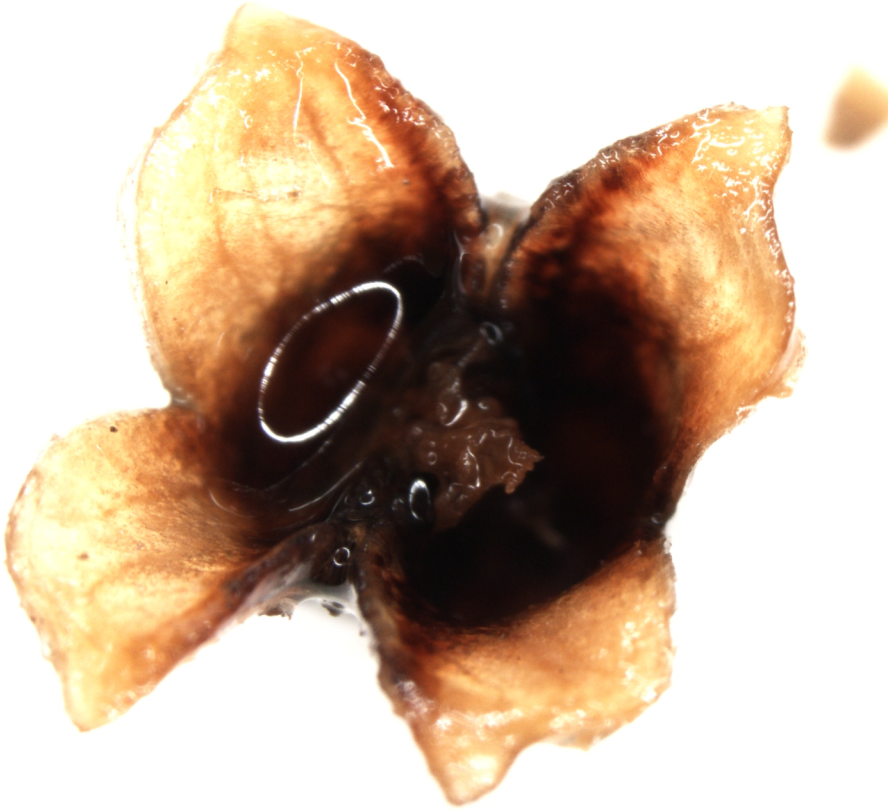
The Evolution and Ecology of Hygrochastic Capsule Dehiscence



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ABSTRACT

This dissertation aims to explore hygrochasy in different genera of various habitats by investigating biomechanics, challenging accepted hypotheses and broadening the knowledge of the ecology and evolution of this dispersal mechanism. Hygrochasy, the dehiscence of capsules in response to moisture, is a specialized plant movement that facilitates primary dispersal by raindrops. This research enhances the understanding of this intriguing plant behaviour with a multidisciplinary approach outlined in the following paragraphs.

Hygrochastic New Zealand *Veronica* (Plantaginaceae) have been identified and investigated in regards to the anatomy and biomechanics of their opening mechanism and comparisons to related ripening dehiscent species have been drawn. Light microscopy has been used to analyse the capsule anatomy and function, while multivariate methods have been used to explore the data and associations with other characters. A swelling tissue in the septum, which absorbs water quickly and expands and a lignified resistance tissue have been found to cause the opening of hygrochastic capsules. This imbibition mechanism can be found in a number of hygrochastic genera in different habitats but the position of involved tissues due to capsule anatomy is unique for New Zealand *Veronica*. Morphological analysis revealed that hygrochasy in *Veronica* is most likely associated with solitary, erect, narrowly angustiseptate capsules on short peduncles of creeping subshrubs or cushions.

The hypothesis that hygrochasy in alpine *Veronica* is an adaptation to ensure short distance dispersal to safe sites is explored. Dispersal distances were measured in the field and in laboratory experiments and habitat patch size was measured for hygrochastic and related non-hygrochastic species. Habitat patches for alpine hygrochastic *Veronica* are small and distinctly different from surrounding habitat. They provide safe sites due to their microtopography and the presence of adult cushion plants. Hygrochastic capsules facilitate ombrohydrochory by raindrops, which is an antitelechoric strategy previously reported from desert plant species. For the first time directed short distance dispersal to safe sites could be demonstrated in alpine hygrochastic species.

Additionally, environmental attributes for known locations of hygrochastic and related non-hygrochastic *Veronica* were obtained from LENZ IV in arcGIS. These have been used to identify the environmental amplitude for each species as well as variations in habitat. Non-hygrochastic species show a higher environmental amplitude and grow in a wider range and variety of habitats than hygrochastic species. Hygrochastic *Veronica* are specialists with a narrow ecological niche and are usually confined to small habitat patches in specific alpine habitats.
By combining both approaches I show that hygrochasy in alpine *Veronica* not only supports safe site strategies in seed dispersal but that hygrochastic *Veronica* are limited to special habitats requiring specific edaphic conditions. Short-distance dispersal also ensures the persistence of existing populations in these rare habitats.

Opening of some sessile New Zealand *Colobanthus* capsules during rain has been observed in the field and I carried out investigations regarding hygrochastic movements in this genus. Various staining and sectioning techniques for light microscopy have been carried out and scanning electron microscopy has been used to further analyse capsule anatomy. Statistical analysis similar to the investigation of *Veronica* capsules was employed. In contrast to other species with hygrochastic capsules, *Colobanthus* capsules are not lignified. Here, opening under wet conditions is a result of a combination of imbibition and cohesion mechanisms. Outer cells of the capsule have a thickened outer cell wall, which absorbs moisture, whereas the inner cells have thin cell walls and the cell lumen swells when water is absorbed. Interestingly, all *Colobanthus* species have the same capsule anatomy and are therefore capable of hygrochastic opening.

Earlier it was assumed that only *Colobanthus* species with sessile capsules might potentially be hygrochastic. In order to understand the relations between those species and other *Colobanthus* and to investigate whether this genus is monophyletic, I attempted to solve the phylogeny of this genus.
I used the nuclear marker *ITS* and the chloroplast markers *rps16* and *trnT-trnE* to investigate the phylogeny with parsimony and Bayesian analyses. A number of outgroups in the family of Caryophyllaceae were used to test for monophyly of *Colobanthus*. Analyses of combined datasets show that the genus *Colobanthus* is monophyletic with *Sagina* as sister clade. *Colobanthus* forms a crown clade with no

distinct differences between species. Results suggest a very recent speciation but further study with different markers or AFLPs is warranted, since the markers used in this study showed very little variation.

Hygrochasy has previously been reported and described to some extent in some North American *Oenothera* (Onagraceae) of subclade B, characterized by winged fruits. Here, I use the same methods employed by Poppendieck to extend the list of known hygrochastic *Oenothera* and I also describe xerochasy in one additional species. The position of the swelling tissue and resistance tissue is the same in all hygrochastic *Oenothera*, whereas the positions of these tissues are reversed in the xerochastic species. Hygrochastic movement was also observed in a ripening dehiscent species of subclade B, which is characterized by lanceoloid fruits. Here, hygrochasy occurs when the exocarp disintegrates and the endocarp expands after water absorption, similar to hygrochastic species of subclade B. However, due to the morphology of the capsule, the opening of the fruit does not resolve in a wide splash cup.

Hypotheses for hygrochastic capsules have mostly been developed for plants in arid regions. The most prevalent theories are that hygrochasy restricts dispersal in time by limiting dispersal events to rainfall events and therefore favourable germination conditions. Also, hygrochasy restricts seed dispersal to short distances, which increases the survival chance of seeds in the very local parental habitat, rather than surrounding harsh environments. However, hygrochasy occurs in a wide range of unrelated genera in a variety of habitats. Here, I investigate whether the widely accepted hypotheses for arid species also apply to hygrochastic *Oenothera* in North America.

Dispersal experiments, cluster analysis of morphological traits and the analysis of environmental and distribution data were used in this study and compared with similar data for hygrochastic *Veronica* in New Zealand and hygrochastic Aizoaceae in Southern Africa. Character evolution was also investigated using the latest published phylogenies of *Oenothera* and *Veronica*.

Results indicate that none of the hypotheses for hygrochasy applies to current day *Oenothera*. However, it appears that hygrochasy evolved only once in this genus and previous research implies that *Oenothera* have evolved as part of the Madro-Tertiary flora in the mid- to late Miocene. The Madro-Tertiary flora evolved in a dry, highly

seasonal climate. Possessing hygrochastic capsules would be advantageous to restrict dispersal to rare rainfall events in the wet seasons.

It therefore appears that at least the temporal restriction hypothesis applies to *Oenothera* at the time of their evolution. Other, unknown factors might play a role in the persistence of this character.

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TABLE OF CONTENTS

TABLE OF FIGURES

GLOSSARY

Definitions are adapted from Van der Pijl (1982) and Van Oudtshoorn and Van Rooyen (1999).

Amphicarpy :	burying of a number of diaspores near the mother plant
Anemochory :	dispersal by wind
Antitelechory :	avoidance of long–distance dispersal by initial placement of diaspores or morphological characteristics
Atelechory :	diaspores lack morphological characteristics promoting long–distance dispersal
Autochory:	dispersal of diaspores by the plant itself
Basicarpy:	formation of fruits immediately above ground level
Barochory:	dispersal of the diaspore by weight alone
Baryspermy:	same as barochory
Bradyspory:	dispersal of diaspores from parent plant is delayed and spread over a significant time.
Bythisochory:	diaspores are transported by water currents on the bottom of the water body, also includes non-floating seeds
Carpospermy:	monospermy (one–seededness) without dispersal
Chiropterochory:	dispersal by bats
Cohesion tissue:	plant tissue capable of absorbing water in the lumen of its cells
Dehiscence:	the splitting open along predetermined lines
Diaspore:	the plant part to be dispersed
Diplochory:	diaspores being dispersed by two different agents
Dissemination:	discharge or liberation of seeds
Dysozoochory:	process, whereby diaspores are destroyed (e.g. eaten), but some of the diaspores are dropped by accident and are therefore dispersed
Endocarp:	the innermost layer of the pericarp of an angiosperm fruit
Endozoochory:	dispersal of diaspores inside an animal
Epizoochory:	diaspores carried accidentally outside the animal
Exocarp:	the outermost layer of the pericarp of an angiosperm fruit

Geocarpy:	production of diaspores beneath the soil surface
Heterocarpy:	production of two or more morphologically distinct diaspores by an individual plant
Heterodiaspory:	same as heterocarpy
Hydroballochory:	rain ballism as dispersal mechanism (using the kinetic energy of the falling rain drops)
Hydrochory:	dispersal of diaspores by water
Hygrochasy:	opening of the capsule in response to moisture and closing when drying out
Ichthyochory:	dispersal of diaspores by fish
Mammaliochory:	dispersal of diaspores by mammals
Mesocarp:	middle layer of the pericarp of an angiosperm fruit
Mucilage:	substance that swells in water to form slimy/sticky solution
Myrmecochory:	dispersal of diaspores by ants
Myxospermy:	an anchorage mechanism, where mucilage is produced after wetting
Nautohydrochory:	diaspores capable of floating are dispersed by water currents on the water surface
Ornithochory:	dispersal of diaspores by birds
Ombrohydrochory:	dispersal of diaspores by rain
Passive ballist:	action of an outside agent releases internal tension within dead tissue in the fruit and provides the energy for the action, propelling out the seeds
Pericarp:	wall of a fruit, derived from the maturing ovary wall
Polychory:	diaspores are dispersed by more than two agents
Rain-ballist:	plant in which falling raindrops provide energy to activate a lever mechanism by which diaspores are ejected from their conatiner
Saurochory:	dispersal of diaspores by reptiles
Stomatochory:	transport of diaspores in the mouth, diaspores are not swallowed
Synaptospermy:	keeping or bringing together many seeds until germination
Syncarpous:	developed from an ovary with two or more carpels
Synzoochory:	deliberate transport of diaspores externally by an animal

Tachyspory:	diaspores are released immediately after maturation
Telechory:	long-distance dispersal of diaspores
Trypanocarpy:	form of diaspore or its appendages favour burial in the soil upon contact
Trypanospermy:	same as trypanocarpy
Vivipary:	the germination of seeds within the fruit prior to abscission from the maternal plant
Xerochasy:	opening of fruits in response to dry conditions and infolding when wet
Zoochory:	dispersal of diaspores by animals

CHAPTER ONE

General Introduction

1.1 Abstract

Plants are not able to move in the sense that most animals can. Often, their only means of establishing populations and colonizing new habitats is seed and spore dispersal, which together with pollination play important roles in gene flow. The variety of different dispersal strategies is astonishing. Some diaspores lack special adaptations and are moved in random dispersal events by various agents. Other dispersal units show adaptations, for example seeds with appendages to be carried by wind, fleshy fruits attracting specific animals to eat them and disperse the seeds and diaspores that are able to float and be transported by water. Plants also show adaptations that react to a specific cue or trigger and only then disperse their seeds, which usually restricts dispersal to a specific dispersal agent. One of these specific plant responses to a trigger is hygrochasy, the opening of a fruit in response to moisture. When rainfall occurs, hygrochastic capsules open, exposing seeds in a splash cup from where raindrops are able to splash the seeds out and disperse them over short distances.

Hygrochasy has mostly been reported for plants growing in arid habitats, where it ensures that seeds are dispersed only during favourable germination conditions. Until dispersal, seeds are protected within the capsule against extreme climatic conditions and seed predators. In recent decades, hygrochasy has been discovered in various taxa and different habitats, raising the question why this specific mechanism has evolved

in various unrelated species and what advantages it poses in diverse environmental conditions. This work takes the opportunity to explore that question and to also investigate hygrochasy in detail in several plant groups.

This first chapter reviews different dispersal strategies in more detail, paying special attention to dispersal by water. The basic principles of hygrochasy are explained and taxa used in this study are introduced. The research hypothesis and related questions are presented, which will be addressed in the data chapters.

1.2 Plant dispersal and evolution of dispersal strategies

Plant dispersal is concerned with the ways and means used to reach sites where a new generation can establish (Van der Pijl, 1982). It is the key biological process responsible for the distribution of species in the world, may explain their presence and absence in different localities, changes the flora at different periods, and it is also a vital factor in the evolution of species, promoting gene flow in and between populations (Ridley, 1930; Van der Pijl, 1982; Webb, 1998). It also helps escape from specialist predators and pathogens supported by the parent, and prevents competition between parents and offspring (Fenner and Thompson, 2005), although some pathogens are seed-borne and rely on the host's seeding for their own dispersal (Gopalakrishnan and Valluvaparidasan, 2009; Sharma and Gour, 2009; Afzal et al., 2010). Plant dispersal is usually achieved by either spores, seeds, fruits or the entire plant, which leads to generative, recombinative dispersal (Van der Pijl, 1982). The next section takes a closer look at dispersal units, which can be immensely complex and variable, whereas the following sections give a brief overview of dispersal agents, classes and strategies and introduce the genera and habitats investigated in this study.

1.2.1 DISPERSAL STRUCTURES

The dispersal unit or dispersal organ is referred to as diaspore (Sernander, 1927), which can be everything from spores or single seeds to the entire plant (e.g. tumbleweeds (Mehlman, 1993)). Here, I will pay special attention to the dispersal of seeds and fruits in Angiosperms. Fruit systems can be classified based on morphology (Levina, 1961; Van der Pijl, 1982), which includes literally hundreds of terms to describe fruits and is not always appropriate. Here, I will follow Bell (1991). Unlike the common belief, a fruit is not always edible, it is rather a term that is applied to a seed bearing structure. Simple fruits can either develop from single flowers, then representing either the single carpel (e.g. hazelnut) or a single compound ovary (e.g. melon – syncarpous fruit). Aggregate fruits can derive from one apocarpous flower, in which the carpels are not united (e.g. raspberry). Sometimes structures other than the gynoecium are involved in fruit formation, such as the receptacle, hypanthium or perianth members. Both Bell (1991) and Van der Pijl (1982) distinguish between fleshy, dry, dehiscent and indehiscent fruits; those classifications are largely based on morphology and dispersal type. For more information on different fruit types, see Bell (1991).

The fruit wall is the pericarp, which is made up of exo- or epicarp (outer layer), mesocarp and endocarp (inner layer) and is derived from the ovary wall. Depending on the type of fruit (e.g. drupe, berry or nut), either epicarp, mesocarp or endocarp can be changed dramatically. For example, in the peach, the skin of the fruit is the epicarp, the flesh the mesocarp and the stone the endocarp. Inside the stone the thin brown layer is the testa surrounding the embryo (Bell, 1991).

1.2.2 DISPERSAL AGENTS AND CLASSES

The transport of the diaspore from the parent plant to a new location can happen via numerous agents, e.g. animals (zoochory) including birds (ornithochory), mammals (mammalochory) or reptiles (saurochory). Abiotic dispersal agents include wind (anemochory) and water (hydrochory) (Van der Pijl, 1982). A comprehensive overview is given in Fig. 1.1, but this list is by no means complete. Almost every category of dispersal class can be specified further and more often than not diaspores can be transported by more than one agent (polychory).

There is evidence that most zoochorous diaspores and their dispersal agents co-evolved (Stapanian and Smith, 1978; Janzen, 1980; Stiles, 1980; Herrera and Jordano, 1981; Tombac, 1982; Wheelwright and Orians, 1982; Janzen, 1983; Rogers and Applegate, 1983; Howe and Westley, 1986; Midgley and Bond, 1991; Dean and Milton, 2003; Gove et al., 2007; Siepielski and Benkman, 2007; Lomascolo and Schaefer, 2010). Fruits with a bird-syndrome usually possess an attractive edible part with signalling colours, outer protection against premature eating, and inner protection of the seed against digestion (Oppel and Mack, 2010; Rumeu et al., 2009; Lomascolo and Schaefer, 2010) but some birds prove to be effective dispersers by caching seeds (Pons and Pausas, 2007). Frugivorous bats respond to exposed fruits that have a rancid odour and easily digestible juice (chiropterochory) (Van der Pijl, 1982). Anemochorous diaspores are usually structures where the surface area to weight ratio is greatly increased. Examples are dust-like seeds, light enough to be carried by wind and balloon fruits, winged or plumed diaspores (Van der Pijl, 1982). Despite various examples of specialisations there are numerous generalists among plant diaspores, which are not adapted to a specific transport agent. Wind and water dispersed species belong to that group as well as plants whose diaspores are dispersed

externally, such as trample burrs and diaspores with other adhesive features (Van der Pijl, 1982). There are numerous examples of diaspores and their dispersal agents, some of which are astonishing. However, since it is such a wide field to cover I refer to the cited literature and textbooks for more in depth information.

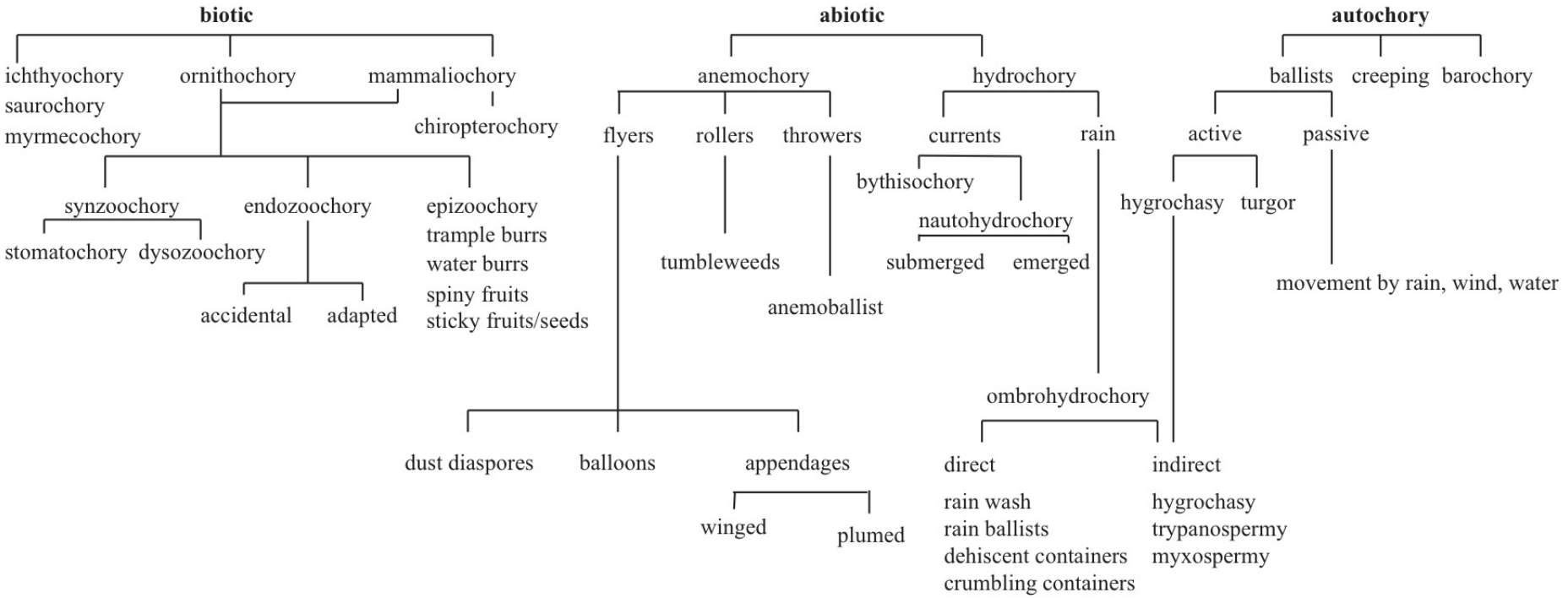

FIG. 1.1 Ecological dispersal classes based on dispersal agents adapted from Van der Pijl (1982), Van Oudtshoorn and Van Rooyen (1999) and Parolin (2006). See Glossary for definitions of dispersal classes.

1.2.3 DISPERSAL STRATEGIES

Long-distance dispersal (telechory) has been essential for colonizing habitats and distributing plant species, which has already been recognized by Darwin (1859). However, for a long time the importance of long-distance dispersal has been rarely studied, partly due to the fact that observing and documenting it is almost impossible (Cain et al., 2000). Cain et al. (2000) define long-distance as being over 100 m, and it is stated that most seeds do not travel further than a few metres (Harper, 1977; Howe and Smallwood, 1982; Willson, 1993; Cain et al., 1998; Cheplick, 1998). However, those short distances cannot account for the re-colonization of areas following the retreat of glaciers or the colonization of remote islands (Cain et al., 2000). Long-distance dispersal can occur in various ways, which have been described by Darwin (1859), Van der Pijl (1982), Janzen (1984) and others in great detail. Well recorded are also extraordinary long-range dispersal events, such as the colonization of Krakatau (Ernst, 1934; Docters van Leeuwen, 1936), the dispersal of the coconut *Cocos nucifera* (Van der Pijl, 1982) and increasingly prevalent conclusion that the New Zealand flora is largely long distance dispersed (Raven, 1973; Pole, 1994; Macphail, 1997; Jordan, 2001; Sanmartin et al., 2007).

As important as long-distance dispersal is for most plant species, avoidance of dispersal (atelechory) also plays a significant role in deserts and semi-deserts (Ellner and Shmida, 1981; Van der Pijl, 1982; Gutterman, 1994; Fenner and Thompson, 2005). Especially for species with very specific ecological conditions or the mother plant being in a favourable niche surrounded by hostile conditions like arid zones or islands, long distance dispersal is rather disadvantageous (Fahn and Werker, 1972). Bolker & Pacala (1999) developed models that suggested local dispersal of the

majority of diaspores, which can retain the benefits of the parent's environment, combined with long-distance dispersal of a few diaspores as a successful strategy.

Gutterman (1994) describes the protection strategy of a large number of desert plant species, where ombrohydrochory is the main dispersal mechanism and the seeds are protected against seed predators until a rainfall event that facilitates dispersal and quick germination thereafter. Another suggested reason for atelechory is the limitation of suitable germination sites, which plays an important role in extreme habitats such as deserts (Ellner and Shmida, 1981), mangrove forests (Lee and Harmer, 1980; Elmquist and Cox, 1996) or alpine and arctic environments (Scherff et al., 1994; Elmquist and Cox, 1996). Here, the dispersal is limited to the already occupied (therefore suitable) site (Van der Pijl, 1982). There are different mechanisms that lead to atelechory, e.g. synaptospermy, hygrochasy, basicarpy and vivipary (Zohary, 1962) (see glossary for definition of terms).

A plant species might not be restricted to one dispersal agent alone. Sometimes two or more mechanisms are combined, which often happens incidentally (Van der Pijl, 1982). Diplochory or polychory has the advantage of possessing two aspects, which are important for the plant survival. One might be vital for the dispersal of the species, whereas the second agent may play an important role in the establishment of the seedling in a suitable habitat. Examples are the genera *Avena*, *Erodium*, and *Stipa*. Their diaspores are dispersed by wind but when they land in suitable habitats (loose soil), their hygroscopic boring action by alternation of wetting and drying fixes the seed in the soil (Zohary, 1937). Other, rather accidental polychorous events might occur when seeds that have been dispersed by wind are transported further by rain-wash (Van der Pijl, 1982) or other anemochorous seeds might be carried underground

by ants and germinate there (Ridley, 1930). An overview of dispersal strategies combined with dispersal classes is given in Fig. 1.2.

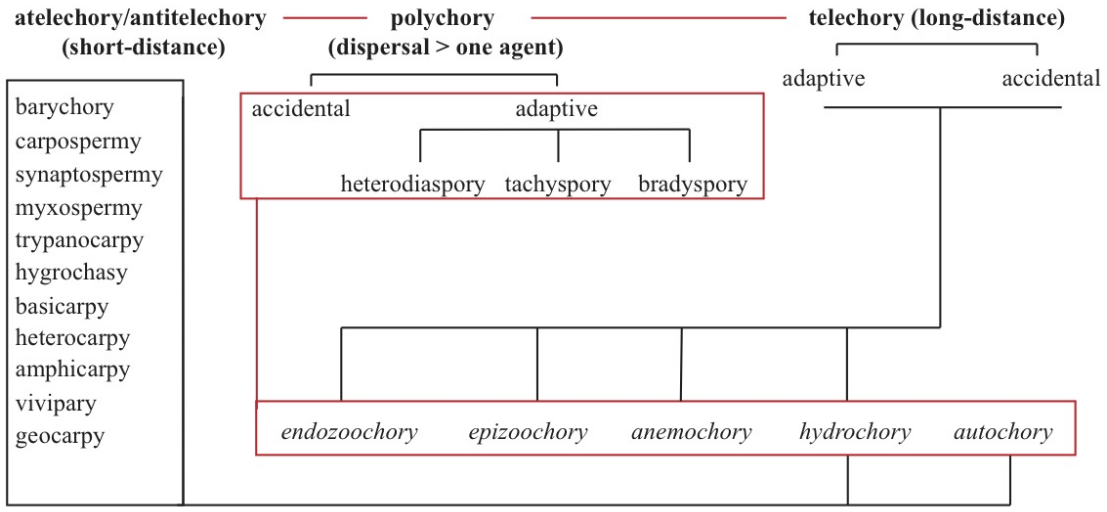

FIG 1.2 Dispersal strategies (BOLD) and associated dispersal classes (*italic*) adapted from Gutterman (1994), Van der Pijl (1982) and Poppendieck (1995). See the Glossary for definition of dispersal strategies and classes. The red lines indicated that every dispersal class and strategy can be part of polychory.

Dispersal is not only concerned with the transport of the diaspore to a suitable habitat; it is a process that is firstly prepared by the detachment or dehiscence of the diaspore, allowing travel by providing structural and physiological protective devices. Travel is then followed by settlement and germination (Van der Pijl, 1982). Often, the means of detachment and the dispersal strategy are closely linked, such as passive ballists dispersed by passing animals (Van der Pijl, 1982) or hygrochasy and ombrohydrochory (Van der Pijl, 1982; Gutterman, 1994).

1.3 Ombrohydrochory and splash cup dispersal

Dispersal by water can be split into three categories: ombrohydrochory, nautohydrochory (or nautochory), and bythisochory. Both latter dispersal modes have water currents as transport agents, but nautohydrochory refers to diaspores capable of floating on the water surface, whereas bythisochory describes transport of diaspores on the bottom of the water body (Mueller-Schneider, 1983; Bonn and Poschlod, 1998).

Bythisochory and nautohydrochory lead to long-distance dispersal along waterways, often playing a role as secondary dispersal mechanism (dichory or polychory). Van der Pijl (1982) also includes ombrohydrochory as part of di- or polychory, combined with dispersal strategies such as anemochory or autochory, whereas Parolin (2006) states that ombrohydrochory is only part of dichory when it is paired with nautochory, as is the case in *Caltha* (Bonn and Poschlod, 1998). Ombrohydrochory (dispersal by water drops, which can activate a ballistic mechanism in the plant) can be split into further categories, where the diaspore could either be washed away from the mother plant (rain wash) or be dispersed by ballistic forces, when raindrops propel seeds out of the fruit (Brodie, 1951, 1955; Parolin, 2006).

As stated before, ombrohydrochory is considered to promote atelechory by restricting seed transport to raindrops as dispersal agents (Zohary and Fahn, 1941; Fahn and Werker, 1972; Van der Pijl, 1982; Van Oudtshoorn and Van Rooyen, 1999; Parolin, 2006) and it is the favoured dispersal strategy in very wet or very dry environments (Zohary and Fahn, 1941; Van Oudtshoorn and Van Rooyen, 1999). Also, plants growing along mountain torrents on rocks, where splash water could easily be caught, profit from being ombrohydrochorous (Nakanishi, 2002). They mostly possess splash cups, from which seeds get expelled by the ballistic forces of splash water. Nakanishi

(2002) found that, although only considered to be of minor importance, splash cup dispersal appears to be far more common and widespread. It can be found in various genera across a wide range of habitats (even temperate regions), where plants grow in sparse vegetation. However, he considered dispersal by rain as disadvantageous in dense vegetation (Nakanishi, 1988). More evidence for ombrohydrochory in temperate regions has been given by Walck & Hidayati (2007), who investigated the dispersal of two *Oenothera* (Onagraceae) species. They found that not only are hygrochastic capsules ombrohydrochoric (*Oenothera triloba*) but also that the xerochastic *Oenothera macrocarpa* is indirectly ombrohydrochoric by opening wider in dry conditions after rain.

Even in tropical rainforest, dispersal by rain is evident in some species. *Bertolonia mosenii* (Melastomataceae), a Neotropical rainforest herb, exhibits yet another mechanism operated by rain, the so-called "squirt-corner". When raindrops hit the capsule, they force seeds outwards to the angles of the triangular fruit, where they are released (Pizo and Morellato, 2002). Some species of the pan-tropical subfamily Acanthoideae (Acanthaceae) are also dispersed by rain (Witzum and Schulgasser, 1995).

1.4 Hygrochasy, hygrochastic capsule dehiscence and examples

When dealing with the dispersal of plants, most authors refer mainly to the morphology of the diaspore (Ridley, 1930; Van der Pijl, 1982). Those morphologies are the results of anatomical changes in the diaspore. Sometimes the whole mechanism of diaspore dispersal is based only on the ultrastructure of the cell walls in the dispersal unit (Fahn and Werker, 1972). Different dehiscence types in fruits may be an example of the profound effect of only slight anatomical changes.

Dehiscence usually involves the splitting of a fruit along specific areas and can be caused by various chemical, anatomical and seasonal changes. It affects dry fruits that either developed from a single carpel, e.g. follicles and legumes, or syncarpous fruits, e.g. siliques and capsules (Fahn, 1990).

Hygrochastic capsule opening is defined as the dehiscence of a fruit in response to the presence of moisture (Van der Pijl, 1982). It most likely aids dispersal by rain (ombrohydrochory) (Van der Pijl, 1982; Parolin, 2001, 2006), but Fahn & Werker (1972) consider it to be an autochorous function, which is triggered by an external agent, in this case, water. Parolin (2006) also clearly separates the different forms of hydrochory as being either telechorous (nautohygrochory and bythisochory) or atelechorous (ombrohydrochory).

Hygrochasy is also considered to be one of the most efficient mechanisms for ombrohydrochory since it restricts seed dispersal to rain events. It is especially advantageous for desert species, giving the seeds protection from predators, spreading the risk of dispersal and germination over a long period and depositing the seeds in a suitable location (Van Oudtshoorn and Van Rooyen, 1999). In contrast to dispersal by turgor mechanism, which is telechoric and involves living cells, hygrochastic mechanisms can be found for both telechoric and atelechoric dispersal strategies and action takes place in cell walls of dead cells (Fahn and Werker, 1972).

Hygrochastic capsule opening is predominantly based on the imbibition mechanism. The movement of fruit parts in this mechanism results from the action of walls of cells belonging to two antagonistic groups. With the loss or uptake of water a cell wall shrinks or expands, respectively, in a direction perpendicular to that of cellulose microfibrils. If the orientations of those microfibrils in different cells or the orientation of those cells vary, the direction of movement in response to moisture will

be different. The movement of the cells and tissues involved is either bending or torsion (Fahn and Werker, 1972). The imbibition mechanism acts both in xerochastic and hygrochastic fruits as both dehiscence types react in response to the change of moisture.

Only a few authors have provided anatomical descriptions of hygrochastic and xerochastic fruits (Steinbrinck, 1883, 1888; Von Guttenberg, 1926; Straka, 1955; Roth, 1977), which Poppendieck (1995) summarized in his paper about hygrochastic capsules in *Oenothera*. In order to be classified as hygrochastic, two conditions must be met. Firstly, the swelling tissue must be composed of fibres whose volume increases in wet conditions at right angles to their longest dimension. Secondly, different tissues must act in an antagonistic fashion to be reversible – the fibres must be either arranged at cross-angles or a differentiation is given between prosenchymatic cells of a swelling tissue and lignified cells of a resistance tissue. There might be variations in the swelling capacity of the tissue, which depends on the wall thickness and its chemical components. Swelling capacity of cell walls usually increases from lignin over cellulose and hemicellulose to pectins (Poppendieck, 1995).

In his study of the hygrochastic capsules of *Oenothera* (evening primroses), Poppendieck (1995) found a sequence of increasing complexity among the fruits he investigated and he also proved the existence of the proposed resistance and swelling tissues in different species, e.g. *Oenothera fruticosa* subsp. *glauca*, *O. triloba*, *O. acaulis* and *O. perennis*. For example, *O. triloba* only needs a few raindrops to open the capsule. The swelling tissue is located in the septum, where its cell walls take up the water, add it to the micellar structure of the cellulose contained in the wall and increase the volume. Consequently, the free parts of the valves are forced outwards

and uncover the seeds, which can then be splashed out by raindrops. The hygrochastic response of *Oenothera triloba* and its consequences for seed dispersal have also been investigated by Walck and Hidayati (2007), but with little regard to the capsule anatomy.

The most studied family in regard to hygrochastic capsule opening and its implications is the ice-plant family Aizoaceae. The majority of the almost 2000 species are hygrochastic with various degrees and specifications (Parolin, 2001). Different species show different seed expulsion mechanisms, depending on their morphological and anatomical details of the capsules. Varying degrees of seed retention and atelechory are achieved by an increasing closure of the inner parts of the capsule (Schwantes, 1952; Straka, 1955; Bittrich, 1986). Hartmann (1988) investigated the relation of the shape and size of closing bodies, covering membranes, and the expanding mechanism to the dispersal syndrome. Another manipulative study by Parolin (2001) showed that the complex structures of different Aizoaceae are responsible not only for an effective seed expulsion but also for the retention of seeds in the capsule. Here, the capsule acts as seed bank and attributes such as closing bodies, covering membranes, and/or funicles ensure the release of seeds only when sufficient rain has fallen; they may also allow the storage of some seeds for further rain events (Parolin, 2001).

Other hygrochastic plants can be found in many different Angiosperm orders but their hygrochastic mechanism is not always related to capsule dehiscence as is the case in the examples described above. One interesting example is *Anastatica hierochuntica* (Brassicaceae), a desert annual, whose whole dead skeleton possesses hygrochastic branches. In dry conditions they curl up, thus protecting the fruits. Seeds are released either after uncurling during dew or rainfall through raindrops or through repeated

curling and uncurling (Hegazy et al., 2006). The mechanics of another hygrochastic mechanism is decribed by Witzum and Schulgasser (1995) on the example of *Ruellia britonniana* (Acanthaceae subf. Acanthoideae). Again, an active and a resistance layer with differently lignified cell walls are involved in the movement, which allows for different water absorption, resulting in tension between the layers. The tension is released explosively with a raindrop hitting the capsule and the seeds are expelled with a catapult action (Witzum and Schulgasser, 1995).

1.5 Potential hygrochastic genera in New Zealand and North America

Traditionally the study of hygrochastic capsules has focussed on Aizoaceae in southern Africa, but in recent decades studies reporting hygrochasy in other families and places have been published. In my dissertation I wanted to extend understanding of the anatomy, function, evolution and ecology of hygrochastic plants by studying New Zealand alpine plants. During the study, an opportunity arose to include work on *Oenothera* in North America.

1.5.1 *VERONICA*

Veronica L., with approximately 450 species of various life forms, is the largest genus within the *Plantaginaceae* (Angiosperm Phylogeny Group, 1998, 2003), having been formerly placed in the *Scrophulariaceae* (Albach et al., 2004; Albach and Meudt, 2010). Species are distributed mainly in temperate regions of the northern hemisphere and in Australasia (Albach et al., 2004; Garnock-Jones et al., 2007) and occur in a wide range of habitats. The genus *Veronica* is divided into numerous sections and subsections (Juan et al., 1997b; Martinez-Ortega, 1999; Garnock-Jones et al., 2007;

Munoz-Centeno et al., 2007) but in this study, I concentrate on 23 Southern Hemisphere *Veronica* species, mostly formerly treated as *Parahebe* W.R.B. Oliv. and *Chionohebe* B. Briggs et Ehrend., which are now included in *Veronica* sect. *Hebe* (Garnock-Jones et al., 2007). Species formerly treated under *Parahebe* and *Chionohebe* are now arranged in two informal groups (speedwell hebes and snow hebes) that do not have the same circumscription as the original genera (Albach and Meudt, 2010).

The generalised *Veronica* fruit is a capsule formed by two locules separated by a linear septum. All capsules have bilateral symmetry. The epicarp and mesocarp are usually thin, whereas the endocarp can be thickened and/or lignified (Juan et al., 1997a).

If 11 New Guinea species are included, the speedwell hebe group comprises ca. 24 species, which are subshrubs with decussate leaves and racemose inflorescences with numerous short-tubed flowers on long slender pedicels. The capsules, developed from two carpels, are only weakly laterally compressed with a quite broad septum and open both at the apex by septicidal (between the carpels) and loculicidal (along the dorsal bundle of each carpel) splits and may gape at the septum by a septicidal split (Garnock-Jones and Lloyd, 2004). However, the position of *V. linifolia* Hook.f., *V. colostylis* Garn.-Jones and *V. cheesemanii* Benth. within the group is still not entirely resolved and the placement of *V. planopetiolata* G.Simpson & J.S.Thomson differs among markers (Albach and Meudt, 2010). The recently described *V. jovellanoides* Garn.-Jones & de Lange (Davidson et al. , 2009) is most similar to the speedwell hebe group.

The snow hebes (Albach and Meudt, 2010; Taskova et al., 2010) include species formerly placed in *Chionohebe* and alpine *Parahebe*. They comprise four species of

high alpine cushion plants and four species of alpine low subshrubs, both on DNA evidence (Albach and Meudt, 2010) and morphology (Garnock-Jones, 1993; Garnock-Jones and Lloyd, 2004; Garnock-Jones et al., 2007). These are found in alpine areas of the South Island of New Zealand, although one cushion plant extends to Tasmania and one subshrub to the Australian Alps (Meudt, 2008; Meudt and Bayly, 2008). Compared to other Australasian *Veronica*, snow hebes have a compact habit, rather symmetrical 5-(6-) merous calyces and corolla and the capsules are laterally compressed with a narrow septum (Meudt and Bayly, 2008). Similar to the speedwell hebes, the capsules of snow hebes also open septicidally and loculicidally, but without gaping at the septum. The flowers are mostly solitary and either sunk in the cushion or set on greatly reduced peduncles. One additional species, *V. zygantha* Garn.-Jones, shares some morphological features with snow hebes (Garnock-Jones and Lloyd, 2004) but a close relationship has not been demonstrated.

So far, the Australasian *Veronica* species have received little attention regarding their fruit anatomy and dispersal strategies, but a number of papers have been published on Mediterranean species (Juan et al., 1997a; Juan et al., 2000). They showed that the dehiscence type is closely related to the anatomical structures of the investigated capsules, with the compression of the capsules strongly correlating with the strength of the cohesion tissue.

1.5.2 *COLOBANTHUS*

The genus *Colobanthus* Bartl. (Caryophyllaceae subf. Alsinoideae) is restricted to the Southern Hemisphere, consisting of approximately 20 species. Thirteen (14) of those are indigenous to New Zealand; of those, all but two are endemic (Allan, 1961; Sneddon, 1999). The genus covers a wide spectrum of habitats, occurring in S.E.

Australia, Tasmania, New Zealand, in the Kerguelen biological region as well as the Andes, Antarctic Peninsula and sub-Antarctic Islands across a wide range of latitudinal, longitudinal, and vertical gradients. *Colobanthus quitensis* is one of the only two higher plants present in Antarctica (Convey, 1996), the other being *Deschampsia antarctica*. The genus *Colobanthus* has not been monographed to date and very little is known about the phylogeny of the genus (Sneddon, 1999). Not many studies have been carried out about all *Colobanthus* species, but a strong research interest exists in *C. quitensis*, due to its wide range and Antarctic distribution (e.g. Corner, 1971; Edwards, 1972, 1974, 1975; Edwards and Smith, 1988; Convey, 1996; Gianoli et al., 2004).

The flowers of the New Zealand *Colobanthus* are hermaphrodite and solitary, with 4–5 sepals and no petals. There are as many stamens as sepals, alternating with them. The ovary is one-celled and there are as many carpels and styles as stamens. Capsules are herbaceous and ovoid and the opening takes place with as many teeth (valves) as sepals. The placenta is a free standing column and there is no septum. The plants are small, densely leafy, glabrous herbs with narrow, exstipulate leaves that are connate at the base. The most cushions have a strong taproot (Allan, 1961). Observations of opening of some *Colobanthus* capsules during rainfall (Bill Malcolm, pers. comm.) led to the assumption that those species might be hygrochastic. They are also similar to *Veronica* with respect to capsule position and splash cup forming, which adds to the possibility that alpine *Colobanthus* also exhibit hygrochasy, hence they were included in this study.

1.5.3 *OENOTHERA*

The genus *Oenothera* L. (Onagraceae) consists of approximately 145 species native to South and North America (Wagner et al., 2007). Literally hundreds of papers have been published concerning *Oenothera*, its ecology and evolution. Some of the interest results from the tendency of *Oenothera* to form interesting chromosome rearrangements and led to the pioneering genetic work of de Vries and the origin of the term "mutation" (de Vries, 1905). I will therefore only give a very brief description of the genus and refer for further studies to the references mentioned therein.

The species range from being annual, biennial, and perennial herbaceous plants, which can vary in size from small sprawling herbs (*O. acaulis* in Chile) to plants up to 3 m tall (*O. stubbei* in Mexico). The range of habitats extends from coastal areas up to the timberline. The leaves form a basal rosette at ground level and spiral up the flowering stems; they can be dentate or deeply lobed. The common name Evening Primrose derives from the opening time of the flowers, which are mostly yellow, but white, purple, pink, or red flowers are also known. The number of petals is four and the stigma has four branches, forming a distinctive cross (Petersen and McKenny, 1968). The fruits of *Oenothera* are capsules with different dehiscence types, such as opening with ripening, xerochastic or hygrochastic opening in varying degrees. Capsule types vary greatly within *Oenothera*, the most prominent difference can be found between species of subclades A and B (Tobe et al., 1987; Wagner et al., 2007). Subclade A includes species with terete or linear capsules, whereas species in subclade B exhibit winged or angled capsules. The differentiation into these two subclades is also supported to some extent by molecular analysis (Levin et al., 2004).

Oenothera appears to have diverged from *Eremothera* about 10 Ma (Sytsma et al., 2004), which is consistent with an origin in the mid- to late Miocene by most literature (Raven and Axelrod, 1974; Raven and Raven, 1976; Raven and Axelrod, 1978; Raven, 1979; Wagner et al., 1985; Wagner, 2005).

Oenothera may have originated in Mexico and Central America, from where it spread north and southwards, colonizing temperate, arid and alpine habitats (Katinas et al., 2004; Wagner et al., 2007). Nowadays, various *Oenothera* species can be found across the world in temperate regions, including New Zealand, due to international travel and horticulture (Webb et al., 1988; Thompson, 1990; Sun et al., 1992; Dhaliwal and Sharma, 1995; Deng et al., 2001; Hosking et al., 2003; Mihulka et al., 2006; Gfutte et al., 2007; Lambdon et al., 2008). The revised classification of Onagraceae now also includes *Gaura*, *Stenosiphon* and *Calylophus* in *Oenothera* (Wagner et al., 2007).

1.6 The New Zealand alpine region

Most of the studies concerned with hygrochastic capsules concentrate on plants in arid regions. However, in the last decades hygrochastic species were reported in species of other habitats in temperate regions. In my dissertation I investigate hygrochasy in alpine plant species native to New Zealand. Therefore it is essential to give an introduction to the New Zealand alpine region.

1.6.1 HISTORY

Most of the South Island of New Zealand is dominated by the Southern Alps, which run almost the entire length of the island, whereas alpine regions on the North Island are restricted to Mount Taranaki, the volcanic plateau and a few high peaks of the axial ranges. All mountains of New Zealand are geologically young and very dynamic and the present relief is very heterogenous, with rocks differing regionally. An important feature of the Southern Alps is the Alpine Fault, where the Australian and Pacific Plates are in collision, which resulted in the uplifting of the Southern Alps during the Pliocene (5-2 million years ago) and a lateral displacement of related rocks by 450 km.

1.6.2 CLIMATE

Since New Zealand is relatively small and located in the southern ocean its climate is oceanic although prevailing westerly winds combine with New Zealand mountains to impose a strong west-east gradient. The more exposed mountains receive more than 4000 mm precipitation annually with local records on the South Island of up to 12000 mm on the western slopes (McSaveney, 1978). However, areas in the rain shadow often record rainfall of only 1000 – 1500 mm annually (Mark, 1965; Mark and

Rowley, 1976; McCracken, 1980). Air temperatures vary greatly between the seasons as well as between night and day (Mark and Dickinson, 1997). At high altitudes only a few days in summer are frost free and freeze-thaw cycles occur frequently but permafrost is not recorded in New Zealand. Wind is a recurrent feature of the New Zealand mountains, especially in the rain shadow region of the South Island, where westerly gales are common.

1.6.3 ALPINE ZONES

In New Zealand, the alpine zone starts with the low-alpine generally at about 1100m in the south and at 1500m on the North Island (Mark and Dickinson, 1997). The low-alpine zone is between 300 and 500m in vertical extent and is characterized by tall grassland, dominated by the largely endemic genus *Chionochloa*. Bogs and mires of various types are a common feature and the plant cover comprises mostly cushion plants and low trailing subshrubs and herbs, carnivorous species, lichens, sedges, and rushes. A century of European pastoralism has clearly influenced species composition in some parts.

The high-alpine zone contains smaller grasses, forbs, and low shrubs. Four distinctive natural types can be found throughout New Zealand's high-alpine plant communities: fell-field, scree, cushion field, and snowbank. Each has very distinctive characteristics and associated vegetation (Enting and Molloy, 1982).

Fell-field can usually be found on relatively stable rocks and consists of sparsely vegetated communities. A distinction can be made between wet and dry fell-field, differing in species composition (Mark and Dickinson, 1997). Scree only occurs on steep greywacke mountains in the rain shadow region of the South Island. Plant species found on scree are usually endemic to this habitat, morphologically different

from near relatives, and have a suite of common characteristics, such as fleshy glaucous leaves. Cushion fields are found in Central Otago, mostly on broad plateau summits. The vegetation is characterized by extreme dwarfism (Mark and Dickinson, 1997). Snowbanks occur only locally in the high alpine zone, where snow accumulates and persists well into summer. They also show very adapted vegetation, which differs greatly even locally (Mark and Dickinson, 1997). They are generally differentiated based on climate, topography and lithology. The permanent snow line forms the upper limit of the high alpine zone. It decreases with latitude from 2400m on the central North Island to about 2000m in the south.

1.7 Key questions, thesis outline and style

The key questions my thesis addresses are concerned with the morphology, anatomy and biomechanics of hygrochastic capsules and the application of traditional hypotheses developed for hygrochasy in arid areas to hygrochastic species in other habitats.

1.7.1 KEY QUESTIONS

- *The first key question is concerned with the mechanism itself – how does it operate in the investigated species and what are the differences regarding capsule anatomy in different hygrochastic species and between hygrochastic species and their non-hygrochastic relatives?*
- *Secondly, how does hygrochasy effect dispersal in New Zealand alpine plants? As stated before, the main reason for desert plants to be hygrochastic is thought to be protection against seed predators and drought and optimal timing for germination, but those reasons might not be as important for New Zealand alpine*

hygrochastic species, where water is a continually available resource. I test the idea that hygrochasy in alpine species is foremost an aid in dispersal by rain ballistic forces and a strategy to inhibit long distance dispersal and quickly occupy a suitable germination site in a restricted area.

- *Thirdly, what are the reasons for hygrochasy in plants of different habitats? Desert plants open rather slowly and only when a certain amount of rain has fallen, whereas New Zealand alpine plants open quickly and with a minimal amount of rain needed (desert plants: Parolin, 2001; Poppendieck, 1995; alpine plants: pers. comm. P.J. Garnock-Jones & W.M. Malcolm, own observations). Hygrochastic species can also be either very limited in their distribution (Aizoaceae) or very widespread (Oenothera).*
- *Lastly, I propose that despite quite complex structural changes, hygrochasy might be quite labile in its evolution. Phylogenetic analysis can show how many times a feature has evolved and whether the change is reversible. It may also indicate suitable precursor conditions from which the feature arose.*

1.7.2 THESIS OUTLINE AND STYLE

This thesis comprises five research chapters that are prepared in the style of research papers, which are either already in press, submitted or formatted for submission to peer reviewed journals. Chapter Three and Four are each split into two subchapters due to the close relation and complexity of the topics. Each chapter starts with an abstract, followed by introduction, materials and methods, results and discussion and references. Chapters with subchapters have an overview and a summary describing both subchapters. This style inevitably results in some repetition, especially in introductory sections and descriptions of plant species and some experiments. The

advantage of this style is the production of separate studies whilst addressing the overall hypotheses and research questions. Figures, photographs, tables and appendix numbers are linked with chapter numbers. These separate studies explore hygrochasy in previously unknown habitats, investigate existing hypotheses for hygrochasy in arid regions and add significantly to the knowledge of the evolution of hygrochastic capsule dehiscence. To achieve this I have:

1. Investigated and described in detail hygrochasy in New Zealand *Veronica*, with the use of various microscopy techniques and multivariate analysis of anatomical and morphological traits (Chapter Two),
2. Explored traditional hypotheses that have been used to explain the evolution of hygrochasy in arid habitats with regard to New Zealand alpine *Veronica* by investigating dispersal distances, habitat sizes and ecological amplitudes (Chapter Three),
3. Investigated capsule opening in *Colobanthus* with methods similar to techniques used in Chapter Two. I also conducted research towards a molecular phylogeny of the genus *Colobanthus*, through sequencing of nuclear and chloroplast markers, with the intention of using it to study evolution of hygrochasy (Chapter Four)
4. Identified additional hygrochastic and xerochastic *Oenothera* species and described their opening mechanism (Chapter Five),
5. Used experimental data about dispersal distance, opening time and seed retention as well as environmental and distribution data of hygrochastic *Veronica*, *Oenothera* and Aizoaceae to study the application of traditional hypotheses to previously unknown species in various habitats. I also studied character evolution by using existing phylogenies of *Veronica* and *Oenothera*, (Chapter Six) and

6. Summarized the results of the studies and included an outlook on future research for hygrochastic dispersal (Chapter Six).

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CHAPTER TWO

Hygrochastic Capsule Dehiscence in New Zealand Alpine *Veronica* (Plantaginaceae)

This chapter is published in the American Journal of Botany under the title "Hygrochastic capsule dehiscence in New Zealand alpine *Veronica* (Plantaginaceae)" by Pufal, G., K.G. Ryan and P. Garnock-Jones. The co-authors' role was restricted to supervision and advice and P. Garnock-Jones designed the drawings in Fig 2.2. G. Pufal collected all data, carried out the analysis and wrote the manuscript.

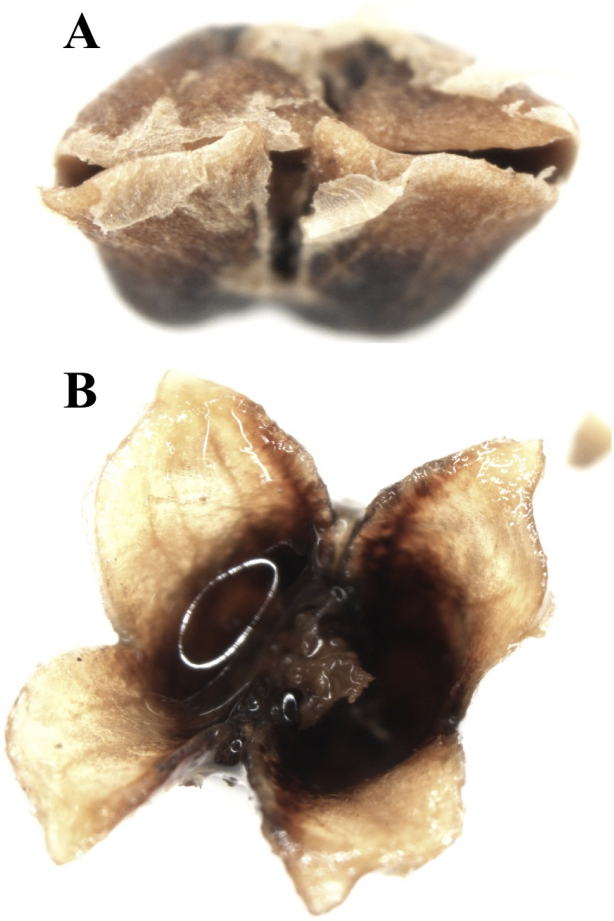

FIG 2.1 Dry (A) and wet (B) capsule of *Veronica densifolia*.

2.1 Abstract

- *Premise of the study*: Plant movement is more widespread than often recognized, involving different organs and mechanisms. Hygrochasy (opening in response to moisture) is a capsule opening movement that is widely believed to be predominantly a feature of plants of desert and arid zones, where it may protect against seed predators and harsh climatic conditions and restrict dispersal to favourable germination times and sites. However, recently it has been reported from a wider range of environments. Here we describe hygrochasy for the first time for plants of alpine habitats.
- *Methods*: Capsules of 23 species of New Zealand *Veronica* were collected and we used light microscopy to investigate the anatomy and biomechanics responsible for the opening mechanism. Additionally, we collected morphological data to identify common traits of hygrochastic species.
- *Key results*: Hygrochastic capsule dehiscence was found in ten alpine *Veronica* species. The opening mechanism is based on an antagonistic reaction between a non-lignified swelling tissue and a lignified resistance tissue. In *Veronica*, hygrochasy is associated with erect, narrowly angustiseptate capsules on short peduncles of creeping subshrubs or cushion plants.
- *Conclusion:* Hygrochasy is a common dehiscence type in New Zealand alpine *Veronica* and for the first time this mechanism is described in detail for plants in alpine habitats. We propose that hygrochasy provides an effective seed dispersal mechanism in solitary capsules embedded in cushion plants and may restrict dispersal within habitat patches.

Keywords: alpine, cushion plants, hygrochasy, New Zealand Alps, rain dispersal, *Veronica*

2.2 Introduction

The inability of plants to move is one of the fundamental differences between them and animals, yet many animals (e.g. corals, sponges) are sessile and in some way plant-like, while many plants can move at least some of their organs. Plants lack nerves, yet are able to detect environmental changes and although they have no muscles they can sometimes move in response. Biologists have been fascinated for centuries by plant movement (Darwin, 1865; Darwin and Darwin, 1880; Edwards and Moles, 2009) and people generally are attracted to plant movements in carnivorous plants (e.g. *Dionaeae*) or flowers that open suddenly (e.g. *Oenothera*). Plants achieve movement either by differential growth or by differential changes in volume of cells and tissues, sometimes through the actions of cells that are already dead. Movements in plants provide interesting questions for plant anatomy (Parolin 2001, 2006), ecology (Gutterman, 1990; Pufal & Garnock-Jones, in press), evolution, and developmental biology. The three main types of movement in plants are (1) circumnutation, a movement associated with growth (Karban, 2008), (2) tropism, a dictated movement in response to stimuli (light, gravity or contact) and (3) nasticism, a movement independent of the direction of the stimulus (Darwin and Darwin, 1880; Karban, 2008). Circumnutation usually takes place in the growing tips of roots and stems, whereas tropism and nasticism function on a larger scale, such as leaves or entire plants.

Even though movements occur in plants, the vast majority of plants are sessile and have to utilize other means to venture into new areas. For a sessile organism, dispersal plays a crucial role in colonizing new habitats, promoting gene flow and extending species distribution. A large number of plant species rely on agents such as animals, wind or water (Van der Pijl, 1982) for successful dispersal and their diaspores (seed

dispersal unit (Fahn and Werker, 1972)) may be highly adapted to their specific dispersal modes. However, autochorous plants are able to disperse their seeds without the aid of external agents, possessing autonomous mechanisms that facilitate dispersal (Fahn and Werker, 1972). Here, movement can be achieved by either change in turgor pressure (e.g. capsules of *Impatiens parviflora*), an imbibition mechanism or cohesion mechanism (Fahn and Werker, 1972); all of them operated by changes in water content and pressure either in the cell lumen (turgor and cohesion mechanism) or cell walls (imbibition mechanism).

Hygrochasy is defined as the opening of a structure in reaction to moisture. The opposite of the process; opening when dry and infolding when wet, is referred to as xerochasy (Fahn and Werker, 1972). Different organs on a plant may be involved in hygrochastic movement, for example parts of the inflorescence (Fahn and Werker, 1972; Gutterman, 1990, 1994; Gutterman and Ginott, 1994), the fruit (Garside and Lockyer, 1930; Ellner and Shmida, 1981; Van der Pijl, 1982; Ihlenfeldt, 1983; Thulin, 1987; Gutterman, 1994; Poppendieck, 1995; Nakanishi, 2002; Parolin, 2006) or even the entire plant (Hegazy et al., 2006). In this paper, we are concerned only with species that possess hygrochastic capsular fruits.

In general, hygrochasy is based on an imbibition mechanism, which involves only the walls of dead cells (Fahn and Werker, 1972). The shrinking and swelling in this mechanism is based on an antagonistic action of cell walls of two contrasting cell types. Enlarged cell walls shrink with water loss and expand with its uptake, in a direction perpendicular to the orientation of cellulose microfibrils. Commonly, increases in cell size in the swelling tissue are converted into bending or torsion by the presence of an attached lignified non-swelling resistance tissue (Fahn and Werker, 1972). Variations in the expanding capacity of the swelling tissue depend on the cell

wall thickness and its chemical components (Poppendieck, 1995). However, size, location, arrangement and cell properties can also vary greatly between genera or even between related species (Garside and Lockyer, 1930; Poppendieck, 1995) and influence the extent, duration and speed of the capsule opening.

Hygrochastic dehiscence has generally been associated with plants of arid environments (Steinbrinck, 1883; Zohary and Fahn, 1941; Van Oudtshoorn and Van Rooyen, 1999). The best-known example is the Aizoaceae in southern Africa, where 98% of the highly diverse family exhibit hygrochastic capsules (Parolin, 2006). Hygrochastic species also occur in other arid regions of the world, belonging to a variety of genera and families (Ellner and Shmida, 1981; Thulin, 1987; Gutterman, 1994; Poppendieck, 1995; Van Oudtshoorn and Van Rooyen, 1999). It is widely recognized that hygrochastic capsule opening is positively linked to delay and restriction of dispersal (Fahn and Werker, 1972; Ellner and Shmida, 1981; Van der Pijl, 1982; Ihlenfeldt, 1983; Parolin, 2006). Thus, hygrochasy not only aids seed dispersal by rain, but also limits it to periods of adequate rainfall. This ensures favourable germination conditions (sufficient moisture) following dispersal. Furthermore, the seeds are splashed out of the capsules by falling raindrops and only travel a short distance, thus restricting dispersal to safe sites. The closed woody capsules additionally protect seeds against predators and harsh climatic conditions during dry periods in extreme habitats such as deserts and may even function after the parent plant is dead (Steinbrinck, 1883; Ellner and Shmida, 1981; Ihlenfeldt, 1983).

In recent years, hygrochastic capsules have also been reported in plants from other habitats, such as wetlands (Van der Pijl, 1982; Parolin, 2006), vegetation along water torrents (Nakanishi, 2002), and in low vegetation in temperate regions (Nakanishi,

2002; Walck and Hidayati, 2007), where wind as the dispersal agent is not readily available but rain and/or drops falling from higher vegetation are frequent.

These studies show that hygrochasy might be more widespread than previously assumed but little to no attention has been paid to the anatomical background and the biomechanics behind the mechanism. However, functioning of hygrochastic capsules in some Aizoaceae (Garside and Lockyer, 1930; Parolin, 2001) and *Oenothera* (Poppendieck, 1995) is well understood and clearly shows the difference in capsule reaction to water depending on tissue arrangement.

Hygrochasy has been reported in a number of cushion-forming species of New Zealand *Veronica* L. (Garnock-Jones, 1993; Garnock-Jones and Lloyd, 2004, as *Parahebe* and *Chionohebe*), but details about the biomechanics and hypotheses for the occurrence of hygrochasy in those species have not been investigated. Most hygrochastic species are found in high alpine areas of the Southern Alps on the South Island, but one species is present in high alpine areas on the volcanic plateau on the North Island. For a description of New Zealand alpine environments, see Wardle (1991).

Veronica is the largest genus within the Plantaginaceae (Angiosperm Phylogeny Group, 2003) with approximately 450 species (Albach et al., 2004; Albach and Meudt, 2010) of various life forms. Species are distributed mainly in temperate regions of the northern hemisphere and in Australasia (Albach et al., 2004; Garnock-Jones et al., 2007) and occur in a wide range of habitats. In this work, we concentrate on 23 Southern Hemisphere *Veronica* species, mostly formerly treated as *Parahebe* W.R.B. Oliv. and *Chionohebe* B. Briggs et Ehrend., which are now included in *Veronica* sect. *Hebe* (Garnock-Jones et al., 2007). Both *Parahebe* and *Chionohebe* are

now arranged in two groups, informally named speedwell hebes and snow hebes, respectively (Albach and Meudt, 2010).

Together with potentially 11 New Guinea species, the speedwell hebe group comprises ca. 24 species, which are subshrubs with decussate leaves and racemose inflorescences with numerous short-tubed flowers on long slender pedicels. The capsules, developed from two carpels, are only weakly laterally compressed with a quite broad septum and open both at the apex by septicidal (between the carpels) and loculicidal (along the dorsal bundle of each carpel) splits and gape at the septum by a septicidal split (Garnock-Jones and Lloyd, 2004). However, the position of *V. linifolia* Hook. f., *V. colostylis* Garn.-Jones and *V. cheesemanii* Benth. within the group is still not entirely resolved and the placement of *V. planopetiolata* G. Simpson & J.S. Thomson differs among markers (Albach and Meudt, 2010). The recently described *V. jovellanoides* Garn.-Jones & de Lange (Davidson et al., 2009) is most similar to the speedwell hebe group. The snow hebes (Albach and Meudt, 2010) include species formerly placed in *Chionohebe* and alpine *Parahebe*. They comprise four species of high alpine cushion plants and four species of alpine low subshrubs, both on DNA evidence (Albach and Meudt, 2010) and morphology (Garnock-Jones, 1993; Garnock-Jones and Lloyd, 2004; Garnock-Jones et al., 2007). These are found in alpine areas of the South Island of New Zealand, although one cushion plant extends to Tasmania and one subshrub to the Australian Alps (Meudt, 2008). Compared to other Australasian *Veronica*, snow hebes have a compact habit, 5-(6-)merous calyces and corolla and the capsules are laterally compressed with a narrow septum (Meudt and Bayly, 2008). Similar to the speedwell hebes, the capsules of snow hebes also open septicidally and loculicidally, but without gaping at the septum. The flowers are solitary and either sunk in the cushion or set on greatly reduced peduncles. One

additional species, *V. zygantha* Garn.-Jones, shares some morphological features with snow hebes (Garnock-Jones and Lloyd, 2004) but a close relationship has not been demonstrated.

So far, the Australasian *Veronica* species have received little attention regarding their fruit anatomy and dispersal strategies, but a number of papers have been published on Mediterranean species (Juan et al., 1997; Juan et al., 2000). They showed that the dehiscence type is closely related to the anatomical structures of the capsules, with the compression of the capsules strongly correlating with the strength of the cohesion tissue, which is the tissue influencing movement within the capsule (Fahn and Werker, 1972).

We document which species of New Zealand *Veronica* possess hygrochastic capsules and describe the anatomy and biomechanics of hygrochastic capsule dehiscence in *Veronica* for the first time. We also introduce hypotheses to explain the investment of alpine plants in hygrochastic opening.

2.3 Materials and Methods

Fresh samples of 11 *Veronica* species were collected in various field sites in New Zealand. Dried capsule samples from a further six species were obtained from the Allan Herbarium at Landcare Research Center in Lincoln, New Zealand (CHR). Annotated voucher specimens of the collected samples are held in the herbarium at Victoria University of Wellington (WELTU) (Appendix 1).

To detect hygrochasy, dry capsules were immersed in water for about 10 min and their reaction was observed. If they opened, they were classified as hygrochastic. In addition, cryostat sections (see below) were exposed to water and observed under a microscope. If there was dramatic swelling of cells they were also classified as

hygrochastic. Capsules that were already open and did not change in response to water, were classified as 'ripening dehiscent' (e.g., capsules open with the ripening process and stay open). Capsules that were closed and remained closed were classified based on previous observations by Garnock-Jones and Lloyd (1994) and/or cryostat analysis.

To investigate the anatomy of the capsules, transverse sections of the capsules of all species were made using a range of embedding and sectioning methods.

For frozen sections (cryostat sections), dried capsule material was placed in a -80ºC freezer for several days and then transferred to a cryostat (Leica CM 3050S). Here, the capsules were embedded in Jung tissue freezing medium and cut with carbon knives, resulting in sections between 5 and 35 µm thick. The advantage of cryostat sections is that the plant tissue is not infiltrated by any medium or stain, therefore preserving a natural state of the material. These sections were used to observe and measure the reaction of cells to water by inserting water under the cover slip and measuring changes in cell size and shape after water absorption. If tissue expanded after water absorption, it was classified as swelling tissue (ST) and the species was considered hygrochastic. Since cryostat sections were relatively thick, it was difficult to distinguish individual cells, and the changes occurring during water uptake were therefore measured as percentage change for the tissue. Ten measurements per species were made, when possible from different capsules. Following these trials, histochemistry was investigated in cryostat sections. Phloroglucinol and hydrochloric acid were used as a sensitive stain for lignin. Unfortunately the use of highly concentrated hydrochloric acid destroyed the samples within a few minutes (Gurr, 1953). In younger samples, Safranin and Light Green were used to stain for lignified substances and cellulose (Gurr, 1963).

Capsules were also embedded in epoxy resin, which allowed for much thinner sections (ultratome sections). Following a dehydration alcohol series, the samples were placed in 100% absolute alcohol and propylene oxide for 30 min each, left overnight in 50:50 propylene oxide : resin and then transferred to 100% resin for at least three to four hours. They were then arranged in resin-filled moulds and dried overnight. Sections (2 – 4 μm) were cut with glass knives in a Leica Ultracut E ultratome and stained with Toluidine Blue. These sections were used to measure cell size and cell wall thickness in ten samples per species, when possible from different capsule samples. The following cell measurements were made both in the septum, where the swelling tissue (ST) is located and on cells in the valve tissue, where the resistance tissue (RT) is located in hygrochastic *Veronica*: length of cells in the ST (STL), wall thickness of cells in the ST (STCW), the percentage change of the length of the ST after water absorption (change), width of cells in the ST (STW), diameter of cells in the RT (RTDIA), and wall thickness of cells in the RT (RTCW). A description of the origin and number of capsules per species used for the microscopical investigation can be found in Appendix 1.

Measurements from ultratome sections were used in a Principal Components Analysis (PCA) in order to find anatomical traits associated with dehiscence type. In the PCA ordination, two clusters were retrieved. One cluster contained species that were classified hygrochastic based on the water test on whole capsules and on the water absorption test in cryostat sections. The other cluster contained 'ripening dehiscent' species, identified by the water test. All measurements were compared between hygrochastic and 'ripening dehiscent' species using Shapiro-Wilks test to test for normality and subsequently Wilcoxon Rank test to test for significant differences between the groups.

In order to identify common characters in hygrochastic species and to use these to predict hygrochasy in species without capsule material, a hierarchical cluster analysis was performed. Usually, hierarchical cluster analysis is used to show which samples are most similar to each other and subsequently group them in the same cluster. The similarity is based on Euclidean distances from every sample to every other sample in an m-dimensional space defined by the m variables (quantitative dissimilarity matrix) (Crawley, 2007). Here, we use cluster analysis slightly differently. Our aim was to identify characters that hygrochastic species have in common. Based on observations of positively identified hygrochastic species we chose life form, capsule position, number of inflorescences, capsule compression and capsule orientation as characters which are important for the dispersal by raindrops. A morphological dataset for a total of 23 *Veronica* was compiled with data from field observations, the Allan Herbarium database, herbarium material and literature (Garnock-Jones and Lloyd, 2004; Meudt, 2008; Davidson et al., 2009). This dataset includes 17 species for which capsule samples were available (Appendix 1) as well as six additional *Veronica* species to include all *Veronica* of the snow hebe and speedwell hebe groups. The traits were scored as character states and a hierarchical cluster analysis using Euclidean distances was performed. If hygrochastic species clustered together and 'ripening dehiscent' species were in a different cluster the chosen morphological characters can serve as likely indicators for hygrochasy. Statistical analyses were carried out using R (R Development Core Team, 2005).

2.4 Results

Six *Veronica* species had capsules that opened when submerged in water and closed again when they dried and were therefore classified as hygrochastic. Seven species had capsules that remained open in the water and were classified as 'ripening dehiscent'. Capsule samples of four species remained closed, these were classified as hygrochastic based on water absorption in cryostat section. These observations are summarized in Table 2.1. The classifications of Garnock-Jones and Lloyd (1994) are also given in the table, where it is apparent that *V. colostylis* and *V. lilliputiana* do not match our classification.

Table 2.1 Classification of *Veronica* with different experimental treatments.
Note: Statistical analysis is based on the classification after the investigation of cryostat sections. For these, an expansion of tissue after water absorption was used to identify hygrochastic and 'ripening dehiscent' species. The classifications from Garnock-Jones and Lloyd (1994) are included for comparison.

	cryostat sections	water test	Garnock-Jones and Lloyd (1994)
V. catarractae	'ripening dehiscent'	'ripening dehiscent'	xerochastic
V. cheesemanii	hygrochastic	hygrochastic	hygrochastic
V. ciliolata	hygrochastic	-	-
V. colostylis	hygrochastic	-	xerochastic
V. decora	'ripening dehiscent'	'ripening dehiscent'	xerochastic
V. densifolia	hygrochastic	hygrochastic	-
V. hookeriana	'ripening dehiscent'	'ripening dehiscent'	xerochastic
V. lanceolata	'ripening dehiscent'	'ripening dehiscent'	xerochastic
V. lilliputiana	hygrochastic	-	xerochastic
V. lyallii	'ripening dehiscent'	'ripening dehiscent'	xerochastic
V. melanocaulon	'ripening dehiscent'	'ripening dehiscent'	xerochastic
V. planopetiolata	hygrochastic	hygrochastic	hygrochastic
V. pulvinaris	hygrochastic	hygrochastic	-
V. senex	'ripening dehiscent'	'ripening dehiscent'	xerochastic
V. spathulata	hygrochastic	hygrochastic	hygrochastic
V. thomsonii	hygrochastic	hygrochastic	-
V. trifida	hygrochastic	-	hygrochastic

Cryostat sections were used to test the reaction of cells to water absorption. After water was injected under the cover slip, tissue at the septum of ten species expanded noticeably, whereas in the septum of the remaining seven species a distinct expansion was not observed. The tissue showing expansion was identified as swelling tissue (ST) and all ten species expressing swelling were classified as hygrochastic. When transverse ultratome sections were analysed, the ST could be identified in hygrochastic species as possessing distinctly different cells. Using sections from three orthogonal planes, the shape of the cells was determined. The ST was located in the septum of the capsule and consisted of one cell layer. It comprised elongated cylindrical cells with thick cell walls. These were aligned so that the longest cell dimension was orthogonal to the outer edge of the tissue (Fig. 2.2 A).

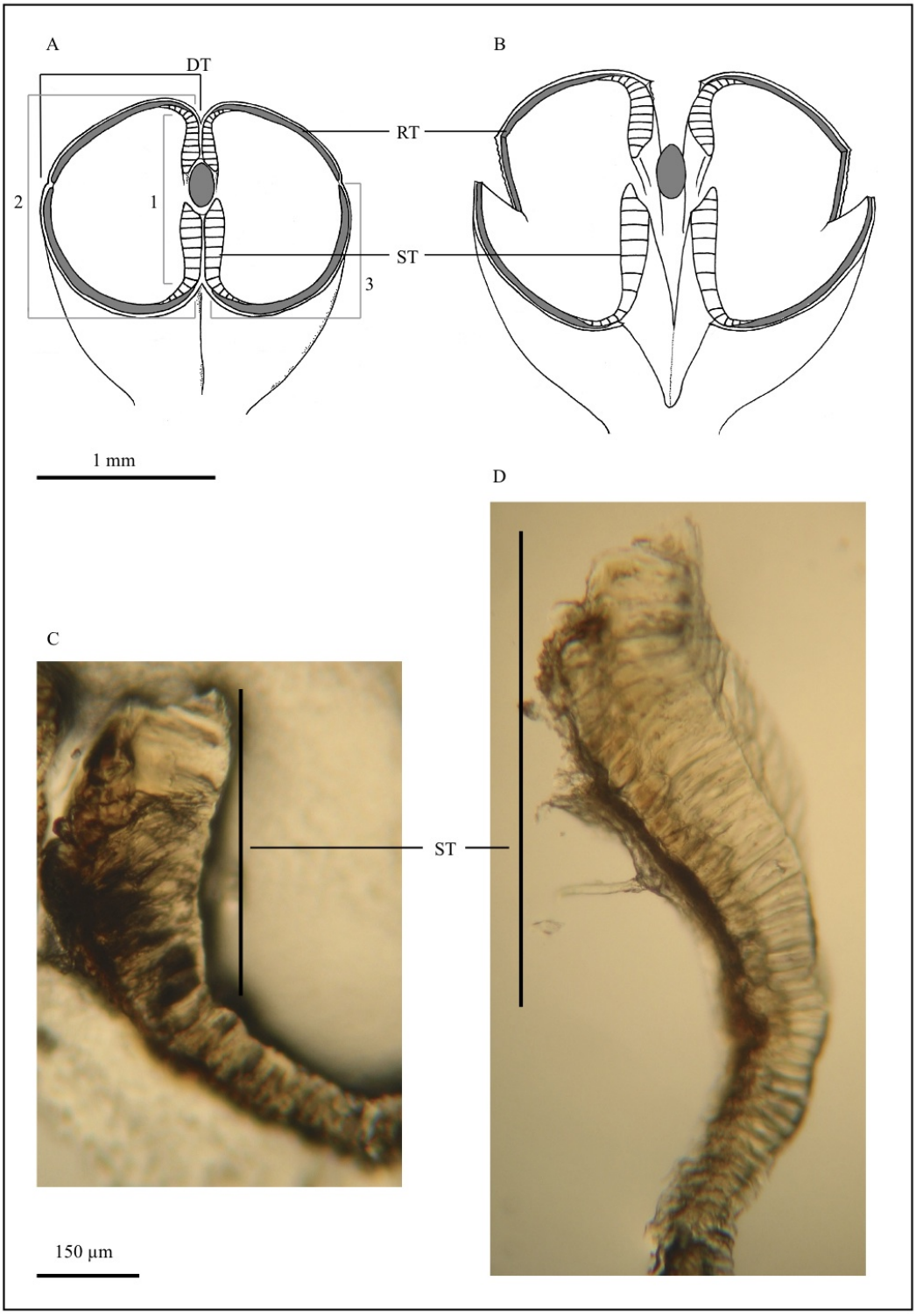

FIG 2.2 Differences in the swelling tissue (ST) between dry and wet capsules.
(A) Diagram of a transverse section of a dry (closed) hygrochastic *Veronica* capsule, (B) Diagram of a transverse section of a wet (open) hygrochastic *Veronica* capsule, (C) micrograph (16x) of an unstained septum of *Veronica spathulata* (dry), (D) micrograph (16x) of an unstained septum of *Veronica spathulata* (wet), Resistance tissue (RT) in the diagrams is shown in grey, swelling tissue (ST) in the diagram is shown in striped, the dehiscence tissue (DT) is indicated by black lines. The grey lines show the septum (1), a locule (2) and the valve of a locule (3).

The RT was present throughout the valves of each carpel and two, sometimes three cell layers thick. RT cells were spherical and generally smaller than the cylindrical cells of the ST. Phloroglucinol staining of cryostat sections revealed that lignin was only present in walls of cells in the RT and was absent in the ST (Fig. 2.3). In contrast, 'ripening dehiscent' *Veronica* showed spherical cells with thick cell walls in the valves as well as in the septum, with all cell walls being completely lignified (similar to RT in hygrochastic species) (Fig. 2.3). Throughout the capsule the cells appeared in layers of two to three cells.

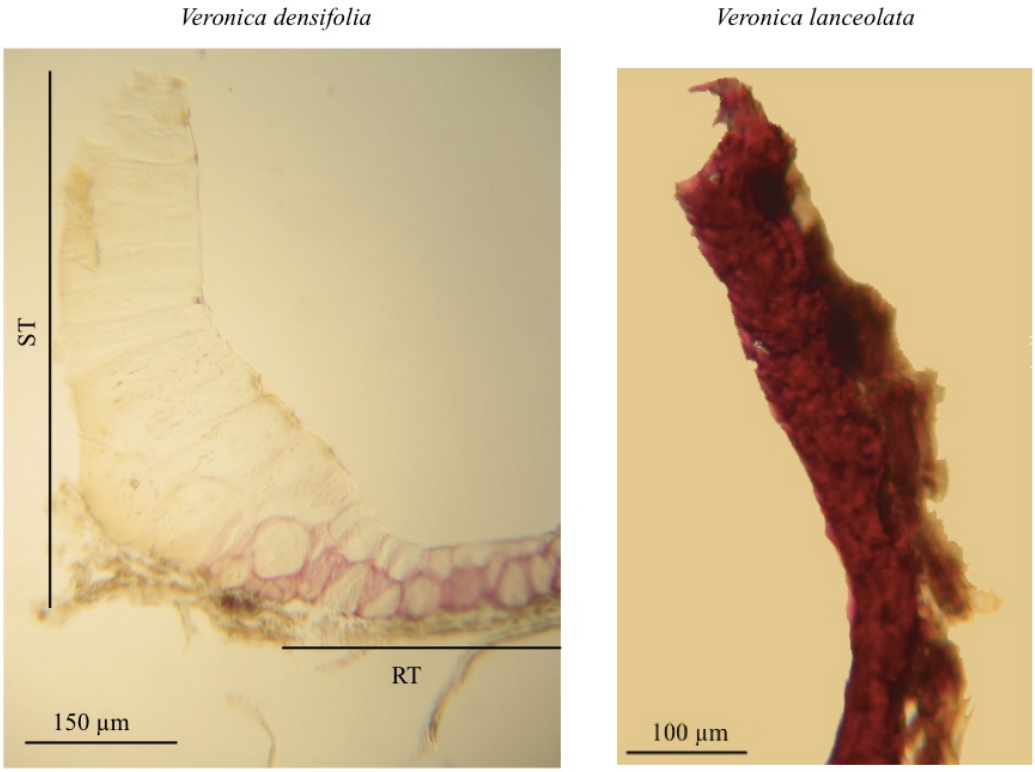

FIG 2.3 Septum of *Veronica densifolia* (hygrochastic) and *Veronica lanceolata* ('ripening dehiscent'). Magnification 16x, Phloroglucinol was used to stain for lignin (red). Section were made using a cryostat, with the *V. densifolia* sample being 12µm and *V. lanceolata* 25 µm thick. The location of the swelling tissue (ST) and resistance tissue (RT) are indicated in *Veronica densifolia*.

Principal Components Analysis (PCA) of cell measurements and change after water absorption was run to determine which of the measurements were the most important in distinguishing between hygrochastic and 'ripening dehiscent' species (Table 2.2). Measurements for each species are given in Appendix 2.2. The first three components of the PCA accounted for 83.5% of the variation among the samples.

Table 2.2 Principal Component Analysis (PCA). Loadings were based on measurements made from ultratome sections of 'ripening dehiscent' species and hygrochastic species, identified based on anatomy.
STL – length of cells in swelling tissue (ST); STCW – thickness of cell walls in ST; change – percentage change of the length of ST after water absorption; STW – width of cells in the ST; RTDIA – diameter of cells in the resistance tissue (RT); RTCW – thickness of cell walls in the RT.

	Comp. 1	Comp. 2	Comp. 3	Comp. 4	Comp. 5	Comp. 6
eigenvalues	2.103	1.632	1.273	0.496	0.315	0.182
St. dev	1.450	1.277	1.128	0.704	0.561	0.427
Proportion of variance	0.350	0.272	0.212	0.083	0.052	0.030
Cumulative proportion	0.350	0.622	0.835	0.917	0.970	1.000
			loadings			
STL	0.606	-0.259		-0.162	-0.176	0.712
STCW	0.586		-0.356	-0.110	-0.349	-0.629
Change	0.378	-0.459	0.388	0.311	0.581	-0.250
STW	0.196	0.467	-0.580	0.240	0.569	0.161
RTDIA	0.237	0.509	0.429	0.617	-0.341	
RTCW	0.228	0.496	0.447	-0.653	0.266	

The variables STL, STCW and change were associated with Axis 1 and STW, RTDIA and RTCW with Axis 2 and are shown in Fig. 2.4. 'ripening dehiscent' and hygrochastic species split into two distinct groups roughly along the first axis. In the PCA (Fig. 2.4, clear oval) the four species that did not open in the water test, but were classified hygrochastic based on cryostat section analysis, fell within the hygrochastic

cluster. However, some measurements for *V. lilliputiana* Stearn lay between the groups.

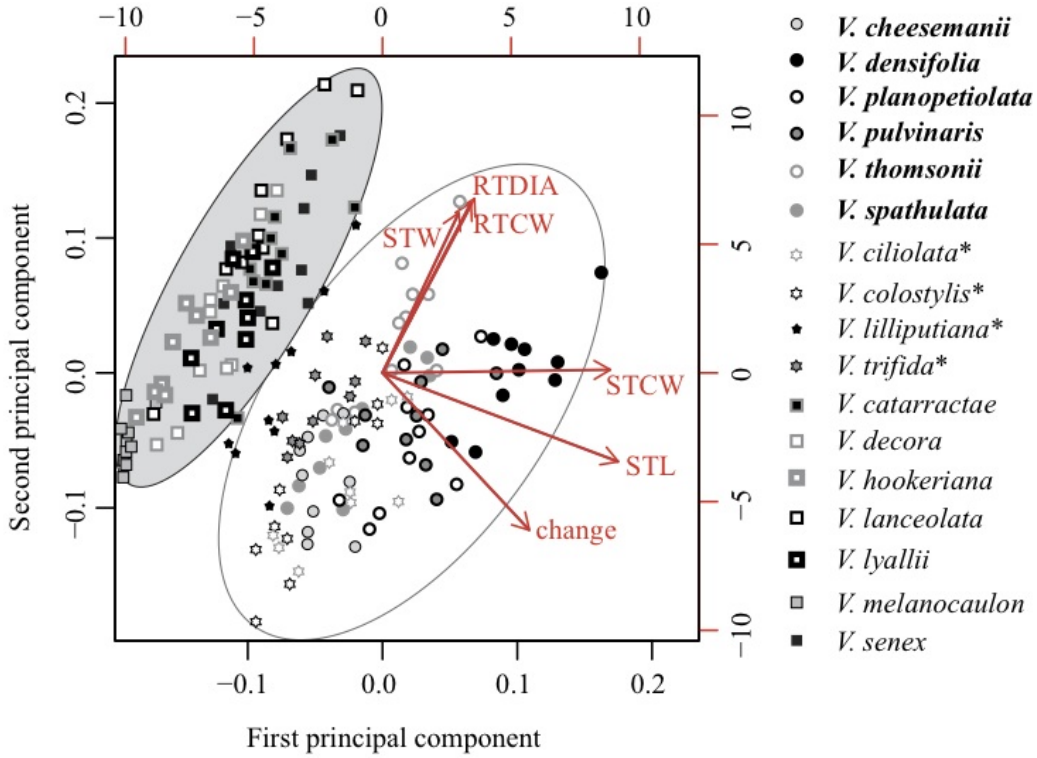

FIG 2.4 Scores for all species on the first two PCA axes. Vector directions/lengths represent eigenvectors of measurements (loadings) for each axis.

Circle symbols and bold font represent known hygrochastic species; star symbols and stars behind the name indicate hygrochastic species inferred from anatomy; square symbols are 'ripening dehiscent' species. The grey shaded oval indicates the group 'ripening dehiscent' species, the clear oval indicates the group hygrochastic species.

STL – length of cells in swelling tissue (ST); STCW – thickness of cell walls in ST; change – percentage change of the length of ST after water absorption; STW – width of cells in the ST; RTDIA – diameter of cells in the resistance tissue (RT); RTCW – thickness of cell walls in the RT.

To investigate the difference between 'ripening dehiscent' and hygrochastic species further, the three influential variables in the first component (STL, STCW and change) were compared separately between both groups ('ripening dehiscent' and hygrochastic) (Fig. 2.5).

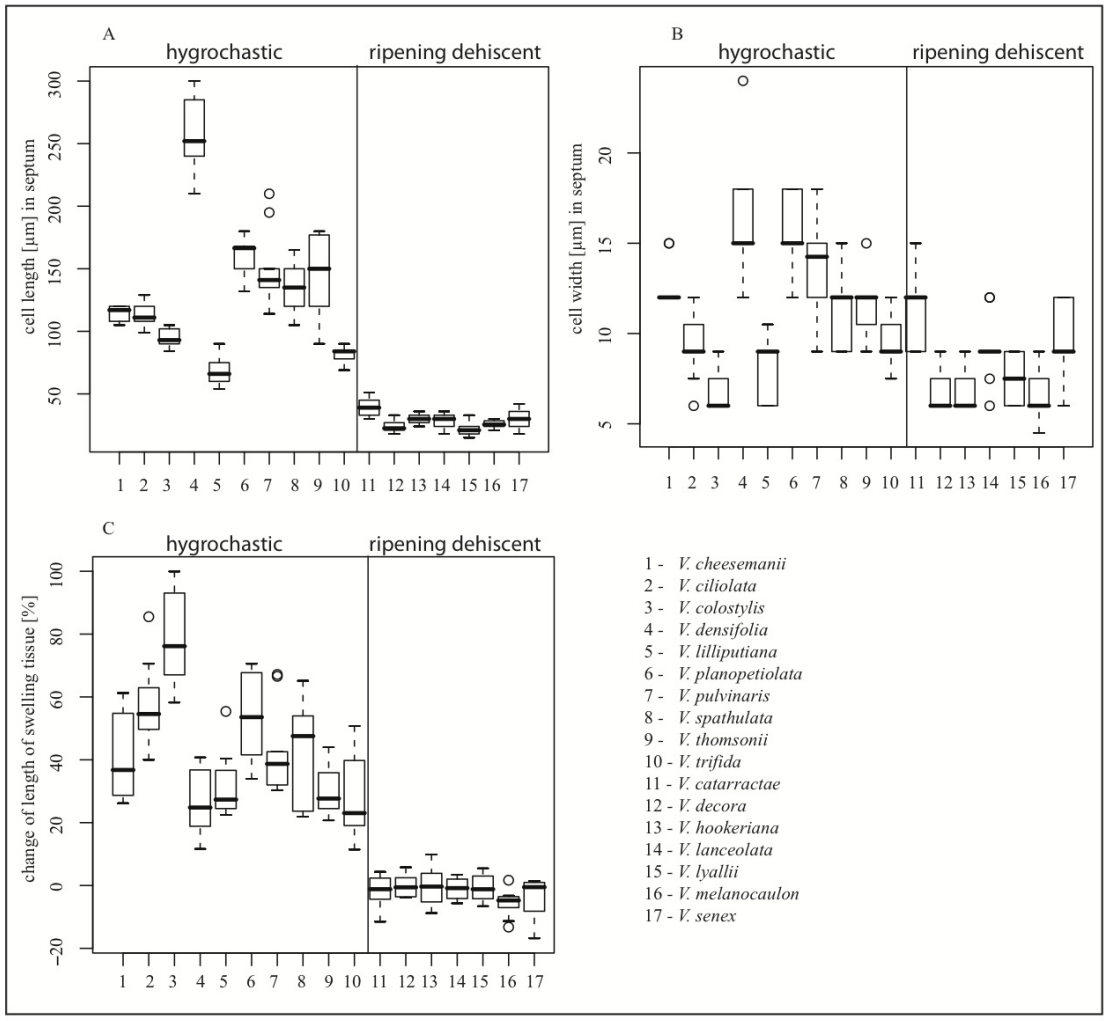

FIG 2.5 Box-whisker plots of (A) Length of cells in the septum (STL), (B) width of cells in the septum (STW), (C) change of length of the swelling tissue after water absorption. Hygrochastic species as identified by anatomy based on the PCA (1-10) and 'ripening dehiscent' species (11-17) are separated by a vertical line.

A Wilcoxon Rank test showed that hygrochastic species possess significantly longer cells (A) with thicker cell walls in the swelling tissue (B), which expand significantly in length (C) compared to 'ripening dehiscent' capsules, when water is absorbed (Table 2.3, Fig. 2.5). The width of cells in the swelling tissue (STW) is significantly different (Table 2.3) between both groups as well but is associated with the second principal component.

Table 2.3 Wilcoxon rank test between hygrochastic and 'ripening dehiscent' *Veronica* species. See Table 2.2 for abbreviations.

Measurement	W	P-value
STL	7000	<0.001***
STCW	5485	<0.001***
change	7000	<0.001***
STW	2643	0.006**
RTDIA	3682.5	0.559
RTCW	3379.5	0.688

In younger fruits of all species, a dehiscence tissue (DT) was found between the carpels along the septum, the carpel midribs and between the valves of each locule (Fig.2.2 A), comprising small round cells with thin cellulose cell walls (stained green with Light Green). The DT is formed as an outer cell layer of the endocarp. Those cells disintegrate with ripening; leaving a small split both at the septicidal and loculicidal end of the capsule. In older fruits that had previously been opened, the DT was usually absent or only visible as ripped cells still attached to the valves.

Based on opening experiments and observation of water absorption in cryostat slides, all hygrochastic *Veronica* capsules have been found to function in the same way, only differing in size of the swelling tissue cells and the degree of swelling tissue increase after water absorption. When a ripe hygrochastic capsule is exposed to water, the cell walls of the elongated cylindrical cells in the swelling tissue rapidly take up moisture

resulting in an increase in diameter of the cylindrical cells (Fig. 2.2 C and D). This leads to an extension of the height and length of the septum, but not its thickness (Fig. 2.2 B). Due to the expansion of the septum the split resulting from the previously disintegrated DT widens and at the same time the valves are pushed towards the loculicidal splits. This opening widens and the four valves move outward, forming a splash cup. When the capsule dries again, the swelling recedes and the valves return to their previous position. Since the DT has been destroyed small splits remain at both the septicidal and loculicidal end.

In 'ripening dehiscent' *Veronica,* the capsules open with the ripening of the fruit, and the DT disintegrates. The lignified cells in the septum and valves most likely shrink in all planes on drying and a pore-like gap is formed at the capsule apex as the locule walls pull apart. This gap is big enough to release seeds when shaken in the wind. Additionally in the speedwell hebes (e.g. *Veronica catarractae* G.Forst.), the septum is pulled apart slightly and is open in the mature fruit. This appears to be reversible (xerochastic movement) in at least some species of New Zealand *Veronica*.

Table 2.4 Characters and character states of New Zealand *Veronica* species.
Note: Information adapted from own observations, Allan (1961); Mark and Adams (1995); Garnock-Jones and Lloyd (2004); Meudt (2008), Davidson et al. (2009).

Character		Character state and codes
1	Life form	Cushion (1), creeping subshrub (2), creeping herb (3), subshrub (4)
2	Number of inflorescences	Solitary (1), <4 (2), <8 (3), >8 (4)
3	Position of capsule	Sessile (1), peduncle < 2cm (2), peduncle > 2cm (3)
4	Orientation of capsule	Erect (1), facing down (2), both (3)
5	Capsule compression	Narrowly angustiseptate (1), broadly angustiseptate (2)

To identify common traits for hygrochastic *Veronica*, morphological characters of 23 species were given character states and are presented in Table 2.4. A cluster analysis

of these was performed to group species based on Euclidean distances between the character states in a dissimilarity matrix. (Fig. 2.6).

Based on the chosen characters the species were split into two distinct groups. With the exception of *V. colostylis* Garn.-Jones, all hygrochastic species were grouped in one cluster. The cluster analysis also incorporated species for which capsule material was not available (*V. linifolia*, *V. zygantha*, *V. spectabilis* (Garn.-Jones) Garn.-Jones, *V. chionohebe* Garn.-Jones, *V. birleyi* N.B. Br., *V. jovellanoides*).

Of these, *V. chionohebe*, *V. zygantha*, *V. spectabilis*, *V. birleyi* and *V. jovellanoides* fell into the hygrochastic group based on their morphological characters. The second group comprised all 'ripening dehiscent' species.

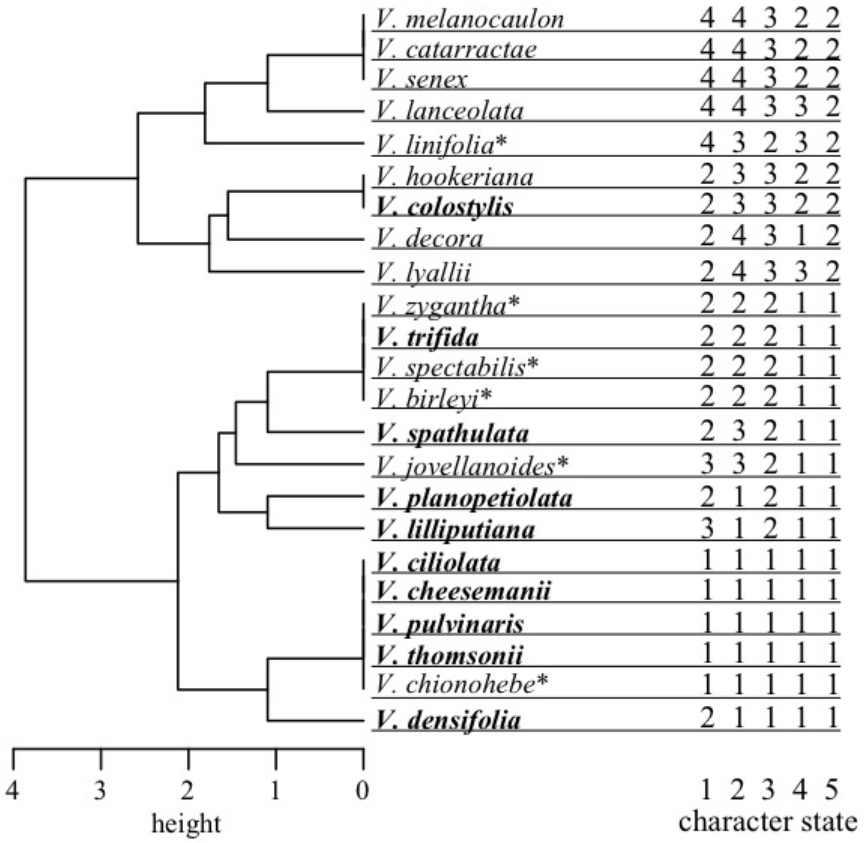

FIG 2.6 Hierarchical cluster analysis of Euclidean distances in a dissimilarity matrix of character states of 23 *Veronica* species. Known hygrochastic species (based on water experiments, anatomical analysis and Garnock-Jones and Lloyd (1994)) are shown in **BOLD**, species without capsule material are marked with *.

2.5 Discussion

Plants, often considered sessile and motionless, are able to move either some of their parts or achieve movements through the dispersal of their diaspores (Darwin, 1865; Darwin and Darwin, 1880; Edwards and Moles, 2009; Van der Pijl, 1982). When differential growth of living tissues is the source of movement, such movements are irreversible, except by further growth of an opposing tissue, which then results in an overall enlargement of the organ. Reversible movement can be achieved by differential enlargement of living cells or tissues, e.g., in the reversible opening of stomata and closing and opening of fly traps in *Dionaea*, or in dead tissues, e.g., in hygrochastic capsules. Here we have shown that in some New Zealand species of *Veronica*, reversible hygrochastic dehiscence of capsules is achieved by differential swelling of thick-walled non-lignified cells of the septum, opposed by a resistance tissue in the lignified cells of the capsule walls.

Based on our observations we have been able to considerably update the number of hygrochastic species in New Zealand. Previously, Garnock-Jones and Lloyd (1994) had reported *V. cheesemanii, V. spathulata* Benth. (speedwell hebes)*, V. birleyi*, *V. spectabilis*, *V. trifida* Petrie, *V. zygantha* (snow hebes) and *V. planopetiolata* as hygrochastic. We are now also able to add *V. colostylis* and *V. lilliputiana* (both in speedwell hebes) and confirm that all snow hebes (Meudt and Bayly, 2008) are also hygrochastic. Our morphological analysis also supports hygrochasy in the newly discovered *V. jovellanoides* (Davidson et al., 2009).

All hygrochastic species, apart from *Veronica lilliputiana* (restricted to lowland to montane lakesides), *V. colostylis* (mostly subalpine but extends to lower and higher altitudes) and *V. jovellanoides* (only found at one side in a lowland forest) were found exclusively in the alpine zone of New Zealand. *Veronica spathulata* Benth. and *V.*

jovellanoides are the only hygrochastic *Veronica* found in the North Island of New Zealand.

Although analysis of capsule sections of *V. colostylis* provides clear evidence that it is hygrochastic, the cluster analysis showed that it does not possess all the morphological characters that are associated in other hygrochastic *Veronica*. This might explain why it was assumed to be xerochastic previously (Garnock-Jones and Lloyd, 2004). Its sister species *V. linifolia* (Garnock-Jones and Lloyd, 1994) has ripening dehiscence. *V. lilliputiana* has not been reported as hygrochastic before but the distinct expansion of the ST after water absorption in cryostat sections as well as measurements of cells in the ST in ultratome sections are good evidence for hygrochasy in this species. However, some of the data points within the PCA are closer to the cluster of 'ripening dehiscent' species, but this is probably due to the smaller cell size in the small capsule. The cluster analysis also grouped *V. lilliputiana* with hygrochastic species.

Although we did not have capsule material to test hygrochastic opening or to investigate capsule anatomy in *V. chionohebe* , *V. jovellanoides*, *V. birleyi*, *V. spectabilis* and *V. zygantha* in this study, the hierarchical cluster analysis suggests that they are highly likely to show it. Garnock-Jones and Lloyd (2004) described the last three as hygrochastic and opening after wetting was also observed in *V. jovellanoides* (Davidson et al., 2009). According to the cluster analysis, erect narrowly angustiseptate capsules, a short peduncle and solitary and/or few-flowered inflorescences, as seen in all these species seem to be characters associated with hygrochasy in *Veronica*. Juan et al. (1997, 2000) found that the compression of the capsule is strongly related to the cohesion tissue. According to Fahn and Werker (1972) a cohesion mechanism is based on the water content of the cell lumen, the

change of which also results in tissue movement. We assume that the cohesion tissue described by Juan et al. (1997) might in fact be swelling tissue, since its description is similar to that of swelling tissue in hygrochastic *Veronica* and our preliminary studies indicate that *V. arvensis* is hygrochastic as well. It appears that the traits associated with hygrochasy in New Zealand *Veronica* are a syndrome of the functionally related hygrochastic species. Narrowly angustiseptate capsules only occur in hygrochastic species regardless of their position in the phylogeny (Albach and Meudt, 2010) and cushion plants can be found both in the speedwell hebe and snow hebe clade (Albach and Meudt, 2010).

Although the snow hebe and speedwell hebe clades both contain species with hygrochastic capsules, no studies have demonstrated a sister relationship for them (Garnock-Jones, 1993; Wagstaff and Garnock-Jones, 2000; Wagstaff et al., 2002; Albach and Meudt, 2010). Further, although all snow hebes have hygrochastic capsules, only a few speedwell hebes are hygrochastic and these species do not form a subclade or a basal grade within that group. The snow hebe and speedwell hebe clades diverge from adjacent nodes on the stem leading to the crown hebe clade (Albach and Meudt, 2010). It is thus unclear whether hygrochasy in New Zealand *Veronica* has evolved once and been lost at least once or has evolved independently in two or more lineages. The possible hybrid origin of morphologically anomalous species in the speedwell hebe clade, such as the tetraploid *V. spathulata*, has not been investigated in detail. The presence of hygrochastic dehiscence in other groups of *Veronica*, e.g. *V. arvensis* (subg. *Chamaedrys*) suggests the character is labile in the genus but further study is needed to confirm this.

The biomechanics of hygrochastic capsule opening in *Veronica* is based on a simple swelling tissue. The direction of the swelling is governed by the arrangement of

swelling and resistance tissue. Our findings correspond with earlier studies of hygrochastic movements and are based on the same general principles (Fahn and Werker, 1972; Poppendieck, 1995). However, in contrast to previously studied hygrochastic species from arid areas (Poppendieck, 1995; Parolin, 2001), the capsules of the different hygrochastic *Veronica* are all similar, exhibit a much simpler mechanism and open in relatively short time (unpublished data). For example, hygrochastic *Oenothera* species show different structural complexity, which, depending on position of the swelling and resistance tissue influences the speed of the opening and closing of the capsules (Poppendieck, 1995). Furthermore, Aizoaceae in southern Africa exhibit a range of hygrochastic fruits, from simple fruits (e.g. in *Mesembryanthemum*) to very complex dispersal units (e.g. in *Ruschia*). The more complex systems have features such as closing bodies, funicles, valve wings, membranous locule lids, seed pockets and expanding keels, which mainly ensure the retention of some seeds in the capsule, and the release of seeds only after a certain amount of rain (Van Oudtshoorn and Van Rooyen, 1999; Parolin, 2001). At the same time, these features allow the seeds to be dispersed over greater distances compared to the more simple fruits (Parolin, 2006).

The development of structures that retain seeds or only release a certain number of seeds over time appears to be an evolutionary response to the harsh environment where those hygrochastic species are found (Van Oudtshoorn and Van Rooyen, 1999). The seeds are stored on the plant until release and are therefore protected against seed predators, drought and unfavourable germination conditions.

However, it has also been proposed that restriction in space might be another advantage of being dispersed by raindrops. The distance seeds can travel with raindrops is very limited, even if fruit structures enable jet dispersal, which increases

dispersal distance (Parolin, 2006). The guarantee of short-distance dispersal enables the seedling to establish in close proximity to the parent plant. Although this carries a risk of parent-offspring competition, chances are considerably higher that the seed will germinate in a suitable habitat rather than a more distant hostile environment where mortality of seeds is exceptionally high (Steinbrinck, 1883; Zohary, 1962; Ellner and Shmida, 1981; Ihlenfeldt, 1983).

Dispersal restriction in time plays a major role in desert plants but it does not seem to apply for New Zealand alpine plants, for wet germination conditions are available throughout the season (Wardle, 1991) and capsules open quickly after only a small amount of rain. Due to the position of hygrochastic capsules on the plant (mostly embedded in the cushion), wind-mediated dispersal is not available to them and raindrops seem to be the most effective dispersal agents. The capsules face upwards and once they are open and form a splash cup the seeds are exposed to rain and falling drops splash the seeds out.

Space restrictions could be a likely explanation for the evolution of hygrochasy in some alpine plants of New Zealand. The majority of them occupy patchy habitats, which are usually restricted in size and altitude (Mark and Adams, 1995). Dispersal in space is limited for species with simple hygrochastic capsules but the restriction in time is not very pronounced (Van Rooyen et al., 1990) and the simplicity of hygrochastic capsules of alpine *Veronica* might play an important role in restricting dispersal to their specific habitats. In limited patchy habitats, restricted dispersal is likely to be selected, providing an explanation for the evolution of hygrochastic rather than 'ripening dehiscent' species (Pufal and Garnock-Jones, in press). By keeping the seeds in the closed capsule and only exposing them during rainfall events, accidental dispersal by other means such as wind gusts or animals is not available for

hygrochastic capsules and short-distance dispersal with the aid of raindrops highly probable. On the other hand, 'ripening dehiscent' capsules disperse the seeds independently of weather conditions. Since they are facing down and are open, seeds are easily dropped or shaken out by slight movements. The seeds are then exposed to the environment and can be blown away by wind at any time, which results in unpredictable dispersal distances. Tests of these hypotheses are presented in Pufal and Garnock-Jones (in press).

Our results suggest that hygrochasy in *Veronica* is more common than previously thought. Van der Pijl (1982) proposed that *Veronica* species in marshy habitats are hygrochastic as well but Juan et al. (1997) did not find evidence for hygrochasy in their study of Mediterranean *Veronica*. Our preliminary observations indicate that *Veronica javanica* and *Veronica arvensis* also possess hygrochastic capsules. Both species are invasive in New Zealand. Especially for alpine *Veronica* in New Zealand, hygrochasy can play a crucial role in plant dispersal but has so far been overlooked (Thorsen et al., 2009). But as Thorsen et al. (2009) emphasized, further research is necessary to close gaps in the knowledge of dispersal systems in New Zealand.

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CHAPTER THREE

The Influence of Hygrochastic Capsule Dehiscence on Seed Dispersal and Species Distribution of New Zealand Alpine *Veronica*

Chapter 3.2 is published in Annals of Botany with the title "Hygrochastic capsule dehiscence supports safe site strategies in New Zealand alpine *Veronica* (Plantaginaceae)" by G. Pufal and P. Garnock-Jones. The co-authors' role was restricted to supervision and advice.

FIG 3.1 Habitat patch with *Veronica densifolia* and *V. thomsonii* at Blue Lake, Garvie Mountains, New Zealand.

Chapter 3.1

Overview

Chapter 3.1 presents two linked research projects on the association between hygrochastic capsule dehiscence and restricted habitat patches.

In Chapter Two, hygrochasy was described in detail in several New Zealand *Veronica*. The majority of those species occur exclusively in high alpine areas predominantly on scree, in rock crevices or sparsely vegetated cushion fields. Hygrochastic capsule dehiscence is usually associated with plants of arid regions, which likewise grow in harsh environments and are patchily distributed. Hypotheses that were developed for desert species state that hygrochasy is a means of restricting dispersal in time (bradyspory) and space (atelechory). The risks associated with dispersal and germination are spread over several rainfall events and raindrops as dispersal agents ensure that seeds are only transported over a short distance, increasing the chance of germinating in the favourable parental habitat. Being atelechoric is an advantage if hygrochastic species are adapted to very specific edaphic conditions that can only be found in restricted areas.

In Chapter 3.2 (Hygrochastic capsule dehiscence supports safe site strategies in New Zealand alpine *Veronica* (Plantaginaceae)) I investigate the influence of dispersal distance on directed dispersal of seeds to safe sites within small habitat patches. Measurements of dispersal distances of several hygrochastic alpine *Veronica* were analysed in the laboratory and field observations of one species were made as well. Habitat patch size was estimated and compared between several hygrochastic and non-hygrochastic species. I hypothesize that hygrochasy in alpine *Veronica* in New Zealand serves as a spatial restriction of seed dispersal within a small suitable habitat

patch with available safe sites in a heterogenous environment. This part of the chapter is published in Annals of Botany.

In Chapter 3.3 the environmental amplitude of hygrochastic *Veronica* in comparison with related non-hygrochastic species was tested in order to gain knowledge about the width of the ecological niche of hygrochastic *Veronica*. The multivariate analysis is based on environmental variables extracted from LENZ IV, a Landcare Research database used in arcMap. The coordinates used in arcMap are from locations chosen from three different herbarium databases. The aim of this chapter is to establish if hygrochastic species in alpine habitats are specialists and have small environmental niches compared to their non-hygrochastic relatives. I also discuss if LENZ and the use of herbarium records is an appropriate tool to assess ecological niche breadth.

Chapter 3.2

Hygrochastic Capsule Dehiscence supports Safe Site Strategies in New Zealand Alpine *Veronica* (Plantaginaceae)

3.2.1 ABSTRACT

• *Background and Aims* Hygrochasy is a capsule opening mechanism predominantly associated with plants in arid habitats, where it facilitates spatially and temporally restricted dispersal. Recently, hygrochastic capsules were described in detail for the first time in alpine *Veronica* in New Zealand. The aim of the present study was to investigate whether hygrochastic capsules are an adaptation of alpine *Veronica* to achieve directed dispersal to safe sites. We expect that by limiting dispersal to rainfall events, distances travelled by seeds are short and confine them to small habitat patches where both seedlings and adults have a greater chance of survival.

• *Methods* Dispersal distances of five hygrochastic *Veronica* were measured under laboratory and field conditions and the seed shadow was analysed. Habitat patch size of hygrochastic *Veronica* and related non-hygrochastic species were estimated and compared.

• *Key results* Dispersal distances achieved by dispersal with raindrops did not exceed one metre but weather conditions could influence the even distribution of seeds around the parent plant. Compared to related *Veronica* species, hygrochastic *Veronica* mostly grow in small, restricted habitat patches surrounded by distinctly different habitats. These habitat patches provide safe sites for seeds due to their microtopography and occurrence of adult cushion plants. Non-hygrochastic Veronica

can be predominantly found in large habitats without clearly defined borders and can be spread over long distances along rivers.

- *Conclusions* The results suggest that hygrochasy is a very effective mechanism of restricting seed dispersal to rainfall events and ensuring short-distance dispersal within a small habitat patch. It appears that it is an adaptation for directed dispersal to safe sites that only exist within the parent habitat.

Key words: dispersal, habitat patch, hygrochasy, New Zealand Alps, ombrohydrochory, safe sites, *Veronica*

3.2.1 INTRODUCTION

Plant dispersal is one of the most important stages in a plant's life cycle as it fulfils the role of colonizing new habitats, influences density and distribution of species and plays an important role in gene transfer for a usually stationary organism (Webb, 1998). A great variety of processes and strategies are involved in a plant's dispersal, which are usually linked to the morphology of the dispersal unit (Ellner and Shmida, 1981; Higgins et al., 2003; Hughes et al., 2006), i.e. the morphology of the dispersal unit defines the dispersal syndrome. These morphological dispersal syndromes (MDS) can be referred to as standard means of dispersal (Higgins et al., 2003) or classical dispersal syndromes, such as anemochory, zoochory, etc. One rather uncommon MDS is hygrochasy, a specific ballistic response by which dry, closed, woody capsules open under the influence of moisture, e.g. rain, and expose the seeds in a splash cup (Van der Pijl, 1982). Raindrops that fall into open capsules splash droplets out, taking the seeds with them over a short distance that is related to the size of the raindrop (Nakanishi, 2002). This occurs directly when seeds are freely exposed and indirectly

when additional structures in the capsules transfer the raindrop's energy (Ihlenfeldt, 1983; Parolin, 2001). This latter dispersal strategy is known as ombrohydrochory, where both seed release and seed dispersal are triggered by rain (Van Oudtshoorn and Van Rooyen, 1999).

Hygrochasy is most famously associated with the Aizoaceae, with more than 98% of the species exhibiting hygrochastic capsules. It is also described as a characteristic of other plant species in arid regions such as Southern Africa, the Israeli desert or North America (Zohary and Fahn, 1941; Ihlenfeldt, 1983; Gutterman, 1994; Poppendieck, 1995; Parolin, 2001, 2006) and Somalia (Thulin, 1987). Less commonly, hygrochasy has been reported sporadically from plant species of other habitats, such as temperate meadows (Walck and Hidayati, 2007) or along streams (Nakanishi, 2002).

Hygrochastic capsules were also reported in several New Zealand *Veronica* in alpine habitats (Garnock-Jones, 1993; Garnock-Jones and Lloyd, 2004 as *Parahebe* and *Chionohebe*; G. Pufal, VUW, Wellington, New Zealand, unpubl. res.). The mechanism can be found in plants of the speedwell hebe and snow hebe groups (for phylogeny and groups see Albach and Meudt, 2010), which are characterized by either cushion or subshrub morphology and solitary, sessile capsules (Pufal et al., 2010).

New Zealand *Veronica* have evolved rapidly in the last 9 million years, which can partly be attributed to the variety of habitats present in the mountain regions, where the species most likely originated (Wagstaff et al., 2002). Most hygrochastic *Veronica* are found exclusively in the high alpine areas of the New Zealand Alps (exceptions in Pufal et al., 2010) There are four distinct plant community types in the high-alpine zone, which can be localized and heterogenous in distribution (Enting and Molloy, 1982). Fell-field habitats can usually be found on relatively stable rocks and consist of

sparsely vegetated communities. A distinction can be made between wet and dry fell-field, differing in species composition (Mark and Dickinson, 1997). Scree habitats only occur on steep greywacke mountains in the rain shadow region of the South Island. Plant species found on scree are usually endemic to this habitat, being morphologically different from near relatives, and having a suite of common characteristics, such as fleshy glaucous leaves. Cushion fields are found in Central Otago, mostly on broad plateau summits. The vegetation is characterized by extreme dwarfism (Mark and Dickinson, 1997). Snowbanks occur only locally in the high alpine zone, where snow accumulates and persists well into summer. They also show very adapted vegetation, which differs greatly even locally. For more information on the New Zealand alpine zone refer to Mark and Dickinson (1997).

There are several hypotheses why plants evolved hygrochastic capsules; most of these stem from the investigation of species in arid regions. Gutterman (1994) argues that the seeds are protected in the capsule against drought and seed predators, both of which cause seed mortality in deserts. Capsules open only after sufficient rain has fallen and dispersal is therefore restricted in time to adequate rainfall events. The seeds are then dispersed and are able to germinate quickly in the now wet and favourable environment. Other authors also favour temporal restriction as the prevalent hypothesis for the evolution of hygrochastic dehiscence (Ellner and Shmida, 1981; Parolin 2001, 2006; Walck and Hidayati, 2007).

Van Rooyen (1990) classifies hygrochasy as an antitelechoric dispersal strategy, where dispersal is not only restricted in time but also space. According to Murbeck (1919, 1920) and Zohary (1937, 1962), desert species might have developed hygrochastic capsules as an adaptation to the harsh conditions of their environment with high seed mortality and germination success only in rare favourable sites. With

an antitelechoric dispersal strategy the chances of the seeds landing and germinating in a suitable habitat are greatly increased. Especially in southern Africa, the plants are highly adapted to their specific habitat conditions. These can often only be found in small patches, which occur in a highly heterogenous environment of different habitat types (Eccles et al., 1999). The work of Ihlenfeldt (1983), Poppendieck (1995) and Parolin (2006) supports this hypothesis. However, most reports are anecdotal and few investigations have been made regarding dispersal distances in hygrochastic plants (but see Parolin (2001) and Nakanishi (2002)).

For the first time this study aims to apply the traditional hypotheses for hygrochasy in arid regions to hygrochastic species in alpine New Zealand. In alpine areas, reproducing with seeds is a high-risk strategy (Urbanska and Schultz, 1986; Koerner, 1999; Zoller and Lenzin, 2004). The seeds are subjected to numerous threats such as freezing temperatures, unsuitable germination conditions, formation of needle ice or insufficient soil conditions. Safe sites, with favourable conditions for seedlings, such as soil micro-topography, moisture, light conditions or temperature are highly advantageous in successful germination (Harper, 1977). Shelter from adult plants or seedlings dispersed in groups to form mutually sheltering clumps can also create safe sites (Zoller and Lenzin, 2004, 2006).

We propose that hygrochastic capsules of New Zealand alpine *Veronica* enforce short distance dispersal, which greatly increases the chance of the seed to land in a safe site within the parental habitat. We tested this by (1) measuring dispersal distances of several hygrochastic *Veronica* under laboratory and natural conditions, and (2) by comparing habitat patch sizes of hygrochastic *Veronica* with their non-hygrochastic relatives.

3.2.3 MATERIALS AND METHODS

3.2.3.1 Dispersal distance

Veronica cheesemanii, *V. densifolia*, *V. thomsonii*, *V. spathulata* and *V. planopetiolata* were used in this experiment. All five species have been confirmed as hygrochastic by their anatomy, morphology and behaviour, with *V. cheesemanii* and *V. thomsonii* representing cushion plants, whereas *V. densifolia*, *V. spathulata* and *V. planopetiolata* are creeping subshrubs (Pufal et al., 2010).

The fruiting period of alpine *Veronica* is relatively short and varies greatly between locations (pers. obs.). We assumed that seeds were dispersed as soon as the capsule was ripe and rainfall occurred. In order to collect capsules of hygrochastic species still containing seeds, ten individuals of *V. thomsonii* and *V. densifolia* were covered with inverted plastic petri dishes to prevent rain from dispersing seeds. These were lined with shade cloth to limit sun damage, since the plastic of the petri dishes could potentially intensify the sunlight. The petri dishes were high enough that the plants were not harmed and air could move freely. This was done in spring, shortly after pollination and when plants were displaying unripe fruits.

In autumn, intact capsules from covered plants were collected and used *in situ* as follows. Capsules were mounted 2 cm above ground facing up (similar to their height *in vivo*) and were placed in the middle of a white sheet (2.5 × 2.5 m). Water was dropped from a height of 175 cm, simulating rain (Parolin 2001). For a subset of the species the point pattern of expelled seeds on the sheet was noted in Cartesian coordinates (Cousens et al., 2008) and their distance from the capsule measured. Capsules of *V. cheesemanii*, *V. planopetiolata* and *V. spathulata,* which were found still containing seeds either in the field or in herbarium samples, were also included in this experiment. Additionally, this experiment was carried out *in vivo*, i.e. the same

set-up was used outside in natural rain for a capsule of *V. densifolia*. However, due to the difficulties in terrain and under harsh weather conditions, the *in vivo* experiments were abandoned for other species.

The measured dispersal distances were used to create dispersal curves to show the frequency distribution of distances and densities. Here, distances of all seeds were used, even if seeds dispersed in clumps of two or more. Seed shadows were created by using the Cartesian coordinates. If several seeds were dispersed with one raindrop, they were only assigned one set of coordinates but their distance measurements were counted individually. The centroid was calculated to capture direction of dispersal. Descriptive statistics and comparisons between species were carried out using R (R Development Core Team, 2005).

3.2.3.2 Habitat patches

Here, we define habitat patch as the area occupied by the respective investigated species, which is clearly different from the surrounding area. Two different methods were used to assess patch size and occupancy. These are outlined below as Method 1 and Method 2.

Method 1:

Patch size was measured in 13 locations for four hygrochastic species (*V. densifolia*, *V. cheesemanii*, *V. pulvinaris*, *V. thomsonii*) (Appendix 3.2.1). Patches where hygrochastic species could be found were mostly very different from the surrounding vegetation and easy to distinguish. The overall size of the patch was estimated using two perpendicular distances through the patch where the species was found and then calculating the area as an ellipse (Fig. 3.2.1). Following Green (1983) we view the patch as safe site since all individuals of the given species could be found inside the

patch, showing this habitat to be suitable for the species and surrounding area likely to be unsuitable. For comparison, patch size was also measured for five locations of non-hygrochastic species, using the same method (Appendix 3.2.1). Here, the habitat occupied by the respective species was not always easily distinguishable from the surrounding habitat. If the habitat patch was bigger than 100 m in length, we used 100 m as cut-off point and thus our estimates might be too small. Hygrochastic and non-hygrochastic species were compared using Mann-Whitney-U tests.

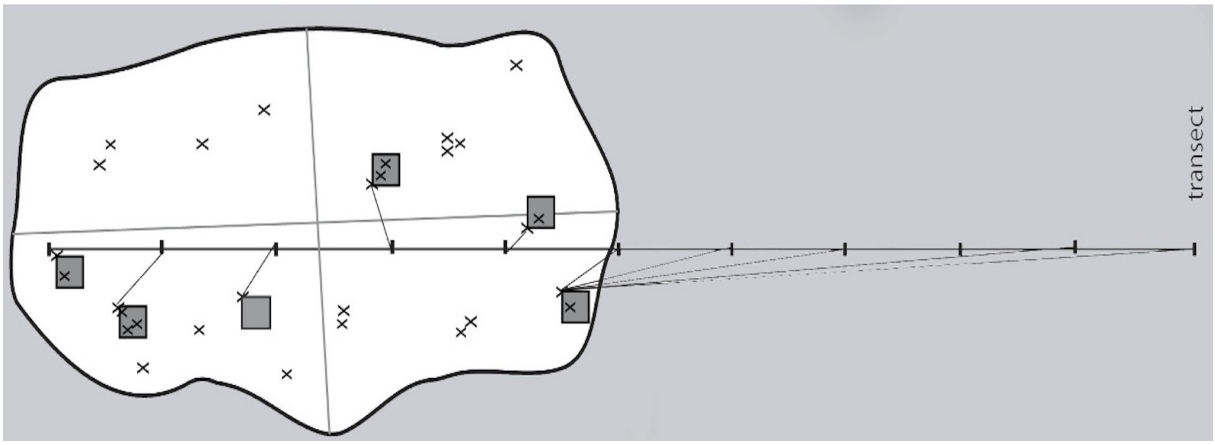

FIG 3.2.1 The sample design for habitat measurement. Distances from fixed points along a 100 m transect (starting at the first individual in the habitat patch) to nearest individuals (x) within a habitat (white area) were measured. Individuals within a $1m^2$ (grey square) were counted and the habitat patch size was estimated using two perpendicular measurements (grey lines) from the borders of the habitat patch to the surrounding habitat (grey area). Note that this drawing is not scaled.

Method 2:

Especially for non-hygrochastic species with large habitat patches, the estimation of patch size area might bias results, therefore we used a different approach to assess patch site and occupancy. Once a patch containing the respective species was identified, a 100 m transect was aligned with the long axis of the population, starting at one outermost individual. Beginning with 0 m and ending with 100 m, 11 points

were fixed along the transect in 10 m steps and the distance from each point to the nearest plant individual was measured (Fig. 3.2.1). The first point at 0 m was excluded, since it was the starting point of the transect and therefore 0. This method was used at all locations for both hygrochastic and non-hygrochastic species. Mean distances from transect points to nearest individuals for each population were compared between hygrochastic and non-hygrochastic species using Mann-Whitney-U tests. From the mean distances we can determine those populations that occupy a small habitat patch. When transect points lie outside a habitat patch, the measurements to plant individuals lead back into the habitat patch, resulting in bigger mean distances between transect points and individuals (Fig. 3.2.1).

To ensure that large mean distances did not result from large populations with single, randomly dispersed individuals, quadrats (1 m^2) were laid out parallel to the transect and individuals within it were counted (Fig. 3.2.1). Nearest individuals to a transect point were used as corner point, resulting in a maximum of 11 quadrats per transect. If habitats were smaller than the transect, a smaller number of quadrats were used, since each quadrat was only counted once.

Densities within the quadrats were compared between hygrochastic and non-hygrochastic species using Mann-Whitney-U tests. Statistical analysis was carried out using R (R Development Core Team, 2005).

In five locations of hygrochastic species, nearest neighbour measurements were carried out to identify the dispersal pattern (Appendix 3.2.1). We used the t-square index (Ludwig and Reynolds, 1988) for ten random points per location. They were chosen by throwing a marker with closed eyes in different directions from the estimated middle of the population. From this marker, the distance to the nearest individual was measured (x_i) and from this individual the distance to the nearest

neighbour was measured as well (y_i). The index of spatial pattern (C) was calculated to find out if the distribution of individuals was random (C = 0.5), uniform (C << 0.5) or clumped (C >> 0.5), using Ludwig and Reynolds (1988) formula. To test for significance, z was calculated and compared with p from a probability table for standard normal distribution (z = 1.96 at p = 0.05).

3.2.4 RESULTS

3.2.4.1 Dispersal distance

In the dispersal distance experiments, seeds were not analysed per capsule but per respective species. Since *Veronica densifolia* and *V. thomsonii* were purposely prepared in the field for seed collection, they retained most of their seeds and have therefore higher seed numbers than *V. cheesemanii, V. spathulata* and *V. planopetiolata* (Table 3.2.1). The furthest dispersal event recorded was for *V. densifolia*, whereas *V. cheesemanii* shows the lowest dispersal distance. Combined, all species disperse their seeds exclusively over a short distance (Table 3.2.1).

Table 3.2.1 Descriptive statistics for dispersal distances [cm] of seeds from 5 hygrochastic *Veronica* species.

Species	#	min	max	med	mean	sd
V. densifolia in situ	208	0.3	110.5	11.85	12.63	9.15
V. thomsonii	55	0.5	38.7	8	10.01	8.79
V. cheesemanii	9	1	12	6	5.54	3.39
V. spathulata	63	0.3	54.9	5.9	10.69	13.54
V. planopetiolata	8	1	23	19.5	15.81	7.59
total	342	0.3	110.5	8.95	13.57	14.52
V. densifolia in vivo	14	3.5	55.2	35	32.16	16.44

A Kruskal-Wallis test showed significant differences in dispersal distances among the hygrochastic species (chi-squared = 15.34, p = 0.005) and subsequently, Mann-Whitney-U tests were conducted between all species. *V. densifolia* and *V. planopetiolata* dispersed their seeds significantly further than the other species (Fig. 3.2.2).

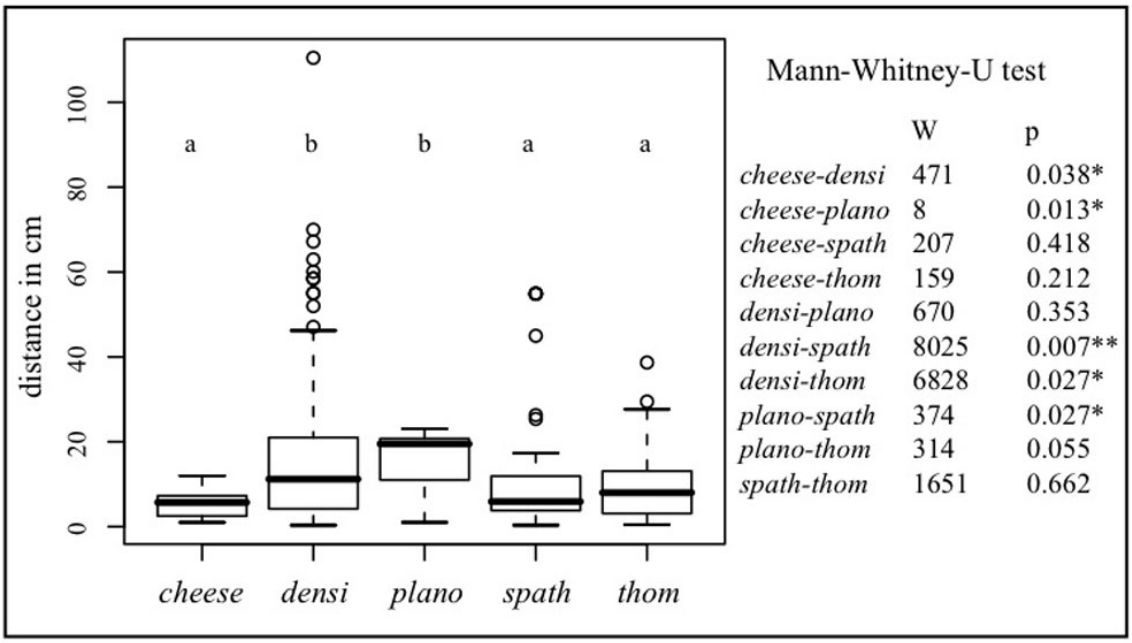

FIG 3.2.2 Boxplots of dispersal distances for 5 hygrochastic *Veronica* and results for Mann-Whitney-U tests between species. Significantly different species are marked with "a" and "b" in the figure. (*V. cheesemanii = cheese, V. densifolia = densi, V. planopetiolata = plano, V. spathulata = spath, V. thomsonii = thom*)

One capsule of *V. densifolia* was used in an outdoor experiment under rain conditions (*V. densifolia* ***in vivo***), which resulted in the dispersal of 14 seeds. Under those conditions, *V. densifolia* dispersed significantly further than under laboratory conditions (Mann-Whitney-U: $W = 382$, $p < 0.0001$).

Frequency distribution and density distribution curves were created for *V. densifolia*, *V. thomsonii* and *V. spathulata* (Fig. 3.3.3). The histogram of the frequency distribution shows that all three species disperse more than 50% of their total seeds less than10cm from the source. All three species follow a Weibull function with a short tail.

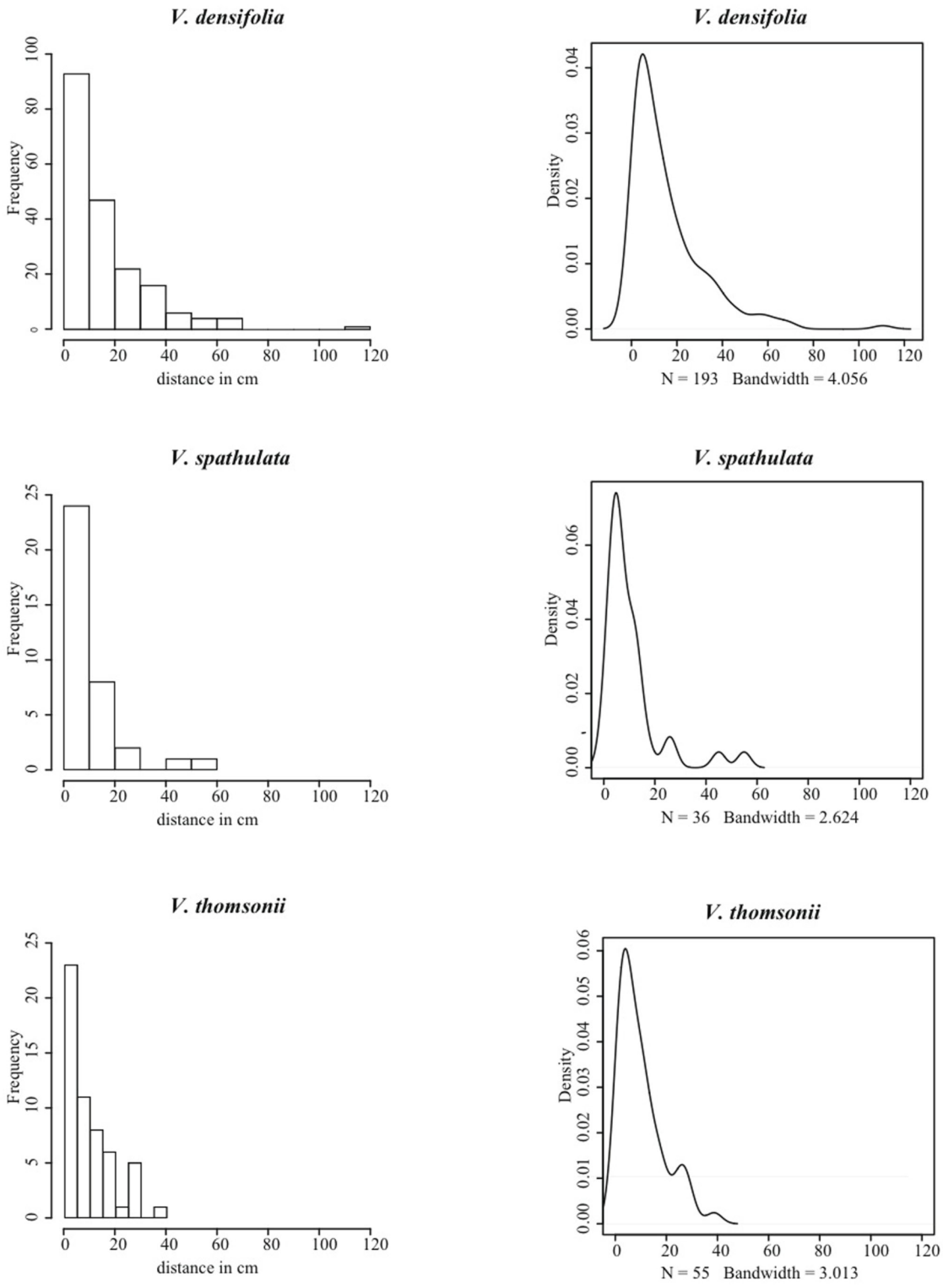

FIG 3.2.3 Histogram of the frequency distribution (A) and line graph of the density distribution (B) of seeds of *V. densifolia, V. spathulata* and *V. thomsonii*. The height of each histogram column represents the number of seeds found within a range of distances (for *V. densifolia* and *V. spathulata* 10 cm per column, *V. thomsonii* 5 cm per column).

Cartesian coordinates from *V. densifolia* and *V. thomsonii* were used to visualize the seed shadow, showing again dispersal distances but also the directions in which seeds were dispersed as well as the 25 %, 50 % and 75 % quartiles as circles around the source (Fig. 3.2.4). The net movement of the seeds away from the source can be measured by the position and distance of the centroid (mean of Cartesian coordinates).

The centroids of *V. thomsonii* and *V. densifolia* ***in vitro*** are 0.81 cm and 1.91 cm away from the source, respectively, whereas the centroid of *V. densifolia* from the outdoor experiment is 32.41 cm away from the source and located in the first quarter (Fig. 3.2.4).

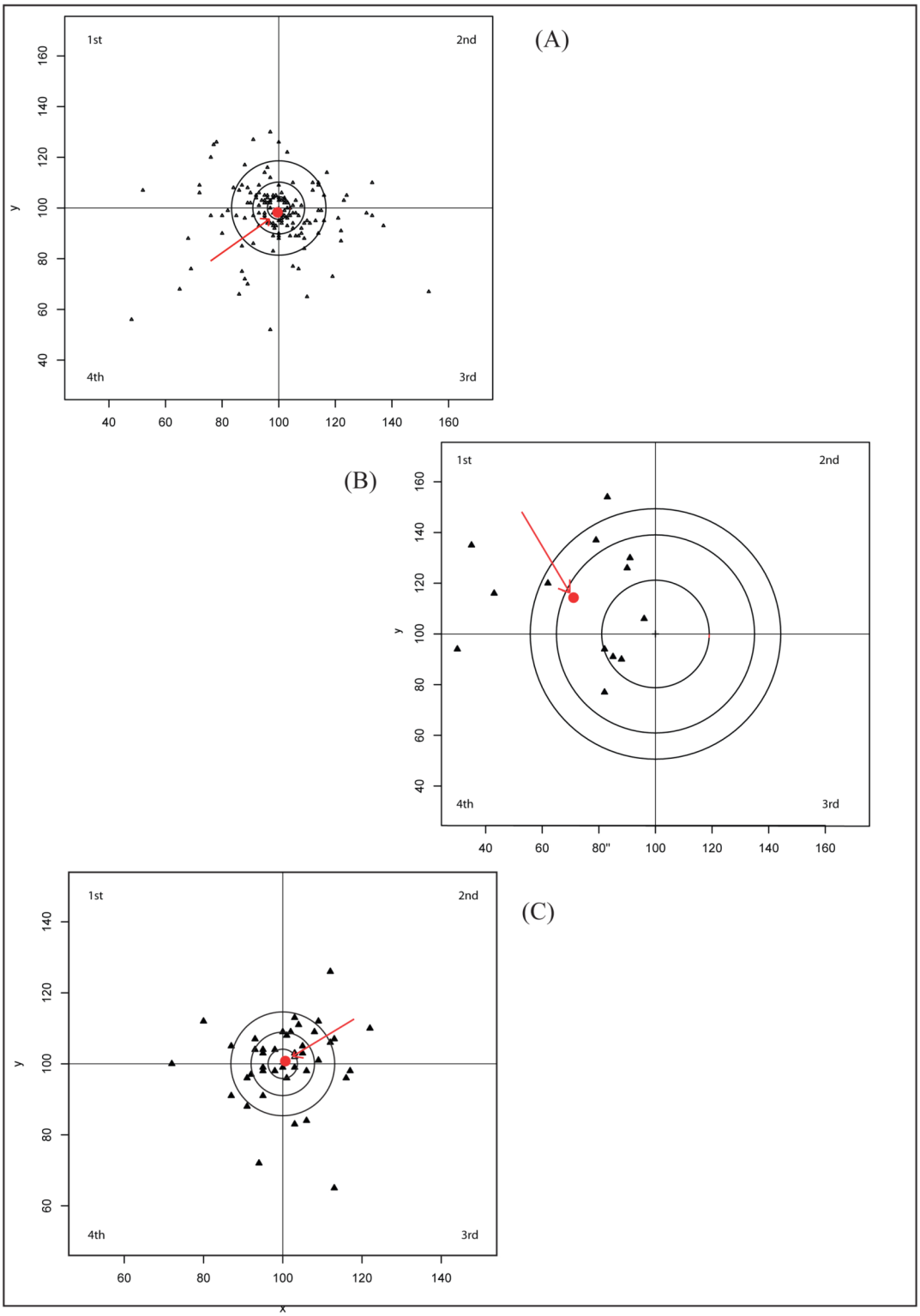

FIG 3.2.4 The seed shadow of *V. densifolia* in situ (A) and in vivo (B) and of *V. thomsonii* (C). The seed source is located at x = 100, y = 100, seed locations are triangles and the centroid is a red circle, pointed out by a red arrow. The area around the source was split into four quarters. Concentric circles show the 25 %, 50 % and 75 % quartiles of dispersal distances from the source. Note that C is scaled differently from A and B.

3.2.4.2 Habitat patches

The habitat patches of hygrochastic species used in this study were between 40 m^2 and 750 m^2 in area, whereas patches of non-hygrochastic species were significantly larger (Mann-Whitney-U: W = 11, p = 0.038) between 270 m^2 and 3920 m^2 (Table 3.2.2). In hygrochastic species, no individuals of the respective species were found outside the habitat patch, but habitats at Arthur's Pass Village, Kea Point, Otira Valley and Fox Glacier were bigger than the 100 m cut-off distance for the patch size. Individuals outside the measured area were not taken into account.

Table 3.2.2 Locations and sizes of habitat patches (area) for hygrochastic species (BOLD) and non-hygrochastic species (detailed location vouchers in appendix 1), mean distance between transect point and random individuals as well as mean number of individuals per quadrat (#).

species	location	habitat area	transect distance	quadrat #
V. cheesemanii	Mt Robert Skifield	314.16	21.5	1.8
V. cheesemanii	Mt Robert Ridgetrack	746.13	2.68	3.8
V. cheesemanii	Mole Tops scree	39.27	50.01	2.5
V. cheesemanii	Mole Tops	530.14	27.28	1.2
V. densifolia	Waiorau Snow Farm	628.32	2.18	2
V. densifolia	Coronet Peak	39.27	39.87	3.5
V. densifolia	Remarkables Skifield	196.35	23.34	6.4
V. pulvinaris	Mt Robert Skifield	471.24	23.33	7
V. pulvinaris	Mole Tops	39.27	45.06	2.5
V. pulvinaris	Mt Robert Ridgetrack	157.08	23.58	4.4
V. thomsonii	Remarkables Skifield	219.91	24.47	2.5
V. thomsonii	Remarkables Skifield	471.24	6.09	2
V. thomsonii	Remarkables track	58.90	50.4	3.5
V. decora	Arthurs Pass village	1570.80	0.43	5.8
V. decora	Kea Point, Mount Cook Village	3926.99	0.13	16
V. lyallii	Klondyke corner Arthurs pass	274.89	9.02	7.8
V. lyallii	Otira valley	392.70	2.37	5.5
V. lyallii	Fox Glacier walking track	2356.19	1.4	6.3

When comparing mean distances from points on the 100 m transect to the nearest individual, hygrochastic species showed significantly longer distances than non-hygrochastic species (Mann-Whitney-U: $W = 5510.5$, $p < 0.0001$). Comparing the number of individuals within quadrats in each location, non-hygrochastic species had significantly more individuals per square (Mann-Whitney-U: $W = 798$, $p < 0.0001$). All squares contained on average more than one individual, which excludes the possibility that large measured distances are due to the occurrence of single widespread individuals.

The t-square index revealed that individuals showed a clumped distribution in all tested sites (Table 3.2.3) except for *V. cheesemanii* at Mt Patriarch, where z is not significant.

Table 3.2.3 The t-square index of nearest neighbour measurements of hygrochastic *Veronica*. For detailed location description see Appendix 3.2.1. Significance is indicated with *.

species	location	C	z
V. cheesemanii	Mt. Patriarch	0.535	0.385
V. cheesemanii	Mt Robert Skifield	0.824	3.546***
V. densifolia	Remarkables Skifield	0.858	3.926***
V. densifolia	Blue Lake	0.875	4.104***
V. pulvinaris	Mt Robert Skifield	0.734	2.561*

3.2.5 DISCUSSION

In this study we tested whether hygrochastic capsules in New Zealand alpine *Veronica* restrict seed dispersal to small safe sites. We were able to confirm in laboratory tests that seeds from hygrochastic capsules only disperse over a short distance. Falling raindrops transport seeds for short distances depending on the raindrop's size (Nakanishi, 2002). In dry weather the capsules stay closed, therefore raindrops become the only available primary dispersal agent. Hygrochastic species

can be found in scree, on rocky surfaces or gravel (Mark and Adams, 1995; Garnock-Jones and Lloyd, 2004; Meudt, 2008). These habitats show a microtopography of small cracks, crevices (rocky surfaces) or small gaps and depressions between stones (scree and gravel), which can trap seeds very easily. Therefore, secondary dispersal is highly unlikely and the dispersal distances achieved with raindrops are probably the absolute dispersal distances. The growth form of hygrochastic *Veronica* also complies with findings by Van Rooyen (1990). She states that most hygrochastic plants in Namaqualand, South Africa are chamaephytes or hemicryptophytes with antitelechory as the prominent dispersal strategy and simple capsules restrict dispersal spatially as opposed to temporally in more complex capsules. Hygrochastic alpine *Veronica* in New Zealand are hemicryptophytes with simple capsules and our data support antitelechory as the primary dispersal strategy.

Although there were differences among species regarding dispersal distance, all distances achieved were less than one metre. The probability density function is a Weibull function with a short tail. In the laboratory the majority of seeds landed very close to the parent plant and dispersed evenly around the source. However, results of an outdoor study of *V. densifolia* indicate that dispersal can become directional depending on the direction of wind and rain. These data suggest that under natural conditions seeds are not dispersed directly next to the parent plant (as was seen under laboratory conditions), preventing possible parent-offspring competition. Nevertheless, the maximum dispersal distance did not increase under natural conditions, confirming exclusive short-distance dispersal as seen in the laboratory studies.

We established that hygrochastic alpine *Veronica* disperse following an antitelechoric strategy as an adaptation to habitats that are small in area and surrounded by stressful environments with harsh conditions for seedling survival. The surrounding area is often strikingly different, densely vegetated and has a very different species composition (Fig 3.2.5).

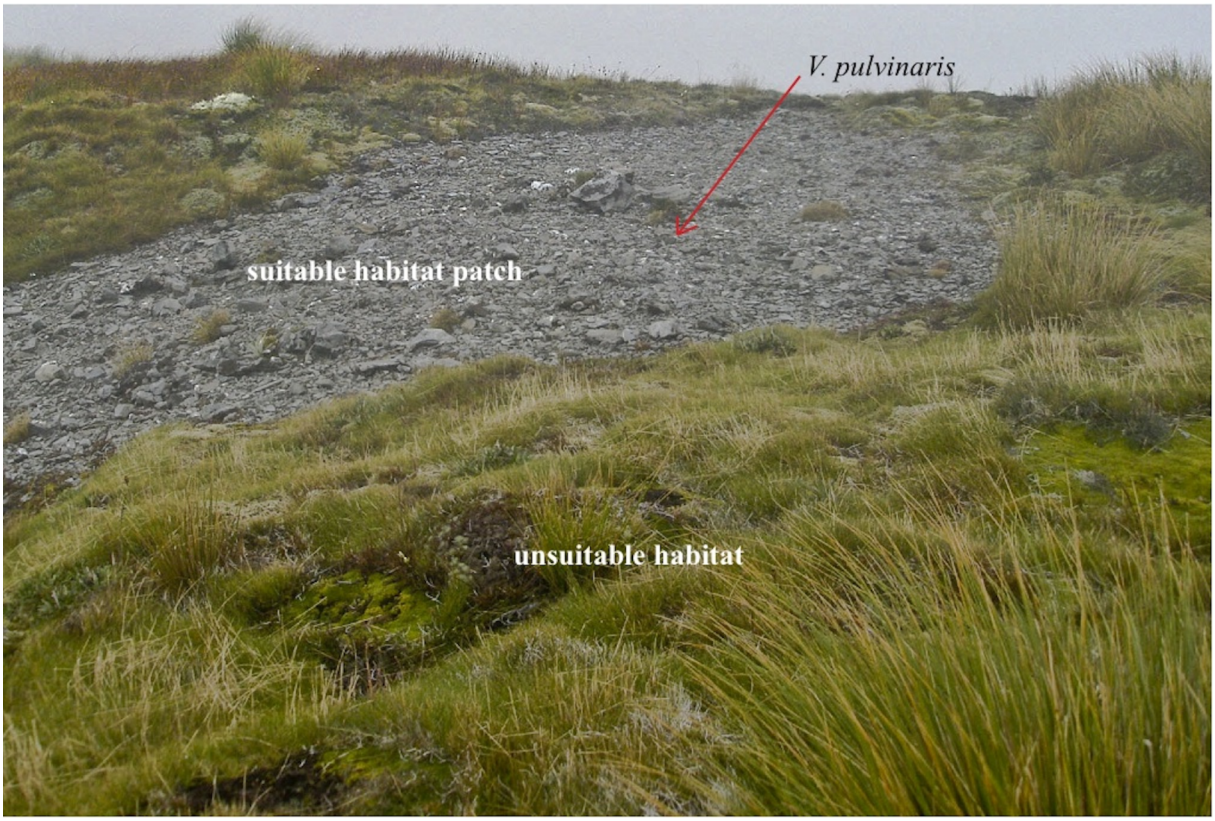

FIG 3.2.5 Habitat patch occupied by *V. pulvinaris* and *V. cheesemanii* on Mole Tops (see Appendix 3.2.1 for location details). One *V. pulvinaris* individual is pointed out with a red arrow, individuals of *V. cheesemanii* are too small and of a grey colour and therefore hard to see.

Our measurements of patch sizes show that they are indeed very restricted, compared to habitat sizes of related non-hygrochastic species. Comparing the distance that can be travelled by seeds dispersed from one plant with the available habitat patch, hygrochasy seems to be a very effective dispersal strategy because it limits seeds to

the suitable site over many generations, sustaining the existing population in that patch. Although most alpine plants are dispersed over a short distance only by wind (Spence, 1990) hygrochastic species have the advantage of avoiding multiple chance dispersal events in very windy conditions by restricting the dispersal to a few rainfall events and protecting the seeds in a closed capsule before dispersal.

There seem to be several reasons why the habitat patches are especially suitable for the species we investigated. Adult individuals seem to be able to survive only within the patch. The complete absence of adult individuals from habitats outside the patch strongly suggests that even occasional dispersal events are most likely not successful. However, we did not investigate the seed bank in adjacent habitats and we also have no evidence that seedlings are unable to survive in habitats other than their parent habitat. We assume that the habitat patch of the extant population has suitable conditions both for seedlings and adults.

Additionally, microsites for seedling establishment seem to be confined to the habitat patch in which adult individuals were found, which by itself is small as well. Dispersal curves with a short tail have been shown to be the most efficacious in this scenario (Geritz et al., 1984). The habitat patches in which the hygrochastic study species are found are usually small, mostly bare and covered in small rocks or scree (pers. obs.). Plants found in those patches are either cushions of the same or another species. The edaphic conditions provide a suitable microtopography for seedlings, providing shelter and reducing the risk of interspecific competition due to the low vegetation density (Chambers et al., 1991; Jumpponen et al., 1999), whereas the adult cushion plants provide sheltered microsites, which support successful recruitment (Zoller and Lenzin, 2004). Choler et al. (2001) found that the effect of neighbours in high altitude environments is facilitation rather than competition. Zoller and Lenzin

(2004) also defined the "incorporating safe site strategy", by which seeds germinating near the border of an adult cushion are included, resulting in a large cushion in a short time. Alpine *Veronica* seem to follow that strategy, which is shown by short dispersal distances in our experiment along with our observations that cushions grow directly next to each other in the field. It has also been shown that dispersal in groups of seeds has a positive effect on recruitment due to the sheltering influence of other seedlings (Carlson and Callaghan, 1991; Moen, 1993; Callaway et al., 2002; Zoller and Lenzin, 2004, 2006). In fact, seeds of hygrochastic *Veronica* often disperse in clumps of two to eight seeds (unpubl. data) and the t-square index showed a clumped distribution of the plants.

Even though hygrochasy restricts the seed dispersal of alpine *Veronica* to small habitat patches, these plants can be found across mountain ranges in New Zealand, albeit confined to certain areas. However, two species have dispersed from New Zealand to Australia (*V. ciliolata* and *V. densifolia*, see Meudt, 2008). This is most likely due to chance secondary dispersal, since rare long distance dispersal is more often not related to the primary dispersal (Higgins et al., 2003). In case of hygrochastic *Veronica* the cause of chance long-distance dispersal might be exceptionally strong winds during rainfall events, which might carry the lightweight seeds further. We also discovered that seeds of *V. densifolia* develop mucilage when wet, which might adhere to feet and feathers of birds, as is the case in arctic and alpine *Bartsia* (Molau, 1990). This might also explain the dispersal across the Tasman Sea. But again we need to stress that long distance dispersal events are probably very rare, which is reflected in the generally narrower distributions of hygrochastic species of *Veronica* compared to their non-hygrochastic relatives across New Zealand (see maps in Garnock-Jones and Lloyd, 2004; Meudt, 2008).

By comparing habitat patch size and effective dispersal distances of hygrochastic and non-hygrochastic species of *Veronica*, we have shown for the first time that hygrochastic capsules and rain-dispersed seeds of alpine cushion plants can effectively restrict dispersal to safe sites. Our work supports the directed dispersal hypothesis (Howe and Smallwood, 1982), according to which the dispersal agents transport the seeds to non-random places that provide suitable conditions for establishment and growth. Habitat patches in which hygrochastic *Veronica* can be found provide safe microsites for seedlings not only with adequate microtopography but also with existing adult cushion plants as incorporating safe sites. This work is in accordance with hypotheses developed for hygrochastic plants in arid regions but until now was never actually investigated in hygrochastic taxa of any habitat type.

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Chapter 3.3

Do Hygrochastic *Veronica* inhabit Restricted Ecological Niches?

3.3.1 ABSTRACT

In the Aizoaceae more than 98% of species possess hygrochastic capsules, making this family the most widely known example of hygrochasy in arid environments. Another striking phenomenon is the distribution of most taxa; the areas single species or even entire genera inhabit are very small; they sometimes only cover a few square kilometres. One can interpret this as a high degree of ecological specialization, with species existing in very narrow ecological niches. The dispersal strategy of restricting dispersal to raindrops as dispersal agents and therefore limiting the seed transport close to the parent plant might explain the distribution pattern in Aizoaceae.

Hygrochastic *Veronica* are mostly found in high alpine areas of New Zealand, where they seem to be restricted to small habitat patches with specific characteristics. The aim of this study is to investigate whether hygrochastic *Veronica* have a narrow ecological niche compared to closely related non-hygrochastic species. The environmental amplitudes of hygrochastic and non-hygrochastic species are compared using data from Land Environments of New Zealand (LENZ IV) based on locations extracted from three herbarium databases.

There is convincing evidence that the ecological niche for hygrochastic *Veronica* is narrower in some environmental variables than for non-hygrochastic relatives, by definition making the hygrochastic species specialists.

This work strongly supports the hypothesis that hygrochasy spatially restricts seed dispersal for plants with little variation in their environmental niches and small habitat patches. The use of GIS based data for small habitats is discussed.

Keywords: ecological niche, GIS, hygrochasy, LENZ IV, specialists, range, *Veronica*

3.3.2 INTRODUCTION

In his investigation of Aizoaceae (as Mesembryanthemaceae, see Bittrich and Hartmann (1988) for taxonomic details) Ihlenfeldt (1983) stressed that most hygrochastic species only occur in very small areas and sometimes even entire genera might be confined to only a few square kilometres (Ihlenfeldt, 1978). He interpreted this as a high degree of ecological specialization, i.e. these species are only able to compete in a very narrow ecological niche.

The succulent Karoo Biome is a region in southern Africa with a remarkably high level of endemism, which has led to its recognition as one of 25 global biodiversity hotspots (Myers et al., 2000). The majority of Aizoaceae are found there (Hilton-Taylor, 1994; Cowling et al., 1998); this family comprises mostly hygrochastic species. Possible explanations for the level of endemism are smallscale habitat heterogeneity (Juergens, 1986), climatic fluctuations in recent geological time frames (Midgley et al., 2000), peculiar substrates (Cowling and Hilton-Taylor, 1997) and incredibly fast speciation amongst the Aizoaceae (Ihlenfeldt, 1994; Klak et al., 2004).

Another arid region in southern Africa with high endemism is the "Sperrgebiet"; most endemics are Aizoaceae with canopy seed storage and short-range dispersal, i.e. hygrochastic species. Most of the habitat patches in these areas are small and mosaic-like distributed and the plants are highly adapted to their specific habitat conditions (Eccles et al., 1999). Again, hygrochastic species in this area can be described as specialists with a narrow ecological niche.

The niche concept is essential in ecology. The "concluding remarks" by Hutchinson (1957) were probably the most important contribution to the concept. Hutchinson (1957) describes the ecological niche as an n–dimensional hypervolume where the axes represent environmental variables, i.e. resources. Along each of the axes a

population or species can display a wider or narrower tolerance. Specialization and generalization can then be defined with reference to certain axes, i.e. environmental variables (Futuyama and Moreno, 1988). Ecological specialization can be defined as a restricted ecological niche width of a given species (Futuyama and Moreno, 1988).

Another important aspect of the niche concept is the distinction between realized and fundamental (i.e. potential) niche (Hutchinson, 1957). The realized niche represents the niche that is actually occupied by a species or population whereas the fundamental niche is the niche where the species can potentially be found because it fulfils all requirements the species has of its niche space. However, the concept of the fundamental and realized niche is often only applied to the physical space a species occupies since other factors such as food resources or competition are hard to measure as axes in the niche space (Colwell and Rangel, 2009).

In general, the quantitative measuring of niche width can be difficult (Colwell and Futuyama, 1971; Feinsinger et al., 1981) and more often than not literature resorts to non-quantitative contrasts between specialists and generalists.

Estimating a niche based on presence data can be cheap and effective (Soberon and Peterson, 2005; Pearson et al., 2007; Soberon, 2007) and one way of gauging niche width. However, this approach might also be regarded as merely describing the characteristics of an environment that species may occupy for several reasons (Kearney, 2006).

In Chapter 3.2 I explored the hypothesis that hygrochasy is an adaptation of alpine *Veronica* to direct dispersal to safe sites within a small habitat patch. This study was carried out on the basis that a similar hypothesis was developed for hygrochastic species in arid areas (predominantly Aizoaceae) (Ihlenfeldt, 1983). From now on I will refer to this hypothesis as ‘spatial restriction hypothesis’. However, this

hypothesis is extended for desert species as it also states that most of them have a very small ecological niche and that hygrochasy is a strategy that supports the maintenance of populations in small areas that represent the ecological niche (Ihlenfeldt, 1983).

There are some astounding similarities between hygrochastic Aizoaceae and hygrochastic *Veronica* in New Zealand. The areas where both taxa can be found show a high degree of heterogeneity (Mark and Dickinson, 1997; Eccles et al., 1999) and most of the *Veronica* species are localized or are endemic to a small region (although *V. ciliolata* and *V. densifolia* show dispersal across the Tasman Sea, but they occupy small regions in both Australia and new Zealand (Meudt, 2008)). Lastly, there is evidence for recent speciation in both the Aizoaecae (Klak et al., 2004) and *Veronica* (Wagstaff et al., 2002; Albach and Meudt, 2010). It seems therefore plausible that both aspects of the spatial restriction hypothesis apply to hygrochastic *Veronica* in alpine New Zealand.

This study attempts to identify the breadth of the ecological niche of hygrochastic *Veronica* and compare it to related non-hygrochastic species. I predicted that alpine hygrochastic *Veronica* are specialists that occupy only specific habitats, which are restricted to certain areas. I tested this by comparing them to their non-hygrochastic relatives to see if they show a small ecological amplitude. If so, hygrochasy might be a dispersal strategy that restricts dispersal to sites within the specific habitats.

The question remains whether the localized extent of hygrochastic *Veronica* is a result of restricted short-distance dispersal or whether the restricted dispersal ability of those species results from their specialized habitats. To answer this question the use of the fundamental niche gives valuable information, i.e. is the distribution of hygrochastic *Veronica* limited by their fundamental niches or their dispersal ability?

Presence-only data from herbarium or museum records is increasingly being used for predictive species distribution modelling (Elith et al., 2006; Elith and Leathwick, 2007; Miller et al., 2007; Loarie et al., 2008; Loiselle et al., 2008) and might therefore also be a valuable tool in comparing the distribution of study species in this work. The Land Environments of New Zealand database IV (LENZ IV) is an environmental classification of New Zealand with numerical data layers that describe multiple aspects of the country's climate, landforms and soils and it also uses a computerised classification procedure, which can identify similar environments (Leathwick et al., 2002). The variables that were used in the data layers were selected using results from an extended study of relationships between forest patterns and environments, including plot data, climate estimates from long-term meteorological station data and soil data from the New Zealand Land Resource Inventory. LENZ level IV has the highest standard of classification detail and contains 500 environments nationally.

For this study, environmental data contained in LENZ IV are used as measurements for the niche width, based on location points from the herbarium databases as presence data. This chapter will also be used as a platform to discuss the use of LENZ as a tool to measure ecological niche width of specialist species.

3.3.3 MATERIAL AND METHODS

In this study we investigate *Veronica* species from the informal speedwell hebe and snow hebe groups (for phylogeny and groups see Albach and Meudt, 2010). Of these, 15 have been identified as being hygrochastic (Pufal et al., 2010) whereas 8 are classified as 'ripening dehiscent' (here referred to as non-hygrochastic).

Databases comprising 22 *Veronica* species were obtained from New Zealand's three large herbaria WELT, AK and CHR (for herbarium acronyms see Thiers [continuously updated]). Double entries, unidentified specimen and specimen without location information were not considered and were omitted from future analysis. For each species in the combined database, the number of entries was counted and compared between the groups hygrochastic and non-hygrochastic using a Mann-Whitney-U test. The data were subsequently used to analyse environmental conditions and identify the ecological amplitude of species at those locations.

All databases were searched for entries with accurate coordinates (degrees, minutes, seconds) to find exact sites. Specimens with insufficient coordinates but with very specific location descriptions (e.g. 20 m south of trig, Mount Arthur) were also included and Google Earth 5.1 (http://earth.google.com) has been used to find correct coordinates.

For the analysis of environmental parameters and population locations, species with 20 or more accurate locations were included. Species for which we could not find this number of locations were omitted and for species with more than 20 locations a random set of 20 was chosen for analysis. The coordinates of each location were used in arcMap 9.3 (ESRI, 1999-2008) to obtain environmental characters from the Land Environment of New Zealand database (LENZ). LENZ level IV is the layer with the finest scale (25 m x 25 m) and the most accurate environmental description including

data for 16 environmental variables (Table 3.3.1). Locations were mapped and the range between the locations that were furthest apart was measured in a rough N-S orientation.

Table 3.3.1 List of variables obtained from the environmental layer IV of the LENZ database. The asterisk indicates constant variables excluded from analysis.

	Environmental Variables from LENZ (level IV)
1	Elevation (m)
2	Mean annual temperature (°C)
3	Minimal temperature of the coldest month (°C)
4	Mean annual solar radiation ($MJ/m^2/day$)
5	Winter solar radiation ($MJ/m^2/day$)
6	October vapour pressure deficit (kPa)
7	Water balance ratio
8	Soil water deficit (mm)
9	Slope (°)
10	Soil drainage (soil description)
11	Soil Age*
12	Chem_Limit*
13	Acid soluble phosphorus in the soil (Mg/100g)
14	Exchangeable calcium in the soil (Mg/100g)
15	Soil induration
16	Soil particle size (mm)

In LENZ IV, the specific combination of environmental variables for every selected point on a map is given a specific value number, i.e. each value number within LENZ IV stands for a specific habitat type. The number of values for the 20 locations for each species were counted and compared between the groups. If species within a group had more value numbers for the same number of locations it means that they occur in more different habitat types. Using LENZ IV, the total available potential habitat for each species was calculated and compared with the total area of New Zealand as well as between hygrochastic and non-hygrochastic groups.

For each species, the Coefficient of Variation (CV) of every environmental parameter among locations was calculated (CV = sd/mean) and general linear models were

conducted on these with dispersal group used as fixed factor to find significant differences between the groups.

Statistical analysis was conducted in SPSS 16.0 and R (SPSS Inc., 1989-2007; R Development Core Team, 2005).

3.3.4 RESULTS

The combined herbarium databases showed no significant differences between hygrochastic and non-hygrochastic species for the number of locations per species (Mann-Whitney-U test; W = 84.5, p = 0.056) (Table 3.3.2).

Table 3.3.2 number of locations per species in a combined database from WELT, AUK, CHR. **BOLD** species were used in further statistical analysis.

Hygrochastic species	locations	Non-hygrochastic species	locations
V. birleyi	24	***V. catarractae***	41
V. cheesemanii	43	***V. decora***	89
V. chionohebe	9	***V. hookeriana***	102
V. ciliolata	57	***V. lanceolata***	276
V. colostylis	36	***V. linifolia***	83
V. densifolia	70	***V. lyallii***	288
V. lilliputiana	28	*V. melanocaulon*	32
V. planopetiolata	8	*V. senex*	8
V. pulvinaris	109		
V. spathulata	36		
V. spectabilis	1		
V. thomsonii	54		
V. trifida	17		
V. zygantha	13		

Accurate locations appropriate for the use in arc GIS were found in six hygrochastic species and six non-hygrochastic species (Table 3.3.2). These were divided into two dehiscence groups (hygrochastic and non-hygrochastic), which were used in all following statistical analysis.

Each location in each species has a certain set of environmental variables assigned by arcGIS and is given a value number. The more value numbers are listed for a species, the more habitat types the species can be found in.

When comparing value numbers between hygrochastic and non-hygrochastic groups, hygrochastic species showed significantly fewer values compared to non-hygrochastic species (Mann-Whitney-U test; $W = 2.5$; $p = 0.016$*)(Table 3.3.3). It is evident that all hygrochastic species included occur exclusively at high altitudes, whereas the non-hygrochastic species were found over a wide range of elevations, from lowland to high alpine areas (Table 3.3.3). Since hygrochastic *Veronica* have a lower number of different habitats, the variation in some environmental variables might thus be restricted. General linear models were therefore conducted on the Coefficient of Variation, which was calculated from the means and standard deviation in Table 3.3.3.

Table 3.3.3 Environmental variables for 12 *Veronica* species extracted from LENZ IV. Given are mean with standard error, minimum, maximum and standard deviation. At the end of the table, the number of different values (habitat type) for each species is given. Hygrochastic species are shown in BOLD.

Species	Elevation (m)				Mean annual temperature (°C)				Minimal temperature of the coldest month (°C)			
	mean±se	min	max	sd	mean	min	max	sd	mean	Min	max	sd
V. cheesemanii	1438.3±45.97	1041	1836	205.56	4.97±0.25	2.8	7.2	1.1	-3.78±0.2	-4.6	-2	0.91
V. ciliolata	1247.35±41.5	1037	1768	185.58	4.96±0.17	3.2	6.3	0.76	-2.68±0.21	-4.3	-1.6	0.93
V. densifolia	1344.25±33.6	962	1674	150.25	4.44±0.18	3.1	6.6	0.79	-3.98±0.12	-4.3	-2.5	0.55
V. pulvinaris	1523.45±45.7	1208	1836	204.37	4.38±0.23	2.8	6.1	1.04	-4.1±0.11	-4.6	-2.9	0.51
V. spathulata	1518.1±74.82	1028	1827	334.61	5.25±0.41	3.6	8.1	1.84	-1.65±0.23	-2.5	0.1	1.02
V. thomsonii	1467.75±37.9	1347	1836	169.51	3.94±0.13	2.8	4.3	0.58	-4.24±0.04	-4.6	-3.8	0.2
V. catarractae	424.85±59.79	95	1290	267.4	8.19±0.27	4.5	10.2	1.22	0.5±0.21	-2.6	1.7	0.96
V. decora	904.45±77.09	292	1387	344.75	7.33±0.4	4.2	10.5	1.8	-2.28±0.25	-3.8	0.3	1.1
V. hookeriana	1154.3±63.05	867	1827	281.97	7.44 ±0.4	3.6	9.1	1.78	-0.06±0.33	-2.5	2.1	1.48
V. lanceolata	423.9±77.29	34	1330	345.65	11.32±0.42	6.1	14	1.87	2.7±0.38	-1.4	5.9	1.7
V. linifolia	993.8±81.09	292	1828	362.66	6.89±0.42	2.8	10.5	1.86	-2.3±0.29	-4.5	0.3	1.29
V. lyallii	812.25±87.73	159	1508	392.35	7.82±0.44	4.6	11.8	1.96	-1.46±0.36	-4.5	1.7	1.6
	Mean annual solar radiation ($MJ/m^2/day$)				Winter solar radiation ($MJ/m^2/day$)				October vapour pressure deficit (kPa)			
V. cheesemanii	14.1±0.12	12.9	14.7	0.52	4.63±0.05	4	5.1	0.24	0.19±0.01	0.11	0.27	0.05
V. ciliolata	12.62±0.13	11.9	13.7	0.6	3.61±0.12	3	4.5	0.53	0.1±0.02	0	0.24	0.08
V. densifolia	13.2±0.01	13.1	13.3	0.05	3.69±0.02	3.6	3.9	0.1	0.23±0.01	0.08	0.29	0.04
V. pulvinaris	13.66±0.1	12.9	14.7	0.43	4.5±0.07	3.6	5.1	0.32	0.2±0.02	0.02	0.33	0.07
V. spathulata	14.13±0.04	14	14.5	0.19	5.04±0.02	5	5.2	0.08	0.15±0.02	0.07	0.32	0.1
V. thomsonii	13.28±0.04	13.2	13.8	0.18	3.79±0.07	3.6	4.7	0.32	0.21±0.01	0.08	0.25	0.06
V. catarractae	12.2±0.05	11.8	12.7	0.23	3.15±0.05	2.8	3.8	0.21	0.15±0.02	0	0.27	0.07
V. decora	13.76±0.1	128	14.5	0.46	4.36±0.09	3.6	4.8	0.4	0.31±0.03	0.11	0.46	0.11

V. hookeriana	14.33±0.07	13.9	14.8	0.31	5.26±0.11	4.6	6.1	0.47	0.22±0.02	0.07	0.32	0.07
V. lanceolata	14.47±0.08	137	15	0.35	5.15±0.11	4.6	6.2	0.49	0.32±0.01	0.18	0.48	0.06
V. linifolia	13.5±0.16	11.9	14.7	0.71	4.17±0.1	3	4.8	0.45	0.23±0.03	0.02	0.45	0.13
V. lyallii	13.65±0.2	12	15.3	0.88	4.2±0.11	3.1	4.8	0.51	0.25±0.03	0.02	0.44	0.12
	Water balance ratio				Soil water deficit (mm)				Slope (°)			
V. cheesemanii	8.16±0.61	4.6	15.7	2.73	0.02±0.01	0	0.13	0.04	29.31±0.42	27.3	35.2	1.86
V. ciliolata	13.3±1.15	4.5	19.9	5.16	0.001±0.001	0	0.01	0.002	31.01±1.24	13.2	42.9	5.56
V. densifolia	4.94±0.33	3.4	10.4	1.46	0.22±0.06	0	0.9	0.26	15.45±2.09	7.3	29.9	9.35
V. pulvinaris	9.31±1.04	4.4	22.8	4.67	0.09±0.04	0	0.63	0.2	28.19±1.18	7.3	35.1	5.27
V. spathulata	7.51±0.46	4.1	9.3	2.07	0.11±0.05	0	0.56	0.23	18.43±1.37	9.5	24.4	6.11
V. thomsonii	5.75±0.49	4.4	10.4	2.21	0.12±0.03	0	0.25	0.12	15.87±2.18	7.3	29.9	9.74
V. catarractae	11.69±0.85	5.9	19.8	3.81	0.001±0.001	0	0.02	0.004	22.47±2.67	1.8	42.9	11.96
V. decora	5.05±0.49	1.6	9	2.2	19.12±8.4	0	141.57	37.59	16.66±2.54	0	31.3	11.36
V. hookeriana	6.05±0.34	4.1	9.3	1.51	0.23±0.08	0	0.79	0.34	19.17±1.58	5.7	27.8	7.06
V. lanceolata	4.02±0.24	2.5	6.8	1.05	11.22±4.36	0	78.69	19.48	13.51±1.96	0.2	26.3	8.78
V. linifolia	9.4±1.3	1.7	22.8	5.81	16.55±9.68	0	175.53	43.3	24.65±2.25	0	35.1	10.06
V. lyallii	8.28±1.08	3.1	19.9	4.84	7.07±4.31	0	75.39	19.27	23.03±2.28	0	33.6	10.21
	Soil drainage (soil description)				Acid soluble phosphorus in the soil (Mg/100g)				Exchangeable calcium in the soil (Mg/100g)			
V. cheesemanii	3.78±0.19	3	5	0.85	2.79±0.13	1.1	3	0.58	1.22±0.1	1	2.5	0.46
V. ciliolata	4.52±0.14	3	5	0.63	2.37±0.21	1	3.7	0.92	1.33±0.07	1	1.9	0.33
V. densifolia	3.9±0.21	3	5	0.93	3±0	3	3	0	1.71±0.08	1	2	0.35
V. pulvinaris	3.8±0.22	3	5	0.98	2.98±0.01	2.9	3	0.04	1.06±0.05	1	2	0.23
V. spathulata	4.85±0.03	4.7	5	0.15	3.06±0.24	1	3.8	1.09	1.39±0.05	1	1.6	0.24
V. thomsonii	4.06±0.22	3	5	0.99	2.99±0.01	2.9	3	0.03	1.72±0.08	1	2	0.35
V. catarractae	3.74±0.2	2.7	5	0.92	1.31±0.16	1	3.8	0.73	1.11±0.06	1	1.9	0.28
V. decora	4.24±0.34	0	5	1.53	2.96±0.25	0	4	1.11	1.21±0.13	0	2	0.56

V. hookeriana	4.76±0.07	4.1	5	0.33	2.1±0.23	1	3.8	1.01	1.28±0.06	1	1.7	0.29
V. lanceolata	4.65±0.2	1.9	5	0.9	1.99±0.23	1	4.9	1.01	1.49±0.15	1	3.7	0.69
V. linifolia	4.27±0.25	0	5	1.12	2.57±0.23	0	3.9	1.04	1.43±0.13	0	2.5	0.59
V. lyallii	4.25±0.28	0	5	1.25	2.42±0.24	0	3.8	1.06	1.39±0.13	0	2.3	0.56
	Soil induration				Soil particle size (mm)				values			
V. cheesemanii	4±0.01	3.9	4	0.02	4.04±0.02	3.9	4.3	0.1	8			
V. ciliolata	4.31±0.1	3.9	5	0.44	4.38±0.1	4	5	0.45	10			
V. densifolia	3.99±0.01	3.9	4	0.03	4±0	4	4	0	6			
V. pulvinaris	3.98±0.01	3.9	4	0.04	3.98±0.01	3.9	4	0.04	8			
V. spathulata	3.72±0.05	3.4	4	0.23	3.62±0.18	2.4	4.2	0.82	3			
V. thomsonii	3.99±0.01	3.9	4	0.03	3.99±0.01	3.9	4	0.03	5			
V. catarractae	4.17±0.29	2	5	1.31	4.44±0.2	3	5	0.88	11			
V. decora	3.12±0.3	0	4.1	1.36	3.27±0.29	0	4.7	1.27	14			
V. hookeriana	3.19±0.19	2	4	0.87	2.67±0.31	1	4.2	1.38	8			
V. lanceolata	3.09±0.23	1.1	4	1.01	2.99±0.35	1	5	1.56	18			
V. linifolia	3.64±0.24	0	5	1.07	3.79±0.22	0	5	0.99	15			
V. lyallii	3.75±0.24	0	5	1.07	3.83±0.25	0	5	1.1	18			

Significant differences were found in five environmental variables (Table 3.3.4). Variation in the topographic variable elevation as well as in the soil fertility variables soluble phosphorus, soil induration, exchangeable calcium and particle size is significantly greater in non-hygrochastic species than it is for hygrochastic species (Fig. 3.3.1). Climatic variables and other soil layers were not significantly different in their variation between groups.

Table 3.3.4 General Linear Model (GLM) comparison between CV of environmental variables of hygrochastic and non-hygrochastic species. Significance is indicated by asterix ($p<0.05$*, $p<0.005$**, $p<0.0005$***).

	df	F	R^2	p
Elevation (m)	1	14.778	0.556	0.003**
Mean annual temperature (°C)	1	0.035	-0.096	0.855
Minimal temperature of the coldest month (°C)	1	0.844	-0.014	0.380
Mean annual solar radiation ($MJ/m^2/day$)	1	1.128	0.012	0.313
Winter solar radiation ($MJ/m^2/day$)	1	1.992	0.083	0.188
October vapour pressure deficit (kPa)	1	0.039	-0.096	0.848
Water balance ratio	1	0.336	-0.064	0.575
Soil water deficit (mm)	1	0.132	-0.086	0.724
Soil drainage (soil description)	1	0.832	-0.015	0.383
Slope (°)	1	2.759	0.138	0.128
Acid soluble phosphorus in the soil (Mg/100g)	1	14.465	0.55	0.003**
Exchangeable calcium in the soil (Mg/100g)	1	6.24	0.323	0.032*
Soil induration	1	93.522	0.894	0.000***
Soil particle size (mm)	1	20.072	0.634	0.001**

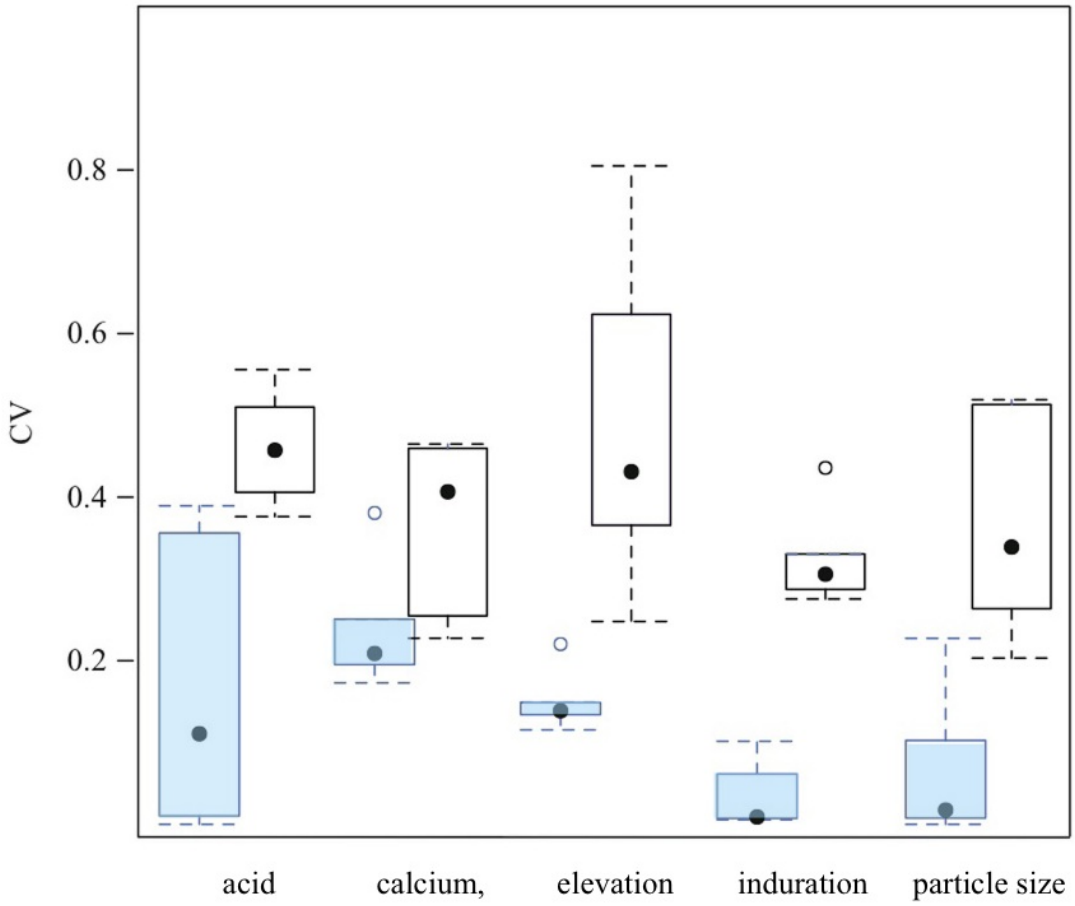

FIG. 3.3.1 Coefficient of variation in five environmental variables of hygrochastic *Veronica* (blue) and non-hygrochastic *Veronica* (black) shown as box and whisker plots. Dark circles indicate the median of the CV.

An advantage of LENZ IV is that the New Zealand wide area of each habitat type is given as well. Values associated with one species were classified as potential available habitats and the area was calculated for each species. Potential available habitats for all species only cover a small percentage of New Zealand (Table 3.3.5). Non-hygrochastic species occur in a wider range of habitat types and the area of suitable habitats for occupation is larger than for hygrochastic species, albeit not significantly (MWU: $W = 30$, $p = 0.06$). When comparing the rough N-S range of 20 locations per species (Table 3.3.5), no significant difference was found between both dehiscence groups (Mann-Whitney-U test; $W = 27.5$, $p = 0.15$).

Table 3.3.5 Potential suitable habitats for 12 *Veronica* species as inferred from values from LENZ IV. Also given is the rough N-S range inferred from 20 locations per species.

species	Area in ha	% of NZ area	N-S range in km
V. cheesemanii	739719	2.82	240
V. ciliolata	1332458	5.08	520
V. densifolia	737841	2.81	210
V. pulvinaris	1067606	4.07	510
V. spathulata	163616	0.62	90
V. thomsonii	586154	2.24	220
V. catarractae	926536	3.53	210
V. decora	1906326	7.27	590
V. hookeriana	627046	2.39	290
V. lanceolata	2665700	10.16	490
V. linifolia	2206221	8.41	660
V. lyallii	2970458	11.33	660

An example of potentially suitable habitat based on LENZ IV values and the distribution of 20 locations per species is given in Fig. 3.3.2, which depicts the South Island of New Zealand and localities for *V. thomsonii* and *V. lyallii*. Similar maps can be drawn for all other investigated species but will not be shown here. Actual distribution maps for the species used in this study can be found in Garnock-Jones and Lloyd (2004) and Meudt and Bayly (2008). Although the map presented here is based on a small dataset (20 locations) it presents the overall distribution of the species well (compare to Meudt and Bayly, 2008).

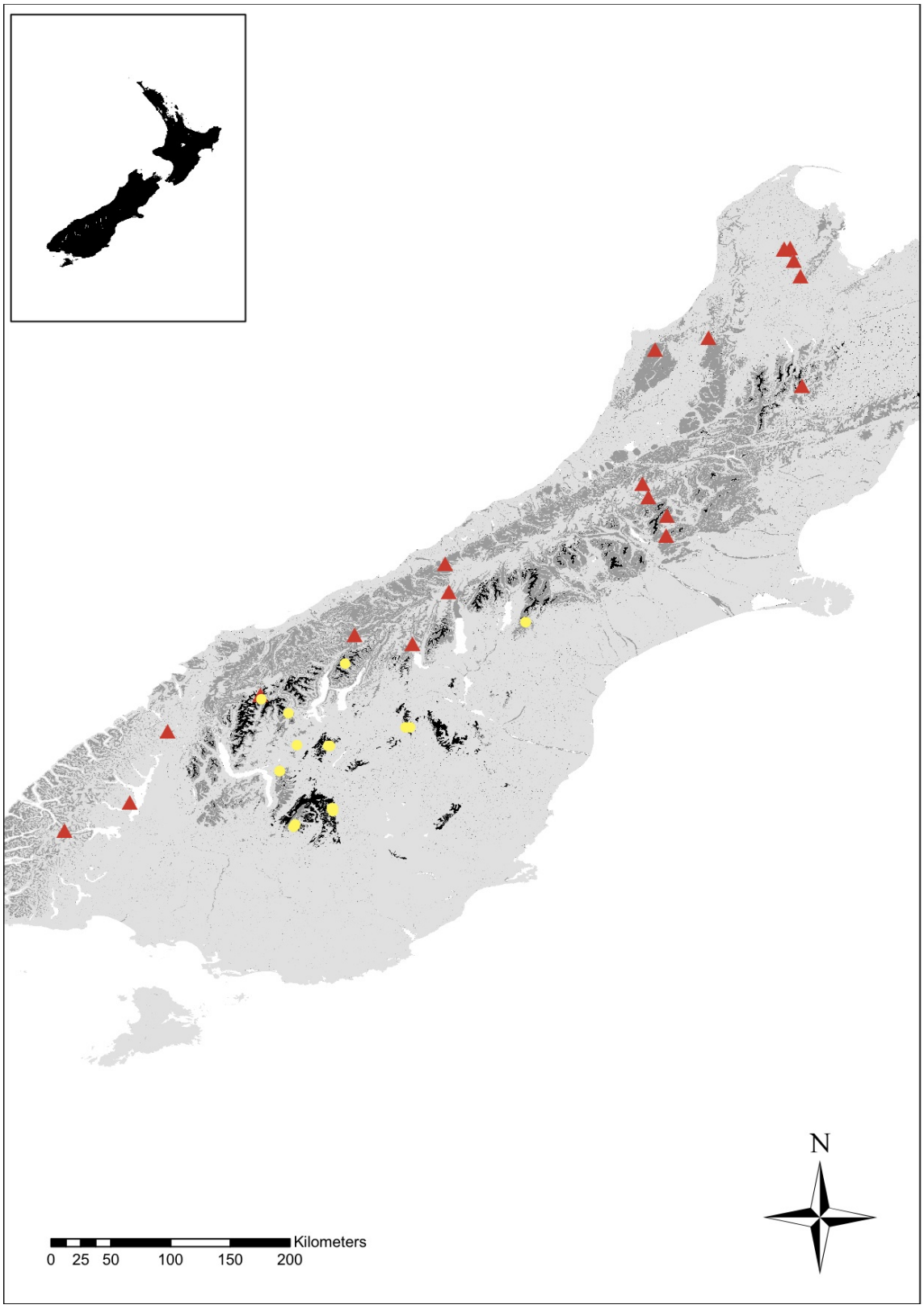

FIG 3.3.2 Potentially suitable habitat for *V. lyallii* (grey) and *V. thomsonii* (black) and locations of *V. thomsonii* (yellow circles) and *V. lyallii* (red triangles) used in the analysis. Shown is the South Island in the main map and New Zealand in the inset.

3.3.5 DISCUSSION

Results show that non-hygrochastic species use a significantly higher number of different habitats compared to the number of habitats utilized by hygrochastic species. The analysis of environmental layers in LENZ IV showed highly significant differences in the variation of five variables associated with altitude and soil fertility among hygrochastic and non-hygrochastic species. In general, hygrochastic species occur in a restricted high altitudinal belt on strongly indurated gravel substrates with low fertility, whereas non-hygrochastic *Veronica* can be found at varying altitudes and with more widely varying environmental variables. According to Hutchinson (1957) we can view the five significantly different variables as axes in a five-dimensional hypervolume, where the hypervolume represents the ecological space. The occurrences of the species within the ecological space essentially represent its niche (Calenge and Basille, 2008). By showing a wide tolerance for these variables, non-hygrochastic species occur in a larger niche, whereas hygrochastic species exhibit a narrow tolerance in a small niche, making them effectively specialists (Futuyama and Moreno, 1988) with a small fundamental niche.

My findings are similar to those proposed by Ihlenfeldt (1983) for Aizoaceae in South Africa but with the added support of GIS-based analyses. Like hygrochastic desert species, alpine *Veronica* are restricted to certain habitat types (Ihlenfeldt, 1983; Desmet and Cowling, 1999). By investigating the distribution of suitable habitat it is evident that not all available sites are occupied (Fig. 3.3.2). Here, the available or potentially suitable sites represent the fundamental niche, whereas the range of the species is equal to the realized niche. This local distribution pattern is most likely due to the short distance dispersal in hygrochastic species (Pufal and Garnock-Jones, 2010). Since suitable sites are located on mountaintops, dispersal to these can only

happen by secondary chance dispersal, e.g. strong winds or storms, since valleys provide an efficient barrier for continuous seed dispersal (McGlone et al., 2001). The limited distribution of hygrochastic *Veronica* is therefore not necessarily a result of the limited distribution of their niche but rather the result of their dispersal ability. Nevertheless, compared to other species and to all available habitat in New Zealand, the fundamental niche of hygrochastic *Veronica* is restricted in size.

The width of the ecological niche regarding 16 environmental variables for New Zealand *Veronica* was identified by using LENZ IV. Based on the narrow amplitude of five variables for hygrochastic species, the occurrence of those species in specific habitats is evident. However, there are several reasons why this result needs to be treated with caution.

From the available 22 *Veronica* species (see Pufal et al., 2010 for details on phylogeny) I was able to use six hygrochastic and six non-hygrochastic species with enough localities. Although this is only a subset of the original species pool, these examples nevertheless provide a good average – hygrochastic species from both North and South Island are included as well as both cushion and subshrub growth form. Non-hygrochastic species are also from both islands and represent the general sub-shrub growth form.

Herbarium databases are increasingly being used as presence only data to model species distribution (Elith et al., 2006; Elith and Leathwick, 2007; Miller et al., 2007; Loarie et al., 2008; Loiselle et al., 2008; Riordan and Rundel, 2009). However, datasets used in those studies were usually much larger than data available for this study. Another limiting factor was the accuracy of locations, which in turn limited the number of localities used in the statistical analysis. Instead of choosing as many locations as possible for each species, I decided to use 20 per species to provide

balanced sampling (Dytham, 2005). This sampling strategy might have influenced the results by not including certain habitat types or over-emphasizing others. However, especially for the low numbers of hygrochastic species in the databases, this sampling effort is reasonable.

Although the LENZ IV dataset is the most accurate system available with 25x25 m squares, it is still rather coarse for my purposes. Most habitat patches for hygrochastic *Veronica* are smaller than the grid provided by LENZ (Chapter 3.2) and much higher spatial resolution is needed in alpine areas due to rugged topography. LENZ also poses a source of circularity since some parameters are based on the interpolation of remote vegetation mapping (Leathwick et al., 2002).

The LENZ database uses climatic layers and soil layers, which are based on different sources. The climatic variables are used directly or indirectly from mathematical surfaces that use information about the climate, elevation and location from a large number of meteorological stations across New Zealand (Leathwick et al., 2002). The soil layers are derived largely from the New Zealand Land Resource Inventory (NZLRI) and the New Zealand Soils database. Information from NZLRI is based on soil maps, whereas the information from the New Zealand Soils database comes from soil samples from close to 1500 locations throughout New Zealand. The soil layers in LENZ are especially prone to error, largely due to 1) variation in the map scale, 2) variation in the size of units mapped in the NZLRI database, where the lowest resolution occurs in montane areas; 3) older NZLRI mapping lacks substantial topographic details, 4) the quality of data sources varies greatly and 5) the sample points used by the New Zealand Soils database are unevenly distributed across the country (Leathwick et al., 2002).

Overall, the use of herbarium data in combination with LENZ IV seems an appropriate tool to identify ecological niches for plant species in New Zealand, but errors due to sample size and accuracy can easily occur.

In the introduction I highlighted that this work is used to explore whether the spatial restriction hypothesis is as important for hygrochastic *Veronica* as it is for hygrochastic Aizoaceae in South African desert regions. In particular, this study focussed on the argument that most hygrochastic desert species have a narrow ecological niche and are specialists in their habitat (Ihlenfeldt, 1983; Eccles et al., 1999). This work supports the spatial restriction hypothesis by providing good evidence that hygrochastic *Veronica* have restricted ecological niches compared to related non-hygrochastic species and are specialists in alpine habitats. Furthermore, the localized extent of hygrochastic *Veronica* is most likely the result of restricted short-distance dispersal through hygrochasy rather than the availablility of fundamental niche space. The spatial restriction hypothesis was previously only associated with species in arid areas but this study provides some indication that this holds true for species in alpine areas as well.

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Chapter 3.4

Summary and Conclusion

Chapter Three is presented as an in depth analysis of the role of hygrochastic dehiscence in the seed dispersal and plant distribution of hygrochastic *Veronica* in New Zealand. I explored the spatial restriction hypothesis, which relates this dispersal strategy to atelechory, directed dispersal, safe site strategies and habitat specialization. In the species presented here, hygrochasy confines seed transport to raindrops as sole dispersal agents, resulting in a very short dispersal distance. I found convincing evidence that this dispersal strategy directs dispersal to safe sites for seeds within the parental habitat, which is otherwise surrounded by a heterogeneous environment. The analysis of herbarium records of both hygrochastic and non-hygrochastic *Veronica* supports the hypothesis that hygrochastic *Veronica* are specialists with a small ecological niche and being hygrochastic supports the persistence of populations in high alpine habitats. Short-distance dispersal within small suitable habitat patches results in the survival of more offspring because seeds germinate in a favourable habitat.

However, the selection of locations from herbarium databases and the use of LENZ IV are open to criticism, because they might not provide accurate small–scale information. Nevertheless, field observations support the analysis of herbarium databases with LENZ IV; the species I investigated in this study are confined to certain habitats, e.g. gravel substrates of a certain size and low vegetation cover.

Overall, this work strongly supports the spatial restriction hypothesis that has so far only been applied to hygrochastic species in arid regions. In New Zealand alpine *Veronica*, ombrohydrochory with hygrochasy appears to be an adaptation to ensure short distance dispersal to safe sites for species with a narrow ecological niche.

CHAPTER FOUR

An Investigation of Hygrochasy and Species Relationships in *Colobanthus* (Caryophyllaceae)

FIG 4.1 Wet open *Colobanthus apetalus* capsules at Blue Lake, Garvie Mountains.

Chapter 4.1

Overview

In this dissertation I was able to show that hygrochasy, which is predominantly associated with plants in arid regions, also occurs in New Zealand *Veronica*, mostly in alpine areas. A common syndrome for alpine hygrochastic species is apparent in the investigated species; hygrochasy seems to be associated with low subshrub or cushion plants and solitary, sessile capsules.

Additional studies (see Chapter Three) also show that hygrochasy in alpine *Veronica* is of advantage for plants in small, specialized habitats and it fascilitates short-dsitance dispersal to safe sites within the parent habitat. These findings raise the question, whether other alpine species in New Zealand might be hygrochastic as well.

A large number of New Zealand alpine species has a cushion or low, creeping life form and capsule fruits can be found in a number of species (Mark and Adams, 1995). Often, these species are found at high altitudes on scree or in fellfield, where it is of advantage to disperse seeds only over a short distance to avoid seedling germination in densely vegetated tussock fields.

One alpine genus, which has potentially hygrochastic capsules, is *Colobanthus* (Caryophyllaceae). Opening of capsules has been observed in some individuals (B.V. Sneddon, pers. comm.; P. Garnock-Jones and W.M. Malcolm, pers. comm.) and further research is warranted to investigate all New Zealand species regarding their dehiscence mechanism. *Colobanthus* is a strictly southern hemispheric genus and consists of approximately 20 species across temperate regions in the Southern Hemisphere. So far, very little is known about the taxonomy and species relationships within the genus. When investigating the capsule dehiscence in the majority of *Colobanthus*, an understanding of the species relationships and lineages would be of

great advantage to infer whether hygrochasy evolved once within the genus or is a labile character associated with occurrence in alpine habitats.

In Chapter 4.2 I investigate capsule anatomy in ten *Colobanthus* species implementing methods successfully used for the investigation of hygrochasy in *Veronica* (Pufal et al., 2010). I used additional species for scanning electron microscopy and analysis of morphological characteristics. I hypothesize that cushion species with sessile capsules are hygrochastic, since they correspond to the syndrome discovered for hygrochastic New Zealand *Veronica*. However, the arrangement of tissues is likely to differ due to genus-specific capsule anatomy. In this Chapter I plan to identify capsule dehiscence mechanisms in all available species.

Chapter 4.3 represents a molecular approach to solve the phylogeny of *Colobanthus*. Taxonomic treatments based on morphology are treated with care, especially in the Caryophyllaceae (Harbaugh et al., 2010) and species identification based on morphology is extremely difficult in *Colobanthus*. One nuclear marker (ITS) and two chloroplast markers (rps16 and trnE-trnTr) are used to study the phylogeny of New Zealand and South American species. The resulting phylogeny can then be used to map hygrochasy as character and trace its evolution within the genus.

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Chapter 4.2

Hygrochastic Movement in Capsules of the Southern Hemisphere Genus *Colobanthus* (Caryophyllaceae)

4.2.1 ABSTRACT

Hygrochasy is a capsule opening mechanism that responds to moisture and is increasingly being found in a diverse range of plants and habitats. In general, it is based on an antagonistic movement, where a swelling tissue absorbs water and expands while the direction of the movement is controlled by an unmoving resistance tissue. Following recent work on hygrochastic capsules in New Zealand alpine *Veronica*, observations of capsule movement in some New Zealand *Colobanthus* also suggested hygrochastic capsules in this genus. I investigated 15 species with regards to their capsule opening mechanism. Based on techniques used for hygrochastic *Veronica*, I employed various light microscopy techniques, staining procedures and SEM micrographs to identify capsule anatomy and cell arrangement. Morphological traits and additional information on capsule structures were used in multivariate analysis to explore relations between species based on dispersal strategy.

In contrast with other genera with hygrochastic capsules, no resistance tissue was found in *Colobanthus* and none of the cells were lignified. However, the outer cell layer of capsules showed thickened cell walls capable of swelling. In contrast to woody hygrochastic capsules, which work with an imbibition mechanism, hygrochasy in *Colobanthus* is based on a combination of imbibition and cohesion mechanism within the capsule. Results suggest that all species in this genus are capable of hygrochastic movement.

Keywords: cohesion mechanism; *Colobanthus*, hygrochasy, imbibition mechanism, microscopy, New Zealand, southern hemisphere

4.2.2 INTRODUCTION

Recently, Thorsen et al. (2009) published a comprehensive study on the seed dispersal systems in the New Zealand Flora. They attempted to assign dispersal mechanisms to the entire flora, based on literature and observations but also acknowledge the lack of data for a number of species. Especially in species with inconspicuous fruits, dispersal strategies were assigned with uncertainty if literature data could not be supported by observations.

Two of the groups Thorsen et al. (2009) listed as uncertain are *Veronica* (as *Parahebe* and *Chionohebe*, for phylogeny and nomenclature see Albach and Meudt, 2010 and Garnock-Jones et al., 2007). The authors assume that the primary dispersal agent is wind and secondary dispersal could be carried out by water.

Some of these species have been previously characterised as being either hygrochastic or xerochastic (Garnock-Jones, 1993; Garnock-Jones and Lloyd, 2004) but until now the hygrochastic movement and its implications for primary dispersal by rain have not been studied in more detail (but see Pufal et al., 2010 and Pufal and Garnock-Jones, 2010). The common morphological traits associated with hygrochasy in *Veronica* were a cushion or subshrub growth form with solitary sessile capsules that stayed closed until wetted. When they are wetted in rainfall events, they open to form a splash cup and seeds are subsequently dispersed by falling raindrops. The mechanism is based on an antagonistic movement between a swelling tissue and a resistance tissue, where the swelling tissue absorbs water in its cell walls and stretches, whereas the resistance tissue is lignified and not capable of moving (Fahn and Werker, 1972).

Dispersal by raindrops alone results in short-distance dispersal, mostly not further than one meter. This atelechoric strategy is most likely an adaptation to ensure directed dispersal within a small suitable habitat patch with available safe sites for seedlings (Pufal and Garnock-Jones, 2010). Most hygrochastic *Veronica* (for exceptions see Pufal et al., 2010) occur exclusively in high alpine areas, where they can be found in small habitat patches of cushion field, fellfield or in snowbank vegetation. An atelechoric dispersal strategy seems favourable in a restricted alpine habitat (Chapter Three) and the idea arose that hygrochasy might well exist in other plants in alpine habitats with similar growth forms and sessile capsule fruits. Potential candidate genera other than *Veronica* are *Colobanthus*, *Donatia* and *Dracophyllum*. Incidentally, opening under wet conditions was observed in some New Zealand *Colobanthus* species (B.V. Sneddon, pers. comm.; P. Garnock-Jones and W.M. Malcolm, pers. comm.) (Fig. 4.2.1).

Colobanthus (Caryophyllaceae subfamily Alsinoideae) is a southern hemispheric genus with approximately 20 species found in South America, Kerguelen Islands, Falkland Islands, South Georgia, Antarctica, Australia, Tasmania and New Zealand. The diversity centre is New Zealand with 13 species, ten of them in alpine habitats and all but two of them endemic (Allan, 1961). To this date the genus has not been monographed and the phylogeny remains unresolved.

Colobanthus is a small perennial plant characterized by a cushion or tufted life form with inconspicuous apetalous flowers that are either sessile or on short pedicels. The fruits are small papery capsules with either four or five valves. When open, the capsules resemble splash cups.

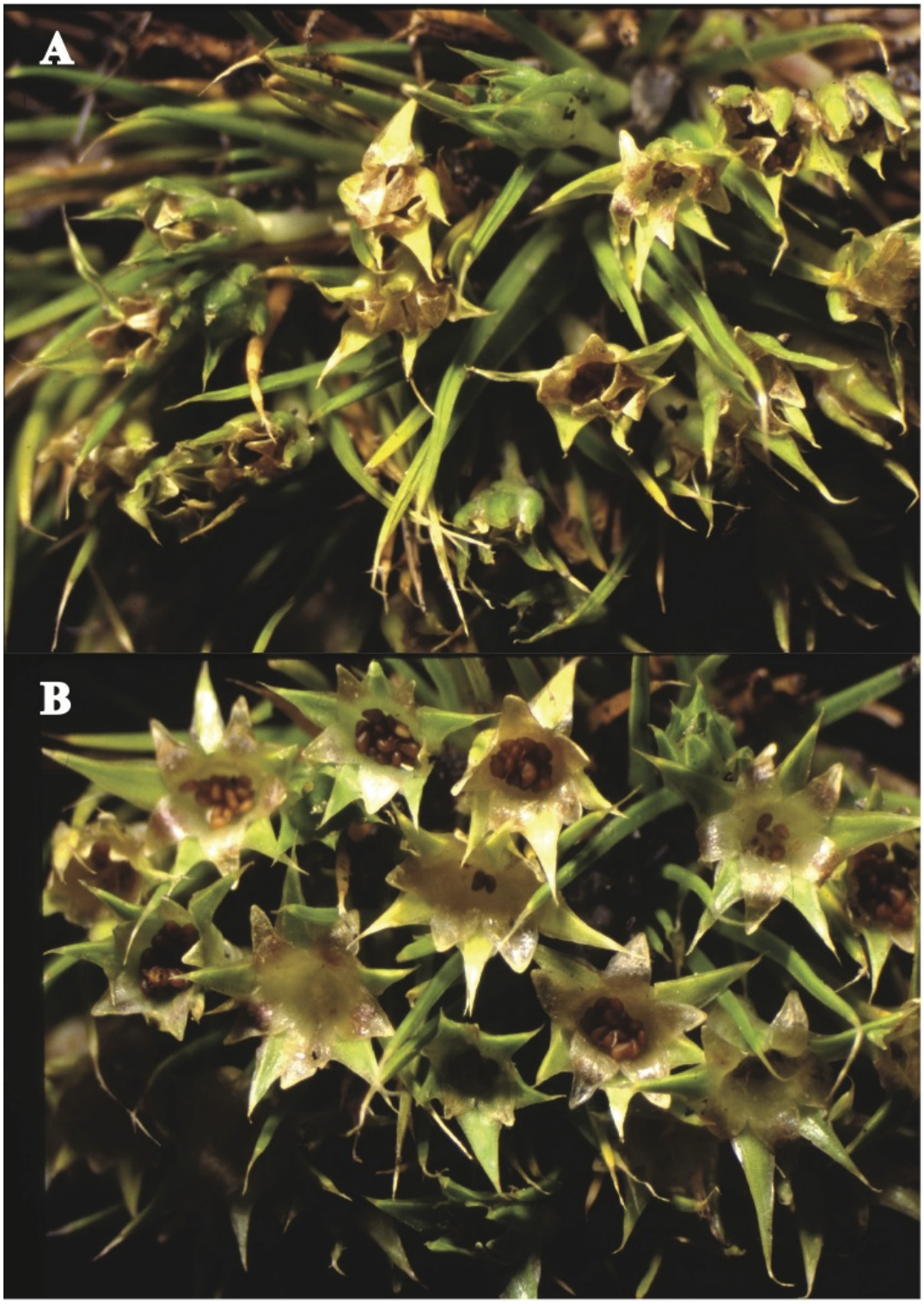

FIG 4.2.1 Dry (A) and wet (B) capsules of *Colobanthus strictus*. Photographs by W.M. Malcolm.

Comparatively little research has been carried out on *Colobanthus*. Most focus lies on *C. quitensis*, which is one of the two only flowering plants in Antarctica and therefore interesting in regards to cold resistance and photosynthesis (Ruhland and Day, 2001; Gianoli et al., 2004; Bravo and Griffith, 2005; Piotrowicz-Cieslak et al., 2005; Bravo et al., 2007; Sierra-Almeida et al., 2007; Gielwanowska et al., 2008; Zuniga-Feest et al., 2009). Subantarctic species received some attention due to their location (Van der Putten et al., 2010) and the seed ecology of *Colobanthus kerguelensis* has been investigated to some extent (Hennion and Walton, 1997). However, New Zealand species are underrepresented in the literature (but see Sneddon, 1999).

The aim of this study is to investigate the dispersal mechanism of New Zealand *Colobanthus* species to broaden the knowledge of New Zealand seed dispersal systems. Special attention is paid to the anatomy and morphology of *Colobanthus* capsules with regards to their opening mechanism since opening under wet conditions had been reported. I attempt to find the underlying biomechanics of the hygrochastic movement in *Colobanthus* and draw parallels to alpine *Veronica*. Methods that have been proved to be appropriate for *Veronica* in Pufal et al. (2010) (light microscopy, different staining techniques) were applied here as well, but in addition scanning electron microscopy was carried out. Additional to the anatomical data, morphological data were also integrated in a hierarchical cluster analysis to attempt the identification of groupings related to the dehiscence mechanism in the genus.

4.2.3 METHODS

Dried capsule samples were obtained from the Allan Herbarium at Landcare Research Centre in Lincoln, New Zealand (CHR) and additional fresh samples were collected at various field sites on South Island, New Zealand (see Appendix 4.2.1 for location

details and voucher numbers). To detect hygrochasy, dry capsules were submerged in water and their reaction was observed. If an opening movement of capsules could be observed, they were classified as hygrochastic. If no movement was observed, they were not classified until further anatomical studies were carried out.

For anatomical studies, capsules were embedded in epoxy resin, which allowed for very thin ultratome sections. Following a dehydration alcohol series, the samples were placed in 100% absolute ethanol and propylene oxide for 30 min each, left overnight in 50:50 propylene oxide : resin and then transferred to 100% resin for at least three to four hours. They were then arranged in resin-filled moulds and dried overnight. Sections (2 – 4 μm) were cut with glass knives in a Leica Ultracut E ultratome and stained with Toluidine Blue. However, most *Colobanthus* specimen proved to be too brittle for this method and only a few samples could be used for measurements. Therefore additional sections were made using paraffin embedded samples following a modified protocol of Jensen (1962). Samples were dehydrated in an alcohol series from water to 100% ethanol in 5 steps, each step lasting about one hour. Tissue was then placed in a 50:50 mix of xylene : 100% ethanol for 1.5 hours. Samples were then transferred to 100% xylene for 2 hours. A small glass jar partly filled with paraffin was topped up with 100% xylene including the tissue sample (resulting in approximately 50:50 of paraffin : xylene) and placed in an oven at 60ºC for 6 hours for infiltration. The paraffin was tipped out, leaving the sample in the jar and the jar was then topped up with another change of paraffin and placed in the oven for four hours. A third change was made and after four hours the sample was mounted in fresh paraffin on a wooden block.

A rotary microtome was used to make sections of 12 – 15μm thickness. Sections were made in two orthogonal planes (cross-sections and longitudinal sections). The

paraffin infiltration was reversed by washing the sections in 100 % xylene and then reversing the ethanol series to the desired ethanol concentration. Sections were stained with Ruthenium Red for pectinous substances (Gurr, 1953) and hydrocholic acid and Phloroglucinol for lignified substances (Gurr, 1953). The following measurements were made on the cells in the capsule wall in ultratome sections and paraffin sections: length, width and cell wall thickness of cells in the inner layers of the capsule (random cells), length, width and cell wall thickness at the outer edge of the outer cell layer of the capsule. The thickness of the capsule wall was also measured. The measurements were used in a Principal Component Analysis (PCA) in order to find anatomical traits associated with dehiscence type. All measurements were compared between the species using Kruskal Wallis tests and subsequent Mann-Whitney-U tests were used to identify significant differences between the species.

Cross sections of single valves made by hand were used to observe change in valve width after water absorption. The valve pieces were placed on glass slides and measured with a moticam (Motic Images Plus 2.0) at 6.3x magnification under a Zeiss microscope before and after water was applied. The change was recorded in percentages.

Scanning electron microscopy was used to collect additional information about capsule anatomy and tissue arrangement. Samples were prepared by mounting dry capsules and capsule cuttings on carbonfoil and coating them with gold (12 nm thick) using a sputter coater (Poleron SC500). Those samples were then carbon coated and viewed with a JEOL JSM-5300LV scanning microscope.

The morphological characters measured for all *Colobanthus* species were based on the *Veronica* treatment in Chapter Two. In addition, the number of valves and the relationship between valves and sepals were also coded as character states (Table

4.2.1). All character states were used in a hierarchical cluster analysis to identify characters separating hygrochastic from non-hygrochastic species. The cophenetic correlation coefficient was calculated to determine how well the dendrogram represents the Euclidean distance matrix. Statistical analyses were carried out using R (R Development Core Team, 2005).

Table 4.2.1 Characters and character states for morphological traits of *Colobanthus* species.

Character		Character states and codes
1	life form	cushion (1), tufted (2), flat rosette (3)
2	number of inflorescences	solitary (1), 2 – 4 (2)
3	position of capsule	sessile (1), peduncle < 2cm (2), peduncle > 2cm (3)
4	orientation of capsule	erect (1), facing down (2), both (3)
5	number of valves	4 valves (1), 5 valves (2), both (3)
6	valves vs. sepals	valves shorter (1), valves longer (2), same or both (3)

4.2.4 RESULTS

Nine *Colobanthus* species were used in the main part of this study, eight from New Zealand and one South American species (*C. subulatus*). Additional species were used for scanning electron microscopy. Obvious opening in water was observed in *C. buchananii*, *C. canaliculatus*, *C. muelleri* and *C. wallii* and these species were classified as hygrochastic. Capsules of *C. affinis*, *C. acicularis* and *C. apetalus* remained closed and only little change could be observed in capsules of *C. subulatus* and *C. strictus*, which showed splits in the capsule before wetting. These species were not classified regarding their opening mechanism.

All *Colobanthus* capsules show the same structure, only differing slightly in size, number of valves and length of valves. The capsules are made up of either four or five valves and a free – standing placental column with no septum. There are nor dorsal

bundles in the valves, but weakly developed veins are present. Once seeds break off the funicles they lie unordered in the capsule cavity.

Sections showed that all species had more or less irregularly shaped rhomboidal cells and the cell walls of the cells on the outer side of the capsule were significantly thicker than cell walls of other cells in the capsule (Mann-Whitney-U test: p-values for all species < 0.002). Staining with Ruthenium Red showed that all cell walls contained pectinous substances but no lignin was detected in any of the capsules when staining with HCl and Phloroglucinol.

Measurements of cell traits and of valve change with water absorption were used in a PCA to reveal grouping amongst the species and to ascertain if some variables were more influential than others. The first three components of the PCA explain 66 % of the variation and all variables are influential in the first three components. When displaying the first two components graphically, no grouping can be observed amongst the species (Table 4.2.2; Fig. 4.2.2). However, samples from the same species tend to group together due to their similar cell size.

A noticeable increase in valve size after water absorption occurred in all tested species (for raw data, see Appendix 4.2.2). However, compared to hygrochastic *Veronica* (Pufal et al., 2010), the change is significantly smaller (Mann-Whitney-U test: $W = 1661$, $p < 0.0001$).

Table 4.2.2 Eigenvalues and loadings for PCA. Change of valve size after water absorption (change), capsule wall thickness (wall thickness), the length (length in), width (width in) and cell wall thickness (cell wall in) of cells in the inner layers of the capsule as well as length (length out), width (width out) and cell wall thickness (cell wall out) of the outer cell layer of the capsule.

Components	1	2	3	4	5	6	7	8
Eigenvalues	2.744	1.432	1.12	0.976	0.751	0.471	0.294	0.212
St. dev.	1.657	1.197	1.058	0.988	0.867	0.686	0.542	0.461
Proportion of variance	0.343	0.179	0.14	0.122	0.094	0.059	0.037	0.027
Cumulative proportion	0.343	0.522	0.662	0.784	0.878	0.937	0.973	1
loadings								
Length in	-0.39	-0.328	-0.393		0.126	0.677	-0.189	-0.274
Width in	-0.301	-0.418	-0.116	0.376	0.573	-0.404	0.26	0.143
Cell wall in	-0.38		-0.357	-0.543	-0.279	-0.201	0.558	
Length out	-0.465	-0.236	0.18	-0.168	-0.274	-0.436	-0.631	
Width out	-0.402	0.312	0.482	0.232			0.286	-0.609
Cell wall out	-0.464	0.348	0.263		0.115	0.302		0.698
Wall thickness		0.619	-0.399	-0.161	0.495	-0.218	-0.319	-0.19
Change	-0.132	0.24	-0.46	0.675	-0.494			

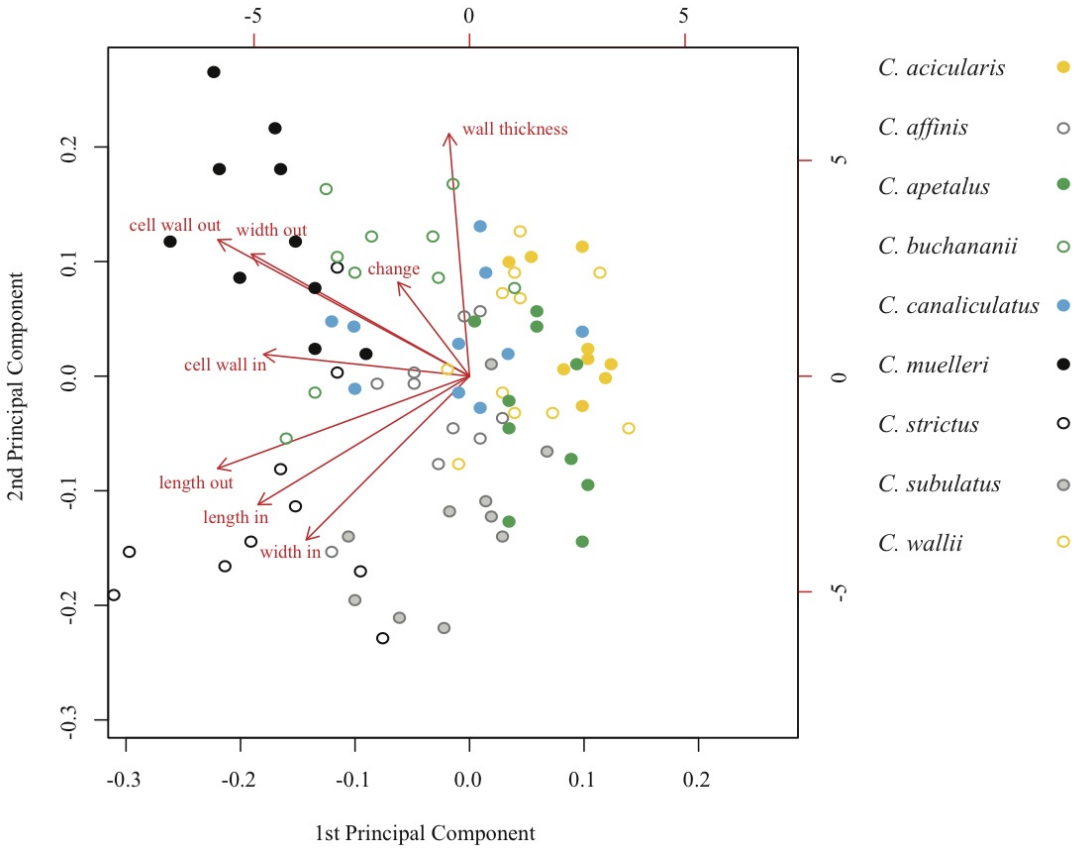

FIG. 4.2.2 Principal Component Analysis of measurements in *Colobanthus* capsules. Red arrows represent the eigenvalues of the change of valve size after water absorption (change), capsule wall thickness (wall thickness), the length (length in), width (width in) and cell wall thickness (cell wall in) of cells in the inner layers of the capsule as well as length (length out), width (width out) and cell wall thickness (cell wall out) of the outer cell layer of the capsule.

When comparing each measured variable between species in a Kruskal Wallis test and subsequent Mann-Whitney-U tests, differences were significant but not consistent between variables (Appendix 4.2.3).

Morphological characters were coded as character states and used in a hierarchical cluster analysis. Characters that were identified as common characters for hygrochastic *Veronica* (Chapter Two), such as solitary capsules and short pedicels, were equally present in all investigated *Colobanthus* species (Fig. 4.2.3).

Clustering into groups is very well supported with a cophenetic value of 0.904. Species split into clusters because of the different life forms; therefore three distinct groups can be found (cushion species - I, tufted species - II and *C. muelleri* - III, which forms a rosette).

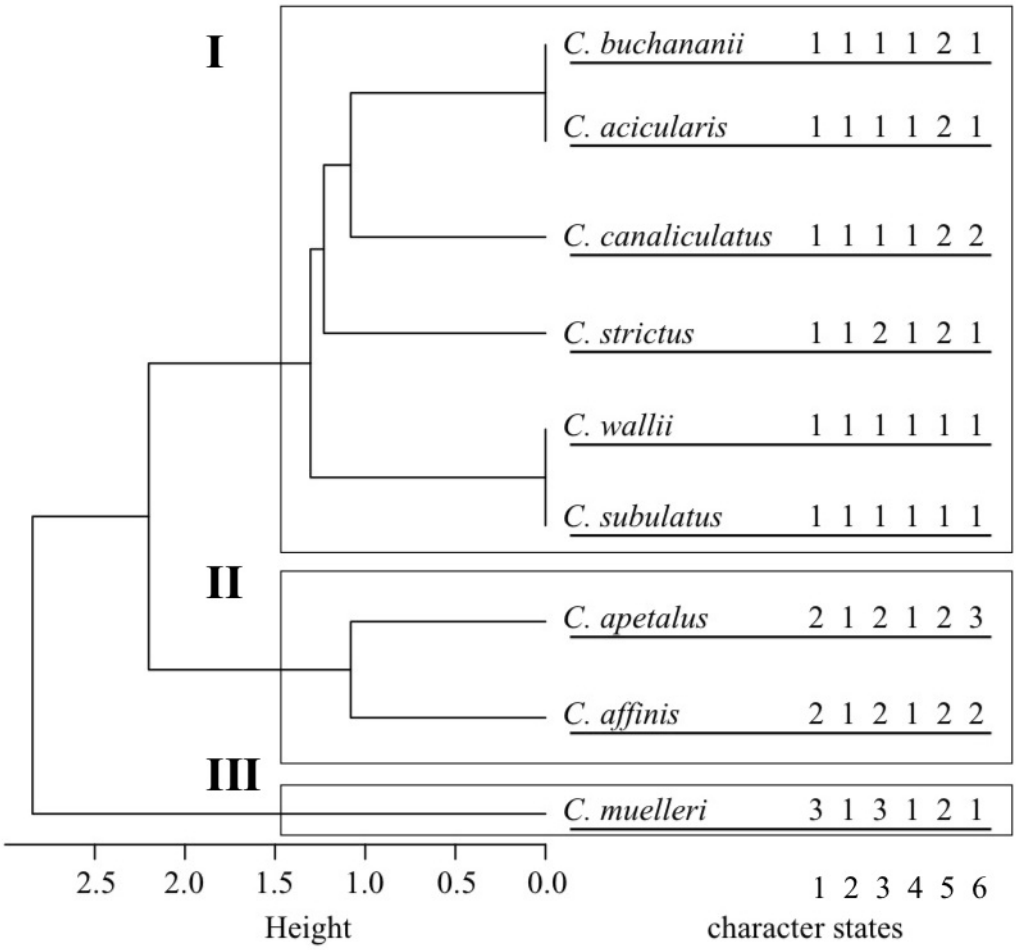

FIG. 4.2.3 Hierarchical Cluster Analysis of character states for nine *Colobanthus* species. Coding for character states can be found in Table 4.2.1. Cluster I includes cushion species, cluster II tufted species and *C. muelleri* forming a rosette stands separately (III).

For SEM micrographs a total of 13 species was used (see Appendix 4.2.1 for location details and voucher specimen) to capture the capsule morphology of as many species as possible. All *Colobanthus* capsules have a ridged surface on the outside of the capsule (Fig. 4.2.4 and Fig. 4.2.5) but vary in pattern and depth of ridges. Thickened

valve apices are also present in all species and are shown in some micrographs (Fig.4.2.4 B and F, Fig. 4.2.5 B, D, E, G). Again, all investigated species appeared to be similar, except for capsule shape and occasional number of valves (Fig. 4.2.4 and Fig. 4.2.5).

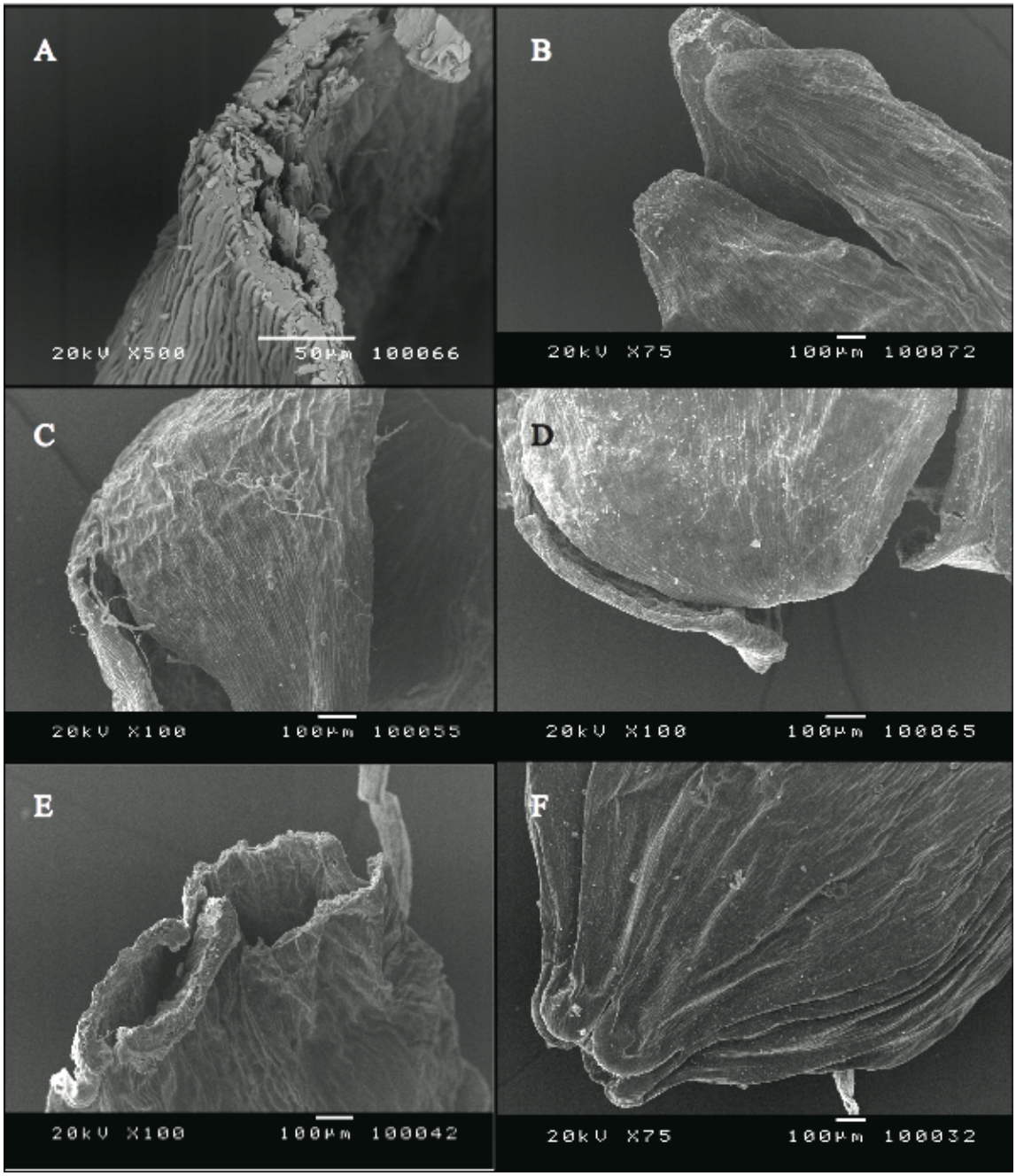

FIG. 4.2.4 SEM micrographs of *Colobanthus* capsules. (A) *C. buchananii* cut valve, (B) *C. apetalus* valve apices, (C) *C. strictus* valve, (D) *C. wallii* valve, (E) *C. canaliculatus* cut capsule apex, (F) *C. masoniae* closed capsule apex.

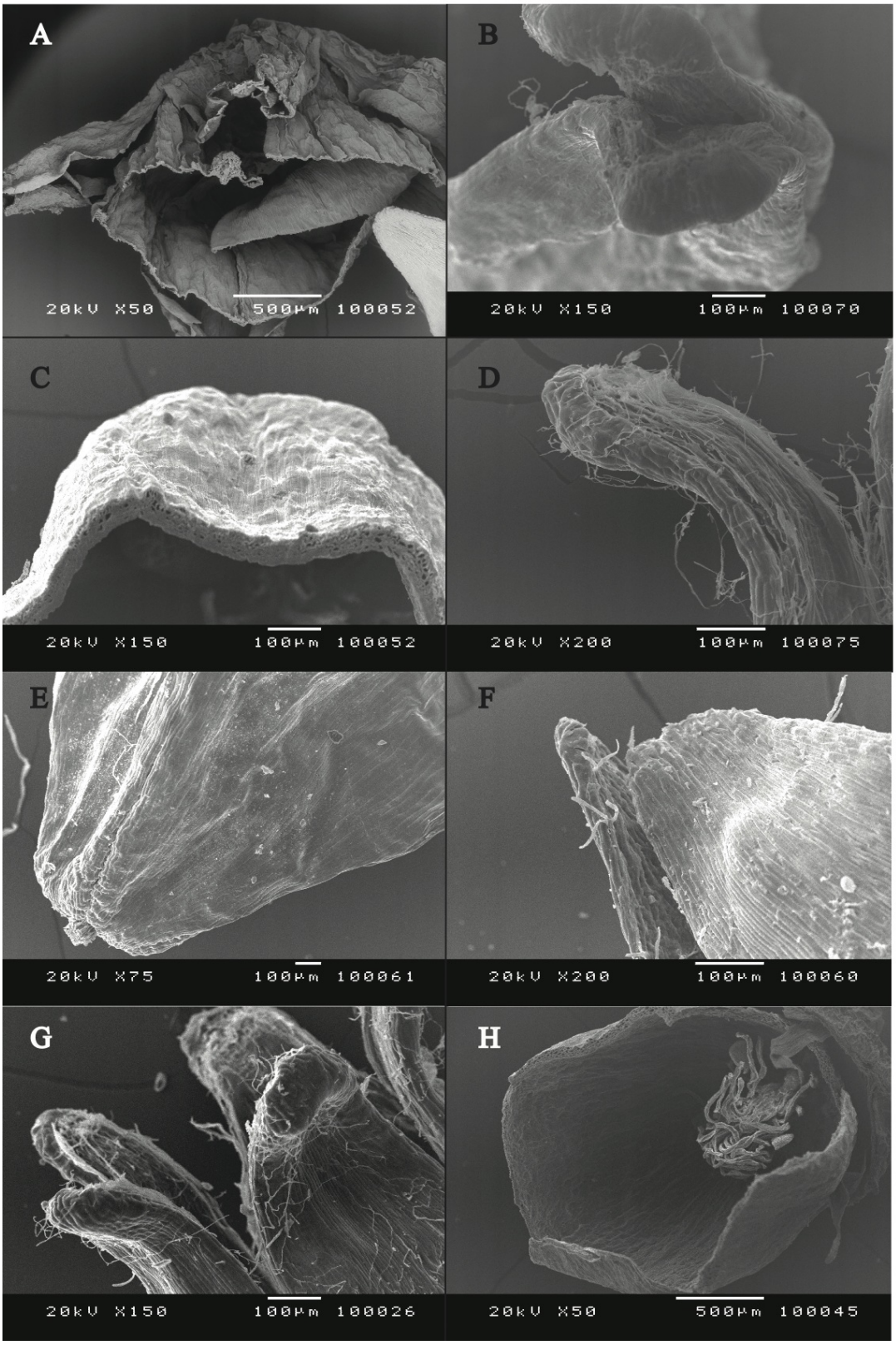

FIG. 4.2.5 SEM micrographs of *Colobanthus* capsules. (A) *C. muelleri* cut capsule top, (B) *C. acicularis* valve apex, (C) *C. brevisepalus* cross-cut capsule, (D) *C. buchananii* valve apex, (E) *C. kerguelensis* closed capsule apex, (F) *C. lycopodioides* cut valve, (G) *C. monticola* valve apices, (H) *C. quitensis* cut capsule with placenta.

4.2.5 DISCUSSION

In this study, the main aim was to test the association between hygrochastic capsules, cushion or mat habit and sessile fruits observed in alpine *Veronica*, by close examination of a phylogenetically independent genus. A secondary aim was to add information on the poorly studied genus *Colobanthus* to the study by Thorsen et al. (2009). The focus of this study was to investigate capsule dehiscence in *Colobanthus* species on the basis that opening of capsules was observed in wet conditions. Submerging capsules in water resulted in the capsule opening of some species. However, detailed analysis of capsule sections, and size change in valve pieces revealed that all investigated species are capable of opening to some degree. The capsules that remained closed during submersion in water were unripe and the valves still completely attached to each other.

Contrary to 'classic' hygrochastic capsules, where a swelling tissue and a resistance tissue are present to direct the swelling movement (Steinbrinck, 1883; Garside and Lockyer, 1930; Fahn and Werker, 1972; Poppendieck, 1995; Pufal et al., 2010), no resistance tissue could be located in the studied *Colobanthus* species. All cells seem to be capable of some swelling, the outer cells more so since their pectinous cell walls are overly thickened and hence probably able to absorb more water than inner cells, which have much thinner cell walls. The PCA did not reveal any grouping based on cell measurements and changes after water absorption, which implies that dehiscence in all investigated species is similar. SEM pictures showed the ribbed outside of capsules and the partly folded and crumbled structure of some valves. After observing capsules during wetting, analysing cross sections and longitudinal section of valves as well as studying SEM micrographs I propose the hygrochastic movement of *Colobanthus* capsules works as follows:

Upon wetting, the pectinous and lignin-free cell walls of cells in the valves absorb water quickly and start to swell. This swelling causes the cells to stretch in all directions to their maximum as they have been irregularly shaped and somewhat shrunk before. Cells on the outside of the capsule have thickened cell walls capable of absorbing substantial amounts of water. The cells line up in a ribbed pattern, directing the swelling to stretch the entire capsule. Within a few minutes the valves open out in a star shape and seeds are exposed in a splash cup to falling raindrops (Fig. 4.2.1).

Part of the movement is an imbibition mechanism, which is based on antagonistic actions (Fahn and Werker, 1972). In most hygrochastic capsules, a swelling and a resistance tissue are involved but imbibition can also function when cell walls of the same cell act as antagonistic groups (Fahn, 1967; Fahn and Werker, 1972). When a cell wall imbibes water, it usually swells in a direction perpendicular to that of cellulose microfibrils. Therefore, if the angles of microfibrils within cell walls varies, their reaction towards moisture will be different. The outer cells of *Colobanthus* capsules show a significant thickening of their outer cell wall compared to the cell wall on the inside of the cells. Here, the thickened cell wall acts as swelling tissue, whereas the inner cell walls of the outer cells act as resistance tissue.

Fahn and Werker (1972) also describe the cohesion mechanism as another movement acting in dead cells, but here the cell lumen is involved. Cell walls of cells involved in this mechanism are usually thin and the cells wrinkle with water loss. When saturated with water, this tissue can expand considerably. Inner cells of *Colobanthus* capsules have thin cell walls and appear wrinkled and irregular when dry, which also leaves the capsule tissue somewhat crumpled when viewed under the microscope or in SEM (Fig. 4.2.3 and 4.2.4). After water absorption, the cells have a regular rhomboid shape and capsules stretch.

Results strongly suggest that the hygrochastic dehiscence of all investigated *Colobanthus* species is most likely based on a combination of imbibition and cohesion mechanisms. The concurrence of imbibition and cohesion mechanisms was observed in a number of plants, where it causes short-distance dispersal (Steinbrinck and Schinz, 1908; Zohary and Fahn, 1941; Hegazy et al., 2006). However, so far it has never been observed within the same fruit.

Unfortunately I was not able to use all known *Colobanthus* species in this study and of the species I investigated only some of them were used in all analyses. I did not include any Australian species since I was not able to procure any specimens. *Colobanthus* capsules are extremely fragile and brittle and prove to be quite a challenge in different embedding techniques. This is the reason why some species could not be used in the anatomical analysis, because their capsules did not deliver good enough sections. However, both New Zealand and South American species uniformly show hygrochastic dehiscence to some extent, which leads me to the assumption that Australian species are highly likely to be hygrochastic as well.

This study extends the list of hygrochastic genera in New Zealand and also includes a coastal species as opposed to exclusively montane species. In contrast to Thorsen et al. (2009) who assumed that *Colobanthus* seeds might be wind dispersed, hygrochasy implies dispersal by raindrops, since fruits expose the seeds in a splash cup. The tufted *Colobanthus* species grow along rivers and streams, which provide splash water as a reliable dispersal agent (Nakanishi, 2002). For the coastal species *C. muelleri*, splash water from the surf is also a highly likely dispersal agent. However, some fruits are slightly open even when dry, which certainly exposes seeds to wind. Whether this exposure is sufficient for dispersal remains to be investigated.

This study demonstrates that small plants with inconspicuous fruits can reveal a relatively uncommon phenomenon on closer inspection. Usually fruits with no apparent adaptation for dispersal are labelled as generalists (Fahn and Werker, 1972; Van der Pijl, 1982) but *Colobanthus* is clearly hygrochastic and most likely disperses by raindrops and subsequent rainwash (ombrohydrochory).

My findings are a further indication that hygrochasy might be a more common dispersal strategy in alpine plants, which was not known before. Alpine habitats are restricted and I showed previously that hygrochasy is an adequate dispersal strategy to maintain populations in those habitats (Chapter Three).

Further examination of dehiscence and associated dispersal mechanism in alpine plants is warranted, not only in New Zealand but in other alpine regions as well.

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Chapter 4.3

A Molecular Approach to the Phylogeny of the Southern Hemisphere Genus *Colobanthus* (Caryophyllaceae)

4.3.1 ABSTRACT

Colobanthus (Caryophyllaceae) is a genus of approximately 20 species occurring in Australasia, South America, Antarctica and various subantarctic islands. The plants are small perennials with a cushion or caespitose growth form and inconspicuous flowers. Although a number of genera in the Caryophyllaceae have received intense phylogenetic treatment, *Colobanthus* has so far been overlooked and relationships within the genus as well as species delimitation are unclear. The aim of this study is to investigate whether *Colobanthus* is a monophyletic genus, how New Zealand and South American species relate to each other and whether species with different growth forms belong to different lineages. To resolve the phylogeny of *Colobanthus* and to answer the research questions, a molecular approach was taken, since morphological characters used to identify species are few, vary within species and are likely to be homoplastic. After running primer assays on 13 primer pairs, ITS, *rps16* and *trn*T-*trn*E were chosen as markers in this study. Parsimony and Bayesian analyses of nrDNA (ITS) and cpDNA (*rps16* and *trn*T-*trn*E) sequence data from New Zealand, South American and subantarctic species were used to build phylogenetic trees of *Colobanthus* and its near and distant relatives within the Caryophyllacae. All sequences tested in this study have single base mutations and show generally very little variation within *Colobanthus*, resulting in a largely unresolved crown clade for the genus. It appears from this lack of resolution that species delimitation is still problematic and no distinct sequence differences between species with different

growth forms or from different continents could be found. However, monophyly of *Colobanthus* within Caryophyllaceae received moderate support with *Sagina* as sister clade. The low resolution for the three markers is suspected to be an indicator for very recent speciation following long distance dispersal events. Additional molecular methods such as AFLPs or microsatellites together with analysis of morphological characteristics are recommended to resolve the problematic phylogeny of this genus.

Keywords: *Colobanthus*, phylogeny, recent speciation, Southern Hemisphere, ITS, *rps16*, *trn*T-*trn*E

4.3.2 INTRODUCTION

The family Caryophyllaceae Juss. includes around 3000 species in 88 genera (Rabeler and Hartmann, 2005). Although cosmopolitan, the main distribution of the family is in the Holarctic, with centres of diversity in the Mediterranean and Southwest Asia (Bittrich, 1993). The relationships within the family have been difficult to understand and numerous studies have been undertaken to solve the problematic phylogeny (Harbaugh et al., 2010; Oxelman et al., 2000; Oxelman et al., 2002; Smissen et al., 2002; Scheen et al., 2004; Popp et al., 2005; Applequist et al., 2006; Fior et al., 2006; Sakai et al., 2006; Fior and Karis, 2007; Frajman and Oxelman, 2007; Kool et al., 2007). Many of the genera are not well defined morphologically (Bittrich, 1993) and monophyletic groups are hard to identify due to the difficulties in determining phylogenetically useful characters. Therefore the use of molecular phylogenetic data is essential to understand the relationships within the family (Smissen et al., 2002; Fior et al., 2006; Fior and Karis, 2007). To date, most phylogenetic studies have concentrated on the relationships between families within the Caryophyllales (Rettig

et al., 1992; Downie and Palmer, 1994; Downie et al., 1997; Cuenoud et al., 2002) and a number of studies on genera in the Caryophyllaceae have been completed (Bacchetta et al., 2010 ; Harbaugh et al., 2010 ; Oxelman et al., 2002; Smissen and Garnock-Jones, 2002; Smissen et al., 2003; Popp et al., 2005; Minuto et al., 2006; Sakai et al., 2006; Sosa et al., 2006; Tzvelev, 2006; Fior and Karis, 2007; Frajman and Oxelman, 2007; Kool et al., 2007; Adams et al., 2008; Schaeferhoff et al., 2009).

These studies have shown that none of the three traditional subfamilies (Alsinoideae, Caryophylloideae and Paronychioideae) is monophyletic (Smissen et al., 2002; Fior et al., 2006). A new study by Harbaugh et al. (2010) proposes to abandon the classification of subfamilies, since they are not natural groups. They recommend recognition of at least eleven tribes, which are based on well-supported lineages. However, further study of diverse genera within the family is necessary to increase the understanding of the relationships within the newly proposed tribes.

The southern hemisphere *Colobanthus* is a genus traditionally placed in the subfamily Alsinoideae (Bittrich, 1993) but according to the new classification belongs to the tribe Sagineae (Harbaugh et al., 2010). The genus comprises ca. 20 species that are distributed across the southern temperate region. The centre of diversity is in New Zealand, with 14 indigenous species, eight of these endemic (Sneddon, 1999). Australia has five species: of these, *Colobanthus pulvinatus* is endemic, *C. nivicola* is endemic to the mainland and *C. curtisiae* to Tasmania (Gilfedder and Kirkpatrick, 1996). Three species are found on the different subantarctic islands, of these *C. muscoides* and *C. hookeri* only occur in the subantarctic region, while *C. apetalus* is more widespread. *Colobanthus kerguelensis* is endemic to the South Indian Ocean province, which includes Kerguelen Island, Heard Island, Crozet Island and the Marion and Prince Edward Islands (Van der Putten et al., 2010). In the South America

region *C. quitensis* (syn. *C. crassifolius*) is the species with the widest distribution, ranging from Mexico, northern South America, along the Andes to Tierra del Fuego, the Falkland Islands, South Georgia Island and the Scotia Basin. The other South American species is *C. subulatus*, which occurs in southern South America, Falkland Islands and South Georgia. Variants of this species have also been described as *C. lycopodioides* (Moore, 1983).

Plants are perennial herbs with an often caespitose or cushion habit, usually with a strong taproot. The solitary flowers are hermaphrodite and inconspicuous and are either sessile or borne on short pedicels (Allan, 1961; Moore, 1983). Petals are absent and capsules dehisce by as many valves as there are sepals.

In Chapter 4.2 I investigated capsule dehiscence in *Colobanthus* under the premise that species with sessile capsules are hygrochastic. At the onset of the study I expected only New Zealand alpine species to be hygrochastic and coastal species as well as South American species to be 'ripening dehiscent'. So far, no studies had been published regarding the phylogeny of *Colobanthus* but to answer questions about the relatedness of suspected hygrochastic and 'ripening dehiscent' species and also to trace character evolution a phylogeny was warranted and work on it commenced simultaneously with the analysis of Chapter 4.2.

Results from Chapter 4.2 indicate that all *Colobanthus* species are hygrochastic but the more general need for a resolved phylogeny has not diminished. It could now be used to investigate how closely related the species are, since they all have the same specialized opening mechanism. Questions about the monophyly of the genus, the relationships between South American and New Zealand species as well as species delimitation are still unanswered. Additionally, a total of five species names have been proposed for South American *Colobanthus* species but it is strongly suspected

that these only describe two species (Moore, 1983). A molecular phylogeny can be used to address those issues.

As with most genera within the former subfamily Alsinoideae (Endress, 1996; Hufford, 1996), morphological traits are very similar within *Colobanthus* and species identification remains difficult (B. Sneddon, pers. comm.). In the Flora of New Zealand (Allan, 1961), habit, flowers, sepals and leaf lengths are predominantly used to identify the species and group them into two informal groups but even here, the differences are usually minute and can often be misinterpreted. The phylogeny should therefore be examined by use of molecular characters.

In this study, a combination of nuclear markers and chloroplast markers is chosen to construct the phylogeny. The nuclear DNA (nrDNA) is independent from the chloroplast DNA (cpDNA) and they are differently inherited. Chloroplast DNA is generally maternally inherited and may therefore be able to show genetic relatedness better than nrDNA, which is inherited from both parents and undergoes recombination (Mort et al., 2007). Another advantage of using both nrDNA and cpDNA is the detection of hybridisation, which can be a reason for conflicting signals from the different genomes (Lockhart et al., 2001; Albach and Chase, 2004; Smissen et al., 2005). If no conflict between markers can be detected, datasets can be concatenated to result in a longer sequence. These can then be used to build more robust trees and improve the power of analysis (Cummings et al., 1995).

Internal transcribed spacer regions (ITS) have previously been successfully used in phylogenetic studies in many angiosperm families, including the Caryophyllaceae (Baldwin et al., 1995). Recently, ITS sequences were used to investigate the origin for *Scleranthus* (Caryophyllaceae) (Smissen et al., 2003), which is in the sister tribe (Scleranthae) to Saginae (Harbaugh et al.). ITS was also successfully used in studies

of *Moehringia* (Fior and Karis, 2007), *Cardia* (Sosa et al., 2006), *Silene* (Popp et al., 2005) and *Gymnocarpos* (Oxelman et al., 2002). The chloroplast *rps*16 intron is another marker that has been used in phylogenies of genera within the Caryophyllaceae (Oxelman et al., 2002; Kool et al., 2007). Lastly, the chloroplast *trn*T-*trn*E region was also chosen as a result of successful primer assays. This region has been developed for phylogenetic studies in Poaceae (Doyle et al., 1992) but was also successfully implemented in the Gesnerioideaa (Zimmer et al., 2002) and in a *Plantago* study (Tay, 2008).

4.3.3. MATERIALS AND METHODS

4.3.3.1 Study group

Fresh samples were collected at various sites on the South Island (New Zealand) and in Patagonia (Chile) and leaf samples were stored in silica gel. Table 4.3.1 shows all sample species and their origin. For common species several individuals from different locations were collected. In New Zealand, two undescribed species (*C. 'marble'* and *C. 'ultramafic'*, B. Sneddon, pers. comm.) were also included in the analysis. Herbarium samples were obtained from CHR, WELTU and MERL (Thiers, [continuously updated]). Furthermore, samples from three species were taken from live collections at the greenhouse at Victoria University of Wellington and used fresh. This sampling resulted in sequences from 100% of New Zealand species and 100% of South American species. Samples of South American species include individuals referable to four of the five accepted names (excluding *C. cherlerioides*). Unfortunately *Colobanthus kerguelensis* and Australian species could not be included since fresh samples could not be obtained and herbarium samples did not yield sufficient DNA samples.

Sagina apetala was selected as sister group (Harbaugh et al., 2010). Sequences from *Bufonia tenuifolia*, *B. paniculata*, *Geocarpon minimum*, *Minuartia rossii*, *Schiedea viscosa*, *Scleranthus biflorus* and *Stellaria media* (Harbaugh et al., 2010; Smissen et al., 2002) were obtained from GenBank as more distant outgroups to test for the monophyly of *Colobanthus* (Table 4.3.1). For the *trn*T-E region, no further outgroup sequences were available.

Table 4.3.1 Collection details, herbarium voucher numbers and GenBank accession numbers for species included in this study. Collectors: MNC – M.N. Correa; PGJ – Phil Garnock-Jones; OM – O. Magens; GP – Gesine Pufal; BS – Barry Sneddon.

Names used in analysis	Species	Location	collector	*ITS*	*rps16*	*trnE-T*	Herbarium voucher	GenBank accession
aci_si_seal	*C. acicularis*	Sealey Range, Aoraki National Park, **NZ**	GP (2008)	1	1	1	WELTU20254	
aci_si_blue	*C. acicularis*	Blue Lake, Garvie Mountains, Central Otago, **NZ**	GP (2009)	1		1	WELTU20255	
aci_si	*C. acicularis*		GP (2008)	1	1	1	WELTU20275	
affi_si_arth	*C. affinis*	Mt. Arthur, Kahurangi National Park, **NZ**	GP (2008)	2	1	1	WELTU20256	
ape_si_blue	*C. apetalus*	Blue Lake, Garvie Mounatins, Central Otago, **NZ**	GP (2009)	1	1	1	WELTU20257	
ape_2519	*C. apetalus*	Garvie Mts, route from Blue Lake to Lake Laura, Otago, **NZ**	PGJ 2005)	1	1		PGJ2519	
ape_green	*C. apetalus*	Greenhouse, VUW, **NZ**	BS	1	1	1		
C_brevisepalus	*C. brevisepalus*			1				AY286511.1
buch_si_dob	*C. buchananii*	Mount Dobson Skifield, Canterbury, **NZ**	GP	3	3	1	WELTU20248	
buch_si_blue	*C. buchananii*	Blue Lake, Garvie Mountains, Central Otago, **NZ**	GP (2009)	1	1	1	WELTU20258	
buch_si_red	*C. buchananii*	Red Peak, Mt Aspiring National Park, **NZ**	GP (2009)	3	3	1	WELTU20259	
buch_2507	*C. buchananii*	Garvie Mts, route from Blue Lake to Lake Laura, Otago, **NZ**	PGJ (2005)	1	1		PGJ2507	
buch_2533	*C. buchananii*	Garvie Mts, ridge east of Blue Lake, Otago, **NZ**	PGJ (2005)	1	1		PGJ2533	
canal_si_green	*C. canaliculatus*	Greenhouse, VUW, **NZ**	BS	1	2	2		
canal_si_kakapo	*C. canaliculatus*	Kakapo Peak, Kahurangi National Park, **NZ**	GP (2009)	1	1	1	WELTU20260	
hook_sub_2484	*C. hookeri*	Campbell Island, west shoulder of Mt Lyall, **NZ**	PGJ (2004)	1	1		PGJ2484	
marble_si_arth	*C. 'marble'*	Mt Arthur, Kahurangi National Park, **NZ**	GP (2008)	1	1		WELTU20261	
mas_si_kakapo	*C. masoniae*	Kakapo Peak, Kahurangi National Park, **NZ**	GP (2009)	2	2	1	WELTU20250	
mont_si_muel1	*C. monticola*	Sealey Range, Aoraki National Park, **NZ**	GP (2008)	2	2		WELTU20262	
muell_si_green	*C. muelleri*	Greenhouse, VUW, **NZ**	BS	1	1	1		
muell_si_cape	*C. muelleri*	Cape Foulwind, Westcoast, **NZ**	GP (2007)	1	1		WELTU20263	
C_muscoides	*C. muscoides*				1			FJ404901.1
squarr_si_sun	*C. squarrosus*	Sunshine Ridge, Kahurangi National Park, **NZ**	GP (2007)	1	1	1	WELTU20264	
stric_SI_blue	*C. strictus*	Blue Lake, Garvie Mountains, Central Otago, **NZ**	GP (2009)	3	3		WELTU20265	
strict_si_patr	*C. strictus*	Mt Patriarch, Richmond Forest Park, Nelson, **NZ**	GP (2008)	1	1		WELTU20266	

strict_si_port	*C. strictus*	Port Hills, Canterbury, **NZ**	PGJ	1	1			
strict_si_robert	*C. strictus*	Mt. Robert, Nelson Lakes National Park, **NZ**	GP (2009)	1	1	1	WELTU20267	
strict_si_arth	*C. strictus*	Mt. Arthur, Kahurangi National Park, **NZ**	GP (2008)	1	2	1	WELTU20252	
ultra_si_asbest	*C. 'ultramafic'*	Asbestos Mine, Kahurangi National Park, **NZ**	GP (2009)	2	2	1	WELTU20268	
wall_si_arth	*C. wallii*	Mt. Arthur, Kahurangi National Park, **NZ**	GP (2008)	2	2	1	WELTU20253	
sealey	*Colobanthus sp.*	Sealey range, Aoraki National Park, **NZ**	GP (2008)	3	3	2	WELTU20269	
redpeak1	*Colobanthus sp.*	Red Peak, Mt Aspiring National Park, **NZ**	GP (2009)	1	1		WELTU20270	
blackbirch1	*Colobanthus sp.*	Blackbirch Creek, Aoraki National Park, **NZ**	PGJ	1	1			
crassi_sa_tierra	*C. crassifolius*	Tierra del Fuego, **Argentina**	OM (1956)	1			WELTU20273	
lyco_sa_laguna	*C. lycopodioides*	Laguna Cabeza de Mar, Patagonia, **Chile**	GP (2008)	1	1		WELTU20271	
lyco_sa_morro	*C. lycopodioides*	Morro Chico, Patagonia, **Chile**	GP (2008)	2	1		WELTU20249	
quit_sa_lake	*C. quitensis*	Lake close to Pali Aike, Patagonia, **Chile**	GP (2008)	3	1	1	WELTU20272	
quit_SA_punta	*C. quitensis*	Punta Arenas, Patagonia, **Chile**	GP (2008)	2	2		WELTU20251	
sub_sa	*C. subulatus*	San Gregorio, Patagonia, **Chile**	GP (2008)	1	1		WELTU20274	
sub_sa_28026	*C. subulatus*	Guer Aike, Cordillera, **Argentina**	MNC (1978)	1	1		MERL28026	
sape_2556_PGJ	*Sagina apetalus*	Cass Railway Station, Canterbury, **NZ**	PGJ	1	1		PGJ2556	
Sape_21	*Sagina apetalus*		PGJ	1	1	1		
Bufonia_ paniculata	*Bufonia paniculata*				1			FJ404897
Bufonia_ tenuifolia	*Bufonia tenuifolia*			1				AY936238.1
Geocarpon_ minimum	*Geocarpon minimum*			1	1			AY517648.1 FJ404906.1
Minuartia_rossii	*Minuartia rossii*			1	1			AY517649.1 FJ404921.1
Schiedea_viscosa	*Schiedea viscosa*			1	1			AY517655.1 FJ404942.1
Scleranthus_ biflorus	*Scleranthus biflorus*				1			FJ404944.1
Stellaria_media	*Stellaria media*			1	1			AY0.5472.1 FJ404953.1

4.3.3.2 Primer assays and genetic markers

A number of both nuclear and organellar DNA markers have been successfully used in building phylogenies of plant species based on molecular characters. The majority of studies on genera in the Caryophyllaceae in the last decade used universal markers such as ITS, *mat*K or *rps16* but additional primers already available to me were tested in this study as well (Table 4.3.2).

Primer assays were run on several pairs of primers to test their usefulness for the genus *Colobanthus* (Table 4.3.2) These were usually run on a subset of species from New Zealand and South America and when clear single bands could be viewed in the agarose gel after amplification, sequencing was carried out for some species.

DNA extractions were performed following a modified cetyltrimethylammonium bromide (CTAB) protocol by Doyle and Doyle (1990), after tissue was finely ground with a pestle and mortar.

PCR amplification was performed with the Eppendorf Mastercycler ep gradient S (Hamburg, Germany). Samples used contained the following: 15.60 µl H_2O, 2.5 µl 10x ThermoPol reaction buffer (10 mM KCl, 10 mM $(NH_4)2SO_4$, 20 mM Tris-HCl (pH 8.8), 2 mM $MgSO_4$, 0.1% Triton X-100) (New England BioLabs), 1 µl each BSA and dNTPs (250 µmol), 1µl each primer, 0.15 µl *Taq* DNA polymerase (New England BioLabs) and 2 µl DNA template. A general thermocycling profile was used for the amplification of all primer pairs and was optimised in some cases using the following PCR protocol with a temperature gradient for the annealing temperature (Table 4.3.2) The PCR protocol was carried out as follows: an initial 2 min at 95ºC, followed by 35 cycles of 1 min at 95ºC, 1 min at 49ºC and 1.5 min at 72ºC, ending with a final extension time of 5 min at 72ºC. Amplified lengths of all PCR products were viewed using a 100 base pair (bp) DNA ladder (Roche, Penzberg, Germany) on a 1.5%

agarose gel. Samples that resulted in single bands were cleaned with ExoSAP-IT (GE Life Sciences) and sequenced with an ABI3730 Genetic Analyzer by the Allan Wilson Centre Genome Service (Massey University, Palmerston North, New Zealand). Primer pairs that resulted in clean sequences were subsequently used in the phylogenetic analysis (Table 4.3.2)

Table 4.3.2 primer pairs. S = single banded PCR product; M = multiple bands in PCR product; – = no amplified product; + = successful sequencing; / = unsuccessful sequencing; – = no sequencing carried out. Markers used in the study are highlighted in **BOLD**.

	region	genome	primer sequences		PCR results	sequence	annealing temp.	base pairs	literature
1	**ITS**	nuclear	ITS28cc ITS5	CGC CGT TAC TAG GGG AAT CCT TGT AAG GGA AGT AAA AGT CGT AAC AAG G	S	+	50 ºC	~ 750	Wagstaff and Garnock-Jones, 1998; White et al., 1990
2	waxy	nuclear	waxy7F waxy13R	GYY TTS TGC ATC CAC AAC ATT GC GGA GTG GCR ACG TTT TCC TT	M	–	49ºC		Olmstead et al., 1999)
3	*mat*K	chloroplast	934F 1470R	ATT TTG GTT ATG ACA ATA A AAG ATG TTG AT[T/C] GTA AAT GA	–	–	50 ºC		Johnson and Soltis, 1994
4	***rps16***	chloroplast	*rps16*F *rps16*R	AAA CGA TGT GGT ARA AAG CAA C AAC ATC WAT TGC AAS GAT TCG ATA	S	+	48 ºC	~ 920	Shaw et al., 2005
5	*trn*Lc-*trn*Lf	chloroplast	C f	CGA AAT CGG TAG ACG CTA CG ATT TGA ACT GGT GAC ACG AG	M	–	50 ºC		Taberlet et al., 1991
6	*trn*s-G spacer	chloroplast	$trnS^{GCU}$ $3'trnG^{UUC}$	AGA TAG GGA TTC GAA CCC TCG GT GTA GCG GGA ATC GAA CCC GCA TC	M	–	49 ºC		Shaw et al., 2005
7	*rbcL*	chloroplast	aF cR	ATG TCA CCA CAA ACA GAG ACT AAA GC GCA GCA GCT AGT TCC GGG CTC CA	–	–	49 ºC		Hasebe et al., 1994
8	***trn*T-*trn*E spacer**	chloroplast	trnE trnTr	GCC TCC TTG AAA GAG AGA TG TAC CAC TGA GTT AAA AGG GC	S	+	52 ºC	~ 650	Doyle et al., 1992
9	*psb*A-*trn*K	chloroplast	trnK3F PsbAR	CCG ACT AGT TCC GGG TTC GAA TC CGC GTC TCT CTA AAA TTG CAG TCA T	S	/	49 ºC		Winkworth et al., 2002a
10	*rpo*B-*trn*C	chloroplast	trnC-gcaR rpoB	CAC CCR GAT TYG AAC TGG GG CKA CAA AAY CCY TCR AAT TG	M	–	57 ºC		Shaw et al., 2005
11	*psb*A-*trn*H spacer	chloroplast	$trnH^{GUG}$ psbA	CGC GCA TGG TGG ATT CAC AAT CC GTT ATG CAT GAA CGT AAT GCT C	S	/	49 ºC		Tate and Simpson, 2003; Sang, Crawford, and Stuessy, 1997
12	Atp1	mitochondrial	F83-atp1 R725-atp1	ATG AGG TCG GTC GAG TGR T GGA TCC GAA GCM GTG GCT GCT AC	–	–	49 ºC		Wikstrom and Pryer, 2005
13	NIA3	mitochondrial	NIA-i3F NIA-i3R	AAR TAY TGG TGY TGG TGY TTY TGG TC GAA CCA RCA RTT GTT CAT CAT DCC	–	–	49 ºC		Howarth and Baum, 2005

4.3.3.3 Molecular techniques

DNA extractions were performed following a modified cetyltrimethylammonium bromide (CTAB) protocol by Doyle and Doyle (1990), after tissue was finely ground with a pestle and mortar. For older herbarium specimen, a CTAB protocol by Loockerman and Jansen (1996) was used with some success. Primers used to amplify DNA regions were: ITS*28CC* (Wagstaff and Garnock-Jones, 1998) and ITS*5* (White et al., 1990); *trn*E and *trn*Tr (Doyle et al., 1992); *rps16*F and *rps16*R (Shaw et al., 2005).

PCR amplification was performed similar to PCR amplification for the primer assays. The amplification for *ITS28CC* and *ITS5* was carried out with a thermocycling profile of an initial 2 min at 95ºC, followed by 35 cycles of 1 min at 95ºC, 1 min at 50ºC and 1.5 min at 72ºC, ending with a final extension time of 5 min at 72ºC. The PCR protocol for *rps16* differed from the ITS protocol only in the annealing temperature, which was 48ºC instead of 50ºC and the extension time in the cycle, which was 1.5 min at 72ºC. The PCR protocol for *trn*T-*trn*E had the following thermocycling profile: initial 2 min at 92ºC, followed by 32 cycles of 30 sec at 95ºC, 30 sec at 52ºC and 1 min at 72ºC, ending with a final extension time of 5 min at 72ºC. PCR products were mixed with blue juice and viewed on 1.5% agarose gels and subsequently cleaned with ExoSAP-IT (GE Life Sciences).

The cleaned PCR products were sequenced in the forward direction by the Allan Wilson Centre Genome Service (Massey University, Palmerston North, New Zealand).

4.3.3.4 Dataset alignment and analysis

The program Geneious (Drummond et al., 2009) was used to assemble and align sequences for each accession. Different individuals from the same location were removed if sequences were identical but included in the analysis if some differences were detected in the sequences. Sequences were aligned with the default Geneious align option with a cost matrix of 65 % similarity, gap open penalty of 9.9 and gap extension penalty of 2.9. A global alignment with free end gaps was performed. Since sequencing was only done in the forward direction, sequences became difficult to read reliably towards the end. Ends were therefore cut off, which resulted in sequences of even lengths for samples sequenced for this study. Additional to those sequences, *C. muscoides* and *C. brevisepalus* and some outgroup species were added from GenBank (Table 4.3.1). The ITS dataset included 63 individuals of 25 species and three unidentified samples. Of these, two were undescribed New Zealand species, six outgroup species and four South American species. The *rps16* dataset included 60 individuals of 25 species and three unidentified samples. Of these species, seven were part of the outgroup, three were South American species and of the 15 New Zealand species two were undescribed. The *trn*T-*trn*E dataset comprised 23 individuals of 12 species and one unidentified sample (Table 4.3.1) Of the 12 species, one was *Sagina* as part of the outgroup, one was South American and one was an undescribed species. Because both chloroplast datasets had a very low number of variable sites (tested in previous analysis, not presented here), they were concatenated to one chloroplast dataset. Additionally, the ITS dataset and the chloroplast dataset were combined into one concatenated set after a Hompart test was performed in PAUP*.

Maximum parsimony (MP) analysis was conducted using PAUP* v4.b10 (Swofford, 2002). A heuristic search was conducted under a MP criterion using 10,000 replicates of random sequence addition and tree-bisection-reconnection (TBR) branch swapping and gaps were coded as missing. Bootstrap support was assessed using 1000 replicates and 10 random addition sequences.

Modeltest v3.7 (Posada and Crandall, 1998) was used to identify the appropriate models of evolution for the respective datasets. The program tests the best fit among a number of different models of different complexity using a hierarchical likelihood ratio test. Models were then selected using Akaike Information Weights criterion (AIC) (Posada and Buckley, 2004). Table 4.3.3 contains a summary of the statistics for all three datasets used in the study.

Table 4.3.3 Summary of statistics for the datasets used in this study.

	ITS	*chloroplast*	*ITS* and choloroplast
base frequencies of all sites (%)	A = 0.2228 C = 0.2703 G = 0.2813 T = 0.2256	A = 0.3678 C = 0.1290 G = 0.1615 T = 0.3417	A = 0.3179 C = 0.1841 G = 0.2010 T = 0.2970
sequence length including outgroups (bp)	986	1638	2624
no. of variable parsimony informative sites including outgroups (only ingroup)	138(18)	129(18)	267(36)
model of evolution (AIC)	GTR+G	K81uf+G	GTR+I+G
gamma shape estimate	0.4098	0.6872	1.0713
pinvar	0	0	0.3239

MrBayes v3.1.1 (Huelsenbeck and Ronquist, 2001) was used to conduct heuristic searches, while implementing the AIC model that was chosen by Modeltest for the respective dataset (see Table 4.3.3). Each dataset was initially analysed with four chains and 4,000,000 generations. Additional generations were added if necessary to achieve a standard deviation of split frequencies of less than 0.01. For ITS, 6,000,000

generations were run and 30% of the trees were excluded as burn-in. The chloroplast DNA dataset was run for 14,000,000 generations and 10% of the trees were excluded as burn-in. The combined ITS and chloroplast dataset was run for 6,000,000 generations and 5% of trees were excluded as burn-in.

Phylogenetic trees from both analyses had congruent topology and it was therefore decided to only display one example. Trees obtained from MrBayes were visualized in Figtree v1.3.1 (Morariu et al., 2008) and posterior values were added as well as bootstrap support obtained from PAUP*. Branches with posterior probabilities of less than 50% were collapsed in the phylogeny.

SplitsTree vs 4.11.3 was used to visualise incompatible splits in each of the three datasets (Hudson, 1998; Hudson and Bryant, 2006). Because of long distances between the outgroups and *Colobanthus* and very short distances within *Colobanthus*, outgroups were removed from the datasets to concentrate on conflicting signals within *Colobanthus*. Datasets were then transformed to uncorrected p-distances with the MatchStates option. Split decomposition networks were constructed using the equal-angle-splits transformation. If connections between species are shown as boxes, it indicates conflicting signals within the dataset, e.g. incompatible splits.

Pairwise genetic distances were calculated using uncorrected p-distances in PAUP*. These were used to assess species delimitation/discrimination by comparing interspecific and intraspecific distances. Species discrimination was considered when the minimum uncorrected interspecific p-distance involving one species was larger than the maximum uncorrected intraspecific p-distance of that species (CBOL Plant Working Group, 2009). For this analysis ITS, *rps*16 and *trn*T-*trn*E datasets were analysed separately and only species were used that had more than one sample per species. Unidentified specimens (e.g. blackbirch1, see Table 4.3.1) were excluded.

4.3.4 RESULTS

4.3.4.1 ITS dataset

The ITS dataset contained 63 sequences, 55 of which were generated for this study and eight obtained from GenBank (Table 4.3.1). The alignment consisted of 986 characters, of which 138 were parsimony informative but only 18 of these are informative within the ingroup (Table 4.3.3). Gaps were treated as missing. Bayesian and parsimony analysis showed similar results and a phylogenetic tree for ITS in *Colobanthus* is shown in Fig. 4.3.1. All *Colobanthus* species form an unresolved crown clade with one backbone, which has weak (62%) bootstrap support for monophyly. Two samples of *Sagina apetala* form the well-supported sister clade to the crown clade. There is a 99% bootstrap support for the *Colobanthus*/*Sagina* clade, indicating that the low support for the *Colobanthus* clade results from almost entirely alternative trees where *Sagina apetala* is not distinguished from the *Colobanthus* clade. Within *Colobanthus*, no support is given for a distinction between South American and New Zealand species or species with sessile fruits versus species with longer pedicels. Several smaller clades branch of the crown clade but these have low bootstrap support and posterior values and never contain all samples of one species.

The splits graph network shows a large number of incompatible splits within *Colobanthus*, visualized by boxes (Fig. 4.3.2). Longer branches represent longer genetic distances from the core of the network. However, all species at the end of longer branches are also represented in the boxy core of the network. Analysing the South American species and the species with longer pedicels separately (splits graphs not shown) resulted in graphs with boxes in the cores, indicating incompatible splits within these sample subsets.

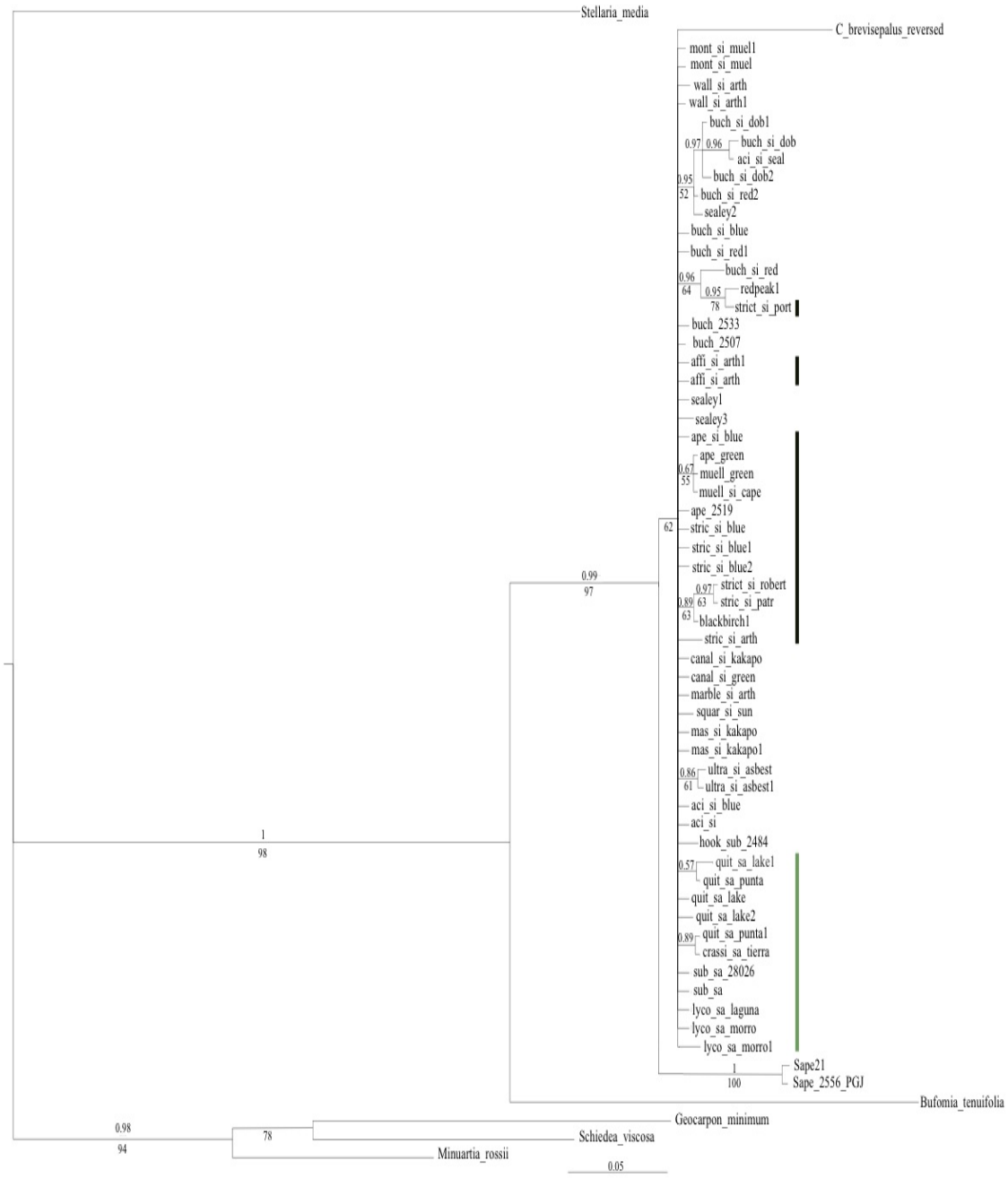

FIG 4.3.1 50% consensus tree using Bayesian analysis of the ITS dataset. Branch lengths are proportional to the number of changes. Numbers above the branches are Bayesian posterior probabilities (numbers < 0.5 not shown). Numbers below branches show parsimony bootstrap support (values < 50% not shown). Matching species names to species abbreviations can be found in Table 4.3.1. Numbers behind species abbreviations indicate different voucher specimen. The green bar highlights South American species, whereas the black bars signify species with longer pedicels.

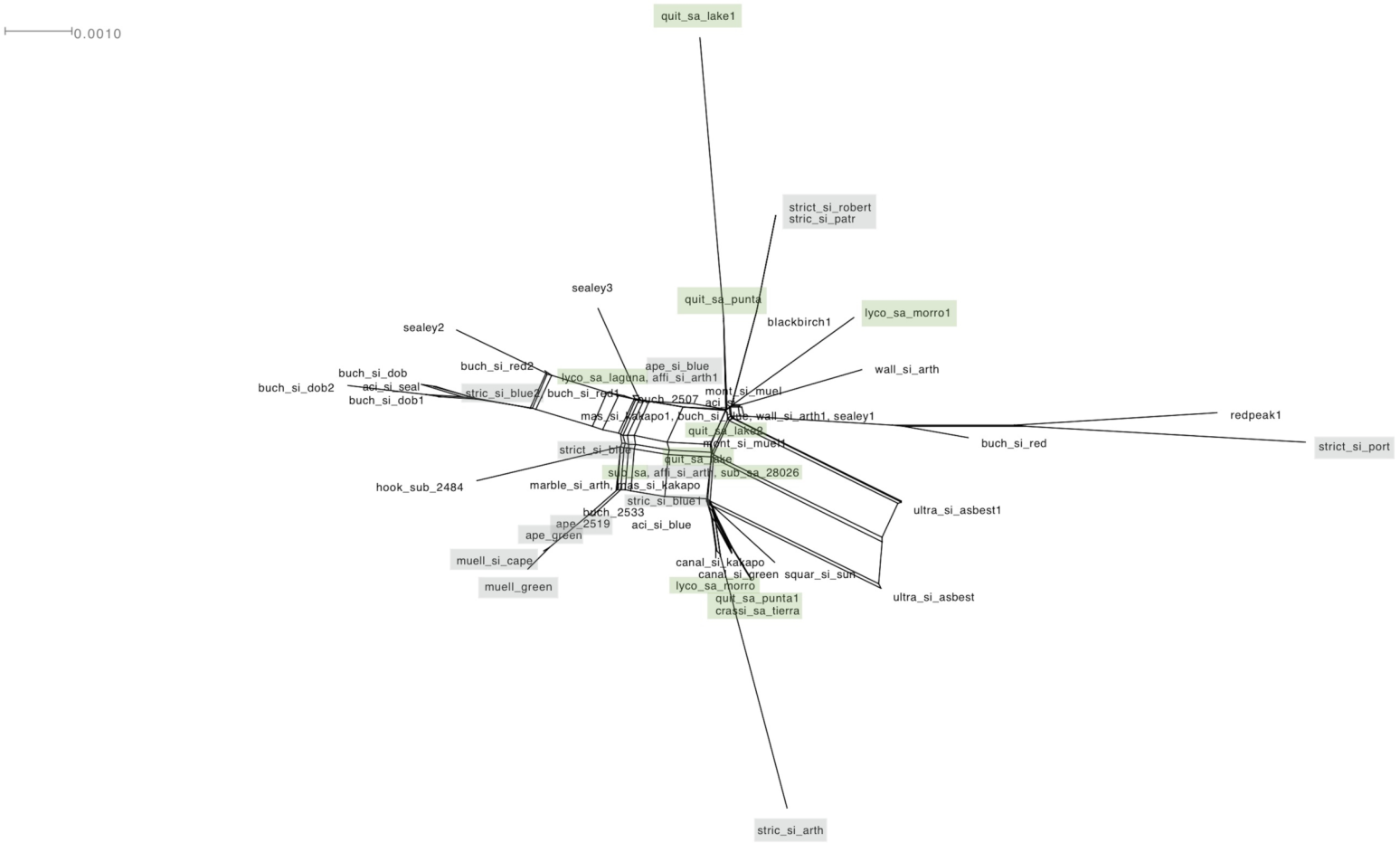

FIG 4.3.2 Splits graph network illustrating relationships among the ITS sequences of *Colobanthus*. Branch lengths are drawn proportionally to the genetic distances, a scale is provided at the top left corner. South American species are highlighted in green and species with longer pedicels are highlighted in grey.

4.3.4.2 Chloroplast dataset

The chloroplast dataset is a concatenated dataset of *rps*16 and *trn*T-*tnr*E sequences. In total, 63 sequences are analysed; seven of these were obtained from GenBank and 55 were generated for this study (Table 4.3.1). The *rps*16 dataset contained 60 sequences including all GenBank samples, whereas the *trn*T-*trn*E dataset comprised 23 sequences, all generated for this study. The concatenated chloroplast alignment contained 1638 characters, 129 of which were parsimony informative (including outgroup). When the outgroup was excluded, 18 characters were parsimony informative (Table 4.3.3).

Parsimony and Bayesian analyses showed similar results and topography of inferred trees, therefore only the 50 % consensus tree from the Bayesian analysis is presented with posterior values and bootstrap support from the MP analysis in PAUP* is added (Fig. 4.3.3). The tree shows an arrangement of outgroups similar to the phylogeny from a combined chloroplast dataset presented in Harbaugh et al. (2010). These clades show both high bootstrap support as well as high posterior values. A clade representing *Sagina* and *Colobanthus* is well supported by bootstrap (90%) and posterior values (1). Within this clade, monophyly for *Colobanthus* is poorly supported by a bootstrap value of 57%. All *Colobanthus* species form an unresolved and weakly supported crown clade with a well supported *Sagina apetala* clade (bootstrap 93%, posterior value 1) as sister group. Within the crown clade there is very little resolution and neither South American species or species with longer pedicels are grouped with each other.

A splits graph network of all *Colobanthus* species (Fig. 4.3.4) shows a high number of incompatible splits within the core group, visualized by the high number of boxes between the species. South American species are concentrated on one side of the core

network on two different clades, whereas species with longer pedicels are scattered throughout the network. Separate analyses of the South American species and species with longer pedicels (splits graphs not shown) show connections between species as boxes in each dataset. These indicate conflicting signals in the chloroplast dataset.

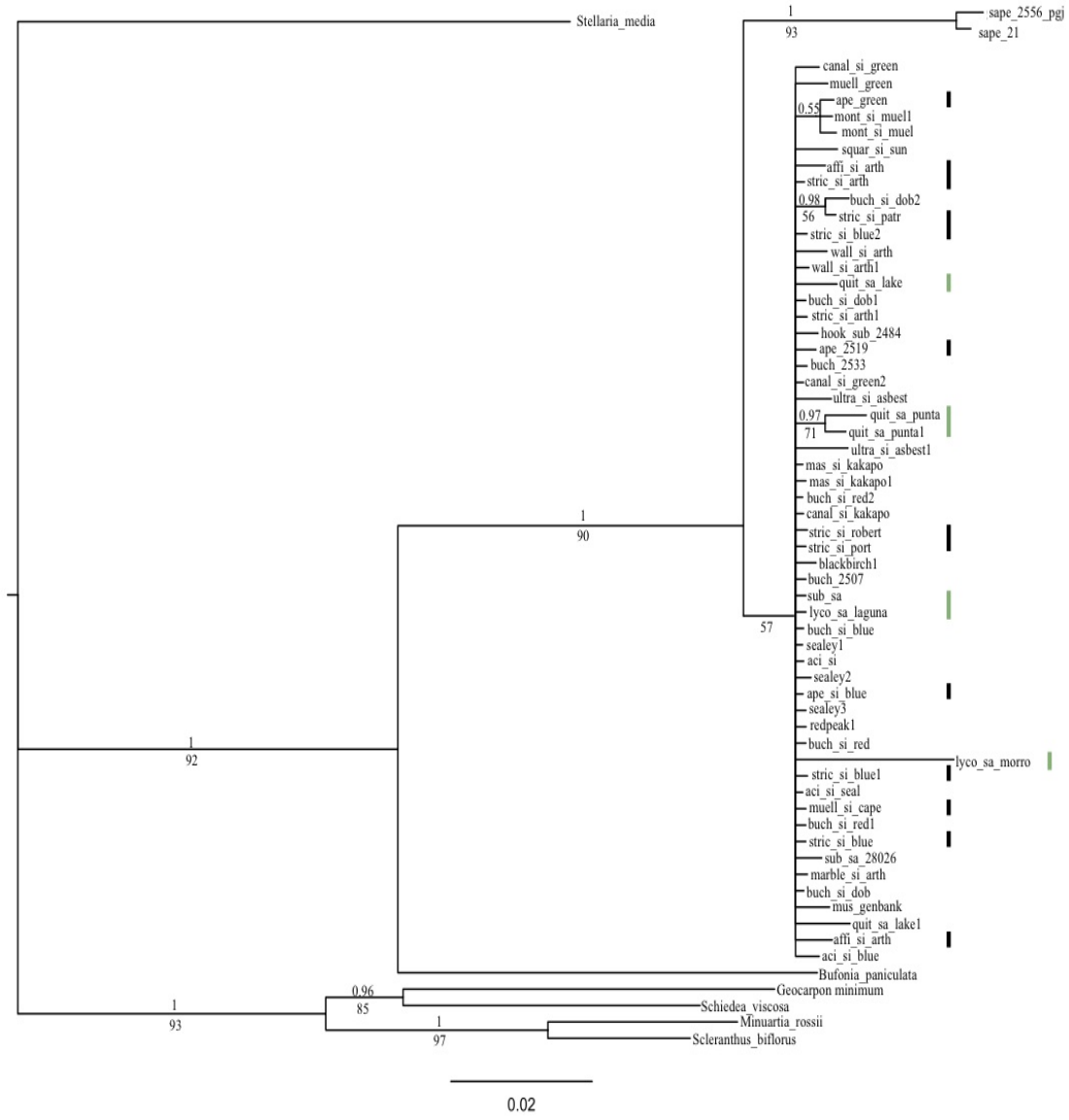

FIG 4.3.3 50% consensus tree using Bayesian analysis of the combined chloroplast dataset (*rps16* and *trn*T-*trn*E). Branch lengths are proportional to the number of changes. Numbers above the branches are Bayesian posterior probabilities (numbers < 0.5 not shown). Numbers below branches show parsimony bootstrap support (values < 50% not shown). Matching species names to species abbreviations can be found in Table 4.3.1. Numbers behind species abbreviations indicate different voucher specimen. The green bars highlight South American species, whereas the black bars signify species with longer pedicels.

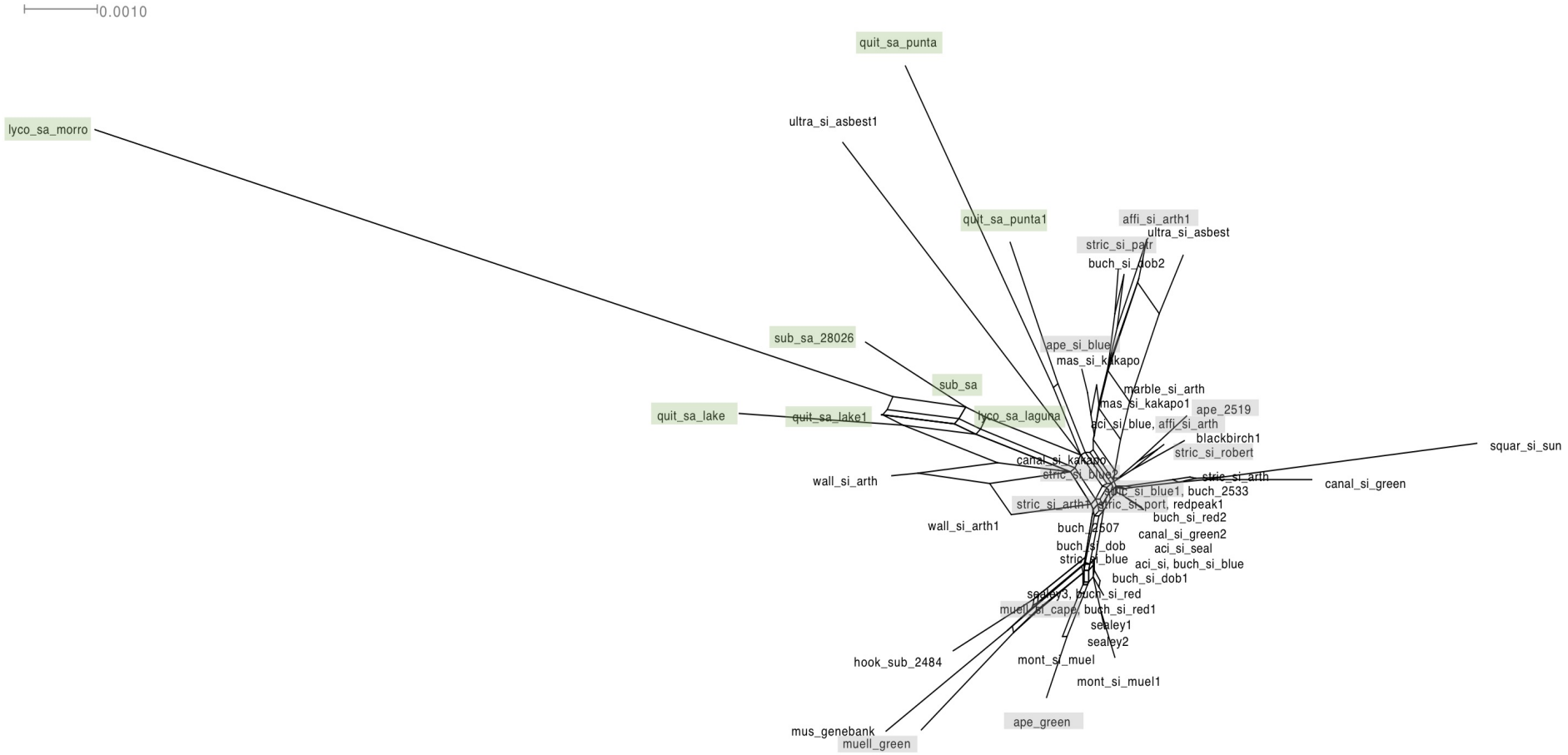

FIG 4.3.4 Splits graph network illustrating relationship among the chloroplast sequences of *Colobanthus*. Branch lengths are drawn proportionally to the genetic distances, a scale is provided in the upper left corner of the figure. South American species are highlighted in green and species with longer pedicels are highlighted in grey.

4.3.4.3 Combined ITS and chloroplast dataset

The combined dataset includes all sequences from the ITS dataset as well as all sequences from the concatenated chloroplast dataset. The final alignment included 2624 characters with 267 parsimony informative sites (including outgroups). When restricted to *Colobanthus* sequences, the number of parsimonious sites was 36.

Both parsimony analysis and Bayesian analysis resulted in similar phylogenetic trees and were therefore combined in the figure, which shows a 50% majority-rule consensus tree with bootstrap support and posterior values (Fig. 4.3.5). The arrangement of the outgroups inferred from the combined dataset is similar to the arrangement of these genera in phylogenetic trees presented by Harbaugh et al. (2010). *Colobanthus* and *Sagina* are sister groups and together with *Bufonia* form a clearly separated clade from the other Caryophyllaceae with strong bootstrap support (98%) and posterior values (1). Monophyly of *Colobanthus* is only weakly supported by a bootstrap value of 65%. All *Colobanthus* species form an unresolved crown clade with one backbone. Within the crown clade, smaller clades branch off with weak support values. These include species that are also part of the backbone of the crown clade.

The splits graph of the combined *Colobanthus* sequences (excluding outgroups) shows a high number of boxes in the core and very short genetic distances (Fig. 4.3.6), indicating conflict in the dataset and very little variation among sequences. Subsets of only South American species and species with longer pedicels (splits graphs not shown) also indicate incompatible splits by forming boxes instead of branches.

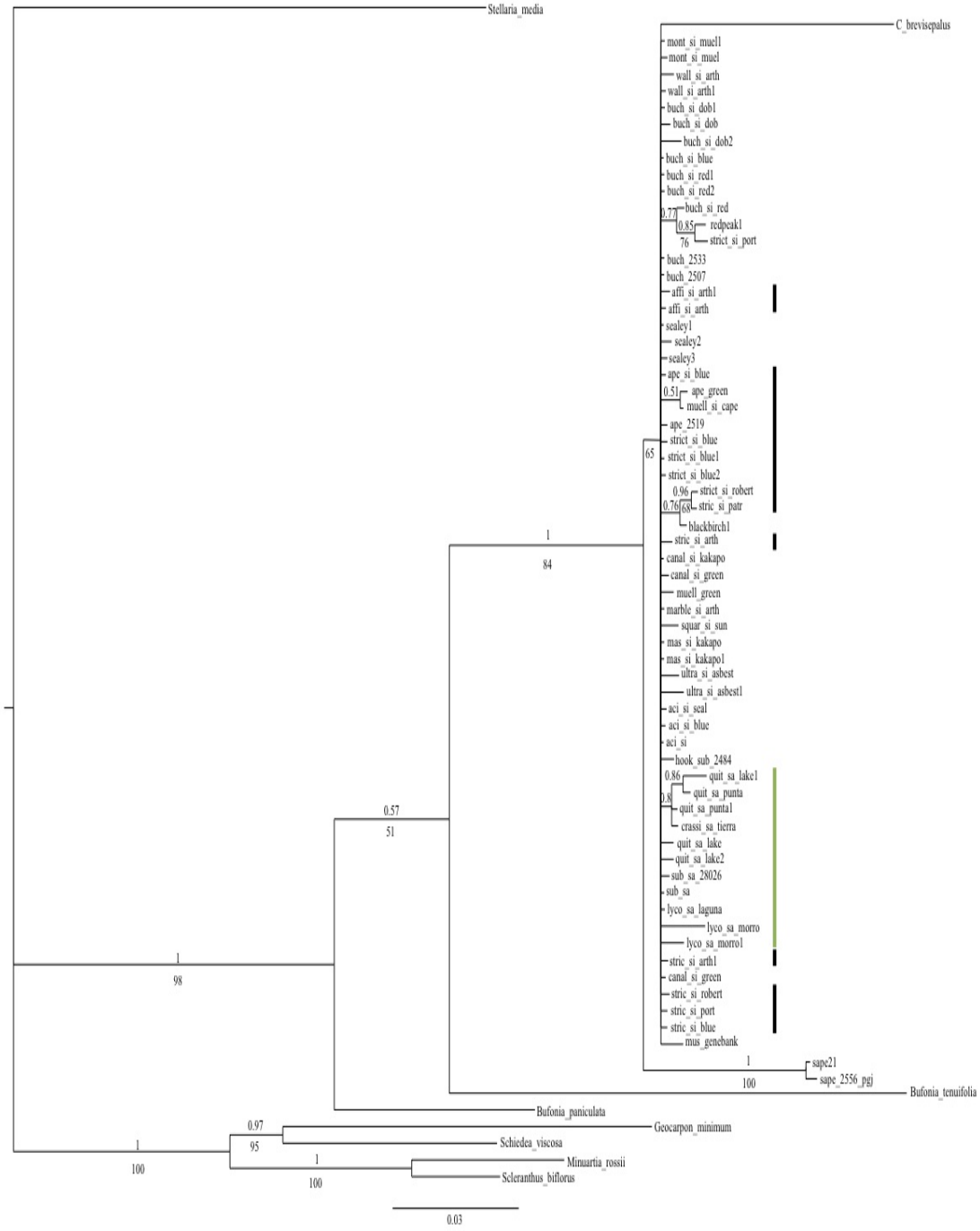

FIG 4.3.5 50% consensus tree using Bayesian analysis of the combined dataset (ITS, *rps*16 and *trn*T-*trn*E). Branch lengths are proportional to the number of changes. Numbers above the branches are Bayesian posterior probabilities (numbers < 0.5 not shown). Numbers below branches show parsimony bootstrap support (values < 50% not shown). Matching species names to species abbreviations can be found in Table 4.3.1. Numbers behind species abbreviations indicate different voucher specimen. The green bars highlight South American species, whereas the black bars signify species with longer pedicels.

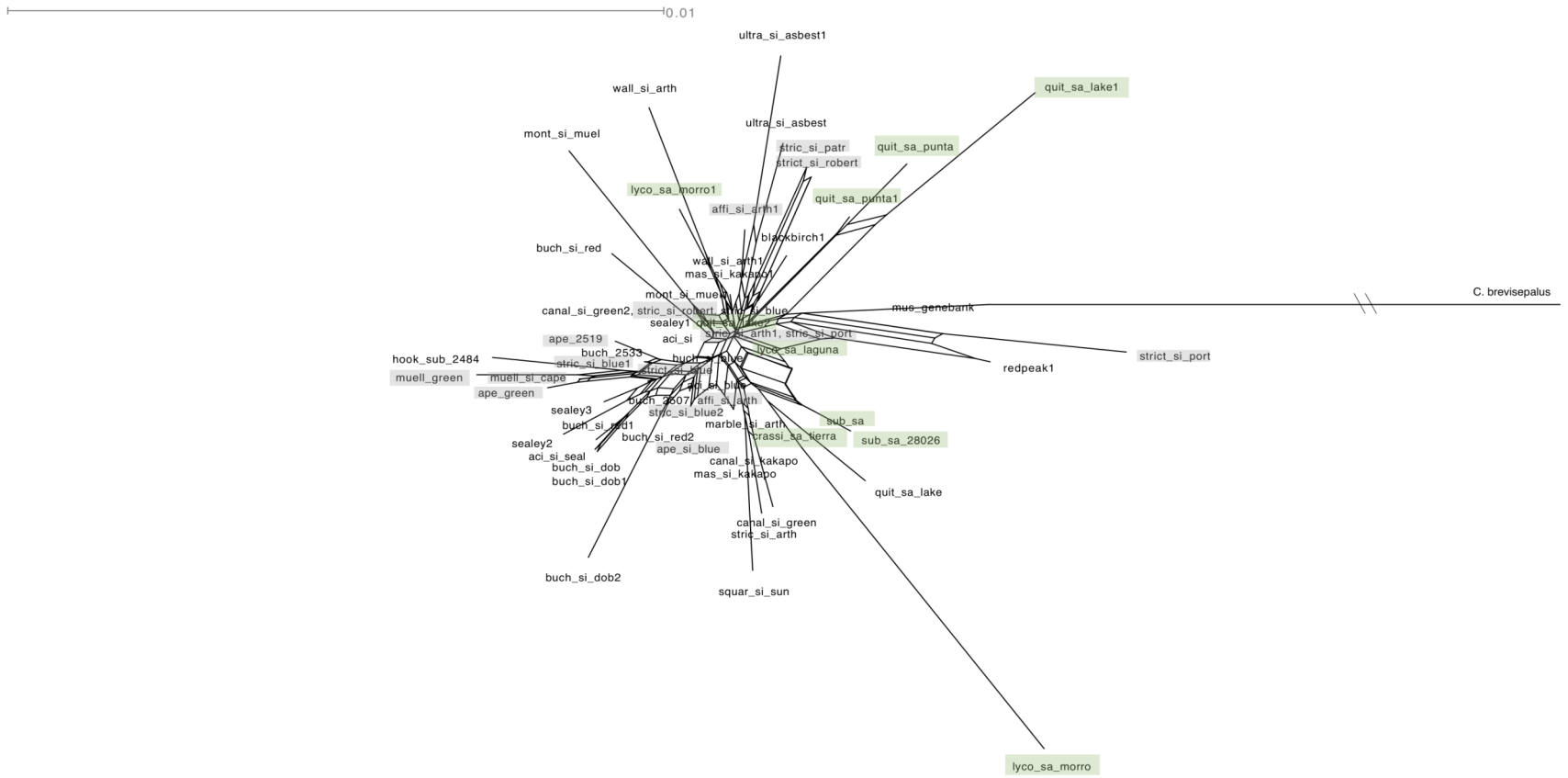

FIG 4.3.6 Splits graph network illustrating relationship among the combined nuclear and chloroplast dataset of *Colobanthus*. Branch lengths are drawn proportionally to the genetic distances, a scale is provided in the upper left corner of the figure. South American species are highlighted in green and species with longer pedicels are highlighted in grey. The branch of *C. brevisepalus* is cut in order to be included in the splits graph.

4.3.4.4 Uncorrected p-distances

Uncorrected p-distances were calculated separately for the ITS, *rps16* and *trn*T-*trn*E datasets. Species with multiple samples were included and unidentified specimens were excluded.

In the ITS dataset, uncorrected p-distances were high between *Colobanthus* and its outgroups (13.5% – 19.6%) as well as within the outgroup (12.3%. – 26.1%). Between *Sagina* and *Colobanthus*, uncorrected p-distances range from 2.9% to 10%. Within *Colobanthus*, uncorrected p-distances are very low (0% – 6.8%). The same distances are also given for comparisons between New Zealand and South American species as well as species with sessile capsules and species with capsules on longer pedicels. Within the South American species, the highest distance is 0.7%.

Table 4.3.4 contains uncorrected p-distances between ITS sequences of *Colobanthus* species to test for species delimitation. Overall, the p-distances are very low and mostly the minimum interspecific distances are smaller than the maximum intraspecific distances. *Colobanthus* 'ultramafic' is the only species that is discriminated from all other species by its uncorrected p-distances and *C. muelleri* is discriminated from all species except *C. apetalus*. South American species cannot be discriminated based on uncorrected p-distances.

In the *rps*16 dataset, distances between *Colobanthus* and the outgroup ranged from 7.6% to 14.4%, within the outgroup distances were 3.5% – 12.9%.

Between *Colobanthus* and *Sagina* uncorrected p-distances ranged from 2.7% to 3.1%. Within *Colobanthus*, distances were generally very low (0% – 1.4%), as were the distances between species with sessile fruits or capsules on longer pedicels (0% – 0.6%), distances between New Zealand and South American species (0.1% – 2.5%) and within South American species (0% – 2.2%).

The *trn*T-*trn*E dataset did not contain further outgroups, therefore uncorrected p-distances could only be calculated within *Colobanthus* and between *Colobanthus* and *Sagina*. Within *Colobanthus*, distances ranged from 0% to 2.3%. These distances include calculations between one South American and the New Zealand species (0.7% – 1.9%) as well as species with sessile fruits and capsules on longer pedicels (0% – 2.3%). Between *Colobanthus* and *Sagina*, uncorrected p-distances of 2.3% to 3.4% were calculated.

Tables 4.3.5 and 4.3.6 contain uncorrected p-distances for rps16 sequences (Table 4.3.5) and trnT-E sequences (Table 4.3.6) to test for species discrimination. Both tables have lower numbers of samples since less species had more than one sample per species compared to the ITS dataset. No clear species delimitation can be detected with the very low uncorrected p-distances although *C. masoniae* is discriminated from all other species except C. apetalus in the rps16 dataset. In the *trn*T-E dataset (Table 4.3.6), only *C. muelleri*, *C. quitensis* and *C.* 'ultramafic' showed slightly higher values of uncorrected p-distances, all other species are similar. Since no intraspecific distances are given for these species, a conclusion about species discrimination cannot be drawn.

Table 4.3.4 Uncorrected p-distances between ITS sequences of *Colobanthus*. Maximum intraspecific distances are in **BOLD**, minimum interspecific distances larger than the max. intraspecific distances are in *ITALICS*. The top row contains only species with more than one sample per species, therefore not all species have maximum intraspecific distances.

	acicularis	*affinis*	*apetalus*	*buchananii*	*canaliculatus*	*lycopodioides*	*masoniae*	*monticola*	*muelleri*	*quitensis*	*strictus*	*subulatus*	*ultra*
acicularis	**0.004**	0	0	0	0	0	0	0	*0.001*	0	0	0	*0.003*
affinis	0	**0.001**	0.001	0	0	0	0	0	*0.003*	0	0.001	0	*0.003*
apetalus	0	0.001	**0.001**	0	*0.001*	0.001	0.001	*0.001*	0	0.001	0	*0.001*	*0.004*
brevisepalus	*0.051*	*0.056*	*0.057*	*0.051*	*0.061*	*0.051*	*0.056*	*0.051*	*0.068*	*0.051*	*0.051*	*0.057*	*0.056*
buchananii	0	0	0	**0.01**	*0.001*	0	0	0	*0.001*	0.001	0	*0.001*	*0.003*
canaliculatus	0	0	0	0.001	**0**	0	0	*0.003*	*0.003*	0	0.001	0	*0.003*
crassifolius	0.003	0.001	0.003	0.003	*0.001*	0.001	0.001	*0.003*	*0.004*	0	0.003	*0.001*	*0.004*
hookeri	0.003	*0.004*	*0.003*	0.003	*0.004*	0.004	*0.004*	*0.004*	*0.004*	0.004	0.003	*0.004*	*0.007*
lycopodioides	0	0	0.001	0	0	**0.005**	0	0	*0.003*	0.001	0.001	0	*0.003*
marble	0	0	0.001	0.001	0	0	0	*0.001*	*0.003*	0	0.001	0	*0.004*
masoniae	0	0	0.001	0	0	0	**0.001**	0	*0.003*	0	0.001	0	*0.003*
monticola	0	0	*0.003*	0	*0.003*	0	0	**0**	*0.004*	0.001	0.001	*0.001*	*0.004*
muelleri	0.003	*0.003*	0	0.001	*0.003*	0.003	*0.003*	*0.004*	**0**	0.003	0.001	*0.003*	*0.006*
quitensis	0	0	0.001	0.001	0	0	0	*0.001*	*0.003*	**0.007**	0.001	0	*0.004*
squarrosus	0.003	0.001	*0.003*	0.003	*0.001*	0.001	0.001	*0.003*	*0.004*	0.004	0.003	*0.001*	*0.004*
strictus	0	0.001	0	0	*0.001*	0.001	0.001	*0.001*	*0.001*	0.001	**0.012**	*0.001*	*0.004*
subulatus	0	0	0.001	0.001	0	0	0	*0.001*	*0.003*	0	0.001	**0**	*0.004*
ultra	0.003	*0.003*	*0.004*	0.003	*0.003*	0.003	*0.003*	*0.004*	*0.006*	0.004	0.004	*0.004*	**0.001**
wallii	0	0	0.001	0	*0.004*	0	0	0	*0.004*	0.001	0.001	*0.001*	*0.003*

Table 4.3.5 Uncorrected p-distances between *rps*16 sequences of *Colobanthus*. Maximum intraspecific distances are in **BOLD**, minimum interspecific distances larger than the max. intraspecific distances are in *ITALICS*. The top row contains only species with more than one sample per species, therefore not all species have maximum intraspecific distances.

	acicularis	*apetalus*	*buchananii*	*canaliculatus*	*lycopodioides*	*masoniae*	*monticola*	*muelleri*	*quitensis*	*strictus*	*ultra*
acicularis	**0**	0.001	0	0	0.001	*0.001*	0.001	0.001	0.005	0	0.002
affinis	*0.003*	0.002	0.003	0.003	0.006	*0.001*	*0.006*	*0.005*	0.006	0.003	0.002
apetalus	*0.001*	**0.004**	0	0.001	0.001	0	*0*	0	0.005	0.001	0.004
buchananii	0	0	**0.007**	0	0.001	*0.001*	0	*0*	0.005	0	0.003
canaliculatus	0	0.001	0	**0.004**	0.001	*0.001*	0.001	0.001	0.005	0	0.003
hookeri	*0.003*	0.004	0.002	0.003	0.006	*0.005*	*0.005*	0.002	0.007	0.002	0.006
lycopodioides	*0.001*	0.001	0.001	0.001	**0.015**	*0.004*	*0.004*	*0.009*	0.004	0.001	*0.009*
marble	0	0.001	0	0	0.001	*0.001*	0.001	0.001	0.003	0	0.003
masoniae	*0.001*	0	0.001	0.001	0.004	**0**	*0.002*	0.002	0.005	0.001	0.002
monticola	*0.001*	0	0	0.001	0.004	*0.002*	**0.001**	*0*	0.005	0.001	0.005
muelleri	*0.001*	0	0	0.001	0.009	*0.002*	*0*	**0.004**	0.005	0.001	*0.009*
muscoides	*0.004*	0.004	0.004	0.004	0.008	*0.007*	*0.004*	0.004	0.009	0.004	*0.008*
quitensis	*0.005*	*0.005*	0.005	*0.005*	0.004	*0.005*	*0.005*	*0.005*	**0.012**	0.002	*0.009*
squarrosus	*0.006*	*0.007*	0.004	0.004	0.009	*0.007*	*0.009*	*0.007*	0.009	0.004	*0.009*
strictus	0	0.001	0	0	0.001	*0.001*	0.001	0.001	0.002	**0.004**	0.002
subulatus	*0.001*	0.004	0.001	0.001	0	*0.004*	*0.004*	0.004	0.004	0.001	*0.008*
ultra	*0.002*	0.004	0.003	0.003	0.009	*0.002*	*0.005*	0.004	0.009	0.002	**0.007**
wallii	*0.001*	0.003	0.001	0.001	0.003	*0.003*	*0.003*	0.003	0.001	0.001	0.004

Table 4.3.6 Uncorrected p-distances between *trn*T-E sequences of *Colobanthus*. Maximum intraspecific distances are in **BOLD**, minimum interspecific distances larger than the max. intraspecific distances are in *ITALICS*. The top row contains only species with more than one sample per species, therefore not all species have maximum intraspecific distances.

	acicularis	*apetalus*	*buchananii*	*canaliculatus*	*strictus*
acicularis	**0**	0	0	0	0
affinis	0	0	0	0	0
apetalus	0	**0.003**	0	0	0
buchananii	0	0	**0**	0	0
canaliculatus	0	0	0	**0**	0
masoniae	0	0	0	0	0
muelleri	*0.003*	0.003	*0.003*	*0.003*	0
quitensis	*0.009*	*0.011*	*0.009*	*0.009*	*0.007*
squarrosus	0	0	0	0	0
strictus	0	0	0	0	**0.003**
ultra	*0.011*	*0.006*	*0.009*	*0.009*	*0.009*
wallii	0	0	0	0	0

4.3.5 DISCUSSION

Both nuclear and chloroplast sequences show similar results, albeit very little variation. The phylogenies emerging from the ITS and chloroplast sequences as well as the combined dataset recognize *Colobanthus* as one weakly supported and unresolved crown clade with *Sagina* forming the sister group. The selected outgroups have been confirmed as outgroups consistent with Smissen et al. (2002) and Harbaugh et al. (2010). Especially the combined dataset shows very strong support for the outgroup topography in the phylogenetic tree.

All three datasets place all *Colobanthus* species in one clade separately from *Sagina*, potentially suggesting monophyly. However, bootstrap support is generally weak and no posterior values are given, even though the bootstrap values increased slightly in the combined dataset. Therefore the monophyly of *Colobanthus* cannot clearly be inferred from the three sequenced genes and further sampling of *Sagina* is warranted to resolve the relationship between these two related genera.

Colobanthus species are presented as a crown clade and splits graphs visualize a high number of conflicting signals among the sequences in the genus. To investigate

species delimitation further, interspecific and intraspecific uncorrected p-distances have been calculated. Two questions were asked concerning species relationships within *Colobanthus* – (1) how many South American species are there and (2) are species delimitations in New Zealand *Colobanthus* appropriate?

In general, uncorrected p-distances are larger in the ITS dataset compared to the chloroplast datasets, which also contain fewer species. However, within *Colobanthus* distances are very low and therefore results have to be treated with caution. Often the maximum intraspecific distances equal 0%, especially when two samples are from the same location. Maximum distances are usually larger when samples of the same species are compared between different locations.

South American species cannot be discriminated based on uncorrected p-distances, since the minimum interspecific distances are never larger than the maximum intraspecific distance (CBOL Plant Working Group, 2009) of any South American species in the ITS dataset. The use of five different names should therefore be abandoned. However, the names that are currently used (*C. quitensis* and *C. subulatus*) apply to two morphologically very different species (Moore, 1983; own obs.) and I propose to maintain the names based on the morphological differences.

Within New Zealand, very few differences could be found between the species. This suggests that speciation and morphological divergence have been achieved without corresponding divergence of ITS, *rps*16 and *trn*T-E sequences. *Colobanthus brevisepalus* has large uncorrected p-distances to all other species. The sample used in this analysis was taken from GenBank and mistakes might have been made in the alignment of this sample with the other specimens. Further sequencing is recommended to confirm the difference to other tested species. *Colobanthus* 'ultramafic' can be discriminated from all other species based on the p-distances in

the ITS dataset and its position on phylogenetic trees inferred from ITS and chloroplast markers (but not the combined dataset) also indicate larger genetic differences to other *Colobanthus* species. This undescribed species can only be found on ultramafic rocks in two locations in the North of the South Island (B. Sneddon, pers. comm.; own obs.). Previously it was identified as *C. strictus* (Robinson et al., 1997) but results of the phylogenetic analysis might indicate that C. 'ultramafic' might be a different species.

Uncorrected p-distances between South American and New Zealand species do not show species discrimination between most species and species delimitation should be reconsidered. Comparing species with sessile capsules and species with capsules on longer pedicels, only *C. muelleri* stands out because it can be clearly discriminated from all other species except *C. apetalus* and this difference is constricted to the ITS dataset. This species is also the only coastal species and therefore ecologically separated from other New Zealand species (Allan, 1961). It is also morphologically very distinct as it is the only species that forms small rosettes. Phylogenetic analyses of the ITS and chloroplast dataset (but not the combined dataset) also indicate that *C. muelleri* is separate from other *Colobanthus* species.

Another potential aim of the molecular analysis of the phylogeny of *Colobanthus* was the identification of unidentified specimen (e.g. blackbirch, sealey). Some New Zealand *Colobanthus* species are very similar and occur conspecific (Allan, 1961) and species identification is difficult. However, due to the low resolution of all tested genetic markers, I have not been able to identify these unknown specimens.

Within *Colobanthus*, the lack of significant sequence divergence and the formation of an unresolved crown clade in all analysed sequence data support a closely related

southern hemisphere genotype and suggest very recent and rapid dispersal and speciation.

The lack of resolution in molecular datasets for New Zealand plant species is not uncommon and well documented in *Sophora* (Mitchell and Heenan, 2002), *Veronica* (Wagstaff and Garnock-Jones, 2000; Meudt and Bayly, 2008), *Hoheria* (Wagstaff et al., 2010), *Scleranthus* (Smissen et al., 2003) , Gnaphalieae (Smissen et al., 2004), *Abrotanella* (Wagstaff et al., 2006) and *Plantago* (Tay, 2008). These unresolved clades can be explained by the natural history of New Zealand. In recent years it became evident that a large part of the New Zealand flora was formed after recent long-distance dispersal events followed by rapid diversification after the tertiary uplift of the mountains and Quaternary climate change (Raven, 1973; Pole, 1994; Macphail, 1997; Swenson and Bremer, 1997; Winkworth et al., 1999; Winkworth et al., 2002b). Even though recent long-distance dispersal presents a possible explanation for the low variation between South American and New Zealand species, the dispersal mechanism and seed size (Allan, 1961; Bergstrom, 1986; Hennion and Walton, 1997; Webb and Simpson, 2001; Chapter 4.2) seem unlikely to support this hypothesis. *Colobanthus kerguelensis* seeds have been found to be able to float in freshwater for up to 24 hours but dispersal agents between islands have still not been identified (Hennion and Walton, 1997; Van der Putten et al., 2010). Nevertheless, long distance dispersal has been proposed in other plants with hygrochastic capsules (e.g. two *Veronica* species of the snow hebe clade occur in New Zealand and Australia (Meudt, 2008)).

However, the extremely low resolution in the tested nuclear and chloroplast sequences is exceptional since not even wide geographic separation, e.g. between South American and New Zealand species seem to have resulted in considerable

sequence divergence. Although some species are morphologically distinct (e.g. species with longer pedicels and caespitose growth form compared to cushion plants with sessile capsules), these differences do not show in the phylogenetic analysis of nuclear and chloroplast sequences. This might suggest that morphological evolution is proceeding at a rate greater than the molecular evolution of the ITS, *rps*16 and *trn*T-*trn*E regions (Hurr et al., 1999; Mitchell and Heenan, 2002).

Similar difficulties associated with establishing sound relationships among species using molecular phylogenies also occur in other genera within the Caryophyllaceae (Smissen et al., 2003; Fior and Karis, 2007; Kool et al., 2007). Likewise, morphological homoplasy is common in the Caryophyllaceae and makes species discrimination problematic (Endress, 1996; Hufford, 1996; Smissen et al., 2002; Fior et al., 2006).

The phylogenies presented here are based on one nuclear and two chloroplast sequences and are by no means sufficient to clearly resolve interspecific relationships in *Colobanthus*. Genetic variation in all three markers is extremely low and restricted to single point mutations to different bases in different specimens, which might also have contributed to the conflicting signals in the splits graph analysis. Reasons for the low resolution the molecular datasets could be very recent speciation and subsequent radiation into new habitats.

There are other, as of yet unexplored ways to solve the phylogeny of this genus. This could include the use of different markers with higher variation or the combination of molecular and morphological data, which has proved to be successful in other Caryophyllaceae (Fior and Karis, 2007) or other New Zealand taxa with otherwise low genetic variation ((Meudt, 2008). Chromosome counts are useful in assessing polyploidy, which might have an influence on incompatible splits in splits graphs

(Tay, 2008; Wagstaff et al., 2010). Other appropriate molecular methods also include AFLPs or microsatellites, which can reveal genetic variation not seen in DNA sequences from a small number of regions (Ellis et al., 2006; Selkoe and Toonen, 2006; Meudt and Bayly, 2008).

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Chapter 4.4

Summary

Contrasting to earlier assumptions, only one dehiscence type can be found across all *Colobanthus* species. Slight differences from species to species are the size of cells and number of valves, but in general, all species are capable of opening upon wetting by using a combination of imbibition and cohesion mechanism in the capsule wall. Unlike most hygrochastic capsules, which are closed when dry, most *Colobanthus* species are partly open due to splits between the valves of the capsules.

Some species can be found in alpine habitats, along water currents and in coastal areas, where splash water as a dispersal agent is readily available. However, some South American species grow on the plains of Patagonia, where rain is scarce. Here, heavy winds can probably contribute to the dispersal of the seeds by shaking the entire plant and seeds escaping through the small slits between the valves.

A molecular approach was taken to investigate the phylogeny of *Colobanthus*. Due to very little variation in the tested genetic markers, the phylogeny remains unresolved. This is probably due to very recent speciation. Since no noticeable genetic differences could be found between South American, subantarctic and New Zealand species, long-distance dispersal between those locations might be a possible explanation for the close interspecific relationships. However, in Chapter 4.2 I established that all *Colobanthus* species are hygrochastic, which implies short distance dispersal as the primary dispersal strategy (see also Chapter Three). Although the occurrence of the same specific dehiscence mechanism in all species supports the recent speciation hypothesis, it appears to contradict the long-distance dispersal theory. However, most *Colobanthus* capsules stay slightly open once they are ripe, which might allow very strong winds to blow seeds out of the capsules and disperse them over wider

distances. The capsules themselves are quite fragile since they do not contain any lignin and could be destroyed easily, which might also release seeds independently from water drops.

Further research is warranted to investigate the chance events that allow seeds of hygrochastic species to be dispersed over long-distances and colonize new habitats. *Colobanthus* might be an extreme example of possibly several recent long-distance dispersal events across the southern hemisphere.

Incidental to the main aims of this chapter, phylogenetic analysis suggests species limits in *Colobanthus* might be too narrowly delineated. Molecular methods that are more sensitive and a wider sampling of *Sagina* are recommended as approaches to taxonomic questions raised here.

CHAPTER FIVE

Capsule Dehiscence Types in *Oenothera* (Onagraceae)

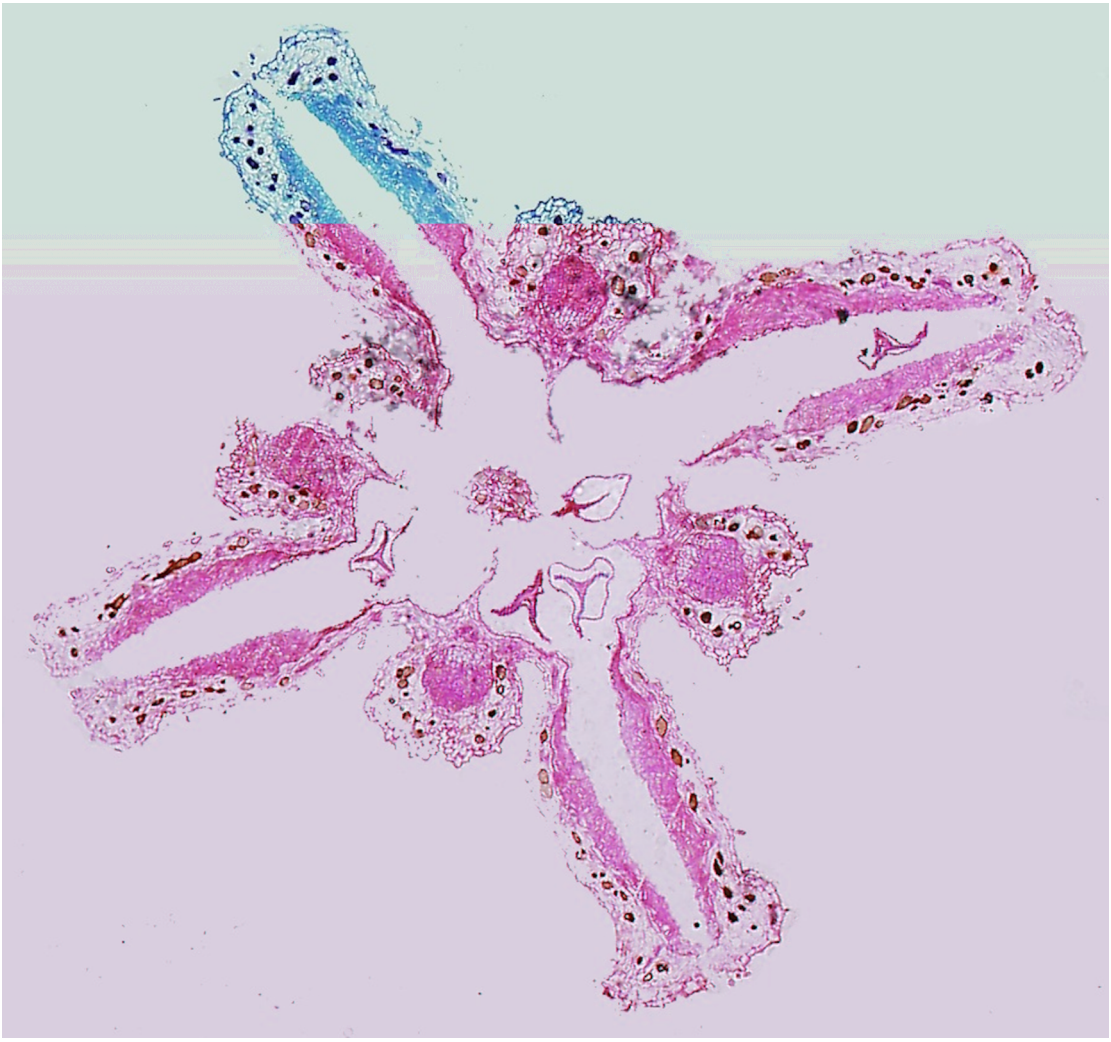

FIG 5.1 Micrograph of cross section through an *Oneothera speciosa* capsule, stained with Toluidine Blue at 4x magnification.

5.1 Abstract

Oenothera is a species-rich genus and best known for its showy flowers that open in the evening, hence the common name evening primrose. However, fruits in this genus prove to be just as fascinating, with the capsule exhibiting a range of dehiscence mechanisms, such as xerochasy and hygrochasy. Hygrochastic capsules stay closed until they are moistened during rainfall and open in the form of a splashcup, whereas xerochastic opening occurs during drying out and is reversed when moistened. This phenomenon has been described in a small number of the 145 species of this genus, all of them in a subclade characterized by winged fruits. I investigate additional *Oenothera* species regarding their dehiscence type using observations of the opening and detailed anatomical study. *Oenothera fruticosa*, *O. speciosa* and *O. perennis* exhibit hygrochasy, whereas *O. howardii* is likely to have xerochastic capsules. *Oenothera pallida*, a 'ripening dehiscent' species, showed hygrochastic movement of the already dehisced fruit. In all investigated species, hygrochastic movement is achieved by directional swelling of the endocarp of the valves and the dorsal bundle together with the exocarp act as resistance tissue. This work extends the list of described hygrochastic and xerochastic *Oenothera* species to nine species and provides the base for further research on the connection between dispersal strategies, systematics and habitat type in several hygrochastic genera.

Keywords: capsule anatomy, hygrochasy, evening primrose, *Oenothera*, North America, rain dispersal

5.2 Introduction

Oenothera is the second largest genus in the Onagraceae with 145 species in 18 sections (Wagner et al., 2007). The genus has a wide distribution across temperate and subtropical areas of North and South America with a few species in Central America. Habitats range from sea level up to 5000 m elevation. Plants can often be found in open or disturbed habitats. A number of species are invasive or naturalized in Europe, Asia, Australia and New Zealand (Webb et al., 1988; Thompson, 1990; Sun et al., 1992; Dhaliwal and Sharma, 1995; Deng et al., 2001; Hosking et al., 2003; Mihulka et al., 2006; Gfutte et al., 2007; Lambdon et al., 2008). The common name evening primrose alludes to the showy flowers opening mostly in the evening and rarely lasting for more than one day. The vespertine flowers are usually pollinated by hawkmoths, whereas diurnal flowers have various pollinators, such as bees, butterflies or hummingbirds (Wagner et al., 2007).

Seed anatomy and capsule morphology have been used with some success to divide the genus roughly into two lineages (Tobe et al., 1987) – lineage A is defined by radially enlarged endotestal cells and lineage B by angled or winged capsules. Molecular evidence more or less supports these two subclades with some differences (Levin et al., 2004). Poppendieck (1995) investigated dispersal strategies in some species of subclade B and found a shift to hygrochastic dispersal in some species and a shift to xerochastic dispersal in *Oenothera macrocarpa*, based on anatomical differences and capsule opening observations. Hygrochasy is a dehiscence mechanism where structures open when moistened and close again when they dry out (Fahn and Werker, 1972). Different plant structures such as bracts, sepals or even entire plants can be involved (Fahn and Werker, 1972; Gutterman, 1990, 1994; Hegazy et al., 2006) but hygrochasy is often restricted to the fruit itself, mostly a woody capsule

(Garside and Lockyer, 1930; Ellner and Shmida, 1981; Van der Pijl, 1982; Ihlenfeldt, 1983; Thulin, 1987; Poppendieck, 1995; Parolin, 2006). Hygrochastic seed dispersal is generally known from plants in arid regions (Ellner and Shmida, 1981; Ihlenfeldt, 1983; Gutterman, 1990, 1994; Van Oudtshoorn and Van Rooyen, 1999) where it serves as a strategy to delay dispersal in time by restricting it to rainfalls. As part of ombrohydrochoric dispersal strategies, hygrochasy also results in atelechoric dispersal since raindrops transport the seeds only over a short distance (Ihlenfeldt, 1983; Hartmann, 1988; Gutterman, 1990; Parolin, 2001; Pufal and Garnock-Jones, 2010). Next to the well-known Aizoaceae, hygrochastic capsule dehiscence has also been reported in various other plant groups and habitats, such as herbs alongside streams in temperate climate (Nakanishi, 2002), *Xylocalyx* (Scrophulariaceae) in Somalia (Thulin, 1987) and alpine *Veronica* in New Zealand (Garnock-Jones, 1993; Garnock-Jones and Lloyd, 2004; Pufal et al., 2010).

Poppendieck's work on *Oenothera* is not the only report of hygrochasy in this genus. In a technical conservation assessment, Ladyman (2005) describes *O. harringtonii* as hygrochastic and Walck and Hidayati (2007) investigated the influence of the dehiscence type on seed dispersal in *O. triloba* (hygrochastic) and *O. macrocarpa* (xerochastic), both described by Poppendieck (1995). Of importance is also the discovery of hygrochasy in *O. rosea* by Brodie (1951). So far, capsule anatomy in hygrochastic *Oenothera* has only been described for three species in greater detail (*O. triloba*, *O. fruticosa* ssp. *glauca*, *O. rosea*) and observations of hygrochasy have been made in two additional species (*O. perennis*, *O. acaulis*) (Poppendieck, 1995; Ladyman, 2005).

In this study I investigate the fruits of several species from subclade B as well as a comparative example belonging to sect. *Anogra* in subclade A regarding their

dispersal mechanism. The aim is to identify and describe their opening mechanism using the methods successfully implemented by Poppendieck (1995). Poppendieck (1995) also proposed a sequence of increasing structural complexity in hygrochastic fruit of *Oenothera* and results from this work are compared with Poppendieck's findings. This study will provide the basis for further research on connections between dehiscence, dispersal mechanisms, distribution and systematics.

5.3 Materials and methods

Material for this study was collected at field sites in the northwestern USA and additional specimens were provided by K. N. Krakos and M. Johnson (St. Louis University) (Table 5.1). The state of the capsules (open/closed) was noted and capsules were then submerged in water. If opening or movement occurred, the time until completion of the movement was recorded. For some species only closed unripe capsule material was available, which was not suitable for this test. However, the opening mechanism of some of them has been described previously by Poppendieck (1995).

Dry capsules of all species were subjected to an ethanol series and embedded in epoxy resin (see Chapter 2.3 for details on this procedure). Capsules of *O. howardii* and *O. macrocarpa* were cut in half lengthwise due to their size and for *O. pallida* single valves were used instead of entire capsules. Sections of 2 – 4 µm thickness were cut using a Leica Ultracut E ultratome and stained using toluidine blue (O'Brien et al., 1964), which stains cell walls. Micrographs were taken in order to describe the arrangement of swelling tissue and resistance tissue, both essential in the hygrochastic opening of capsules. Photographs of all ripe capsules were taken before and after wetting under a stereo microscope with a Panasonic G1 camera.

Table 5.1 Origin of *Oenothera* capsules used in the study. The subclade according to Levin et al (2004) is indicated behind the species name.

species	location	coordinates	collector	voucher #
O. flava (B)	State Highway 70 near Portola, California	N 39º39'33.0" W129º21'29.5"	Gesine Pufal, 22/07/08	WELTU20244
O. fruticosa (B)	Durham Co, North Carolina		Marc Johnson, 27/06/07	
O. gaura (B)	Belchertown, Massachusetts		Kyra N. Krakos, 09/09/07	07KK01
O. howardii (B)	along State Highway 12 near Red Canyon, Utah	N 37º44'29.7" W112º18'00.4"	Gesine Pufal, 17/07/08	WELTU20245
O. linifolia (B)	Nel's Homberg property, Gray Summit, MO		Kyra N. Krakos, 25/06/08	08KK03
O. macrocarpa	Shaw Nature Reserve, Missouri		Kyra N. Krakos, 20/06/08	08KK08
O. perennis (B)	Great Meadows National Forest, Massachusetts		Kyra N. Krakos, 07/07/08	08KK12
O. speciosa (B)	Payson, Arizona	N 34º14'25.1" W 111º19'05.4"	Gesine Pufal, 14/07/08	WELTU20246
O.pallida (A)	Outside fence at north entrance Petrified Forest National Park, Arizona	N 35º03'40.3" W 109º46'58.2"	Gesine Pufal, 15/07/08	WELTU20247

5.4 Results

Capsules were available for nine species, although five of them (*O. howardii*, *O. gaura*, *O. macrocarpa, O. pallida* and *O. flava*) were still unripe and tissues were partly not fully developed (e.g. dorsal bundle not differentiated). *Oenothera macrocarpa* has been described previously by Poppendieck (1995) and is here used as reference in comparison with *O. howardii*. In *O. gaura* and *O. flava* the development of tissues was not completed and they are therefore not used in the analysis of micrographs. In the remaining five *Oenothera* species opening was observed in *O. fruticosa*, *O. linifolia* and *O. perennis* and in some *O. speciosa* capsules, which were already slightly open when received (Table 5.2). Capsules of *O. pallida* were unripe

when collected and split slightly during storage in silica gel. Here, valves moved outward further when submerged in water.

Table 5.2 State of *Oenothera* capsules before and after wetting. If opening occurred the time was noted. Given is the mean opening time for the number of capsules tested.

species	before wetting	after wetting	time	# caps.
O. flava	unripe	NA	NA	10
O. fruticosa	top closed, splits between valves	splash cup	3 min	11
O. gaura	unripe	NA	NA	10
O. howardii	unripe	NA	NA	5
O. linifolia	closed	splash cup	2 min	11
O. macrocarpa	unripe	NA	NA	7
O. pallida	unripe, valves slightly split open	Further opening	2 min	6
O. perennis	closed	splash cup	4.5 min	4
O. speciosa	slightly open	splash cup	5 min	4

Below, the arrangement of tissues in capsules is specified in six species and their role in the movement of the capsules is described.

5.4.1. *OENOTHERA LINIFOLIA* (SECT. *PENIOPHYLLUM*)

Plants of this species are slender erect herbs between 15 and 45 cm tall. The capsules are described as nearly obovoid, sessile, puberulent and sharply four-sided (Britton and Brown, 1970) (Fig. 5.2 A). The wings are the tips of valves folded backward and attached to each other. When dehiscing, the valves separated and the capsules split at the wings. Especially older fruit, which had been open before, opened quickly (Table 5.2) and displayed a very wide splash cup (Fig. 5.2 B). The swelling tissue is located at the inside of the capsule walls and consists of several cell layers (endoderm) (Fig. 5.3 A).

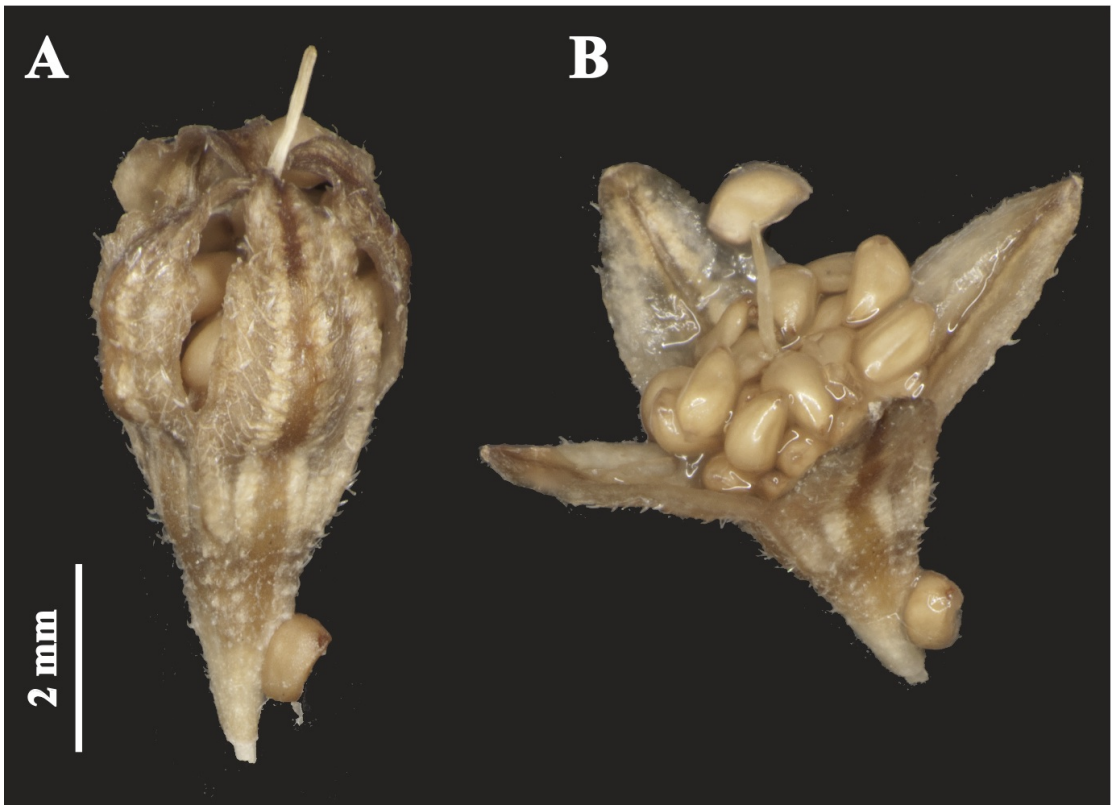

FIG 5.2 Dry (A) and wet (B) capsule of *O. linifolia*. Mucilage on the seeds exposed in the splash cup is visible.

The cells have considerably thickened cell walls compared to the cells in the dorsal bulge around the dorsal bundle (indicated by the strong staining with toluidine blue) (Fig. 5.3 A). After moistening the swelling tissue enlarges lengthwise leading to straightening of the valves and a subsequent split at the wings. Rigidity of the valve is given by the dorsal bulge, which, together with the outer capsule wall, acts as resistance tissue. This resistance forces the straightened valves to move backwards as a whole, resulting in a wide splash cup. Note that the dorsal bulge hardly 'bulges' at all, compared to other *Oenothera* species it is rather flat and the dorsal bundle is embedded in the valve. Another interesting feature is that the seeds develop mucilage immediately upon wetting. When dry, the mucilage acts like glue, keeping the seeds firmly in place.

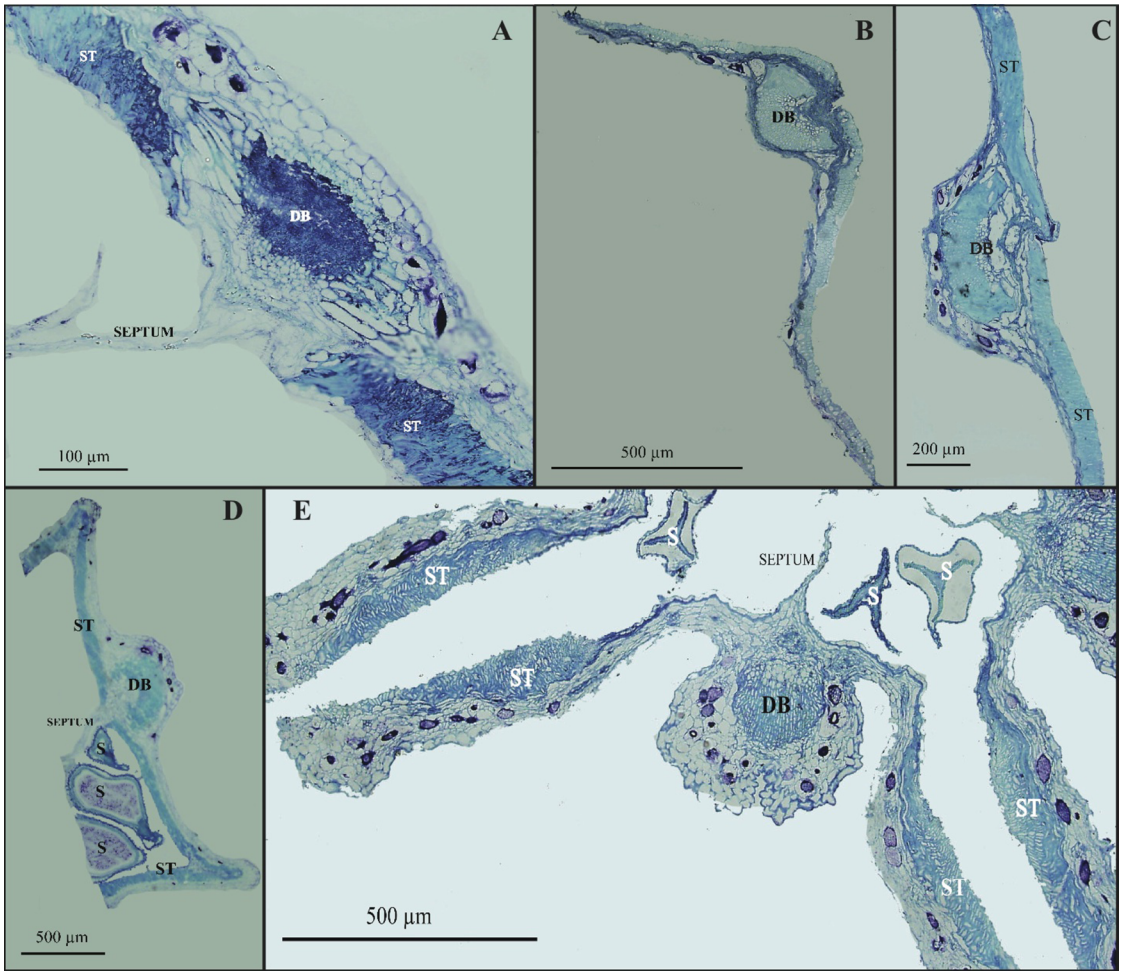

FIG 5.3 Micrographs cross-sections of *Oenothera* capsules, stained with toluidine blue. Species from subclade B are BOLD - (A) ***O. linifolia*** valve, (B) *O. pallida* valve, (C) ***O. fruticosa*** valve, (D) ***O. perennis*** valve (still attached to neighbouring valves) and seeds, (E) ***O. speciosa*** two valves and seeds. DB - dorsal bundle, ST – swelling tissue, S – seed.

5.4.2 *Oenothera perennis* (sect. *Kneiffia*)

This perennial herb is 20 to 75 cm tall and only sparingly branched. Capsules are tetragonal or narrowly winged, approximately 5 – 10 mm long and 2 – 3 mm thick, tapering to a short stipe (Straley, 1977). In contrast to *O. linifolia*, the dorsal bulge is very dominant with a strongly developed dorsal bundle. The swelling tissue is located on the inside of the capsule wall and extends along the entire length of the valve. No swelling tissue can be found on the septum (Fig. 5.3 D). The hygrochastic mechanism in this species works similarly to what has been described in *O. linifolia*. The samples

available were not fully ripe when processed (Fig. 5.4 A). The opening after wetting affects only the upper third of the capsules (Fig. 5.4 B) but might extend further in ripe capsules.

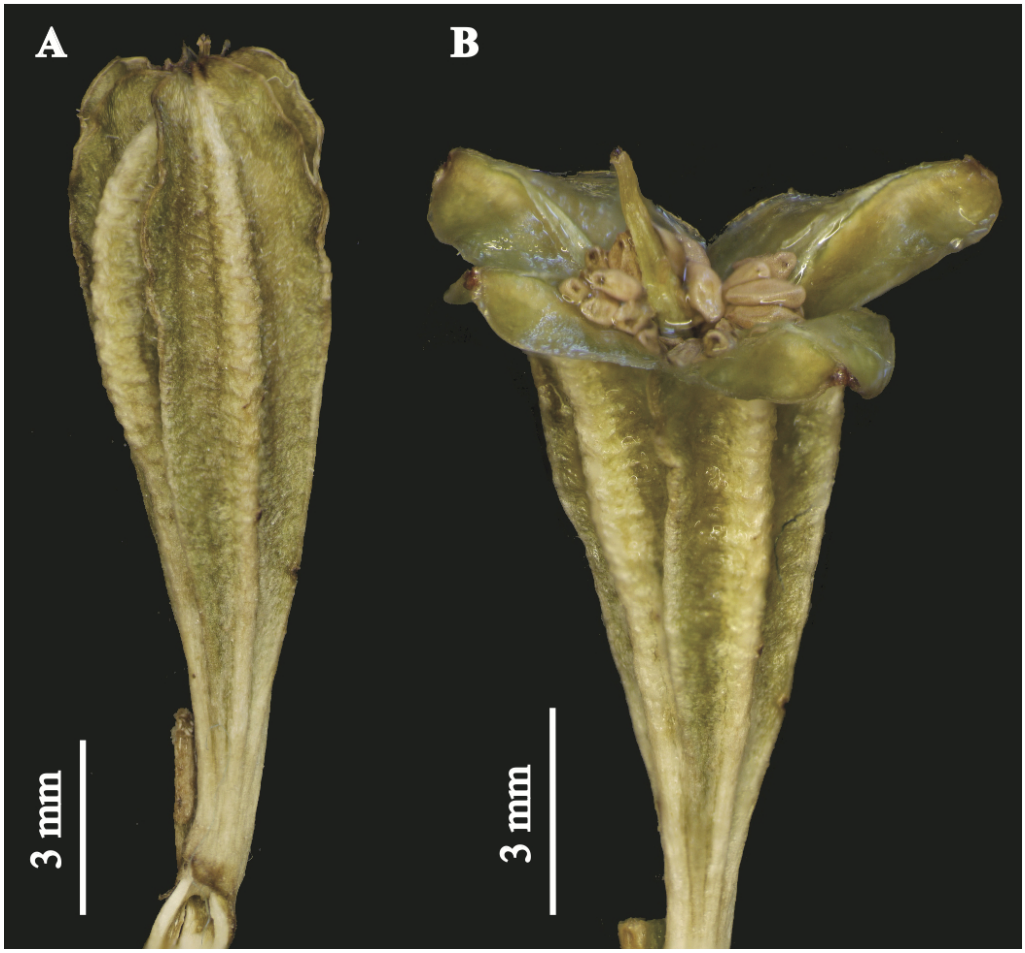

FIG 5.4 Dry capsule (A) of *Oenothera perennis* and same capsule after wetting (B) exposing seeds and placental column.

5.4.3 *OENOTHERA SPECIOSA* (SECT. *HARTMANNIA*)

Plants of this species are erect, ascending or decumbent perennials between 15 and 95 cm tall. The capsules are more or less clavate, strongly four-ribbed and four-winged and sit on a short pedicel (Britton and Brown, 1970). Here, the swelling tissue does not cover the entire inside of the valve; rather, when viewed in transverse sections, it is very prominent in the middle with several cell layers and disappears towards the

end of the valve (Fig. 5.3 E). The dorsal bulge is extremely thick, which is additionally accentuated by the thinness of the valve around the dorsal bulge (Fig. 5.3 E). In dry conditions the valves are bent backwards, which causes the very prominent wings of the capsule. Of the material available the capsules closest to ripening did not open. However, some unripe capsules that dried in the silica gel opened slightly during the drying process (Fig. 5.5 A). After wetting, the four valves of those capsules opened in a splash cup in the upper third of the capsule (Fig. 5.5 B). Since the valves are bent back in dry conditions, the stretching of the swelling tissue after wetting leads to forward motion of the valves, basically unfolding the wings and straightening the valves. The dorsal bundle and the cells surrounding it act as resistance tissue and the valves stretch and open into a splash cup.

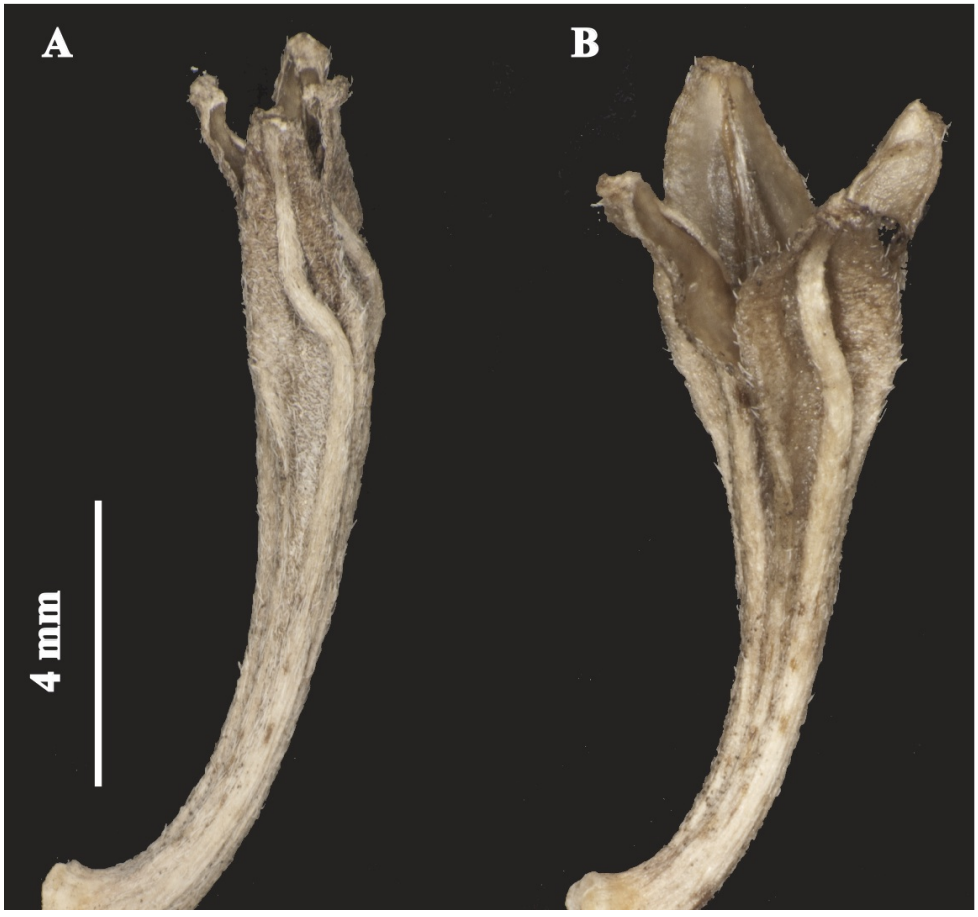

FIG 5.5 Dry capsule (A) of *Oenothera speciosa* and same capsule after wetting (B).

5.4.4 *OENOTHERA FRUTICOSA* (SECT. *KNEIFFIA*)

This erect and branched herb is about 30 to 95 cm tall and pubescent. Capsules are sessile or sit on very short pedicels, oblong and prominently winged (Britton and Brown, 1970) (Fig. 5.6 A). Poppendieck (1995) sectioned *O. fruticosa* subsp. *glauca* 'Highlight" and found swelling tissue only at the inner wall of the capsule, which can also be confirmed for wild material of *O. fruticosa*. In contrast to *O. speciosa* but similar to *O. linifolia* and *O. perennis*, the swelling tissue occupies the entire length of the inner valve, resulting in a very wide stretch of the valve (Fig 5.3 C). The dorsal bulge is prominent but not as much as in *O. speciosa*. Poppendieck (1995) noted that the capsules did not open widely in *O. fruticosa* subsp. *glauca* 'Highlight' but in wild *O. fruticosa* a wide splash cup is formed with the valve splitting more than halfway down the capsule (Fig 5.6 B).

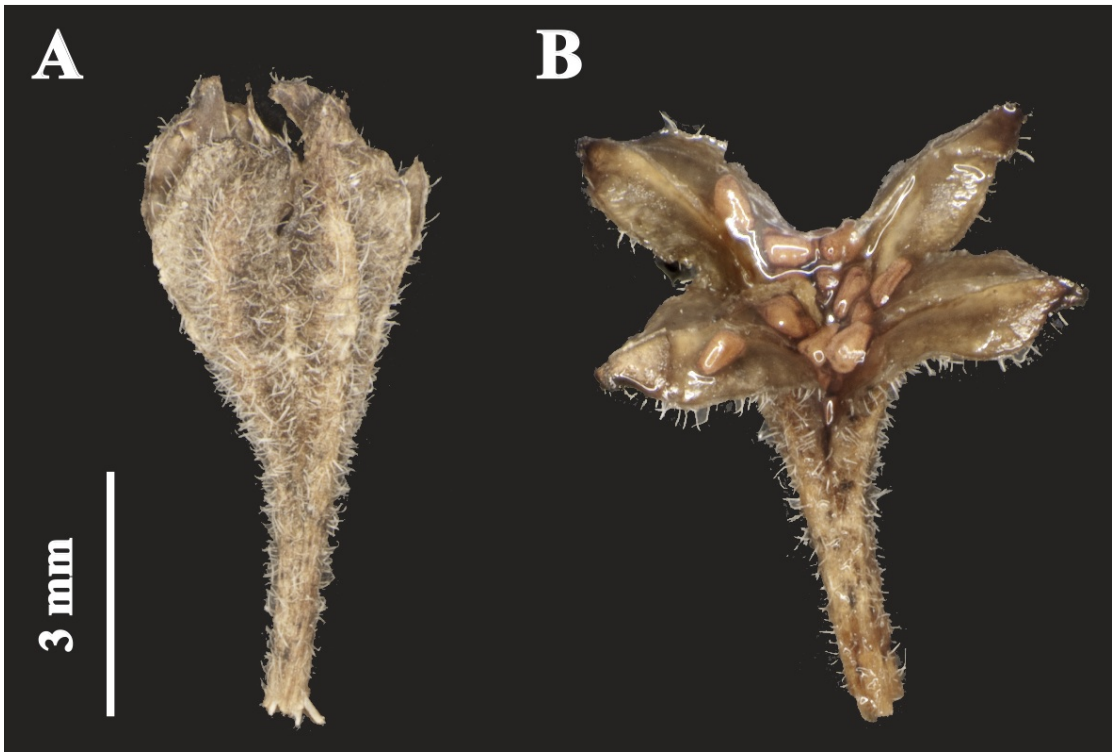

FIG 5.6 Dry capsule (A) of *Oenothera fruticosa* and same capsule after wetting (B), exposing seeds and the collapsed placental column.

5.4.5 *OENOTHERA PALLIDA* (SECT. *ANOGRA*)

This species represents the only sample from subclade A (Tobe et al., 1987; Levin et al., 2004). Plants are perennial herbs without basal rosettes of leaves and their fruits are sessile, linear capsules (Taylor, 1998; Wagner et al., 2007). Ripe fruits are pale brown in colour and dehisce during the ripening process about half the length of the capsule and seeds can fall out. Specimens in this study were still unripe but slight splits were visible after drying in silica gel (Fig. 5.7 A). However, an opening of the valves after wetting was observed (Fig. 5.7 B). The micrograph shows a prominent dorsal bundle not unlike *O. perennis* (Fig. 5.3 B) and the valves are bent back substantially in dry conditions, leading to the split of the capsule. The endocarp in the micrograph appears also very similar to the swelling tissue of species in the subclade B but the exocarp in the sample presented in Fig. 5.3 B is disintegrating. *Oenothera pallida* dehisces very similarly to *O. biennis* during ripening, which has been described by Ridley in great detail (1930).

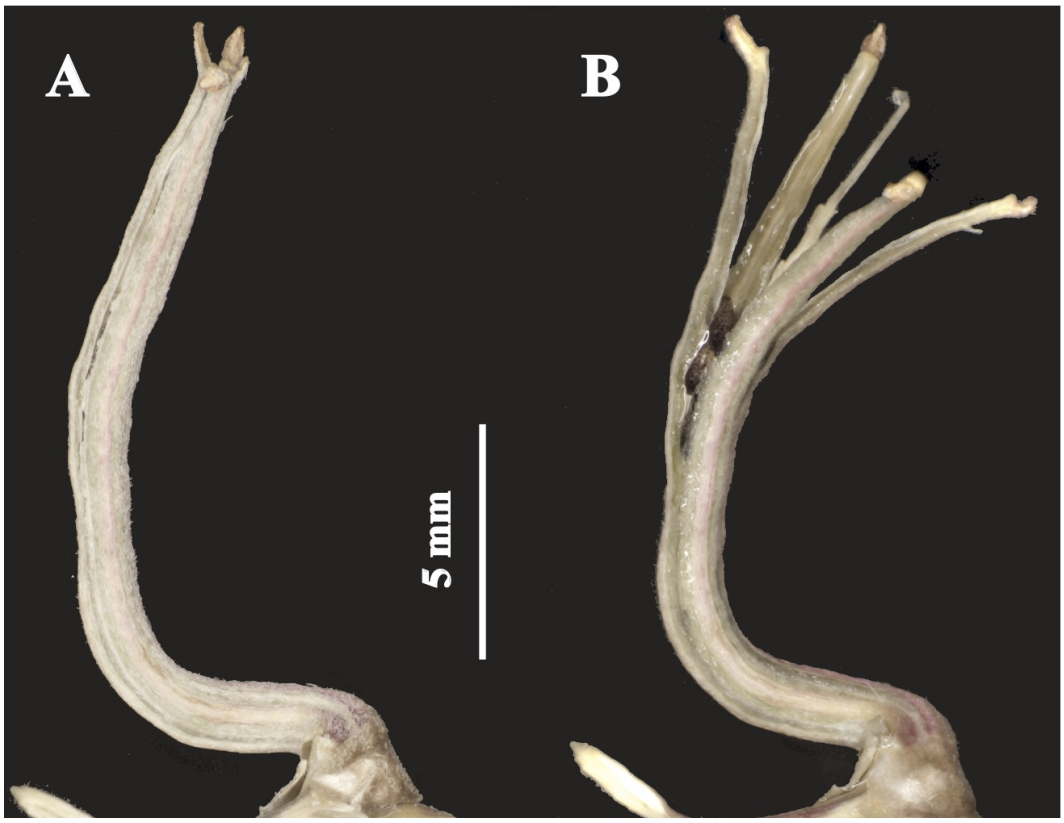

FIG 5.7 Unripe, dry capsule (A) of *Oenothera pallida* with splits between valves visible and same capsule after wetting (B), exposing seeds and placental column.

However, the valves curl back substantially when wetted but unlike the hygrochastic species from subclade B, the valves do not possess wings that bend back and form a splash cup. Rather, the bending of the valves away from the placental column leads to a deeper split of the capsule but due to the lanceolid shape of the capsule the deeper split does not result in a splash cup. This curling motion is probably due to the swelling capacity of the endocarp but since the exocarp disintegrates, a sufficient resistance tissue is absent.

5.4.6 *OENOTHERA HOWARDII* (SECT. *MEGAPTERIUM*)

This species is one of four species in sect. *Megapterium* and is described as a perennial herb with a branching caudex arising from a taproot (Wagner et al., 2007). Capsules are winged and appear quadrangular in cross-section.

Comparing cross-sections, *Oenothera howardii* is very similar to *O. macrocarpa*, which has been described as xerochastic (Poppendieck, 1995) (Fig. 5.8 A). It is therefore highly likely that *O. howardii* is xerochastic as well, given the systematic background.

According to Poppendieck (1995), *O. macrocarpa* opens when dry and closes when wet due to fibres at cross-angles in the wings (valves), which make the wings coil when dry. This leads to a widening of the capsule cavity. Additionally, the exocarp acts as swelling tissue and bulges outward when dry. The septum and the dorsal bulge have the function of resistance tissue. The collenchyma acting as swelling tissue can be seen in the micrograph of *O. howardii* (Fig. 5.8 B), but in this section it has partly separated from the capsule wall.

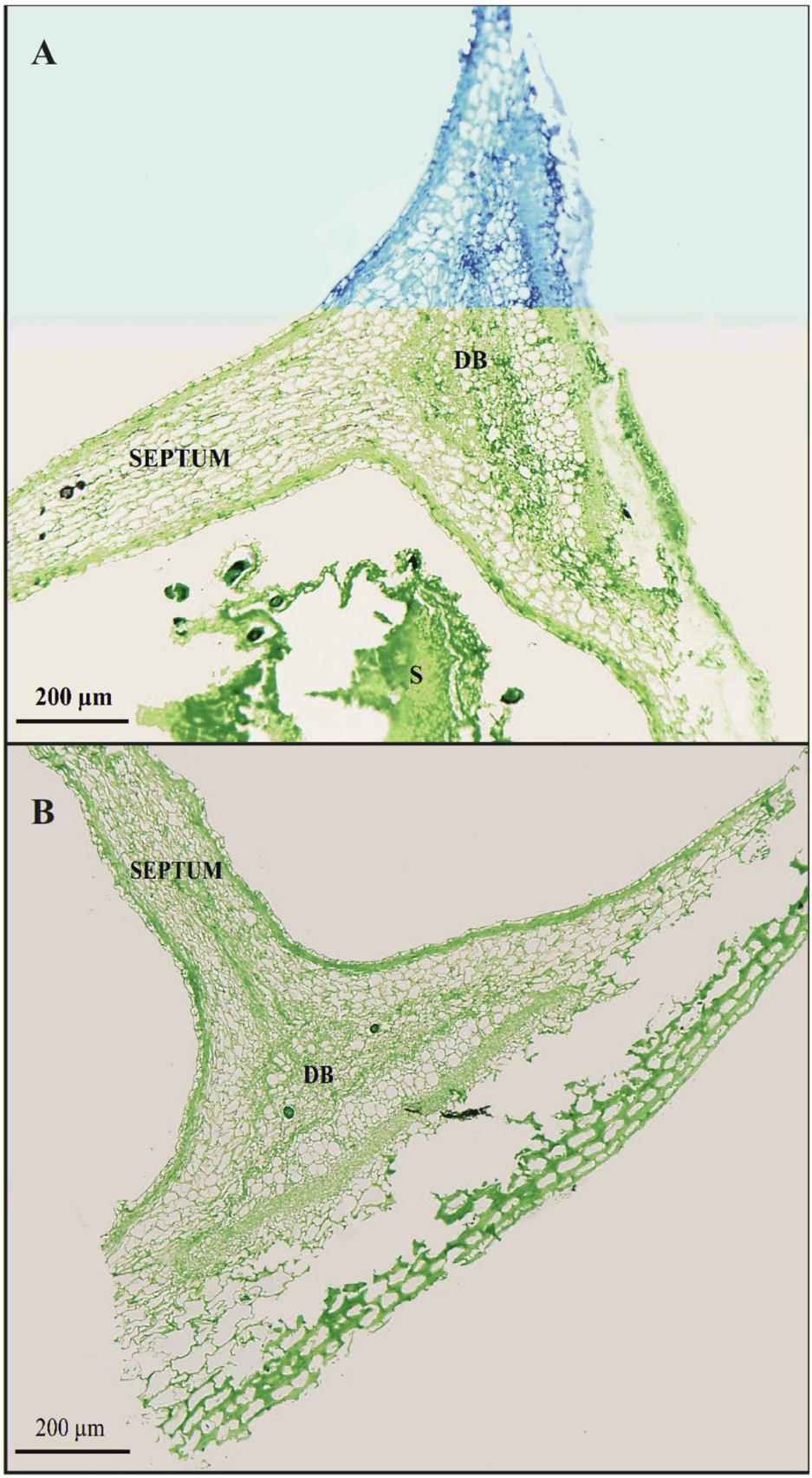

FIG 5.8 Micrographs of *O. macrocarpa* (A) and *O. howardii* (B) stained with Toluidine Blue. S = seed, DB = dorsal bundle. Magnification is 10x.

5.5 Discussion

Additional to the observations on the dehiscence mechanisms in some *Oenothera* (Poppendieck, 1995), hygrochasy was observed and described in four more species and xerochasy in *O. howardii* is also strongly suspected. Previously, three sections within subclade B were known to include hygrochastic species (Poppendieck, 1995); with *O. speciosa*, the section *Xylopleurom* can also be recognized as including plants with hygrochastic capsules.

Like other hygrochastic woody capsules (Steinbrinck, 1883; Garside and Lockyer, 1930; Ihlenfeldt, 1978; Parolin, 2001; Pufal et al., 2010) the opening of hygrochastic *Oenothera* is caused by an imbibition mechanism depending on the interaction of swelling tissue and resistance tissue (Fahn and Werker, 1972). In every species tested, the dorsal bundle acts as resistance tissue whereas the endocarp of the valves (or wings) represents the swelling tissue, having thick cell walls capable of absorbing water. Xerochasy is also based on an imbibition mechanism, but in contrast to hygrochasy, the opening of the fruit occurs in dry conditions and closing commences with moistening (Fahn and Werker, 1972). Here, the swelling tissue is located in the exocarp of the capsule, resulting in an outward bulge as the exocarp shrinks in dry conditions and therefore a wider opening of the capsule (Poppendieck, 1995). Similar to hygrochastic *Oenothera*, the dorsal bulge fulfils the role of resistance tissue, but here, the septum is involved as well. Due to the position of the swelling tissue the movement occurs in the opposite direction compared to hygrochastic species.

Oenothera pallida represents the only species sampled from subclade A and has capsules that dehisce with ripening. Here, the exocarp disintegrates during ripening but the endocarp remains as capsule wall. As in hygrochastic species from subclade B, the endocarp seems to be capable of absorbing water and during rainfall events this

can lead to a further splitting of the already open capsule. However, this movement probably only occurs in capsules, that are already dehisced and therefore *O. pallida* is still classified as dehiscing during ripening ('ripening dehiscent').

Hygrochasy as part of ombrohydrochory is assumed to result in atelechoric dispersal strategies (Fahn and Werker, 1972; Ellner and Shmida, 1981; Ihlenfeldt, 1983; Gutterman, 1994; Poppendieck, 1995; Pufal and Garnock-Jones, 2010; Chapter 3.3), since raindrops transport the seeds only a short distance. On the other hand, xerochasy is viewed as a telechoric mechanism (Fahn and Werker, 1972) since a number of dispersal agents, such as wind and animals can carry the seeds over large distances. However, Walck and Hidayati (2007) describe the xerochastic capsules of *O. macrocrapa* as indirect ombrohydrochoric. Capsules close during rain but open fully when moisture decreases after rain whereas opening without previous rainfall was not as prominent.

As in Aizoaceae (Van Oudtshoorn and Van Rooyen, 1999), Poppendieck (1995) proposed a sequence of increasing structural complexity in *Oenothera*, but unlike in the Aizoaceae it is unclear whether this sequence adequately reflects the phylogeny of the genus. Nevertheless, the species investigated here can be arranged seamlessly within the proposed sequence. Thus, *O. pallida* represents terete capsules from subclade A, *O. howardii* is most likely a xerochastic species similar to *O. macrocarpa* and *O. linifolia, O. perennis* and *O. fruticosa* have simpler hygrochastic capsules with the swelling tissue covering the entire length of the valve. Lastly, *O. speciosa* has a more complex hygrochastic capsule with the swelling tissue only extending to parts of the valve.

Including the results of this study, *Oenothera* is now known to comprise at least eight hygrochastic species and two xerochastic species, all in subclade B (Wagner et al.,

2007). There is a high likelihood that more species in this subclade exhibit either of the investigated dehiscence types, especially species from the same section. Despite broadening the knowledge about hygrochastic *Oenothera* with this work, there are still many unresolved questions regarding this dispersal strategy. Poppendieck (1995) mentioned that interpreting hygrochasy as an atelechoric mechanism is justified in *Oenothera* but does not give an explanation for this statement. Based on previous studies (Ellner and Shmida, 1981; Ihlenfeldt, 1983; Gutterman, 1994; Pufal and Garnock-Jones, 2010; Chapter 3.3), I would predict that habitat characteristics of hygrochastic *Oenothera* play an important role in the evolution of hygrochasy. Additionally, the taxonomy of this genus will likely be of interest in investigating dehiscence mechanisms in greater detail, given the sequence of structural complexity (Poppendieck, 1995). The following chapter aims to address these questions by investigating whether the hypotheses developed for hygrochastic capsules in plants of arid and alpine regions also apply to *Oenothera* by using a multidisciplinary approach.

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CHAPTER SIX

Are Hypotheses for Hygrochasy in Plants of Arid Regions also Applicable for Unrelated Genera in Different Habitats?

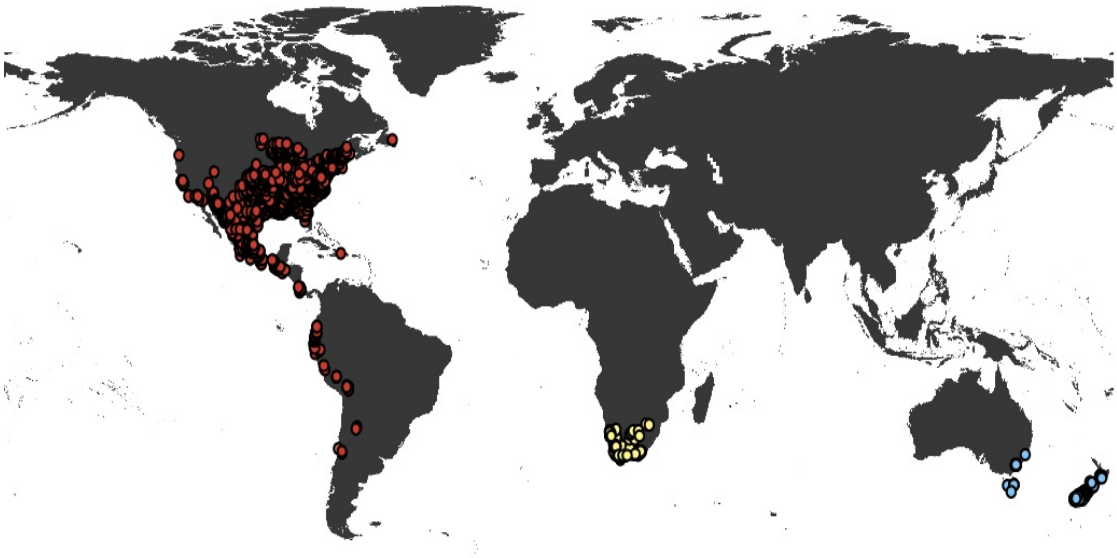

FIG 6.1 Worldwide distribution of hygrochastic *Veronica* (blue dots), *Oenothera* (red dots) and Aizoaceae (yellow dots).

6.1 Abstract

Hygrochasy, the opening of capsules in response to moisture and the subsequent dispersal of seeds by raindrops from the open splash cup (ombrohydrochory) has evolved in several unrelated genera in very different habitats.

The main theories for this dispersal strategy commonly associated with plants in arid environments are that (1) hygrochasy restricts dispersal spatially to the suitable parent habitat in a harsh environment and (2) it also restricts dispersal temporally to times when rainfall is sufficient for seed germination and seedling establishment. In this study I investigate whether hypotheses postulated for hygrochasy in arid areas are also applicable for hygrochastic *Oenothera* in North America. At least seven species of *Oenothera* in one subclade of the genus are hygrochastic. They have different life and growth forms and occur in a wide range of habitats across North America, some of them extend into Central and South America.

I tested the application of known hypotheses for hygrochasy in *Oenothera* by using various methods such as laboratory dispersal experiments, cluster analysis of morphological data, analysis of environmental and distribution data as well as character evolution. Examples of hygrochastic Aizoaceae and *Veronica* are used in comparison, since the hypotheses have been successfully implemented for those species.

Analysis revealed that none of the proposed hypotheses applies for present day hygrochastic *Oenothera*. However, it is highly likely that hygrochasy evolved only once in the genus. The hygrochastic species possibly evolved as part of the Madro-Tertiary flora, when the temporal restriction hypothesis might have played an important role in response to dry, highly seasonal climate with unpredictable rainfall. The time since then to present date was sufficient for the species to spread across the

continent, since they do not seem to have specialized ecological niches. Therefore, hygrochasy seems to be an ancient relict, that is still present in most species of one *Oenothera* subclade, but in others a shift to xerochasy or indehiscent capsules took place.

Key words: Aizoaceae, alpine, arid, character evolution, dispersal, hygrochasy, Madro-Tertiary flora, North America, *Oenothera*, splash cup, *Veronica*

6.2 Introduction

Hygrochasy is defined as the opening of a structure in response to moisture and its closing on drying (Fahn and Werker, 1972). It can be found in a variety of plant parts, such as parts of the inflorescence (Fahn and Werker, 1972; Gutterman, 1990, 1994; Gutterman and Ginott, 1994), or branches, leading to hygrochastic movement of the entire plant (Hegazy et al., 2006). Most commonly, hygrochasy can be found as a specialized dehiscence mechanism in woody dehiscent capsules (Garside and Lockyer, 1930; Ellner and Shmida, 1981; Van der Pijl, 1982; Ihlenfeldt, 1983; Thulin, 1987; Gutterman, 1994; Poppendieck, 1995; Parolin, 2006). In contrast to most capsular fruits that dehisce during the ripening process, hygrochastic capsules stay closed until they are wetted sufficiently. Opening results in a so-called splash cup, which exposes the seeds to falling raindrops. These subsequently disperse the seeds by splashing them out (ombrohydrochry) (Brodie, 1951; Van der Pijl, 1982; Nakanishi, 2002; Parolin, 2006).

The opening of hygrochastic capsules is usually based on an imbibition mechanism, where two antagonistic tissues interact with each other. A swelling tissue is capable of absorbing water and increasing considerably in size (usually in one direction),

whereas the resistance tissue is unable to swell and instead directs the movement of the increase of the swelling tissue. In most fruits, two different tissues are involved but cases of swelling and resistance happening in opposite cell walls of the same cells are also known (Fahn and Werker, 1972). However, a combination of imbibition and cohesion mechanisms can also be found in hygrochastic capsules of *Colobanthus* (Caryophyllaceae) (see Chapter four).

In the Aizoaceae in southern Africa more than 98% of the species are hygrochastic with a multitude of different capsule morphologies (Garside and Lockyer, 1930; Ihlenfeldt, 1983; Van Rooyen, 1990; Van Oudtshoorn and Van Rooyen, 1999; Parolin, 2001, 2006). Hygrochastic capsules are generally woody and contain several locules, depending on the species. Species in the core Ruschioideae also exhibit special features such as covering membranes or closing bodies, which retain seeds in the capsule over several rainfalls and also allow for slightly further dispersal distances (Parolin, 2001; Klak et al., 2004). The specialisation and increasing complexity reflects the phylogeny of the family, with the species-poor Mesembryanthemeae having simpler capsules, whereas Ruschioideae show more complex structures (Van Oudtshoorn and Van Rooyen, 1999; Klak et al., 2004).

Other examples of plants in arid areas include some *Xylocalyx* species in Somalia (Thulin, 1987), and desert plants in the Middle Eastern flora (Zohary and Fahn, 1941; Ellner and Shmida, 1981; Gutterman, 1990, 1994).

Poppendieck (1995) investigated hygrochastic capsules in some *Oenothera* of North American origin and Ladyman (2005) reported an additional *Oenothera* species from the US as hygrochastic. *Oenothera* is a species rich genus and in recent years the phylogeny has been changed and updated several times (Tobe, et al., 1987; Levin et al., 2004; Wagner, 2005; Wagner et al., 2007). Hygrochastic species are placed in

subclade B of *Oenothera*, which is characterized by angled or winged fruits (Tobe et al., 1987). They occur across North America well into South America in varying habitats.

In hygrochastic *Oenothera*, the swelling tissue consists of cells in the endocarp with thickened cell walls capable of swelling (Poppendieck, 1995; see also Chapter Five). Poppendieck (1995) also observed that in *O. triloba* the septum contributed to the swelling tissue as well. The role of resistance tissue is fulfilled by the lignified vascular bundle of the dorsal bulge and sometimes the outside of the valves. All *Oenothera* open to form a splash cup but seeds might be stacked differently, leading to seed retention in some species (Poppendieck, 1995).

Veronica in alpine New Zealand (Garnock-Jones, 1993; Garnock-Jones and Lloyd, 2004; Meudt, 2008) with a cushion or subshrub habit and solitary capsules exhibit hygrochasy, which I investigated in regards to their anatomy and biomechanics (Pufal et al., in press). Capsules vary in size, but they are all narrowly angustiseptate. In all investigated species, the endocarp in the septum acts as swelling tissue, having elongated cylindrical cells with an enlarged cell wall capable of absorbing water. The resistance tissue is the endocarp of the valves and has lignified, thickened cell walls (Pufal et al., 2010).

Not surprisingly, hypotheses for the evolution of hygrochastic capsule dehiscence stem from work with species in arid habitats Table 6.1. In the following paragraphs I will introduce these hypotheses; additionally, an overview can be found in Table 6.1.

Temporal Restriction Hypothesis

Hygrochastic capsules stay closed until they are sufficiently wetted. Therefore, dispersal is restricted to rain events, which is essentially a restriction in time and guarantees favourable germination conditions at time of dispersal (Fahn and Werker,

1972; Ellner and Shmida, 1981; Van der Pijl, 1982; Ihlenfeldt, 1983; Van Oudtshoorn and Van Rooyen, 1999; Parolin, 2006), especially in regions where rainfall events are rare and unpredictable.

Spreading Dispersal Hypothesis

The 'temporal restriction hypothesis' can also be specified – most Aizoaceae (core Ruschioidae (Klak et al., 2004)) possess specialized structures that retain seeds in the capsules over several rainfall events and only release a small percentage of seeds with single rainfalls (Parolin, 2001). This enables seed release over time, therefore spreading the risk at germination over time as well (Van Rooyen et al., 1980; Van Oudtshoorn and Van Rooyen, 1999; Parolin, 2001; Klak et al., 2004; Burke, 2005).

Spatial Restriction Hypothesis and Safe Site Hypothesis

Hygrochastic capsules form a splash cup when wetted by rain and raindrops act as the predominant dispersal agent. Dispersal by raindrops results in very short dispersal distances for seed, which results in spatial restriction. This plays an important role in a highly heterogenous environment, where hygrochastic species only occupy a small, specialized niche (Ellner and Shmida, 1981; Ihlenfeldt, 1983; Gutterman, 1994; Van Oudtshoorn and Van Rooyen, 1999) or to support a safe site strategy to ensure higher survival rates for seedlings (Pufal and Garnock-Jones, 2010).

Protection Hypothesis

Hygrochastic capsules are only open during rainfall events, in between rainfalls they stay closed. Especially in arid regions, they can stay closed for very long times but since seeds are stored in the capsule, they are protected against seed predators and harsh environmental conditions. In case of a sufficient rainfall event, seeds are released and germinate quickly, therefore avoiding seed predators that use the soil

seed bank (Steinbrinck, 1883; Ellner and Shmida, 1981; Ihlenfeldt, 1983; Van Oudtshoorn and Van Rooyen, 1999).

Suitable Strategy Hypothesis

The forming of a splash cup is also advantageous for small herbaceous species, where water drops from taller plants act as dispersal agents (Nakanishi, 2002). Here, hygrochasy is not essential but hygrochastic species were identified that comply with this hypothesis. Why those species are hygrochastic is not yet fully understood. It was also thought that splash cups are the only available form of dispersal, when capsules are sessile or sunken in a cushion (P. Garnock-Jones, pers. comm.), e.g. in hygrochastic alpine *Veronica* that have a cushion growth form. But again, hygrochasy is not necessary in forming a splash cup; other species (e.g. *Sagina decumbens* (Brodie, 1951) or *Chrysosplenium* species (Nakanishi, 2002)) dehisce to form a splash cup during the ripening process.

Hygrochastic capsule dehiscence evolved independently in a number of unrelated families and is therefore a homoplastic character. Convergent evolution of a character is due to adaptation to similar environments or similar functions. Therefore, the same hypotheses for the evolution of hygrochastic capsules should apply to all species, regardless of their origin. While most studies focussed on plants of arid zones (Ellner and Shmida, 1981; Van der Pijl, 1982; Ihlenfeldt, 1983; Van Oudtshoorn and Van Rooyen, 1999; Parolin, 2001, 2006), I have added anatomical and ecological information on temperate alpine plants (*Veronica* and *Colobanthus*) in previous chapters.

Table 6.1 Traditional hypotheses for hygrochastic capsules.

hypothesis	description	references
Temporal restriction (Bradyspory)	restriction seed dispersal to favourable germination conditions, e.g. sufficient rain	Fahn and Werker (1972); Ellner and Shmida (1981); Van der Pijl (1982); Ihlenfeldt (1983); Van Oudtshoorn and Van Rooyen (1999); Parolin (2006)
Spreading dispersal over time	Only a few seeds are released at one rainfall event, more are saved in the capsule for future rainfall events – this spreads the risk of germination over time.	Van Rooyen, Barkhuizen, and Myburgh (1980); Van Oudtshoorn and Van Rooyen (1999); Parolin (2001); Klak, Reeves, and Hedderson (2004); Burke (2005)
spatial restriction (atelechory)	restricting dispersal to suitable habitat close to the parent plant in an otherwise heterogenous hostile environment	Ellner and Shmida (1981); Ihlenfeldt (1983); Gutterman (1994); Van Oudtshoorn and Van Rooyen (1999), Chapter 3.3
safe site strategy	ensuring dispersal to safe sites, such as nurse plants, or creating safe sites by dispersing seeds in clumps	Pufal and Garnock-Jones, 2010
protection	When closed, capsules protect seeds against predators and harsh environmental conditions.	Steinbrinck (1883); Ellner and Shmida (1981); Ihlenfeldt (1983); Van Oudtshoorn and Van Rooyen (1999)
suitable dispersal strategy	splash cup only effective dispersal mechanism for plant morphology, hygrochasy not essential	P. Garnock-Jones, pers. comm.
	splash cup effective along water streams, hygrochasy not essential	Nakanishi (2002)

To test for a universal explanation of hygrochasy, the North American genus *Oenothera* (Onagraceae) is used as study group. At least seven species in the subclade B are hygrochastic (Poppendieck, 1995; Ladyman, 2005; Wagner et al., 2007; see also Chapter Five) and they occur in a wide range of habitats across northern America. Different approaches such as laboratory dispersal experiments, analysis of morphological data, analysis of environmental, climatic and distributional variables and the investigation of character evolution in combination with phylogenetic data are used to test to application of common hypotheses for hygrochasy. The well studied Aizoaceae (see references above) and *Veronica* (Pufal and Garnock-Jones, 2010; Pufal et al., 2010) are used as references for the compliance with the hypotheses.

6.3 Materials and methods

To infer whether hypotheses that were developed for the evolution of hygrochasy in plants of arid region can also explain hygrochasy in unrelated genera in different habitat types, a multidisciplinary approach was chosen using laboratory experiments, occurrence data and climatic variables in arcGIS (gbif and worldclim), plant morphology and phylogenetic information. The global biodiversity information facility (gbif) (http://data.gbif.org) provides occurrence and distribution data for most plants, animals, fungi or micro-organisms as well as inventory lists for countries or entire datasets, which are based on records provided by organization such as museums, herbaria or societies. WorldClim is a dataset of global climate layers, which is also available online (www.worlclim.org) (Hijmans et al., 2005).

The main hypotheses tested are the delay of dispersal in time (temporal restriction hypothesis) and the restriction of dispersal in space (spatial restriction hypothesis). Each approach was conducted separately and explained in detail in the following sections.

6.3.1 Dispersal and seed retention

In arid conditions it is favourable to delay dispersal over time and only release a few seeds per rainfall to spread the risk of germination failure (Parolin, 2006). In Aizoaecae, structures such as funicles, closing membranes or closing bodies are present. These structures prevent all seeds dispersing at one time but their presence also increases the dispersal distances of seeds that are dispersed. In this part of the study dispersal distances of some *Veronica*, *Oenothera* and Aizoaceae are compared and the differences between seed retention in those species is investigated (Table 6.2).

In order to achieve a relevant comparison with previously investigated Aizoaceae species, dispersal experiments were modelled on Parolin (2001). Different plant heights were taken into account to simulate natural conditions. Dispersal distances were measured and number of seeds retained after 100 drops were counted. In addition to Parolin's experiments (2001), we also timed the opening of capsules. Kruskal-Wallis and Mann-Whitney-U tests were used to compare all species with each other. Genera were grouped and compared with each other as well. Statistical analysis was carried out in R (R Development Core Team, 2005).

Table 6.2 Species used in various analyses for this study.

species	dispersal	seed retention	opening time	morphology	habitat
Acrodon subulatus	yes	yes	no	yes	yes
Argyroderma fissum	yes	yes	no	yes	yes
Bergeranthus scapiger	yes	yes	no	yes	yes
Cephalophyllum spissum	yes	yes	no	yes	yes
Dracophilus dealbatus	yes	yes	no	yes	yes
Drosanthemum globosum	yes	yes	no	yes	yes
Drosanthemum hispidum	yes	yes	no	yes	yes
Drosanthemum schoenlandianum	yes	yes	no	yes	yes
Ebracteola wilmaniae	yes	yes	no	yes	yes
Phyllobolus spinuliferus	yes	yes	no	yes	yes
Rhombophyllum dolabriforme	yes	yes	no	yes	yes
Oenothera fruticosa	no	no	yes	yes	yes
Oenothera harringtonii	no	no	no	yes	yes
Oenothera linifolia	yes	yes	yes	yes	yes
Oenothera perennis	yes	yes	yes	yes	yes
Oenothera rosea	no	no	no	yes	yes
Oenothera speciosa	no	no	yes	yes	yes
Oenothera triloba	no	no	yes	yes	yes
Veronica birleyi	no	no	no	yes	yes
Veronica cheesemanii	yes	yes	yes	yes	yes
Veronica ciliolata	no	no	yes	yes	yes
Veronica densifolia	yes	yes	yes	yes	yes
Veronica planopetiolata	yes	yes	yes	yes	yes
Veronica pulvinaris	no	no	yes	yes	yes
Veronica thomsonii	yes	yes	yes	yes	yes
Veronica spathulata	yes	yes	yes	no	yes

6.3.2 PLANT MORPHOLOGY

In order to identify a syndrome or syndromes for hygrochasy in unrelated species, morphological characters deemed important for dispersal (e.g., plant height, length of pedicel) were assessed for all species. This analysis was carried out previously for *Veronica* (Pufal et al., 2010) but not all characters used there could be used in this analysis. Traits such as capsule shape are mostly genus-specific and were therefore not taken into consideration. Twelve traits were considered but only seven were used in statistical analysis. Seed size, seed shape, seed number per capsule and development of mucilage were omitted from analysis due to missing values in some species. Additionally, retention mechanisms could not be used since they were only found in the Aizoaceae. Characters were either coded as binary or multistate or left as continuous variables (e.g. max capsule height) (Table 6.3).

Table 6.3 Characters and character states used in the cluster analysis of morphological characters in hygrochastic plants.

	character	character states
1	Pedicel length	sessile (1), < 1cm (2), < 2cm (3), 2-4 cm (4), > 4cm (5)
2	number of capsules	solitary (1), 1-3 (2), 3 and more (3)
3	orientation of capsules	erect(1), hanging down (2), both (3)
4	plant habit	cushion (1), tufted/caespitose (2), acaulescent (3), creeping susbshrub (4), subshrub (5), herb (6), shrub (7)
5	max. capsule height above ground	max plant height in cm
6	capsules at different heights on plant	no (0), yes (1)
7	life form	perennial (1), annual (2), both (3)

The final matrix was converted into a distance matrix using Gower's coefficient in the function 'daisy' in the R package 'cluster' (Gower, 1971; Kaufman and Rousseeuw, 1990). Gower's coefficient has the ability to convert mixed matrices into a distance

matrix and was therefore chosen for this analysis. The resulting distance matrix was analysed with cluster analysis after Ward's method (Ward, 1963).

To interpret how well the dendrogram imposes on the Gower's distance matrix, the cophenetic correlation coefficient was calculated. All statistical analysis was carried out in R (R Development Core Team, 2005).

6.3.3 HABITAT QUALITY, CLIMATE CONDITIONS AND SPECIES DISTRIBUTION

Hypotheses for hygrochasy in plants state, that this dehiscence type delays dispersal until favourable germination conditions are present and/or restricts dispersal in space to safe sites and specific habitats (Table 6.1). In order to investigate if any of these hypotheses apply to *Oenothera*, species distribution and climatic conditions were assessed for hygrochastic *Oenothera* and compared with hygrochastic New Zealand *Veronica* and some Aizoaceae in South Africa.

The distribution of species was measured as the number of grid cells occupied by the respective species based on the gbif database (for citations on the origin of data see Appendix 6.1). Occurrences in habitats due to cultivation and spread by humans were not taken into consideration and only occurrences with coordinates were used. All known hygrochastic species in *Oenothera* and *Veronica* are used in this part of the study. Aizoaceae species investigated by Parolin (2001), for which sufficient information was available in the gbif database, were also included.

Precipitation data were used from the worldclim database (Hijmans et al., 2005) and the climatic layers annual precipitation, driest quarter as well as seasonality (Coefficient of Variation) were imported in arcGIS. Occurrences with geospatial information for all species were imported from the gbif database and precipitation

values for the locations were recorded. If data points were very close together only one record was taken for those sites.

The number of grid cells occupied by species in the gbif database was used as a measure of distribution for the spatial hypothesis. All variables were standardized using the scale function in R (R Development Core Team, 2005) and converted into a distance matrix using Euclidean distances. Ward's hierarchical cluster analysis was performed. The cophenetic correlation coefficient was calculated to determine how well the dendrogram represents the original Euclidean distance matrix.

It was not possible to assess the habitat size and ecological niche breadth on location, hence literature was used to make a qualitative assessment of these variables. They were used as additional information for the hierarchical cluster analysis.

6.3.4 SYSTEMATICS AND CHARACTER EVOLUTION

Hygrochastic capsules in the Aizoaceae show an array of capsule structures from simple to complex, with modifications such closing bodies and covering membranes to retain seeds (Van Oudtshoorn and Van Rooyen, 1999; Parolin, 2001). It appears that, at least in the Aizoaceae, hygrochasy evolved once and varying complexity can be traced in the phylogeny of the family (Van Oudtshoorn and Van Rooyen, 1999; Klak et al., 2004).

In order to test if hygrochasy is a trait that evolved only once or if it is a rather labile trait, parts of published phylogenies of *Oenothera* (Levin et al., 2004; Wagner et al., 2007) and *Veronica* (Albach and Meudt, 2010) were used in MacClade 4.08 (Maddison and Maddison, 2001). In *Oenothera*, a consensus tree based on ITS and cpDNA sequences was used (Levin et al., 2004) with updated names according to Wagner et al. (2007). Here, only the genus *Oenothera* was used for the analysis. Only

New Zealand *Veronica* were used from an ITS phylogeny (Albach and Meudt, 2010) in the analysis of character evolution. The dehiscence type of species (hygrochastic, xerochastic, 'ripening dehiscent', indehiscent, unknown) was traced on the phylogeny in MacClade 4.08 to investigate character evolution. It was traced as an unordered character and polytomies were interpreted as soft polytomies. In this interpretation, the character's evolution is traced and steps counted assuming that the polytomy is resolved in the most favourable way for the character.

6.4 Results

6.4.1 Dispersal and seed retention

Dispersal distances from laboratory experiments were available for five *Veronica* species, two *Oenothera* and 11 Aizoaceae (Parolin, 2001) (Table 6.4). Significant differences were detected amongst the *Veronica* species (Kruskal-Wallis-test: chi-squared = 15.34, df = 4, p = 0.004), the *Oenothera* species (Mann-Whitney-U test: W = 1924.5, p = 0.008) but not among the Aizoaceae (Kruskal-Wallis-test: chi-squared = 8.06, df = 10, p = 0.623).

Table 6.4 Opening times (min), dispersal distances (cm) and percentage of expelled seeds after 100 water drops from 175 cm. Data for Aizoaceae were provided by P. Parolin (see also Parolin, 2001) and opening times for *O. fruticosa* ssp. *glauca* and *O. triloba* are taken from Poppendieck (1995). For full species names see Table 6.2.

species	opening time	min	max	mean	sd	% expelled
Ac. subulatus	NA	8	45	26	18.71	14.3
Ar. fissum	NA	7	116	42.96	27.25	7.4
B. scapiger	NA	20	70	41	25.94	8.3
C. spissum	NA	6	116	42.15	22.7	13
Dr. globosum	NA	3	79	42	37.16	57.1
Dr. hispidum	NA	7	7	7	0	20
Dr. schoenlandianum	NA	13	117	58.83	36.47	15.8
Draco. dealbatus	NA	11	152	44.58	41.46	26
E. wilmaniae	NA	11	124	49.13	25.11	9.4
Phy. spinuliferus	NA	28	28	28	0	7.7
Rh. dolabriforme	NA	2	86	35.25	39.83	6.7
O. fruticosa ssp. glauca	285	NA	NA	NA	NA	NA
O. linifolia	112.8	0.1	59	16.01	12.57	94.5
O. perennis	277	1	66.7	24.64	18.28	62.1
O. speciosa	298	NA	NA	NA	NA	NA
O. triloba	300	NA	NA	NA	NA	NA
O. fruticosa	181.6	NA	NA	NA	NA	NA
V. cheesemanii	90	1	12	5.49	3.62	69.2
V. ciliolata	158.43	NA	NA	NA	NA	NA
V. densifolia	234.3	0.3	110.5	15.6	16.04	73.1
V. planopetiolata	107	1	23	15.81	7.59	100
V. pulvinaris	139.1	NA	NA	NA	NA	NA
V. spathulata	220.3	0.3	54.9	10.69	13.54	100
V. thomsonii	162	0.5	38.7	10.01	8.79	93.5

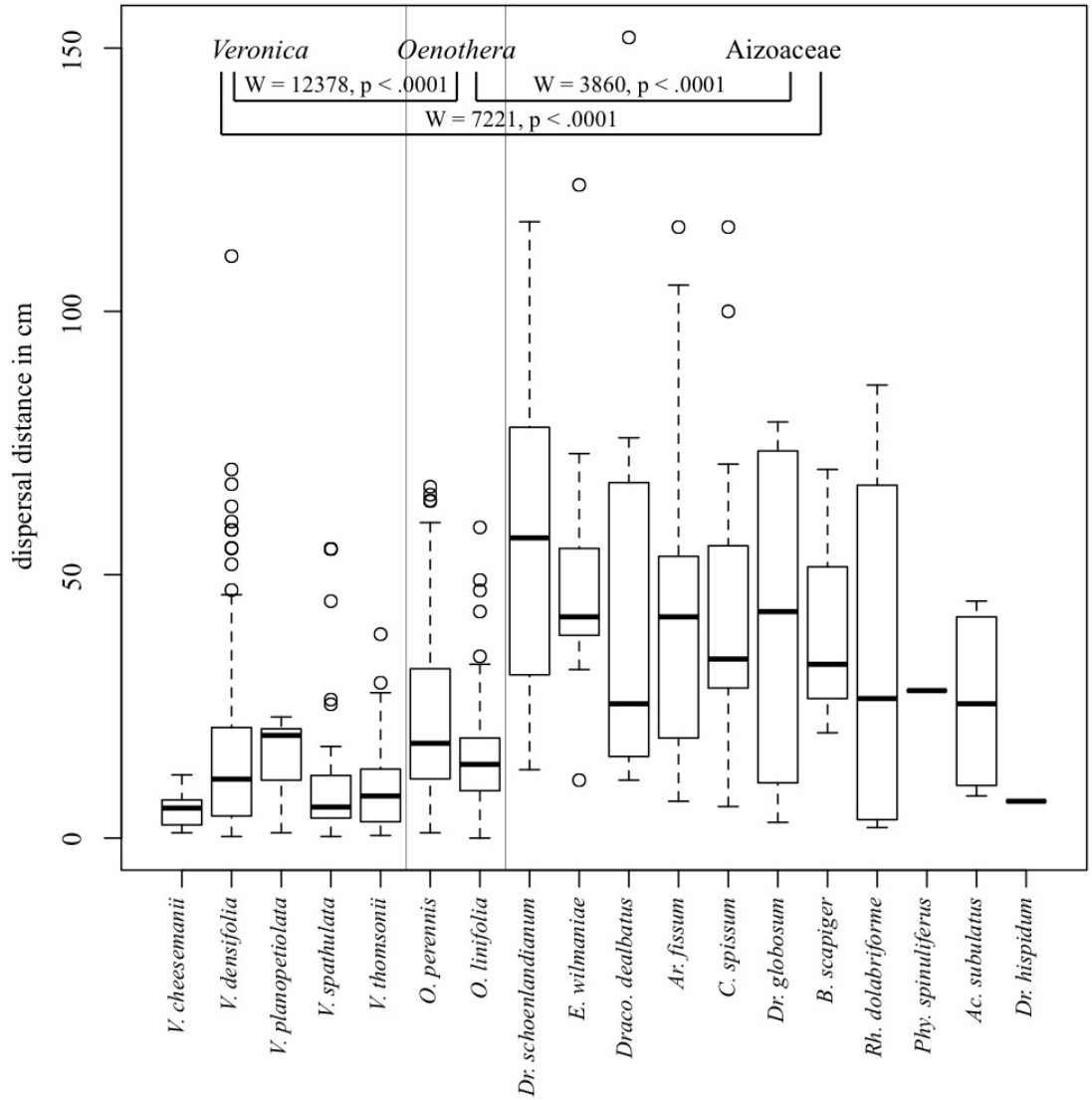

FIG 6.2 Dispersal distances of hygrochastic species. Significant differences between *Veronica*, *Oenothera* and Aizoaceae are given as results of Mann-Whitney-U tests.

Further statistical tests were carried out among the genera and families, respectively. Significant differences exist between all groups, with the Aizoaceae having the longest dispersal distances, followed by *Oenothera* and then *Veronica* with the shortest distances in comparison (Fig. 6.2).

Capsule opening times were only available for seven *Veronica* species and six *Oenothera* species. Of these, *Oenothera fruticosa* ssp. *glauca* and *O. triloba* were taken from Poppendieck (1995) (Table 6.4). Kruskal-Wallis tests showed significant

differences among *Veronica* (chi-squared =31.6, df = 6, $p < 0.0001$) and *Oenothera* species (chi-squared = 17.27, df = 3, p = 0.0006), respectively.

However, when both genera were compared with each other, no significant differences were found (Mann-Whitney-U test: W = 969.5, p = 0.55).

After 100 drops of water from a height of 175 cm, the majority of *Veronica* seeds were dispersed, regardless of species (Table 6.3). Both *Oenothera* species tested showed similar high numbers of expelled seeds, whereas all Aizoaceae species (except *Drosanthemum globosum*) only dispersed a small proportion of their seeds.

6.4.2 PLANT MORPHOLOGY

The cluster analysis of morphological traits of hygrochastic species shows three distinct clusters with good support (cophenetic value = 0.723) (Fig. 6.3).

The first cluster includes species with solitary, erect capsules on short plants with all capsules at the same height (Table 6.5). Except *Oenothera triloba*, all species are perennial plants.

The second cluster comprises species with solitary erect capsules on taller plants, where the capsules are located at different heights on the plant. The third cluster is distinguished from the other groups by species having between one and three flowers in the inflorescence.

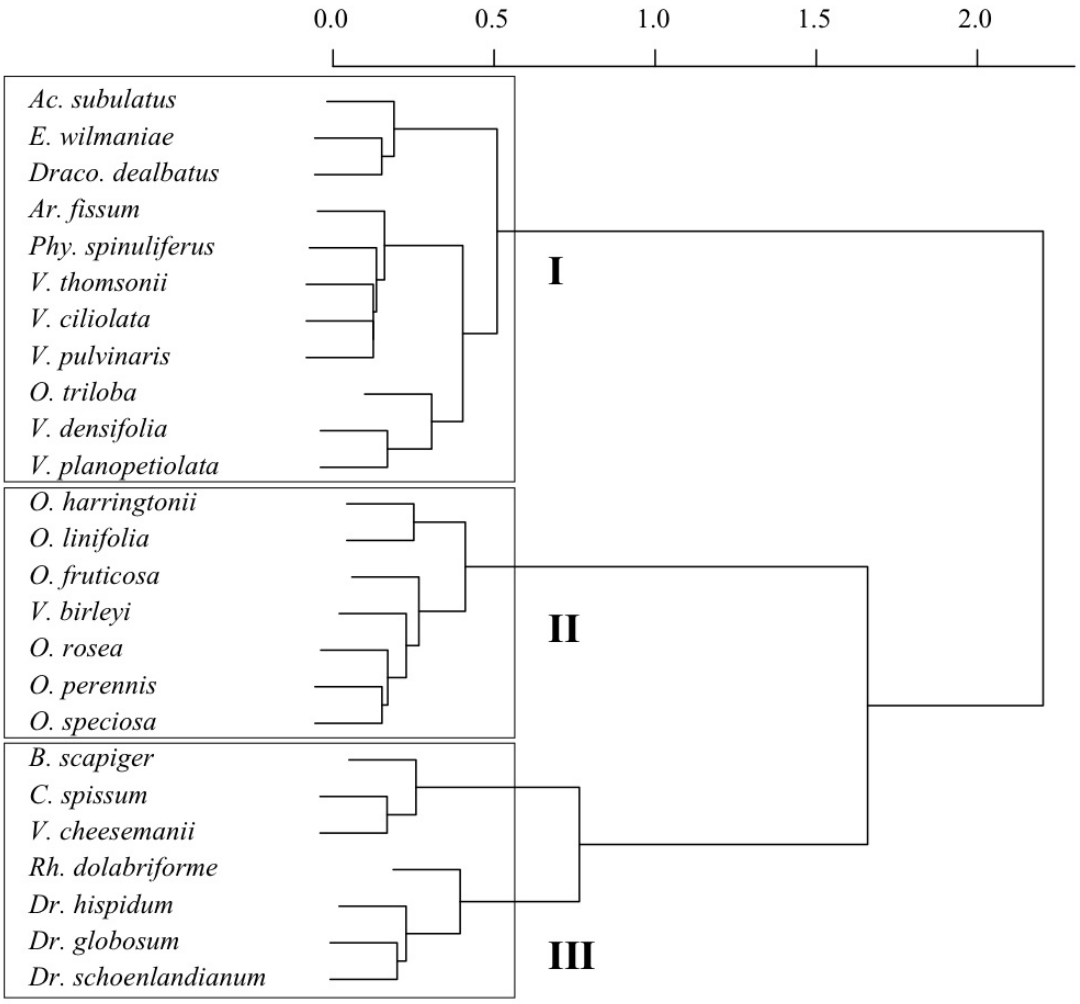

FIG 6.3 Ward's cluster analysis of morphological characters in hygrochastic species, based on a distance matrix between character states using Gowers' coefficient (Table 6.3 and Table 6.5).

Table 6.5 Morphological traits of hygrochastic species coded as character states based on Table 6.2. References: (1) Jacobsen (1954), (2) Meudt (2008), (3) Walck and Hidayati (2007), (4) Wagner et al. (2007), (5) Garnock-Jones and Lloyd (2004), (6) Ladyman (2005), (7) Straley (1977), (8) Poppendieck (1995), (9) Britton and Brown (1970), (10) Wolin et al. (1984).

	Cluster	1	2	3	4	5	6	7
Acrodon subulatus [1]		4	1	1	2	10	0	1
Ebracteola wilmaniae [1]		3	1	1	2	10	0	1
Dracophilus dealbatus [1]		3	1	1	1	5	0	1
Argyroderma fissum [1]		1	1	1	2	5	0	1
Phyllobolus spinuliferus [1]		1	1	1	1	10	0	1
Veronica thomsonii [2]	I	1	1	1	1	4	0	1
Veronica ciliolata [2]		1	1	1	1	4	0	1
Veronica pulvinaris [2]		1	1	1	1	4	0	1
Oenothera triloba [3, 4, 9]		1	1	1	4	5	0	3
Veronica densifolia [2]		1	1	1	4	7	0	1
Veronica planopetiolata [5]		2	1	1	4	5	0	1
Oenothera harringtonii [6]		1	1	1	6	30	1	3
Oenothera linifolia [7]		2	1	1	6	50	1	2
Oenothera fruticosa [7]		2	1	1	6	120	1	1
Veronica birleyi [5]	II	1	1	1	5	20	1	1
Oenothera rosea [4, 8]		1	1	1	6	100	1	1
Oenothera perennis [7]		1	1	1	6	75	1	1
Oenothera speciosa [4, 9, 10]		1	1	1	6	50	1	1
Bergeranthus scapiger [1]		4	2	1	2	10	0	1
Cephalophyllum spissum [1]		3	2	1	4	5	0	1
Veronica cheesemanii [5]		2	2	1	4	4	0	1
Rhombophyllum dolabriforme [1]	III	1	2	1	2	30	1	1
Drosanthemum hispidum [1]		4	2	1	7	60	1	1
Drosanthemum globosum [1]		2	2	1	7	45	1	1
Drosanthemum schoenlandianum [1]		3	2	1	7	15	1	1

6.4.3 HABITAT QUALITY, CLIMATE CONDITIONS AND SPECIES DISTRIBUTION

Precipitation, distribution and altitude information was obtained for a total of 26 species (Table 6.6). A hierarchical cluster analysis resulted in a dendrogram with three distinctive clusters, which is moderately supported with a cophenetic value of 0.638 (Fig. 6.4). The first cluster represents alpine species with high annual rainfall, no obvious seasonality and a small distribution. This cluster exclusively contains New Zealand *Veronica*.

The second cluster contains all Aizoaceae and two *Oenothera* species. It represents species with a restricted distribution (except *O. triloba*), low annual rainfall and/or very dry seasons and/or high seasonality. Altitudinal ranges vary between species. Cluster three contains the remaining *Oenothera* species, which occur in a wide range of areas with high annual rainfall and little seasonality. *Oenothera rosea* shows a very high seasonality and has also the highest altitudinal range.

Table 6.6 Precipitation, altitudinal and distribution information for hygrochastic species based on the worldclim (mean annual rain, seasonality, altitude range, altitude mean) and gbif database (distribution). For full species names see Table 6.2.

species	Mean annual rain	seasonality	Min. driest quarter	distribution	Altitude range	Altitude mean
V. densifolia	1602	13	191	7	791	1339
V. pulvinaris	2318	15	205	10	1176	1400
V. ciliolata	3033	12	318	12	897	1397
V. thomsonii	1983	11	321	2	595	1440
V. cheesemanii	2340	16	270	3	649	1306
V. birleyi	3489	12	603	3	1017	1969
V. planopetiolata	2948	11	497	2	686	1362
V. spathulata	3030	19	480	6	901	1413
P. spinuliferus	77	38	10	1	0	349
D. globosum	352	19	56	1	279	482
R. dolabriformes	611	21	107	1	0	940
A. subulatus	618	33	65	5	868	465
D. dealbatus	64	38	6	2	842	517
B. scapiger	504	43	28	2	975	679
O. triloba	913	36	17	93	1815	481
D. hispidum	300	42	4	33	1571	668
E. wilmaniae	453	78	4	5	343	1404
O. harringtonii	351	57	21	4	698	1671
D. schoenlandianum	210	65	11	2	811	540
A. fissum	171	68	10	2	141	81
C. spissum	165	66	11	1	0	146
O. rosea	1092	82	6	150	4115	1584
O. speciosa	1059	31	2	302	1910	433
O. linifolia	1211	20	72	96	580	242
O. fruticosa	1193	17	130	190	1218	367
O. perennis	1023	19	46	162	1116	331

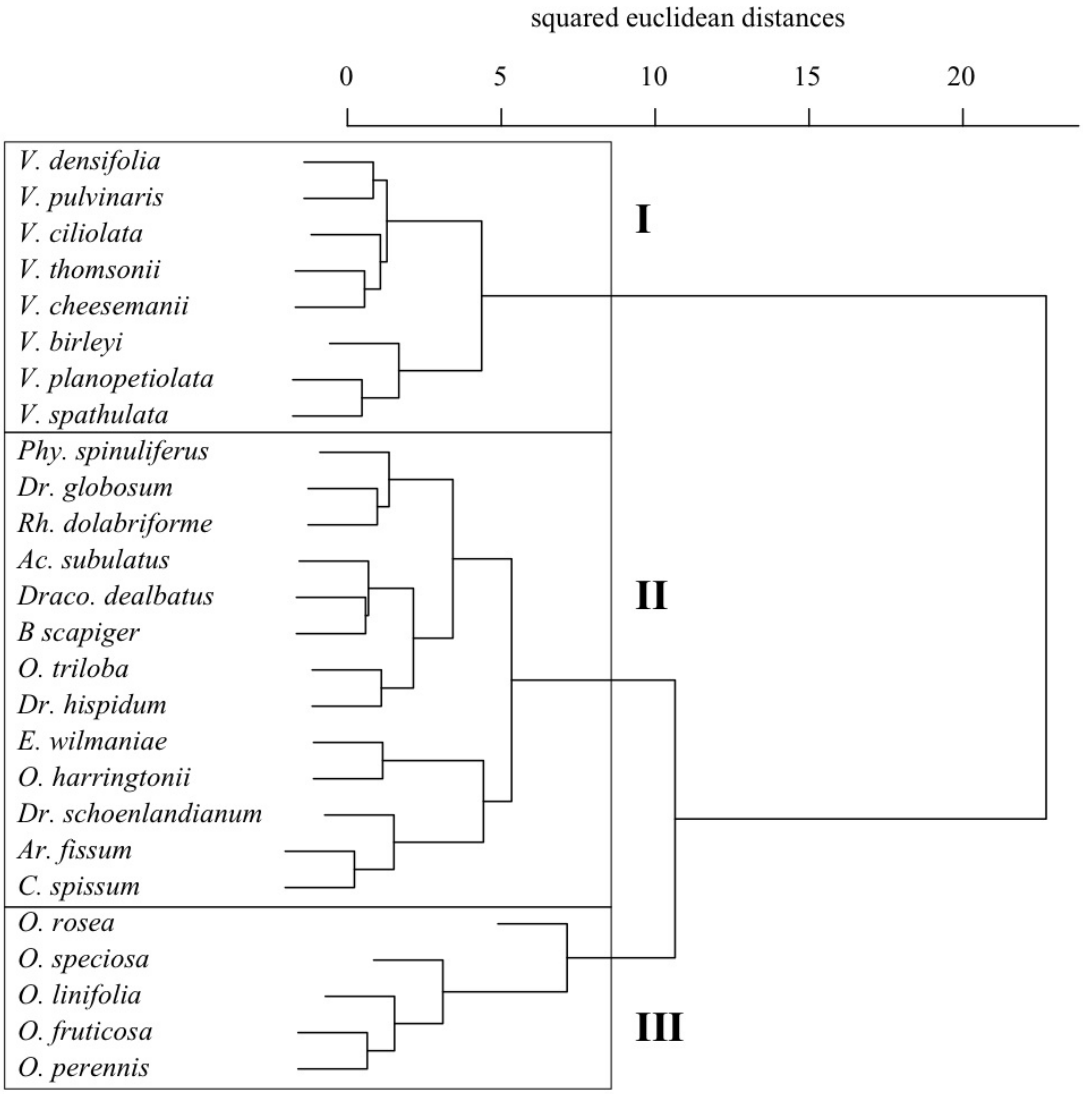

FIG 6.4 Ward's hierarchical cluster analysis of precipitation, distribution and altitude in hygrochastic species.

6.4.4 SYSTEMATICS AND CHARACTER EVOLUTION

To investigate the evolution of hygrochasy in *Oenothera*, part of the phylogeny of Onagraceae published by Levin et al. (2004) and updated by Wagner et al. (2007) was used. The strict consensus is based on ITS (nuclear), *trnL-trnF* and *rps16* (both chloroplast) data. Here, I present the *Oenothera* clade with the subclades A and B (Fig. 6.5). Some species used in this study were not presented in the phylogeny but have putative close relatives of the same section that were included. For these, the name of the species tested for capsule dehiscence is given in brackets behind the relative's name in the phylogenetic tree. Hygrochasy and xerochasy only occur in subclade B, whereas 'ripening dehiscent' fruits can be found in subclade A. Hygrochasy is also present in species of sect. *Lavauxia* (*O. triloba* and *O. acaulis*) and sect. *Pachylophis* (*O. harringtonii*), both outside the main subclade B. Sections *Gaura* and *Gauropsis* comprise species with indehiscent capsules (Wagner et al., 2007), marked by black branches in the dendrogram (Fig. 6.5). These form one clade within subsection B.

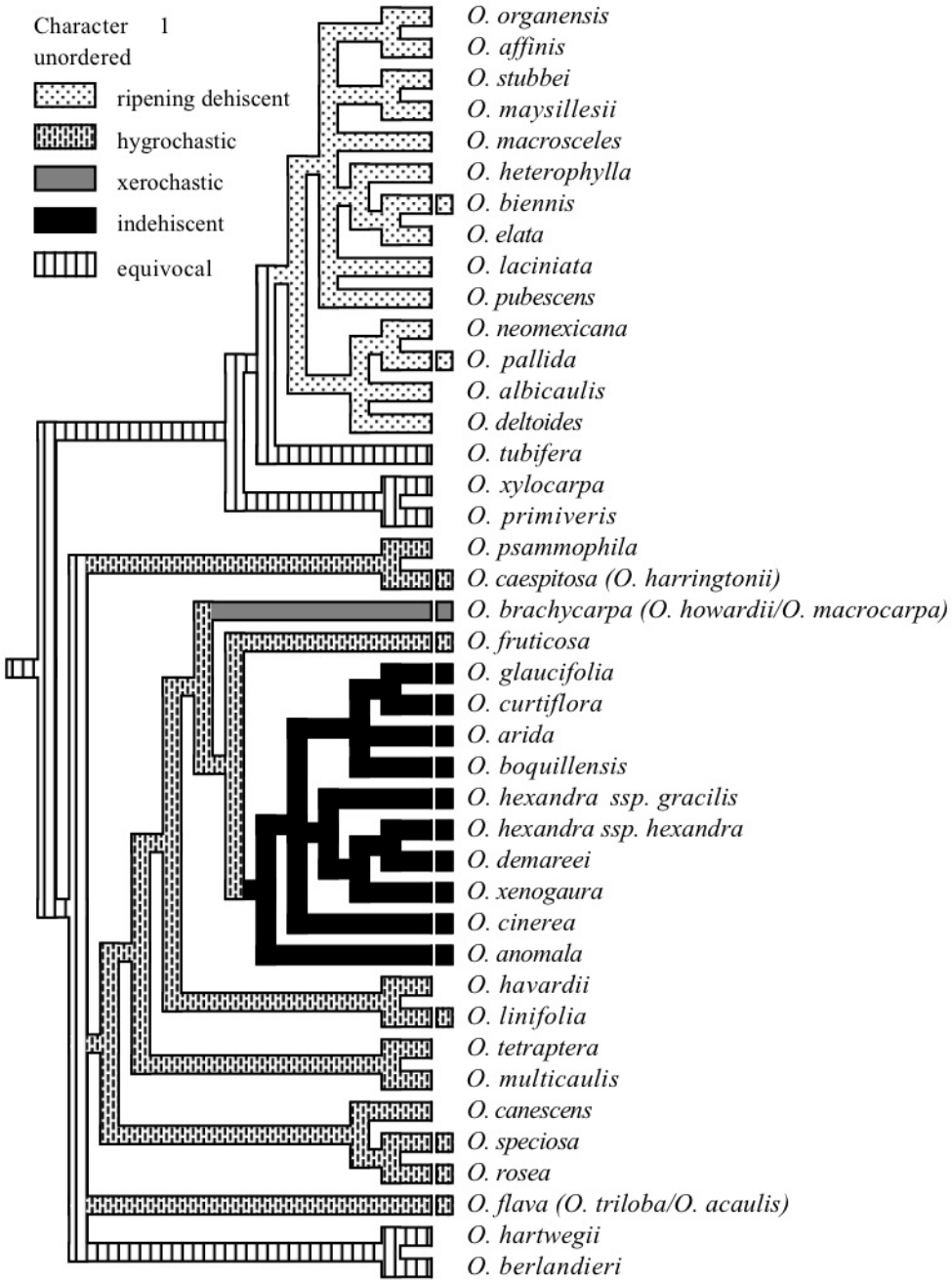

FIG 6.5 Updated strict consensus tree of combined ITS, trnL-trnF and rps16 data, reproduced from Wagner et al. (2007). The two main subclades of *Oenothera* are indicated as A and B. The character 'capsules dehiscence' is traced on the phylogeny using MacClade 4.08. Species for which concrete information was available are marked with a box at the branch end.

For the investigation of hygrochastic capsule evolution in *Veronica*, only the New Zealand section of this genus was used (Albach and Meudt, 2010). The phylogenetic tree is based on the combined ITS and cpDNA data and is well supported (Albach and Meudt, 2010) (Fig. 6.6). This study focussed on the speedwell hebe and snow hebe clades which contain hygrochastic species. The shrubby hebe clade with ripening dehscent capsules is represented by 5 of its 85 species (*V. elliptica* to *V. salicornioides* in Fig. 6.6). Except for *Veronica tubata*, the dehiscence mechanism is known in all presented species. Hygrochasy is present in the entire snow hebe group and as well as in some speedwell hebes and in *V. planopetiolata*. All other species have capsules that dehisce during the ripening process.

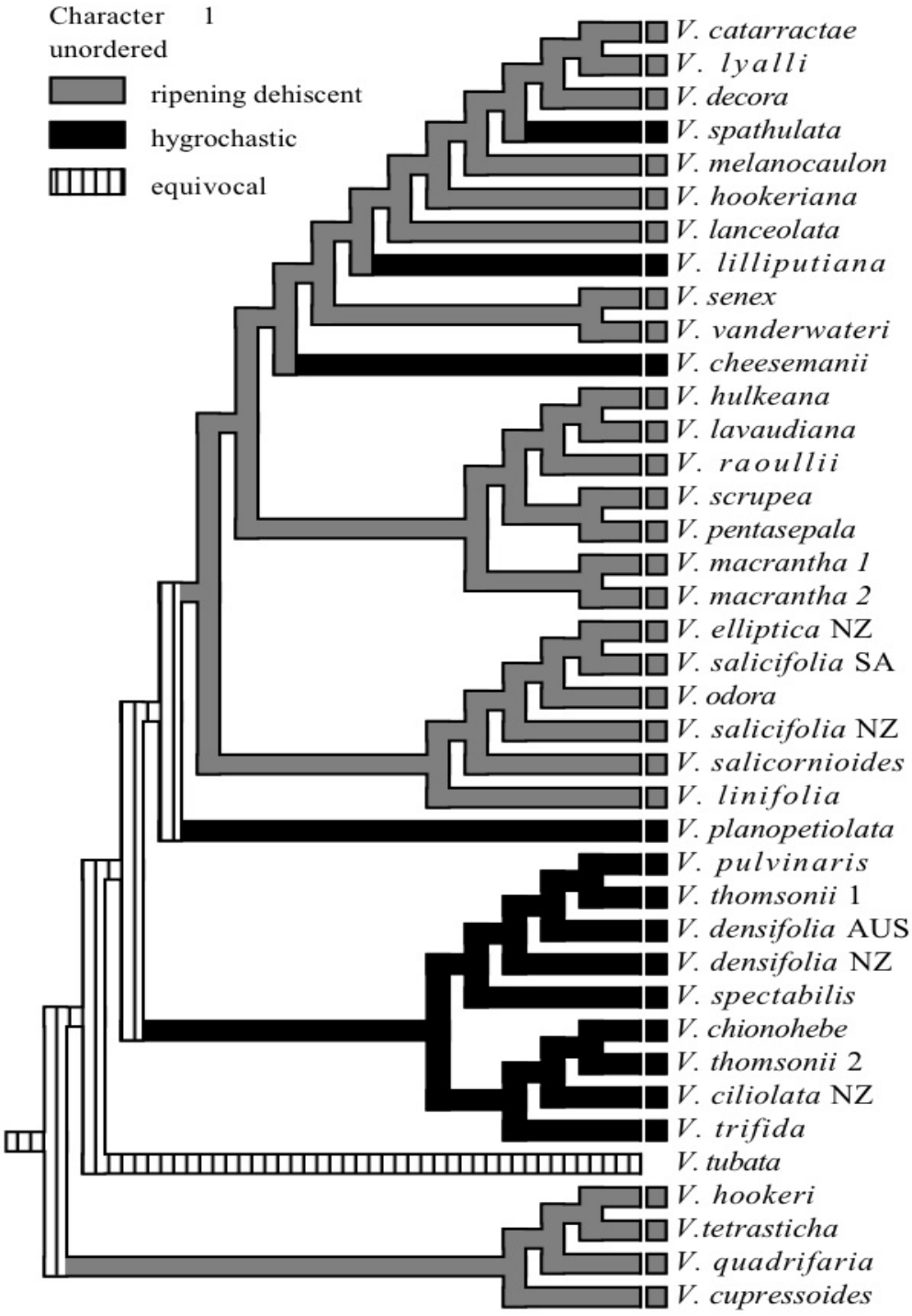

FIG 6.6 Combined phylogeny (ITS and cpDNA) of *Veronica* sect. Hebe, reproduced from Albach and Meudt (2010). The character capsule dehiscence is traced on the phylogeny, species with definite information are indicated with a box at the branch end.

6.5 Discussion

There are several hypotheses for the evolution of hygrochastic capsule dehiscence, most of them developed for plant species in arid areas (Table 6.1). In this study I explore whether the two main hypotheses apply to genera outside arid areas as well. Both the temporal and spatial restriction hypotheses are discussed in hygrochastic North American *Oenothera* species and a possible explanation for hygrochasy in this genus is sought.

6.5.1 Dispersal and seed retention

All species tested show short dispersal distances with the longest distances not further than 1.5 m (*Dracophilus dealbatus*). It is generally known that hygrochasy restricts dispersal to raindrops, which cannot transport seeds very far (Ihlenfeldt, 1983; Van Oudtshoorn and Van Rooyen, 1999; Parolin, 2001; Nakanishi, 2002) and this is certainly true for the tested *Oenothera* species as well. Overall, seeds of Aizoaceae species were dispersed furthest, followed by *Oenothera*; *Veronica* species had the shortest dispersal distances. Data for Aizoaceae species was provided by P. Parolin, who used the same data in a study of the influence of specialized capsule parts in dispersal distance and seed retention (Parolin, 2001). She showed that covering membranes and closing bodies successfully retain seeds in the capsule, so as not to disperse all of them in one rainfall, spreading the risk of germination over time. As a trade-off these appendages enhance the dispersal distance as well. Here, membranes transfer energy of falling drops, whereas closing bodies restrict the outlet, causing ejected water drops to be squirted further through a smaller aperture, taking single seeds with them.

In the laboratory test of seed dispersal, the capsules of both tested *Oenothera* species were mounted 30 cm above the ground to simulate realistic conditions of capsules on the plants. This most likely resulted in the larger dispersal distances for those species (Parolin, 2001). Since all tested species are definitely short distance dispersed, the spatial restriction hypothesis is a possible explanation for hygrochasy in *Oenothera*. Habitat and distribution data are used to explore this idea further.

Restriction of dispersal in time has been explored extensively in Aizoaceae and also in other plants of arid regions (Fahn and Werker, 1972; Ellner and Shmida, 1981; Van der Pijl, 1982; Ihlenfeldt, 1983; Van Rooyen, 1990; Van Oudtshoorn and Van Rooyen, 1999; Parolin, 2006). It is very pronounced in Aizoaceae, at least for species in the core Ruschioideae (Klak et al., 2004), which have been tested in Parolin's work (2001). The risk of germination is spread over time, since only a few seeds are released at a rainfall event, preserving more seeds in the capsule for future rainfall (Van Oudtshoorn and Van Rooyen, 1999). In *Oenothera*, temporal restriction does not seem to be as important, with the majority of the seeds dispersed after only a small amount of rain (Table 6.3). However, Poppendieck (1995) observed that in *Oenothera triloba* only a few seeds at a time are dispersed, since seeds are arranged in vertical piles and not easily splashed out of the capsule. Hygrochasy certainly restricts dispersal to rainfall events, which is a form of temporal restriction but it is not evident in *Oenothera* if dispersal and henceforth the germination risk is spread over time.

6.5.2 PLANT MORPHOLOGY

Splash cup dispersal and henceforth also hygrochasy could potentially be associated with plant morphology. Nakanishi (2002) stated that small herbaceous plants profit from waterdrops, which act as dispersal agents when falling from taller plants;

especially in vegetation where wind cannot reach all fruits. In alpine cushion plants, splash cups also seem to be an advantage for sessile fruits that are embedded in the cushion (P. Garnock-Jones, pers. comm.). Plant morphology of all hygrochastic species in this study was therefore investigated regarding traits that might be of importance for hygrochasy (suitable dispersal hypothesis). In a previous study (Pufal et al., 2010), a syndrome for hygrochastic *Veronica* was identified. Here, hygrochasy is associated with either solitary or few erect, narrowly angustiseptate capsules on short peduncles of creeping subshrubs or cushion plants.

Other than erect capsules and fewer than three capsules in one cluster, no common morphological traits were found for hygrochastic species of unrelated genera. Sessile fruits or fruits on short pedicels were predominantly found in *Veronica* and *Oenothera*, but Aizoaceae exhibit a range of pedicel lengths. Nakanishi's (2002) argument of splash cups in small herbaceous plants could also not be supported. Especially in *Oenothera* and Aizoaceae, hygrochastic species represent a range of life forms and can be quite tall. In prairies, which are a common habitat for *Oenothera* (Britton and Brown, 1970; Straley, 1977; Wolin et al., 1984; Wagner et al., 2007), individuals are mostly not overshadowed by other vegetation and capsules are easily exposed to wind. However, Cluster One in the analysis is associated with small plants (either cushion or rosettes) (Fig. 6.3), which complies with the suitable dispersal hypothesis. At least for *Oenothera triloba*, which is included in this cluster, this theory might be applicable. For all other hygrochastic *Oenothera* species, the suitable dispersal hypothesis of splash cups in small herbaceaous plants has to be rejected (Cluster 2 in Fig. 6.3).

6.5.3 HABITAT QUALITY AND SPECIES DISTRIBUTION

Most hygrochastic Aizoaecae are found in arid and semi arid regions in Southern Africa and often have a small and patchy distribution (Ihlenfeldt, 1983) (spatial restriction hypothesis). The spatial restriction hypothesis also applies to hygrochastic New Zealand *Veronica*, which only occur in small habitat patches in the alpine zone. Here, the short-distance dispersal heightens the chances for seeds to land in safe sites within a suitable habitat (Pufal and Garnock-Jones, 2010; Chapter 3.2).

A hierarchical cluster analysis based on environmental and distribution data groups all species in three distinctive clusters (Fig. 6.4). Cluster One corresponds to the spatial restriction hypothesis – it includes all hygrochastic *Veronica* species, which receive abundant rainfall year round but have a small distribution and are restricted to the alpine zone. Cluster Two includes all Aizoaceae species and two species of *Oenothera* and represents species for which the temporal restriction hypothesis applies. Here, either low annual rainfall or dry seasons with very little rain are common, seasonality occurs for some species and altitude varies greatly. Except for *Oenothera triloba*, all species have a narrow distribution – for those, the spatial restriction hypothesis applies as well. *Oenothera triloba* can be found in this group since it receives very little rainfall in the dry season. It would therefore be favourable to only disperse seeds in the wet season, when germination conditions are favourable. *Oenothera harringtonii* can also be found in this group. It only occurs in Colorado (Ladyman, 2005) and is subjected to a dry climate and high seasonality.

The last cluster includes the remaining *Oenothera* species, which receive abundant rainfall and have a wide distribution with a range of different habitats at varying altitudes (Straley, 1977; Wolin et al., 1984; Wagner et al., 2007). Even though *Oenothera rosea* shows a very high seasonality and very little precipitation in the

driest quarter, it clusters with the other *Oenothera* due to its great range and distribution. It also needs to be taken into account that the minimum driest quarter value only represents the minimum for some locations out of a pool of hundreds. This species is also the only alpine species in this cluster (according to average altitude) but specimens can also be found at sea level.

At least for two *Oenothera* species, the temporal restriction hypothesis seems to apply and *O. harringtonii* is also a good candidate for the spatial restriction hypothesis. However, for the other *Oenothera* species neither hypothesis seems to be applicable, at least not with the data analysed here.

Nakanishi (2002) also mentioned that species with splash cups can be found along streams or water torrents, where the splash water is an effective dispersal agent. The predominant habitat of *Oenothera triloba* (Wagner et al., 2007) is along streams and the suitable dispersal hypothesis could be relevant for this species in this regard.

6.5.4 SYSTEMATICS AND CHARACTER EVOLUTION

Recent phylogenetic trees of *Oenothera* and *Veronica* were used to trace the evolution of hygrochastic capsules in these genera and to find out whether the trait evolved once or several times. It appears that in the New Zealand *Veronica*, hygrochasy has evolved independently several times (Fig. 6.6). This might be due to homoplasy – the character hygrochasy is mostly restricted to cushion plants or low subshrubs in alpine habitat. In fact, homoplasy is relatively common in *Veronica* and has previously led to difficulties in resolving the phylogeny (Meudt, 2008; Albach and Meudt, 2010). Other reasons for previously poorly resolved trees might be a rapid species radiation in the New Zealand *Veronica* (Albach et al., 2004; Albach et al., 2004a; Albach et al., 2004b; Albach and Meudt, 2010). According to Wagstaff et al. (2002) rapid

speciation of *Veronica* (as *Hebe*) in New Zealand occurred in the late Tertiary and plants radiated from alpine areas into the lowland. Hence, hygrochastic *Veronica* are relatively young and the rapid speciation in a highly heterogenous alpine environment combined with a short distance dispersal mechanism might be an explanation for the restricted distributions of those species. Exceptions are *V. densifolia* and *V. ciliolata*, which can also be found in Australia and Tasmania (Meudt, 2008; Meudt and Bayly, 2008). This is most likely due to unexplained long distance dispersal events (Meudt, 2008). However, *Veronica densifolia* possesses mucilaginous seeds (own obs.), which could easily stick to feathers of birds when wet and be dispersed over long distances.

Aizoaceae, with more than 98% of their species being hygrochastic, also have a history of rapid speciation. The rapid radiation of the core Ruschioideae (approximately 1500 species) is unprecedented. They diversified over the last 3.8 to 8.7 Myr with a diversification rate per lineage of only 0.77 to 1.75 Myr (Klak et al., 2004). The morphology of hygrochastic capsules in the core Ruschioideae is very specialized, which might have played an important role in the diversification. The capsules have retention features such as closing bodies or funicles, which are lacking in the species-poor Mesembryanthemoideae. These features allow the plants to spread dispersal over time, whereas the Mesembryanthemoideae disperse their seeds in only a few rainfall events. The spreading of dispersal over time is highly advantageous in regions with erratic rainfall. It reduces the chance of secondary dispersal, thereby reducing gene flow distances and potentially leading to isolation of populations in space (Parolin, 2001). Burke (2005) also suggested that hygrochasy might play an important role in the evolution of endemic species in Namibia.

In *Oenothera*, hygrochasy seems to have evolved only once in the subclade B and parallel in sect. *Lavauxia* and *Pachylophus* (Fig. 6.5). According to new findings,

these sections can also be included in subclade B (K. N. Krakos, pers. comm.), leading effectively to one shift from 'ripening dehiscent' species to hygrochastic species. According to the analyses presented so far, none of the hypotheses proposed for hygrochastic plants in arid regions, alpine areas or the splash cup dispersal in herbaceous vegetation seems to apply to *Oenothera*, with a few exceptions (*O. triloba*, *O. harringtonii*).

For *Oenothera*, the centre of diversity lies in the semiarid to subhumid mountains and plains of interior North America (Tobe et al., 1987; Katinas et al., 2004). The genus *Oenothera* has diverged from *Eremothera* about 10 Myr ago (Sytsma et al., 2004). Raven and Axelrod (1978) postulate that the early diversification of Onagraceae tribe Onagreae took place in the Madrean vegetation of western North America as part of the Madro-Tertiary flora (Axelrod, 1958). This flora was derived mainly from subtropical to temperate groups, which evolved in response to the expansion of a new adaptive zone with much drier climate. Katinas et al. (2004) postulate that the current distribution of Onagrae can be partly attributed to either short distance dispersal into geographically contiguous but unrelated areas or Quaternary migrations into areas, where the previous flora had been eliminated by glacial or preglacial conditions (Grichuk, 1984).

A Madro-Tertiary origin might explain the evolution of hygrochasy in *Oenothera* in combination with the temporal restriction hypothesis. Compared to Aizoaceae and *Veronica*, *Oenothera* are comparatively old and might have had time to spread across the continent despite having restricted dispersal distances. Hygrochasy in *Oenothera* can be most parsimoniously interpreted as a single evolution in the ancestor of subclade B (Fig. 6.5). Hence, hygrochastic dehiscence does not necessarily relate to any living species.

6.6 Conclusion

When analysing the dispersal distances, opening times, plant morphology and distribution and climatic conditions of hygrochastic *Oenothera*, no apparent link could be found to common hypotheses for hygrochasy in other genera. Exceptions are *Oenothera triloba* and *O. harringtonii*, for which the spatial restriction hypothesis, the suitable dispersal hypothesis as well as the temporal restriction hypothesis might apply. However, most hygrochastic *Oenothera* are widespread, occur in areas with sufficient rainfall and low seasonality and seem to have no specialized habitat preferences. However, the phylogeny revealed that hygrochastic capsule dehiscence most likely evolved once with a one-time shift to indehiscence and likewise to xerochasy. The evolutionary history of the tribe Onagreae suggests an origin as part of the Madro-Tertiary flora, where hygrochasy could have been an advantage by delaying the dispersal and germination of seeds to favourable conditions in a dry and seasonal climate. Given the approximate age of the genus *Oenothera* (10 Myr) and the distribution theories of Katinas et al (2004), short-distance dispersal by hygrochastic capsules does not negate the wide distribution of hygrochastic *Oenothera*.

With the available dataset I was only able to explore four out of six potential hypotheses for hygrochasy in *Oenothera*. The safe site strategy might be a possibility, since seeds occasionally get dispersed in clumps of two or more (unpubl. data) and seedlings could shelter each other (Pufal and Garnock-Jones, 2010). However, I was only able to test seed dispersal in two species and more data are needed on the specific habitats and competition between seedlings for all hygrochastic *Oenothera* species.

The protection hypothesis might also be possible explanation for hygrochasy in *Oenothera*. Seed predation can play an important role in herbaceous vegetation, such as deserts or grassland (Brown and Heske, 1990; Price and Joyner, 1997). Storing seeds in the canopy (e.g. hygrochastic capsules) would therefore be of advantage compared to a soil seedbank. Support for this hypothesis can be found in *Oenothera triloba*. The seeds are nondormant (Walck and Hidayati, 2007) and would therefore germinate quickly after dispersal (mostly within one week), decreasing the chance of being destroyed by seed predators. However, more information is needed on seed predators in the diverse habitats of hygrochastic *Oenothera* and dormancy of seeds.

In conclusion I propose that hygrochasy in *Oenothera* is probably an ancient relict from the mid- to late Miocene, when hygrochasy might have evolved in species of the subclade B according to the temporal restriction hypothesis. The spatial restriction hypothesis probably did not play a role, since most hygrochastic *Oenothera* occur in a wide range of habitats and do not seem to have a restricted ecological niche. However, both the protection hypothesis and safe site strategy might play a role in present day hygrochastic *Oenothera*, but more information is needed to investigate these hypotheses in more detail.

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CHAPTER SEVEN

SUMMARY AND CONCLUSION

7.1 Summary of key findings

In this dissertation, I report a series of multidisciplinary studies that answer key questions about the evolution and ecology of hygrochastic capsule dehiscence. Furthermore, the biomechanics and anatomy of hygrochastic capsules have been discovered and described in a number of species of three unrelated genera. The following sections re-introduces the key questions and presents key findings from associated chapters.

- *How does hygrochasy operate in the investigated species and what are the differences regarding capsule anatomy in different hygrochastic species and between hygrochastic species and their non-hygrochastic relatives?*

In **Chapter Two**, capsule anatomy of 17 New Zealand *Veronica* was desribed using different light microscopy techniques and subsequent analysis of cell properties. Additional vegetative and reproductive morphological data of a total of 23 New Zealand *Veronica* were collected and analysed. With this dataset I identified 15 species of *Veronica* as hygrochastic in the speedwell hebe and snow hebe clades (for phylogeny see Albach and Meudt, 2010) whereas eight related species were shown not to be hygrochastic. The hygrochastic movement is based on an imbibition mechanism (Fahn and Werker, 1972). In hygrochastic *Veronica*, a swelling tissue in the septum, consisting of elongated cells with non-lignified, thickened cell walls,

absorbs water and subsequently expands. The expansion of the swelling tissue is converted into an opening movement by a lignified resistance tissue in the capsule valve, which does not change its size or shape. The general biomechanics and involved tissues responsible for the movement are consistent with findings from other species (Steinbrinck, 1883; Garside and Lockyer, 1930; Zohary and Fahn, 1941; Fahn and Werker, 1972; Poppendieck, 1995) but the position of tissues is unique to *Veronica*.

Hygrochasy was previously suspected in some New Zealand *Colobanthus* species with sessile capsules. Capsule material was collected in the field and from herbarium specimen **(Chapter Four)** and analysed using similar methods to those used in Chapter Two. Phylogenetic relationships between sessile species were suspected and samples from herbaria and field sites were analysed using nuclear and chloroplast markers to construct a phylogeny of this genus.

Contrary to previous assumptions, hygrochasy was detected in all investigated *Colobanthus* species and is suspected to occur in every species of the genus. Compared to woody hygrochastic capsules, which function with an imbibition mechanism (Fahn and Werker, 1972), the non-lignified *Colobanthus* capsules open through a combination of imbibition and cohesion mechanism. Here, the outer cells of the capsule have thickened cell walls on the outside, which absorb water and expand. The inner cell walls of those cells are thin and act as resistance tissue. The cohesion mechanism takes place in the inner cells of the capsule, where the cell lumen takes up water and the entire cells expand in size.

The phylogenetic analysis remains inconclusive but shows that *Colobanthus* is probably monophyletic within the Caryophyllaceae. No significant genetic differences

could be detected among *Colobanthus* species, which might be due to recent rapid speciation after long distance dispersal events. The occurrence of hygrochasy in all *Colobanthus* species might be further evidence that species are very closely related and species delimitation is discussed.

I studied capsule material of nine *Oenothera* species from North America using microscopic sections and observations of movement after water absorption **(Chapter Five)**. Additionally to previously known hygrochastic *Oenothera* (Brodie, 1951; Poppendieck, 1995), I confirmed three more species from subclade B (Wagner et al., 2007) as hygrochastic (*O. fruticosa, O. linifolia, O. speciosa*) and one xerochastic species (*O. howardii*), also from subclade B. Interestingly, results show that *Oenothera pallida*, a ‘‘ripening dehiscent’’ species of subclade A, has a similar water absorbent endocarp to hygrochastic species. The capsules exhibit post-dehiscence hygrochastic movement, when the exocarp has disintegrated and the endocarp swells due to water absorption.

- *How does hygrochasy affect dispersal in New Zealand alpine plants?*

This question has been addressed in **Chapter Three**. Population size measurements and dispersal distance data have been collected for a number of hygrochastic and non-hygrochastic New Zealand *Veronica* to explore explanations for hygrochasy in alpine areas in New Zealand. Additionally, environmental data (LENZ IV) for a sample of collection sites were used in arcGIS to identify the width of the ecological niche of those species. This study shows that seeds of hygrochastic *Veronica* are primarily dispersed by raindrops, which results in only short dispersal distances. Secondary dispersal is highly unlikely due to the microtopography of the ground cover (e.g.

crevices, gravel or rocky substrate). Hygrochastic alpine *Veronica* can only be found in small habitat patches that are distinctly different from the surrounding environment. Environmental details from the LENZ IV database provide evidence that hygrochastic *Veronica* in New Zealand are specialists with a small ecological niche compared to related non-hygrochastic species which may be found in a wide variety of habitats. This study shows that hygrochasy is an advantageous strategy for alpine *Veronica* with a narrow ecological niche to restrict seed dispersal to safe sites within small parental habitat patches.

- *What are the reasons for hygrochasy in plants of different habitats and what is the evolutionary history of hygrochasy?*

To answer this question, a meta–dataset of dispersal distances, seed retention and capsule opening times was used together with distribution data, environmental data and character evolution analysis to test whether traditional hypotheses for hygrochasy apply to plants of varying habitats **(Chapter Six)**. Hygrochastic North American *Oenothera* were used as study group in comparison with hygrochastic New Zealand alpine *Veronica* and some hygrochastic South African Aizoaceae. Capsule opening times were faster in *Veronica* and *Oenothera* than Aizoaceae and all three study groups show short-distance dispersal. There was no common syndrome for hygrochastic plants across different families other than three or fewer capsules in one infrutescence and with an erect posture. Both alpine *Veronica* in New Zealand as well as Aizoaceae occur in small, restricted habitats. However, *Veronica* occur in a very humid environment with frequent rainfalls all year round, whereas most Aizoaceae can be found in arid areas with often highly seasonal rainfall. In contrast, most *Oenothera* are very widespread and are present in a range of different habitats with

varying rainfall. Neither of the traditional hypotheses for hygrochasy (restriction in time or space) applied to the widespread *Oenothera* species of present times. However, patterns of character evolution, ages of clades and geological and climate histories suggests that *Oenothera* might have evolved as part of the Madro-Tertiary flora (Raven and Axelrod, 1978; Tobe et al., 1987; Katinas et al., 2004; Sytsma et al., 2004), where hygrochasy could have posed an advantage to restrict dispersal to rare, seasonal rainfall events.

7.2 Conclusion and future directions

In this dissertation I have been able to answer a number of question regarding the evolution and ecology of hygrochastic capsule dehiscence in a range of species.

Hygrochasy is a specialised capsule opening mechanism that restricts seed dispersal by using raindrops as primary dispersal agents, resulting in short-distance dispersal (atelechory). Traditionally, hygrochasy has been mostly associated with plants of arid regions, especially Aizoaceae in Southern Africa (Ellner and Shmida, 1981; Ihlenfeldt, 1983; Van Oudtshoorn and Van Rheede, 1999; Parolin, 2001, 2006). Here, it was thought that it not only restricts dispersal in space but also in time, by limiting dispersal to times of rainfall, when germination conditions are favourable (Van Oudtshoorn and Van Rheede, 1999). The studies presented in my dissertation show that hygrochasy is not only a feature of desert plants but can also play an important role in alpine areas of New Zealand. Additionally, I was able to identify and describe hygrochasy in a number of species previously not known to be hygrochastic. The hypothesis of hygrochasy as an atelechoric strategy applies to hygrochastic *Veronica* in alpine areas but the wide distribution of hygrochastic *Oenothera* across Northern America and into South America defies traditional hypotheses associated with

hygrochasy at first glance. However, they might have played a role when *Oenothera* first emerged as part of the Madro–Tertiary flora, when climatic circumstances were very different from the present day climate.

In a broader context my research shows that hygrochasy is not as rare and restricted as previously thought. It occurs in a wide range of habitats and in very different unrelated genera; some of them have never been associated with hygrochasy until now (e.g. *Colobanthus*).

I have shown that hygrochasy can play an important role for the dispersal of some alpine species and it is highly likely that other alpine cushion plants with sessile capsules might exhibit hygrochasy. For example, some *Myosotis* and *Sagina* in Japan were reported as hygrochastic (Nakanishi, 2002) and their alpine relatives might show this dehiscence type as well. Further research in alpine areas, not only in New Zealand but also elsewhere is warranted.

Occurrence data and associated GIS–based environmental data are increasingly being used in investigating ranges and distribution of plant species. In this study I take a novel approach by testing hypotheses about dispersal strategies with the use of these data. GIS–based information is increasingly updated and refined and often freely available online. These resources can provide tools to explore different theories and hypotheses about dispersal, distribution and analyse relationships between occurrence data, plant traits and habitats.

Larger data sets and more accurate location information of hygrochastic species would provide a clearer picture about the role of hygrochasy in plant dispersal and distribution.

It appears that hygrochasy is a labile character and occurs in many unrelated genera. However, even within genera, the evolution of hygrochasy is diverse – it might have evolved only once in *Oenothera* and has then shifted to other mechanisms, whereas in *Veronica* it evolved several times independently but always shows the same structure. Further investigations especially in *Colobanthus* are worth persuing. The phylogeny is still unresolved and should be approached with different methods, such as AFLPs or microsatellites. It is important to explore the chance of long-distance dispersal between South America, Antarctica, the subantarctic Islands and New Zealand and Australia, especially since all species are hygrochastic and therefore predominantly short-distance dispersed.

I hope that the studies presented in this dissertation will inspire researchers to investigate not only hygrochasy but also other, little known dispersal strategies and their important role in plant distribution. Often the inconspicuous and overlooked details are very intriguing and evoke the desire to learn more about them. And sometimes these inconspicuous things, such as small capsules embedded in cushion plants on the top of mountains prove to be a lot more interesting than they appear to be at first glance. They mark the beginning of a study that spans continents and vastly different plants with very different capsules. Yet they are united by one small movement – they open when it rains.

7.3 References

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APPENDIX

Appendix 2.1

List of species used in the study. Given are origin of capsules (with voucher identification) and number of capsules used for different experiments. Species with a WELTU voucher number were collected for this study.

species	location	altitude	coordinates	voucher	water test	cryostat	ultratome
V. catarractae	Hawkes Bay, North Island			CHR535074	5	3	2
V. cheesemanii	Mt Patriarch, Richmond Forest Park, South Island	1590 m	S 41º36'34.8" E 173º13'28.4"	WELTU20233	5	1	2
V. ciliolata	Sealey Range, Mt Cook Aoraki National Park, South Island	1782 m	S 43º43'02.5"; E 170º03'53.1"	WELTU20231	4	1	1
V. colostylis	Franz Joseph Glacier, South Island			CHR140140	2	1	1
V. decora	Lakeside Track, Lake Rotoiti, Nelson, South Island	625 m	S 41º52'06.8"; E 172º49'18.2"	WELTU20231	5	2	3
V. densifolia	Remarkables Skifield, Central Otago, South Island	1719 m	S 45º03'32.1" E 168º49'06.2"	WELTU20234	6	3	3
V. hookeriana	Mt Ruapehu Skifield, North Island	1615 m	S 39º18'20.4"; E 175º31'32.4"	WELTU20238	6	3	3
V. lanceolata	Waterfall track, granite cliff, Mt Ruapheu. North Island	1222 m	S 39º19'51.8"; E 175º29'51.9"	WELTU20242	5	3	2
V. lilliputiana	Ashburton River, Lake Camp, South Island	685 m		CHR403927	4	1	1
V. lyallii	Fox Glacier walking track, Fox Glacier, South Island	244 m	S 43º29'50.5" E 170º02'50.5"	WELTU20235	6	3	3
V. melanocaulon	Blue Mountains Station, Waimara River, South Island	100 m	S 41º52'; E 173º49'	CHR517481	2	1	1
V. planopetiolata	Mount Dick, Kingston, South Island			CHR512626	2	1	1
V. pulvinaris	Mt Robert Ridgetrack, Nelson Lakes National Park, South Island	1649 m	S 41º50'59.9" E 172º47'14.5"	WELTU20241	6	2	2
V. senex	Webb Stream, Anatori Valley, Nelson, South Island	6 m	S 40º42.76'; E 177º22'	CHR547075B	2	1	1
V. spathulata	Mt Ruapehu Skifield, North Island	1620 m	S 39º18'20.4"; E 175º31'32.4"	WELTU20239	6	3	3
V. thomsonii	Mt Dobson Skifield, South Island	1760 m	S 43º56'36.1"; E 170º39'55.1"	WELTU20237	6	3	3
V. trifida	Blue Lake, Garvie Mountains, South Island	1344 m	S 45º28'33.4"; E 168º56'11.5"	WELTU20240	6	2	1

Appendix 2.2

Measurements of cells in ultratome sections and change of ST length in cryostat sections. Measuremenst are given in μm, the change is measured in %. STL – length of cells in swelling tissue (ST); STW – width of cells in the ST; STCW – thickness of cell walls in ST; RTDIA – diameter of cells in the resistance tissue (RT); RTCW – thickness of cell walls in the RT; change – percentage change of the length of ST after water absorption.

species	STL	STW	STCW	RTDIA	RTCW	change
V. cheesemanii	105	30	12	27	6	30.43
V. cheesemanii	117	33	12	30	6	41.33
V. cheesemanii	105	27	12	27	6	28.40
V. cheesemanii	117	36	15	18	3	26.19
V. cheesemanii	120	33	12	21	3	51.20
V. cheesemanii	120	30	12	27	6	61.25
V. cheesemanii	120	27	12	24	6	28.69
V. cheesemanii	114	30	12	30	6	32.20
V. cheesemanii	120	30	15	21	4.5	59.40
V. cheesemanii	108	27	12	24	4.5	54.80
V. ciliolata	129	30	12	30	9	49.69
V. ciliolata	111	24	9	18	6	56.03
V. ciliolata	108	24	9	24	9	50.29
V. ciliolata	120	21	6	18	9	53.12
V. ciliolata	108	24	10.5	21	9	62.49
V. ciliolata	120	27	10.5	24	9	85.55
V. ciliolata	111	24	9	24	9	70.56
V. ciliolata	99	27	7.5	30	9	62.93
V. ciliolata	120	27	12	27	10.5	46.32
V. ciliolata	105	24	9	18	6	40.00
V. colostylis	105	24	9	36	12	67.03
V. colostylis	90	24	9	36	9	85.86
V. colostylis	84	18	6	36	12	97.40
V. colostylis	105	24	6	42	9	99.94
V. colostylis	90	15	6	30	7.5	79.61
V. colostylis	99	24	6	18	9	67.99
V. colostylis	102	21	6	24	9	58.23
V. colostylis	90	21	6	24	6	65.99
V. colostylis	96	21	6	21	7.5	93.06
V. colostylis	90	21	7.5	15	6	72.71
V. densifolia	240	36	18	30	9	11.63
V. densifolia	249	36	18	30	9	12.11
V. densifolia	240	42	18	27	6	18.86
V. densifolia	210	48	24	30	9	20.51
V. densifolia	285	36	15	30	9	20.60
V. densifolia	300	33	15	30	12	36.77
V. densifolia	300	36	15	33	9	38.10
V. densifolia	255	30	15	24	9	30.25
V. densifolia	255	30	12	33	12	29.02
V. densifolia	240	30	12	27	9	40.74
V. lilliputiana	60	21	9	36	9	26.83

V. lilliputiana	75	24	7.5	30	4.5	22.40
V. lilliputiana	75	42	10.5	36	9	22.67
V. lilliputiana	90	27	9	24	4.5	40.39
V. lilliputiana	60	33	9	36	9	33.34
V. lilliputiana	72	27	6	33	6	55.43
V. lilliputiana	60	27	6	27	6	36.64
V. lilliputiana	54	27	6	36	6	27.85
V. lilliputiana	75	30	9	30	4.5	26.25
V. lilliputiana	60	36	9	36	3	24.48
V. planopetiolata	180	36	18	33	9	39.48
V. planopetiolata	147	39	18	24	6	42.33
V. planopetiolata	168	42	15	27	6	55.93
V. planopetiolata	156	36	15	27	9	33.92
V. planopetiolata	168	36	15	30	6	41.57
V. planopetiolata	165	30	12	24	6	51.21
V. planopetiolata	168	30	12	24	6	70.55
V. planopetiolata	150	36	15	27	6	63.99
V. planopetiolata	132	30	15	24	6	67.73
V. planopetiolata	171	42	18	24	4.5	68.20
V. pulvinaris	138	36	15	30	9	42.26
V. pulvinaris	144	36	15	24	9	33.93
V. pulvinaris	135	27	12	27	9	35.95
V. pulvinaris	135	30	12	27	9	67.19
V. pulvinaris	129	30	9	27	9	30.36
V. pulvinaris	114	36	16.5	27	6	42.57
V. pulvinaris	210	30	15	24	6	41.46
V. pulvinaris	195	36	18	27	9	30.68
V. pulvinaris	150	27	12	30	6	31.99
V. pulvinaris	150	27	13.5	30	7.5	66.59
V. spathulata	120	36	15	30	9	21.95
V. spathulata	105	30	12	39	9	49.45
V. spathulata	165	30	12	36	9	56.30
V. spathulata	150	27	9	30	9	45.63
V. spathulata	120	24	12	42	12	53.97
V. spathulata	165	39	12	33	9	23.66
V. spathulata	135	27	9	39	9	65.15
V. spathulata	150	30	12	48	12	35.14
V. spathulata	135	30	12	36	12	22.57
V. spathulata	114	27	9	30	9	51.42
V. thomsonii	177	36	15	24	9	20.71
V. thomsonii	174	30	12	18	6	24.47
V. thomsonii	174	39	12	27	9	26.56
V. thomsonii	180	36	12	30	9	35.88
V. thomsonii	177	33	9	24	9	28.77
V. thomsonii	120	24	12	27	6	25.72
V. thomsonii	90	24	12	24	6	30.29
V. thomsonii	126	24	10.5	21	6	23.18
V. thomsonii	105	24	12	27	9	39.78

V. thomsonii	120	21	9	30	9	43.98
V. trifida	78	30	9	33	6	24.23
V. trifida	69	30	10.5	27	4.5	22.69
V. trifida	78	27	10.5	30	9	39.78
V. trifida	84	30	9	30	9	18.98
V. trifida	84	36	12	33	6	23.38
V. trifida	87	24	9	30	6	11.45
V. trifida	84	24	7.5	33	6	40.94
V. trifida	90	24	9	27	6	20.92
V. trifida	84	27	9	30	9	50.78
V. trifida	75	24	12	30	6	19.07
V. catarractae	36	36	9	30	12	2.35
V. catarractae	30	30	12	30	9	-11.51
V. catarractae	45	27	12	24	6	4.35
V. catarractae	45	45	12	33	12	-1.03
V. catarractae	42	42	9	30	9	3.15
V. catarractae	51	30	15	36	12	-1.32
V. catarractae	33	33	12	33	9	-2.80
V. catarractae	36	36	9	30	9	1.39
V. catarractae	30	30	9	39	13.5	-4.35
V. catarractae	42	42	12	21	9	-8.13
V. decora	33	33	9	24	6	-3.64
V. decora	24	24	6	24	6	5.79
V. decora	24	24	6	24	4.5	-3.92
V. decora	27	27	6	30	6	3.19
V. decora	30	30	9	27	6	-1.88
V. decora	21	36	7.5	36	9	-3.59
V. decora	21	30	6	27	9	-1.44
V. decora	18	45	6	24	6	2.46
V. decora	21	36	6	30	7.5	0.81
V. decora	21	36	7.5	36	10.5	0.28
V. hookeriana	24	24	6	24	6	-3.82
V. hookeriana	33	33	6	33	6	-5.22
V. hookeriana	27	27	6	27	6	3.88
V. hookeriana	33	33	6	33	6	1.75
V. hookeriana	30	30	7.5	30	7.5	9.87
V. hookeriana	24	24	7.5	24	7.5	-8.78
V. hookeriana	30	30	6	30	6	-5.23
V. hookeriana	30	30	9	30	9	-2.43
V. hookeriana	36	36	7.5	36	7.5	5.65
V. hookeriana	27	27	6	27	6	2.70
V. lanceolata	24	30	6	21	6	2.03
V. lanceolata	30	30	9	30	12	3.13
V. lanceolata	36	27	9	33	12	-2.93
V. lanceolata	21	39	9	27	9	-0.03
V. lanceolata	33	30	12	27	9	3.40
V. lanceolata	18	30	9	36	12	-5.64
V. lanceolata	24	30	9	36	15	-4.55

V. lanceolata	30	30	7.5	30	10.5	-4.14
V. lanceolata	33	33	12	42	15	-1.63
V. lanceolata	30	42	9	36	15	1.93
V. lyallii	18	33	6	24	6	-6.59
V. lyallii	21	42	9	27	7.5	-2.05
V. lyallii	24	24	7.5	24	6	-6.02
V. lyallii	18	36	6	30	9	5.46
V. lyallii	15	39	6	30	7.5	-4.21
V. lyallii	33	33	9	18	6	-2.46
V. lyallii	15	39	6	21	7.5	3.35
V. lyallii	21	30	7.5	36	6	-0.33
V. lyallii	24	42	9	24	4.5	0.56
V. lyallii	24	39	7.5	27	6	3.01
V. melanocaulon	27	27	6	18	6	-13.21
V. melanocaulon	24	24	6	15	6	-3.56
V. melanocaulon	24	24	6	15	6	-11.33
V. melanocaulon	30	27	7.5	18	6	-6.42
V. melanocaulon	27	27	6	21	7.5	-4.49
V. melanocaulon	30	30	9	21	6	-7.04
V. melanocaulon	28.5	27	7.5	18	6	-5.00
V. melanocaulon	21	18	4.5	15	6	1.71
V. melanocaulon	24	24	6	18	6	-4.00
V. melanocaulon	24	24	4.5	18	6	-3.23
V. senex	24	51	9	30	9	0.98
V. senex	33	54	12	30	9	-16.82
V. senex	36	36	12	27	9	-10.75
V. senex	42	42	12	27	6	-0.39
V. senex	30	30	9	21	6	-8.19
V. senex	18	33	6	33	9	-5.77
V. senex	27	27	9	30	9	-0.65
V. senex	36	36	9	27	9	1.40
V. senex	30	42	9	33	9	1.48
V. senex	24	33	6	30	7.5	0.56

Appendix 3.2.1
Coordinates and associated elevation of locations used for experiments in habitat patches (transect and quadrats) and t-square index (TS). Hygrochastic species are shown in bold.

#	species	location	coordinates	Elevation (m)	experiment
1	***V. cheesemanii***	Mt Robert Skifield	S 41º50'42.1" E 172º47'59.6"	1507	transect/quadrats/TS
2	***V. cheesemanii***	Mt Robert Ridgetrack	S 41º50'49.4" E 172º47'30.7"	1601	transect/quadrats
3	***V. cheesemanii***	Mole Tops scree	S 41º57'59.5" E 172º34'51.2"	1556	transect/quadrats
4	***V. cheesemanii***	Mole Tops	S 41º58'05.4" E 172º34'54.4"	1644	transect/quadrats
5	***V. cheesemanii***	Mt. Patriarch	S 41º36'34.8" E 173º13'28.4"	1590	TS
6	***V. densifolia***	Waiorau Snow Farm	S 44º52'11.5" E 169º06'20.3"	1560	transect/quadrats
7	***V. densifolia***	Coronet Peak	S 44º55'33.1" E 168º44'11.6"	1237	transect/quadrats
8	***V. densifolia***	Remarkables Skifield	S 45º03'32.1" E 168º49'06.2"	1719	transect/quadrats/TS
9	***V. densifolia***	Blue Lake	S 45º29'05.3" E 168º55'45.1"	1412	TS
10	***V. pulvinaris***	Mt Robert Skifield	S 41º50'38.1" E 172º48'04.5"	1565	transect/quadrats/TS
11	***V. pulvinaris***	Mole Tops	S 41º58'05.4" E 172º34'54.4"	1644	transect/quadrats
12	***V. pulvinaris***	Mt Robert Ridgetrack	S 41º50'59.9" E 172º47'14.5"	1649	transect/quadrats
13	***V. thomsonii***	Remarkables Skifield	S 45º03'32.1" E 168º49'06.2"	1719	transect/quadrats
14	***V. thomsonii***	Remarkables Skifield	S 45º03'40.1" E 168º49'00.0"	1737	transect/quadrats
15	***V. thomsonii***	Remarkables track	S 45º03'26.4" E 168º49'04.8"	1667	transect/quadrats
16	*V. decora*	Arthurs Pass village	S 42º56'03.6" E 171º33'34.8"	771	transect/quadrats
17	*V. decora*	Kea Point, Mount Cook Village	S 43º42'55.1" E 170º05'09.2"	309	transect/quadrats
18	*V. lyallii*	Klondyke corner, Arthurs pass	S 43º00'01.9" E 171º35'26.2"	722	transect/quadrats
19	*V. lyallii*	Otira valley	S 42º53'43.4" E 171º32'29.9"	991	transect/quadrats
20	*V. lyallii*	Fox Glacier walking track	S 43º29'50.5" E 170º02'50.5"	244	transect/quadrats

Appendix 4.2.1

Location details and experiment information for species used in this study. Shown is the number of capsules that were used for cell measurements and SEM micrograpahs.

species	location	voucher number	measurements	SEM
C. acicularis	Mt Kaweka, Kaweka Range, New Zealand	CHR358804	3	1
C. affinis	Egmont, New Zealand	CHR86723	4	0
C. apetalus	Blue Lake, Garvie Mountains, New Zealand	WELTU20257	3	1
C. brevisepalus	Lake Tekapo, New Zealand	WELTU17486	0	1
C. buchananii	Mt Dobson Skifield, Canterbury, New Zealand	WELTU20248	6	2
C. canaliculatus	Mt Peel, Tasman Range, New Zealand	CHR75580	5	1
C. kerguelensis	Marion and Prince Edward Islands	CHR2042	0	1
C. lycopodioides	Morro Chico, Patagonia, Chile	WELTU20249	0	1
C. masonae	Kakapo Peak, Kahurangi National Park, New Zealand	WELTU20250	0	1
C. monticola	Mt Gow, Landsborough Valley, New Zealand	CHR223853	0	1
C. muelleri	Cape Palliser, New Zealand	CHR146055	3	1
C. quitensis	Punta Arenas, Patagonia, Chile	WELTU20251	0	1
C. strictus	Mt Arthur, Kahurangi National Park, New Zealand	WELTU20252	4	1
C. subulatus	Isla Capitan Aracena, Tierra del Fuego, Chile	CHR259749	4	0
C. wallii	Mt Arthur, Kahurangi National Park, New Zealand	WELTU20253	4	1

Appendix 4.2.2

Measurements of cells in paraffin sections and change of ST length in hand sectioned valves. Measuremenst are given in µm, the change is measured in %.
lin – length of inner cells; win – width of inner cells; cwin – thickness of cell walls of inner cells; lout – length of cells of outer capsule wall; wout – width of cells of outer capsule wall; cwout – cell wall thickness of cells of outer capsule wall; capswall – thickness of capsule wall; change – percentage change width of valve piece after water absorption.

species	lin	win	cwin	lout	wout	cwout	capswall	change
C. acicularis	11.65	5.60	1.76	6.21	5.28	2.32	72.54	28.84
C. acicularis	7.33	4.80	1.34	6.21	4.96	2.49	50.10	22.01
C. acicularis	14.45	6.81	0.77	5.28	5.92	1.87	50.03	32.24
C. acicularis	8.96	7.14	0.88	7.22	6.19	2.26	59.99	10.14
C. acicularis	11.80	4.35	1.73	6.83	6.36	2.35	60.56	49.25
C. acicularis	8.84	5.99	0.77	6.06	4.62	2.01	43.04	20.36
C. acicularis	9.74	6.21	1.33	4.63	4.88	2.09	73.83	31.83
C. acicularis	11.80	6.52	1.14	7.39	5.10	2.14	56.47	28.37
C. acicularis	8.88	4.35	1.32	6.85	4.01	1.94	51.82	24.02
C. acicularis	9.70	7.09	0.92	7.41	3.76	1.95	64.91	14.86
C. affinis	19.19	10.35	1.88	19.42	10.94	2.19	38.16	19.63
C. affinis	10.08	7.63	1.25	10.35	8.84	2.81	51.43	52.17
C. affinis	10.83	9.76	1.56	12.17	12.17	2.60	42.21	42.20

C. affinis	12.89	11.96	1.56	12.87	11.75	3.44	52.82	13.22
C. affinis	11.10	9.37	0.63	9.40	10.98	2.94	40.30	15.45
C. affinis	7.41	6.50	0.94	11.22	15.73	2.83	43.08	13.39
C. affinis	8.01	10.21	1.36	12.00	9.37	2.65	41.32	16.15
C. affinis	8.81	8.68	0.66	15.70	9.02	4.00	39.13	15.68
C. affinis	12.15	7.18	2.10	10.00	11.59	3.29	41.67	6.78
C. affinis	12.75	12.66	1.25	11.12	8.95	3.52	43.78	8.99
C. apetalus	16.21	7.09	0.49	7.84	5.47	1.49	65.92	39.72
C. apetalus	13.87	14.90	0.49	6.87	4.68	1.71	51.44	7.05
C. apetalus	20.46	17.70	0.94	4.75	5.55	1.72	58.51	20.37
C. apetalus	9.56	8.75	0.89	6.71	8.88	2.74	61.59	12.47
C. apetalus	9.81	10.25	1.72	9.66	9.69	2.03	66.74	16.88
C. apetalus	13.67	11.96	0.95	7.36	8.75	2.18	54.07	13.77
C. apetalus	16.00	8.49	1.00	6.64	9.89	2.27	52.21	12.55
C. apetalus	10.31	9.41	1.34	5.78	8.33	2.03	63.54	16.94
C. apetalus	10.47	7.65	0.78	6.56	8.31	2.06	35.19	17.18
C. apetalus	13.98	10.05	0.49	8.52	5.99	1.44	51.63	14.58
C. buchananii	22.02	13.56	1.27	12.74	10.42	3.55	64.64	24.18
C. buchananii	18.84	8.55	1.30	8.35	10.49	4.38	58.71	43.97
C. buchananii	9.56	9.00	0.42	6.67	7.92	3.96	59.40	21.03
C. buchananii	13.83	5.81	2.08	8.33	7.88	3.03	72.37	25.20
C. buchananii	8.20	4.16	1.51	10.46	9.42	3.44	68.06	33.15
C. buchananii	16.80	11.88	1.47	13.08	10.09	3.33	77.41	45.32
C. buchananii	13.21	9.59	1.56	12.92	13.14	4.15	79.69	13.79
C. buchananii	19.40	20.02	2.21	10.71	8.77	3.43	65.95	20.01
C. buchananii	15.37	7.71	1.46	10.02	9.67	4.25	64.85	39.78
C. buchananii	9.43	9.87	1.47	8.40	7.19	4.35	63.57	16.06
C. canaliculatus	16.96	14.72	1.10	12.02	10.92	3.79	75.15	11.83
C. canaliculatus	9.87	13.16	0.34	8.31	10.75	2.96	47.79	16.41
C. canaliculatus	7.69	10.69	0.94	9.95	10.59	2.89	38.90	35.90
C. canaliculatus	8.89	7.89	0.47	7.48	11.06	2.96	44.34	20.41
C. canaliculatus	15.11	12.93	1.71	8.45	10.53	3.76	61.05	23.91
C. canaliculatus	9.19	9.11	0.47	6.99	11.37	3.29	59.71	20.66
C. canaliculatus	7.96	9.52	0.64	7.44	10.87	3.24	68.76	24.83
C. canaliculatus	9.03	15.22	1.25	16.19	11.18	3.02	60.68	15.39
C. canaliculatus	6.08	17.33	0.47	9.89	10.31	3.29	66.28	10.89
C. canaliculatus	4.89	8.75	0.47	4.49	9.03	2.71	49.45	13.29
C. muelleri	12.77	8.86	2.68	12.09	15.50	5.62	77.71	30.42
C. muelleri	11.13	11.46	1.25	15.09	14.67	4.62	45.16	14.83
C. muelleri	22.02	6.59	2.10	10.94	10.15	4.55	63.71	19.91
C. muelleri	14.14	12.53	0.81	13.00	13.38	5.73	59.87	35.58
C. muelleri	22.34	14.19	1.47	9.30	17.07	6.70	52.69	39.78
C. muelleri	19.12	7.97	1.33	8.40	14.88	6.15	63.71	21.65
C. muelleri	12.24	8.25	0.83	14.13	13.54	4.81	43.02	6.03
C. muelleri	16.20	10.87	1.79	13.32	11.14	5.31	91.35	24.97
C. muelleri	17.27	13.15	1.40	13.76	14.03	4.31	58.40	131.10
C. muelleri	12.09	9.05	1.63	19.66	13.58	5.73	56.40	13.74
C. strictus	27.60	18.08	1.97	13.76	10.13	3.17	48.73	37.29
C. strictus	21.15	9.79	2.37	16.33	9.67	3.94	45.82	11.15
C. strictus	24.79	13.52	2.29	24.38	12.50	4.62	40.87	9.28
C. strictus	27.22	25.92	2.33	18.83	11.62	4.05	48.57	41.97
C. strictus	16.77	20.60	1.84	20.96	10.25	3.77	39.88	35.45
C. strictus	12.53	6.45	1.77	20.01	12.12	3.26	52.47	10.93
C. strictus	12.04	19.42	1.04	19.42	9.47	2.52	43.08	29.08

C. strictus	16.88	7.56	1.25	13.07	12.31	4.83	61.75	16.69
C. strictus	19.72	18.25	1.32	12.89	11.14	4.24	40.38	29.13
C. strictus	21.83	24.11	1.33	9.19	6.77	2.92	48.82	6.42
C. subulatus	26.76	11.40	0.96	14.60	7.29	3.09	37.97	23.81
C. subulatus	23.00	12.35	1.67	13.98	6.83	2.92	49.33	19.13
C. subulatus	18.15	6.64	0.93	11.46	9.29	2.88	34.78	5.08
C. subulatus	7.77	6.18	1.26	11.65	8.10	2.81	50.31	14.19
C. subulatus	18.17	7.80	0.83	7.41	8.62	2.38	29.94	25.70
C. subulatus	9.59	12.97	1.04	11.38	6.04	2.37	37.93	14.23
C. subulatus	19.05	12.91	1.12	9.25	7.23	2.43	22.44	27.74
C. subulatus	6.67	8.63	1.25	9.14	6.36	2.29	41.19	11.63
C. subulatus	12.18	6.68	1.27	13.78	6.46	2.33	37.51	14.32
C. subulatus	21.90	15.84	0.86	13.05	6.43	2.40	38.43	34.03
C. wallii	7.35	14.85	0.67	5.53	11.31	2.45	61.88	54.89
C. wallii	13.86	11.75	0.30	6.26	11.08	2.86	68.18	20.40
C. wallii	10.00	11.71	0.88	6.38	9.07	2.00	47.08	28.93
C. wallii	5.27	13.91	0.95	8.95	9.33	3.09	41.88	22.09
C. wallii	13.86	15.86	0.64	7.54	12.37	3.19	43.54	6.72
C. wallii	6.72	6.39	0.64	9.22	12.66	3.63	43.45	23.30
C. wallii	11.37	13.42	0.43	10.94	12.56	3.30	50.63	29.17
C. wallii	7.08	7.14	1.28	6.65	10.90	2.89	59.42	29.67
C. wallii	14.83	7.67	1.85	8.13	8.02	2.02	56.63	7.81
C. wallii	9.06	4.13	0.53	6.77	10.01	1.95	58.00	29.95

Appendix 4.2.3

Kruskal-Wallis test for cell measurements of nine *Colobanthus* species and subsequent Mann-Whitney-U tests for measurements between species. Given are chi-squared, df and p-values for the Kruskal-Wallis test and W and p-values for mann-Whitney-U test. Significant results are highlighted in **BOLD.**

LIN – length of inner cells; WIN – width of inner cells; CWIN – thickness of cell walls of inner cells; LOUT – length of cells of outer capsule wall; WOUT – width of cells of outer capsule wall; CWOUT – cell wall thickness of cells of outer capsule wall; CHANGE – percentage change of the width of valve pieces after water absorption; CAPSWALL – thickness of capsule wall.

LIN	**Chi-squared = 52.99**				**Df = 9**		**p-value < 0.0001**									
	C. acicularis		*C. affinis*		*C. buchananii*		*C. canaliculatus*		*C. wallii*		*C. apetalus*		*C. subulatus*		*C. strictus*	
	W	p	W	p	W	p	W	P	W	p	W	p	W	p	W	p
C. affinis	42	0.57														
C. buchananii	**23**	**0.05**	27	0.089												
C. canaliculatus	61	0.43	68	0.19	**82**	**0.015**										
C. wallii	55	0.73	61	0.43	**78**	**0.038**	47	0.85								
C. apetalus	**21**	**0.03**	31	0.17	54	0.796	**18**	**0.015**	**23**	**0.05**						
C. subulatus	26	0.08	32	0.19	43	0.63	**20**	**0.023**	**23**	**0.05**	38	0.39				
C. strictus	**2**	**0.0003**	**9**	**0.0011**	24	0.052	**6**	**0.0003**	**6**	**0.001**	**15**	**0.007**	35	0.28		
C. muelleri	**8**	**0.002**	**17**	**0.012**	43	0.63	**11**	**0.002**	**14**	**0.007**	31	0.166	51	0.97	70	0.14
WIN	**Chi-squared = 39.03**				**Df = 9**		**p-value < 0.0001**									
	C. acicularis		*C. affinis*		*C. buchananii*		*C. canaliculatus*		*C. wallii*		*C. apetalus*		*C. subulatus*		*C. strictus*	
	W	p	W	p	W	p	W	p	W	p	W	p	W	p	W	p
C. affinis	**4**	**0.0006**														
C. buchananii	**16**	**0.0113**	51	0.97												
C. canaliculatus	**0**	**0.0002**	26	0.075	33	0.22										
C. wallii	**15**	**0.009**	40	0.48	45	0.74	59	0.53								
C. apetalus	**1**	**0.0002**	43	0.63	44	0.68	63	0.35	50	1						
C. subulatus	**11**	**0.004**	45	0.74	47	0.85	69	0.17	56	0.68	55	0.74				
C. strictus	**4**	**0.0006**	**22**	**0.035**	25	0.06	29	0.12	24	0.052	26	0.075	**22**	**0.035**		
C. muelleri	**3**	**0.0004**	40	0.48	44	0.68	66	0.25	54	0.796	50	1	45	0.74	75	0.063

CWIN	**Chi-squared = 52.49**		**Df = 9**		**p-value < 0.0001**							
	C. acicularis	*C. affinis*	*C. buchananii*		*C. canaliculatus*		*C. wallii*		*C. apetalus*		*C. subulatus*	*C. strictus*

	W	p	W	p	W	p	W	p	W	p	W	p	W	p	W	p
C. affinis	42	0.57														
C. buchananii	30	0.14	40	0.47												
C. canaliculatus	**79**	**0.03**	**82**	**0.017**	**84**	**0.01**										
C. wallii	**79**	**0.03**	**78**	**0.037**	**83**	**0.014**	46	0.79								
C. apetalus	68	0.185	75	0.063	**81**	**0.03**	34	0.24	41	0.52						
C. subulatus	57	0.623	62	0.3843	**82**	**0.017**	24	0.053	24	0.054	31	0.16				
C. strictus	**20**	**0.026**	27	0.089	38	0.38	**6**	**0.001**	**8**	**0.002**	**8**	**0.002**	**11**	**0.0036**		
C. muelleri	30	0.14	39	0.43	52	0.91	**13**	**0.006**	**15**	**0.009**	**19**	**0.02**	26.5	0.082	60.5	0.45
LOUT	**Chi-squared = 70.25**				**Df = 9**		**p-value < 0.0001**									
	C. acicularis		*C. affinis*		*C. buchananii*		*C. canaliculatus*		*C. wallii*		*C. apetalus*		*C. subulatus*		*C. strictus*	
	W	p	W	p	W	p	W	p	W	p	W	p	W	p	W	p
C. affinis	**0**	**0.0002**														
C. buchananii	**5**	**0.0008**	69	0.17												
C. canaliculatus	**13**	**0.0058**	**83**	**0.012**	67	0.22										
C. wallii	28	0.104	**97**	**< 0.0001**	**82**	**0.015**	67	0.22								
C. apetalus	37	0.34	**99**	**< 0.0001**	**88**	**0.003**	76	0.052	57	0.63						
C. subulatus	**1**	**0.0002**	53	0.85	31	0.17	26	0.075	**8**	**0.0007**	**5**	**0.0002**				
C. strictus	**0**	**0.0002**	**18**	**0.015**	**9**	**0.001**	**7**	**0.0005**	**2**	**< 0.0001**	**1**	**< 0.0001**	**18**	**0.015**		
C. muelleri	**0**	**0.0002**	41	0.53	**20**	**0.023**	**17**	**0.0115**	**5**	**0.0002**	**3**	**< 0.0001**	38	0.39	73.5	0.082
WOUT	**Chi-squared = 71.88**				**Df = 9**		**p-value < 0.0001**									
	C. acicularis		*C. affinis*		*C. buchananii*		*C. canaliculatus*		*C. wallii*		*C. apetalus*		*C. subulatus*		*C. strictus*	
	W	p	W	p	W	p	W	p	W	p	W	p	W	p	W	p
C. affinis	**0**	**< 0.0001**														
C. buchananii	**0**	**< 0.0001**	71	0.12												
C. canaliculatus	**0**	**< 0.0001**	55	0.74	**17**	**0.012**										
C. wallii	**0**	**< 0.0001**	47	0.85	29	0.12	44	0.68								
C. apetalus	**15**	**0.0068**	**91**	**0.001**	75	0.063	**98**	**< 0.0001**	**90**	**0.0015**						
C. subulatus	**3**	**< 0.0001**	**97**	**< 0.0001**	**88**	**0.0023**	**99**	**< 0.0001**	**96**	**0.0001**	54	0.80				
C. strictus	**0**	**< 0.0001**	45	0.74	30	0.14	48	0.91	51	0.97	**10**	**0.0015**	**6**	**0.0003**		
C. muelleri	**0**	**< 0.0001**	**17**	**0.012**	**4**	**0.0001**	**11**	**0.002**	**10**	**0.0015**	**0**	**< 0.0001**	**0**	**< 0.0001**	**10.5**	**0.003**
CWOUT	**Chi=squared = 78.56**				**Df = 9**		**p-value < 0.0001**									
	C. acicularis		*C. affinis*		*C. buchananii*		*C. canaliculatus*		*C. wallii*		*C. apetalus*		*C. subulatus*		*C. strictus*	
	W	p	W	p	W	p	W	p	W	p	W	p	W	p	W	p
C. affinis	**4**	**0.0001**														
C. buchananii	**0**	**< 0.0001**	**14.5**	**0.008**												

C. canaliculatus	**0**	**< 0.0001**	38	0.38	**87**	**0.0058**										
C. wallii	**21**	**0.029**	61	0.44	**92**	**0.0007**	71	0.12								
C. apetalus	65	0.27	**96**	**0.0006**	**100**	**0.0002**	**99**	**0.0002**	**81**	**0.021**						
C. subulatus	**9**	**0.001**	75	0.063	**99**	**< 0.0001**	**91**	**0.0022**	60	0.48	6	**0.001**				
C. strictus	**0**	**< 0.0001**	**24**	**0.05**	55	0.74	28	0.104	**15**	**0.007**	1	**0.0002**	**5.5**	**0.0009**		
C. muelleri	**0**	**0.0002**	**0**	**0.0002**	**2**	**0.0003**	**0**	**0.0002**	**0**	**0.0002**	**0**	**0.0002**	**0**	**0.0002**	**6.5**	**0.001**
CHANGE	**Chi-squared = 35.17**				**Df = 9**		**p-value < 0.0001**									
	C. acicularis		*C. affinis*		*C. buchananii*		*C. canaliculatus*		*C. wallii*		*C. apetalus*		*C. subulatus*		*C. strictus*	
	W	p	W	p	W	p	W	p	W	p	W	p	W	p	W	p
C. affinis	71	0.123														
C. buchananii	46	0.80	24	0.052												
C. canaliculatus	69	0.17	42	0.59	75	0.06										
C. wallii	51	0.97	33	0.22	55	0.74	35	0.28								
C. apetalus	**78**	**0.035**	50	1	**83**	**0.01**	60	0.48	74	0.08						
C. subulatus	71	0.123	47	0.85	72	0.10	51	0.97	66	0.25	43	0.63				
C. strictus	55	0.74	47	0.85	64	0.32	46	0.80	53	0.85	46	0.80	43	0.63		
C. muelleri	53	0.85	34	0.25	58	0.58	35	0.28	49	0.97	28	0.10	34	0.25	43	0.63
CAPSWALL	**Chi-squared = 63.62**				**df = 9**		**p-value < 0.0001**									
	C. acicularis		*C. affinis*		*C. buchananii*		*C. canaliculatus*		*C. wallii*		*C. apetalus*		*C. subulatus*		*C. strictus*	
	W	p	W	p	W	p	W	p	W	p	W	p	W	p	W	p
C. affinis	**91**	**0.001**														
C. buchananii	**25**	**0.063**	**0**	**< 0.0001**												
C. canaliculatus	52	0.91	**15**	**0.0068**	73	0.089										
C. wallii	64	0.32	**15**	**0.0068**	**89**	**0.002**	64	0.315								
C. apetalus	51	0.97	**13**	**0.0039**	**85**	**0.007**	41	0.97	38	0.39						
C. subulatus	**96**	**0.0001**	**80**	**0.023**	**100**	**< 0.0001**	**92**	**0.0007**	**92**	**0.0007**	**93**	**0.0005**				
C. strictus	**84**	**0.009**	34	0.25	**98**	**< 0.0001**	74	0.075	71	0.12	**81**	**0.019**	**19**	**0.019**		
C. muelleri	47	0.85	**7**	**0.001**	73	0.0889	46	0.79	33	0.21	42	0.57	**4**	**0.0006**	**17**	**0.014**

Appendix 6.1
Citation records for data used from the gbif database.

species	accessed through gbif portal	occurences
Acrodon subulatus	HBGSpermatophyta - Herbarium Hamburgense, http://data.gbif.org/datasets/resource/1604 SABIF Resource, http://data.gbif.org/datasets/resource/8051	9(14)
Argyroderma fissum	EURISCO, The European Genetic Resources Search Catalogue, http://data.gbif.org/datasets/resource/1905 HBGSpermatophyta - Herbarium Hamburgense, http://data.gbif.org/datasets/resource/1604) University of California Botanical Garden DiGIR provider, http://data.gbif.org/datasets/resource/1412 SysTax, http://data.gbif.org/datasets/resource/1875 Royal Botanic Gardens, Kew, http://data.gbif.org/datasets/resource/629 SABIF Resource, http://data.gbif.org/datasets/resource/8051	10(91)
Bergeranthus scapiger	BoGART, http://data.gbif.org/datasets/resource/1087 HBGSpermatophyta - Herbarium Hamburgense, http://data.gbif.org/datasets/resource/1604 Nationaal Herbarium Nederland, http://data.gbif.org/datasets/resource/1211 Universidad Polit√©cnica de Madrid, Dpto. Biolog√≠a Vegetal, Banco de Germoplasma, http://data.gbif.org/datasets/resource/1521 University of California Botanical Garden DiGIR provider, http://data.gbif.org/datasets/resource/1412 SysTax, http://data.gbif.org/datasets/resource/1875 EURISCO, The European Genetic Resources Search Catalogue, http://data.gbif.org/datasets/resource/1905 Phanerogamic Botanical Collections (S), http://data.gbif.org/datasets/resource/8113 RBGE Living Collections, http://data.gbif.org/datasets/resource/9167 SABIF Resource, http://data.gbif.org/datasets/resource/8051	3(34)
Cephalophyllum spissum	HBGSpermatophyta - Herbarium Hamburgense, http://data.gbif.org/datasets/resource/1604 Phanerogamic Botanical Collections (S), http://data.gbif.org/datasets/resource/8113 SABIF Resource, http://data.gbif.org/datasets/resource/8051	4(16)
Dracophilus dealbatus	HBGSpermatophyta - Herbarium Hamburgense, http://data.gbif.org/datasets/resource/1604 Herbarium Berolinense, http://data.gbif.org/datasets/resource/1095 SysTax, http://data.gbif.org/datasets/resource/1875 SABIF Resource, http://data.gbif.org/datasets/resource/8051 Phanerogamic Botanical Collections (S), http://data.gbif.org/datasets/resource/8113	10(93)
Drosanthemum globosum	Royal Botanic Gardens, Kew, http://data.gbif.org/datasets/resource/629 SABIF Resource, http://data.gbif.org/datasets/resource/8051	5(8)

Drosanthemum hispidum	HBGSpermatophyta - Herbarium Hamburgense, http://data.gbif.org/datasets/resource/1604 Nationaal Herbarium Nederland - Leiden Branch, http://data.gbif.org/datasets/resource/1085 University and Jepson Herbaria DiGIR provider, http://data.gbif.org/datasets/resource/1413 USDA PLANTS Database, http://data.gbif.org/datasets/resource/1066 Real Jardin Botanico (Madrid), Vascular Plant Herbarium (MA), http://data.gbif.org/datasets/resource/240 Phanerogamic Botanical Collections (S), http://data.gbif.org/datasets/resource/8113 EURISCO, The European Genetic Resources Search Catalogue, http://data.gbif.org/datasets/resource/1905 SABIF Resource, http://data.gbif.org/datasets/resource/8051 Consortium of California Herbaria, http://data.gbif.org/datasets/resource/9153	67(105)
Drosanthemum schoenlandianum	HBGSpermatophyta - Herbarium Hamburgense, http://data.gbif.org/datasets/resource/1604 Royal Botanic Gardens, Kew, http://data.gbif.org/datasets/resource/629 Phanerogamic Botanical Collections (S), http://data.gbif.org/datasets/resource/8113 SABIF Resource, http://data.gbif.org/datasets/resource/8051	4(9)
Ebracteola wilmaniae	HBGSpermatophyta - Herbarium Hamburgense, http://data.gbif.org/datasets/resource/1604) SysTax, http://data.gbif.org/datasets/resource/1875) SABIF Resource, http://data.gbif.org/datasets/resource/8051)	6(16)
Phyllobolus spinuliferus	Phanerogamic Botanical Collections (S), http://data.gbif.org/datasets/resource/8113 EURISCO, The European Genetic Resources Search Catalogue, http://data.gbif.org/datasets/resource/1905	1(3)
Rhombophyllum dolabriforme	SysTax, http://data.gbif.org/datasets/resource/1875 HBGSpermatophyta - Herbarium Hamburgense, http://data.gbif.org/datasets/resource/1604 Nationaal Herbarium Nederland, http://data.gbif.org/datasets/resource/1211 Herbarium Berolinense, http://data.gbif.org/datasets/resource/1095 Universidad Polit√©cnica de Madrid, Dpto. Biolog√≠a Vegetal, Banco de Germoplasma, http://data.gbif.org/datasets/resource/1521 Phanerogamie, http://data.gbif.org/datasets/resource/1506 Phanerogamic Botanical Collections (S), http://data.gbif.org/datasets/resource/8113 RBGE Living Collections, http://data.gbif.org/datasets/resource/9167 EURISCO, The European Genetic Resources Search Catalogue, http://data.gbif.org/datasets/resource/1905 Real Jardin Botanico (Madrid), Vascular Plant Herbarium (MA), http://data.gbif.org/datasets/resource/240 SBT-Living, http://data.gbif.org/datasets/resource/7962	2(25)

	California State University, Chico, http://data.gbif.org/datasets/resource/737 NatureServe Network Species Occurrence Data, http://data.gbif.org/datasets/resource/607 SysTax, http://data.gbif.org/datasets/resource/1875 Herbarium of Oskarshamn (OHN), http://data.gbif.org/datasets/resource/1024 Missouri Botanical Garden, http://data.gbif.org/datasets/resource/621 Nationaal Herbarium Nederland, http://data.gbif.org/datasets/resource/1211 Nationaal Herbarium Nederland - Leiden Branch, http://data.gbif.org/datasets/resource/1085 United States National Plant Germplasm System Collection, http://data.gbif.org/datasets/resource/1429 USDA PLANTS Database, http://data.gbif.org/datasets/resource/1066 Botanic Garden of Finnish Museum of Natural History, http://data.gbif.org/datasets/resource/2406 Botany (UPS), http://data.gbif.org/datasets/resource/1045 CSU Herbarium, http://data.gbif.org/datasets/resource/7892 Harvard University Herbaria, http://data.gbif.org/datasets/resource/1827 E.C. Smith Herbarium, http://data.gbif.org/datasets/resource/1829 Botanical garden, University of Hohenheim, Germany, http://data.gbif.org/datasets/resource/1855 IPK Genebank, http://data.gbif.org/datasets/resource/1851 Plants of Papua New Guinea, http://data.gbif.org/datasets/resource/969 USU-UTC Specimen Database, http://data.gbif.org/datasets/resource/1508 Fairchild Tropical Botanic Garden Virtual Herbarium Darwin Core format, http://data.gbif.org/datasets/resource/202 EURISCO, The European Genetic Resources Search Catalogue, http://data.gbif.org/datasets/resource/1905 NMNH Botany Collections, http://data.gbif.org/datasets/resource/1874 CONN GBIF data, http://data.gbif.org/datasets/resource/7857 Oklahoma Vascular Plants Database Provider, http://data.gbif.org/datasets/resource/2558 EKY_Darwincore, http://data.gbif.org/datasets/resource/7894 MISS_DC_01MAR2006, http://data.gbif.org/datasets/resource/7895 Herbarium Berolinense, http://data.gbif.org/datasets/resource/1095 Herbarium, http://data.gbif.org/datasets/resource/7984 Phanerogamic Botanical Collections (S), http://data.gbif.org/datasets/resource/8113 Peabody Paleobotany DiGIR Service, http://data.gbif.org/datasets/resource/8141 Peabody Paleoportal DiGIR Service (PB), http://data.gbif.org/datasets/resource/8176 Herbarium of The New York Botanical Garden, http://data.gbif.org/datasets/resource/8967 RBGE Living Collections, http://data.gbif.org/datasets/resource/9167 Herbarium (UNA), http://data.gbif.org/datasets/resource/775 Botany Vascular Plant Collection, http://data.gbif.org/datasets/resource/7915	
Oenothera fruticosa	SBT-Living, http://data.gbif.org/datasets/resource/7962	786(1126)

Oenothera harringtonii	NatureServe Network Species Occurrence Data, http://data.gbif.org/datasets/resource/607 USDA PLANTS Database, http://data.gbif.org/datasets/resource/1066 Botany (UPS), http://data.gbif.org/datasets/resource/1045 CSU Herbarium, http://data.gbif.org/datasets/resource/7892 USU-UTC Specimen Database, http://data.gbif.org/datasets/resource/1508 Specimen Database of Colorado Vascular Plants, http://data.gbif.org/datasets/resource/1832 NMNH Botany Collections, http://data.gbif.org/datasets/resource/1874, UA Herbarium, http://data.gbif.org/datasets/resource/7900 Botany Vascular Plant Collection, http://data.gbif.org/datasets/resource/7915 Phanerogamic Botanical Collections (S), http://data.gbif.org/datasets/resource/8113 Herbarium of The New York Botanical Garden, http://data.gbif.org/datasets/resource/8967	11(103)
Oenothera linifolia	NatureServe Network Species Occurrence Data, http://data.gbif.org/datasets/resource/607 Missouri Botanical Garden, http://data.gbif.org/datasets/resource/621, USDA PLANTS Database, http://data.gbif.org/datasets/resource/1066 Harvard University Herbaria, http://data.gbif.org/datasets/resource/1827 NMNH Botany Collections, http://data.gbif.org/datasets/resource/1874 Oklahoma Vascular Plants Database Provider, http://data.gbif.org/datasets/resource/2558 MISS_DC_01MAR2006, http://data.gbif.org/datasets/resource/7895 Botany Vascular Plant Collection, http://data.gbif.org/datasets/resource/7915 Herbarium, http://data.gbif.org/datasets/resource/7984 Phanerogamic Botanical Collections (S), http://data.gbif.org/datasets/resource/8113 RBGE Herbarium (E), http://data.gbif.org/datasets/resource/8402 Herbarium (UNA), http://data.gbif.org/datasets/resource/775 Herbarium of The New York Botanical Garden, http://data.gbif.org/datasets/resource/8967	316(686)

	Royal Botanical Gardens Herbarium, http://data.gbif.org/datasets/resource/512 herbario, http://data.gbif.org/datasets/resource/566 Database Schema for UC Davis [Herbarium Labels], http://data.gbif.org/datasets/resource/734 California State University, Chico, http://data.gbif.org/datasets/resource/737 NatureServe Network Species Occurrence Data, http://data.gbif.org/datasets/resource/607 SysTax, http://data.gbif.org/datasets/resource/1875 Missouri Botanical Garden, http://data.gbif.org/datasets/resource/621 Lund Botanical Museum (LD), http://data.gbif.org/datasets/resource/1028 USDA PLANTS Database, http://data.gbif.org/datasets/resource/1066 United States National Plant Germplasm System Collection, http://data.gbif.org/datasets/resource/1429 Plant Specimens of Kurashiki Museum of Natural History, http://data.gbif.org/datasets/resource/599 NMNH Botany Collections, http://data.gbif.org/datasets/resource/1874 MISS_DC_01MAR2006, http://data.gbif.org/datasets/resource/7895 Phanerogamic Botanical Collections (S), http://data.gbif.org/datasets/resource/8113 Herbarium (UNA), http://data.gbif.org/datasets/resource/775 Vascular Plant Specimen Database of Kanagawa Prefectural Museum of Natural History, http://data.gbif.org/datasets/resource/8011 Ibaraki Nature Museum, Vascular Plants collection (1), http://data.gbif.org/datasets/resource/8030 Gunma Museum of Natural History, Vascular Plant Specimen, http://data.gbif.org/datasets/resource/8018 Botany Vascular Plant Collection, http://data.gbif.org/datasets/resource/7915 CONN GBIF data, http://data.gbif.org/datasets/resource/7857	
Oenothera perennis	Canadian Museum of Nature Herbarium, http://data.gbif.org/datasets/resource/123	420(583)

Plant Specimens of Kurashiki Museum of Natural History, http://data.gbif.org/datasets/resource/599
herbario, http://data.gbif.org/datasets/resource/566
MEXU/Plantas Vasculares, http://data.gbif.org/datasets/resource/780
SysTax, http://data.gbif.org/datasets/resource/1875
Herbarium of Oskarshamn (OHN), http://data.gbif.org/datasets/resource/1024
Missouri Botanical Garden, http://data.gbif.org/datasets/resource/621
Lund Botanical Museum (LD), http://data.gbif.org/datasets/resource/1028
Herbario de la Universidad de Sevilla, SEV, http://data.gbif.org/datasets/resource/283
Herbarium WU, http://data.gbif.org/datasets/resource/1496
Herbario de la Universidad de Arizona, EUA, http://data.gbif.org/datasets/resource/2479
United States National Plant Germplasm System Collection, http://data.gbif.org/datasets/resource/1429
USDA PLANTS Database, http://data.gbif.org/datasets/resource/1066
Herbario de la Universidad de Sevilla, SEV-Historico, http://data.gbif.org/datasets/resource/284
Direcci√≥n General de Investigaci√≥n, Desarrollo Tecnol√≥gico e Innovaci√≥n de la Junta de Extremadura(DGIDTI): HSS, http://data.gbif.org/datasets/resource/291
Hortus Botanicus Sollerensis Herbarium (FBonaf√®), http://data.gbif.org/datasets/resource/300
Botany (UPS), http://data.gbif.org/datasets/resource/1045
Universidad de M√É¬°laga: MGC-Cormof, http://data.gbif.org/datasets/resource/8105
Herbarium Specimens of Museum of Nature and Human Activities, Hyogo Pref., Japan, http://data.gbif.org/datasets/resource/589
SANT herbarium vascular plants collection, http://data.gbif.org/datasets/resource/222
Phanerogamie, http://data.gbif.org/datasets/resource/1506
Botanical specimens database of Mr. Jiro Ito collection, Shizuoka Prefecture Museum of Natural History, http://data.gbif.org/datasets/resource/1811
USU-UTC Specimen Database, http://data.gbif.org/datasets/resource/1508
Universidad de Oviedo. Departamento de Biolog√É¬≠a de Organismos y Sistemas: FCO, http://data.gbif.org/datasets/resource/245
Herbario del Instituto de Ecolog√≠a, A.C., M√©xico (IE-BAJIO), http://data.gbif.org/datasets/resource/1595
MEXU/Colecci√≥n Hist√≥rica, http://data.gbif.org/datasets/resource/1984
Herbario de la Universidad de Salamanca: SALA, http://data.gbif.org/datasets/resource/239
Real Jardin Botanico (Madrid), Vascular Plant Herbarium (MA), http://data.gbif.org/datasets/resource/240
Herbarium of Kitakyushu Museum of Natural History and Human History, http://data.gbif.org/datasets/resource/606
NMNH Botany Collections, http://data.gbif.org/datasets/resource/1874
UA Herbarium, http://data.gbif.org/datasets/resource/7900
Herbarium Specimens of Museum of Nature and Human Activities, Hyogo Prefecture, Japan, http://data.gbif.org/datasets/resource/1958
Herbarium Berolinense, http://data.gbif.org/datasets/resource/1095
EURISCO, The European Genetic Resources Search Catalogue, http://data.gbif.org/datasets/resource/1905
Phanerogamic Botanical Collections (S), http://data.gbif.org/datasets/resource/8113
Vascular Plants Collection of Sagamihara City Museum, http://data.gbif.org/datasets/resource/1809
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Herbarium of The New York Botanical Garden, http://data.gbif.org/datasets/resource/8967
Fundaci√É¬≥n Biodiversidad, Real Jard√É¬≠n Bot√É¬°nico (CSIC): Anthos. Sistema de Informaci√É¬≥n de las plantas de Espa√É¬±a, http://data.gbif.org/datasets/resource/9090
Consortium of California Herbaria, http://data.gbif.org/datasets/resource/9153
Royal Botanic Gardens, Kew, http://data.gbif.org/datasets/resource/629
Gunma Museum of Natural History, Vascular Plant Specimen, http://data.gbif.org/datasets/resource/8018
Herbario XAL del Instituto de Ecolog√É¬≠a, A.C., M√É¬©xico (IE-XAL), http://data.gbif.org/datasets/resource/10980
Herbarium de Geo. B. Hinton, M√©xico, http://data.gbif.org/datasets/resource/1594
Herbario IEB del Instituto de Ecolog√É¬≠a, A.C., M√É¬©xico (IE-BAJ√É¬≠O), http://data.gbif.org/datasets/resource/11106

herbario, http://data.gbif.org/datasets/resource/566
Database Schema for UC Davis [Herbarium Labels], http://data.gbif.org/datasets/resource/734
California State University, Chico, http://data.gbif.org/datasets/resource/737
Herbarium (UNA), http://data.gbif.org/datasets/resource/775
MEXU/Plantas Vasculares, http://data.gbif.org/datasets/resource/780
SysTax, http://data.gbif.org/datasets/resource/1875
Utah Valley State College Herbarium, http://data.gbif.org/datasets/resource/1013
Missouri Botanical Garden, http://data.gbif.org/datasets/resource/621
Nationaal Herbarium Nederland, http://data.gbif.org/datasets/resource/1211
Bishop Museum Natural History Specimen Data, http://data.gbif.org/datasets/resource/54
USDA PLANTS Database, http://data.gbif.org/datasets/resource/1066
Botany (UPS), http://data.gbif.org/datasets/resource/1045
Herbarium Specimens of Museum of Nature and Human Activities, Hyogo Pref., Japan, http://data.gbif.org/datasets/resource/589
University and Jepson Herbaria DiGIR provider, http://data.gbif.org/datasets/resource/1413
Botanical specimens database of Mr. Jiro Ito collection, Shizuoka Prefecture Museum of Natural History, http://data.gbif.org/datasets/resource/1811
USU-UTC Specimen Database, http://data.gbif.org/datasets/resource/1508
Fairchild Tropical Botanic Garden Virtual Herbarium Darwin Core format, http://data.gbif.org/datasets/resource/202
Herbario del Instituto de Ecolog√≠a, A.C., M√©xico (IE-BAJIO), http://data.gbif.org/datasets/resource/1595
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Herbarium Specimens of Tokushima Prefectural Museum, Japan, http://data.gbif.org/datasets/resource/600
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Oenothera triloba	Herbarium (UNA), http://data.gbif.org/datasets/resource/775)	209(479)
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Veronica birleyi	New Zealand National Plant Herbarium (CHR), http://data.gbif.org/datasets/resource/474	16(18)
Veronica cheesemanii	New Zealand National Plant Herbarium (CHR), http://data.gbif.org/datasets/resource/474	34(41)
	NSW herbarium collection, http://data.gbif.org/datasets/resource/968 New Zealand National Plant Herbarium (CHR), http://data.gbif.org/datasets/resource/474 RBGE Living Collections, http://data.gbif.org/datasets/resource/9167 Royal Botanic Gardens, Kew, http://data.gbif.org/datasets/resource/629 New Zealand Biodiversity Recording Network, http://data.gbif.org/datasets/resource/7910	
Veronica ciliolata	Australian National Herbarium (CANB), http://data.gbif.org/datasets/resource/47	39(54)
	NSW herbarium collection, http://data.gbif.org/datasets/resource/968 New Zealand National Plant Herbarium (CHR), http://data.gbif.org/datasets/resource/474 SysTax, http://data.gbif.org/datasets/resource/1875 RBGE Living Collections, http://data.gbif.org/datasets/resource/9167	
Veronica densifolia	Australian National Herbarium (CANB), http://data.gbif.org/datasets/resource/47	58(85)
Veronica planopetiolata	New Zealand National Plant Herbarium (CHR), http://data.gbif.org/datasets/resource/474	4(5)

Veronica pulvinaris	NSW herbarium collection, http://data.gbif.org/datasets/resource/968 New Zealand National Vegetation Survey Databank, http://data.gbif.org/datasets/resource/473 New Zealand National Plant Herbarium (CHR), http://data.gbif.org/datasets/resource/474 RBGE Living Collections, http://data.gbif.org/datasets/resource/9167 EURISCO, The European Genetic Resources Search Catalogue, http://data.gbif.org/datasets/resource/1905 Royal Botanic Gardens, Kew, http://data.gbif.org/datasets/resource/629	59(92)
Veronica thomsonii	NSW herbarium collection, http://data.gbif.org/datasets/resource/968 New Zealand National Plant Herbarium (CHR), http://data.gbif.org/datasets/resource/474 RBGE Living Collections, http://data.gbif.org/datasets/resource/9167 Royal Botanic Gardens, Kew, http://data.gbif.org/datasets/resource/629	10(24)
Veronica spathulata	SysTax, http://data.gbif.org/datasets/resource/1875 New Zealand National Plant Herbarium (CHR), http://data.gbif.org/datasets/resource/474 RBGE Herbarium (E), http://data.gbif.org/datasets/resource/8402	7(30)