore 1961), "distal cauline leaves" is here used to refer to what Moore (1961) called bracts, "trichomes" is used here instead of "hairs", and the word "ribbed" is used to described the nutlet margins, rather than "winged" (Webb and Simpson (2001). Author names and journal abbreviations follow the International Plant names Database (IPNI; http://www.ipni.org/). Phenology and habitat information were taken from all data-based pygmy forget-me-not herbarium specimens housed at AK, CHR, CONC, K, OTA, S, UPS and WELT. Chromosome counts were taken from published papers (Beuzenberg and Hair 1983; Murray and de Lange 2013). Botanical drawings were done by myself in ink on tracing paper. The habit drawings were based on photographs of live specimens in the field, whereas close ups were based on photographs of live plants grown in the growth room (Chapter 2), or in some instances were based on herbarium specimens. The ink drawings were coloured with pencils after being scanned, resized electronically, and scale bars added.

Determining threat status and rarity type

The threat status of each newly circumscribed species and subspecies was assessed following the guidelines of the New Zealand Threat Classification System (NZTCS; Townsend et al., 2008). Data required to determine the threat status, i.e. population size and area of occupancy of each population, was recorded in the field when on collecting trips between 2011 and 2015. Each species' extent of occurrence (EOO) and overall area of occupancy (AOO) were measured using the GeoCAT online tool developed by Kew's Spatial Analysis team (http://geocat.kew.org/editor). The niche modelling occurrence points were uploaded to the website in .csv file format, and the "grid size", which defines how large an area around each occurrence point the species inhabits, was estimated to be the smallest unit allowed by the online tool (100 m^2) based on the average area of occupancy of populations in the field (see Tables 4.4 and 5.5). The number of additional populations not visited was estimated based on herbarium records from AK, CHR, OTA and WELT. Levels of decline were estimated based on the number of historical locations visited at which target plants could no longer be located.

In addition to threat status, rarity type was assessed following Rabinowitz (1981) and Reilly (2010). Rarity type differs from threat status in that it is conceptualising the way a species is rare, but not attempting to describe that species' risk of extinction, so not taking into account population size, or trends over time. Additionally, rarity type takes into account niche breadth, which is not explicitly taken into account in the NZTCS. Never-the-less there is a relationship between rarity type and threat status, whereby rarity types 1–5 (see Table 1.1) were found to be more threatened (Reilly 2010). Reilly (2010) developed a framework to quantifiably assess the rarity type of a species using measures of range, abundance and habitat specificity. However, a part of her methodology requires abundance (presence, absence and frequency count) data from a series of vegetation survey plots. The data from such plot systems are available in New Zealand (e.g. Land Use and Carbon Analysis System (LUCAS) plots https://www.mfe.govt.nz/sites/default/files/measuring-carbon-emissions.pdf and the New Zealand Department of Conservation (DOC) Tier One biodiversity monitoring and reporting system http://www.doc.govt.nz/our-work/monitoring-and-reporting-system/). However, pygmy forget-me-nots (and *Myosotis* in general) have been recorded in those plots so few times due to their frequently sparse distributions (Brandon 2001) as to make the sample size too small to be of use. Furthermore, Reilly (2010) did not assign concrete number values to range size, abundance and habitat specificity for each category, instead making the point that the transition between "large" and "small" for each criterion is

relative and depends on the scale and flora of the area under study. For the pygmy forget-me-not group, abundance was estimated from average population size data, range size was measured as their extent of occurrence (see previous paragraph), and habitat specificity was approximated in two ways. Firstly, habitat specificity was quantified from niche breadth estimates calculated in ENMTools (Nakazato et al., 2010; Warren et al., 2010), and secondly by quantifying the number of environments that each species is found in, as defined by the LENZ environmental classification levels I-IV available from the New Zealand Ministry for the Environment (https://data.mfe.govt.nz/). Information about how the levels were generated is available in the technical guide (Leathwick et al., 2002). Local population sizes were considered small if they comprised ≤ 500 individuals, or large if they had > 500 individuals, based on the cut-off criteria between Threatened and Not Threatened categories in the New Zealand Threat Classification manual (Townsend et al., 2008). The criteria for deciding whether a geographic range was large or small was set to 20,000 km^2, based on the cut-off between "Vulnerable" and "Near threatened" in the IUCN Red list threat criteria (as extent of occupancy is not explicitly taken into account in the NZTCS). Whether habitat specificity was wide or narrow was taken from the niche breadth estimates; niche breadth ≥ 0.5 was considered broad.

As another measure of potential risk to extinction, the percentage of populations for each species that are growing on land managed by the New Zealand Department of Conservation (DOC) was calculated. The GIS layer "DOC public conservation areas" was downloaded from https://koordinates.com/.

Assessing genetic structure and variation

Previously, microsatellite markers were developed (Chapter 3) and 497 pygmy forget-me-not group individuals were genotyped at 12 loci (Chapter 4). To assess genetic variation for each species and subspecies newly circumscribed in this chapter, the following were calculated in GenAlEx 6 (Peakall and Smouse 2006; Peakall and Smouse 2012): average observed number of alleles (N_A), the effective number of alleles (N_E), expected heterozygosity (H_E), observed heterozygosity (H_O), F_{IS} (observed heterozygosity of an individual relative to the expected heterozygosity of individuals in the population), F_{ST} (expected heterozygosity of individuals within a subpopulation relative to the total expected heterozygosity of individuals across all populations), and the percentage of polymorphic loci (%P). This data was used here to look for differences in population genetic variation between species with different rarity and threat types.

Results

Ecological niche modelling

Different environmental variables contributed differently to each species' modelled niche (Figure 5.2). In many instances the environmental variable that was most important to estimating a species' niche was a variable for which that species had a relatively extreme mean value. For example "Biol1 mean annual temperature" was important to building the niche of *M. antarctica* subsp. *antarctica* (contributing 45%), and the mean for that entity was the lowest 6.6 °C vs. 7.0–10.2 °C for the other pygmy forget-me-nots (Figure 5.2). A second example is that of the importance of "Soil particle size" to estimating the niche of *M*. "Volcanic Plateau" (contributing 37%). The mean for that entity was 2.0 vs. 3.0–3.6 for the other species (Figure 5.2).

Model fit under different datasets and background sampling strategies

Area under the receiving operating characteristic curve (AUC) values ranged from 0.75–0.98 for all models generated in MaxEnt (Table 5.2). As a rough guide scores from 0.7–0.8 are often considered "fair", 0.8–0.9 "good" and 0.9–1 "excellent". As predicted, species and subspecies with narrower ranges tended to have higher AUC values. For example, the average AUC for North Island *M*. "Volcanic Plateau" was 0.98 vs. 0.76 for *M. antarctica* subsp. *traillii* (Table 5.2). The differences in AUC values between niches modelled using the different background sampling methods were small, with AUC values either increasing or decreasing as the area the background points were sampled from was reduced (Table 5.2). In some cases, the species, subspecies and tag-names with the highest AUC rates, e.g. *M.* "Volcanic Plateau", actually showed a relatively poor fit to the model as assessed by commission/omission rates (Figure 5.4), probably due to the small sample size. The AUC values did not differ much between the LENZ only, LENZ+WorldClim and WorldClim only datasets (Table 5.2), although the LENZ+WorldClim models usually had the highest AUC score. However, the niches modelled sometimes differed markedly, e.g. for *M.* "Volcanic Plateau" (see maps in Figure 5.3). This is likely due to the importance of one of the LENZ layers (soil particle size) to building the model when that environmental layer was included (Figure 5.2). The projected maps of modelled niches for each species and subspecies built using default background sampling points and the LENZ+WorldClim layers (in most cases) can be seen in Figure 5.3.

Niche overlap

Niche overlap between the niches modelled using the LENZ+WorldClim layers ranged from 0.11 to 0.62 (Table 5.3). Niche overlap between the niches modelled using the LENZ only dataset could not be calculated due to computer constraints (the input and output files for that dataset frequently exceeded 20GB each). Niche overlap between *M.* "Volcanic Plateau" and *M. antarctica* subsp. *antarctica* increased from 0.17 to 0.56 when analysing the WorldClim data only as compared to the LENZ+WorldClim layers (Table 5.3). The niche identity test (based on the LENZ+WorldClim data) found that the niches of *M. brevis* and *M. glauca* were identical, but no other tested pairs were (data not shown). The niche background similarity test found that the *M.* "Volcanic Plateau" niche was not significantly different from that of *M. antarctica* subsp. *antarctica*. However, this is a pairwise test, and the opposite test, found the niche of *M. antarctica* subsp. *antarctica* to be significantly differentiated from that of *M.* "Volcanic Plateau".

The PCAs of each dataset are shown in Figure 5.5. As was found in the niche overlap analyses above, all species showed high levels of overlap. The distribution of points can be linked to geographic location, i.e. in Figures 5.5B and C the y-axis corresponds to latitude from south (below) to north (above) whereas the x-axis appears to correlate loosely to whether plants are growing coastally (left) or inland (right). In Figure 5.5A the y-axis corresponds to latitude from south (above) to north (below) and the x-axis is similar to Figures 5.5B and C. The "mclust" analyses found between six to eight significant groups in each dataset, but none of these correspond to species or lineages identified in Chapter 4 (data not shown). Only Campbell Island *M. antarctica* plants formed a distinctive geographic group in one of the datasets (visibly separated in Figure 5.5C).

Integrated analyses

The integrated analyses including morphological, molecular and ecological data for the 31 individual dataset found two groups; these nearly correspond to *M. brevis* vs. the remaining samples (Figure 5.6). When the ecological data was based on the LENZ layers (therefore excluding the four *M. antarctica* samples from Campbell Island), two populations not identified as *M. brevis* also grouped with the *M. brevis* cluster (LT & CL). The population LT was identified as "*M. drucei*" (i.e. *M. antarctica* subsp. *antarctica*) based on morphology (Chapter 2), but genetically was affiliated to *M. brevis* (Chapter 4). The population CL was identified as *M. glauca*?. When the concatenated ecological data was based on the seven WorldClim layers (therefore including the four *M. antarctica* samples from Campbell Island) the *M. drucei* LT population no longer clustered with *M. brevis*. The

M. glauca? CL population did, but with high uncertainty (0.47). When both ecological datasets were concatenated just with the molecular data, no groups were identified in either analysis (data not shown). When just the morphological and ecological datasets were concatenated, two different groups were identified based on the LENZ and WorldClim data (data not shown). In the analyses with the WorldClim layers, the four *M. antarctica* samples from Campbell Island formed a group (CT, AZ, HB & HW). In the analyses with the LENZ layers, four *M. antarctica* subsp. *antarctica* samples form a cluster (TP, CO, MM, MA); there are five additional populations identified as *M. antarctica* subsp. *antarctica* in this dataset that fall into the other group, but these are all assigned with uncertainty (P1&2, CP, BP, & M3).

Assessing threat status and rarity type

Taking into account evidence of census size and area of occupation, *Myosotis antarctica* subsp. *antarctica* is recommended to be classified as Naturally Uncommon, whereas *M. antarctica* subsp. *traillii*, *M. brevis* and *M. glauca* are all recommended to be classified as Nationally Vulnerable (Table 5.5). *Myosotis glauca* and *M. antarctica* subsp. *traillii* fit the criteria for Nationally Vulnerable based on both census size and small areas of occupancy. *Myosotis brevis* has a large enough census size that it could be considered Naturally Uncommon, but its small area of occupancy (Table 5.4) and fluctuating population size (Rogers et al., 2002) means it is better placed in Naturally Vulnerable (see Table 5.5 for details).

Myosotis brevis was found to have the largest estimated census size (17,600) even though it had the smallest number of populations (35). Conversely *M. antarctica* subsp. *antarctica* has the highest number of estimated populations (299), but the smallest estimated average population size (40) (Table 5.5).

The hypothesised rarity type for each species or subspecies is listed in Table 5.4. The geographic range of each species, as measured by extent of occupancy, ranged from 14,000 km^2 for *M. glauca*, up to 244,000 km^2 for *M. antarctica* subsp. *antarctica*, when specimens from South America were excluded (Table 5.5). Abundance, as measured by average population size (see Table 4.4 for individual population estimates), ranged from 40 plants per population for *M. antarctica* subsp. *antarctica*, up to 500 plants per population for *M. brevis* (Table 5.5). The narrowest niche breadth belonged to *M. glauca*, at 0.24, whereas *M. antarctica* subsp. *traillii* had a niche breadth of 0.59. *M. glauca* also inhabited the least number of environments when considering any of the four levels of the LENZ classifications, whereas *M. antarctica* subsp. *antarctica* inhabited the most (Table

5.5). The percentage of populations growing on DOC managed land ranged from 24–72 % (Table 5.5).

Assessing genetic structure and variation

The genetic structuring (Chapter 4) of individuals within the pygmy forget-me-not group is summarized in Figure 5.7. The genetic variation contained in each newly circumscribed species is detailed in Table 5.6. All species or subspecies had lower observed heterozygosity than expected, and overall *M. brevis* had the lowest observed heterozygosity, and highest F-statistics (Table 5.6). *Myosotis glauca* had the lowest percentage of polymorphic loci, but also the smallest number of sampled populations (Table 5.6).

Discussion

Considering data from morphological, genetic and ecological niche modelling sources to delimit species within the pygmy forget-me-not group helps overcome the difficulties associated with species delimitation in a species radiation that has occurred over a relatively short time-frame (Winkworth et al., 2002; Meudt et al., 2015). Taking into account the ecological niche modelling data presented here, and in conjunction with morphological data (Chapter 2) and molecular data (Chapter 4), three species are recognised within the pygmy forget-me-not species group: *Myosotis antarctica*, *M. glauca* and *M. brevis*. Two subspecies of *M. antarctica* are also here recognised: *M. antarctica* subsp. *antarctica* and *M. antarctica* subsp. *traillii*. Note that *M. antarctica* is now circumscribed to include *M. antarctica* (sensu Moore 1961), *M. drucei* and *M. pygmaea* (Table 5.7). Furthermore *M. antarctica* subsp. *traillii* corresponds to the entity known by New Zealand botanists as *M. pygmaea*, although as the notes sections below the relevant description explains, that name has been misapplied. *Myosotis brevis*, *M. glauca* and *M. antarctica* subsp. *traillii* are all assessed as being Threatened (Nationally Vulnerable), whereas *M. antarctica* subsp. *antarctica* is assessed as being At Risk (Naturally Uncommon). For all of these species the majority of their genetic variation is partitioned between rather than within populations, meaning that conserving as many populations as possible should be the priority to minimise risk of extinction (Frankham et al., 2010).

Modelling the niches of pygmy forget-me-nots

In general, the environmental space inhabited by pygmy forget-me-nots was not found to be highly variable, probably reflecting the oceanic climate they inhabit (Figure 5.2). For example, mean precipitation seasonality only ranged from 13.1–20.5 mm, compared to

43.4–129.3 mm for a similar study of wild tomatoes from South America (Nakazato et al., 2010). Similarly, the range in mean annual temperature was only 3.6 °C (6.6 – 10.6 °C) for the pygmy forget-me-nots vs. 8 °C for the tomatoes (13.8 – 21.8 °C). Overall the niches of *M. brevis* and *M. glauca* are characterised by low slope, relatively high mean temperature of the wettest quarter, and low mean annual rainfall (Figure 5.2). Even though their niches were found to be identical, their projected distributions were different, particularly around the coastal areas of southern North Island (Figure 5.3). The niche of *M.* "Volcanic Plateau" most obviously differed from that of *M. antarctica* subsp. *antarctica* based on the average soil particle size, but given the background test of niche similarity found that *M.* "Volcanic Plateau" did not differ from *M. antarctica* subsp. *antarctica*, there is not evidence that the two entities inhabit different niches. The niches of *M. antarctica* subsp. *antarctica* and *M. antarctica* subsp. *traillii* differ based on slope and mean annual temperature (Figure 5.2). However, they share quite high niche overlap (0.35; Table 5.3), even though their niches were not found to be identical. When the niche of *M. antarctica* subsp. *antarctica* was modelled across its entire range (i.e. including Chile) the projected maps do not fit the known distribution well (map for Chile shown in Figure 5.3G), probably due to the low number of locations able to be included in the model.

The success and accuracy of niche modelling depends heavily on the underlying data (Warren 2012). Both the occurrence points and the environmental layers used bring their own sources of error. Deciding which environmental layers to use, and whether these are a good estimation of the niche of the species of interest, is difficult to assess. Furthermore, each environmental layer has itself been modelled, which brings additional sources of error. The size of the grid used can also have important implications. For organisms that are large and mobile (such as a large mammal), 1 km^2 grids could well be a good estimation, but for organisms that are small and less mobile (such as pygmy forget-me-nots), such a scale is most likely concealing some of the important micro-habitat variation. Even a 25 m^2 grid may be too coarse. In cases when the niche has been modelled well, the projected inhabited area can still be very different to a species' geographic distribution. This difference can be due to dispersal barriers, or the difference between the fundamental (or ideal) and realized niche (Warren 2012). Nevertheless, the ecological niches modelled here do appear to have some biological meaning in that they match the current known geographic distributions (as confirmed by the generally high AUC scores [Table 5.2] and the maps in Figure 5.3). However, the 25 m^2 or 1 km^2 grids that the models are based on are likely too coarse. The problem of scale has been noted by other

researchers attempting to model the niches of New Zealand herbaceous plants, even when 25 m^2 scale is being used (Lehnebach 2008; Pufal 2010).

Using ecological niche modelling data for species delimitation

Niche modelling data has been used in species delimitation in the past (Raxworthy et al., 2007) but there is still some debate over how useful a data source it is (Godsoe 2010; Tocchio et al., 2015). Tocchio et al. (2015) argue that niche modelling rarely offers informative data to aid in questions of species delimitation, as follows (see their Figure 6). When geographic distributions of two potentially different species are disjunct, ecological niche modelling provides no extra information to address taxonomic questions. In this case, niche differences may represent vicariant speciation in allopatry, or they may simply indicate that the species inhabits a broad niche (Godsoe, 2010). By contrast, when geographic distributions are contiguous and ecological niches are different, interruption of gene flow and species-level differentiation can be inferred. Finally, when geographic distributions are contiguous but niches are similar (as is the case here, see next paragraph), conclusions are limited: either no ecological differentiation has taken place, or ecological differentiation exists that has not been expressed in the niche model, so niche modelling is therefore not helpful for species delimitation. As such, Tocchio et al. (2015) suggest that ecological niche modelling approaches are best used to explore speciation mechanisms, rather than being applied to questions of setting species limits.

In the system explored here, the ranges of *Myosotis antarctica* subsp. *antarctica*, *M. antarctica* subsp. *traillii*, *M. glauca* and *M. brevis* are overlapping, and their niches are also overlapping (ranging from 0.30–0.61; Table 5.3). Ecological niche differences therefore have not manifested or were not captured by the environmental layers assessed here, and ecological niche modelling does not contribute any data to the question of species delimitation for these entities. Whether or not the niche of *M.* "Volcanic Plateau" is different to that of *M. antarctica* subsp. *antarctica* is theoretically harder to assess given their ranges are disjunct (Tocchio et al., 2015). Nevertheless, the background test for niche similarity found no difference between the niche of *M.* "Volcanic Plateau" and *M. antarctica* subsp. *antarctica*. However, this is a pairwise test, and the opposite test, comparing *M. antarctica* subsp. *antarctica* to *M.* "Volcanic Plateau" was found to be significantly differentiated. Interpreting the results here based on how similar results have been interpreted in other studies (e.g., wild tomatoes; Nakazato et al., 2010), means that the area inhabited by *M.* "Volcanic Plateau" contains a higher proportion of desirable habitat for both entities, and is not evidence that the two occupy different niches. Therefore, given

the lack of morphological (Chapter 2), genetic (Chapter 4) and ecological differentiation, there is no evidence to continue to separate pygmy forget-me-not plants from the central North Island (known as *M.* "Volcanic Plateau") from *M. antarctica* subsp. *antarctica*.

Given niche modelling has been shown to provide little useful data for species delimitation in the pygmy forget-me-not group, future niche modelling research could focus on gathering more fine-scale ecological data. For example, data recording boxes could be installed at relevant sites to help assess whether the WorldClim and LENZ layers used in this study are adequately describing the niches of the pygmy forget-me-not group.

Threat status, rarity type, and genetic variation present in the pygmy forget-me-nots: implications for conservation

Myosotis glauca, M. brevis and *M. antarctica* subsp. *traillii* are all assessed as "Nationally Vulnerable", with rarity types 1, 6 and 6 (Table 1.1; Reilly, 2010) respectively (see Table 5.5). By contrast, *M. antarctica* subsp. *antarctica* is best considered as being "At Risk – Naturally Uncommon", but also has a rarity type of 6. Rarity type numbers of 1–4 correspond to the more threatened species in Reilly (2010), so it is interesting here that the three entities that have been assessed as either Threatened or At Risk all share one of the less threatened rarity types (i.e. 6).

Previously it has been suggested that natural rarity (i.e. not declining due to anthropogenic factors) is associated with "old" species nearing extinction (Fiedler 1986) or with newly formed species (Willis 1922). But it is also accepted that species of any age can be rare, having persisted with low numbers for a long time (Stebbins 1980). Both recently expanding and declining populations are more likely to display low genetic diversity, because they are limited by either the few founding or the remaining survivor individuals (Ellstrand and Elam 1993). However, it may be possible to distinguish between rapidly declining vs. expanding scenarios. Binks et al. (2015) argue that declining species are likely to show evidence of recent bottlenecks, while expanding species often exhibit star-shaped haplotype networks. Star-shaped NeighborNet networks appear to be a feature of not only the pygmy forget-me-not group (Figure 5.6), but all of the New Zealand *Myosotis* (Meudt et al., 2015). Taken in conjunction with molecular dating of the genus which estimate the origin of the New Zealand radiation at ~2mya, it is hypothesised that the whole *Myosotis* radiation in New Zealand is relatively young, and the high number of rare and threatened species in the genus is a reflection of that. However, not all species radiations in New Zealand conatin such a high percentage of rare and threatened species,

so other factors, such as pollination and dispersal mechanisms, may be influencing their rarity also.

The three species belonging to the pygmy forget-me-not group are all self-fertilizing, which contributes to the patterns seen in their morphological (Chapter 2) and genetic data (Chapter 4). For all three species, the same pattern whereby most of the genetic variation is partitioned between, rather than within subpopulations is evident (Table 5.6 this chapter, Table 4.4) and is characteristic of selfing species (Frankham 1995; Frankham et al., 2010). The levels of genetic variation partitioned between populations seen here are high compared even to other self-fertilizing plants (e.g. Chen et al. 2008), which could be an indication of low levels of dispersal. The dispersal mechanisms of the pygmy forget-me-nots are most likely water splash, wind, or possibly "foliage as fruit" (Thorsen et al., 2009). Foliage as fruit refers to seeds being eaten and dispersed incidentally by herbivores, and evidence for this has been found in the seeds of *Myosotis* having been located in moa coprolites (Wood et al., 2012). As moa are extinct it is possible that seed dispersal in New Zealand *Myosotis* has declined as a result. The population genetic metrics are similar between the two subspecies of *M. antarctica*, despite the differences in their overall census sizes and their rarity types, except for F_{IS} which is counter intuitively reduced in the threatened *M. antarctica* subsp. *traillii* (Table 5.6). A reduction in % polymorphic loci is evident in the Nationally Vulnerable *M. glauca* as compared to *M. antarctica*, though this could be an artefact of the small number of *M. glauca* populations sampled. *Myosotis brevis* has very low observed heterozygosity compared to the other species and subspecies. This could indicate an even higher rate of selfing than in the other pygmy forget-me-not species; *M. brevis* does have even smaller corollas than the already miniscule corollas of the other species (0.5–1.5 vs. 1.5–4.0 mm diameter). Alternatively—or additionally—the patterns of genetic variation in *M. brevis* could be influenced by their annual life cycle, which in conjunction with their fluctuating populations sizes (Rogers et al., 2002), could lead to high levels of genetic drift and corresponding fixation of alleles (Ellstrand and Elam 1993).

Selfing species require a greater emphasis on conservation of multiple populations than do outcrossers—due to most of their genetic variation being partitioned between populations. Additionally, mutational accumulation is more of a threat (Paland and Lynch 2006), meaning larger population sizes should be conserved when possible (Frankham et al., 2010). This could be challenging for the threatened pygmy forget-me-nots, given their usually small population size to begin with (Table 5.5). Another potential challenge to managing the conservation of pygmy forget-me-nots is that not all populations are found

on land managed by DOC (Table 5.5). Unsurprisingly, the entity that is least threatened, *M. antarctica* subsp. *antarctica*, has the highest proportion of populations growing on land managed by DOC (75 %; Table 5.5). *Myosotis antarctica* is also the species with the highest elevational range (sea level to 2300 m). This too is not an unexpected pattern; it has often been recognised that lowland plants are most at risk in New Zealand, due to greater levels of habitat modification (Rogers and Walker 2002). Most of the protected land in New Zealand is at higher elevation, and is less attractive for development. The pygmy forget-me-not that has the highest rate of decline, *M. antarctica* subsp. *traillii* (Table 5.5), inhabits the lowland for most its range (sea level to 250 m), and has a relatively low percentage (ca. 25 %) of populations growing on DOC managed land. Populations that are at particular risk, or may be particularly important to conserve due to being genetically or morphologically unusual, are indicated in the notes section of the taxonomic treatment below.

Summary and conclusions

Integrating data from multiple sources is once again shown to be a useful method for delimiting species, even in recently radiating species groups. Although niche modelling was shown to be not particularly useful for species delimitation in the pygmy forget-me-nots, the method of integrating data from the same individuals is found to be an effective method for lineage discovery. Multiple lines of evidence have been analysed to study the New Zealand native pygmy forget-me-not group, and as a result a taxonomic revision in which three species are recognised (with two subspecies) has been produced. This is both a taxonomic reduction relative to the taxonomy currently in use, which comprises five species and several tag named entities (de Lange et al., 2010), as well as a taxonomic increase from the latest *Flora* (Moore 1961), which included two species and three varieties. Given the integrated analyses revealed two lineages (*M. brevis* vs. the remainder of the pygmy forget-me-nots; Figure 5.6) only recognising two species was considered. However, the morphological and genetic evidence that unites *M. glauca,* a lineage that is sympatric with respect to *M. antarctica* (both are present in Central Otago), meant that species level was considered the appropriate level to recognise this entity (Steussy 2009). By contrast, the two lineages recognised at subspecies rank are distinguished based only on small morphological differences, are not genetically differentiated, and their ranges are mostly allopatric (inland vs. coastal on the North and South Islands), meaning that subspecies rank is more appropriate (Steussy 2009). The informal name for the group was previously the "*Myosotis pygmaea* species group", but as *M. pygmaea* is no longer recognised, I now refer to this group as the pygmy forget-me-nots. All of the pygmy forget-

me-nots have a sparse distribution, which in conjunction with their small population size, and in some cases reduced geographic extent, means none of these species is common.

Taxonomic treatment

The pygmy forget-me-not group is a subgroup of the bracteate-prostrate group (Robertson 1989; Meudt et al., 2015), the limits of which are currently being addressed elsewhere as part of an ongoing project to revise the taxonomy of all native New Zealand *Myosotis* (Meudt et al. unpubl. data). The key and descriptions are based on herbarium material and live plants grown under standardised conditions in the growth room (see Methods). This key is for plants in the pygmy forget-me-not group only, and requires flowering or fruiting material.

All pygmy forget-me-not plants have the following characteristics: decumbent annual, biennial or perennial rosette herbs, with multiple prostrate flowering and fruiting branches upon which each flower is associated with a cauline leaf (i.e., "bracteate prostrate"). Rosette leaves are obovate, with lamina (excluding the petiole) ranging from 2.0–68.0 mm long × 0.8–27.0 mm wide. Flowers, calyces and nutlets are small: corolla diameter 0.5–4.0 mm, corolla lobe length < 1.5 mm, corolla tube length < 3.0 mm; anther placement usually wholly (but at least partly) below the faucal scales, anthers < 1.0 mm long, anthers (sub)sessile (i.e. filament length of 0–0.3 mm); style length < calyx length, calyx lobed about half way to the base, calyx length at flowering < 3.5 mm; pedicel length at fruiting < 2.0 mm. Nutlets 4, 0.9–1.9 mm long × 0.5–1.2 mm wide, margins scarcely forming ribs, sometimes only at apex, glossy brown-black when mature. Trichomes are densely distributed and antrorse on leaves, stems and calyces; they can be straight, flexuous or curved, and can vary from appressed to erect. Retrorse trichomes are never present on the leaves, but are occasionally a feature of the calyces. Hooked trichomes are not present anywhere on pygmy forget-me-nots. Number of branches and flowers per branch are not distinguishing characteristics, and leaf size, branch length and internode length are also highly plastic. The plasticity of these characters was revealed when growing plants in the common garden (Chapter 2), whereby plants in the common garden grow much larger than their parent plants in the field: this is demonstrated in Figure 2.7 and Appendix 3. Measurements at the extreme high end of ranges given in brackets in the descriptions below are almost always from growth room grown plants.

Key

1a. Corollas 0.5–1.5 mm in diameter; calyx at flowering 0.7–1.7 mm long, at fruiting 1.7–3.7 mm long; nutlets 0.9–1.2 × 0.5–0.8 mm → 1. *Myosotis brevis*

1b. Corollas 1.5–4.0 mm in diameter; calyx at flowering 1.7–3.5 mm long, at fruiting 3.0–7.8 mm long; nutlets 1.2–1.9 × 0.8–1.2 mm → 2

2a. Trichomes on leaves, calyces and stems straight and appressed; leaves glaucous; Otago and Canterbury only → 2. *M. glauca*

2b. Trichomes on leaves, calyces and stems flexuous or curved, and patent to erect; leaves green to brown; throughout New Zealand (North, South, Stewart, and Campbell Islands), also southern Chile → 3. *M. antarctica*

1. *Myosotis brevis* de Lange & Barkla in de Lange et al., *Threat. Pl. New Zealand,* 437 (2010)

≡ *Myosotis pygmaea* var. *minutiflora* G.Simpson & J.S.Thomson, *Trans. & Proc. Roy. Soc. New Zealand* 73: 161 (1943)

=*Myosotis pygmaea* var. *imbricata* Cockayne, *Veg. N.Z.,* 396 (1928) nom. nud.

TYPE: Lake Lyndon, Canterbury, moist gravel at lake shores, *G.Simpson & J.S.Thomson* (HOLOTYPE: CHR 75725!).

Description: Rosette plants with multiple prostrate branches up to 5(–11) cm long. Rosette leaves 1–9; petioles (0–)0.7–7.0(–11.6) mm long; lamina usually flat, oblanceolate to broadly obovate, 1.1–8.7(–19.2) mm long × 0.9–4.3(–6.8) mm wide (length:width ratio 1.2–2.4(–3):1), green to brown; apex obtuse (or occasionally acute) and mucronate; trichomes densely distributed and often overlapping, flexuous, antrorse, appressed to erect, spreading or sometimes appressed on leaf margins, distributed evenly on leaf adaxial surface, but sparsely distributed, or on leaf midribs only, or glabrous on leaf abaxial surface, (0.2–)0.4–0.9(–1.6) mm long, deciduous with age. Basal cauline leaves not subtending flowers 1–5 per branch, lamina similar in size and shape to the rosette leaves, with petioles up to 2.7 mm; distal cauline leaves subtending flowers up to 30 per branch, lamina 1.3–6.2(–18.1) mm long × 0.5–2.2 mm wide, usually sessile. Flowers up to 30 per branch; pedicels up to 0.7 mm long (flowering) or 0.8 mm long (fruiting). Calyx 0.7–1.7 mm long (flowering) increasing to 1.7–3.7 mm long (fruiting), 0.9–3.2(–4.9) mm wide at the top at fruiting, lobed to 1/3–2/3 the length of the calyx; with trichomes usually of

uniform length, denser along calyx ribs, occasionally of two different lengths, longer and antrorse on ribs vs. shorter and retrorse between ribs and near the base. Corolla 0.5–1.5(–2.0) mm in diameter, white or cream, occasionally pale blue or cream striped with blue; faucal scales yellow; corolla lobes 0.2–0.5(–0.7) mm long × 0.2–0.4(–0.7) mm wide; corolla tube 0.3–0.5(–0.9) mm wide at faucal scales, 0.8–1.6 mm long from base to faucal scales, narrow cylindric. Stamens 5, included; filaments attached below faucal scales, 0–0.1 mm long; anthers 0.2–0.3(–0.5) mm long. Style 0.5–1.2 mm long (flowering) to 0.5–1.6(–2) mm long (fruiting). Nutlets 4, 0.9–1.2(–1.4) mm long × 0.5–0.8 mm wide.

Illustrations: Figures 2.1E–F, 2.6E–G, 2.7A–B & D–E, 5.8. *Threat. Pl. New Zealand* (de Lange et al., 2010:300–301). *Seeds of New Zealand* (Webb and Simpson 2001:142) as *M. pygmaea* var. *minutiflora.*

Phenology: Flowering September–April. Fruiting October–April. Peak flowering and fruiting October–December.

Chromosome number: Unknown.

Distribution: North Island: Taranaki, Wairarapa and Wellington coasts; South Island: Canterbury and Otago; see map in Figure 5.3D.

Habitats: North Island: shore platforms, cliff top herb fields and turfs, or beach gravels. South Island, Canterbury: in shingle or mud at seasonally inundated lake or tarn edges; Otago: dry, exposed, sunny, sometimes seasonally moist, alpine fell field, cushion field, eroded pasture, or turf. Elevation: sea level to 1900 m.

Representative specimens: see Appendices 2 and 7.

Recommended conservation status: Threatened, Nationally Vulnerable: Extreme Fluctuations, Sparse (see Table 5.5).

Notes:

Identification: *Myosotis brevis* is the smallest New Zealand forget-me-not. Plants of this species can be distinguished from all other *Myosotis,* including other pygmy forget-me-nots, based on their smaller corolla diameter of 0.5–1.5(–2.0), smaller calyx length at flowering of 0.7–1.7 mm and smaller nutlet size of 0.9–1.2(–1.4) mm long × 0.5–0.8 mm wide. When not in flower or fruit, plants of *M. brevis* can be difficult to distinguish from small plants of *M. antarctica* subsp. *antarctica*, as plants of both species have flexuous

trichomes. However, plants of *M. brevis* are usually spring annuals (Rogers et al., 2002; de Lange et al., 2010), and it is rare to find plants that are not in either flower or fruit.

Taxonomic history: *M. brevis* was first described as a variety of *M. pygmaea* (as var. *minutiflora*; Simpson and Thomson 1943). It was then elevated to species rank based on its morphological distinctiveness (de Lange et al., 2010). Species is considered the best rank to recognise this entity given the multiple discrete morphological characters and molecular evidence that unites it (see below). The morphological description given here differs subtly to that given by de Lange et al. (2010). For example, the description here records rosette leaves as being smaller (1.1–8.7 × 0.9–4.3 vs. 8–25 × 4–10 mm), and calyx length at both flowering (0.7–1.7 vs. 2–3 mm) and fruiting (1.7–3.7 vs. 3–5 mm) as being shorter. Additionally the description here allows for slightly larger corolla diameter (0.5–1.5 vs. 0.5–1 mm). These differences are considered minor.

Patterns in the data: *M. brevis* specimens are united both morphologically (Chapter 2) and genetically (Chapter 4). In the nMDS analyses of morphological characters measured on herbarium specimens, all samples of *M. brevis* group together (Figure 2.3A). All plants of *M. brevis* were even more obviously differentiated from other pygmy forget-me-nots when grown in the growth room (Figure 2.5A). Multiple morphological characters were found to significantly differentiate *M. brevis* from other pygmy forget-me-not species in both the herbarium and growth room datasets, e.g., length of calyx at fruiting, floral lobe length and nutlet length (Tables 2.5 and 2.6). When molecular data from microsatellites was integrated with the morphological data from herbarium specimens, all populations identified as *M. brevis* formed a significantly differentiated cluster (Figure 4.5A). When analysing just the molecular data, all *M. brevis* populations fall into a single cluster in the Structure analyses of K = 3 (Figure 4.3), and most populations of *M. brevis* form a group in the NeighborNet network (Figure 4.4).

However, plants from three populations not identified as *M. brevis* also grouped genetically with *M. brevis* in the Structure analyses of K = 3 (Figure 4.3): H1-3 (*M. antarctica* subsp. *traillii*, WELT SP090629, SP090631 and SP090634), LT (*M. antarctica* subsp. *antarctica*, WELT SP100425) and HB & HW (*M. antarctica* subsp. *antarctica*. WELT SP102779 and SP102780) (see Table 4.4 or Appendix 2 for explanation of population codes). This may be due to an effect similar to that of long branch attraction (Chapter 4). The population from Lake Tennyson (LT) was identified as *M. antarctica* subsp. *antarctica* (not *M. brevis*) due to nutlet size (1.4 × 1.0 mm vs. the range for *M. brevis* nutlets of 0.9–1.2 × 0.5–0.8 mm), but given it shares a habitat and morphology otherwise similar to *M. brevis*,

and plants at this location have been identified as *M. brevis* previously (Rogers et al., 2002), this is a difficult population to classify.

Threats: The main threats to *M. brevis* are habitat loss, and invasive weeds leading to overshading (de Lange et al., 2010). North Island populations of *M. brevis* are more at risk than the South Island populations. The North Island populations are smaller on average (190 vs. 1000 plants per population), and cover a smaller area on average (34 × 34 m vs. 83 × 83 m). The *M. brevis* bare pavement habitat in Otago may be increasing (Rogers et al., 2002), whereas the coastal habitat in the North Island is at risk, e.g., the populations PU, NG and TO (WELT SP090361, SP090545 and SP090550) grow in LENZ habitat types C and J which are considered acutely threatened (DOC 2014). Furthermore, none of the North Island populations inhabit Department of Conservation managed land (Table 5.5). The populations around the Taranaki coast appear particularly precarious; for example the population visited at Puketapu Road (PU; WELT SP090361) is less than 2 m from the edge of an eroding cliff, but the population cannot migrate inland due to farmland (pers. obs. 2011). At least one population that has recently (~2005) gone extinct in the East Cape was most likely *M. brevis*; its habitat is thought to have been destroyed by wild goats (G. Atkins pers. comm. 2012). The two most genetically distinct *M. brevis* populations are one from the North Island (ST; WELT SP090543) and one from the South Island (BE; WELT SP102760) (Figure 4.4); these could be prioritised when it comes to potential conservation effort.

2. ***Myosotis glauca*** (G.Simpson & J.S.Thomson) de Lange & Barkla in de Lange et al., *Threat. Pl. New Zealand,* 438 (2010)

TYPE: Base of Mt Ida at 500 m altitude, grassland, *Simpson & Thomson* (HOLOTYPE: CHR 75722!)

≡ *Myosotis pygmaea* var. *glauca* G.Simpson & J.S.Thomson, *Trans. & Proc. Roy. Soc. New Zealand* 72: 26 (1942)

Description: Rosette plants with multiple prostrate branches up to 12 cm long. Rosette leaves 4–15(–100); petioles 1.5–8.9(–22.5) mm long; lamina usually flat, curling when grown in the growth room, narrowly oblanceolate to broadly obovate, 3.7–16.9(–52.4) mm long × 1.5–7.0(–15.3) mm wide (length:width ratio 1.3–3.6(–5.9):1), dull greyish green (glaucous) or occasionally bright green; apex obtuse and mucronate; trichomes sparsely distributed, straight, antrorse, appressed to patent, appressed on leaf margins, distributed evenly on leaf adaxial surface, but usually glabrous, occasionally sparsely

distributed and on leaf ribs only on leaf abaxial surface, (0.2–)0.4–0.8(–1.2) mm long, deciduous with age. Basal cauline leaves not subtending flowers 1–5 per branch, lamina similar in size and shape to the rosette leaves, with petioles up to 7.3 mm; distal cauline leaves subtending flowers up to 19 per branch, lamina 1.8–11.4 mm long × 1.0–4.9 mm wide, usually sessile. Flowers up to 19 per branch; pedicels up to 1 mm long (flowering) or 1.8 mm long (fruiting). Calyx 1.6–3.3 mm long (flowering) increasing to 2.5–7.8 mm long (fruiting), 1.3–4.3 mm wide at the top at fruiting, lobed to 1/4–1/2 the length of the calyx, with trichomes usually only along ribs both inside and outside the calyx, but occasionally present in between ribs. Corolla (1.0–)1.4–4.0 mm in diameter, white; faucal scales yellow; corolla lobes 0.3–1.3 mm long × 0.2–1.0 mm wide; corolla tube 0.4–1.1 mm wide at faucal scales, 1.2–2.5(–3.2) mm long from base to faucal scales, narrow cylindric. Stamens 5, included; filaments attached below faucal scales, 0–0.1 mm long; anthers 0.4–0.9 mm long; style 0.8–2.3 mm long (flowering) to 0.9–2.8 mm long (fruiting). Nutlets 4, (1.0–)1.2–1.5 mm long × (0.7–)0.8–1.2 mm wide.

Illustration: Figures 2.1A–B, 2.6A–D, 5.9. *Threat. Pl. New Zealand* (de Lange et al., 2010:404–405). *Seeds of New Zealand* (Webb and Simpson 2001:142) as *M. pygmaea* var. *glauca*. *Above the Treeline* (Mark 2012:257). *Flora of New Zealand* (Moore 1961:808) as *M. pygmaea* var. *glauca*.

Phenology: Flowering September–March. Fruiting October–April. Peak flowering and fruiting December–January.

Chromosome number: Unknown.

Distribution: South Island, Otago and Canterbury. See map in Figure 5.3A.

Habitats: Fine semi-consolidated gravels on lake, tarn or stream edges, erosion fans, the base of tors, or old mine tailings. Depleted tussock-grassland, low grass turf. Elevation: 180–1500 m.

Representative specimens: See Appendix 2 (specimens identified as *M. glauca* and *M.* aff. *glauca*) and Appendix 8.

Conservation status: Threatened, Nationally Vulnerable, Range restricted, Sparse (see Table 5.5)

Notes:

Identification: *Myosotis glauca* plants can be distinguished from other pygmy forget-me-nots by their straight, appressed trichomes and glaucous grey leaves. *M. glauca* as here circumscribed is only known from Central Otago and southern Canterbury. Specimens identified as *M. glauca* collected from the North Island Central Plateau (e.g. CHR 252337) do not solely have the straight, appressed leaf trichomes that unite all plants that fall under this species. Instead, a small number of straight, appressed trichomes are mixed with flexuous, patent trichomes, and therefore these specimens are better placed as *M. antarctica* subsp. *antarctica*. While most plants of *M. glauca* have glaucous green to grey leaves, some plants with brighter green leaves from the Pisa Range (previously identified as *M.* aff. *glauca*, e.g. WELT SP089898) are otherwise unable to be distinguished from the remainder of *M. glauca.* Leaf colour variation is known from other pygmy forget-me-nots—most notably *M. brevis* (Figure 2.1F)—and thus these *M.* aff. *glauca* specimens are considered here to be *M. glauca,* which is variable in leaf colour. Recent collections of *M. glauca?* (CL; WELT SP103892) from the Clutha outwash are difficult to place due to their unusual combination of glaucous leaf colour with flexuous trichomes and deserve further study (more details below).

Taxonomic history: *M. glauca* was first described as a variety of *M. pygmaea* (as var. *glauca*; Simpson and Thomson 1942). It was then elevated to species rank due to its morphological distinctiveness (de Lange et al., 2010). Species is considered the best rank to recognise this entity given the morphological and molecular evidence that unites it (see below). The morphological description given here differs subtly to that given by de Lange et al. (2010:405). Specifically, two characters they identified as distinguishing *M. glauca* where not found here to be diagnostic, i.e., "…inner calyx surface midline of *M. glauca* is furnished with 4–5 shortly erect, stiff hairs", and "broadly ovate rather than narrowly ovate nutlets (seeds)". The surface of the inner calyx of *M. glauca* specimens was found to be sometimes glabrous, sometimes covered in short stiff hairs, and sometimes as described above by de Lange et al. (2010) (data not shown). The length to width ratio of *M. glauca* nutlets was not found to differ from that of *M. antarctica*, though nutlets of *M. brevis* did have a slightly higher length to width ratio on average (visible in Figure 5.7 vs. 5.8). Additionally the description here has slightly longer corolla tubes to that reported by de Lange et al. (2010) (1.2–2.5 vs. 0.4–0.6 mm). In other respects the descriptions are similar.

Patterns in the data: Specimens of *M. glauca* are united by morphological (Chapter 2) and genetic (Chapter 4) data. In the nMDS analyses of morphological characters measured on

herbarium specimens all samples of *M. glauca* group together (Figure 2.3B). Qualitative morphological characters differentiate *M. glauca* from all other pygmy forget-me-nots i.e., leaf colour (usually glaucous-green to grey), and trichomes that are straight and appressed on the leaf blade and leaf margins (Figures 2.4F and 2.6). In the analyses of microsatellite data all populations of *M. glauca* form a cluster in the Structure analyses above K = 10 (Figure 4.3), and these populations group together in the NeighborNet network also (Figure 4.4).

Specimens identified as *M.* aff. *glauca* (n = 5, see Appendix 2) cluster with those identified as *M. glauca* based on morphological data (Figure 2.3B), and appear to differ only by having brighter green leaves than is usual for *M. glauca*. Only one individual identified as *M.* aff. *glauca* was included in the genetic dataset (RM; WELT SP093282), so little is known regarding genetic relationships. Specimens identified as *M.* aff. *glauca* are therefore here considered part of *M. glauca* based on morphological similarity. Recent collections identified as *M. glauca?* (CL; WELT SP103892) from the Clutha outwash appear to be morphologically intermediate between *M. glauca* and *M. antarctica* subsp*. antarctica* or *M. brevis,* having a glaucous leaf colour yet flexuous trichomes. The nMDS analyses of morphological characters measured on herbarium specimens placed this sample within the cluster containing *M. antarctica* subsp. *antarctica* + *M. brevis* and not *M. glauca*, although with high uncertainty (Figure 2.3). Genetically *M. glauca?* does not appear to be affiliated to *M. glauca* based on the NeighborNet network of microsatellite data (Figure 4.4). This entity possibly represents a hybrid between *M. glauca* and *M. antarctica* subsp. *antarctica* or *M. brevis*; plants of the latter of which have been collected from nearby (WELT SP103893); further collections and research are encouraged.

Threats: The main threat to *M. glauca* is considered to be weed invasion (de Lange et al., 2010). *Myosotis glauca* is the rarest pygmy forget-me-not based on estimated census size, it is only found in Central Otago and southern Canterbury, and only five of its populations (31%) grow on Department of Conservation managed land (Table 5.5). At one of those populations (Lake Ohau, AK 280800), plants of *M. glauca* were not found in 2013 and further searches are recommended. Populations from two locations included in the molecular data set (N1&2; WELT SP093284&5 and M1&2; WELT SP100497), are from areas both managed by DOC. With the recent decision to reject a proposal to dam the Nevis Valley (Environment Court decision, 2013, available at http://www.nzlii.org/cgi-bin/sinodisp/nz/cases/NZEnvC/2013/131.html?query=nevis), the future of populations N1&2 (WELT SP093284&5) has become more secure.

3. ***Myosotis antarctica*** Hook.f. *Bot. Antarct. Voy. I. (Fl. Antarct.) Part I,* 57, t. 38 (1844)

TYPE: "Campbell's Island; on the debris at the base of precipices and in the most exposed places along with *Cardamine stellata* and in clefts of rock on the very summits of mountains" (Hooker, 1844). (LECTOTYPE here designated: K000787899!). Moore (1961) identified that the type specimen was housed at K, but did not indicate a particular specimen. There are two specimens at K collected by Hooker: K000787899 and K000787901. The specimen designated as the lectotype, K000787899, has a "Herbariorum hookerianum 1867" stamp, and a note that reads "1609 *Myosotis antarctica* Hook.f. On rocky debris near the sea and at considerable elevation (1000 ft) Campbells Island Dec 1840". There is also a pencil illustration pinned to the sheet with the number "1609" in the corner. The drawing consists of recognisable drafts of the colour plate published in the *Bot. Antarct. Voy. I. (Fl. Antarct.)* (reproduced here as Figure 5.10). "TYPE specimen!" is written on the sheet in a different pen. There are five plants making up the specimen, which is clearly distinguishable from the other specimen on the sheet (K000787898; collected by J. Kirk, 1884, from "Dog Island"). The second specimen, K000787901, which is on a separate sheet, has a "Herbariorum benthamianum" stamp on it, and a note that reads "*Myosotis antarctica* Hook.f. Fl. Ant. p. 57 & 305 Campbell Island Hooker 1845". Hooker therefore collected these two specimens on the same visit to Campbell Island, but the specimen designated as the lectotype is more clearly linked to the publication of the name.

Description: Rosette plants with multiple prostrate branches up to 15(–31) cm long. Rosette leaves (0–)4–22(–40); petioles 1.0–19.6(–26.0) mm long; lamina margins and apex sometimes curling under, especially in common garden conditions, narrowly oblanceolate to very broadly obovate, 3.1–26.1(–67.5) mm long × 1.5–11.2(–26.9) mm wide (length:width ratio 1.1–3.9(–6.4):1), bright to dull green to reddish–brown, often with red–brown petioles and midveins; apex obtuse and mucronate; trichomes densely distributed and often overlapping, curved or flexuous, antrorse, patent to erect, appressed or spreading on leaf margins, distributed evenly on leaf adaxial surface, but sparsely distributed, or on leaf ribs only, or glabrous on leaf abaxial surface, (0.2–)0.5–1.1(–2.0) mm long, deciduous with age. Basal cauline leaves not subtending flowers 1–5 per branch, lamina similar in size and shape to the rosette leaves, with petioles up to 8.8 mm; distal cauline leaves subtending flowers up to 46(–75) per branch, lamina 1.4–16.0(–31.2) mm long × 0.8–6.5 mm wide, usually sessile. Flowers up to 46(–75) per branch; pedicels up to 1.2 mm long (flowering) or 1.9 mm long (fruiting). Calyx (1.2–)2.0–3.0(–3.5) mm long (flowering) increasing to (2.1)–3.0–6.2(–8.0) mm long (fruiting), 1.5–5.8(–10.2) mm wide

at the top at fruiting, lobed to 1/3–3/4 the length of the calyx; with trichomes sometimes of two lengths, longer and antrorse on ribs vs. shorter and retrorse in between ribs and near the base (in other instances the two length classes are not so obvious, and retrorse trichomes are not always present). Corolla (1.0–)1.5–4.0 mm in diameter, white, cream, blue or occasionally orange; faucal scales yellow; corolla lobes (0.3–)0.5–1.5 mm long × (0.2–)0.4–1.1(–1.3) mm wide; corolla tube 0.5–1.2(–1.5) mm wide at faucal scales, 1.2–2.8(–3.2) mm long from base to faucal scales, narrow cylindric. Stamens 5, included; filaments attached below faucal scales, 0–0.3 mm long; anthers 0.3–0.9 mm long; style (0.7–)1.1–2.3 mm long (flowering) to (0.8–)1.1–2.8(–4.8) mm long (fruiting). Nutlets 4, (1.1–)1.2–1.9 mm long × (0.7–)0.8–1.2 mm wide.

Two subspecies of *M. antarctica* are recognised:

1a. Trichomes on rosette leaves flexuous, patent to erect on blade and margins; usually found at inland localities of the North and South Islands, can be coastal in Fiordland, Campbell Island and Chile → 3a. *Myosotis antarctica* subsp. *antarctica*.

1b. Trichomes on rosette leaves curved, and appressed to patent on lamina surfaces and margins; coastal localities of North, South and Stewart Islands (rarely inland). → 3b. *Myosotis antarctica* subsp. *traillii*

3a. *Myosotis antarctica* subsp. *antarctica*.

= *Myosotis pygmaea* Colenso, *Trans. & Proc. New Zealand Inst.* 16: 334 (1883 [1884])

= *Myosotis ramificata* G.Simpson, *Trans. Roy. Soc. New Zealand* 79: 426 (1952)

= *Myosotis pygmaea* var. *drucei* L.B.Moore in Allan, *Fl. New Zealand 1,* 816, 973 (1961)

= *Myosotis drucei* (L.B.Moore) de Lange & Barkla in de Lange et al., *Threat. Pl. New Zealand,* 438 (2010)

Description: same as for *M. antarctica* except rosette leaf trichomes are flexuous and patent to erect, not curved or appressed.

Illustration: Figures 2.1G–J, 2.6H–K, 2.7C, F, G & J, 5.10 and 5.11. *Above the Treeline* (Mark 2012:256) as *M. drucei. Seeds of New Zealand* (Webb and Simpson 2001:142) as *M.*

pygmaea var. *drucei*. *Wild Plants of Mt Cook National Park* (Wilson 1996:221) as *M. pygmaea* var. *drucei. Stewart Island Plants* (Wilson 1994:245) as *M. pygmaea* var. *drucei. New Zealand Alpine Plants* (Mark and Adams 1973:87) as *M. pygmaea* s.l. *Flora of New Zealand* (Moore 1961:808) as *M. pygmaea* var. *drucei. Die Gefässpflanzen der Magellansländer* (Dusen 1900:134) as *M. albiflora. Bot. Antarct. Voy. I. (Fl. Antarct.) Part I,* (Hooker 1844) plate 38 (reproduced here as Figure 5.10).

Phenology: Flowering August–April. Fruiting September–April. Peak flowering and fruiting December–January.

Chromosome number: Counts from two individuals have been undertaken: 1. as *M. pygmaea* s.l., *n* = 24, CHR 101449 (Beuzenberg and Hair 1983); 2. as *M.* aff. *drucei / M.* "Volcanic Plateau", *n* = 22, AK 331000 (Murray and de Lange 2013).

Distribution: North Island, South Island, Stewart Island, Campbell Island; southern Chile (Magallanes Region).

Habitats: From coastal turf to sub-alpine damp semi-stable scree, cliff faces, incised runnels and fell-fields. Elevation: sea level to 2200 m.

Representative specimens: See Appendix 2 (specimens listed as *M. antarctica*, *M. drucei*, *M.* "intermedia" and *M.* "Volcanic Plateau") and Appendix 8.

Conservation status: At Risk, Naturally Uncommon, Threatened Overseas (see Table 5.5).

Notes:

Identification: *M. antarctica* subsp. *antarctica* can be distinguished from *M. glauca* and *M. antarctica* subsp. *traillii* based on its flexuous, patent to erect trichomes. It can be separated from *M. brevis* due to its generally larger size; e.g. corolla diameter of (1.0–)1.5–4.0 mm, calyx length at flowering of (1.2–)2.0–3.0(–3.5) mm long and nutlets of (1.0–)1.2–1.9 mm long × (0.7–)0.8–1.2 mm wide.

Taxonomic history: *Myosotis antarctica* was first published by Hooker in 1844, from collections he had made on Campbell Island. The name was then also applied to specimens collected from the rest of New Zealand (e.g., WELT SP043359 collected by T. Kirk, 1877, Otago, Dart Valley, identified as *M. antarctica*, and UPS V-702353 collected by J. Hector, collection date unknown but likely in the 1860s, from Mt Aspiring Range, identified as *M. antarctica* Hook.f.). The name was first applied to Chilean specimens by Skottsberg (1915).

In 1884 the name *M. pygmaea* was published by Colenso, based on specimens he collected in the North Island. Although a few specimens collected from mainland New Zealand after this time were still identified as *M. antarctica* (e.g. WELT SP002665 collected by F. Gibbs in 1894, Western Nelson, Mt Arthur, identified as *M. antarctica*), it became common usage to reserve the name of *M. antarctica* for plants from Campbell Island, and use *M. pygmaea* for all plants from the North, South and Stewart Islands, which was formalised in the treatment by Moore (1961). For example, the T. Kirk 1877 specimen mentioned above (WELT SP043359) was re-identified later as *M. pygmaea*. Several varieties of *M. pygmaea* were also published in the 1940s and 1960s, and elevated to species rank by de Lange et al. in 2010 (i.e., *M. pygmaea* var. *glauca* → *M. glauca*, *M. pygmaea* var. *minutiflora* → *M. brevis* and *M. pygmaea* var. *drucei* → *M. drucei*). Two of those entities are recognised here at species rank (see *M. glauca* and *M. brevis* above), but *M. pygmaea*, *M. drucei* and *M. antarctica* are not considered sufficiently differentiated morphologically or genetically to individually warrant that rank, and instead are considered to make up a single species, for which *M. antarctica* is the earliest published name. The specimens that were previously identified as *M. antarctica* and *M. drucei* are considered best recognised as a single entity at the rank of subspecies, as *M. antarctica* subsp. *antarctica*, given they are united by morphological (Chapter 2) but not genetic data (Chapter 4), and they are mostly allopatric with respect to *M. antarctica* subsp. *traillii* (which is made up of specimens that were previously identified as *M. pygmaea*, see below) (Figure 5.3B, E, H).

Not only does this circumscription of *M. antarctica* subsp. *antarctica* comprise several previously described species, it also subsumes two informal tag-named entities currently in use by some New Zealand botanists. Those examined which are here considered to be part of this enlarged *M. antarctica* subsp. *antarctica* are *M.* "Volcanic Plateau" (e.g., CHR 244442) and *M.* "intermedia" (e.g., WELT SP089911). An additional published name, which has not been applied since its publication, *M. ramificata* (Simpson 1952) was also unable to be distinguished morphologically from this circumscription of *M. antarctica* subsp. *antarctica* (Figure 2.3).

Patterns in the data: Specimens of *M. antarctica* subsp. *antarctica* are united morphologically (Chapter 2) but not genetically (Chapter 4). In the nMDS analyses of morphological characters measured on herbarium specimens all samples of *M. antarctica* subsp. *antarctica* group together (identified as *M. antarctica*, *M. drucei*, *M.* "intermedia" and *M.* "Volcanic Plateau"; Figure 2.3A). All plants of *M. antarctica subsp. antarctica* were even more obviously differentiated from other pygmy forget-me-nots when grown in the growth room (Figure 2.5A). Qualitative morphological characters found in both the

herbarium and growth room datasets differentiate *M. antarctica* subsp. *antarctica* from *M. glauca* and *M. antarctica* subsp. *traillii,* i.e., trichomes that are flexuous and patent to erect on the leaf blade and leaf margins (Figures 2.4F and 2.6). Despite the similarity in trichome types, multiple quantitative morphological characters distinguish this entity from *M. brevis* (see notes under that species).

The only morphological character found to distinguish between specimens identified as *M. drucei* and an earlier narrow circumscription of *M. antarctica* that referred to plants from Campbell Island and Chile only, was that of trichome density. However, the ranges in trichome density overlap considerably, and so this character is not considered useful in this context (Table 2.5). Furthermore, no morphological characters were found to differentiate specimens identified as *M.* "Volcanic Plateau" or *M*. "intermedia" (Chapter 2), and so these tag-names are no longer considered to refer to separate entities.

In the analyses of microsatellite data, not all populations of *M. antarctica* subsp. *antarctica* form a cluster in any Structure analyses (Figure 4.3), and neither do these populations group together in the NeighborNet network (Figure 4.4). There is geographic structuring present in the genetic data, whereby populations that grow closer together are often more closely related, but this pattern is not universal (Figure 4.2). For example, some of the populations from Central Otago cluster together in the Structure and NeighborNet network (e.g. CO; WELT SP091599 and C1&C2; WELT SP093286&291), but the populations collected from Campbell Island (CT; WELT SP102775, AZ; WELT SP102777, HB&HW; WELT SP102779&80) are not more closely related to each other than they are to other populations on the North and South Islands. Some of the populations identified as *M.* "Volcanic Plateau" do cluster together genetically (CP; WELT SP089738; T1&T2 WELT SP089909; see Figure 4.3, K = 24), but given the lack of morphological differentiation, and the presence of geographic clustering in the genetic data discussed already, this is not considered sufficient evidence to recognise this entity. The two populations identified as *M.* "intermedia" (BP; WELT SP093292 and M3; WELT SP100498) included in the microsatellite dataset do not cluster together genetically (Figure 4.3).

Threats: This subspecies is not threatened in New Zealand, but is listed as At Risk: Naturally Uncommon, Threatened Overseas. Over 70% of the populations on the North, South and Campbell Islands are growing on DOC managed land. However, little is known regarding the Chilean populations. A total of nine herbarium specimens are known from southern Chile (Magallanes Region), and these have only been collected from two locations. Six of these specimens represent two collection events of *M. antarctica* from

Punta Arenas (Lechler, 1852: S15-37467, S15-37492 and K000573650; Dusén, 1895: UPS V-702363, UPS V-702365 and UPS V-702371). Therefore, the most recent collection of *M. antarctica* from Punta Arenas was by Per Dusén in 1895. Punta Arenas is now a city with over 100,000 inhabitants and this population and any suitable habitat may no longer exist. There is a later collection possibly from the same area but with the less precise locality information of "Magellans Land" collected by Andersson in 1905 (S15-37494). By contrast, when Carl Skottsberg visited the second known location, Puerto Altamirano, and collected two specimens in 1908 (UPS V-702372 and S15-37481), he encountered "…a resident (at that time the only one) in Puerto Altamirano…" (Skottsberg 1941), and the area is still sparsely populated today. The modelled niche for this species, projected into southern Chile, does not fit the known distribution in that region well at all, but nevertheless does suggest there may be additional suitable habitat for this species (Figure 5.3G). As most recent herbarium specimen was collected over 100 years ago in Magallanes, *M. antarctica* must be considered data poor in Chile, and potentially at a severe risk of extinction there. Botanists, landowners and conservation staff working in the area are encouraged to look for populations of *M. antarctica* at Puerto Altamirano and other locations nearby.

3b. *Myosotis antarctica* Hook.f. subsp. *traillii* Kirk in *Trans. & Proc. New Zealand Inst.* 16: 373 (1883 [1884]).

≡ *Myosotis pygmaea* var. *traillii* (Kirk) Cockayne in *Veg. N.Z.* 1921, 69, 72 and index.

TYPE: "Sandy places on west coast of Stewart Island" *T. Kirk*, 13 Jan 1882, W 2666!

Description: Same as for *M. antarctica* except rosette leaf trichomes are always appressed to patent and curved, never flexuous or erect; corollas are cream or white, not blue or orange.

Illustration: Figures 2.1D, 2.6L–M, 2.7H–I & K–L, 5.12. *Above the Treeline* (Mark 2012:256) as *M. pygmaea. Seeds of New Zealand* (Webb and Simpson 2001:142) as *M. pygmaea* var. *pygmaea. Stewart Island Plants* (Wilson 1994:245) as *M. pygmaea* var. *pygmaea* (with note "=*M. antarctica* var*. traillii*"). *Flora of New Zealand* (Moore 1961:808) as *M. pygmaea* var. *pygmaea.*

Chromosome number: A count from one individual has been undertaken (as *M. pygmaea*), *n* = 22, AK 303514 (Murray and de Lange 2013).

Distribution: North Island and South Island, mostly coastal.

Habitats: Coastal turfs, sand dunes, fell fields, river terraces, and rock tors. Elevation: sea level to 250(–1500) m.

Representative specimens: See Appendix 2 (as *M. pygmaea*) and Appendix 8.

Conservation status: Threatened, Nationally Vulnerable, Sparse (see Table 5.5).

Notes:

Identification: Plants of *M. antarctica* subsp. *traillii* can be distinguished from *M. glauca* and *M. antarctica* subsp. *antarctica* based on their curved, appressed to patent trichomes. Like *M. antarctica* subsp. *antarctica*, it can be separated from *M. brevis* due to its generally larger size, e.g. corolla diameter of (1.0–)1.5–4.0 mm, calyx length at flowering of (1.2–)2.0–3.0(–3.5) mm long and nutlets of (1.0–)1.2–1.9 mm long × (0.7–)0.8–1.2 mm wide. Plants of *M. antarctica* subsp. *traillii* usually grow coastally (Figure 5.3B), but 11 inland populations with curved, appressed to patent trichomes have been identified (Appendix 8), these are the populations that reach the higher elevations indicated above.

Taxonomic history: *M. antarctica* subsp. *traillii* was first published by Kirk (1884). The name was not often applied to herbarium specimens (though see CHR 357370 collected by L. Cranwell in 1940), and in the *Flora* treatment Moore (1961) considered it a synonym of *M. pygmaea*. However, the vast majority of specimens identified as *M. pygmaea* do not match the type specimen of *M. pygmaea* (WELT SP004743!), which has flexuous trichomes (Figure 2.3). The name *M. pygmaea* was published on an earlier page of the same journal as *M. antarctica* subsp. *traillii*, and Lucy Moore noted the apparent similarities in the *descriptions* of these two entities (Moore 1961: 815), but did not discuss the differences in the trichomes between the two type specimens themselves. Thus the epithet "pygmaea" is unable to be used to name this subspecies, as it has previously been applied to a type specimen that falls within my circumscription of the other subspecies, *M. antarctica* subsp. *antarctica*. The original description of *M. antarctica* subsp. *traillii* mentions the trichomes are "appressed" which matches those plants generally identified as *M. pygmaea* in recent years (trichomes are appressed to patent, Table 5.7). Furthermore in nMDS analyses of morphological characters, the type of *M. antarctica* subsp. *traillii* clusters with all other specimens identified as "*M. pygmaea*" apart from the type specimen of *M. pygmaea* (Figure 2.3).

Additional characters identified by Moore (1961) as characteristic of *M. pygmaea* var. *pygmaea* vs. *M. pygmaea* var. *drucei* e.g., protruding nutlets, were not found to vary

significantly between plants identified as *M. pygmaea* and those identified as *M. drucei* (Chapter 2). Given there is only a single morphological character that does distinguish these two subspecies, the possibility of not recognising them at any taxonomic rank, or recognising them at the rank of variety was considered. However, given the morphological differentiation seen here is usually correlated with allopatry (i.e. inland vs. coastal on the North and South Islands), it was decided that it was appropriate to recognise these entities at subspecies rank. This decision follows the common practise identified by Hamilton and Reichard (1992), whereby the rank of subspecies is most often used for lineages united by morphological and either evolutionary OR ecogeographic data (see also Meudt 2006; Stuessy 2009).

Patterns in the data: All specimens of *M. antarctica* subsp. *traillii* are united morphologically (Chapter 2) but not genetically (Chapter 4). In the nMDS analyses of morphological characters measured on herbarium specimens, all samples of *M. antarctica* subsp. *traillii* group together (identified as *M. pygmaea,* excluding the *M. pygmaea* type specimen*;* Figure 2.3B). All plants of *M. antarctica* subsp. *traillii* were even more obviously differentiated from other pygmy forget-me-nots when grown in the growth room (identified as *M. pygmaea*; Figure 2.5A). Qualitative morphological characters found in both the herbarium and growth room dataset differentiate *M. antarctica* subsp. *antarctica* from *M. glauca* and *M. antarctica* subsp. *traillii,* i.e., trichomes that are curved and appressed to patent on the leaf blade and leaf margins (as *M. pygmaea*; Figures 2.4F and 2.6).

In the Structure analyses of microsatellite data not all populations of *M. antarctica* subsp. *traillii* form a cluster (as *M. pygmaea*; Figure 4.3), and neither do these populations group together in the NeighborNet network (as *M. pygmaea*; Figure 4.4). There is geographic structuring present in the genetic data, whereby populations that grow closer together are often more closely related, but this pattern is not universal (Figure 4.2). Five populations from the northwest South Island and coastal Taranaki are united genetically (SC, WELT SP100460; PR, WELT SP100462; AR, WELT SP090542; MN, WELT SP090544; OK, WELT SP090540); these land areas would have been connected during the last glacial maxima (Lewis et al., 1994), so this can be interpreted as a geographic pattern. No morphological characters were found to unite these five populations.

Conservation: It has been recognised that *M. antarctica* subsp. *traillii* is declining (de Lange et al., 2009; de Lange et al., 2013). As is the case with *M. brevis* (see above)*,* the North Island populations are most at risk, as none of them inhabit DOC managed land, and

the same pressures of cliff edge erosion and farmland proximity were seen at coastal Taranaki populations i.e. OK (WELT SP090540), AR (WELT SP090542) and MN (WELT SP090544). Two populations previously collected from the Wairarapa and Taranaki coasts (e.g. CHR 245912 and WELT SP095607) were not found when searching for them in 2011. The most genetically distinct *M. antarctica* subsp. *antarctica* populations that could be considered a priority for conservation are from the North Island Hawke's Bay region (H1-3; WELT SP090629, 31 & 34; Figure 4.3), where they grow on rock outcrops on privately owned farmland.

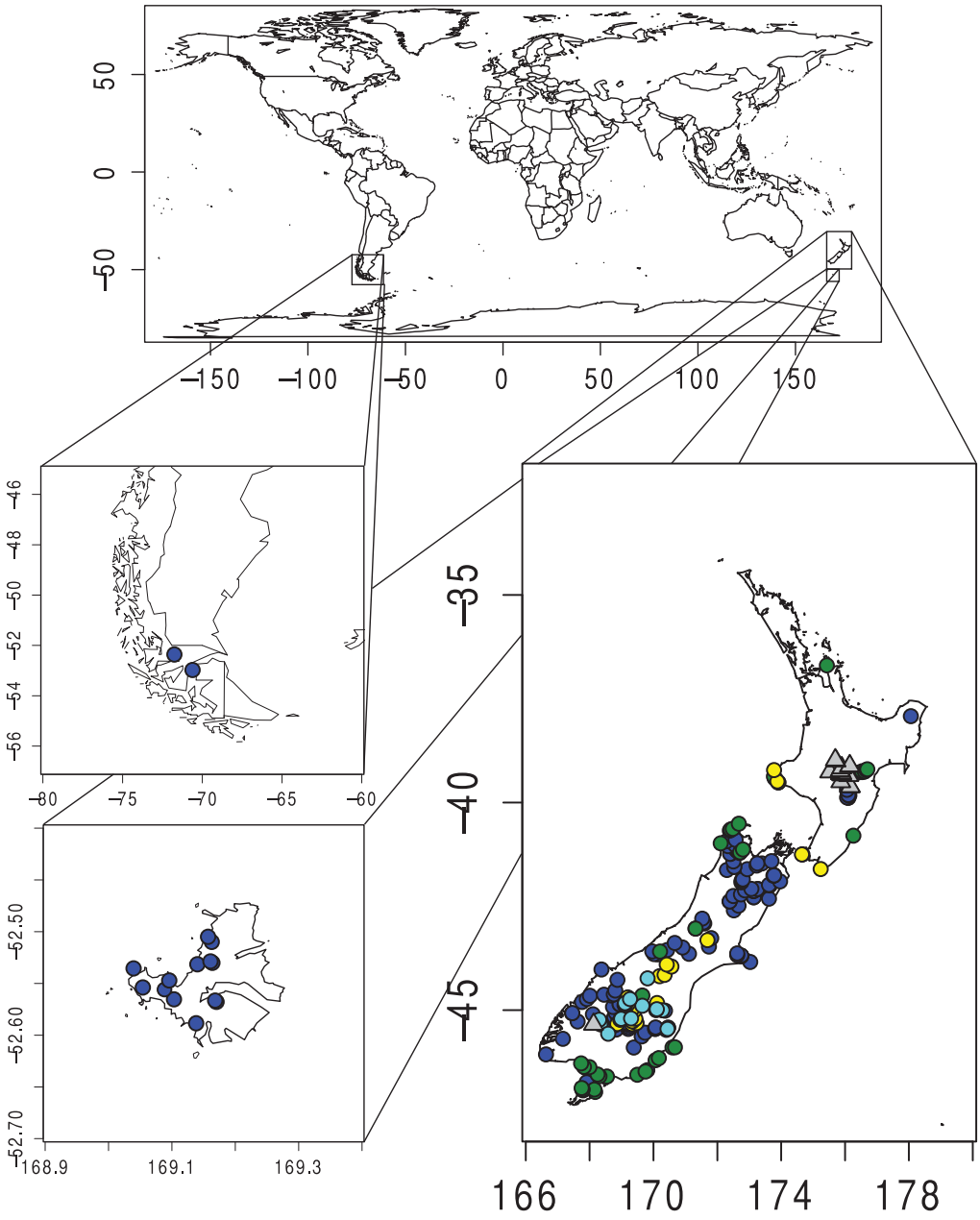


Figure 5.1 Maps displaying all 290 occurrence points used for *Myosotis* pygmy species group niche modelling (Appendix 8). Maps, clockwise from top: World, New Zealand, Campbell Island, and southern South America. Dark blue circles = *Myosotis antarctica* subsp. *antarctica*. Grey triangles = *M.* "Volcanic Plateau" (= *M. antarctica* subsp. *antarctica*). Green circles = *M. antarctica* subsp. *traillii*. Yellow circles = *M. brevis*. Light-blue circles = *M. glauca*.

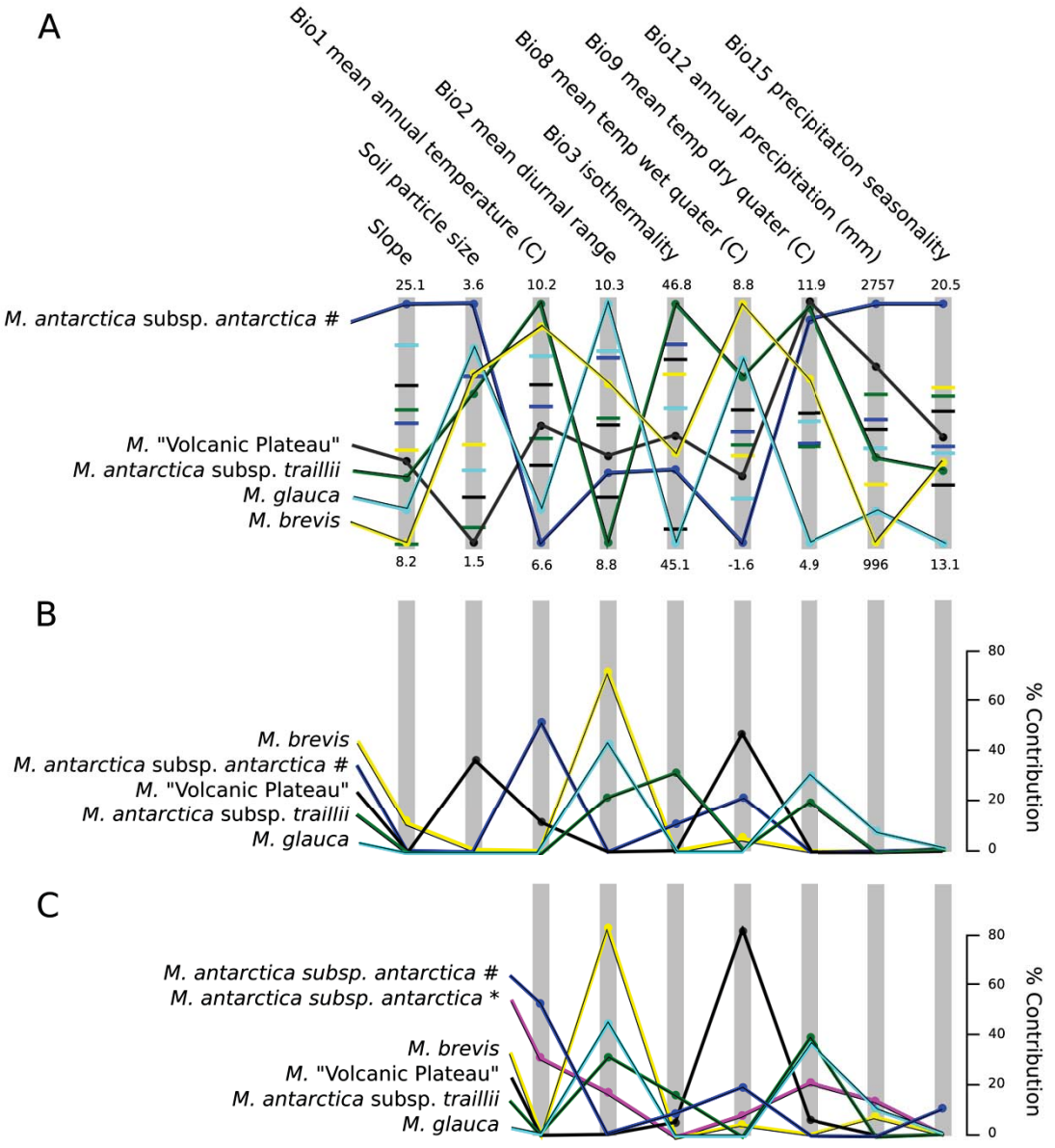


Figure 5.2 Ecological niche modelling results for pygmy forget-me-nots. Different colours represent different species. Vertical grey bars represent the 9 (or 7) environmental variables studied. A number symbol (#) indicates samples from Campbell Island and Chile are excluded, whereas an asterisk (*) indicates those samples are included. **A:** Environmental variation among pygmy forget-me-not species and subspecies. Population mean values for each species indicated where each species line crosses the vertical bar. Numbers indicate the maximum and minimum of the mean species values for each environmental variable. Short horizontal lines on the grey bars show the 1st standard deviation of the environmental values for species with respective colours (lines above and below the maximum and minimum values are omitted). **B** & **C:** Contributions of each environmental variable to MaxEnt predictions for pygmy forget-me-nots. Percentage contributions of each environmental variable to MaxEnt predictions for each species are indicated where each species line crosses the vertical bar, as indicated by the scale for percentage contribution given on the right hand side.

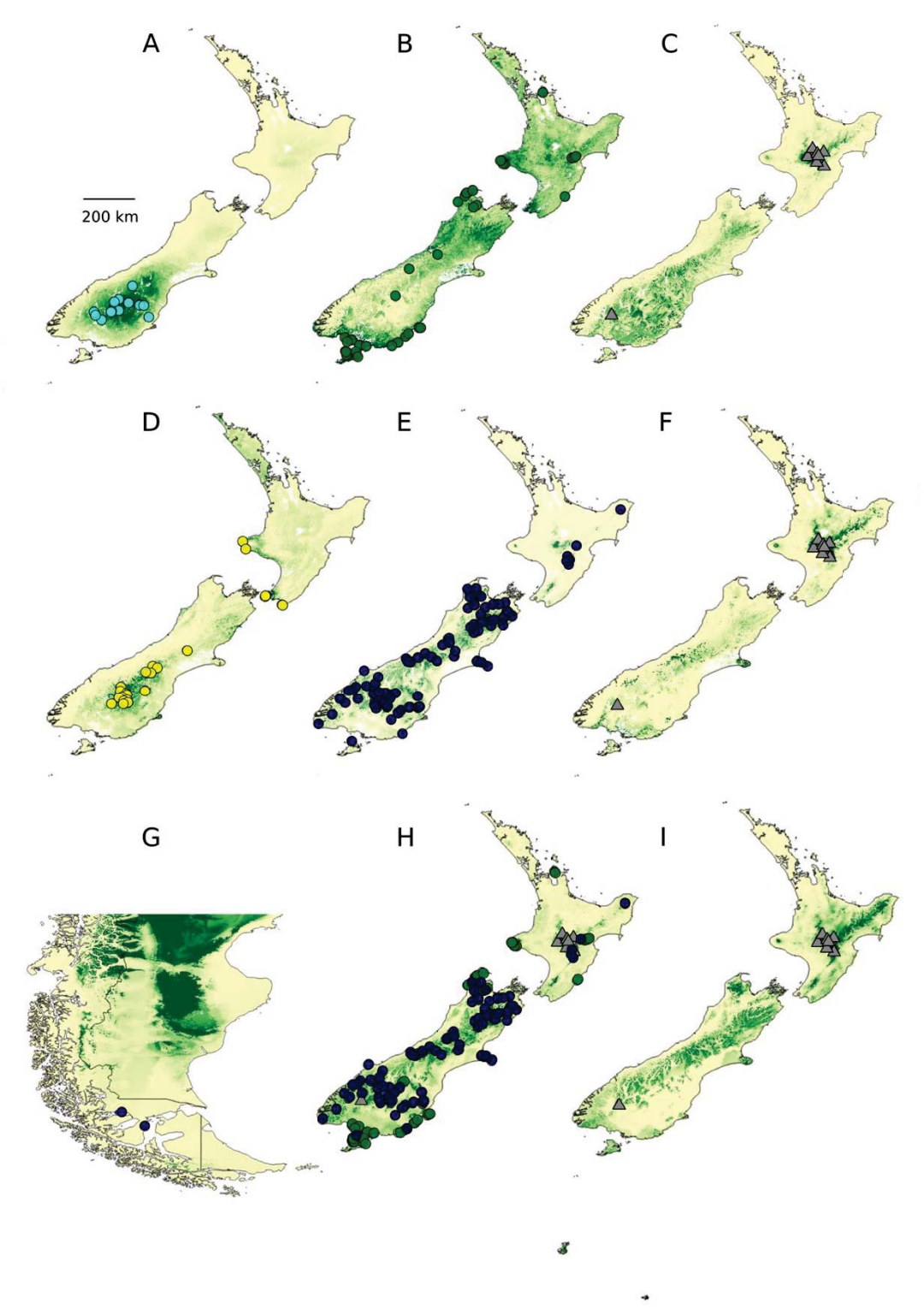


Figure 5.3 Maps of MaxEnt niche models for pygmy *Myosotis* in New Zealand and southern South America. **A:** *Myosotis glauca* (light blue circles). **B:** *M. antarctica* subsp. *traillii* (green circles). **C, F, I:** *M.* "Volcanic Plateau" (grey triangles). **D:** *M. brevis* (yellow circles). **E:** *M. antarctica* subsp. *antarctica* (dark blue circles; excluding Subantarctic locations and individuals identified as *M.* "Volcanic Plateau"). **G:** *M. antarctica* (dark blue circles; Chilean locations), note scale is the same as for maps of New Zealand. **H:** *M. antarctica* subsp. *antarctica* (dark blue circles and grey triangles) and *M. antarctica* subsp. *traillii* (green circles). A, B, D, E, F & H are models based on nine LENZ+WorldClim environmental layers (see Table 5.1), C is based on four LENZ layers at higher resolution (Table 5.1), G&I are based on seven WorldClim layers.

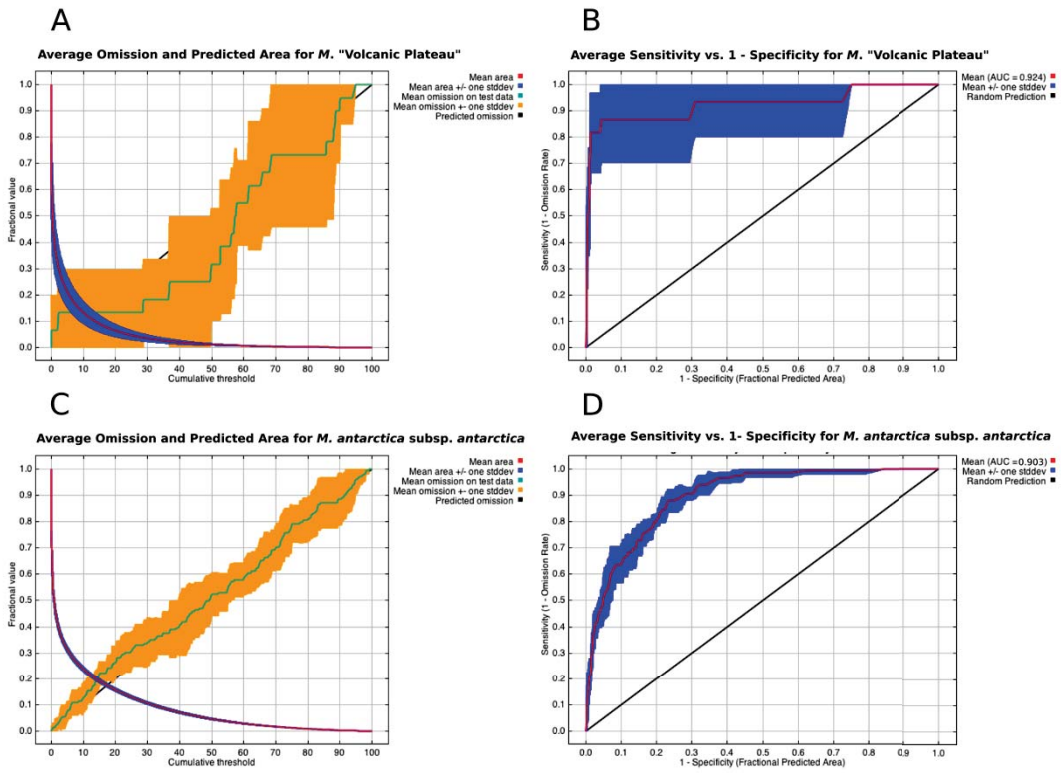


Figure 5.4 Plots displaying omission/commission values (A & C) and area under the receiving operating characteristic curve (AUC) curves (B & D) for two pygmy forget-me-not entities: *M.* "Volcanic Plateau" (A & B) and *M. antarctica* subsp. *antarctica* (C & D) modelled using MaxEnt and all nine environmental layers for the New Zealand extent.

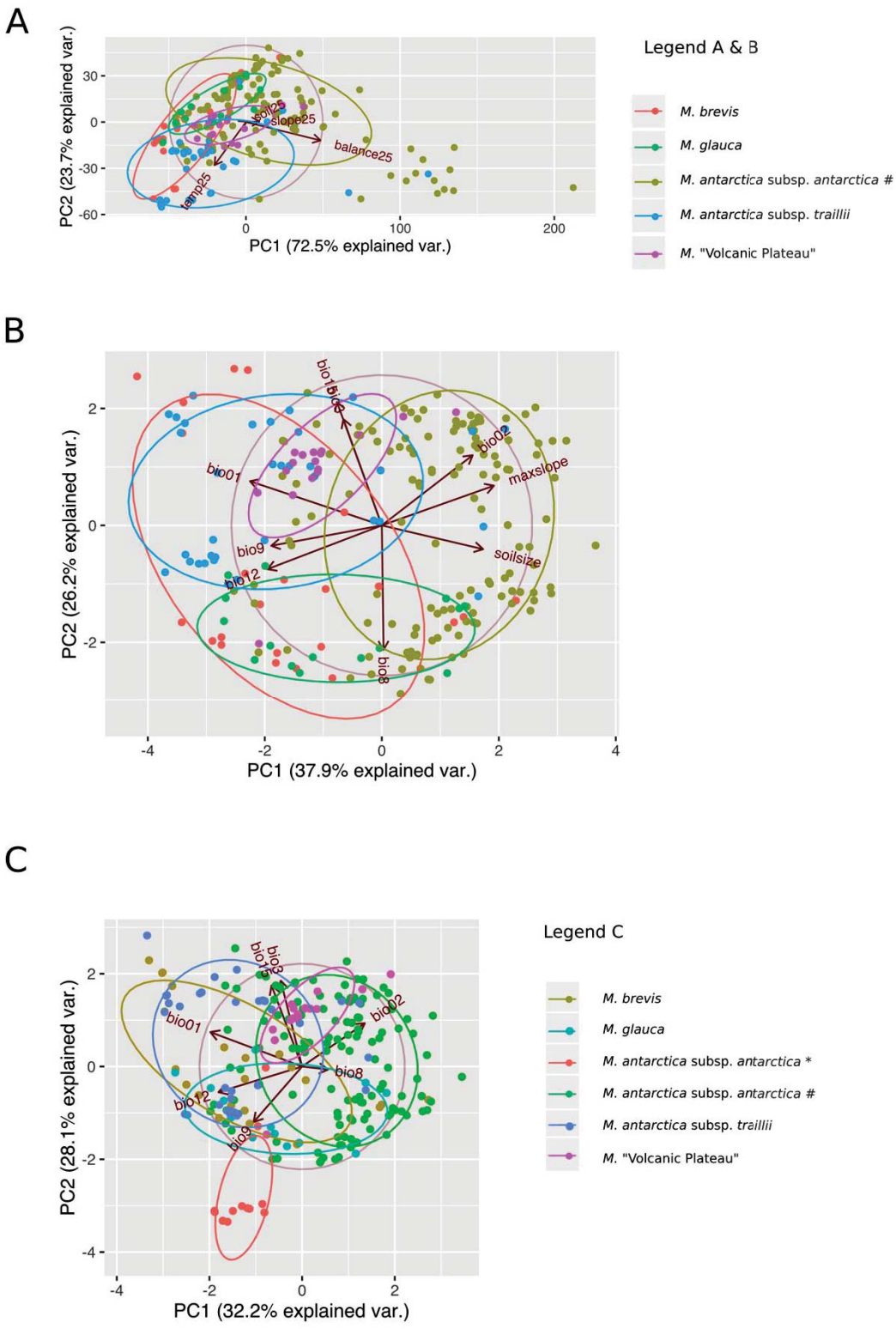


Figure 5.5 PCA of environmental variables, showing high degree of niche overlap between species within the pygmy forget-me-not group**. A.** LENZ only dataset (four layers). **B.** LENZ+WorldClim dataset (nine layers) and **C.** WorldClim only dataset (seven layers). For explanation of datasets see Table 5.1. A number symbol (#) indicates samples from Campbell Island and Chile were excluded, whereas an asterisk (*) indicates only samples from Campbell Island and Chile are included. Ovals represent normal data ellipses for each species.

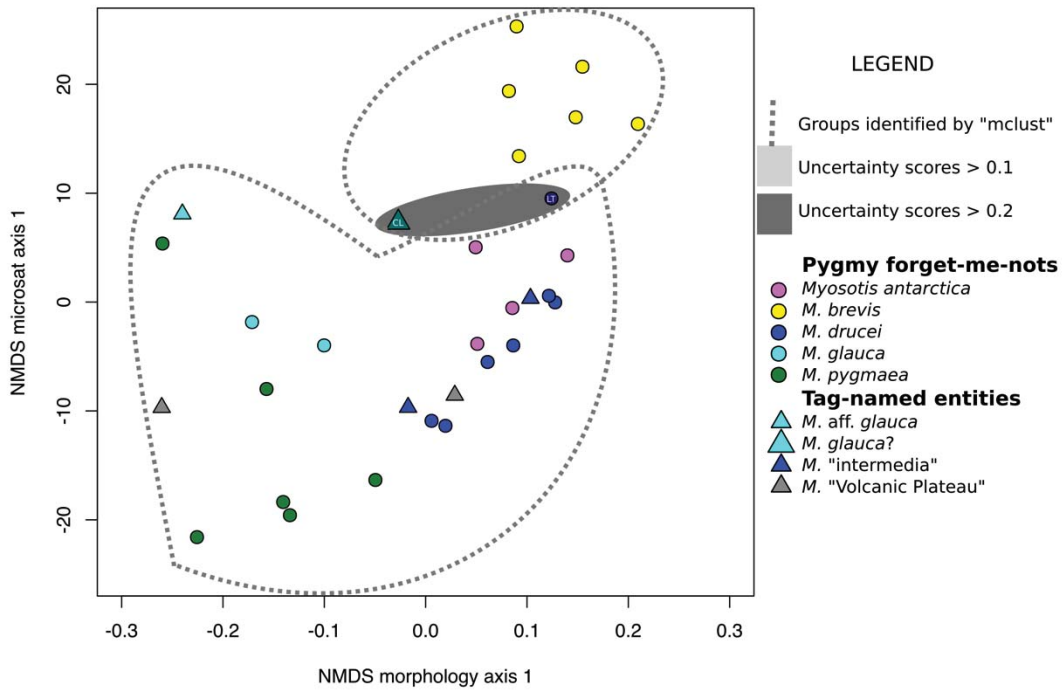


Figure 5.6 Integrated analysis of morphological, molecular and environmental datasets of 31 populations of pygmy forget-me-nots. Non-metric multidimensional scaling (nMDS) plot showing the first dimension of the morphological data vs. the first dimension of the molecular data. Points are colour coded by morphological species. Groups identified by "mclust" when analysing the nMDS points of molecular, morphological and environmental datasets combined are shown with the grey dotted lines. Population LT is shown as belong to both clusters, as the analyses based on LENZ environmental layers clustered it with the *M. brevis* populations, whereas the analyses based on WorldClim environmental layers clustered it with the remainder of the pygmy forget-me-nots. See Table 4.4 and Appendix 2 for explanation of population codes and voucher information. Compare to Figure 4.5A.

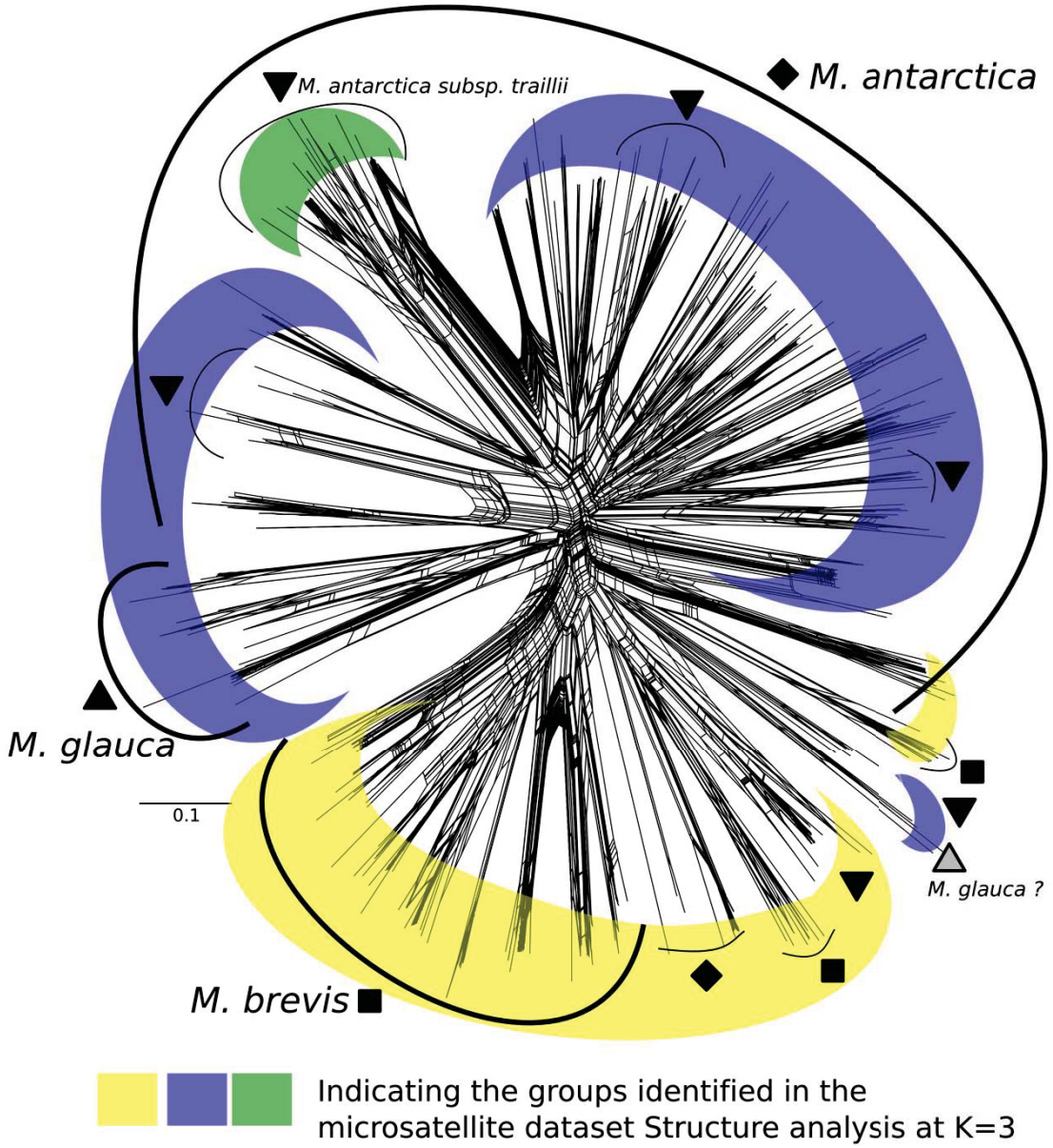


Figure 5.7 Summary of the morphological (Chapter 2) and molecular (Chapter 4) data pertaining to the pygmy forget-me-nots. A NeighborNet network of 497 individuals based on 12 microsatellite loci, groups identified at K = 3 of a Structure run with the same dataset are indicated in colours. Morphological groupings that are recognised with taxonomic ranks are indicated with black lines and symbols as follows: black squares = *M. brevis,* black upward triangles = *M. glauca,* black diamonds = *M. antarctica* subsp. *antarctica*, black downward triangles = *M. antarctica* subsp. *traillii,* and grey upward triangle = *M. glauca?*

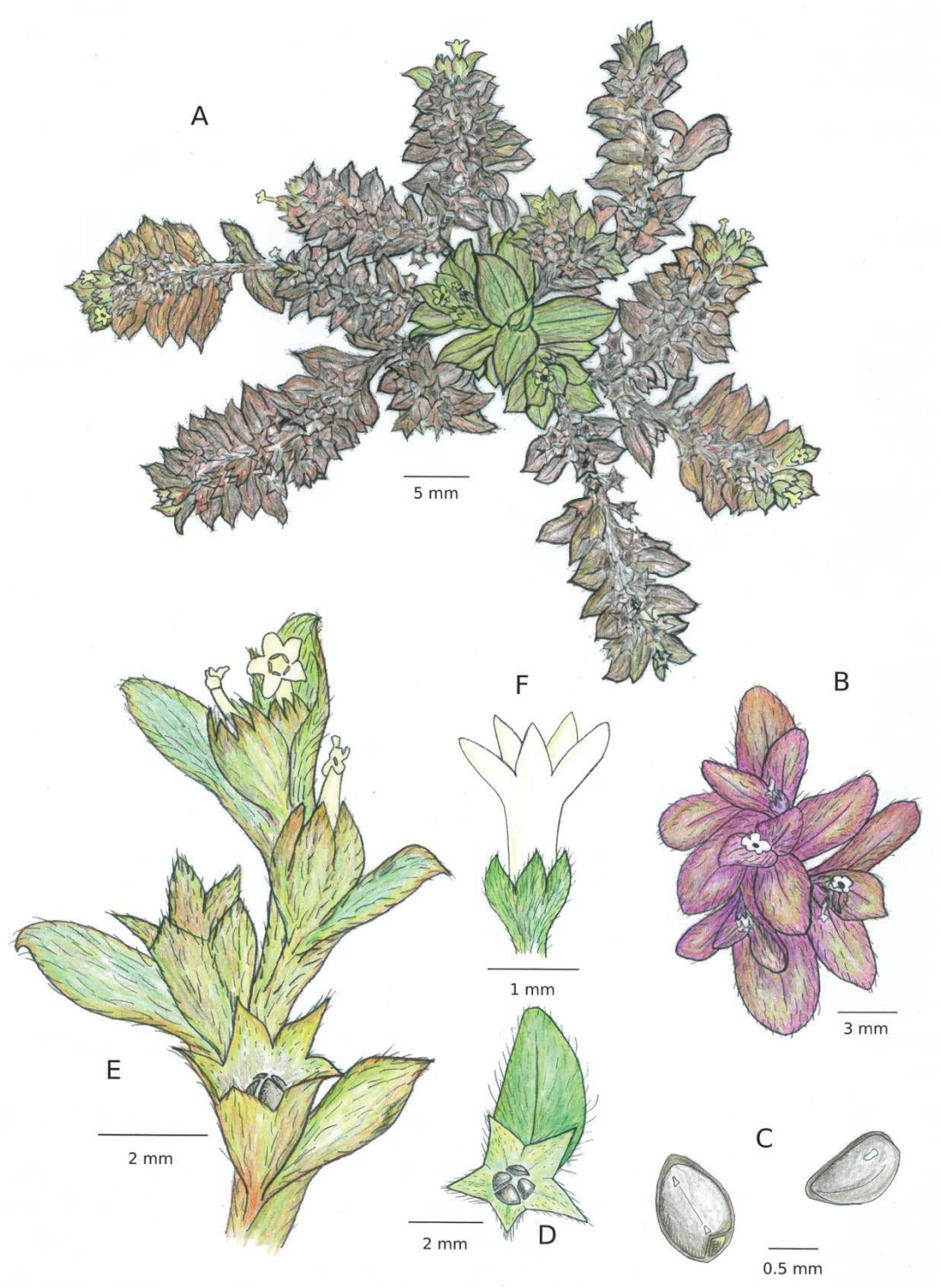


Figure 5.8 *Myosotis brevis* **A:** Habit, from photograph of plant growing at Lake Lyndon, Canterbury, South Island late in the season (February, population voucher WELT SP093294). **B:** Habit, from photograph of plant growing at Stent Rd, coastal Taranaki, North Island early in the season (October, population voucher WELT SP090543). **C:** Nutlets. **D:** Calyx containing nutlets and cauline leaf. **E:** Branch showing extended internodes when grown in cultivation compared to A. **F:** Corolla and calyx at flowering. C-F from plant grown in growth room, originally collected at Lake Lyndon (WELT SP104515). Original photos and all drawings by JMP.

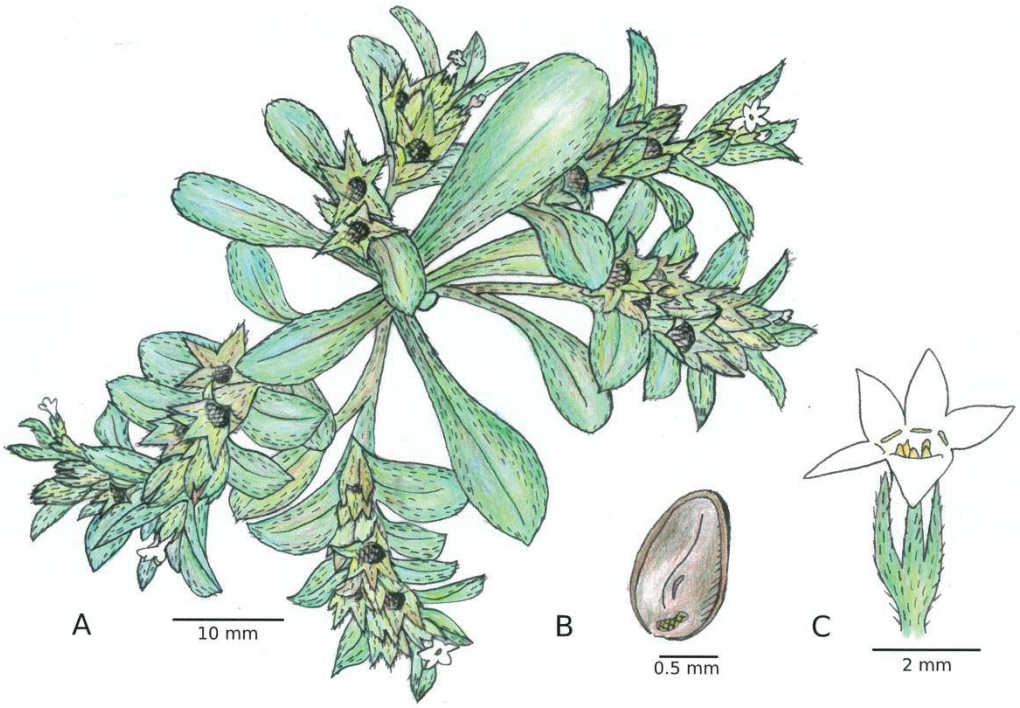


Figure 5.9 *Myosotis glauca* **A:** Habit, from photograph of plant grown in cultivation (photo by John Barkla accessed on www.nzpcn.org.nz). **B:** Nutlet. **C:** Corolla and calyx. B & C from the type specimen (CHR 75722). Drawings by JMP.

Figure 5.10 *Myosotis antarctica* subsp. *antarctica.* Illustration reproduced from *Bot. Antarct. Voy. I. (Fl. Antarct.) Part I,* plate 38 (Hooker 1844). Draft pencil drawings for this figure are attached to the type specimen of *M. antarctica* (K00078799) which was collected by J. D. Hooker from Campbell Island. Illustration by W. H. Fitch.

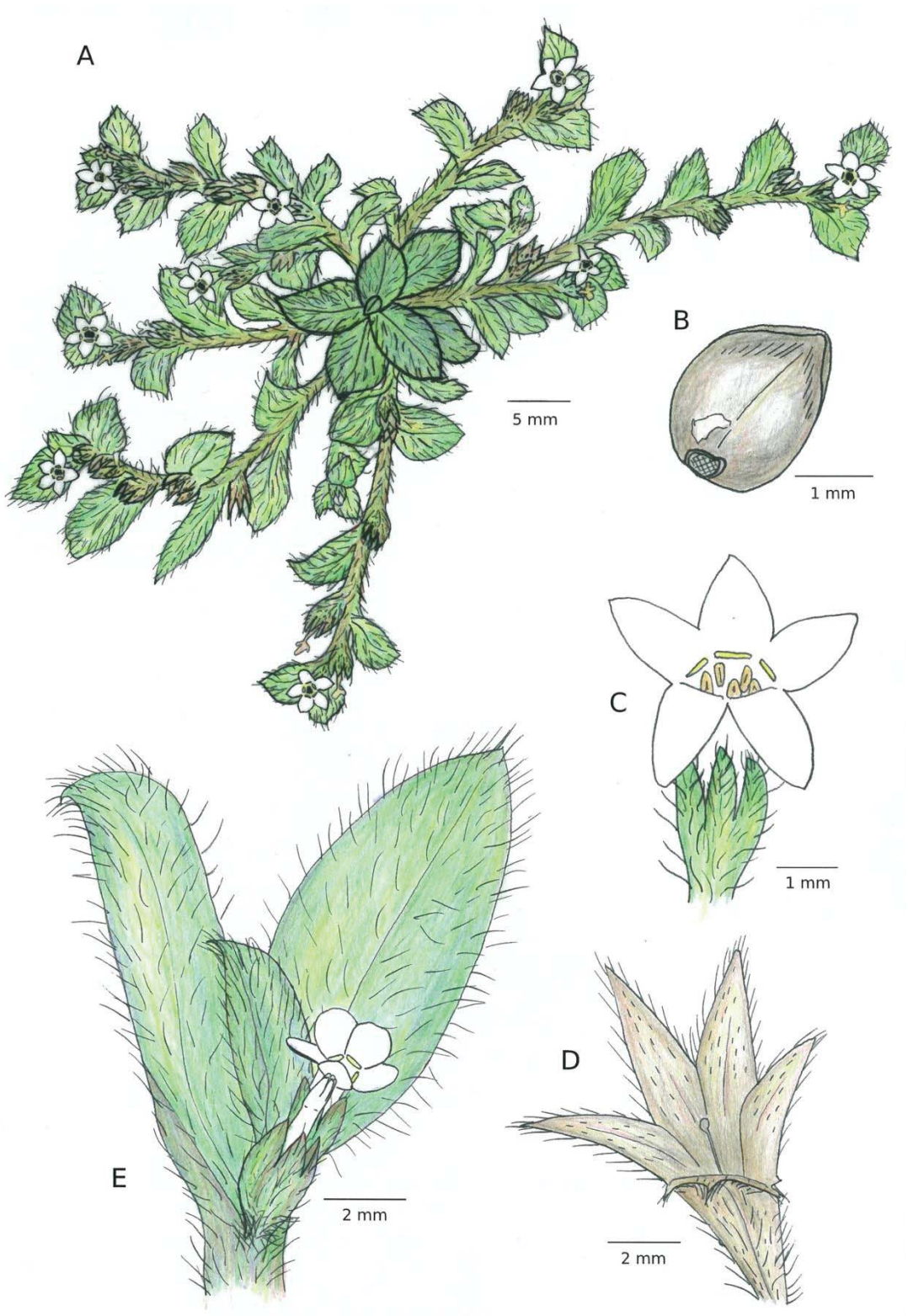


Figure 5.11 *Myosotis antarctica* subsp. *antarctica*. **A:** Habit, from photograph of plant growing at Mt Maungamahue, Ruahine Ranges, North Island, which is the type locality of *M. drucei* (WELT SP100445). **B:** Nutlet. **C:** Corolla and calyx from type specimen (CHR 76820). **D:** Calyx at fruiting. **E:** Flowering stem. B, D, E from plant grown in growth room, originally collected from Mt Peel, Kahurangi National Park, north western South Island (WELT SP104500). Original photos and all drawings by JMP.

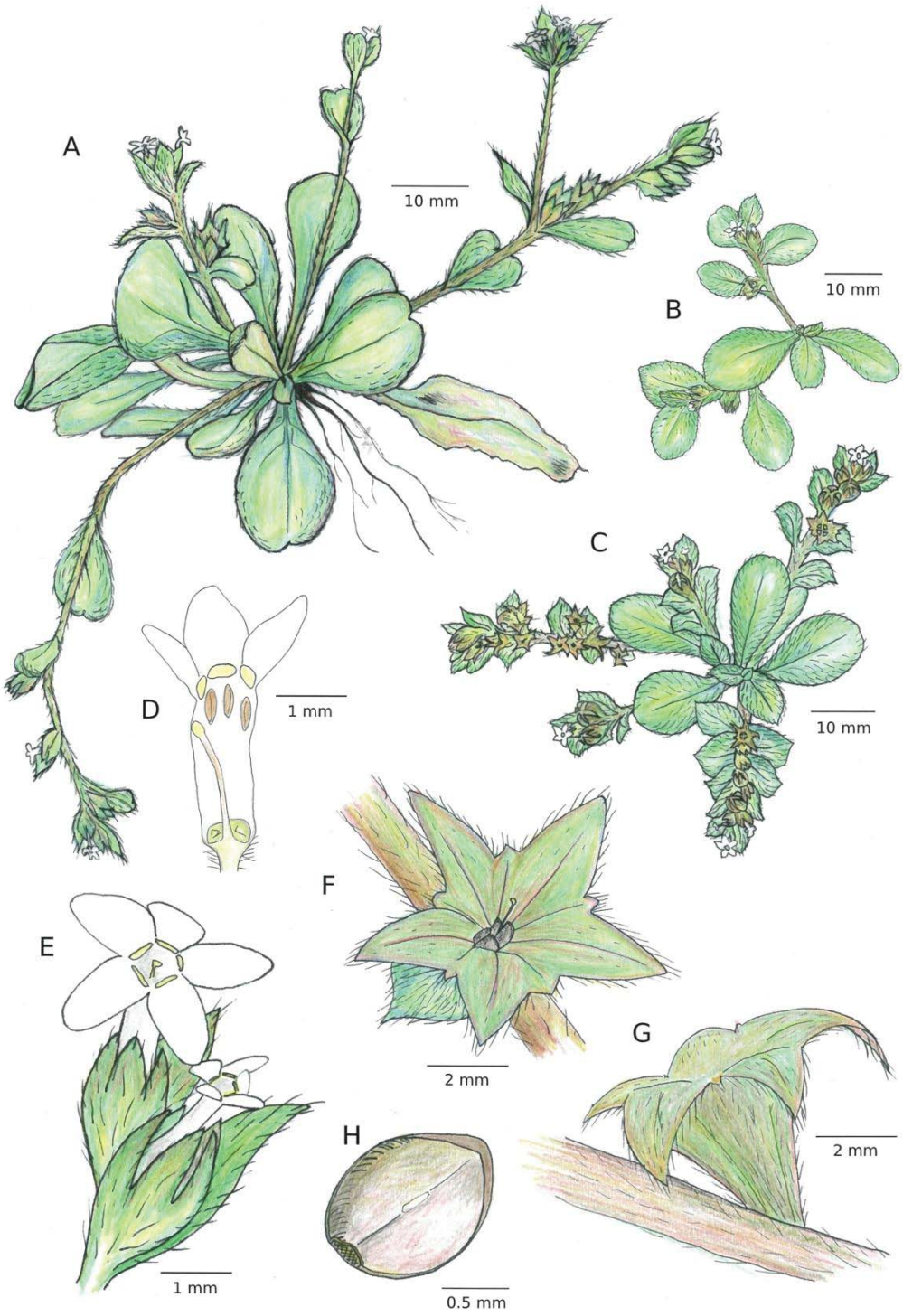


Figure 5.12 *Myosotis antarctica* subsp. *traillii.* **A:** Habit **B:** Habit, from photograph of plant growing at Arawhata Rd, coastal Taranaki, North Island early in the season (October, population voucher WELT SP090542). **C:** Habit, from plant growing at Waituna, Southland, later in the season (February, WELT SP100487). **D:** Flower cut in half from photograph by Carlos Lehnebach taken of a plant growing in cultivation at Percy's Reserve, Wellington (no voucher). **E:** Flowering branch. **F** & **G:** Calyx at fruiting. **H:** Nutlet. A, E-H from plant grown in growth room, originally collected from Stewart Island (WELT SP104518). Original photos and all drawings by JMP.

Table 5.1 Environmental layers trialled for niche modelling of the pygmy forget-me-not species group. When layers were found to be correlated, only one of the correlated layers was retained. A plus sign (+) indicates the layer was included in that dataset. An asterisk (*) indicates layers not used because they contributed little to the models during trial runs.

Layer	Description	Source	Correlated layer (> 0.8 Pearson's coefficient)	LENZ dataset	LENZ+ WorldClim dataset	WorldClim dataset
Bio1	Annual mean temperature	WorldClim	Altitude, Bio5, Bio6, Bio10, Bio11, Temperature		+	+
Bio2	Mean diurnal range (mean of monthly temperature (max temp - min temp))	WorldClim	Bio4, Bio7		+	+
Bio3	Isothermality (Bio2/Bio7) (* 100)	WorldClim			+	+
Bio4	Temperature seasonality (standard deviation *100)	WorldClim	Bio2			
Bio5	Maximum temperature of warmest month	WorldClim	Bio1			
Bio6	Minimum temperature of coldest month	WorldClim	Bio1			
Bio7	Temperature annual range (Bio5-Bio6)	WorldClim	Bio2			
Bio8	Mean temperature of wettest quarter	WorldClim			+	+
Bio9	Mean temperature of driest quarter	WorldClim			+	+
Bio10	Mean temperature of warmest quarter	WorldClim	Bio1			
Bio11	Mean temperature of coldest quarter	WorldClim	Bio1			
Bio12	Annual precipitation	WorldClim	Bio13, Bio14, Bio16, Bio17, Bio18, Bio19, Balance, October (negative correlation)		+	+
Bio 13	Precipitation of wettest month	WorldClim	Bio12			
Bio14	Precipitation of driest month	WorldClim	Bio12			
Bio15	Precipitation seasonality (coefficient of	WorldClim			+	+

Layer	Description	Source	Correlated layer (> 0.8 Pearson's coefficient)	LENZ dataset	LENZ+ WorldClim dataset	WorldClim dataset
	variation)					
Bio16	Precipitation of wettest quarter	WorldClim	Bio12			
Bio17	Precipitation of driest quarter	WorldClim	Bio12			
Bio18	Precipitation of warmest quarter	WorldClim	Bio12			
Bio19	Precipitation of coldest quarter	WorldClim	Bio12			
Elevation	Altitude	WorldClim	Bio1			
Soil	Soil particle size	LENZ		+	+	
Slope	Maximum slope	LENZ		+	+	
Balance	Monthly water balance ratio	LENZ	Bio12, October	+		
Temp	Mean annual temperature	LENZ	Bio1	+		
October	October vapour pressure deficit	LENZ	Bio12, Balance			
Winter	Mean winter solar radiation	LENZ	*			
Calcium	Exchangeable calcium	LENZ	*			
Phos	Acid soluble phosphorous	LENZ	*			
Chem	Chemical limitations to plant growth	LENZ	*			
Water	Annual water deficit	LENZ	*			
Age	Soil age	LENZ	*			
Annual	Mean annual solar radiation	LENZ	*			
Drain	Soil drainage	LENZ	*			

Table 5.2 Average AUC value of 5 runs for each species and subspecies of *Myosotis* showing different datasets, different background sampling strategies, and different species and subspecies sampled for the ecological niche modelling.

	LENZ (4 layers)	**LENZ (2 layers)+ WorldClim (7 layers)**			**WorldClim (7 layers)**
Background extent	Default	Default	200 km	80 km	Default
***M. antarctica* subsp. *antarctica* #**	0.890	0.903	0.827	0.834	0.896
***M. antarctica* subsp. *antarctica* ***	NA	NA	NA	NA	0.878
M. antarctica* subsp. *traillii	0.617	0.756	0.773	0.794	0.761
M. brevis	0.874	0.927	0.930	0.911	0.926
M. glauca	0.922	0.922	0.804	NA	0.925
***M.* "Volcanic Plateau"**	0.915	0.924	0.936	0.931	0.914

Note: a number symbol (#) indicates samples from Campbell Island and Chile are excluded, whereas an asterisk (*) indicates those samples are included as was environmental data from southern South America. Samples identified as *M.* "Volcanic Plateau" were excluded from all *M. antarctica* subsp. *antarctica* datasets and analysed separately.

Table 5.3 Niche overlap as calculated using the D statistic (Warren et al. 2008) between species and subspecies pairs in the pygmy forget-me-not group. Niches compared in this table were modelled using the nine-layer dataset (LENZ+WorldClim) with default background sampling, and in brackets the overlap between niches modelled using only the 7 WorldClim layers are given. An asterisk (*) indicates the pair of niches were found to be indistinguishable using the niche identity test. Samples identified as *M.* "Volcanic Plateau" were excluded from all *M. antarctica* subsp. *antarctica* datasets and analysed separately.

	M. antarctica* subsp. *antarctica	***M. antarctica* subsp. *traillii***	***M. brevis***	***M. glauca***	***M.* "Volcanic Plateau"**
M. antarctica* subsp. *antarctica	-	0.35 (0.33)	0.37 (0.38)	0.37 (0.48)	0.17 (0.56)
M. antarctica* subsp. *traillii		-	0.46 (0.51)	0.30 (0.26)	0.20 (0.50)
M. brevis			-	0.62* (0.57)	0.17 (0.35)
M. glauca				-	0.11 (0.30)
***M.* "Volcanic Plateau"**					-

Table 5.4 Rarity type, which is assessed using geographic range (based on the extent of occupancy), abundance (based on the average population size) and habitat specificity (based on the niche breadth and number of occupied habitats) for each species or subspecies of pygmy forget-me-not. For explanation of rarity number and type see Table 1.1.

	M. antarctica* subsp. *antarctica	***M. antarctica* subsp. *traillii***	***Myosotis brevis***	***M. glauca***
Extent of occupancy (km^2)[1]	7,403,000 (NZ, CI & SA); 244,000 (NZ only)	214,000	109,000	14,000
Geographic Range[2]	Large	Large	Large	Small
Abundance[3]	40	50	500	70
Local population size[4]	Small, non-dominant	Small, non-dominant	Small, non-dominant	Small, non-dominant
Niche breadth[5]	0.50	0.59	0.53	0.24
Number of LENZ environments occupied[6]	61, 36, 27, 11	24, 18, 23, 10	16, 13, 10, 8	12, 9, 7, 2
Habitat specificity[7]	Wide	Wide	Wide	Narrow
Rarity number and type, see Table 1.1 for explanation	6: constantly sparse over a large range and in several habitats	6: constantly sparse over a large range and in several habitats	6: constantly sparse over a large range and in several habitats	1: constantly sparse and geographically restricted in a specific habitat

1. Extent of occupancy was calculated using an online tool (http://geocat.kew.org/editor). 2. Geographic range was interpreted as being "large" if the area of occupancy was greater than 20,000 km^2. 3. Abundance is the average population size in number of adult individuals (see Table 4.4). 4. Local population size was considered "small" if the average was less than or equal to 500 individuals. 5. Niche breadth was calculated using ENMTools. 6. Numbers of LENZ environments inhabited are given at Levels 4, 3, 2, 1 (out of a possible 500, 200, 100, 20). 7. Habitat specificity was considered "wide" if niche breadth was greater than or equal to 0.5; see methods for more details

Table 5.5 Historical and suggested threat classifications of the pygmy forget-me-not group, and the data used to determine the current threat status. Note De = declining, DP = data poor, EF = extreme fluctuations, IE = island endemic, OL = one location, RR = range restricted, Sp = sparse, TO = threatened overseas.

Name used in 2008 threat listing (de Lange et al. 2009)	*Myosotis antarctica* Hook.f.	*Myosotis pygmaea* var. *drucei* L.B.Moore	*Myosotis* aff. *pygmaea* (CHR 244566; Volcanic Plateau)	***Myosotis pygmaea* Colenso var. *pygmaea***	***Myosotis pygmaea* var. *minutiflora* G.Simpson et J.S.Thomson**	***Myosotis pygmaea* var. *glauca* G.Simpson et J.S.Thomson**
2008 threat listing (de Lange et al. 2009)	Naturally Uncommon	Not listed (i.e. Not Threatened)	Naturally Uncommon	At Risk - declining	Nationally Endangered	Nationally Vulnerable
Qualifiers 2008 threat listing (de Lange et al. 2009)	IE, OL	n/a	EF, RR, Sp	Sp	DP, EF	De, DP, Sp
Name used in 2012 threat listing (de Lange et al. 2013)	*Myosotis antarctica* Hook.f.	*Myosotis drucei* (L.B.Moore) de Lange & Barkla	*Myosotis* aff. *pygmaea* (CHR 244566 "Volcanic Plateau")	*Myosotis pygmaea* Colenso	*Myosotis brevis* de Lange & Barkla	*Myosotis glauca* (G.Simpson & J.S.Thomson) de Lange & Barkla
2012 threat listing (de Lange et al. 2013)	Naturally Uncommon	Not Threatened	Naturally Uncommon	At Risk - declining	Nationally Vulnerable	Nationally Vulnerable
Qualifiers 2012 threat listing (de Lange et al. 2013)	IE, OL	n/a	EF, RR, Sp	Sp	EF, RR, Sp	De, DP, Sp
Notes 2012 threat listing (de Lange et al. 2013)	n/a	n/a	n/a	B(1/1) 20 000–100 000 mature individuals, predicted decline 10–50 %	C(3/1) Total area of occupancy ≤ 100 ha (1 km2), predicted decline 10–50 %	B(3/1) Total area of occupancy ≤ 100 ha (1 km2), stable population
Name used in this chapter	***Myosotis antarctica* Hook.f. subsp. *antarctica***			***Myosotis antarctica* subsp. *traillii* Kirk**	***Myosotis brevis* de Lange & Barkla**	***Myosotis glauca* (G.Simpson & J.S.Thomson) de Lange & Barkla**
Average population size (based on populations visited 2011–2015; see Table 4.4)	40			50	190 (NI); 1000 (SI); 500 (overall)	70

Range in population size (based on populations visited 2011–2015; see Table 4.4)	6–150	5–200	50–2000	5–2000
Number of populations visited 2011–2015; see Table 4.4	20	13	10	5
Total number of populations estimated to be extant[1]	299	58	35	38
TOTAL estimated census size based on average population size × number of populations	12006	2875	17600	4601
Number of populations known to be no longer extant	2[2]	3–4[3]	2[4]	1[5]
Population trend	Stable	Declining 10–20 %	Fluctuates (Rogers et al., 2002)	Stable
Area of occupancy (AOO)	1.69 km^2	0.35 km^2	0.25 km^2	0.17 km^2
Suggested threat status	Naturally Uncommon	Nationally Vulnerable	Nationally Vulnerable	Nationally Vulnerable
Suggested qualifiers	Sp, TO	Sp, De	EF, Sp	RR, Sp,
Comments on suggested threat status		C(1/1) 1000–5000 mature individuals, predicted decline 10–50 %, C(3/1) area of occupancy < 100 ha (1 km^2)	C(3/1) predicted decline 10–50 %, area of occupancy < 100 ha (1 km^2)	B(1/1) 1000–5000 mature individuals, B(3/1) stable population, area of occupancy < 100 ha (1 km^2)
% Populations on DOC managed land (Appendix 8)	72	26	24	31

[1]Calculated from the number of population locations identified, based on herbarium specimens at AK, CHR, OTA and WELT. [2]Campbell Island, Windlass Bay; Hawke's Bay, Matamau. [3]Wairarapa, Castle Point; Taranaki, Puketapu Road; Northwest Nelson, Whararíki Beach; Coastal Otago, Chrystall Beach (In 2013, two plants were found at Chrystall Beach, so may represent a fluctuation of population size rather than an extinct population). [4]East Cape, Canterbury Plains. [5]Canterbury, Lake Ohau.

Table 5.6 Frequency statistics by pygmy forget-me-not species or subspecies based on 12 microsatellite loci, only including populations of n > 5. For details of the frequencies statistics for individual populations see Table 4.4.

	Myosotis antarctica	*M. antarctica* subsp. *antarctica*	*M. antarctica* subsp. *traillii*	*M. brevis*	*M. glauca*
Total no. of pops	30	19	11	10	3
Total no. of individuals	328	200	128	128	35
N population average	10.26	9.84	10.99	12.13	10.81
% P	33.05	36.40	27.27	28.33	19.44
N_A	1.400	1.469	1.280	1.408	1.222
N_E	1.246	1.279	1.189	1.177	1.069
H_O	0.060	0.061	0.059	0.005	0.033
H_E	0.131	0.147	0.105	0.097	0.044
F_{IS}	0.653	0.704	0.499	0.856	0.586
F_{ST}	0.803	0.778	0.818	0.823	0.826

Note: For each species the following are detailed: number of populations, number of individuals, average population size (missing data taken into account), % polymorphic loci (%P), number of alleles (N_A), number of effective alleles (N_E), observed (H_O) and expected (H_E) heterozygosity, observed heterozygosity of an individual relative to the expected heterozygosity of individuals in the population (F_{IS}), and expected heterozygosity of individuals within a subpopulation relative to the total expected heterozygosity of individuals across all populations (F_{ST})

Table 5.7 Summary table showing how pygmy *Myosotis* names have been applied historically, over the course of this thesis, and in this chapter, and whether each dataset analysed provides support for each species or not. NA = not assessed.

Names in Cockayne (1921)	Names in Moore (1961)	Name in Chapters 1–4 of this thesis (following current usage)	Name used in this revision (Chapter 5)	Chromosome counts	Morphology (Chapter 2)		Molecular (Chapter 4)	Niche (This Chapter)	Three datasets integrated (N=31)	Distinguishing morphological characteristics			
					Herbarium data	Growth room data				Corolla diameter	Nutlet size	Trichomes	Leaf colour
M. antarctica	*M. antarctica*	*M. antarctica*	*M. antarctica* subsp. *antarctica*	NA	YES	NA	YES	YES	YES	1.5–4.0	1.2–1.9 × 0.8–1.2 mm	Flexuous, patent to erect	Green to brown
M. pygmaea	*M. pygmaea* var. *drucei*	*M. drucei*		*n* = 24 CHR 101449		YES							
		M. "Volcanic Plateau"		*n* = 22 AK 331000		NA	YES						
M. pygmaea var. *traillii*	*M. pygmaea* var. *pygmaea*	*M. pygmaea*	*M. antarctica* subsp. *traillii*	*n* = 22 AK 303514	YES	YES						Curved, appressed to patent	
M. pygmaea	*M. pygmaea* var. *glauca*	*M. glauca*	*M. glauca*	NA	YES	NA	YES	YES				Straight, appressed	Glaucous to occasionally bright green
	M. pygmaea var. *minutiflora*	*M. brevis*	*M. brevis*	NA	YES	YES	YES		YES	0.5–1.5	0.9–1.2 × 0.5–0.8 mm	Flexuous, appressed to erect	Green to brown

References

Ahmadzadeh F, Flecks M, Carretero MA, Mozaffari O, Bohme W, Harris DJ, Freitas S, Rodder D (2013) Cryptic speciation patterns in Iranian rock lizards uncovered by integrative taxonomy. *PLOS One* 8: e80563.

Beuzenberg EJ, Hair JB (1983) Contributions to a chromosome atlas of the New Zealand flora—25. Miscellaneous species. *New Zealand Journal of Botany* 21: 13-20.

Binks RM, Millar MA, Byrne M (2015) Not all rare species are the same: Contrasting patterns of genetic diversity and population structure in two narrow-range endemic sedges. *Biological Journal of the Linnean Society* 114: 873-886.

Bourg NA, McShea WJ, Gill DE (2005) Putting a cart before the search: Successful habitat prediction for a rare forest herb. *Ecology* 86: 2793-2804.

Brandon AM (2001) *Breeding systems and rarity in New Zealand Myosotis*. PhD Thesis. Palmerston North: Massey University.

Buckley TR, Marske K, Attanayake D (2010) Phylogeography and ecological niche modelling of the New Zealand stick insect *Clitarchus hookeri* (White) support survival in multiple coastal refugia. *Journal of Biogeography* 37: 682-695.

Cameron KM (2010) On the value of taxonomy, phylogeny, and systematics to orchid conservation: Implications for China's Yachang Orchid Reserve. *Botanical Review* 76: 165-173.

Cockayne L (1921) *The Vegetation of New Zealand*. Leipzig, Germany: Wilhelm Engelmann.

de Lange PJ, Heenan P, Norton D, Rolfe J, Sawyer J (2010) *Threatened Plants of New Zealand*. Christchurch: Canterbury University Press.

de Lange PJ, Norton DA, Courtney SP, Heenan PB, Barkla JW, Carmeron EK, Hitchmough R, Townsend AJ (2009) Threatened and uncommon plants of New Zealand (2008 revision). *New Zealand Journal of Botany* 47: 61-69.

de Lange PJ, Rolfe JR, Champion PD, Courtney SP, Heenan PB, Barkla JW, Cameron EK, Norton DA, Hitchmough RA (2013) *Conservation Status of New Zealand Indigenous Vascular Plants, 2012*. Wellington: New Zealand Department of Conservation.

de Queiroz K (2007) Species concepts and species delimitation. *Systematic Biology* 56: 879-886.

DOC (2014) *Department of Conservation Biodiversity Indicators: 2014 Assessment—Supplementary Material*. Wellington: Department of Conservation.

Dusen P (1900) *Die Gefässpflanzen der Magellansländer; nebst einem Beitrage zur Flora der Ostküste von Patagonien*. Stockholm: Norstedt.

Edwards DL, Knowles LL (2013) Species detection and individual assignment in species delimitation: Can integrative data increase efficacy? *Proceedings of the Royal Society B: Biological Sciences* 281:20132765.

Elith J, Graham C, Anderson R, Dudík M, Ferrier S, Guisan A, Hijmans RJ, Huettmann F, Leathwick JR, Lehmann A, Li J, Lohmann LG, Loiselle BA, Manion G, Moritz C, Nakamura M, Nakazawa Y, Peterson AT, Phillips SJ, Richardson K, Scachetti-Pereira R, Schapire RE, Soberón J, Williams S, Wisz MS, Zimmermann NE (2006) Novel methods improve prediction of species' distributions from occurrence data. *Ecography* 29: 129-151.

Ellstrand NC, Elam DR (1993) Population genetic consequences of small population-size – implications for plant conservation. *Annual Review of Ecology and Systematics* 24: 217-242.

Fiedler P (1986) Concepts of rarity in vascular plant species, with special reference to the genus *Calochortus* Pursh (Liliaceae). *Taxon* 35: 1-18.

Fraley C, Raftery AE (2002) Model-based clustering, discriminant analysis and density estimation. *Journal of the American Statistical Association* 97: 611-631.

Fraley C, Raftery AE, Murphy TB, Scrucca L (2012) Mclust version 4 for R: Normal mixture modeling for model-based clustering, classification, and density estimation technical report no. 597, Department of Statistics, University of Washington.

Frankham R (1995) Conservation Genetics. *Annual Review of Genetics* 29: 305-327.

Frankham R, Ballou JD, Briscoe DA (2010) *Introduction to Conservation Genetics*. Cambridge: Cambridge University Press.

Godsoe W (2010) Regional variation exaggerates ecological divergence in niche models. *Systematic Biology* 59: 298-306.

Greenberg JA (2014) spatial.tools: R functions for working with spatial data.. R package version 1.4.8. http://CRAN.R-project.org/package=spatial.tools.

Hamilton W, Reichard SH (1992) Current practice in the use of subspecies, variety, and forma in the classification of wild plants. *Taxon* 41: 485-498.

Hijmans RJ (2015) Raster: Geographic data analysis and modeling. R package version 2.4-15. http://CRAN.R-project.org/package=raster.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25: 1965-1978.

Joly S, Heenan PB, Lockhart P (2014) Species radiation by niche shifts in New Zealand's rockcresses (*Pachycladon*, Brassicaceae). *Systematic Biology* 63: 192-202.

Knowlton N, Jackson JBC (1994) New taxonomy and niche partitioning on coral reefs: jack of all trades or master of some? *Trends in Ecology & Evolution* 9: 7-9.

Leathwick J, Morgan F, Wilson G, Rutledge D, McLeod M, Johnston K (2002) *Land Environments of New Zealand: A Technical Guide*. Wellington, New Zealand: Ministry for the Environment

Lehnebach CA (2008) *Phylogenetic affinities, species delimitation and adaptive radiation of New Zealand Ranunculus*. PhD Thesis. Palmerston North: Massey University.

Lewis KB, Carter L, Davey FJ (1994) The opening of Cook Strait – interglacial tidal scour and aligning basins at a subduction to transform plate edge *Marine Geology* 116: 293-312.

Mark A (2012) *Above the Treeline: a Nature Guide to Alpine New Zealand*. Nelson, New Zealand: Craig Potton Publishing.

Mark AF, Adams NM (1973) *New Zealand Alpine Plants*. Auckland: Reed Methuen Publishers

Meudt HM (2006) Monograph of *Ourisia* (Plantaginaceae). *Systematic Botany Monographs* 77: 1-188.

Meudt HM, Prebble JM, Lehnebach CA (2015) Native New Zealand forget-me-nots (*Myosotis*, Boraginaceae) comprise a Pleistocene species radiation with very low genetic divergence. *Plant Systematics and Evolution* 301: 1455–1471.

Meudt HM, Prebble JM, Stanley RJ, Thorsen MJ (2013) Morphological and amplified fragment length polymorphism (AFLP) data show that New Zealand endemic *Myosotis petiolata* (Boraginaceae) comprises three rare and threatened species. *Australian Systematic Botany* 26: 210-232.

Moore LB (1961) *Boraginaceae*. In: Allan H, editor. *Flora of New Zealand. Vol. 1*. Wellington, New Zealand: PD Hasselberg, Government Printer. p. 806-833.

Murray BG, de Lange PJ (2013) Contributions to a chromosome atlas of the New Zealand flora—40. Miscellaneous counts for 36 families. *New Zealand Journal of Botany* 51: 31-60.

Nakazato T, Warren DL, Moyle LC (2010) Ecological and geographic modes of species divergence in wild tomatoes. *American Journal of Botany* 97: 680-693.

Oksanen J, Blanchet FG, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2015) vegan: Community Ecology Package. R package version 2.3-0. http://CRAN.R-project.org/package=vegan.

Paland S, Lynch M (2006) Transitions to asexuality result in excess amino acid substitutions. *Science* 311: 990-992.

Peakall R, Smouse PE (2006) GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288-295.

Peakall R, Smouse PE (2012) GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28: 2537-2539.

Phillips S (2010) A brief tutorial on MaxEnt. *Lessons in Conservation*: 107-135.

Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling* 190: 231-259.

Phillips SJ, Dudik M, Schapire RE (2004) A maximum entropy approach to species distribution modeling. *Pages 655-662 in Proceedings of the 21st International Conference on Machine Learning. ACM Press, New York*.

Pufal G (2010) *The evolution and ecology of hygrochastic capsule dehiscence*. PhD Thesis. Wellington: Victoria University.

Pyšek P, Hulme PE, Meyerson LA, Smith GF, Boatwright JS, Crouch NR, Figueiredo E, Foxcroft LC, Jarošik V, Richardson DM, Suda J, Wilson JRU (2013) Hitting the right target: taxonomic challenges for, and of, plant invasions. *AOB Plants* 5: 1-25.

Rabinowitz D (1981) *Seven Forms of Rarity*. In: Synge H, editor. *The Biological Aspects of Rare Plants Conservation*. p. 205-217.

Radosavljevic A, Anderson RP (2014) Making better MaxEnt models of species distributions: complexity, overfitting and evaluation. *Journal of Biogeography* 41: 629-643.

Raxworthy C, Ingram C, Rabibisoa N, Pearson R (2007) Applications of ecological niche modeling for species delimitation: A review and empirical evaluation using day geckos (*Phelsuma*) from Madagascar. *Systematic Biology* 56: 907-923.

RCoreTeam (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Reeves PA, Richards CM (2011) Species delimitation under the general lineage concept: An empirical example using wild North American hops (Cannabaceae: *Humulus lupulus*). *Systematic Biology* 60: 45-59.

Reginato M (2016) MonographaR: An R package to facilitate the production of plant taxonomic monographs. *Brittonia* DOI 10.1007/s12228-015-9407-z: 1–5.

Reilly LA (2010) *A quantitative approach for defining rarity*. PhD Thesis. Chapel Hill: University of North Carolina.

Rissler LJ, Apodaca JJ (2007) Adding more ecology into species delimitation: Ecological niche models and phylogeography help define cryptic species in the black salamander (*Aneides flavipunctatus*). *Systematic Biology* 56: 924-942.

Robertson A (1989) *Evolution and pollination of New Zealand Myosotis (Boraginaceae).* PhD Thesis. Christchurch: University of Canterbury.

Rogers G, Walker S (2002) Taxonomic and ecological profiles of rarity in the New Zealand vascular flora. *New Zealand Journal of Botany* 40: 73-93.

Rogers G, Walker S, Tubbs M, Henderson J (2002) Ecology and conservation status of three "spring annual" herbs in dryland ecosystems of New Zealand. *New Zealand Journal of Botany* 40: 649-669.

Simpson G (1952) Notes on some New Zealand plants and descriptions of new species (No. 5). *Transactions and Proceedings of the Royal Society of New Zealand* 79: 426.

Skottsberg C (1915) Notes on the relations between the floras of Subantarctic America and New Zealand. *The Plant World* 18: 129-142.

Skottsberg C (1941) *Communities of Marine Algae in Subantarctic and Arctic waters*. Stockholm, Sweden: Almqvist & Wiksells Boktryckeri-A.B.

Stebbins G (1980) Rarity of plant species: a synthetic viewpoint. *Rhodora* 82: 77-86.

Stuessy TF (2009) *Plant Taxonomy: The Systematic Evaluation of Comparative Data*. New York: Columba University Press.

Thiers B (2016) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. http://sweetgum.nybg.org/science/ih/. Continuously updated, accessed May 2016.

Thorsen MJ, Dickinson KJM, Seddon PJ, Dickinson KJ (2009) Seed dispersal systems in the New Zealand flora. *Perspectives in Plant Ecology, Evolution and Systematics* 11: 285-309.

Tocchio LJ, Gurgel-Goncalves R, Escobar LE, Peterson AT (2015) Niche similarities among white-eared opossums (Mammalia, Didelphidae): Is ecological niche modelling relevant to setting species limits? *Zoologica Script* 44: 1-10.

Townsend AJ, de Lange PJ, Duffy CAJ, Miskelly CM, Molloy J, Norton DA (2008) *New Zealand Threat Classification System Manual*. Wellington: Science and Technical Publishing, Department of Conservation.

VanDerWal J, Shoo LP, Graham C, Williams SE (2009) Selecting pseudo-absence data for presence-only distribution modeling: How far should you stray from what you know? *Ecological Modelling* 220: 589-594.

Warren D, Glor R, Turelli M (2008) Environmental niche equivalency versus conservatism: Quantitative approaches to niche evolution. *Evolution* 62: 2868–2883.

Warren DL (2012) In defense of 'niche modeling'. *Trends in Ecology & Evolution* 27: 497-500.

Warren DL, Glor RE, Turelli M (2010) ENMTools: A toolbox for comparative studies of environmental niche models. *Ecography* 33: 607-611.

Webb CJ, Simpson MJA (2001) *Seeds of New Zealand Gymnosperms & Dicotyledons*. Christchurch: Manuka Press.

Weigend M, Selvi F, Thomas DC, Hilger HH (2016) *Boraginaceae*. In: Kadereit JW, Bittrich V, editors. *Flowering plants. Eudicots, the families and genera of vascular plants 14*. Switzerland: Springer International Publishing.

Willis JC (1922) *Age and Area*. Cambridge, England: Cambridge University Press.

Wilson H (1994) *Field Guide: Stewart Island Plants*. Christchurch, New Zealand: Manuka Press.

Wilson H (1996) *Wild Plants of Mt Cook National Park*. Christchurch, New Zealand: Manuka Press.

Winkworth R, Grau J, Robertson A, Lockhart P (2002) The origins and evolution of the genus *Myosotis* L. (Boraginaceae). *Molecular Phylogenetics and Evolution* 24: 180-193.

Winkworth RC, Wagstaff SJ, Glenny D (2005) Evolution of the New Zealand mountain flora: Origins, diversification and dispersal. *Organisms Diversity & Evolution* 5: 237-247.

Wood JR, Wilmshurst JM, Worthy TH, Cooper A (2012) First coprolite evidence for the diet of *Anomalopteryx didiformis*, an extinct forest ratite from New Zealand. *New Zealand Journal of Ecology* 36: 164-170.

Wooten JA, Gibbs HL (2012) Niche divergence and lineage diversification among closely related *Sistrurus* rattlesnakes. *Journal of Evolutionary Biology* 25: 317-328.

Zare R, Gams W, Schroers H-J (2004) The type species of *Verticillium* is not congeneric with the plant-pathogenic species placed in *Verticillium* and it is not the anamorph of *'Nectria' inventa*. *Mycological Resources* 108: 675-582.

Chapter 6 general conclusions and future directions

In this thesis, morphological, population genetic and ecological niche modelling data were integrated to identify lineages within the pygmy forget-me-not group. The work presented here expands our understanding of evolution, systematics, taxonomy, rarity and conservation, particularly as these pertain to the New Zealand native pygmy forget-me-nots (Chapters 2–5).

The morphological research involved measuring herbarium specimens from both field-collected plants and those grown in a common garden. This approach revealed high levels of morphological plasticity and highlighted the importance of undertaking common garden experiments to understand which morphological characters have a more genetic vs. environmental basis, and therefore are potentially most useful for delimiting species (Chapter 2). The population genetic research involved developing 12 microsatellite markers (Chapter 3) and genotyping over 500 specimens (Chapter 4). This research represents an increase in our knowledge of how population genetic data can be used for species delimitation, and specifically emphasises the benefits of integrating data for species delimitation (Chapters 4 & 5). A taxonomic revision of the pygmy forget-me-nots comprised three species, one divided into two subspecies. The taxonomic revision of the New Zealand native pygmy forget-me-nots is a major output of this thesis, and is of importance from a biodiversity as well as conservation standpoint. For each of the pygmy forget-me-nots, their ecological niches were modelled, rarity type determined, and threat status assessed. Chapter 5 is thus an example of implementing a rigorous, objective method for determining rarity type and threat status, a process for which the methodologies are often so clearly defined. The population genetic data generated also was used for assessing differences in genetic variation and structure between species with different rarity types.

The aims for this thesis that were introduced in Chapter 1 mostly related to this final point, i.e., the relationships between population genetic variation and rarity. Below I briefly summarise how each of those aims was addressed by this thesis, and highlight conclusions that can be drawn relevant to those aims. A section highlighting the implications of this research for conservation management is included. Finally in this chapter there is a section on potential future research projects inspired by the work undertaken for this thesis.

Aims of the thesis

The main aims of this thesis were to address the two following questions: 1) How can population genetic information be used to inform species delimitation and conservation management in rare plants? And 2) How is population genetic variation structured in rare plants? Specifically are there differences in the population genetic structure or variation between naturally uncommon species and those that are thought to be rare due to human-influenced decline?

To understand the relevance of population genetic data to species delimitation (Aim 1), 12 microsatellite loci were developed for the pygmy forget-me-not group (Chapter 3). Over 500 individuals were then genotyped, and the population genetic data was used to delimit lineages within the pygmy forget-me-nots using both iterative and integrative taxonomic approaches (Chapter 4). Population genetic variation was shown to be most useful for informing species delimitation when integrated with data from additional sources, and morphological data (Chapter 2) was shown to be particularly useful in this regard (Chapters 4 & 5). The thesis concludes with a taxonomic revision (Chapter 5), in which the lineages discovered in previous chapters were translated directly into taxonomic changes, which has practical implications for conservation (Kim and Byrne 2006). Taxonomic revision is therefore one of the chief means by which population genetic data can also be used to inform conservation management, as threat classification systems are based upon taxonomy at the species level (Townsend et al., 2008). In general, most of the genetic variation found in the pygmy forget-me-nots is partitioned between, rather than within, populations (Chapters 4 & 5). This pattern most likely reflects high levels of self-fertilisation coupled with low levels of seed dispersal (Loveless and Hamrick 1984). Interpreting the implications of this pattern for conservation management, the conclusion is that each population is equally important in terms of its contribution to the genetic diversity of each species. A more detailed interpretation of the conservation implications was included in the notes sections of the taxonomic revision, and is summarised in the section below.

To understand in more detail the pattern of genetic structuring found in naturally uncommon vs. rare plants (Aim 2), the population genetic metrics from the same 500+ individual microsatellite dataset of 12 loci were analysed for *M. antarctica* subsp. *antarctica* (Naturally Uncommon) vs. other pygmy forget-me-nots (*M. antarctica* subsp. *traillii, M. brevis* and *M. glauca,* all Nationally Vulnerable). *Myosotis antarctica* subsp. antarctica does have higher % polymorphic loci than the other pygmy forget-me-nots, and

a slightly higher number of effective alleles (Table 5.6), however no other general trends are evident. Indeed *M. antarctica* subsp. *traillii* was counter intuitively found to have lower F_{IS} than *M. antarctica* subsp. *antarctica* (Table 5.6), indicating higher levels of observed heterozygosity relative to expected heterozygosity. Lower levels of observed heterozygosity in *M. brevis* and *M. glauca* may be due to their smaller number of populations relative to *M. antarctica* subsp. *antarctica*, but could also be due to a different life history (in the case of the annual *M. brevis*) or lower numbers of populations sampled (in the case of *M. glauca*). To tease apart the effects of pollination syndrome, life cycle and rarity on the population genetic structure and variation of New Zealand *Myosotis*, additional *Myosotis* species, including species considered to be "Common" (i.e. not threatened) could be studied in a comparative framework as outlined below under the future directions section. It should also be noted that inclusion of a "Common" species was planned for this thesis to more fully assess Aim 2. Before the taxonomic revision and threat classification were undertaken (Chapter 5), *M. drucei* was thought to be a "Common" pygmy forget-me-not species. However, after the taxonomic revision and threat classification assessment*, M. drucei* became a synonym of *M. antarctica* subsp. *antarctica,* which was reassessed as "At Risk, Naturally Uncommon".

Implications for conservation management

Each of the species and subspecies of pygmy forget-me-nots assessed in this study were found to be Threatened or At Risk in some way, but some were found to be more at risk than others. In terms of practical management implications of this research, there are different recommendations for each entity, and these are summarised below. For more details regarding specific populations referred to please see the notes sections of the taxonomic revision. In general, most of the genetic diversity across all species in the group is partitioned between, rather than within populations. As discussed in chapter 4 this is most likely due to the selfing nature of the pygmy forget-me-nots, coupled with low levels of seed dispersal. The main implication is that an emphasis on conservation of multiple populations of each species and subspecies is a priority in order to maintain genetic diversity. For each entity, the number of populations known to exist, the percentage of populations growing on DOC managed land, the observed rate of decline and any observed threats were taken into account to propose the following recommendations regarding which entities and which populations may require more active management.

Myosotis antarctica subsp. *antarctica* is the least threatened entity of the group and is considered Naturally Uncommon despite being fairly widespread, due to the small

estimated overall census of ~12000 plants. However, this could well be an underestimate, as new populations are often discovered when subalpine areas are surveyed in detail (JMP pers. obs). Approximately 70% of known populations are growing on land managed by DOC, which offers them some protection. The sites visited were not usually weedy (JMP pers. obs.), and other than recording new locations for this species, and recording the persistence of populations when they are re-visited, no other direct management requirements are recommended in New Zealand. However, this species was last recorded in southern Chile in 1908, and botanists, landowners and conservation staff are strongly encouraged to look for populations at Puerto Altamirano, Punta Arenas and surrounding areas with suitable habitat.

In contrast, *M. antarctica* subsp. *traillii* is considered to be Nationally Vulnerable with an overall census of ~2900 plants, and has the highest observed rate of decline (Table 5.5), coupled with a relatively low percentage (ca. 25 %) of populations growing on DOC managed land. None of the populations growing on the North Island inhabit DOC managed land, and the coastal populations of Taranaki are at risk from cliff edge erosion and farmland proximity. These populations are known to DOC, and if the landowners are willing, it is recommended that the populations on the edge of farmland be monitored regularly and protected from farming activities.

Myosotis brevis is also considered to be Nationally Vulnerable with an overall census of ~17600 plants, and as with *M. antarctica* subsp. *traillii* it is also the North Island populations that are most at risk. Again none of the North Island populations inhabit DOC managed land, and the coastal habitat they grow in is considered acutely threatened. *Myosotis brevis* is also at risk of overshading due to invasive weeds, and it is recommended that the North Island populations be monitored and weeding trials implemented if necessary. In particular the population at Stent Road on the Taranaki Coast is genetically distinct compared to other North Island populations and could therefore be prioritised.

The final entity, *M. glauca*, is also considered Nationally Vulnerable based on estimated census size (~4600 plants), and only four known populations (31 %) grow on DOC managed land. Efforts to find, record, and protect more populations are a priority, as is monitoring known populations to assess the effects of invasive weeds.

Future directions

While undertaking this research, several projects were initiated or identified, but for various reasons were unable to be included or completed in the thesis. Three of these represent potential future directions for research, and are briefly outlined below.

Chromosome counts

There are only three published chromosomes counts of pygmy forget-me-nots (Table 5.7), and these potentially show two different chromosome races within *Myosotis antarctica* (*n* = 22 and *n* = 24). The advantages to making additional counts was recognised while undertaking the morphological research, and are discussed in the introduction to Chapter 2. With the help of Prashant Joshi (Massey University, Palmerston North) counts were attempted both from floral bud tissue and root tip tissue, taken from additional plants grown in the growth chamber (under the same conditions used for plants grown for the common garden experiment). To collect root tips, seeds were germinated on filter paper in Petri dishes, and harvested between 0900–1000 into colchicine 0.05% (w/v*)* as soon as the root tip was visible. The root tips remained in colchicine in the dark for 3 hours before being fixed in 3:1 ethanol:acetic acid fixative. Floral buds were harvested directly into in 3:1 ethanol:acetic acid fixative; buds were collected at a range of ages for comparison. We found that it was easier to use tissue taken from root tips of just germinated seeds rather than floral buds, due to difficulties in determining the best time to fix the buds. Overall, 150 root tips were fixed from 11 populations of pygmy forget-me-nots, representing *M. antarctica* subsp. *antarctica* (five populations), *M. antarctica* subsp. *traillii* (four populations) and *M. brevis* (two populations); see Table 6.1 for details. Several photographs of squashes were taken, however, we struggled to get photographs showing sufficient separation of the chromosomes in good focus. The small size of the chromosomes of New Zealand *Myosotis* present challenges (e.g., Figure 2B; Murray and de Lange 2013), but nevertheless future endeavours to count the chromosomes of pygmy forget-me-nots would be beneficial and is encouraged.

Sequencing microsatellite loci

In Chapter 3, the development of 12 microsatellite markers was outlined, and over 500 forget-me-nots were genotyped using those markers in Chapter 4. The number of alleles found at each locus varied between 5 and 19 (when considering just the pygmy-plus dataset; Table 4.3). In a number of instances, those alleles differed by a single base pair in size, despite the microsatellite repeat being di- or even tri-nucleotide (data not shown). When microsatellite alleles differ by numbers other than the size of the nucleotide repeat,

it can be due to mutations other than those altering the number of microsatellite repeats having occurred, for example small indels in either the microsatellite itself, or in the flanking regions (Adams et al., 2004). Sequencing the microsatellites allows the detection of such mutations, with the additional benefit of being able to assess whether individuals presenting with the same allele when genotyped are indeed homologous (Adams et al., 2004). Although sequencing microsatellite regions has long been an option using Sanger sequencing, the process was time consuming and costly, whereas now with the advent of high-throughput sequencing technologies it can be undertaken in a more efficient and cost-effective manner (e.g., Germain-Aubrey et al., 2016). Using high-throughput sequencing, microsatellites have been sequenced for New Zealand *Veronica* (Mayland-Quellhorst, Meudt and Albach, under review) and New Zealand *Myosotis* could be a good contender for the method, particularly as microsatellite loci have already been developed.

Comparisons between different types of rarity and different breeding systems

The patterns of genetic variation within and between populations in the pygmy forget-me-nots are reflective of high levels of self-fertilisation coupled with low levels of seed dispersal (Chapter 5). The one species with a different life cycle (*M. brevis* is a spring annual vs. other pygmy forget-me-nots are usually biennial or perennial), also had on average larger population sizes, and in conjunction with self-fertilization these influences appeared to have a greater effect on the population genetic structure and variation than the rarity type of each species (Chapter 5). To help identify the different effects of breeding system, population size and rarity type on genetic variation and structure, population genetic studies of New Zealand *Myosotis* species with different breeding systems and rarity types could be undertaken. To this end, specimens from populations of outcrossing species (*M. macrantha* and *M. arnoldii*) as well as species with unknown outcrossing rates (*M. matthewsii* and *M. spathulata*) were also collected during field seasons from 2011–2015. In the outcrossing pair, *M. macrantha* is widespread and Not Threatened, whereas *M. arnoldii* is a basicole known only from two locations and is classified as At Risk – Naturally Uncommon, Range Restricted. *Myosotis spathulata* and *M. matthewsii* both have exserted stamens, but their outcrossing rates are unknown. Neither species is common in this pair, but *M. spathulata* has a widespread distribution (Naturally Uncommon, Data Poor, Extreme Fluctuations, Sparse), whereas the Nationally Endangered *M. matthewsii* is only known for certain from a single population near Kaitaia, Northland. The number of recent collections of these species is indicated in Table 6.2, along with the number of specimens for which their DNA has already been extracted. The 12 microsatellite loci developed for the pygmy forget-me-nots were trialled on *M. macrantha*

(n = 6), *M. arnoldii* (n = 6) and *M. spathulata* (n = 3) in Chapter 3. The amplification rates were relatively low (*M. spathulata* 50%; *M. arnoldii* 62.5%; *M. macrantha* 73.6%) and often produced alleles that were of different sizes to those found in the pygmy group (data not shown). Therefore genotyping these species using the 12 published microsatellites may not be the best method for generating population genetic data for these outcrossing comparisons, and alternative genetic methods, e.g. GBS or Rad-Seq, may need to be pursued (Hodel et al., 2016).

Concluding remarks

Rarity is a complex concept; there are many ways to "be" a rare plant, as well as many factors influencing how and why a species may be rare. Understanding the effects of different types of rarity on genetic variation and structure, especially if this can be linked to extinction risk, will have useful implications for conservation. New Zealand *Myosotis* is an eminently suitable study system to address these questions, given the range of rarity types, breeding systems and life history traits exhibited. This thesis has laid the groundwork and addressed these questions for New Zealand *Myosotis* by developing a genetic resource and describing in detail how rarity and threat classifications can be undertaken in a rigorous way. By utilising the genetic resource for both species delimitation and to help answer questions regarding rarity, the research in this thesis has also helped to highlight the importance of taxonomy, and methods whereby taxonomic questions can be addressed alongside more theoretical questions.

Table 6.1 Number of seeds germinated and root tips fixed for chromosome counts of pygmy forget-me-nots (*Myosotis*). NI = North Island, SI = South Island.

Species	Location	Voucher No.	Age of seed at planting (months)	No. of seeds sown	No. of seeds germinated and root tips fixed	% germination	Length of time to germination (days)		
							Average	First seed	Last seed
M. brevis	SI, Canterbury, Lake Lyndon	WELT SP093294	8	20	20	100	4.0	4	4
M. brevis	cult. ex NI, Taranaki, Stent Rd	WELT SP104495	5	20	6	30	34.5	30	45
***M. brevis* total**			5 to 8	40	26	65	19.3	4	45
M. antarctica* subsp. *antarctica	cult. ex SI, Fiordland, Routeburn	Pending	5	20	19	95	5.7	3	9
M. antarctica* subsp. *antarctica	NI, Ruahines, Maungamahue	WELT SP100445	17	20	19	95	7.8	4	8
M. antarctica* subsp. *antarctica	cult. ex SI, Fiordland, Beansburn	WELT SP104526	1	20	10	50	15.6	9	45
M. antarctica* subsp. *antarctica	cult. ex SI, Southland, Livingstone Mts	WELT SP104503	1	10	9	90	10.1	8	12
M. antarctica* subsp. *antarctica	cult. ex SI, NW Nelson, Lake Peel	WELT SP104500	1	2	2	100	18.0	17	19
***M. antarctica* subsp. *antarctica* total**			1 to 17	72	59	82	11.4	3	45
M. antarctica* subsp. *traillii	cult. ex NI, Taranaki, Arawhata Rd	WELT SP104505	3	20	20	100	22.9	15	45
M. antarctica* subsp. *traillii	SI, NW Nelson, Paturau River	WELT SP100462	8	20	6	30	21.7	8	45
M. antarctica* subsp. *traillii	cult. ex SI, Coastal Otago, Tahakopa Bay	WELT SP104498	3	20	19	95	14.1	8	19
M. antarctica* subsp. *traillii	cult. ex Stewart Is, Mason Bay	WELT SP104501	1	20	20	100	22.4	15	32
***M. antarctica* subsp. *traillii* total**			1 to 8	80	65	81	20.3	8	45
Total			1 to 17	192	150	78	16.0	3	45

Table 6.2 Collections of outcrossing New Zealand *Myosotis.* Threat status from the latest New Zealand threat classification (de Lange et al., 2013). NI = North Island, SI = South Island.

Species	Threat status	Location	Collector (collection number)	Collection date	Voucher	No. leaves into silica	No. individuals, DNA extracted
M. arnoldii	Naturally Uncommon	SI, NW Nelson, Hoary Head	JM Prebble, M Prebble & LM Bagnall (JMP13006)	21/01/13	WELT SP100473	30 (a-dd)	30
M. arnoldii	Naturally Uncommon	SI, Marlborough, Mt Benmore	JM Prebble, J Clayton-Green et al. (JMP13023)	7/02/13	WELT SP100439	29 (a-ac)	29
M. macrantha	Not Threatened	SI, NW Nelson, Mt Arthur	JM Prebble, M Prebble & LM Bagnall (JMP13010)	22/01/13	WELT SP100479	10 (a-j)	10
M. macrantha	Not Threatened	SI, NW Nelson, Lake Peel	JM Prebble, M Prebble & LM Bagnall (JMP13015)	25/01/13	WELT SP100468	30 (a-dd)	30
M. macrantha	Not Threatened	SI, Marlborough, Mt Weld	JM Prebble & J Clayton-Green (JMP13030)	12/02/13	WELT SP100418	10 (a-j)	10
M. macrantha	Not Threatened	SI, Otago, Moke Ck	JM Prebble, N Simpson et al. (JMP13036)	28/02/13	WELT SP100494	19 (a-s)	19
M. macrantha	Not Threatened	SI, Mt Cook NP, Hooker Valley	JM Prebble & TM Herleth (JMP13043)	7/10/13	WELT SP102794	4 (a-d)	4
M. macrantha	Not Threatened	SI, Southland, Livingstone Mts	JM Prebble & R Hindmarsh-Walls (JMP14008)	7/02/14	WELT SP102189	9 (a-i)	0
M. matthewsii	Nationally Endangered	NI, Northland, Warawara forest	JM Prebble et al. (JMP12010)	8/03/12	WELT SP093687	11 (a-f, h-l)	3
M. spathulata	Naturally Uncommon	NI, Wairarapa, Mairangai	HM Meudt, JM Prebble, T Silbery et al. (HMM316)	2/11/11	WELT SP090548	5 (a-e)	0
M. spathulata	Naturally Uncommon	NI, Hawke's Bay, Hukanui	HM Meudt, JM Prebble & MJ Thorsen (HMM331)	12/12/11	WELT SP090628	22 (a-v)	10
M. spathulata	Naturally Uncommon	NI, Hawke's Bay, Waipuna	HM Meudt, JM Prebble & MJ Thorsen (HMM334)	13/12/11	WELT SP090633	15 (a-q)	0
M. spathulata	Naturally Uncommon	SI, Arthur's Pass, Prebble Hill	JM Prebble, MJ Thorsen et al. (JMP12008)	21/02/12	WELT SP093293	6 (a-f)	0
M. spathulata	Naturally Uncommon	NI, Ruahines, Potae	JM Prebble (JMP12014)	7/11/12	WELT SP100400	15 (a-o)	0
M. spathulata	Naturally Uncommon	NI, Ruahines, Potae	JM Prebble (JMP12015)	7/11/12	WELT SP100406	5 (a-e)	0

References

Adams RI, Brown KM, Hamilton MB (2004) The impact of microsatellite electromorph size homoplasy on multilocus population structure estimates in a tropical tree (*Corythophora alta*) and an anadromous fish (*Morone saxatilis*). *Molecular Ecology* 13: 2579-2588.

de Lange PJ, Rolfe JR, Champion PD, Courtney SP, Heenan PB, Barkla JW, Cameron EK, Norton DA, Hitchmough RA (2013) *Conservation Status of New Zealand Indigenous Vascular Plants, 2012*. Wellington: New Zealand Department of Conservation.

Germain-Aubrey CC, Nelson C, Soltis DE, Soltis PS, Gitzendanner MA (2016) Are microsatellite fragment lengths useful for population-level studies? The case of *Polygala lewtonii* (Polygalaceae). *Applications in Plant Sciences* 4: 1500115.

Hodel RGJ, Segovia-Salcedo MC, Landis JB, Crowl AA, Miao Sun XL, Gitzendanner MA, Douglas NA, Germain-Aubrey CC, Chen S, Soltis DE, Soltis PS (2016) The report of my death was an exaggeration: A review for researchers using microsatellites in the 21st century. *Applications in Plant Sciences* 4: 1600025.

Kim KC, Byrne LB (2006) Biodiversity loss and the taxonomic bottleneck: Emerging biodiversity science. *Ecological Research* 21: 794-810.

Loveless MD, Hamrick JL (1984) Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* 15: 65-95.

Murray BG, de Lange PJ (2013) Contributions to a chromosome atlas of the New Zealand flora—40. Miscellaneous counts for 36 families. *New Zealand Journal of Botany* 51: 31-60.

Townsend AJ, de Lange PJ, Duffy CAJ, Miskelly CM, Molloy J, Norton DA (2008) *New Zealand Threat Classification System Manual*. Wellington: Science and Technical Publishing, Department of Conservation.

Appendix 1 Reproduction of Chapter 3, as published in *Applications in Plant Sciences*

DRC 16

MASSEY UNIVERSITY
GRADUATE RESEARCH SCHOOL

STATEMENT OF CONTRIBUTION
TO DOCTORAL THESIS CONTAINING PUBLICATIONS

(To appear at the end of each thesis chapter/section/appendix submitted as an article/paper or collected as an appendix at the end of the thesis)

We, the candidate and the candidate's Principal Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of Candidate: Jessie Prebble

Name/Title of Principal Supervisor: Vaughan Symonds

Name of Published Research Output and full reference:
Prebble JM, Tate JA, Meudt HM, Symonds, VV. 2015. Microsatellite markers for the New Zealand endemic Myosotis pygmaea species group (Boraginaceae) amplify across species. Applications in Plant Sciences 3:6 DOI: http://dx.doi.org/10.3732/apps.1500027

In which Chapter is the Published Work: Chapter 3, and reproduced as Appendix 1

Please indicate either:

- The percentage of the Published Work that was contributed by the candidate: 90%

  and / or

- Describe the contribution that the candidate has made to the Published Work:

Jessie Prebble — Digitally signed by Jessie Prebble DN: cn=Jessie Prebble, o=Massey University, ou=Institute of Fundamental Sciences, email=jessie.prebble@gmail.com, c=NZ Date: 2016.07.06 11:02:31 +12'00'

Candidate's Signature — 06/07/2016 — Date

V. Vaughan Symonds — Digitally signed by V. Vaughan Symonds DN: cn=V. Vaughan Symonds, o=Massey University, ou=IFS, email=v.v.symonds@massey.ac.nz, c=NZ Date: 2016.07.06 09:43:55 +12'00'

Principal Supervisor's signature — 06/07/2016 — Date

GRS Version 3– 16 September 2011

Applications in Plant Sciences 2015 3(6): 1500027

PRIMER NOTE

MICROSATELLITE MARKERS FOR THE NEW ZEALAND ENDEMIC *MYOSOTIS PYGMAEA* SPECIES GROUP (BORAGINACEAE) AMPLIFY ACROSS SPECIES[1]

JESSICA M. PREBBLE[2,3,4], JENNIFER A. TATE[2], HEIDI M. MEUDT[3], AND V. VAUGHAN SYMONDS[2]

[2]Institute of Fundamental Sciences, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand; and [3]Museum of New Zealand Te Papa Tongarewa, P.O. Box 467, Cable Street, Wellington 6140, New Zealand

- *Premise of the study:* Microsatellite loci were developed as polymorphic markers for the New Zealand endemic *Myosotis pygmaea* species group (Boraginaceae) for use in species delimitation and population and conservation genetic studies.
- *Methods and Results:* Illumina MiSeq sequencing was performed on genomic DNA from seedlings of *M. drucei*. From trimmed paired-end sequences >400 bp, 484 microsatellite loci were identified. Twelve of 48 microsatellite loci tested were found to be polymorphic and consistently scorable when screened on 53 individuals from four populations representing the geographic range of *M. drucei*. They also amplify in all other species in the *M. pygmaea* species group, i.e., *M. antarctica*, *M. brevis*, *M. glauca*, and *M. pygmaea,* as well as 18 other *Myosotis* species.
- *Conclusions:* These 12 polymorphic microsatellite markers establish an important resource for research and conservation of the *M. pygmaea* species group and potentially other Southern Hemisphere *Myosotis*.

Key words: Boraginaceae; forget-me-nots; microsatellites; *Myosotis*; New Zealand; threatened species.

Forget-me-nots (*Myosotis* L., Boraginaceae) are found in both the Northern and Southern Hemispheres, with a center of diversity in New Zealand. The *M. pygmaea* species group (Meudt et al., 2015) comprises *M. antarctica* Hook. f., *M. brevis* de Lange & Barkla, *M. drucei* (L. B. Moore) de Lange & Barkla, *M. glauca* (G. Simpson & J. S. Thomson) de Lange & Barkla, and *M. pygmaea* Colenso, all native to New Zealand. Questions persist regarding the delimitation of these morphologically similar species (de Lange et al., 2010), four of which appear on the New Zealand threatened species list (de Lange et al., 2013). Indeed, of the 44 endemic New Zealand *Myosotis* taxa, 32 are considered threatened or at risk (de Lange et al., 2013). A priority in the conservation management of members of this genus is to both accurately delimit species and understand the levels and structure of genetic diversity present. Low genetic diversity in New Zealand *Myosotis*, as evidenced by previous studies (Meudt et al., 2013, 2015), suggests that additional molecular markers are needed.

Here we report the development of 12 polymorphic microsatellite markers for the *M. pygmaea* species group, which will be used in future studies of species delimitation and population genetic research. Additionally, we evaluate the utility of these loci in 18 other *Myosotis* species.

METHODS AND RESULTS

Sibling individuals were selected from the type locality of *M. drucei* as the source DNA for marker development (WELT SP100445; Appendix 1). Genomic DNA was extracted from fresh young leaf tissue from 15 seedlings using a modified cetyltrimethylammonium bromide (CTAB) method (Shepherd and McLay, 2011). To generate sufficient template for the requirements of Illumina MiSeq library preparation, extracted DNA was pooled and amplified using a REPLI-g kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. DNA was quantified using a Qubit 2.0 Fluorometer (Thermo-Fisher Scientific, Waltham, Massachusetts, USA), and a genomic library was prepared using the TruSeq Library Preparation Kit (Illumina, San Diego, California, USA) by the Massey Genome Service (Massey University, Palmerston North, New Zealand). The indexed library was pooled with three other libraries in equal concentration and sequenced using the paired-end 250-bp chemistry on a MiSeq (Illumina) by the Massey Genome Service. The resulting 2.7 million sequences were trimmed of low-quality results using a 0.01 quality cut-off in DynamicTrim in SolexaQA (Cox et al., 2010), which yielded 1,449,369 trimmed paired-end sequences with an average length of 380 bp, ranging in size from 11–492 bp. Paired-end sequences were joined using the program FLASH (Magoc and Salzberg, 2011).

The paired-end sequences were then imported into Geneious 6.1.5 (Biomatters, Auckland, New Zealand), where only sequences >400 bp were retained. Organellar sequences were removed by performing a local BLAST search of the *M. drucei* sequences against the phylogenetically closest relatives (Soltis et al., 2011) with the most complete mitochondrial and chloroplast sequences from GenBank. The chloroplast genomes used were: *Nicotiana undulata* Ruiz & Pav. NC_016068 (Solanaceae), *Olea europaea* L. subsp. *maroccana* (Greuter & Burdet) P. Vargas, J. Hess, Muñoz Garm. & Kadereit NC_015623 (Oleaceae), *Coffea arabica* L. NC_008535 (Rubiaceae), and *Arabidopsis thaliana* (L.) Heynh. NC_000932 (Brassicaceae). The mitochondrial genomes used were: *N. tabacum* L. NC_006581, *A. thaliana* NC_001284, and *Vigna radiata* (L.) R. Wilczek NC_015121 (Fabaceae). The remaining 397,224 sequences were split into four groups (due to computer memory constraints), and the first group of 99,999 sequences was searched for perfect di- to hexanucleotide microsatellite

[1]Manuscript received 18 March 2015; revision accepted 27 April 2015.

The authors thank Te Papa and Massey University for funding, including a Massey University Vice-Chancellor's Doctoral Scholarship to J.M.P. Fieldwork was facilitated by the Australasian Systematic Botany Society Eichler Award, the Royal Society of New Zealand's Hutton Fund, and the New Zealand Department of Conservation (permit number CA-31615-OTH). This research was supported by core funding for Crown Research Institutes from the Ministry of Business, Innovation and Employment's Science and Innovation Group.

[4]Author for correspondence: jessie.prebble@gmail.com

doi:10.3732/apps.1500027

TABLE 1. Primer sequences and characteristics of 12 microsatellite loci developed in *Myosotis drucei*.

Locus	Primer sequences (5′–3′)	Fluorescent dye (pooling group)	Repeat motif	Allele size range (bp)[a]	T_a (°C)	GenBank accession no.
MYPY-4	F: TATGCTCGTACCGAAACAC	NED (2)	$(TGT)_8$	248–255	53	KP861356
	R: AGTGCTTATGTTTGCCCTC					
MYPY-10	F: GCGACATTGCAACTGATAC	VIC (1)	$(GAT)_{10}$	312–345	53	KP861353
	R: TACCTCATCGCTCAATACC					
MYPY-14	F: AAGAACATTTTGCCACAGC	VIC (2)	$(GAA)_7$	211–217	53	KP861350
	R: TTAAATCATTGCACGTCCG					
MYPY-17	F: CCTCTCTCTATATGTCGCG	VIC (3)	$(ATA)_{12}$	273–311	53	KP861357
	R: GGATTACCTTGAGGCAGTG					
MYPY-20	F: GTTGAGAGAGCTCTACTGC	FAM (4)	$(AT)_9$	228–236	53	KP861359
	R: GTACCCAGCATTAACCAGG					
MYPY-26	F: ACTTGGAGAACGATTTGTCCG	NED (3)	$(TC)_7$	374–477	53	KP861355
	R: AACCGCCGCAAAATTCAAAC					
MYPY-28	F: TGACTCTGGACAATGATGAGAGAG	VIC (4)	$(TA)_9$	341–357	53	KP861352
	R: CGGCTGTTTTAGAACCACCC					
MYPY-29	F: GGTTCAGTGATAATGTTCGAGCC	FAM (2)	$(AC)_9$	334–342	53	KP861351
	R: CACAGGAAGGATCAATGACTGC					
MYPY-36	F: GTTGTGCTTGATGGTGACCC	NED (4)	$(GAT)_{10}$	259–296	53	KP861360
	R: CCCATCCTTCTTCTCCACCC					
MYPY-40	F: CTGCCTCATTATTCTCTGGG	FAM (1)	$(AG)_7$	261	53	KP861358
	R: CACGACCATTCCATGTTAAC					
MYPY-41	F: CTTCTTGACGCTTTTGCTAC	NED (1)	$(TG)_8$	269–271	53	KP861354
	R: TTCAGAATAGCAATTGTCGC					
MYPY-48	F: ATTCGACGTAGATCTTGTGC	FAM (3)	$(GATGAA)_7$	251–275	53	KP861349
	R: AAAGAAAACTGCAGAACGTG					

[a]Fragment size range based on 53 *Myosotis drucei* samples from four populations: WELT SP091599, WELT SP100445, WELT SP100440, and WELT SP100428; voucher information in Appendix 1.

repeats with a minimum of seven uninterrupted repeat units using a search tool in Geneious (Phobos plugin; Mayer, 2010), which identified 484 repeats. Sequences were removed from consideration if the paired-end sequences were found to be overlapping only in the repeat region, if regions near the microsatellite contained other microsatellite loci or single base pair repeats >4 bp, or if there were greater than 14 repeats. After removing unsuitable loci, primers were designed for 147 microsatellite regions using Primer3 within Geneious (Untergasser et al., 2012). The default settings were used except for: product size = 100–400 bp with a 50-bp buffer on both sides of the target region; primer size = 18 bp (minimum)–20 bp (optimal)–22 bp (maximum); melting temperature (T_m) = 47–55–60°C; 3′ GC content = 40–50–60%; maximum T_m difference = 10°C; GC clamp = 1; max poly N = 4. An M13 tag (CACGACGTTGTAAAACGAC) was added to the 5′-end of the forward primer for each locus, and a PIG-tail sequence (GTTTCTT; Brownstein et al., 1996) was added to the 5′-end of each reverse primer.

For reasons of practicality, 48 primer pairs were chosen to trial a range of: uninterrupted number of repeats, types of microsatellites (e.g., di-, tri-, tetra-, penta-, and hexa-), and PCR product sizes. These 48 were initially trialed on seven individuals from five populations of four *M. pygmaea* group species (Appendix 1). Each locus was amplified individually in 10-μL PCR reactions that contained 1 μL of a 1:50 dilution of template DNA (5–50 ng), 0.02 μM forward primer, 0.45 μM reverse primer, 0.45 μM M13 primer (labeled with FAM, NED, or VIC), 1.5 mM $MgCl_2$, 1× buffer BD (Solis BioDyne, Tartu, Estonia), 250 μM of each dNTP, and 1 unit FIREPol *Taq* polymerase (Solis BioDyne). PCRs were carried out with the following cycling program: an initial denaturation of 95°C for 3 min; 40 cycles of 95°C for 30 s, 53°C for 40 s, and 72°C for 1 min; and a final extension at 72°C for 10 min. A volume of 0.75 μL of each PCR product for three loci, each with a different fluorophore, was added to 9 μL of Hi-Di formamide (Applied Biosystems, Carlsbad, California, USA) premixed with a ROX-labeled CASS ladder (Symonds and Lloyd, 2004) for

TABLE 2. Summary statistics of microsatellite polymorphism determined by screening 53 *Myosotis drucei* samples from four populations; three from the South Island and one from the North Island of New Zealand.[a]

	South Island									North Island			
	Coronet Peak (N = 13)			Tapuae-o-Uenuku (N = 14)			Mt. Altimarlock (N = 11)			Ruahine Ranges (N = 15)			Total (N = 53)
Locus	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A	H_o	H_e	A_T
MYPY-4	2	0.077	0.204	2	0.000	0.375	1	0.000	0.000	1	0.000	0.000	2
MYPY-10	3	0.000	0.462	3	0.000	0.500	2	0.091	0.351	1	0.000	0.000	7
MYPY-14	1	0.000	0.000	2	0.000	0.408	1	0.000	0.000	2	0.000	0.391	3
MYPY-17	2	0.077	0.074	1	0.000	0.000	1	0.000	0.000	1	0.000	0.000	4
MYPY-20	2	0.000	0.153	2	0.000	0.408	3	0.100	0.515	1	0.000	0.000	4
MYPY-26	2	0.000	0.142	2	0.000	0.408	1	0.000	0.000	3	0.000	0.561	5
MYPY-28	2	0.000	0.500	2	0.000	0.355	2	0.091	0.087	1	0.000	0.000	4
MYPY-29	2	0.000	0.165	3	0.667	0.667	2	1.000	0.500	2	0.600	0.420	4
MYPY-36	3	0.077	0.210	2	0.000	0.408	1	0.000	0.000	1	0.000	0.000	4
MYPY-40	2	0.000	0.165	1	0.000	0.000	1	0.000	0.000	1	0.000	0.000	2
MYPY-41	1	0.000	0.000	2	0.000	0.142	1	0.000	0.000	1	0.000	0.000	2
MYPY-48	2	0.000	0.473	2	0.000	0.408	1	0.000	0.000	2	0.000	0.337	4

Note: A = number of alleles; A_T = total number of alleles; H_e = expected heterozygosity; H_o = observed heterozygosity; N = sample size for each population.

[a]South Island: Coronet Peak = WELT SP091599, Tapuae-o-Uenuku = WELT SP100440, Mt. Altimarlock = WELT SP100428; North Island: Ruahine Ranges = WELT SP100445. See Appendix 1 for voucher information.

TABLE 3. Cross-amplification of 12 novel microsatellite loci in 22 *Myosotis* species.[a]

Species name	Voucher no.[b]	*N*	Location[c]	MYPY-4	MYPY-10	MYPY-14	MYPY-17	MYPY-20	MYPY-26	MYPY-28	MYPY-29	MYPY-36	MYPY-40	MYPY-41	MYPY-48
***Myosotis pygmaea* species group**															
M. antarctica	SP102775	12	CI	2	1	2	1	2	1	2	1	1	1	1	1
M. brevis	SP090361	25	NZ	1	1	1	1	2	2	1	1	1	1	1	1
M. glauca	SP093284	17	NZ	1	1	1	1	1	1	2	1	1	2	1	1
M. pygmaea	SP090540	13	NZ	1	1	1	1	1	1	2	1	1	1	1	1
Other New Zealand *Myosotis*															
M. arnoldii	SP100473	3	NZ	6	8	5	6	1	2	+	3	2	3	—	4
	SP100439	3													
M. chewesmanii	SP092210	1	NZ	+	+	+	+	—	—	—	+	+	+	—	—
M. colensoi	SP092419	1	NZ	+	—	—	+	—	—	—	+	+	+	—	—
M. forsteri	SP089691	1	NZ	2	1	2	2	—	2	—	2	3	1	1	1
	SP089928	1													
	SP092179	1													
M. glabrescens	SP089801	1	NZ	+	+	2	+	—	—	—	+	+	+	—	—
M. macrantha	SP100468	3	NZ	3	7	4	4	2	1	2	3	4	2	3	3
	SP100494	3													
M. pansa subsp. *pansa*	SP089670	2	NZ	2	1	2	2	—	—	1	1	—	1	—	—
	SP089674	1													
M. pansa subsp. *praeceps*	SP089685	2	NZ	2	1	3	—	—	—	—	1	—	2	—	—
	SP089686	1													
M. petiolata	SP089853	3	NZ	2	1	2	2	—	—	—	1	—	1	1	—
M. potsiana	SP089687	2	NZ	1	2	1	2	—	1	1	1	—	2	1	—
	SP089689	1													
M. pulvinaris	SP092196	1	NZ	—	2	+	+	—	—	+	2	+	+	+	+
M. "small white"	SP090247	1	NZ	2	1	1	2	—	1	—	1	3	1	1	—
	SP090251	1													
M. spathulata	SP090628	2	NZ	2	1	1	1	—	—	1	1	—	2	1	—
	SP092757	1													
M. tenericaulis	SP092404	1	NZ	2	—	+	+	—	—	—	+	—	+	—	—
Other *Myosotis*															
M. arvensis	SP094173	1	Euro	—	—	+	+	—	—	—	—	—	—	—	—
M. australis	MPN44757	2	Aust	1	—	1	2	—	—	—	—	1	2	—	—
M. discolor	SP089930	1	Euro	—	—	—	+	—	—	+	+	—	+	—	—
M. laxa	SP090206	1	Euro	—	—	+	+	—	—	—	—	—	—	—	—

Note: *N* = number of individuals trialed from each population.

[a]Number of amplified alleles are indicated, + = amplified with unknown levels of polymorphism as only one allele in one individual amplified, — = no amplification.

[b]See Appendix 1 for voucher information.

[c]Aust = Australian native; CI = Campbell Island native; Euro = European native growing in New Zealand; NZ = New Zealand endemic.

subsequent fragment separation on an ABI 3730 Genetic Analyzer (Applied Biosystems) by the Massey Genome Service.

Alleles were visualized and scored using GeneMapper version 3.7 (Applied Biosystems). Of the 48 primer pairs tested, 25 were polymorphic, two were monomorphic, seven were unscorable, and 14 did not amplify. Twenty-four of the polymorphic loci were further tested using the above PCR conditions on 15 individuals from five *Myosotis* species. The 12 markers (Table 1) with the best amplification rates were selected for further investigation using four populations of *M. drucei* to demonstrate the utility of the markers in a population genetic framework. For these four populations, Table 2 shows the number of alleles, and observed (H_o) and expected (H_e) heterozygosities, which were determined using GenAlEx (Peakall and Smouse, 2012). The average number of observed alleles per locus was 3.75, and average H_o was 0.059 (Table 2). H_o was typically lower than H_e, which matches the hypothesized mostly selfing nature of the *M. pygmaea* species group (Robertson and Lloyd, 1991; Brandon, 2001). The 12 markers amplified well across the other four species (one population each) in the *M. pygmaea* group (voucher information in Appendix 1) and were also trialed in an additional 18 species of *Myosotis*, 14 endemic to New Zealand, one from Australia, and three introduced to New Zealand from Europe. Amplification rates and polymorphism are reported in Table 3.

CONCLUSIONS

We describe 12 polymorphic microsatellite loci that will be useful for exploring species limits within the *M. pygmaea* species group, as well as determining the population genetic variation within and among other species of Southern Hemisphere *Myosotis*.

LITERATURE CITED

Brandon, A. M. 2001. Breeding systems and rarity in New Zealand *Myosotis*. Ph.D. Thesis, Massey University, Palmerston North, New Zealand.

Brownstein, M. J., J. D. Carpten, and J. R. Smith. 1996. Modulation of non-templated nucleotide addition by Taq DNA polymerase: Primer modifications that facilitate genotyping. *BioTechniques* 20: 1004–1010.

Cox, M. P., D. A. Peterson, and P. J. Biggs. 2010. SolexaQA: At-a-glance quality assessment of Illumina second-generation sequencing data. *BMC Bioinformatics* 11: 485.

de Lange, P. J., P. B. Heenan, D. A. Norton, J. R. Rolfe, and J. Sawyer. 2010. Threatened plants of New Zealand. Canterbury University Press, Christchurch, New Zealand.

de Lange, P. J., J. R. Rolfe, P. D. Champion, S. P. Courtney, P. B. Heenan, J. W. Barkla, E. K. Cameron, et al. 2013. Conservation status of New Zealand indigenous vascular plants, 2012. New Zealand Department of Conservation, Wellington, New Zealand.

Magoc, T., and S. Salzberg. 2011. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* 27: 2957–2963.

Mayer, C. 2010. Phobos Version 3.3.11. http://www.ruhr-uni-bochum.de/spezzoo/cm/cm_phobos.htm [accessed 21 May 2015].

Meudt, H. M., J. M. Prebble, R. J. Stanley, and M. J. Thorsen. 2013. Morphological and amplified fragment length polymorphism (AFLP) data show that New Zealand endemic *Myosotis petiolata* (Boraginaceae) comprises three rare and threatened species. *Australian Systematic Botany* 26: 210–232.

Meudt, H. M., J. M. Prebble, and C. A. Lehnebach. 2015. Native New Zealand forget-me-nots (*Myosotis*, Boraginaceae) comprise a Pleistocene species radiation with very low genetic divergence. *Plant Systematics and Evolution* 301: 1455–1471. 10.1007/s00606-014-1166-x .

Peakall, R., and P. E. Smouse. 2012. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—An update. *Bioinformatics* 28: 2537–2539.

Robertson, A. W., and D. G. Lloyd. 1991. Herkogamy, dichogamy and self-pollination in six species of *Myosotis* (Boraginaceae). *Evolutionary Trends in Plants* 5: 53–63.

Shepherd, L. D., and T. G. B. McLay. 2011. Two micro-scale protocols for the isolation of DNA from polysaccharide-rich plant tissue. *Journal of Plant Research* 124: 311–314.

Soltis, D. E., S. A. Smith, N. Cellinese, K. J. Wurdack, D. C. Tank, S. F. Brockington, N. F. Refulio-Rodriguez, et al. 2011. Angiosperm phylogeny: 17 genes, 640 taxa. *American Journal of Botany* 98: 704–730.

Symonds, V. V., and A. M. Lloyd. 2004. A simple and inexpensive method for producing fluorescently labeled size standard. *Molecular Ecology Notes* 4: 768–771.

Untergasser, A., I. Cutcutache, T. Koressaar, J. Ye, B. C. Faircloth, M. Remm, and S. G. Rozen. 2012. Primer3—New capabilities and interfaces. *Nucleic Acids Research* 40: e115.

APPENDIX 1. Voucher and location information for all *Myosotis* populations used in this study.

Species	Location[a]	Voucher no.[b]
***Myosotis pygmaea* species group**		
Myosotis antarctica Hook. f.	New Zealand, Campbell Island, cliffs near Menhir	WELT SP102775
Myosotis brevis de Lange & Barkla	New Zealand, Coastal Taranaki, Puketapu Rd. end*	WELT SP090361
Myosotis brevis de Lange & Barkla	New Zealand, Coastal Taranaki, Stent Rd.	WELT SP090543
Myosotis drucei (L. B. Moore) de Lange & Barkla	New Zealand, North Island, Ruahine Ranges, near Mt. Maungamahue*	WELT SP100445
Myosotis drucei (L. B. Moore) de Lange & Barkla	New Zealand, South Island, Marlborough, Tapuae-o-Uenuku	WELT SP100440
Myosotis drucei (L. B. Moore) de Lange & Barkla	New Zealand, South Island, Central Otago, Coronet Peak	WELT SP091599
Myosotis drucei (L. B. Moore) de Lange & Barkla	New Zealand, South Island, Marlborough, Mt. Altimarlock*	WELT SP100428
Myosotis glauca (G. Simpson & J. S. Thomson) de Lange & Barkla	New Zealand, South Island, Central Otago, Nevis Valley*	WELT SP093284
Myosotis pygmaea Colenso	New Zealand, North Island, Coastal Taranaki, Opunake treatment ponds	WELT SP090540
Myosotis pygmaea Colenso	New Zealand, South Island, Northwest Nelson, near Sandhill Creek river mouth*	WELT SP100460
Other New Zealand *Myosotis*		
Myosotis arnoldii L. B. Moore	New Zealand, South Island, Marlborough, Mt. Benmore	WELT SP100439
Myosotis arnoldii L. B. Moore	New Zealand, South Island, Northwest Nelson, Hoary Head	WELT SP100473
Myosotis cheesemanii Petrie	New Zealand, South Island, Central Otago, Pisa Range	WELT SP092210
Myosotis colensoi (Kirk) J. F. Macbr.	New Zealand, cultivated (Origin: South Island, Canterbury, Castle Hill)	WELT SP092419
Myosotis forsteri Lehm.	New Zealand, North Island, Kaweka Ranges	WELT SP089928
Myosotis forsteri Lehm.	New Zealand, North Island, Raukumara, Waioeka Conservation Area	WELT SP089691
Myosotis forsteri Lehm.	New Zealand, South Island, Northwest Nelson, Kahurangi National Park	WELT SP092179
Myosotis glabrescens L. B. Moore	New Zealand, South Island, Central Otago, Hector Mountains	WELT SP089801
Myosotis macrantha (Hook. f.) Benth. & Hook. f.	New Zealand, South Island, Central Otago, Queenstown, Moke Creek	WELT SP100494
Myosotis macrantha (Hook. f.) Benth. & Hook. f.	New Zealand, South Island, Northwest Nelson, Lake Peel	WELT SP100468
Myosotis pansa (L. B. Moore) Meudt, Prebble, R. J. Stanley & Thorsen subsp. *pansa*	New Zealand, North Island, Auckland Region, Anawhata stream	WELT SP089670
Myosotis pansa (L. B. Moore) Meudt, Prebble, R. J. Stanley & Thorsen subsp. *pansa*	New Zealand, North Island, Auckland Region, Pararaha Valley	WELT SP089674
Myosotis pansa subsp. *praeceps* Meudt, Prebble, R. J. Stanley & Thorsen	New Zealand, North Island, Taranaki, Paraninihi/White Cliffs	WELT SP089686
Myosotis pansa subsp. *praeceps* Meudt, Prebble, R. J. Stanley & Thorsen	New Zealand, North Island, Waikato, Ngarupupu Point	WELT SP089685
Myosotis petiolata Hook. f.	New Zealand, North Island, Hawkes Bay, Te Waka Range	WELT SP089853
Myosotis pottsiana (L. B. Moore) Meudt, Prebble, R. J. Stanley & Thorsen	New Zealand, North Island, Bay of Plenty, Ohutu Stream	WELT SP089689
Myosotis pottsiana (L. B. Moore) Meudt, Prebble, R. J. Stanley & Thorsen	New Zealand, North Island, Bay of Plenty, Waikokopu Stream	WELT SP089687
Myosotis pulvinaris Hook. f.	New Zealand, South Island, Central Otago, Pisa Range	WELT SP092196
Myosotis "small white"	New Zealand, South Island, Northwest Nelson, Kahurangi National Park	WELT SP090251
Myosotis "small white"	New Zealand, South Island, Northwest Nelson, Kahurangi National Park	WELT SP090247
Myosotis spathulata G. Forst.	New Zealand, North Island, Hawkes Bay	WELT SP090628
Myosotis spathulata var. *radicata* L. B. Moore	New Zealand, cultivated, origin Kaweka Ranges, North Island	WELT SP092757
Myosotis tenericaulis Petrie	New Zealand, South Island, Northwest Nelson, Kahurangi National Park	WELT SP092404
Myosotis uniflora Hook. f. aff.	New Zealand, South Island, Central Otago, Pisa Flats	WELT SP089883
Other *Myosotis*		
Myosotis arvensis (L.) Hill	New Zealand, North Island, Wellington, Karori	WELT SP094173
Myosotis australis R. Br.	Australia, New South Wales, Barrington Tops National Park	MPN 44757
Myosotis discolor Pers.	New Zealand, South Island, Central Otago, Ranfurly Holiday Park	WELT SP089930
Myosotis laxa Lehm.	New Zealand, South Island, Canterbury, Arthurs Pass	WELT SP090206

[a] A written description of the population location is included rather than GPS locations due to the threatened status of these species. An * indicates the five populations on which the markers were initially trialed.

[b] One voucher was collected for each population used; all vouchers are deposited in the herbaria of the Museum of New Zealand Te Papa Tongarewa (WELT) or Massey University (MPN).

Appendix 2 Voucher table with information about *Myosotis* specimens included in the morphological analyses (Chapter 2) and microsatellite analyses (Chapter 4). Specimens are listed alphabetically under four subheadings: *Myosotis pygmaea* group; Other bracteate-prostrate species; Tag-named entities associated with the *M. pygmaea* group; and Other bracteate-prostrate tag-named entities. For each specimen the herbarium and accession number, location information, collection date, collector and collector's number are given. If latitude and longitude are given, then that specimen was included in the ecological niche modelling dataset (Chapter 5). Latitude and longitude of all specimens included in the niche modelling analyses are also given in Appendix 8. If a population was included in the microsatellite analyses, the population code and number of individuals included is given. A "Y" indicates that the herbarium specimen was measured for the herbarium morphological dataset, an "S" that the population was the source of seed for specimens measured as part of the growth room dataset (more details in Table 2.3). A number symbol (#) indicates a specimen was included in the integrated analyses. An asterisk (*) indicates a type specimen; and a "^" indicates the type specimen of *M. antarctica* subsp. *traillii*.

Species/ tag name	Voucher number	Location information	Collection date	Collector (collector's number)	Latitude	Longitude	Microsatellite population code (n)	Herbarium dataset	Growth room dataset
***Myosotis pygmaea* group**									
M. antarctica	KEW K000787899*	Campbell Island	?/12/1840	J. D. Hooker				Y	
M. antarctica	CHR 269135	Campbell Island	?/?/1947	W. B. Brockie				Y	
M. antarctica	WELT SP102775	Campbell Island, cliff top between Menhir and Mt Dumas	27/12/2013	J. M. Prebble (JMP12063)	-52.5599	169.0818	CT (12)	Y #	
M. antarctica	CHR 308180	Campbell Island, Eboule Peak	13/01/1976	D. R. Given	-52.5928	169.1312		Y	
M. antarctica	CHR 117901	Campbell Island, Lyall Ridge	6/01/1961	F. J. Fuher				Y	
M. antarctica	WELT SP102777	Campbell Island, Mt Azimuth	27/12/2013	J. M. Prebble and A. J. Fergus (JMP13065)	-52.5093	169.1499	AZ (10)	Y (2) #	
M. antarctica	WELT SP002687	Campbell Island, Mt Azimuth	20/03/1947	J. H. Sorensen				Y	
M. antarctica	CHR 284737	Campbell Island, Mt	10/01/1976	D. R. Given	-52.5710	169.0990		Y	

Species/ tag name	Voucher number	Location information	Collection date	Collector (collector's number)	Latitude	Longitude	Microsatellite population code (n)	Herbarium dataset	Growth room dataset
		Dumas							
M. antarctica	CHR 284740	Campbell Island, Mt Fizeau	7/01/1976	D. R. Given	-52.5142	169.1558		Y	
M. antarctica	WELT SP102779	Campbell Island, Mt Honey, blue flowers	28/12/2013	J. M. Prebble and A. J. Fergus (JMP13067)	-52.5721	169.1634	HB (10)	Y #	
M. antarctica	WELT SP102780	Campbell Island, Mt Honey, white flowers	28/12/2013	J. M. Prebble and A. J. Fergus (JMP13068)	-52.5721	169.1634	HW (9)	Y #	
M. antarctica	OTA 60937	Campbell Island, Mt Lyall	24/02/1971	C. Meurk				Y	
M. antarctica	WELT SP002676	Campbell Island, Top of Col Peak	23/12/1946	W. B. Brockie				Y	
M. antarctica	WELT SP002680	Campbell Island, Windlass Bay	20/03/1947	J. H. Sorensen				Y	
M. antarctica	UPS V-702363	Chile, Patagonia, Punta Arenas	12/12/1895	P. K. H. Dusén (173)				Y	
M. antarctica	UPS V-702372	Chile, Patagonia, Skyring Sound, Puerto Altamirano	22/04/1908	C. Skottsberg	-52.5500	-72.0333		Y	
M. antarctica	K 000573650	Chile, Punta Arenas	?/?/1852	W. Lechler	-53.1634	-70.8923		Y (2)	
M. brevis	CHR 245911	NI, Coastal Taranaki, Puketapu Rd end	?/11/1971	A. P. Druce	-39.5174	173.9119		Y	
M. brevis	WELT SP090361	NI, Coastal Taranaki, Puketapu Rd end	5/10/2011	H. M. Meudt, J. M. Prebble et al. (HMM308)	-39.5193	173.9165	PU (16)		
M. brevis	WELT SP090543	NI, Coastal Taranaki, Stent Rd	5/10/2011	H. M. Meudt, J. M. Prebble et al. (HMM311)	-39.2186	173.7777	ST (13)	Y #	S
M. brevis	WELT SP090545 A	NI, Wairarapa, Kawakawa Rocks, near Ngawi	1/11/2011	H. M. Meudt, J. M. Prebble et al. (HMM313)	-41.6020	175.2367	NG (20)	Y #	
M. brevis	WELT	NI, Wellington South	7/11/2011	H. M. Meudt, J. M.	-41.2467	174.6654	TI (7)	Y #	

Species/ tag name	Voucher number	Location information	Collection date	Collector (collector's number)	Latitude	Longitude	Microsatellite population code (n)	Herbarium dataset	Growth room dataset
	SP090549	Coast, Te Ikaamaru Bay		Prebble et al. (HMM317)					
M. brevis	WELT SP090550 A	NI, Wellington South Coast, Te Ohau Bay	7/11/2011	H. M. Meudt, J. M. Prebble et al. (HMM318)	-41.2449	174.6506	TO (10)	Y	
M. brevis	WELT SP093294	SI, Canterbury, Lake Lyndon	21/02/2012	J. M. Prebble, M. Thorsen et al. (JMP12009)	-43.3130	171.6910	LL (8)	Y #	S
M. brevis	WELT SP002641	SI, Canterbury, Lake Lyndon	?	T. Kirk				Y	
M. brevis	CHR 208536	SI, Canterbury, Lake Lyndon	?/11/1971	A. P. Druce				Y	
M. brevis	CHR 75725*	SI, Canterbury, Lake Lyndon	?	G. Simpson and J. S. Thomson				Y	
M. brevis	WELT SP102761	SI, Otago, Banockburn	8/10/2013	J. M. Prebble et al. (JMP13045)	-45.0858	169.1355	BA (16)	Y #	
M. brevis	WELT SP102760	SI, Otago, Bendigo	8/10/2013	J. M. Prebble et al. (JMP13044)	-44.9409	169.3716	BE (19)	Y #	
M. brevis	WELT SP102762	SI, Otago, Chapman Rd Reserve	9/10/2013	J. M. Prebble et al. (JMP13046)	-45.2677	169.3452	CH (10)		
M. brevis	WELT SP093486	SI, Otago, Hawkdun Range	8/12/2011	J. Barkla				Y	
M. brevis	CHR 476031	SI, Otago, Nevis Valley	?/02/1992	A. P. Druce	-45.3333	168.8667		Y	
M. brevis	WELT SP102763	SI, Otago, Springvale Reserve	9/10/2013	J. M. Prebble et al. (JMP13047)	-45.2041	169.4341	SP (9)		
M. drucei	CHR 252337	NI, Moawhango, Kaimanawas	?/01/1974	A. P. Druce				Y	
M. drucei	CHR 86262	NI, Mt Hikurangi	?/01/1954	A. P. Druce				Y	
M. drucei	CHR 190683	NI, Ruahine Range, Te Hekenga	?/02/1968	A. P. Druce	-39.8833	176.1000		Y	
M. drucei	CHR 190682	NI, Ruahine Range, Trig	?/02/1968	A. P. Druce				Y	

Species/ tag name	Voucher number	Location information	Collection date	Collector (collector's number)	Latitude	Longitude	Microsatellite population code (n)	Herbarium dataset	Growth room dataset
		Ck							
M. drucei	WELT SP100445	NI, Ruahine Ranges, near Mt Maungamahue	23/12/2012	J. M. Prebble et al. (JMP12017-2)	-39.8767	176.0635	MM (15)	Y #	S
M. drucei	CHR 76820*	NI, Ruahine Ranges, Maungamahue	21/01/1952	J. A. Hay				Y	
M. drucei	WELT SP104524	SI, Fiordland, Beansburn	?	B. D. Rance					S
M. drucei	WELT SP104523	SI, Fiordland, Merrie Range, Tamatea Peak	?/03/2014	R. Hindmarsh-Walls	-45.6917	167.1649	MR (5)		
M. drucei	WELT SP104519	SI, Fiordland, Routeburn	2/12/2014	A. F. Fergus					S
M. drucei	WELT SP100428	SI, Marlborough, Altimarlock	8/02/2013	J. M. Prebble and C. Jones (JMP13026)	-41.7524	173.7756	MA (12)	Y #	S
M. drucei	WELT SP100425	SI, Marlborough, Lake Tennyson	11/02/2013	J. M. Prebble and J. Clayton-Green (JMP13027)	-42.2123	172.7397	LT (15)	Y #	
M. drucei	WELT SP002647	SI, Marlborough, Seaward Kaikoura Mountains, Mt Fyffe	10/02/1892	L. C. Cockayne	-42.3167	173.6167		Y	
M. drucei	WELT SP100440	SI, Marlborough, Tapuae-o-Uenuku	6/01/2013	J. M. Prebble et al. (JMP13002)	-41.9961	173.6487	TP (14)	Y #	
M. drucei	CHR 386879	SI, Marlborough, Tapuae-o-Uenuku	?/02/1981	B. Molloy				Y	
M. drucei	CHR 142854	SI, NW Nelson, Cobb Valley, near Lake Sylvester	13/01/1962	R. Melville and H. Telbot (Melville No 5961)				Y	
M. drucei	WELT SP100465	SI, NW Nelson, ridge above Lake Peel	25/01/2013	J. M. Prebble et al. (JMP13017)	-41.1421	172.5982	L1 (5)		
M. drucei	WELT SP100466	SI, NW Nelson, ridge above Lake Peel	25/01/2013	J. M. Prebble et al. (JMP13018)	-41.1440	172.6115	L2 (8)		S
M. drucei	WELT	SI, Otago, Coronet Peak	7/02/2012	H. M. Meudt et al.	-44.9232	168.7328	CO (16)	Y #	

Species/ tag name	Voucher number	Location information	Collection date	Collector (collector's number)	Latitude	Longitude	Microsatellite population code (n)	Herbarium dataset	Growth room dataset
	SP091599			(HMM344)					
M. drucei	WELT SP089892	SI, Otago, Dunstan	?	M. Thorsen	-45.0667	169.3322		Y	
M. drucei	WELT SP093291	SI, Otago, Mt Hocken, Pisa Range	16/02/2012	J. M. Prebble et al. (JMP12006)	-44.9923	168.9600	C2 (4)		
M. drucei	WELT SP093286	SI, Otago, Mt Hocken, Pisa Range	16/02/2012	J. M. Prebble et al. (JMP12005)	-44.9948	168.9516	C1 (5)		
M. drucei	CHR 75720	SI, Otago, near Luggate	28/12/1947	G. Simpson				Y	
M. drucei	WELT SP102783	SI, Otago, Rock and Pillar Range	2/02/2014	J. M. Prebble and E. Connor (JMP14002)	-45.4481	170.0600	P2 (10)	Y #	
M. drucei	WELT SP102782	SI, Otago, Rock and Pillar Range, trig rock circle	2/02/2014	J. M. Prebble and E. Connor (JMP14001)	-45.4362	170.0767	P1 (1)	Y #	
M. drucei	WELT SP100492	SI, Otago, The Remarkables ski area	27/02/2013	J. M. Prebble, N. Simpson et al. (JMP13034)	-45.0597	168.8191	RE (5)		S
M. drucei	OTA 37124	SI, Otago, Treble Cone	5/01/1978	A. F. Mark				Y	
M. drucei	WELT SP102785	SI, Southland, Livingstone Mountains	5/02/2014	J. M. Prebble and A. J. Fergus (JMP14004)	-45.0993	168.1043	LM (6)		S
M. drucei	AK 251910	Stewart Island, Mount Anglem	6/01/2000	P. J. de Lange (4109)	-44.7333	167.9167		Y	
M. glauca	WELT SP081872	SI, Canterbury Plains OR Otago, Kurow	?	D. Petrie and J. F. von Haast				Y	
M. glauca	AK 280800	SI, Canterbury, Lake Oahu	27/10/2002	A. E. Wright (12963)	-44.2333	169.8167		Y	
M. glauca	AK 210591	SI, Otago, base of Mt Ida	?	G. Simpson and J. S. Thomson				Y	
M. glauca	CHR 75722*	SI, Otago, base of Mt Ida	?	G. Simpson and J. S. Thomson				Y	
M. glauca	WELT SP089837	SI, Otago, Dunstan	17/01/2006	M. Thorsen				Y	

Species/ tag name	Voucher number	Location information	Collection date	Collector (collector's number)	Latitude	Longitude	Microsatellite population code (n)	Herbarium dataset	Growth room dataset
M. glauca	WELT SP089838	SI, Otago, Kyeburn Diggings	9/12/2006	M. Thorsen				Y	
M. glauca	WELT SP100497	SI, Otago, Macraes flats	1/03/2013	J. M. Prebble and K. Pilkington (JMP13039)	-45.4518	170.4403	M2 (8)	Y #	
M. glauca	WELT SP100415	SI, Otago, Macraes flats	14/03/2012	G. Loh			M1 (5)		
M. glauca	WELT SP081871	SI, Otago, mountains of Vincet County	?	D. Petrie				Y	
M. glauca	WELT SP089836	SI, Otago, Nevis Valley	25/04/2004	M. Thorsen				Y	
M. glauca	WELT SP093284	SI, Otago, School House Flat, Nevis Valley	15/02/2012	J. M. Prebble et al. (JMP12003)	-45.2092	168.9884	N1 (10)		S
M. glauca	WELT SP093285	SI, Otago, School House Flat, Nevis Valley	15/02/2012	J. M. Prebble et al. (JMP12004)	-45.2326	168.9591	N2 (12)	Y #	
M. glauca	CHR 191750	SI, Otago, Tourist Spur on Mt Ida	26/04/1969	L. B. Moore	-44.9758	170.1057		Y	
M. pygmaea	No voucher	cult. Otari-Wilton's Bush, ex Stewart Island, Mason Bay	?	R. Elliot					S
M. pygmaea	WELT SP090542	NI, Coastal Taranaki, Arawhata Rd end	5/10/2011	H. M. Meudt, J. M. Prebble et al. (HMM310)	-39.4172	173.7984	AR (12)		S
M. pygmaea	WELT SP090544	NI, Coastal Taranaki, Manihi Rd end	6/10/2011	H. M. Meudt, J. M. Prebble et al. (HMM312)	-39.3715	173.7753	MN (8)		
M. pygmaea	WELT SP090540	NI, Coastal Taranaki, Opunake water treatment ponds	5/10/2011	H. M. Meudt, J. M. Prebble et al. (HMM309)	-39.4461	173.8318	OK (14)	Y #	
M. pygmaea	CHR 245912	NI, Coastal Taranaki, Puketapu Rd end	?/11/1971	A. P. Druce	-39.5174	173.9119		Y	

Species/ tag name	Voucher number	Location information	Collection date	Collector (collector's number)	Latitude	Longitude	Microsatellite population code (n)	Herbarium dataset	Growth room dataset
M. pygmaea	WELT SP089920	NI, Hawke's Bay, Chrystall's Beach	27/12/2004	M. Thorsen	-46.2045	170.0704		Y	
M. pygmaea	WELT SP090629	NI, Hawke's Bay, Hukanui Station	13/12/2011	H. M. Meudt, J. M. Prebble and M. J. Thorsen (HMM332)	-39.2485	176.5316	H1 (5)		
M. pygmaea	CHR 246383	NI, Hawke's Bay, Te Waka Range	?/01/1972	A. P. Druce	-39.2167	176.6667		Y	
M. pygmaea	WELT SP090634	NI, Hawke's Bay, Waipuna Station	13/12/2011	H. M. Meudt, J. M. Prebble and M. J. Thorsen (HMM335)	-39.2478	176.6127	H3 (15)		
M. pygmaea	WELT SP090631	NI, Hawke's Bay, Waipuna Station	13/12/2011	H. M. Meudt, J. M. Prebble and M. J. Thorsen (HMM333)	-39.2552	176.6084	H2 (5)	Y #	
M. pygmaea	WELT SP004743*	NI, heath near Matamau	02/11/1883	W. Colenso				Y	
M. pygmaea	CHR 245193	SI, Cape Farewell, Whararkiki Beach	?/11/1971	A. P. Druce	-40.5048	172.6732		Y	
M. pygmaea	No voucher	SI, Coastal Otago, the Catlins, Tahakopa Bay	24/08/2012	J. M. Prebble (JMP12012)	-46.5571	169.4989	TB (5)		S
M. pygmaea	WELT SP100402	SI, Coastal Otago, The Chasm, Otago Peninsula	23/08/2012	J. M. Prebble (JMP12011)	-45.8942	170.6799	OP (15)		
M. pygmaea	WELT SP2650A	SI, NW Nelson, Gordon's Knob	5/02/1910	D. Petrie				Y	
M. pygmaea	WELT SP100472	SI, NW Nelson, Hoary Head	21/01/2013	J. M. Prebble et al. (JMP13007)	-41.1308	172.8047	HH (13)	Y #	S
M. pygmaea	CHR 313155	SI, NW Nelson, N of Heaphy River	?/11/1977	A. P. Druce	-40.9820	172.1052		Y	
M. pygmaea	WELT SP100460	SI, NW Nelson, near Sandhill Ck river mouth	26/01/2013	J. M. Prebble et al. (JMP13022)	-40.6721	172.3913	SC (9)	Y #	
M. pygmaea	WELT SP100477	SI, NW Nelson, ridge track to Mt Arthur	22/01/2013	J. M. Prebble et al. (JMP13009)	-41.1998	172.7102	AR (7)		S

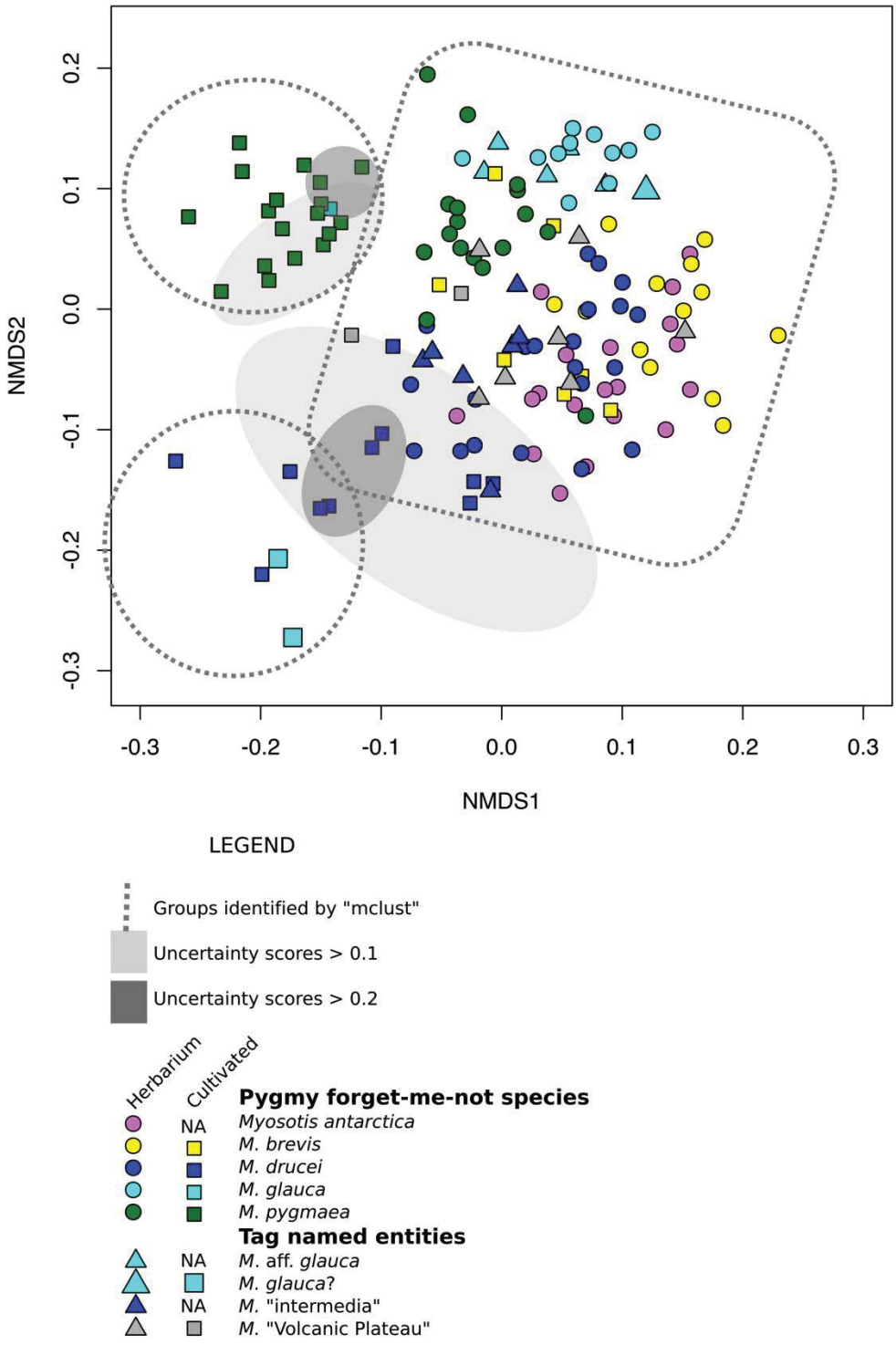
Species/ tag name	Voucher number	Location information	Collection date	Collector (collector's number)	Latitude	Longitude	Microsatellite population code (n)	Herbarium dataset	Growth room dataset
M. pygmaea	WELT SP100462	SI, NW Nelson, south of Paturau River mouth	26/01/2013	J. M. Prebble et al. (JMP13020)	-40.6503	172.4188	PR (10)	Y #	S
M. pygmaea	OTA 44898	SI, Otago, Eyre ecological district	6/01/1987	A. F. Mark				Y	
M. pygmaea	CHR 541256	SI, Southland, Oraka Point	17/01/2000	B. D. Rance	-46.3904	167.8803		Y	
M. pygmaea	AK 231694	SI, Southland, Waituna	8/01/1995	P. J. de Lange	-46.5500	168.2500		Y	
M. pygmaea	WELT SP100487	SI, Southland, Waituna Beach	25/02/2013	J. M. Prebble and K. Pilkington (JMP13031)	-46.5988	168.5457	WA (15)	Y #	
M. pygmaea	WELT SP002666	Stewart Island, Mason Bay	13/01/1882	T. Kirk				Y	
M. pygmaea	AK 7443^	Stewart Island, Mason Bay	13/01/1882	T. Kirk				Y	
M. pygmaea	No voucher	Stewart Island, Smoky Beach	?	C. Lehnebach			SI (1)		
Other bracteate-prostrate group									
M. albiflora	UPS V-702387	Chile, Tierra del Fuego, Porvenir	22/12/1895	P. K. H. Dusén				Y	
M. albiflora	UPS V-702392	Chile, Tierra del Fuego, Rio Azopardo	29/2/1896	P. K. H. Dusén				Y	
M. cheesemanii	CHR 475919	SI, Otago, Pisa Range	?/01/ 1992	A. P. Druce (APD1655)				Y	
M. colensoi	WELT SP090553	SI, Marlborough, Chalk Range	24/11/2011	H. M. Meudt et al. (HMM321A)				Y	
M. colensoi	WELT SP043858	SI, Canterbury, Broken River basin	13/12/1947	W. R. B. Oliver				Y	
M. elderi	WELT SP103839	SI, Marlborough, Mt St Patrick	17/12/2014	H.M. Meudt et al. (HMM384G)				Y	
M. elderi	CHR 198128	SI, Marlborough, Mt Murphy	4/12/1969	L. Moore (5)				Y	

Species/ tag name	Voucher number	Location information	Collection date	Collector (collector's number)	Latitude	Longitude	Microsatellite population code (n)	Herbarium dataset	Growth room dataset
M. elderi	CHR 295316	SI, Otago, Ben Lomond, Lake Wakatipu	?/01/ 1932	Wall				Y	
M. elderi	AK 46591	SI, Canterbury, Mt Captain, Hanmer	?	Christensen (Cockayne 5723)				Y	
M. glabrescens	WELT SP089801	SI, Otago, Hector Mts	30/01/2007	M. Thorsen (012/07)				Y	
M. lyallii	WELT SP104522	SI, Fiordland, Merrie Range, Lake Story, Plot K165	?/03/2014	R. Hindmarsh-Walls			LY (3)	Y	
M. lyallii	OTA 0218910	SI, Fiordland, Gertrude Saddle	12/01/1968	A. Mark and N. Adams				Y	
M. lyallii var. *lyallii*	CHR 223932	SI, Landsborough, Mt Gow	21/02/1972	Wardle				Y	
M. matthwesii	CHR 469691	cult. Hutt Valley, ex Warawara Forest, North Auckland	?/05/1989	A. P. Druce (APD716)				Y	
M. matthwesii	WELT SP043854	NI, Taranga Island	?/12/1924	W. R. B. Oliver				Y	
M. pulvinaris	WELT SP103819	SI, Otago, Dunstan Mountains	11/12/2014	H. M. Meudt et al. (HMM365A)				Y	
M. pulvinaris	OTA 037097	SI, Otago, Treble Cone	5/01/1978	A. Mark				Y	
M. spathulata	WELT SP090628	NI, Hawkes Bay, Maungaharuru Range	12/12/2011	H. M. Meudt et al. (HMM331B)				Y	
M. spathulata	WELT SP002565	SI, Salt's Gully, Lyttelton	15/02/1918	R. Laing				Y	
M. tenericaulis	WELT SP089834/A	Stewart Is, Mt Allen, Rakeakua River	16/02/2010	M. Thorsen (012/10)				Y	
M. tenericaulis	WELT SP095613	SI, Marlborough, Boulder Creek	5/01/1982	W. D. Burke				Y	
M. tenericaulis	CHR 80161	NI, NW Ruahine Mts, Reporoa bog	8/01/1948	A. P. Druce				Y	

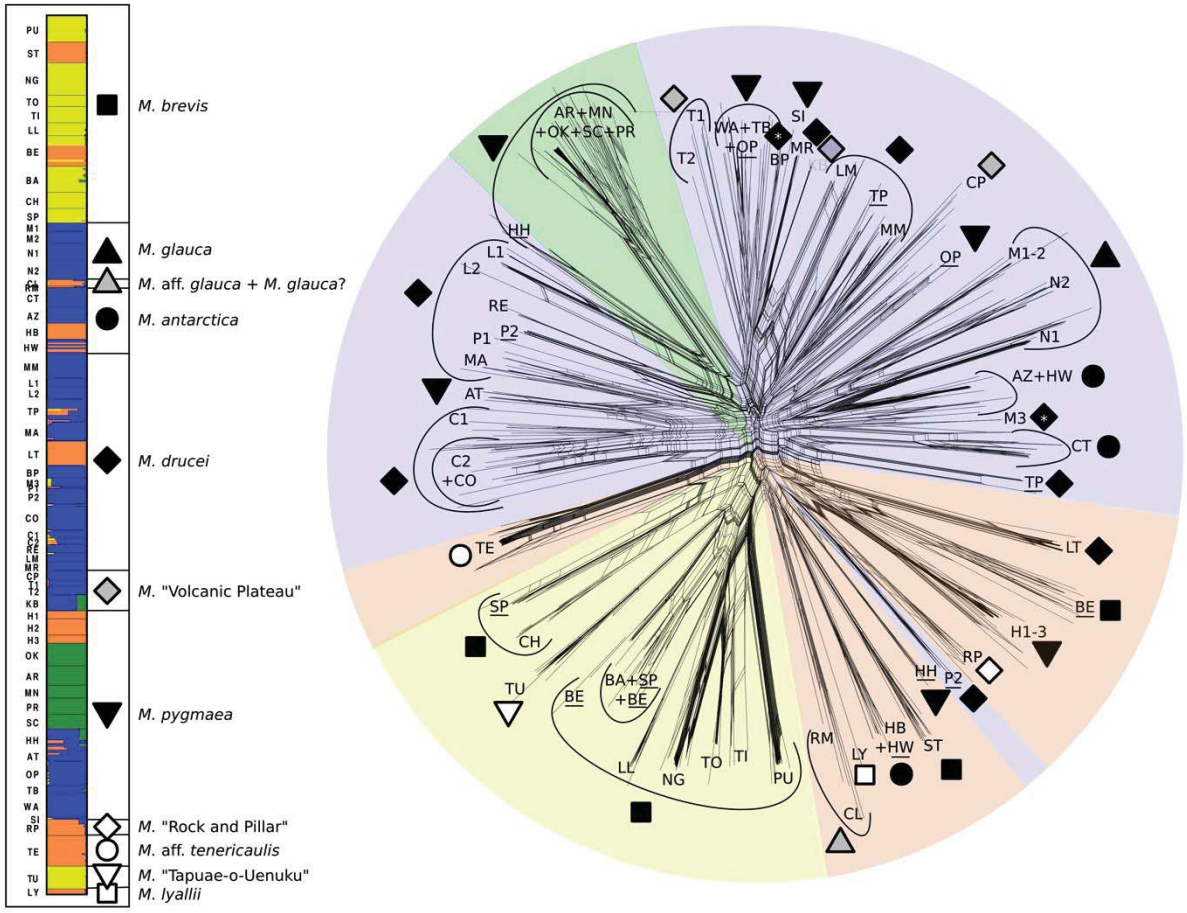
Species/ tag name	Voucher number	Location information	Collection date	Collector (collector's number)	Latitude	Longitude	Microsatellite population code (n)	Herbarium dataset	Growth room dataset
M. uniflora	CHR 467435A	SI, Canterbury, Waimakariri River	21/10/1990	Douglass (90/26)				Y	
M. uniflora	WELT SP103845	SI, Tasman River delta	18/10/2014	M. Thorsen (151/14)				Y	
M. uniflora	WELT SP089883	SI, Pisa Flats	18/10/2010	M. Thorsen				Y	
Tag-named entities associated with the *M. pygmaea* group									
M. "intermedia"	CHR 201540	SI, Canterbury, Banks Peninsula, Herbert Peak	22/01/1970	B. P. J. Molloy	-43.6896	172.7412		Y	
M. "intermedia"	WELT SP093292	SI, Canterbury, Banks Peninsula, Port Hills, Trig O	19/02/2012	J. M. Prebble et al. (JMP12007)	-43.6738	172.6219	BP (8)	Y #	
M. "intermedia"	WELT SP089913	SI, Otago, Macraes	30/11/2002	M. Thorsen (176/07)	-45.4753	170.4033		Y (2)	
M. "intermedia"	WELT SP100498	SI, Otago, Macraes flat	1/03/2013	J. M. Prebble and K. Pilkington (JMP13040)	-45.4217	170.4561	M3 (5)	Y #	
M. "intermedia"	WELT SP089911	SI, Otago, Rock and Pillar Range	31/12/2005	M. Thorsen	-45.4325	170.0728		Y	
M. "intermedia"	WELT SP089916	SI, Otago, Taieri River, Beaumont Station	15/12/2009	M. Thorsen (129/09)	-45.5425	169.7355		Y	
M. "intermedia"	WELT SP089909	SI, Otago, The Remarkables, Glen Roy Station	5/12/2005	M. Thorsen	-45.1328	168.8803		Y	
M. "Volcanic Plateau"	WELT SP100412	cult. Dunedin, ex Ruahine Ranges, Makirkiri tarns	31/10/2012	G. Rogers			T1 (4)	Y #	
M. "Volcanic Plateau"	CHR 131697	cult. ex NI, NW Ruahine Ranges, Makirikiri tarns	?/10/1962	A. P. Druce				Y	
M. "Volcanic Plateau"	WELT SP089738	NI, Central Plateau, Waipakihi River	15/02/2011	N. Singers	-39.1653	175.8975	CP (6)	Y #	

Species/ tag name	Voucher number	Location information	Collection date	Collector (collector's number)	Latitude	Longitude	Microsatellite population code (n)	Herbarium dataset	Growth room dataset
M. "Volcanic Plateau"	CHR 86263	NI, Kaimanawa Mountains	?/06/1948	A. P. Druce				Y	
M. "Volcanic Plateau"	CHR 65075	NI, Kaimanawas, Ngarururoro	?/12/1948	A. P. Druce	-39.1173	176.1484		Y	
M. "Volcanic Plateau"	CHR 273143	NI, Mt Tihia, SW of L Taupo	?/04/1974	A. P. Druce	-39.0000	175.7167		Y	
M. "Volcanic Plateau"	CHR 244442	NI, Patutu, Kaimanawa Huts	?/01/1973	A. P. Druce	-39.2333	175.8500		Y	
M. "Volcanic Plateau"	CHR 310610	NI, Ruahine Ranges, Makirikiri tarns	?/01/1977	A. P. Druce				Y	
M. "Volcanic Plateau"	No voucher	NI, Ruahine Ranges, Makirkiri tarns	6/11/2012	J. M. Prebble (JMP12013)	-39.6190	176.1603	T2 (5)		
M. "Volcanic Plateau"	CHR 310192	NI, Ruahines, Makirikiri tarns	?/01/1977	A. P. Druce				Y	
M. "Volcanic Plateau"	No voucher	SI, Southland, Kiwi Burn, near Mavora Lakes	?/02/2013	G. Rogers	-45.3427	168.1242	KB (10)		
M. aff. *glauca*	WELT SP089898	SI, Otago, Pisa Range, around snowfarm	23/01/2006	M. Thorsen	-44.8317	169.1103		Y (2)	
M. aff. *glauca*	WELT SP093282	SI, Otago, Pisa Range, Roaring Meg	14/02/2012	J. M. Prebble et al. (JMP12002)	-44.8672	169.1231	RM (1)	Y #	
M. aff. *glauca*	CHR 586018	SI, Otago, western Pisa Range	26/01/2006	M. Thorsen	-44.8309	169.1103		Y	
M. aff. *glauca*	OTA 34535	SI, Otago, western slopes of The Remarkables	25/01/1972	C. Meurk				Y	
M. glauca ?	WELT SP103892	SI, Lake Wanaka, Clutha River outflow	13/10/2014	G. Rogers	-44.6921	169.1940	CL (4)	Y #	S
Other bracteate-prostrate tag-named entities									
M. "non-pulvinaris"	CHR 272747	SI, Lyell Bay, Thompson Sound	?/01/1958	Metcalf s.n.				Y	
M. "non-	OTA 043909	SI, Umbrella Mountains,	11/12/1985	K. Dickinson and A.				Y	

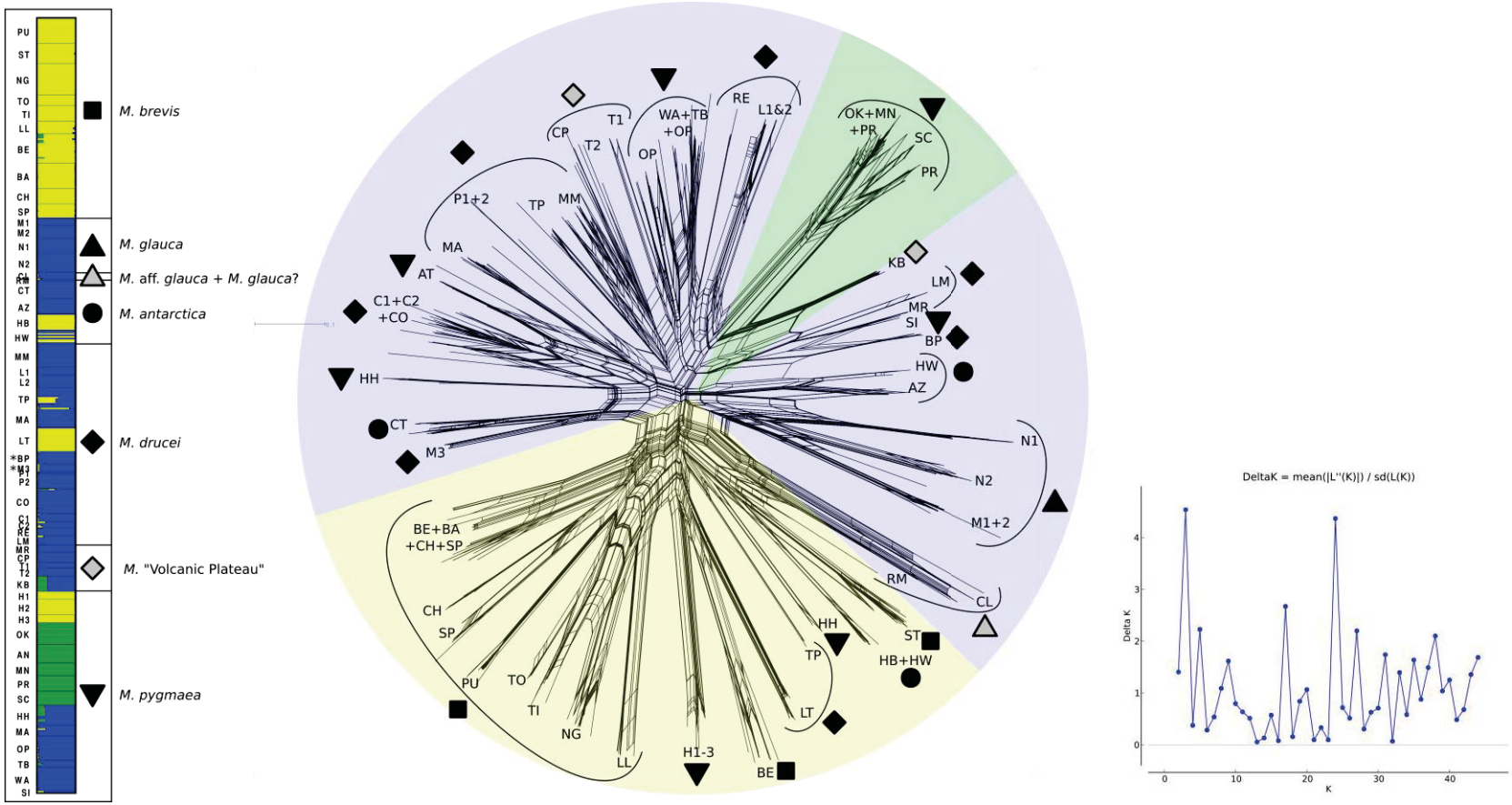
Species/ tag name	Voucher number	Location information	Collection date	Collector (collector's number)	Latitude	Longitude	Microsatellite population code (n)	Herbarium dataset	Growth room dataset
pulvinaris"		Gem Lake		Mark					
M. "Rock & Pillar"	WELT SP102784	SI, Otago, Rock and Pillar Range	2/02/2014	J. M. Prebble and E. Connor (JMP14003)	-45.4291	170.0717	RP (10)	Y	
M. "Rock & Pillar"	WELT SP089905	SI, Otago, Rock and Pillar Range	31/12/2005	M. Thorsen				Y	
M. "Rock & Pillar"	WELT SP089903	SI, Otago, Rock and Pillar Range	24/01/2008	M. Thorsen				Y	
M. "Rock & Pillar"	WELT SP089904	SI, Otago, Rock and Pillar Range	1/01/2006	M. Thorsen				Y	
M. "Tapuae-o-Uenuku"	WELT SP103922	SI, Marlborough, Mt Giles	13/01/2015	J. Clayton-Green				Y	
M. "Tapuae-o-Uenuku"	CHR 386966	SI, Marlborough, Tapuae-o-Uenuku	1/02/1981	B. J. P Molloy				Y	
M. "Tapuae-o-Uenuku"	CHR 386966	SI, Marlborough, Tapuae-o-Uenuku	1/02/1981	B. J. P Molloy				Y	
M. "Tapuae-o-Uenuku"	WELT SP100449	SI, Marlborough, Tapuae-o-Uenuku, near Staircase Ck	6/01/2013	J. M. Prebble et al. (JMP13003)	-41.9946	173.6482	TU (14)	Y	
M. aff. *tenericaulis*	WELT SP089696	SI, Otago, Kakanui Mountains, Siberia Hill	7/12/2010	J. Barkla				Y	
M. aff. *tenericaulis*	WELT SP089802	SI, Otago, Lammerlaw Range	16/12/2009	M. Thorsen				Y	
M. aff. *tenericaulis*	WELT SP093492	SI, Otago, Nevis Valley, School House Ck	10/11/2011	J. Barkla				Y	
M. aff. *tenericaulis*	WELT SP103811	SI, Otago, Old Man Range	9/12/2014	H. M. Meudt et al. (HMM357A)	-45.4484	169.2135		Y	
M. aff. *tenericaulis*	WELT SP089803	SI, Otago, Old Woman Range	8/04/2007	M. Thorsen				Y	
M. aff. *tenericaulis*	WELT SP091590	SI, Otago, The Remarkables	5/02/2012	H. M. Meudt et al. (HMM336)	-45.0623	168.8188	TE (19)	Y	



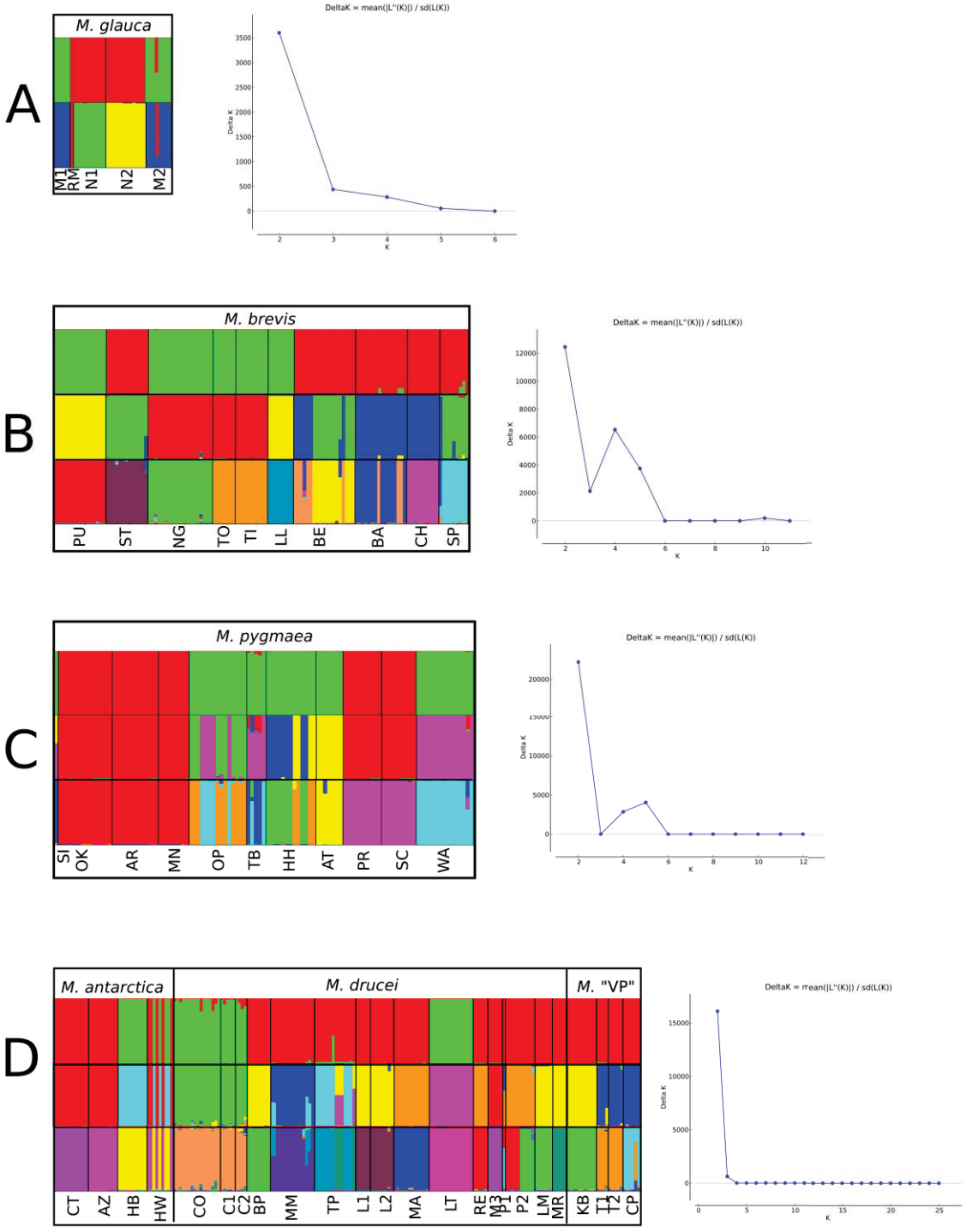
Appendix 3 Non-metric multidimensional scaling (nMDS) plot of combined herbarium and growth room data for the *Myosotis pygmaea* species group based on 52 characters and two dimensions retained.



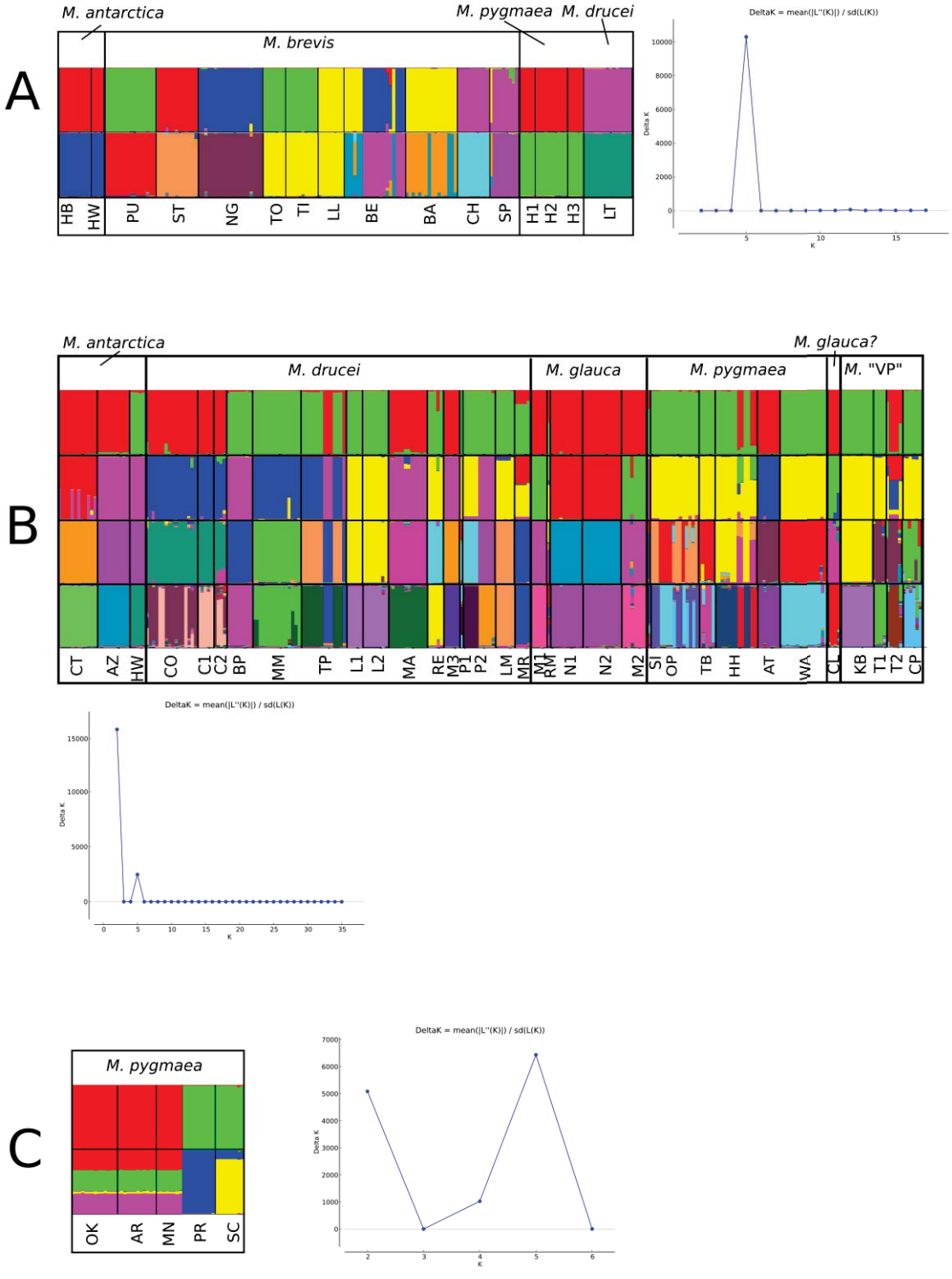
Appendix 4 Structure plot (K = 4) and Neighbornet network based on the Kosman and Leonard (2005) distance matrix for the *Myosotis* "pygmy-plus" microsatellite dataset of 58 populations (543 individuals). Population codes are shown in Figure 4.2 and Table 4.4, voucher details are in Appendix 2, and more details about each dataset is in Table 4.1.



Appendix 5 Structure plot (K = 3) and Neighbornet network based on the POSA distance matrix for the *Myosotis* "pygmy-only" microsatellite dataset of 54 populations (497 individuals). Delta K plot for structure runs shown on right. Population codes are shown in Figure 4.2 and Table 4.4, voucher details are in Appendix 2, and more details about each dataset is in Table 4.1.



Appendix 6 Structure plots of multiple datapartitions based on morphological clusters recovered in Chapter 2. Plots shown are of the K identified following the Evanno method (see each corresponding deltaK plot) with higher Ks shown that are of interest. **A** *M. glauca* morpho groups, K = 2 & 4. **B** *M. brevis* morpho group, K = 2, 4 & 10. **C** *M. pygmaea* morpho group, K = 2, 5 & 7. **D** *M. drucei*-plus morpho group, K = 2, 7 & 15. Population codes are shown in Figure 4.2 and Table 4.4, voucher details are in Appendix 2, and more details about each dataset is in Table 4.1.



Appendix 7 Structure plots of multiple datapartitions based on clusters recovered in the Structure analyses of the "pygmy-only" datasets at K = 3. Plots shown are of the K identified following the Evanno method (see each corresponding deltaK plot) with higher Ks shown that are of interest. **A** *M. brevis*-plus Structure group, K = 5 & 12. **F** *M. drucei*-plus Structure group, K = 2, 5, 13 & 28. **G** *M. pygmaea*-reduced Structure group, K = 2 & 5. Population codes are shown in Figure 4.2 and Table 4.4, voucher details are in Appendix 2, and more details about each dataset is in Table 4.1.

Appendix 8 Herbarium and locality information for all *Myosotis* voucher specimens used in the ecological niche modelling analyses. These specimens are also representative specimens for the taxonomic treatment, listed in alphabetical order by species, main area and region. The Land Environments of New Zealand (LENZ) environment is listed for mainland New Zealand specimens (see Leathwick [2002] for interpretation; note specimens from Chile, Campbell Island and some coastal and lakeside populations are excluded, this happens when the layers are not modelled with identical extents). The type and name of Department of Conservation (DOC) reserves for each specimen (if relevant) is also listed.

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
M. antarctica* subsp. *antarctica	CHR	308180	Given DR 9189	13 January 1976	Campbell Island	Campbell Island	Eboule Peak, south ridge and summit above western cliffs	-52.5928	169.1312		S20_NATURE_RESERVE	Campbell Island Nature Reserve
M. antarctica* subsp. *antarctica	WELT	SP102780	Prebble JM 13068; Fergus AJ	28 December 2013	Campbell Island	Campbell Island	Western slopes of Mt Honey	-52.5721	169.1634		S20_NATURE_RESERVE	Campbell Island Nature Reserve
M. antarctica* subsp. *antarctica	CHR	284737	Given DR 9136	10 January 1976	Campbell Island	Campbell Island	Mount Dumas, summit area	-52.5710	169.0990		S20_NATURE_RESERVE	Campbell Island Nature Reserve
M. antarctica* subsp. *antarctica	WELT	SP102781	Prebble JM 13069; Fergus AJ	28 December 2013	Campbell Island	Campbell Island	Western slopes of Mt Honey just below summit rocks	-52.5709	169.1654		S20_NATURE_RESERVE	Campbell Island Nature Reserve
M. antarctica* subsp. *antarctica	CHR	303843	Given DR 9208	15 January 1976	Campbell Island	Campbell Island	Mount Honey summit area, west of highest point	-52.5702	169.1634		S20_NATURE_RESERVE	Campbell Island Nature Reserve
M. antarctica* subsp. *antarctica	CHR	308158	Given DR 9137	10 January 1976	Campbell Island	Campbell Island	Mount Dumas	-52.5697	169.0962		S20_NATURE_RESERVE	Campbell Island Nature Reserve
M. antarctica* subsp. *antarctica	WELT	SP102775	Prebble JM 13063	27 December 2013	Campbell Island	Campbell Island	Cliff tops on ridge between Menhir and Mt Dumas	-52.5599	169.0818		S20_NATURE_RESERVE	Campbell Island Nature Reserve
M. antarctica* subsp. *antarctica	CHR	303850	Given DR 9076	December 1975	Campbell Island	Campbell Island	Mount Paris, south east ridge from Rocky Bay, summit	-52.5582	169.0470		S20_NATURE_RESERVE	Campbell Island Nature Reserve

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
							ridge					
M. antarctica* subsp. *antarctica	CHR	87559	Sorensen JH	4 February 1946	Campbell Island	Campbell Island	Windlass Bay	-52.5508	169.0909		S20_NATURE_RESERVE	Campbell Island Nature Reserve
M. antarctica* subsp. *antarctica	CHR	284739	Given DR 9319	30 January 1976	Campbell Island	Campbell Island	Yvon Villarceau Peak, summit area adjacent to prominent rock outcrops	-52.5398	169.0324		S20_NATURE_RESERVE	Campbell Island Nature Reserve
M. antarctica* subsp. *antarctica	CHR	308148	Given DR 9100	7 January 1976	Campbell Island	Campbell Island	St Col Peak, Mount Azimuth Ridge	-52.5358	169.1331		S20_NATURE_RESERVE	Campbell Island Nature Reserve
M. antarctica* subsp. *antarctica	CHR	303759	Given DR 9174	19 December 1975	Campbell Island	Campbell Island	Mount Lyall, west end of ridge, 1km from summit	-52.5344	169.1567		S20_NATURE_RESERVE	Campbell Island Nature Reserve
M. antarctica* subsp. *antarctica	CHR	233441	Brockie WB	9 March 1947	Campbell Island	Campbell Island	East of Lyall ridge	-52.5330	169.1538		S20_NATURE_RESERVE	Campbell Island Nature Reserve
M. antarctica* subsp. *antarctica	CHR	284740	Given DR 9431	7 January 1976	Campbell Island	Campbell Island	Mount Fizeau, summit area and north slopes	-52.5142	169.1558		S20_NATURE_RESERVE	Campbell Island Nature Reserve
M. antarctica* subsp. *antarctica	WELT	SP102777	Prebble JM 13065; Fergus AJ	27 December 2013	Campbell Island	Campbell Island	Slopes of Mt Azimuth	-52.5093	169.1499		S20_NATURE_RESERVE	Campbell Island Nature Reserve
M. antarctica* subsp. *antarctica	UPS	V-702372	Skottsberg C	March 1905	Chile	Magallanes	Seno Skyring, Puerto Altamirano	-52.5500	-72.0333			
M. antarctica* subsp. *antarctica	K	000573650	Lechler W	December 1905	Chile	Magallnaes	Punta Arenas	-53.1634	-70.8923			
M. antarctica* subsp. *antarctica	CHR	116218	Druce AP	January 1954	North Island	East Cape	Mount Hikurangi	-37.9212	178.0631	D4.1c		
M. antarctica* subsp. *antarctica	CHR	74274	Druce AP	January 1952	North Island	Southern North Island	Ruahine Ranges, Te Hekenga	-39.8833	176.1000	P8.2a	S19_CONSERVATION_PARK	Ruahine Forest Park
M. antarctica* subsp. *antarctica	WELT	SP100445	Prebble JM 12017-2	23 December	North Island	Southern North Island	Ruahine Ranges, Small peak to the	-39.8767	176.0635	P4.1a	S19_CONSERVATIO	Ruahine Forest Park

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
				2012			north of Maungamahue, Whanahuia Range				N_PARK	
M. antarctica* subsp. *antarctica	WELT	SP091832	Sneddon BV	10 February 1968	North Island	Southern North Island	Ruahine Ranges	-39.8319	176.1134	P8.2a	S19_CONSERVATION_PARK	Ruahine Forest Park
M. antarctica* subsp. *antarctica	CHR	42329	Zotov VD	4 January 1944	North Island	Southern North Island	Ruahine Rangess, Rangioteatua	-39.8212	176.1355	P8.2a	S19_CONSERVATION_PARK	Ruahine Forest Park
M. antarctica* subsp. *antarctica	WELT	SP091832	Sneddon BV	10 February 1968	North Island	Southern North Island	Ruahine Ranges, Trig Creek off Kawhatau River	-39.8205	176.1023	P8.2a	S19_CONSERVATION_PARK	Ruahine Forest Park
M. antarctica* subsp. *antarctica	CHR	74299	Druce AP	February 1951	North Island	Southern North Island	Ruahine Ranges, Mokai Patea	-39.6939	176.0601	F7.3a		
M. antarctica* subsp. *antarctica	CHR	358805	Druce AP	April 1980	North Island	Volcanic Plateau	Kaweka Range, Makahu spur	-39.2833	176.3833	P7.1a	S19_CONSERVATION_PARK	Kaweka Forest Park
M. antarctica* subsp. *antarctica	CHR	533101	Wilson HD	14 February 1999	South Island	Canterbury	Banks Peninsula, Stony Bay Valley, SW of Trig P	-43.8408	173.0297	F3.1b		
M. antarctica* subsp. *antarctica	WELT	SP092448	Carson C; Garnock-Jones PJ	3 December 2010	South Island	Canterbury	Banks Peninsula, Mount Herbert, growing on south facing bluff with clump of moss.	-43.6974	172.7531	P5.2a	S19_1_A_SCENIC_RESERVE	Mt Herbert Scenic Reserve
M. antarctica* subsp. *antarctica	CHR	295301	Wall A	April 1921	South Island	Canterbury	Banks Peninsula, Mount Herbert	-43.6968	172.7452	F3.3a	S19_1_A_SCENIC_RESERVE	Mt Herbert Scenic Reserve
M. antarctica* subsp. *antarctica	CHR	201540	Molloy BPJ	22 January 1970	South Island	Canterbury	Banks Peninsula, Herbert Peak (summit)	-43.6896	172.7412	P5.2a		
M. antarctica* subsp. *antarctica	CHR	280352	Thompson J	1 March 1976	South Island	Canterbury	Banks Peninsula, Mount Herbert, summit	-43.6833	172.7500	F3.3b		
***M. antarctica* subsp.**	WELT	SP093292	Prebble JM	19	South	Canterbury	Banks Peninsula,	-43.6738	172.6219	F3.3b		

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
antarctica			12007	February 2012	Island		on rock outcrop just south of Trig "O"					
M. antarctica* subsp. *antarctica	CHR	481283	Wilson HD BP325	23 November 1984	South Island	Canterbury	Southern end of Port Hills near Trig O	-43.6733	172.6233	F3.3a		
M. antarctica* subsp. *antarctica	CHR	252811	Logeman W; Wilson H	22 November 1970	South Island	Canterbury	East Hooker terraces	-43.6570	170.1278	R1.1a	S4_NATIONAL_PARK	Aoraki Mount Cook National Park
M. antarctica* subsp. *antarctica	CHR	87571	Macmillan B	3 November 1956	South Island	Canterbury	Lake Emma	-43.6415	171.1111	E4.1b	S19_CONSERVATION_PARK	Hakatere Conservation Park
M. antarctica* subsp. *antarctica	CHR	94349	Shalland P; Moar TS829	30 August 1961	South Island	Canterbury	Port Hills, Sign of the Bellbird	-43.6321	172.6252	F3.3b		
M. antarctica* subsp. *antarctica	CHR	584557	Wilson HD	6 February 1971	South Island	Canterbury	Mt Cook, Head of Rutherford Stream, Godley	-43.5525	170.4270	R1.1c	S4_NATIONAL_PARK	Aoraki Mount Cook National Park
M. antarctica* subsp. *antarctica	WELT	SP103832	Meudt HM HMM378; Kusabs A	15 December 2014	South Island	Canterbury	Mt Potts	-43.4913	170.9121	P1.2d	S25_STEWARDSHIP_AREA	Mt Potts Conservation Area
M. antarctica* subsp. *antarctica	CHR	295302	Wall A	October 1925	South Island	Canterbury	Hills near Whitecliffs, High Peak	-43.4681	171.7395	P1.2d		
M. antarctica* subsp. *antarctica	CHR	261228	Given DR 72051; Wilson H	January 1972	South Island	Canterbury	Havelock Valley, small gorge upstream of Veil Creek bivouac, true right bank	-43.3833	170.6667	R1.1d	S25_STEWARDSHIP_AREA	Rangitata/Rakaia Head Waters Conservation Area
M. antarctica* subsp. *antarctica	CHR	295305	Wall A	October 1919	South Island	Canterbury	Mount Torlesse	-43.2666	171.8087	P1.2d	S19_CONSERVATION_PARK	Korowai Torlesse Tussocklands Park
M. antarctica* subsp. *antarctica	CHR	569097	Huber AM	1 February 1984	South Island	Canterbury	River bed of Wilberforce River,	-43.0348	171.3185			

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
							Urquharts Hut					
M. antarctica* subsp. *antarctica	WELT	SP002670	Oliver WRB	20 January 2028	South Island	Canterbury	Blimit	-42.9122	171.5898	R1.2a	S4_NATIONAL_PARK	Arthur's Pass National Park
M. antarctica* subsp. *antarctica	WELT	SP089919	Thorsen MJ	23 December 2007	South Island	Canterbury	Kelly Range	-42.7953	171.5292	R1.1e	S25_STEWARDSHIP_AREA	Conservation Area - Wanganui / Otira Catchments
M. antarctica* subsp. *antarctica	CHR	568890	Abraham H	1 April 1980	South Island	Canterbury	Kakapo Brook, lower end	-42.5975	172.5169	E1.4d		
M. antarctica* subsp. *antarctica	WELT	SP002643	Morrison W	Jan 1914	South Island	Canterbury	Amuri Co. summit of Mt. Miro Miro [Amuri County, summit of Mount Miromiro],	-42.4833	172.6667	P1.2c	S19_CONSERVATION_PARK	Hanmer Forest Park
M. antarctica* subsp. *antarctica	CHR	221969	Kelly OC	22 December 1971	South Island	Canterbury	Lewis Pass Scenic Reserve	-42.3809	172.3878	P2.1a	S19_1_A_SCENIC_RESERVE	Lewis Pass Scenic Reserve
M. antarctica* subsp. *antarctica	AK	284629	Wright AE 12896	2 March 2002	South Island	Canterbury	Wairau-Hanmer Springs Hydro Road, head of valley immediately south of Mt Weld	-42.1167	172.8667	P2.1b	17_RECREATION_RESERVE	Molesworth Recreation Reserve
M. antarctica* subsp. *antarctica	WELT	SP002647	Cockayne L	10 February 1892	South Island	Marlborough	Seaward Kaikoura Mts [Range], Mt [Mount] Fyffe.	-42.3167	173.6167	P1.2a	S19_CONSERVATION_PARK	Ka Whata Tu o Rakihouia Conservation Park
M. antarctica* subsp. *antarctica	CHR	295320	Wall A	December 1926	South Island	Marlborough	Mount Halfmoon	-42.2833	173.1333	E1.4b	17_RECREATION_RESERVE	Molesworth Recreation Reserve
M. antarctica* subsp. *antarctica	CHR	309606	Williams PA	December 1980	South Island	Marlborough	Seaward Kaikoura range, Mount Saunders	-42.2790	173.1444	E1.4b	17_RECREATION_RESERVE	Molesworth Recreation Reserve
***M. antarctica* subsp.**	WELT	SP100425	Prebble JM	11	South	Marlborough	Lake Tennyson,	-42.2123	172.7397			

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
antarctica			13027; Clayton-Greene J	February 2013	Island		gravels at moraine end of lake - cross stream from car park					
M. antarctica* subsp. *antarctica	CHR	73460	Allan HH	16 November 1944	South Island	Marlborough	Molesworth station, hill behind Acheron Hut	-42.1305	173.1365	E1.4b	17_RECREATION_RESERVE	Molesworth Recreation Reserve
M. antarctica* subsp. *antarctica	CHR	73461	Allan HH	16 November 1944	South Island	Marlborough	Molesworth station, hill behind Acheron Hut	-42.1305	173.1365	E1.4b	17_RECREATION_RESERVE	Molesworth Recreation Reserve
M. antarctica* subsp. *antarctica	CHR	87566	Moore LB	27 October 1951	South Island	Marlborough	Awatere valley, Molesworth	-42.0833	173.2500	E4.1a	17_RECREATION_RESERVE	Molesworth Recreation Reserve
M. antarctica* subsp. *antarctica	CHR	77226	Allan HH	5 November 1946	South Island	Marlborough	Muller Station, Airstrip terrace, upper Awatere Valley	-42.0833	173.2833	B8.1a		
M. antarctica* subsp. *antarctica	CHR	494246	Given DR 12254	19 February 1980	South Island	Marlborough	Mount Severn	-42.0786	173.0667	P1.2b	17_RECREATION_RESERVE	Molesworth Recreation Reserve
M. antarctica* subsp. *antarctica	WELT	SP100440	Prebble JM JM13002	6 January 2013	South Island	Marlborough	Cultivated at Newtown, Wellington, ex side of trail from Hodder huts to top of Tapuae-o-Uenuku, next to seep	-41.9961	173.6487	P1.2b	S19_1_A_SCENIC_RESERVE	Tapuae O Uenuku Scenic Reserve
M. antarctica* subsp. *antarctica	CHR	387014	Druce AP	February 1981	South Island	Marlborough	Inland Kaikoura range, Hodder valley	-41.9833	173.6167	P1.2b	S19_1_A_SCENIC_RESERVE	Tapuae O Uenuku "Scenic" Reserve Addition
M. antarctica* subsp. *antarctica	CHR	295291	Wall A	January 1930	South Island	Marlborough	Isolated Hill	-41.9000	173.9667	E1.2b	S19_1_A_SCENIC_RE	Isolated Hill Scenic Reserve

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
											SERVE	
M. antarctica* subsp. *antarctica	CHR	215862	Canterbury Botanical Society 83	8 January 1971	South Island	Marlborough	Black Birch Range, Mount Harkness near observatory	-41.7617	173.7541	P1.2a	S25_STEWARDSHIP_AREA	Conservation Area - Ferny Gair
M. antarctica* subsp. *antarctica	CHR	176970	Moore KB	25 March 1967	South Island	Marlborough	Black Birch Range, Mount Altimarlock	-41.7526	173.7756	P1.2b	S25_STEWARDSHIP_AREA	Conservation Area - Ferny Gair
M. antarctica* subsp. *antarctica	CHR	215857	Canterbury Botanical Society 80	8 January 1971	South Island	Marlborough	Blackbirch Range, Altimarlock	-41.7526	173.7756	P1.2b	S25_STEWARDSHIP_AREA	Conservation Area - Ferny Gair
M. antarctica* subsp. *antarctica	WELT	SP100428	Prebble JM 13026; Jones C	8 February 2013	South Island	Marlborough	Mt Altimarlock, near summit	-41.7524	173.7756	P1.2b	S19_1_A_SCENIC_RESERVE	Black Birch Scenic Reserve
M. antarctica* subsp. *antarctica	CHR	87563	Gibbs FG	Unknown	South Island	Marlborough	Mount Richmond Forest Park, Mount Rintoul	-41.5167	173.2333	P2.1b	S19_CONSERVATION_PARK	Mount Richmond Forest Park
M. antarctica* subsp. *antarctica	CHR	283749	Webb CJ 7498; Webb TH	29 December 1974	South Island	Marlborough	Mount Richmond	-41.4667	173.4000	P6.2b	S19_CONSERVATION_PARK	Mount Richmond Forest Park
M. antarctica* subsp. *antarctica	CHR	223074	Given DR 71549	18 December 1971	South Island	Marlborough	Richmond Forest Park, Mount Starveall, north of point	-41.4654	173.2519	P2.1b	S19_CONSERVATION_PARK	Mount Richmond Forest Park
M. antarctica* subsp. *antarctica	CHR	36289	Healy AJ	15 November 1942	South Island	Marlborough	Summit of Mount Riley	-41.4101	173.6960	P1.1b	S19_CONSERVATION_PARK	Mount Richmond Forest Park
M. antarctica* subsp. *antarctica	WELT	SP095605	Burke W	17 January 1982	South Island	Otago	Catlins	-46.4485	169.8090	Q4.1a	S22_GOVERNMENT_PURPOSE_RESERVE	Nugget Point Lighthouse Reserve
M. antarctica* subsp. *antarctica	CHR	417882	Johnson PN 331	16 January 1985	South Island	Otago	Lammerlaw Range, Teviot Swamp Millers Flat	-45.6333	169.6333	Q3.3c		
M. antarctica* subsp. *antarctica	WELT	SP089916	Thorsen MJ	15 December	South Island	Otago	Taieri River, Beaumont [Station]	-45.5425	169.7355	Q3.3b	S22_GOVERNMENT_	Canadian Flats Wildlife

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
				2009							PURPOSE_RESERVE	Management Reserve
M. antarctica* subsp. *antarctica	WELT	SP089913	Thorsen MJ	30 November 2002	South Island	Otago	[Emerald Creek], Falcon Nest	-45.4753	170.4033	Q4.3b	S19_1_A_SCENIC_RESERVE	Redbank Scenic Reserve
M. antarctica* subsp. *antarctica	CHR	395334	Druce AP	March 1987	South Island	Otago	Garvie Mountains, Dome Burn	-45.4667	168.8333	Q1.1c		
M. antarctica* subsp. *antarctica	WELT	SP089884	Thorsen MJ	23 January 2009	South Island	Otago	Old Man Range, Boulder Creek	-45.4528	169.2108	Q3.3a		
M. antarctica* subsp. *antarctica	WELT	SP089866	Thorsen MJ	January 2006	South Island	Otago	Rock and Pillar Range	-45.4509	170.0489	Q3.3a		
M. antarctica* subsp. *antarctica	WELT	SP102783	Prebble JM 14002; Conner E	2 February 2014	South Island	Otago	Rock and Pillar Range, near the Stonehenge rock outcrop	-45.4481	170.0600	Q3.3a	S25_STEWARDSHIP_AREA	Rock and Pillar Conservation Area
M. antarctica* subsp. *antarctica	WELT	SP102782	Prebble JM 14001; Conner E	2 February 2014	South Island	Otago	Rock and Pillar Range, near Trig rock circle above Big Hut	-45.4362	170.0767	Q3.3a	S25_STEWARDSHIP_AREA	Rock and Pillar Conservation Area
M. antarctica* subsp. *antarctica	WELT	SP089911	Thorsen MJ	31 December 2005	South Island	Otago	Rock and Pillar Range, near Big Hut	-45.4325	170.0728	Q3.3a	S25_STEWARDSHIP_AREA	Rock and Pillar Conservation Area
M. antarctica* subsp. *antarctica	WELT	SP089914	Thorsen MJ	7 November 2008	South Island	Otago	Waikouaiti stream [River]	-45.4262	170.4605	Q4.3b		
M. antarctica* subsp. *antarctica	WELT	SP100498	Prebble JM 13040; Pilkington K	1 March 2013	South Island	Otago	Lammerlaw, Macraes flat, side of small stream near the second gate on the road from the DOC field office to the skink enclosure	-45.4217	170.4561	N3.1e		
M. antarctica* subsp. *antarctica	CHR	318764	Anderson J	1 December	South Island	Otago	Rock and Pillar Range	-45.4167	170.0667	Q3.3a		

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
				1977								
M. antarctica* subsp. *antarctica	WELT	SP103810	Meudt HM HMM356 et al.	9 December 2014	South Island	Otago	Old Man Range	-45.4029	169.2085	Q3.3a	S25_STEWARDSHIP_AREA	Bain Block (Old Man Range) Conservation Area
M. antarctica* subsp. *antarctica	WELT	SP089895	Thorsen MJ	2 February 2007	South Island	Otago	Hector Mountains	-45.3667	168.7742	Q1.2a		
M. antarctica* subsp. *antarctica	CHR	73836	Moore LB	19 December 1969	South Island	Otago	Old Man range, high slopes above Fruitlands	-45.3445	169.2329	Q1.1a		
M. antarctica* subsp. *antarctica	AK	304723	de Lange PJ 7809; Thorsen MJ	18 January 2008	South Island	Otago	Old Man Range, Symes Road (near summit)	-45.3436	169.2133	Q3.3a		
M. antarctica* subsp. *antarctica	WELT	SP089888	Thorsen MJ	18 January 2008	South Island	Otago	Old Man Range, Symes Road	-45.3422	169.2153	Q3.3a		
M. antarctica* subsp. *antarctica	WELT	SP089896	Thorsen MJ	8 April 2007	South Island	Otago	Old Woman Range	-45.3345	169.1430	Q3.3a	S25_STEWARDSHIP_AREA	Kopuwai Conservation Area
M. antarctica* subsp. *antarctica	WELT	SP089910	Thorsen MJ	Unknown	South Island	Otago	Old Man Range, [Fraser Basin]	-45.3230	169.2050	Q3.3a	S25_STEWARDSHIP_AREA	Kopuwai Conservation Area
M. antarctica* subsp. *antarctica	CHR	417847	West J	December 1984	South Island	Otago	Old Man Range, top of Shingle Creek Road	-45.3167	169.2333	Q1.1c		
M. antarctica* subsp. *antarctica	WELT	SP089938	Barkla J	16 March 2011	South Island	Otago	Old Man Range, Fraser Basin	-45.2905	169.1178	Q3.3a	S25_STEWARDSHIP_AREA	Kopuwai Conservation Area
M. antarctica* subsp. *antarctica	WELT	SP089885	Thorsen MJ	30 January 2007	South Island	Otago	Hector Mountains, Loch Linne [Linnhe], Staircase Creek	-45.2375	168.8372	Q1.2a		
M. antarctica* subsp. *antarctica	WELT	SP089889	Thorsen MJ	19 October 2005	South Island	Otago	Nevis Burn	-45.2133	168.9167	Q1.1c	S25_STEWARDSHIP_	Remarkables Conservation

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
											AREA	Area
M. antarctica* subsp. *antarctica	WELT	SP089908	Thorsen MJ	29 January 2007	South Island	Otago	Hector Mountains	-45.1658	168.7708	Q1.1c		
M. antarctica* subsp. *antarctica	WELT	SP089909	Thorsen MJ	5 December 2005	South Island	Otago	Glen Roy station, Left Branch Doolans [Creek]	-45.1328	168.8803	Q1.1c	S25_STEWARDSHIP_AREA	Remarkables Conservation Area
M. antarctica* subsp. *antarctica	WELT	SP089892	Thorsen MJ	Unknown	South Island	Otago	Dunstan Mountins, Fairfax [Spur]	-45.0667	169.3322	Q1.1c		
M. antarctica* subsp. *antarctica	WELT	SP089872	Thorsen MJ	9 March 2006	South Island	Otago	Dunstan Mountains	-45.0630	169.3250	Q1.1a		
M. antarctica* subsp. *antarctica	WELT	SP100492	Prebble JM 13034 et al.	27 February 2013	South Island	Otago	Remarkables ski area, on old 4WD track to Lake Alta	-45.0597	168.8191	Q3.3a	17_RECREATION_RESERVE	Rastus Burn Recreation Reserve
M. antarctica* subsp. *antarctica	WELT	SP089912	Thorsen MJ	6 December 2006	South Island	Otago	Mount Nobbler, Shortlands station	-45.0117	170.3628	Q1.2a		
M. antarctica* subsp. *antarctica	WELT	SP103817	Meudt HM HMM363 et al.	10 December 2014	South Island	Otago	Dunstan Mountains	-45.0056	169.3794	Q3.3a	S25_STEWARDSHIP_AREA	Bendigo Conservation Area
M. antarctica* subsp. *antarctica	WELT	SP092186	Lehnebach CA; Kusabs A	14 February 2011	South Island	Otago	Pisa Conservation Area, track to Mt Hocken	-44.9950	168.9517	Q1.1a	S25_STEWARDSHIP_AREA	Pisa Conservation Area
M. antarctica* subsp. *antarctica	WELT	SP093286	Prebble JM 12005	16 February 2012	South Island	Otago	Pisa Range, near track towards Mt Hocken	-44.9948	168.9516	Q1.1a	S25_STEWARDSHIP_AREA	Pisa Conservation Area
M. antarctica* subsp. *antarctica	WELT	SP093291	Prebble JM 12006	16 February 2012	South Island	Otago	Pisa Range, track to Mt Hocken	-44.9923	168.9600	Q1.1c	S25_STEWARDSHIP_AREA	Pisa Conservation Area
M. antarctica* subsp. *antarctica	WELT	SP103813	Meudt HM HMM359; Kusabs A; Thorsen MJ	10 December 2014	South Island	Otago	Dunstan Mountains	-44.9702	169.4365	Q1.1b		
M. antarctica* subsp. *antarctica	WELT	SP089907	Rogers G	7 November	South Island	Otago	Bendigo	-44.9287	169.3447	N4.1e		

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
				2007								
M. antarctica* subsp. *antarctica	WELT	SP089873	Thorsen MJ	23 March 2006	South Island	Otago	Bendigo	-44.9285	169.3448	N4.1e		
M. antarctica* subsp. *antarctica	WELT	SP091599	MeudtHM HMM344; Garnock-Jones PJ; Simpson N	7 February 2012	South Island	Otago	Coronet Peak Skifield, near 4WD track ca. 10 min drive above skifield buildings	-44.9232	168.7328	Q1.2a	17_RECREATION_RESERVE	Coronet Peak Recreation Reserve
M. antarctica* subsp. *antarctica	WELT	SP089871	Thorsen MJ	19 January 2006	South Island	Otago	Dunstan Mounatins	-44.8968	169.5470	Q1.1c		
M. antarctica* subsp. *antarctica	WELT	SP093490	Barkla J	7 April 2011	South Island	Otago	Dunstan Mountains, Wainui Creek	-44.8927	169.4942	Q2.2a		
M. antarctica* subsp. *antarctica	WELT	SP089886	Thorsen MJ	14 January 2010	South Island	Otago	Pisa Range, Roaring Meg	-44.8672	169.1230	Q3.3a	S25_STEWARDSHIP_AREA	Pisa Conservation Area
M. antarctica* subsp. *antarctica	WELT	SP092204	Lehnebach CA; Kusabs A	16 February 2011	South Island	Otago	Pisa Range, Snow Farm	-44.8663	169.1794	Q1.2a		
M. antarctica* subsp. *antarctica	WELT	SP092222	Lehnebach CA; Kusabs A	17 February 2011	South Island	Otago	Mt Cardrona	-44.8591	168.9436	Q1.2a		
M. antarctica* subsp. *antarctica	WELT	SP092223	Lehnebach CA; Kusabs A	17 February 2011	South Island	Otago	Mt Cardrona	-44.8586	168.9427	Q1.2a		
M. antarctica* subsp. *antarctica	CHR	283526	Johnson PN	January 1977	South Island	Otago	Tutoko Valley	-44.6531	168.0094	R2.1b	S4_NATIONAL_PARK	Fiordland National Park
M. antarctica* subsp. *antarctica	WELT	SP089864	Thorsen MJ	24 February 2009	South Island	Otago	Mt Greenland	-44.6424	168.6785	R1.1b		
M. antarctica* subsp. *antarctica	WELT	SP089865	Thorsen MJ	25 February 2009	South Island	Otago	Mt Greenland	-44.6424	168.6785	R1.1b		
***M. antarctica* subsp.**	CHR	475920	Druce AP	February	South	Otago	Forbes Mountains,	-44.6267	168.4533	O2.3b	S4_NATIO	Mount

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
antarctica			APD1656	1992	Island		Mount Earnslaw, Kea Basin				NAL_PARK	Aspiring National Park
M. antarctica* subsp. *antarctica	WELT	SP089915	Thorsen MJ	26 February 2009	South Island	Otago	Harris Mountains, Mount Seton	-44.6183	168.7597	R1.1b		
M. antarctica* subsp. *antarctica	CHR	78858	H PJ	29 December 1952	South Island	Otago	Northwest Otago, Mount Ragan, Wilkin Valley	-44.2700	168.8722	R1.2b	S4_NATIONAL_PARK	Mount Aspiring National Park
M. antarctica* subsp. *antarctica	CHR	310920	Johnson PN	3 November 1976	South Island	Southland	Fiordland, Preservation Inlet, Te Whara beach	-46.0667	166.6333		S4_NATIONAL_PARK	Fiordland National Park
M. antarctica* subsp. *antarctica	CHR	518613	Rogers GM	13 January 1997	South Island	Southland	Summit of Blue Mountains	-45.9000	169.3833	Q1.1d	S25_STEWARDSHIP_AREA	Conservation Area - Blue Mountains Forest
M. antarctica* subsp. *antarctica	WELT	SP104523	Hindmarsh-Walls R	March 2014	South Island	Southland	Tamatea Peak, Merrie Range	-45.6917	167.1649	R2.1b	S4_NATIONAL_PARK	Fiordland National Park
M. antarctica* subsp. *antarctica	WELT	SP006454	Oliver WRB	20 February 1952	South Island	Southland	Takahe Valley cirque	-45.2751	167.6264	R2.1f	S4_NATIONAL_PARK	Fiordland National Park
M. antarctica* subsp. *antarctica	WELT	SP089906	Thorsen MJ	1 February 2007	South Island	Southland	Loch Linne [Linnhe]	-45.2728	168.8192	Q1.2a		
M. antarctica* subsp. *antarctica	CHR	395549	Druce AP	March 1985	South Island	Southland	Hector Mountains, Two-mile valley	-45.1667	168.8167	Q1.2a	S25_STEWARDSHIP_AREA	Remarkables Conservation Area
M. antarctica* subsp. *antarctica	WELT	SP102785	Prebble JM 14004; Fergus A	5 February 2014	South Island	Southland	Mavora, edge of stable scree above a tributary of the Windon Burn	-45.0993	168.1043	Q1.2b	S25_STEWARDSHIP_AREA	Conservation Area - Mavora Park
M. antarctica* subsp. *antarctica	CHR	520288	Platt KH	13 March 1985	South Island	Southland	Wapiti Lake, Doon Catchment, Fiordland	-45.0742	167.4486	R2.2a	S4_NATIONAL_PARK	Fiordland National Park
M. antarctica* subsp. *antarctica	CHR	520287	Platt KH	14 January 1985	South Island	Southland	Wapiti Lake, Doon River, Fiordland	-45.0712	167.4598	R2.1c	S4_NATIONAL_PAR	Fiordland National Park

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
											K	
M. antarctica* subsp. *antarctica	CHR	76092	Gibbs FG	DATE	South Island	Southland	Fiordland, McKinnon Pass	-44.8026	167.7661	R2.2a	S4_NATIONAL_PARK	Fiordland National Park
M. antarctica* subsp. *antarctica	CHR	357853	Meurk CD; Wilson HD 789-565	25 January 1979	South Island	Stewart Island	Summit of Western peak of Mount Anglem	-46.7376	167.9058	O5.2b	S4_NATIONAL_PARK	Rakiura National Park
M. antarctica* subsp. *antarctica	CHR	521249	Simpson MJA; Moss IB	January 1959	South Island	Western Nelson	Hopeless Creek, Travers Valley	-41.9230	172.7519	P2.1b	S4_NATIONAL_PARK	Nelson Lakes National Park
M. antarctica* subsp. *antarctica	CHR	355139	Druce AP	March 1979	South Island	Western Nelson	Matiri Range, head of Bay Creek	-41.6250	172.3017	P3.2b	S4_NATIONAL_PARK	Kahurangi National Park
M. antarctica* subsp. *antarctica	CHR	355137	Druce AP	March 1979	South Island	Western Nelson	Matiri range, head of Bay creek	-41.6169	172.3046	P3.2b	S4_NATIONAL_PARK	Kahurangi National Park
M. antarctica* subsp. *antarctica	WELT	SP002650/A	Petrie D	5 February 2010	South Island	Western Nelson	Gordon's Knob	-41.6058	172.9402	P2.1a	S19_CONSERVATION_PARK	Mount Richmond Forest Park
M. antarctica* subsp. *antarctica	CHR	249602	Druce AP	January 1972	South Island	Western Nelson	Mount Owen	-41.5500	172.5333	P1.1c	S4_NATIONAL_PARK	Kahurangi National Park
M. antarctica* subsp. *antarctica	CHR	389081	Druce AP	February 1982	South Island	Western Nelson	South Arthur Range, Mount Patriarch	-41.4167	172.5000	P1.1a	S4_NATIONAL_PARK	Kahurangi National Park
M. antarctica* subsp. *antarctica	CHR	551328	Courtney SP	18 December 2003	South Island	Western Nelson	Garibaldi Plateau	-41.2367	172.4097	P1.1c	S4_NATIONAL_PARK	Kahurangi National Park
M. antarctica* subsp. *antarctica	CHR	358525	Druce AP	March 1980	South Island	Western Nelson	Garibaldi ridge	-41.2333	172.4000	P1.1c	S4_NATIONAL_PARK	Kahurangi National Park
M. antarctica* subsp. *antarctica	CHR	77000	Hay JA	13 April 1952	South Island	Western Nelson	Mount Arthur summit ridge	-41.2145	172.6999	P2.1b	S4_NATIONAL_PARK	Kahurangi National Park
***M. antarctica* subsp.**	CHR	76994	Hay JA	13 April	South	Western	Mount Arthur, near	-41.1994	172.6914	P1.1a	S4_NATIO	Kahurangi

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
antarctica				1952	Island	Nelson	saddle from Gordon Pyramid				NAL_PARK	National Park
M. antarctica* subsp. *antarctica	CHR	277570	Druce AP	January 1975	South Island	Western Nelson	Mount Arthur Tableland	-41.1833	172.6500	P3.2a	S4_NATIONAL_PARK	Kahurangi National Park
M. antarctica* subsp. *antarctica	WELT	SP091883	Sneddon BV	8 January 1968	South Island	Western Nelson	Mt Lodestone nr summit	-41.1703	172.7459	P1.1b	S4_NATIONAL_PARK	Kahurangi National Park
M. antarctica* subsp. *antarctica	WELT	SP100466	Prebble JM 13018	25 January 2013	South Island	Western Nelson	Ridge above Lake Peel to the west	-41.1440	172.6115	P1.1c	S4_NATIONAL_PARK	Kahurangi National Park
M. antarctica* subsp. *antarctica	WELT	SP100465	Prebble JM 13017	25 January 2013	South Island	Western Nelson	Ridge above Lake Peel to the west	-41.1421	172.5982	P1.1c	S4_NATIONAL_PARK	Kahurangi National Park
M. antarctica* subsp. *antarctica	CHR	269153	Talbot H	1 January 1948	South Island	Western Nelson	Mount Peel	-41.1378	172.5922	P1.1c	S4_NATIONAL_PARK	Kahurangi National Park
M. antarctica* subsp. *antarctica	CHR	311791	Druce AP	1 February 1977	South Island	Western Nelson	Cobb Valley	-41.1000	172.5833	K1.1c	S4_NATIONAL_PARK	Kahurangi National Park
M. antarctica* subsp. *antarctica	CHR	387645	Druce AP	January 1982	South Island	Western Nelson	Lockett Range, Mount Lockett	-41.0735	172.6176	P1.1c	S4_NATIONAL_PARK	Kahurangi National Park
M. antarctica* subsp. *antarctica	CHR	310484	Druce AP	January 1977	South Island	Western Nelson	Above Lake Aorere	-41.0500	172.3333	P3.1a	S4_NATIONAL_PARK	Kahurangi National Park
M. antarctica* subsp. *antarctica	CHR	401041	Druce AP	February 1964	South Island	Western Nelson	Douglas Range	-41.0167	172.5500	P1.1c	S4_NATIONAL_PARK	Kahurangi National Park
M. antarctica* subsp. *antarctica	CHR	197031	Druce AP	January 1969	South Island	Western Nelson	Gouland Downs	-40.9333	172.2833	P5.1a	S4_NATIONAL_PARK	Kahurangi National Park
M. antarctica* subsp. *antarctica	CHR	283086	Druce AP	February 1976	South Island	Western Nelson	Boulder Lake	-40.8977	172.5753	P3.2a	S4_NATIONAL_PARK	Kahurangi National Park

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
M. antarctica* subsp. *antarctica	WELT	SP089891	Thorsen MJ	2 February 2005	South Island	Westland	Mount Aspiring, Fog Peak	-44.5263	168.8028	T1.1a	S25_STEWARDSHIP_AREA	Black Peak Conservation Area
M. antarctica* subsp. *antarctica	CHR	310966	Johnson PN	30 March 1977	South Island	Westland	Cascade River mouth	-44.0200	168.3712	O1.3a	S25_STEWARDSHIP_AREA	Conservation Area - Arawhata
M. antarctica* subsp. *antarctica	CHR	203417	Wardle P	16 February 1970	South Island	Westland	Copland Valley, Douglas Rock	-43.6500	170.0167	O2.3a	S4_NATIONAL_PARK	Westland / Tai Poutini National Park
M. antarctica* subsp. *antarctica	CHR	214903	Wardle P	7 February 1971	South Island	Westland	Westland National Park, head of Architects Creek	-43.5854	169.9631	R1.2b	S4_NATIONAL_PARK	Westland / Tai Poutini National Park
M. antarctica* subsp. *antarctica	WELT	SP002662	Cockayne L 5705	Jan 1898	South Island	Westland	Hill's [Hills] Peak	-42.8871	171.5668	R1.1e	S4_NATIONAL_PARK	Arthur's Pass National Park
M. antarctica* subsp. *antarctica	CHR	323547	Druce AP	1 January 1978	South Island	Westland	West of Lewis Pass	-42.3833	172.3833	P2.1a	S19_CONSERVATION_PARK	Lake Sumner Forest Park
M. antarctica* subsp. *antarctica	CHR	420758	Burke WD 190	12 February 1985	South Island	Westland	Fairie Queen, basin on north west side	-42.2500	172.5000	P2.1a	S4_NATIONAL_PARK	Nelson Lakes National Park
M. antarctica* subsp. *antarctica	CHR	127828	Simpson MJA 3908	24 April 1962	South Island	Westland	Nelson Lakes National Park, Travers Range, Fifth Basin	-41.8817	172.7620	P2.1a	S4_NATIONAL_PARK	Nelson Lakes National Park
M. antarctica* subsp. *antarctica	CHR	129050	Druce AP	23 April 1962	South Island	Westland	Nelson Lakes National Park, Travers Range, Third Basin	-41.8589	172.7835	P2.1a	S4_NATIONAL_PARK	Nelson Lakes National Park
M. antarctica* subsp. *antarctica	CHR	204877	Raren P	10 October 1969	South Island	Westland	Nelson Lakes National Park, Travers Range, Robert Ridge, Second Basin	-41.8500	172.8000	P2.1a	S4_NATIONAL_PARK	Nelson Lakes National Park
***M. antarctica* subsp.**	AK	251910	de Lange	6 January	Stewart	Stewart	Mount Anglem	-44.7333	167.9167	R2.1b	S4_NATIO	Fiordland

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
antarctica			PJ 4109	2000	Island	Island					NAL_PARK	National Park
***M. antarctica* subsp. *antarctica* (as *M.* "Volcanic Plateau")**	CHR	65075	Druce AP; Elder NL	December 1948	North Island	Southern North Island	Kaimanawa ranges, Ngarururoro River	-39.1173	176.1484	F7.1b		
***M. antarctica* subsp. *antarctica* (as *M.* "Volcanic Plateau")**	CHR	86270	Druce AP	January 1950	North Island	Volcanic Plateau	Hawke's Bay, Kaimanawa Mountains, Te Rei	-39.5000	175.8667	F1.3c		
***M. antarctica* subsp. *antarctica* (as *M.* "Volcanic Plateau")**	CHR	323790	Druce AP	December 1978	North Island	Volcanic Plateau	Kaimanawa Mountains, Moawhango river	-39.3333	175.9000	F7.3a		
***M. antarctica* subsp. *antarctica* (as *M.* "Volcanic Plateau")**	CHR	402357	Druce AP	January 1988	North Island	Volcanic Plateau	Kaimanawa Mountains, Moawhango Valley	-39.3061	175.8616	F7.3a		
***M. antarctica* subsp. *antarctica* (as *M.* "Volcanic Plateau")**	CHR	323791	Druce AP	December 1978	North Island	Volcanic Plateau	Kaimanawa Mountains, Moawhango West River	-39.3000	175.8667	P7.1c		
***M. antarctica* subsp. *antarctica* (as *M.* "Volcanic Plateau")**	CHR	402369	Rogers GM	1 November 1983	North Island	Volcanic Plateau	Kaimanawa Mountains, North of Ngamatea East Swamp	-39.3500	176.1167	F7.3b		
***M. antarctica* subsp. *antarctica* (as *M.* "Volcanic Plateau")**	CHR	244442	Druce AP	January 1973	North Island	Volcanic Plateau	Kaimanawa Mountains, Patutu	-39.2333	175.8500	P7.1c		
***M. antarctica* subsp. *antarctica* (as *M.* "Volcanic Plateau")**	CHR	243775	Elder NL 311/28	31 December 1948	North Island	Volcanic Plateau	Kaweka Range, Lower Waiotupuritia	-39.1286	176.1428	F7.1c		
***M. antarctica* subsp. *antarctica* (as *M.* "Volcanic Plateau")**	AK	331000	de Lange PJ	22 May 2012	North Island	Volcanic Plateau	Mt Ruapehu, Upper Makatote	-39.2638	175.5195	P4.1b	S4_NATIONAL_PARK	Tongariro National Park
***M. antarctica* subsp. *antarctica* (as *M.* "Volcanic Plateau")**	CHR	473272	de Lange PJ 755	6 April 1991	North Island	Volcanic Plateau	Northwest Ruahines, Makirikiri Plateau	-39.6167	176.1667	F7.3a		
***M. antarctica* subsp.**	CHR	273143	Druce AP	1 April	North	Volcanic	Southwest of Lake	-39.0000	175.7167	P7.1c	S4_NATIO	Tongariro

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
***antarctica* (as *M.* "Volcanic Plateau")**				1974	Island	Plateau	Taupo, Mount Tihia				NAL_PARK	National Park
***M. antarctica* subsp. *antarctica* (as *M.* "Volcanic Plateau")**	WELT	SP089738	Singers NJ	15 February 2011	North Island	Volcanic Plateau	Volcanic Plateau, Waipakihi Valley	-39.1653	175.8975	P7.1c	S19_CONSERVATION_PARK	Kaimanawa Forest Park
***M. antarctica* subsp. *antarctica* (as *M.* "Volcanic Plateau")**	No voucher		Rogers GM	December 2013	South Island	Southland	Kiwi Burn	-45.3427	168.1242	Q4.2a	S25_STEWARDSHIP_AREA	Conservation Area - Snowdon Forest
***M. antarctica* subsp. *antarctica* (as *M.* "Volcanic Plateau")"**	CHR	512853	Ogle CC 3219	25 February 1997	North Island	Volcanic Plateau	Rangitikei, Moawhango River, upriver from Ngawakaakauae Stream	-39.3000	175.9000	P7.1c		
***M. antarctica* subsp. *antarctica* (as *M.* "Volcanic Plateau")"**	CHR	273144	Robins I	1 April 1974	North Island	Volcanic Plateau	Southwest of Lake Taupo, Mount Kakaramea	-38.9748	175.6942	P4.1a		
M. antarctica* subsp. *traillii	AK	167155	Wright AE 5794	30 August 1983	North Island	Auckland	Off Papaaroha, Moturua (Rabbit) Island	-36.6996	175.3942	D1.1a		
M. antarctica* subsp. *traillii	AK	167143	Wright AE 5782	30 August 1983	North Island	Auckland	Coromandel Ecological Region, Colville Ecological District, off Papaaroha, Moturua (Rabbit) Island	-36.6942	175.3927	A7.1a		
M. antarctica* subsp. *traillii	AK	167142	Wright AE 5781	30 August 1983	North Island	Auckland	Coromandel Ecological Region, Colville Ecological District, off Papaaroha, Moturua (Rabbit) Island	-36.6940	175.3930			
***M. antarctica* subsp.**	WELT	SP090631	Meudt HM	13	North	Hawkes Bay	Waipuna Station,	-39.2552	176.6084	F6.2a		

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
traillii			HMM333 et al.	December 2011	Island		south end of Te Waka Range on bluffs					
M. antarctica* subsp. *traillii	WELT	SP090629	Meudt HM HMM332 et al.	12 December 2011	North Island	Hawkes Bay	Hukanui Station	-39.2485	176.5316	E1.1d		
M. antarctica* subsp. *traillii	WELT	SP090634	Meudt HM HMM335 et al.	13 December 2011	North Island	Hawkes Bay	Waipuna Station, south end of Te Waka Range	-39.2478	176.6127	F6.2a		
M. antarctica* subsp. *traillii	WELT	SP095607	Park G	19 December 1966	North Island	Southern North Island	Eastern Wairarapa	-40.7986	176.2618	F1.2c		
M. antarctica* subsp. *traillii	CHR	208728	Druce AP	March 1973	North Island	Southern North Island	Mount Hukanui	-39.2667	176.5333	F6.1c		
M. antarctica* subsp. *traillii	CHR	246383	Druce AP	January 1972	North Island	Southern North Island	Te Waka Range	-39.2167	176.6667	D4.1e		
M. antarctica* subsp. *traillii	CHR	208826	Druce AP	December 1972	North Island	Southern North Island	Maungaharuru Range	-39.2000	176.7000	P7.1a		
M. antarctica* subsp. *traillii	CHR	245912	Druce AP	1 November 1971	North Island	Taranaki	Coast, Pihama, Puketapu road	-39.5174	173.9119	F5.2c		
M. antarctica* subsp. *traillii	WELT	SP090540	Meudt HM HMM309 et al.	5 October 2011	North Island	Taranaki	Near Opunake water treatment pools	-39.4461	173.8318	C1.3a		
M. antarctica* subsp. *traillii	CHR	131076	Druce AP	May 1964	North Island	Taranaki	Coast, near Heimama Stream	-39.4459	173.8318	C1.3a		
M. antarctica* subsp. *traillii	WELT	SP090542	Meudt HM HMM310 et al.	5 October 2011	North Island	Taranaki	Arawhata Rd end	-39.4172	173.7984	F5.2c		
M. antarctica* subsp. *traillii	WELT	SP090544	Meudt HM HMM312 et al.	6 October 2011	North Island	Taranaki	Manihi Rd end	-39.3715	173.7753	G1.1d		
M. antarctica* subsp. *traillii	CHR	252804	Wilson HD	13 December 1971	South Island	Canterbury	Foot of Haast ridge	-43.5891	170.2056	R1.1d	S4_NATIONAL_PARK	Aoraki Mount Cook National Park

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
M. antarctica* subsp. *traillii	CHR	568877	Huber AM	15 February 1980	South Island	Canterbury	Wilberforce River, near Urqharts Hut	-43.0348	171.3185			
M. antarctica* subsp. *traillii	AK	234644	de Lange PJ 3421	12 November 1997	South Island	Otago	Chrystalls Beach	-46.2057	170.0679	Q4.3c		
M. antarctica* subsp. *traillii	WELT	SP089920	Thorsen MJ	27 December 2004	South Island	Otago	Cooks Tooth [Cooks Head Rock], Chrystalls Beach	-46.2045	170.0704	Q4.3c		
M. antarctica* subsp. *traillii	AK	234869	de Lange PJ 3439	12 November 1997	South Island	Otago	Milton, Watson Road, south of Quoin Point	-46.1544	170.1686	L4.1a		
M. antarctica* subsp. *traillii	WELT	SP089918	Thorsen MJ	18 March 2008	South Island	Otago	Otago Penninsula, Highcliff.	-45.9025	170.5958			
M. antarctica* subsp. *traillii	WELT	SP100402	Prebble JM 12011	23 August 2012	South Island	Otago	The Chasm, Otago Peninsula	-45.8942	170.6799	Q4.3d	17_RECREATION_RESERVE	Sandymount Recreation Reserve
M. antarctica* subsp. *traillii	CHR	570356	Rance BD	28 January 2004	South Island	Otago	Chain Hills, Morven Hills Station, Lindis Pass	-44.6478	169.6511	Q1.2a		
M. antarctica* subsp. *traillii	CHR	357370	Cranwell LM	14 January 1940	South Island	Southland	Rabbit Island	-46.9124	168.1511	O5.2a	S19_1_A_SCENIC_RESERVE	Native Island Scenic Reserve
M. antarctica* subsp. *traillii	WELT	SP100487	Prebble JM; Pilkington K	25 February 2013	South Island	Southland	Tiwai Point, on beach where the 4WD track meets the beach at the Waituna lagoon end	-46.5988	168.5457	L1.1d	S21_SCIENTIFIC_RESERVE	Waituna Wetlands Scientific Reserve
M. antarctica* subsp. *traillii	CHR	87569	Moore LB	25 January 1957	South Island	Southland	Bluff, Ocean Beach	-46.5919	168.3096	L1.1d		
M. antarctica* subsp. *traillii	WELT	SP089940	Barkla J	17 February 2011	South Island	Southland	Catlins, Tahakopa Bay	-46.5573	169.4932	Q4.2c	S19_1_A_SCENIC_RESERVE	Tahakopa Bay Scenic Reserve
***M. antarctica* subsp.**	AK	231694	de Lange	8 January	South	Southland	Makarewa	-46.5500	168.2500	Q4.1a		

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
traillii			PJ	1995	Island		Ecological Region, Waituna Ecological District, Omaui, Three Sisters Dune					
M. antarctica* subsp. *traillii	WELT	SP089917	Thorsen MJ	31 August 2008	South Island	Southland	False Islet.	-46.4775	169.7513	L1.1d		
M. antarctica* subsp. *traillii	WELT	SP089922	Thorsen MJ	5 May 2005	South Island	Southland	near False Islet, Cannibal Bay.	-46.4738	169.7528	L1.1d		
M. antarctica* subsp. *traillii	CHR	541256	Rance BD	17 January 2000	South Island	Southland	Oraka Point, Western Southland	-46.3904	167.8803	Q4.2b		
M. antarctica* subsp. *traillii	WELT	SP090029	Perrie LR; Shepherd LD; Glenny D	5 December 2010	South Island	Southland	Riverton, coast in front of Mores Bush Scenic Reserve	-46.3776	167.9937	Q4.2b		
M. antarctica* subsp. *traillii	WELT	SP006441	Oliver WRB	7 November 1940	South Island	Southland	Wakapatu Bay	-46.3696	167.8236	L1.1d		
M. antarctica* subsp. *traillii	CHR	358007	Powell R	DATE	South Island	Southland	Orepuki	-46.2833	167.7333	Q4.2c		
M. antarctica* subsp. *traillii	CHR	357851	Wilson HD 789-551	30 January 1979	South Island	Stewart Island	Ocean Beach	-46.9702	168.1765	L6.1a	S19_1_A_SCENIC_RESERVE	Glory Cove Scenic Reserve
M. antarctica* subsp. *traillii	CHR	355428	Wilson HD 789-170	29 November 1978	South Island	Stewart Island	Sand east of Big Sandhill, Mason Bay	-46.9305	167.7993	L6.1a	S4_NATIONAL_PARK	Rakiura National Park
M. antarctica* subsp. *traillii	CHR	283985	Webb CJ; Webb TH	30 December 1977	South Island	Stewart Island	Mason Bay, north of Duck creek	-46.9190	167.7716	L6.1a	S4_NATIONAL_PARK	Rakiura National Park
M. antarctica* subsp. *traillii	WELT	SP100477	Prebble JM 13009	22 January 2013	South Island	Western Nelson	Side of track (and on track itself) from Mt Arthur Hut to Mt Arthur	-41.1998	172.7102	P2.1b	S4_NATIONAL_PARK	Kahurangi National Park
M. antarctica* subsp. *traillii	WELT	SP100472	Prebble JM 13007	21 January 2013	South Island	Western Nelson	Northwest Nelson, Hoary Head	-41.1308	172.8047	P1.1a	S4_NATIONAL_PARK	Kahurangi National Park

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
M. antarctica* subsp. *traillii	CHR	313155	Druce AP	1 November 1977	South Island	Western Nelson	Northwest Nelson, north of Heaphy River	-40.9820	172.1052	F1.1a	S4_NATIONAL_PARK	Kahurangi National Park
M. antarctica* subsp. *traillii	WELT	SP100460	Prebble JM 13022	26 January 2013	South Island	Western Nelson	Past the headland just south of Sandhill Creek River mouth	-40.6721	172.3913	F1.1a		
M. antarctica* subsp. *traillii	WELT	SP100462	Prebble JM 13020	26 January 2013	South Island	Western Nelson	1.3 K south of Paturau River mouth	-40.6503	172.4188	F1.1a		
M. antarctica* subsp. *traillii	AK	303514	de Lange PJ 7462	11 September 2008	South Island	Western Nelson	Cult. ex Northwest Nelson, Mangarakau	-40.6333	172.4833	P6.2c		
M. antarctica* subsp. *traillii	CHR	245193	Druce AP	1 November 1971	South Island	Western Nelson	West of Cape Farewell, Whararikibeach	-40.5048	172.6732	F1.1a	17_RECREATION_RESERVE	Puponga Farm Park
M. antarctica* subsp. *traillii	WELT	SP089710	Rance B	DATE	Stewart Island	Stewart Island	Mason Bay	-46.9108	167.7908	L6.1a	S4_NATIONAL_PARK	Rakiura National Park
M. antarctica* subsp. *traillii	WELT	SP089921	Thorsen MJ	13 February 2010	Stewart Island	Stewart Island	Mason Bay, Big Sand pass	-46.9108	167.7855	L6.1a	S4_NATIONAL_PARK	Rakiura National Park
M. antarctica* subsp. *traillii	CHR	189693	Ritchie IM	February 1967	Stewart Island	Stewart Island	Mason Head	-46.8743	167.7530	O5.2d	S4_NATIONAL_PARK	Rakiura National Park
M. brevis	WELT	SP090545/B	Meudt HM HMM313 et al.	1 November 2011	North Island	Southern North Island	Kawakawa rocks, past Ngawi on road to Cape Palliser	-41.6020	175.2367	J4.3b		
M. brevis	CHR	369772	Druce AP	August 1981	North Island	Southern North Island	Wairarapa, Ngawihi point, west of Cape Palliser	-41.6017	175.2359	J4.3b		
M. brevis	WELT	SP090549	Meudt HM HMM317 et al.	7 November 2011	North Island	Southern North Island	Te Ikaamaru Bay near rocky beach, Terawhiti Station (	-41.2467	174.6654	F1.2c		

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
M. brevis	WELT	SP090550/A	Meudt HM HMM318 et al.	7 November 2011	North Island	Southern North Island	Te Ohau Bay ca. 20-30 m from beach, Terawhiti Station	-41.2449	174.6506	C2.1e		
M. brevis	WELT	SP090361	Meudt HM HMM308 et al.	5 October 2011	North Island	Taranaki	Puketapu Road end	-39.5193	173.9165	F5.2c		
M. brevis	CHR	131005	Druce AP	December 1964	North Island	Taranaki	Coast, near Taungatara Stream	-39.4894	173.8834	J4.2a		
M. brevis	WELT	SP090543/A	Meudt HM HMM311 et al.	5 October 2011	North Island	Taranaki	Stent Rd end near car park	-39.2186	173.7777	H1.3a		
M. brevis	CHR	243750	Macmillan BH	7 January 1973	South Island	Canterbury	Lake Pukaki	-44.1817	170.1915	E4.1b		
M. brevis	CHR	496538	Ford KA 18/93	19 October 1993	South Island	Canterbury	Mackenzie Basin, Maryburn Station	-44.1500	170.3500	E4.1b	S25_STEWARDSHIP_AREA	Conservation Area - Maryburn
M. brevis	CHR	243997	Anderson J	18 October 1977	South Island	Canterbury	Lake Tekapo, Mount Hay	-43.9510	170.5650	E4.2b		
M. brevis	CHR	320144	Johnson PN	27 November 1977	South Island	Canterbury	Mount St John, near Lake Tekapo	-43.9000	170.4167	E4.1b		
M. brevis	WELT	SP093294	Prebble JM 12009; Thorsen MJ	21 February 2012	South Island	Canterbury	Lake Lyndon, the shore at the south end	-43.3130	171.6910	E4.2a	S24_3_FIXED_MARGINAL_STRIP	Lake Lyndon Marginal Strip
M. brevis	WELT	SP089902	Thorsen MJ	12 February 2004	South Island	Otago	Flat top hill, near Alexandra (cultivated)	-45.3370	169.3280	N4.1b		
M. brevis	CHR	476031	Druce AP APD1767	1 February 1992	South Island	Otago	Nevis Valley	-45.3333	168.8667	K3.2a	S25_STEWARDSHIP_AREA	Remarkables Conservation Area
M. brevis	CHR	48081	Simpson G	DATE	South Island	Otago	Little river near Alexandra	-45.3095	169.4711	N5.1a		
M. brevis	WELT	SP102762	Prebble JM 13046	9 October 2013	South Island	Otago	Chapman Rd Scientific Reserve	-45.2677	169.3452	N4.1e		

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
M. brevis	CHR	480321	Patrick B	1 October 1992	South Island	Otago	Conroys Road	-45.2667	169.3333	N8.1b		
M. brevis	WELT	SP089900	Thorsen MJ	18 October 2008	South Island	Otago	Springvale, "McDonnell's"	-45.2050	169.4358	N8.1b		
M. brevis	WELT	SP102763	Prebble JM 13047	9 October 2013	South Island	Otago	Springvale Scientific Reserve	-45.2041	169.4341	N8.1b		
M. brevis	WELT	SP102761	Prebble JM 13045	8 October 2013	South Island	Otago	Bannockburn slucings, Stewart Town, on flat areas above slucings	-45.0858	169.1355	N8.1b		
M. brevis	CHR	474620	Patrick B	30 September 1991	South Island	Otago	Dunstan Gorge	-45.0833	169.2667	N4.1e		
M. brevis	WELT	SP103814	Meudt HM HMM360 et al.	10 December 2014	South Island	Otago	Dunstan Mountains	-45.0025	169.4073	Q1.1a	S25_STEWARDSHIP_AREA	Bendigo Conservation Area
M. brevis	WELT	SP102760	Prebble JM 13044	8 October 2013	South Island	Otago	Bendigo historic reserve, near Welshtown car carpark.	-44.9409	169.3716	N4.1b	S18_HISTORIC_RESERVE	Bendigo Historic Reserve
M. brevis	CHR	605688	Barkla J	1 October 2008	South Island	Otago	Bendigo, Aurora Creek	-44.9393	169.3707	N4.1b	S18_HISTORIC_RESERVE	Bendigo Historic Reserve
M. brevis	WELT	SP089894	Thorsen MJ	23 March 2006	South Island	Otago	Near Bendigo, Dunstan Mountains.	-44.9287	169.3447	N4.1e		
M. brevis	CHR	586032	Thorsen M	24 January 2006	South Island	Otago	Cliff Burn cirque, Pisa Range	-44.8554	169.1973	Q1.2a		
M. brevis	WELT	SP093498	Barkla J	8 December 2011	South Island	Otago	Hawkdun Mountains	-44.8270	170.1126	Q1.1a	S25_STEWARDSHIP_AREA	Mount Ida Conservation Area (ex Mt Ida Syndicate)
M. brevis	WELT	SP103893	Rogers G	13 October 2014	South Island	Otago	Clutha River, near Lake Wanaka	-44.6921	169.1940	N5.1c		
M. glauca	AK	280800	Wright AE	27 October	South	Canterbury	Lake Ohau, shores	-44.2333	169.8167		S25_STEW	Ruataniwha

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
			12963	2002	Island		of lake, below Lake Ohau lodge, c.2 cm above water level				ARDSHIP_AREA	Conservation Area
M. glauca	WELT	SP100497	Prebble JM 13039; Pilkington K	1 March 2013	South Island	Otago	Macraes flat, side of road between the DOC field office and the skink enclosure	-45.4518	170.4403	N3.1e	S19_1_A_SCENIC_RESERVE	Redbank Scenic Reserve
M. glauca	WELT	SP093285	Prebble JM 12004	15 February 2012	South Island	Otago	Nevis Valley, old mine tailings	-45.2326	168.9591	N6.2a	S25_STEWARDSHIP_AREA	Remarkables Conservation Area
M. glauca	WELT	SP093284	Prebble JM 12003	15 February 2012	South Island	Otago	Nevis Valley, Schoolhouse Flat ponds, old mine tailings	-45.2092	168.9884	N6.1b		
M. glauca	CHR	87808	McNeur IA	25 September 1949	South Island	Otago	Hill above Fraser Domain	-45.2000	169.3000	N4.1e		
M. glauca	WELT	SP089838	Thorsen MJ	9 December 2006	South Island	Otago	Donseys Pass, Kyeburn Diggings	-45.0037	170.2783	N3.1d		
M. glauca	CHR	363622	Johnson PN	15 November 1979	South Island	Otago	West Eweburn Dam	-45.0000	170.0833	N3.1d		
M. glauca	CHR	191750	Moore LB	26 April 1969	South Island	Otago	Near Naseby, Mount Ida, Tourist Spur	-44.9758	170.1057	Q2.1b		
M. glauca	WELT	SP089837	Thorsen MJ	17 January 2006	South Island	Otago	Dunstan Mts. [Mountains], Lauder [Creek]	-44.8945	169.6512	Q1.1c	S25_STEWARDSHIP_AREA	Lauder Basin Conservation Area
M. glauca	CHR	586018	Thorsen M	26 January 2006	South Island	Otago	Western Pisa Range	-44.8309	169.1103	Q1.1a		
M. glauca	CHR	87636	Allan HH	4 December 1948	South Island	Otago	Luggate	-44.7333	169.2667	N5.1c		

Species name as used in niche modelling	Herbarium	Accession Number	Collector	Collection Date	Main area	Region	Locality	Latitude	Longitude	LENZ environment	DOC Reserve type	Reserve Name
M. glauca	CHR	72448	Allan HH	28 January 1948	South Island	Southland	North of Lumsden, Mid-Dome	-45.5667	168.5833	Q2.1a		
M. glauca	CHR	514987	Johnson PN 1393	29 January 1998	South Island	Southland	Von Valley near Oreti head	-45.2333	168.3000	Q3.1a		
M. glauca **(as *M.* aff. *glauca*)**	WELT	SP089897	Thorsen MJ	14 January 2010	South Island	Otago	Pisa Range, Snowfarm	-44.8778	169.0933	Q3.3a		
M. glauca **(as *M.* aff. *glauca*)**	WELT	SP093282	Prebble JM 12002	14 February 2012	South Island	Otago	Waiorau Snowfarm, near the Roaring Meg stream	-44.8672	169.1231	Q3.3a	S25_STEWARDSHIP_AREA	Pisa Conservation Area
M. glauca **(as *M.* aff. *glauca*)**	WELT	SP089898	Thorsen MJ	23 January 2006	South Island	Otago	Pisa Range, Robrosa pastoral lease	-44.8317	169.1103	Q1.1a		
M. glauca **(as *M.* aff. *glauca*)**	OTA	44898	Mark AF	6 January 1987	South Island	Southland	Little Jungle Creek, headwaters of Eyre Creek	-45.3403	168.3446	Q1.1d	S19_CONSERVATION_PARK	Eyre Mountains/Taka Ra Haka Conservation Park