Taxonomic delimitation and the evolutionary history of the Australasian Lautusoid group of *Senecio* (Asteraceae)

A thesis submitted in partial fulfillment of the requirements for the

Degree of Doctor of Philosophy in Evolutionary Biology

of the University of Canterbury

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University of Canterbury 2016

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ACKNOWLEDGEMENTS

To Pieter and Peter, my supervisors, thank you for guiding me through my study and this thesis. Without your patience and dedication, I would not have been able to do it. To the curators and managers of AD, AK, CANU, CHR, BRI, MEL, PERTH, and WELT, thank you for approving my herbarium loans and giving me permission to sample tissue materials. A special shout out to all the kind souls at the Allan Herbarium (CHR): Mary, Debby, Sue, Charles, Kerry, Rob, and especially Ines, who endured so many requests from me and without whom my research would not have progressed. To numerous people from different institutions who have been lending me a hand with collecting samples: Shannel Courtney, Nicholas Head, John Barkla, Jeremy Rolfe, Daniel Kimber, Hugh Wilson, Mike Thorsen, Mike Harding, Alice Shanks, Miles and Gillian Giller, Di Carter, Trevor Partridge and Peter Johnson, thank you so much. I would not have been able to carry out my research if it was not for your help. Thanks to Maggie, Craig, Claire, Nicole, Gavin, Nicki, Penny and Thomas at the UC School of Biological Sciences, who helped me in various ways during my study. Thanks to Tim, who kindly contributed his R code and detailed instructions of how to draw pie charts on a map and being a friend throughout my study. Special thanks to Rachel, Ilina, Natalie, Michael, Stephanie, Jacob, Jasper, Sarah, Stephen, Denise, Ayla, and Valentin, thank you for being my lab and office buddies. Your support and encouragement in times of need are what kept me going and are what I missed the most since leaving New Zealand. Also thanks to Tammy and Marie, who provided much needed guidance, assurance and help from time to time. I am absolutely grateful and can not thank Mom and Dad enough, who are monumental in my journey of doing a PhD. Thanks for getting me home, keeping me alive and meeting all my needs while I wrestled with my thesis. Your unconditional love and support is what kept me motivated and inspired in life. Also thanks to my brothers, I have come a long way under your love and support. Thanks for providing me a place to stay and feed me when needed throughout my thesis writing. Derek, your patience and love keep me forward when it is hard. Thanks for all the treats and love you lots. Funding for this research project was provided by a University of Canterbury Doctoral Scholarship and the School of Biological Sciences Strategic Investment Fund to Pieter Pelser.

ABSTRACT

Taxonomic delimitation can be a challenging task for systematists, because of the dynamic and complex evolutionary processes that shape patterns of biodiversity. Yet, it is an essential aspect of biology, because it defines units of evolutionary significance, which form the basis for studying all aspects of biodiversity. In this thesis, I studied the taxonomic delimitation and evolutionary history of the Australasian Lautusoid group of *Senecio* at the infrageneric, species, and infraspecific level. Members of the Lautusoid group are morphologically very diverse and occupy a wide array of habitats. Moreover, the Lautusoid group has a large diversity of chromosome profiles compared to other Australasian *Senecio*, which indicates the possible occurrence of hybridization in its evolutionary history. These patterns of diversity make it an attractive system for various evolutionary and ecological studies. Despite these interesting characteristics and the inclusion of members of the Lautusoid group in a number of taxonomic treatments, it is not known how many and which species form the Lautusoid group. To determine the delimitation of the Lautusoid group and to investigate the origin of Lautusoid species with higher chromosome numbers, a molecular phylogenetic study was carried out. The results of this study indicate that the group is a morphologically and phylogenetically distinct *Senecio* lineage with an Australasian distribution. These results also highlight the important role of hybrid speciation in the evolutionary history of the Lautusoid group by identifying allopolyploid hybrids between members of the Lautusoid group and members of other Australasian lineages. An allopolyploid species complex that was found to be affiliated with the Lautusoid group, *S. glaucophyllus*, was the focus of subsequent studies. *Senecio glaucophyllus* and a morphologically similar informally named taxon, *S.* aff. *glaucophyllus*, were examined to determine if they are distinct species. The results confirm that the two taxa are indeed morphologically and genetically distinct. However, against expectation, this study revealed that *S.* aff. *glaucophyllus* is the true *S. glaucophyllus* and that the plants that were called *S. glaucophyllus* belong to a species that is presently unnamed. This taxon, tentatively called *S.* "pseudoglaucophyllus", aligns with *S. glaucophyllus* sensu Ornduff excluding *S. glaucophyllus* Cheeseman. In order to revisit the current classification of recognizing four infraspecific groups for *S.* "pseudoglaucophyllus" and to propose taxonomic recommendations, studies that look into its morphological and genetic diversity were performed. The results of these studies show that patterns of morphological variation in *S.* "pseudoglaucophyllus" are not congruent with patterns of genetic variation and that neither supports the current classification in which four

infraspecific groups are recognized. Because infraspecific taxon boundaries cannot be unambiguously determined for *S.* “pseudoglaucophyllus”, this species is therefore best regarded as a single variable New Zealand species for which infraspecific groups should not be formally recognized.

CHAPTER 1: Introduction

1.1. Thesis overview

This PhD thesis presents the results of my study of aspects of the taxonomy and evolutionary history of the Lautusoid *Senecio* group, which is an Australasian lineage of tribe Senecioneae (Asteraceae). In this first chapter, I introduce my study system and discuss the research questions that I am addressing in my thesis. Because my research largely focused on taxonomic delimitation at the infrageneric, species, and infraspecific levels, I subsequently briefly discuss these topics as a broader introduction to the research chapters of my thesis. Chapter 2 presents the results of a molecular phylogenetic study into the taxonomic delimitation of the Lautusoid group and the evolutionary origin of taxa with high chromosome numbers. In Chapter 3, the focus switches to an allopolyploid species complex that was found to be affiliated with the Lautusoid group. In this study, I aimed to determine if *S. glaucophyllus* Cheeseman and a morphologically similar undescribed taxon, *S.* aff *glaucophyllus*, are distinct species. In the later part of the thesis, I investigate the morphological (Chapter 4) and genetic (Chapter 5) diversity of *S.* "pseudoglaucophyllus", which is an unnamed species that aligns with *S. glaucophyllus* sensu Ornduff excluding *S. glaucophyllus* Cheeseman. At the end of Chapter 5, I use the findings of these studies to inform the infraspecific taxonomic delimitation of this species. Chapter 6 presents a general overview of the findings of my thesis

1.2. Background and aims of this study

Senecio is one of the largest genera of flowering plants with over 1000 species. It has a nearly worldwide distribution except Antarctica (Nordenstam, 2007; Pelser et al., 2007; Nordenstam et al., 2009; Calvo et al., 2013). In addition to its huge size, *Senecio* is also morphologically and ecologically diverse (i.e., Pelser et al., 2002, 2004; Radford et al., 2004; Roda et al., 2013a). The occurrence of hybridization is also well-documented in *Senecio*'s evolutionary history (e.g., Abbott & Lowe, 2004; Abbott et al., 2009; Pelser et al., 2010a, 2012; Calvo et al., 2013; James & Abbott, 2005).

Senecio has been the focus of many taxonomic studies especially in Africa and Europe (i.e., Jeffrey et al., 1977; Jeffrey, 1979, 1992; Pelser & Houchin, 2004; Pelser et al., 2012; Calvo et al., 2013, 2015; Kandziora, 2016a, b), and more recently in the Americas (Calvo, 2015; Calvo et al., 2016). Comparatively, there are few taxonomic treatments of *Senecio* in

Australasia. Within the region, taxonomic studies have often been carried out in isolation (i.e., Ornduff, 1960; Lawrence, 1980; Belcher, 1992). Australasian *Senecio* were included in Pelser et al. (2007)'s molecular phylogenetic study of the tribe Senecioneae and *Senecio*. This study found that *Senecio* has dispersed to the region in at least three separate events (Pelser et al., 2007). *Senecio lautus* G.Forst. ex Willd. was one of the Australasian species included in this study and grouped with two other Australian species in a clade (see Fig. 1I in Pelser et al., 2007). Because of the few representatives found in the clade (Australian *Senecio* clade clade 3 in Fig. 1I, Pelser et al., 2007), it is largely unknown of which and how many species would be found in the same clade if more *Senecio* species were included.

1.2.1. The Lautusoid group of *Senecio*

The focus group of this PhD study is the Lautusoid group of *Senecio* L. In the literature, this Australasian group is also commonly referred to as the *S. lautus* complex or alliance (e.g., Ali, 1964, 1969; Belcher, 1993, 1994; Thompson, 2005b; Roda et al., 2013a, b) or the *S. pinnatifolius* A.Rich. complex (e.g., Radford & Cousens, 2000; Radford et al. 2004; Thompson, 2005b). The Lautusoid group is one of the eight infrageneric morphological groups of *Senecio* recognized by Thompson in his revision of Australian Senecioneae (2004a, b, c, 2005a, b, 2006). In the current study, the Lautusoid group of *Senecio* is considered in a broader sense than Thompson considered it for Australia. It is extended to also include closely related species from other parts of Australasia. In total, 23 species have been associated with *S. lautus* in previous taxonomic studies of Australasian *Senecio* (Ornduff, 1960; Belcher, 1992b; de Lange & Murray, 2003; Thompson, 2006; de Lange et al., 2014), because of their morphological similarities to *S. lautus* and each other (e.g., Ornduff, 1960; Webb, 1988; Thompson, 2005b, 2006; de Lange et al., 2014), and this therefore brings the total number of putative members of the Lautusoid *Senecio* group to 23 (Table 1.1).

Table 1.1. Putative members of the Lautusoid group of *Senecio*, their general locality, and chromosome number, if known (see Table 2.1 for a more detailed version of this table). Chromosomes of specimens of *S. australis* Willd. from both New Zealand and Norfolk Island were counted and 2n = 80 were obtained for materials from both places (de Lange & Murray, 2003; de Lange et al., 2014).

Species	**Locality**	**Chromosome number (2*n*)**
Senecio brigalowensis I.Thomps.	Australia	
Senecio condylus I.Thomps.	Australia	
Senecio depressicola I.Thomps.	Australia	

Senecio eremicola I.Thomps.	Australia	
Senecio hamersleyensis I.Thomps.	Australia	
Senecio lacustrinus I.Thomps.	Australia	
Senecio pinnatifolius A.Rich.	Australia	40
Senecio spanomerus I.Thomps.	Australia	
Senecio spathulatus A.Rich.	Australia	40
Senecio warrenensis I.Thomps.	Australia	
Senecio carnosulus (Kirk) C.Webb	New Zealand	80
Senecio esperensis (Sykes) de Lange	New Zealand	40
Senecio glaucophyllus Cheeseman	New Zealand	100
Senecio lautus G.Forst. ex Willd.	New Zealand	40
Senecio marotiri C.Webb	New Zealand	80
Senecio radiolatus F.Muell.	New Zealand	40
Senecio repangae de Lange & B.G.Murray	New Zealand	100
Senecio sterquilinus Ornduff	New Zealand	40
Senecio australis Willd.	New Zealand & Norfolk Island	80
Senecio evansianus Belcher	Norfolk Island	
Senecio hooglandii Belcher	Norfolk Island	80
Senecio howeanus Belcher	Lord Howe Island	
Senecio pauciradiatus Belcher	Lord Howe Island	

Taxonomic studies of the Lautusoid *Senecio* group have thus far mostly been done in regional isolation (e.g., Australia: Ali, 1964; Thompson, 2005b, 2006; New Zealand: Ornduff, 1960; Sykes, 1971; Webb, 1988; de Lange et al., 2014; and Norfolk Island and Lord Howe Island: Belcher, 1992b). Particularly in Australia, the Lautusoid group of *Senecio* has a long and complicated taxonomic history, which is nicely summarized by Belcher (1992a) and Thompson (2005b). The delimitation of *S. lautus* was one of the main issues of contention (Thompson, 2005b). This species was once very broadly defined (Bentham, 1867) and included New Zealand as well as Australian plants. Later authors (Ornduff, 1960; Ali, 1964), however, considered New Zealand plants taxonomically distinct from Australian plants, but disagreed about whether these differences should be recognized at the species-level (Ornduff, 1960) or at the level of subspecies (Ali, 1964). So, whereas Ornduff (1960) and others proceeded with resurrecting previously used names and describing new species in the process of narrowing the delimitation of *S. lautus* for New Zealand, Ali (1969) accommodated the Australian plants in various subspecies of *S. lautus*. Belcher (1992a, 1993) studied the Australian Lautusoid taxa in detail and presented characters that separate the Australian taxa

from the native New Zealand taxa at the species-level. Because the type of *S. lautus* is a New Zealand plant, he concluded that the name *S. pinnatifolius* A.Rich. is perhaps best used for the Australian plants formerly placed in *S. lautus*. Thompson (2005b) concurred and contributed to the taxonomic delimitation of the Lautusoid species by describing several new Australian species that he considered morphologically distinct from *S. pinnatifolius*. Prior to Thompson's (2005b, 2006) revision, *S. pinnatifolius* and *S. spathulatus* A.Rich. were the only native Australian Lautusoid taxa that were recognized as distinct species. In addition to these two species, Thompson (2005b, 2006) recognized eight Lautusoid species that he newly described from plants that were previously recognized as part of *S. lautus*: *S. brigalowensis* I.Thomps., *S. condylus* I.Thomps., *S. depressicola* I.Thomps., *S. eremicola* I.Thomps., *S. hamersleyensis* I.Thomps., *S. lacustrinus* I.Thomps., *S. spanomerus* I.Thomps., and *S. warrenensis* I.Thomps. Thompson, however, expressed some doubt as to whether *S. condylus* is truly Lautusoid (Thompson, 2005b, 2006), because it appears to be morphologically associated with both the Lautusoid and Glossanthus groups. In addition, he (Thompson, 2005b, 2006) included the introduced *S. madagascariensis* Poir. in the Lautusoid group because it is morphologically similar to the Australian Lautusoid taxa.

In New Zealand, Ornduff's (1960) treatment of the Lautusoid group recognized five New Zealand species: *Senecio antipodus* Kirk, *S. lautus*, *S. glaucophyllus* Cheeseman, *S. radiolatus* F.Muell., and *S. sterquilinus* Ornduff. *Senecio antipodus* was later reduced to a subspecies of *S. radiolatus* (Connor & Edgar, 1987). A year later, Webb (1988) added two species to the Lautusoid *Senecio* group. The first species, *S. carnosulus* (Kirk) C.Webb, was previously treated as a subspecies of *S. lautus* by Ornduff (1960). The second species, *S. marotiri* C.Webb, was a newly described species from northern New Zealand offshore islands (Webb, 1988). De Lange & Murray (1998) added another species to the Lautusoid group, *S. repangae* de Lange & B.G.Murray, which was described from plants that were previously identified as *S. lautus*. The latest additions to the Lautusoid *Senecio* group in New Zealand are *S. australis* Willd. (de Lange et al., 2014) and *S. esperensis* (Sykes) de Lange (Sykes, 1971; de Lange et al., 2015). *Senecio australis* is a recent arrival in New Zealand from Norfolk Island (de Lange et al., 2014) and *S. esperensis* was elevated from a subspecies of *S. lautus* to the species rank by de Lange et al. (2015). The results of the phylogenetic studies presented in Chapter 2 of this thesis contributed to confirming the presence of *S. australis* in New Zealand and provided support for the revised taxonomy of *S. esperensis*, but the

resulting scientific publications (de Lange et al., 2014, 2015) are not included as parts of this thesis.

In the South Pacific Ocean, two of the three Norfolk Island *Senecio* species (Belcher 1992b), *S. australis* and *S. evansianus* Belcher were identified as part of the Lautusoid group by de Lange et al. (2014). The third species, *S. hooglandii* Belcher was thought to be closely affiliated to *S. australis* and *S. evansianus* because of morphological similarities (Belcher, 1992b) and is here therefore also regarded a putative member of the Lautusoid group. Belcher (1992b) also noted morphological similarities between Lautusoid species and *S. howeanus* Belcher and *S. pauciradiatus* Belcher from Lord Howe Island.

One of the remarkable aspects of the Lautusoid *Senecio* group is that it displays considerable variation in chromosome numbers compared with the other morphological infrageneric groups that Thompson (2006) recognized. Even though many Lautusoid *Senecio* species have a chromosome profile of $2n = 40$, many New Zealand species have higher chromosome numbers of $2n \geq 80$ (Table 1.1). This indicates the autopolyploid or allopolyploid origin of some members of the Lautusoid group (Lawrence, 1980; de Lange & Murray, 1998).

In addition to displaying substantial variation in chromosome numbers, members of the Lautusoid group of *Senecio* display a considerable range of morphological diversity and have colonized a wide range of habitats. For example, the Lautusoid species exhibit substantial variation in leaf morphology, sometimes within a single population or even a single plant (Burns, 2005; Thompson, 2005b). *Senecio pinnatifolius*, for example, consists of eight varieties that are morphologically highly variable and occupy habitats ranging from arid to high rainfall and coastal to alpine environments (Radford et al., 2004; Thompson, 2005b). These patterns of morphological and ecological diversity have inspired many ecological and evolutionary studies (Ornduff, 1956; Thompson, 2005b). Examples include a study on the life span, weight variation of fruit and seed, and reproductive capacity of Australian Lautusoid species (Ali, 1968), a study on the plastic heteroblasty of *S. lautus* in response to environmental factors (Burns, 2005), a study by Melo et al. (2014) on the ecological and genetic mechanisms that prevent gene flow in parapatric populations of Australian Lautusoid species, and an investigation of the interaction between *S. lautus*, the tephritid herbivore *Sphenella fascigera* (Malloch), and the parasitic wasp *Pteromalus* sp. (Krejcek et al., 2015).

1.2.2. The delimitation and evolution of the Lautusoid group (Chapter 2)

Despite the interest of various researchers in members of the Lautusoid group of *Senecio* and multiple taxonomic treatments of species in the Lautusoid group (e.g., Ornduff, 1960; Webb et al., 1988; Thompson, 2005b; de Lange et al., 2014), its delimitation and the evolutionary relationships of its members are currently not known. For example, it is not clear if the New Zealand and Australian Lautusoid taxa are indeed closely related despite their morphological similarities. This issue is further complicated by the diversity of chromosome profiles in the Lautusoid group (Table 1.1), which indicates autopolyploidy or interspecific hybridization in the evolutionary history of this taxon (Lawrence, 1980). Chapter 2 therefore aims to determine the delimitation of the Lautusoid group, to identify species that are most closely related to *S. lautus* and to better understand the evolutionary origins of the Lautusoid taxa with higher chromosome numbers (2*n* = 80 and 100).

1.2.3. Resolving the *Senecio glaucophyllus* complex (Chapter 3)

The focus of this study switches to the *Senecio glaucophyllus* complex in Chapter 3. This species is hypothesized to be an allopolyploid affiliated with the Lautusoid *Senecio* group (Chapter 2). *Senecio glaucophyllus* is a New Zealand endemic that exhibits a wide range of morphological and ecological diversity (Ornduff, 1960), much like *S. pinnatifolius* in Australia (Radford et al., 2004; Thompson, 2005b). In its current delimitation, *S. glaucophyllus* consists of four subspecies: subsp. *glaucophyllus*, subsp. *basinudus*, subsp. *discoideus* and subsp. *toa* (Ornduff, 1960; Connor & Edgar, 1987). However, de Lange et al. (2013a) informally recognized an additional taxon that is morphologically similar to *S. glaucophyllus*: *S.* aff. *glaucophyllus*. Both *S. glaucophyllus* subsp. *glaucophyllus* and *S.* aff. *glaucophyllus* occur in the northwestern part of the South Island, particularly in the Nelson region and grow in sympatry (Fig. 3.8; Courtney, pers. comm.; data in Chapter 3). A better understanding of the taxonomic status of *S. glaucophyllus* subsp. *glaucophyllus* and *S.* aff. *glaucophyllus* is, amongst others, needed to inform their conservation management. *Senecio* aff. *glaucophyllus* is currently assessed as "Threatened" with the category "Nationally vulnerable" and *S. glaucophyllus* subsp. *glaucophyllus* has a conservation status of "At Risk" with the category "Naturally Uncommon" under the New Zealand Threat Classification System (Molloy et al., 2002; Townsend et al., 2008; de Lange et al., 2013a). In Chapter 3, I therefore aimed to resolve the taxonomic status of *S.* aff. *glaucophyllus* by studying the genetic and morphological differences within the *Senecio glaucophyllus* complex.

1.2.4. Testing the infraspecific delimitation of *Senecio* "pseudoglaucophyllus" (Chapters 4 & 5)

The results of Chapter 3 indicate that *Senecio* aff. *glaucophyllus* and *S. glaucophyllus* are distinct species and that specimens of *S.* aff. *glaucophyllus* are conspecific with the types of *S. glaucophyllus*. This discovery renders the plants that were mistakenly called *S. glaucophyllus* nameless. These plants (plants that used to be known as *S. glaucophyllus* subsp. *glaucophyllus* pro parte, subsp. *basinudus*, subsp. *discoideus* and subsp. *toa*) are tentatively and collectively referred to as *S.* "pseudoglaucophyllus" in Chapters 4 & 5. Each of these four *S.* "pseudoglaucophyllus" groups is given the following tag names to ease communication in these chapters: *S. glaucophyllus* subsp. *glaucophyllus* sensu Ornduff (1960) excl. *S. glaucophyllus* Cheeseman is referred to as the Nelson-group and the remaining three subspecies are simply referred to as subsp. *basinudus*, subsp. *discoideus*, and subsp. *toa*.

The intraspecific delimitation of *Senecio* "pseudoglaucophyllus" has long been a topic of discussion among taxonomists (Ornduff, 1960; Ali, 1964; Webb, 1988; Webb et al., 1988). This is due to the great amount of morphological variation within *S.* "pseudoglaucophyllus", sometimes even within a single individual or among individuals of the same population (Ornduff, 1960, 1962). For example, Ali (1964) considered the infraspecific morphological differences of a clinal nature and therefore argued that infraspecific taxa should not be formally recognized, whereas Webb et al. (1988) suggested the recognition of additional intraspecific taxa to resolve the existing taxonomic problems. Chapter 4 presents the results of a detailed morphometric analysis of the four *S.* "pseudoglaucophyllus" groups that was carried out to study the morphological dis(similarities) of these infraspecific groups.

A molecular genetic study similar in nature to the morphometric study in Chapter 4 is presented in Chapter 5 to investigate the genetic structure of the four *Senecio* "pseudoglaucophyllus" groups with the aim of further informing the infraspecific delimitation of *S.* "pseudoglaucophyllus". Sequences of the nuclear ribosomal internal transcribed spacer (ITS) region and amplified fragment length polymorphism (AFLP) data were used for this purpose. The results of the multivariate and Bayesian STRUCTURE analyses of these data were subsequently used together with the results of the morphometric study of Chapter 4 to discuss the patterns of morphological and molecular genetic variation present in *S.* "pseudoglaucophyllus" and to make a recommendation about its infraspecific taxonomic delimitation.

1.2.5. Aims of this thesis

Using molecular genetic markers (DNA sequences of nuclear and chloroplast regions and AFLP data) and morphometric data, the current study aims to contribute to the taxonomic treatment of the Lautusoid group of *Senecio*. More specifically, it aims:

1. To delimit the Lautusoid group of *Senecio* by identifying Australasian species that are most closely-related to *S. lautus* and to investigate the origins of putative Lautusoid species with chromosome numbers of $2n = 80$ and $2n = 100$ (Chapter 2).
2. To determine if the two cryptic taxa in the *S. glaucophyllus* complex are distinct species by investigating their genetic and morphological differences (Chapter 3).
3. To evaluate the current morphology-based infraspecific classification of *S.* "pseudoglaucophyllus" using a morphometric approach (Chapter 4).
4. To investigate the genetic structure of the four infraspecific groups of *S.* "pseudoglaucophyllus" and to combine the findings of the morphometric (Chapter 4) and genetic studies to propose a revised taxonomic treatment for *S.* "pseudoglaucophyllus" (Chapter 5).

1.3. Taxonomic delimitation

1.3.1. Species delimitation

Systematists discover, formally describe, and classify species that serve as the foundation for all biological research (de Queiroz, 2005; Schlick-Steiner et al., 2010). All formally described species have scientific names, which are labels on groups of organisms that are defined in accordance with a chosen species concept. These scientific names therefore become the tools that enable biologists across different disciplines to communicate effectively about their research subjects and to ensure the consistent application of names to particular groups of organisms (Patterson et al., 2010; Hardisty et al., 2013). Species description by systematists is not merely a service of systematists to the wider biological community but has a major impact on our knowledge of biological diversity, species conservation, resource management, and environmental sustainability (Costello et al., 2013; Hardisty et al., 2013). The number of formally described species provides a direct measurement of our progress in exploring and documenting the Earth's biodiversity (Wheeler, 2008; Costello et al., 2013). Species that are not discovered stay undescribed and cannot be subjected to further biological studies (Costello et al., 2013). Moreover, conservation, resource, and environmental management

depends on our knowledge of biodiversity (Hardisty et al., 2013). Especially in the face of a growing human population, the need for managing our environment and natural resources in a sustainable way becomes increasingly pressing (Hardisty et al., 2013). Without named species, none of this is possible.

Species delimitation is the part of the taxonomic process in which a systematist needs to consider whether to treat a group of organisms as a distinct species. The inference of species boundaries is dependent on a chosen species concept, and this has long since been a topic of intense discussion (e.g., Sokal & Crovello, 1970; Donoghue, 1985; Baum & Donoghue, 1995; Mayden, 1997; Coyne & Orr, 2004; de Queiroz, 2005, 2007). The most popular species concept, especially among ecologists, conservationists, and some evolutionary biologists, is the biological species concept (Mayr, 1940, 1942, 1963), in which species are defined as ‘‘groups of actually or potentially interbreeding natural populations, which are reproductively isolated from other such groups’’ (Mayr, 1942). Critics of the biological species concept argue that the ability to interbreed should not be used as the criterion to distinguish species (e.g., Donoghue, 1985; Mallet, 1995), because of the existence of asexually reproducing organisms and, at least for plants, the prevalence of hybridization (Sokal & Crovello, 1970; Mayden, 1997; Rieseberg & Carney, 1998; Soltis & Soltis, 2009). A number of species concepts have been developed based on the idea that species are evolutionary groups, such as the evolutionary species concept (Simpson, 1961; Mayden, 1997) and the various forms of phylogenetic species concepts (Hennig, 1966; Donoghue, 1985; Coyne & Orr, 2004). An example of a phylogenetic species concept is the history-based phylogenetic species concept or genealogical species concept (Baum & Donoghue, 1995; Coyne & Orr, 2004). In this species concept, species are delineated using the historical property of ‘exclusivity’. In this context, a species is defined as “a basal, exclusive group of organisms all of whose genes coalesce more recently with each other than with those of any organisms outside the group, and that contains no exclusive group within it” in one version of this species concept (PSC3/GSC sensu Coyne & Orr, 2004). For the purpose of this study, I have chosen the PSC3/GSC species concept, because it aligns with my aim of understanding the evolutionary history of an infrageneric group of Australasian *Senecio* (Chapter 2).

1.3.2. Supra-specific and infraspecific taxonomic delimitation

The Linnaean classification system does not provide criteria for determining at which taxonomic rank a group of organisms should be recognized. Decisions such as whether a

group is best classified as a section or a genus are therefore at the discretion of individual systematists (Bertrand et al., 2006; Zhao et al., 2016). Despite the absence of criteria for assigning taxonomic rank, many systematists (but see, for example, Hörandl & Stuessy (2010) for a different view), however, prefer to only formally recognize monophyletic groups, at least above species-level. Monophyletic groups are composed of an ancestral species and all its descendants. Therefore, all taxa in a monophyletic group are more closely related to each other than to taxa that are not included in the group. Recognizing only monophyletic taxa keeps classifications informative, because they convey consistent information about evolutionary relationships (Potter & Freudenstein, 2005). In addition to monophyly, decisions about taxonomic rank allocation are commonly based on other considerations such as the size of the groups and the existing rank allocation of related taxa (Bertrand et al., 2006). These decisions are often closely tied to conventions shaped throughout the taxonomic history of the group in question. This is especially true for taxonomic ranks that are higher (e.g., section or genus) or lower (e.g., subspecies or variety) than species rank (Hamilton & Reichard, 1992; Bertrand et al., 2006), because the species rank is generally considered unique among taxonomic ranks in approaching objective biological reality (e.g., Mace, 2004) and is therefore more precisely defined in the form of an explicit species concept. Despite being seemingly arbitrary, taxonomic ranks in addition to the species rank have their merits. They, for example, enable researchers to develop appropriate sampling strategies by identifying closely related taxa by their classification.

For example, the recognition of infrageneric groups (e.g., sections) is particularly useful in resolving phylogenies of large genera, such as *Senecio* (>1,000 species), because this allows for a compartmentalized approach to tackling these genera, in which species-level relationships are resolved one section at a time. In addition, they provide a way for researchers to communicate about groups of related species that are of a size that is meaningful for the specific questions that are asked. The Lautusoid *Senecio* group, which is the focus group of my thesis research, is a good example of a group of species that might benefit from a clear delimitation and associated formal taxonomic recognition at a rank in the Linnaean classification system. However, currently, this group is only known under various informal names (e.g., *S. lautus* group, *S. lautus* complex, *S. lautus* alliance, *S. pinnatifolius* complex; Ornduff, 1964; Sykes, 1971; Webb, 1988; Thompson, 2005b) and it is unknown how many and which species it contains. This taxonomic gap stands, for instance, in the way of studies aimed at understanding the genomic events that underlie the progress of speciation

using an approach in which the genomes of increasingly divergent taxa are compared and for which *S. lautus* has been flagged as a powerful system (Roda et al., 2013a).

Similarly, the recognition of infraspecific ranks can assist researchers in many fields of biology to study groups of organisms at a level of resolution that is appropriate for their studies and to communicate about them. These ranks can for instance be particularly valuable in formulating hypotheses in ecological or population genetic studies. In Chapters 4 & 5, I used a genotypic cluster species concept (Mallet, 1995) as an operational infraspecific concept for the study of the infraspecific delimitation of *Senecio* "pseudoglaucophyllus". The genotypic cluster species concept defines species as "distinguishable groups of individuals that have few or no intermediates when in contact" (Mallet, 1995; Coyne & Orr, 2004). Mallet (1995) views the rank of subspecies as similar to that of a species with the exception of the former's ability to produce intermediates in areas of sympatry. To quantify "distinguishable" in this definition, I follow the recommendation by Braby et al. (2012) and Ellison et al. (2014) that infraspecific taxa should have "at least one fixed diagnosable character state". Below species-level, the ranks of subspecies and variety are more commonly used than other ranks, such as that of forma (Hamilton & Reichard, 1992; Ellison et al., 2014). However, just like above species-level, there are no universally accepted criteria for assigning taxonomic ranks at the infraspecific level and conventions within a particular taxonomic group or within a particular geographic region often dictate whether and how the ranks of subspecies and variety are used (Hamilton & Reichard, 1992; Ellison et al., 2014). In addition, most taxonomists do not explicitly mention the criteria that they used to decide at which infraspecific rank to recognize a group of organisms (Ellison et al., 2014) and some consider that subspecies and varieties are mostly interchangeable in practice (Hamilton & Reichard, 1992). Following the recommendations of Stuessy (2009) and Ellison et al. (2014), I will therefore only use the rank of subspecies for infraspecific taxa in my study, if my results indicate that these should be recognized for *S.* "pseudoglaucophyllus".

1.3.3. Taxonomic delimitation in the presence of hybrids

Interspecific hybridization is a common theme in plant speciation (Stebbins, 1950; Grant, 1975, 1981; Rieseberg & Carney, 1998; Rieseberg et al., 2007). For example, over 20% of extant flowering plant species are known to be hybridizing (e.g., 25% according to Mallet, 2005, 2007; 30-35% estimated by Stebbins, 1971; Rieseberg & Carney, 1998). Species that originated from hybridization display complex morphological patterns (Macdonald et al.,

1988; Rieseberg, 1995; Rieseberg & Carney, 1998; Soltis & Soltis, 2009). Contrary to the popular belief that hybrids are usually morphologically intermediate (Rieseberg, 1995), they often display a mosaic of parental, intermediate, and extreme characters (Rieseberg & Carney, 1998; Soltis & Soltis, 2009). Surprisingly, hybrids can also exhibit characters that are not found in their parental species (Rieseberg, 1995; Rieseberg & Carney, 1998; Soltis & Soltis, 2009). In addition, some hybrids display greater phenotypic and genomic plasticity than their non-hybrid relatives (Hegarty et al., 2006; Leitch & Leitch, 2008; Jackson & Chen, 2010; Hahn et al., 2012). For example, Hahn et al. (2012) studied the extent of phenotypic plasticity in *Centaurea stoebe* L. of which both diploids and allotetraploids are found in the European native range and allotetraploids are found in the North American invasive range. Their common garden experiment simulating conditions in native and introduced ranges resulted in the discovery of increased phenotypic plasticity levels in the allotetraploids compared to the diploids in response to different climatic conditions, especially in traits essential for rapid growth and phenological development (Hahn et al., 2012). The unpredictability and complex morphological patterns of hybrid species can confound species delimitation if a morphological species concept is used (Soltis & Soltis, 2009). In addition, interspecific hybrids challenge the biological species concept (Soltis & Soltis, 2009), because it does not accept interspecific hybridization as a biological process. By complicating phylogeny reconstruction, hybridization can also make taxonomic delimitation difficult above species-level.

Species with hybrid origins may cause topological conflicts among phylogenies generated from different genic regions or genomic sources (e.g., biparently inherited nuclear genome vs. maternally inherited chloroplast genome in plants; Wendel, 1989; Soltis & Kuzoff, 1995; Fehrer et al., 2007; Pelser et al., 2010a; Särkinen et al., 2015). For example, in a study in which two nuclear DNA regions are sequenced, it is possible that a paternal copy of a hybrid is obtained from one region, and a maternal copy from the other. If the parental species of this hybrid are not each other's closest relatives, this will result in phylogenetic incongruence between the two gene trees. These incongruent phylogenetic patterns among gene trees complicate the reconstruction of species trees and therefore make taxonomic inferences based on these phylogenies difficult (Linder & Rieseberg, 2004; Rønsted et al., 2006). However, incongruent phylogenetic signals can also be used to identify lineages of hybrid origin and their parental lineages (Linder & Rieseberg, 2004; Knowles & Carstens, 2007; Nakhleh, 2013; O'Malley, 2016). For example, Pelser et al. (2007) found topological incongruence among

ITS and plastid *Senecio* phylogenies regarding the phylogenetic position of *S. massaicus* (Maire) Maire, a species from Morocco and the Canary Islands. Although phylogenetic incongruence can also be the result of incomplete lineage sorting or undetected paralogous sequences (Pamilo & Nei, 1988; Doyle, 1992; Maddison, 1997; Álvarez & Wendel, 2003; Knowles & Carstens, 2007; Liu & Pearl, 2007; Pelser et al., 2010a), further studies revealed patterns of ITS polymorphism within *S. massaicus* and ITS recombination patterns that are compatible with hybridization between two different *Senecio* lineages, confirming the hybrid origin of *S. massaicus* (Pelser et al., 2012).

Senecio is one of several large genera (e.g., *Rhododendron* L., Milne et al., 1999; *Solanum* L., Volkov et al., 2003; *Eryngium* L., Calviño et al., 2008; *Onopordum* L., Balao et al., 2015) for which the prevalence of hybridization is supported by an increasing number of studies (e.g., Abbott & Lowe, 2004; James & Abbott, 2005; Kadereit et al., 2006; Raudnitschka et al., 2007; Pelser et al., 2010a, 2012; Brennan et al., 2013; Calvo et al., 2013). The results of molecular phylogenetic analyses indicate that hybridization has been common throughout the evolutionary history of *Senecio* and Senecioneae (Pelser et al., 2010a) and this has resulted in complex patterns of phylogenetic incongruence that complicate infrageneric taxonomic delimitation. Such patterns might also affect the delimitation of the Lautusoid *Senecio* group and identifying lineages of hybrid origin that are affiliated with this group will therefore be an important aspect of my taxonomic studies of Lautusoid *Senecio*.

1.3.4. Species delimitation in the presence of morphologically cryptic or complex species

Alpha taxonomy is the field of systematics in which species are discovered, identified, described, and classified (Schlick-Steiner et al., 2010). Traditional alpha taxonomy is mostly morphology based. In this approach, organisms are grouped using the (dis)similarity of their morphological characteristics (Mayden, 1997; Seifert et al., 2014; Decraemer & Backeljau, 2015) and are assigned a name following the conventions of the Linnaean taxonomic classification system. Morphology-based alpha-taxonomy is highly important in the wider field of systematics because it links nominal species to name-bearing type specimens (Schlick-Steiner et al., 2007). Molecular phylogenetic studies that are aimed at revising taxonomic classifications cannot always include type specimens in their studies (Schlick-Steiner et al., 2007), because sampling of tissue for DNA extraction might result in too much damage to type specimens and therefore jeopardize their function as nomenclatural anchors, or because they are too old to yield DNA of a suitable quality for molecular genetic analyses

(Schlick-Steiner et al., 2007; Seifert et al., 2014). If the need to revise the classification of a particular group arises as a result of a molecular phylogenetic study, close examinations and comparisons between specimens that were included in the study and type specimens are therefore often required before taxonomic changes can be recommended (Steiner et al., 2009). Morphology therefore continues to be a key source of data in taxonomic studies.

A potential problem of morphology-based alpha taxonomy, however, is the occurrence of species complexes (groups of species with ambiguous morphological boundaries) and cryptic species (Mayden, 1997). Cryptic species are "two or more distinct species that are, or have been, classified as a single nominal species because they are at least superficially morphologically indistinguishable" (Bickford et al., 2006). For taxonomic delimitation within these groups, non-morphological characters such as molecular genetic and biochemical data are often needed (e.g., Schönrogge et al., 2002; Endersby et al., 2013; Vigalondo et al., 2015; Egea et al., 2016). An integrative approach in resolving species complexes and cryptic species has therefore been advocated (Dayrat, 2005; Padial et al., 2010; Schlick-Steiner et al., 2010) and is progressively adopted by systematists (Pante et al., 2015). The integrative approach "aims to delimit the units of life's diversity from multiple and complementary perspectives (phylogeography, comparative morphology, population genetics, ecology, development, behaviour, etc.)" (Dayrat, 2005). The integrative approach is seen as the solution to the limitations of morphology-based traditional taxonomy. For example, Wachter et al. (2015) examined the morphology-based species delimitation of a group of closely related *Megabunus* harvestmen endemic to the European Alps. Their integrative approach utilized mitochondrial and nuclear DNA data, morphology, and biochemistry and this resulted in the discovery of two cryptic species complexes (of two and three species) among the five nominal species (Wachter et al., 2015). These species complexes and the full diversity of the European Alps *Megabunus* harvestmen would never have been revealed if a single source of data was used (Wachter et al., 2015). For species that are difficult to delineate (e.g. low resolution using a single data source and cryptic or morphologically complex species), an integrative approach in which congruent findings among different data sources reinforce biological inferences (Will et al., 2005) is therefore a suitable strategy (Dayrat, 2005; Schlick-Steiner et al., 2010; Yeates et al., 2011) and has been successfully applied to morphologically complex plant species such as *Anthyllis montana* L. (Kropf, 2008), *Cardamine amara* L. (Lihová et al., 2004), and *Myosotis petiolata* Hook.f. (Meudt et al., 2013). This approach is for that reason used in this thesis for resolving the *Senecio*

glaucophyllus complex (Chapter 3) and the infraspecific relationships of *S.* "pseudoglaucophyllus" (Chapter 5).

CHAPTER 2: The delimitation and evolutionary history of the Australasian Lautusoid group of *Senecio* (Asteraceae; Senecioneae)

To reviewers: This chapter is written as a manuscript to be submitted to the journal *Taxon*. Thus, it has a slightly different format than the rest of the thesis.

2.1. ABSTRACT

Senecio (Asteraceae; Senecioneae) is one of the largest genera of flowering plants and its infrageneric delimitation has been impeded by its large size (>1,000 species), large morphological variation and widespread incongruence between phylogenies derived from different data sets. As part of efforts to improve our understanding of the evolutionary relationships among infrageneric *Senecio* groups, nuclear (nrITS and ETS) and plastid (*psb*A-*trn*H, *trn*L and *trn*L-F) DNA sequence data were used to study the delimitation of the Australasian Lautusoid group of *Senecio*. These data were also used to understand the evolutionary origins of polyploid species that have been placed in this informally recognized group. The results of our phylogenetic analyses indicate that Australasian *Senecio* compose four separate and distantly related lineages, which are here informally named the Disciform s.s., Lautusoid, Odoratus s.l., and Quadridentatus groups. A new delimitation of the Lautusoid group is presented that includes species previously placed in this group on the basis of morphological similarities, as well as some that were previously assigned to other informally recognized *Senecio* groups. This brings the total number of confirmed members of the Lautusoid group to 15 species. Six allopolyploid species were identified that resulted from hybridization between members of the Lautusoid group and species of the three other Australasian *Senecio* lineages. Our findings indicate that hybridization has played an important role in the evolutionary diversification of Australasian *Senecio* and provide a framework for further studies into their evolutionary history.

2.2. INTRODUCTION

Senecio L. (Senecioneae; Asteraceae) is a large genus that shows considerable morphological and ecological diversity. It consists of 1,000−1,250 species and has a nearly worldwide distribution (Nordenstam, 2007; Pelser et al., 2007; Nordenstam et al., 2009; Calvo et al. 2013). Its large size, morphological diversity, but particularly the prevalence of topological incongruence between nuclear and plastid DNA sequence phylogenies have been identified as impediments to understanding its evolutionary history and the processes that resulted in its

biological diversity (Pelser et al., 2007; Pelser et al., 2010a). Although incomplete lineage sorting could be an alternative explanation for some of the incongruence between nuclear and plastid phylogenies, evidence supporting a significant role of hybridization in the evolutionary history of *Senecio* and other Senecioneae genera is accumulating (e.g., Abbott & Lowe, 2004; Abbott et al., 2009; Pelser et al., 2010a, 2012; Calvo et al., 2013; James & Abbott, 2005). Aside from patterns of phylogenetic incongruence that can be explained by hybridization, putative hybrids have been identified in karyological studies (e.g., Beuzenberg, 1975; Lawrence, 1980; de Lange & Murray, 1998), through the identification of chimeric DNA sequences (Pelser et al., 2012), DNA sequence polymorphism (e.g., Mas De Xaxars et al., 2015), additive AFLP profiles (Kirk et al., 2004), and by observing plants that are morphologically intermediate between putative parental species (e.g. Belcher, 1956; Calvo et al., 2015).

Species-level molecular phylogenies are powerful tools for developing infrageneric classifications. They, for instance, allow us to test if traditional morphology-based infrageneric taxa (e.g., subgenera and sections) constitute evolutionary lineages that merit taxonomic recognition. These infrageneric classifications facilitate a compartmentalized approach to further resolving phylogenetic relationships (van Welzen et al., 2009). This is particularly important in large, widespread, and complex genera such as *Senecio* (Frodin, 2004; van Welzen et al., 2009), because financial limitations and time restrictions often prevent researchers from using a genus-wide taxon sampling strategy. In addition, infrageneric classifications that reflect evolutionary relationships provide useful frameworks for biological studies that are outside the field of systematics, but do require a taxon sampling that includes the closest relatives of a focal species or group of species (Radford et al., 2004; Pelser et al., 2005; Prentis et al., 2007; Langel et al., 2011; Roda et al., 2013a, b; Melo et al., 2014; Ahrens & James, 2015; Nardin et al., 2015). A well-resolved *Senecio* phylogeny is therefore not only important for understanding the evolutionary history and processes that led to its incredible biological diversity, but also to facilitate such studies. The complex patterns of phylogenetic incongruence between *Senecio* phylogenies have thus far however prevented a genus-wide phylogenetic hypothesis of the relationships between its species and species groups, although progress towards this has been made. For example, molecular phylogenetic studies resulted in a new, monophyletic delimitation of *Senecio* (Pelser et al., 2007), the identification of lineages and patterns of phylogenetic incongruence in *Senecio* and Senecioneae (Pelser et al., 2010a, 2012; Calvo et al., 2013), greater phylogenetic resolution

within several *Senecio* lineages (Pelser et al., 2010b, 2012; Calvo et al., 2013, 2015; Kandziora et al., 2016a), and a better understanding of its biogeographic history and diversification (Pelser et al., 2007; Kandziora et al., 2016a,b). The present study aims to contribute further to this process by providing a new taxonomic delimitation of an Australasian *Senecio* species group.

The Lautusoid group is one of the eight informal infrageneric morphological groups in Thompson's treatment of *Senecio* in Australia (Thompson, 2004a, b, c, 2005a, b, 2006). This group was first coined by Belcher (1993) and is also known as the *S. lautus* G.Forst. ex Willd. complex or alliance (Ornduff, 1964; Ali, 1964, 1969; Belcher, 1993, 1994; Roda et al., 2013a; Thompson, 2005b) and the *S. pinnatifolius* A.Rich. complex (Radford et al., 2004; Radford et Cousens, 2000; Thompson, 2005b). It is an assemblage composed of *S. lautus* and Australasian species (i.e. species native to Australia, New Guinea, New Zealand, and nearby islands in the Pacific Ocean) that are morphologically similar to it. A total of 23 species (Table 1) that are recognized in recent treatments of *Senecio* in Australasia (e.g., de Lange et al., 2014; Thompson, 2005b, 2006) have at some point in their taxonomic history been associated with *S. lautus* by one or more authors and are therefore putative members of the Lautusoid group. However, although species of this group have been the topic of several studies (e.g., Ornduff, 1962, 1964; Ali, 1964, 1968; Radford et al., 2004; Burns, 2005; Roda et al., 2013a, b; Melo et al., 2014; Krejcek et al., 2015), including regional taxonomic treatments (e.g., Ornduff, 1960; Webb et al., 1988; Belcher, 1992b; Thompson, 2005b, 2006), there are no recent comprehensive taxonomic accounts of the Lautusoid group and its exact species composition and delimitation is presently unknown.

The Lautusoid group stands out from other infrageneric species groups of *Senecio* by considerable variation in chromosome numbers. Most species for which chromosome numbers are known are $2n = 40$, but higher chromosome numbers ($2n = 80, 100$) are also reported (e.g., Beuzenberg, 1975; Lawrence, 1980, 1985a; de Lange & Murray, 1998; Ahrens & James, 2015). These higher chromosome numbers indicate that these species are of polyploid origin (Lawrence, 1980; de Lange & Murray, 1998). It is, however, mostly unknown if the $2n = 80$ and $2n = 100$ Lautusoid species are of autopolyploid or allopolyploid origin and, if the latter, what their parental species are. The Lautusoid group might therefore be another *Senecio* lineage in which hybridization has played an important role in its diversification.

The aim of this molecular phylogenetic study is to better understand the delimitation and the evolutionary history of the Australasian Lautusoid group of *Senecio* by addressing these questions: (1) Which *Senecio* species are most closely related to *S. lautus*? (2) What is the evolutionary origin of putative Lautusoid species with chromosome numbers of $2n = 80$ and $2n = 100$?

Table 2.1

All 23 putative species of the Lautusoid group of *Senecio* and their country of origin, chromosome number (if known) and examples of studies in which they were considered members of the Lautusoid group.

Species	Country	Chromosome number (2*n*)	Ornduff, 1960	Thompson, 2006	de Lange et al., 2014	de Lange & Murray 2003/ Belcher 1992b
S. brigalowensis I.Thomps.	Australia			Lautusoid		
S. condylus I.Thomps.	Australia			Lautusoid		
S. depressicola I.Thomps.	Australia			Lautusoid		
S. eremicola I.Thomps.	Australia			Lautusoid		
S. hamersleyensis I.Thomps.	Australia			Lautusoid		
S. lacustrinus I.Thomps.	Australia			Lautusoid		
S. pinnatifolius A.Rich.	Australia	40		Lautusoid		
S. spanomerus I.Thomps.	Australia			Lautusoid		
S. spathulatus A.Rich.	Australia	40		Lautusoid		
S. warrenensis I.Thomps.	Australia			Lautusoid		
S. carnosulus (Kirk) C.Webb	New Zealand	80			Lautusoid	
S. esperensis (Sykes) de Lange	New Zealand	40			Lautusoid	
S. glaucophyllus Cheeseman	New Zealand	100	Lautusoid			
S. lautus G.Forst. ex Willd	New Zealand	40	Lautusoid		Lautusoid	
S. marotiri C.Webb	New Zealand	80			Lautusoid	
S. radiolatus F.Muell.	New Zealand	40	Lautusoid			
S. repangae de Lange & B.G.Murray	New Zealand	100			Lautusoid	
S. sterquilinus Ornduff	New Zealand	40	Lautusoid		Lautusoid	
S. australis Willd.	New Zealand & Norfolk Island	80			Lautusoid	Lautusoid
S. evansianus Belcher	Norfolk Island				Lautusoid	Lautusoid
S. hooglandii Belcher	Norfolk Island	80				Lautusoid
S. howeanus Belcher	Lord Howe Island					Lautusoid
S. pauciradiatus Belcher	Lord Howe Island					Lautusoid

2.3. MATERIALS & METHODS

2.3.1. Taxon sampling

Using taxonomic and phylogenetic treatments that include *S. lautus* or formulate hypotheses about the identity of its closest relatives (Ornduff, 1960; Sykes, 1971; Lawrence, 1980, 1985a, 1985c, 1985d; Webb et al., 1988; Belcher, 1992a, b; de Lange & Murray, 1998; Thompson, 2005a, b, 2006; de Lange et al., 2014), a total of 23 *Senecio* species were identified as putative Lautusoid species (Table 2.1). A total of 18 of these were included in our molecular phylogenetic study (Table S1). Specimens of *S. evansianus* Belcher, *S. howeanus* Belcher, *S. pauciradiatus* Belcher and *S. warrenensis* I.Thomps. were not available to us because of the lack or limited collection (<5 specimens) of these species and the lack of resources to carry out field collection. *Senecio eremicola* I.Thomps. was not included due to the poor quality of the DNA samples that were obtained from the available specimens. *Senecio madagascariensis* Poir. is a South African species that is placed in the Lautusoid group by Thompson (2005b, 2006). We have, however, not included it in our study, because previous studies have shown that it is a member of the *S. inaequidens* DC. clade (Pelser et al., 2012; represented in this study by *S. inaequidens*), which is only distantly related to *Senecio* clades that contain Australasian species (Pelser et al., 2007). For most species, up to three specimens were sequenced. However, additional specimens were included for some of the species to represent their varieties or subspecies.

In addition to the 18 putative Lautusoid species, three out of the four members of Thompson's Glossanthus group (Thompson, 2005a, 2005b, 2006; Table S1) were included in our studies, because of morphological similarities between both groups (Thompson, 2006). Specimens of *S. productus* I.Thomps. (the fourth member of the Glossanthus group) were not available to us. We also included 45 non-Lautusoid native Australasian *Senecio* species to represent other Australasian lineages (Tables S1 & S2). Representatives of *Senecio* lineages from elsewhere in the world were included in this study to provide the phylogenetic context needed to determine if the Lautusoid group of *Senecio* is monophyletic. Taxon sampling for this purpose focused on including representatives of lineages that were resolved as most closely related to Australasian taxa in previous phylogenetic studies (Pelser et al., 2007, 2010a, 2012). *Kleinia neriifolia* Haw. was chosen as the outgroup in our phylogenetic analyses (Pelser et al., 2002, 2003, 2004, 2007, 2010a, b, 2012).

Identifications of herbarium specimens that were used for this study (Table S2) were confirmed using identification keys and morphological descriptions provided by Thompson (2005a, b, 2006) for the Australian species, Ornduff (1960), Allan (1961), Webb (1988), Webb et al. (1988), de Lange & Murray (1998), and de Lange et al. (2014, 2015) for the New Zealand species and Belcher (1992b) and de Lange et al. (2014) for the Norfolk Island species.

2.3.2. DNA extraction, PCR amplification and sequencing

A total of 449 DNA sequences were generated for this study. This data set was complemented with DNA sequences that were obtained from GenBank. Most specimens of the Lautusoid group used for sequencing were herbarium specimens from AD, AK, BRI, CANU, CHR, MEL and PERTH, but also freshly collected specimens were used. Less than 10mg of dried leaf tissue per specimen was used for DNA extraction. This tissue was grinded to a fine powder with a RETSCH Mixer Mill MM 400 (Dusseldorf, Germany) before DNA extraction using the Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia, California, U.S.A.). Two nuclear (ITS and ETS) and three plastid (*psbA-trnH* intergenic spacer, *trnL* intron and *trnL-F* intergenic spacer) regions were PCR-amplified. Amplification of the ITS region followed Pelser et al. (2002, 2007). The ETS region was amplified with primers listed by Pelser et al. (2010a) and with the following PCR conditions: 30 cycles of denaturation (94°C for 2 min), annealing (55°C for 30 s), and extension (72°C for 1 min) and a final 10 min extension cycle at 72°C. The primer annealing temperature was reduced to 53°C or 54°C for specimens that were more difficult to amplify. PCR amplification of the *psbA-trnH*, *trnL* and *trnL-F* plastid regions followed Pelser et al. (2002, 2003). PCR products were examined on 1% agarose gels and successful amplifications were cleaned with the Promega Wizard SV Gel and PCR Clean-up System. Cycle sequencing followed protocols developed by Applied Biosystems (Foster City, California, U.S.A.) for the BigDye Terminator v3.1 Cycle Sequencing Kit. DNA sequencing was done by an Applied Biosystems 3130xL Genetic Analyzer at the University of Canterbury. Because of the breakdown of the genetic analyzer at the University of Canterbury during the course of this study, some of the cleaned PCR products were sent to Macrogen® Korea and sequenced through the company's standard-sequencing service. Geneious 6.1.7 (Biomatters Ltd., Auckland, New Zealand) was used to examine and edit sequencing trace files. DNA sequences were manually aligned using Se-Alv2.0a11 (Rambaut, 1996). A python script (gapcode.py by Richard Ree), available from http://rree.fastmail.fm/gapcode.py, was used to code gaps in the alignment as binary

presence-absence characters using the 'simple indel coding' method as described by Simmons & Ochoterena (2000). Sequencing of different specimens of some taxa resulted in identical sequences. In these cases, only a single sequence was included in our phylogenetic analyses. When sequences of different specimens of the same taxon formed a clade in preliminary phylogenetic analyses (methods outlined below) with individual accessions having a low number (≤ 3) of unique single-nucleotide polymorphisms (SNPs), a consensus sequence was generated for subsequent phylogenetic analyses. This approach was used to reduce the computational time for the final phylogenetic analyses. Each DNA region was treated as an individual data set for reconstructing gene trees. In addition, combined nuclear (ITS and ETS) and plastid (*psbA-trnH*, *trnL* and *trnL-F*) data sets were generated for phylogeny reconstruction.

Nucleotide substitution models for individual data sets were selected using jModelTest2.1.7 (Guindon & Gascuel, 2003; Darriba et al., 2012) on an IBM POWER7cluster at University of Canterbury High Performance Computing (UC HPC) center. For the combined nuclear and plastid data sets, sequence alignments of individual regions were concatenated before they were analyzed in jModelTest. Table 2.2 summarizes patterns of variation in the different DNA sequence data sets and the nucleotide substitution models selected by the Akaike Information Criterion (AIC) in jModelTest.

2.3.3. Recombination detection

The presence of recombination in the nuclear data sets (ITS and ETS) was tested using RDPv4.43 (Martin et al., 2010). Screenings were performed with the RDP (Martin & Rybicki, 2000), GENECONV (Padidam et al., 1999), MAXCHI (Smith, 1992), CHIMAERA (Posada & Crandall, 2001), BOOTSCAN (Martin et al., 2005), SISCAN (Gibbs et al., 2000) and 3SEQ (Boni et al., 2007) methods. "Auto mask for optimal recombination detection" was used to exclude sequences that were too similar.

2.3.4. Phylogeny reconstruction

Phylogeny reconstruction was done using Bayesian inference (BI) and maximum parsimony (MP) for individual and combined nuclear (ITS and ETS) and plastid (*psbA-trnH*, *trnL* and *trnL-F*) data sets. BI and MP analyses were conducted using the parallel version of MrBayes (Ronquist et al., 2012) on an IBM POWER7cluster at UC HPC and TNT 1.1 (Goloboff et al., 2008) on a personal computer, respectively.

In MrBayes, the models that are most similar to those selected by jModelTest (see Table 2.2) were used if these models were not supported by the program. Gaps were treated as restriction (binary) data. Two independent simultaneous runs were carried out with four chains and one tree was sampled every 500 generations. The runs were terminated when the average standard deviation of split frequencies between them dropped below 0.01. Burn-in values were determined empirically using the plot of the generation number versus the log likelihood values generated by the 'sump' command. To determine if the analyses converged and if there was adequate sampling of the posterior probability (PP) distribution, the Potential Scale Reduction Factor (PSRF) values were examined (Ronquist et al., 2011).

In TNT, MP bootstrap analyses were executed with Poisson independent reweighting for 1000 replicates using the New Technology Search. The trees were generated under the Driven Search option with 10 sequence addition replicates used to build the starting trees and until the search hit the minimum length for 5 times using the default settings for Sectorial Searches (RSS, CSS and XSS), Ratchet, Tree Drifting, and Tree Fusing methods.

In addition to separate analyses of the ITS and ETS data sets, phylogenetic analyses of a combined nuclear data set were performed. Pairwise comparisons of the ITS and ETS consensus trees, however, indicated that accessions of one specimen of an Australasian species were in well-supported (defined in this study as having a BS value of > 80% or PP of > 0.95) incongruent phylogenetic positions. Separate ITS-only and ETS-only accessions of this specimen were included in a combined ITS-ETS data set using the method outlined by Pelser et al. (2010a). This approach was used to improve phylogenetic resolution and nodal support, benefiting from an increase in the number of variable characters by combining the ITS and ETS data sets, while retaining as many relevant taxa as possible in the analyses. Phylogenetic trees of individual plastid regions did not display well-supported incongruence. Phylogenetic analyses of a combined plastid data set were therefore also performed.

Chromosome data of Australasian *Senecio* were compiled from the literature (Table S1 in Appendix) to identify putative allopolyploid taxa by determining if taxa in incongruent phylogenetic positions have high ($2n$ = 80 or $2n$ = 100) chromosome numbers.

2.3.5. Testing topological hypotheses

The majority of the species that were identified as putative members of the Lautusoid group (Table 2.1) form a polytomy with several other Australasian *Senecio* species and two South

African species in the combined plastid phylogeny (Fig. 2.2). Using Bayes factor comparisons, we tested the hypothesis that Australasian members of this polytomy form a monophyletic group (H_0) against the hypothesis that these species do not form a monophyletic group (H_1). A method that estimates marginal likelihoods, the stepping-stone sampling method (Xie et al., 2011) as implemented in MrBayes, was used for this purpose. Two analyses, one with positive and one with negative constraints, were run using the GTR + Γ model (Table 2.2). The positive constraint analysis sampled only trees in which the aforementioned Australasian species form a monophyletic group. The negative constraint analysis sampled only those in which they do not form a clade. The stepping-stone sampling analyses were executed with two independent simultaneous runs of 50 steps with 200,000 generations within each step (a total of 10 million generations) and the power posterior distributions were sampled once every 1000 generations. Ten thousand samples were obtained and these fell into 50 bins, one of which was the burn-in and was discarded. Convergence among independent runs of each steps of the stepping-stone sampling was checked by examining the estimated marginal log likelihood values of the runs (Ronquist et al., 2011).

Table 2.2 Details of the DNA sequence data sets: number of OTUs, alignment length, number of variable and phylogenetically informative sites (with and without gaps), average pairwise sequence identity and nucleotide substitution model.

Data set (no. of OTUs)	**Alignment length**	**No. and % of variable sites**	**No. of phylogenetically informative sites**		**Average sequence identity %**	**Model selected**
			(incl. gaps)	**(without gaps)**		
nuclear (131)	1155	841 (73%)	309	303	92.3	GTR + I + G
ITS (131)	722	543 (75%)	202	197	92.9	GTR + I + G
ETS (118)	433	302 (70%)	107	106	90.6	TIM2 + G
plastid (124)	1526	955 (63%)	184	98	95.5	GTR + G
psbA-trnH	591	371 (63%)	88	20	92.1	TPM1uf + G
(114)	935	584(18%)	96	78	96.7	GTR + G
trnL-LF **(123)**						

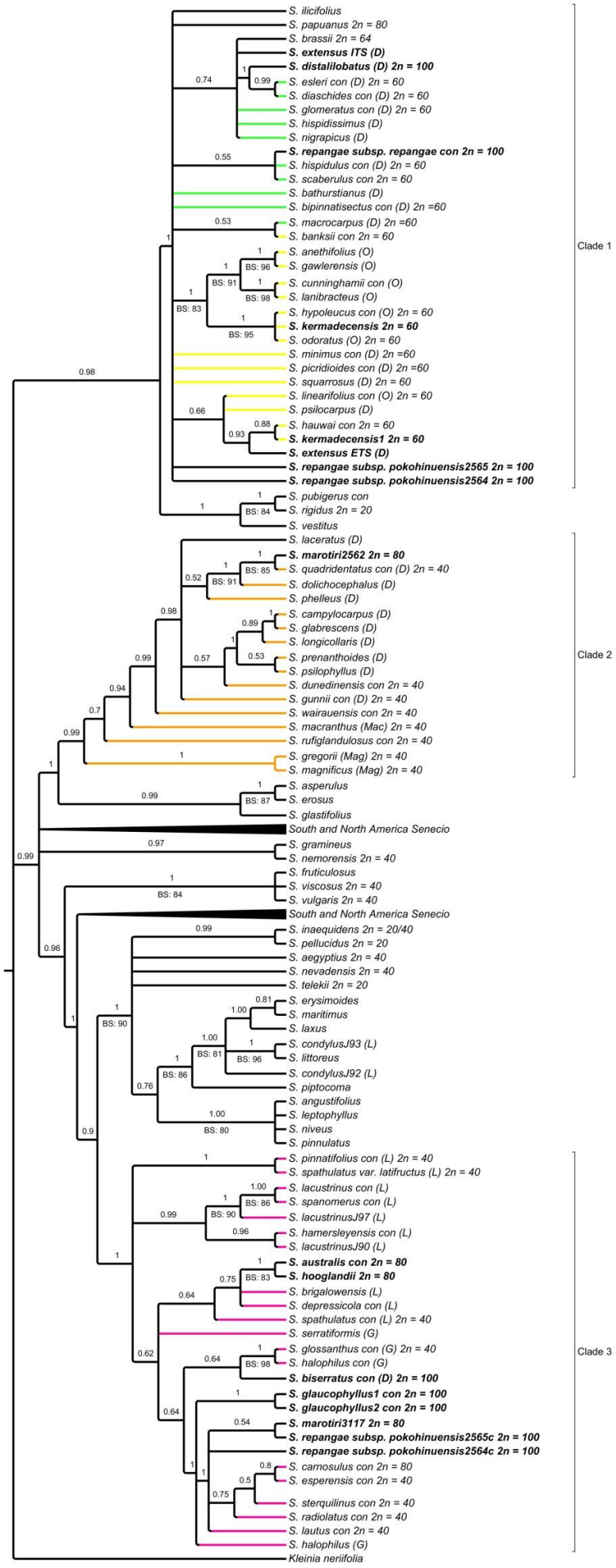

S. ilicifolius
S. papuanus 2n = 80
S. brassii 2n = 64
S. extensus ITS (D)
S. distalilobatus (D) 2n = 100
S. esleri con (D) 2n = 60
S. diaschides con (D) 2n = 60
S. glomeratus con (D) 2n = 60
S. hispidissimus (D)
S. nigrapicus (D)
S. repangae subsp. repangae con 2n = 100
S. hispidulus con (D) 2n = 60
S. scaberulus con 2n = 60
S. bathurstianus (D)
S. bipinnatisectus con (D) 2n =60
S. macrocarpus (D) 2n =60
S. banksii con 2n = 60
S. anethifolius (O)
S. gawlerensis (O)
S. cunninghamii con (O)
S. lanibracteus (O)
S. hypoleucus con (O) 2n = 60
S. kermadecensis 2n = 60
S. odoratus (O) 2n = 60
S. minimus con (D) 2n =60
S. picridioides con (D) 2n =60
S. squarrosus (D) 2n = 60
S. linearifolius con (O) 2n = 60
S. psilocarpus (D)
S. hauwai con 2n = 60
S. kermadecensis1 2n = 60
S. extensus ETS (D)
S. repangae subsp. pokohinuensis2565 2n = 100
S. repangae subsp. pokohinuensis2564 2n = 100
S. pubigerus con
S. rigidus 2n = 20
S. vestitus
S. laceratus (D)
S. marotiri2562 2n = 80
S. quadridentatus con (D) 2n = 40
S. dolichocephalus (D)
S. phelleus (D)
S. campylocarpus (D)
S. glabrescens (D)
S. longicollaris (D)
S. prenanthoides (D)
S. psilophyllus (D)
S. dunedinensis con 2n = 40
S. gunnii con (D) 2n = 40
S. wairauensis con 2n = 40
S. macranthus (Mac) 2n = 40
S. rufiglandulosus con 2n = 40
S. gregorii (Mag) 2n = 40
S. magnificus (Mag) 2n = 40
S. asperulus
S. erosus
S. glastifolius
South and North America Senecio
S. gramineus
S. nemorensis 2n = 40
S. fruticulosus
S. viscosus 2n = 40
S. vulgaris 2n = 40
South and North America Senecio
S. inaequidens 2n = 20/40
S. pellucidus 2n = 20
S. aegyptius 2n = 40
S. nevadensis 2n = 40
S. telekii 2n = 20
S. erysimoides
S. maritimus
S. laxus
S. condylusJ93 (L)
S. littoreus
S. condylusJ92 (L)
S. piptocoma
S. angustifolius
S. leptophyllus
S. niveus
S. pinnulatus
S. pinnatifolius con (L) 2n = 40
S. spathulatus var. latifructus (L) 2n = 40
S. lacustrinus con (L)
S. spanomerus con (L)
S. lacustrinusJ97 (L)
S. hamersleyensis con (L)
S. lacustrinusJ90 (L)
S. australis con 2n = 80
S. hooglandii 2n = 80
S. brigalowensis (L)
S. depressicola con (L)
S. spathulatus con (L) 2n = 40
S. serratiformis (G)
S. glossanthus con (G) 2n = 40
S. halophilus con (G)
S. biserratus con (D) 2n = 100
S. glaucophyllus1 con 2n = 100
S. glaucophyllus2 con 2n = 100
S. marotiri3117 2n = 80
S. repangae subsp. pokohinuensis2565c 2n = 100
S. repangae subsp. pokohinuensis2564c 2n = 100
S. carnosulus con 2n = 80
S. esperensis con 2n = 40
S. sterquilinus con 2n = 40
S. radiolatus con 2n = 40
S. lautus con 2n = 40
S. halophilus (G)
Kleinia neriifolia
Clade 1
Clade 2
Clade 3

Fig. 2.1. Bayesian inference phylogeny of the combined ITS and ETS data sets. Posterior probabilities (PP) are labeled above and bootstrap support (BS > 75) below the branches. Chromosome numbers (if known) follow taxon names. 'con' following species names indicates consensus sequences of multiple accessions. Species in bold indicate species of putative hybrid ancestry. Letters in brackets indicate to which of Thompson's (2006) infrageneric groups the species belongs: (D) – Disciform group, (O) – Odoratus group, (Mag) – Magnificus group, (Mac) – Macranthus Group, (G) – Glossanthus group, (L) - Lautusoid group. Colored branches indicate in which Australasian lineage a species is placed: green – Disciform s.s. group, yellow – Odoratus s.l. group, orange – Quadridentatus group, pink – Lautusoid group. Numbers and letters following taxon names are used to distinguish multiple accessions of the same taxon (Table S2). ITS and ETS sequences of a specimen of *S. extensus* were included as separate accessions, because these were resolved in incongruent phylogenetic positions (see Materials and Methods). Two South and North American *Senecio* clades were collapsed to enhance the presentation of the cladogram.

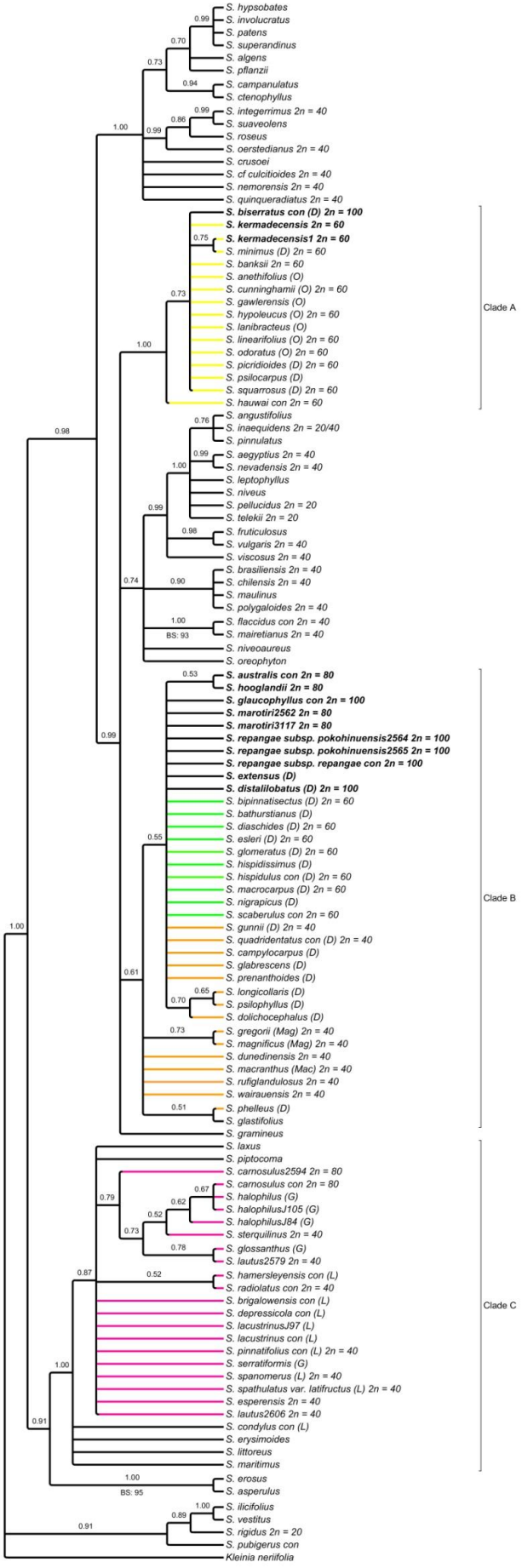

S. hypsobates
S. involucratus
S. patens
S. superandinus
S. algens
S. pflanzii
S. campanulatus
S. ctenophyllus
S. integerrimus 2n = 40
S. suaveolens
S. roseus
S. oerstedianus 2n = 40
S. crusoei
S. cf culcitioides 2n = 40
S. nemorensis 2n = 40
S. quinqueradiatus 2n = 40
S. biserratus con (D) 2n = 100
S. kermadecensis 2n = 60
S. kermadecensis1 2n = 60
S. minimus (D) 2n = 60
S. banksii 2n = 60
S. anethifolius (O)
S. cunninghamii (O) 2n = 60
S. gawlerensis (O)
S. hypoleucus (O) 2n = 60
S. lanibracteus (O)
S. linearifolius (O) 2n = 60
S. odoratus (O) 2n = 60
S. picridioides (D) 2n = 60
S. psilocarpus (D)
S. squarrosus (D) 2n = 60
S. hauwai con 2n = 60
Clade A
S. angustifolius
S. inaequidens 2n = 20/40
S. pinnulatus
S. aegyptius 2n = 40
S. nevadensis 2n = 40
S. leptophyllus
S. niveus
S. pellucidus 2n = 20
S. telekii 2n = 20
S. fruticulosus
S. vulgaris 2n = 40
S. viscosus 2n = 40
S. brasiliensis 2n = 40
S. chilensis 2n = 40
S. maulinus
S. polygaloides 2n = 40
S. flaccidus con 2n = 40
S. mairetianus 2n = 40
S. niveoaureus
S. oreophyton
S. australis con 2n = 80
S. hooglandii 2n = 80
S. glaucophyllus con 2n = 100
S. marotiri2562 2n = 80
S. marotiri3117 2n = 80
S. repangae subsp. pokohinuensis2564 2n = 100
S. repangae subsp. pokohinuensis2565 2n = 100
S. repangae subsp. repangae con 2n = 100
S. extensus (D)
S. distalilobatus (D) 2n = 100
S. bipinnatisectus (D) 2n = 60
S. bathurstianus (D)
S. diaschides (D) 2n = 60
S. esleri (D) 2n = 60
S. glomeratus (D) 2n = 60
S. hispidissimus (D)
S. hispidulus con (D) 2n = 60
S. macrocarpus (D) 2n = 60
S. nigrapicus (D)
S. scaberulus con 2n = 60
S. gunnii (D) 2n = 40
S. quadridentatus con (D) 2n = 40
S. campylocarpus (D)
S. glabrescens (D)
S. prenanthoides (D)
S. longicollaris (D)
S. psilophyllus (D)
S. dolichocephalus (D)
S. gregorii (Mag) 2n = 40
S. magnificus (Mag) 2n = 40
S. dunedinensis 2n = 40
S. macranthus (Mac) 2n = 40
S. rufiglandulosus 2n = 40
S. wairauensis 2n = 40
S. phelleus (D)
S. glastifolius
Clade B
S. gramineus
S. laxus
S. piptocoma
S. carnosulus2594 2n = 80
S. carnosulus con 2n = 80
S. halophilus (G)
S. halophilusJ105 (G)
S. halophilusJ84 (G)
S. sterquilinus 2n = 40
S. glossanthus (G)
S. lautus2579 2n = 40
S. hamersleyensis con (L)
S. radiolatus con 2n = 40
S. brigalowensis con (L)
S. depressicola con (L)
S. lacustrinusJ97 (L)
S. lacustrinus con (L)
S. pinnatifolius con (L) 2n = 40
S. serratiformis (G)
S. spanomerus (L) 2n = 40
S. spathulatus var. latifructus (L) 2n = 40
S. esperensis 2n = 40
S. lautus2606 2n = 40
S. condylus con (L)
S. erysimoides
S. littoreus
S. maritimus
S. erosus
S. asperulus
Clade C
S. ilicifolius
S. vestitus
S. rigidus 2n = 20
S. pubigerus con
Kleinia neriifolia
BS: 93
BS: 95

Fig. 2.2. Bayesian inference phylogeny of the combined *psbA-trnH* and *trnL-LF* data sets. Posterior probabilities (PP) are labeled above and bootstrap support (BS > 75) below the branches. Chromosome numbers (if known) follow taxon names. 'con' following species names indicates consensus sequences of multiple accessions. Species in bold indicate species of putative hybrid ancestry. Letters in brackets indicate to which of Thompson's (2006) infrageneric groups the species belongs: (D) – Disciform group, (O) – Odoratus group, (Mag) – Magnificus group, (Mac) – Macranthus Group, (G) – Glossanthus group, (L) - Lautusoid group. Colored branches indicate in which Australasian lineage a species is placed: green – Disciform s.s. group, yellow – Odoratus s.l. group, orange – Quadridentatus group, pink – Lautusoid group. Numbers and letters following taxon names are used to distinguish multiple accessions of the same taxon (Table S2).

2.4. RESULTS

2.4.1. Phylogenetic analyses of the nuclear regions

Recombination detection analyses in RDPv4.43 did not reveal evidence of recombination in the ITS and ETS data sets. Phylogenetic analyses of these data sets resulted in phylogenetic trees with a very similar topology. Accessions of a specimen of S. *extensus* I.Thomps were, however, found in conflicting well-supported (PP > 0.95 or BS > 80%) phylogenetic positions in the ITS and ETS trees (not shown). This species was therefore included in the combined ITS-ETS data set as separate ITS-only and ETS-only accessions to resolve its alternative phylogenetic positions using a larger number of phylogenetically informative characters. In this combined nuclear phylogeny (Fig. 2.1), most Australasian *Senecio* taxa are found in three main clades, which are each more closely related to *Senecio* lineages from elsewhere in the world than to each other.

Clade 1 (PP = 1; BS = 60%) consists of Australasian *Senecio* species that are placed in a polytomy with *S. ilicifolius* L. from South Africa. All included members of Thompson's Odoratus group are placed in this clade as well as several taxa of his Disciform group (Thompson, 2006). Several accessions of both subspecies of *S. repangae* de Lange & B.G.Murray, a putative New Zealand Lautusoid species (de Lange & Murray, 1998; Webb et al., 1998), are also placed in Clade 1. It also contains four other species that are endemic to New Zealand (*S. banksii* Hook.f., *S. hauwai* Sykes, *S. kermadecensis* Belcher, *S. scaberulus* (Hook.f.) D.G.Drury) and two species that are endemic to New Guinea (*S. brassii* Belcher, *S. papuanus* (Lauterb.) Belcher), which were not included in Thompson's revision. All species in this clade for which chromosome data are available are 2*n* = 60, except for *S. brassii* (2*n* = 64), *S. papuanus* (2*n* = c. 80) and *S. repangae* (2*n* = 100) (Table S1).

Clade 2 (PP = 0.99; BS = 55%) is solely composed of Australasian *Senecio* and includes species that are included in Thompson's Disciform group as well as several taxa from the Macranthus and Magnificus groups, and accessions of three species that are endemic to New Zealand (*S. dunedinensis* Belcher, *S. rufiglandulosus* Colenso, *S. wairauensis* Belcher). In addition to these, Clade 2 contains one of two accessions of *S. marotiri* C.Webb, which is a putative Lautusoid species from New Zealand (Webb, 1988). Within Clade 2, all species for which chromosome data is available have a chromosome number of 2*n* = 40 with the exception of *S. marotiri* (2*n* = 80).

All remaining accessions of putative members of the Lautusoid group of *Senecio* included in our studies (Table 2.1) are placed in Clade 3 (PP = 1.0, BS = 62%) with the exception of *S. condylus* I.Thomps. (Fig. 1). The latter species is placed in the sister clade of Clade 3 (PP = 1.0, BS = 90%), which consists of African and European taxa (Fig. 2.1). In addition to New Zealand and Norfolk Island Lautusoid taxa, Clade 3 contains members of Thompson's Glossanthus and Lautusoid groups and *S. biserratus* Belcher from the Disciform group (Fig. 2.1). Various accessions of several species (i.e., *S. halophilus* I.Thomps., *S. lacustrinus* I.Thomps., *S. pinnatifolius*, *S. spathulatus* A.Rich.) have different phylogenetic placements within the clade. In addition, Clade 3 contains accessions of two taxa of which other accessions are placed in Clade 1 (*S. repangae* subsp. *pokohinuensis*) and Clade 2 (*S. marotiri*). Accessions of most New Zealand Lautusoid taxa (*S. carnosulus* (Kirk) C.Webb, *S. esperensis* (Sykes) de Lange, *S. glaucophyllus* Cheeseman, *S. lautus*, *S. marotiri* p.p., *S. radiolatus* F.Muell., *S. repangae* subsp. *pokohinuensis* p.p., *S. sterquilinus* Ornduff) are found nested deep within Clade 3 and form a well-supported sub-clade with one of three included accessions of *S. halophilus* from Australia (PP = 1.0; BS = 58%). Clade 3 displays a wide range of chromosome numbers (2*n* = 40, 80, and 100).

2.4.2. Phylogenetic analyses of the plastid regions

Visual inspection of phylogenetic trees obtained from the individual plastid data sets (*psbA-trnH* , *trnL* and *trnL*-F; not shown) did not reveal well-supported incongruence and these were therefore concatenated into a single combined plastid data set. This data set has a slightly smaller taxon sampling than the combined nuclear data set (nuclear: 131 OTUs vs. plastid: 124 OTUs; Table 2.2) but contains the same number of putative members of the Lautusoid group. In the combined plastid phylogenies (Fig. 2.2), most Australasian *Senecio*

can be found in three main clades, which are different in species composition than those of the nuclear trees.

Clade A (PP = 1.0, BS = 52%) consists of three endemic New Zealand taxa (*S. banksii* Hook.f., *S. hauwai* Sykes, *S. kermadecensis* Belcher), all members of Thompson's Odoratus group that were included in our analyses, and five Disciform species. With the exception of *S. biserratus* (2*n* = 100), all taxa in Clade A have a chromosome number of 2*n* = 60, although chromosome numbers are not known for four species of this clade.

The monophyly of Clade B is poorly supported (PP = 0.61, BS = <50%) and it is poorly resolved. Clade B contains many of Thompson's Disciform taxa and all Macranthus and Magnificus taxa that were included in the analyses. In addition, it also contains most of the putative Lautusoid species that have chromosome numbers of 2*n* = 80 and 100 (*S. australis* Willd., *S. glaucophyllus*, *S. hooglandii* Belcher, *S. marotiri*, *S. repangae*) and a species that is native to South Africa (*S. glastifolius* L.f.). Of the three plastid clades in which Australasian species are found, Clade B shows the most diversity in chromosome numbers (2*n* = 40, 60, 80, and 100).

Clade C (PP = 1, BS = 69%) contains many putative Lautusoid taxa with a chromosome number of 2*n* = 40 and a putative Lautusoid species with a chromosome profile of 2*n* = 80 (*S. carnosulus*), five South African *Senecio* species (*S. erysimoides* DC., *S. laxus* DC., *S. littoreus* Thunb., *S. maritimus* L.f., *S. piptocoma* O.Hoffm.), *S. condylus* and all species of Thompson's Glossanthus group that were included in our studies.

2.4.3. Australasian *Senecio* lineages

There is extensive incongruence between the nuclear (Fig. 2.1) and plastid (Fig. 2.2) phylogenies in the position of individual *Senecio* species as well as of clades of species and several of these incongruent patterns are well supported. These patterns are found for Australasian taxa as well as for species from other parts of the world. Despite these incongruent patterns, four Australasian lineages can be identified that are each composed of *Senecio* species that are resolved as closely related in both the nuclear and plastid phylogenies. These are referred to in this paper as the Disciform s.s., Lautusoid, Odoratus s.l., and Quadridentatus groups (Table 2.4). They are largely composed of species that are either 2*n* = 40 (Lautusoid and Quadridentatus groups) or 2*n* = 60 (Disciform s.s. and Odoratus s.l. groups).

The Odoratus s.l. group contains 14 species (Table 2.4) that are placed in Clade A in the plastid phylogenies and together with members of the Disciform s.s. group in Clade 1 in nuclear trees. It contains all members of Thompson's Odoratus group included in our study as well as four species that Thompson (2006) included in his Disciform group and three New Zealand endemics. Nine of the other Disciform species, and an endemic New Zealand species form the Disciform s.s. group (Table 2.4). Although members of this group are closely related to those of the Odoratus s.l. group in the nuclear trees (Clade 1), they are more closely related to those of the Quadridentatus group in the plastid phylogenies (Clade B). The 15 members of the Quadridentatus group (Table 2.4) are placed in nuclear Clade 2. This group contains species assigned by Thompson (2006) to his Disciform, Macranthus, and Magnificus groups, as well as three endemic New Zealand species. The Lautusoid group contains species that are placed in nuclear Clade 3 and plastid Clade C. It consists of the majority of the putative Lautusoid species (Table 2.1) and all included representatives of Thompson's (2006) Glossanthus group.

The relationships between the four Australasian *Senecio* groups identified in this study are incongruent between the nuclear and plastid phylogenies. For example, the Quadridentatus group is more closely related to the Lautusoid group (PP = 0.99, BS <50%) than to both other groups in the nuclear trees, whereas it is more closely related to the Disciform s.s group and the Odoratus s.l group (PP = 0.99; BS <50%) in the plastid trees. In addition, although most Australasian *Senecio* species can be classified into one of the four Australasian groups, accessions of eight species (*S. australis, S. biserratus, S. distallilobatus* I.Thomps., *S. extensus* I.Thomps., *S. glaucophyllus, S. hooglandii, S. marotiri, S. repangae*) are placed with members of different groups in the nuclear trees than in the plastid trees. Seven of these stand out from most other Australasian species by having high chromosome numbers (2*n* = 80 and 100; *S. australis, S. biserratus, S. distallilobatus, S. glaucophyllus, S. hooglandii, S. marotiri, S. repangae*).

The nuclear phylogeny indicates that *S. condylus* is not closely related to other Australasian *Senecio* species. It is instead nested within a clade of five South African species in the nuclear trees (PP = 1; BS = 86%). *Senecio condylus* also groups with these taxa in a basal position in Clade C in the plastid phylogenies (PP = 1; BS = 69%).

2.4.4. Topological test for the monophyly of the Lautusoid group in the plastid phylogeny

In the nuclear phylogenies, the Lautusoid group forms a well-supported clade (defined in this study as having a BS value of > 80% or PP of > 0.95) with accessions of *S. biserratus* and a few other Australasian species with high chromosome numbers that were identified as putative Lautusoid species in previous studies (Table 2.1, Fig. 2.1; Clade 3; PP = 1, BS = 62%). The Lautusoid group is however unresolved and placed in a polytomy with two South African species (*S. laxus, S. piptocoma*) in the plastid trees (Fig. 2.2). In order to test the monophyly of the Lautusoid group in the plastid phylogeny, we conducted a Bayes factor analysis in which trees in which the Lautusoid group is constrained to be monophyletic (positive constraint) are compared with trees in which this clade is constrained to be non-monophyletic (negative constraint). The natural log of the model likelihood values for the positive and negative constraint analyses were -4167.72 and -4199.30, respectively. Using a log difference above 5 as an indication of very strong evidence of support (Kass & Raftery, 1995; Ronquist et al., 2011), the hypothesis that the Lautusoid group is monophyletic according to the plastid data is therefore very strongly supported relative to the hypothesis that it is not monophyletic.

2.5. DISCUSSION

The Lautusoid group of *Senecio* is an Australasian lineage composed of the closest relatives of *S. lautus*. As part of ongoing efforts to better understand the evolution of *Senecio* and to contribute to an infrageneric classification of this large genus, we studied the delimitation of the Lautusoid group and aimed to determine the evolutionary origin of putative Lautusoid species that have higher chromosome numbers (i.e., 2*n* = 80, 100) than found in the majority of other Australasian *Senecio* species (i.e., 2*n* = 40, 60).

2.5.1. An explanation for phylogenetic incongruence between plastid and nuclear *Senecio* phylogenies

Despite widespread and sometimes well-supported phylogenetic incongruence, most Australasian *Senecio* species included in our study can be placed in four lineages (the Disciform s.s, Lautusoid, Odoratus s.l., and Quadridentatus groups) that are each composed of species that are close relatives in both the nuclear and plastid phylogenies. Eight Australasian species (*S. australis, S. biserratus, S. distalilobatus, S. extensus, S.*

glaucophyllus, *S. hooglandii*, *S. marotiri*, *S. repangae*) could however not be assigned to any of the four Australasian lineages because of their different phylogenetic positions in the nuclear and plastid phylogenies. This suggests that they might be the results of hybridization between species belonging to different Australasian lineages (Table 2.3). This hypothesis is supported for seven of these species (*S. australis*, *S. biserratus*, *S. distalilobatus*, *S. glaucophyllus*, *S. hooglandii*, *S. marotiri*, *S. repangae*) by high chromosome numbers that are compatible with allopolyploid hybridization. For example, *S. biserratus* has a chromosome number of 2*n* = 100 and is affiliated with the Lautusoid group (2*n* = 40) in the nuclear phylogeny but with the Odoratus s.l. (2*n* = 60) group in the plastid phylogeny. Similarly, *S. marotiri* (2*n* = 80) is potentially an allopolyploid hybrid between a paternal parent from the Lautusoid group (2*n* = 40) and a maternal parent from the Quadridentatus group (2*n* = 80), according to the placements of its accessions in the nuclear and plastid phylogenies.

The different phylogenetic positions of accessions of a *S. extensus* specimen in the ITS and ETS phylogenies suggest that this species might also be of hybrid origin (Table 2.3). This hypothesis, however, needs to be tested in future studies, because the phylogenetic positions of the accessions of this species in the nuclear phylogenies are only poorly supported (Fig. 2.1). If it is indeed of hybrid origin, our phylogenetic results suggest that it might be a hybrid between species of the Disciform s.s. and Odoratus s.l. groups.

Accessions of two *S. kermadecensis* specimens are placed in well-supported phylogenetic positions with different members of the Odoratus s.l. group (i.e., *S. hypoleucus* F.Muell. ex Benth. and *S. odoratus* Horn. vs. *S. hauwai*) in the nuclear phylogenies (Fig. 2.1). This species might therefore be a hybrid between two species of the Odoratus s.l. group. *Senecio kermadecensis* has 2*n* = 60 chromosomes (Beuzenberg & Groves, 1974; Murray & de Lange, 2013). If this species is indeed of hybrid origin, it is therefore not an allopolyploid. *Senecio kermadecensis* is morphologically most similar to *S. minimus* Poir. and this species should therefore be included in studies aimed at further resolving its evolutionary history.

Table 2.3. List of putative hybrid species of Australasian *Senecio* identified in this study, their chromosome number and hypotheses of their parentage.

Putative hybrid	Chromosome number	Hypotheses of parentage

Senecio australis & *Senecio hooglandii*	2*n* = 80	Lautusoid (2*n* = 40) × Quadridentatus (2*n* = 40) *both species may have originated following the same hybridization event
Senecio biserratus	2*n* = 100	Lautusoid (2*n* = 40) × Odoratus s.l. (2*n* = 60)
Senecio distalilobatus	2*n* = 100	Disciform s.s. (2*n* = 60) × Quadridentatus (2*n* = 40)
Senecio extensus	?	Odoratus s.l. (2*n* = 60) × Disciform s.s. (2*n* = 60)?
Senecio glaucophyllus	2*n* = 100	Lautusoid (2*n* = 40) × Disciform s.s. (2*n* = 60)
Senecio kermadecensis	2*n* = 60	Odoratus s.l. (2*n* = 60) × Odoratus s.l. (2*n* = 60)
Senecio marotiri	2*n* = 80	Lautusoid (2*n* = 40) × Quadridentatus (2*n* = 40)
Senecio repangae	2*n* = 100	Lautusoid (2*n* = 40) × Disciform s.s. (2*n* = 60)

In addition to hybridization, phylogenetic incongruence can be a result of incomplete lineage sorting (Gurushidze et al., 2010; Blair et al., 2012; Zhang et al., 2015) or a failure to distinguish paralogous from orthologous sequences when using multi-copy markers such as ITS and ETS (Álvarez & Wendel, 2003). However, we consider these alternative explanations less plausible than hybridization because they do not account for the higher chromosome numbers of most taxa that were found to have incongruent phylogenetic positions in the nuclear and plastid phylogenetic trees. The allopolyploid origin of some of these incongruent taxa is further supported by other lines of evidence, such as cytological (e.g. de Lange & Murray, 1998) and morphological observations made during this study (see below).

Three non-Lautusoid Australasian *Senecio* species (*S. brassii*, *S. laceratus* (F.Muell.) Belcher, *S. papuanus*) were only represented by nuclear DNA sequences in our phylogenetic analyses. It is therefore at present not possible to determine if they are of hybrid origin. *Senecio brassii* and *S. papuanus* from New Guinea are resolved as closely related to members of the Disciform s.s. group in the nuclear phylogeny (Clade 1; Fig. 2.1). *Senecio brassii* is morphologically quite similar to *S. glomeratus* Desf. ex Poir. of the Disciform s.s. group (Belcher, 1982) and therefore potentially a member of this group, but Borgmann (1964)

reported a chromosome count of 2*n* = 64 for this species, which, if correct, deviates from the 2*n* = 60 counts that are known from other Disciform s.s. species (Table S1). Also for *S. papuanus* a chromosome number has been obtained that is different from that of the Disciform s.s. species for which chromosome numbers are known (2*n* = c. 80; Borgmann, 1964). *Senecio papuanus* is morphologically similar to *S. laceratus* from Australia (Belcher, 1956). The latter species is, however, resolved among species of the Quadridentatus group (Clade 2; Fig. 2.1). Pending future studies into the evolutionary history and relationships of these three species, they are not assigned to one of the four Australasian *Senecio* lineages in this study.

2.5.2. The taxonomic delimitation of the Lautusoid group

By identifying the main Australasian *Senecio* lineages and species that are putative hybrids between them, we developed a better understanding of the identity of the species that form the Lautusoid group and those that evolved through hybridization between Lautusoid species and members of other Australasian lineages. Of the 23 species that we identified as putative Lautusoid species prior to our study (Table 2.1), we included 18 in our studies. Our results show that 12 of these are indeed members of the Lautusoid group (Table 2.4). Three species of Thompson's Glossanthus group (*S. glossanthus* (Sond.) Belcher, *S. halophilus*, *S. serratiformis* I.Thomps.) are resolved as most closely related to these 12 species. For example, an accession of *S. halophilus* forms a well-supported clade (PP = 1.0, BS 58%) with most New Zealand Lautusoid species in the nuclear phylogeny (Fig. 2.1) and multiple accessions of this species form a deeply-nested clade with New Zealand *S. carnosulus* in the plastid phylogeny (Fig. 2.2; PP = 0.62, BS < 50%). The close phylogenetic relationship between Thompson's Lautusoid and Glossanthus groups is supported by their morphological similarities (Thompson 2006), although Glossanthus species have radiate florets with much shorter ligules (often < 2 vs. 4−25 mm for Australian Lautusoid species) and achenes that are usually dimorphic (vs. usually homomorphic; Thompson, 2005a,b, 2006). We therefore consider Thompson's Glossanthus group as part of an expanded Lautusoid group.

Thompson considered *S. condylus* a member of his Lautusoid group (Thompson, 2005b, 2006), but our results show that it is not placed among Australasian species. It is instead found to be more closely related to South African species, especially *S. erysimoides*, *S. laxus*, *S. littoreus*, and *S. maritimus* (Figs. 2.1 and 2.2). The distribution of *S. condylus* is mostly limited to south-western Western Australia and particularly the Perth metropolitan area

(Australia's Virtual Herbarium, 2016; Thompson, 2005b). Its presence in a metropolitan area suggests that this species might not be native to Australia and, instead, have arrived in Australia from Africa in recent times. A taxonomic study of *S. condylus* and morphologically similar South African species is needed to determine if *S. condylus* is a synonym of a previously described African species that has naturally dispersed or become naturalized, in the same way that other African plants have in Western Australia (e.g., St. George, 1996; de Lange et al., 2011). Because *S. condylus* is not a member of the clades formed by the Lautusoid species, it is here excluded from this group.

Five species that were identified as putative Lautusoid species at the onset of our studies (*S. australis*, *S. glaucophyllus*, *S. hooglandii*, *S. marotiri*, and *S. repangae*; Table 2.1) are allopolyploid hybrids between members of the Lautusoid group and the other three Australasian *Senecio* lineages. These species are therefore excluded from the Lautusoid group. Hypotheses regarding their hybrid origin are provided below.

2.5.3. Morphological differences between the Lautusoid group and other Australasian *Senecio* lineages

The species that form the Lautusoid group (incl. former Glossanthus species) form a lineage that is characterized by annual or short-lived perennial plants with a herbaceous growth form (although some plants of *S. lacustrinus* are semi-shrubs) and that are not glaucous (except for some plants of *S. glaucophyllus*) and have radiate capitula (*S. radiolatus* ssp. *antipodus* (Kirk) C.J.Webb being the sole exception) and leaves that are commonly slightly to strongly fleshy. This combination of characters distinguishes members of the Lautusoid group from most species that were resolved as members of the other three Australasian *Senecio* lineages and helps to identify potential additional members of the Lautusoid group among the species that were not included in our studies.

Previous taxonomic classifications of Australasian *Senecio* species predominantly relied on capitulum morphology. Belcher (1956) and Lawrence (1980), for example, divided Australasian *Senecio* into three groups: those with radiate capitula (‘radiate *Senecio*’; capitula with an outer whorl of zygomorphic pistillate florets with ligules), discoid capitula (‘discoid *Senecio*’; capitula with an outer whorl of actinomorphic bisexual florets without ligules), and disciform capitula (‘erechtitoid *Senecio*’; capitula with an outer whorl of actinomorphic pistillate florets without ligules). Also Thompson used these three capitula types in his classification (Thompson, 2004a, b, c, 2005a, b, 2006). The present study indicates that

capitulum morphology is still of critical importance for distinguishing Lautusoid *Senecio* from those of the three other groups. In contrast to most other Australasian *Senecio* species that were included in our study, members of the Lautusoid group have radiate capitula. However, some species of the three other Australasian *Senecio* lineages are also radiate. Some of these species might therefore be confused with Lautusoid species, although examination of other morphological characters signals their closer affinities with non-Lautusoid lineages in most cases.

Senecio condylus is one of the non-Lautusoid species that has radiate capitula. It can, however, be distinguished from the species formerly assigned to Thompson's Glossanthus group by having longer ligules (> 4 mm vs. < 2 mm) and more supplementary bracts (*S. condylus*: 8−12 vs. Glossanthus group: 2−6; Thompson, 2005a). It is different from the other Lautusoid species by having dimorphic achenes (those of the radiate florets are c. 1mm longer than those of disc florets) and somewhat persistent and coarse trichomes on the abaxial leaf surface, whereas these Lautusoid species commonly have homomorphic achenes and a glabrous abaxial leaf surface (Thompson, 2005a, b).

Thompson placed most of the Australian non-Lautusoid radiate *Senecio* in his Magnificus and Macranthus groups (Thompson, 2004c, 2006), of which three species were included in our analyses and resolved as members of the Quadridentatus group: *S. gregorii* F.Muell., *S. macranthus* A.Rich., and *S. magnificus* F.Muell. *Senecio macranthus* and the other species of Thompson's Macranthus group can be distinguished from Lautusoid species by having relatively large (usually >4 vs. 0.5−4.5 mm long) supplementary bracts that are strap-shaped or narrow oblong instead of broadly ovate to narrow lanceolate (Thompson, 2004c, 2005b, 2006). *Senecio magnificus* and *S. gregorii* and the other species of Thompson's Magnificus group can be differentiated from the Lautusoid species by often being glaucous and having capitula with distally dilated peduncles (Thompson 2006). *Senecio magnificus* has relatively few supplementary bracts (0−4 vs. 4−18 for Australian Lautusoid species) and persistent pappus (vs. caducous pappus; persistent only in *S. spathulatus*) and *S. gregorii* has ecalyculate (vs. calyculate) capitula with fused (vs. free) phyllaries.

Senecio linearifolius A.Rich. is the only additional non-Lautusoid radiate species that Thompson included in his classification, in which he placed it in his Odoratus group. In our study, *Senecio linearifolius* and the New Zealand *S. banksii* are the only two radiate species that were resolved as members of the Odoratus s.l. group. *Senecio linearifolius* is a highly

variable species of which some forms might be confused with Lautusoid species. However, *S. linearifolius* can be identified as a non-Lautusoid species by having leaves that are occasionally glaucous and having capitula with relatively few radiate florets (5−6) compared to most Lautusoid species ((4−)5−13(−28)). *Senecio banksii* is a perennial herb that is woody at the base. In addition, its leaves are distinctly glaucous, not fleshy and have an abaxial leaf surface that is moderately to densely hairy at maturity. It is morphologically similar to *S. colensoi* Hook.f. (Sykes, 1987), which is the only New Zealand radiate *Senecio* that is not included in our study. Because of these similarities, and the morphological similarities between *S. colensoi* and *S. hauwai* (Odoratus s.l. group; Sykes, 1987), *S. colensoi* is most probably also a member of the Odoratus s.l. group. *Senecio colensoi* can be differentiated from New Zealand Lautusoid species by its semi-woody perennial growth habit and by its leaves, whose surfaces are moderately to densely covered in lanate trichomes – so imparting a silvery appearance.

Senecio rufiglandulosus Colenso is a New Zealand non-Lautusoid radiate species that was resolved as a member of the Quadridentatus group in our studies. It has radiate florets with relatively long ligules (5.5−14.0 mm) compared to most New Zealand Lautusoid species (1−5 mm) and it too is a long-lived perennial that has a woody base, whereas most Lautusoid species are short-lived and herbaceous (Webb et al., 1988).

Six putative Lautusoid *Senecio* species were not included in our analyses: *S. eremicola*, *S. evansianus*, *S. howeanus*, *S. pauciradiatus*, *S. productus* and *S. warrenensis*. Whereas these species fall within the morphological variation of the Lautusoid group and are therefore potentially Lautusoid species, *S. evansianus* and *S. howeanus* differ from members of the Lautusoid group in, amongst others, having disciform instead of radiate flower heads (Belcher, 1992b; Green, 1994). Just like the two other native *Senecio* species on Norfolk and Lord Howe Islands (*S. australis* and *S. hooglandii*), these species might be hybrids between the Lautusoid group and one of the three other Australasian *Senecio* lineages.

The results of our study show that the Australasian Lautusoid group of *Senecio* can be distinguished from other Australasian lineages using phylogenetic and morphological evidence and this group is therefore a distinct *Senecio* lineage. We, however, refrain from formally recognizing the Lautusoid group as a section until sufficient morphological data is available to provide a comprehensive taxonomic description of this group. Although detailed morphological descriptions are available for the Australian members of the Lautusoid group,

descriptions of many New Zealand Lautusoid species lack detail and some characters that might be diagnostic for the Lautusoid group and that were recorded for Australian Lautusoid species have not yet been studied for New Zealand species.

2.5.4. At the periphery of the Lautusoid group: hybrids with other Australasian *Senecio* lineages

The results of our study suggest that allopolyploid hybridization plays an important role in the evolution of the Lautusoid group, because six out of seven of the allopolyploid species discovered in our study (Table 2.3) are hybrids between the Lautusoid group and one of three other Australasian *Senecio* lineages: *S. australis, S. biserratus, S. glaucophyllus, S. hooglandii, S. marotiri* and *S. repangae*. Although the identity of the parental species of these putative allopolyploid species is difficult to determine, because of a lack of resolution and support in parts of the phylogenies (Figs. 2.1 and 2.2), hypotheses regarding the parentage of these species are here provided using the available evidence.

The Norfolk Island native *S. australis* and endemic *S. hooglandii* (both $2n = 80$) are hypothesized as hybrids between a paternal parent from the Lautusoid group ($2n = 40$) and a maternal parent from the Quadridentatus group ($2n = 40$). It is not clear if these two species originated as a result of separate hybridization events or if they diverged from a common allopolyploid ancestor, because they are resolved as each other's closest relatives in our nuclear and plastid phylogenies. The identity of the parental species of *S. australis* and *S. hooglandii* in the Lautusoid and Quadridentatus groups is presently unknown. There is now no other native Australasian *Senecio* on Norfolk Island than *S. evansianus* (de Lange & Murray, 2003; de Lange et al., 2005), which might also be of allopolyploid origin. *Senecio australis* also grows in New Zealand (de Lange et al., 2014), but it is more likely that it arrived there relatively recently from Norfolk Island by means of bird-assisted seed dispersal than that it originated in New Zealand following hybridization between members of the Lautusoid and Quadridentatus group and subsequently dispersed to Norfolk Island (de Lange et al., 2014). Morphological evidence that supports the allopolyploid origin of *S. hooglandii* and *S. australis* is that the former has disciform instead of radiate capitula. *Senecio australis* has fewer (< 5 vs. 5−18 in most Lautusoid species) and linear-lanceolate instead of broadly ovate to narrowly lanceolate supplementary bracts, although *S. esperensis* has only 3−5 supplementary bracts, which are linear-lanceolate (Belcher, 1992a, 1992b; Thompson, 2006; de Lange et al., 2014, 2015).

Senecio biserratus (2*n* = 100), a disciform species that is native to New Zealand and Australia, is postulated to be a hybrid between a paternal parent from the Lautusoid group (2*n* = 40) and a maternal parent from the Odoratus s.l. group (2*n* = 60). The maternal parent of *S. biserratus* is possibly *S. minimus* (2*n* = 60) because *S. biserratus* is morphologically very similar to *S. minimus*, although they are different in achene length and indumentum, the shape of leaf margin, and the type of indumentum on the leaves. The morphological similarities between both species are also evident from the fact that *S. biserratus* was once included in *S. minimus* (Belcher, 1956). Moreover, the two species have overlapping distributions in Australia and New Zealand. This hypothesis is, however, not supported by our phylogenetic data, because *S. minimus* was not resolved as the closest relative of *S. biserratus* in the plastid phylogenies (Fig. 2.2). The identity of the paternal parent of *S. biserratus* from the Lautusoid group is not known because none of the Lautusoid species has a close morphological resemblance to *S. biserratus*. In fact, affinities of this species with Lautusoid species have not been suggested prior to this study.

The New Zealand endemic *S. glaucophyllus* (2*n* = 100) is hypothesized to be a hybrid of a paternal parent from the Lautusoid group (2*n* = 40) and a maternal parent from the Disciform s.s. group (2*n* = 60). *Senecio glaucophyllus* is a highly variable species that displays both typical Lautusoid (e.g., radiate capitula and non-glaucous fleshy leaves) as well as non-Lautusoid (e.g., discoid capitula and less fleshy and glaucous leaves) features. The paternal parent of *S. glaucophyllus* is hard to determine, because of the lack of apparent similarities with any of the New Zealand Lautusoid species and *S. glaucophyllus*. One possible candidate is *S. lautus*, which distribution overlaps with a few of the subspecies of *S. glaucophyllus* (e.g., subsp. *basinudus*, *toa* and *discoideus*) in South Island (Ornduff, 1960; Allan, 1961; de Lange, 1998; de Lange et al., 2011). The maternal parent of S. *glaucophyllus* is perhaps *S. hispidulus A.Rich.* or *S. glomeratus*, because of the presence of these two Disciform s.s. species within the current distribution area of *S. glaucophyllus*.

Senecio marotiri (2*n* = 80) is a New Zealand endemic that has a natural distribution range that is restricted to islands in the north-eastern part of the North Island and to the Chatham Island group (Webb, 1988; de Lange, 1998; de Lange et al., 2011; de Lange et al., 2014). This species is postulated to be a hybrid between a paternal parent from the Lautusoid group (2*n* = 40) and a maternal parent from the Quadridentatus group (2*n* = 40). The most probable parental species of *S. marotiri* are *S. lautus* (paternal) and *S. quadridentatus* Labill. (maternal). These species are sympatric with *S. marotiri*, which is morphologically

intermediate between the radiate and mostly glabrous *S. lautus* and the disciform and lanate *S. quadridentatus* by having radiate flowers with reduced ligules and by being glabrescent. The hypothesis that *S. quadridentatus* is the maternal parent of *S. marotiri* is supported by our phylogenetic data, because one of the two nuclear accessions of *S. marotiri* is well resolved as sister to *S. quadridentatus* (Fig. 2.1; PP = 1, BS = 83%).

Senecio repangae (2*n* = 100) is another New Zealand endemic and is restricted to the north-eastern part of the North Island. de Lange & Murray (1998) recognize two subspecies of *S. repangae*, both of which are represented in our analyses. The phylogenetic positions of accessions of both taxa suggest that they are hybrids between a paternal parent from the Lautusoid group (2*n* = 40) and a maternal parent from the Disciform s.s. group (2*n* = 60). Although very poorly supported (PP = 0.55; BS <50%; Fig. 2.1), the results of our phylogenetic analyses of the nuclear data suggest that *S. repangae* subsp. *repangae* is more closely related to accessions of *S. hispidulus* and *S. scaberulus* (Hook.f.) D.G.Drury than subsp. *pokohinuensis*. Because of this and the morphological differences between the two subspecies (de Lange & Murray, 1998), it is possible that they have originated from different hybridization events, involving different parental species in the Lautusoid and Disciform s.s. groups. The morphological characteristics of subsp. *repangae* suggest that the best candidates for its paternal and maternal parents are *S. lautus* (Lautusoid) and *S. scaberulus* or *S. hispidulus* (Disciform s.s.). Subspecies *repangae* has radiate florets with reduced ligules and pilose leaves. These characters make it morphologically intermediate between the mostly glabrous and radiate *S. lautus* and the hispid and disciform *S. scaberulus* and *S. hispidulus*. The morphological similarities with *S. scaberulus* are also evident from the fact that many older herbarium specimens of *S. repangae* have been misidentified as *S. scaberulus* (de Lange & Murray, 1998). In addition, subsp. *repangae* is sympatric with *S. lautus*, *S. scaberulus* and *S. hispidulus* (de Lange & Murray, 1998), although *S. hispidulus* is a relatively recent arrival in New Zealand (Belcher, 1956; Drury, 1974) and therefore perhaps less likely to be one of the parents of *S. repangae* subsp. *repangae*. Plants of subsp. *pokohinuensis* are glaucous and glabrescent and this makes it unlikely that *S. scaberulus* or *S. hispidulus* is its maternal parent. Future studies could focus on testing the hypothesis that the two subspecies of *S. repangae* have separate origins and, if so, on identifying the maternal parent of subsp. *pokohinuensis*.

2.5.5. Evolutionary relationships among Lautusoid species

Relationships among members of the Lautusoid group are not fully resolved in this study, because of the low resolution within Clade 3 in the nuclear phylogeny and Clade C in the plastid phylogeny (Figs. 2.1 and 2.2). In Clade 3 of the nuclear phylogeny, however, several well-supported sub-clades are found (Fig. 2.1). For example, one sub-clade consists of accessions of *S. glossanthus* and *S. halophilus* (PP = 1.0, BS = 98%). The close relationship between these two species is not unexpected because they are morphologically similar to each other by having capitula with 4−8 female florets and longer achenes of female florets than those of bisexual florets (Thompson, 2005a).

Another sub-clade of Clade 3 consists of most New Zealand endemic species and one accession of *S. halophilus* (PP = 1.0, BS = 58%). This indicates that New Zealand Lautusoid species most probably originated from one most recent common ancestor following a single colonization event from Australia. Within this sub-clade, four coastal New Zealand species with radiate flower heads and fleshy leaves (*S. carnosulus, S. esperensis, S. radiolatus, S. sterquilinus*) form a deeply-nested clade (Fig. 2.1, PP = 0.75, BS < 50%). Of these, *S. esperensis, S. radiolatus, S. sterquilinus* are known to be associated with guano-rich soil and seabird colonies (Norton et al., 1997; de Lange & Murray, 1998; Ornduff, 1960; de Lange & Murray, 1998; Sykes, 1971; de Lange et al., 2015) and this suggests that they evolved from a common ancestor with that habitat preference.

Several Australian Lautusoid species have accessions that are placed in different phylogenetic positions within Clade 3 of the nuclear tree (*S. halophilus, S. lacustrinus, S. pinnatifolius, S. spathulatus*; Fig. 2.1) or Clade C of the plastid tree (*S. carnosulus, S. lautus*; Fig. 2.2). Because of the poor resolution and support for their phylogenetic positions, our data do not allow us to determine if these findings indicate that the taxonomic delimitation of these taxa needs to be revised or if these species are hybridizing with other Lautusoid members. Among these species is *S. carnosulus*, which has a high chromosome number ($2n$ = 80) compared to the other Lautusoid species ($2n$ = 40). Studies targeted at resolving the evolutionary history of this species are needed to determine if *S. carnosulus* is an autopolyploid or an allopolyploid that resulted from hybridization between two Lautusoid species.

Table 2.4.

The species composition of the four Australasian *Senecio* groups as identified in the current study. Abbreviations in brackets indicate Thompson's infrageneric groups (for Australian species; Thompson, 2006) followed by their countries of origin (only for *Senecio* species found outside of Australia): D - Disciform group, O - Odoratus group, Mag - Magnificus group, Mac - Macranthus Group, G - Glossanthus group, L - Lautusoid group. NZ - New Zealand, Aus - Australia. * 2*n* = 80 for *S. carnosulus*.

Odoratus s. l. group (2*n* = 60)	**Disciform s.s. group (2*n* = 60)**	**Quadridentatus group (2*n* = 40)**	**Lautusoid group (2*n* = 40)**
S. anethifolius (O)	*S. bathurstianus* (D)	*S. campylocarpus* (D)	*S. brigalowensis* (L)
S. banksii (NZ)	*S. bipinnatisectus* (D- Aus & NZ)	*S. dolichocephalus* (D)	*S. carnosulus* (NZ)*
S. cunninghamii (O)	*S. diaschides* (D- Aus & NZ)	*S. dunedinensis* (NZ)	*S. depressicola* (L)
S. gawlerensis (O)	*S. esleri* (D)	*S. glabrescens* (D)	*S. esperensis* (NZ)
S. hauwai (NZ)	*S. glomeratus* (D- Aus & NZ)	*S. gregorii* (Mag)	*S. glossanthus* (G)
S. hypoleucus (O)	*S. hispidissimus* (D)	*S. gunnii* (D)	*S. halophilus* (L)
S. kermadecensis (NZ)	*S. hispidulus* (D- Aus & NZ)	*S. longicollaris* (D)	*S. hamersleyensis* (L)
S. lanibracteus (O)	*S. macrocarpus* (D)	*S. macranthus* (Mac)	*S. lacustrinus* (L)
S. linearifolius (O)	*S. nigrapicus* (D)	*S. magnificus* (Mag)	*S. lautus* (NZ)
S. minimus (D- Aus & NZ)	*S. scaberulus* (NZ)	*S. phelleus* (D)	*S. pinnatifolius* (L)
S. odoratus (O)		*S. prenanthoides* (D)	*S. radiolatus* (NZ)
S. picridioides (D)		*S. psilophyllus* (D)	*S. serratiformis* (G)
S. psilocarpus (D)		*S. quadridentatus* (D- Aus & NZ)	*S. spanomerus* (L)
S. squarrosus (D)		*S. rufiglandulosus* (NZ)	*S. spathulatus* (L)
		S. wairauensis (NZ)	*S. sterquilinus* (NZ)

2.6. CONCLUSION

We provide the first comprehensive taxonomic delimitation of the Australasian Lautusoid group of *Senecio* with evidence that it is a phylogenetically and morphologically distinct lineage. A total of 15 *Senecio* species are identified as members of the Lautusoid group (Table 2.1 and 2.4) with the exclusion of *S. condylus*, *S. madagascariensis*, and five allopolyploid species that were previously associated with the Lautusoid group (Table 2.1) and the addition of species previously placed in Thompson's Glossanthus group (Thompson, 2005a). In addition to the Lautusoid group, three additional Australasian *Senecio* lineages were identified: the Odoratus s.l., Disciform s.s., and Quadridentatus groups. The present study used topological conflicts between molecular phylogenies derived from different genomes to unveil patterns of reticulate evolution in the history of allopolyploid Australasian *Senecio* species (Table 2.3). These patterns demonstrate allopolyploid hybridization between members of the Lautusoid group and all three other Australasian *Senecio* lineages. Our study thereby highlights the prevalence of hybridization in the evolutionary history of the Lautusoid group of *Senecio* and provides further evidence for the importance of hybridization in the diversification of *Senecio* and Senecioneae (e.g., Abbott & Lowe, 2004; Calvo et al., 2013; Kadereit et al., 2006; Pelser et al., 2010a, 2012).

CHAPTER 3: Hiding in plain sight: cryptic species in the *Senecio glaucophyllus* complex

3.1. ABSTRACT

Progress in documenting and understanding the diversity of life is challenged by the presence of cryptic species, which often go undetected. This study aims to resolve a New Zealand species complex for which in its current delimitation, *Senecio glaucophyllus* is a species that shows substantial morphological diversity. However, plants that have been collected in North-West Nelson (South Island) are of a taxon that appears somewhat different from *Senecio glaucophyllus*. Specimens of this taxon have often been filed in herbaria as *Senecio glaucophyllus* subsp. *glaucophyllus* with which it is sympatric. However, more recently, this form has been informally recognized as *S.* aff. *glaucophyllus*. The results of a PCoA of a morphometric data set and phylogenetic studies of an ITS DNA sequence data set show that the two taxa are morphologically distinct and only distantly related to each other. Random Forest analyses resulted in the discovery of several diagnostic morphological characters for the taxa. The inclusion of type specimens of *S. glaucophyllus* in the PCoA analyses show that specimens of *S.* aff. *glaucophyllus* are conspecific with *S. glaucophyllus* whereas those of *S. glaucophyllus* in its current delimitation belong to an undescribed species. I further discuss the impact of these results on the conservation status of the taxa involved.

3.2. INTRODUCTION

Cryptic species ("two or more distinct species that are erroneously classified (and hidden) under one species name"; Bickford et al., 2006) pose challenges to documenting, understanding, and conserving biodiversity. If undetected, the presence of cryptic species in biodiversity studies can result in an underestimation of species diversity (e.g. Bickford et al., 2006; Buhay et al., 2007; Rato et al., 2016) or can negatively impact pest management, disease vector control, conservation planning, and other activities for which accurate species identification is important.

Cryptic species complexes have been found in agricultural pests, such as in the whitefly *Bemisia tabaci* (Gennadius) (Frewin et al., 2014). Because cryptic species require different management strategies, misidentifications reduce management efficacy and result in monetary loss (Frewin et al., 2014). Cryptic species complexes are also of concern in efforts aimed at improving human health. For example, the vector of malaria in Zambia, mosquitos

of the genus *Anopheles* Meigen (Lobo et al., 2015), consist of several cryptic species complexes of which members can not be reliably distinguished using morphological data. This hampers efforts to eliminate the disease, because the different cryptic species vary in bionomic traits, such as feeding behavior and insecticide resistance, and therefore require different control strategies (Lobo et al., 2015).

In addition to agricultural pest control and disease elimination, the presence of cryptic species can have an impact on conservation and biosecurity. For example, Williams et al. (2012) discovered that the commercially valuable bumblebee *Bombus hypocrite* Pérez, known to be present in North China and Japan, is actually composed of two cryptic species. They found that the bumblebees in North China are members of the widespread Russian *B. patagiatus* Nylander, whereas the bumblebees in Japan are true *B. hypocrite* (Williams et al., 2012). Their study prevented the possibly dire consequences of introducing non conspecific bumblebees into areas where they are not native to. Another challenge that cryptic species pose to conservation is if they are endangered, but remain undetected and erroneously mistaken for a common species (e.g., Sattler et al., 2007; Murphy et al., 2011) or if cryptic species within an endangered cryptic species complex are overlooked. The threatened obligately parasitic hoverfly *Microdon mutabilis* Linnaeus is an example of the latter (Schönrogge et al., 2002). The discovery of two cryptic species in what was originally thought to be a single species indicates that these hoverfly species have smaller populations and a more restricted range than was previously thought (Schönrogge et al., 2002). Because of the host specificity of these two cryptic parasitic species, each has different conservation management needs which could only be assessed accurately once the cryptic species are recognized (Schönrogge et al., 2002).

The problem of cryptic speciation and poorly resolved taxonomy is as much a New Zealand problem as it is a worldwide one. Increasingly, with the better tools now available for exploring taxonomic issues, new species are being segregated from within traditionally accepted ‘variable’ New Zealand species. The situation with New Zealand *Lepidium* L. exemplifies the probably of cryptic species (de Lange et al, 2013b). The last Flora treatment of the New Zealand species recognized seven species, six endemic (Webb et al., 1988). Now following detailed molecular and morphological investigation, twenty species (18 endemic) are recognized, ten of these segregated from the already threatened *Lepidium oleraceum* G.Forst ex Sparrm. (de Lange et al. 2013b). The conservation implications of these studies are significant, of the 20 species now accepted for New Zealand, two are now extinct (one of

these was recognized from historical collections only), and the rest considered ‘Threatened’ (de Lange et al., 2013a, b). A molecular and morphological study of the endemic New Zealand *Corybas trilobus* complex also resulted in the segregation of a number of new species, some believed to be highly threatened (Lehnebach et al., 2016). In this chapter, I present the results of a study into the delimitation of the New Zealand endemic *S. glaucophyllus* Cheeseman that lead to the discovery that it is a cryptic species complex. I will also discuss the conservation implications of the findings of this discovery.

Senecio glaucophyllus is an endemic New Zealand species. It was described by Cheeseman (1895) from plants from Mt. Arthur (North-West Nelson, South Island). He mentioned in the protologue that this species as “a very curious plant, its dense habit of growth and glaucous leaves giving it a very different appearance from any of its allies” (Cheeseman, 1895, p. 536). Ornduff (1960) considerably expanded the morphological delimitation of *S. glaucophyllus* by merging it with *S. lautus* var. *montanus* Cheeseman (1906), a taxon that is sympatric with *S. glaucophyllus* sensu Cheeseman on Mt. Arthur. Ornduff (1960) further expanded *S. glaucophyllus* by incorporating *S. lautus* var. *discoideus* Cheeseman (1906) as *S. glaucophyllus* subsp. *discoideus* (Cheeseman) Ornduff, and by erecting two subspecies to accommodate morphological forms that he considered allied but different to the nominal subspecies and subsp. *discoideus*: *S. glaucophyllus* subsp. *basinudus* Ornduff and *S. glaucophyllus* subsp. *raoulii* (Hook.f.) Ornduff. The latter name, however, later proved invalid, because the type of *S. lautus* var. *raoulii* Hook.f., on which this name is based, belongs to *S. glaucophyllus* subsp. *basinudus* (Connor & Edgar, 1987). Needing a name for subsp. *raoulii* sensu Ornduff, Webb described *S. glaucophyllus* subsp. *toa* C.J.Webb for these plants. This broader delimitation of *S. glaucophyllus* in which four subspecies are recognized is followed to this day (including in Chapter 2 of this thesis) and is referred to in this paper as *S. glaucophyllus* sensu Ornduff.

Senecio glaucophyllus sensu Ornduff is a morphologically very variable species and some plants ascribed to this taxon can not be confidently accommodated in any of the four currently recognized subspecies. One of these forms is morphologically similar to (Fig. 3.1) and sympatric with (Fig. 3.7) subsp. *glaucophyllus* sensu Ornduff. This form has been referred to as ‘*S.* aff. *glaucophyllus*’ (AK253477 ; Mt. Burnett) (e.g., de Lange et al., 2009, 2013a) and herbarium specimens of it have also been filed as *Senecio* ‘Mt. Burnett’. *Senecio* aff. *glaucophyllus* has thus far only been reported from North-West Nelson and this is also the area from which the nominal subspecies of *S. glaucophyllus* is best known (Fig. 3.7). This

study aims to establish the taxonomic status of *S.* aff. *glaucophyllus* by determining if this cryptic form is morphologically and genetically distinct from *S. glaucophyllus* and to identify its diagnostic morphological characters if it indeed merits taxonomic recognition.

It is important to resolve the delimitation of *Senecio glaucophyllus* subsp. *glaucophyllus* sensu Ornduff and *S.* aff. *glaucophyllus*, because both are of conservation interest and of the need to apply conservation assessments to the correct name. Subspecies *glaucophyllus* sensu Ornduff is classified as 'At Risk / Naturally Uncommon' and *S.* aff. *glaucophyllus* is designated as 'Threatened / Nationally Vulnerable' under the New Zealand Threat Classification System (Molloy et al., 2002; Townsend et al., 2008; de Lange et al., 2010, 2013a). Both taxa are classified with the qualifier Data Poor and this highlights the urgency of a taxonomic study that clarifies their delimitation.

Fig. 3.1. *S. glaucophyllus* subsp. *glaucophyllus* (left) and *S.* aff. *glaucophyllus*(right) in the field. The similarities of the general appearance and the habitats of the two taxa can be seen in these pictures. Photo credit: Shannel Courtney.

3.3. MATERIALS AND METHODS

3.3.1. Sampling and morphometric data collection

Most specimens selected for the morphometric analyses are of *Senecio* aff. *glaucophyllus* and *S. glaucophyllus* subsp. *glaucophyllus* sensu Ornduff, because *S.* aff. *glaucophyllus* is morphologically more similar, and therefore more commonly confused with subsp. *glaucophyllus* sensu Ornduff than with the three other subspecies of Ornduff's *S. glaucophyllus*. The specimens were identified to *S.* aff. *glaucophyllus* and subsp. *glaucophyllus* sensu Ornduff using putatively diagnostic characters (selection criteria described below) in Table 3.1 following close examination of the specimens. A total of 34

herbarium specimens (all available specimens) of *S.* aff. *glaucophyllus* and 34 specimens of subsp. *glaucophyllus* sensu Ornduff from AK, CANU, CHR and WELT were examined for this study (Table S3). The specimens for the latter were selected to represent its morphological variation. The selected specimens include the lectotype and three isolectotypes of subsp. *glaucophyllus.* Two out of the 34 *S.* aff *glaucophyllus* specimens were examined but could not be included in the morphometric study because they are too incomplete or of poor quality (these are marked with * in Table S3). In order to be able to determine the taxonomic affinities of *S.* aff. *glaucophyllus* and subsp. *glaucophyllus* sensu Ornduff with the other three subspecies of Ornduff's *S. glaucophyllus*, the holotype (two sheets (A and B) of the same specimen) and an isotype of subsp. *basinudus*, the lectotype of subsp. *discoideus*, and the holotype of subsp. *toa* were included in the PCoA analyses (methodology outlined below). In addition, five representative specimens of *S. lautus* var. *montanus* were studied. Cheeseman did not designate types when he described this variety (Cheeseman, 1906), but four of these five specimens were collected and identified by him as *S. lautus* var. *montanus* and are therefore suitable representatives of this taxon. Details for all specimens used in the analyses are provided in Table S3.

Potentially diagnostic characters for distinguishing *Senecio* aff. *glaucophyllus* and subsp. *glaucophyllus* sensu Ornduff were selected as part of a broader effort to identify informative characters for resolving patterns of morphological variation in *S.* aff. *glaucophyllus* and *S. glaucophyllus* sensu Ornduff (see Chapter 4 for details). Of a total of 93 morphological characters, three qualitative and seven quantitative characters (Table 3.1) were selected from those that displayed the largest differences between the two taxa in a comparison that included 19 specimens (12 of *S.* aff. *glaucophyllus* and 7 of *S. glaucophyllus* subsp. *glaucophyllus* sensu Ornduff). Because of the importance of including type specimens in the morphometric analyses, characters were selected for which character states could be determined without destructive sampling. The specimens were examined and measured using a caliper and under a dissecting microscope if magnification was required.

Table 3.1. Morphological characters selected for the morphometric analyses.

Character	**Type**
Mid-cauline leaf	
1. Length (mm)	Quantitative (numeric)
2. Width (mm)	Quantitative (numeric)
3. Length (1) /width (2) ratio	Quantitative (numeric)
4. Shape of leaf margin	Qualitative (single serrate or dentate /

	double-serrate or dentate)
5. No. of leaf dissections per one side of the leaf	Quantitative (numeric)
6. No. of dissections on one side of the leaf (5) / leaf length (3)	Quantitative (numeric)
Inflorescence	
7. Peduncle length (mm)	Quantitative (numeric)
Capitulum	
8. No. of involucral bracts	Quantitative (numeric)
9. Apex of involucral bracts	Qualitative (acute / acuminate)
10. Woolly trichomes at the apex of involucral bracts	Qualitative (present / absent)

3.3.2. Morphometric data analysis

3.3.2.1. PCoA analyses

Principal Coordinate Analyses (PCoA; Gower, 2015) of the morphological data set were used to examine if *Senecio* aff. *glaucophyllus* and subsp. *glaucophyllus* sensu Ornduff are morphologically distinct. PCoA is an ordination method derived from Principal Component Analysis (PCA; Gower, 1966). Ordinations are techniques that enable relationships of multidimensional data points to be inspected along two- or three axes that explain most of the variation among these data points (Gauch, 1982). PCoA enables the visualization of morphological differences of individual specimens in distance-based, multivariate space on a plot with axes indicating the percentage of variation explained. Because of this, PCoA is one of the most popular methods for exploring morphometric and genetic distances (e.g., Martínez-Ortega et al., 2004; Henderson, 2006; Dufresne et al., 2014; Ahrens & James, 2015; Shepherd et al., 2015). Gower's dissimilarity coefficient was used to calculate pairwise morphometric distances among all specimens (Gower, 1971; Podani, 1999) and these formed the input for the PCoA analyses. One strength of Gower's distance is its ability to tolerate missing values in the data matrix (Gower, 1971; Podani, 1999). In addition to that, Gower's distance is also suitable for data sets that contain both qualitative and quantitative characters (Gower, 1971; Crisp & Weston, 1993), such as the data set of the present study. Because of these properties, Gower's distance is frequently used for morphometric studies (e.g., Drury & Randal, 1969; Crisp & Weston, 1993; Binns et al., 2002; Mrinalini et al., 2015). Gower's distance among all 72 specimens was calculated using the function 'daisy' in the package CLUSTER (Maechler et al., 2015) in R version 3.2.4 Revised (R Core Team, 2016) using the program RStudio (RStudio Team, 2015). Some of these specimens were not in flower and

their reproductive characters could therefore not be scored. Character 2 (width of the mid-cauline leaf) was excluded in the calculation of Gower's distances, because Character 1 (length of the mid-cauline leaf) and 2 are not independent if Character 3 (length/width ratio of the mid-cauline leaf) is also included in the analyses (Pelser & Houchin, 2004; Meudt et al., 2013). Instead of Character 5 (number of dissections of the leaf), the frequency of dissections of the leaf as measured by the ratio (Character 6; Table 3.1), which is comparable across leaves of different sizes, was used in the analyses. The PCoA analyses were executed using the function 'cmdscale' (Mardia, 1978) in the package STATS (of base R) in R.

3.3.2.2. Random Forest analysis

Random Forest (RF) classification (Breiman, 2001) was used (1) to examine if the ten putative diagnostic characters (Table 3.1) are able to effectively differentiate *Senecio* aff. *glaucophyllus* and subsp. *glaucophyllus* sensu Ornduff and (2) to identify the characters that are the most informative for distinguishing both taxa. The RF method has previously been used for multivariate data exploration in, for instance, genetic, epidemiological, and medicinal studies (e.g. Strobl et al., 2009; Touw et al., 2012) and has recently also been adopted in taxonomic studies (e.g. Shipunov et al., 2011; Skoracka et al., 2014; Moffat et al., 2015). In morphometric applications, the RF method predicts to which taxon each specimen belongs given the morphometric data, while simultaneously assessing which characters contribute to this prediction. In addition, it calculates the probabilities of taxon membership for each specimen. This approach also provides a way to confirm the identity of specimens that might have been misidentified or to identify morphologically intermediate specimens. The RF method accommodates mixed-type variables, which are typical for morphometric data, because these are often composed of both quantitative and qualitative characters, and assesses the importance of variables in the presence of covaried variables (Strobl et al., 2007, 2008; Moffat et al., 2015). RF and its predecessor, bagging (Breiman, 1996, 1998), are ensemble methods. The power of such methods is based on the aggregation of a committee of de-correlated classification trees (Hastie et al., 2009; Strobl et al., 2009). In simpler terms, in the RF method, an ensemble of classification trees each cast a vote on which group (e.g., taxon) a subject (e.g., specimen) belongs to a given set of predictor variables (morphological characters in this study). A detailed and schematic description of the RF method and its properties is presented by Touw et al. (2012). Strobl et al. (2009) used example data sets to illustrate the underlying mechanisms of classification trees, bagging and RF. Strobl et al. (2007, 2008) explain the importance of accounting for variable types and covariation among

variables in variable importance measures. In the current study, seven specimens with missing data for some of the characters were excluded from the RF analysis, because the variable importance measure cannot account for variables with missing data (Strobl et al., 2009). In order to rank the ten putative diagnostic characters in terms of their efficacy in distinguishing *S. aff. glaucophyllus* and subsp. *glaucophyllus* sensu Ornduff, each specimen included in the analysis needed to be assigned to a group. This was done on the basis of patterns of clustering in the results of the PCoA analyses (Figs. 3.2 and 3.3). The RF analysis was carried out using the 'cforest' function in the package PARTY (Hothorn et al., 2006; Strobl et al., 2007, 2008) in conjunction with the package RANDOMFOREST (Liaw & Wiener, 2002). The optimal number of randomly preselected splitting variables for the RF analysis was determined with the function 'tuneRF' in the RANDOMFOREST package using 100 iterations. This optimal number was used to build the forest of trees using the 'cforest' function, with the number of trees set at 1000. The output of the RF procedure was then used to generate a confusion matrix, which is used to calculate the misclassification rates for *S. aff. glaucophyllus* and subsp. *glaucophyllus* sensu Ornduff in order to examine how useful the ten characters are in differentiating the two taxa. The output of the RF analysis was also used to calculate the 'variable importance' of the ten characters in order to determine which characters are the most informative. This calculation was done with the function 'varimp' in the PARTY package, which computes the mean decrease in accuracy, for which more important variables would have higher values. To account for covaried variables, this calculation was done with the argument conditional = TRUE (Strobl et al., 2008).

3.3.3. Molecular phylogenetic analyses

To determine if *Senecio* aff. *glaucophyllus* and subsp. *glaucophyllus* sensu Ornduff are genetically distinct, phylogenetic analyses of an ITS DNA sequence data set were performed in which four ITS accessions of *S. aff. glaucophyllus* (three newly generated and one obtained from GenBank; Table S3), two accessions of subsp. *glaucophyllus* sensu Ornduff, three of subsp. *basinudus*, two of subsp. *discoideus*, and two of subsp. *toa* were included. A subset of accessions of the ITS data set used in Chapter 2 were used to determine the phylogenetic affinities of *S.* aff. *glaucophyllus* and subsp. *glaucophyllus* sensu Ornduff (see Table S2 for voucher details). DNA extractions, PCR amplification of the ITS region, sequencing of cleaned PCR products and phylogeny reconstruction using Bayesian inference (BI) followed the methods outlined in Chapter 2.

3.4. RESULTS

3.4.1. PCoA analyses

A PCoA analysis of a data set composed of Gower's distances among 72 specimens of *Senecio* aff. *glaucophyllus* and *S. glaucophyllus* sensu Ornduff resulted in a bidimensional plot (PCoA axis 1: 18.2%, PCoA axis 2: 4.9%) in which most specimens are placed in one of two distinct clusters (Fig. 3.2). One of these two clusters (Cluster 1) is centered around the type specimens of subsp. *basinudus* and subsp. *toa*, and the five representative specimens of *S. lautus* var. *montanus.* This cluster contains most of the specimens that have been identified as subsp. *glaucophyllus* sensu Ornduff and none of the *S.* aff. *glaucophyllus* specimens. The second cluster (Cluster 2) contains the lectotype and one of the three isolectotypes of *S. glaucophyllus* that were included in our studies, but does not contain any specimens of subsp. *glaucophyllus* sensu Ornduff. However, this cluster is also composed of all but one of the *S.* aff. *glaucophyllus* specimens. However, there are a number of specimens that are quite isolated from the two clusters. A closer inspection reveals that some of these specimens are non-flowering specimens and that they are therefore missing data for floral characters (four out of ten characters; Table 3.1). The bidimensional plots of the first axis vs. third axis and the second axis vs. third axis show similar patterns as the plot of the first axis versus second axis and are therefore not shown.

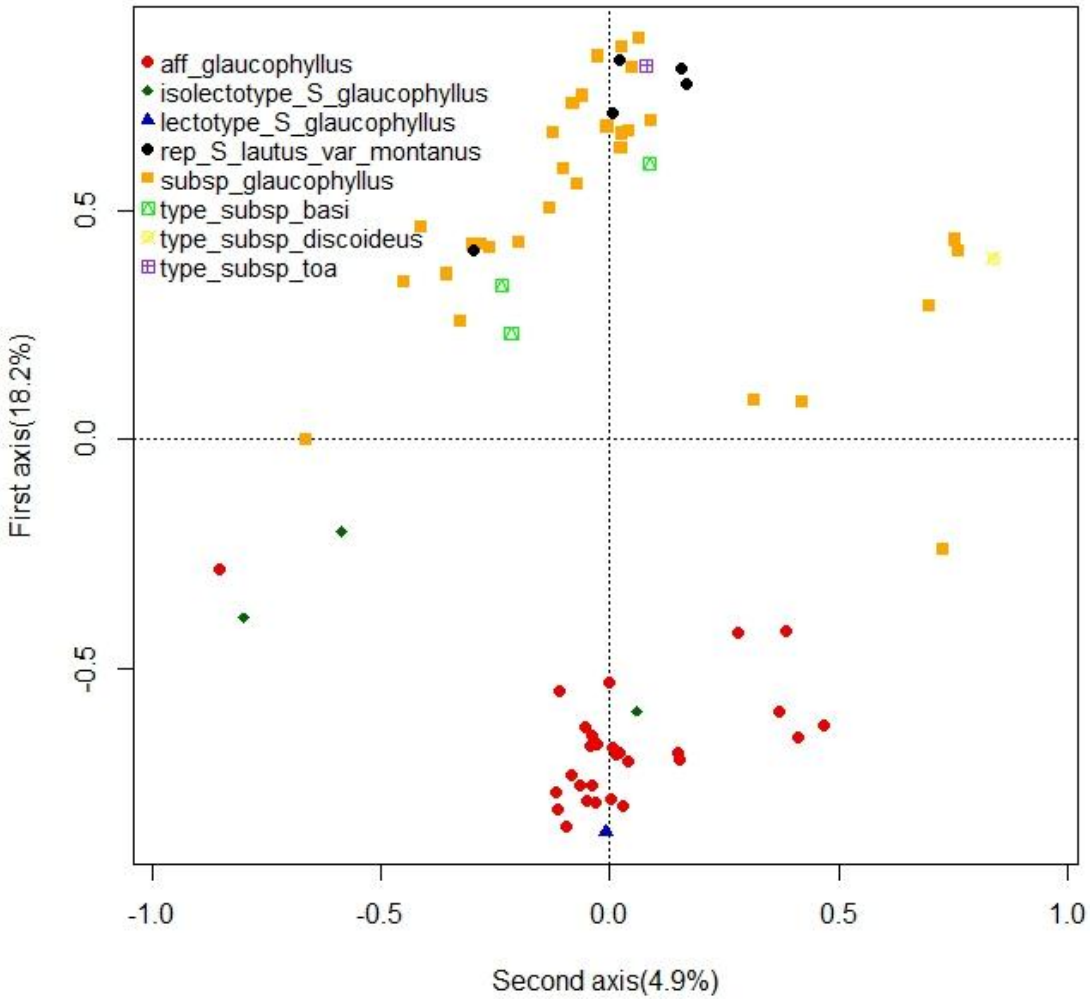


Fig. 3.2. Bidimensional plot of the first and second axes from a PCoA analysis of 72 specimens of *Senecio* aff. *glaucophyllus* and *S. glaucophyllus* sensu Ornduff including specimens that are not flowering. Different colors and symbols indicate the respective taxon that the specimens are putatively identified to and the type specimens.

In order to determine the effect of missing data on the computation of Gower's distances, five non-flowering specimens (four of *Senecio* aff. *glaucophyllus* and one of subsp. *glaucophyllus* sensu Ornduff) were removed from the morphometric data set and the analyses were rerun. The resulting PCoA bidimensional plot contains the same two clusters, but shows an increase in the variation explained by the first and second axes of the PCoA analysis (before removal (Fig. 3.2), first axis: 18.2%, second axis: 4.9%; after removal (Fig. 3.3), first axis: 30.7%, second axis: 6.5%). In addition, there are fewer specimens with ambiguous positions in the PCoA plot.

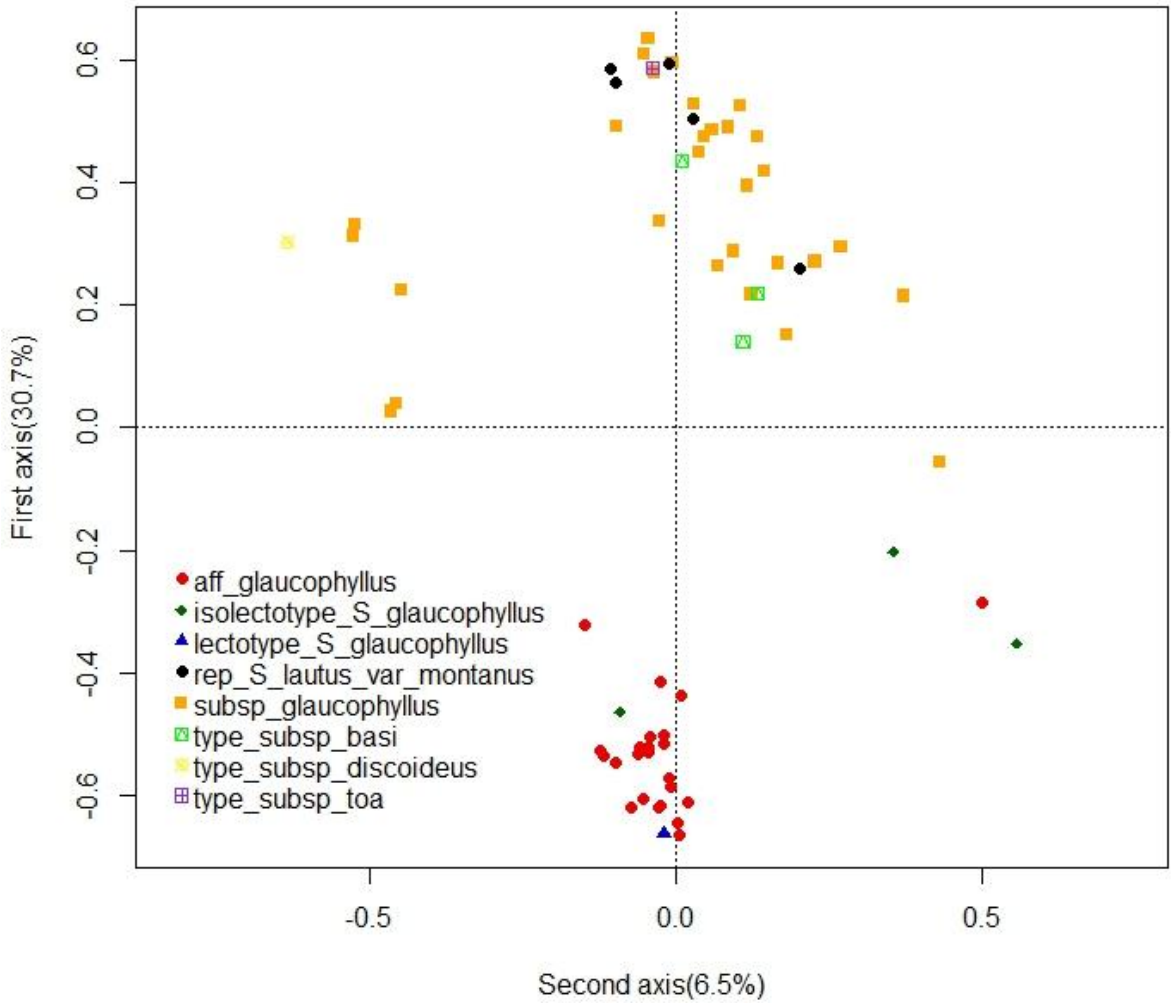


Fig. 3.3. Bidimensional plot of the first and second axes from a PCoA analysis of 67 specimens of *Senecio* aff. *glaucophyllus* and *S. glaucophyllus* excluding specimens that are not flowering. Different colors and symbols indicate the respective taxon that the specimens are putatively identified to and the type specimens.

3.4.2. Random Forest analysis

3.4.2.1. Predictive power of the morphological data

Six specimens of *Senecio* aff. *glaucophyllus* and one specimen of subsp. *glaucophyllus* sensu Ornduff were excluded from the RF analysis because of the presence of missing values. All morphological characters from Table 3.1 were included in the analysis, because RF can account for covaried variables. The results of the RF classification show that specimens identified as either *S.* aff. *glaucophyllus* or subsp. *glaucophyllus* sensu Ornduff are assigned correctly to their putatively identified taxon or classified group 98.5% of the time (i.e., a misclassification rate of 1.5%). The only specimen that was misclassified is a *S.* aff. *glaucophyllus* specimen (Table 3.2).

Table 3.2. Confusion matrix of *Senecio* aff. *glaucophyllus* and subsp. *glaucophyllus* sensu Ornduff.

	Predicted	
True	Subsp. *glaucophyllus* sensu Ornduff	*S.* aff. *glaucophyllus*
Subsp. *glaucophyllus* sensu Ornduff (PCoA Cluster 1)	39	0
***S.* aff. *glaucophyllus* (PCoA Cluster 2)**	1	25

3.4.2.2. Diagnostic characters

Among the ten characters included in this study, the number of involucral bracts (Character 8) is the most important diagnostic character as identified by the variable importance measure in the RF analysis (Fig. 3.4). *Senecio* aff. *glaucophyllus* has capitula with (10–)19(–22) involucral bracts, whereas subsp. *glaucophyllus* sensu Ornduff has capitula with fewer involucral bracts: (10–)13(–18) (Figs. 3.5a and 3.8). The second most diagnostic character is the shape of apex of the involucral bracts (Character 9), which is acuminate in *S.* aff. *glaucophyllus* (Fig. 3.8) and acute in subsp. *glaucophyllus* sensu Ornduff. The third highest ranked character is the ratio of the number of dissections per one side of the leaf and the mid-cauline leaf length (Character 6). Similar sized mid-cauline leaves of subsp. *glaucophyllus* sensu Ornduff have twice the number of dissections than those of *S.* aff. *glaucophyllus* (Fig. 3.5b). The fourth-most diagnostic character is the shape of the leaf margin of the mid-cauline leaves (Character 4). It is double serrate in subsp. *glaucophyllus* sensu Ornduff and single serrate to sinuate-dentate in *S.* aff. *glaucophyllus*.

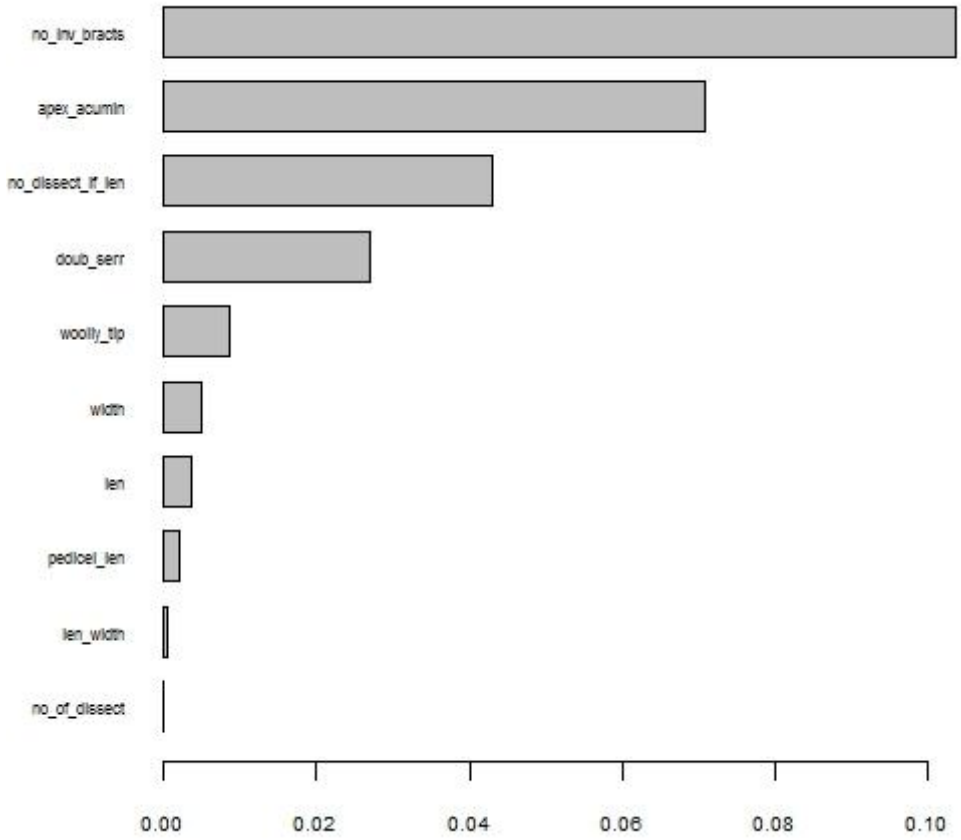


Fig. 3.4. Random Forest variable importance of the putative diagnostic characters of *Senecio* aff. *glaucophyllus* and subsp. *glaucophyllus* sensu Ornduff in decreasing order.

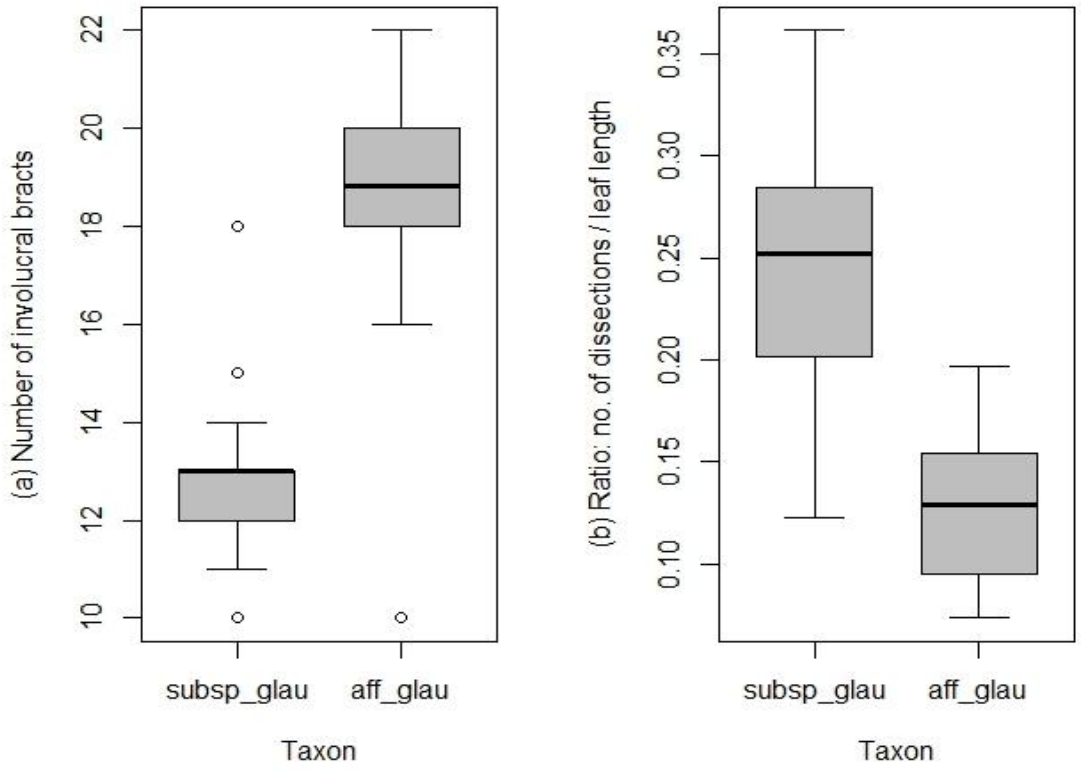


Fig. 3.5. Box plots illustrating the variation in the two most diagnostic quantitative characters for *Senecio* aff. *glaucophyllus* and subsp. *glaucophyllus* sensu Ornduff. a) Number of involucral bracts (Character 8) and b) the ratio of number of dissections on one side of the leaf and the mid-cauline leaf length (Character 6). The median (bisecting each box), lower and upper quartiles (box), minimum and maximum values (whiskers) and outliers (open circles) are shown.

3.4.3. Molecular phylogenetic analyses

The BI ITS phylogeny shows that accessions of *Senecio* aff. *glaucophyllus* and subsp. *glaucophyllus* sensu Ornduff group with different Australasian *Senecio* lineages (Fig. 3.6). Accessions of *S.* aff. *glaucophyllus* form a clade (posterior probability (PP) = 1.0) that is sister to *S. rufiglandulosus* Colenso (PP = 0.62) within Clade 2 (PP = 0.98). In contrast, accessions of the four subspecies of *S. glaucophyllus* including those of subsp. *glaucophyllus* sensu Ornduff form a clade (PP = 0.99) within the sub-clade of New Zealand Lautusoid species and an accession of *S. halophilus* I.Thomps. (PP = 1.0) in Clade 3 (PP = 0.99). ITS sequences (ITS1, 5.8S, ITS2) of the four accessions of *S.* aff. *glaucophyllus* share five synapomorphic nucleotide positions (1 in ITS1 and 4 in ITS2). These character states are not present in any of the other *Senecio* species included in the ITS alignment. The ITS sequences of *S.* aff. *glaucophyllus* are different from those of subsp. *glaucophyllus* in four insertions/deletions and 27 nucleotide substitutions.

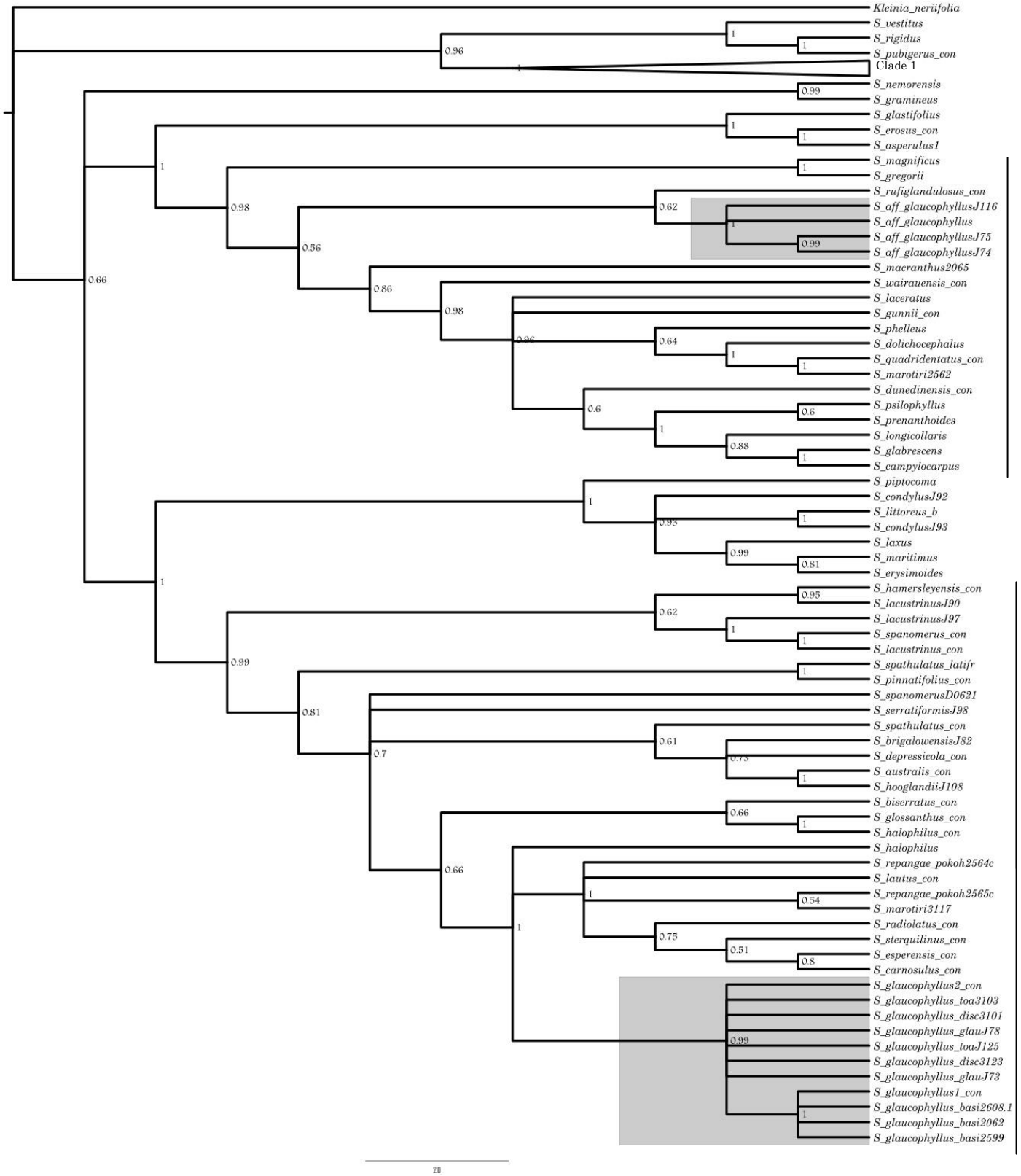


Fig. 3.6. Bayesian Inference majority rule consensus ITS phylogeny of Australasian *Senecio* and other relevant lineages. Clade labels correspond to those used in Chapter 2 (Fig. 2.1). Clades containing specimens of *S.* aff. *glaucophyllus* and *S. glaucophyllus* sensu Ornduff are highlighted in grey. "con" following species names indicates a consensus sequence of multiple accessions (see Materials and Methods of Chapter 2 for details). Numbers and letters following taxon names are used to distinguish multiple accessions of the same taxon (Table S3). Abbreviations for subspecies of *S. glaucophyllus* sensu Ornduff: _toa - subsp. *toa*, _disc - subsp. *discoideus*, _glau - subsp. *glaucophyllus* and _basi - subsp. *basinudus*. Voucher details of accessions that were also included in the phylogenetic analyses presented in Chapter 2 are presented in Table S2.

3.5. DISCUSSION

3.5.1. Distinguishing *Senecio* aff. *glaucophyllus* from *S. glaucophyllus subsp. glaucophyllus* sensu Ornduff

3.5.1.1. Morphological differences

Senecio aff. *glaucophyllus* and *S. glaucophyllus* subsp. *glaucophyllus* sensu Ornduff are morphologically similar. They are both herbs with radiate capitula and have a similar leaf shape (i.e., oblanceolate to obovate). This gives them a similar 'Gestalt' and they are therefore, without careful examination, morphologically cryptic. Especially juvenile plants of *S.* aff. *glaucophyllus* are often almost indistinguishable from plants of subsp. *glaucophyllus* sensu Ornduff (pers. obs.). Because of these morphological similarities and the fact that they occupy similar habitats (e.g. limestone rock crevices and outcrops), *S.* aff. *glaucophyllus* has often been mistaken for subsp. *glaucophyllus* sensu Ornduff. As a result, most specimens of *S.* aff. *glaucophyllus* are filed in herbaria as *Senecio glaucophyllus*.

Despite their morphological similarities, the results of the PCoA analyses of the morphometric data set show that *Senecio* aff. *glaucophyllus* and subsp. *glaucophyllus* sensu Ornduff are morphologically distinct, although a few specimens show a somewhat intermediate morphology (Figs. 3.2 and 3.3). Subspecies *glaucophyllus* sensu Ornduff appears to be morphologically more diverse than *S.* aff. *glaucophyllus*, because specimens of this taxon occupy a wider area of the morphospace (Figs. 3.2 and 3.3). The selected ten putative diagnostic characters proved useful in differentiating specimens of *S.* aff. *glaucophyllus* and subsp. *glaucophyllus* sensu Ornduff as shown by the presence of distinct clusters in the PCoA and the low misclassification rate for specimens of both taxa in the RF analyses (Table 3.2). These characters and several others resulting from further examination of herbarium specimens (Table 3.3) can be used to reliably distinguish *S.* aff. *glaucophyllus* from *S. glaucophyllus* subsp. *glaucophyllus* sensu Ornduff. All of these characters can be easily observed in a field setting with the use of a hand lens.

Table 3.3. Diagnostic morphological characters for differentiating between *Senecio aff. glaucophyllus* and *S. glaucophyllus subsp. glaucophyllus* sensu Ornduff.

Characters	subsp. *glaucophyllus* sensu Ornduff	*S.* aff. *glaucophyllus*
Vegetative:		
Leaf arrangement	Leaves widely spaced	Leaves densely spaced, especially in the middle section of the stem

Leaf glaucous	No	Yes
Mid-cauline leaf		
[$]Leaf margin	Double serrate, rarely single serrate	Single serrate to sinuate-dentate
Difference in leaf shape	Mostly oblong to oblanceolate	Mostly ovate to obovate
Size (mm)	(20.3–)42.7(–75.3) × (5.5–)12.5(–19.0)	(47.1–)67.9(–96.7) × (11.7–)20.4(–29.4)
[$]no. of dissections on one side of the leaf / length of the leaf	(0.12–)0.25(–0.36)	(0.07–)0.12(–0.20)
length of incision / width of the leaf	(0.04–)0.15(–0.46)	(0.02–)0.06(–0.11)
Uppermost leaf		
Size (mm)	(6.8–)18.7(–33.5) × (1.2–)5.3(–11.6)	(26.7–)42.2(–53.2) × (5.6–)9.9(–13.2)
no. of dissections on one side of the leaf / length of the leaf	(0–)0.32(–0.62)	(0.12–)0.17(–0.38)
length of incision	(0–)1.2(–6.2)	(0.45–)0.6(–0.75)
length of incision / width of the leaf	(0–)0.17(–0.53)	(0.04–)0.06(–0.11)
***Floral*:**		
Capitula		
no. of capitula per inflorescence	(3–)5(–8)	(6–)14(–34)
size of capitulum (mm)	(7.2–)8.2(–9.2) long × (5.3–)5.9(–7.2) diam.	(6.9–)7.4(–8.3) long × (6.7–)7.7(–8.6) diam.
ratio of length to diam. of capitulum	(1.17–)1.42(–1.72)	(0.87–)0.97(–1.10)
Radiate florets		
ligules (mm)	(2.3–)4.3(–6.1) x (1.1–)1.5(–2.1)	(6.3–)6.5(–7.1) x (1.7–)2.0(–2.2)
Size of corolla tube of radiate florets (mm)	(2.8–)3.5(–4.0) long × (0.25–)0.3(–0.4) diam.	(2.0–)2.3(–2.7) long × (0.3–)0.5(–0.7) diam.
ratio of length to diam. of corolla tube of radiate florets	(8.0–)11.4(–13.2)	(3.5–)5.1(–7.3)
Disc florets		
no. of disc florets	(36–)45(–53)	(59–)65(–69)
Immature achene length (mm)	(1.4–)1.6(–2.0)	(0.8–)0.93(–1.1)
Involucral bracts		
[$]Shape	Linear to lanceolate, apex acute *	Linear, apex acuminate up to about one half of the bracts
Indumentum	Glabrous, but short trichomes sometimes present, especially at the apex	A tuft of woolly trichomes at the apex
[$]no. of involucral bracts	(10–)13(–18)	(10–)19(–22)

* immature flower heads sometimes also have quite long tapering involucral bracts, although not as long as *S.* aff. *glaucophyllus*. [$] the four most diagnostic characters as identified by the RF analyses (Fig. 3.4)

3.5.1.2. Molecular phylogenetic differences

A total of three specimens of *Senecio* aff. *glaucophyllus* and one of subsp. *glaucophyllus* sensu Ornduff were included in the morphometric studies, as well as the molecular phylogenetic analyses. These specimens allowed me to determine how the morphometric clusters align with the two clades in which specimens of each taxon are resolved.

All sequenced specimens of the *Senecio* aff. *glaucophyllus* morphometric cluster align with species of the Quadridentatus group (sensu Chapter 2) in the ITS phylogeny (Fig. 3.6). In a plastid (psb*A*-trn*H*, trn*L* and trn*L*-*F*) phylogeny in which three specimens of this taxon were included (not shown), these specimens are also resolved as members of the Quadridentatus group. All species of this group for which chromosome number are known are 2*n* = 40. This suggests that *S.* aff. *glaucophyllus* might have this chromosome number as well, although this needs to be confirmed in future studies.

Accessions of subsp. *glaucophyllus* sensu Ornduff are affiliated with very different Australasian lineages as indicated by the ITS phylogeny (Fig. 3.6) and the phylogenetic findings presented in Chapter 2. In agreement with the results of the morphometric analyses, subsp. *glaucophyllus* sensu Ornduff is more closely related to the three other subspecies of *Senecio glaucophyllus* that Ornduff recognized (subsp. *basinudus*, subsp. *discoideus* and subsp. *toa*) than to *S.* aff. *glaucophyllus*. In agreement with the results of the morphometric analyses, this indicates that *S.* aff. *glaucophyllus* and *S. glaucophyllus* sensu Ornduff are distinct, but superficially cryptic, taxa. As outlined in Chapter 2, *Senecio glaucophyllus* sensu Ornduff is an allopolyploid (2n = 100) hybrid between the Lautusoid and Disciform s.s. groups.

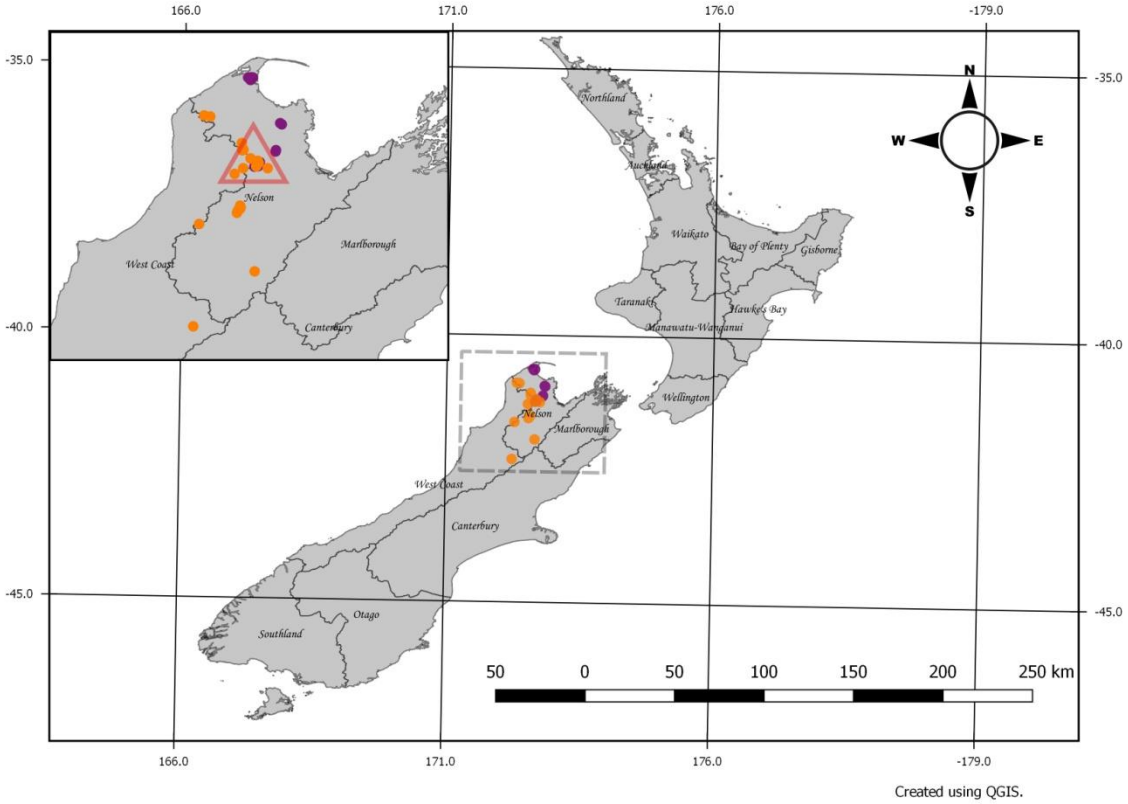


Fig. 3.7. Distribution map of *Senecio* aff. *glaucophyllus* and *S. glaucophyllus* subsp. *glaucophyllus* sensu Ornduff based on the locality data of the examined herbarium specimens. Orange: *S. glaucophyllus* subsp. *glaucophyllus* sensu Ornduff; Purple: *S.* aff. *glaucophyllus*; Red Triangle: Mt. Arthur. Coordinates of some of the specimens, especially old specimens, are approximated from Google Map©2016 from the locality data of herbarium specimens and might not be completely accurate.

3.5.2. Taxonomic realignment of *Senecio* aff. *glaucophyllus* and *S. glaucophyllus* sensu Ornduff

The lectotype and isolectotypes of *Senecio glaucophyllus* subsp. *glaucophyllus* are shown to bear more morphological resemblance to specimens of *S.* aff. *glaucophyllus* than to specimens of *S. glaucophyllus* subsp. *glaucophyllus* sensu Ornduff and the other three subspecies of Ornduff's (1960) infraspecific classification (Figs. 3.2 and 3.3). This implies that, in its current delimitation (Ornduff, 1960) the name *S. glaucophyllus* is misapplied to an undescribed species (including in Chapter 2 of this thesis) and that *S.* aff. *glaucophyllus* is the true *S. glaucophyllus*. The undescribed taxon is referred to as *S.* "pseudoglaucophyllus" in the remainder of this thesis.

In New Zealand, tag names have long been used to refer to entities that are deemed distinct by a panel of expert but are yet to be furnished with a formal name (Cameron et al, 1995;

Townsend et al, 2008). However, the usage of tag names can create conservation and taxonomic issues (Leschen et al., 2009). One of the consequences of using tag names for taxonomy is the lack of formal description, diagnostic feature and voucher specimen for these tag name entities. These create confusion about the taxonomic "reality" of these tag name entities because it is not clear if these entities are demed distinct by a single person or have been well-studied by a range of specialists (Leschen et al., 2009). In addition, the non-regulation of these entities by nomenclatural codes implies that more than one tag name might be attached to a single species of plant, therefore causes taxonomic confusion (Leschen et al., 2009). An example of such instance in the genus *Hebe* Comm. ex Juss. is the species *Hebe tairawhiti* Clarkson & Garnock-Jones, which was formerly known by two different tag names (Clarkson and Garnock-Jones, 1996). Tag names are usually enclosed in inverted commas, such as *Senecio* "pseudoglaucophyllus". From a nomeclatural perspective, the usage of tag names increases the risk of introducing *nomina nuda* when the inverted commas are accidentally omitted (Leschen et al., 2009). The potential taxonomic issues created by tag names have real conservation implications. For example, tag names, if not used consistently, will affect conservation management because it is not clear which groups of plants the names refer to (Leschen et al., 2009). In parallel with Leschen et al. (2009), I am of the view that tag names should not be used if possible and if unavoidable, a voucher or reference specimen deposited at an accredited institution is essential. In the literature, if tag names are to be included, conventions of those of Leschen et al. (2009) are recommended to ensure nomenclatural consistency. The undescribed taxon found as a result of this chapter was given a tag name, *S.* "pseudoglaucophyllus". The usage of this tag name and the repeated explanation of the taxonomic issues of *S. glaucophyllus* and *S.* "pseudoglaucophyllus" for the remaining of my thesis are unavoidable because of the lack of an official name for the undescribed species.

Senecio "pseudoglaucophyllus" finds its origin in Cheeseman's *Senecio lautus* var. *discoideus* and var. *montanus* (1906), and Hooker's *Senecio lautus* var. *raoulii* (Hooker, 1853). Cheeseman considered *S. glaucophyllus* and *S.* "pseudoglaucophyllus" distinct. This is not only evident from his taxonomic treatment of these taxa, but also from his annotations of specimens collected by him from Mt. Arthur. AK and WELT contain six of these specimens, of which four were annotated by him as *S. glaucophyllus* (Cheeseman s.n. Jan-1886: AK 10601, AK 10602, AK 10604, WELT SP043140) and two as *Senecio lautus* var. *montanus* (Cheeseman s.n. Jan-1886: AK 10591, AK 10592). This suggests that Cheeseman was well

aware of the morphological differences between both taxa. Although Cheeseman's description of *S. glaucophyllus* contains several of the diagnostic characters that also came to light in this study (e.g., glaucous leaves, dense foliage, and acuminate involucral bracts with woolly trichomes at the apex), Ornduff (1960) seemed to have overlooked these features when he prepared his taxonomic treatment of this species or considered them uninformative.

Before *Senecio* "pseudoglaucophyllus" can be formally named and described, more information about its delimitation and morphological diversity is needed. This requires a study of the patterns of morphological and genetic diversity of the four subspecies that Ornduff (1960) recognized for this taxon. This study is the topic of Chapters 4 & 5.

In addition to identifying *Senecio* "pseudoglaucophyllus" as a new and undescribed species, this study also revealed another taxonomic issue that needs to be addressed. Although Herrick & Cameron (1994) list seven syntypes for *S. lautus* var. *montanus* in AK and these specimens have been annotated as such, Cheeseman (1906) did not mention any specimens in his protologue of *S. lautus* var. *montanus*. This means that the plants that Herrick & Cameron (1994) identified as syntypes are, at best, representative specimens of this taxon. This indicates the need to lectotypify *S. lautus* var. *montanus*. Although I am planning to do this in the published version of this chapter, I refrain from presenting the lectotypification in this thesis, because this would not constitute valid publication and would therefore potentially create confusion if my thesis is made publicly available online prior to the publication of this chapter in a scientific journal.

3.5.3. Geography, ecology, and conservation

Senecio glaucophyllus and subsp. *glaucophyllus* sensu Ornduff are taxa with a rather small distribution area. The former is restricted to North-West Nelson and the latter has a distribution area that extends from this area into bordering areas, such as West Coast (Fig. 3.7). *Senecio glaucophyllus* does not only have a smaller distribution area than subsp. *glaucophyllus* sensu Ornduff, but is also known from fewer populations. Only five populations of *S. glaucophyllus* are currently known: Kahurangi National Park (Arthur Range, Mt. Arthur, The Twins and Hoary Head), NW Nelson Forest Park (Mt. Burnett) and Abel Tasman National Park (The Gorge Creek in East Takaka) (Fig. 3.7), whereas subsp. *glaucophyllus* sensu Ornduff is known from at least 18 populations. *Senecio glaucophyllus* is therefore more rare than subsp. *glaucophyllus* sensu Ornduff. Habitat data from the examined herbarium specimens show that both taxa are basicolous species favoring base-rich substrates

such as limestone, dolomite, dolomite marble and marble, often in rock crevices, on exposed rock outcrops and on rock taluses. Because they grow in similar habitats, conservation management strategies aimed at one of these taxa will also benefit the other.

The results of this study have implications for the conservation of *Senecio glaucophyllus* and subsp. *glaucophyllus* sensu Ornduff. Because of the misapplication of the name *S. glaucophyllus* and in contrast to the conservation assessment by de Lange et al. (2013a), *S. glaucophyllus* should have the conservation status of Nationally Vulnerable and subsp. *glaucophyllus* sensu Ornduff the status of Naturally Uncommon under the New Zealand Threat Classification System. Both taxa are currently considered Data Poor. This study addressed this knowledge gap by providing new information about their morphological diversity and evolutionary relationships and resulted in the discovery of diagnostic characters for *S. glaucophyllus* and subsp. *glaucophyllus* sensu Ornduff, which can be used to identify these taxa in future conservation-relevant studies.

Because *Senecio* "pseudoglaucophyllus" is revealed to be an undescribed species, it will be treated as a 'taxonomically indeterminate' taxon and therefore will need a reference specimen in order to be included in future conservation assessments (Townsend et al., 2008). In addition, reference specimens would need to be selected for any infraspecific taxa of *S.* "pseudoglaucophyllus" that might need to be recognized. Because the infraspecific classification of *S.* "pseudoglaucophyllus" is in need of revision and this is the topic of Chapters 4 & 5, I refrain from selecting these specimens here.

3.6. CONCLUSION

The results of this study show that *Senecio glaucophyllus* sensu Ornduff (1960) is a cryptic species complex composed of two superficially similar species. Plants of one of these species form an undescribed, yet well-known species to which the name *S. glaucophyllus* has been misapplied. This species contains a plant group that is currently referred to as *S. glaucophyllus* subsp. *glaucophyllus* sensu Ornduff. Of the four subspecies that Ornduff recognized, this subspecies is morphologically most similar to the true *S. glaucophyllus*. The results of my morphometric and molecular phylogenetic studies also show that *S. glaucophyllus* and subsp. *glaucophyllus* sensu Ornduff are morphologically and genetically distinct. Furthermore, I discovered several diagnostic characters that can be used to reliably differentiate the two taxa. The clarification of the taxonomic status and delimitation of the two taxa will be especially helpful for field workers who survey and monitor the populations

of *S. glaucophyllus* and subsp. *glaucophyllus* sensu Ornduff and will thereby contribute to their conservation management. In the next two chapters, I aim to revise the infraspecific classification of the unnamed species, which is here informally named *S.* "pseudoglaucophyllus".

Fig. 3.8. Flower heads of representative specimen of *Senecio glaucophyllus* subsp. *glaucophyllus* sensu Ornduff (left) and *S. glaucophyllus* (right). Note: more numerous and acuminate involucral bracts and longer ligules in *S. glaucophyllus* compared to subsp. *glaucophyllus* sensu Ornduff. Photo credit: Allan Herbarium©.

CHAPTER 4: Patterns of morphological diversity in *Senecio* "pseudoglaucophyllus"

4.1. ABSTRACT

Senecio "pseudoglaucophyllus" is a tag name for an unnamed, but well-known, New Zealand species. This species composes the larger part of *S. glaucophyllus* sensu Ornduff, but does not include the type of *S. glaucophyllus* Cheeseman. *Senecio* "pseudoglaucophyllus" is a morphologically very variable species for which currently four infraspecific groups are recognized. However, some specimens, including those of two morphological forms from Marlborough cannot be unambiguously assigned to any of these groups. The aim of this study was to use a morphometric phenetic approach to determine if patterns of morphological variation within *S.* "pseudoglaucophyllus" support the formal taxonomic recognition of the four infraspecific groups, to identify their diagnostic characters, and to resolve the taxonomic status of the two Marlborough morphotypes. PCA and Random Forest analyses identified 16 morphological characters that are most informative for studying patterns of morphological diversity in *S.* "pseudoglaucophyllus". The results of multivariate (PCoA, NMDS, hierarchical cluster analyses, ANOSIM) and univariate phenetic analyses of a morphometric data set obtained from these 16 characters show patterns of morphological similarity that do not support formal taxonomic recognition of the four infraspecific groups in their current delimitation. Instead, these patterns reveal the existence of two poorly defined morphological groups with many intermediate specimens. A Mantel test further showed the presence of geographical structuring in the morphological data. These results are used to discuss if the two infraspecific groups of *S.* "pseudoglaucophyllus" merit formal taxonomic recognition or, alternatively, if this species is best considered as a taxon that displays large but near-continuous morphological variation and for which infraspecific taxa should not be recognized.

4.2. INTRODUCTION

The results of Chapter 3 reveal that Ornduff (1960) and subsequent authors who adopted his classification used a taxonomic concept of *Senecio glaucophyllus* Cheeseman that includes two species: *S. glaucophyllus* Cheeseman and *Senecio* "pseudoglaucophyllus". They also show that these two species are only distantly related to each other and morphologically different. In addition, these results demonstrate that *S. glaucophyllus* subsp. *glaucophyllus* sensu Ornduff p.p. (excl. *S. glaucophyllus* sensu Cheeseman, 1895), *S. glaucophyllus* subsp. *basinudus* Ornduff, *S. glaucophyllus* subsp. *toa* C.J.Webb, and *S. glaucophyllus* subsp. *discoideus* (Cheeseman) Ornduff are infraspecific taxa of *S.* "pseudoglaucophyllus". These

findings leave *S.* "pseudoglaucophyllus" and its four subspecies in nomenclatural limbo, because *S.* "pseudoglaucophyllus" is a taxon that is unnamed at the species-level. The taxonomy of *S.* "pseudoglaucophyllus" is further complicated by substantial morphological variation within some of the subspecies and plants that are morphologically intermediate between subspecies (Ornduff, 1960). These issues highlight the need to revisit the current infraspecific classification of *S.* "pseudoglaucophyllus" and to recommend taxonomic changes based on the findings of this study. For the purpose of this chapter, *S. glaucophyllus* subsp. *glaucophyllus* sensu Ornduff p.p. (excl. *S. glaucophyllus* sensu Cheeseman) will be referred to as the Nelson-group and the remaining three subspecies within *S.* "pseudoglaucophyllus" as subsp. *basinudus*, subsp. *discoideus*, and subsp. *toa*.

4.2.1. The taxonomic history of *Senecio* "pseudoglaucophyllus"

Senecio "pseudoglaucophyllus" finds its taxonomic origin in *S. lautus* G.Forst. ex Willd. when the latter was delimited as a hyper variable species native to Australia and New Zealand (Hooker, 1853; Cheeseman, 1906; this delimitation included most species of the Lautusoid group as outlined in Chapter 2). Ornduff (1960) preferred a narrower delimitation of *S. lautus.* He reinstated a species that was subsumed in *S. lautus* by others (i.e., *S. radiolatus* F.Muell.) and elevated an infraspecific taxon that was recognized for *S. lautus* to species level (i.e., *S. sterquilinus* Ornduff; Ornduff 1960). In addition, he transferred *S. lautus* var. *montanus* Cheeseman to *S. glaucophyllus*, expanding Cheeseman's (1906) concept of this species. Ornduff (1960) considered the former a composite taxon that is in part synonymous with *S. glaucophyllus* subsp. *glaucophyllus* and in part synonymous with his newly named *S. glaucophyllus* subsp. *raoulii* (Hook.f.) Ornduff. In addition to subsp. *glaucophyllus* and subsp. *raoulii*, Ornduff (1960) recognized two other subspecies: *S. glaucophyllus* subsp. *discoideus* for *S. lautus* var. *discoideus* Cheeseman, and *S. glaucophyllus* subsp. *basinudus* for a group of plants that he considered conspecific with *S. glaucophyllus* but regarded morphologically and ecologically distinct from his other three subspecies (Ornduff, 1960). Ornduff's (1960) infraspecific classification into four subspecies is followed until this day (including in Chapter 2 of this thesis), although subsp. *raoulii* was renamed as subsp. *toa* C.J.Webb by Webb in Connor & Edgar (1987) when the lectotype selected by Ornduff (1960) for subsp. *raoulii* was found to be a specimen of subsp. *basinudus* and the name *S. glaucophyllus* subsp. *raoulii* (Hook.f.) Ornduff therefore could not be used for Ornduff's subspecies.

4.2.2. The infraspecific groups of *Senecio* "pseudoglaucophyllus"

All four infraspecific groups of *Senecio* "pseudoglaucophyllus" are present in the South Island while subsp. *discoideus* and subsp. *toa* are also found in the North Island (Ornduff, 1960; Webb et al., 1988, Fig. 4.7). The distribution areas of three of the four infraspecific groups are almost non-overlapping and largely parapatric in the South Island (Fig. 4.7). The distribution of subsp. *toa* in the South Island, however, overlaps with that of subsp. *basinudus* and subsp. *discoideus*. While in the mountain ranges of central and southern North Island, subsp. *toa* and subsp. *discoideus* also co-occur.

The Nelson-group and subsp. *basinudus*. The results of Chapter 3 indicate that the Nelson-group is a taxon that was previously treated by Cheeseman (1906) as part of a broader delimited *Senecio lautus* var. *montanus.* Cheeseman (1906) described *S. lautus* var. *montanus* as an erect, quite simple or sparingly branched plant with oblong to spathulate leaves that have an entire or dentate margin or are shallowly pinnatifid, and have radiate capitula with revolute rays and that are 12–19 mm in diameter. Cheeseman's (1906) concept of *S. lautus* var. *montanus* includes radiate plants that grow in mountain ranges in North and South Island. In his treatment, Ornduff (1960) segregated *S. lautus* var. *montanus* into those plants without pinnatifid leaves and restricted to northern South Island, which he included in his concept of *S. glaucophyllus* as *S. glaucophyllus* subsp. *glaucophyllus*, and those with pinnatifid leaves as *S. glaucophyllus* subsp. *raoulii* Ornduff (1960) (now subsp. *toa*).

According to Ornduff (1960), *Senecio glaucophyllus* subsp. *glaucophyllus* sensu Ornduff (1960) is most similar to subsp. *basinudus* and this also holds true for the Nelson-group, which has a narrower delimitation than subsp. *glaucophyllus* sensu Ornduff (1960). Plants of the Nelson-group and subsp. *basinudus* both lack deeply pinnatifid leaves, but can be distinguished from each other by other morphological characters; the former are erect and branch only at the base and the latter are erect or prostrate and also branch freely above the base; the inflorescences of the Nelson-group are loose corymbs and those of subsp. *basinudus* are loose panicles; the ligules are longer in the former (2.3–6.1 mm) than in the latter (nearly absent to 3.5 mm).

Although the Nelson-group and subsp. *basinudus* share morphological similarities, they have very different habitat preferences: plants of the Nelson-group grow in calcareous montane to alpine habitats, whereas those of subsp. *basinudus* mostly grow on basalt rock outcrops and rubble slopes (less commonly on associated sand dunes), usually near the coast. The Nelson-

group is only known from mountains (600–1700m) in North-West Nelson and in the adjacent northeastern part of West Coast (Ornduff, 1960; data from Chapter 3; Figs. 3.7 and 4.7). Subspecies *basinudus* is most commonly collected from the Port Hills, Banks Peninsula, and Otago Peninsula and extends as far south as the Catlins in Southland (Ornduff, 1960; Webb et al., 1988; J. Liew, unpubl. data; Fig. 4.7).

Subspecies *discoideus.* Cheeseman (1906) described subsp. *discoideus* plants (as *Senecio lautus* var. *discoideus*) as rarely erect or more commonly prostrate or decumbent plants that branch sparingly and have very fleshy, obovate or spathulate leaves that are "coarsely toothed or lobed, sometimes pinnatifid below" and have large capitula of 12–19 mm in diameter that lack radiate flowers. However, Ornduff's (1960) description of subsp. *discoideus* does not mention if the leaves of this taxon are fleshy and he presented capitulum diameter measurements of subsp. *discoideus* that are a lot smaller (6–9 mm) compared to what Cheeseman (1906) recorded for this taxon (12–19 mm). He, however, agreed with Cheeseman regarding the plant's habit and presence of discoid capitula (Ornduff, 1960). Ornduff (1960) characterized subsp. *discoideus* as "an ecotype adapted to unstable scree" and, in combination with discoid capitula, this habitat character differentiates subsp. *discoideus* from the other three infraspecific groups. Other than in the mountain ranges in the central and south of the North Island, subsp. *discoideus* can also be found in montane to subalpine areas in the Southern Alps and along the east coast of South Island (Ornduff, 1960; Webb et al., 1988, Fig. 4.7).

Subspecies *toa.* Subspecies *toa* is characterized by deeply pinnatifid mid-cauline leaves, which differentiate it from the three other subspecies (Connor & Edgar, 1987). In addition, it differs from subsp. *discoideus* by having radiate capitula (Connor & Edgar, 1987). Distribution and habitat data gathered from examined herbarium sheets indicate that subsp. *toa* colonizes a range of substrates in open habitats and can be found from western Hawkes Bay, shore of Lake Taupo and the adjacent eastern Central North Island ranges whence it is then absent until it reappears in the north eastern South Island extending to South Canterbury. In these places it has been collected from coastal sites up to an elevation of 1550m. Ornduff (1960) noted that subsp. *toa* (as subsp. *raoulii*) grows at lower elevations in the South Island compared to the North Island and that the plants of this subspecies are more uniform in the North Island than those in the South Island. He also observed that subsp. *toa* "appears to merge with other subspecies on the periphery of its South Island range" (Ornduff, 1960).

4.2.3. Problems with the infraspecific taxonomic delimitation of *Senecio* "pseudoglaucophyllus"

As outlined in the previous section, Ornduff's (1960) infraspecific delimitation of *Senecio* "pseudoglaucophyllus" is based on differences in distribution, ecology and morphology between the four infraspecific groups. However, there are plants that cannot confidently be assigned to one of these four groups, because they are morphologically intermediate (Ornduff, 1960, 1962). Ali (1964) was therefore of the opinion that the morphological variation observed in *S.* "pseudoglaucophyllus" is of a clinal nature and that infraspecific taxa therefore should not be recognized. Webb (1988) and Webb et al. (1988) also acknowledged the presence of taxonomic problems in *S.* "pseudoglaucophyllus" and suggested that there might be a need for revisiting its delimitation. Some examples of problematic plants include those from Marlborough as highlighted by both Ornduff (1960) and Webb et al. (1988), particularly plants that grow in coastal areas between Blenheim and Kaikoura, which show an admixture of diagnostic characteristics of three of the four groups within the complex. In addition, short-rayed plants of subsp. *discoideus* and rayless plants of the other subspecies have also been observed in the field (Webb et al., 1988; pers. obs., Fig. 4.6), blurring the distinction between this subspecies and the other infraspecific groups. Moreover, Ornduff (1960) noted the presence of morphological intermediates on the seaward side of the range of subsp. *discoideus* between this subspecies and perhaps subsp. *basinudus* or *toa*. A final example that illustrates these taxonomic problems is the existence of Marlborough plants that resemble subsp. *toa*, but have larger capitula (Druce & Williams, 1989).

4.3. AIMS

The aim of this study is to use a morphometric phenetic approach to 1) revisit Ornduff's (1960) amended infraspecific classification of *Senecio* "pseudoglaucophyllus" into subsp. *basinudus*, subsp. *discoideus*, subsp. *toa* and the Nelson-group and to 2) identify morphological characters that are of diagnostic value in distinguishing these four groups. This study also aims to contribute to resolving the taxonomic status of problematic and intermediate Marlborough plants by including representatives of two Marlborough morphotypes (*S.* aff. *glaucophyllus* "South Marlborough" and *S.* aff. *glaucophyllus* "Cape Campbell") in the morphometric analyses.

4.4. MATERIALS AND METHODS

4.4.1. A phenetic approach to testing morphology-based taxonomical hypotheses

Phenetic analyses determine relationships among operational taxonomic units (OTUs) based on similarities of observable properties (e.g., morphological, genetic, physiological, and biochemical characters; Sokal & Crovello, 1970) and are widely employed in numerical taxonomy (Sneath & Sokal, 1973; Sneath, 1995; Cron et al., 2007; Jensen, 2009). In such studies, these analyses use patterns of phenotypic similarities and differences to identify groups of related individuals under the assumption that phenotypic similarity is a suitable proxy for evolutionary relatedness (Jensen, 2009).

There might be differences in species recognition in botany and zoology. For example, Luckow (1995) examined the application of species concepts in practice by sampling botanical and zoological papers published during 1989-1993 in three journals (*Systematic Botany/Zoology/Biology*). She found that "Phylogenetic", "Quantitave" and "Phenetic" species concept were more common among the botanical literature while "Biological" and "Monophyletic" species concepts were used in majority of the zoological papers (Luckow, 1995). However, Sangster (2014) obtained incongruent results in surveying >1000 avian taxonomic studies. He discovered that criterion for species recognition under the Phylogenetic Species Concept was more frequently applied than the criterion for species recognition under Biological Species Concept (Sangster, 2014) for avian taxonomy. On the other hand, McDade (1995) surveyed 104 botanical monographs from three journals between the years 1984-1993. Her study found that most botanists did not discuss which species concepts were used and for those who did, most employed a "Morphological" or "Taxonomic" species concepts (McDade, 1995).

In this study of *Senecio* "pseudoglaucophyllus", a morphometric phenetic approach is used and a genotypic cluster species concept (Mallet, 1995; Coyne & Orr, 2004) is chosen as an operational taxonomic concept for recognizing infraspecific taxa. The genotypic cluster species concept defines species as "distinguishable groups of individuals that have few or no intermediates when in contact" and can be applied to both morphological and genetic data sets (Mallet, 1995; Coyne & Orr, 2004). To quantify "distinguishable" in this definition, I follow the subspecies concept for botanists recommended by Ellison et al. (2014) (modified from the zoological subspecies concept suggested by Braby et al. (2012)) which states that infraspecific taxa should have "at least one fixed diagnosable character state". Mallet (1995)

views the rank of subspecies as similar to that of a species with the exception of the former's ability to produce intermediates in areas of sympatry. Therefore, if the morphometric analyses of this study would show that the four infraspecific groups are each composed of individuals that are morphologically more similar to each other than to individuals of the other three groups, this would be considered as evidence supporting Ornduff's amended infraspecific classification. However, if distinct groups of individuals would be discovered that do not align with those recognized in Ornduff's amended infraspecific classification, than these would be considered as candidates for taxonomic recognition. Finally, if morphometric analyses would not recover distinct groups of individuals with or without a few individuals of intermediate morphology, this is taken as evidence that infraspecific taxa should not be recognized for *S.* "pseudoglaucophyllus".

4.4.2. Morphometric data collection

Herbarium specimens of the Nelson-group, subsp. *basinudus*, subsp. *discoideus*, and subsp. *toa* were selected to maximize representation of populations throughout their distribution ranges and of their morphological diversity in the analyses. Herbarium specimens of the Nelson-group (n = 29), subsp. *basinudus* (n = 40), subsp. *discoideus* (n = 28) and subsp. *toa* (n = 26) from AK, CANU, CHR, and WELT (Table S4) were included in the studies. These specimens included type specimens of *Senecio glaucophyllus* subsp. *basinudus*, subsp. *discoideus*, and subsp. *toa*, which were also included in the analyses presented in Chapter 3. Among the examined specimens of subsp. *basinudus*, 11 herbarium sheets from the same population (Sykes 496/69, Okains Bay, Banks Peninsula, CHR194703A–K) were included as a case study to investigate the extent of morphological variation within a single population. Ornduff's (1960) identification key and the diagnostic characters for the Nelson-group as identified in Chapter 3 were used to assign each specimen to one of the four infraspecific groups. In addition to herbarium specimens of the four groups of *S.* "pseudoglaucophyllus", seven problematic and intermediate Marlborough plants of two informally recognized morphotypes (*S.* aff. *glaucophyllus* "South Marlborough" (possibly *S. glaucophyllus* ssp. (b) in Druce & Williams, 1989) and *S.* aff. *glaucophyllus* "Cape Campbell") and one specimen from North Canterbury that could not be identified to any of the four infraspecific groups were also included in the morphometric data set (Table S4). Among the examined specimens from CANU, some specimens were previously collected as voucher specimens for the molecular genetic analyses presented in Chapters 3 and 5. These specimens were mostly collected by staff of the New Zealand Department of Conservation (Canterbury: Nicholas

Head, Daniel Kimber; Nelson: Shannel Courtney; Otago: John Barkla), QE II National Trust (Private properties in Canterbury: Alice Shanks and Miles & Gillian Giller), with permission from private land owners in Banks Peninsula and Otago Peninsula and a Christchurch City Council permit for collecting flora and fauna in public land around Banks Peninsula and the Port Hills. Collection details of these specimens are presented in Chapters 3 and 5.

A list of characters chosen for preliminary phenetic analyses was compiled from those proven to be useful at the infraspecific level in other Senecioneae species (e.g., Pelser et al., 2004; Pelser & Houchin, 2004; Hodálová et al., 2007; Pelser et al., 2012; Lowe & Abbott, 2015), characters that have been used to distinguish Australasian *Senecio* species (Ali, 1964; Belcher, 1993; Radford et al., 2004; Thompson, 2005), and most importantly, characters that Ornduff (1960) used in his descriptions of the four subspecies of *S. glaucophyllus* sensu Ornduff and in his identification key. The preliminary analyses included 39 floral and 54 qualitative and quantitative vegetative characters. Following the scoring of these 93 characters from 44 herbarium specimens, 48 characters were excluded from the data set, because these resulted in too many missing characters in the data set (e.g., missing characters of the radiate florets, because of their absence in most specimens of subsp. *discoideus*), because of difficulties with scoring due to variation between specimens due to differences in their age (e.g., coloration of leaf surfaces), because some characters were considered uninformative because they displayed too much variation within an individual specimen (e.g., shape of midrib), and because the basal part of the plant was lacking in many specimens (e.g., missing characters of lower leaves and of branching patterns). A data set of 45 characters was subsequently used to perform preliminary analyses to identify the most informative characters for documenting patterns of morphological variation within *S.* "pseudoglaucophyllus". A principal component analysis (PCA) was used to identify the ten characters that contributed most to the variance along the first three principal component axes by examining character loadings along these three axes (Table S5). This PCA analysis was done in R using the function "prcomp" with the argument of "scale = TRUE" to obtain unit variance for all 45 characters. In addition to this approach, I identified the most informative characters among the selected 45 by performing a Random Forest (RF) analysis. For this, first a principal coordinate analysis (PCoA) using the method outlined in Chapter 3 was carried out to visualize the morphospace of the initial data set. This analysis indicated that the specimens that were included in the preliminary phenetic analyses group into two indistinct and adjacent clusters along principal component axis 1 (Fig. S1). Membership to these clusters was used as input for a RF analysis. Specimens that could

not unambiguously be placed in one of the two PCoA clusters (due to an intermediate morphology) were grouped with the cluster to which the nearest specimen in the PCoA plot was assigned. The RF analysis was executed using the method outlined in Chapter 3. Thirteen characters were deemed informative (Table S6) in the RF analysis and these characters also ranked highly (among the top ten) when the PCA approach was used (Table 4.1). One of the characters (achene length to width ratio) that was identified as informative was excluded from subsequent morphometric analyses, because it could not be scored without dissecting capitula and would therefore result in too much damage to specimens. The combined results of the PCA and RF analyses were used to select a total of 16 characters (Table 4.1) for studying a larger number of herbarium specimens than the 44 specimens included in the preliminary study (i.e., n = 130).

Table 4.1. Final list of characters chosen for morphometric analyses of *Senecio* "pseudoglaucophyllus". Characters selected by the RF and the PCA analyses are marked with * and $^{\$}$ respectively.

Code	Character	Type
	Upper leaf (leaf subtending the inflorescence)	
1*$^{\$}$	Leaf division (considered as divided if incision length >30% of total leaf width)	Qualitative: undivided (1), divided (0)
2*$^{\$}$	Leaf length/width ratio	Quantitative: numeric
3$^{\$}$	No. of dissections on one side of the leaf divided by leaf length (to standardize for leaf size)	Quantitative: numeric
4*$^{\$}$	Degree of leaf incision (length of incision divided by leaf width)	Quantitative: numeric
5*$^{\$}$	Double serrate leaf margin	Qualitative: present (1), absent (0)
	Mid-cauline leaf	
6*$^{\$}$	Leaf division (considered as divided if incision length >30% of total leaf width)	Qualitative: undivided (1), divided (0)
7*$^{\$}$	Leaf length (mm)	Quantitative: numeric
8*$^{\$}$	Degree of leaf incision (length of incision divided by leaf width)	Quantitative: numeric
9*$^{\$}$	Double serrate leaf margin	Qualitative: present (1), absent (0)
10*	Petiole	Qualitative: present (1), absent (0)
	Floral characters	
11*$^{\$}$	Flower head radiate	Qualitative: yes (1), no (0)
12$^{\$}$	No. of involucral bracts	Quantitative: numeric
13*$^{\$}$	Length of involucral bracts (mm)	Quantitative: numeric
14*	Trichomes at the base of receptacle	Qualitative: present (1), absent (0)
15$^{\$}$	Length of supplementary bracts (mm)	Quantitative: numeric

16[$]	Length/width ratio of supplementary bracts	Quantitative: numeric

4.4.3. Morphometric data analysis

4.4.3.1. Multivariate analyses

Multivariate analyses (PCoA, non-metric multidimensional scaling **(**NMDS), cluster analyses, and an analysis of similarities (ANOSIM)) were used to study the delimitation of the four infraspecific groups of *Senecio* "pseudoglaucophyllus".

A PCoA was carried out using the method outlined in Chapter 3 using Gower's distances computed from the data of 130 examined specimens. In addition to PCoA, another ordination method (NMDS) was used. NMDS has been shown to outperform PCoA in some ecological (Minchin, 1987) and taxonomical (Crisp & Weston, 1993; Pimental, 1981) studies. NMDS is a rank-order ordination that possesses all benefits of PCoA (e.g., tolerance to missing data and mixed data types) without making assumptions about the nature of data (e.g., data can be non-linear and non-metric) (Pimental, 1981; Minchin, 1987; Crisp & Weston, 1993). Unlike ordination techniques like PCA and PCoA which seek to explain the most variance in the first few axes, NMDS uses a number of axes (dimensions) that is provided by the user and iteratively tries to find a solution specified by the user (e.g., a given threshold of stress value or iteration until convergence is reached) and terminates the computation when the given threshold of "stress" value is achieved or when "stress" values of runs with random starting points converge (Oksanen et al., 2016). In short, NMDS attempts to reconstruct pairwise morphometric dissimilarities between two specimens in a low dimensional space that best match the observed pairwise Gower's distances. Using the same Gower's distance dissimilarity matrix as that used for the PCoA analysis, an NMDS analysis was conducted in R using the function "metaMDS" in the package VEGAN (Oksanen et al., 2016). In order to choose the appropriate number of dimensions (K) for the morphometric data set, twenty NMDS runs with random starting points were done iteratively for K = 1–10 and the observed stress values for each K were visualized on a scree plot. The optimal value for K was determined by the highest reduction in "stress" value and the non-metric and linear fit R^2 values in the "Goodness of Fit or Shepard" plot (VEGAN::stressplot). The metaMDS ordination procedure was then carried out with the selected K dimensions and a maximum number of iterations of 100. The resulting metaMDS axes were rotated in such a way to maximize the variation observed between points by arranging the NMDS axes in hierarchical order (VEGAN::postMDS (pc = TRUE)). Confidence ellipses (95%, based on standard errors)

were added to NMDS ordination plots to aid visualization of the boundaries among groups by estimating the group mean (centroid) given the data of the collected samples (e.g. Owen & Chmielewski, 1985; Krauss, 1996; Mráz et al., 2011; Wachter et al., 2015). Linear vector-fitting (VEGAN::envfit) to the ordination was done with 999 permutations to examine how each morphological character contributed to the ordination.

Hierarchical clustering is routinely employed in morphometric studies to determine the number and composition of morphological groups (e.g., Krauss, 1996; Mráz et al., 2011; Mapaya & Cron, 2016). In this study, cluster analysis was performed using the function "hclust" (available in standard R) to hierarchically cluster the specimens using the average linkage clustering method. The cophenetic correlation coefficient (Sokal & Rohlf, 1962; Sneath & Sokal, 1973) between the resulting hierarchical structure and the Gower's dissimilarity matrix was used to assess how well the results of the clustering analysis represent the actual pairwise distances.

ANOSIM is regularly employed in morphometric studies to examine the extent of morphological variation within and among groups using distance matrices of choice (Clarke, 1993; Hammer et al., 2001; Jolles, 2015; Shepherd et al., 2015; Wachter et al., 2015). An ANOSIM was carried out in Paleontological Statistics (PASTv3.12; Hammer et al., 2001) with 9999 permutations using the Gower's dissimilarity matrix generated for the multivariate analyses to determine if the Nelson-group, subsp. *basinudus*, subsp. *discoideus*, subsp. *toa*, and the two morphotypes from Marlborough are statistically distinct from each other in their morphology. ANOISM is a non-parametric test that uses any distance measure by converting global and pairwise among group and within group distances to ranks to evaluate if two or more groups are significantly different (Clarke, 1993; Hammer et al., 2001).

4.4.3.2. Univariate analyses

In order to examine the extent and distribution of morphological variation of nine quantitative characters (Table 4.1) within and among the four infraspecific groups of *Senecio* "pseudoglaucophyllus", univariate analyses (analysis of variance (ANOVA)) at the 5% significance level were conducted in PAST. Specimens of the two morphotypes of Marlborough plants were excluded from the univariate analysis because of their small sample size ($n < 5$). Data for each character were checked for normality using "Normality tests" in PAST. Seven of the nine characters failed the test of normality: characters 2–4, 7, 8, 12, and 15 (Table 4.1). Therefore, analysis of variance for these characters were done using a

Kruskal-Wallis test, which is a non-parametric version of ANOVA. Homogeneity of variance for each character was assessed with Levene's test using a "One-way ANOVA" in PAST. If the result of Levene's test was significant, the unequal-variance (Welch) version of ANOVA was used instead. Tukey-Kramer and Mann-Whitney pairwise post-hoc tests were used to test if variation among groups exceeded variation expected by chance for ANOVA and Kruskal-Wallis tests respectively.

4.4.3.3. Geographical patterns in the morphometric data set

The results of the multivariate analyses show clustering patterns that are indicative of the presence of geographical signal in the morphometric data set. To test the hypothesis that there is a positive correlation between geographic distance and morphological dissimilarity, a Mantel test between Gower's pairwise distances computed from the 16 morphological characters included in the morphometric data set and pairwise Euclidean distances computed from geographical coordinates of specimens was carried out. Euclidean geographical distances were calculated using the function "dist" in the package STATS (in standard R). A Mantel test was carried out using the function "mantel" (Legendre & Legendre, 1998) in the package VEGAN with 999 permutations in R.

4.5. RESULTS

4.5.1. Multivariate analyses

4.5.1.1. PCoA and NMDS analyses

Stress values of NMDS ordination reduce the most (from 0.19 to 0.12; Fig. 4.1, left) when three instead of two dimensions are used to reflect variation in the morphometric data set. Even though K = 4 appears to be the 'breakpoint' in the scree plot of stress vs. number of dimensions (Fig. 4.1, left), the reduction in stress (from 0.12 for K = 3 to 0.09 for K = 4) is half of that from K = 2 to K = 3 (a reduction of 0.07). A stress value of 0.12 is considered as 'fair', indicating a 'fair' fit between Gower's dissimilarities and ordination distance (Kruskal, 1964). K = 3 was therefore selected for subsequent analyses. A Shepard stressplot for K = 3 (Fig. 4.1, right) shows high R^2 values for both the non-metric (R^2 = 0.985) and linear (R^2 = 0.905) fit between pairwise dissimilarities observed in Gower's distance and plotted ordination distance.

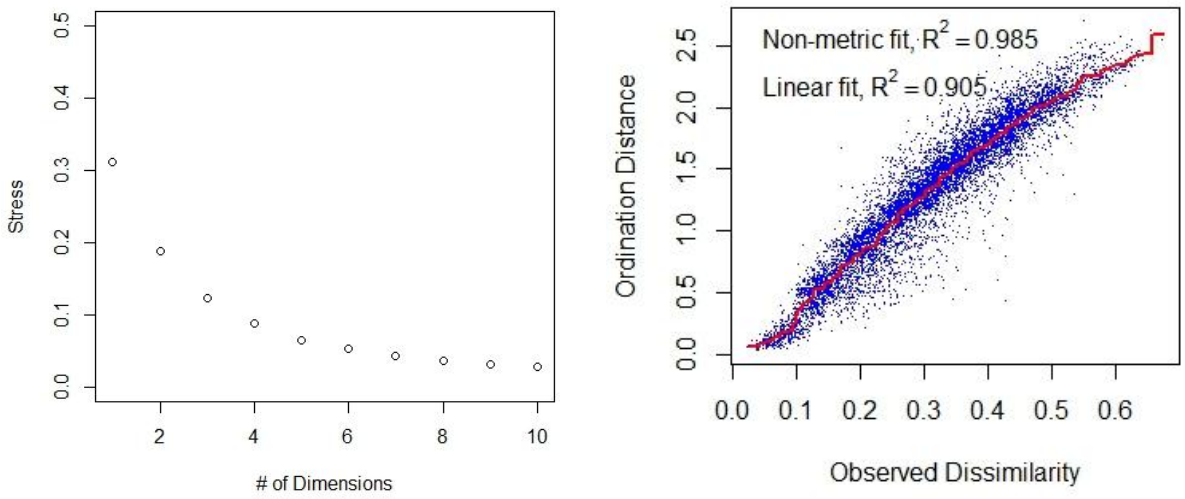
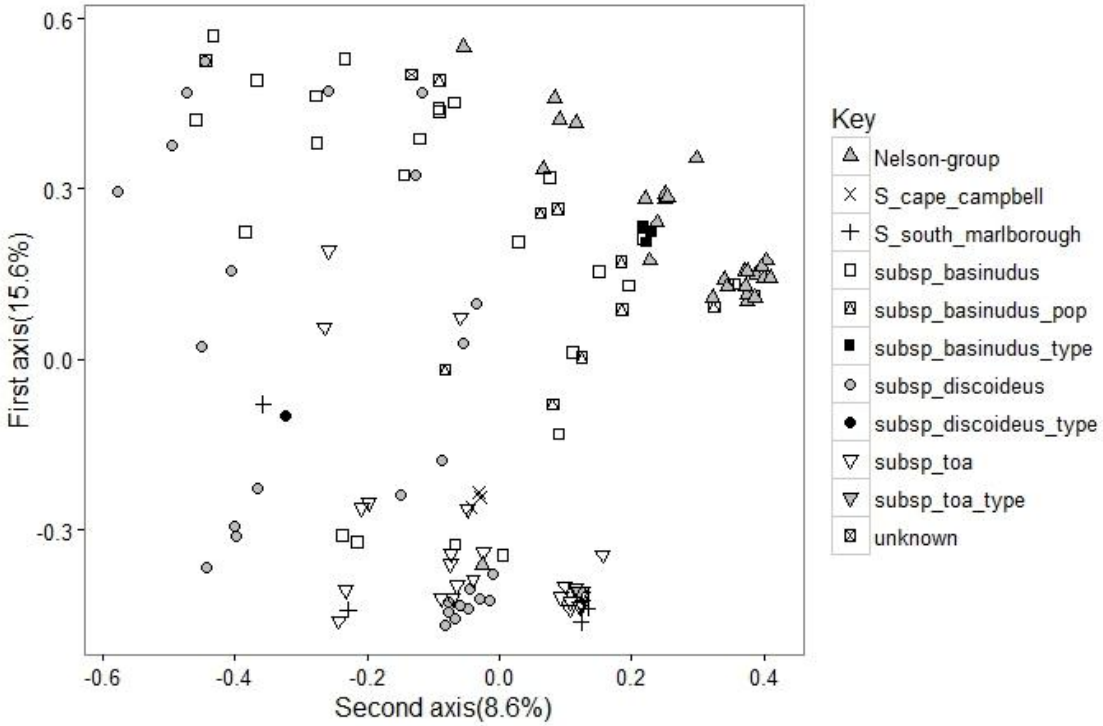


Fig. 4.1. Choosing the best K. (Left) A scree plot showing the ordination stress for ordination dimension (K) of 1–10 for the morphometric data set. (Right) A Shepard stressplot (K = 3) showing the relationship between the pairwise distances of Gower's dissimilarity matrix and the NMDS ordination distances of the morphometric data set.



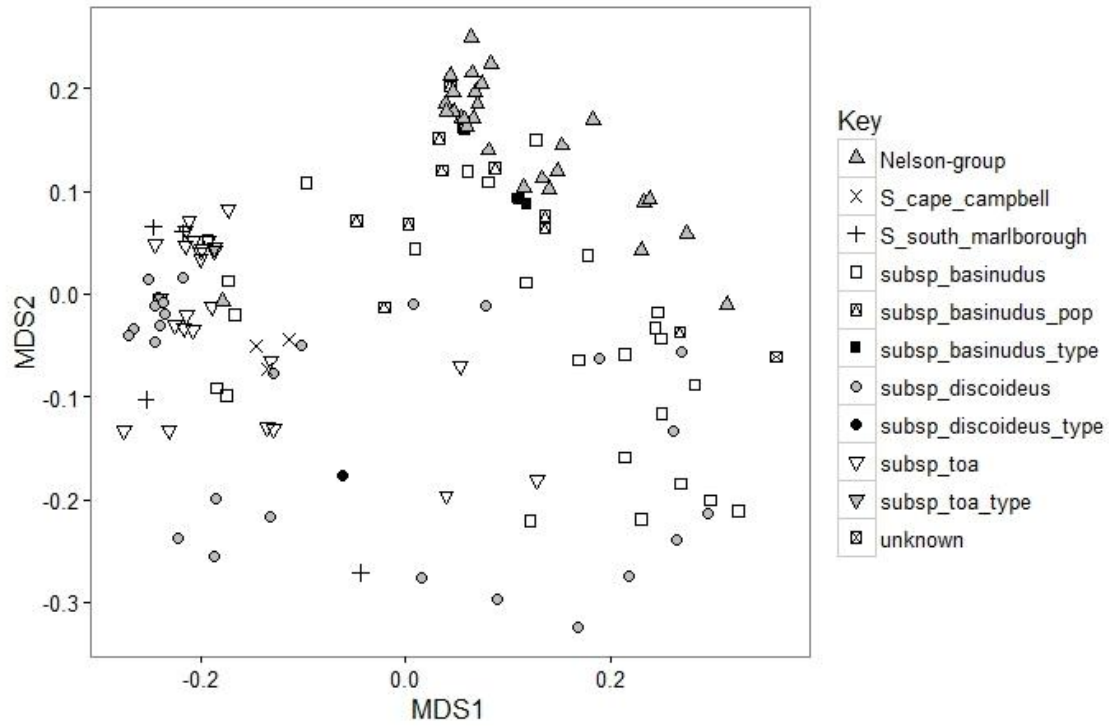


Fig. 4.2. Bidimensional plots of the first and second axes of the PCoA (above) and the NMDS (below) analyses of *Senecio* "pseudoglaucophyllus". Different symbols indicate the four subgroups of *S.* "pseudoglaucophyllus", type specimens of subsp. *basinudus*, subsp. *discoideus*, and subsp. *toa*, 11 specimens of a population of subsp. *basinudus*, and two morphotypes of Marlborough plants: *S.* aff. *glaucophyllus* "South Marlborough" and *S.* aff. *glaucophyllus* "Cape Campbell" and a specimen of an unknown plant. Legend: subsp_basinudus_pop - specimens of a single population of subsp. *basinudus* from Okains Bay and unknown- specimens of unknown identity.

Bidimensional plots of the first and second axes of the PCoA and NMDS analyses show very similar clustering patterns (Fig. 4.2). PCoA bidimensional plots of 1st vs. 3rd axes and 2nd vs. 3rd axes are not shown because they are very similar to the plot of 1st vs. 2nd axes. The first and second axes of the PCoA together explain 24.2% of the variation in the morphometric data set (Fig. 4.2). Unlike in the PCoA, axes in the NMDS do not account for variation in the data set in a decreasing order, which means that each MDS axis may be of equal importance in describing overall variation in the morphometric data set. Bidimensional plots of 1st vs. 3rd axes and 2nd vs. 3rd axes of the NMDS analysis are presented with 95% confidence ellipses (based on standard errors) added for the centroids of each of the four subgroups of *Senecio* "pseudoglaucophyllus" as well as the two informally recognized morphotypes from Marlborough (Fig. 4.3).

Specimens of none of the four infraspecific groups of *Senecio* "pseudoglaucophyllus" form distinct clusters to the exclusion of specimens belonging to other subgroups in the

bidimensional plots of the 1st vs. 2nd axes of the PCoA and the NMDS analyses (Fig. 4.2). However, most of the specimens of the Nelson-group and subsp. *toa* form two poorly defined clusters that don't overlap with each other. Specimens of subsp. *basinudus* and subsp. *discoideus* are found scattered across the plots, with the former mostly intermingled with specimens of the Nelson-group and a subset of the latter grouping with subsp. *toa* (Fig. 4.2). Type specimens of subsp. *basinudus* are placed in close proximity to specimens of the Nelson-group. Lectotypes of subsp. *toa* and subsp. *discoideus* fall within a tight cluster of subsp. *toa* specimens and between the clusters of subsp. *toa* and the Nelson group respectively. Specimens of the two morphotypes of Marlborough plants (*S.* aff. *glaucophyllus* "South Marlborough" and *S.* aff. *glaucophyllus* "Cape Campbell") are placed within the vicinity of the subsp. *toa* cluster (Fig. 4.2). The 11 specimens of subsp. *basinudus* from the same population, which are included as a case study, do not form a cluster to the exclusion of other specimens and are placed with Nelson-group specimens, other specimens of subsp. *basinudus*, and between the subsp. *toa* and Nelson-group clusters.

Even though specimens of the Nelson-group, subsp. *basinudus*, subsp. *discoideus*, and subsp. *toa* do not form discrete clusters in the NMDS and PCoA bidimensional plots, there is no overlap of the 95% confidence ellipses of the four groups in the MDS1 vs. MDS2 and MDS1 vs. MDS3 plots (Fig. 4.3a, b) and for subsp. *discoideus* and the Nelson-group in the MDS2 vs. MDS3 plot (Fig. 4.3c). The non-overlapping of these confidence ellipses shows that the morphometric means of the four groups are significantly different at $P \leq 0.05$. However, the 95% confidence ellipses of the two morphotypes of Marlborough plants both overlap with those of subsp. *discoideus* and *toa* in the MDS1 vs. MDS2 and MDS1 vs. MDS3 plots. In addition, the 95% confidence ellipse of *Senecio* aff. *glaucophyllus* "South Marlborough" overlaps with those of subsp. *basinudus*, subsp. *discoideus*, and subsp. *toa* in the MDS2 vs. MDS3 plot (Fig. 4.3).

The contribution of each morphological character to various MDS axes, based on rank-order (dis)similarities, is given as vector correlations, of which the vector length indicates the extent of influence (Table 4.2). In my 3-dimensional NMDS analysis, the characters that contribute the most to MDS1 (Fig. 4.3a, b) are 1 and 6, followed by 5, 9, 8, and 10 (Table 4.2). MDS2 and MDS3 are driven by variation in characters 10 and 14, and character 14, respectively (Fig. 4.3a–c; Table 4.2).

All morphological characters can be fitted to the three-dimensional NMDS ordination with significant P-values ($P < 0.05$; Table 4.3). Many foliar characters (1–4, 6 and 8) are strongly associated with MDS1 (Fig. 4.3d, e; Table 4.3). Characters 7 and 10 are the only characters that plot very well along MDS2 (Fig. 4.3d, e; Table 4.3). Most floral characters (Characters 13–16) fit MDS3 relatively well and explain variation not explained by MDS1 and MDS2 (Fig. 4.3e, f; Table 4.3).

Table 4.2. Contributions of the 16 morphological characters to the NMDS ordination. Characters in bold indicate strong contributions (>0.5 or <-0.5) to the MDS1, MDS2 and MDS3 axes.

Character	MDS1	MDS2	MDS3
Upper leaves (first leaf from inflorescence)			
Leaf division [1]	**1.0796**	0.4269	0.1159
Leaf length/width ratio [2]	0.1419	-0.0562	-0.0244
No. of dissections on one side of the leaf/ leaf length ratio [3]	-0.2409	-0.0425	0.0483
Degree of leaf incision [4]	-0.4899	-0.2275	0.0352
Presence of a double serrated leaf margin [5]	**-0.8101**	0.4520	0.4935
Mid-cauline leaves			
Leaf division [6]	**1.0729**	0.2955	0.1765
Leaf length (mm) [7]	-0.0062	0.0401	0.0253
Degree of leaf incision [8]	**-0.5856**	0.2698	0.0645
Presence of a double serrated leaf margin [9]	**-0.5140**	0.3402	0.0430
Presence of petiole [10]	**0.7875**	**-1.2346**	0.1134
Floral characters			
Flower head radiate [11]	0.0607	-0.3701	-0.4444
No. of involucral bracts [12]	-0.0340	0.0941	0.0096
Length of involucral bracts (mm) [13]	-0.0178	0.0424	0.0446
Trichomes at the base of receptacle [14]	-0.1330	**-0.5566**	**0.8210**
Length of supplementary bracts (mm) [15]	0.0284	0.0295	0.0910
Length/width ratio of supplementary bracts [16]	0.0007	0.0884	0.0320

4.5.1.2. Cluster analysis

The results of the hierarchical cluster analysis are similar to those of the PCoA and NMDS, in which none of the four infraspecific groups of *Senecio* “pseudoglaucophyllus” forms a distinct cluster to the exclusion of specimens of the other three groups (Fig. 4.4). Most of the specimens of the Nelson-group and many specimens of subsp. *basinudus* form one of four main clusters that can be recognized in the cluster dendrogram (Cluster 1; Fig. 4.4). Cluster 2 consists of a mixture of specimens of subsp. *basinudus*, subsp. *discoideus*, the Nelson-group, a specimen of subsp. *toa*, one of *S.* aff. *glaucophyllus* "South Marlborough", and the unknown specimen. Cluster 3 is made up of only two specimens of subsp. *toa*. The majority of the specimens of subsp. *toa* form Cluster 4 together with many specimens of subsp. *discoideus*, a few specimens of subsp. *basinudus*, one specimen of the Nelson-group, and specimens of *S.* aff. *glaucophyllus* "South Marlborough" and *S.* aff. *glaucophyllus* “Cape Campbell”. The cluster analysis has a cophenetic correlation coefficient (R) of 0.76, which indicates a good representation of the actual pairwise dissimilarities by the dendrogram.

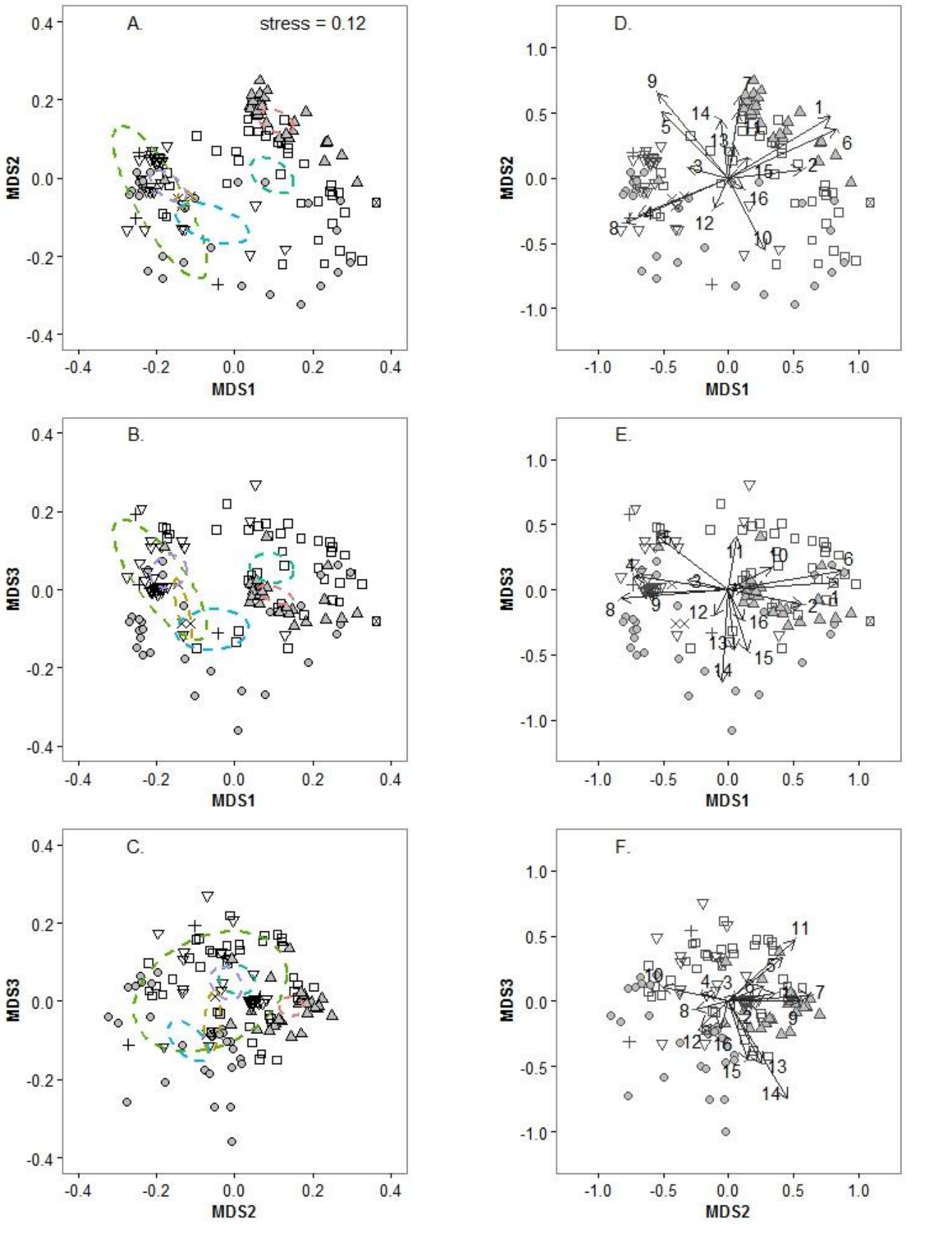

A.
stress = 0.12
B.
C.
D.
E.
F.
MDS1
MDS2
MDS3
Key
Nelson-group
S_cape_campbell
S_south_marlborough
subsp_basinudus
subsp_discoideus
subsp_toa
unknown
group
Nelson-group
S_cape_campbell
S_south_marlborough
subsp_basinudus
subsp_discoideus
subsp_toa

Fig. 4.3. Plots of three NMDS dimensions for 16 morphological characters used in the morphometric study showing 95% confidence ellipses for each taxon (a–c) and the contribution of each character to the ordination via linear vector analysis (d–f). Character numbers correspond to those in Table 4.1.

Table 4.3. Linear vector-fitting of the 16 morphological characters to the 3-dimensional NMDS ordination fits the vector of each character in a way that best correlates with the placements of specimens in the ordination space. Values in bold indicate a strong character influence on the ordination axes. P-values are based on 999 permutations (significance codes: 0 '***', 0.001 '**', 0.01 '*') and R^2 (squared correlation coefficient) values are goodness of fit statistics.

Character	MDS1	MDS2	MDS3	R^2	Pr (>r)	
Upper leaves (first leaf from inflorescence)						
Leaf division [1]	**0.8454**	-0.5191	-0.1261	0.8330	0.001	***
Leaf length/ width ratio [2]	**0.9673**	-0.1478	0.2063	0.3457	0.001	***
No. of dissections on one side of the leaf/ leaf length ratio [3]	**-0.9282**	-0.1739	-0.3290	0.1218	0.003	**
Degree of leaf incision [4]	**-0.9116**	0.4006	-0.0918	0.5703	0.001	***
Presence of a double serrated leaf margin [5]	-0.6155	-0.5577	-0.5569	0.6947	0.001	***
Mid-cauline leaves						
Leaf division [6]	**0.8899**	-0.4076	-0.2048	0.8470	0.001	***
Leaf length (mm) [7]	0.1404	**-0.9766**	-0.1633	0.4065	0.001	***
Degree of leaf incision [8]	**-0.9038**	0.4024	0.1459	0.7418	0.001	***
Presence of a double serrated leaf margin [9]	-0.6545	-0.7519	-0.0797	0.7084	0.001	***
Presence of petiole [10]	0.4638	**0.8809**	-0.0943	0.4018	0.001	***
Floral characters						
Flower head radiate [11]	0.0965	-0.6824	-0.7246	0.4682	0.001	***
No. of involucral bracts [12]	-0.3480	0.6583	0.6675	0.0952	0.010	**
Length of involucral bracts (mm) [13]	0.0876	-0.5752	**0.8133**	0.2783	0.001	***
Trichomes at the base of receptacle [14]	-0.0740	-0.5805	**0.8109**	0.7844	0.001	***
Length of supplementary bracts (mm) [15]	0.2956	-0.3950	**0.8698**	0.2582	0.001	***
Length/width ratio of supplementary bracts [16]	0.42311	0.21548	**0.88008**	0.0701	0.035	*

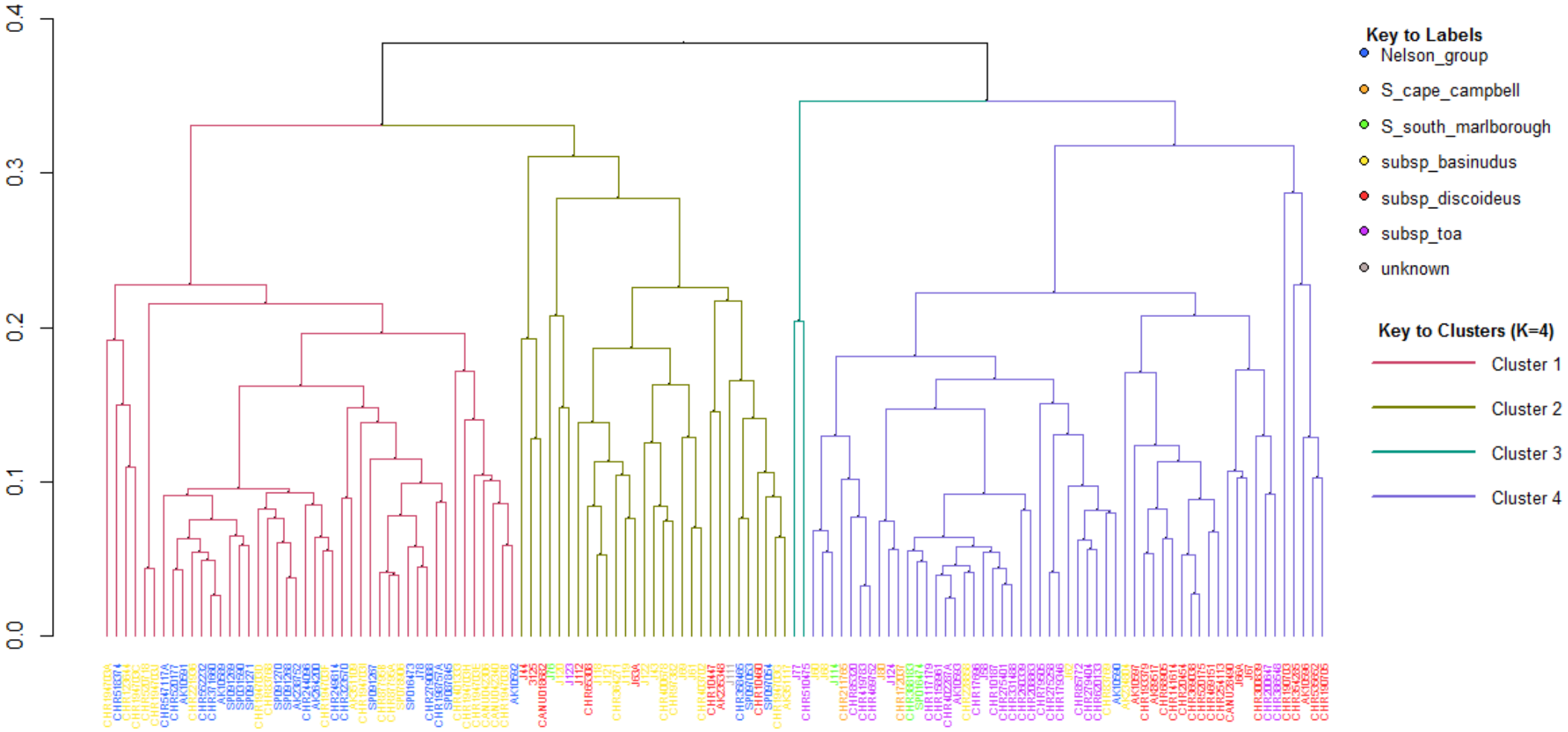


Fig. 4.4. Dendrogram resulting from hierarchical clustering of Gower's dissimilarities of the 130 specimens using the average linkage clustering method, R = 0.76.

4.5.1.3. ANOSIM

The results of an ANOSIM using the 16 selected morphological characters show that there is significantly more morphological variation among the Nelson-group, subsp. *basinudus*, subsp. *discoideus*, subsp. *toa*, and the two Marlborough morphotypes than within these groups (R = 0.4156, P < 0.001). Pairwise comparisons of the four infraspecific groups of *Senecio* "pseudoglaucophyllus" indicate that subsp. *basinudus* and the Nelson-group are the only two groups that are not significantly different from each (Table 4.4). The two Marlborough morphotypes (*S.* aff. *glaucophyllus* "South Marlborough" and *S.* aff. *glaucophyllus* "Cape Campbell") are not significantly different from subsp. *discoideus* and subsp. *toa* and each other, but differ from the Nelson-group while the former also differs from subsp. *basinudus*.

Table 4.4. ANOSIM results. Bonferroni-corrected P-values of pairwise comparisons of the four infraspecific groups of *Senecio* "pseudoglaucophyllus" and the two morphotypes of Marlborough plants. P-values of pairs with significant differences are in bold (P < 0.05).

	subsp. *basinudus*	**subsp. *discoideus***	**Nelson-group**	**subsp. *toa***	***S.* "Sth. Marlborough"**	***S.* "Cape Campbell"**
subsp. *basinudus*		**0.0015 (R = 0.3811)**	0.1560	**0.0015 (R = 0.4661)**	**0.0060 (R = 0.5094)**	0.5325
subsp. *discoideus*			**0.0015 (R = 0.6489)**	**0.0030 (R = 0.2218)**	1	1
Nelson-group				**0.0015 (R = 0.8131)**	**0.0015 (R = 0.9036)**	**0.0345 (R = 0.7806)**
subsp. *toa*					1	1
***S.* "Sth. Marlborough"**						1
***S.* "Cape Campbell"**						

4.5.2. Univariate analysis

Box and bar plots of the nine quantitative and seven qualitative characters are presented to show the distribution of their variation for each of the four *Senecio* "pseudoglaucophyllus" groups (Figs. 4.5 and 4.6). There is substantial overlap in variation among subsp. *basinudus*, subsp. *discoideus*, subsp. *toa*, and the Nelson-group in all characters, especially in characters 3, 12, and 16 (Fig. 4.5). Of the four groups, subsp. *toa* and the Nelson-group are the most different from each other. This is most noticeable in characters 1, 4, 6, 8, and 13.

Despite considerable within group variation, all nine quantitative characters show more among-group than within-group variation (Fig. 4.5; Table 4.5). One-way ANOVA and

pairwise post-hoc tests indicate that the Nelson-group, subsp. *basinudus*, subsp. *discoideus*, and subsp. *toa* are significantly different from each other in individual characters (all characters have P-values < 0.05; Table 4.5). Five out of the nine characters (Characters 4, 7, 8, 12, and 13) vary significantly among all group pairs with the exceptions of one or two pairs (Table 4.5).

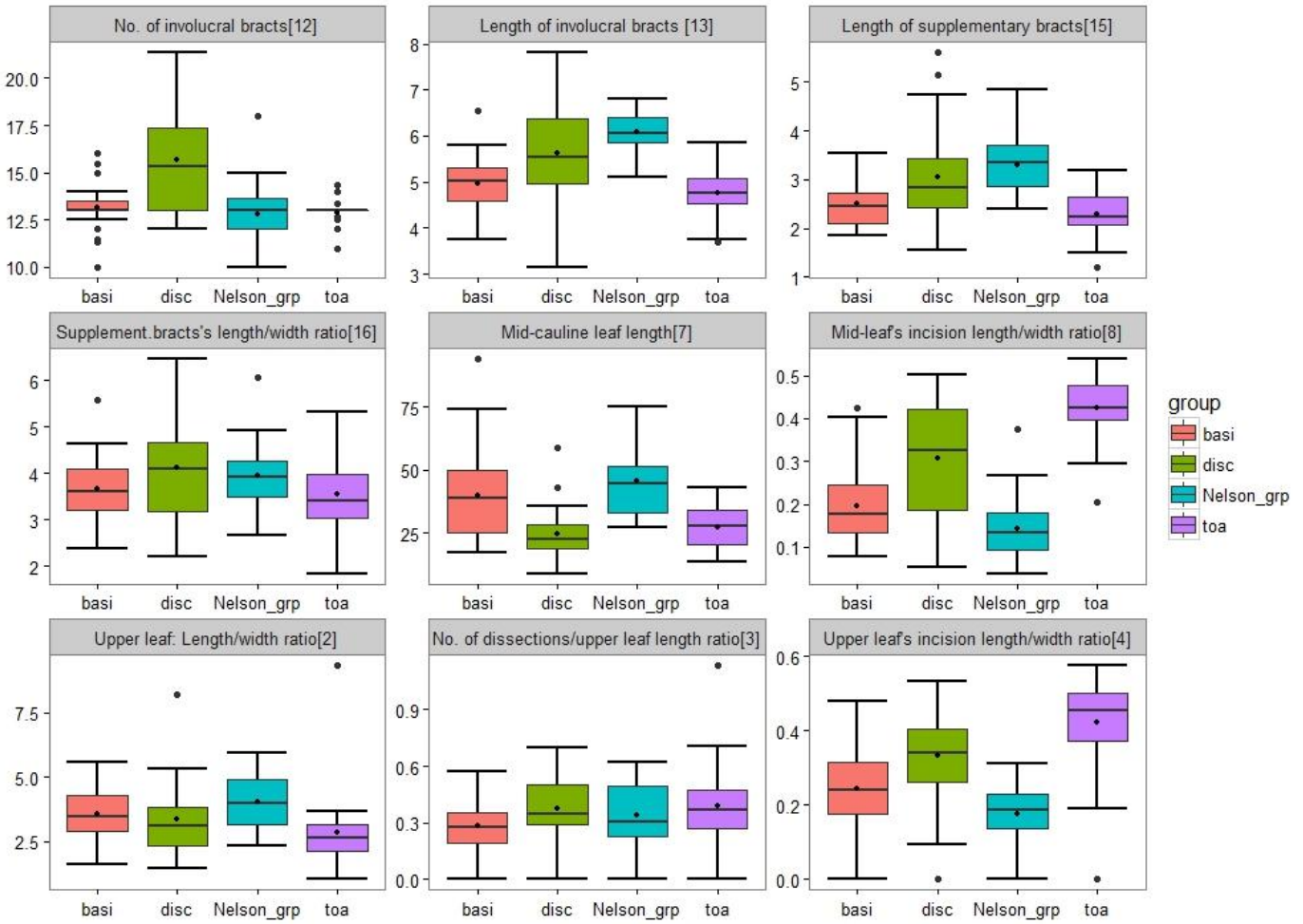


Fig. 4.5. Boxplots showing median, upper and lower quartiles, minimum and maximum values for all nine quantitative morphometric characters for the four *Senecio* "pseudoglaucophyllus" groups. Outliers are plotted as single points beyond whiskers and means as the single black dots within each box. Legend: basi - subsp. *basinudus*, disc - subsp. *discoideus*, Nelson_grp - Nelson-group, toa - subsp. *toa*.

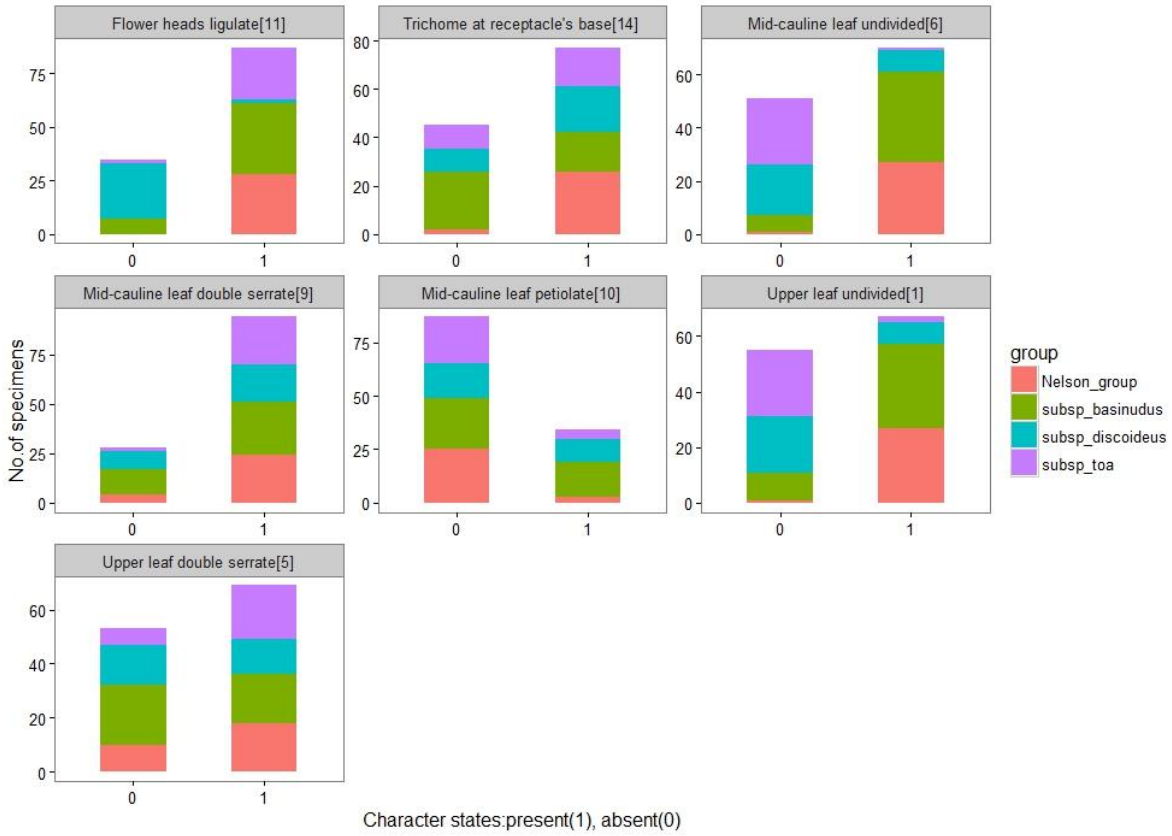


Fig. 4.6. Histograms showing the number of specimens with each character state for each infraspecific group of *Senecio* "pseudoglaucophyllus" for all seven qualitative morphological characters (Table 4.1).

4.5.3. Correlation between geographic distance and morphological dissimilarity

The results of a Mantel test show that there is a significant positive correlation between morphological dissimilarity and geographical distance (R = 0.1527, P = 0.001; Fig. 4.8). Geographical patterns in the morphometric data were further explored by plotting individuals belonging to the four clusters in the dendrogram of hierarchical clustering analysis (Fig. 4.4) on the distribution map (Fig. 4.7) of *Senecio* "pseudoglaucophyllus" (Fig. 4.9). Specimens in Cluster 1 form two allopatric groups: Group1: Nelson and Group 2: the Port Hills and Banks Peninsula and adjacent parts of Canterbury (Fig. 4.9). Cluster 2 has a distribution range that extends from central North Island to Southern Otago and that overlaps with the other three clusters, except in coastal Otago (Fig. 4.9). Cluster 3 and 4 consist of mostly inland specimens in the North and South Island with distribution ranges stretching from central North Island to Southland (Cluster 4, Fig. 4.9).

Table 4.5. Variation in the nine quantitative morphological characters (Table 4.1): mean, standard deviation and range for the Nelson-group (n = 29), subsp. *basinudus* (n = 40), subsp. *discoideus* (n = 28) and subsp. *toa* (n = 26). Results of univariate analysis (ANOVA) for the four infraspecific groups are also presented, which include the F- (ANOVA) or H- (Kruskal-Wallis test) ratio with corresponding P-values and results of pairwise post-hoc tests that indicate which group pairs (in bold) have significantly different variances with corresponding P-values.

	Nelson-group (*n*)	**subsp. *basinudus* (*b*)**	**subsp. *discoideus* (*d*)**	**subsp. *toa* (*t*)**	**ANOVA**		
	Mean ± SD (Min-Max)	Mean ± SD (Min-Max)	Mean ± SD (Min-Max)	Mean ± SD (Min-Max)	*F*/*H*-ratio	*P*	Tukey-Kramer/ Mann-Whitney tests
2	3.94 ± 1.11 (1.84–5.92)	3.58 ± 0.98 (1.61–5.56)	3.48 ± 1.60 (1.46–8.19)	2.84 ± 1.45 (1.05–9.33)	18.28	0.0004	< 0.05 **b × t, d × n, n × t**
3	0.34 ± 0.16 (0.00–0.62)	0.28 ± 0.13 (0.00–0.58)	0.36 ± 0.16 (0.00–0.70)	0.39 ± 0.22 (0.00–1.14)	7.99	0.046	< 0.01 **d × b**
4	0.18 ± 0.10 (0.00–0.53)	0.25 ± 0.11 (0.00–0.48)	0.31 ± 0.14 (0.00–0.53)	0.42 ± 0.12 (0.00–0.58)	45.11	< 0.05	<0.01, **all**
7	44.26 ± 14.34 (20.25–75.25)	39.94 ± 16.57 (17.40–93.95)	24.62 ± 9.69 (9.00–58.90)	27.21 ± 8.86 (13.63–42.73)	40.82	< 0.0001	< 0.01 **all except b × n, d × t**
8	0.15 ± 0.09 (0.04–0.38)	0.19 ± 0.09 (0.08–0.42)	0.28 ± 0.14 (0.07–0.50)	0.42 ± 0.07 (0.20–0.54)	61.28	< 0.05	< 0.01 **all**
12	12.81 ± 1.54 (10.00–18.00)	13.14 ± 1.01 (10.00–16.00)	15.60 ± 2.72 (12.00–21.33)	12.88 ± 0.83 (11.00–14.33)	28.35	< 0.0001	< 0.01 **all except b × n**
13	6.08 ± 0.40 (5.10–6.80)	4.97 ± 0.55 (3.77–6.55)	5.56 ± 1.03 (3.15–7.80)	4.76 ± 0.55 (3.70–5.85)	43.63	< 0.0001	< 0.01 **all except t × b**
15	3.25 ± 0.62 (2.05–4.85)	2.49 ± 0.46 (1.85–3.55)	3.00 ± 1.02 (1.55–5.60)	2.29 ± 0.46 (1.20–3.20)	35.70	< 0.0001	< 0.01 **b × n, d × t, n × t**
16	3.90 ± 0.70 (2.65–6.06)	3.65 ± 0.64 (2.39–5.57)	4.14 ± 1.04 (2.20–6.46)	3.56 ± 0.76 (1.85–5.33)	3.48	0.040	< 0.05 **d × t**

4.6. DISCUSSION

4.6.1. Patterns of morphometric variation in *Senecio* "pseudoglaucophyllus"

In this study, phenetic analyses were employed to determine if the infraspecific classification of *Senecio* "pseudoglaucophyllus" into subsp. *basinudus*, subsp. *discoideus*, subsp. *toa*, and the Nelson-group is supported by patterns of morphometric variation. The results of the PCoA and NMDS analyses indicate that the specimens of *S.* "pseudoglaucophyllus" that were studied cluster into two poorly defined primary clusters, with a large number of specimens occupying the morphospace between the two clusters (Figs. 4.2 and 4.3). One of these clusters roughly aligns with plants ascribed to subsp. *basinudus* and the Nelson-group (Basinudus-Nelson cluster), and the other with those identified as subsp. *discoideus*, subsp. *toa*, and the two Marlborough morphotypes (Discoideus-Toa cluster). The dendrogram resulting from the hierarchical cluster analysis shows a congruent pattern, in which Cluster 1 aligns with the Basinudus-Nelson cluster of the PCoA and NMDS analyses, Cluster 4 corresponds to the Discoideus-Toa cluster, and morphologically intermediate specimens group in Clusters 2 and 3.

In most of the NMDS ordination plots, 95% confidence ellipses of the four infraspecific groups are not overlapping within the Basinudus-Nelson and Discoideus-Toa clusters, although some cluster in close vicinity of each other in morphometric space. However, when plants belonging to the two Marlborough morphotypes are considered, 95% confidence ellipses within the Discoideus-Toa cluster are overlapping (Fig. 4.3). These results indicate that the recovered morphometric patterns somewhat align with the four infraspecific groups of *Senecio* "pseudoglaucophyllus", but that these groups are morphologically very similar and that specimens of intermediate morphology within the Discoideus-Toa cluster blur the distinction between subsp. *discoideus* and subsp. *toa*. The ANOSIM provided additional support for the presence of patterns of variation that somewhat align with the four groups, because significantly more morphological variation among the groups of *S.* "pseudoglaucophyllus" was found than within them ($R = 0.4156$, $P < 0.001$), but show that not all groups are significant different from each other (Table 4.4). In contrast to the NMDS analyses, however, the ANOSIM did not reveal significant differences between subsp. *basinudus* and the Nelson-group (Table 4.4).

4.6.2. Morphological delimitation and diversity of the infraspecific groups of *Senecio* "pseudoglaucophyllus"

The Nelson-group is one of the most morphologically uniform groups among the four infraspecific groups of *Senecio* "pseudoglaucophyllus" as shown by its small 95% confidence ellipses in Figs. 4.3a–c and the relatively small size of the box plots of the quantitative characters in Fig. 4.5. This group is characterized by having radiate flower heads and mostly undivided mid-cauline and upper leaves. In plants with divided leaves, the leaves are less deeply incised than what is observed in the other three subspecies. The Nelson group usually has trichomes at the base of the receptacle (Figs. 4.5 and 4.6; Table 4.5).

Subspecies *basinudus* is morphologically more variable than the Nelson-group as shown by the relatively large size of its 95% confidence NMDS ellipses (Fig. 4.3) and in the results of the univariate analyses (Figs. 4.5 and 4.6; Table 4.5), as well as by the large morphospace that this group occupies in the PCoA and NMDS plots (Fig. 4.2). This is also evident from a case study in which 11 specimens from the same population of subsp. *basinudus* (Sykes 496/69; Table S4) were included to determine the extent of variation within this population (Figs. 4.10–4.20). These 11 specimens occupy a substantial portion of the morphospace in the PCoA and NMDS ordinations (Fig. 4.2), indicating that the amount of variation within this population is large compared to the total amount of morphological diversity in *Senecio* "pseudoglaucophyllus". One of the specimens from this population (CHR194703I) has non-radiate capitula, in contrast to the other ten specimens (Fig. 4.16). Moreover, these 11 specimens have leaves with highly variable shapes (from obovate to ovate), sizes (21–94 mm long × 6–34 mm wide) and depth of incisions of the leaf margin (0.65–6.45 mm). This shows that morphological variation within populations sometimes exceeds that between the four infraspecific groups of *S.* "pseudoglaucophyllus" and confirms Ornduff's (1962) observation that there is considerable variation within his subspecies.

Subspecies *basinudus* appears to lack diagnostic morphological characters (Figs. 4.5 and 4.6) and its specimens are found throughout the morphospace of the PCoA and NMDS plots (Fig. 4.2). Many, however, have close morphological affinities with specimens of the Nelson-group. This pattern is also observed in the dendrogram produced from the cluster analysis, in which subsp. *basinudus* specimens fall in three clusters with many of the specimens grouping with those of the Nelson-group (Fig. 4.4). The results of the ANOSIM also support Ornduff's hypothesis that subsp. *basinudus* and the Nelson-group (as *Senecio glaucophyllus* subsp.

glaucophyllus sensu Ornduff; Ornduff 1960, 1962) are morphologically similar, with the two being the only two groups that are not significantly different from each other if the two Marlborough morphotypes are not considered (Table 4.4).

Subspecies *toa* is characterized by (mostly) radiate flower heads, divided and double serrate mid-cauline and upper leaves, and deeply incised mid-cauline and often upper leaves (Figs. 4.5 and 4.6; Table 4.5). In the results of the multivariate and cluster analyses, the majority of the specimens of subsp. *toa* form a cluster with many specimens of subsp. *discoideus*, specimens of the Marlborough morphotypes, and a few specimens of subsp. *basinudus* and the Nelson-group. Three specimens of subsp. *toa* (Liew 77, Liew 123 and Ogle 3088) are morphologically intermediate between clusters of subsp. *toa* and the Nelson-group in the ordination plots and the dendrogram.

Subspecies *discoideus* is, in part, morphologically similar to subsp. *toa*, but some specimens of subsp. *discoideus* are placed in the morphospace relatively distant from the clusters of subsp. *toa* and the Nelson-group (Figs. 4.2–4.4). Subspecies *discoideus* exhibits the largest range of variation for all examined characters among the four subspecies of *Senecio* "pseudoglaucophyllus" as illustrated by its large 95% confidence ellipses (Fig. 4.3) and box plots (Fig. 4.5). The results of the current study indicate that other than the characters that Ornduff (1960) used to distinguish subsp. *discoideus* from the other subspecies ("ligules absent and plants of scree"), subsp. *discoideus* is very similar to subsp. *toa* in measurements and character states of all 16 examined characters (Figs. 4.5 and 4.6). Their distributions and habitats largely overlap. For example, subsp. *discoideus* and subsp. *toa* are sympatric in the North Island and parts of the mountainous regions of the South Island (Fig. 4.7) and grow at similar elevations (subsp. *discoideus*: 281–1524m; subsp. *toa*: 457–1550m).

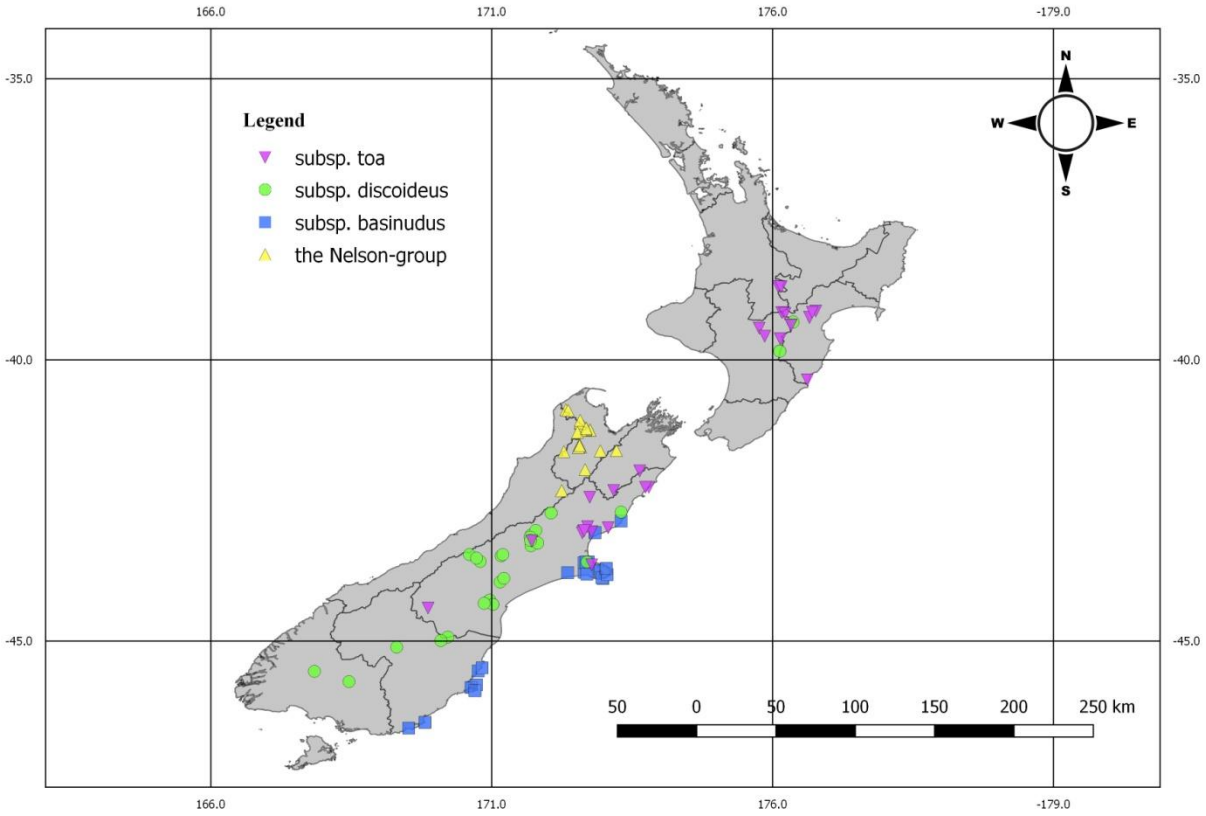


Fig. 4.7. Distribution map of the Nelson-group, subsp. *basinudus*, subsp. *discoideus*, and subsp. *toa* drawn from locality data on herbarium sheets. Coordinates of some of the specimens, especially old specimens, are approximated from Google Map©2016 from the locality data of herbarium specimens and might not be completely accurate.

4.6.3. The delimitation and morphological affinities of the two Marlborough morphotypes

One of the aims of this study was to contribute to resolving the taxonomic status of two morphotypes of Marlborough plants (*Senecio* aff. *glaucophyllus* "South Marlborough" and *S.* aff. *glaucophyllus* "*Cape* Campbell"). These two morphotypes are informally recognized on herbarium labels for plants that cannot unambiguously be accommodated in any of the four infraspecific groups of *S.* "pseudoglaucophyllus", although they do resemble some of these. For example, *S.* aff. *glaucophyllus* "South Marlborough" and subsp. *toa* both have pinnatifid to pinnatisect mid-cauline leaves, even though the depth of the incisions and their leaf sizes are very different. *Senecio* aff. *glaucophyllus* "South Marlborough" is characterized by leaves that are lobed when young but irregularly pinnatisect when mature, large capitula of up to 28mm in diameter compared to the four subspecies in *S.* "pseudoglaucophyllus", and is confined to limestone in South Marlborough (Druce & Williams, 1989; pers. obs.). *Senecio* aff. *glaucophyllus* "Cape Campbell" is distinguished by a low-spreading habit, glaucous to

almost black plants, small and spathulate leaves with variously serrated leaf margins and grows on calcareous mudstones and siltstones. The results of the morphometric analyses confirm previous observations that *S.* aff. *glaucophyllus* "South Marlborough" is morphologically similar to subsp. *toa*. They also show that *S.* aff. *glaucophyllus* "Cape Campbell" shares close similarities with subsp. *toa* and further indicate morphological similarities between these two Marlborough morphotypes and subsp. *discoideus* (Figs. 4.3–4.4). Both morphotypes show considerable morphological diversity as is evident by the size of their 95% confidence ellipses in the NMDS ordination plots (Fig. 4.3) and by the position of individual specimens in the morphometric space shown in the NMDS and PCoA plots (Fig. 4.2). The 95% confidence ellipses of *S.* aff. *glaucophyllus* "South Marlborough" and *S.* aff. *glaucophyllus* "Cape Campbell" are overlapping those of subsp. *discoideus* and subsp. *toa* (Fig. 4.3). They are, however, more distant from those of the Nelson-group and subsp. *basinudus* (Fig. 4.3). The more distant morphological affinities of the two morphotypes with the Nelson-group and subsp. *basinudus* are also supported by the results of the ANOSIM, which show that the two Marlborough morphotypes are significantly different from the Nelson-group and that *S.* aff. *glaucophyllus* "South Marlborough" is also significantly different from subsp. *basinudus*. The results of the morphometric studies therefore indicate that *S.* aff. *glaucophyllus* "South Marlborough" and *S.* aff. *glaucophyllus* "Cape Campbell" are not morphologically distinct from subsp. *discoideus* and subsp. *toa.*

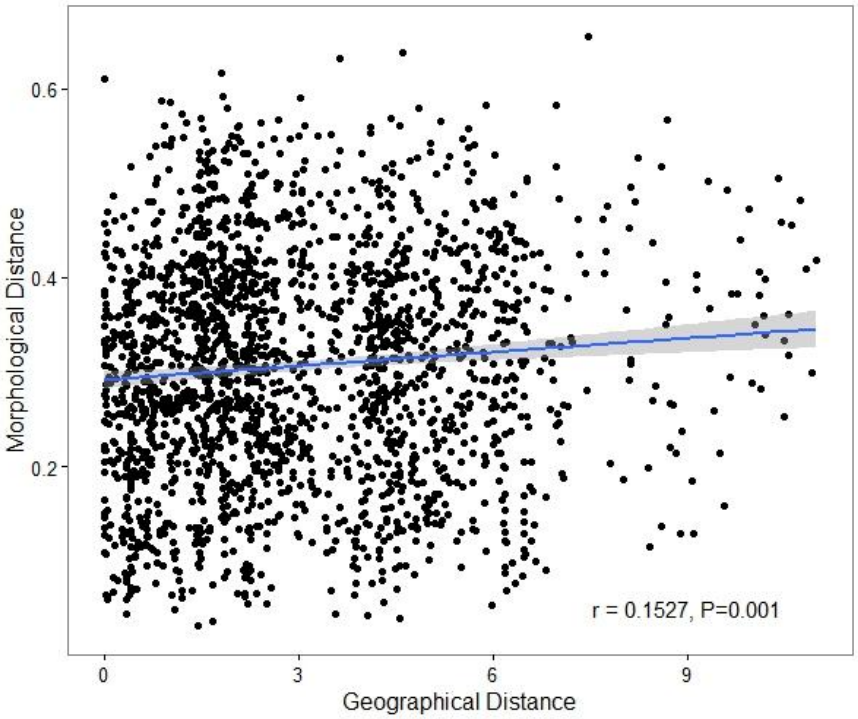


Fig. 4.8. A plot of pairwise Euclidean geographical distance and pairwise Gower's distances for *Senecio* "pseudoglaucophyllus". A regression line is added with standard error. Results of the Mantel test with 999 permutations are shown at the bottom right of the plot, which indicate a statistically significant positive correlation (R = 0.1527, P = 0.001).

4.6.4. Geographical patterns in the morphological data

Although a Mantel test indicated a statistically significant positive correlation between geographic distance and morphological dissimilarity, further inspection of geographical patterns by overlaying the hierarchical clustering patterns of the morphometric data (Fig. 4.4) on the distribution map of *Senecio* "pseudoglaucophyllus" does not suggest that these patterns are very pronounced. For example, the four clusters have mostly overlapping distributions (Fig. 4.9). However, the presence of a geographical signal in the morphological variation of *S.* "pseudoglaucophyllus" cannot be completely ruled out. For example, specimens of Cluster 1 form two isolated and rather tight-knit allopatric groups (Fig. 4.9).

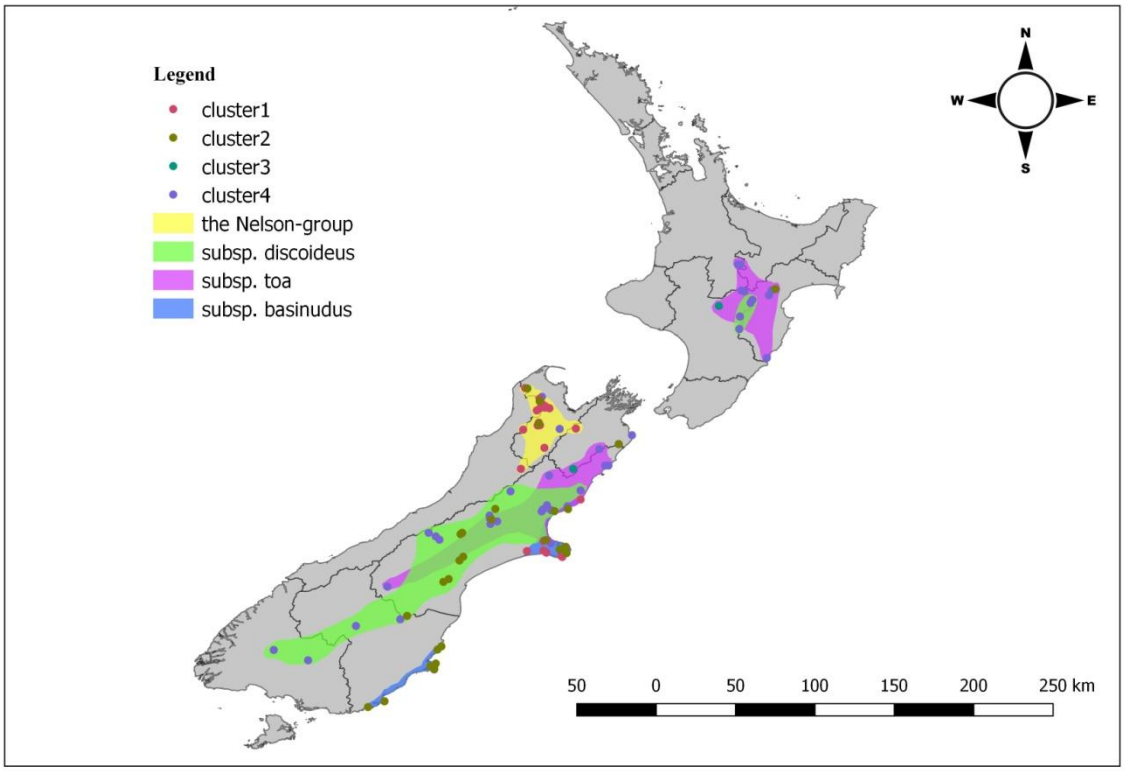


Fig. 4.9. Individuals belonging to the four clusters in dendrogram generated by a hierarchical clustering analysis (Fig. 4.4) are plotted against the distribution range of *Senecio* "pseudoglaucophyllus" (Fig. 4.7). The colors of the clusters follow those in the dendrogram (Fig. 4.4) and the colors of distribution ranges follow those in the distribution map (Fig. 4.7).

4.6.5. The infraspecific classification of *Senecio* "pseudoglaucophyllus"

Using morphometric analyses, this study aimed to assess Ornduff's (1960) amended infraspecific classification of *Senecio* "pseudoglaucophyllus" into four infraspecific taxa: subsp. *basinudus*, subsp. *discoideus*, subsp. *toa*, and the Nelson-group. Despite the non-overlapping 95% confidence ellipses in some of the NMDS ordination plots (Fig. 4.3) and significant differences among some of the four groups resulting from an ANOSIM (Table 4.4), the results of this study do not support an infraspecific classification into the three infraspecific groups that Ornduff (1960) recognized (subsp. *basinudus*, subsp. *discoideus*, subsp. *toa*) and a fourth that aligns with the Nelson group (i.e., *S. glaucophyllus* subsp. *glaucophyllus* sensu Ornduff p.p. (excl. *S. glaucophyllus* sensu Cheeseman)). None of these groups is composed of specimens that are morphologically more similar to each other than to specimens of other groups in the results of any of the multivariate analyses, (PCoA, NMDS, and hierarchical clustering; Figs. 4.2–4.4). In addition, the results of the univariate analyses show that the four infraspecific groups cannot be distinguished by unique combinations of

character states (Figs. 4.5 and 4.6), although some pairs of groups are statistically different from each other in individual characters (Table 4.5).

Instead of supporting an infraspecific classification of *Senecio* "pseudoglaucophyllus" into four groups, the results of this study suggest that an alternative classification into two infraspecific groups should be considered. One of these two morphological groups corresponds to subsp. *basinudus* and the Nelson group (Basinudus-Nelson group), and the other to subsp. *discoideus*, subsp. *toa*, *S.* aff. *glaucophyllus* "South Marlborough", and *S.* aff. *glaucophyllus* "Cape Campbell" (Discoideus-Toa group). Such a classification would reflect the lack of diagnostic characters between subsp. *discoideus* and subsp. *toa* (other than that plants of the former have non-radiate capitula and are restricted to unstable scree habitat), as well as the mostly overlapping measurements for quantitative characters of both groups, their sympatric distributions, and their close affinities with the two Marlborough morphotypes. Similarly, this classification into two infraspecific groups would communicate the similarities between subsp. *basinudus* and the Nelson-group as acknowledged by Ornduff (1960) and as indicated in the results of the morphometric analyses of this study. The Basinudus-Nelson group and Discoideus-Toa group can be differentiated by differences in leaf morphology, although many specimens with an intermediate morphology exist (Figs. 4.2–4.4). Plants of the Discoideus-Toa group mostly have divided mid-cauline leaves, whereas those of the Basinudus-Nelson group are usually undivided. Furthermore, the Discoideus-Toa group has shorter mid-cauline leaves (25.92 ± 9.28mm) than the Basinudus-Nelson group (42.1 ± 15.46 mm). Finally, the mid-cauline leaves of the Discoideus-Toa group are more deeply incised (incision/leaf width ratio: 0.35 ± 0.11mm) than those of the Basinudus-Nelson group (0.17 ± 0.09mm) (Figs. 4.3, 4.5, and 4.6; Table 4.5).

The results of the present study could also be interpreted as evidence against formally recognizing infraspecific taxa for *Senecio* "pseudoglaucophyllus". Instead, these results could be seen as support for considering *S.* "pseudoglaucophyllus" as a species that displays extensive, but near-continuous, morphological variation. This view was advocated by Ali (1964) and is supported in this study by the absence of definitive diagnostic characters (both qualitative and quantitative) for any of the infraspecific groups (Figs. 4.5 and 4.6; Table 4.5), the absence of clear discontinuities in morphometric space (Figs. 4.2 and 4.3), and the presence of extensive morphological variation within a single population of *S.* "pseudoglaucophyllus" (subsp. *basinudus*: Sykes 496/69; Figs. 4.10–4.20). Also the finding that some infraspecific groups are statistically significantly different from each other in their

morphology (Tables 4.4 and 4.5) is compatible with this hypothesis, because this is to be expected for highly variable species of which some forms are morphologically very different from each other if morphologically intermediate forms are not considered. These differences in morphology could be explained by phenotypic plasticity or localized selection in response to environmental factors (e.g., elevation and substrate). In addition, or alternatively, also geographic differentiation could be an underlying factor for some of the patterns of morphological variation (Thorpe, 1976; Krauss, 1996; de Queiroz, 2007). This finds some support in the significant positive correlation between geographic distance and morphological dissimilarity (Fig. 4.8).

4.7. CONCLUSION

This study set out to use a morphometric phenetic approach to evaluate the classification of *Senecio* "pseudoglaucophyllus" into four infraspecific groups: subsp. *basinudus*, subsp. *discoideus*, subsp. *toa*, and the Nelson-group, and to resolve the taxonomic status of two morphotypes from Marlborough. *Senecio* "pseudoglaucophyllus" is morphologically and ecologically very diverse and has a widespread distribution. Also some of the infraspecific groups that have been formally and informally recognized for this species exhibit considerable morphological variation. The results of the morphometric studies do not support the infraspecific classification into the four groups and also show that the two Marlborough morphotypes do not warrant taxonomic recognition. Instead, a classification into two groups composed of 1) subsp. *basinudus* and the Nelson-group and 2) subsp. *discoideus*, subsp. *toa*, and the two Marlborough morphotypes could be considered as an alternative classification. However, the morphometric patterns could also be interpreted as evidence of a single variable species that displays near-continuous morphological variation and for which infraspecific taxa cannot be unambiguously recognized. In Chapter 5, I aim to further contribute to resolving the infraspecific classification of *S.* "pseudoglaucophyllus" by determining if patterns of morphological variation within *S.* "pseudoglaucophyllus" are congruent with patterns of molecular genetic data, because such groups could be considered as diagnosable evolutionary units that merit formal taxonomic recognition (Braby et al., 2012; Ellison et al., 2014).

Fig. 4.10. Sheet A of K of the single population of subsp. *basinudus* that was included in the morphometric study as a case study of how much variation can be observed within a population**.** Photo credit: Allan Herbarium©.

Fig. 4.11. Sheet B of K of the single population of subsp. *basinudus* that was included in the morphometric study as a case study of how much variation can be observed within a population**.** Photo credit: Allan Herbarium©.

Fig. 4.12. Sheet C of K of the single population of subsp. *basinudus* that was included in the morphometric study as a case study of how much variation can be observed within a population**.** Photo credit: Allan Herbarium©.

Fig. 4.13. Sheet D of K of the single population of subsp. *basinudus* that was included in the morphometric study as a case study of how much variation can be observed within a population**.** Photo credit: Allan Herbarium©.

Fig. 4.14. Sheet E of K of the single population of subsp. *basinudus* that was included in the morphometric study as a case study of how much variation can be observed within a population**.** Photo credit: Allan Herbarium©.

Fig. 4.15. Sheet F of K of the single population of subsp. *basinudus* that was included in the morphometric study as a case study of how much variation can be observed within a population**.** Photo credit: Allan Herbarium©.

Fig. 4.16. Sheet G of K of the single population of subsp. *basinudus* that was included in the morphometric study as a case study of how much variation can be observed within a population**.** Photo credit: Allan Herbarium©.

Fig. 4.17. Sheet H of K of the single population of subsp. *basinudus* that was included in the morphometric study as a case study of how much variation can be observed within a population**.** Photo credit: Allan Herbarium©.

Fig. 4.18. Sheet I of K of the single population of subsp. *basinudus* that was included in the morphometric study as a case study of how much variation can be observed within a population**.** Photo credit: Allan Herbarium©.

Fig. 4.19. Sheet J of K of the single population of subsp. *basinudus* that was included in the morphometric study as a case study of how much variation can be observed within a population**.** Photo credit: Allan Herbarium©.

Fig. 4.20. Sheet K of K of the single population of subsp. *basinudus* that was included in the morphometric study as a case study of how much variation can be observed within a population**.** Photo credit: Allan Herbarium©.

CHAPTER 5: An integrative approach to revisiting the infraspecific classification of *Senecio* "pseudoglaucophyllus"

5.1. ABSTRACT

At least in the first instance, morphology plays a key role in the discovery of most new plant taxa and their subsequent description and taxonomic classification. Morphology is therefore an important source of data for documenting botanical biodiversity. However, taxonomic studies that only use morphological characters are not always able to identify evolutionary significant units that merit formal taxonomic recognition. This highlights the importance of incorporating multiple lines of evidence in taxonomic delimitation: an integrative approach. The morphometric study of *Senecio* "pseudoglaucophyllus" in Chapter 4 revealed patterns of morphological diversity that could be used to inform the infraspecific classification of this species, but it remains to be tested if these patterns are congruent with patterns of genetic diversity. In this chapter, phylogenetic analyses of ITS DNA sequence data, and model-based Bayesian clustering, multivariate analyses, and AMOVA of AFLP data were therefore used to study patterns of genetic diversity within *S.* "pseudoglaucophyllus". The resulting genetic patterns do not support the formal taxonomic recognition of subsp. *basinudus*, subsp. *discoideus*, subsp. *toa*, and the Nelson-group and instead show a strong geographic signal. The morphological and genetic patterns of diversity are largely incongruent, and neither source of data supports the recognition of the four infraspecific groups as distinct evolutionary units with diagnostic characters. Unambiguous support for alternative intraspecific classifications is similarly lacking and *S.* "pseudoglaucophyllus" is therefore best considered as a morphologically variable species for which infraspecific morphological forms should not be formally recognized.

5.2. INTRODUCTION

Morphology has traditionally been (Mayden, 1997) and continues to be the most commonly used data source for describing new plant species and infraspecific taxa. Although taxonomic delimitations based on morphology are often confirmed in subsequent research projects by other sources of evidence, particularly molecular genetic data (Bond et al., 2012; Zuccarello et al., 2015), there are some limitations to morphology as a source of data for delimiting taxa (Dayrat, 2005; Schlick-Steiner et al., 2010). For example, morphologically complex species (e.g., species with substantial intraspecific variation, or cryptic species) can be difficult to delineate using morphology alone. In addition, phenotypic plasticity and convergent evolution of morphological traits under selective pressure may confound taxonomic

delimitations that reflect evolutionary history (Mrinalini et al., 2015; Vigalondo et al., 2015). In such cases, integrative taxonomy, which combines multiple, complementary lines of evidence has been proven to be a powerful taxonomic delimitation approach (Dayrat, 2005; Schlick-Steiner et al., 2010). Indeed, many botanists use an integrative approach in delimiting difficult plant groups at various taxonomic levels, using multiple data sources, including karyotypic, molecular genetic, and morphological evidence (e.g., Lihová et al., 2004; Martínez-Ortega et al., 2004; Kropf, 2008; Pessoa et al., 2012; Meudt et al., 2013; Caković et al., 2015; Loeuille et al., 2015; Moffat et al., 2015) and this approach has been demonstrated to have a greater potential for taxonomic delimitation than approaches that only use a single source of data (Hillis, 1987; Page et al., 2005). Because taxonomists agree that species hypotheses formed through the evaluation of several lines of evidence are more robust (Pante et al, 2015), integrative systematic is becoming more popular among systematic studies compared to the traditional morphology-based approach. When multiple types of data are involved, characters from various sources might not always result in congruent patterns of diversity (Caković et al., 2015; Moffat et al., 2015). This in itself, however, can provide valuable insights into the evolutionary processes that have resulted in these incongruent patterns (Schlick-Steiner et al., 2010; Andújar et al., 2014; Schlick-Steiner et al., 2014; Wachter et al., 2015).

Senecio "pseudoglaucophyllus" is a tag name for an unnamed, but well-known, New Zealand species. This species composes the larger part of *S. glaucophyllus* sensu Ornduff, but does not include the type of *S. glaucophyllus* Cheeseman (Chapter 2). *Senecio* "pseudoglaucophyllus" is a morphologically very variable species for which currently four infraspecific groups are recognized: subsp. *basinudus*, subsp. *discoideus*, subsp. *toa*, and the Nelson-group (Chapters 3 and 4). In Chapter 4, a morphometric phenetic approach was used to determine if patterns of morphological variation within *S.* "pseudoglaucophyllus" support the formal taxonomic recognition of these four infraspecific groups. Although the results of the morphometric analyses did not support this classification, patterns of morphological variation were recovered that instead indicate the presence of two morphological groups that might merit formal taxonomic recognition (Figs. 4.2 and 4.3a,b). However, many specimens were found that are morphologically intermediate between these two groups and this might instead indicate that *S.* "pseudoglaucophyllus" is best considered as a taxon that displays large but near-continuous morphological variation and for which infraspecific taxa should not be recognized. Because morphological data alone could not unambiguously resolve the issues

regarding the infraspecific delimitation of *S. "*pseudoglaucophyllus", an integrative approach was used in the study presented in this chapter. In this approach, patterns of genetic diversity were resolved and compared with patterns of morphological variation to inform the infraspecific taxonomic classification of *S. "*pseudoglaucophyllus".

Following the publication of his intraspecific classification of *Senecio glaucophyllus* sensu Ornduff (1960), Ornduff published his findings of a study in which he made artificial hybrids between his four subspecies (subsp. *basinudus*, subsp. *discoideus*, subsp. *glaucophyllus*, subsp. *toa*) to investigate subspecific genetic affinities and the genetic nature of morphologically intermediate populations (Ornduff, 1962). These artificially produced F1 hybrids did not show a reduction in fertility compared to their parental subspecies (Ornduff, 1962). Moreover, Ornduff (1962) did not find lower fertility in specimens resulting from crosses between specimens from different geographical origins or from different habitats than between those from nearby areas or similar habitats. From Ornduff's hybridization experiments, it is clear that the four *S.* "pseudoglaucophyllus" groups have very close genetic affinities and might readily hybridize (Ornduff, 1962). However, his results failed to provide more detailed information about the infraspecific genetic structure of *S.* "pseudoglaucophyllus". Fortunately, with the advancement of molecular genetic techniques, it is now possible to employ sensitive genetic markers such as DNA sequence and Amplified Fragment Length Polymorphism (AFLP) data to further examine the genetic structure of this species.

DNA sequences are routinely employed to study the taxonomic delimitation of taxa at different taxonomic levels (e.g., Bayer et al., 2002; Dillenberger & Kadereit, 2013; Ohlsen et al., 2014) and have contributed to resolving problematic taxa for which morphological analyses alone provided insufficient resolution (e.g., Pessoa et al., 2012; Egea et al., 2016). In cases where sequencing of DNA regions does not provide enough resolution to resolve taxonomic boundaries, multilocus genetic fingerprinting methods are often employed, because of their ability to yield data from a larger number of loci and therefore to enhance resolution (Rønsted et al., 2006). AFLP and microsatellite data are some of the most popular fingerprinting data and these markers are commonly utilized to study infraspecific genetic structure and diversity (Meudt & Clarke, 2007; Dufresne et al., 2014), especially in plants (Bensch & Åkesson, 2005). Even though, in contrast to microsatellite data, AFLP genotyping results in dominant instead of co-dominant data and therefore does not result in direct estimates of heterozygosity, this technique is commonly applied to study inter- and

infraspecific genetic structure. It is particularly popular, because, in contrast to microsatellites, AFLP markers are anonymous and AFLP studies therefore do not require the development of species-specific primers, making this approach more cost- and time-effective. In addition, the dominant nature of AFLP data allows for studies involving polyploids (Meudt & Clarke, 2007; Dufresne et al., 2014). Because microsatellite primers for *S.* "pseudoglaucophyllus" have not yet been developed and because of the polyploid origin of this species (Chapter 2), AFLP rather than microsatellite markers were selected for this study of the infraspecific genetic structure of *S.* "pseudoglaucophyllus".

5.3. AIMS

The aims of this study are 1) to determine if patterns of morphological variation of *Senecio* "pseudoglaucophyllus" as detailed in Chapter 4 are congruent with patterns of molecular genetic diversity and 2) to use the results of the morphometric and genetic studies to determine if the four infraspecific groups of *S.* "pseudoglaucophyllus" (subsp. *basinudus*, subsp. *discoideus*, subsp. *toa*, and the Nelson-group) should be formally recognized as distinct taxa or if an alternative infraspecific classification is more strongly supported. For this, a genotypic cluster concept (Mallet, 1995) is chosen as an operational species concept. The genotypic cluster species concept defines species as "distinguishable groups of individuals that have few or no intermediates when in contact" and can be applied to both morphological and genetic data sets (Mallet, 1995; Coyne & Orr, 2004). To quantify "distinguishable" in this definition, I follow the subspecies concept for botanists recommended by Ellison et al. (2014) (modified from the zoological subspecies concept suggested by Braby et al. (2012)) which states that infraspecific taxa should have "at least one fixed diagnosable character state". Mallet (1995) views the rank of subspecies as similar to that of a species with the exception of the ability of the former to produce intermediates in areas of sympatry. Under the genotypic cluster species concept, the four currently recognized infraspecific groups of *S.* "pseudoglaucophyllus" should only be considered for formal taxonomic recognition if they are found to form four genetically distinct groups with few or no genetically intermediate specimens. Similarly, the alternative infraspecific delimitation into two groups would only be supported if these two groups are shown to be genetically distinct.

5.4. MATERIALS AND METHODS

5.4.1. Sampling and DNA extraction

Specimens for the genetic analyses were selected to represent *Senecio* "pseudoglaucophyllus" populations across its geographical and ecological range as well as its morphological diversity. Species lists from regional surveys (e.g., Wilson, 1992), knowledge of personnel from the New Zealand Department of Conservation (DoC) and the QE II National Trust and other botanists, and the Allan Herbarium database were used to locate populations of the four groups of *S.* "pseudoglaucophyllus" (subsp. *basinudus*, subsp. *discoideus*, subsp. *toa,* Nelson-group) for collecting tissue samples and vouchers for the genetic analyses. Because of the need for high quality DNA for AFLP analyses, freshly-collected tissue samples instead of tissue from herbarium specimens were used when possible. These samples were collected by myself with others or by others for me (John Barkla, Shannel Courtney, Daniel Kimber and Nicholas Head of DoC, Alice Shanks and Miles and Gillian Giller of QE II) in the summers (December to March) of 2014 and 2015. A permit to collect flora and fauna was obtained from Christchurch City Council to collect in public land around Banks Peninsula and the Port Hills. Fresh specimens from the North Island could not be obtained and recently collected herbarium specimens were used instead (Populations 1−3) after confirming that non-degraded DNA was obtained from these specimens. Specimens from two of these populations (Population 1 and 3) were contributed by Mike Thorsen, who collected these plants as private collections. One or more voucher specimens per population were collected for morphometric studies (Chapter 4) if the population contained more than five individuals. Voucher specimens were not collected from smaller populations to avoid negative impacts on these populations due to over-collecting.

A total of 58 specimens (Table S7) from 29 populations of subsp. *basinudus* (n = 12), subsp. *discoideus* (n = 12), subsp. *toa* (n = 7), and the Nelson-group (n = 13) were included in this study (Fig. 5.1; Table 5.1). In addition, four specimens of *Senecio* aff. *glaucophyllus* "South Marlborough" (Population 12), one specimen of *S.* aff. *glaucophyllus* "Cape Campbell" (Population 11), and nine unknown specimens (eight from Population 5 and one from Population 16) were sampled. The identities of the aforementioned nine specimens are unknown because voucher specimens were not collected and plants could not be identified to one of the four groups with certainty in the field (Population 5) or because the quality of the voucher specimen is too poor for identification (Population 16). The taxonomic identities of the collected specimens were determined using Ornduff's (1960) identification key and the diagnostic characters for the Nelson-group as identified in Chapter 3. A few leaves were taken from one or more plants per population as tissue samples for DNA extraction and these

were preserved on silica gel (Table 5.1). DNA was extracted following the protocols presented in Chapter 2.

Table 5.1. Sampled populations with the number of *Senecio* "pseudoglaucophyllus" individuals included in the analyses (n) listed from North to South. Some latitudes and longitudes were approximated using Google Map©2016. Elevation was sometimes estimated from locality data. Populations are identified to the following groups: subsp. *basinudus* - Pop 18, 21, 22, 23, 27, 28, 29; subsp. *discoideus* - Pop 15, 19, 20, 21, 24, 25, 26; subsp. *toa* – Pop 1, 2, 3, 13, 14, 17; Nelson-group - Pop 4, 6, 7, 8, 9, 10; unknown - Pop 5 and 16; *S.* aff. *glaucophyllus* "Cape Campbell" - Pop 11; *S.* aff. *glaucophyllus* "South Marlborough" - Pop 12.

Pop	Location	Latitude	Longitude	Elevation	n
1	Hawke's Bay, Maungaharuru Range	-39.1344	176.7686	870m	1
2	Hawke's Bay, Ngaruroro River	-39.1710	176.1718	1000m	1
3	Hawke's Bay, Te Waka Range	-39.2450	176.6555	793m	1
4	Nelson, Gouland Downs (cultivated)	-40.8907	172.3531	633m	1
5	Nelson, Cundy Creek	-41.1847	172.6257	1259m	8
6	Nelson, The Twins	-41.2383	172.6592	1737m	1
7	Nelson, Mt. Owen	-41.5165	172.5669	1500m	2
8	Nelson, Haystack Creek	-41.5449	172.3442	1270m	1
9	Nelson, Southern Mt Owen	-41.5517	172.5408	1864m	6
10	Nelson, 1000 Acres Plateau of the Matiri Plateau	-41.6293	172.2835	1090m	2
11	Marlborough, Mussel Point	-41.7275	174.2187	5m	1
12	Marlborough, Isolation Creek	-41.8855	173.9832	160-200m	4
13	Marlborough, Rough Creek	-42.3257	173.1720	1550m	2
14	Canterbury, inland Waikari	-42.9694	172.7058	232m	1
15	Canterbury, Mt. Sugarloaf	-43.0353	171.7875	1347m	1
16	Canterbury, Motunau	-43.0387	173.0815	20m	1
17	Canterbury, Mt. Brown	-43.0742	172.6321	210m	1
18	Canterbury, Mt. Cass	-43.0754	172.8390	500-600m	4
19	Canterbury, Craigieburn Forest Park	-43.1183	171.7015	1250m	1
20	Canterbury, Castle Hill	-43.2240	171.7181	762m	2
21	Canterbury, The Tors	-43.5919	172.6956	448m	2
22	Canterbury, Witch Hill	-43.5933	172.6775	406m	1
23	Banks Peninsula, Akaroa	-43.8193	173.0558	392m	1
24	Canterbury, Rockdale	-44.2791	170.9579	281m	4
25	Canterbury, Taiko	-44.3477	171.0217	196m	2
26	Otago, Mt. Buster	-44.9328	170.2189	1315m	1
27	Otago, Shag Point	-45.4742	170.8290	15m	1
28	Otago, Tavora Beach	-45.5304	170.7595	5m	1
29	Otago Peninsula, Allans Beach	-45.8749	170.7013	5m	3

5.4.2. ITS sequencing and phylogeny construction

To study patterns of genetic diversity within *Senecio* "pseudoglaucophyllus", ITS sequences for 25 of the 58 specimens were generated. Amplification and sequencing of the ITS region followed the protocols presented in Chapter 2. These ITS sequences were added to the global ITS data alignment of Chapter 2 for phylogeny reconstruction using Bayesian inference (BI), following the methodology outlined in Chapter 2. In addition to these 25 specimens, eight ITS sequences generated for members of *S.* "pseudoglaucophyllus" in Chapter 3 and one ITS sequence of the Nelson-group (EU812813) obtained from GenBank were included in the phylogenetic analyses.

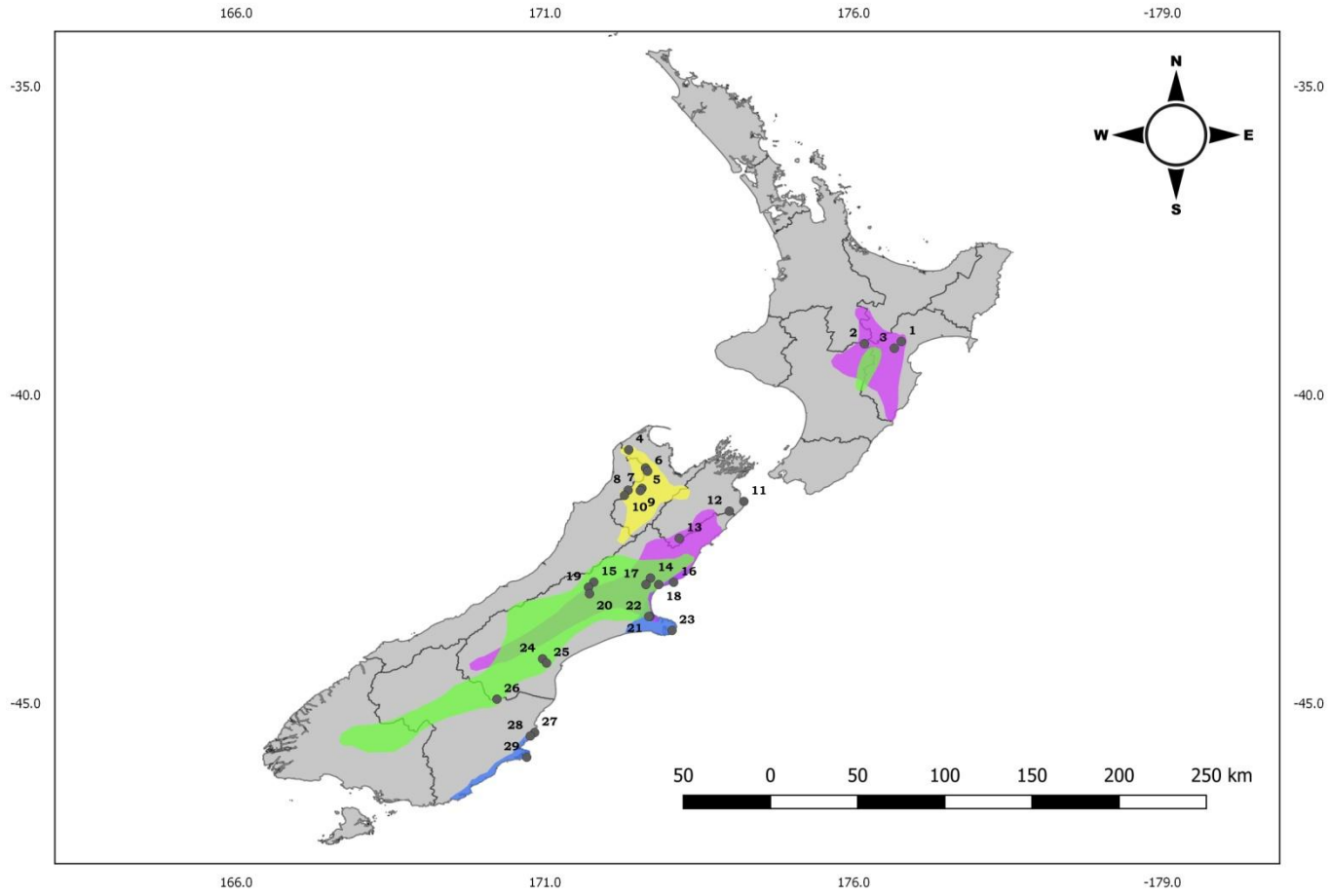


Fig. 5.1. Locations of the 29 sampled *Senecio* "pseudoglaucophyllus" populations. Numbers on the map correspond to population numbers (Table 5.1). Colored areas indicate the distribution ranges of the four currently recognized *S.* "pseudoglaucophyllus" groups drawn from the locality data of herbarium specimens examined in Chapter 4 (Fig. 4.7). Blue: subsp. *basinudus*; green: subsp. *discoideus*; purple: subsp. *toa*; yellow: the Nelson-group. Populations 11 (*S.* aff. *glaucophyllus* "Cape Campbell") and 12 (*S.* aff. *glaucophyllus* "South Marlborough") are not within the colored areas because ranges of these groups were not plotted in Chapter 4 due to the small number of specimens examined.

5.4.3. AFLP analysis

AFLP is a DNA fingerprinting technique, in which restriction fragments throughout the genome are selectively amplified to produce a restriction fragment profile (Meudt & Clarke, 2007). AFLP protocol version 4 of Clarke & Meudt (2005) (accessed at http://clarkeresearch.org/aflp_2012-01-26/AFLP_Protocol.pdf), a modified protocol based on Vos et al. (1995), was used for the current study. Because using degraded DNA in AFLP analyses might result in null-alleles (Bensch & Åkesson, 2005), genomic DNA was visualized on 1% agarose gels to assess the DNA quality of each sample. If a single, high molecular weight band (indicating non-degraded DNA) was not observed, DNA extractions were repeated and the quality of the genomic DNA was reassessed. If good quality DNA was not obtained after a second extraction, the relevant specimen was excluded from the analyses. DNA of three samples (5.4%, randomly selected) was extracted twice and these duplicates were used as samples for genotyping error rate checking as recommended by Bonin et al. (2004) and Pompanon et al. (2005) and outlined in section 5.4.4 of this chapter. A negative control was also included at every step of the AFLP procedure (restriction, ligation, pre-selective and selective amplifications), which included all reagents except for DNA to check for exogenous contamination. The first three steps of the AFLP procedure: DNA restriction, ligation and pre-selective amplification were done in one day to prevent non-specific restriction and degradation of ligation products (Clarke & Meudt, 2005).

5.4.3.1. Restriction

Genomic DNA was restricted using *Eco*R I and *Mse* I restriction enzymes (Table 5.2). The reaction mixture consisted of 5µl of 5x reaction buffer (250mM potassium acetate (KOAc), 50mM magnesium acetate (MgOAc) and 50mM Tris-HCL[pH 7.5]), 1µl of Roche *Eco*R I (10U/µl), 1µl of NEB *Mse* I (10U/µl), ~250ng of DNA and Milli-Q water to a total volume of 25µl. The restriction reaction was carried out with incubation at 37°C for 3 hours, followed by incubation at 70°C for 15 min to denature the restriction enzymes. To check if restriction was complete, digested DNA was run on a 1% agarose gel next to a control of undigested DNA. A smear of up to ~750bp was regarded as evidence that DNA samples are completely digested.

5.4.3.2. Linker ligation

The restricted DNA was ligated with double-stranded *Eco* and *Mse* linkers (Table 5.2) that have complementary sticky ends to those of the restriction fragments. The 20µl reaction ligation cocktail was made up of 2µl of Roche 10x ligation buffer, 1µl of Roche T4 DNA ligase, 5µl of restricted DNA sample, 1µl each of *Eco* and *Mse* linkers and 10µl of Milli-Q water. Ligation reactions were then incubated at 37°C for 3 hours.

Table 5.2. Enzymes and oligonucleotide sequences used in the AFLP analyses. ^ indicates where the restriction enzymes are cutting ● indicates the fluorescently labeled primer (6FAM). **Bold** type indicates selective nucleotides.

	Sequence (5' – 3')
Restriction enzymes	
***Eco*R I**	G^AATTC
	CTTAA^G
***Mse* I**	T^TAA
	AAT^T
Linkers	
***Eco* Linker I**	CTCGTAGACTGCGTACC
***Eco* Linker II**	AATTGGTACGCAGTCTAC
***Mse* Linker I**	GACGATGAGTCCTGAG
***Mse* Linker II**	TACTCAGGACTCAT
Pre-selective primers	
***Eco* + A**	GACTGCGTACCAATTC**A**
***Mse* + C**	GATGAGTCCTGAGTAA**C**
Selective primers	
● ***Eco* + ACT**	●GACTGCGTACCAATTC**ACT**
***Mse* + CAA**	GATGAGTCCTGAGTAA**CAA**
***Mse* + CCC**	GATGAGTCCTGAGTAA**CCC**
***Mse* + CCG**	GATGAGTCCTGAGTAA**CCG**
***Mse* + CTA**	GATGAGTCCTGAGTAA**CTA**
***Mse* + CGG**	GATGAGTCCTGAGTAA**CGG**
***Mse* + CTC**	GATGAGTCCTGAGTAA**CTC**
***Mse* + CAG**	GATGAGTCCTGAGTAA**CAG**
***Mse* + CTGG**	GATGAGTCCTGAGTAA**CTGG**

5.4.3.3. Pre-selective amplification

Restriction fragments ligated with linker sequences were subjected to pre-selective amplification, which reduces the number of fragments by amplifying only fragments that have complementary sequences to the pre-selective primers (Table 5.2). PCR reactions had a total volume of 20µl, which consisted of 1µl of ligated DNA, 0.2µl of Taq polymerase (5U/µl) (Roche), 2µl of 10x PCR buffer (Roche), 1µl of *Eco* + A primer (10pmol/µl), 1µl of *Mse* + C primer (10pmol/µl), 0.25mM of dNTPs, 1M of betaine and 8.3µl of Milli-Q water. The PCR

program for pre-selective amplification followed 20 cycles of denaturation at 94°C for 30 sec, annealing at 56°C for 1 min and extension at 72°C for 1 min with ramping speed limited to 1°C/sec. The pre-selective amplification PCR products were run on a 1% agarose gel to confirm that the PCR resulted in DNA amplification. PCR was done using an Eppendorf thermocycler Mastercycler® ep gradient S.

5.4.3.4. Selective amplification

Selective amplification was performed in 8µl reactions, consisting of 1µl of pre-amplification product, 0.08µl of Taq polymerase (5U/µl) (Roche), 0.8µl of 10x PCR buffer (Roche), 0.4µl of 6Fam-labelled *Eco* + ACT primer (10pmol/µl), 0.4µl of *Mse* + CNN primer (10pmol/µl), 1µl of 2mM dNTPs, 1µl of 25mM $MgCl_2$ and 3.32µl of Milli-Q water. The amplification was conducted using the following touchdown program: initial incubation for 2 min at 94°C to activate the Taq polymerase followed by 10 cycles of denaturation at 94°C for 30 sec, annealing at (65−56°C) for 30 sec and extension at 72°C for 1 min. The annealing temperature (starting from 65°C) was reduced by 1°C per cycle and reached 56°C at the end of the tenth cycle. This was followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 56°C for 30 sec and extension at 72°C for 1 min. The program ended with a final extension at 72°C for 30 min. Ramping speed for the selective amplification was limited to 1°C/sec.

As this study is the first AFLP study of *Senecio* "pseudoglaucophyllus", a screening of selective amplification primers was carried out to select primer pairs that produce scorable and reproducible profiles. Eight primer combinations (Table 5.2) were screened using six specimens, a replicate and a negative control. Following the screening, three primer pairs were selected for the analysis: *Eco* + ACT / *Mse* + CAA, *Eco* + ACT / *Mse* + CTA and *Eco* + ACT / *Mse* + CTGG. The primer combinations that were not selected either yielded little or no amplification product, had a very low number of peaks and were therefore deemed too uninformative, or displayed AFLP profiles that were too complex to be reliably scored (e.g., groups of fragments of very similar sizes).

5.4.4. Genotyping and scoring of AFLP fragments

Samples for genotyping consisted of 2µl of selective amplification product, 10µl of HiDi Formamide (Applied Biosystems) and 0.4µl of GeneScanTM 500 Liz Size Standard (Applied

Biosystems). Samples were denatured at 95°C for 4 min. before genotyping them using an ABI 3130xl Genetic Analyzer (Applied Biosystems) at University of Canterbury.

AFLP profiles were successfully produced for 55 of the 58 specimens selected for this study. The three remaining specimens were excluded from the analyses because they failed to amplify or produced AFLP profiles that were of poor quality. A total of 50 of the 55 remaining samples yielded good quality AFLP profiles for all primer combinations used in the current study. Raw electropherograms were analyzed in Peak Scanner 2 (Applied Biosystems) to detect, visualize, and calculate the size of AFLP fragments using the default settings of the software except for a light peak smoothing to smooth out jagged, small secondary peaks due to background noise, and using a minimum peak height of 100 Relative Fluorescent Units (RFU). Size standards were checked and adjusted manually to respective fragment lengths if needed. Scoring is reported as one of the most error-prone steps in the AFLP procedure and especially manual scoring is prone to arbitrary and subjective decisions (Bonin et al., 2004). A semi-automated approach was therefore used in the current study as recommended by Bonin et al. (2004) and Papa et al. (2005). Scoring of AFLP restriction fragments to produce a binary presence/absence matrix was done using the automated scoring package RawGeno (Arrigo, 2013) in R (R Core Team, 2016) and the scored bins were manually reviewed after scoring. The scoring parameters were as follows: scoring range of 100 bp – maximum fragment length (≤ 500bp), minimum intensity of 100 RFU, minimum bin width of 0 bp, maximum bin width of 2 bp and reproducibility of 80%. Fragments of smaller sizes (< 100 bp) were not scored because small fragments are more likely to be homoplasious (Vekemans et al., 2002). The bins scored by the RawGeno binning algorithm were reviewed by confirming the presence of peaks in scored bins, by adjusting the position of bins towards the center of respective peaks, and by eliminating bins with very similar sizes. In addition, monomorphic (peaks present in all individuals) and singleton (the presence of peaks in only a single individual) loci were removed from the data set. The removal of singleton loci has shown to decrease error rate and improve the signal of population structure (Crawford et al., 2011). The information content per bin (Ibin) was subsequently calculated in RawGeno. Ibin is an "optimality criterion" introduced by Arrigo et al. (2009) and defined as M_sampling/nbin where "M_sampling is the average number of mismatches between the considered sample and the other samples of the data set and nbin is the total number of bins in the data set". The resulting matrix was exported to Microsoft Excel where the mean error rate per locus (Bonin et al., 2004; Pompanon et al., 2005) and mean genotyping error per

primer pair were calculated. The calculation of mean error rate per locus followed Pompanon et al. (2005): error rate, $e = m / nt$, which m is "the number of single-locus genotypes including at least one allelic mismatch, and nt, the number of replicated single-locus genotypes". The percentage of polymorphic loci (PLP) was computed using the function "Diversity" in AFLPdat (Ehrich, 2006) in R. Potential homoplasy due to co-migrating non-homologous fragments was detected by assessing if there is a negative correlation between fragment size and frequency (Vekemans et al., 2002) by doing a linear regression with a significance test using the function "lm" (in standard R).

All molecular genetic data sets include genotyping errors (Bonin et al., 2004). For example, AFLP data sets of plant taxa typically have genotyping error rates of up to 5%, although usually lower than 2% (Jones et al., 1997; Hansen et al., 1999; Bonin et al., 2004; Zhang & Hare, 2012). When addressing the issue of genotyping errors, there is a trade-off between minimizing overall genotyping error by removing markers or loci with relatively high error rates from a data set, and increasing the potential of recovering stronger population genetic signal by retaining as many markers and loci as possible (Bonin et al., 2004; Zhang & Hare, 2012). For example, Zhang & Hare (2012) investigated the effects of varying degrees of genotyping error on the study of population structure of two oyster species and found that data sets with 0–2% error rates failed to recover known population structure, whereas data sets with 3 and 4% error rates yielded results that were more congruent with known patterns of genetic diversity. This finding highlights the importance of taking the trade-off between reducing genotyping error and increasing population genetic signal into account in molecular genetic studies. In this study, a similar strategy to that of Zhang & Hare (2012) was adopted to examine the consequences of genotyping error rates on the inference of genetic structure in *Senecio* "pseudoglaucophyllus". In this approach, first a liberal strategy was used in which the number of loci that were included in the data set was maximized by relaxing the reproducibility parameter of the automated scoring of the RawGeno algorithm at the expense of higher error rates. This was done by using a reproducibility parameter of 60% instead of the default setting of 80%. Subsequently, nested data sets with error rates of 2% (referred to as the 2% error data set) and 4% (4% error data set) were created from RawGeno's AFLP matrix by progressively removing high error loci following the method described by Zhang & Hare (2012). The error rate level of 2% was selected because an error rate of about or less than 2% is typically observed in AFLP studies (e.g., Bonin et al., 2004; Moffat et al., 2015). The more liberal error rate of 4% was chosen because Zhang & Hare (2012) found that data

sets with 3% and 4% error rates produced results that match biological expectations in their study. The results of analyses of both data sets were compared to identify the data set that is most powerful in resolving patterns of genetic diversity in *S.* "pseudoglaucophyllus" and to examine the robustness of the patterns of genetic diversity that were recovered against genotyping errors.

5.4.5. AFLP data analyses

5.4.5.1. Bayesian inference of genetic structure

A Bayesian model-based clustering method was used to infer the genetic structure of *Senecio* "pseudoglaucophyllus" using the program STRUCTURE v2.3.4 (Pritchard et al., 2000; Falush et al., 2003, 2007). This program determines the number of distinct genetic groups (K) from allele frequencies (Pritchard et al., 2000). Individuals are then assigned probabilistically to one or more of these K groups based on their genotypes (Pritchard et al., 2000). STRUCTURE analyses were run with an admixture model and correlated allele frequencies using the complete AFLP binary matrix of 55 individuals with the 2% (194 loci) and 4% (202 loci) error data sets to investigate the most probable number of distinct genetic groups in *S.* "pseudoglaucophyllus" (i.e., K) and its genetic structure. The analyses were done for K = 1–10 for 10 iterations each and with a burn-in period of 20,000 MCMC replicates followed by 100,000 replicates. The output of the STRUCTURE analyses was summarized using STRUCTURE HARVESTER (Earl & VonHoldt, 2012) to determine the number of K that best explains the genetic structure in the data sets. Two "ad hoc" estimates were used to determine the best K. The first is the average Ln posterior probability of each K, L (K), which is included in the simulation summary of STRUCTURE (Pritchard et al., 2000; Falush et al., 2003). In this approach, the K value with the highest L (K) is interpreted to be the most probable K (Pritchard et al., 2010). Evanno et al. (2005), however, argued that L (K) does not always reflect the real number of genetic groups and proposed another estimate, Delta K (ΔK). Delta K is associated with the rate of change of the second order likelihood function of K and its modal value might indicate the real K (Evanno et al., 2005). When an admixture model is run in STRUCTURE, it is possible that a sample is assigned to more than one genetic group based on its genotype (Pritchard et al., 2000). Q is an estimate of the proportion of an individual's genotype to K genetic groups or an estimate of membership probabilities (Pritchard et al., 2000; Jakobsson & Rosenberg, 2009). The CLUster Matching and Permutation Program Version 1.1.2 (CLUMPP; Jakobsson & Rosenberg, 2007) was used to calculate Q values for each sample, using the replicate runs for the best K and the Greedy

algorithm with 1000 permutations. The results of CLUMPP were visualized in DISTRUCT Version 1.1 (Rosenberg, 2004) and R.

5.4.5.2. Multivariate analyses

Jaccard pairwise (dis)similarities are commonly used for dominant markers such as AFLP in taxonomic studies (e.g. Pelser et al., 2003; Brysting et al., 2004; Devey et al., 2007; Arrigo et al., 2010; Caković et al., 2015). This measure of genetic distance is deemed appropriate for AFLP data because it only uses shared presence of fragments as evidence of genetic similarity between samples (Bonin et al., 2007; Meudt & Clarke, 2007; Dufresne et al., 2014). This reduces the impact of null-alleles on analyses that aim to resolve genetic structure. Five individuals with missing data (for one primer pair) due to technical difficulties were excluded from the computation of Jaccard similarities. Jaccard distances were calculated for 50 samples for both the 2% and 4% error data sets using the function "dist.binary" (Gower & Legendre, 1986) in the package ADE4 (Dray & Dufour, 2007) in R (R Core Team, 2016). The resulting pairwise similarity matrices were used for subsequent Principal Coordinate Analyses (PCoAs) which were performed following the procedures described in Chapter 3.

5.4.5.3. Testing for isolation by distance

The presence of isolation by distance was tested with a Mantel test using the Jaccard pairwise similarities computed from the 2% and 4% error data sets and Euclidean distances computed from the geographical coordinates of the collected samples. The Euclidean geographical distance was calculated using the function "dist" in the package STATS (in standard R). The Mantel test was carried out using the function "mantel" (Legendre & Legendre, 1998) with 999 permutations in the R package VEGAN (Oksanen et al., 2016).

5.4.5.4. Analysis of molecular variance (AMOVA)

Patterns of genetic differentiation among subsp. *basinudus*, subsp. *discoideus*, subsp. *toa*, the Nelson-group, and *Senecio* aff. *glaucophyllus* "South Marlborough" were studied with an AMOVA (Excoffier et al., 1992). This analysis was performed with 999 random permutations in Genetic Analysis in Excel, version 6.5 (GENAlEx; Peakall & Smouse, 2006, 2012) to calculate Φ_{PT} and pairwise Φ_{PT} among the groups. *Senecio* aff. *glaucophyllus* "Cape Campbell" was excluded from the analyses because only one specimen of this morphotype was included in the data set. Φ-Statistics are analogous to F-Statistics (Wright, 1951, 1965) and are used to partition genetic variation hierarchically between species, populations and

individuals (Excoffier et al., 1992; Maguire et al., 2002). Φ_{PT} was calculated to determine whether the four infraspecific groups of *S.* "pseudoglaucophyllus" and *S.* aff. *glaucophyllus* "South Marlborough" are significantly genetically differentiated (Maguire et al., 2002). Φ_{PT} was standardized (Φ'_{PT}) for within-group diversity by dividing it by the maximum Φ_{PT} (Hedrick et al., 2000; Meirmans & Hedrick, 2011).

5.5. RESULTS

5.5.1. ITS data

ITS sequences of the 34 specimens of *Senecio* "pseudoglaucophyllus" that were included in the analyses are on average 98.6% similar and seven genotypes were recovered. The *S.* "pseudoglaucophyllus" accessions form a poorly supported clade (posterior probability (PP) = 0.85) within Clade 3 (clade numbering follows that of Chapter 2). Most accessions are positioned in a basal polytomy within the *S.* "pseudoglaucophyllus" clade, but 13 accessions group into two clades (Clade A and B; Fig. 5.2). Clade A (PP = 1.0) consists of accessions of specimens from two of the four groups (subsp. *basinudus* and subsp. *discoideus*) and most of these were collected from the Port Hills and Banks Peninsula in Canterbury except for specimen Liew J120 (subsp_basiJ120.1; Otago Peninsula; Fig. 5.2). Clade B has a posterior probability of 0.66 and contains specimens of subsp. *toa* and *S.* aff. *glaucophyllus* "South Marlborough", and a specimen of subsp. *basinudus* (Fig. 5.2). Specimens in Clade B were collected in South Marlborough and North and Central Canterbury.

5.5.2. AFLP data

AFLP profiles were successfully obtained from 55 out of 58 specimens. For a total of 50 of these, all three primer combinations successfully amplified. RawGeno analyses of the AFLP data of the three primer pairs resulted in the identification of between 146 (*Eco*-ACT / *Mse*-CTGG) and 192 (*Eco*-ACT/*Mse*-CAA) initial bins per primer pair ('initial bins', Table 5.3). Subsequent removal of low intensity, non-replicable and rare frequency bins by the RawGeno binning algorithm reduced the number of bins ('final bins', Table 5.3) to between 44 (*Eco*-ACT / *Mse*-CTGG) and 93 (*Eco*-ACT/*Mse*-CAA). The information content per bin (Ibin, Table 5.3) for the three primer pairs is similar (0.17–0.18). A liberal scoring approach in RawGeno, which was used to retain the maximum number of potentially informative loci, yielded AFLP binary matrices with relatively high error rates: 8.6% for *Eco*-ACT/*Mse*-CAA, 6.7% for *Eco*-ACT/*Mse*-CTA and 9.1% for *Eco*-ACT/*Mse*-CTGG (Table 5.3). These matrices were subsequently filtered by progressively weeding out loci with high error rates (>

0.1 as recommended by Bonin et al. (2007)) to obtain nested data sets with 2% and 4% error rates (Table 5.4). The results of linear regression analyses for each of the three primer pairs do not show a significant negative correlation between fragment size and band frequency for the 2% and 4% error data sets (data not shown), which suggests that there is no evidence that these data sets exhibit pronounced size homoplasy.

Table 5.3. Bin statistics as produced from RawGeno. Initial bin numbers when first scored, final bin numbers after removal of low intensity, non-replicable and rare frequency bins by the RawGeno binning algorithm with specified parameters and manual bin review. Ibin: information content per bin (Arrigo et al., 2009). Error rate: mean genotyping error rate (Bonin et al., 2004; Pompanon et al., 2005) of each primer pair calculated using the final bin selection.

	***Eco*-ACT/*Mse*-CAA**	***Eco*-ACT/*Mse*-CTA**	***Eco*-ACT/*Mse*-CTGG**
Initial Bin no.	192	181	146
Final Bin no.	93	75	44
Ibin	0.18	0.18	0.17
Error rate	8.6%	6.7%	9.1%

Fig. 5.2. Bayesian ITS phylogeny that includes specimens of *Senecio* "pseudoglaucophyllus". The *S.* "pseudoglaucophyllus" clade is highlighted in grey. Posterior probabilities (PP) are presented on the nodes. Nelson_group – the Nelson-group, subsp_basi – subsp. *basinudus*, subsp_toa – subsp. *toa*, subsp_disc – subsp. *discoideus*, S sth Marl – *S.* aff. *glaucophyllus* "South Marlborough". Collection numbers follow the taxon abbreviations (see Tables S2, S3 and S7 for specimen details).

Table 5.4. Number of loci and percentage of polymorphic loci (PLP) of the 2% and 4% error data sets and the original ('final bin') RawGeno AFLP matrices (after removal of low intensity, non-replicable and rare frequency bins). n: number of specimens that were genotyped.

	n	**2% Error**		**4% Error**		**Original**	
		No. of loci	PLP (%)	No. of loci	PLP (%)	No. of loci	PLP (%)
subsp. *basinudus*							
***Eco*-ACT/*Mse*-CAA**	9	61	57%	66	58%	71	59%
***Eco*-ACT/*Mse*-CTA**	9	54	54%	56	55%	58	55%
***Eco*-ACT/*Mse*-CTGG**	9	23	48%	24	49%	27	50%
Total		138		146		156	
subsp. *discoideus*							
***Eco*-ACT/*Mse*-CAA**	12	64	60%	69	61%	74	63%
***Eco*-ACT/*Mse*-CTA**	10	50	52%	52	53%	54	53%
***Eco*-ACT/*Mse*-CTGG**	12	28	63%	29	63%	32	66%
Total		142		150		160	
subsp. *toa*							
***Eco*-ACT/*Mse*-CAA**	6	60	52%	64	53%	69	56%
***Eco*-ACT/*Mse*-CTA**	7	49	54%	50	53%	52	53%
***Eco*-ACT/*Mse*-CTGG**	7	23	53%	24	54%	27	57%
Total		132		138		148	
the Nelson-group							
***Eco*-ACT/*Mse*-CAA**	13	58	54%	63	57%	67	58%
***Eco*-ACT/*Mse*-CTA**	12	53	54%	54	53%	56	53%
***Eco*-ACT/*Mse*-CTGG**	13	25	55%	26	56%	29	59%
Total		136		143		152	
***S.* aff. *glaucophyllus* "South Marlborough"**							
***Eco*-ACT/*Mse*-CAA**	4	53	37%	56	39%	61	42%
***Eco*-ACT/*Mse*-CTA**	3	39	24%	40	23%	42	24%
***Eco*-ACT/*Mse*-CTGG**	3	18	30%	19	32%	20	30%
Total		110		115		123	

5.5.3. AFLP analyses

5.5.3.1. Bayesian clustering using STRUCTURE

5.5.3.1.1. Number of genetic clusters (K)

Samples of Populations 22 (Liew J69) and 28 (Liew J121) (Table 5.1) were not included in the AFLP analyses because of amplification problems and these populations are therefore not represented in the STRUCTURE results. Examination of the results of the STRUCTURE analyses for K = 1–10 in STRUCTURE HARVESTER suggests that the number of genetic clusters that best represents the genetic structure in *Senecio* "pseudoglaucophyllus" is five (Fig. 5.3). This value of K was obtained from the mean Ln probabilities of K (L (K)) and the delta K values (ΔK) of the 2% error data set and from the ΔK values of the 4% error data set. However, for the 4% error data set, K = 7 has a larger L (K) than K = 5 (Fig. 5.3c). Because

the three other analyses (Fig. 5.3a, b, d) indicate that the best value of K is five and because the differences in L (K) between K = 5 and K = 7 for the 4% error data set are small (Fig. 5.3c), a value of K = 5 was used for the subsequent STRUCTURE analyses.

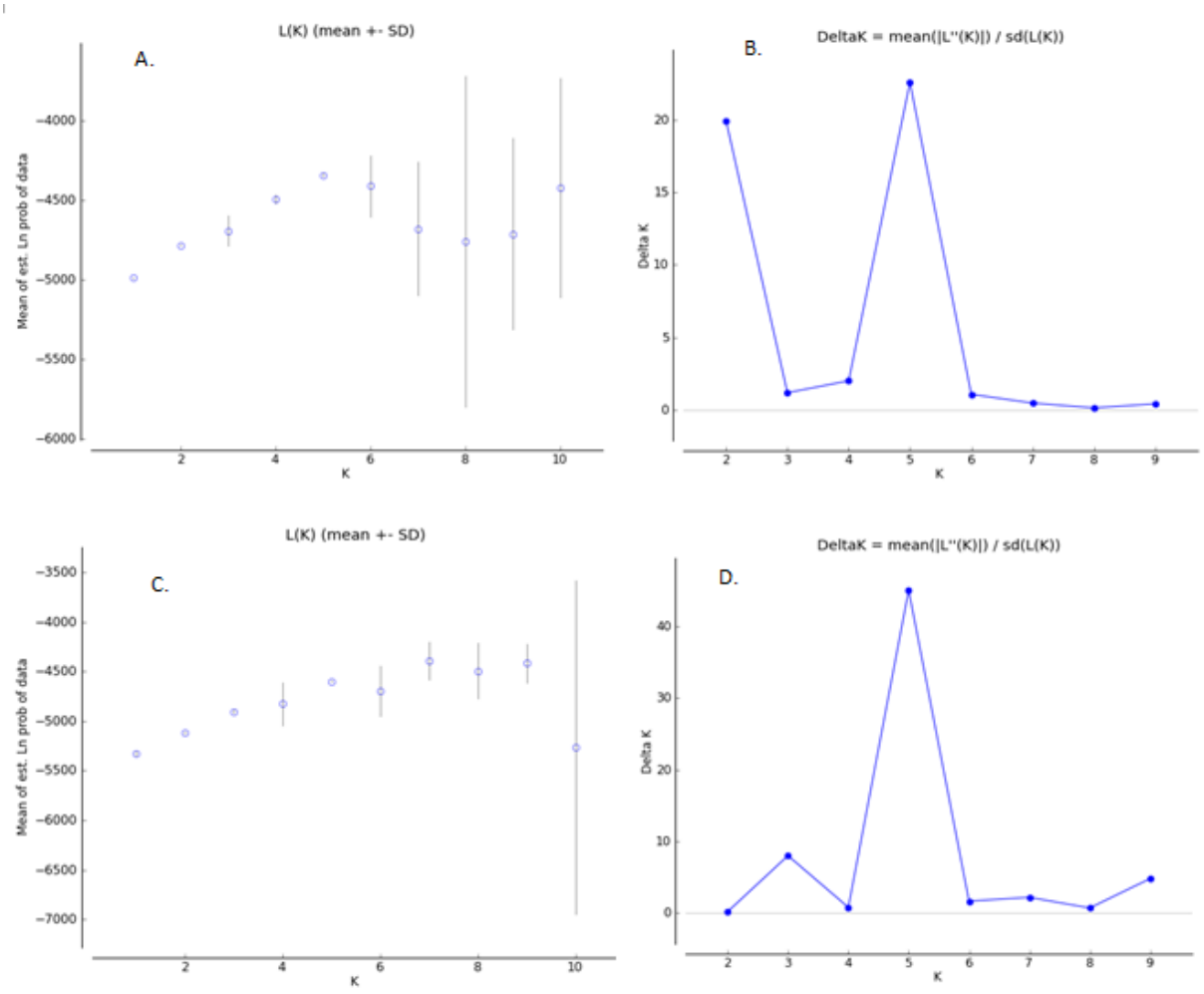


Fig. 5.3. Mean Ln probabilities, L(K) and values of ΔK for the 2% (A, B) and the 4% (C, D) error data sets for K = 1–10.

5.5.3.1.2. Genetic structure of *Senecio* "pseudoglaucophyllus"

Both the 2% and 4% error data sets yield similar membership coefficients for all samples included in the STRUCTURE analyses (Figs. 5.4a, b). The four infraspecific taxonomic groups that are currently recognized in the amended version of Ornduff's classification (subsp. *basinudus*, subsp. *discoideus*, subsp. *toa*, Nelson-group; Ornduff, 1960; Chapter 3) align with at least two of the five genetic clusters that are recovered by STRUCTURE. These four groups, as well as those that belong to *Senecio* aff. *glaucophyllus* "South Marlborough" and *S.* aff. *glaucophyllus* "Cape Campbell", include relatively many specimens that show a

large degree of admixture, except for specimens of subsp. *basinudus*. With the exception of the Nelson-group, of which many specimens align with the green STRUCTURE cluster, the infraspecific groups of *S.* "pseudoglaucophyllus" do not align well with the genetic clusters recovered by STRUCTURE (Fig. 5.4).

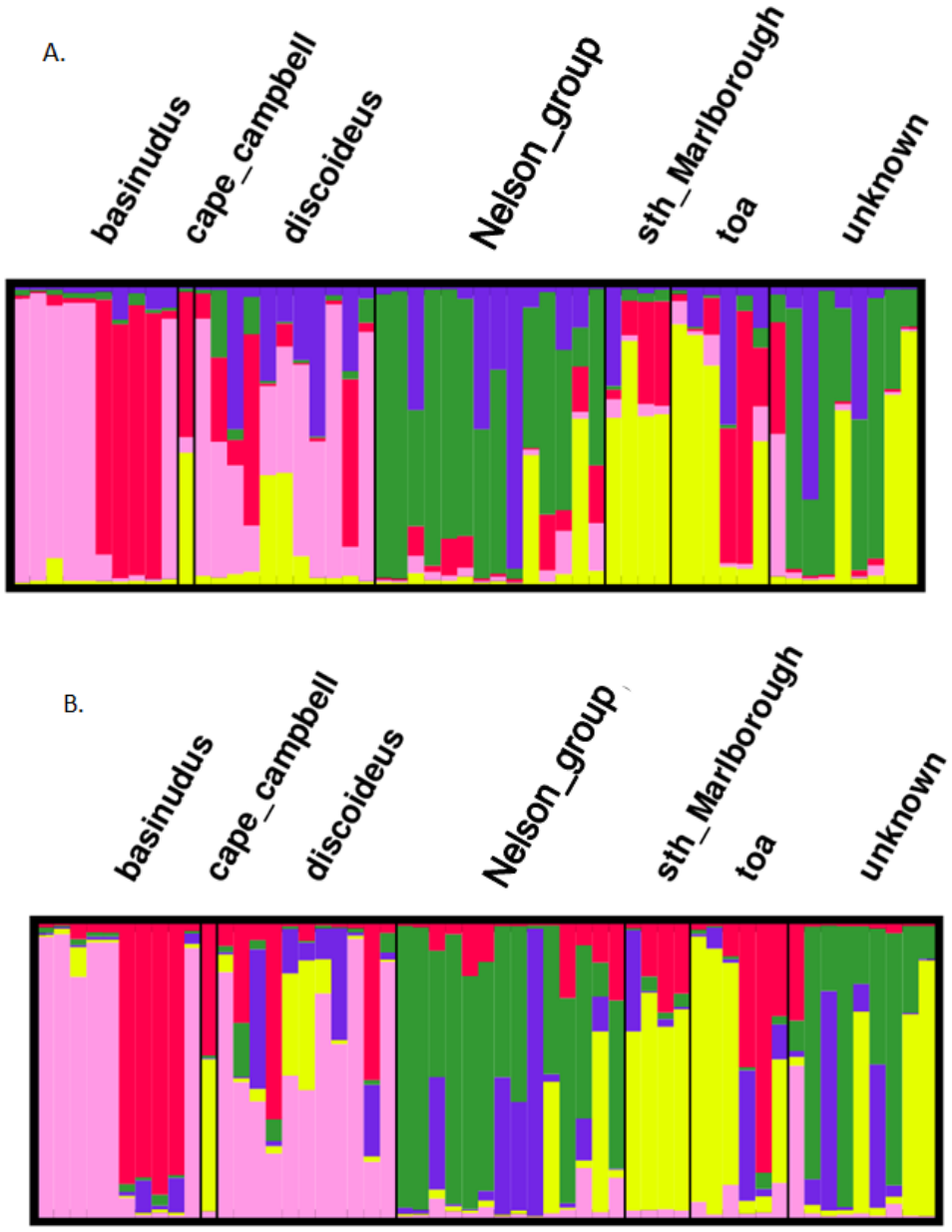


Fig. 5.4. DISTRUCT histograms showing results of STRUCTURE analyses with K = 5 for (A) the 2% error data set and (B) the 4% error data set. Each bar represents an individual from the four currently recognized infraspecific taxonomic groups of *Senecio* "pseudoglaucophyllus" (subsp. *basinudus*: basinudus, subsp. *discoideus*: discoideus, subsp. *toa*: toa, the Nelson-group: Nelson_group), and the *S.* aff. *glaucophyllus* "Cape Campbell" (cape_campbell) and *S.* aff. *glaucophyllus* "South Marlborough" (sth_Marlborough) morphotypes. These groups are separated by single black lines.

5.5.3.2. Geographical structure of AFLP data

Genetic clustering of members of *Senecio* "pseudoglaucophyllus" demonstrates a strong geographical structure (Fig. 5.5). For example, populations in the North Island and Marlborough have high Q values corresponding to the yellow genetic cluster (Cluster 2; Fig. 5.5). In addition, populations from Central Canterbury and Otago have high Q values for membership to the pink cluster (Cluster 5). High Q values for Cluster 1 (the purple cluster) appear to be more common in specimens from inland populations than in those at the coast (Fig. 5.5). Plants from North Canterbury (especially populations 14, 17, 18) have genetic profiles that correspond for a large part to the red genetic cluster (Cluster 4). Finally, the green cluster (Cluster 3) is largely localized in Nelson, especially Northwest Nelson (Populations 4–10; Fig. 5.5).

Mantel tests for isolation by distance were carried out to determine if there is a positive correlation between pairwise Jaccard distances and Euclidean geographical distances. The results of these Mantel tests show a significant, positively correlated relationship between the genetic and geographical distance for both the 2% and 4% error data sets (2%: $r = 0.4587$, $P = 0.001$, 4%: $r = 0.4528$, $P = 0.001$; Fig. 5.7).

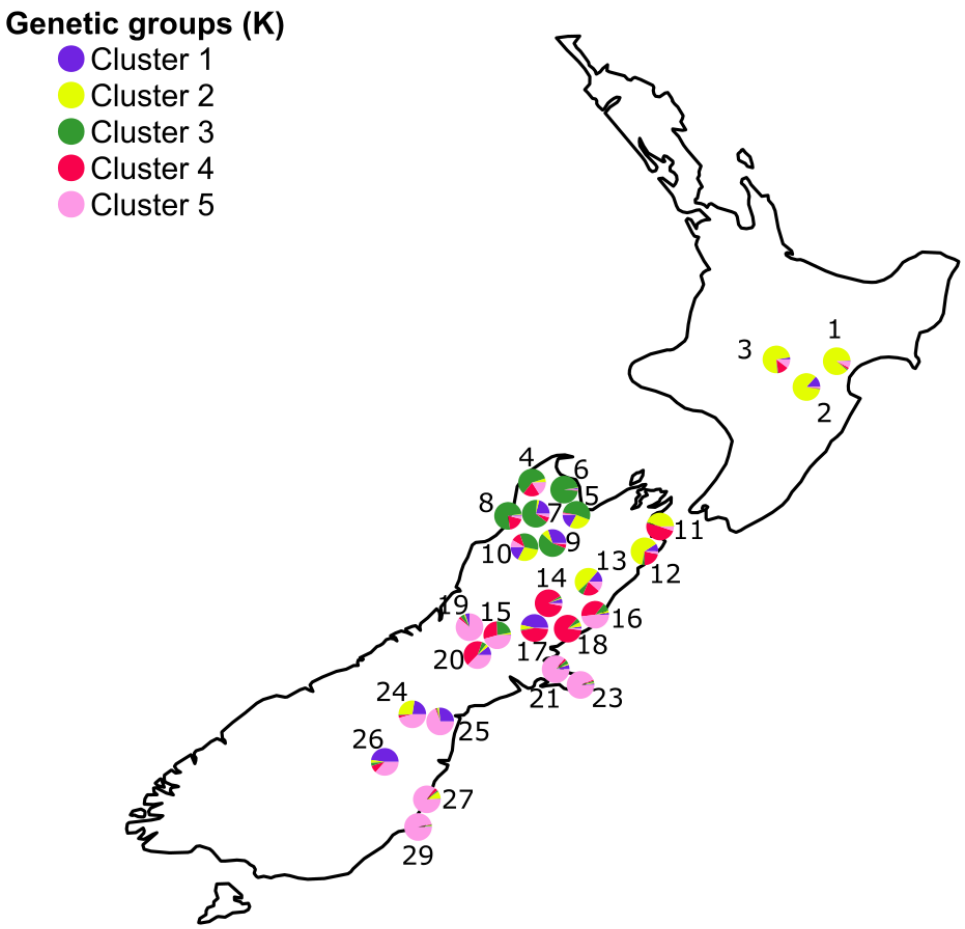


Fig. 5.5. Map of sampled populations with pie charts indicating Q values (membership probabilities) to genetic groups as assigned by STRUCTURE when K = 5 (2% error data set, data for the 4% error data set not shown). If more than one individual was sampled per population, Q values were averaged across all sampled individuals. To ensure visibility of all populations, positions of pie charts have been adjusted accordingly. Population numbers correspond to those in Table 5.1.

5.5.3.3. Multivariate analyses

5.5.3.3.1. PCoA

The PCoA bidimensional plots of the first and second PCoA axes of the 2% and 4% error data sets are nearly identical, therefore only one of them is shown (2% error data set; Fig. 5.6). The bidimensional plots of second vs. third and first vs. third axes for both data sets are very similar to those in which the first and second axes are shown and are therefore also not presented. The total percentages of variation explained by the first and second PCoA axes of the 2% and 4% error data sets are 14.4% and 14.3% respectively. The PCoA plots do not reveal distinct genetic clusters (Fig. 5.6). Instead, the specimens form a single large cluster in which specimens of the Nelson-group and unidentified specimens from the Cundy Creek population (Population 5) in northwest Nelson loosely cluster together with relatively little overlap with the similarly loose cluster composed of specimens from other parts of New Zealand (Fig. 5.6).

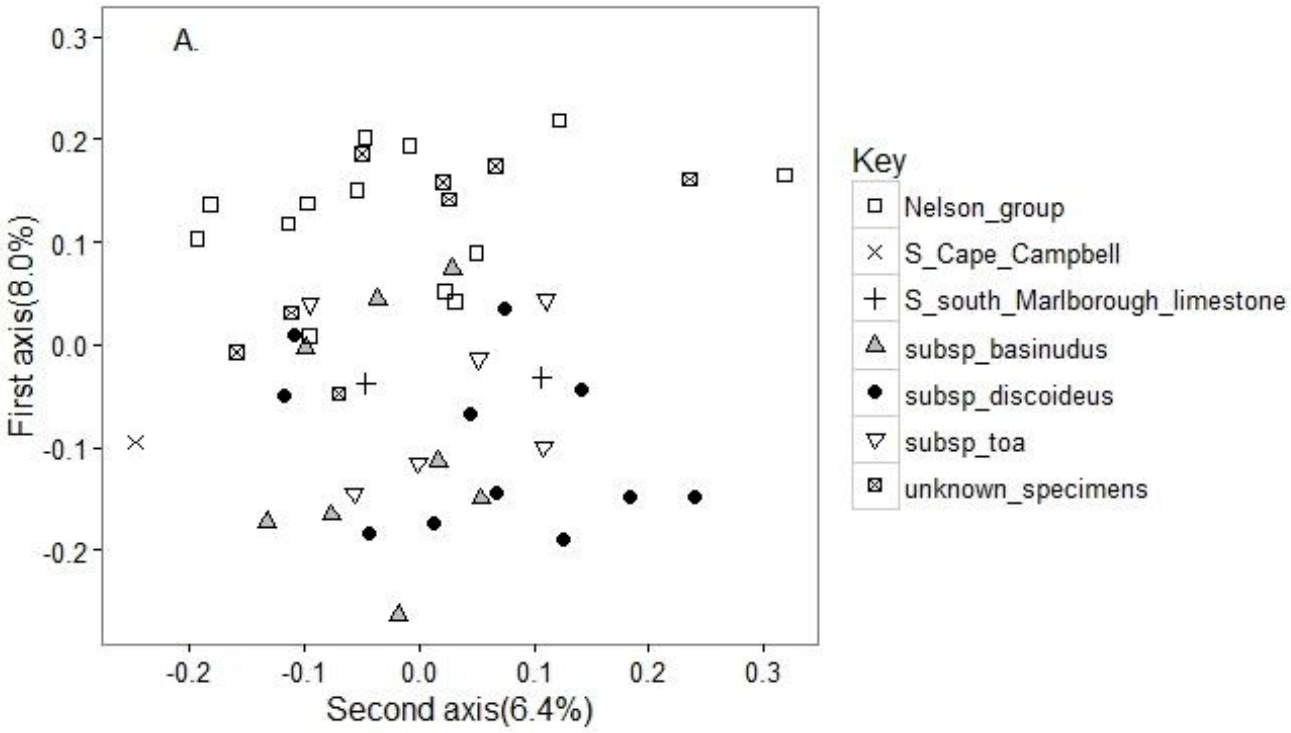


Fig. 5.6. PCoA bidimensional plots for the first vs. second axis of the 2% error AFLP data set generated from Jaccard distances computed from 50 specimens.

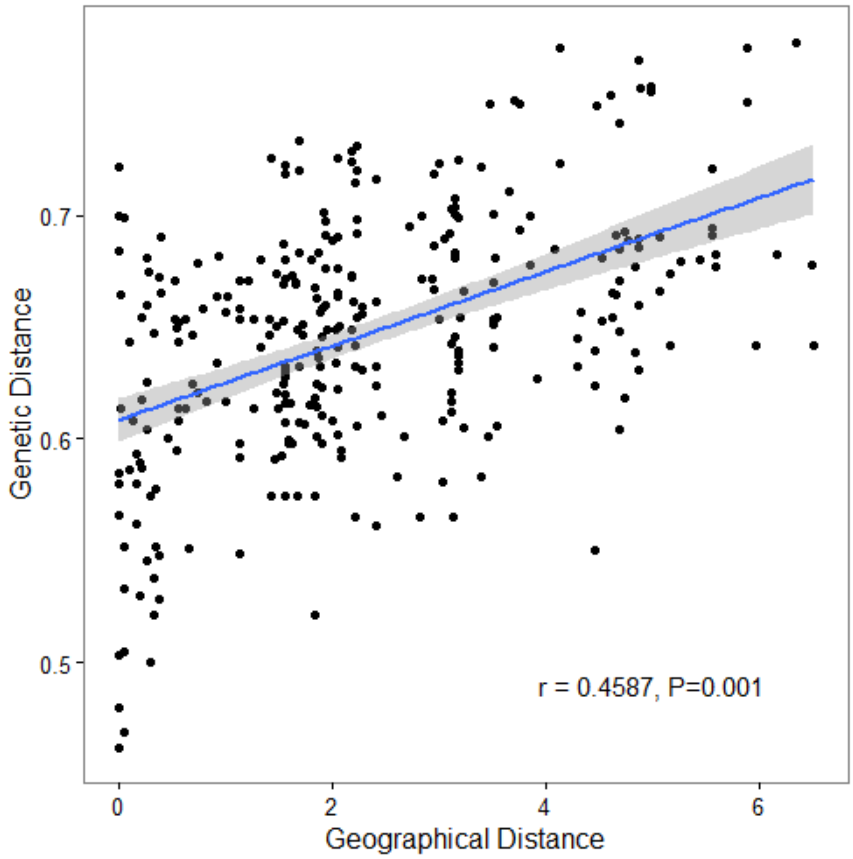


Fig. 5.7. A plot of pairwise Euclidean geographical distance and pairwise Jaccard dissimilarities for the 2% error data set. The blue line is the linear regression line. The grey shaded area indicates the standard error associated with the regression line.

5.5.3.3.2. AMOVA

The results of the AMOVA of the 2% error data set indicate no or negligible genetic differentiation ($\Phi_{PT} = 0.027$, $\Phi'_{PT} = 0.034$, P = 0.191) among subsp. *basinudus*, subsp. *discoideus*, and subsp. *toa*, the Nelson-group, and *Senecio* aff. *glaucophyllus* "South Marlborough", whereas the standardized Φ_{PT} (Φ'_{PT}) of the 4% error data set suggests moderate differentiation among these *S.* "pseudoglaucophyllus" groups ($\Phi_{PT} = 0.129$, $\Phi'_{PT} = 0.160$, P = 0.002) (Tables 5.5 and 5.6). In both data sets, most of the variation detected by the AMOVA is found within the infraspecific groups (2% - WP = 97%, 4% - WP = 87%; Table 5.5). Standardized pairwise Φ_{PT} (Φ'_{PT}) values for the 4% error data set indicate that the Nelson-group is genetically differentiated from the remaining groups (Table 5.6). In addition to the Nelson-group, *S.* aff. *glaucophyllus* "South Marlborough" is moderately differentiated from subsp. *basinudus* (Table 5.6).

Table 5.5. AMOVA statistics for the 2% and 4% error data sets, which include genetic variation within and among subsp. *basinudus*, subsp. *discoideus*, and subsp. *toa*, the Nelson-group, and *Senecio* aff. *glaucophyllus* "South Marlborough", estimated total molecular variance, overall PhiPT (Φ_{PT}) with P-values and standardized PhiPT (Φ'_{PT}).

	2% error	**4% error**
% variance among groups (AP)	3%	13%
% variance within groups (WP)	97%	87%
Estimated variance	20.7	22.8
PhiPT (Φ_{PT})	0.027 (P = 0.191)	0.129 (P = 0.002)
Phi'PT (Φ'_{PT})	0.034	0.160

Table 5.6. Standardized pairwise Φ_{PT} (Φ'_{PT}) values based on 999 permutations for 2% error (lower left) and 4% error (upper right) data sets. $\Phi_{PT} > 0.25$ (** great differentiation); Φ_{PT} = 0.15–0.25 (* moderate differentiation); $\Phi_{PT} < 0.015$ (negligible differentiation) (Wright, 1978). Only pairwise Φ_{PT} values that show moderate or great differentiations with significant P-values (P < 0.05) are marked with asterisks.

4% / 2%	**subsp. *basinudus***	**subsp. *discoideus***	**Nelson-group**	**subsp. *toa***	**"South Marlborough"**
subsp. *basinudus*		0.085	0.202*	0.134	0.235*
subsp. *discoideus*	0.049		0.190*	0.111	0.144
Nelson-group	0.056	0.015		0.174*	0.258**
subsp. *toa*	0.019	0.000	0.031		0.013
"South Marlborough"	0.127	0.100	0.057	0.086	

5.6. DISCUSSION

5.6.1. Patterns of genetic diversity in *Senecio* "pseudoglaucophyllus"

The results of a phylogenetic analysis of ITS DNA sequence data indicate that *Senecio* "pseudoglaucophyllus" specimens are genetically very similar to each other and this analysis therefore failed to provide much phylogenetic resolution (Fig. 5.2). This finding supports the results of Ornduff's (1962) hybridization experiments, which indicated that the four infraspecific taxa of this species might readily hybridize. Despite of the lack of resolution in the ITS cladogram, some specimens of two of the currently recognized infraspecific groups of *S.* "pseudoglaucophyllus" (subsp. *basinudus* and subsp. *discoideus*) are strongly supported to be more closely related to each other than to other specimens of the same groups (Fig. 5.2).

This suggests that subsp. *basinudus* and subsp. *discoideus* are not genetically distinct from each other.

Analyses of two AFLP data sets that have different genotyping error rates (the 2% and 4% error data sets) provided very similar patterns of genetic diversity. For example, similar values of K (Fig. 5.3) and genetic structure profiles (Fig. 5.4) were obtained in the STRUCTURE analyses and the PCoA analyses of both data sets also resulted in nearly identical PCoA ordination plots (Fig. 5.6). In line with other studies (e.g., Zhang & Hare, 2012), however, the data set in which a higher error rate was accepted (4% error data set) showed somewhat stronger genetic structuring (AMOVA; Tables 5.5 and 5.6). In agreement with the results of the phylogenetic analysis of the ITS data set, the results of the STRUCTURE and PCoA analyses of the AFLP data sets show that several members of the four infraspecific groups (subsp. *basinudus*, subsp. *discoideus*, subsp. *toa*, Nelson-group) are genetically most similar to members of other groups (Figs. 5.4 and 5.6). The results of the STRUCTURE analyses further indicate that the genetic variation within *Senecio* "pseudoglaucophyllus" is best structured into five genetic groups (Fig. 5.3), and that these five groups are not congruent with the currently used infraspecific classification of *S.* "pseudoglaucophyllus" (Fig. 5.4). AMOVA of the AFLP data sets also failed to provide support for a classification of this species into the four currently recognized groups (Table 5.6).

Although the AFLP data does not support subsp. *basinudus*, subsp. *discoideus*, and subsp. *toa* as genetically distinct groups, the results of an AMOVA of the 4% error data set suggest that the Nelson-group is genetically differentiated from the other groups (Table 5.6). This hypothesis also finds support in the results of the STRUCTURE analyses (Fig. 5.4), which show that the Nelson-group is mostly composed of specimens that have high Q values for the green STRUCTURE cluster and that none of the specimens assigned to the other groups have high Q values for this cluster. In addition, most specimens of the Nelson-group cluster fairly closely together in the PCoA plots (Fig. 5.6). The genetic distinctiveness of the Nelson-group is, however, not very strongly supported by the data. This is evident from the failure to find support for recognizing the Nelson-group as distinct from the other groups in the AMOVA of the 2% error data set (Table 5.6) and the presence of many specimens of the Nelson-group with admixed (Fig. 5.4) or intermediate (Fig. 5.6) genetic signatures.

Instead of providing evidence for the current classification of *Senecio* "pseudoglaucophyllus" into subsp. *basinudus*, subsp. *discoideus*, subsp. *toa*, and the Nelson-group or strongly supporting an alternative infraspecific classification, our results suggests that the patterns of genetic diversity that were resolved in this study are primarily a consequence of isolation by distance. This is evident from the results of a Mantel test, which show that specimens that are in geographic proximity of each other are genetically more similar than those further away (Fig. 5.7). This pattern is also clear from Fig. 5.5, which shows that populations with similar genetic profiles are mostly located in the same part of New Zealand. Although some specimens included in the analyses could not be identified to one of the four infraspecific groups, also their genetic profiles are most similar to plants from nearby areas. For example, most specimens of unknown identity were from Population 5 in the eastern part of Nelson and this population shows a genetic signature that is intermediate between that of the more westerly Nelson populations and that found in Marlborough and the central North Island (Figs. 5.4 and 5.5). The strong geographic signal in the patterns of genetic diversity of *S.* "pseudoglaucophyllus" is also clear in the ITS phylogeny, in which five of the six specimens of the only well-supported clade were collected from the same area (the Port Hills and Banks Peninsula).

5.6.2. A comparison of morphological and genetic patterns of diversity

The patterns of morphological and genetic diversity within *Senecio* "pseudoglaucophyllus" show both similarities and differences. Neither data source provides support for a formal taxonomic classification of *S.* "pseudoglaucophyllus" into subsp. *basinudus*, subsp. *discoideus*, subsp. *toa*, and the Nelson-group, because the morphological and genetic data sets fail to show the presence of four distinct morphometric or genetic groups that are congruent with this classification. In addition, both data sources show a pattern of more or less continuous variation and a statistically positive correlation between morphological/genetic distance and geographical distance, although this geographical signal is much more evident in the genetic data set. Furthermore, in agreement with the results of the morphometric analyses, the two informally recognized morphotypes from Marlborough (*S.* aff. *glaucophyllus* "South Marlborough", and *S.* aff. *glaucophyllus* "Cape Campbell") are genetically most similar to subsp. *discoideus* and subsp. *toa* (Table 5.6).

Although both data sources show patterns of diversity that indicate some morphometric and genetic structure that could be used as evidence in support of recognizing infraspecific taxa,

these patterns are incongruent. The morphometric diversity of *Senecio* “pseudoglaucophyllus” is structured into two indistinct groups, of which one aligns with subsp. *basinudus* and the Nelson-group and the other with subsp. *discoideus*, subsp. *toa*, *S.* aff. *glaucophyllus* "South Marlborough", and *S.* aff. *glaucophyllus* “Cape Campbell”. This contrasts with the genetic patterns, which provide some support for recognizing two different infraspecific taxa: one that aligns with the Nelson-group and one that is composed of all the other morphological forms of *S.* “pseudoglaucophyllus”.

It is not uncommon to find incongruent morphological and genetic patterns such as those identified in this study (e.g., Caković et al., 2015; Moffat et al., 2015) or to find a stronger geographic signal than taxonomic signal in genetic data sets (e.g., Resetnik et al., 2016). For example, very similar patterns of morphological and genetic diversity were obtained in a recent study of another New Zealand species complex (Millar, 2014). In that study, Millar (2014) found that patterns of morphological diversity in a group of five species of rosette-*Brachyglottis* (a genus in the same tribe as *Senecio*) show a continuum of morphological variation and are incongruent with similarly continuous patterns of genetic variation. Instead, a strong correlation between genetic similarity and geographic proximity was discovered. Likewise, Roda et al. (2013a) found that patterns of genetic diversity reflect geographic proximity better than morphological similarity in an Australasian Lautusoid *Senecio* species complex that is closely related to *S.* “pseudoglaucophyllus”.

The lack of congruence between morphological and genetic patterns of diversity in *Senecio* “pseudoglaucophyllus” demonstrates that morphological similarity is not a good proxy for inferring evolutionary relatedness within this species. This is, for example, well-illustrated by subsp. *basinudus* and the Nelson-group. Subspecies *basinudus* is morphologically most similar to the Nelson-group (Figs. 4.2 and 4.3; Ornduff, 1960), but genetically more similar to subsp. *discoideus*, subsp. *toa*, and the two Marlborough morphotypes (Fig. 5.4). The incongruence between patterns of morphological and genetic diversity might indicate local selection on ecologically relevant traits, but the incongruent patterns in combination with the considerable morphological variation within populations as indicated in Chapter 4 for a population of subsp. *basinudus* (Fig. 4.10–20), suggests that some of the morphological diversity in *S.* “pseudoglaucophyllus” is due to phenotypic plasticity.

Phenotypic plasticity can be considerable in polyploid species (Leitch & Leitch, 2008; Jackson & Chen, 2010; Hahn et al., 2012). It is therefore not unexpected if this were in part

responsible for the morphological diversity of *Senecio* "pseudoglaucophyllus", which is a species of allopolyploid origin (2*n* = 100; Chapter 2, as *S. glaucophyllus*). This phenotypic plasticity might be adaptive and has potentially helped *S.* "pseudoglaucophyllus" to colonize the diverse and sometimes extreme habitats (e.g., high elevation scree slopes, coastal habitats) in which it is currently found, as has been shown in studies of other plant taxa (Levin, 1983; Otto & Whitton, 2003; Soltis et al., 2014; Segraves & Anneberg, 2016). If the diversification of *S.* "pseudoglaucophyllus" was rapid (as found for other New Zealand plant taxa, such as *Myositis* L. and *Veronica* L.; Wagstaff & Garnock-Jones, 1998; Winkworth et al., 1999; Winkworth et al., 2002) this might explain the relatively limited genetic diversity of this species compared to its morphological diversity (McBreen et al., 2003). More fine-scaled morphological and genomic studies such as those by Roda et al. (2013a) for an Australian Lautusoid *Senecio* lineage are, therefore, needed to understand the processes responsible for the patterns of morphological diversity of *S.* "pseudoglaucophyllus".

5.6.3. CONCLUSION and taxonomic implications

The results of the morphometric and genetic studies of *Senecio* "pseudoglaucophyllus" do not support the formal taxonomic recognition of subsp. *basinudus*, subsp. *discoideus*, subsp. *toa*, and the Nelson-group, because these do not meet the criterion of being "distinguishable groups of individuals that have few or no intermediates when in contact" (Mallet, 1995). In addition, they are not characterized by "at least one fixed diagnosable character state" as is recommended by Ellison et al. (2014) (subspecies concept modified from Braby et al. (2012)) for infraspecific taxa. Unambiguous support for alternative intraspecific classifications is similarly lacking and *S.* "pseudoglaucophyllus" is therefore best considered as a morphologically variable species for which infraspecific morphological forms should not be formally recognized as subspecies or varieties.

Senecio "pseudoglaucophyllus" is presently an unnamed taxon. In the manuscript version of Chapter 3 of this thesis, this species will be formally named and a morphological description will be provided. In the manuscript version of the present chapter, the following taxa will subsequently be synonymized with *Senecio* "pseudoglaucophyllus":

Senecio lautus var. *raoulii* Hook.f., Flora Novae-Zelandiae, Vol. 2, Part 1 (1853) 145. HOLOTYPE: Raoul s.n. "Senecio Raouli (Spach) Akaroa" (K 852333, Photo!).

> ≡*Senecio glaucophyllus* subsp. *raoulii* (Hook.f.) Ornduff, Transactions and Proceedings of the Royal Society of New Zealand 88 (1960) 72.

=*Senecio glaucophyllus* subsp. *basinudus* Ornduff, Transactions and Proceedings of the Royal Society of New Zealand 88 (1960) 71. HOLOTYPE: R. Ornduff s.n., 18-Jun-1955 (cultivated from J.W. Dawson s.n., Jul-1954) (CHR 87795!).

Senecio lautus var. *discoideus* Cheeseman, Manual of the New Zealand Flora (1906) 374. LECTOTYPE: T.F. Cheeseman s.n., Jan-1888 (AK 10596!).

≡*Senecio glaucophyllus* subsp. *discoideus* (Cheeseman) Ornduff, Transactions and Proceedings of the Royal Society of New Zealand 88 (1960) 73.

Senecio lautus var. *montanus* Cheeseman, Manual of the New Zealand Flora (1906) 373. LECTOTYPE: to be designated (see Chapter 3).

Senecio glaucophyllus subsp. *toa* C.J.Webb in Connor & Edgar, New Zealand Journal of Botany 25 (1987) 148. HOLOTYPE: K. W. Allison s.n., 18-Dec-1934 (CHR 17696!).

CHAPTER 6: Summary and conclusions

6.1. Overview

Taxonomic delimitation delineates groups of organisms that are of evolutionary significance and it is therefore an important aspect of describing the diversity of life and understanding its origin (Bertrand et al., 2006; Andújar et al., 2014). In this thesis, I aimed to contribute to the taxonomic delimitation of *Senecio* at the infrageneric, specific, and infraspecific levels by studying evolutionary patterns and processes in an informal group of Australasian *Senecio*, the Lautusoid group (i.e., Ornduff, 1960; Belcher, 1992b; Thompson, 2005b; de Lange et al., 2014). I used a combination of genetic and morphological approaches to (1) delimit the Lautusoid group by identifying Australasian species that are most closely related to *S. lautus* and to investigate the evolutionary origins of putative Lautusoid species with chromosome numbers of $2n = 80$ and $2n = 100$, (2) determine if the two cryptic taxa in the *S. glaucophyllus* complex are distinct species, and (3) revisit the current infraspecific classification of *S.* "pseudoglaucophyllus".

Taxonomic delimitation can be particularly challenging if the targeted taxonomic group is large (i.e., Frodin, 2004; Linder et al., 2005; Rønsted et al., 2006; van Welzen et al., 2009; Pick et al., 2010; Mansion et al., 2012), has experienced extensive interspecific hybridization (i.e., Linder & Rieseberg, 2004; Rønsted et al., 2006), and contains morphologically complex or cryptic taxa (i.e., Serb et al., 2003; Bickford et al., 2006; Rato et al., 2016). These three challenges also had to be overcome in my research project.

It can be quite time consuming and expensive to reconstruct comprehensive phylogenies for large genera, such as *Senecio* (Rønsted et al., 2006). For example, it can be challenging to obtain a representative taxon and character sampling, because of the sheer number of species that make up the genus (Frodin, 2004). This is especially the case if it has a widespread distribution (van Welzen et al., 2009). Even for a moderately sized genus, it would be too costly and time consuming to rely on fresh-collected tissue samples for molecular phylogenetic studies (van Welzen et al., 2009). However, even if one primarily relies on tissue from herbarium specimens, it can take a considerable amount of time to locate specimens of the taxa of interest, to receive these specimens on loan from the herbaria where they are lodged, to confirm their identifications, and to select those that have been preserved well-enough to yield DNA of sufficient quality for molecular genetic analyses. Despite that these analyses have become cheaper and easier to perform in the last two decades, the costs

and the hands-on time involved would be prohibitive for most research groups when a comprehensive taxon sampling is desired (Mansion et al., 2012). This is especially a concern for large genera that have started diversifying relatively recently, because sequences from multiple DNA regions might need to be generated to provide enough phylogenetic resolution and statistical support (Rønsted et al., 2006). Systematists can in part work around these problems by using a compartmentalized approach that focuses on resolving phylogenetic relationships one clade at a time (e.g., *Allium* L.: Gurushidze et al., 2010; *Veronica* L.: Kosachev et al., 2016; *Inga* Mill.: Richardson et al., 2001; *Scrophularia* L.: Scheunert & Heubl, 2014). In *Senecio*, this approach consisted of first identifying its main lineages by using a wide sampling approach that aimed to represent geographic, morphological, and taxonomic diversity and that primarily relied on herbarium specimens as a source of DNA (Pelser et al., 2007). Subsequent studies used this 'skeleton phylogeny' to inform taxon sampling for more focused and detailed phylogenetic studies (e.g., Pelser et al., 2010b, 2012; Calvo et al. 2013; Kandziora et al., 2016a,b). Likewise, I used this skeleton phylogeny and a wide sampling of herbarium specimens of Australasian *Senecio* species to arrive at a taxon sampling that is appropriate for the research questions that I wanted to address.

Senecio is not only a large genus, but it has also experienced widespread hybridization throughout its evolutionary history (e.g., Abbott & Lowe, 2004; James & Abbott, 2005; Abbott et al., 2009; Pelser et al., 2010a, 2012; Calvo et al., 2013). This further complicates efforts aimed at arriving at an infrageneric taxonomic delimitation for *Senecio*, because the presence of hybrids in phylogenetic analyses is one of the possible causes of phylogenetic incongruence (Maddison, 1997; Knowles & Carstens, 2007). This incongruence hinders the reconstruction of well-resolved species-level phylogenies, which are important in facilitating taxonomic inferences (Linder & Rieseberg, 2004; Rønsted et al., 2006). However, if incongruent phylogenetic patterns are sufficiently well resolved and supported and if hybridization can be distinguished from other causes of phylogenetic incongruence (e.g., incomplete lineage sorting, undetected paralogous sequences), they can instead inform taxonomic delimitation by identifying species or lineages of hybrid origin and their parental species (Linder & Rieseberg, 2004; Knowles & Carstens, 2007; Nakhleh, 2013; O'Malley, 2016). The latter approach was used to inform the taxonomic delimitation of the Lautusoid group in my study.

Morphologically cryptic species (Bickford et al., 2006; Mayden, 1997) or species complexes that are composed of taxa with ambiguous morphological boundaries (Mallet, 2008; Padial et

al., 2010) can form another challenge to taxonomic delimitation. An integrative approach to taxonomy is often employed to better resolve these morphologically difficult taxa (Dayrat, 2005; Schlick-Steiner et al., 2010; Yeates et al., 2011). I therefore used both morphological and genetic data to study the taxonomic delimitation of the *S. glaucophyllus* complex and the infraspecific taxa of *S.* "pseudoglaucophyllus".

6.2. The delimitation and evolution of the Lautusoid group of *Senecio* (Chapter 2)

As part of efforts to improve our understanding of the evolutionary relationships among infrageneric *Senecio* groups and to inform their taxonomic delimitation, I used phylogenetic analyses of nuclear (nrITS and ETS) and plastid (*psb*A-*trn*H, *trn*L and *trn*L-F) DNA sequence data to study the delimitation and the evolutionary history of the Australasian Lautusoid group of *Senecio*. Previous taxonomic studies of putative members of the Lautusoid group often had a regional focus (Ornduff, 1960; Belcher, 1992b; Thompson, 2005b, 2006) and a comprehensive study has never been attempted. Prior to my studies, it was therefore not known which and how many species compose the Lautusoid group and how this group is related to other Australasian lineages.

The results of my phylogenetic analyses indicate that in spite of widespread phylogenetic incongruence, most Australasian *Senecio* species that were included in my study can be placed in four distantly related lineages (the Disciform s.s., Lautusoid, Odoratus s.l., and Quadridentatus groups; Table 2.4). The Lautusoid group is both phylogenetically and morphologically distinct from the other three groups. Of the 18 putative Lautusoid species that were included in my study (of 23 species that were hypothesized to be associated with *S. lautus* in previous taxonomic treatments; Table 2.1), 12 were confirmed to be members of the Lautusoid group. Also three members of Thompson's (2005a) Glossanthus group (*S. glossanthus* (Sond.) Belcher, *S. halophilus* I.Thomps., *S. serratiformis* I.Thomps.) were placed in the Lautusoid group and this brings the total number of Lautusoid species to 15. Five putative Lautusoid species and one species of the Glossanthus group could, however, not be included in my analyses and future studies are therefore needed to determine if they belong to the Lautusoid group.

Senecio condylus was tentatively included in the Lautusoid group by Thompson (2005b, 2006), but is here excluded, because it was found to be more closely related to a lineage of African *Senecio* species. Five other species that were previously associated with the Lautusoid group (*S. australis* Willd., *S. glaucophyllus* Cheeseman, *S. hooglandii* Belcher, *S.*

marotiri C.Webb, and *S. repangae* de Lange & B.G.Murray; Table 2.1) are also excluded. The results of Chapter 3 indicate that the name *S. glaucophyllus* has, in part, been misapplied to plants belonging to a taxon that is unnamed at the species level and for which the tag-name *S.* "pseudoglaucophyllus" is used in this thesis, pending formal description and naming in a publication resulting from this thesis. Whereas *S. glaucophyllus* is resolved as a member of the Quadridentatus group, patterns of phylogenetic incongruence in combination with karyotypic data suggest that *S.* "pseudoglaucophyllus" ($2n = 100$) is an allopolyploid that originated from hybridization between the Disciform s.s group ($2n = 60$) and the Lautusoid group ($2n = 40$; as *S. glaucophyllus* in Table 2.3). Similarly, also *S. australis*, *S. biserratus* Belcher, *S. hooglandii*, *S. marotiri*, and *S. repangae* were identified as allopolyploid hybrids between a member of the Lautusoid group and one of the other Australasian lineages. In addition, *S. distalilobatus* I.Thomps. and *S. extensus* I.Thomps. are most likely allopolyploid hybrids between non-Lautusoid lineages (Table 2.3). Due to the generally low phylogenetic resolution at the interspecific level, however, more detailed studies are needed to identify the parental species of the hybrids that were discovered in my study.

Using the results of my study, I proposed a new delimitation of the Lautusoid group. However, at this stage, its formal taxonomic recognition as a section of *Senecio* is not recommended until more comprehensive and detailed morphological studies of the Lautusoid species are completed. These should be especially targeted at determining if the six putative Lautusoid species that could not be included in my analyses (*S. evansianus* Belcher, *Senecio eremicola* I.Thomps., *S. howeanus* Belcher, *S. pauciradiatus* Belcher, *S. productus* I.Thomps., and *S. warrenensis* I.Thomps.) are members of the Lautusoid group. They should also focus on providing detailed morphological descriptions of Lautusoid species for which these are currently lacking (especially the New Zealand species), so that a comprehensive morphological description of the Lautusoid group can accompany its recognition as a section of *Senecio*. The results of my study also provide an explanation for the origin of putative Lautusoid species with high chromosome numbers ($2n = 80$ and $2n = 100$) by indicating that these are the result of allopolyploid hybridization between members of the Lautusoid group ($2n = 40$) and the Disciform s.s. ($2n = 60$), Odoratus s.l. ($2n = 60$), and Quadridentatus ($2n = 40$) groups. In addition, they highlight the prevalence of hybridization in the evolutionary history of the Lautusoid group and provide further evidence for the importance of hybridization in the diversification of *Senecio* and Senecioneae (e.g., Abbott & Lowe, 2004; Kadereit et al., 2006; Pelser et al., 2010a, 2012; Calvo et al., 2013).

6.3. Resolving the *Senecio glaucophyllus* complex (Chapter 3)

Cryptic species pose challenges to biodiversity studies and can thereby hinder the progress of documenting biodiversity (e.g., Bickford et al., 2006; Buhay et al., 2007; Rato et al., 2016). The presence of cryptic species complexes, if undetected, may also have serious consequences on activities that depend on accurate species identification, such as conservation planning and management (Bickford et al., 2006). *Senecio glaucophyllus* sensu Ornduff (1960) is a morphologically variable New Zealand species that is morphologically similar to a taxon that is informally known as *S.* aff. *glaucophyllus* (de Lange et al., 2013a). *Senecio* aff. *glaucophyllus* is only found in North-West Nelson in New Zealand's South Island and most closely resembles *S. glaucophyllus* subsp. *glaucophyllus* sensu Ornduff (1960; Fig. 3.1), with which it overlaps in distribution (Fig. 3.7). Perhaps because of the morphological similarities between both taxa, their sympatry (e.g., on Mt. Arthur; Fig. 3.7), the substantial morphological diversity of *S. glaucophyllus* sensu Ornduff, and because *S.* aff. *glaucophyllus* has not been formally named and described, plants of *S.* aff. *glaucophyllus* have been collected and filed in herbaria as *S. glaucophyllus*. Using nuclear ITS sequence data and morphometric data obtained from herbarium specimens of *S.* aff. *glaucophyllus* and *S. glaucophyllus* sensu Ornduff, I investigated if the two taxa are morphologically and genetically distinct. The results of principal coordinate analyses of the morphometric data set indicate that the two taxa are morphologically distinctly different and, in fact, not as cryptic as they might appear at first sight. Similar results were obtained from the phylogenetic analyses of the ITS data set. These also indicate that *S.* aff. *glaucophyllus* and *S. glaucophyllus* sensu Ornduff are only distantly related to each other. A Random Forest analysis was carried out to identify the morphological characters that are most diagnostic for distinguishing the two species. This analysis revealed that the number and shape of the involucral bracts and the number of dissections and shape of the leaf margin of the mid-cauline leaves are the most informative characters for differentiating *S.* aff. *glaucophyllus* and *S. glaucophyllus* subsp. *glaucophyllus* sensu Ornduff.

One surprising finding from this study is the close resemblance of the type specimens of *Senecio glaucophyllus* to specimens of *S.* aff. *glaucophyllus*. This implies that, in its current delimitation (Ornduff, 1960), the name *S. glaucophyllus* has been misapplied to an unnamed species and that *S.* aff. *glaucophyllus* is the true *S. glaucophyllus*. This unnamed taxon is referred to as *S.* "pseudoglaucophyllus" in my thesis. Because of the misapplication of the name *S. glaucophyllus*, the conservation status of the two taxa should be reversed (de Lange

et al., 2013a). *Senecio glaucophyllus* should have the conservation status of Nationally Vulnerable and subsp. *glaucophyllus* sensu Ornduff the status of Naturally Uncommon under the New Zealand Threat Classification System. *Senecio* "pseudoglaucophyllus" will be formally named and described at the species-level in a publication that will result from this thesis. In this way, my findings will contribute to future biological studies of both species and their conservation management.

6.4. Testing the infraspecific delimitation of *Senecio* "pseudoglaucophyllus" (Chapters 4 & 5)

Following Ornduff's (1960) infraspecific delimitation of *Senecio glaucophyllus* sensu Ornduff and a subsequent nomenclatural amendment by Webb (in Connor & Edgar, 1987), four subspecies of this taxon are currently recognized: subsp. *glaucophyllus*, subsp. *basinudus*, subsp. *discoideus*, and subsp. *toa*. However, because of the misapplication of the name *S. glaucophyllus* as revealed in Chapter 3, there are currently no formal names for subsp. *basinudus*, subsp. *discoideus*, and subsp. *toa*, and a taxon that conforms to subsp. *glaucophyllus* sensu Ornduff p.p. (excl. *S. glaucophyllus* sensu Cheeseman, 1895). In Chapters 4 & 5, these four infraspecific taxa of *S.* "pseudoglaucophyllus" are therefore simply referred to as subsp. *basinudus*, subsp. *discoideus*, subsp. *toa*, and the Nelson-group, respectively. These four groups display considerable morphological variation and morphological intermediates between them have made it difficult to assign some plants to these four infraspecific groups (Ornduff, 1960, 1962). I therefore revisited the infraspecific delimitation of *S.* "pseudoglaucophyllus".

The results of a morphometric study of *Senecio* "pseudoglaucophyllus" using herbarium specimens (Chapter 4) showed that patterns of morphological variation within this species do not support the formal taxonomic recognition of the four infraspecific groups. Instead, they suggest the presence of two poorly defined morphological groups with many intermediate specimens. One of these two groups corresponds to subsp. *basinudus* and the Nelson group and the other to subsp. *discoideus*, subsp. *toa*, and two informally recognized forms from the Marlborough area. Although these findings could be interpreted as providing some support for recognizing two subspecies instead of four, they can also be interpreted as support for considering *S.* "pseudoglaucophyllus" a species with near-continuous morphological variation and for which infraspecific taxa should not be recognized.

Taxonomic delimitation of species that show great morphological diversity and seemingly continuous variation is difficult if only morphological data are used (e.g., Dayrat, 2005; Pessoa et al., 2012). An integrative approach that incorporates data from more than one source of evidence can be a more powerful strategy in taxonomic studies of morphologically complex species (Dayrat, 2005; Pessoa et al., 2012) and this approach was therefore applied to *Senecio* "pseudoglaucophyllus" in Chapter 5. In agreement with the results of the morphometric analyses, the results of phylogenetic analyses of ITS DNA sequence data, model-based Bayesian clustering, multivariate analyses, and AMOVA of AFLP data do not support the formal taxonomic recognition of subsp. *basinudus*, subsp. *discoideus*, subsp. *toa*, and the Nelson-group, because they are not genetically distinct. Instead, patterns of genetic variation within *S.* "pseudoglaucophyllus" can mostly be explained by isolation by distance as indicated by a positive Mantel test between genetic and geographical distance and geographic clustering of similar genetic profiles (Fig. 5.5). In addition, patterns of genetic variation are largely incongruent with patterns of morphological variation. Morphological similarity is therefore not a good indicator of genetic similarity in *S.* "pseudoglaucophyllus. Because my morphological and molecular genetic analyses do not support the formal taxonomic recognition of subsp. *basinudus*, subsp. *discoideus*, subsp. *toa* and the Nelson-group and do not provide unambiguous support for alternative intraspecific classifications, *S.* "pseudoglaucophyllus" is best considered as a morphologically variable species for which infraspecific morphological forms should not be formally recognized.

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APPENDIX

Table S1. (Chapter 2) All known *Senecio* species from Australia, New Guinea, and New Zealand, their general distribution area, chromosome numbers (if known), and their affiliation with the four Australasian lineages as identified in the present study. For Australian species, their classification according to Thompson (2006) is indicated. Species in bold were included in our phylogenetic analyses. **Senecio colensoi* is treated as a synonym of *S. banksii* by Webb et al. (1988), but Allan (1961) considered *S. colensoi* and *S. banksii* as distinct species. Critical study of the two species is required to determine their taxonomic status.

Species	Distribution	Chromosome number	Thompson's classification	Phylogenetic affinities as per the present study
Senecio albogilvus	Australia		Macranthus	
Senecio amygdalifolius	Australia	2n = 38 (Lawrence, 1985a)	Macranthus	
Senecio anethifolius	**Australia**	**2n = 60 (Turner, 1970)**	**Odoratus**	**Odoratus s.l.**
Senecio australis	**New Zealand and Norfolk Island**	**2n = 80 (de Lange & al., 2004)**		**Lautusoid × Quadridentatus**
Senecio banksii	**New Zealand**	**2n = 60 (Beuzenberg, 1975)**		**Odoratus s.l.**
Senecio barkhausioides	Australia		Ramosissimus	
Senecio bathurstianus	**Australia**	**2n = 60 (Lawrence, 1980; Thompson, 2004a)**	**Disciform**	**Disciform s.s.**
Senecio behrianus	Australia		Odoratus	
Senecio bipinnatisectus	**Australia and New Zealand**	**2n = 60 (Beuzenberg & Groves, 1974; Lawrence, 1985a)**	**Disciform**	**Disciform s.s.**
Senecio biserratus	**Australia and New Zealand**	**2n = 100 (Beuzenberg & Groves, 1974; Lawrence, 1980)**	**Disciform**	**Lautusoid × Odoratus s.l.**
Senecio brassii	**New Guinea**	**2n = 64 (Borgmann, 1964)**		**Aff. Disciform s.s.**
Senecio brigalowensis	**Australia**		**Lautusoid**	**Lautusoid**
Senecio campylocarpus	**Australia**		**Disciform**	**Quadridentatus**
Senecio carnosulus	**New Zealand**	**2n = 80 (Beuzenberg, 1975; Webb, 1988)**		**Lautusoid**
*Senecio colensoi**	New Zealand	2n = 60 (Beuzenberg, 1975)		
Senecio condylus	**Australia**		**Lautusoid**	**Affiliated with South African species**
Senecio conferruminatus	Australia		Magnificus	
Senecio cunninghamii	**Australia**	**2n = 60 (Lawrence, 1980)**	**Odoratus**	**Odoratus s.l.**

Senecio daltonii	Australia		Macranthus	
Senecio depressicola	**Australia**		**Lautusoid**	**Lautusoid**
Senecio diaschides	**Australia**	**2n = 60 (Lawrence, 1980; Thompson, 2004a)**	**Disciform**	**Disciform s.s.**
Senecio distalilobatus	**Australia**	**2n = 100 (Lawrence, 1980; Thompson, 2004a)**	**Disciform**	**Disciform s.s. × Quadridentatus**
Senecio dolichocephalus	**Australia**		**Disciform**	**Quadridentatus**
Senecio dunedinensis	**New Zealand**	**2n = 40 (Beuzenberg & Groves, 1974)**		**Quadridentatus**
Senecio eremicola	Australia		Lautusoid	
Senecio esleri	**Australia and New Zealand**	**2n = 60 (Webb, 1989)**	**Disciform**	**Disciform s.s.**
Senecio esperensis	**New Zealand**	**2n = 40 (Sykes, 1971; Murray & de Lange, 2013; de Lange & al., 2015)**		**Lautusoid**
Senecio euclaensis	Australia		Odoratus	
Senecio evansianus	Norfolk Island			
Senecio extensus	**Australia**		**Disciform**	**Odoratus s.l. × Disciform s.s.**
Senecio garlandii	Australia		Odoratus	
Senecio gawlerensis	**Australia**		**Odoratus**	**Odoratus s.l.**
Senecio georgianus	Australia		Disciform	
Senecio gilbertii	Australia		Ramosissimus	
Senecio glabrescens	**Australia**		**Disciform**	**Quadridentatus**
Senecio glaucophyllus	**New Zealand**	**2n = 100 (Beuzenberg, 1975)**		**Lautusoid × Disciform s.s.**
Senecio glomeratus	**Australia and New Zealand**	**2n = 60 (Lawrence, 1980; Murray & de Lange, 2013)**	**Disciform**	**Disciform s.s.**
Senecio glossanthus	**Australia**	**2n = 40 (Lawrence, 1980)**	**Glossanthus**	**Lautusoid**
Senecio gnoma	New Guinea	2n = 84 (Borgmann, 1964)		
Senecio gregorii	**Australia**	**2n = 40 (Lawrence, 1985a)**	**Magnificus**	**Quadridentatus**
Senecio gunnii	**Australia**	**2n = 40 (Lawrence, 1980)**	**Disciform**	**Quadridentatus**
Senecio gypsicola	Australia		Magnificus	
Senecio halophilus	**Australia**		**Glossanthus**	**Lautusoid**
Senecio hamersleyensis	**Australia**		**Lautusoid**	**Lautusoid**
Senecio hauwai	**New Zealand**	**2n = 60 (Beuzenberg, 1975; Sykes, 1987)**		**Odoratus s.l.**
Senecio helichrysoides	Australia		Disciform	

Senecio hispidissimus	**Australia**	**2n = 60 (Lawrence, 1980; Thompson, 2004a)**	**Disciform**	**Disciform s.s.**
Senecio hispidulus	**Australia and New Zealand**	**2n = 60 (Lawrence, 1980)**	**Disciform**	**Disciform s.s.**
Senecio hooglandii	**Norfolk Island**	**2n = 80 (de Lange & Murray, 2003)**		**Lautusoid × Quadridentatus**
Senecio howeanus	Lord Howe Island			
Senecio hypoleucus	**Australia**	**2n = 60 (Lawrence, 1980)**	**Odoratus**	**Odoratus s.l.**
Senecio interpositus	Australia		Disciform	
Senecio kermadecensis	**New Zealand**	**2n = 60 (Beuzenberg & Groves, 1974, Murray & de Lange, 2013)**		**Odoratus s.l. × Odoratus s.l.**
Senecio laceratus	**Australia**		**Disciform**	**Aff. Quadridentatus**
Senecio lacustrinus	**Australia**		**Lautusoid**	**Lautusoid**
Senecio lageniformis	Australia		Disciform	
Senecio lanibracteus	**Australia**		**Odoratus**	**Odoratus s.l.**
Senecio lautus	**New Zealand**	**2n = 40 (Beuzenberg, 1975; Webb, 1988)**		**Lautusoid**
Senecio leptocarpus	Australia		Macranthus	
Senecio leucoglossus	Australia		Ramosissimus	
Senecio linearifolius	**Australia**	**2n = 60 (Lawrence, 1980)**	**Odoratus**	**Odoratus s.l.**
Senecio longicollaris	**Australia**		**Disciform**	**Quadridentatus**
Senecio longipilus	Australia		Disciform	
Senecio macranthus	**Australia**	**2n = 40 (Lawrence, 1980)**	**Macranthus**	**Quadridentatus**
Senecio macrocarpus	**Australia**	**2n = 60 (Ahrens & James, 2015)**	**Disciform**	**Disciform s.s.**
Senecio magnificus	**Australia**	**2n = 40 (Lawrence, 1985a)**	**Magnificus**	**Quadridentatus**
Senecio marotiri	**New Zealand**	**2n = 80 (Webb, 1988; Murray & de Lange, 1999)**		**Lautusoid × Quadridentatus**
Senecio megaglossus	Australia		Magnificus	
Senecio microbasis	Australia		Disciform	
Senecio minimus	**Australia and New Zealand**	**2n = 60 (Lawrence, 1980)**	**Disciform**	**Odoratus s.l.**
Senecio multicaulis	Australia		Disciform	
Senecio murrayanus	Australia		Magnificus	
Senecio nigrapicus	**Australia**		**Disciform**	**Disciform s.s.**
Senecio niveoplanus	Australia		Disciform	
Senecio odoratus	**Australia**	**2n = 60 (Lawrence, 1980)**	**Odoratus**	**Odoratus s.l.**

Senecio oldfieldii	Australia		Disciform	
Senecio papillosus	Australia		Macranthus	
Senecio papuanus	**New Guinea**	**2n = c. 80 (Borgmann, 1964)**		**Aff. Disciform s.s.**
Senecio pauciradiatus	Lord Howe Island			
Senecio pectinatus	Australia	2n = 80 (Lawrence, 1980; Lawrence, 1985a)	Macranthus	
Senecio phelleus	**Australia**		**Disciform**	**Quadridentatus**
Senecio picridioides	**Australia**	**2n = 60 (Lawrence, 1980)**	**Disciform**	**Odoratus s.l.**
Senecio pilosicristus	Australia		Magnificus	
Senecio pinnatifolius	**Australia**	**2n = 40 (Lawrence, 1980)**	**Lautusoid**	**Lautusoid**
Senecio platylepis	Australia		Magnificus	
Senecio prenanthoides	**Australia**	**2n = 40 (Lawrence, 1980; Thompson, 2004a)**	**Disciform**	**Quadridentatus**
Senecio primulifolius	Australia		Macranthus	
Senecio productus	Australia		Glossanthus	
Senecio psilocarpus	**Australia**		**Disciform**	**Odoratus s.l.**
Senecio psilophyllus	**Australia**		**Disciform**	**Quadridentatus**
Senecio quadridentatus	**Australia and New Zealand**	**2n = 40 (Beuzenberg & Groves, 1974; Lawrence, 1980)**	**Disciform**	**Quadridentatus**
Senecio queenslandicus	Australia		Disciform	
Senecio radiolatus	**New Zealand**	**2n = 40 (Beuzenberg, 1975; Murray & de Lange, 2013)**		**Lautusoid**
Senecio ramosissimus	Australia		Ramosissimus	
Senecio repangae	**New Zealand**	**2n = 100 (de Lange & Murray, 1998; Murray & de Lange, 1999)**		**Lautusoid × Disciform s.s.**
Senecio rufiglandulosus	**New Zealand**	**2n = 40 (Beuzenberg, 1975)**		**Quadridentatus**
Senecio runcinifolius	Australia	2n = 40 (Lawrence, 1985a)	Disciform	
Senecio scaberulus	**New Zealand**	**2n = 60 (Beuzenberg & Groves, 1974; Drury, 1974)**		**Disciform s.s.**
Senecio scabrellus	Australia	2n = 60 (Lawrence, 1980; Thompson, 2004a)	Disciform	
Senecio serratiformis	**Australia**		**Glossanthus**	**Lautusoid**
Senecio spanomerus	**Australia**		**Lautusoid**	**Lautusoid**
Senecio spathulatus	**Australia**	**2n = 40 (Beuzenberg, 1975; Lawrence, 1980)**	**Lautusoid**	**Lautusoid**
Senecio squarrosus	**Australia**	**2n = 60 (Lawrence, 1980)**	**Disciform**	**Odoratus s.l.**

Senecio sterquilinus	**New Zealand**	**2n = 40 (Beuzenberg, 1975)**		**Lautusoid**
Senecio tasmanicus	Australia		Disciform	
Senecio tenuiflorus	Australia		Disciform	
Senecio tuberculatus	Australia		Magnificus	
Senecio vagus	Australia	2n = 98 (Lawrence, 1980, 1985a; Robinson & al., 1997)	Macranthus	
Senecio velleioides	Australia	2n = 38 (Lawrence, 1980, 1985a; Robinson & al., 1997)	Magnificus	
Senecio wairauensis	**New Zealand**	**2n = 40 (Beuzenberg & Groves, 1974)**		**Quadridentatus**
Senecio warrenensis	Australia		Lautusoid	

Table S2. (Chapter 2) Australasian specimens used in the current study of the Lautusoid group of *Senecio*. For sequences obtained from GenBank, location, voucher and herbarium information are not listed. Also included are the labels of each specimen in the nuclear (Fig. 2.1) and plastid (Fig. 2.2) phylogenies. For the purpose of this PhD thesis, location, voucher and sequence information of the non-Australasian species are not included in the following table. Readers are referred to Pelser et al. (2002), (2003), (2007), (2010a,b) and (2012) for details of these specimens. These data will be presented in the published version of Chapter 2.

Species	Location	Voucher	Herbarium	Sequenced regions	Label in Fig. 2.1	Label in Fig. 2.2
Senecio anethifolius	Australia, South Australia	R.D. Pearce 134	MSC	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. anethifolius*	*S. anethifolius*
Senecio australis	New Zealand, North Island	P.J. de Lange 5514	AK259121	ITS, ETS, psbA-trnH & trnL-F	*S. australis con*	*S. australis con*
Senecio australis	New Zealand, Fanal Island	P.J. de Lange 5514	AK283447	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. australis con*	*S. australis con*
Senecio australis	Norfolk Island, Rocky Point	P.J. de Lange 4304	AK251840	ITS	*S. australis con*	
Senecio banksii				ITS (EF538305)	*S. banksii con*	
Senecio banksii		Druce s.n.	CHR402420	ITS	*S. banksii con*	
Senecio banksii	New Zealand, Gisborne	I. Breitwieser 2190 with K. Ford & S. Wagstaff	CHR570581	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. banksii con*	*S. banksii*
Senecio bathurstianus	Australia, Victoria	I.R. Thompson 910	MEL	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. bathurstianus*	*S. bathurstianus*
Senecio bipinnatisectus	New Zealand, Auckland	R.O. Gardner 1392	MO	ITS	*S. bipinnatisectus con*	
Senecio bipinnatisectus	New Zealand, North Auckland	E.B. Bangerter 5409	CHR421754	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. bipinnatisectus con*	*S. bipinnatisectus*
Senecio biserratus	Australia, Victoria	I.R. Thompson 923	MEL	ITS, ETS, trnL & trnL-F	*S. biserratus con*	*S. biserratus con*
Senecio biserratus	New Zealand, Canterbury	A.E. Memory 8	CANU	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. biserratus con*	*S. biserratus con*
Senecio biserratus	New Zealand, Fiordland	B.D. Rance s.n.	CHR585596	ITS	*S. biserratus con*	
Senecio brassii	New Guinea	Shea 71022	S	ITS (EF538307)	*S. brassii*	
Senecio brigalowensis	Australia, Queensland	A.B. Pollock ABP698 & M. Edginton	AQ678675	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. brigalowensis*	*S. brigalowensis con*

Senecio brigalowensis	Australia, Queensland	J. W. Noble HB	AQ544457	trnL		*S. brigalowensis con*
Senecio campylocarpus	Australia, Victoria	I.R. Thompson 917	MEL	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. campylocarpus*	*S. campylocarpus*
Senecio carnosulus				ITS (EU331121)	*S. carnosulus con*	
Senecio carnosulus	New Zealand, Canterbury	I. Hanken s.n.	CHR595309A	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. carnosulus con*	*S. carnosulus2594*
Senecio carnosulus	New Zealand, Otago	J. Barkla s.n.	CHR595292A	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. carnosulus con*	*S. carnosulus con*
Senecio carnosulus	New Zealand, Otago	M. Thorsen	CHR574452	ITS, psbA-trnH & trnL	*S. carnosulus con*	*S. carnosulus con*
Senecio condylus	Australia, Western Australia	A. Bellman 27A	PERTH05701627	ITS, ETS & trnL	*S. condylusJ92*	*S. condylus con*
Senecio condylus	Australia, Western Australia	G. Davies 89	PERTH05911796	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. condylusJ93*	*S. condylus con*
Senecio cunninghamii				ITS (EF538323)	*S. cunninghamii con*	
Senecio cunninghamii	Australia, Victoria	I.R. Thompson 911	MEL	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. cunninghamii con*	*S. cunninghamii*
Senecio depressicola	Australia, South Australia	F.J. Badman 1290	AD98449178	ITS, ETS, psbA-trnH	*S. depressicola con*	*S. depressicola con*
Senecio depressicola	Australia, South Australia	D.J. Duval 1139 & T.S. Te & R. J. Bates	AD223507	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. depressicola con*	*S. depressicola con*
Senecio depressicola	Australia, South Australia	P.K. Latz 23574	AD228204	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. depressicola con*	*S. depressicola con*
Senecio diaschides	Australia, Victoria	I.R. Thompson 976	MEL	ITS, ETS	*S. diaschides con*	
Senecio diaschides	New Zealand, Auckland	P.J. de Lange 1879	CHR482945	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. diaschides con*	*S. diaschides*
Senecio diaschides		Mason & Esler 11399	CHR214311	ITS, ETS	*S. diaschides con*	
Senecio distalilobatus	Australia, Victoria	I.R. Thompson 947	MEL	ITS, ETS, trnL & trnL-F	*S. distalilobatus*	*S. distalilobatus*
Senecio dolichocephalus	Australia, Victoria	I.R. Thompson 987	MEL	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. dolichocephalus*	*S. dolichocephalus*
Senecio dunedinensis				ITS (AY554109)	*S. dunedinensis con*	

Senecio dunedinensis	New Zealand, Southland	Wardle 96/29 with R.P. Buxton	CHR511331	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. dunedinensis con*	*S. dunedinensis*
Senecio dunedinensis	New Zealand, Canterbury	J. Sullivan JJS-111007-51	Lincoln Uni. Herbarium	ITS	*S. dunedinensis con*	
Senecio esleri	New Zealand, Auckland	W.R. Sykes 491/87	CHR458931	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. esleri con*	*S. esleri*
Senecio esleri	New Zealand, Hamilton	P.J. de Lange 7031 with T.J. & F.J.T. de Lange	CHR552563	ITS, ETS	*S. esleri con*	
Senecio esperensis				ITS (AY554113)	*S. esperensis con*	
Senecio esperensis	New Zealand, cultivated	W.R. Sykes 894/K	CHR194652A	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. esperensis con*	*S. esperensis*
Senecio esperensis	New Zealand, Kermadec Islands	R. Williams s.n.	CHR518159	ITS	*S. esperensis con*	
Senecio extensus	Australia, Victoria	I.R. Thompson s.n.	MEL	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. extensus ITS, S. extensus ETS*	*S. extensus*
Senecio gawlerensis	Australia, South Australia	D.E. Symon 8046A	MSC	ITS, psbA-trnH, trnL & trnL-F	*S. gawlerensis*	*S. gawlerensis*
Senecio glabrescens	Australia, Victoria	N. Middleton s.n.	MEL	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. glabrescens*	*S. glabrescens*
Senecio glaucophyllus	New Zealand, Canterbury	A.E Memory 6	CANU	ITS	*S. glaucophyllus1 con*	
Senecio glaucophyllus subsp. *basinudus*	New Zealand, Banks Peninsula	W.R. Sykes 496/69	MSC	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. glaucophyllus1 con*	*S. glaucophyllus con*
Senecio glaucophyllus subsp. *basinudus*	New Zealand, Banks Peninsula	A.E. Memory 7	CANU	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. glaucophyllus1 con*	*S. glaucophyllus con*
Senecio glaucophyllus subsp. *basinudus*	New Zealand, Banks Peninsula	A.E. Memory 42	CANU	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. glaucophyllus2 con*	*S. glaucophyllus con*
Senecio glaucophyllus subsp. *discoideus*	New Zealand, Canterbury		CHR469151	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. glaucophyllus2 con*	*S. glaucophyllus con*
Senecio glaucophyllus subsp. *glaucophyllus*				ITS (EU812813)	*S. glaucophyllus2 con*	
Senecio glaucophyllus subsp. *glaucophyllus*	New Zealand, Canterbury	I. Hanken s.n.	CHR595308A	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. glaucophyllus1 con*	*S. glaucophyllus con*

Senecio glaucophyllus subsp. *toa*	New Zealand, Wellington	C.C. Ogle 3088	CHR510475	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. glaucophyllus2 con*	*S. glaucophyllus con*
Senecio glomeratus				ITS (AY554111)	*S. glomeratus con*	
Senecio glomeratus				ITS (EU331117)	*S. glomeratus con*	
Senecio glomeratus				ITS (EU331106)	*S. glomeratus con*	
Senecio glomeratus	New Zealand, Marlborough	A.E. Memory 3	CANU	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. glomeratus con*	*S. glomeratus*
Senecio glomeratus	New Zealand, Banks Peninsula	A.E. Memory 14	CANU	ITS, ETS	*S. glomeratus con*	
Senecio glomeratus	Australia, Victoria	I.R. Thompson 909	MEL	ITS	*S. glomeratus con*	
Senecio glossanthus	Australia, New South Wales	W. Greuter 20849	B	ETS	*S. glossanthus con*	
Senecio glossanthus	Australia, Western Australia	A. Markey & S. Dillon 3295	PERTH07455615	ITS	*S. glossanthus con*	
Senecio glossanthus	Australia, Western Australia	C. D. Turley & R. M. Hoggart 3/912	PERTH08394288	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. glossanthus con*	*S. glossanthus*
Senecio gregorii				ITS (GU818651), ETS (GU818263), psbA-trnH (GU818448), trnL & trnL-F (GU818069)	*S. gregorii*	*S. gregorii*
Senecio gunnii				ITS (EF538343)	*S. gunnii con*	
Senecio gunnii	Australia, Victoria	I.R. Thompson 948	MEL	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. gunnii con*	*S. gunnii*
Senecio halophilus	Australia, Victoria	I.R. Thompson 902	MEL2334195A	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. halophilus*	*S. halophilus*
Senecio halophilus	Australia, Victoria	V. Stajsic 5151	MEL2334245	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. halophilus con*	*S. halophilusJ84*
Senecio halophilus	Australia, South Australia	R. J. Bates 73930	AD226417	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. halophilus con*	*S. halophilusJ105*
Senecio hamersleyensis	Australia, Western Australia	S. van Leeuwen 3556	MEL2196397	ITS, ETS	*S. hamersleyensis con*	
Senecio hamersleyensis	Australia, Western Australia	A.A. Mitchell PRP1195	PERTH05221366	ITS, psbA-trnH	*S. hamersleyensis con*	*S. hamersleyensis con*

Senecio hamersleyensis	Australia, Western Australia	S. van Leeuwen 3556	PERTH06230555	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. hamersleyensis con*	*S. hamersleyensis con*
Senecio hauwai	New Zealand, Marlborough	P.J. de Lange 1912	CHR482837	ITS, trnL	*S. hauwai con*	*S. hauwai con*
Senecio hauwai	New Zealand, Marlborough	P.J. de Lange 1020 with P. Simpson	CHR473607	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. hauwai con*	*S. hauwai con*
Senecio hispidissimus				ITS (GU818653), ETS (GU818266), psbA-trnH (GU818450), trnL & trnL-F (GU818071)	*S. hispidissimus*	*S. hispidissimus*
Senecio hispidissimus				ITS (GU818654)	*S. hispidissimus*	
Senecio hispidissimus				ITS (GU818658)	*S. hispidissimus*	
Senecio hispidissimus				ITS (GU818659)	*S. hispidissimus*	
Senecio hispidissimus				ITS (GU818660)	*S. hispidissimus*	
Senecio hispidissimus				ITS (GU818655)	*S. hispidissimus*	
Senecio hispidissimus				ITS (GU818656)	*S. hispidissimus*	
Senecio hispidissimus				ITS (GU818657)	*S. hispidissimus*	
Senecio hispidulus	New Zealand, Marlborough	D.G. Drury s.n.	CHR603495	ITS, trnL-F	*S. hispidulus con*	*S. hispidulus con*
Senecio hispidulus				ITS (EU331118)	*S. hispidulus con*	
Senecio hispidulus	Australia, Victoria	I.R. Thompson 908	MEL	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. hispidulus con*	*S. hispidulus con*
Senecio hispidulus	New Zealand, Marlborough	A.E. Memory 47	CANU	ITS	*S. hispidulus con*	
Senecio hispidulus	New Zealand, Canterbury	A.E. Memory 48	CANU	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. hispidulus con*	*S. hispidulus con*
Senecio hooglandii	Norfolk Island, Bloody Bridge	P.J. de Lange NF 196	AK238304	ITS, ETS, psbA-trnH & trnL	*S. hooglandii*	*S. hooglandii*
Senecio hypoleucus	Australia, Victoria	I.R. Thompson 979	MEL	ITS, ETS	*S. hypoleucus con*	
Senecio hypoleucus	New Zealand, cultivated	D. Barwick s.n.	CHR567254	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. hypoleucus con*	*S. hypoleucus*
Senecio hypoleucus	New Zealand, cultivated	D. Barwick s.n.	CHR567255	ITS	*S. hypoleucus con*	

Senecio kermadecensis	New Zealand, Raoul Island	W.R. Sykes 1183/K	US	ITS	*S. kermadecensis*	*S. kermadecensis*
Senecio kermadecensis	New Zealand, Kermadec Islands	J. Parkes s.n.	CHR491761	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. kermadecensis1*	*S. kermadecensis1*
Senecio laceratus	Australia, South Australia	J.S. Womersley 373 & D.E. Symon	SIU	ITS	*S. laceratus*	
Senecio lacustrinus	Australia, Western Australia	D.J. Edinger 1783	PERTH05730090	ITS	*S. lacustrinusJ90*	
Senecio lacustrinus	Australia, Western Australia	J.M. Collins 550	PERTH08080690	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. lacustrinusJ97*	*S. lacustrinusJ97*
Senecio lacustrinus	Australia, South Australia	F.J. Badman 7073	AD99409105	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. lacustrinus con*	*S. lacustrinus con*
Senecio lacustrinus	Australia, South Australia	H.P. Vonow & N.R. Neagle BS721-136	AD241264	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. lacustrinus con*	*S. lacustrinus con*
Senecio lanibracteus	Australia, South Australia	R. Merrill King 9627 & L. Haegi	US	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. lanibracteus*	*S. lanibracteus*
Senecio lautus				ITS (EU812814)	*S. lautus con*	
Senecio lautus	New Zealand, Marlborough	A.E. Memory 5	CANU	ITS, ETS, trnL & trnL-F	*S. lautus con*	*S. lautus2606*
Senecio lautus	New Zealand, Auckland	W.R. Sykes 310/90	CHR473716	ITS	*S. lautus con*	
Senecio lautus	New Zealand, Nelson	C.J. Webb & M. O'Brian s.n.	CHR468744	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. lautus con*	*S. lautus2579*
Senecio lautus	New Zealand, Porirua	J. R. Rolfe	CANU	ITS	*S. lautus con*	
Senecio linearifolius				ITS (EF538301)	*S. linearifolius con*	
Senecio linearifolius				ITS (EF538302)	*S. linearifolius con*	
Senecio linearifolius	Australia, New South Wales	W.T. Stearn 5	MO	ITS	*S. linearifolius con*	
Senecio linearifolius var. *denticulatus*	Australia, Victoria	I.R. Thompson 914	MEL	ITS	*S. linearifolius con*	
Senecio linearifolius var. *linearifolius*	Australia, Victoria	I.R. Thompson 919	MEL	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. linearifolius con*	*S. linearifolius*
Senecio longicollaris	Australia, Victoria	I.R. Thompson 766	MEL	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. longicollaris*	*S. longicollaris*

Senecio macranthus	Australia, New South Wales	N.S. Lander 505	MSC	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. macranthus*	*S. macranthus*
Senecio macrocarpus	Australia, Victoria	I.R. Thompson 658	MEL	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. macrocarpus*	*S. macrocarpus*
Senecio magnificus	Australia, South Australia	P. Short 749	MSC	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. magnificus*	*S. magnificus*
Senecio marotiri		P.J. de Lange CH585 with P.B. Heenan	CHR551988	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. marotiri2562*	*S. marotiri2562*
Senecio marotiri	New Zealand, Motukino Island	E.K. Cameron 7721 with P.J. de Lange	CHR486220	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. marotiri3117*	*S. marotiri3117*
Senecio minimus				ITS (AY554114)	*S. minimus con*	
Senecio minimus				ITS (EU331119)	*S. minimus con*	
Senecio minimus	Australia, Victoria	I.R. Thompson 935	MEL	ITS, ETS	*S. minimus con*	
Senecio minimus	New Zealand, Canterbury	A.E. Memory 4	CANU	ITS	*S. minimus con*	
Senecio minimus	New Zealand, Canterbury	A.E. Memory 59	CANU	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. minimus con*	*S. minimus*
Senecio nigrapicus	Australia, Victoria	I.R. Thompson 760a	MEL	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. nigrapicus*	*S. nigrapicus*
Senecio odoratus	Australia, Victoria	I.R. Thompson 906	MEL	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. odoratus*	*S. odoratus*
Senecio phelleus	Australia, Victoria	I.R. Thompson 903	MEL	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. phelleus*	*S. phelleus*
Senecio picridioides	Australia, South Australia	D.R. Symon 8616	B	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. picridioides con*	*S. picridioides*
Senecio picridioides	Australia, Victoria	I.R. Thompson 941	MEL	ITS	*S. picridioides con*	
Senecio pinnatifolius	Australia, Victoria	I.C. Clarke 2318	MO	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. pinnatifolius con*	*S. pinnatifolius con*
Senecio pinnatifolius var. *lanceolatus*				ITS (GU818671), ETS (GU818287), psbA-trnH (GU818460), trnL & trnL-F (GU818081)	*S. pinnatifolius con*	*S. pinnatifolius con*
Senecio pinnatifolius var. *lanceolatus*				ITS (GU818672)	*S. pinnatifolius con*	

Senecio pinnatifolius var. *lanceolatus*	ITS (GU818673)	*S. pinnatifolius con*	
Senecio pinnatifolius var. *lanceolatus*	ITS (GU818674)	*S. pinnatifolius con*	
Senecio pinnatifolius var. *lanceolatus*	ITS (GU818675)	*S. pinnatifolius con*	
Senecio pinnatifolius var. *lanceolatus*	ITS (GU818676)	*S. pinnatifolius con*	
Senecio pinnatifolius var. *lanceolatus*	ITS (GU818677)	*S. pinnatifolius con*	
Senecio pinnatifolius var. *lanceolatus*	ITS (GU818678)	*S. pinnatifolius con*	
Senecio pinnatifolius var. *lanceolatus*	ITS (GU818679)	*S. pinnatifolius con*	
Senecio pinnatifolius var. *lanceolatus*	ITS (GU818680)	*S. pinnatifolius con*	
Senecio prenanthoides	ITS (GU818681), ETS (GU818289), psbA-trnH (GU818462), trnL & trnL-F (GU818083)	*S. prenanthoides*	*S. prenanthoides*
Senecio psilocarpus	ITS (GU818682), ETS (GU818290), psbA-trnH (GU818463), trnL & trnL-F (GU818084)	*S. psilocarpus*	*S. psilocarpus*
Senecio psilocarpus	ITS (GU818683)	*S. psilocarpus*	
Senecio psilocarpus	ITS (GU818688)	*S. psilocarpus*	
Senecio psilocarpus	ITS (GU818689)	*S. psilocarpus*	
Senecio psilocarpus	ITS (GU818690)	*S. psilocarpus*	
Senecio psilocarpus	ITS (GU818691)	*S. psilocarpus*	
Senecio psilocarpus	ITS (GU818692)	*S. psilocarpus*	
Senecio psilocarpus	ITS (GU818684)	*S. psilocarpus*	
Senecio psilocarpus	ITS (GU818685)	*S. psilocarpus*	
Senecio psilocarpus	ITS (GU818686)	*S. psilocarpus*	

Senecio psilocarpus				ITS (GU818687)	*S. psilocarpus*	
Senecio psilophyllus	Australia, New South Wales	I.R. Thompson 790a	MEL	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. psilophyllus*	*S. psilophyllus*
Senecio quadridentatus				ITS (AF422134)	*S. quadridentatus con*	
Senecio quadridentatus	Australia, Victoria	I.R. Thompson 899	MEL	ITS, ETS	*S. quadridentatus con*	
Senecio quadridentatus	New Zealand, Banks Peninsula	A.E. Memory 33	CANU	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. quadridentatus con*	*S. quadridentatus con*
Senecio quadridentatus	New Zealand, Banks Peninsula	A.E. Memory 40	CANU	ITS	*S. quadridentatus con*	
Senecio quadridentatus	New Zealand, Canterbury	A.E. Memory 58	CANU	ITS, trnL	*S. quadridentatus con*	*S. quadridentatus con*
Senecio radiolatus	New Zealand, Chatham Islands	W.R. Sykes 186/07	CHR607538	ITS, ETS, psbA-trnH & trnL	*S. radiolatus con*	*S. radiolatus con*
Senecio radiolatus subsp. *radiolatus*	New Zealand, Chatham Islands		CHR301153	trnL & trnL-F	*S. radiolatus con*	*S. radiolatus con*
Senecio radiolatus subsp. *radiolatus*		W.R. Sykes s.n.	CHR201175	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. radiolatus con*	*S. radiolatus con*
Senecio radiolatus subsp. *radiolatus*		W.R. Sykes 431/93	CHR4976759	ITS, trnL	*S. radiolatus con*	*S. radiolatus con*
Senecio repangae subsp. *pokohinuensis*	New Zealand, Motukino Island	P.J. de Lange s.n.	CHR486230	ITS, trnL & trnL-F	*S. repangae subsp. pokohinuensis2564, S. repangae subsp. pokohinuensis2564c*	*S. repangae subsp. pokohinuensis2564*
Senecio repangae subsp. *pokohinuensis*		P.J. de Lange 5374	CHR549560	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. repangae subsp. pokohinuensis2565, S. repangae subsp. pokohinuensis2565c*	*S. repangae subsp. pokohinuensis2565*
Senecio repangae subsp. *repangae*	New Zealand, Auckland	P.J. de Lange 3740	CHR493856	ITS2 & psbA-trnH	*S. repangae subsp. repangae con*	*S. repangae subsp. repangae con*
Senecio repangae subsp. *repangae*		W.R. Sykes 438/71	CHR224804	ITS, psbA-trnH, trnL & trnL-F	*S. repangae subsp. repangae con*	*S. repangae subsp. repangae con*
Senecio repangae subsp. *repangae*	New Zealand, Cuvier Island	I.E.A. Adkinson s.n.	CHR216206	ITS, ETS, psbA-trnH & trnL	*S. repangae subsp. repangae con*	*S. repangae subsp. repangae con*

Senecio rufiglandulosus				ITS (AF422135)	*S. rufiglandulosus con*	
Senecio rufiglandulosus	New Zealand, Wellington	D. Glenny 6796	CHR530476	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. rufiglandulosus con*	*S. rufiglandulosus*
Senecio rufiglandulosus	New Zealand, Nelson	A.P. Druce s.n.	CHR395675	ITS	*S. rufiglandulosus con*	
Senecio scaberulus				ITS (EF538377)	*S. scaberulus con*	
Senecio scaberulus				ITS (EF538378)	*S. scaberulus con*	
Senecio scaberulus				ITS (EU331120)	*S. scaberulus con*	
Senecio scaberulus	New Zealand, Auckland	P.J. de Lange 1827	CHR483072	ITS, ETS, psbA-trnH & trnL	*S. scaberulus con*	*S. scaberulus con*
Senecio scaberulus	New Zealand, cultivated	P.J. de Lange 5379	CHR574261B	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. scaberulus con*	*S. scaberulus con*
Senecio serratiformis	Australia, South Australia	P. Coombe	AD98671653	ITS, psbA-trnH, trnL & trnL-F	*S. serratiformis*	*S. serratiformis*
Senecio spanomerus	Australia, Victoria	I.R. Thompson 657	MEL2334175A	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. spanomerus con*	*S. spanomerus*
Senecio spanomerus	Australia, Victoria	N. G. Walsh 7466 (Birch, J.L.; Gallagher, C.; Stewart, S.)	MEL2357326	ITS, ETS	*S. spanomerus con*	
Senecio spathulatus var. *attenuatus*	Australia, New South Wales	W. Cherry 504 & I. R. Thompson	MEL2233944	ITS, ETS	*S. spathulatus con*	
Senecio spathulatus var. *latifructus*	Australia, Victoria	I.R. Thompson 953	MEL2334173A	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. spathulatus var. latifructus*	*S. spathulatus var. latifructus*
Senecio spathulatus var. *spathulatus*	Australia, Tasmania	M. Wapstra MW7	MEL	ITS	*S. spathulatus con*	
Senecio spathulatus var. *spathulatus*	Australia, Tasmania	D. Rathbone	MEL2334163	ITS, ETS	*S. spathulatus con*	
Senecio squarrosus				ITS (GU818698), ETS (GU818296), psbA-trnH (GU818465), trnL & trnL-F (GU817964)	*S. squarrosus*	*S. squarrosus*
Senecio squarrosus				ITS (GU818699)	*S. squarrosus*	

Senecio squarrosus				ITS (GU818700)	*S. squarrosus*	
Senecio squarrosus				ITS (GU818701)	*S. squarrosus*	
Senecio squarrosus				ITS (GU818702)	*S. squarrosus*	
Senecio squarrosus				ITS (GU818703)	*S. squarrosus*	
Senecio squarrosus				ITS (GU818704)	*S. squarrosus*	
Senecio sterquilinus				ITS (EU331122)	*S. sterquilinus con*	
Senecio sterquilinus	New Zealand, Somes Island	P.J. de Lange 1041	CHR474957	ITS	*S. sterquilinus con*	
Senecio sterquilinus	New Zealand, Mokopuna Island	P.J. de Lange 1516 with G.M. Crawcroft	CHR479560	ITS	*S. sterquilinus con*	
Senecio sterquilinus	New Zealand, Nelson	C.J. Webb & M. O'Brien	CHR468743	ITS, ETS, trnL & trnL-F	*S. sterquilinus con*	*S. sterquilinus*
Senecio sterquilinus	New Zealand, Westland	P.J. de Lange 1479	CHR479217	ITS	*S. sterquilinus con*	
Senecio wairauensis				ITS (EF538397)	*S. wairauensis con*	
Senecio wairauensis				ITS (EU812817)	*S. wairauensis con*	
Senecio wairauensis				ITS (EU812816)	*S. wairauensis con*	
Senecio wairauensis				ITS (EU812811)	*S. wairauensis con*	
Senecio wairauensis	New Zealand, Canterbury	D.G. Drury 175201	US	ITS	*S. wairauensis con*	
Senecio wairauensis	New Zealand, Canterbury	A.E. Memory 1	CANU	ITS	*S. wairauensis con*	
Senecio wairauensis	New Zealand, Canterbury	A.E. Memory 31	CANU	ITS, ETS, psbA-trnH, trnL & trnL-F	*S. wairauensis con*	*S. wairauensis*

Table S3. (Chapter 3) Specimens of *Senecio* aff. *glaucophyllus* and *S. glaucophyllus* used in the study of the *S. glaucophyllus* complex. Taxon names as included in the analyses (do not represent the specimen names on herbarium sheets) are those that are identified by me using my knowledge on the two taxa following the initial screening of a subset of the specimens.

Specimens in bold are used for both the phylogenetic and morphometric studies. All specimens were included in the morphometric study except those marked with * and #. Specimens marked with asterisk (*) are incomplete or poor quality specimens that were examined but could not be used for the morphometric study. Specimens marked with # are those used only for the phylogenetic analyses.

Species as identified in this study	Taxon name as included in the analyses	Current identification in herbarium	Location	Specimen	Herbarium accession number	Notes
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Burnett	F. Soper Jr. s.n., 20-Feb-1965	AK 104586	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Arthur, 4000ft	T.F. Cheeseman s.n., Jan-1886	AK 10601	Lectotype of S. glaucophyllus (Ornduff, 1960)
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Arthur, 4000ft	T.F. Cheeseman s.n., Jan-1886	AK 10602	Isolectotype of S. glaucophyllus (Ornduff, 1960)
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Arthur, 4000ft	T.F. Cheeseman s.n., Jan-1886	AK 10604	Isolectotype of S. glaucophyllus (Ornduff, 1960)
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Arthur	T.F. Cheeseman & J. Adams s.n.	AK 15729*	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Burnett	R.O. Gardner 7612	AK 224249	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Burnett	P.J. de Lange 3291 & P.B. Heenan	AK 232597	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Burnett	P.J. de Lange 4938	AK 253477	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Hoary Head	E.A. Brown s.n., 26-Feb-1986	AK 275780	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Arthur	J. Adams s.n.	AK 35335*	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Arthur	D.A. Norton DN 1865	CANU 37162	

S. glaucophyllus	**S. aff. glaucophyllus**	**Senecio 'Burnett'**	**North-West Nelson, Mt. Burnett**	**S. Walls s.n., 12-May-2014**	**CANU 42528**	**S_aff_glaucophyllusJ74**
S. glaucophyllus	**S. aff. glaucophyllus**	**Senecio 'Burnett'**	**North-West Nelson, Mt. Burnett**	**S. Walls s.n., 12-May-2014**	**CANU 42529**	**S_aff_glaucophyllusJ75**
S. glaucophyllus	S. aff. glaucophyllus	Senecio 'Burnett'	North-West Nelson, The Twins	S.P. Courtney s.n., 12-Feb-2015	No voucher specimen #	S_aff_glaucophyllusJ116
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Burnett	F.G. Soper s.n., 28-Feb-1965	CHR 155457	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Arthur	A.P. Druce s.n., Jan-1975	CHR 277586	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus	North-West Nelson, Mt. Arthur	M.J.A. Simpson 7523	CHR 278338	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, The Gorge Stream	A.P. Druce s.n., Feb-1976	CHR 286508	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, The Gorge Creek	A.P. Druce s.n., Jan-1979	CHR 365568	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Burnett	A.P. Druce s.n., Jan-1979	CHR 365602	
S. glaucophyllus	**S. aff. glaucophyllus**	**S. glaucophyllus**	**North-West Nelson, Mt. Arthur**	**K.A. Ford 7/99**	**CHR 489460**	**S_aff_glaucophyllus (AY554110)**
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus	North-West Nelson, The Twins	S.P. Courtney s.n., 17-Jan-2008	CHR 552233	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus	North-West Nelson, The Twins	S.P. Courtney s.n., 17-Jan-2008	CHR 552252	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus	North-West Nelson, Mt. Burnett	S.P. Courtney s.n., 20-Jan-2006	CHR 552987	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus	North-West Nelson, Mt. Arthur	S.P. Courtney s.n., 13-Jan-2004	CHR 596951	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus	North-West Nelson, Mt. Arthur	S.P. Courtney s.n., 13-Jan-2004	CHR 596952	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus	North-West Nelson, Mt. Arthur	S.P. Courtney s.n., 13-Jan-2004	CHR 596953	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus	North-West Nelson, Mt. Arthur	S.P. Courtney s.n., 13-Jan-2004	CHR 596954	

S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus	North-West Nelson, Mt. Arthur	S.P. Courtney s.n., 13-Jan-2004	CHR 596955	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Arthur	T.F. Cheeseman s.n.	WELT SP031588	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Arthur	T.F. Cheeseman s.n., Jan-1886	WELT SP043140	Isolectotype of S. glaucophyllus (Ornduff, 1960)
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Burnett	P.J. de Lange 4938	WELT SP082670	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Hoary Head	B.V. Sneddon s.n., 23-Jan-1970	WELT SP091272	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Burnett	W. Burke s.n., 3-Feb-1973	WELT SP097050	
S. glaucophyllus	S. aff. glaucophyllus	S. glaucophyllus?	North-West Nelson, Mt. Burnett	W. Burke s.n.	WELT SP097052	
S. pseudoglaucophyllus	S. glaucophyllus subsp. basinudus	S. glaucophyllus subsp. basinudus	Canterbury, cultivated from J.W. Dawson s.n., Jul-1954	R. Ornduff s.n., 18-Jun-1955	CHR 87795A of B	Holotype of S. glaucophyllus subsp. basinudus (Ornduff, 1960)
S. pseudoglaucophyllus	S. glaucophyllus subsp. basinudus	S. glaucophyllus subsp. basinudus	Canterbury, cultivated from J.W. Dawson s.n., Jul-1954	R. Ornduff s.n., 18-Jun-1955	CHR 87795B of B	Holotype of S. glaucophyllus subsp. basinudus (Ornduff, 1960)
S. pseudoglaucophyllus	S. glaucophyllus subsp. basinudus	S. glaucophyllus subsp. basinudus	Canterbury, cultivated from J.W. Dawson s.n., Jul-1954	R. Ornduff s.n., 18-Jun-1955	WELT SP078906	Isotype of S. glaucophyllus subsp. basinudus (Ornduff, 1960)
S. pseudoglaucophyllus	S. glaucophyllus subsp. basinudus	Senecio glaucophyllus subsp. basinudus	New Zealand, Banks Peninsula	W.R. Sykes 496/69	MSC #	S_glaucophyllus_basi2062
S. pseudoglaucophyllus	S. glaucophyllus subsp. basinudus	Senecio glaucophyllus subsp. basinudus	New Zealand, Banks Peninsula	A.E. Memory 7	CANU #	S_glaucophyllus_basi2608.1
S. pseudoglaucophyllus	S. glaucophyllus subsp. basinudus	Senecio glaucophyllus subsp. glaucophyllus	New Zealand, Canterbury	I. Hanken s.n.	CHR595308A #	S_glaucophyllus_basi2599
S. pseudoglaucophyllus	S. glaucophyllus subsp. discoideus	S. glaucophyllus subsp. discoideus	Canterbury, Mt. Torlesse	T.F. Cheeseman s.n., Jan 1888	AK 10596	Lectotype of S. lautus var. discoideus (Ornduff, 1960)
S. pseudoglaucophyllus	Senecio glaucophyllus subsp. discoideus	Senecio glaucophyllus subsp. discoideus	New Zealand, Canterbury		CHR469151 #	S_glaucophyllus_disc3101

S. pseudoglaucophyllus	Senecio glaucophyllus subsp. discoideus	Senecio glaucophyllus subsp. discoideus	New Zealand, Craigieburn Forest Park	P.B. Pelser 3123	CANU42563 #	S_glaucophyllus_disc3123
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Owen	W.L. Townson 612	AK 10589	Representative specimen of S. lautus var. montanus
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Gordon's Knob	T.F. Cheeseman s.n., Jan-1882	AK 10590	Representative specimen of S. lautus var. montanus
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Arthur, 4000ft	T.F. Cheeseman s.n., Jan-1886	AK 10591	Representative specimen of S. lautus var. montanus
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Arthur, 4000ft	T.F. Cheeseman s.n., Jan 1886	AK 10592	Representative specimen of S. lautus var. montanus
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Arthur	F.G. Gibbs s.n.	AK 10605	
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Arthur	F.G. Gibbs s.n.	AK 10607	
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Arthur	F.G. Gibbs s.n.	AK 10609	
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Replica Hill	W.R. Sykes 126/98	AK 238752	
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	Nelson, Tableland Caves	J.A. Rattenbury s.n., Dec-1952	AK 264200	
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Arthur	J.A. Rattenbury s.n., Dec-1952	AK 264203	
S. pseudoglaucophyllus	**S. glaucophyllus subsp. glaucophyllus**	**S. glaucophyllus subsp. glaucophyllus**	**North-West Nelson, Matiri Plateau**	**S.P. Courtney s.n., 20-Feb-2014**	**CANU 42531**	**S_glaucophyllus_glauJ78**
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Haystack Creek	A. Shanks s.n., 3-May-2014	CANU #	S_glaucophyllus_glauJ73
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Owen	S.P. Courtney s.n., 10-Dec-2013	CANU 42530	
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus		P.H. Raven 25652	CHR 198757A	
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	Senecio glaucophyllus ssp. glaucophyllus	North-West Nelson, cultivated from Gouland-Downs	A.P. Druce s.n., 1-Dec-1969	CHR 244096	

S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Blue Creek	A.P. Druce s.n., Jan-1972	CHR 249814
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Thorns Creek	A.P. Druce s.n., Apr-1969	CHR 279088
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	Nelson, Springs Junction	A.P. Druce s.n., Jan-1978	CHR 323570
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Mytton	A.P. Druce s.n., Mar-1980	CHR 358465
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus	North-West Nelson, Cobb Valley	I.M. Ritchie s.n., 3-Jan-1970	CHR 371660
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Replica Hill	W.R. Sykes 126/98	CHR 518374
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	West Nelson, Mt. Misery	K.H. Platt s.n., 21-Feb-1983	CHR 520177
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus	North-West Nelson, The Twins	S.P. Courtney s.n., 17-Jan-2008	CHR 547117A
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus	North-West Nelson, The Twins	S.P. Courtney s.n., 17-Jan-2008	CHR 552232
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	Senecio	North-West Nelson, Mt. Arthur	J.A. Hay s.n., 13-Apr-1952	CHR 75531
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. discoideus	North-West Nelson, Mt. Owen	W.L. Townson s.n.	WELT SP016473
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Arthur	F. Gibbs s.n.	WELT SP031590
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	Nelson, Baton Spur	W.R.B. Oliver s.n., 24-Jan-1956	WELT SP087845
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	Nelson, Mt. Patriarch	B.V. Sneddon s.n., 10-Feb-1970	WELT SP091267
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Mytton	B.V. Sneddon s.n., 28-Mar-1981	WELT SP091268
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Benson	B.V. Sneddon s.n., 26-Jan-1980	WELT SP091269
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Olive	B.V. Sneddon s.n., 14-Mar-1983	WELT SP091270

S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Mt. Arthur	B.V. Sneddon s.n., 19-Jan-1965	WELT SP091271	
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Granity Pass	C. Bell s.n., 27-Feb-1968	WELT SP097053	
S. pseudoglaucophyllus	S. glaucophyllus subsp. glaucophyllus	S. glaucophyllus subsp. glaucophyllus	North-West Nelson, Gouland Downs	A. McNeill-Adams s.n., 22-Jan-1969	WELT SP097054	
S. pseudoglaucophyllus	S. glaucophyllus subsp. toa	S. glaucophyllus subsp. toa	North Island, Taupo	K. W. Allison s.n., 18-Dec-1934	CHR 17696	Holotype of S. glaucophyllus subsp. toa (Connor & Edgar, 1987)
S. pseudoglaucophyllus	S. glaucophyllus subsp. toa	S. glaucophyllus subsp. raoulii	North Island, Taupo, Mt. Tauhara	T.F. Cheeseman s.n., Jan-1889	AK 10593	Representative specimen of S. lautus var. montanus
S. pseudoglaucophyllus	S. glaucophyllus subsp. toa	S. glaucophyllus subsp. toa	New Zealand, Wellington	C.C. Ogle 3088	CHR510475 #	S_glaucophyllus_toa3103
S. pseudoglaucophyllus	S. glaucophyllus subsp. toa	S. glaucophyllus subsp. toa	North Island, Ngaruroro River	T. Lawson 201501798	CANU #	S_glaucophyllus_toaJ125

Table S4. (Chapter 4) Specimen details for the morphometric study of *Senecio* "pseudoglaucophyllus".

Taxon	Location	Voucher	Herbarium	Notes
The Nelson-group	North-West Nelson, Mt. Owen	S.P. Courtney s.n., 12-Dec-2013	CANU42530	
The Nelson-group	North-West Nelson, Matiri Plateau	S.P. Courtney s.n., 20-Feb-2014	CANU42531	
The Nelson-group	North-West Nelson, The Twins	S.P. Courtney s.n., 17-Jan-2008	CHR552232	
The Nelson-group	North-West Nelson, Cobb Valley	I.M. Ritchie s.n., 3-Jan-1970	CHR371660	
The Nelson-group	North-West Nelson, Blue Creek	A.P. Druce s.n., Jan-1972	CHR249814	
The Nelson-group	North-West Nelson, cultivated from Gouland-Downs	A.P. Druce s.n., 1-Dec-1969	CHR244096	
The Nelson-group	North-West Nelson, Mt. Mytton	A.P. Druce s.n., Mar-1980	CHR358465	
The Nelson-group	North-West Nelson, The Twins	S.P. Courtney s.n., 17-Jan-2008	CHR547117A	
The Nelson-group	Nelson, Springs Junction	A.P. Druce s.n., Jan-1978	CHR323570	
The Nelson-group	North-West Nelson, Mt. Benson	B.V. Sneddon s.n., 26-Jan-1980	WELT SP091269	
The Nelson-group	Nelson, Mt. Patriarch	B.V. Sneddon s.n., 10-Feb-1970	WELT SP091267	
The Nelson-group	North-West Nelson, Mt. Mytton	B.V. Sneddon s.n., 28-Mar-1981	WELT SP091268	
The Nelson-group	North-West Nelson, Replica Hill	W.R. Sykes 126/98	AK238752	
The Nelson-group	North-West Nelson, Granity Pass	C. Bell s.n., 27-Feb-1968	WELT SP097053	
The Nelson-group	North-West Nelson, Gouland Downs	A. McNeill-Adams s.n., 22-Jan-1969	WELT SP097054	
The Nelson-group	North-West Nelson, Mt. Arthur	F. Gibbs s.n.	WELT SP031590	
The Nelson-group	Nelson, Tableland Caves	J.A. Rattenbury s.n., Dec-1952	AK264200	
The Nelson-group	North-West Nelson, Mt. Arthur	P.H. Raven 25652	CHR198757A	
The Nelson-group	North-West Nelson, Mt. Arthur	B.V. Sneddon s.n., 19-Jan-1965	WELT SP091271	
The Nelson-group	North-West Nelson, Mt. Olive	B.V. Sneddon s.n., 14-Mar-1983	WELT SP091270	
The Nelson-group	Nelson, Baton Spur	W.R.B. Oliver s.n., 24-Jan-1956	WELT SP087845	
The Nelson-group	North-West Nelson, Mt. Owen	W.L. Townson s.n.	WELT SP016473	
The Nelson-group	North-West Nelson, Thorns Creek	A.P. Druce s.n., Apr-1969	CHR279088	
The Nelson-group	North-West Nelson, Replica Hill	W.R. Sykes 126/98	CHR518374	
The Nelson-group	West Nelson, Mt. Misery	K.H. Platt s.n., 21-Feb-1983	CHR520177	

The Nelson-group	North-West Nelson, Mt. Arthur	T.F. Cheeseman s.n., Jan-1886	AK10591	Representative specimen of S. lautus var. montanus
The Nelson-group	North-West Nelson, Gordon's Knob	T.F. Cheeseman s.n., Jan-1882	AK10590	Representative specimen of S. lautus var. montanus
The Nelson-group	North-West Nelson, Mt. Owen	W.L. Townson 612	AK10589	Representative specimen of S. lautus var. montanus
The Nelson-group	North-West Nelson, Mt. Arthur	T.F. Cheeseman s.n., Jan 1886	AK10592	Representative specimen of S. lautus var. montanus
subsp. basinudus	Canterbury, Mt. Cass	J. Liew J60	CANU42541	
subsp. basinudus	Canterbury, Mt. Cass	N. Head s.n., 19-Feb-2014	CANU42542	
subsp. basinudus	Canterbury, Mt. Cass	J. Liew J62 & N. Head	CANU42551	
subsp. basinudus	Canterbury, Port Hills	J. Liew J68	CANU42533	
subsp. basinudus	Canterbury, Port Hills	J. Liew J69	CANU42546	
subsp. basinudus	Otago Peninsula, Allans Beach	J. Liew J118 & J. Barkla	CANU42553	
subsp. basinudus	Otago Peninsula, Allans Beach	J. Liew J119 & J. Barkla	CANU42537	
subsp. basinudus	Otago Peninsula, Allans Beach	J. Liew J120 & J. Barkla	CANU42552	
subsp. basinudus	North Otago, Tavora Beach	J. Liew J121 & J. Barkla	CANU42536	
subsp. basinudus	North Otago, Shag Point	J. Liew J122 & J. Barkla	CANU42535	
subsp. basinudus	Canterbury, Port Hills	A.E. Memory 46	CANU042340	
subsp. basinudus	Banks Peninsula, Akaroa	A.E. Memory 42	CANU042306	
subsp. basinudus	Canterbury, cultivated from J.W. Dawson s.n., Jul-1954	R. Ornduff s.n., 18-Jun-1955	CHR87795A and B	Holotype of S. glaucophyllus subsp. basinudus (Ornduff, 1960)
subsp. basinudus	Otago, Pilot Bay	W.R. Sykes 11/83	CHR400678	
subsp. basinudus	Canterbury, cultivated from J.W. Dawson s.n., Jul-1954	R. Ornduff s.n., 18-Jun-1955	WELT SP078906	Isotype of S. glaucophyllus subsp. basinudus (Ornduff, 1960)
subsp. basinudus	Southland, Tahakopa Bay	D.R. Given 13164 & H.K. Hall	CHR403702	
subsp. basinudus	Otago, Quarantine Island	P.N. Johnson s.n., 27-Nov-1981	CHR364271	
subsp. basinudus	North Canterbury, Gore Bay	B.H. Macmillan 87/42	CHR401333	
subsp. basinudus	Canterbury, Lyttelton Hills	H.H. Allan s.n., 23-Oct-1947	CHR83769	
subsp. basinudus	Banks Peninsula, Kaituna Valley	L.B. Moore s.n., 14-Oct-1961	CHR123644	

subsp. basinudus	Banks Peninsula, Duvauchelle	L.B. Moore s.n., 6-Sep-1961	CHR97382
subsp. basinudus	Canterbury, Port Hills	H.H. Allan s.n., 19-Dec-1940	CHR83768
subsp. basinudus	Canterbury, Port Hills	W.R. Sykes 314/72	CHR228858
subsp. basinudus	Banks Peninsula, Lake Forsyth		CHR520718
subsp. basinudus	Banks Peninsula, Dan Rogers Creek	D. Kelly s.n., 11-Nov-1971	CHR221936
subsp. basinudus	Otago Peninsula, Allans Beach	P.J. de Lange 3492 & GMC	AK234804
subsp. basinudus	Catlins, Nugget point	A.E. Wright 14090	AK351109
subsp. basinudus	Catlins, Nugget point	A.E. Wright 14093	AK351117
subsp. basinudus	Banks Peninsula, Akaroa	J. Liew J43	CANU42550
subsp. basinudus	Banks Peninsula, Okains Bay	W.R. Sykes 496/69	CHR194703A-K
subsp. discoideus	Canterbury, Castle Hill	N. Head s.n., 11-Dec-2013	CANU42549
subsp. discoideus	Canterbury, Cass	P.B. Pelser 3125	CANU42548
subsp. discoideus	South Canterbury, Sterndale Stream	J. Liew J63A	CANU42540
subsp. discoideus	Canterbury, Castle Hill	D. Kimber s.n., 21-Feb-2014	CANU42556
subsp. discoideus	Canterbury, Port Hills	J. Liew J67	CANU42543
subsp. discoideus	North Otago, Mt. Buster	P.J. de Lange 12510	CANU42539
subsp. discoideus	Canterbury, Lake Heron	A.T. Dobson s.n., 20-Dec-1972	CANU018682
subsp. discoideus	Canterbury, Rangitata	B.A. Fineran 66030	CANU28490
subsp. discoideus	North Canterbury, Parnassus	I. Robins s.n., 6-Jan-1969 & A.R. Mitchell	CHR193379
subsp. discoideus	Canterbury, Lake Sumner	A.D. Campbell s.n., 20-Feb-1979 & B.P.J. Molloy	CHR354285
subsp. discoideus	Hawke's Bay, Cooks Horn Basin	N.L. Elder 673/5	CHR535652
subsp. discoideus	Wellington, Ruahine Range	A.P. Druce s.n., 1-Feb-1968	CHR190705
subsp. discoideus	Canterbury, Mt. Hay	A.J.D. Barker 362	CHR20454
subsp. discoideus	Canterbury, Mt. Sugarloaf	H.H. Allan s.n., 24-Jan-1919	CHR10460
subsp. discoideus	Canterbury, Lake Lyndon	H. Talbot s.n.	CHR300839
subsp. discoideus	Otago, Cromwell Gorge	I.A. McNeur s.n., 25-Dec-1949	CHR68905
subsp. discoideus	Hawke's Bay, Ruahine Range	A.P. Druce s.n., 1-Feb-1968	CHR190703
subsp. discoideus	Otago, Naseby Forest	B.H. Macmillan 79/295	CHR369038

subsp. discoideus	Canterbury, Mt. Peel	H.H. Allan s.n., 3-Jan-1919	CHR10447	
subsp. discoideus	Canterbury, Two Thumb Range	A.P. Druce APD226	CHR469151	
subsp. discoideus	South Canterbury, Pareora Gorge	R. Mason s.n., 11-Feb-1945	CHR65308	
subsp. discoideus	Canterbury, Broken River	K.H. Platt s.n., 18-Feb-1987	CHR520175	
subsp. discoideus	Canterbury, McKinnon Stream	H.D. Wilson s.n., 12-Jan-1971	CHR254113	
subsp. discoideus	Southland, Wilderness	L.B. Moore s.n., 1-Jan-1957	CHR141614	
subsp. discoideus	Otago, Lumsden	J. E. Attwood s.n., Jan-1940	AK89517	
subsp. discoideus	Canterbury, Mt. Peel	P.J. de Lange 2824	AK235348	
subsp. discoideus	Canterbury, Castle Hill	T. F. Cheeseman s.n., Jan-1883	AK10597	
subsp. discoideus	Canterbury, Mt. Torlesse	T.F. Cheeseman s.n., Jan 1888	AK10596	Lectotype of S. lautus var. discoideus (Ornduff, 1960)
subsp. toa	Canterbury, Waikari	M. & G. Giller s.n., 20-Dec-2013	CANU42547	
subsp. toa	South Marlborough, Clarence River	S.P. Courtney s.n., 26-Feb-2014	CANU42544	
subsp. toa	Hawke's Bay, Maungaharuru Range	M. Thorsen s.n., 12-Dec-2011	CANU42534	
subsp. toa	Hawke's Bay, Te Waka Range	M. Thorsen s.n., 2-Jan-2007	CANU42532	
subsp. toa	Marlborough, Kaikoura	P. Wardle s.n., 1-Feb-1961	CHR117179	
subsp. toa	Marlborough, Hodder Valley	R. Mason & D.R. McQueen 2828	CHR85320	
subsp. toa	Hawke's Bay, Ngaruroro River	A.P. Druce s.n., 1-Jan-1976	CHR279404	
subsp. toa	Hawke's Bay, Ngaruroro River	A.P. Druce s.n., 1-Jan-1975	CHR275401	
subsp. toa	North Canterbury, Lower Waipara	A.W. Robertson s.n., 14-Jan-1986	CHR419783	
subsp. toa	North Island, Taupo	K. W. Allison s.n., 18-Dec-1934	CHR17696	Holotype of S. glaucophyllus subsp. toa (Connor & Edgar, 1987)
subsp. toa	South Hawke's Bay, Cooks Tooth	A.P. Druce s.n., 1-Jan-1966	CHR158961	
subsp. toa	Wellington, Te Rakaunuiakura	A.P. Druce s.n., 1-Dec-1973	CHR260339	
subsp. toa	Hawke's Bay, Maungaharuru Range	A.P. Druce s.n., 1-Dec-1972	CHR208863	
subsp. toa	Canterbury, Castle Hill	E.J. Beuzenberg s.n., 18-Mar-1970	CHR200647	
subsp. toa	Marlborough, Puhi Puhi River	A. Wall s.n., 1-Dec-1929	CHR331488	
subsp. toa	North Canterbury, Mt. St. Patrick	A.P. Druce s.n., 1-Apr-1975	CHR275258	
subsp. toa	Marlborough, Boundary Creek	H.H. Allan s.n., 5-Jan-1929	CHR10187	

subsp. toa	Marlborough, Waipara	H.H. Allan s.n., 28-Jan-1941	CHR85772	
subsp. toa	Otago, Bendhu Reserve	B. Molloy s.n., 25-Feb-1977	CHR386548	
subsp. toa	North Canterbury, Waipara Valley	A.P. Druce s.n., 1-May-1967	CHR179346	
subsp. toa	Canterbury, North Dean	B.P.J. Molloy s.n., 1-Jun-1991	CHR469752	
subsp. toa	Wellington, Moawhango River	C.C. Ogle 3088	CHR510475	
subsp. toa	Hawke's Bay, The Harkness Valley	A.P. Druce s.n., 1-Jan-1985	CHR402287A	
subsp. toa	Banks Peninsula, Mt. Evans	S. Wiser s.n., 18-Jan-2001 , R. Buxton & N. Zvigina	CHR620133	
subsp. toa	Wellington, Moawhango River	A.P. Druce s.n., 12-Mar-1953 & B.G. Hamlin	CHR79505	
subsp. toa	North Island, Taupo, Mt. Tauhara	T.F. Cheeseman s.n., Jan-1889	AK10593	Representative specimen of S. lautus var. montanus
S. "pseudoglaucophyllus"	North Canterbury, Motunau	M. Giller s.n., 21-Feb-2015	CANU42555	
S. aff. glaucophyllus "South Marlborough"	South Marlborough, Isolation Creek	C. Jones CJ14/01	CANU42545	
S. aff. glaucophyllus "South Marlborough"	South Marlborough, Isolation Creek	C.E. Ecroyd s.n., 28-Mar-2014	CANU42538	
S. aff. glaucophyllus "South Marlborough"	Marlborough, Blue Duck Reserve	B. Molloy s.n., 19-Mar-1975	CHR388183	
S. aff. glaucophyllus "South Marlborough"	Nelson, Mt. Owen	W. Townson s.n.	WELT SP016474	
S. aff. glaucophyllus “Cape Campbell”	South Marlborough, Mussel Point	S.P. Courtney s.n., 11-Apr-2014	CANU42554	
S. aff. glaucophyllus “Cape Campbell”	Marlborough, Marfells Beach	M.J.A. Simpson 5040	CHR172037	
S. aff. glaucophyllus “Cape Campbell”	Marlborough, Cape Campbell	W.R. Sykes 502/70	CHR211765	

Table S5. The ten morphological characters with the highest loadings on the first three PCA axes resulting from an initial screening of informative morphological characters. Characters selected for subsequent morphometric analyses are printed in bold.

PC1	PC2	PC3
Length of involucral bracts	**Mid-cauline leaf incision length/ leaf width**	Capitulum length/diameter
Diameter of capitulum	**Mid-cauline leaf undivided**	**Length of mid-cauline leaf**
Length of corolla tube of disc florets	**Upper leaf incision length/ leaf width**	No. of dissections on one side of mid-cauline leaf/leaf length
Length of pappus	**Upper leaf undivided**	Achene length of disc florets
Length/ width ratio of upper leaf	**Mid-cauline leaf double serrate**	Length/width ratio of achene of disc florets
Capitulum length	**Upper leaf double serrate**	**Capitulum radiate**
Length/width ratio of supplementary bracts	**Capitulum radiate**	**No. of dissections on one side of upper leaf/leaf length**
Number of disc florets	No. of dissections on one side of mid-cauline leaf/leaf length	The presence of trichomes on lower leaf surface of upper leaf
Width of upper leaf	No. of capitula per inflorescence	**Number of involucral bracts**
Length/width ratio of involucral bracts	Length of pappus	**Upper leaf undivided**

Table S6. The results of a Random Forest analysis showing "mean decrease of accuracy" importance scores for 45 characters of the initial data set. Characters with higher scores are more important. Characters selected for the final analyses are printed in bold.

Characters	Importance scores
Mid-cauline leaf undivided	8.615385e-02
Mid-cauline leaf margin double serrate	6.726154e-02
Mid-cauline leaf incision length/ leaf width	3.993077e-02
Upper leaf undivided	2.651538e-02
Upper leaf double serrate	2.536154e-02
Upper leaf incision length/ leaf width	1.356923e-02
Mid-cauline leaf petiolate	1.946154e-03
Length of mid-cauline leaf	1.523077e-03
Length/width ratio of achenes of disc florets	1.453846e-03
Trichomes at the base of receptacle	7.692308e-04
Capitulum radiate	4.692308e-04
Length/width ratio of upper leaf	2.538462e-04
Length of involucral bracts	2.538462e-04
Purplish lower leaf surface of mid-cauline leaf	1.923077e-04
Length/width ratio of mid-cauline leaf	3.076923e-05
Length of corolla tube of disc floret	3.076923e-05
No. of dissections on one side of mid-cauline leaf/leaf length	7.692308e-06
Purplish lower leaf surface of upper leaf	0.000000e+00
The presence of trichomes on lower leaf surface of upper leaf	0.000000e+00
Upper leaf glaucous	0.000000e+00
No. of dissections on one side of upper leaf/leaf length	0.000000e+00
Width of upper leaf	0.000000e+00
Length of upper leaf	0.000000e+00
The presence of trichomes on lower leaf surface of mid-cauline leaf	0.000000e+00
Mid-cauline leaf glaucous	0.000000e+00
Achenes of disk florets completely covered in trichomes	0.000000e+00
Achenes of disc florets with trichomes restricted to longitudinal grooves	0.000000e+00
Achenes of disc florets glabrous	0.000000e+00
The presence of extra involucral bracts	0.000000e+00
Length/width ratio of supplementary bracts	0.000000e+00
Length of supplementary bracts	0.000000e+00
Number of involucral bracts	0.000000e+00
Achene length of disc florets	0.000000e+00
Length/width ratio of corolla tube of disc florets	0.000000e+00
Incision length of corolla of disc florets	0.000000e+00
Number of disc florets	0.000000e+00
Length/diameter ratio of capitulum	0.000000e+00
Capitulum length	0.000000e+00
Capitulum diameter	0.000000e+00

Ratio of ray floret number to involucral bract number	-6.923077e-05
Number of supplementary bracts	-1.307692e-04
Length/width ratio of involucral bracts	-1.538462e-04
Achene of disc floret less than half covered in trichomes	-1.615385e-04
Length of pappus	-1.769231e-04
Number of capitula per inflorescence	-2.307692e-04

Table S7. (Chapter 5) Specimen details for the molecular genetic study. Details include the location of where the specimens were collected, the collector and collecting number (if assigned), voucher herbarium accession number (if present), population number following Table 5.1, number of individuals collected from each population (n), and the type of molecular genetic data generated.

Taxon	Location	Collector	Voucher	Population	n	Data
S. "pseudoglaucophyllus"	North Canterbury, Motunau	M. Giller s.n., 21-Feb-2015	CANU 42555	Population 16		AFLP
S. "pseudoglaucophyllus"	North-West Nelson, Cundy Creek	S.P. Courtney s.n., 12-Feb-2015	No voucher	Population 5	8	AFLP
S. "pseudoglaucophyllus"	North-West Nelson, Cundy Creek	S.P. Courtney s.n., 12-Feb-2015	No voucher	Population 5		AFLP
S. "pseudoglaucophyllus"	North-West Nelson, Cundy Creek	S.P. Courtney s.n., 12-Feb-2015	No voucher	Population 5		ITS (aff_Nelson_groupJ117-3), AFLP
S. "pseudoglaucophyllus"	North-West Nelson, Cundy Creek	S.P. Courtney s.n., 12-Feb-2015	No voucher	Population 5		ITS (aff_Nelson_groupJ117-4), AFLP except the primer pair of Eco + ACT / Mse + CTA
S. "pseudoglaucophyllus"	North-West Nelson, Cundy Creek	S.P. Courtney s.n., 12-Feb-2015	No voucher	Population 5		AFLP
S. "pseudoglaucophyllus"	North-West Nelson, Cundy Creek	S.P. Courtney s.n., 12-Feb-2015	No voucher	Population 5		AFLP
S. "pseudoglaucophyllus"	North-West Nelson, Cundy Creek	S.P. Courtney s.n., 12-Feb-2015	No voucher	Population 5		AFLP
S. "pseudoglaucophyllus"	North-West Nelson, Cundy Creek	S.P. Courtney s.n., 12-Feb-2015	No voucher	Population 5		AFLP
S. aff. glaucophyllus "South Marlborough"	South Marlborough, Isolation Creek	C. Jones CJ14/01	CANU 42545	Population 12	2	ITS (S_sth_marlJ76A), AFLP
S. aff. glaucophyllus "South Marlborough"	South Marlborough, Isolation Creek	C. Jones CJ14/01	CANU 42545	Population 12		ITS (S_sth_marlJ76B), AFLP except the primer pair of Eco + ACT / Mse + CTGG
S. aff. glaucophyllus "South Marlborough"	South Marlborough, Isolation Creek	C.E. Ecroyd s.n., 28-Mar-2014	CANU 42538	Population 12		AFLP
S. aff. glaucophyllus "South Marlborough"	South Marlborough, Sawcut Gorge	C.E. Ecroyd s.n., 28-Mar-2014	CANU 42562	Population 12		AFLP except the primer pair of Eco + ACT / Mse + CTA
S. aff. glaucophyllus “Cape Campbell”	South Marlborough, Mussel Point	S.P. Courtney s.n., 11-Apr-2014	CANU 42554	Population 11		AFLP
subsp. basinudus	Banks Peninsula, Akaroa	J. Liew J43	CANU 42550	Population 23		AFLP

subsp. basinudus	Canterbury, Mt. Cass	J. Liew J60	CANU 42541	Population 18		ITS (subsp_basiJ60), AFLP
subsp. basinudus	Canterbury, Mt. Cass	J. Liew J60_J61_J62	No voucher	Population 18		AFLP
subsp. basinudus	Canterbury, Mt. Cass	J. Liew J62 & N. Head	CANU 42551	Population 18		AFLP except the primer pair of Eco + ACT / Mse + CTA
subsp. basinudus	Canterbury, Mt. Cass	N. Head s.n., 19-Feb-2014	CANU 42542	Population 18		AFLP
subsp. basinudus	Canterbury, The Tors	J. Liew J68	CANU 42533	Population 21		AFLP
subsp. basinudus	Canterbury, Witch Hill	J. Liew J69	CANU 42546	Population 22		ITS (subsp_basiJ69)
subsp. basinudus	North Otago, Shag Point	J. Liew J122 & J. Barkla	CANU 42535	Population 27		ITS (subsp_basiJ122), AFLP
subsp. basinudus	North Otago, Tavora Beach	J. Liew J121 & J. Barkla	CANU 42536	Population 28		ITS (aff_subsp_basiJ121), AFLP
subsp. basinudus	Otago Peninsula, Allans Beach	J. Liew J118 & J. Barkla	CANU 42553	Population 29		ITS (subsp_basiJ118.1), AFLP
subsp. basinudus	Otago Peninsula, Allans Beach	J. Liew J119 & J. Barkla	CANU 42537	Population 29		ITS (subsp_basiJ119.1), AFLP
subsp. basinudus	Otago Peninsula, Allans Beach	J. Liew J120 & J. Barkla	CANU 42552	Population 29		ITS (subsp_basiJ120.1), AFLP
subsp. discoideus	Canterbury, Castle Hill	D. Kimber s.n., 21-Feb-2014	CANU 42556	Population 20	2	AFLP
subsp. discoideus	Canterbury, Castle Hill	N. Head s.n., 11-Dec-2013	CANU 42549	Population 20		AFLP
subsp. discoideus	Canterbury, Craigieburn Forest Park,	P.B. Pelser 3123	CANU 42563	Population 19		AFLP
subsp. discoideus	Canterbury, Mt. Sugarloaf	P.B. Pelser 3125	CANU 42548	Population 15		AFLP
subsp. discoideus	Canterbury, The Tors	J. Liew J67	CANU 42543	Population 21		ITS (subsp_discJ67), AFLP
subsp. discoideus	North Otago, Mt. Buster	P.J. de Lange 12510	CANU 42539	Population 26		ITS (subsp_discJ112), AFLP
subsp. discoideus	South Canterbury, Sterndale Stream	J. Liew J63A	CANU 42540	Population 24	2	AFLP
subsp. discoideus	South Canterbury, Sterndale Stream	J. Liew J63B	CANU 42559	Population 24		ITS (subsp_discJ63B)
subsp. discoideus	South Canterbury, Sterndale Stream	J. Liew J64A	CANU 42558	Population 24	2	AFLP
subsp. discoideus	South Canterbury, Sterndale Stream	J. Liew J64B	CANU 42560	Population 24		AFLP
subsp. discoideus	South Canterbury, Taiko	J. Liew J65	CANU 42561	Population 25	2	AFLP
subsp. discoideus	South Canterbury, Taiko	J. Liew J65	CANU 42557	Population 25		AFLP

subsp. toa	Canterbury, Mt. Brown	J. Liew J1 & M. Giller	No voucher	Population 17		ITS (subsp_toaJ1), AFLP
subsp. toa	Canterbury, Waikari	M. & G. Giller s.n., 20-Dec-2013	CANU 42547	Population 14		ITS (subsp_toaJ58), AFLP
subsp. toa	Hawke's Bay, Maungaharuru Range	M. Thorsen s.n., 12-Dec-2011	CANU 42534	Population 1		ITS (subsp_toaJ123), AFLP
subsp. toa	Hawke's Bay, Ngaruroro River	T. Lawson 201501798	CANU 42392	Population 2		ITS (subsp_toaJ125), AFLP
subsp. toa	Hawke's Bay, Te Waka Range	M. Thorsen s.n., 2-Jan-2007	CANU 42532	Population 3		AFLP
subsp. toa	South Marlborough, Rough Creek	S.P. Courtney s.n., 26-Feb-2014	CANU 42544	Population 13		ITS (subsp_toaJ77A)
subsp. toa	South Marlborough, Rough Creek	S.P. Courtney s.n., 26-Feb-2014	CANU 42544	Population 13		ITS (subsp_toaJ77B), AFLP
The Nelson-group	North-West Nelson, Gouland Downs (cultivated)	S.P. Courtney s.n., 24-Feb-2014	No voucher	Population 4		AFLP
The Nelson-group	North-West Nelson, Haystack Creek	A. Shanks s.n., 3-May-2014	CANU 42564	Population 8		ITS (Nelson_groupJ73), AFLP
The Nelson-group	North-West Nelson, Matiri Plateau	S.P. Courtney s.n., 20-Feb-2014	CANU 42531	Population 10	2	ITS (Nelson_groupJ78A), AFLP
The Nelson-group	North-West Nelson, Matiri Plateau	S.P. Courtney s.n., 20-Feb-2014	CANU 42531	Population 10		ITS (Nelson_groupJ78B), AFLP
The Nelson-group	North-West Nelson, Mt. Owen	S.P. Courtney s.n., 12-Dec-2013	No voucher	Population 7		AFLP
The Nelson-group	North-West Nelson, Mt. Owen	S.P. Courtney s.n., 12-Dec-2013	CANU 42530	Population 7	2	AFLP
The Nelson-group	North-West Nelson, Southern Mt. Owen	A. Shanks s.n., 5-Jan-2014	CANU 42565	Population 9	6	AFLP
The Nelson-group	North-West Nelson, Southern Mt. Owen	A. Shanks s.n., 5-Jan-2014	CANU 42565	Population 9		ITS (Nelson_groupJ57-3), AFLP
The Nelson-group	North-West Nelson, Southern Mt. Owen	A. Shanks s.n., 5-Jan-2014	CANU 42565	Population 9		AFLP
The Nelson-group	North-West Nelson, Southern Mt. Owen	A. Shanks s.n., 5-Jan-2014	CANU 42565	Population 9		AFLP except the primer pair of Eco + ACT / Mse + CTA
The Nelson-group	North-West Nelson, Southern Mt. Owen	A. Shanks s.n., 5-Jan-2014	CANU 42565	Population 9		ITS (Nelson_groupJ57-6), AFLP

The Nelson-group	North-West Nelson, Southern Mt. Owen	A. Shanks s.n., 5-Jan-2014	CANU 42565	Population 9	AFLP
The Nelson-group	North-West Nelson, The Twins	S.P. Courtney s.n., 12-Feb-2015	No voucher	Population 6	AFLP

Fig. S1. Bidimensional PCA plot obtained from a Gower's distance matrix computed from 44 specimens used in the initial screening of 45 morphological characters.