Phylogenetic relationships and evolutionary history of the southern hemisphere genus *Leptinella* Cass. (Compositae, Anthemideae)

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Chapter 1

General Introduction

Evolution of New Zealand plant groups

New Zealand has been isolated by a distance of c. 1500 km from its closet landmass Australia after the break-up of Gondwana 80 million years ago (Cooper and Miller 1993, McLoughlin 2001). After the break-up, New Zealand had undergone several dramatic geologic and climatic events that formed a very diverse topography with a high diversity of biomes (Winkworth et al 2005, Linder 2008). Large parts (or the entire archipelago) of New Zealand were inundated during the Oligocene (Cooper and Millener 1993, Winkworth et al. 2002, Trewick and Morgan-Richards 2005). The uplift of the Southern Alps is dated to c. 12 Ma, but the alpine habitat was established only during the last 5 Ma (Chamberlain and Poage 2000, Winkworth et al. 2005). In the Pleistocene, the glacial cycles and volcanism played an important role in the evolution of the environment of New Zealand (Winkworth et al. 2005).

In the past, the biogeography of the southern hemisphere plant groups has received much attention by biologists and the origin of its flora and fauna was extensively discussed. Two contradictory concepts exit about the origin of the southern hemisphere plant groups - vicariance or long distance dispersal (see reviews by Pole 1994, McGlone 2005, Trewick et al. 2007, Goldberg et al. 2008). Recent studies using molecular data suggest that long distance dispersal is more prevalent than vicariance, at least as far as the New Zealand plant and animal lineages are concerned (e.g. Pole 1994, Sanmartin and Ronquist 2004, Winkworth et al. 2005, Sanmartin et al. 2007, Goldberg et al. 2008). Several molecular phylogenies show that the divergence times of many groups are too recent to explain the observed geographic patterns by vicariance (e.g. von Hagen and Kadereit 2001, Swenson et al. 2001, Knapp et al. 2005, Wagstaff et al. 2006, Mitchell et. 2009). However, there is evidence that some New Zealand plant groups originated from before the Gondwana break-up (e.g. *Agathis*; Stöckler et al. 2002, Knapp et al. 2007).

Long distance dispersal events were suggested from New Zealand to Australia, New Guinea, South America, southern Africa, the sub-Antarctic islands, the northern hemisphere, and vise versa (e.g. Winkworth et al. 2005, Sanmartin and Ronquist 2004, Sanmartin et al. 2007, Goldberg et al. 2008, Bergh and Linder 2009). For instance, several proven dispersal events from Australia to New Zealand are thought to be connected to the predominant West Wind Drift and the westerly sea current between these landmasses. Likewise, several cases for long distance dispersal in the reverse direction have been proven as well (reviewed in Winkworth et al. 2002, Sanmartin and Ronquist 2004,

Sanmartin et al. 2007, Goldberg et al. 2008). The mechanisms involved in such transoceanic long distance dispersal events are discussed in the recent literature (e.g. Wagstaff et al. 2006, Ford et al. 2007, Goldberg et al. 2008, Bergh and Linder 2009). Animals, water, and wind are the suggested dispersal vectors between the southern hemisphere continents and islands. Additionally, dispersal via stepping stones, for example from South America to New Zealand via Antarctica or the sub-Antarctic islands, was proposed by some authors (e.g. *Abrotanella*, Wagstaff et al. 2006).

Many of the so far investigated plant groups of New Zealand evolved in the Miocene, Pliocene and Pleistocene after arriving by long distance dispersal, and conclusive evidence for rapid radiation could be presented (e.g. Wagstaff et al. 2006, Bergh and Linder 2009). These radiation processes were often associated with speciation and adaptation to newly emerged habitats after the uplift of the Southern Alps or during the glaciations cycles, respectively (e.g. Wagstaff and Garnock-Jones 1998, Lockhardt et al. 2001, Winkworth 2002b, Trewick and Morgan-Richards 2005).

Species delimitation in New Zealand plant lineages is often complicated, especially due to processes of recent and rapid speciation by adaptive radiation. As a consequence, the taxonomic description of the flora of New Zealand is yet incomplete. Druce (1993) mentioned c. 2000 described species and a further c. 500 informal, undescribed entities that might warrant taxonomic recognition. Additionally, hybridization, introgression, and polyploidyzation are common in many New Zealand groups (reviewed in Morgan-Richards et al. 2009).

A further problem when dealing with plants from New Zealand is that, although many groups show large morphological variation among and within species, they show unexpected low sequence variation (e.g. Breitwieser et al. 1999, Mitchell and Heenan 2000, Lockhart et al. 2001, Wagstaff and Wege 2002, Wagstaff and Breitwieser 2004, Meudt and Simpson 2006, Ford et al. 2007, Mitchell et al. 2009b). For example, Winkworth et al. (2002b) found very low sequence variation in the morphologically diverse *Myosotis* taxa from New Zealand as compared with the morphologically more uniform taxa from the northern hemisphere.

In the last years, the number of published molecular phylogentic analyses dealing with plant groups of New Zealand has increased. Such studies have been used to clarify the taxonomy of plant groups (e.g. Albach et al. 2005, Heenan et al. 2006, de Lange et al. 2009), for the dating of lineages (e.g. Wagstaff et al. 2006, Barker et al. 2007, Knapp et al. 2005, 2007, Perrie and Brownsey 2007, Mitchell et al. 2009), to investigate biogeography

(e.g. Wagstaff and Wege. 2002, Wagstaff et al. 2006, Meudt and Simpson 2006, Sanmartin et al. 2007), and to reconstruct character evolution (e.g. Mitchell et al. 2009a). Several authors have employed molecular data to disentangle reticulate evolution, hybridization, and polyploidzation (e.g. Breitwieser et al. 1999, Perrie and Brownsey 2005, Meudt and Bayly 2008, reviewed by Morgan-Richards et al. 2009). Additionally, molecular phylogenies were used in conservation biology, for example to clarify the taxonomic status of threatened taxa (e.g. de Lange et al. 2008).

Although there are several recent molecular studies that are dealing with New Zealand plant lineages, there is still a lack of knowledge about the phylogeny, taxonomy, origin, biogeography, and divergence time of many groups of the New Zealand flora. One of these so far not investigated groups is the species rich southern hemisphere genus *Leptinella,* which has its centre of distribution in New Zealand.

Dimorphic sex expression

Since Darwin (1877), there has been a continuing interest by biologists in the evolution of dimorphic sex expressions in plants such as dioecy (female and male plants), gynodioecy (female and hermaphrodite plants), or androdioecy (male and hermaphrodite plants). Many authors argued that such systems evolved as a mechanism to promote outcrossing (reviewed in Thomson and Brunet 1990, Sakai and Weller 1999). Shifts in resource allocation is another explanation for the origin of dimorphic sex expression (Webb 1999).

There are several studies dealing with the different pathways that lead to dimorphic sex expression (reviewed in Webb 1999), the genetic of such systems (reviewed in Grant 1999, Ainsworth 2000, Ming et al. 2007), the evolutionary theories (reviewed in Charlesworth 1999), the secondary sexual dimorphism in plants (reviewed in Lloyd and Webb 1977, Geber 1999), or the correlations of gender dimorphism (reviewed in Renner and Ricklefts 1995, Sakai and Weller 1999). For example, it was suggested that dioecy and related systems are correlated with ecological traits such as fleshy fruits, insect pollination by small generalists, wind pollination, woodiness, or climbing growth habit (Sakai and Weller 1999).

Yampolsky and Yampolsky (1922) provided the first overview of the distribution of different sex expression systems in flowering plants. A new review was present by Renner and Ricklefs (1995), taking into account more recent finding concerning the phylogeny of higher plants. Around 7 % of all plant species have a dimorphic sex expression (14,620 of

c. 240,000 species), and 7.1 - 7.6 % of all genera contain dioecious taxa (959 of 12,650 or 13,479 genera; the two differing statements on genera number depending on different taxonomic concepts). Dioecy is the most common mode of dimorphic sex expression. Therefore, most studies that deal with dimorphic sex expression are concerned with dioecy. Interestingly, nearly half of all families contain dimorphic species suggesting its independent origin in several lineages (Renner and Ricklefs 1995).

Many authors pointed to the high levels of dimorphic plants on islands, especially the Hawaiian Islands (20.7 % of genera, 14.7 % of species; Sakai et al. 1995a,b) and New Zealand (23 % of genera, 12-13 % of species; Godley 1979, Webb and Kelly 1993, Webb et al. 1999). On the other hand, there are several islands with a lower number of dioecious plants, e.g. the Galapagos Islands, Iceland, and the Azores (2-3 % of species; Baker and Cox 1984). The high frequency of dioecy on some islands has fascinated numerous botanist, and many hypotheses have been put forward to explain this correlation (see Baker 1967, Baker and Cox 1984, Sakai et al. 1995a,b, Webb 1999).

Studies of New Zealand species with sexual dimorphism based on morphology was done in *Leptinella* (Lloyd 1972a,b,c, 1975a,b, 1980), *Melicytus* (Beuzenberg 1961), *Hebe* (*Veronica* s.l.; Delph 1990a,b, Delph and Lloyd 1991), and several genera of Apiaceae (Webb 1979, 1992). However, until now only one molecular phylogenetic study carried out in these groups that takes into consideration the evolution of sex expression (*Melicytus*, Mitchell et al. 2009, see below). Nevertheless, there are several studies dealing with groups outside New Zealand that contain dioecious species (e.g. *Lycium,* Miller and Venable 2000; *Rumex*, Navajas-Perez et al. 2005; Cucurbitales, Zhang et al. 2006; *Bouteloua,* Kinney et al. 2007; *Bryonia*, Volz and Renner 2008; *Carex*, Guibert et al. 2009; Inuleae, Torices et al. 2009). These studies provided information about the origin, the evolutionary pathways that led to dioecy, or correlations of dioecy with other characters. The results differ among the study groups, and no general patterns have been found.

One remarkable point is the assumed connection of dioecy and polyploidy. Until now, there are only few molecular studies that deal with this subject (e.g. *Bryonia*, Volz and Renner 2008; *Lycium*, Miller and Venable 2000, Yeung et al. 2005; *Melicytus*, Mitchel et al. 2009, *Mercurialis*, Pannell et al. 2004, Obbard et al. 2006).

Miller and Venable (2000) suggested that polyploidy is a trigger of unrecognized importance for the evolution of gender dimorphism, which disrupted self-incompatibility and lead to inbreeding depression. Subsequently, dioecy may evolve to recover outcrossing. The authors could show that gender dimorphism in North American *Lycium*

(Solanaceae) evolved in polyploid, self-compatible taxa while the closest relatives are hermaphrodite, self-incompatible diploids. Additionally, they presented evidence for this pathway for further 12 genera. However, other authors suggested that polyploidization may break down dimorphic breeding systems (Westergaard 1958, Smith 1958, 1969, Richards 1997). Mitchell et al. (2009) found that in *Melicytus* the change in sex expression is from dioecism and mostly tetraploid (functionally diploid) to hermaphroditism and predominately octoploid, which suggests a break down of dimorphic sex expression after polyploidization. However, there are three exceptions: two hermaphrodite tetraploids and one dioecious octoploid taxon. Also, Volz and Renner (2008) and Pannell et al. (2008) found no strong correlation between sexual system and ploidy level.

Molecular phylogeny and genetic markers

Systematics is a synthetic science, drawing up data from fields as diverse as morphology, anatomy, cytology, genetics, cytogenetics, chemistry, and molecular biology (Stuessey 2009). Of all the different data sources currently used in plant systematics, molecular biological data are most intriguing, exciting, and conspicuous. Phylogenetic analyses using molecular data are extremely useful and widely used in plant systematics on different taxonomic levels from studies on relationships among families to studies within species. When working on molecular phylogenies, the choice of markers is an important issue. The marker should be variable enough to get a well resolved phylogeny. At the same time, the marker should not to be too variable, which would cause problems with the homology of sequences or fragments obtained by fingerprint analyses. Which molecular markers should be used in a phylogenetic study depends on the taxonomical level of the study group.

Coding DNA markers (i.e. genes) are usually used in the case of molecular phylogentic studies on relationships among families, tribes, or subtribes. Frequently used genes are *ndh*F and *rbc*L. Non-coding DNA markers are mostly used for studies among and within genera, because at this taxonomic level these markers provide more informative characters (synaphomorphies). The internal transcribed spacer (ITS) is such a non-coding marker from the nuclear genome, and it is widely applied in phylogenetic studies from fungi to higher plants. Another common nuclear marker is the external transcribed spacer (ETS). ITS and ETS are high-copy DNA markers. In the last years, a set of single or low copy markers were used (e.g. Joly et al. 2006, Brysting et al. 2007). Likewise, the chloroplast genome provides a large quantity of non-coding markers (e.g. *psb*A-*trn*H, *trn*L-

*trn*F, *trn*C-*pet*N; see Taberlet et al. 1991, Hamilton 1998, Shaw et al. 2005, 2007). DNA fingerprint methods have been for population genetic studies, in phylogentic studies of closely related species, and within genera. Amplified fragment length polymorphism analysis (AFLP) or microsatellites are commonly used fingerprint methods in such molecular studies (e.g. Pelser et al. 2003, Guo et al. 2005, Edwards et al. 2008, Koopman et al. 2008, Meudt and Bayly 2008, Pleines and Blattner 2008, Schenk et al. 2008).

Several markers from independently evolving genomes should be used to gernerate species trees instead of gene trees. In phylogenetic analyses of plant groups, most researchers use markers from the nuclear (biparental inherited) and chloroplast (mostly maternal inherited) genome. Additionally, the use of several independent markers will possibly allow visualization of processes such as hybridization, introgression, reticulate evolution, and polyploidization (e.g. Jolly et al. 2006, Shepherd et al. 2008a,b, Peterson et al. 2009).

Introduction to the study group: *Leptinella* Cass.

Description: The southern hemisphere genus *Leptinella* comprises 42 taxa. It consists of small perennial and procumbent herbs (Fig. 1-2). The capitula are pedunculate, the corollas are inflated, and the outer disc florets are female and the inner ones functionally male (Fig. 1-3a). The female florets have bifid styles. The styles of the functionally male florets are undivided, with an expanded saucer-shaped apex, which pushes the pollen beyond the anthers and presents it above the corolla. The male florets have a longer corolla and a shorter sterile ovary than the female florets (Lloyd 1975b; Fig. 1-3b). There are no hermaphrodite florets in the genus. The leaf shape is an important diagnostic feature for the identification of taxa (Fig. 1-4). It ranges from linear to tri-pinnate. Palmate leaves occur in *L. goyenii*.

Distribution: *Leptinella* is a southern hemisphere genus occurring in Australia, New Guinea, New Zealand, South America, and on the Chatham Islands or sub-Antarctic islands. New Zealand is clearly the centre of diversity with 29 taxa being endemic. The distribution area and the numbers of taxa in each area are shown in Fig. 1-1. Tab. 1-1 provides information on the distribution areas for all taxa.

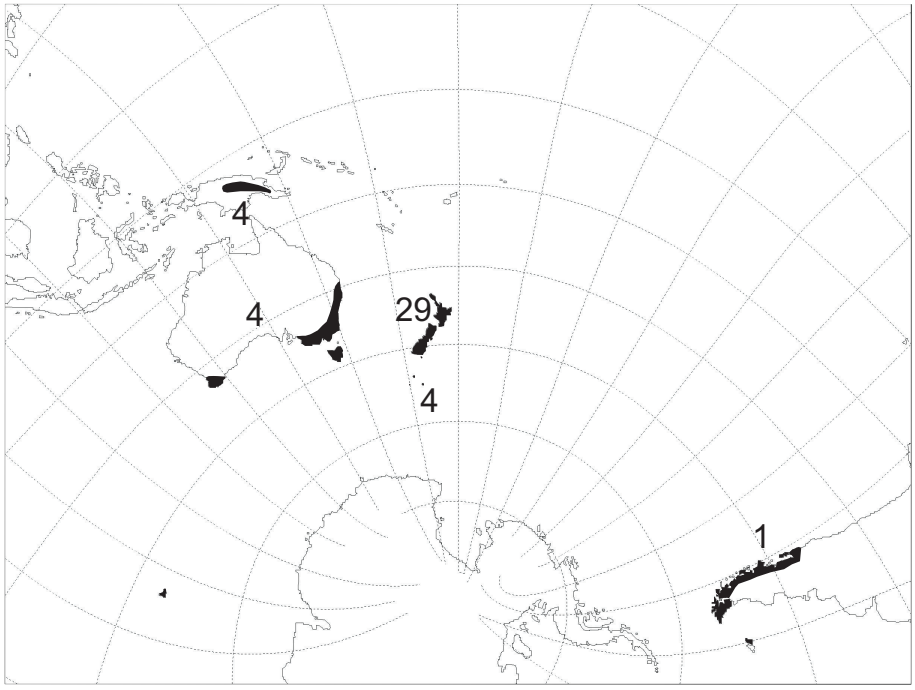

Fig. 1-1: Distribution of *Leptinella* based on Lloyd (1972c), van Royen and Lloyd (1975), and Thompson (2007). The numbers of taxa in each area are indicated.

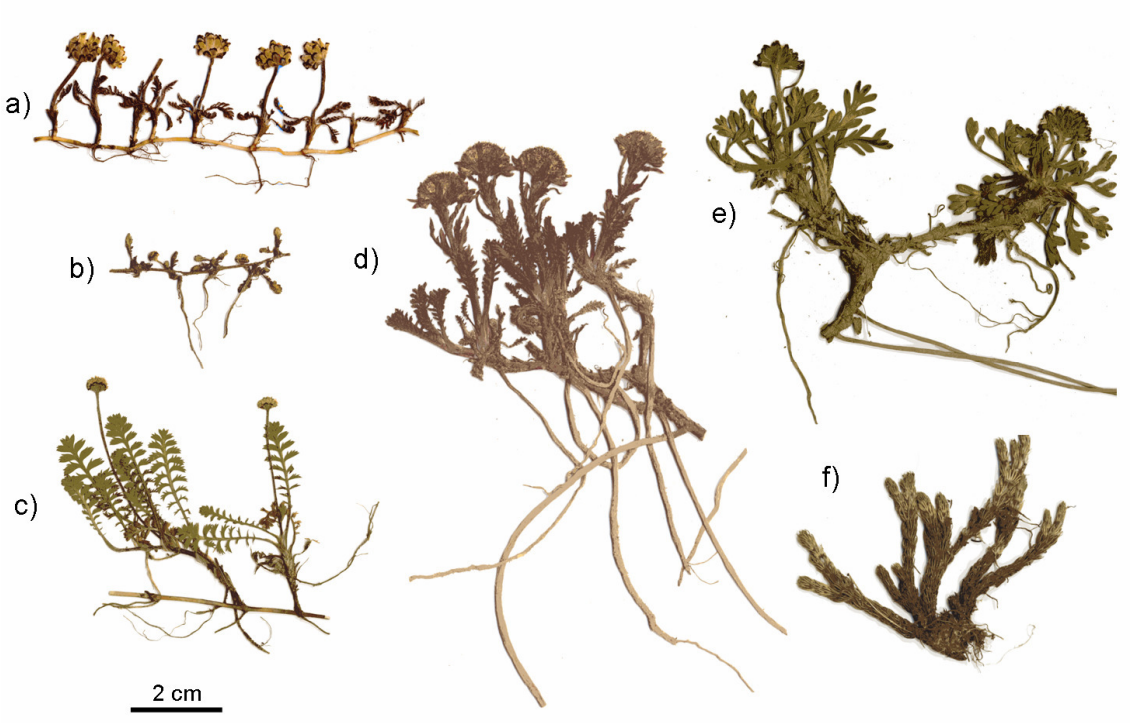

Fig. 1-2: Variation of plants in *Leptinella.*
subgenus *Leptinella*
a) *L. pusilla* - Rupprecht and Himmelreich NZ 29 (CHR)
b) *L. squalida* subsp. *squalida* - Rupprecht and Himmelreich NZ 12 (CHR)
c) *L. squalida* subsp. *mediana* - Rupprecht and Himmelreich NZ 20 (CHR)
subgenus *Radiata*
d) *L. dendyi* - Rupprecht and Himmelreich NZ 27 (CHR)
e) *L. pyrethrifolia* var. *pyrethrifolia* - Himmelreich and Rupprecht NZ B6 (CHR)
f) *L. goyenii* - Barkla (CHR)

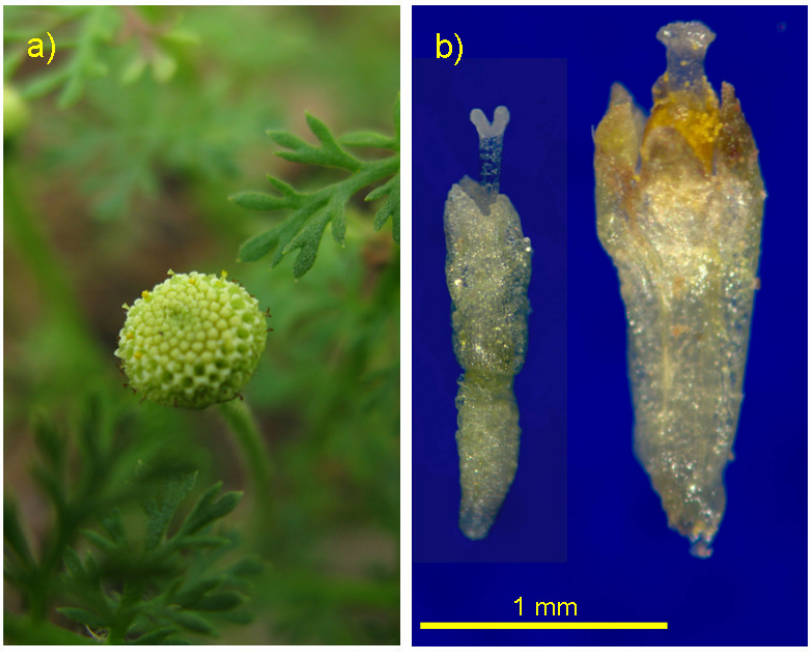

Fig. 1-3: Capitula and florets of *Leptinella*
a) Capitulum of *L. drummondii* with outer female and inner male florets (photo by S. Tausch, Germany)
b) Female floret (left) and male floret (right) of *L. dioica* subsp. *dioica.*

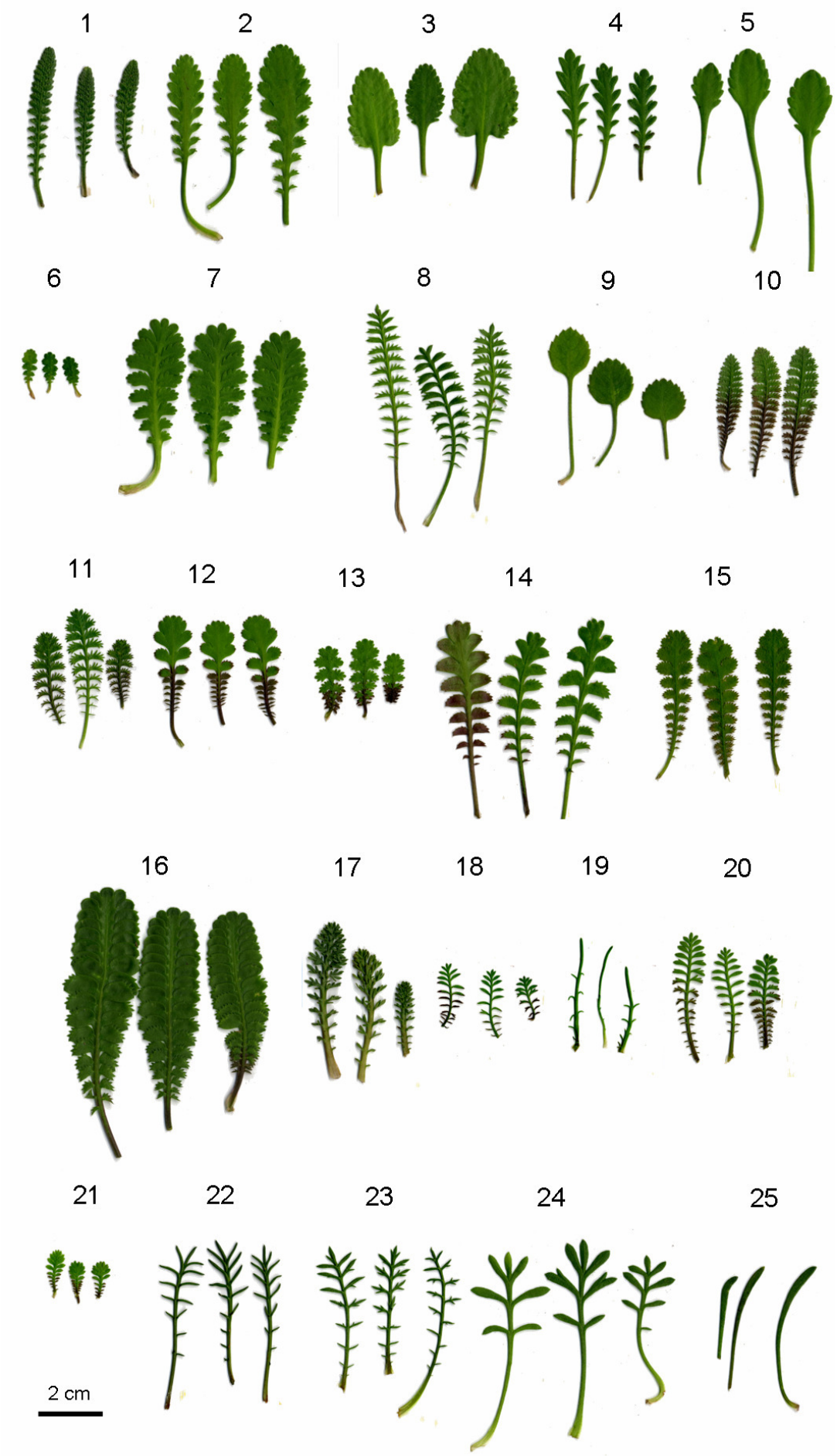
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Fig. 1-4 (previous page): Leaves from different *Leptinella* taxa from cultivated plants.

Subgenus *Leptinella*
1) *L. calcarea* - Rupprecht & Himmelreich NZ 16 (CHR)
2) *L. dioica* subsp. *dioica* (Canterbury, Banks Peninsula) - Rupprecht & Himmelreich NZ 02 (CHR)
3) (Marlborough, Molesworth Station) - Rupprecht & Himmelreich NZ 28 (CHR)
4) (Southland, near Invercargill) - Rupprecht & Himmelreich NZ 39 (CHR)
5) *L. dioica* subsp. *manoica* - Rupprecht & Himmelreich NZ 05 (CHR)
6) *L. dispersa* subsp. *rupestris* - Ogle, Rupprecht & Himmelreich NZ 07 (CHR)
7) *L. potentillina* - Baird (CHR)
8) *L. pusilla* - Rupprecht & Himmelreich NZ 30 (CHR)
9) *L. rotundata* - Rupprecht & Himmelreich NZ 54 (CHR)
10) *L. serrulata* - Rupprecht & Himmelreich NZ 32 (CHR)
11) *L. squalida* subsp. *mediana* - Rupprecht & Himmelreich NZ 20 (CHR)
12) *L. squalida* subsp. *squalida* - Rupprecht & Himmelreich NZ 08 (CHR)
13) *L. tenella* - Rupprecht & Himmelreich NZ 09 (CHR)
14) *L. traillii* subsp. *pulchella* - Rupprecht & Himmelreich NZ 44B (CHR)
15) *L. traillii* subsp. *traillii* - Rupprecht & Himmelreich NZ 40 (CHR)
16) *L.* „Seal“ - Korver (CHR)
Subgenus *Radiata*
17) *L. dendyi* - Rupprecht & Himmelreich NZ 27 (CHR)
18) *L. filiformis* - Rupprecht & Himmelreich NZ 52 (CHR)
19) *L. maniototo* - Korver (CHR)
20) *L. minor* - Rupprecht & Himmelreich NZ 04 (CHR)
21) *L. nana* - Rupprecht & Himmelreich NZ 03 (CHR)
22) *L. pectinata* subsp. *pectinata* - Rupprecht & Himmelreich NZ 26 (CHR)
23) *L. pectinata* subsp. *villosa* - Rupprecht & Himmelreich NZ 31 (CHR)
24) *L. pyrethrifolia* var. *pyrethrifolia* - Rupprecht & Himmelreich NZ 24 (CHR)
25) *L. pyrethrifolia* var. *linearifolia* - Korver (CHR)

Taxonomy:

Intergeneric relationships: The genus *Leptinella* belongs to the tribe Anthemideae of the sunflower family (Compositae). The relationship of *Leptinella* within the tribe has been discussed by several authors (Lloyd 1972c, Heywood and Humphries 1977, Lloyd and Webb 1987, Bruhl and Quinn 1990, 1991). Bremer and Humphries (1993) included the genus in their subtribe Matricarineae that consist of 25 genera from the southern as well as the northern hemisphere. The subtribe was considered to be characterized by the apomorphies of the arrangement of myxogenic cells on the achenes and the possession of an adaxially long pappus. However, Bremer and Humphries (1993) also report an equally parsimonious reconstruction based on morphological data that found no synapomorphies for the subtribe. Recent molecular phylogenetic studies (Watson et al. 2000, Oberprieler 2004a,b, 2005) demonstrated the non-monophyly of most of these subtribes (including Matricarinae). These molecular studies within the tribe Anthemideae have concentrated either on tribal overview (Watson et al. 2000), on geographical subgroups (i.e. Mediterranean area: Francisco-Ortega et al. 1997, Oberprieler and Vogt 2000, Oberprieler 2004a,b, 2005), or on several Mediterranean or Eurasian taxonomic subgroups (e.g. Oberprieler 2001, Vallès et al. 2003, Guo et al. 2004, Vogt and Oberprieler 2006, Tkach et

al. 2007, Lo Presti and Oberprieler 2009). While a complete sampling on the generic level was achieved for the Mediterranean and Eurasian part (Oberprieler 2004a,b, 2005), the southern hemisphere genera are still far from being completely sampled.

The only study which includes several genera from the southern hemisphere was done by Watson et al. (2000); they included representatives of 16 of the 29 genera in a phylogenetic study based only on cpDNA *ndh*F sequence variation. Their reconstructions showed that there is a distinct biogeographical pattern in the evolutionary history of the tribe, with a basal grade of southern hemisphere genera followed by a grade of genera from Asia and southern Africa and a monophyletic crown group of Mediterranean and Eurasia Anthemideae representatives. Unfortunately, Watson et al. (2000) did not include *Leptinella* in their study; therefore, the phylogenetic position of *Leptinella* is still unclear. Close relationships of *Leptinella* with the southern hemisphere genus *Cotula* and the South American genus *Soliva* were suggested by several authors, but the relationships among these genera remain unclear (e.g. Lloyd and Webb 1987, Bruhl and Quinn 1990, 1991, Bremer and Humphries 1993, Oberprieler et al. 2006).

Leptinella was described as a genus by Cassini (1822), but was later reduced to infrageneric rank within *Cotula* by Hooker (1864). Bentham (1867) recognized three sections within the genus *Cotula* (sect. *Cotula*, sect. *Strongylosperma*, sect. *Leptinella*), and this has been followed with minor changes by most of the subsequent authors. However, Lloyd and Webb (1987) reinstated *Leptinella* at generic rank, primarily because of the inflated corollas of the female disc florets and the basic chromosome number of $x = 13$ which is unique within the tribe Anthemideae.

Infrageneric relationships: The first study of *Leptinella* in New Zealand was done by Edgar (1958). She divided the genus into two informal groups based on stem anatomy. In the first group, stem sections showed a ring of 8 vascular bundles (Fig. 4A in Edgar 1958). The second group had 4 vascular bundles (Fig. 4B in Edgar 1958). Later Lloyd (1972c) studied the New Zealand, sub-Antarctican and South American members of *Leptinella* (as *Cotula* subgenus *Leptinella)*. He divided *Leptinella* into three subgenera: *Oligoleima* (Australia, New Guinea; seeds compressed and with broad margin), *Leptinella* (New Zealand, South America, sub-Antarctic islands; seeds not compressed, branches single, rhizome internodes long; Fig. 1-2a-c), and *Radiata* (New Zealand, sub-Antarctic islands; seeds not compressed, branches usually clustered, rhizome internodes often short; Fig. 1-2d-f). The subgenera *Leptinella* and *Radiata* are more or less identical with Edgar's (1958) informal groups based on stem anatomy.

Taxa within *Leptinella:* Allan (1961) accepted 21 species of *Leptinella* (as *Cotula*) in his Flora of New Zealand. In his revision, Lloyd (1972c) described several new species from New Zealand. A revision of *Leptinella* (as *Cotula*) from New Guinea (van Royen and Lloyd 1975) includes the description of three new species. Lloyd and Webb (1987; see Tab. 1-1) accepted 33 species and additional seven subspecies and one variety of *Leptinella* (41 taxa). The delimitation of these species and subspecies has been discussed by different recent botanists (Druce 1987, 1992, 1993, Wilson 1994, New Zealand Plant Conservation Network 2009, de Lange et al. 2009). For example, the New Zealand Plant Conservation Network (2009) and de Lange et al. (2009) do not regard *Leptinella dioica* subsp. *manoica* as distinct from *L. dioica* subsp. *dioica*. Additionally, some taxa are morphologically and cytologically variable (e.g. *L. squalida* subsp. *mediana*, Lloyd 1972c). Druce (1993; see Tab. 1-1), who made extensive field observations and collected numerous herbarium specimens in New Zealand, listed six informal, undescribed entities that might or might not warrant taxonomic recognition. One of them, *L. conjuncta* (informal tagname *L.* "Clutha"), has been recently described by Heenan (2009).

Hybridization occurs frequently among species of subgenus *Leptinella* and less frequently in subgenus *Radiata* (Lloyd 1972c). Lloyd (1975a) performed 163 crosses between different species and cultivated the resulting progeny. Astonishingly, there was no difficulty in obtaining viable seeds from the majority of these crosses, even from crosses between the subgenera *Leptinella* and *Radiata*.

Chromosome numbers: The lowest chromosome number found in *Leptinella* is $2n = 52$, which would indicate that the basic number for the genus is $x = 26$. However, this is a relatively high number and suggests that this is a secondary basic number, following a polyploid event. The basic number of the genus is therefore $x = 13$ (Hair 1962, Lloyd and Webb 1987). The proposed sister genus *Cotula* has $x = 8, 9, 10$. Several authors speculated on how the basic number of *Leptinella* may have evolved. The number could result from an amphidiploid combination of species of *Cotula* sect. *Strongylosperma* ($x = 9$) and *Cotula* sect. *Cotula* ($x = 5$), with a subsequent reduction in basic number from 14 to 13 (Hair 1962). On the other hand, Turner (1970) suggested, that *Leptinella* could have originated as an amphiploid from a taxon with base number $x = 8$ (*Cenia*, now *Cotula*) and $x = 5$ (*Cotula*). However, *Cotula* species with $n = 5$ are not known, the number based on the presence of two nuclear organizers in the genome of *C. coronopifolia* with $n = 10$ (Turner 1970). To further gain insight the evolution of the basic chromosome number more

chromosome counts are required as well as a complete molecular phylogeny which the chromosome number can be mapped.

Several chromosome counts are reported in the literature for the subgenera *Leptinella* and *Radiata* (Hair 1962, Lloyd 1972c, Moore 1981, Beutzenberg and Hair 1984, Dawson 1995; see Tab. 1-1), with ploidy levels ranging from tetraploid chromosome numbers to chromosome sets of $2n = 24x$. Unfortunately, no chromosome counts are available for the subgenus *Oligoleima* from Australia and New Guinea. The following chromosome numbers are reported for *Leptinella*: $2n = 52$ ($4x$), 104 ($8x$), c. 156 ($12x$), 208 ($16x$), 260 ($20x$), and c. 312 ($24x$). Different chromosome numbers have been found in *L. pectinata* subsp. *villosa* ($4x$, $8x$; Lloyd 1972c), *L. pyrethrifolia* var. *pyrethrifolia* ($12x$, $16x$; Lloyd 1972c, Beuzenberg and Hair 1984), and *L. squalida* subsp. *mediana* ($12x$, $16x$, $20x$; Lloyd 1972c). *Leptinella scariosa* has $2n = 262$ (reported as $n = 131$) and *L. featherstonii* has $2n = 54$ chromosomes (Moore 1981, Dawson 1995). These numbers differ from reported counts for other *Leptinella* species ($2n = 260$ and $2n = 52$).

Sex expression: The breeding system of *Leptinella* was studied intensively in the field and in the glasshouse by the New Zealand botanist David Lloyd. He published his results in a series of papers (Lloyd 1972a,b,c, 1975a,b, 1980). He found that a number of modes of sex expressions are realised in the genus: monoecy, paradioecy, dioecy and a number of different intermediate conditions (Lloyd 1972a). These conditions are unusual in the Anthemideae, the majority of the genera being gynomonoecious or hermaphrodite. The proposed sister genera, *Cotula* and *Soliva*, are hermaphrodite, gynomonoecious, monoecious, or monoecious, respectively (Lloyd 1972a, Bremer and Humphries 1993).Variation in the sex expression is also observed within species (e.g. *L. dioica, L. dispersa, L. pyrethrifolia*; Lloyd 1972a,c, 1975b). A short description of the different sex expressions in *Leptinella* is provided in Tab. 1-2. The type of sex expression is listed for all taxa in Tab. 1-1.

In monoecious species of *Leptinella*, female and male florets occur in the same capitulum. The average percentage of female florets in the capitula was found to range from 20 % in *L. intermedia* to 82 % in *L. nana* (Lloyd 1972b).

The eight species with dioecious populations of subgenus *Leptinella* are all closely related and the sex expression, secondary sex differences, and sex ratio are uniform in these species (Lloyd 1975a). Six of these species have only dioecious populations *(L. calcarea, L. pusilla, L. scariosa, L. serrulata, L. squalida, L. traillii)* while *L. dispersa*

and *L. dioica* have dioecious and monoecious or intermediate populations. Some individuals of both genders bear a small proportion of florets of the opposite sex (inconstant female and male plants), whereas most are constant with florets of one sex only (Lloyd 1975a). From 317 capitula of male plants *(L. dioica, L. pusilla, L. squalida)* grown in the glasshouse, ten capitula were bisexual with 1.6 to 12.5 % female florets. From 306 capitula of female plants, only three were bisexual with 2.3 to 31.6 % male florets (Lloyd 1975a). Inconstant plants are also found in *L. calcarea*, *L. dispersa* and *L. serrulata*. Occasionally, there are some markedly inconstant male or female plants with a higher percentage of florets of the opposite gender. Nevertheless, the inconstancy of both sexes was found to be very low. The florets of the opposite sex of inconstant male and female individuals were found to be as fertile as florets in constant individuals (Lloyd 1975a).

Another dimorphic sex expression found in *Leptinella* is paradioecy. It is found in *L. dendyi, L. goyenii* and partly in *L. pyrethrifolia* (Lloyd 1972a, 1980a). All three species belong to subgenus *Radiata*. Lloyd (1980a) examined 88 capitula from 38 plants of *L. dendyi* and he found male, bisexual and female capitula, but all plants had a clear majority of either female or male florets.

In his fourth paper on the sex expresions in *Leptinella*, Lloyd (1975b) described the diverse breeding systems in *L. dioica, L. dispersa* and *L. rotundata*. While *L. dioica* subsp. *dioica* is dioecious, *L. dioica* subsp. *manoica* is monoecious or complex-monoecious. *L. rotundata*, which is closely related to *L. dioica*, is complex-monoecious. *L. dispersa*, which occurs throughout New Zealand and on the Campbell Islands, has the greatest diversity in sex expression: There are dioecious, 'pseudo-monomorphic dioecious', unisexual male, unisexual female and monoecious populations. The four uncommon classes of sex expression ('pseudo-monomorphic dioecy', unisexual female, unisexual male, complex monoecy) combine in various ways the features of dioecy and monoecy (Lloyd 1975b).

Lloyd (1972a,b, 1975a,b, 1980a) discussed the evolutionary pathways which may have led to the different sex expressions in *Leptinella*. He wrote that there were several independent transitions between different sex expressions (at least 12 within *Cotula* and *Leptinella*). Fig. 1-5 shows his interpretation of these pathways that could lead to the different sex expression in *Leptinella*.

The ancestral breeding system in the genus is monoecy. Dioecy may have evolved from monoecy via paradioecy (paradioecy pathway; Lloyd 1975a, 1980a, Webb 1999). Webb (1999) pointed out that *Leptinella* is the best studied example for this pathway. The

paradioecy pathway starts with populations of monoecious plants in which individual florets are already pollen or seed sterile. Divergence in the ratio of female and male florets may then lead to sex specialization of the plants. Inconstancy in both genders is characteristic for this pathway (Webb 1999).

Lloyd (1975b) highlighted, that the diversity of sex expression in *L. dispersa, L. dioica* and *L. rotundata* as described above, provides a rare opportunity to trace the phylogenetic directions and pathways of these sex expresions. The available evidences indicate that the changes may have occurred independently in the three species on different ploidy levels. Geographical, morphological and genetic data suggested that the direction of changes have been from dioecy to monoecy, and not vice-versa. Monoecy may evolve from dioecy via unisexual male populations with few inconstant male plants by changing of gender ratio. In unisexual female populations, male plants could be established from crosses between female and inconstant female individuals. From the resulting 'pseudo-monomorphic dioecious' populations new dioecious populations could be established by increasing of male plants in a population.

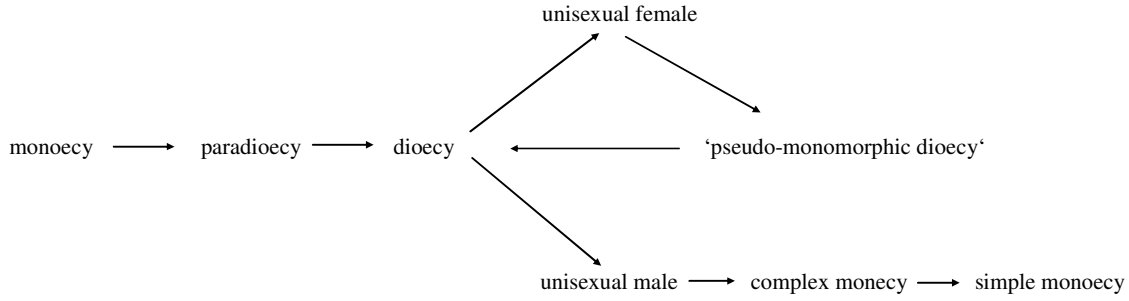

Fig. 1-5: Postulate steps of the evolution of sex expression systems in *Leptinella* (modified from Lloyd 1975b).

In addition to the complex sex expression system, the dioecious species of subgenus *Leptinella* form a remarkable polyploidy series. Two species have the lowest reported chromosome number $2n = 4x$, while the other represent five higher ploidy levels (up to $2n = 24x$). The sex expression, the secondary sexual characters, and the genetic basis of sex determination are similar in all species (Lloyd 1975a). These suggest that dioecy evolved from monoecy at the tetraploid level and was retained during the evolution of the higher ploidy levels. The proposed cycle from dioecy via unisexual female and 'pseudo-monomorphic dioecy' to dioecy could be one way in which the formation of both males

and females at a new ploidy level may be accomplished without breaking down of dioecy after polyploidization (Lloyd 1975a,b).

Little is known about the genetic background of dioecy in *Leptinella*, but artificial crosses gave first evidences (Lloyd 1975a). When monoecious plants of different species were crossed the progeny plants had bisexual heads. Crosses between female and male plants of dioecious species led to female and male offspring. Only male plants occurred from crosses between female florets of an inconstant male plant as ovule parent and male florets of a male plant as pollen donator. Crosses between inconstant female plants as pollen donator and female plants as ovule parents led to female and male plants. These results indicated that female plants are heterogametous and male plants are homogametous.

It is also interesting, that plants of complex-monoecious populations of *L. dioica* subsp. *manoica* and *L. rotundata* behave genetically like males of dioecious populations and not like plants of species with only monoecious populations (Lloyd 1975a). Crosses between individuals of these populations led only to male or inconstant male offspring. This result underlines the suggested origin of complex-monoecious populations from unisexual male population.

Lloyd (1972b) also studied self- vs. cross-pollination in three monoecious species (*L. atrata, L. minor, L. pectinata*). He could show that the seed set and subsequent germination percentages are slightly lower after self-pollination. However, all three species are able to self-pollinate; there are no effective barriers against selfing. In some species, self-pollination within one capitulum is preserved by absence of overlap in the anthesis of the female and male florets (Lloyd 1972a, 1980a).

Tab. 1-1: Taxa of *Leptinella* and information to sex expression, chromosome number and distribution (Lloyd 1972b,c, 1975b, van Royen and Lloyd 1975, Moore 1981, Beuzenberg and Hair 1984, Lloyd and Webb 1987, Druce 1993, Dawson 1995, Thomson 2007, Heenan 2009, New Zealand Plant Conservation Network 2009).

Taxon	Sex expression	Ploidy level	Distribution
Subgenus *Leptinella*			
L. calcarea (D. G. Lloyd) D. G. Lloyd & C. J. Webb	D	8	NZ
L. dioica Hook. f. subsp. *dioica*	D	20	NZ
L. dioica subsp. *monoica* (D. G. Lloyd) D. G. Lloyd & C. J. Webb	M	20	NZ
L. dispersa (D. G. Lloyd) D. G. Lloyd & C. J. Webb subsp. *dispersa*	M/D	4	NZ/SUB
L. dispersa subsp. *rupestris* (D. G. Lloyd) D. G. Lloyd & C. J. Webb	D	n/a	NZ
L. intermedia (D. G. Lloyd) D. G. Lloyd & C. J. Webb	M	12	NZ
L. potentillina F. Muell.	M	4	CHA/SUB
L. pusilla Hook. f.	D	8	NZ
L. rotundata (Cheeseman) D. G. Lloyd & C. J. Webb	M	24	NZ
L. scariosa Cass.	D	20	SAM
L. serrulata (D. G. Lloyd) D. G. Lloyd & C. J. Webb	D	4	NZ
L. squalida subsp. *mediana* (D. G. Lloyd) D. G. Lloyd & C. J. Webb	D	20	NZ
L. squalida Hook. f. subsp. *squalida*	D	12/16/20	CHA/NZ
L. tenella (A. Cunn.) D. G. Lloyd & C. J. Webb	M	4	NZ
L. traillii subsp. *pulchella* (Kirk) D. G. Lloyd & C. J. Webb	D	24	NZ
L. traillii (Kirk) D. G. Lloyd & C. J. Webb subsp. *traillii*	D	n/a	NZ
Subgenus *Radiata* (D. G. Lloyd) D. G. Lloyd & C. J. Webb			
L. albida (D. G. Lloyd) D. G. Lloyd & C. J. Webb	M	4	NZ
L. atrata (Hook. f.) D. G. Lloyd & C. J. Webb subsp. *atrata*	M	4	NZ
L. atrata subsp. *luteola* (D. G. Lloyd) D. G. Lloyd & C. J. Webb	M	4	NZ
L. conjuncta Heenan	M	8	NZ
L. dendyi (Cockayne) D. G. Lloyd & C. J. Webb	PD	4	NZ
L. featherstonii F. Muell.	M	4	CHA
L. filiformis (Hook. f.) D. G. Lloyd & C. J. Webb	M	4	NZ
L. goyenii (Petrie) D. G. Lloyd & C. J. Webb	PD	4	NZ
L. lanata Hook. f.	M	4	SUB
L. maniototo (Petrie) D. G. Lloyd & C. J. Webb	M	4	NZ
L. minor Hook. f.	M	4	NZ
L. nana (D. G. Lloyd) D. G. Lloyd & C. J. Webb	M	4	NZ
L. pectinata (Hook. f.) D. G. Lloyd & C. J. Webb subsp. *pectinata*	M	8	NZ
L. pectinata subsp. *villosa* (D. G. Lloyd) D. G. Lloyd & C. J. Webb	M	4/8	NZ
L. pectinata subsp. *willcoxii* (Cheeseman) D. G. Lloyd & C. J. Webb	M	8	NZ
L. plumosa Hook. f.	M	4	SUB
L. pyrethrifolia var. *linearifolia* (Cheeseman) D. G. Lloyd & C. J. Webb	M/PD	12	NZ
L. pyrethrifolia (Hook. f.) D. G. Lloyd & C. J. Webb var. *pyrethrifolia*	M/PD	12/16	NZ
Subgenus *Oligoleima* Hook. f.			
L. altilitoralis (P. Royen & D. G. Lloyd) D. G. Lloyd & C. J. Webb	M	n/a	NG
L. drummondii (Benth.) D. G. Lloyd & C. J. Webb	M	n/a	AUS
L. filicula (Hook. f.) Hook. f.	M	n/a	AUS
L. leptoloba (Mattf.) D. G. Lloyd & C. J. Webb	M	n/a	NG
L. longipes Hook. f.	M	n/a	AUS
L. reptans (Benth.) D. G. Lloyd & C. J. Webb	M	n/a	AUS
L. sarawaketensis (P. Royen & D. G. Lloyd) D. G. Lloyd & C. J. Webb	M	n/a	NG
L. wilhelminensis (P. Royen) D. G. Lloyd & C. J. Webb	M	n/a	NG
Unnamed taxa			
L. "high altitude"	n/a	n/a	NZ
L. "limestone"	n/a	n/a	NZ
L. "Seal"	D	n/a	NZ
L. "seep"	n/a	n/a	NZ
L. "Volcanic Plateau"	n/a	n/a	NZ

D - dioecy, M - monoecy, PD - paradioecy; AUS - Australia, CHA - Chatham Islands, NG - New Guinea, NZ - New Zealand, SAM - South America, SUB - sub-Antarctic islands

Tab. 1-2: Summary of sex expressions in *Leptinella* according to Lloyd (1972a,b, 1975a,b, 1980a)

Sex expression		Description	Remarks	Species
dimorphic	dioecy	plants with female florets and plants with male florets (F) and (M)	rare inconstant female or male plants (see text)	*L. calcarea, L. dioica* subsp. *dioica, L. dispersa* subsp. *dispersa, L. pusilla, L. scariosa, L. serrulata, L. squalida, L. traillii*
	paradioecy	plants with female florets and plants with male florets, both sexes with a significant level of inconstancy (F, variable M) and (M, variable F)		*L. dendyi, L. goyenii, L. pyrethrifolia*
	‚pseudo-monomorphic dioecy'	female or inconstant female plants and only few male plants (F) and rare (M)		*L. dispersa* subsp. *dispersa*
monomorphic	monoecy	plants with female and male florets, all capitula bisexual (F, M)		*L. dispersa* subsp. *dispersa*, *L. intermedia, L. potentillina, L. tenella* and species of subgenera *Oligoleima* and *Radiata* (excluding *L. dendyi, L. goyenii)*
	complex- monoecy	predominantly male plants with bisexual and male capitula (M, variable F)	resemble the markedly inconstant males from dioecious populations (see text)	*L. dioica* subsp. *manoica, L. rotundata*
	unisexual female	only female plants (F)		*L. dispersa* subsp. *dispersa* *L. dispersa* subsp. *rupestris*
	unisexual male	only male plants (M)		*L. dispersa* subsp. *dispersa* *L. dispersa* subsp. *rupestris*

Terminology following Lloyd (1975b) and Sakai and (Weller 1999). Parentheses refer to the florets found on an individual: M - male florets, F - female florets.

Thesis outlines:

In the present thesis, different molecular methods are used to reconstruct molecular phylogenies of the southern hemisphere genus *Leptinella* and related genera. The obtained molecular phylogenies are then used to a) investigate the intergeneric and infrageneric relationships of *Leptinella*, b) elucidate the origin, the biogeography and the divergence time, and c) reconstruct the evolution of polyploidy and sex expression in *Leptinella*.

Chapter 2 deals with the position of *Leptinella* within the tribe Anthemideae. For this purpose a molecular phylogeny based on one non-coding nuclear marker (ITS) and on one coding chloroplast marker (*ndh*F) for the southern hemisphere members of the tribe were obtained, with the intention to a) reconstruct the evolutionary history of this basal group of the tribe, b) to discuss alternative generic groupings based on the outcome of the analyses, and c) to determine the phylogenetic position of *Leptinella*.

The subsequent three chapters deal with the phylogeny, biogeography, divergence time, and the evolution of dioecy and polyploidy in the genus *Leptinella*. For this purpose, two different molecular methods were used. The results from sequencing of three non-coding DNA markers from the nuclear and chloroplast genome are described in chapter 3. This chapter focuses on the whole genus *Leptinella*. Chapter 4 deals with the AFLP analysis on the monophyletic *Leptinella* main group. The evolution of sex expression and polyploidy in *Leptinella* is outlined in chapter 5.

Finally, chapter 6 summarises the results and discusses than in a synopsis.

Chapter 2

Phylogeny of southern hemisphere Compositae-Anthemideae based on nrDNA ITS and cpDNA *ndh*F sequence information

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Introduction

The tribe Anthemideae Cass. of the sunflower family (Compositae or Asteraceae) comprises 111 genera and around 1800 species that are distributed worldwide (extratropical) but mainly in central Asia, the Mediterranean, and southern Africa (Oberprieler et al. 2006). Twenty-nine of these genera are distributed naturally in the southern hemisphere. The diversity of this plant group is especially pronounced in the southern parts of Africa, with 27 genera and c. 290 species that are mostly restricted to this part of the world (e.g. *Athanasia* L., *Hippia* L., *Osmitopsis* Cass., *Ursinia* Gaertn.). Exceptions are formed by the genus *Cotula* L. that is distributed mainly in S Africa but with some species found in Australia, New Zealand, and S America, two genera that have distributional areas outside S Africa but are restricted to the S hemisphere [i.e. *Leptinella* Cass. (Australia, New Guinea, New Zealand, S America) and *Soliva* Ruiz & Pav. (S America)], and finally some genera which enter the northern hemisphere with only one or two species (i.e., *Cotula*, *Lasiospermum* Lag., *Pentzia* Thunb., *Ursinia*). On the other hand, there is the mainly N hemisphere genus *Artemisia* L. that enters with a few species into the S hemisphere. In recent times, however, some S hemisphere or N hemisphere species (e.g. *Achillea millefolium* L., *Anthemis cotula* L., *Cotula australis* (Spreng.) Hook.f., *Soliva sessilis* Ruiz. & Pav.) are found widespread as weeds in both hemispheres (Bremer and Humphries 1993, Oberprieler et al. 2006).

Former molecular phylogenetic studies have concentrated either on tribal overviews (Watson et al. 2000), or on geographical (e.g. in the Mediterranean area: Francisco-Ortega et al. 1997, Oberprieler and Vogt 2000, Oberprieler 2004a,b, 2005) or taxonomic subgroups of the tribe (e.g. Oberprieler 2001, Vallès et al. 2003, Guo et al. 2004). While a complete sampling on the generic level was achieved for the Mediterranean and Eurasian part (Oberprieler 2004a,b, 2005), the central Asian and the S hemisphere genera are still far from being completely sampled.

Watson et al. (2000) included representatives of 16 of the 29 genera with a predominantly S hemisphere centre of distribution in a phylogenetic study based on cpDNA *ndh*F sequence variation. Their reconstructions showed that there is a distinct biogeographical pattern in the evolutionary history of the tribe, with a basal grade of S hemisphere genera followed by a grade of genera from Asia and S Africa and a monophyletic crown group of Mediterranean and Eurasia Anthemideae representatives.

The basal position of these S hemisphere genera was clearly demonstrated and corroborated in some subsequent studies (e.g. Oberprieler 2005 based on nrDNA ITS).

Members of this geographical group were classified into four (Gonosperminae, Matricariinae, Thaminophyllinae, Ursiniinae) of their 12 subtribes by Bremer and Humphries (1993) who based their subtribal classification on a cladistic study of morphological, anatomical, cytological, and phytochemical characters. Whereas their Thaminophyllinae and Ursiniinae contained only S hemisphere genera, the two other subtribes were made up of representatives of both hemispheres. In the following years, molecular studies (Watson et al. 2000, Francisco-Ortega et al. 2001) demonstrated the non-monophyly of these subtribes. However, due to incomplete sampling of all genera concerned, no alternative generic classifications have been proposed.

As far as the S hemisphere representatives of the tribe are concerned, the generic delimitations and subtribal classification proposed by Bremer and Humphries (1993) largely rested on earlier works of a number of authors: The delimitation and revision of *Osmitopsis* by Bremer (1972, 1976), along with the generic re-classification of S African members of *Chrysanthemum* L. s.l. (Nordenstam 1976) and the results of anatomical studies of fruits in the whole tribe (and some of its S African members) made by Reitbrecht (1974) were incorporated into the treatment of the tribe proposed by Heywood and Humphries (1977). In continuation of this work, further studies concerned the generic delimitation of *Athanasia* (Källersjö 1985) and *Hymenolepis* Cass. (Bremer and Källersjö 1985), the generic re-classifications of S African Members of *Matricaria* L. (Nordenstam 1987) and *Pentzia* (Källersjö 1988), and the delimitation and tribal placement of members of the ´Cotuleae´ (Gadek et al. 1989, Bruhl and Quinn 1990, 1991).

In the last treatment of the Anthemideae Oberprieler et al. (2006) arranged the genera in a geographic order based on the primarily results of Watson et al. (2000): beginning with the S African representatives, followed by the central and eastern Asian ones, and ending with the Eurasian/Mediterranean genera. They mentioned also some informal groups within the S hemisphere members of the tribe (i.e. *Athanasia*-group, *Cotula*-group, *Pentzia*-group, *Phymaspermum*-group), but they mentioned also, that there is still a need for a more comprehensive morphological and molecular study. Therefore, in the present publication we have aimed at a complete sampling of cpDNA *ndh*F and nrDNA ITS sequence variation for all S hemisphere genera of the tribe, with the intention to (1) reconstruct the evolutionary history of this basal group within the Anthemideae and discuss relationships among its members and with the N hemisphere representatives of the tribe,

(2) to clarify the position of yet unsequenced S hemisphere genera, (3) to determine the position of *Cotula* and *Leptinella* for further more detailed species-level phylogenies of these genera, (4) to evaluate the four subtribes Ursiniinae, Gonosperminae, Thaminophyllinae, and Matricariinae sensu Bremer and Humphries (1993) as natural generic groupings, and (5) to discuss alternative generic grouping based on the outcome of the present analyses.

Materials and methods

Plant material. Sixty-two representatives from 61 genera of Compositae-Anthemideae were included in the present analysis. While all 29 S hemisphere genera were covered by the present sampling, Asian and Eurasian/Mediterranean genera of the tribe were represented by 10 (of 42) and 22 (of 38) genera, respectively. Sequence information for cpDNA *ndh*F and nrDNA ITS either came from former publications (Kim and Jansen 1995, Francisco-Ortega et al. 1997, 2001, Kornkven et al. 1998, Oberprieler and Vogt 2000, Watson et al. 2000, 2002, Oberprieler 2001, 2002, 2004a,b, Vallès et al. 2003, Guo et al. 2004, Gemeinholzer et al. 2006) and from unpublished EMBL/GenBank/DDBJ accessions (Tab. 2-1) or was established here for the first time. Sequence information for cpDNA *ndh*F and nrDNA ITS was established newly for 12 and 20 genera, respectively. For ten S hemisphere genera sequence information is presented here for the first time.

In the case of cpDNA *ndh*F, we included representatives of the tribes Astereae Cass., Calenduleae Cass., Gnaphalieae (Cass.) Lecoq & Juillet, and Inuleae Cass. as outgroup taxa, while for the analyses based on nrDNA ITS we omitted members of Inuleae and Gnaphalieae from the data set due to a problematic alignment. All outgroup taxa belong to the subfamily Asteroideae, in which many authors indicated a close relationship among the four tribes Astereae, Calenduleae, Gnaphalieae and Anthemideae, Anthemideae and Astereae having often considered to be sister groups to each other (e.g. Kim and Jansen 1995, Eldenäs et al. 1999, Panero and Funk 2002, Funk et al. 2005).

Tab. 2-1: Species analysed in this study and their accession data.

Taxon	Accession	Genbank accession number		
		ITS1	ITS2	*ndh*F
Anthemideae				
Aaronsohnia pubescens (Desf.) Bremer & Humphries	Watson et al. (2000)			AF153643
	Oberprieler and Vogt (2000)	AJ3296408	AJ3296443	
Achillea millefolium L.	Watson et al. (2000)			AF153633
	Guo et al. (2004)	AY603186		
Adenanthellum osmitoides (Harvey) B. Nord.	South Africa, Natal, Paulpietersburg, 12.12.1975, Hilliard & Burtt 8581 (S)	AM774445		AM900445
Adenoglossa decurrens (Hutch.) B. Nord.	South Africa, Cape Province, Richtersveld, 02.11.1962, Nordenstam 1709 (S)	AM774446		AM900446
Ajania fastigiata (Winkler) Poljakov	Valles et al. (2003)	AF504169	AF504142	
Ajania fruticulosa (Ledeb.) Poljakov	Watson et al. (2000)			AF153657
Anacyclus clavatus (Desf.) Pers.	Watson et al. (2000)			AF153634
	Oberprieler (2004a)	AJ748762	AJ748763	
Arctanthemum arcticum (L.) Tzvelev	Watson et al. (2000)			AF153671
	Francisco-Ortega et al. (1997)	L777756		
Argyranthemum foeniculaceum (Willd.) Webb ex Schultz-Bip.	Francisco-Ortega et al. (1997)	AF155270	AF155307	
Argyranthemum frutescens (L.) Schultz-Bip.	Watson et al. (2000)			AF153637
Artemisia tridentata Nutt.	Watson et al. (2000)			AF153630
	Kornkven et al. (1998)	AF060460	AF061376	
Artemisia vulgaris L.	Watson et al. (2000)			AF153632
	Oberprieler and Vogt (2000)	AJ3296389	AJ3296424	
Athanasia pachycephala DC.	South Africa, Cape Province, road between Heiveld and Kouberg, 10.12.1985, Källersjö 278 (S)	AM774447		AM900447
Chamaemelum nobile (L.) All.	Watson et al. (2000)			AF153655
	Oberprieler and Vogt (2000)	AJ3296382	AJ3296417	
Chrysanthemum x *grandiflorum* Hook.	Kim and Jansen (1995)			L39443
	Zhao et al. (unpubl.)	AF314599		
Cladanthus arabicus (L.) Cass.	Watson et al. (2000)			AF153654
	Oberprieler and Vogt (2000)	AJ3296383	AJ3296418	
Coleostephus multicaulis (Desf.) Durieu	Oberprieler and Vogt (2000)	AJ296393	AJ296428	
Coleostephus myconis (L.) Reichb. f.	Watson et al. (2000)			AF153652
Cota tinctoria (L.) J. Gay	Watson et al. (2000)			AF153636
	Oberprieler (2001)	AJ312802	AJ312831	
Cotula australis (Spreng.) Hook. f.	New Zealand, Canterbury, South Branch Waimakariri, 43°26'S 172°38'E, 29.09.1998, Wagstaff 98.086 (CHR)	AM774448		AM900448
Crossostephium chinense (L.) Makino	Watson et al. (2000)			AF153664
	Watson et al. (2002)	AY127685	AY127686	
Cymbopappus adenosolen (Harvey) B. Nord.	Watson et al. (2000)			AF153658
	South Africa, Boesmansrivier, 17.11.1985, Källersjö 208 (S)	AM774449		
Eriocephalus paniculatus DC.	South Africa, Western Cape, Citrusdal, 13.06.1998, Hanekom 3033 (S)	AM774450		AM900449
Eumorphia sericea J. M. Wood & M. Evans	South Africa, E Cape, Barkly East District, 03.02.1963, Hilliard & Burtt 16369 (S)	AM774451		AM900450

Tab 2-1: Continued

Taxon	Accession	Genbank accession number		
		ITS1	ITS2	*ndh*F
Fovoelina albida (DC.) Källersjö	South Africa, Namaqualand, Springbok, Goegap Nat. Res., W of Klippas, 29-39-42 S 18-00-57 E, 11.08.1997, leRoux & Mucina (S)	AM774452		AM900451
Glebionis coronaria (L.) Spach	Watson et al. (2000)			AF153661
	Francisco-Ortega et al. (1997)	L777741		
Glossopappus macrotus (Durieu) Briq. & Cavill.	Watson et al. (2000)			AF153639
Glossopappus macrotus subsp. *hesperius* (Maire) Jahand. & Maire	Oberprieler and Vogt (2000)	AJ3296394	AJ3296429	
Gonospermum canariense (DC.) Less.	Watson et al. (2000)			AF153665
	Francisco-Ortega et al. (2001)	AF155243	AF155280	
Gymnopentzia bifurcata Benth.	Watson et al. (2000)			AF153622
	South Africa, E Cape, Barkly East District, Hilliard & Burtt 16384 (S)	AM774453		
Heteranthemis viscidehirta Schott	Watson et al. (2000)			AF153638
	Francisco-Ortega et al. (1997)	L777761		
Hilliardia zuurbergensis (Oliver) B. Nord.	South Africa, Natal, near Mt. Alida, Eweka Estates, 16.19.1991, Hilliard & Burtt 19118 (S)	AM774454		AM900452
Hippia pilosa (P. Bergius) Druce	Watson et al. (2000)			AF153646
	South Africa, Cape Province, Rooiberg Mountain, 02.11.1988, Vlok 2041 (S)	AM774455		
Hymenolepis incisa DC.	South Africa, Western Cape Prov., Worcester Distr., Hex River Mountains, along road to Ceres at turnoff to Klipfontein, 05.09.1996, Bayer & Puttock SAF-96115 (S)	AM774456		AM900453
Inezia integrefolia (Klatt) E. Phillips	South Africa, Mpumalanga (Eastern Transvaal), Rosehaugh midway between Sabie and Nelspruit, 700 m, 08.01.1997, Bremer & Bremer 3812 (S)	AM774457		AM900454
Inulanthera leucoclada (DC.) Källersjö	South Africa, Royal Natal National Park, 06.03.1986, Steiner 1221 (S)	AM774458		AM900455
Ismelia carinata (Schousboe) Schultz-Bip.	Watson et al. (2000)			AF153653
	Francisco-Ortega et al. (1997)	L777764		
Kaschgaria komarovii (H. Krasch. & N. Rubtzow) Poljakov	Watson et al. (2000)			AF153631
	Watson et al. (2002)	AY127689	AY127690	
Lasiospermum pedunculare Lag.	South Africa, Cape, Little Karoo (HB Uppsala, cult. HB Jenensis 97-2), Oberprieler 9774 (Herbarium Oberprieler)	AM774459		AM900456
Leptinella pectinata subsp. *villosa* (D. Lloyd) D. Lloyd & C. Webb	New Zealand, Old Woman Range, Otago, 22.12.2004, Heenan (CHR)			AM900457
	New Zealand, The Remarkables (HB Arktisch-Alpiner-Garten Chemnitz, cult. HB Regensburg, Germany), 28.07.2004, Himmelreich 5 (CHR)	AM774460		
Leucanthemella serotina (L.) Tzvelev	Watson et al. (2000)			AF153659
	Francisco-Ortega et al. (1997)	L77766		
Leucanthemum vulgare Lam.	Watson et al. (2000)			AF153640
Leucanthemum vulgare ssp. *pujiulae* Sennen	Oberprieler and Vogt (2000)	AJ3296398	AJ864598	
Leucoptera subcarnosa B. Nord.	South Africa, Cape Province, Vanrhynsdorp Div., 03.09.1974, Nordenstam & Lundgren 1615 (S)	AM774461		AM900458

Tab 3-1: Continued

Taxon	Accession	Genbank accession number		
		ITS1	ITS2	*ndh*F
Lidbeckia pectinata P. Bergius	South Africa, Cape, Tulbagh, middle slopes of Roodsandberg on the farm Twee Jonge-gezellen, 400m, 23.10.1983, Rourke 1812 (S)	AM774462		AM900459
Lonas annua (L.) Vines & Druce	Watson et al. (2000)			AF153651
	Oberprieler and Vogt (2000)	AJ3296411	AJ3296446	
Lugoa revoluta (C. Smith ex Link) DC.	Watson et al. (2000)			AF153660
	Francisco-Ortega et al. (2001)	AF155252	AF155289	
Marasmodes dummeri Bolus ex Hutch.	South Africa, Cape Province, Paarl District, 10.06.1975, Esterhuysen 33883 (S)	AM774463		AM900460
Matricaria discoidea DC.	Watson et al. (2000)			AF153647
	Oberprieler and Vogt (2000)	AJ3296412	AJ3296447	
Mauranthemum gaetulum (Blatt.) Vogt & Oberprieler	Oberprieler and Vogt (2000)	AJ3296399	AJ3296434	
Mauranthemum paludosum (Poir.) Vogt & Oberprieler	Watson et al. (2000)			AF153670
Microcephala discoidea (Ledeb.) Bremer & al.	Watson et al. (2000)			AF153668
	Watson et al. (2002)	AY127677	AY127678	
Myxopappus acutiloba (DC.) Källersjö	Namibia, Ovamboland, 16.04.1968, Kers 3133 (S)	AM774464		AM900461
Nipponanthemum nipponicum (Franchet ex Maxim.) Xitam.	Watson et al. (2000)			AF153662
	Francisco-Ortega et al. (1997)	L77772		
Oncosiphon grandiflorum (Thunb.) Källersjö	Watson et al. (2000)			AF153648
	South Africa, Piekienerskloof Pass, 04.10.1985, Källersjö 46 (S)	AM774465		
Osmitopsis asteriscoides Cass.	South Africa, Western Cape, 29.01.2003, Ueckert & Oberprieler 10279 (Herbarium Oberprieler)	AM774466		
Osmitopsis osmitoides (Less.) Bremer	Watson et al. (2000)			AF153642
Pentzia dentata (L.) OK.	Watson et al. (2000)			AF153649
	Watson et al. (2002)	AY127681	AY127682	
Phymaspermum leptophyllum (DC.) Benth. ex B. D. Jackson	South Africa, Cape Province, Swellendam Div., Wildehondkloof Pass, 44 km E of Montagu, E side of Pass, 08.08.1974, Nordenstam & Lundgren 1194 (S)	AM774467		AM900462
Pseudohandelia umbellifera (Boiss.) Tzvel.	Watson et al. (2000)			AF153629
	Gemeinholzer et al. (2006)	AJ880330		
	Afghanistan, Kataghan, Rechinger 33840_b (B)		AM774468	
Rennera limnophila Merxm.	South Africa, District Grootfontein, 03.08.1974, Volk 01402 (S)	AM774469		AM900463
Rhodanthemum arundanum (Boiss.) Wilcox, Bremer & Humphries	Watson et al. (2000)			AF153641
	Oberprieler and Vogt (2000)	AJ3296405	AJ3296440	
Santolina chamaecyparissus L.	Kim and Jansen (1995)			L39444
	Francisco-Ortega et al. (2001)	AF155276	AF155313	
Schistostephium crataegifolium Fenzl ex Harv. & Sond.	South Africa, Natal, Lions River District, Fort Nottingham Commonage, 04.05.1977, Hilliard & Burtt 10331 (S)	AM774470		
Schistostephium umbellatum (L. f.) Bremer & Humphries	Watson et al. (2000)			AF153650
Soliva sessilis Ruiz & Pav.	USA, California, San Francisco, 06.05.1970, Rose 70037 (S)	AM774471		AM900464

Tab 3-1: Continued

Taxon	Accession	Genbank accession number		
		ITS1	ITS2	*ndh*F
Tanacetum macrophyllum (Waldst. & Kit.) Schultz-Bip.	Watson et al. (2000)			AF153628
	Guo et al. (2004)		AY603262	
Thaminophyllum latifolium Bond	South Africa, Cape Province, Caledon Div., Hermanus, above the houses at Voelklip, 06.09.1974, Esterhuysen 33604 (S)		AM774472	AM900465
Tripleurospermum caucasicum (Willd.) Hayek	Armenia, Aragats, 30.06.2002, Oberprieler 10192 (Herbarium Oberprieler)			AM900466
	Oberprieler (2004b)	AJ864590	AJ864610	
Ursinia anthemoides (L.) Poiret	South Africa, Cape Province, Namakwaland Division, 12.09.1993, Strid & Strid 37382 (S)		AM774473	AM900467
Outgroup				
Anisothrix integra (Compton) Anderb.	Kim and Jansen (1995)			L39437
Antennaria neodioica Greene	Kim and Jansen (1995)			L39436
Antennaria virginica Stebbins	Bayer et al. (1996)	L40851	L40930	
Baccharis neglecta Britton & A. Brown	Kim and Jansen (1995)			L39448
	Morgan (1997)		U97604	
Bellis perennis L.	Kim and Jansen (1995)			L39446
	Noyes and Rieseberg (1999)		AF046950	
Calendula officinalis L.	Kim and Jansen (1995)			L39439
	Wagstaff and Breitwieser (2002)		AF422114	
Callilepis salicifolia Oliver	Anderberg et al. (2005)			AY780851
Conyza sp.	Kim and Jansen (1995)			L39451
Conyza canadensis (L.) Cronquist	Noyes and Rieseberg (1999)		AF046987	
Cratystylis conocephala (F. Muell.) S. Moore	Anderberg et al. (2005)			AY780821
Dielitzia tysonii P. S. Short	Anderberg et al. (2005)			AY780822
Dimorphotheca pluvialis (L.) Moench	Kim and Jansen (1995)			L39438
Epaltes cunninghamii (Hook.) Benth.	Anderberg et al. (2005)			AY780824
Erigeron annuus Pers.	Noyes (2000)		AF118489	
Erigeron hybridus Hieron.	Kim and Jansen (1995)			L39450
Felicia bergeriana O.Hoffm. ex Zahlbr.	Kim and Jansen (1995)			L39445
Felicia echinata Nees	Eastwood et al. (2004)		AY193797	
Nannoglottis ravida (C. Winkl.) Y. L. Chen	Liu et al. (2002)			AY017150
Osteospermum fruticosum (L.) Norlindh	Wagstaff and Breitwieser (2002)		AF422131	
Osteospermum pinnatum (Thunb.) Norlindh	Watson et al. (2000)			AF153669
Pyrrocoma sp.	Kim and Jansen (1995)			L39447
Pyrrocoma lanceolata Greene	Markos and Baldwin (2001).		AF251574	
Rosenia humilis (Less.) Bremer	Eldenäs et al. (1999)			AF063080
Symphyotrichum cordifolium (L.) G.L. Nesom	Kim and Jansen (1995)			L39449
	Kress et al. (2005)		DQ005972	

Voucher information is given for new sequences, for the others the original papers are cited. (Herbarium codes: B - Botanical Garden and Museum, Berlin, Germany; CHR - Landcare Research, Lincoln, New Zealand; S - Swedish Museum of Natural History, Stockholm, Sweden)

DNA isolation, amplification and sequencing. DNA was extracted from leaves taken from herbarium specimens or from material dried in silica gel. Specimens were extracted either following a modified protocol based on the method by Doyle and Doyle (1987) or with the DNEasy plant DNA extraction kit (Qiagen) following the manufacturer's protocol. PCR amplifications of the nrDNA ITS marker were performed using primers 18SF and 26SR (Rydin et al. 2004) or ITS5A (Funk et al. 2004) and ITS4 (White et al. 1990). In some cases ITS1 and ITS2 were amplified separately using primers ITS5A (Funk et al. 2004), ITS2, ITS3, and ITS4 (White et al. 1990). Since in the analysis of *ndh*F we used only the 3'end of the gene, PCR amplifications were carried out with primers ndhF-5b and ndhF-10b (Eldenäs et al. 1999).

Some amplification reactions were performed with 10 µmol/l primers in 25 µl reaction using "Ready-to-go" PCR beads (Amersham Pharmacia Biotech) following the manufacturer's standard protocol. In other cases, PCR amplifications were performed with 0.2 µM dNTP's, 0.02 µM of each primer, 0.2 U Taq polymerase (Qbiogene) in 10 µl 1x Buffer. Amplification of nrDNA ITS (cpDNA *ndh*F) was carried out with the following temperature profile: 2-5min at 95°C, then 35 to 40 cycles of 30s at 95°C, 30s at 50°C, 60(80)s at 72°C, with a final extension of 8 min at 72°C.

The PCR products were purified with QIAquick PCR Purification Kit (Qiagen). Cycle sequencing reactions used the same primers as in the PCR, with the exception of *ndh*F where we used ndhF-1260F (Eldenäs et al. 1999) and ndhF-1700R (Anderberg and Swenson 2003) as internal sequencing primers. The Big Dye Terminator Sequencing Kit (Applied Biosystems) or the DTCS Sequencing Kit (Beckman Coulter) were used following the manufacturer's manual, and the fragments were separated either on an ABI377 or on a CEQ8000 sequencer.

Sequence alignment, phylogenetic reconstructions. Sequences were aligned with BioEdit version 7.05.2 software (Hall 1999). Gaps in the alignments were treated as missing data. In the alignment of ITS2, a 33 bp long sequence (between alignment position 15 and 48) was excluded from the further analyses, because the high variability of this position made an unequivocal alignment impossible. This segment is part of a loop flanked by a conservative stem that is found in many Compositae (Goertzen et al. 2003).

Maximum Parsimony (MP) analyses were performed using the heuristic search algorithm of PAUP* version 4.0b10 (Swofford 2002) with ACCTRAN, MULPARS and TBR branch swapping in action. Character states were specified unordered and

unweighted. One thousand random addition sequence replicates were performed. Support of branches was evaluated using bootstrapping (Felsenstein 1985) and the following settings: 1000 bootstrap replicates, 10 random addition sequence replicates per bootstrap replicate, ACCTRAN, TBR and MULPARS. Limitation of computer capacity made it necessary to set MAXTREE to 5000 for each random addition sequence replicate.

The data sets were also analysed with two model-based approaches to phylogenetic inference, the Maximum-Likelihood (ML) method (Felsenstein 1981, Kishino and Hasegawa 1989) and a Bayesian inference (BI) approach (Lewis 2001). Since both methods are dependent on assumptions about the process of DNA substitution (a model of DNA evolution), the program Modeltest version 3.06 (Posada and Crandall 1998) was used to find the model that best fits the underlying sequence information. This resulted in the acceptance of the Tamura-Nei-model with gamma distribution (TrN+G) for nrDNA ITS and the transversions model (TVM+G) for cpDNA *ndh*F. The base frequencies for nrDNA ITS (and for cpDNA *ndh*F, respectively) being freqA = 0.2490 (0.3081), freqC = 0.2198 (0.1606), freqG = 0.2100 (0.1510) and freqT = 0.3212 (0.3803), a gamma distribution shape parameter of $\alpha = 0.7531$ (0.3474) and a substitution rate matrix with R[A–C] = 1.0 (1.2567), R[A–T] = 1.0 (0.1873), R[C–G] = 1.0 (1.6645), R[G–T] = 1.0 (1.0), R[A–G] = 2.9103 (1.5888) and R[C–T] = 4.2369 (1.5888). Using these parameters, ML searches were performed with Treefinder version June 2004 (Jobb 2004) including bootstrap analyses with 1000 replicates.

The same parameters of the TrN+G (TVM+G) model were also used in the BI approach performed with the software programme MrBayes version 2.01 (Huelsenbeck and Ronquist 2001). Four Metropolis-coupled Markov chain Monte Carlo chains with incremental heating temperature of 0.2 were run for 1.000.000 generations and sampled every 100th generation. The burn-in period was determined graphically, and the first 200 (1000) of the 10.000 trees were discarded. Estimation of tree topology and posterior probabilities (PP) of clades were based on the remaining 9.800 (9.000) trees. Since in Bayesian inference, it is not necessary to fix substitution model parameters and estimation of these parameters is considered usually computationally feasible and theoretically preferable (Ronquist, pers. comm.), the search was repeated with substitution model parameters (basfreq, revmat, shape) estimated from the data and were run for 2.000.000 generation (with burn-in periods of 500 and 1500 trees, respectively).

An incongruence length difference (ILD) test (Farris et al. 1994) as implemented in PAUP* version 4.0b10 (Swofford 2002) was applied to both data sets to test their

congruence. The data sets were combined into a single data matrix with two partitions (*ndh*F nad ITS), invariant characters were excluded, and heuristic searches were conducted with simple addition order, TBR branch swapping, and the MULTREES option in action. Ninety-nine random repartitions of the data were performed with the MAXTREE limit set to 1000. Since the test found that the phylogenetic trees based on cpDNA *ndh*F and nrDNA ITS were significantly incongruent (P = 0.01), we refrained from the joint analysis of the two data sets.

Results

Phylogenetic reconstructions based on cpDNA *ndh*F. The alignment of all 80 cpDNA *ndh*F sequences is 952 bp long with 337 variable positions including 208 parsimony informative characters. The heuristic MP search yielded 493.976 equally most parsimonious trees with a length of 799 steps, a consistency index (CI with autapomorphies excluded) of 0.5183, and a retention index (RI) of 0.8107. The strict consensus tree is shown in Fig. 2-1. The tree received from the ML analysis (lnL = -6231.2828) is depicted in Fig. 2-2. The two BI analyses (with and without a constrained model of DNA evolution) did show comparable results (data not shown) and posterior probabilities drawn from the analysis based on the model-constrained search are shown in Fig. 2-2.

In all the analyses of the *ndh*F sequences, the members of the tribe Anthemideae form a well supported monophyletic group (MP bootstrap 87%, ML bootstrap 90%, PP 1.00), but the sister-group relationship to Calenduleae, Gnaphalieae, or Astereae is not clearly resolved. At the base of the tree, we consistently find *Osmitopsis* and the well supported *Cotula*-group of genera *(Adenanthellum* B. Nord., *Cotula, Hilliardia* B. Nord., *Hippia, Inezia* E. Phillips*, Leptinella, Lidbeckia* P.J. Bergius*, Schistostephium* Less., *Soliva,* and *Thaminophyllum* Harv.; 100%, 100%, 1.00).

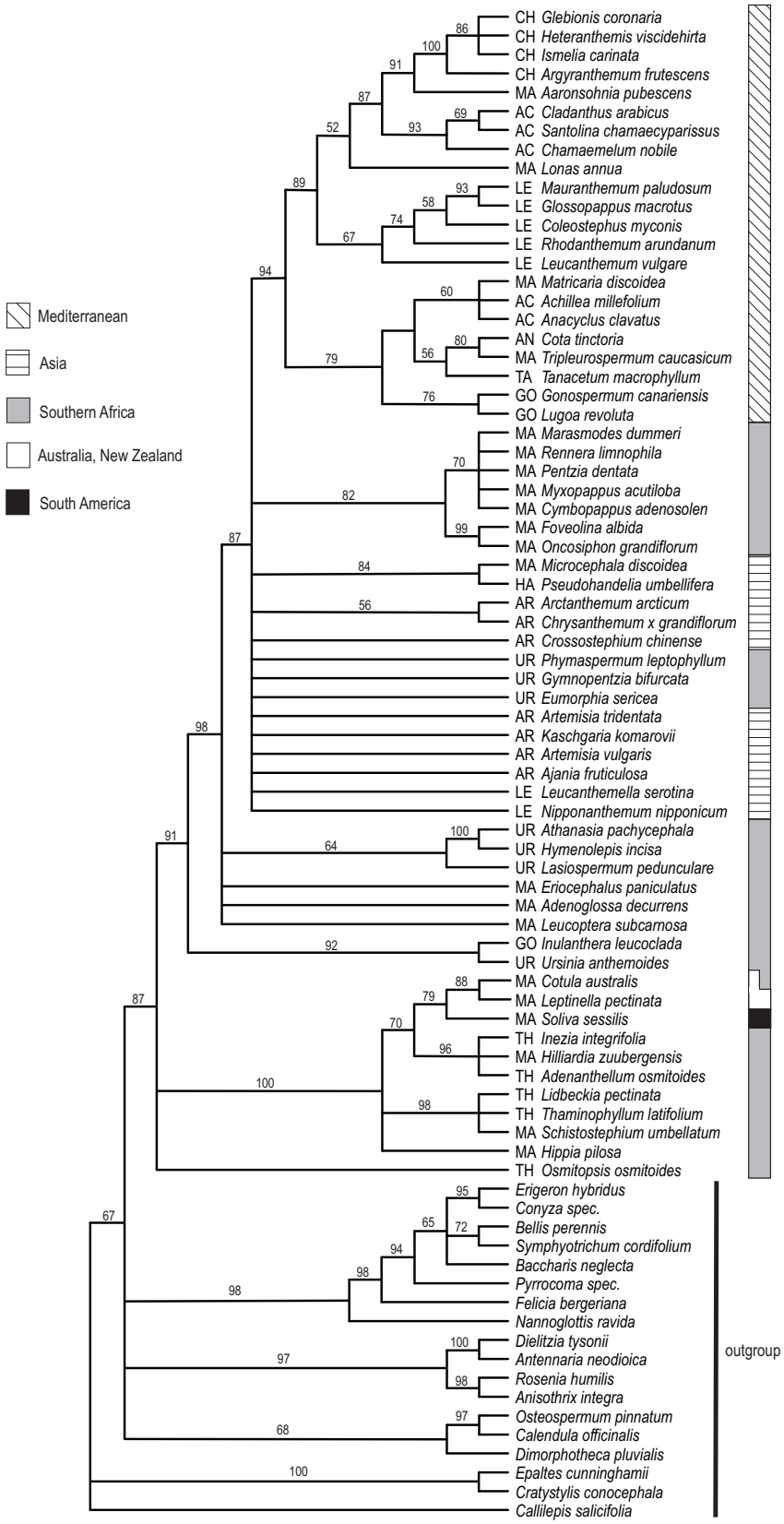

Fig. 2-1: Strict consensus tree of 493.976 equally most parsimonious trees based on cpDNA *ndh*F sequence information. Numbers are bootstrap values, geographical distribution are indicated by bar patterns and subtribal classification according to Bremer and Humphries (1993) by letters (AC - Achilleinae, AR - Artemisiinae, AN - Anthemidinae, CH - Chrysantheminae, GO - Gonosperminae, HA - Handeliinae, LE - Leucantheminae, MA - Matricariinae, TA - Tanacetinae, TH - Thaminophyllinae, UR - Ursiniinae).

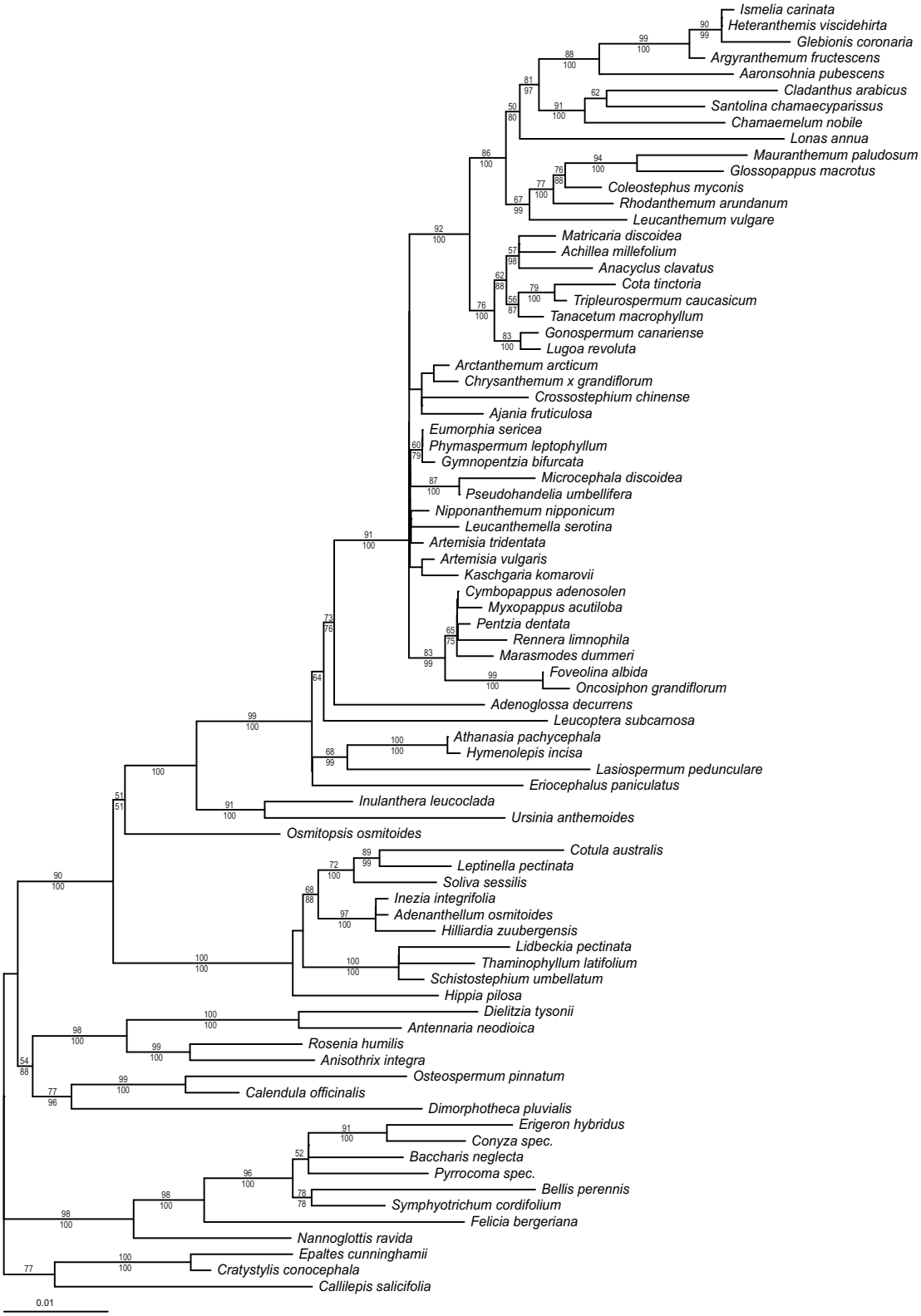

Fig. 2-2: Phylogenetic tree from a Maximum-Likelihood (ML) analysis based on cpDNA *ndh*F sequence information. Numbers above the lines are bootstrap values of the ML analysis, and numbers below the lines are posterior probabilities (PP x 100) of the Bayesian inference (BI) approach (more information is given in the text).

The rest of the tribe (MP 91%, PP 1.00, but no significant support from ML) forms a monophyletic group. Within the latter lineage, the analyses consistently show (a) the sister-group relationship of *Inulanthera* Källersjö + *Ursinia* (92%, 91%, 1.00) and the rest of the clade (98%, 99%, 1.00), (b) a grade of S African and Asian representatives of the tribe *(Marasmodes* DC. through *Leucoptera* B. Nord. in the MP tree), and (c) a well supported (94%, 92%, 1.00) monophyletic group of Mediterranean and Eurasian genera *(Glebionis* Cass. through *Lugoa* DC.). While sister-group relationships within the S African/Asian grade are unresolved in the MP analyses or weakly supported [with the exception of the group of *Athanasia* + *Hymenolepis* (100%, 100%, 1.00), the clade around *Pentzia* (82%, 83%, 0.99), and the sister-group relationship of the Asian genera *Microcephala* Pobed. and *Pseudohandelia* Tzvelev (84%, 87%, 1.00)], the Mediterranean/Eurasian clade is characterised by a comparably well resolved topology.

Phylogenetic reconstructions based on nrDNA ITS. The alignment of all 72 nrDNA ITS sequences is 488 bp long with 356 variable positions including 266 parsimony informative characters. The heuristic MP search yielded 61 equally most parsimonious trees with a length of 1681 steps, a consistency index (CI with autapomorphies excluded) of 0.3405, and a retention index (RI) of 0.6352. The strict consensus tree is shown in Fig. 2-3. The ML tree (lnL = -8531.3763) is shown in Fig. 2-4, together with the posterior probabilities gained from the model constrained BI analysis that did not deviate from the ML tree and from the BI search result constrained to the TrN+G model.

As far as the main branches of the trees are concerned, the resulting phylogenetic reconstructions are both consistent with each other and with the results of the analyses based on cpDNA *ndh*F. Corresponding results comprise (a) the basal split into the isolated genus *Osmitopsis,* the generic group around *Cotula* (76%, 90%, 1.00), and the rest of the tribe (83%, 98%, 1.00), (b) the grade of S African and Asian representatives of the tribe *(Ajania* Poljakow through *Ursinia* in the MP tree), and (c) the monophyly of the Mediterranean/Eurasian group (82%, 88%, 1.00; also supported by a deletion of 19 bp in ITS2 that was omitted from the analyses).

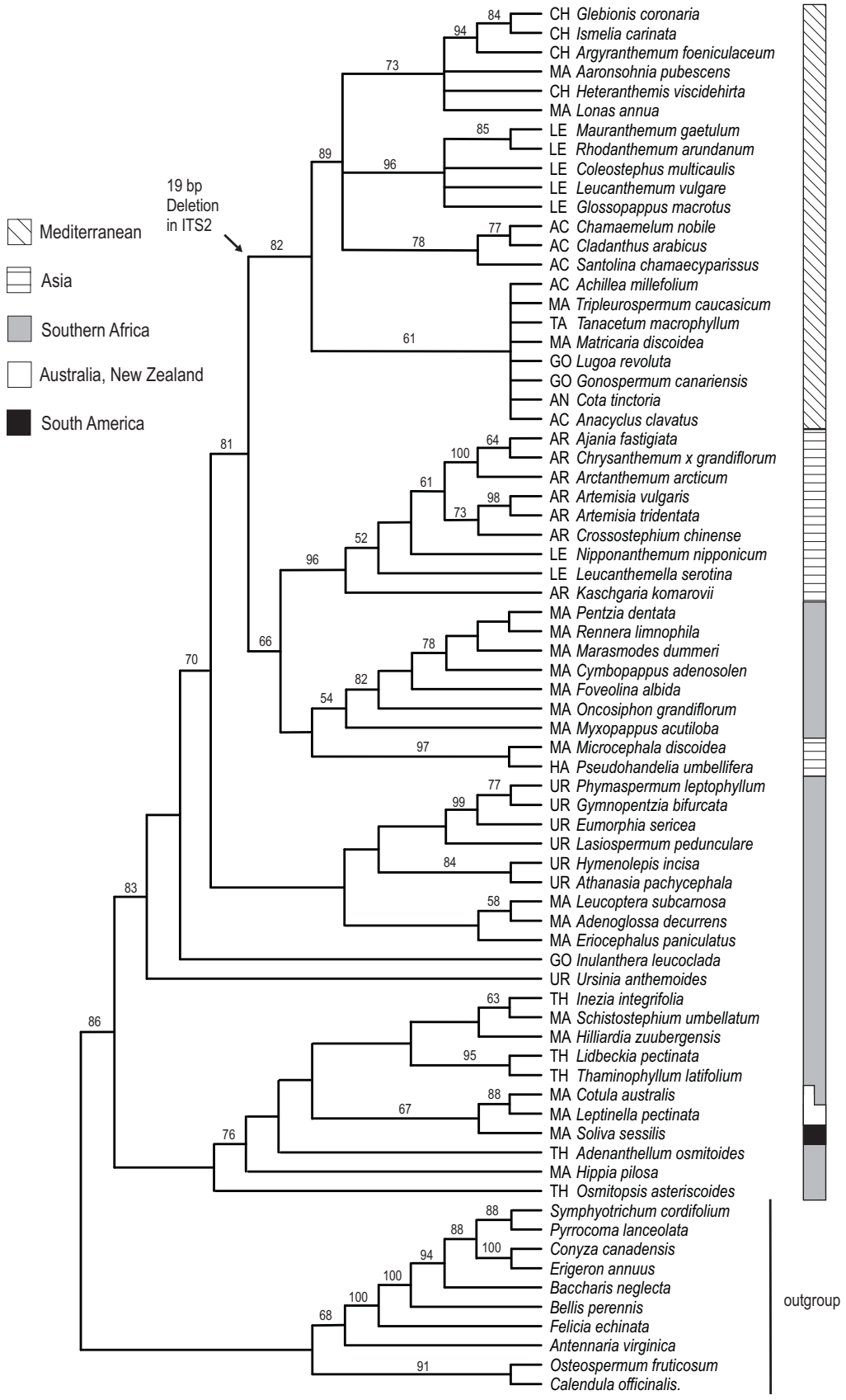

Fig. 2-3: Strict consensus tree of 61 equally most parsimonious trees based on nrDNA ITS sequence information. Numbers are bootstrap values, geographical distribution are indicated by bar patterns and subtribal classification according to Bremer and Humphries (1993) by letters (for explanations see Fig.: 2-2).

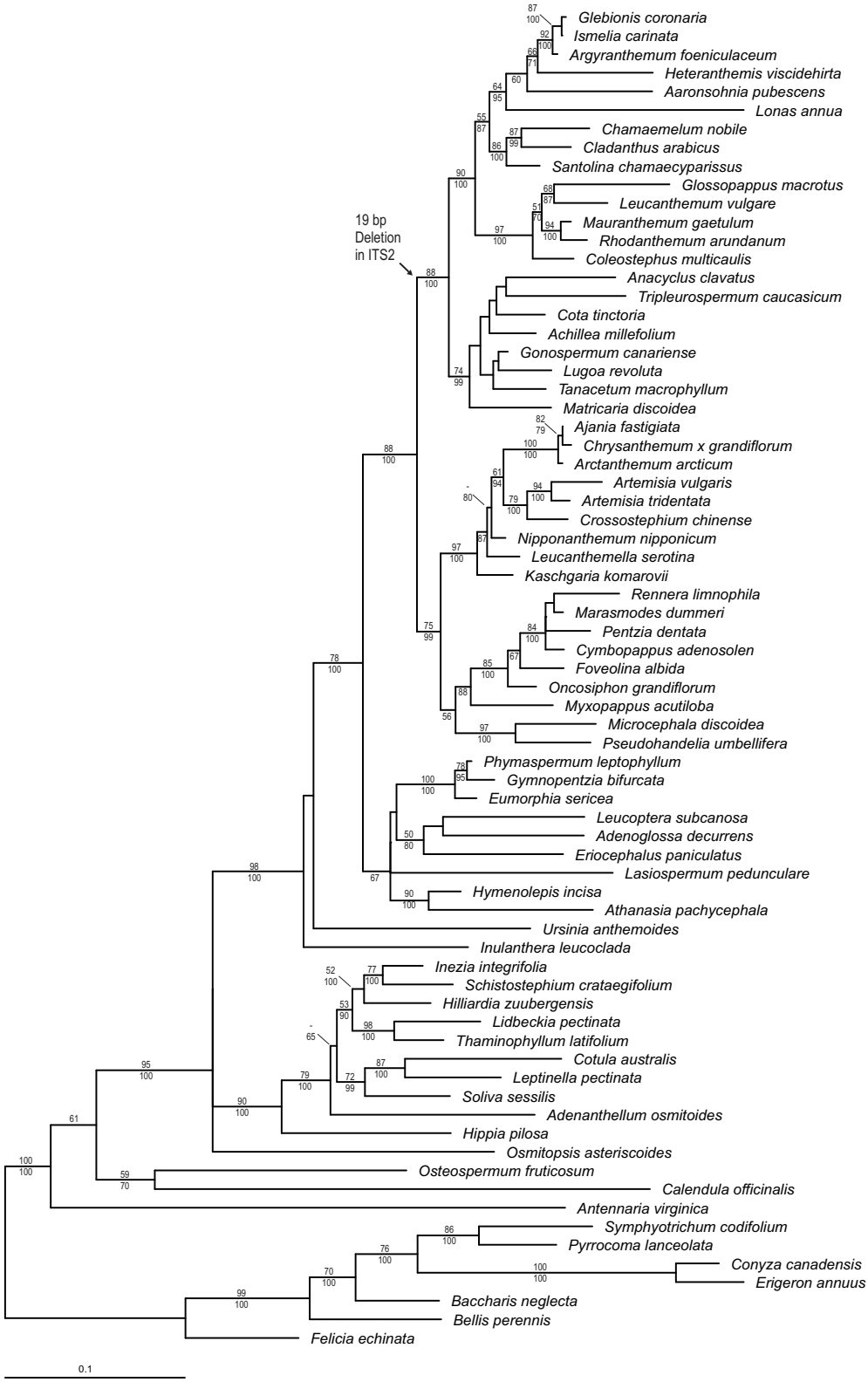

Fig. 2-4: Phylogenetic tree from a Maximum-Likelihood (ML) analysis based on nrDNA ITS sequence information. Numbers above the lines are bootstrap values of the ML analysis, and numbers below the lines are posterior probabilities (PP times 100) of the Bayesian inference (BI) approach (more information is given in the text).

There are three major differences between the analyses based on cpDNA *ndh*F and nrDNA ITS: (1) While *Inulanthera* and *Ursinia* form a well supported clade in the *ndh*F analysis, the two genera are consistently unresolved in the analyses based on nrDNA ITS. (2) The group of *Eumorphia* DC., *Gymnopentzia* Benth., and *Phymaspermum* Less. (not significantly supported as monophyletic in the analyses based on *ndh*F, but strongly supported in the ITS trees) are part of the strongly supported clade (*ndh*F: 90%, 91%, 1.00; ITS: 80%, 88%, 1.00) of Mediterranean / Eurasian + Asian + S African representatives of the tribe when *ndh*F sequence information is considered, but is excluded from this clade in the ITS tree. Here it is found in a more basal position among another part of S African representatives with unclear sister-group relationships. (3) While the relationships of Asian representatives of the tribe are unresolved in the *ndh*F tree, this group is clearly divided into a clade around *Artemisia* (96%, 97%, 1.00) and the group of *Microcephala* and *Pseudohandelia* (97%, 97%, 1.00).

Discussion

Phylogeny and biogeography. Both data sets analysed in the present study unequivocally show a clear biogeographic pattern with a basal position of S hemisphere representatives of the tribe. This is in accordance with former studies based on a less complete data set of cpDNA *ndh*F sequence (Watson et al. 2000) and on nrDNA ITS sequences variation (Oberprieler 2005).

The movement of tribe members into the N hemisphere as a younger event in the evolution of the tribe is also clearly demonstrated by the present data. Sister-group relationships between S and N hemisphere representatives are unclear from the cpDNA *ndh*F analysis (as in the study using the same marker by Watson et al. 2000). However, the phylogenetic reconstructions based on nrDNA ITS sequence variation show that there are close connections between the Asian and Mediterranean members of the tribe with the S African *Pentzia*-clade. It is also demonstrated that the colonization of the N hemisphere may have occurred twice as independent movements into Asia (genera around *Artemisia*) and the Mediterranean region (the group of genera characterised by the 19 bp deletion in ITS2). The same scenario was found in a biogeographic study by Oberprieler (2005) who dated these two dispersal events to the Early Miocene (around 18 to 14 Ma ago) when the collision of the African and Eurasian platforms occurred.

The position of *Cotula, Leptinella,* and *Soliva* in both reconstructions (and more detailed, species-based nrDNA ITS studies, Himmelreich et al., in prep.) indicates that there was a likely dispersal event of *Cotula* out of S Africa into Australia and New Zealand *(Leptinella),* and at two time *(Soliva, Leptinella)* into S America. An African origin and a subsequent dispersal out of S Africa into Eurasia or America is found also in related tribes: The Gnaphalieae have a basal African group and also their greatest diversity in this area (Unwin et al. 2006, Bayer et al. 2002). The Astereae have there origin in S Africa, from where the tribe moved into other parts of the world (Brouillet et al. 2006).

Phylogeny and subtribal classification. With the exception of Cancriniinae (6 genera, 29 species) from Asia, our present data set comprises representatives of all 12 subtribes accepted by Bremer and Humphries (1993) in their generic monograph based on a cladistc analysis of morphological, anatomical, phytochemical, and cytological sources of evidence. As found in several less comprehensive molecular phylogenetic studies based on plastid and nuclear markers (e.g. Francisco-Ortega et al. 1997, Oberprieler and Vogt 2000, Watson et al. 2000, Oberprieler 2004a,b, 2005), our present analyses indicate that only a minority of these subtribes are monophyletic, i.e. the Chrysantheminae sensu Bremer and Humphries. The non-monophyly of subtribes based on morphological data were also found in related tribes of the Compositae: For example, Bayer et al. (2006) mentioned that the subtribes of the Gnaphalieae are non-monophyletic, and will need a re-circumscription. There is also a huge amount of discrepancy reported between morphological and molecular data in the tribe Astereae (Noyes and Rieseberg 1999, Brouillet et al. 2006, Nesom and Robinson 2006).

Our present analyses demonstrate in accordance with the former studies by Watson et al. (2000) and Francisco-Ortega et al. (2001) that all of the four subtribes accommodating S hemisphere genera in the treatment of Bremer and Humphries (1993), i.e. Gonosperminae, Matricariinae, Thaminophyllinae, and Ursiniinae, lack monophyly, with the Matricariinae being the most obvious case with its members scattered throughout the trees irrespective of the molecular marker employed.

The Gonosperminae (3 genera, 15 species), comprising the three genera *Gonospermum* Less. (Canary Islands), *Lugoa* (Canary Islands), and *Inulanthera* (S Africa), were considered a monophyletic group by Bremer and Humphries (1993) based on the alleged synapomorphies of large leaves with rounded lobes, a paleate receptacle, and an achene apex with a corona of small scales terminating each rib. The molecular

phylogenetic study based on nrDNA ITS sequence variation made by Francisco-Ortega et al. (2001) clearly demonstrated, however, that while the two former genera from the Canary Islands show close phylogenetic relationships with representatives of the Eurasian genus *Tanacetum* L. (see also Oberprieler 2005), *Inulanthera* is firmly nested among the S African representatives of the tribe. These positions are clearly corroborated here by the analyses based on the chloroplast marker *ndh*F. Källersjö (1985), when describing the genus *Inulanthera* to accommodate species formerly treated under *Athanasia* and *Pentzia* but deviating mainly due to the possession of tailed anthers, the absence of ellipsoid secretory cavities from all parts of the plant, and the possession of polyacetylenes instead of furanosesesquiterpenes, also speculated on a close relationship of this genus with *Gonospermum* and *Lugoa,* but also noted the differences (tailed anthers and achenes with 8-10 ribs in *Inulanthera* as opposed to rounded anthers and achenes with 5 ribs in the other two genera). It appears well supported by our present analyses that (a) *Inulanthera* is a phylogenetically distinct entity from *Athanasia* or *Pentzia* and that (b) the leaf characters are only superficially pointing to a close phylogenetic relationship between this S hemisphere genus and the two Canary Island genera.

The Matricariinae (25 genera, 265 species) of Bremer and Humphries (1993) constitutes a further problematic and obviously non-monophyletic subtribe, with its members scattered throughout the phylogenetic trees based both on the chloroplast and nuclear marker. The subtribe was considered to be characterised by the apomorphies of the arrangement of myxogenic cells on the achenes (abaxially and on the ribs, but not on the adaxial surface) and the possession of an adaxially long pappus (corona, auricle, or composed of separate scales). But Bremer and Humphries (1993) also report on equally most parsimonious reconstructions based on morphological data that found no synapomorphies for the subtribe.

Despite the clear polyphyly of Matricariinae sensu Bremer and Humphries (1993), at least the bipartition of the subtribe into two generic groups seen in their reconstructions receives some support from our present study: (a) Their *Cotula*-group of genera (with the exception of *Eriocephalus* L.) forms a monophyletic group which includes, however, also the majority of their subtribe Thaminophyllinae *(Adenanthellum, Inezia, Lidbeckia,* and *Thaminophyllum)*, and (b) the *Pentzia*-group of genera which forms a monophyletic group in their phylogenetic reconstructions that includes, however, also the N hemisphere representatives of the subtribe *(Aaronsohnia* Warb. & Eig, *Daveaua* Willk. ex Mariz, *Endopappus* Sch. Bip., *Heteromera* Pomel, *Lonas* Adans., *Matricaria, Microcephala,*

Otospermum Willk., and *Tripleurospermum* Sch. Bip.), and the two S African genera *Adenoglossa* B. Nord. and *Leucoptera* is also, but in a far smaller circumscription, recovered as a monophyletic group in the molecular phylogenies. Obviously, a large number of genera and generic groups of the Anthemideae have their sister-group within the subtribe Maricariinae sensu Bremer and Humphries (1993), pointing to the plesiomorphic or highly homoplastic nature of the morphological characters used to characterise it as a natural group.

The Thaminophyllinae sensu Bremer and Humphries (1993) comprise the five S African genera (17 species) *Adenanthellum, Inezia, Lidbeckia, Osmitopsis,* and *Thaminophyllum* that share a similar habit as being perennial herbs, sub-shrubs or shrubs, a similar foliage with entire to only few-lobed leaves, and similar ray florets with many, branching veins. In the cladistic analysis, the subtribe was considered to form a monophyletic group due to the apomorphies of the lack of resin canals in floral parts and the base chromosome number of $x = 10$ (Bremer and Humphries 1993). It appears obvious from the present analyses and corroborates results based on morphological evidence, that *Osmitopsis* is clearly separate form the other four genera in the subtribe, being found at the base of the tribe in the molecular phylogenies while the others are tightly linked to the genera around the genus *Cotula*. This is in accordance with considerations by Bremer (1972) and Nordenstam (1987) who found that *Osmitopsis* is systematically isolated with no close relatives, and that while the white rays with branching venation and often bilobed apex link the genus with the Thaminophyllinae, other characters like the deviating ligule micromorphology observed by Baagøe (1977), the caudate anthers, and the paleate receptacle distinctly distinguish it from them.

The Ursiniinae sensu Bremer and Humphries (1993) are formed by seven S African genera with around 115 species (two of them also occurring in Ethiopia and on the Sinai Peninsula). The circumscription of this subtribe was mainly based on phytochemical evidence, with a number of publications by Bohlmann and co-workers (Bohlmann et al. 1973, Bohlmann and Rao 1972, Bohlmann and Zdero 1972b, 1974 and 1978a,b, Bohlmann and Grenz 1975) indicating that representatives of these genera possess furanosesesquiterpenes rather than the common polyacetylenes. This in conjunction with morphological and anatomical evidence (a paleate receptacle, ray floret limbs with tabular epidermis cells, and anthers with partly or totally polarised endothecia tissue) was considered to sufficiently underpin the monophyly of the subtribe (Källersjö 1985, Bremer and Humphries 1993) which should even include the carpologically and palynologically

deviating genus *Ursinia.* Due to its anthers with broad ovate apical appendages and balusterform filament collars, the pollen exine without columnar structure, and the achenes with a biseriate pappus formed by scales, this genus had formerly been considered to hold a very isolated position within the tribe Anthemideae (Cassini 1816, Beauverd 1915), or had even been suggested as an independent tribe Ursinieae (Robinson and Brettell 1973) or a member of the Arctoteae (Bentham 1873). Our present analyses corroborate results of Watson et al. (2000) that *Ursinia* is a member of the tribe Anthemideae. It also shows, however, that this genus holds an isolated position from the remaining six members of the subtribe Ursiniinae sensu Bremer and Humphries (1993) for which a status as a monophyletic group is only marginally supported in the MP analysis based on the nuclear marker (Fig. 2-3), but definitively not by the chloroplast marker (Fig. 2-1 and 2-2). This astonishing result is demonstrated by the different positions of the so-called *Phymaspermum*-group, consisting of the closely related genera *Eumorphia, Gymnopentzia,* and *Phymaspermum,* in the analyses based on the two markers: While in the nrDNA ITS data set this generic group is found among the S hemisphere genera of the tribe and distinctively excluded from the well supported (80%, 88%, 1.00) Asian and Eurasian crown group, it is equally well supported (90%, 91%, 1.00) as a member of this crown croup in the cpDNA *ndh*F phylogeny. Two scenarios are conceivable to account for these discrepancies: (a) The progenitor of the *Phymaspermum*-group may have been formed by a hybridisation event between a member of the phylogenetically basal S African group of genera as a paternal partner and either a member of the Asian groups around *Artemisia, Microcephala,* or *Pseudohandelia* or a member of the S African *Pentzia*-group as the maternal (chloroplast contributing) partner, whereby the latter event seems geographically more reasonable. (b) The *Phymaspermum*-group may hold a phylogenetically intermediate and bridging position between the more basal S African members of the tribe and the more advanced crown group consisting of the *Pentzia*-group and all Asian and Eurasian Anthemideae, sharing the rather apomorphic chloroplast type with the latter but a relatively plesiomorphic nrDNA ITS sequence with the former. As a consequence of this scenario, the *Phymaspermum*-group may be a good candidate for the sister-group to the clade of *Pentzia*-group + Asian + Eurasian Anthemideae, while the *Pentzia*-group itself may exhibit a sister group-relationship to the Asian (or the Asian + Eurasian) members of the tribe.

Generic groups and their characterisation. As a consequence of the above described non-monophyletic nature of the subtribes described by Bremer and Humphries (1993) we would like to propose and discuss in the following generic groupings based on our present study that may have more justification as natural groups. In the present contribution we would like to limit our considerations only to the S hemisphere Anthemideae, since for the Mediterranean genera comparable discussion have already been presented elsewhere (Oberprieler 2005) and for the Asian members of the tribe we consider the present sampling of genera and species yet insufficient.

(a) *Osmitopsis*: Our present analyses based on both nuclear and chloroplast DNA sequence variation correspondingly indicate that *Osmitopsis* (with nine species) is a member of the tribe Anthemideae and that it holds a basal and isolated position within this tribe. While Cassini (1817) included the genus into his concept of Anthemideae, Bentham (1873) and Hofmann (1894) considered the tailed anthers of *Osmitopsis* an argument for an inulean affiliation. Palynological evidence (Stix 1960) and further characters like odour, the occurrence of pluriseriate involucral bracts with scarious margins, together with the truncate style and the tendency towards the reduction of the pappus were arguments for Bremer and Humphries (1993) to include the genus into their concept of Anthemideae. Since tailed anthers are also observed in other, unequivocal members of Anthemideae *(Inulanthera, Hippolytia* Poljakov*)* this character does not indicate any close relationship towards the tribe Inuleae and the decision of Bremer and Humphries (1993) is justified.

As Bremer (1972) and Nordenstam (1987) already noted the genus is systematically isolated in the Anthemideae. The inclusion of the paleate genus *Osmitopsis* in their subtribe Thaminophyllinae, together with the more closely related (epaleate) genera *Adenenthellum, Inezia, Lidbeckia,* and *Thaminophyllum* (Bremer and Humphries 1993), was mainly based on a similar habit and similar foliage, the occurrence of many-veined rays and a large stylopodium, the tendency towards the loss of a pappus in some species, and the (not yet corroborated) base chromosome number of $x = 10$. Alternative affiliations were proposed by Reitbrecht (1974) and Baagøe (1977) who considered closer relationships of the genus to *Lasiospermum* (paleate, $x = 9$) based on morphological and ligule micromorphological grounds, respectively, and by Watson et al. (2000) who found a strongly supported sister-group relationship of *Osmitopsis* with *Athanasia* (paleate, $x = 8$) in their molecular study based on cpDNA *ndh*F sequence variation. Since both *Lasiospermum* and *Athanasia* are characterised, however, by deviating base chromosome numbers and anthers with polarised endothecial tissue (unpolarised in *Osmitopsis*), these

alleged relationships are unjustified and can be explained by incomplete taxon sampling and long-branch attraction. Following the present analyses with the unequivocal basal and isolated position of *Osmitopsis* the character expressions of basifixed hairs, paleate receptacles, and anthers with unpolarised endothecial tissue and slender filament collars, together with a base chromosome number of $x = 10$ found in this genus are considered plesiomorphic for the whole tribe and may help in the following to circumscribe the other generic assemblages found in the present study by apomorphic character states.

(b) The *Cotula* clade: This strongly supported monophyletic group of genera consists of members of subtribes Matricariinae *(Cotula, Hilliardia, Hippia, Leptinella, Schistostephium, Soliva)* and Thaminophyllinae *(Adenanthellum, Inezia, Lidbeckia, Thaminophyllum)* sensu Bremer and Humphries (1993). Comprising mainly shrubs and perennial herbs (with annuals occurring in *Cotula, Leptinella,* and *Soliva*) with a plesiomorphic, basifixed indumentum, anthers with unpolarised endothecial tissue and slender filament collars (both conditions plesiomorphic), and the plesomorphic base chromosome number of $x = 10$ (with descending dysploidy in *Cotula* [$x = 8, 9, 10$] but ascending dysploidy in the closely related genus *Leptinella* [$x = 13$]), the monophyly of this clade suggested by our molecular results may be corroborated by the apomorphies of epaleate receptacles and the formation of 4-lobed corollas in tubular florets (with exceptions to this in *Adenanthellum* and *Hippia*).

Further evidence for the unification of members of Bremer´s and Humphries´ (1993) two subtribes into a monophyletic group was suggested by Nordenstam (1987) when describing the new genus *Hilliardia* (Matricariinae) and connecting it with *Adenanthellum* and *Inezia,* (Thaminophyllinae): These genera share ray florets with a bifid or emarginated limb, a branching venation, a papillate upper surface, a reduced tube, and large sessile glands. Additionally, there is further support from phytochemical investigations made by Bohlmann and Zdero (1972a, 1974, 1977, 1982) who found that the guaianolide called zuubergenin from *Hilliardia* is closely related to guaianolides that have been found in *Lidbeckia* and *Inezia* (both Thaminophyllinae) and that *Thaminophyllum* is phytochemically related to *Schistostephium* (Matricariinae).

In fruit-anatomical respects, the *Cotula* clade as circumscribed in our present contribution is highly polymorphic, with a tendency towards the reduction of rib number of achenes from 3-4 (as in *Osmitopsis*) towards 2 and the transition between terete to dorso-ventrally flattened cross-sections. As an exception to this, 10-ribed and only slightly compressed achenes are observed in *Adenanthellum*.

(c) The *Athanasia* grade: This group consists of the rather isolated genera *Inulanthera* and *Ursinia* and a group of members of Matricariinae *(Adenoglossa, Eriocephalus*, *Leucoptera)* and Ursiniinae *(Athanasia, Eumorphia, Gymnopentzia, Hymenolepis, Lasiospermum, Phymaspermum)* sensu Bremer and Humphries (1993) that form a very weakly supported monophyletic group in our nrDNA ITS data analysis, but definitely not in the cpDNA ndh*F* analysis. With the exception of *Eriocephalus* all members of this grade are characterised by the possession of anthers with a polarised endothecial tissue, a character expression apomorphic relative to the unpolarised endothecium found in the more basal *Cotula*-clade and *Osmitopsis,* along with the more advanced genera of the rest of the tribe. Since the analyses of both molecular markers did not reveal the whole group of genera with polarised endothecial tissue as a monophyletic unit, we have to recognise a strong discrepancy between the (micro)morphological and molecular sources of evidence; especially when the strongly deviating position of the clade around *Eumorphia* in the cpDNA *ndh*F analysis is considered.

Within this grade of genera the sister-group relationship of *Athanasia* and *Hymenolepis* receives strong support from the molecular analyses. This corroborates findings of Källersjö (1985, 1991), Bremer and Källersjö (1985), and Bremer and Humphries (1993) who reported a number of synapomorphies for this clade (indumentum of medifixed hairs, funnel-shaped corollas with continuous veins extending into the lobes, floral parts with resin canals, achenes with longitudinal resin ducts in ribs). A further supported monophyletic group consists of the three genera *Eumorphia, Gymnopentzia,* and *Phymaspermum.* Again, there is also strong evidence from morphological studies for this generic grouping because its members share the apomorphies of achenes with 10-12(-18) ribs and a papillose pericarp (Källersjö 1985, Bremer and Humphries 1993). The sister-group relationship of *Ursinia* and *Inulanthera* found in the cpDNA analysis*,* on the other hand, is neither supported by the nrDNA ITS data set nor by any morphological synapomorphies, and is likely to be due the effect of long-branch attraction in the phylogenetic reconstructions.

(d) The *Pentzia* clade: This moderately to strongly supported, monophyletic group of seven genera *(Cymbopappus* B. Nord., *Foveolina* Källersjö, *Marasmodes, Myxopappus* Källersjö, *Oncosiphon* Källersjö*, Pentzia, Rennera* Merxm.) contains further S African members of subtribe Matricariinae sensu Bremer and Humphries (1993). This group was mentioned already by Oberprieler et al. (2006), but they also included *Adenoglossa* and *Leucoptera* of the *Athanasia*-grade. This closely-knit group of genera is characterised by

epaleate receptacles, anthers with unpolarised endothecial tissue and slender filament collars, basifixed hairs (medifixed in *Pentzia*), and a base chromosome number of $x = 9$ (with descending dysploidy in *Myxopappus, Oncosiphon,* and *Pentzia*). It unites genera of shrubby habit *(Cymbopappus, Marasmodes, Pentzia)* and annuals *(Foveolina*, *Myxopappus, Oncosiphon, Rennera*) that were all once united under a broad concept of *Pentzia* which has been dismembered by Källersjö (1988) into the presently acknowledged entities. Following Bremer and Humphries (1993), this group may be supported as monophyletic by achenes with myxogenic cells on the abaxial surface and on the ribs of the adaxial surface (with exceptions in *Oncosiphon* and *Rennera*) and with an adaxially long auricle (with a secondary loss of a corona in *Oncosiphon, Rennera* and some *Pentzia* species). Despite a considerable similarity concerning these achene characters, alleged close relationships with the N hemisphere Matricariinae genera *Matricaria, Otospermum,* or *Tripleurospermum* were not supported by our present analyses.

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Chapter 3

Phylogeny of *Leptinella* (Anthemideae, Compositae) inferred from sequence information

Introduction

New Zealand is an ancient continental landmass, which was separated from Gondwana approximately 80 Ma ago (Cooper and Millener 1993, McLoughlin 2001, Neall and Trewick 2008) and has undergone several geological and climatical events which formed a very diverse topography with a great diversity of biomes (Winkworth et al 2005, Linder 2008). Large parts of New Zealand were inundated during the Oligocene (Cooper and Millener 1993, Trewick and Morgan-Richards 2005). The uplift of the Southern Alps was dated to c. 12 mya, but the alpine habitat arose only in the last 5 my (Chamberlain and Poage 2000, Winkworth et al. 2005). In the Pleistocene the glacial cycles and volcanism played an important role in the evolution of the environment of New Zealand (Winkwoth et al. 2005).

In the past, the origin of the flora and fauna of New Zealand was discussed (see Trewick et al. 2007). Molecular data have shown that many plant and animal groups had reached New Zealand by long-distance dispersal events, Gondwana vicariance on the other hand seems to be less important (for more details see Winkworth et al. 2002a, Sanmartin and Ronquist 2004, Sanmartin et al. 2007, Trewick et al. 2007, Goldberg et al. 2008). Recent molecular studies have illustrated that the evolution of plants from New Zealand is often rapid (e.g. Breitwieser et al. 1999, Mitchel and Heenan 2000, Lockhart et al. 2001, Wagstaff et al. 2002, Wagstaff and Breitwieser 2004, Meudt and Simpson 2006, Ford et al. 2007, Mitchell et al. 2009b). The rapid radiation of New Zealand plants led to morphologically and ecologically diverse but genetic very similar species. Furthermore, the evolutionary history of New Zealand plant groups is often complicated by hybridization and polyploidy (reviewed in Morgan-Richards et al. 2009). Polyploidy in particular is increasingly recognized as an important process in plant evolution and as a major mechanism of adaptation and speciation (Soltis et al. 2004, Seehausen 2004).

One remarkable plant group of the southern hemisphere is the genus *Leptinella* (Compositae, Anthemideae). The genus comprises 42 taxa (34 species and additional seven subspecies and one variety) occurring in Australia, New Guinea, New Zealand, South America, on the Chatham Islands and on the sub-Antarctic islands. In New Zealand, which is clearly the centre of diversity with 29 taxa being endemic, *Leptinella* occurs in open habitats from coastal to high alpine areas. *Leptinella* consists of small perennial and procumbent herbs with pedunculate capitula, inflated female corollas, and outer female and inner functionally male disc florets. Additionally, *Leptinella* is characterised by the basic

chromosome number $x = 13$. The genus forms an impressive polyploid complex with chromosome numbers ranging from tetraploid level to a chromosome set of $2n = 24x$ (Hair 1962, Lloyd 1972c, Beuzenberg and Hair 1984).

Leptinella belongs to the tribe Anthemideae and within it is a member of the basal southern hemisphere subtribe Cotulinae (Oberprieler et al. 2007, Himmelreich et al. 2008). A close relationship of *Leptinella* with *Cotula* and *Soliva* was pointed out by several authors, but the relationships among these genera remains unclear (e.g. Lloyd and Webb 1987, Bremer and Humphries 1993, Oberprieler et al. 2006, Himmelreich et al. 2008). Especially, the separation of *Cotula* and *Leptinella* is questionable (Lloyd 1972c). *Leptinella* was described as a genus by Cassini (1822), but was later reduced to an infrageneric rank within *Cotula* by Hooker (1864) and this concept has been followed by most of the subsequent authors. However, Lloyd and Webb (1987) reinstated *Leptinella* at generic rank, primarily because of the inflated female corollas and the basic chromosome number of $x = 13$. Both characters are unique within the tribe Anthemideae.

Lloyd (1972c) divided *Leptinella* into three subgenera: *Oligoleima* (Australia, New Guinea; seeds compressed and with broad margin), *Leptinella* (New Zealand, South America, sub-Antarctic islands; seeds not compressed, branches single, rhizome internodes long), and *Radiata* (New Zealand, sub-Antarctic islands; seeds not compressed, branches usually clustered, rhizome internodes often short). Within these subgenera, the delimitation in species and subspecies is often difficult and has been discussed by several recent botanists (Lloyd 1972c, Druce 1993, Wilson 1994, New Zealand Plant Conservation Network 2009, de Lange et al. 2009). Hybridization occurs frequently among species of subgenus *Leptinella* and less frequently in subgenus *Radiata* (Lloyd 1972c). Some hybrids are widespread (e.g. *L. dioica* subsp. *dioica* × *L. squalida* subsp. *mediana*). Artificial crosses between different species and even subgenera lead to fertile offspring (Lloyd 1975a). Artificial and natural hybrids between taxa with different chromosome numbers are possible, but there is no information about the ploidy level and the fertility of the offspring (Lloyd 1972c, Lloyd 1975a).

The present study focuses on the intergeneric and infrageneric relationships of *Leptinella*: Is *Leptinella* monophyletic and are the subgenera accepted by Lloyd (1972c) natural groups? What role played hybridization and polyploidization in the evolutionary history of the genus? Secondly, we focus on the divergence time and the biogeographic implications in the genus *Leptinella*. Are the results of this study compatible to previous studies dealing with other plant groups from New Zealand?

Material and methods:

Plant material. In our study, we provide a almost complete sampling of the genus *Leptinella*, by including 59 individuals from 40 taxa out of 42. We included also the hybrid of *L. dioica* subsp. *dioica* and *L. squalida* subsp. *squalida* and the informal undescribed entity *L.* "Seal" (Druce 1993). We analyzed also species of the presumed sister genus *Cotula*, and of all other members of the subtribe Cotuliniae *(Adenanthellum, Hilliardia, Hippia, Inezia, Lidbeckia, Schistostephium, Soliva, Thaminophyllum)*. As outgroup taxa, we chose *Inulanthera, Osmitopsis*, and *Ursinia* (see Oberprieler et al. 2007, Himmelreich et al. 2008). Some ITS sequences of the Cotulineae and outgroup came from a former publication (Himmelreich et al. 2008). Herbarium vouchers and Genebank information are provided in Tab. 3-1.

Marker choice, DNA extraction, PCR amplification, and sequencing. Three markers were used for the present study, from both nuclear and chloroplast DNA. From nrDNA, the internal transcribed spacer (ITS) was chosen. This maker was successfully used in different phylogenetic studies within genera of the tribe Anthemideae (e.g. Watson et al. 2002, Vogt and Oberprieler 2006, Tkach et al. 2007, Lo Presti and Oberprieler 2009). A number of cpDNA markers were screened [*rpl*20-5'*rps*12, *psb*B-*psb*F, *psb*A-*trn*H (Hamilton 1998); *trn*C-*pet*N, *pet*N-*psb*M (Lee and Wen 2004); *trn*L-*trn*K, *trn*L-*trn*F (Taberlet et al. 1991)]. Finally, the *psb*A-*trn*H and *trn*C-*pet*N intergenic spacers were chosen because these markers were phylogenetically most informative and more or less easy to sequence.

DNA was extracted from herbarium specimens or from silica dried leaves using a modification of the CTAB method (Doyle and Doyle 1987). For the amplification of nrDNA ITS we used the same primer and PCR conditions as described in Himmelreich et al. (2008). PCR amplifications of *psb*A-*trn*H were performed using primers *psb*A and *trn*H (Hamilton 1998), and the amplification of the *trn*C-*pet*N intergenic spacer were performed using the primer *trn*C (Demesure et al. 1995) and *pet*N1R or *pet*N2R (Lee and Wen 2004).

PCR amplifications were performed with 0.2 µM dNTP's, 0.02 µM of each primer, 0.2 U Taq polymerase (Qbiogene) in 12.5 µl 1x Buffer. High dilution of the DNA extracts and the addition of Q-solution (Qiagen) improved the amplification results of difficult samples. Amplification of cpDNA markers was carried out with the following temperature profile: 2 min at 94 °C, then 35 to 40 cycles of 1 min at 94 °C, 1 min at 53 °C, 1 min at

Tab. 3-1: Species analysed in this study, their accession data and additional information. ITS sequences marked with an asterisk came from Himmelreich et al. 2008. Cloned individuals are marked with #.

Taxon	Distr.	Accession	Genbank accession number		
			ITS	*psb*A-*trn*H	*trn*C-*pet*N
Leptinella					
L. albida (D. G. Lloyd) D. G. Lloyd & C. J. Webb #	NZ	New Zealand: Otago, Dunstan Mountains, Leaning Rock, 1650m, G41 229650, 02.02.1984, Given 13589 (CHR 416102)	5 clones	x	x
L. altilitoralis (P. Royen & D. G. Lloyd) D. G. Lloyd & C. J. Webb	NG	Indonesia: Irian Jaya, Mt. Trikora, Somalak valley, high cliffs to the west, 3959 m, 10.08.1984, Mangen 959 (L)	x	x	
L. atrata (Hook. f.) D. G. Lloyd & C. J. Webb subsp. *atrata* #	NZ	New Zealand: Canterbury, Brocken River skifield, 1700 m, K34 024 559, 02.03.2005, Heenan (CHR 573419)	4 clones	x	x
L. atrata subsp. *luteola* (D. G. Lloyd) D. G. Lloyd & C. J. Webb	NZ	New Zealand: Marlborough, Seaward Kaikoura Range, Kowhai Stream Headquarters, O31 598 829, 173°36'E 42°15'S, 04.03.1994, Courtney (CHR 515373)	x	x	x
L. calcarea (D. G. Lloyd) D. G. Lloyd & C. J. Webb #	NZ	New Zealand: NW Nelson, Westhaven, cult., 20.12.2004, Heenan (CHR 573524)	5 clones	x	x
L. conjuncta Heenan #	NZ	New Zealand: Cultivation, Landcare Research, ex. Fiddlers Flat, Maniototo, Otago, 09.12.2004, Heenan (CHR 572831)	7 clones	x	x
L. dendyi (Cockayne) D. G. Lloyd & C. J. Webb A #	NZ	Germany: Cultivation, Botanical Garden University Regensburg, 19.09.04, Himmelreich (CHR)	4 clones	x	x
L. dendyi (Cockayne) D. G. Lloyd & C. J. Webb B		New Zealand: Canterbury, Broken River Skifield, screefield, 19.12.2006, Rupprecht & Himmelreich NZ25/02 (CHR)	x	x	x
L. dioica Hook. f. subsp. *dioica* A #	NZ	Germany: Cultivation, Botanical Garden Martin-Luther University Halle-Wittenberg, 01.08.2004, Himmelreich (CHR)	2 clones	x	x
L. dioica Hook. f. subsp. *dioica* B #		New Zealand: Otago, Kakanui, 18.12.2004, Heenan (CHR 573394)	5 clones	x	x
L. dioica Hook. f. subsp. *dioica* C #		New Zealand: Otago, Tunnel Beach Dunedin, 18.12.2004, Heenan (CHR 573393)	3 clones	x	x
L. dioica subsp. *monoica* (D. G. Lloyd) D. G. Lloyd & C. J. Webb #	NZ	New Zealand: Wellington, Hutt (Gracafield), Waiwhetu Stream, 0m, R27 696 950, 41°14'S 174°54'E, 15.06.1993, de Lange 2117 (CHR 497619)	4 clones	x	x
L. cf. *diocia* #	-	Germany: Cultivation, Botanical Garden University Regensburg, 07.06.2004, Himmelreich (CHR)	6 clones	x	x
L. dispersa (D. G. Lloyd) D. G. Lloyd & C. J. Webb subsp. *dispersa* A	NZ Sub	New Zealand: Cultivation, ex Wellington, Baring Head Lakes, Lake Kohangatera, 09.10.2004, de Lange 6262 & de Lange (AK 299438)	x	x	x
L. dispersa (D. G. Lloyd) D. G. Lloyd & C. J. Webb subsp. *dispersa* B		New Zealand: Stewart Island, Mason Bay, way from Mason Bay Hut to beach, before bridge, 01.01.2007, Rupprecht & Himmelreich NZ 42 (CHR)	x	x	x
L. dispersa subsp. *rupestris* (D. G. Lloyd) D. G. Lloyd & C. J. Webb	NZ	New Zealand: Taranaki, Patea, 10m, Q22 372 585, 39°46'S 174°29'E, 10.11.1994, Ogle 2830 & Barkla (CHR 500175)	x	x	x
L. drummondii (Benth.) D. G. Lloyd & C. J. Webb	Aus	Australia: Western Australia, at Blackwood crossing on Great North Road near Hut Pool, 34°5'19.700" S 115°17'30.500" E, 15.12.2003, Hislop 3145 (PERTH 6896642)	x	x	
L. featherstonii F. Muell.	Cha	New Zealand: Rekohu (Chatham Island), Western Reef, 15.01.2006, de Lange CH377 & Sawyer (AK 294924)	x	x	x
L. filicula (Hook. f.) Hook. f. A	Aus	Australia: New South Wales, Northern Tablelands, New England National Park, Banksia Point, 0,5 km SSW of Point Lookout, 1400 m, 30°29'40"S 152°24'20"E, 30.03.2002, Telford 12506 (CANB)	x	x	x
L. filicula (Hook. f.) Hook. f. B		Australia: New South Wales, Southern Tablelands, Northern Kosciusko National Park, junction of Boundary Road and Diggers Creek Road, 1110 m, 35°23'35"S 148°38'46"E, 15.02.1999, Taws 879 (CANB)	x	x	x
L. filiformis (Hook. f.) D. G. Lloyd & C. J. Webb #	NZ	New Zealand: Cultivation, Landcare Research, ex Canterbury, Hanmer, 23.12.2004, Heenan (CHR 573526)	1 clone	x	x
L. goyenii (Petrie) D. G. Lloyd & C. J. Webb	NZ	New Zealand: Central Otago, Queenstown, Remarkables, near Lake Alta, 1850 m, 19.01.1994, Breitwieser & Vogt 1050 (B)	x	x	

Tab 3-1: Continued

Taxon	Distr.	Accession	Genbank accession number		
			ITS	*psb*A-*trn*H	*trn*C-*pet*N
L. intermedia (D. G. Lloyd) D. G. Lloyd & C. J. Webb #	NZ	New Zealand: Canterbury, Grampian Range, above Hakataramea Saddle, 1500m, collected from cultivated plants, 17.03.1971, Lloyd 68044 (CANU 17847)	5 clones	x	
L. lanata Hook. f.	Sub	New Zealand: Campbell Island, St. Col Peak, immediately southwest of summit of ridge leading to sea 0.5km from fence, 21.01.1976, Given 9242 (CHR 303773)	x	x	x
L. longipes Hook. f. A	Aus	Australia: Victoria, East Gippsland, Wallagaraugh River, c. 1 km dowstream from Gipsy Point settlement, W bank, 10 m, 37°29'S 149°41'E, 21.10.1991, Walsh 3136 (S)	x	x	x
L. longipes Hook. f. B		Australia: Australian Commonwealth Territory Jervis Bay, Jevery Bay National Park, foreshore of St Georges Basin, c. 1 km S of Park boundary, 35°07'43"S 150°39'27"E, 2 m, 23.11.1996, Taws 693 (CANB)	x	x	x
L. maniototo (P&rie) D. G. Lloyd & C. J. Webb	NZ	New Zealand: Canterbury, Cass, Cattlehole, alt. 600 m, L34 107 941, Breitwieser 2198, 6.12.2005 (CHR)	x	x	x
L. minor Hook. f. A	NZ	New Zealand: Banks Peninsula, Taylor´s Mistake, N36 927 359, 43°35' 172°47'E, 26.09.1984, Stolp 12 (CHR 418818)	ITS2 fehlt	x	x
L. minor Hook. f. B		New Zealand: Canterbury, Port Hills, Mt Pleasant, Lyttelton Scenic Reserve, below rock face, 02.12.2006, Rupprecht & Himmelreich NZ 04 (CHR)	x	x	x
L. nana (D. G. Lloyd) D. G. Lloyd & C. J. Webb A #	NZ	New Zealand: Nelson, Rai River on Bulford Road, 30m, 23.10.1982, Given 13106, Pankhurst & Hall (CHR 403456)	5 clones	x	x
L. nana (D. G. Lloyd) D. G. Lloyd & C. J. Webb B #		New Zealand: Landcare Research, ex Port Hills, Canterbury, 20.12.2004, Heenan (CHR 573522)	4 clones	x	x
L. pectinata (Hook. f.) D. G. Lloyd & C. J. Webb subsp. *pectinata*	NZ	New Zealand: Marlborough, Murphy, near Molesworth station, Awatere Valley, bare soil (greywacke derived), exposed summit at Murphy, 1820 m, 06.02.2006, Ford 613/06 (CHR)	x	x	x
L. pectinata subsp. *villosa* (D. G. Lloyd) D. G. Lloyd & C. J. Webb A #		Germany: Botanical Garden University Regensburg, ex Arktisch-Alpiner-Garten Chemnitz, ex New Zealand, The Remarkables, 28.07.2004, Himmelreich 5 (CHR)	6 clones AM774460*	x	x
L. pectinata subsp. *villosa* (D. G. Lloyd) D. G. Lloyd & C. J. Webb B #	NZ	New Zealand: Otago, Old Wowan Range, 22.12.2004, Heenan (CHR 573400)	3 clones	x	x
L. cf. *pectinata* subsp. *villosa*		New Zealand: Cultivation, ex Nevis Valley, Otago, E2195207 N5554190, 23.01.2007, Barkla (CHR)	x	x	x
L. pectinata subsp. *willcoxii* (Cheeseman) D. G. Lloyd & C. J. Webb#	NZ	New Zealand: Otago, Richardson Mountains, Invincible Spur, 1800m, 44°42'S 168°32'E, 03.03.1995, Burke 404 (CHR 518999)	2 clones	x	x
L. plumosa Hook. f. A	Sub	Australia: Heard Island, Paddick Valley, XXXX, 04.02.2004 (ADT7536)	x	x	x
L. plumosa Hook. f. B		Australia: Macquarie Island, west side of the Isthmus at edge of beach, 1.5m, 54°30'S 158°51'E, 14.02.1985, Seppelt (ADT)	x	x	x
L. potentillina F. Muell,	Cha Sub	New Zealand: Chatman Islands, Cascade Gorge at mouth of stream, Bank of sandy soil, 3m, 25.02.1985, Given 13926 (CHR 417498)	x	x	x
L. pusilla Hook. f. #	NZ	New Zealand: Westland, Whataroa River, historic gold mine, turf on river terrace, 22.03.2002, Wagstaff (CHR 559133)	5 clones	x	x
L. pyrethrifolia var. *linearifolia* (Cheeseman) D. G. Lloyd & C. J. Webb	NZ	New Zealand: Cultivation, Oratia Native Plant Nursery Auckland, ex Red Hills, 22.02.2007, Korver (CHR)			x
L. pyrethrifolia var. *linearifolia* (Cheeseman) D. G. Lloyd & C. J. Webb #		New Zealand: Marlborough, Red Hills, 41°38'S 173°3'E, Shallow soil, 09.04.1997, Heenan & de Lange (CHR 512605)	7 clones	x	
L. pyrethrifolia (Hook. f.) D. G. Lloyd & C. J. Webb var. *pyrethrifolia* A #	NZ	Germany: Cultivation, Botanical Garden University Regensburg, ex Arktisch-Alpiner-Garten Chemnitz, 12.05.2004, Himmelreich (CHR)	4 clones	x	x
L. pyrethrifolia (Hook. f.) D. G. Lloyd & C. J. Webb var. *pyre*thrifolia B #		New Zealand: Canterbury, Brocken River, Craigieburn Range, 02.03.2005, Heenan (CHR 573418)	5 clones	x	x

Tab 3-1: Continued

Taxon	Distr.	Accession	Genbank accession number		
			ITS	*psb*A-*trn*H	*trn*C-*pet*N
L. reptans (Benth.) D. G. Lloyd & C. J. Webb	Aus	Australia: South Australia, Picaninnie Ponds, c. 30 km direct SSE of Mt Gambier, (38°3'03" S 140°30'56" E), 26.11.2006, Thomson 930	x	x	x
L. rotundata (Cheeseman) D. G. Lloyd & C. J. Webb	NZ	New Zealand: Cultivation, University of Canterbury Christchurch (Lloyd 9106-1), 17.01.2007, Rupprecht & Himmelreich NZ55 (CHR)	x	x	x
L. scariosa Cass. A	SAm	Chile: Los Lagos, Portezuelo Queulat, along road, 01.02.1997, Wardle & Wagstaff 97118 (CHR 514083)	x	x	x
L. scariosa Cass. B		Chile: 39°49'56,1"S, 73°24'28,2"W, ~0m, 26.11.2006, Alvarez (CHR)	x	x	x
L. „Seal“	NZ	New Zealand: Wellington, Petone, Percy Scenic Reserve, cultivated specimen from Seal Island, 17.12.1992, de Lange 1906 (CHR 482831)	x	x	x
L. serrulata (D. G. Lloyd) D. G. Lloyd & C. J. Webb A #	NZ	New Zealand: Canterbury, Land just south of Waikmakariri River, 90m, 43°28'S 172°23'E, farmland, 11.01.1993, Ruth 9/DPR (CHR 506265)	5 clones	x	x
L. serrulata (D. G. Lloyd) D. G. Lloyd & C. J. Webb B		New Zealand: Otago, near Wanaka, Mt Iron, above walkway, 27.12.2006, Rupprecht & Himmelreich NZ32 (CHR)	x	x	x
L. squalida subsp. *mediana* (D. G. Lloyd) D. G. Lloyd & C. J. Webb A #	NZ	Germany: Cultivation, Botanical Garden University Regensburg, 07.06.2004, Himmelreich (CHR)	4 clones	x	x
L. squalida subsp. *mediana* (D. G. Lloyd) D. G. Lloyd & C. J. Webb B #		New Zealand: Canterbury, Craigieburn Forest Park, middle reaches of Basin Creek, 930m, 43°5'S 171°27'E, 13.01.1998, Bellingham 615 (CHR 515342)	4 clones	x	x
L. squalida subsp. *mediana* x *L. dioica* subsp. *dioic*a	NZ	New Zealand: Canterbury, Banks Peninsula, Hinewai Reserve, lawn near the house of the manager Hugh Wilson, 02.12.2006, Rupprecht & Himmelreich NZ01 (CHR)	x	x	x
L. squalida Hook. f. subsp. *squalida* A #	NZ	New Zealand: Gisborne, Waikura Valley, 38°38,4'S 177°42,2'E, 260m, river terrace open forest, 01.1991, Druce APD837 (CHR 469762)	5 clones	x	x
L. squalida Hook. f. subsp. *squalida* B		New Zealand: Mt Taranaki / Egmont, Egmont National Park, Pembroke Road, East Egmont, traffic island with public toilet near Mountain Lodge, 09.12.2006, Rupprecht & Himmelreich NZ10 (CHR)	x	x	x
L. tenella (Cunn.) D. Llyod & C. J. Webb A #	NZ	New Zealand: Aukland, Upper Waitemata Harbour, 36°43,4'S 174°41.7'E, 0m, 08.12.1990, Sykes 258/90, (CHR)	5 clones	x	x
L. tenella (Cunn.) D. Llyod & C. J. Webb B		New Zealand: Taranaki, beside old Powerhouse, near Powerhouse Road between Kakaramea and Patea, meadow, 07.12.2006, Rupprecht & Himmelreich NZ 09 (CHR)	x	x	x
L. traillii (Kirk) D. G. Lloyd & C. J. Webb	NZ	New Zealand: Stewart Island, Mason Bay, way from Mason Bay Hut to Freshwater Hut, 02.01.2007, Rupprecht & Himmelreich NZ 43 (CHR)	x	x	x
L. traillii subsp. *pulchella* (Kirk) D. G. Lloyd & C. J. Webb	NZ	New Zealand: Southland, Bluff (near Invercargill), Bluff Hill, Foveaux walkway, between carpark Stirling Point and Lookout Point, 03.01.2007, Rupprecht & Himmelreich NZ44B (CHR)	x	x	x
L. traillii (Kirk) D. G. Lloyd & C. J. Webb subsp. *traillii*	NZ	New Zealand: Stewart Island, way between Freshwater Hut and Mason Bay Hut, near bridge, 01.01.2007, Rupprecht & Himmelreich NZ 40 (CHR)	x	x	x
L. wilhelminensis (P. Royen) D. G. Lloyd & C. J. Webb	NG	Indonesia: Irian Jaya, Mt. Trikora, east rim of upper Somalak valley, near landslide, alt. 3960 m, 11.08.1984, Mangen 1011 (L)	x	x	x
other Cotuliniae					
Adenanthellum osmitoides (Harvey) B. Nord.	Afr	South Africa: Natal, Paulpietersburg, 12.12.1975, Hilliard & Burtt 8581 (S)	AM774445*	x	x
Cotula abyssinica Sch. Bip. ex A. Rich.	Afr	Tanzania: Arusha Prov., Mt. Meru, E slope, inner slope of N portion of crater, 2700-3050 m, 3°13'S 36°47'E, 17.01.1970, Bremer 38 (S)	x	x	x
Cotula alpina Hook. f.	Aus	Australia: New South Wales, S Tablelands, South along internal road, c. 2 km south of Kydra Reefs, 36°24'22"S 149°20'56"E, 1170 m, 10.03.2002, Coveny 19004 & Orme (NSW 488340)	x (only ITS1)	x	x

Tab 3-1: Continued

Taxon	Distr.	Accession	Genbank accession number ITS	*psb*A-*trn*H	*trn*C-*pet*N
Cotula australis (Spreng.) Hook. f.	Afr Aus NZ	New Zealand: Canterbury, South Branch Waimakariri, 43°26'S 172°38'E, 29.09.1998, Wagstaff 98.086 (CHR)	AM774448*	x	x
Cotula coronopifolia L.	Afr Aus NZ	Greece: Nom. Etolia-Akarnania, Ep. Vonitsis 8,9 km SO Astakos, 10 m, 38°29´30"N 21°8´,14.05.1994, Willing 31547 (B)	x	x	x
Cotula mexicana (DC.) Cabrera	SAm	Bolivia: Dpto. La Paz, Prov. P.D. Murillo, debajo de Pongo, sobre camino antiguo, pasando el rio y subiendo a la cascada, 16°19'S, 67°56'W, 3700m, 12.11.2006, Beck 32503 (LPB)	x	x	x
Cotula turbinata L.	Afr	South Africa: 3418 AD, Cape Point Nature Reserve, 07.10.1985, Källersjö 52 (S)	x	x	x
Hilliardia zuurbergensis (Oliver) B. Nord.	Afr	South Africa: Natal, near Mt. Alida, Eweka Estates, 16.19.1991, Hilliard & Burtt 19118 (S)	AM774454*	x	x
Hippia pilosa (P. Bergius) Druce	Afr	South Africa: Cape Province, Disrict 3321 DA, Rooiberg Mountain, 4200ft, 2.11.1988, Vlok 2041(S)	AM774455*	x	x
Inezia integrifolia (Klatt) E. Phillips	Afr	South Africa: Mpumalanga (Eastern Transvaal), Rosehaugh midway between Sabie and Nelspruit, 700 m, 08.01.1997, Bremer & Bremer 3812 (S)	AM774457*	x	x
Lidbeckia pectinata P. Bergius	Afr	South Africa: Cape, Tulbagh, middle slopes of Roodsandberg on the farm Twee Jongegezellen, 400m, 23.10.1983, Rourke 1812 (S)	AM774462*	x	x
Schistostephium crataegifolium Fenzl ex Harv. & Sond.	Afr	South Africa: Natal, Lions River District, Fort Nottingham Commonage, 04.05.1977, Hilliard & Burtt 10331 (S)	AM774470*	x	x
Soliva mutisii Kunth	SAm	Ecuador: Prov. Azuay, Cuenca, in weedy vegetation, in hard gravelly ground, c. 2550 m, 25.09.1955, Asplund 17806 (S)	x	x	
Soliva pterosperma (Juss.) Less.	SAm	Australia: New South Wales: Mylestom, North Beach Camping Site, Lawns on dune sand, 22.11.1989, Anderberg & Anderberg 7016 (S)	x	x	x
Soliva valdiviana Phil.	SAm	Chile: Region de los Lagos, 39°50'18,4"S, 73°24'02,2", 22m, 26.11.2006, Alvarez (CHR)	x	x	x
Taminophyllum latifolium Bond	Afr	South Africa: Cape Province, Caledon Div., Hermanus, above the houses at Voelklip, 06.09.1974, Esterhuysen 33604 (S)	AM774472*	x	x
other Anthemideae					
Inulanthera leucoclada (DC.) Källersjö	Afr	South Africa: Royal Natal National Park, locally common shrub next to path to Tugela Gorge, 1560 m, 2828 DB Bethlehem, 06.03.1986, Steiner 1221 (S)	AM774458*	x	x
Osmitopsis asteriscoides Cass.	Afr	South Africa: Western Cape, near the entrance to Cape Point National Park, 29.1.2003, Ueckert & Oberprieler 10279 (Herbarium Oberprieler)	AM774466*	x	x
Ursinia anthemoides (L.) Poir.	Afr	South Africa: Cape Province, Namakwaland Division: Kamiesbergpas, c. 5 km ENE of Kamieskroon, 800-1000 m, 30°12'S 17°58'E, 12.09.1993, Strid & Strid 37382 (S)	AM774473*	x	x
Ursinia crithmoides Poir.	Afr	South Africa: Cape Prov., Mossel Bay Div., Robinson Pass, S side, above road, 850 m, 33°54'S 22°2'E, 3322 CC Outdtshoorn, 12.10.1972, Bremer 313 (S)	x	x	x
Outgroup BEAST					
Calendula officinalis L.		Wagstaff and Breitwieser 2002	AF422114		
Erigeron annuus Pers.		Noyes 2000	AF118489		
		Schlaepfer et al. 2008		EU337693	
Helianthus annuus L.		Vischi, M. unpublished	AM490230		
		Ambrosini et al. 1992		X60428	
Helychrysum lanceolatum Kirk		Smissen and Breitwieser (unpublished)	EU007682		
		Ford et al. 2007		EF187698	
Senecio glaberrimus DC.		Pelser et al. 2007	EF538338		
		Pelser et al. 2007		EF538081	
Symphyotrichum cordifolium (L.) G.L. Nesom		Kress et al. 2005	DQ005972		
		Kress et al. 2005		DQ006144	
Tagetes patula L.		Serrato-Cruz et al. (unpublished)	DQ862121		

x - sequences will be submitted to genebank

Distribiution: Afr- Africa, Aus - Australia, Cha - Chatham Islands, NG - New Guinea; NZ - New Zealand, SAm - South America, Sub - sub-Antarctic islands

72° C, with a final extension of 8 min at 72 °C. The PCR products were purified with QIAquick PCR Purification Kit (Qiagen) or with the Agencourt AMPure Kit (Beckman Coulter). Cycle sequencing reactions were carried out with the same primers as in the amplification. The DTCS Sequencing Kit (Beckman Coulter) was used following the manufacturer's manual, and the fragments were separated on a CEQ8000 sequencer.

As polyploid species may often contain several different copies of a nuclear genetic marker, direct sequencing proves to be technically difficult in some cases. For this purpose, cloning of nrDNA ITS is necessary. Permission to destructively sample material from the Allan herbarium (CHR), including own collection was granted only on the condition that cloning not be undertaken with any sample from localities where consulting with local Māori indicated opposition to genetic manipulation of New Zealand native plants proceeding. This condition precluded cloning of nrDNA ITS from some individuals with heterozygotic or partly difficult to read sequence. Cloning of nrDNA ITS was applied for 24 samples (see Tab. 3-1). After amplification, PCR products were excised from agarose gel and cloned into *Escherichia coli*, following the protocol of the manufacturer of the pGEM®-T Easy Vector System I (Promega). Subsequently, clones were picked and up to ten cloned DNA fragments were amplified by colony PCR, purified and sequenced.

Sequence alignment and phylogenetic reconstructions. Sequences were aligned with BioEdit vers. 7.05.2 (Hall 1999). The program GapCoder (Young and Healy 2003) was used to code indels according to the simple gap coding method described by Simmons and Ochoterena (2000). In the alignment of *trn*C-*pet*N, a 20 bp long sequence (between alignment positions 330 and 350) was excluded from further analyses, because this part consists of a variable number of A´s or T´s and an unequivocal alignment was impossible.

In a first step, ITS and the combined cpDNA markers were analysed separately, and then all three markers were combined. Bayesian inference (BI) approach was performed with MrBayes vers. 3.1.2. (Ronquist and Huelsenbeck 2003), with the following initial settings: nst = 6 and rates = invgamma for nucleotide data (GTR+I+Γ model). For the 0/1 matrix resulting from gap coding, the specified model for restriction sites as implemented in MrBayes were used with variable coding. For partitioned analyses, substitution parameters and rates of substitutions were allowed to vary across partitions. Two runs with four chains were performed for 5.000.000 (cpDNA), 8.000.000 (ITS) or 9.000.000 (combined dataset) iterations and sampled every 100th generation. Two independent analyses with different heating temperatures (0.2 and 0.06) were performed. The first 25 %

to 37.5 % trees were discarded as burn-in, this was well after the chains had reached stationary in the likelihood and in all other parameters and the split frequency was < 0.01. A 50 % majority rule consensus tree of the remaining trees was computed.

Alternatively, Maximum Parsimony (MP) analyses were performed using the heuristic search algorithm of PAUP* version 4.0b10 (Swofford 2002) with ACCTRAN, MULPARS and TBR branch swapping in action. Character states were specified unordered and unweighted. 10.000 random addition sequence replicates were performed. Due to the large number of most parsimony trees, nchuck was set to 10. Support of branches was evaluated using bootstrapping (Felsenstein 1985) with the following settings: 10.000 bootstrap replicates, 10 random addition sequence replicates per bootstrap replicate with nchuck set to 10.

Because phylogenetic models assume a hierarchical, bifurcating tree that may not apply to some lineages, we explored an alternative network method that allows reticulate evolution. The networks were constructed to examine more closely the relationship within the species from New Zealand in the *Leptinella* main group (for details see results below). The networks were created with uncorrected p-distances and neighbor-net method using the program SplitsTree vers. 4.10 (Huson and Bryant 2006).

Calibration. BEAST vers. 1.4.6 (Drummond and Rambaut 2007) was used to estimate divergence times from all three markers simultaneously with gaps treated as missing data. The partitionated BEAST input file was created with BEAUti vers. 1.4.6 (Drummond and Rambaut 2007) and edited manually to allow parameters to be estimated independently amongst data partitions. The substitution model was the same as in MrBayes (GTR+I+Γ). An uncorrected lognormal molecular clock model and a Yule prior for branch lengths were used as suggested by the BEAST manual.

Several short runs were performed to examine the optimal performance, and their results were used to adjust the parameters of the two final runs with 12.000.000 generations (sampled every 1000th). Convergence was assessed using Tracer ver. 1.3 (Drummond and Rambaut 2007). After discarding the first 1.000.000 samples as burn-in, the trees and parameters of the two runs were combined. The samples of the posterior were summarised on the maximum clade credibility tree using TreeAnnotator ver. 1.4.6 (Drummond and Rambaut 2007) with a posterior probability limit set to 0.5 and summarizing mean node heights.

As no fossils are known for *Leptinella* or other members of the southern hemisphere Anthemideae, we had to use external and geological calibration. For this purpose, we included members of the tribes Astereae, Calenduleae, Gnaphalineae, Heliantheae, Senecioneae and Tageteae in the analysis (see Tab. 3-1). The calibration of the root node corresponded to the crown age of the subfamily Asteroideae. There are three different age estimations for this group (26 - 29 my, nonparametric rate smoothing dating with outgroup fossil as calibration point, Kim et al. 2005; 29 - 30 Ma, penalized likelihood analysis, Hershkovitz et al. 2006; 35 - 39 Ma, rate calibration using substitution rates from other angiosperm families, Kim et al. 2005). We used a normal distribution with a mean of 31.3 Ma (the midpoint of the three available calibration dates) and a 95 % confidence interval of 26.2 to 36.4 Ma as our prior for the root node.

Ambrosia is a member of the tribe Heliantheae and we used the earliest *Ambrosia*-type pollen to calibrate the age of the node connecting Heliantheae (represented by *Helianthus annus*) and Tageteae (represented by *Tagetes patula*). Graham (1996) estimated the age of the fossil to be 22 - 35 Ma. The 95 % confidence interval for our prior lay between 25.07 - 34.93 Ma with the mean at 30.0 Ma.

Leptinella featherstonii is endemic to the Chatham Islands which emerged from the sea level 4 - 2 Ma ago (Campbell 1998, Campbell et al. 2006, Landis et al. 2008). Genetic evidence from several studies are consistent with this assumption (see Goldberg et al. 2008 and citation within). Since the date may be not accurate, we allowed a greater range with the mean at 3.0 Ma and a 95 % confidence interval between 0.9 - 5.0 Ma for the split between *L. featherstonii* and the remaining members of the *pyrethrifolia*-group (see results).

Results

Phylogenetic analysis and topology. Tab. 3-2 shows the number of sequences/ individuals, number of characters and indel information for each dataset. The table also compares the statistics from the parsimony analyses. The ITS alignment for the complete dataset is 505 bp long, and includes 48.2 % parsimony informative (PI) characters (including gap information). The alignment of the combined cpDNA is two and a half time as long as the ITS dataset (1213 bp), but consists only of one third PI characters (15.4 %). Fewer PI characters of the chloroplast dataset result in a lower resolution of the trees. The combined dataset of all three markers has almost 25 % PI characters. Besides the percentage of PI characters, the cpDNA and the ITS dataset also vary in the indels length: In the two chloroplast markers, the average length of indels is 16.2 bp with indels ranging from 1 to 328 bp. In contrast to this, the average length of indels in the ITS dataset is only 1.3 bp (1-3 bp).

Tab. 3-2: Comparison of phylogenetic analysis statistics for the various molecular datasets analyzed in this study.

	complete dataset			***Leptinella* main group**	
	cpDNA	ITS	combined	cpDNA	ITS
number of individuals	77	81	81	55	57
number of sequences	77	163	163	55	139
base pairs in alignment	1213	505	1718	951	492
number of indels	117	41	158	12	16
length of indels (bp)	1-328	1-3	1-328	1-255	1-2
average length of indels (bp)	16.2	1.3	12.3	32.2	1.2
total number of characters	1328	546	1876	963	508
number parsimony informative (PI) characters *(%)*	204 *(15.4)*	263 *(48.2)*	467 *(24.9)*	17 *(1.8)*	126 *(24.8)*
number of most parsimonious trees	>93980	>11350	>35170	-	-
tree length	533	971	1548	-	-

The topology, posterior probabilities (PP) and branch lengths of the two BI analyses with different heating temperatures are similar (data not shown), therefore only the trees obtained by using the lower temperature are used. The result of the BI analysis from the combined dataset is shown in fig. 3-1 and 3-2. The MP analysis showed comparable results (data not shown). The networks obtained from the ITS and cpDNA dataset are shown in fig. 3-4 and 3-5.

Intergeneric relationships. The clade containing *Cotula*, *Leptinella* and *Soliva* is well supported (PP 1.00, BS 100) and this clade is part of the supported subtribe Cotulineae. The monophyletic group which contains the South American genus *Soliva* and *Cotula mexicana* is sister to *Cotula* and *Leptinella*. The relationship among *Soliva* and *Cotula mexicana* is well supported (PP 1.00, BS 99).

In the well supported clade (PP 1.00, BS 100) that includes species of *Cotula* (excluding *C. mexicana*) and *Leptinella* the situation is complex: The remaining species of *Cotula* do not form a monophyletic group. *Leptinella* is also not monophyletic and is nested within *Cotula*. However, *Leptinella* and *Cotula alpina* form a moderate supported monophyletic clade in the combined dataset (PP 0.93, BS 80). In the cpDNA and nrDNA dataset this group is not monophyletic.

Infrageneric relationships. The division of *Leptinella* into three subgenera according to Lloyd (1972c) is only partially supported; while subgenus *Leptinella* is monophyletic, the subgenera *Oligoleima* and *Radiata* are not (see Fig. 3-1 and 3-2).

All analyses show a clear subdivision of *Leptinella* into two parts: one clade (*filicula*-group) contains species from New Guinea and *L. filicula* from Australia (PP 1.00, BS 100). In the combined and in the cpDNA dataset but not in the nrDNA dataset, *C. alpina* belongs also to this well supported clade. The second group (*Leptinella* main group) includs all remaining *Leptinella* species and is well supported (PP 1.00, BS 100).Within the Leptinella main group there are several subgroups. The relationships among the taxa of *Leptinella* main group are best illustrated in the networks obtained from the ITS dataset (fig. 3-4). In general, there is low sequence variation within these groups and multiple sequences from taxa are not recovered as monophyletic.

The *pyrethrifolia*-group (*L. atrata* subsp. *luteola*, *L. featherstonii, L. pyrethrifolia)* is supported both in the ITS and combined dataset (PP 1.00), but it is not monophyletic in the cpDNA dataset. The *pectinata*-group *(L. albida, L. atrata* subsp. *atrata, L. conjuncta, L. dendyi, L. pectinata)* is only monophyletic in the ITS network of the *Leptinella* main clade. In the phylogenetic trees, this group is not monophyletic. *L. drummondii, L. longipes* and *L. reptans* form a well supported group in all three datasets (*longipes* group). The *dioica*-group, the *minor*-group and the *plumosa*-group form a moderate supported clade (PP 0.99, BS 64). In the cpDNA dataset this group does not exist. Members of the *dioica*- and *minor*-group share a 1 bp deletion in *psb*A-*trn*H and a 255 bp deletion in *trn*C-*pet*N. Both are not found in the *plumosa*-group. The deletion in *trn*C-*pet*N is also found in *C. australis*.

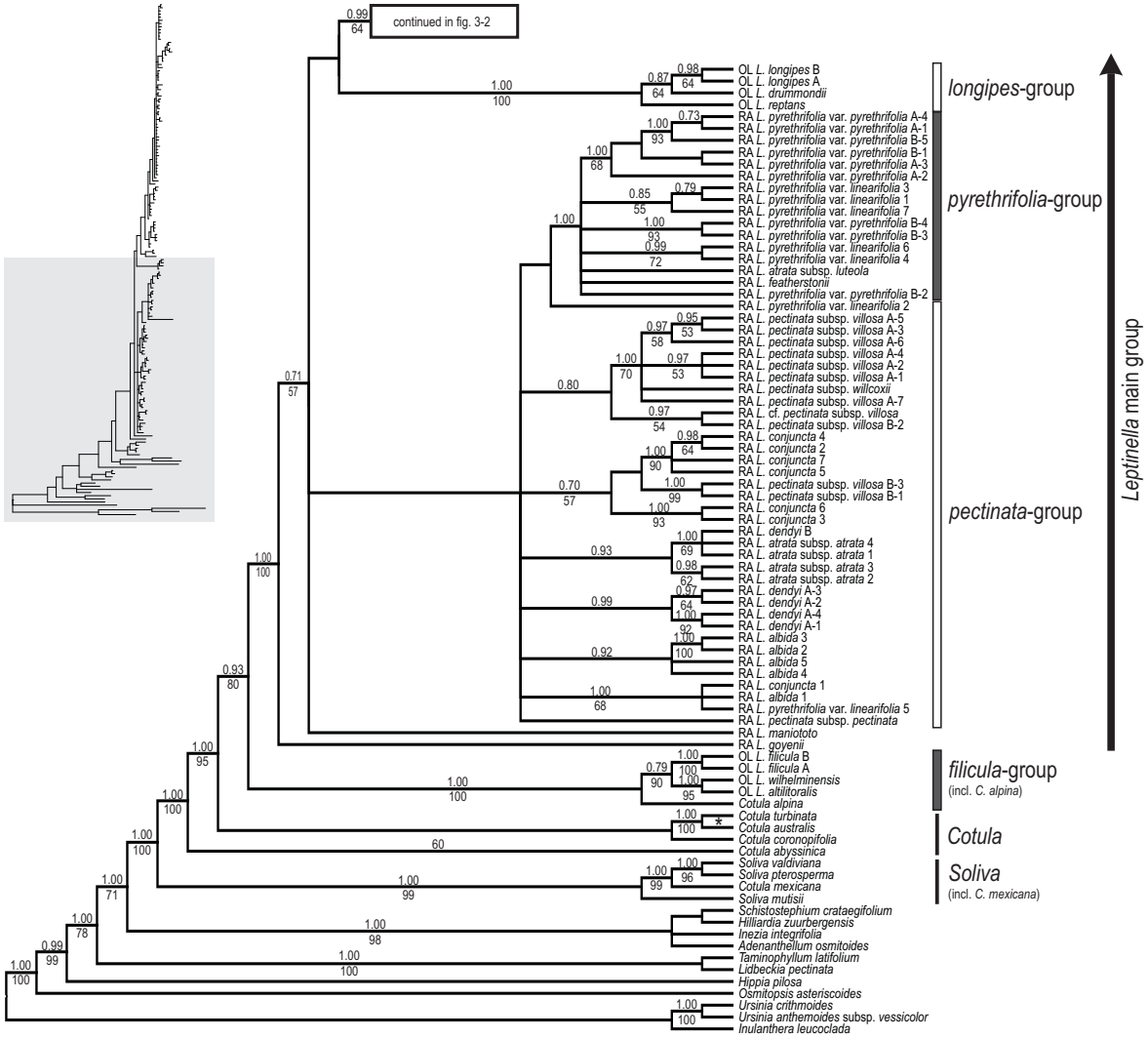

Fig. 3-1: Basal part of the majority rule consensus tree inferred from Bayesian analysis of the combined dataset (ITS, *psba-trn*H, *trn*C-*pet*N). Numbers above the lines are posterior probabilities and numbers below the lines bootstrap values of the maximum parsimony analysis. Letters after the taxa refer to different accessions and the numbers indicate different clones. The current classification is indicated by letters before the taxa (L - *Leptinella*, R - *Radiata*, O - *Oligoleima*). Groups discussed in the text are indicated by bar patterns. The asterics indicate groups with a 328 bp indel in *trn*C-*pet*N.

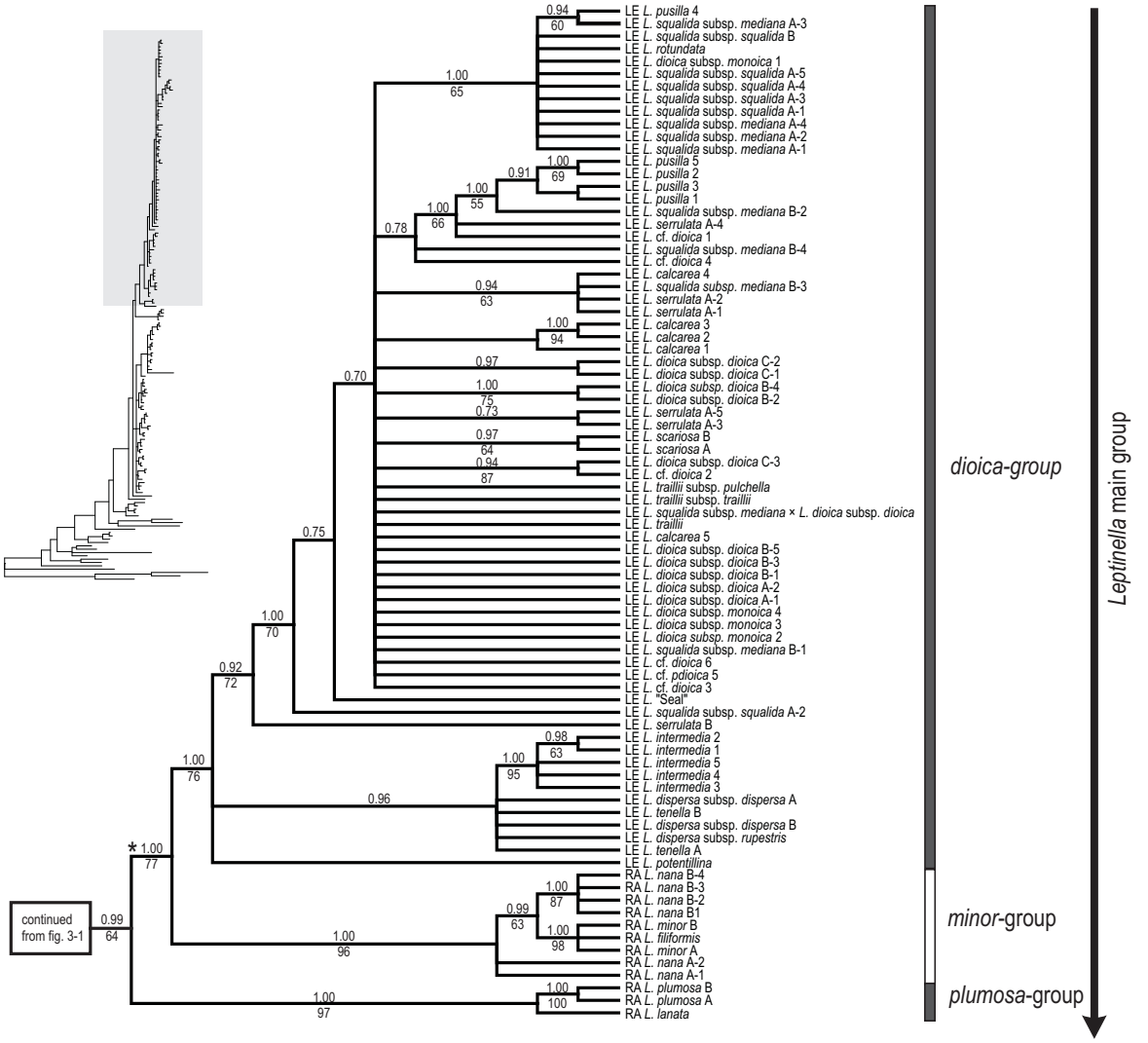

Fig. 3-2: Apical part of the majority rule consensus tree inferred from Bayesian analysis of the combined dataset (ITS, *psba-trn*H, *trn*C-*pet*N). Numbers above the lines are posterior probabilities and numbers below the lines bootstrap values of the maximum parsimony analysis. Letters after the taxa refer to different accessions and the numbers indicate different clones. The current classification is indicated by letters before the taxa (LE - *Leptinella*, RA - *Radiata*, OL - *Oligoleima*). Groups discussed in the text are indicated by bar patterns. The asterisks indicate groups with a 328 bp indel in *trn*C-*pet*N.

The *dioica*-group consists of 11 taxa *(L. calcarea, L. dioica, L. dispersa, L. intermedia, L. pusilla, L. rotundata, L. scariosa, L. serrulata, L. squalida, L. tenella, L.* "Seal"). The three range restricted species of the *minor*-group *(L. filiformis, L. minor, L. nana)* form a supported clade in all analyses. *L. lanata* and *L. plumosa* from the sub-Antarctic islands group also together (*plumosa*-group). *L. goyenii* and L. *maniototo* are isolated in the ITS dataset but show an affinity to other groups in the cpDNA dataset (*L. goyenii* - *pectinata*-group; *L. maniototo* - *minor*- and *dioica*-group).

There are some differences between the cpDNA and ITS dataset in the Leptinella main group (see Fig. 3-4 and 3-5). a) *L. goyenii* is isolated in the ITS and combined dataset, but has a similar haplotype to *L. albida, L. conjuncta, L. pectinata* subsp. *pectinata* and *L. pyrethrifolia* var. *linearifolia*. b) *L. maniototo* clusters with *dioica*- and *minor*-group in the cpDNA dataset, but does not share a 1 bp deletion in *psb*A-*trn*H and a 255 bp deletion in *trn*C-*pet*N. In the ITS data, *L. maniototo* group unsupported with *longipes*-group. c) The ITS data support a relationship of *L. atrata* subsp. *luteola* and *L. featherstonii* to *L. pyrethrifolia*; however this is not substantiated by the chloroplast dataset. d) The ITS clones of *L. pyrethrifolia* var. *linearifolia* cluster with *pyrethrifolia*-group and *pectinata*-group and share a chloroplast type with *L. albida, L. conjuncta, L. goyenii* and *L. pectinata* subsp. *pectinata* (*pectinata*-group).

Sequence variation. Maximal sequence divergences of 9.6 % within *Leptinella* and of 7.4 % within the groups mentioned above were found. Sequence divergences within taxa are summarised in Tab. 3-3. Where more than one accession of a taxa or different clones of one individual were sequenced, the sequence divergence ranged from 0 % *(L. filiformis)* to 4.8 % *(L. pyrethrifolia* var. *linearifolia)*. Different taxa also share identical sequences (e.g. *L. pusilla* clone 4 and *L. squalida* subsp. *mediana* clone A-3). A two base pair indel (alignment position 148 to 149) is shared by clones of three different taxa (*L. albida* clone 1, *L. conjuncta* clone 1, *L. pyrethrifolia* var. *linearifolia* clone 5).

Taxa where more than one accession or clones of ITS were included, do not generally form monophyletic clades (see Tab. 3-3). The analyses of the ITS dataset indicate that of the 24 species of which multiple individuals or clones were sequenced, only 4 are monophyletic *(L. filicula, L. longipes, L. plumosa* and *L. scariosa)*. Additionally, *L. intermedia* is monophyletic in the combined dataset, but not in the ITS analyses. The other species were either paraphyletic or polyphyletic. For species exhibiting non-monophyly, 17 had at least one well supported allele (>0.95 PP), ensuring non-

monophyly. The other paraphyletic or polyphyletic taxa show low sequence variation and differences are not supported (e.g. *L. dispersa* subsp. *dispersa, L. minor, L. tenella).* Most differences are substitutions, but indels also occur. Sequences and clones of the non-monophyletic taxa are not widely scattered throughout the tree, all sequences of a taxon cluster only within a group mentioned above. The only exception is *L. pyrethrifolia* var. *linearifolia* which cluster in two different groups (*pectinata*-group, *pyrethrifolia*-group; see fig. 3-4).

In the chloroplast dataset, the sequence divergences are lower. When more than one accession of taxa was sequenced, the sequences differ in up to two substitutions.

Divergence time estimation: The maximum clade credibility tree from the BEAST analysis is shown in fig. 3-3 together with the distribution area of each taxon. The estimated mean ages and 95 % higher posterior densities (HPD) for relevant groups are shown in Tab. 3-4. The topology of the tree is comparable with the tree from the Bayesian analysis of the combined dataset. However, there are some differences between the two trees: for example, the *pectinata*-group is monophyletic in the tree obtained from BEAST (as in the ITS network of the *Leptinella* main group), but not in the tree obtained from the combined dataset with MrBayes.

The posterior distributions of the root node match their prior quite well (see Tab. 3-4), even if the node which designates the crown age of the Heliantheae is shifted forward in time, and the crown age of the group containing *L. featherstonii* is slightly shifted backwards in time.

The mean number of substitutions per site per million years across the whole tree was estimated to be 0.0037 (0.0027 - 0.0049). The derived crown age of the tribe Anthemideae is 26.0 (17.9 - 34.7) Ma and for clade of *Cotula* (excluding *C. mexicana*) and *Leptinella* the crown age is estimated to be 13.9 (9.1 - 18.7) Ma. Most lineages within *Leptinella* are even much younger and radiated in Pliocene and Pleistocene.

Tab. 3-3: Sequence divergence in the ITS dataset.

Taxa	**Ni**	**Nc**	**Ns**	**differences***	**monophyly #**
L. albida	1	1	5	11 (2.3)	N
L. atrata subsp. *atrata*	1	1	4	9 (1.9)	N
L. calcarea	1	1	5	9 (1.9)	N
L. conjuncta	1	1	7	13 (2.7)	N
L. dendyi	2	1	5	10 (2.1)	N
L. dioica subsp. *dioica*	3	3	10	8 (1.7)	N
L. dioica subsp. *manoica*	1	1	4	8 (1.7)	N
L. dispersa subsp. *dispersa*	2	0	2	0	N
L. filicula	2	0	2	0	Y
L. filiformis	1	1	1	-	-
L. intermedia	1	1	5	4 (0.8)	N
L. longipes	2	0	2	3 (0.6)	Y
L. minor	2	0	2	0	N
L. nana	2	2	6	3 (0.6)	N
L. pectinata subsp. *villosa*	2	2	10	15 (3.1)	N
L. plumosa	2	0	2	1 (0.2)	Y
L. pusilla	1	1	5	14 (2.9)	N
L. pyethrifolia var. *linearifolia*	1	1	7	23 (4.8)	N
L. pyethrifolia var. *pyrethrifolia*	2	2	9	14 (2.9)	N
L. scariosa	2	0	2	0	Y
L. serrulata	2	1	6	14 (2.9)	N
L. squalida subsp. *mediana*	2	2	8	11 (2.3)	N
L. squalida subsp. *squalida*	2	1	6	11 (2.3)	N
L. tenella	2	0	2	3 (0.6)	N

Ni - number of individuals
Nc - number of cloned individuals
Ns - number of distinct sequences
* - highest number of differences between sequences (%)
# - monophyly of taxa in the BI tree of the ITS dataset

Tab. 3-4: Divergence age estimates (crown age).

Node	**Describtion**	**prior**	**posterior**
A	root node (Asteroideae)	31.3 (26.2-36.4)	32.8 (27.4-38.4)
B	Heliantheae	30.0 (25.1-34.9)	25.0 (19.2-30.9)
C	Anthemideae	-	26.0 (17.9-34.7)
D	*Cotula* + *Leptinella* + *Soliva*	-	16.3 (10.9-22.2)
E	*Cotula* (excl. *C. mexicana*) + *Leptinella*	-	13.9 (9.1-18.7)
F	*filicula*-group (incl. *C. mexicana*)	-	6.4 (2.3-10.9)
G	*Leptinella* main group	-	10.3 (6.6-14.1)
H	*pyrethrifolia*-group (*L. featherstonii* on Chatham Islands)	3.0 (0.9-5.0)	4.6 (3.0-6.3)
I	*longipes*-group	-	6.4 (2.-10.9)
J	*pectinata*-group	-	6.5 (3.5-9.8)
K	*plumosa*-group	-	3.6 (1.1-6.3)
L	*minor*-group	-	3.9 (1.4-6.7)
M	*dioica*-group	-	6.6 (4.0-9.6)

Values are in million years before present and represented the mean and 95% HPD for each node.

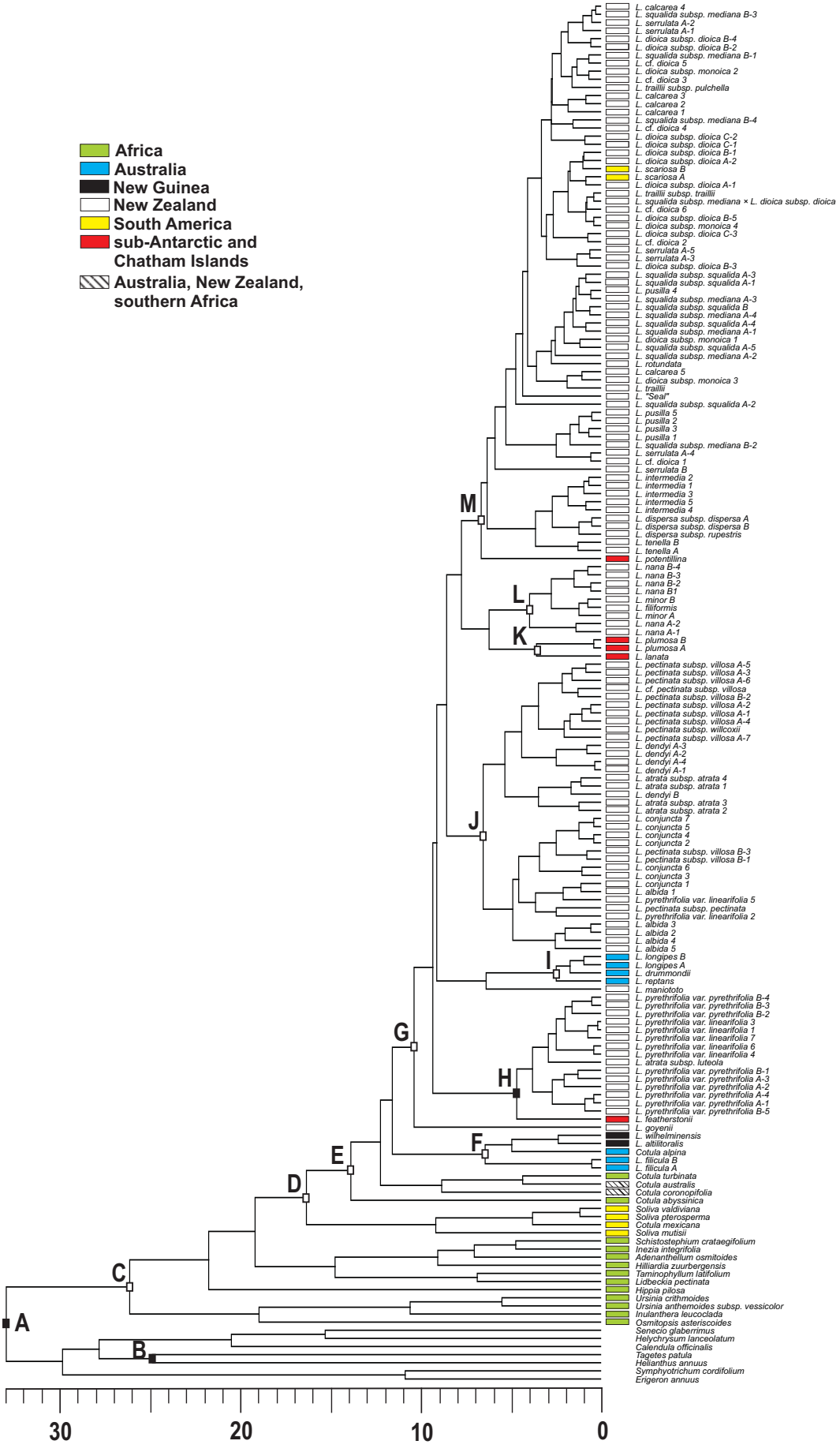
Africa
Australia
New Guinea
New Zealand
South America
sub-Antarctic and Chatham Islands
Australia, New Zealand, southern Africa
A
B
C
D
E
F
G
H
I
J
K
L
M
30
20
10
0
L. calcarea 4
L. squalida subsp. mediana B-3
L. serrulata A-2
L. serrulata A-1
L. dioica subsp. dioica B-4
L. dioica subsp. dioica B-2
L. squalida subsp. mediana B-1
L. cf. dioica 5
L. dioica subsp. monoica 2
L. cf. dioica 3
L. traillii subsp. pulchella
L. calcarea 3
L. calcarea 2
L. calcarea 1
L. squalida subsp. mediana B-4
L. cf. dioica 4
L. dioica subsp. dioica C-2
L. dioica subsp. dioica C-1
L. dioica subsp. dioica B-1
L. dioica subsp. dioica A-2
L. scariosa B
L. scariosa A
L. dioica subsp. dioica A-1
L. traillii subsp. traillii
L. squalida subsp. mediana × L. dioica subsp. dioica
L. cf. dioica 6
L. dioica subsp. dioica B-5
L. dioica subsp. monoica 4
L. dioica subsp. dioica C-3
L. cf. dioica 2
L. serrulata A-5
L. serrulata A-3
L. dioica subsp. dioica B-3
L. squalida subsp. squalida A-3
L. squalida subsp. squalida A-1
L. pusilla 4
L. squalida subsp. mediana A-3
L. squalida subsp. squalida B
L. squalida subsp. mediana A-4
L. squalida subsp. squalida A-4
L. squalida subsp. mediana A-1
L. dioica subsp. monoica 1
L. squalida subsp. squalida A-5
L. squalida subsp. mediana A-2
L. rotundata
L. calcarea 5
L. dioica subsp. monoica 3
L. traillii
L. "Seal"
L. squalida subsp. squalida A-2
L. pusilla 5
L. pusilla 2
L. pusilla 3
L. pusilla 1
L. squalida subsp. mediana B-2
L. serrulata A-4
L. cf. dioica 1
L. serrulata B
L. intermedia 2
L. intermedia 1
L. intermedia 3
L. intermedia 5
L. intermedia 4
L. dispersa subsp. dispersa A
L. dispersa subsp. dispersa B
L. dispersa subsp. rupestris
L. teniella B
L. tenella A
L. potentillina
L. nana B-4
L. nana B-3
L. nana B-2
L. nana B1
L. minor B
L. filiformis
L. minor A
L. nana A-2
L. nana A-1
L. plumosa B
L. plumosa A
L. lanata
L. pectinata subsp. villosa A-5
L. pectinata subsp. villosa A-3
L. pectinata subsp. villosa A-6
L. cf. pectinata subsp. villosa
L. pectinata subsp. villosa B-2
L. pectinata subsp. villosa A-2
L. pectinata subsp. villosa A-1
L. pectinata subsp. villosa A-4
L. pectinata subsp. willcoxii
L. pectinata subsp. villosa A-7
L. dendyi A-3
L. dendyi A-2
L. dendyi A-4
L. dendyi A-1
L. atrata subsp. atrata 4
L. atrata subsp. atrata 1
L. dendyi B
L. atrata subsp. atrata 3
L. atrata subsp. atrata 2
L. conjuncta 7
L. conjuncta 5
L. conjuncta 4
L. conjuncta 2
L. pectinata subsp. villosa B-3
L. pectinata subsp. villosa B-1
L. conjuncta 6
L. conjuncta 3
L. conjuncta 1
L. albida 1
L. pyrethrifolia var. linearifolia 5
L. pectinata subsp. pectinata
L. pyrethrifolia var. linearifolia 2
L. albida 3
L. albida 2
L. albida 4
L. albida 5
L. longipes B
L. longipes A
L. drummondii
L. reptans
L. maniototo
L. pyrethrifolia var. pyrethrifolia B-4
L. pyrethrifolia var. pyrethrifolia B-3
L. pyrethrifolia var. pyrethrifolia B-2
L. pyrethrifolia var. linearifolia 3
L. pyrethrifolia var. linearifolia 1
L. pyrethrifolia var. linearifolia 7
L. pyrethrifolia var. linearifolia 6
L. pyrethrifolia var. linearifolia 4
L. atrata subsp. luteola
L. pyrethrifolia var. pyrethrifolia B-1
L. pyrethrifolia var. pyrethrifolia A-3
L. pyrethrifolia var. pyrethrifolia A-2
L. pyrethrifolia var. pyrethrifolia A-4
L. pyrethrifolia var. pyrethrifolia A-1
L. pyrethrifolia var. pyrethrifolia B-5
L. featherstonii
L. goyenii
L. wilhelminensis
L. altilitoralis
Cotula alpina
L. filicula B
L. filicula A
Cotula turbinata
Cotula australis
Cotula coronopifolia
Cotula abyssinica
Soliva valdiviana
Soliva pterosperma
Cotula mexicana
Soliva mutisii
Schistostephium crataegifolium
Inezia integrifolia
Adenanthellum osmitoides
Hilliardia zuurbergensis
Taminophyllum latifolium
Lidbeckia pectinata
Hippia pilosa
Ursinia crithmoides
Ursinia anthemoides subsp. vessicolor
Inulanthera leucoclada
Osmitopsis asteriscoides
Senecio glaberrimus
Helychrysum lanceolatum
Calendula officinalis
Tagetes patula
Helianthus annuus
Symphyotrichum cordifolium
Erigeron annuus

Fig. 3-3 (previous page): Maximum clade credibility tree from the BEAST analysis. Letters identifying nodes are discussed in the text. Black boxes indicate calibration nodes. The bar pattern indicates the geographical distribution.

Discussion

Sequence variation. When more than one accession of a taxon and/or clones of one individual were sequenced, these different sequences do not form monophyletic groups in the ITS dataset. The only exceptions are the directly sequenced species *L. filicula, L. longipes, L. plumosa* and *L. scariosa*.

A number of factors have been suggested as potential mechanisms for apparent species non-monophyly (Funk and Omland 2003, Syring et al. 2007). As Funk and Omland (2003) pointed out, definitive causes for species polyphyly are difficult to prove.

Inadequate phylogenetic signal could lead to taxon non-monophyly (Funk and Omland 2003): If a marker is evolving too slowly, the obtained sequence data may provide too few synapomorphies to recover the underlying phylogeny. A clear example is *L. minor*, which differs in only one substitution in ITS from *L. filiformis*. The two allopatric species are very similar and differ only in size (Lloyd 1972c). Another example is the group of *L. dispersa, L. intermedia,* and *L. tenella* where only few synapomorphies for the different taxa were found. Little sequence divergence between morphological and ecological more or less distinct taxa is documented for several New Zealand plant groups (e.g. Wagstaff et al. 2006, Ford et al. 2007, Mitchell et al. 2009a). For example, there is given evidence that different alpine lineages of New Zealand are evolutionary young groups that have evolved rapidly in the new mountain habitats within the last five million years (e.g. Lockhart et al. 2001, Winkworth et al. 2005). The young age and the recent radiations of New Zealand plant groups are the reasons why sequencing of different nuclear and chloroplast markers could lead to unsatisfactory results for phylogenetic reconstruction. As *Leptinella* diverged in the last 10 Ma with much younger radiations in most groups, *Leptinella* is a good example for little sequence variation in recent plant lineage as already seen in other New Zealand plant lineages.

However, inadequate phylogenetic information can not explain all of the cases of taxa non-monophyly. There are 17 taxa in *Leptinella* that had one or more alleles in a supported group that ensured allelic non-monophyly. Hybridization may lead to species non-monophyly (Funk and Omland 2003, Syring et al. 2007). Several natural hybrids between different species of *Leptinella* were reported (Lloyd 1972c). Some are rare and

occur only occasionally, but others are widespread (e.g. *L. dioica* subsp. *dioica* × *L. squalida* subsp. *mediana* on the South Island). Artificial hybrids could be obtained from several crosses between different species, even crosses between distinct groups were successful (Lloyd 1972a, Lloyd 1975a). Hybridization may occur in conjunction with polyploidization. The combination of two or more divergent genomes in allopolyploids could lead to species non-monophyly. Ploidy levels higher than tetraploid were found in the *dioica*-group (8*x* to 24*x*). But polyploids are also found in the *pectinata*-group (8*x*) and *pyrethrifolia*-group (12*x,* 16*x*). Artificial and natural crosses between taxa with different ploidy level are possible (Lloyd 1972c, Lloyd 1975a). Unfortunately, no chromosome numbers of the resulting offspring has been reported. An interesting aspect is that our ITS data do not indicate hybridization among the different groups, although hybrids were reported (Lloyd 1972c, 1975a). One exception is the hybrid origin of *L. pyrethrifolia* var. *linearifolia*, where alleles of ITS are found in two different groups (fig. 3-4).

Additionally, since we have only sequenced one to three individuals per taxon and only up to ten clones per individual, it is possible that we have not detected all alleles of one taxon. An increase in number of analysed clones and individuals may lead to the detection of more alleles. The identification of additionally alleles could lead to a more complicated picture, especially if there would be more individuals that have alleles in different groups like *L. pyrethrifolia* subsp. *linearifolia*. On the other side, we used a *Taq* DNA polymerase for amplification of the clones. The DNA polymerase of *Thermus aquaticus* is not endowed with proofreading activity and therefore may produce replication errors. These errors may increase allele variation per species and lead to a more complicated result.

To sum this section up: Hybridization and polyploidization in combination with inadequate phylogenetic signal and technical problems with cloning seem to be the likeliest explanations for species non-monophyly within *Leptinella*. But to clarify the complex history of the genus, it is necessary to increase the phylogenetic signal by sequencing of additional markers or by using faster evolving markers (e.g. AFLP, microsatellites). Both methods were successful used in several studies (e.g. Edwards et al. 2008, Guo et al. 2005, 2008). Another possibility would be the use of nuclear single or low copy genes instead of the multi copy locus ITS as shown in several studies (e.g. Joly et al. 2006, Brysting et al. 2007).

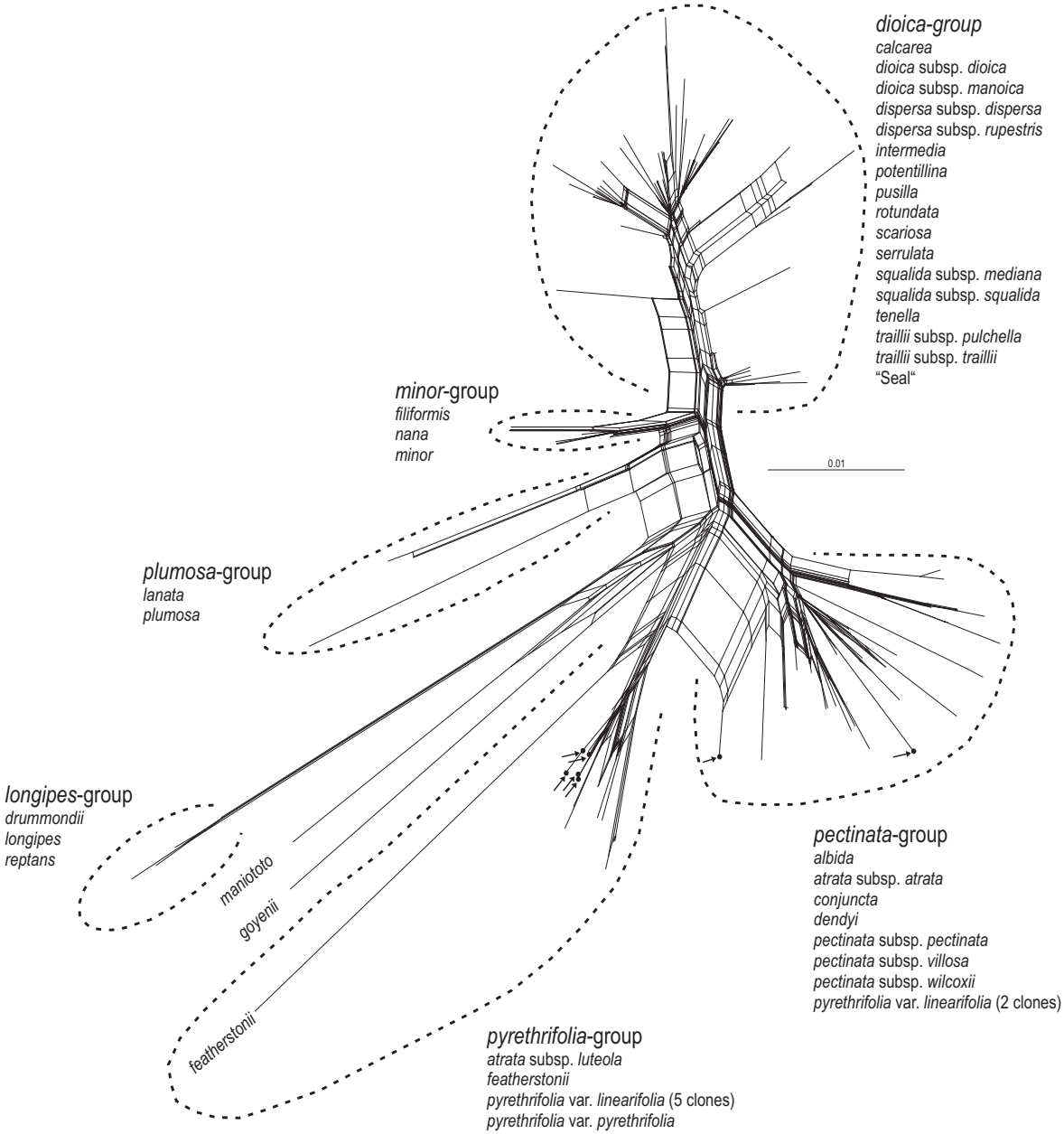

Fig. 3-4: Phylogenetic network based on ITS data of the *Leptinella* main group. Groups and taxa discussed in the text are labelled, for labels of individuals and clones see appendix 3-1. The arrows indicate the position of clones of *L. pyrethrifolia* var. *linearifolia.*

Divergence time estimation and biogeographic implications. The estimated age for the Anthemideae of 26.0 Ma agree roughly with previously inferred age for the tribe obtained via non-parametric rate smoothing (23.1 ± 4.1 Ma; Oberprieler 2005). The age of the tribe is in line with other molecular dating studies for related tribes in the subfamily Asteroideae (Senecioneae, Wagstaff et al. 2006). But Bergh et al. (2009) estimated an older age for the sister tribe Gnaphalieae (34.5 Ma). However, our estimated age of the tribe Anthemideae lies in the range of the major radiations of the family in Southern Africa (2 5- 35 Ma; Funk et al. in press).

According to Himmelreich et al. (2008), the basal Anthemideae (including Cotulineae) originated in Southern Africa. From this area, long distance dispersal events to South America *(Soliva, C. mexicana)* and to Australasia *(Cotula, Leptinella)* have occurred. The group which contains *Cotula, Leptinella* and *Soliva* has originated in the Miocene (16.3 Ma). The age of this group rules out a Gondwana vicariance, because the continents of the southern hemisphere had separated earlier (McLoughlin 2001, Neall and Trewick 2008). Based on our present data, we are not able to decide whether there was a dispersal event from Africa to Australia, New Guinea or New Zealand. Such transoceanic dispersal events in recent times from Africa to Australasia are hypothesised for the subtribe Gnaphalieae of the sunflower family and for at least six other plant groups (Bergh and Linder 2009, and citations within).

The basal filicula-group occurs in New Guinea and Australia. The *Leptinella* main group, which has its centre of diversity in New Zealand, has originated in the Miocene, followed by a rapid diversification in the Pliocene and Pleistocene. This is a period characterized by important geological and climatic changes in New Zealand: the uplift of the Southern Alps and the glaciation cycles (Winkworth et al. 2005).

The rapidly changing environment during the uplift of the Southern Alps may have created a range of new, open habitats that offered opportunities for the establishment of dispersing lineages (Winkworth et al. 2005). *Leptinella* occurs in such open habits from coastal to the alpine area. Furthermore, speciation of *Leptinella* could be enforced by processes suggested by Winkworth et al. (2005) during the glaciation cycles. Cycles of range expansion and contraction of one taxon could result in different local forms. When the local forms interact, hybridization or introgression and polyploidization could have enforced diversification of different lineages.

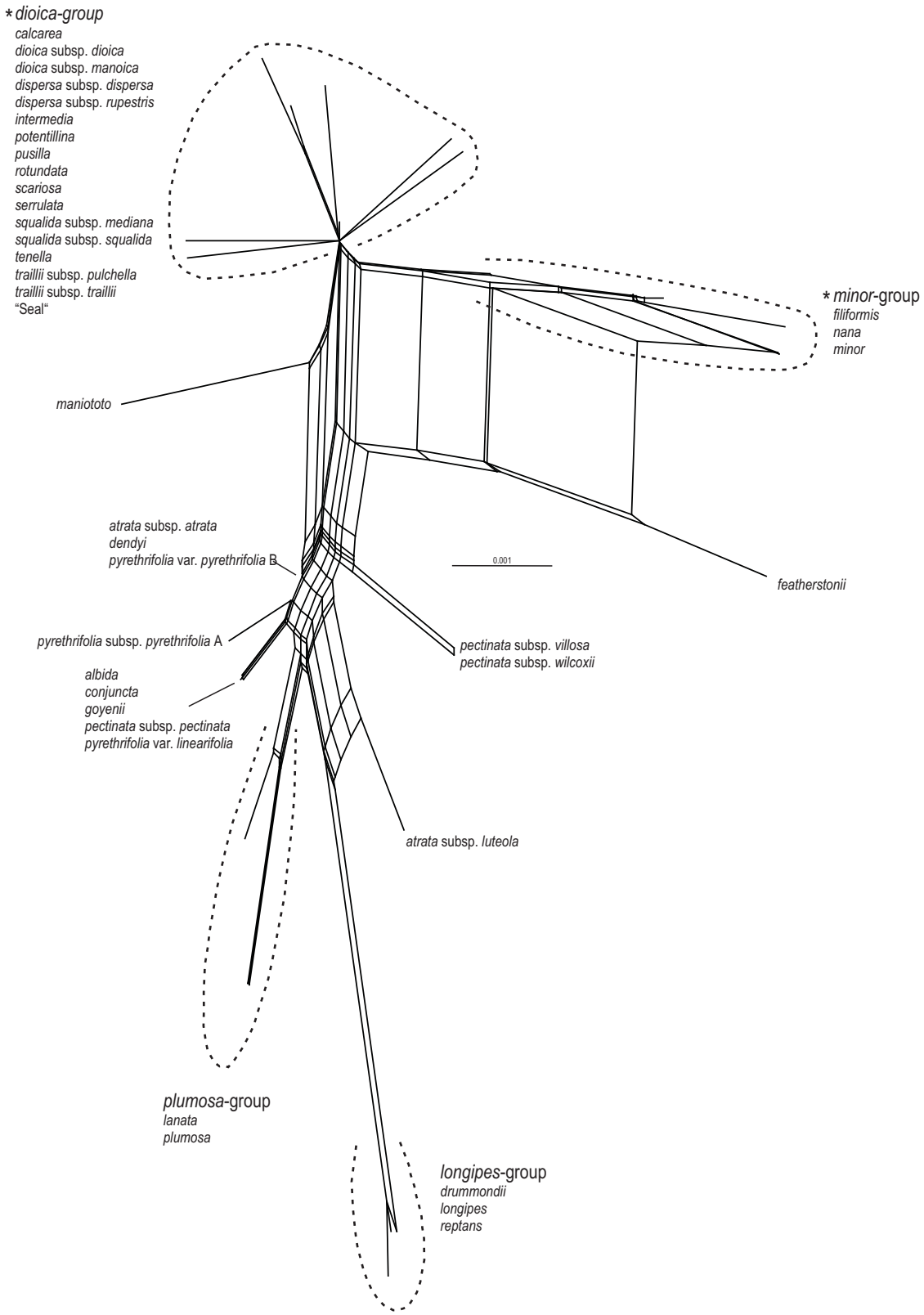

Fig. 3-5: Phylogenetic network based on the combined cpDNA data (*psb*A-*trn*H, *trn*C-*pet*N) of the *Leptinella* main group. Groups and taxa discussed in the text are labelled, for labels of individuals and clones see appendix 3-2. The asterics indicate groups with a 255 bp indel in *trn*C-*pet*N.

Within the *Leptinella* main group, our data indicates five additional long-distance dispersal events in recent time from New Zealand to Australia, South America, the sub-Antarctic islands and Chatham Islands.

The three Australian species of the *longipes*-group *(L. drummondii, L. longipes, L. reptans)* nested among the *Leptinella* main clade, which consists of mainly New Zealand taxa. This result suggests a long-distance dispersal event from New Zealand to Australia against the westerly winds.

The dioecious taxon *L. scariosa* is the only *Leptinella* species in South America. It clusters with species from New Zealand in the *dioica*-group. There is very few sequence variation in all three markers in this group, and the species are morphologically very similar (Lloyd 1972c). Our data, therefore, suggest a long-distance dispersal event from New Zealand to South America. Such dispersal events between New Zealand and South America are documented for several groups (see Winkworth et al 2002a, Sanmartin and Ronquist 2004, Sanmartin et al. 2007).

Six species of the *Leptinella* main clade occur on the Chatham Islands or on the sub-Antarctic islands. These islands are separated from New Zealand or other landmasses by hundreds of kilometres of water. *L. dispersa* subsp. *dispersa*, (Campbell Island) and *L. squalida* subsp. *squalida* (Chatham Island) are also widely distributed on the New Zealand main islands, while the other four [*L. featherstonii* (Chatham Island), *L. lanata* (Auckland, Campbell Islands), *L. plumosa* (Antipodes, Auckland, Campbell, Crozet, Heard, Kerguelen, Macquarie, Marion Islands), *L. potentillina* (Aucklands, Chatham Islands)] are distributed only on the small islands. These species are found to be members of three different groups, suggesting at least three independent dispersal events to the islands (*dioica*-group: *L. dispersa* subsp. *dispersa, L. potentillina, L. squalida* subsp. *squalida; plumosa*-group: *L. lanata, L. plumosa; pyrethrifolia*-group: *L. featherstonii*).

A nice example is *L. plumosa*, which is distributed on many sub-Antarctic islands (Lloyd 1972c). A recent colonisation of Heard Island by *L. plumosa* was documented by Turner et al. (2006). The closest island that is inhabited by *L. plumosa* is the Isle Kerguelen group, approximately 500 km to the northeast. An analysis including samples from Heard, Marion and Macquarie Island, resulted in no sequence differentiation in the ITS and *psb*A-*trn*H markers, although these islands are separated by thousands of kilometres (Himmelreich, unpublished data). This lack of sequence difference suggests a recent dispersal event.

The mechanisms involved in the transoceanic long distance dispersal are discussed in the recent literature (e.g. Winkworth et al. 2002a, Ford et al. 2007, Goldberg et al. 2008, Bergh and Linder 2009). Dispersal by wind has been suggested by different authors for different groups. Munez et al. (2004) documented long distance dispersal by wind for representatives of the extra tropical southern hemisphere lichens, bryophytes and pteridophytes. The strong westerly winds (e.g. roaring forties) in the southern hemisphere might enforce this process. *Leptinella* seeds are small and light, but they have no pappus or other morphological features that might enhance dispersal by wind. Another possibility is transport by water or with floating islands. This would be facilitated by the westerly sea current. Tolerance to sea water would be necessary for this dispersal way, unfortunately this information is still lacking for the Anthemideae.

Long distance dispersal between the southern hemisphere landmasses by attachment of seeds to the feet or feathers of birds is also possible. This mechanism is suggested for *Lepidium* (Mummenhoff et al. 2004), *Zygophyllum* (Beier et al 2003) and for Chenopodiaceae (Kadereit et al. 2006). This dispersal way was also suggested for the spreading of *L. plumosa* to Heard Island (Turner et al. 2006). Since *L. plumosa* has a sticky seed coat (Webb and Simpson 2001), it is possible this kind of surface may assist dispersal through attachment to the feathers or feet of birds. *Leptinella* species from the different islands (*L. featherstonii, L. lanata, L. plumosa)* are associated with bird-influenced vegetations (Lloyd 1972c, New Zealand Plant Conservation Network 2009). Turner (2006) also did not rule out dispersal trough attachment to marine mammals.

It is impossible to determine which mechanisms led to the observed geographical patterns within *Leptinella*. No experimental data are available for dispersal by wind, sea water tolerance or attachment capacity to birds for *Leptinella* or related plant groups. However, long distance dispersal between the southern hemisphere continents and islands seems to be an important process for many plants and even animals that have no obvious adaptation for such process either by wind, water or animals (see Winkworth et al. 2002b, Ford et al. 2007, Goldberg et al. 2008).

Successful long distance dispersal is only the first step for the establishment of plants in a new area. Suitable habitats in the new area are also necessary. For dioecious plants the joint dispersal and the establishment of female and male plants is necessary (Baker and Cox 1984, Sakai et al. 1995a,b). In this context, one interesting example is the dioecious taxon *L. scariosa* from South America. If dioecy has evolved only once in the monophyletic *dioica*-group in New Zealand as suggested by Lloyd (1975b), a dispersal

event of a female and a male plant must have been occurred. Another possibility is the dispersal of only one inconstant female plant to South America, and the establishments of a new dioecious population as described in Lloyd (1975b). Inconstant female plants have a low number of male florets, and are found rarely in populations of dioecious *Leptinella* taxa. Crosses between male florets and female florets of such inconstant female plant lead to female and male offspring (Lloyd 1975a).

Taxonomic implications

Generic relationships: The three genera *Cotula, Leptinella*, and *Soliva* form a strongly supported clade within the subtribe Cotulineae. The occurrence of this group was already pointed out by several authors (Bremer and Humphries 1993, Lloyd 1972c, Lloyd and Webb 1987, Oberprieler et al. 2007, Himmelreich et al. 2008). However, the relationships within this group remained unclear.

In the present analysis, *Soliva* and *Cotula mexicana* form a well supported monophyletic group. Both are the only indigenous taxa of Anthemideae in South America. The position of *Cotula mexicana* within the genus Soliva is unexpected: Whereas *C. mexicana* has pedunculate capitula like other *Cotula* species, all *Soliva* species have sessile capitula (Cabrera 1949, Bremer and Humphris 1993).

The sister group to *Soliva* is *Cotula* (excl. *C. mexicana*) + *Leptinella*, but both genera are not monophyletic. Besides the molecular results, the genus *Leptinella* is morphological and cytological well distinguishable from *Cotula* (Lloyd 1972c, Lloyd and Webb 1987, Bremer and Humphries 1993, Oberprieler et al. 2006), with its inflated female corolla, mostly perennial habit, and the basic chromosome number $x = 13$. To investigate the relationships between *Leptinella* and *Cotula*, more species of *Cotula* have to be included in the analyses. In our present analyses, only six representatives of *Cotula* (out of c. 55) were selected.

Infrageneric relationships: Our results are only partly congruent with the subgenera sensu Lloyd (1972c). The subgenus *Leptinella* is monophyletic in all datasets, but the subgenera *Oligoleima* and *Radiata* are not. All analyses show clearly that *Leptinella* is split into two groups: the *filicula*-group and the *Leptinella* main group.

filicula-group*:* This group comprises species of subgenus *Oligoleima* from New Guinea (*L. altilitoralis, L. wilhelminensis)* and *L. filicula* from Australia. The New Guinean species *L. leptoloba* and *L. sarawaketensis* are morphologically very similar to the sequenced species from New Guinea (van Royen and Lloyd 1975) and may also be

members of this group. The subgenus *Oligoleima* is characterised by distinctly compressed achenes with a broad margin and a persistent corolla of the female florets (Lloyd 1972c, van Royen and Lloyd 1975). These features are not seen in other taxa of *Leptinella*. However, the molecular analyses show that subgenus *Oligoleima* sensu Lloyd (1972c) is not monophyletic. Beside the *filicula*-group, there is another group of three Australian species of subgenus *Oligoleima* within the Leptinella main group (*longipes*-group, see below). We did not found any morphological character that would discriminate the two groups. The New Guinean species however differ from the Australian ones in the consistently lacking of short shoots and in having uniseriate hairs on the achenes, a character expression that is found in many species of *Cotula* (van Royen and Lloyd 1975).

Remarkable is the position of *Cotula alpina*, a native species of Australia. In the combined and chloroplast dataset, *C. alpina* is a member of the well supported *filicula*-group, but not in the ITS tree. For this species we could obtain only ITS 1, so the inconsistence could be the consequence of missing data. In his revision of the Australian Anthemideae, Thompson (2007) discusses the morphological position of *C. alpina*: it has functionally male central florets, multiseriate female florets, glandular leaves, and a prostrate habit as in *Leptinella*, but lacks a corolla on the female florets, as in the genus *Cotula*. Because of the morphological similarity, the taxon is also often confused with *L. filicula. C. alpina* has $2n = 108$ chromosomes ($x = 9$; Hair 1962). This number fits to the basic chromosome numbers of *Cotula* ($x = 8, 9, 10$), whereas *Leptinella* has $x = 13$ (Hair 1962, Lloyd 1972c, Bremer and Humphries 1993). Unfortunately, there are no counts for species from Australia and New Guinea. *C. alpina* could be a link between *Leptinella* and *Cotula*, but again more sequences of *Cotula* are necessary to confirm this scenario.

Leptinella main group: All *Leptinella* species from New Zealand, the sub-Antarctic Islands, South America and three species from Australia form a well supported monophyletic clade in all datasets. All members of subgenera *Leptinella* and *Radiata* and three species of subgenus *Oligoleima* belongs to this group. The *Leptinella* main group consists of five more or less supported groups, which will be discussed in detail below.

longipes-group: This clade includes three other Australian species of subgenus *Oligoleima (L. drummondii, L. longipes, L. reptans).* As described above, the subgenus *Oligoleima* is not monophyletic and is split into the well supported *filicula*- and *longipes*-group.

pectinata-group: This group comprises *L. pectinata* and its allies *(L. albida, L. atrata* subsp. *atrata, L. conjuncta, L. dendyi).* The circumscription of this group is only supported

in the ITS network. The chloroplast data are more complex and may indicate a close connection between the *pectinata*- and *pyrethrifolia*-group. In this group, there are three chloroplast types: *L. dendyi* and *L. atrata* subsp. *atrata* share a haplotype with one accession of *L. pyrethrifolia* var. *pyrethrifolia*. *L. albida, L. conjuncta, L. goyenii*, and *L. pectinata* subsp. *pectinata* share the same haplotype with *L. pyrethrifolia* var. *linearifolia*. The two other subspecies of *L. pectinata* (subsp. *villosa,* subsp. *willcoxii*) have a third haplotype.

There is some morphological evidence that *L. pectinata* consists of two different species: *L. pectinata* s.s. and *L. villosa/wilcoxii* (P. B. Heenan (New Zealand), personal communication). The different chloroplast haplotypes could be an evidence for this, even if they share the same ITS sequence.

The two subspecies of *L. atrata* cluster in two different groups in the ITS data. While *L. atrata* subsp. *atrata* is part of the *pectinata*-group, *L. atrata* subsp. *luteola* is found as a member of the *pyrethrifolia*-group. This result is astonishing, because both subspecies are morphological very similar and difficult to distinguish, especially in the vegetative state (Lloyd 1972c). The two subspecies differ in the convex receptaculum and dark red florets (*atrata*) vs. a conical receptaculum and yellow florets with red tips (*luteola*).

pyrethrifolia-group: This group comprises *L. pyrethrifolia* from the New Zealand mainland. According to the ITS phylogenetic analyses, *L. featherstonii* (Chatham Islands) and *L. atrata* subsp. *luteola* (South Island) belong also to this well supported group. However, these two taxa are isolated from *L. pyrethrifolia* in the cpDNA dataset.

The small erect and woody shrub habit and the entire leaves of *L. featherstonii* are very uncommon in the genus, and this species is not easily to recognize as *Leptinella*. The growth form was interpreted as a secondarily derived feature of insular gigantism on the Chatham Islands by Lloyd (1981, 1982). Having 2n = 54 chromosomes (Dawson 1995), the species differs from the expected 2n = 52 as reported for other tetraploid *Leptinella* species (Hair 1962, Lloyd 1972, Beuzenberg and Hair 1984). However, beside the unique morphological habit and the unusual chromosome number, the analyses show that *L. featherstonii* is correctly classified as a *Leptinella* species.

The position of *L. pyrethrifolia* var. *linearifolia* is another interesting result. This taxon is confined to ultramafic substrate in the Red Hills on the South Island. In contrast to the typical variety *L. pyrethrifolia* var. *linearifolia* has linear leaves. In his checklist of the indigenous vascular plants of New Zealand, Druce (1993) treated it as an independent species. Cloned ITS sequences of this taxa belong to two different groups (*pyrethrifolia*-

group and *pectinata*-group). *L. pyrethrifolia* var. *linearifolia* shares also its chloroplast haplotype with *L. goyenii* and different species of the *pectinata*-group *(L. albida, L. conjuncta, L. pectinata* subsp. *pectinata*). These results may suggest a hybrid origin of this taxon, with a member of the pectinata-group and another of the *pyrethrifolia*-group as potential parents. A more detailed sampling and faster evolving markers (e.g. AFLP fingerprinting, microsatellites) would be necessary to confirm this hypothesis.

The following three groups *(dioica-, minor-, plumosa*-group) form a well supported clade in the ITS data, which is not confirmed in the cpDNA data. The *dioica*- and the *minor*-group share a 1 bp deletion in *psb*A-*trn*H and a 255 bp deletion in *trn*C-*pet*N. The *plumosa*-group does not show these deletions in the cpDNA dataset and its chloroplast haplotype show an affinity to the *longipes*-, *pectinata*- and *pyrethrifolia*-group.

The mentioned 255 bp deletion in *trn*C-*pet*N was also found in *C. australis*, but not in the other analysed *Cotula* species. *C. australis* occurs widespread in the southern hemisphere and is also indigenous in New Zealand (Webb et al. 1988). The shared deletion in both groups could be explained either by parallelism or by hybridization and introgression.

minor-group*:* This monophyletic group comprises the three rare and range restricted species *L. filiformis, L. minor* and *L. nana* from New Zealand. All three species are closely related. Especially, the allopatric taxa *L. filiformis* and *L. minor* are morphological very similar as they differ only in size (Lloyd 1972c). Lloyd (1972c) placed these three species in the subgenus *Radiata*. They also share the same stem anatomy, which is characteristic to the other members of subgenus *Radiata* (Edgar 1958). However, the molecular analyses indicate that there is an affinity to the *dioica*-group (subgenus *Leptinella*).

plumosa-group: This well supported monophyletic group includes *L. lanata* and *L. plumosa.* Both species occur on the sub-Antarctic islands. When they occur together, hybrids are occasionally found between both species (i.e. Auckland and Campbell Islands; Lloyd 1972c).

dioica-group: All species of subgenus *Leptinella* as circumscribed by Lloyd (1972c) belongs to this monophyletic group. The species group is morphologically well-characterised by long rhizome internodes and single branches. It is a rather homogeneous group and most species are very similar to each other and difficult to distinguish. Extensive hybridisation has been observed in this group and hybrids were found to be fertile (Lloyd 1972c, 1975a). Some of these hybrids are widespread and common (e.g. *L. squalida* subsp.

mediana × *L. dioica* subsp. *dioica*; Lloyd 1972c, H. Wilson (New Zealand), personal communication).

In this clade, the tetraploid species *L. dispersa*, *L. potentillina*, and *L. tenella* together with *L. intermedia* (12*x*) form a basal grade, followed by a clade of higher polyploid taxa *(L. calcarea, L. dioica, L. pusilla, L. rotundata, scariosa, L. squalida, L. traillii)*.

Of special interest is the position of *L. intermedia* within the *dioica*-group. *L. intermedia* has been suggested to be a hybrid between a member of subgenus *Leptinella (L. pusilla* or *L. serrulata)* and subgenus *Radiata (L. pectinata* subsp. *pectinata)*, as it shares characteristics of both subgenera (Lloyd 1972c). Artificial F1 hybrids, resulting from crosses between the proposed parental species, were found to be virtually indistinguishable from *L. intermedia*. Since its formal description by Lloyd (1972c), this species has not been collected again, and searches at the only known locality have only resulted in the observation of hybrids between *L. pusilla* and *L. pectinata* subsp. *pectinata* (New Zealand Plant Conservation Network 2009). In our analyses, we found that the chloroplast of *L. intermedia* belongs to the *dioica*-group. We only found ITS sequences which resemble sequences of the *dioica*-group. These were not similar to the sequences of the proposed parental species of subgenus *Leptinella, L. pusilla* or *L. serrulata*. Since, we were only able to sequence few ITS clones of one individual, additional ITS alleles could have remained undetected (see chapter above). So far, our data do not support the suggested hybrid origin of *L. intermedia*.

On the small Seal Island on the West Coast of the South Island, the robust *L.* "Seal" occurs. This plant is superficially similar to *L. potentillina* from Auckland and the Chatham Islands, but differs mainly by its dioecious breeding system, erect and dark green leaves and by the leaf pinnae which are markedly less toothed (Druce 1993, New Zealand Plant Conservation Network 2009). The sequences of these two taxa are different. But further morphological and molecular studies (e.g. AFLP or microsatellites) are necessary to clearify the taxonomic status of *L.* "Seal".

The morphologically distinct species *L. goyenii* and *L. maniototo* hold an isolated position and both taxa show long branches in the obtained ITS network. The palmate leaves and the compact woody branches of *L. goyenii* are unique in the genus. In the ITS data, this taxon show no affinity to other species, but it shares the same chloroplast haplotype with *L. albida, L. conjuncta, L. pectinata* subsp. *pectinata* and *L. pyrethrifolia* subsp. *linearifolia*. With two of them, *L. albida* and *L. pectinata, L. goyenii* grows

intermingled and occasional hybrids were found (Lloyd 1972c). Our data may suggest hybridization and/or introgression among these taxa.

The occasionally annual species *L. maniototo* has narrow linear leaves with minute pinnae and is easily distinguishable from all other taxa. Lloyd (1972) also mentioned the morphologically isolated position of *L. maniototo*, which is also indicated by our ITS data. However, in the phylogenetic analyses of the cpDNA dataset, *L. maniototo* cluster with the *dioica*- and *minor*-group, even if it does not share the 1 bp deletion in *psb*A-*trn*H and the 255 bp deletion in *trn*C-*pet*N with these groups.

Conclusion

This study shows that *Leptinella* radiated in the Pliocene and Pleistocene and that several long-distance dispersal events occurred. Both results are comparable with other molecular studies dealing with New Zealand plant lineages. Furthermore, hybridization and polyploidization have played an important role in the evolution of *Leptinella*. But to look more detailed at the evolutionary history of the genus and to resolve taxonomical problems (e.g. Is *Leptinella* monophyletic?), a more comprehensive sampling and the exploration of additional molecular markers (e.g. low copy genes, AFLP fingerprinting, microsatellites) would be necessary.

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Chapter 4

Phylogenetic relationships in *Leptinella* (Anthemideae, Compositae) inferred from AFLP fingerprinting

Introduction

The southern hemisphere genus *Leptinella* consists of 42 taxa (34 species and additional 7 subspecies and one variety). New Zealand is clearly the centre of diversity of the genus with 29 taxa, the remaining taxa are distributed in Australia (4 taxa), New Guinea (4), South America (1), and on the Chatham Islands or on the sub-Antarctic islands (4). Some of the New Zealand species are morphologically and cytogenetically variable and this variation is not easily partitioned, making species delineation difficult (Lloyd 1972c). Druce (1993) listed six informal, undescribed *Leptinella* entities. One of them, *L. conjuncta* (informal tagname *L.* "Clutha"), has been recently described by Heenan (2009).

Leptinella consists of very small perennial and procumbent herbs with pedunculate capitula. The outer disc florets are female and the inner disc florets are functionally male, or all florets of one plant are either female or male. The female florets are characterised by an inflated corolla. The genus *Leptinella* forms an impressive polyploid-complex with chromosome numbers ranging from tetraploid to a chromosome set of $2n = 24x$ (the chromosome counts for each taxon are summarized in Tab. 4-1. Unfortunately, chromosome counts are not available for all taxa yet.

The genus *Leptinella* belongs to the basal southern hemisphere grade of the tribe Anthemideae (Himmelreich et al. 2008). *Leptinella* was described as a genus by Cassini (1822), but was later sunk to infrageneric rank within *Cotula* by Hooker (1864). Later, Lloyd and Webb (1987) reinstated *Leptinella* on generic rank, mostly because of the inflated corolla of the female florets and the basic chromosome number of $x = 13$ which is unique within the tribe Anthemideae. However, the results of sequencing of nuclear and chloroplast DNA markers had shown that *Leptinella* is nested within *Cotula* and that the genus may be not monophyletic (see Chapter 3).

A first comparative study of taxa of *Leptinella* (as *Cotula*) was done by Edgar (1958). She divided *Leptinella* into two informal groups based on rhizome anatomy. Later Lloyd (1972c) divided *Leptinella* into three subgenera (*Leptinella*, *Oligoleima*, *Radiata*; as series of *Cotula* subgenus *Leptinella*). These three subgenera were found to be only partially monophyletic in a molecular study (chapter 3).

In all analyses, *Leptinella* was split into two subgroups: One group consists of species from New Guinea, *L. filicula*, and *Cotula alpina* from Australia (*filicula*-group; chapter 3). The remaining taxa belong to the monophyletic *Leptinella* main group

(Chapter 3). These taxon group contains 37 species, subspecies or varieties. Members of this group are distributed in Australia (3 taxa), New Zealand (29), South America (1), and on the Chatham and sub-Antarctic islands (4). Several more or less supported groups could be recognized within the *Leptinella* main group (Chapter 3): a) the *dioica*-group *(L. calcarea, L. dioica, L. dispersa, L. intermedia, L. pusilla, L. rotundata, L. scariosa, L. serrulata, L. squalida, L. traillii*), b) the *minor*-group *(L. filiformis, L. minor, L. nana)*, c) the *plumosa*-group *(L. lanata, L. plumosa)*, d) the *pectinata*-group *(L. atrata* subsp. *atrata, L. conjuncta, L. dendyi, L. pectinata)*, e) the *pyrethrifolia*-group *(L. atrata* subsp. luteola, *L. featherstonii, L. pyrethrifolia*), and f) the *longipes*-group *(L. drummondii, L. longipes, L. reptans)*. The morphologically distinct species *L. goyenii* and *L. maniototo* were found to group with no other species. Within these groups little sequence variation was found and many taxa were found to be non-monophyletic, especially in the *dioica*-group. Inadequate phylogenetic signal (the lack of sufficient synapomorphies) may be one reason for this. Hybridization and polyploidization may also have played an imported role in the recent radiation of the Leptinella main group (chapter 3). The *Leptinella* main group is relatively young with a crown age of 10.3 Ma (chapter 3).

Little sequence variation among morphologically and ecologically more or less distinct taxa were also found for other New Zealand plant species (e.g. *Ranunculus*, Lockhard et al. 2001; *Abrotanella*, Wagstaff et al. 2006; *Craspedia*, Ford et al. 2007). For example, within different alpine lineages of New Zealand, there is evidence that these are evolutionary young groups that have evolved rapidly in the new mountain habitats within the last five million years (e.g. Lockhart et al. 2001, Winkworth et al. 2005). Additionally, the evolutionary history could be complicated by hybridization and polyploidization (e.g. Lockhart et al. 2001, Smissen et al. 2004, Meudt and Bayly 2008; reviewed in Morgan-Richards et al. 2009).

The young age of New Zealand plant groups as well as hybridization and polyploidization are the reasons, why sequencing of DNA markers could lead to unsatisfactory results for phylogenetic reconstruction (see *Leptinella* chapter 3). A remedy for this problem may be to choose faster evolving markers that may provide more phylogenetic useful characters. The amplified fragment length polymorphism (AFLP; Vos et al. 1995) could be useful for this purpose, since this method produces a large set of polymorphic markers. Many authors has been successfully used AFLPs in phylogentic studies of closely related species or of genera within different families and different geographical areas (e.g. *Lactuca* (Asteraceae), Koopman et al. 2001; *Pachycladon*

(Brassicaceae), Mitchell and Heenan 2002a; *Senecio* (Asteraceae), Pelser et al. 2003; *Achillea* (Asteraceae), Guo et al. 2005; *Rosa* (Rosaceae), Koopman et al. 2008; *Veronica* (Plantaginaceae), Meudt and Bayly 2008; *Hordeum* (Poaceae), Pleines and Blattner 2008; *Betula* (Betulaceae), Schenk et al. 2008; *Pseudopanax* (Araliaceae), Perrie and Shepherd 2009). For example, Meudt and Bayly (2008) have shown that AFLP is a very useful method for resolving relationships in the Australasian group *Chionohebe* (*Veronica* s.l.) finding well supported groups within this genus. AFLPs seem to be also useful to untangle the complex and reticulate evolution of polyploid complexes (e.g. Hedrén et al. 2001, Guo et al. 2005, 2008, Weiss-Schneeweiss and Tremetsberger 2008).

In the present study we would like to answer following questions: (i) Is the AFLP method suitable for phylogenetic analyses within the *Leptinella* main group and are the results in correspondence to the results of sequence data in chapter 3? (ii) It is possible to discriminate different taxon groups and taxa within the *Leptinella* main group? (iii) Can AFLP data help do elucidate the complex and reticulate relationships among species of this polyploid complex?

Methods

Plant sampling. 236 individuals of 31 taxa of the *Leptinella* main group (see chapter 3) from Australia, New Zealand and South America were analysed. The undescribed taxon *L.* "Seal" and two populations of the hybrid *L. dioica* subsp. *dioica* × *L. squalida* subsp. *mediana* were also included. Five out of six subgroups of the *Leptinella* main clade (chapter 3) were included in our analysis; the *plumose*-group from the sub-Antarctic islands could not be included. Unfortunately, no silica dried material was available of *L. atrata* subsp. *luteola, L. pectinata* subsp. *willcoxii* (New Zealand), *L. drummondii* (Australia), *L. lanata*, and *L. plumosa* (sub-Antarctic islands). Additionally, *L. intermedia* from the South Island was also not included, because since its formal description by Lloyd (1972c) this species was not found again (New Zealand Plant Conservation Network 2009). In general, one to six populations with up to five individuals per taxon were included in the study. For some taxa only one population could be included, because no other populations were found (e.g. *L. scariosa, L. serrulata).* Other taxa are restricted to very small areas were only few populations occur, therefore only one population was included for these taxa (e.g. *L. filiformis, L. minor, L.* "Seal").

Tab. 4-1: Samples include in the AFLP analysis

Taxon	Accession	Ploidy level FCM[1]	Lit[2]		AFLP Code	In.[3]	Gr.[4]
L. albida (D. G. Lloyd) D. G. Lloyd & C. J. Webb	New Zealand, Old Man Range, Symes road, Rupprecht & Himmelreich NZ 37 (CHR)	4	4	L	NZ 37	5	A
L. atrata (Hook. f.) D. G. Lloyd & C. J. Webb subsp. *atrata*	New Zealand, Craigieburn Range, Brocken River, Heenan (CHR)	4	4	L	A419	1	A
	New Zealand, Torlesse Range, Foggy Peak, Heenan (CHR)	4			A757	1	A
	New Zealand, Torlesse Range, Foggy Peak, Rupprecht & Himmelreich NZ 47 (CHR)	4			NZ 47	3	A
L. calcarea (D. G. Lloyd) D. G. Lloyd & C. J. Webb	New Zealand, Cultivation, ex Westhaven, Heenan (CHR)	-	8	L	A420	1	C
	New Zealand, Whariariki Beach, Rupprecht & Himmelreich NZ 16 (CHR)	8			NZ 16	4	C
L. conjuncta Heenan	New Zealand, Cultivation, ex Maryburn Station, Rupprecht & Himmelreich NZ 49 (CHR)	8	8	He	NZ 49	1	A
	New Zealand, Cultivation, ex Manuherikia Valley, Fiddlers Flat, Rupprecht & Himmelreich NZ 50 (CHR)	8			NZ 50	1	A
	New Zealand, Cultivation, ex Nevis River Valley, School House Flat, Rupprecht & Himmelreich NZ 51 (CHR)	-			NZ 51	1	A
L. dendyi (Cockayne) D. G. Lloyd & C. J. Webb	New Zealand, Craigieburn Range, Brocken River, Rupprecht & Himmelreich NZ 25 (CHR)	-	4	B	NZ 25	2	A
	New Zealand, Molesworth Station, Island Saddle, Rupprecht & Himmelreich NZ 27 (CHR)	4			NZ 27	5	A
L. dioica subsp. *manoica* (D. G. Lloyd) D. G. Lloyd & C. J. Webb	New Zealand, Waikanae Beach, Rupprecht & Himmelreich NZ 05 (CHR)	20	20	Ll	NZ 05	4	C
	New Zealand, Tangimoana, Rupprecht & Himmelreich NZ 06 (CHR)	20			NZ 06	5	C
L. dioica Hook. f. subsp. *dioica*	New Zealand, Tunnel Beach, Dunedin, Heenan (CHR)	20	20	L, B	A424	5	C
	New Zealand, Banks Peninsula, Lake Forsyth, Rupprecht & Himmelreich NZ 02 (CHR)	20			NZ 02	4	C
	New Zealand, Whariariki Beach, Rupprecht & Himmelreich NZ 15 (CHR)	20			NZ 15	5	C
	New Zealand, Molesworth Station, Serpentine Creek, Rupprecht & Himmelreich NZ 28 (CHR)	20			NZ 28	5	C
	New Zealand, Invercargill, Sandy Point Reserve, Rupprecht & Himmelreich NZ 38 (CHR)	20			NZ 38	5	C
	New Zealand, Catlin Coast, Curio Bay Reserve, Rupprecht & Himmelreich NZ 45 (CHR)	20			NZ 45	5	C
L. dispersa (D. G. Lloyd) D. G. Lloyd & C. J. Webb subsp. *dispersa*	New Zealand, Lake Wairarapa, Western Lake Road, Rupprecht & Himmelreich NZ 11 (CHR)	4	4	L, B	NZ 11	5	B
	New Zealand, Cultivation, Lake Kohangatera, de Lange 6262 & de Lange (AK)	4			A760	1	B
	New Zealand, Stewart Island, Mason Bay, Rupprecht & Himmelreich NZ 42 (CHR)	4			NZ 42	5	B
L. dispersa subsp. *rupestris* (D. G. Lloyd) D. G. Lloyd & C. J. Webb	New Zealand, Castlecliff, Ogle, Rupprecht & Himmelreich NZ 07 (CHR)	4	-	-	NZ 07	5	B
L. featherstonii Muell.	New Zealand, Chatham Islands, Western Reef, de Lange CH377 & Sawyer (AK)	8	4	D	A710	1	A
L. filiformis (Hook. f.) D. G. Lloyd & C. J. Webb	New Zealand, Cultivation, ex Hanmer Spring, Rupprecht & Himmelreich NZ 52 (CHR)	4	4	L, B	NZ 52	1	A
	New Zealand, Cultivation, ex Hanmer Spring, Rupprecht & Himmelreich NZ 57 (CHR)	-			NZ 57	1	A
L. goyenii (Petrie) D. G. Lloyd & C. J. Webb	New Zealand, Old Woman Range, Nevis Road, Rupprecht & Himmelreich NZ 33 (CHR)	4	4	L, B	NZ 33	4	A

Tab. 4-1: Continued

Taxon	Accession	Ploidy level FCM[1]	Lit[2]		AFLP Code	In.[3]	Gr.[4]
	New Zealand, Cultivation, ex Old Woman Range, Barkla (CHR)	4			NZ 63	1	A
L. longipes Hook. f.	Australia, Victoria, Lake Tyers, Thomson 955	4	-	-	A762	1	A
L. maniototo (Petrie) D. G. Lloyd & C. J. Webb	New Zealand, Cass, Breitwieser 2198 (CHR)	-	4	L, B	A567	1	A
	New Zealand, Cultivation, ex Lake Lyndon, Heenan (CHR)	-			A755	1	A
	New Zealand, Cultivation, ex Upper Clutha terraces, Barkla (CHR)	4			NZ 60	1	A
L. minor Hook. f.	New Zealand, Port Hills, Mt Pleasant, Rupprecht & Himmelreich NZ 04 (CHR)	4	4	H, L, B	NZ 04	4	A
L. nana (D. G. Lloyd) D. G. Lloyd & C. J. Webb	New Zealand, Cultivation, ex Titahi Bay, Heenan (CHR)	4	4	L	A756	1	A
	New Zealand, Port Hills, Mt Pleasant, Rupprecht & Himmelreich NZ 03A (CHR)	4			NZ 03	6	A
L. pectinata (Hook. f.) D. G. Lloyd & C. J. Webb subsp. *pectinata*	New Zealand, Awatere Valley, Ford 613/06	-	8	L	A655	1	A
	New Zealand, Torlesse Range, Foggy Peak, Rupprecht & Himmelreich NZ 26 (CHR)	8			NZ 26	4	A
L. pectinata subsp. *villosa* (D. G. Lloyd) D. G. Lloyd & C. J. Webb	New Zealand, Canterbury, Lake Ohau skifield, Rupprecht & Himmelreich NZ 31 (CHR)	4	4, 8	L, B	NZ 31	4	A
	New Zealand, Carrick Range, Nevis Road, Rupprecht & Himmelreich NZ 34 (CHR)	8			NZ 34	4	A
	New Zealand, Cultivation, ex Dunstan Mountains, Barkla (CHR)	4			NZ 64	1	A
L. cf. *pectinata* subsp. *villosa*	New Zealand, Cultivation, ex Nevis Valley, Barkla (CHR)	8			NZ 59	1	A
L. potentillina Muell.	New Zealand, Chatham Islands, Point Somes, de Lange CH387 (AK)	4	4	H, L, B	A758	1	B
	New Zealand, Chatham Islands, Kaiangaroa, de Lange CH404, Sawyer, Baird (AK)	4			A759	1	B
	New Zealand, Chatham Islands, Otauwe Conservation, Baird (CHR)	4			NZ 61	5	B
L. pusilla Hook. f.	New Zealand, Tasman Valley, Blue Lake, Rupprecht & Himmelreich NZ 29 (CHR)	8	8	L, B	NZ 29	4	C
	New Zealand, Tasman Valley, Rupprecht & Himmelreich NZ 30 (CHR)	8			NZ 30	4	C
L. cf. *pusilla/serrulata*	New Zealand, Old Man Range, Symes Road, Rupprecht & Himmelreich NZ 35 (CHR)	12*			NZ 35	4	C
L. pyrethrifolia var. *linearifolia* (Cheeseman) D. G. Lloyd & C. J. Webb	New Zealand, Cultivation, ex Red Hills, Korver (CHR)	12	12	L	NZ 66	1	A
L. pyrethrifolia (Hook. f.) D. G. Lloyd & C. J. Webb var. *pyrethrifolia*	New Zealand, Craigieburn Range, Brocken River, Heenan (CHR)	12	12, 16	L, B	A417	1	A
	New Zealand, Craigieburn Range, Brocken River, Rupprecht & Himmelreich NZ 24 (CHR)	12			NZ 24	5	A
L. reptans (Benth.) D. G. Lloyd & C. J. Webb	Australia, South Australia, Picaninnie Ponds, Thomson 930	-	-	-	A703	1	A
L. rotundata (Cheeseman) D. G. Lloyd & C. J. Webb	New Zealand, Cultivation, ex Maunganui Bluff, Heenan (CHR)	-	24	L	A761	1	C
	New Zealand, Cultivation, ex Lloyd 7615, Rupprecht & Himmelreich NZ 54 (CHR)	24			NZ 54	1	C
	New Zealand, Cultivation, ex Lloyd 9105-1, Rupprecht & Himmelreich NZ 56 (CHR)	24			NZ 56	1	C
L. scariosa Cass.	Chile, Valdivia, Alvarez (CHR)	16	20	M	NZ 58	5	C
L. serrulata (D. G. Lloyd) D. G. Lloyd & C. J. Webb	New Zealand, near Wanaka, Mt Iron, Rupprecht & Himmelreich NZ 32 (CHR)	4	4	L, B	NZ 32	5	C

Tab. 4-1: Continued

Taxon	Accession	Ploidy level FCM[1]	Lit[2]		AFLP Code	In.[3]	Gr.[4]
L. squalida subsp. *mediana* (D. G. Lloyd) D. G. Lloyd & C. J. Webb	New Zealand, Mt Arthur Hut, Rupprecht & Himmelreich NZ 17 (CHR)	16	12, 16, 20	L, B	NZ 17	5	C
	New Zealand, Red Hill Hut, Rupprecht & Himmelreich NZ 20 (CHR)	12			NZ 20	5	C
	New Zealand, St Arnaud Range, Rainbow Skifield, Rupprecht & Himmelreich NZ 21 (CHR)	16			NZ 21	5	C
	New Zealand, St Arnaud Range, Rainbow Skifield, Rupprecht & Himmelreich NZ 22 (CHR)	12			NZ 22	5	C
	New Zealand, Cultivation, ex Old Man Range, Barkla (CHR)	8-12			NZ 62	1	C
L. squalida subsp. *mediana* × *dioica* subsp. *dioica*	New Zealand, Banks Peninsula, Hinewai Reserve, Wilson, Rupprecht & Himmelreich NZ 01 (CHR)	20	-	-	NZ 01	4	C
	New Zealand, Red Hills, Rupprecht & Himmelreich NZ 18 (CHR)	20			NZ 18	5	C
	New Zealand, Red Hills, Rupprecht & Himmelreich NZ 19 (CHR)	12, 16			NZ 19	4	C
L. squalida Hook. f. subsp. *squalida*	New Zealand, Tangahoe River mouth, Rupprecht & Himmelreich NZ 08 (CHR)	20	20	L	NZ 08	5	C
	New Zealand, Mt Taranaki, Rupprecht & Himmelreich NZ 10 (CHR)	>20			NZ 10	5	C
	New Zealand, Lake Kohangapiripiri, Rupprecht & Himmelreich NZ 12 (CHR)	20			NZ 12	5	C
L. tenella (Cunn.) D. Llyod & C. J. Webb	New Zealand, Powerhouse Road, near Patea, Rupprecht & Himmelreich NZ 09 (CHR)	4	4	L	NZ 09	4	B
L. tenella/dispersa	New Zealand, Lake Kohangapiripiri, Rupprecht & Himmelreich NZ 14 (CHR)	4			NZ 14	5	B
L. traillii subsp. *pulchella* (Kirk) D. G. Lloyd & C. J. Webb	New Zealand, Bluff Hill, Rupprecht & Himmelreich NZ 44 (CHR)	>20	-	-	NZ 44	4	C
	New Zealand, Bluff Hill, Rupprecht & Himmelreich NZ 44B (CHR)	>20			NZ 44B	5	C
L. traillii (Kirk) D. G. Lloyd & C. J. Webb subsp. *traillii*	New Zealand, Stewart Island, near Mason Bay, Rupprecht & Himmelreich NZ 40 (CHR)	24	24	L	NZ 40	3	C
	New Zealand, Stewart Island, Mason Bay, Rupprecht & Himmelreich NZ 41 (CHR)	24			NZ 41	5	C
L. cf. *traillii*	New Zealand, Stewart Island, Mason Bay, Rupprecht & Himmelreich NZ 43 (CHR)	20			NZ 43	4	C
L. "Seal"	New Zealand, Cultivation, ex Seal Island, Korver (CHR)	20	-	-	NZ 67	1	C

[1)] Ploidy level estimation by Flow cytometry (FCM)
[2)] Ploidy level from literature: H - Hair (1962), L - Lloyd (1972c), Ll - Lloyd (1975b), M - Moore (1981), B - Beuzenberg and Hair (1984), D - Dawson (1995), He - Heenan (2009)
[3)] Number of individuals in the AFLP analysis.
[4)] The letter indicates the group membership of the population in the analysis of the whole dataset.
* The ploidy level of this population could not be estimated, the number was estimated for a morphological similar population 100 m apart.

As *Leptinella* plants grow clonally, it was not possible to determine single plants within a population and no information was found in the literature about how large one genet may grow. Hence, only individuals located sufficiently distant from each other were collected to minimize sampling of duplicates. The localities, collectors, sample sizes and population codes are listed in Tab. 4-1. Vouchers of the populations were deposited at AK, CHR or MEL.

Flow cytometry. Fresh leaf samples from cultivated *Leptinella* plants at the Botanical Garden of the University of Regensburg (Germany) or silica dried material were used for this analysis. The dried samples were collected during a field excursion in summer 2006/2007 or from cultivated plants in 2007 or 2008. We used one sample per population; unfortunately, a few populations with too little material had to be excluded. The ploidy levels were estimated by flow cytometry (FCM) with DAPI staining by Plant Cytometry Services (Schijndel, Netherlands).

DNA extraction. A preliminary test had shown that the DNA extraction from silica dried or even fresh leaves of *Leptinella* was a critical point. To find a suitable extraction method we screened several extraction protocols for eight different *Leptinella* species. The following extraction protocols were tested: CTAB protocols with different modifications (according to Doyle and Doyle 1987), a gel extraction following a CTAB extraction, a modified silica extraction using CTAB as lysis buffer (Rogstad 2003), commercial plant extraction kits based either on DNA binding columns (NucleoSpin Plant II, Macherey-Nagel; DNeasy plant mini kit, Quiagen) or on magnetic beads (Chloropure, Agentcourt), and finally a combined CTAB and column based method (Smissen et al. 2006). Additionally, the number of wash steps in each extraction was varied to promote removing of secondary compounds. Following the extraction, the DNA was checked for quality and quantity by agarose gel electropherese and spectrometric measurements with a Nanodrop photometer. Additionally, the quality was checked by an endonuclease restriction reaction with MseI. Fresh leaf material from *Leucanthemum* and *Rosa* was used as control to exclude manual or technical mistakes in the extraction processes.

The gel electrophoresis of the obtained DNA of the several extractions showed a ‘normal’ quantity of DNA, but the spectrometric measurements showed low quality and low quantity of the more or less colourless DNA solution. Complete digestions of DNA in an endonuclease restriction reaction with MseI were mostly not successful. A suitable quality of DNA for the endonuclease restriction reaction was only obtained with a modified protocol of the NucleoSpin Plant II kit (Macherey-Nagel). Lysis Buffer PL2, based on SDS lysis method was used instead of CTAB based buffer, and both wash steps were increased (4 times with optional buffer PW1, and 3 times with buffer PW2).This extraction worked well in all 31 *Leptinella* taxa, which were used in this study.

AFLP procedure. AFLP procedure was carried out according to a modified protocol of Meister et al. (2006). 21 replicates were included to find the optimal scoring parameter and to check reproducibility of the AFLPs. The restriction and ligation reaction contained ~50 ng genomic DNA, 1 µl 10x ligase buffer, 0.1 µl $Nacl_2$ (5 M), 0.5 µl BSA (1 mg/µl), 0.5 µl MseI adaptor (20 µM), 0.5 µl EcoRI adaptor (2 µM), 0.25 µl MseI and EcoRI enzyme (10 U/µl, Fermentas), and 0.5 µl T4-ligase (1 U/µl, Fermentas) in a total volume of 10 µl. The reaction was incubated at 37°C for 4 h. Preselective amplifications contained 0.5 µl 10x buffer S, 0.125 µl MseI+C primer, 0.125 µl EcoRI+A primer (both 10 µM), 0.2µl dNTPs, (5 mM, PeqLab), 0.025 µl Sawady Taq (5 U/µl, PeqLab), and 1 µl ligated DNA in a total volume of 5 µl. The following PCR parameters were used: 2 min at 94 °C, 30 cycles of 20 s at 94 °C, 30 s at 56 °C, and 2 min at 72 °C, and a final step of 30 min at 60 °C. Selective amplifications were performed with the three primer combinations MseI-CTA/EcoRI-AAC, MseI-CTT/EcoRI-ACT, MseI-CAC/EcoRI-AAG with different colour labels. The PCR reaction was carried out in a total volume of 5 µl and contained 0.5 µl 10x buffer S, 0.25 µl of each selective primer (5 µM for MseI, 1 µM for EcoRI), 0.2µl dNTPs (5 mM, PeqLab), 0.025 µl Sawady Taq (5 U/µl, PeqLab) and 0.75 µl of product from the preselective PCR. The reaction was performed with the following touchdown profile: 2 min at 94 °C, 10 cycles of 20 s at 94 °C, 30 s at initially 66 °C and then dropping by 1 °C in each cycle, 2 min at 72 °C, followed by 25 cycles for 20 s at 94 °C, 30 s at 56 °C and 2 min at 72 °C, and a final extension for 30 min at 60 °C.

After DNA precipitation, DNA pellets were vacuum-dried and dissolved with a mixture of Sample Loading Solution (SLS) and CEQ Size Standard 400 (both Beckman Coulter). The pooled fluorescence-labelled selective amplification products were separated by capillary gel electrophoresis on an automated sequencer (CEQ 8000, Beckman Coulter).

Data analysis. Electropherograme data was exported as crv-files, showing virtual gels with AFLP fragments for each primer combination separately from all studied individuals and analyzed in CelCompare II vers. 5.0 (Applied Maths). To determine optimal scoring parameter settings, a modified protocol of Holland et al. (2008) was used. The software GelCompare II version 5.0 was used instead of GeneMapper (Applied Biosystems) or GeneMarker (SoftGenetics) as in Holland et al. (2008). The following parameters were tested to determine the best scoring parameter settings: Firstly, we varied the minimum profiling in the auto search band tool (0.5 %, 1.0 %, 1.5 %, 2.0 %; percents relative to the maximum value of the lane). Secondly, we varied the position tolerance in the band

matching tool (0.02 %, 0.04 %, 0.06 %, 0.08 %, 0.1 %; one bp = c. 0.08 %). Additionally, we conducted three different minimum fragment lengths (80 bp, 90 bp, 100 bp), and maximum fragment length (320 bp, 380 bp, 420 bp). The optimal parameter setting according to the modified protocol of Holland et al. (2008) were found at a minimum profiling of 0.5 %, a position tolerance of 0.06 %, a minimum fragment length of 90 bp, and a maximum fragment length of 320 bp (primer MseI-CTT/EcoRI-ACT) or 380 bp (primer MseI-CTA/EcoRI-AAC, MseI-CAC/EcoRI-AAG).

To further investigate reproducibility and errors in the data set, the placement of the replicates in a neighbour joining (NJ) tree was checked (according to Meudt and Bayly 2008). The NJ tree based on Nei-Li distances (Nei and Li 1979) was reconstructed in PAUP* vers. 4.0b10 (Swofford 2002) with 257 samples (including 21 replicates). Bootstrap support (BS; Felsenstein 1985) was evaluated using 1000 re-samples. Of the 21 replicates, 12 clustered together (7 with 100 % BS, 4 with 75 - 99 % BS, 1 with 60 - 74 % BS). The remaining 11 replicates did not clustered with their original probes, but still clustered with high support in the same clade, as did other individuals from the same population. Once the optimal parameter settings were determined and replicates were checked for errors, all replicates were removed from the dataset.

In a first analysis, we analysed the complete dataset including all taxa and populations (236 individuals). Secondly, we analysed the tetraploid taxa separately. Additionally, we analysed the three groups resulting from the first analysis separately (taxon group A-C, see results below).

Unrooted or midpoint rooted NJ tree based on Nei-Li genetic distance was constructed using PAUP* vers. 4.0b10. To estimate phylogenetic support, a bootstrap analysis was performed with 1000 bootstrap replicates. Additionally, a maximum parsimony analysis was conducted with 1000 random addition sequence replicates, all characters weighted equal, ACCTRAN and TBR branch swapping in action. Bootstrap support was evaluated using 1000 re-samples with the same program settings as before, but with only 10 random addition sequence repeats per replicate.

To illustrate the genetic similarities among the individuals and taxa, a principal coordinate analysis (PCoA) based on the Bray-Curtis distance index (equivalent to the Sørensen similarity index) was calculated and plotted with the software package MVSP vers. 3.1 (Kovach 1999).

Results

Flow cytometry. The results of the flow cytometry analysis of *Leptinella* taxa are summarised in Tab. 4-1. These results of the analysis are not easy to interpret (see discussion). In general, tetraploid, octoploid, and dodecaploid populations are well definable, but the populations with higher ploidy levels are sometimes problematic. Therefore, it was not possible to determine the ploidy level for all populations exactly.

Few results differ from previous literature data (i.e. *L. featherstonii, L. scariosa),* and there are new ploidy level reports for some taxa (i.e. *L. dispersa* subsp. *rupestris, L. longipes, L. squalida* subsp. *squalida, L. traillii* subsp. *pulchella, L.* "Seal"). Where more than one population of one taxon were included in the analysis, these were found to have mostly the same ploidy level. But different ploidy levels within a single taxon were found for *L. pectinata* subsp. *villosa* (4*x*, 8*x*), *L. squalida* subsp. *mediana* (12*x*, 16*x*), and *L. squalida* subsp. *squalida* (20*x*, >20*x*).

AFLP analyses

Relationships within the complete dataset. Using three primer pairs a total of 1053 fragments were scored automatically for the complete dataset of 236 individuals of 31 *Leptinella* taxa. The unrooted NJ tree based on the complete dataset is shown in Fig. 4-1. It shows three main clusters (taxon group A-C), although the support values for these groups are quite modest (53 - 65 % BS). Group A consist of 14 species (*L. albida, L. atrata, L. conjuncta, L. dendyi, L. featherstonii, L. filiformis, L. goyenii, L. longipes, L. maniototo, L. minor, L. nana, L. pectinata*, *L. pyrethrifolia, L. reptans*). The three taxa *L. dispersa, L. potentillina* and *L. tenella* belong to group B. Group C consists of all individuals of *L. calcarea, L. dioica, L. pusilla, L. rotundata, L. scariosa, L serrulata, L. squalida,* and *L. traillii*. The hybrid of *L. squalida* subsp. *mediana* and *L. dioica* subsp. *dioica* and *L.* "Seal" belong also to this group. The tree resulting from the MP analysis shows similar results (not shown), but the three main groups have BS below 50 %.

The first three axes of the PCoA explain only 19.9 % of the variation (9.3 %, 6.0 %, 4.5 %, respectively). The first two components are plotted in Fig. 4-1. The three groups mentioned above are clearly separated from each other on the first two components. Furthermore, group A is split in two subgroups by the third component (not shown; a: *L. filiformis, L. minor, L. nana*; b: the remaining taxa).

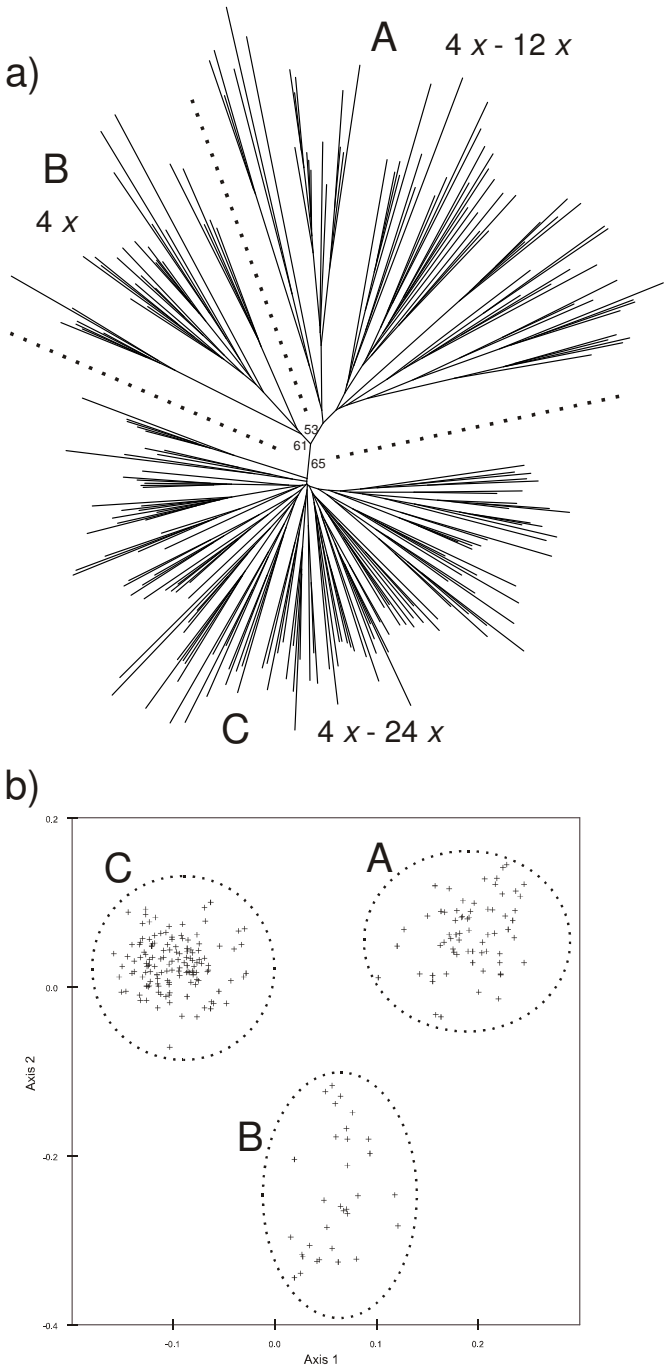

Fig. 4-1: AFLP analysis of all 236 investigated individuals (31 taxa) of *Leptinella* main clade. Letters indicate groups which discussed in the text.
a) Unrooted neighbour-joining tree using Nei-Li distances. Bootstrap values for basal branches are indicated.
b) Axes 1 and 2 of the principal coordinate analysis.

Relationships within the tetraploid taxa. Fifteen tetraploid taxa of *Leptinella* were included in this analysis (*L. albida, L. atrata* subsp. *atrata, L. dendyi, L. dispersa* (both subspecies), *L. filiformis, L. goyenii, L. longipes, L. maniototo, L. minor, L. nana, L. pectinata* subsp. *villosa* (only populations NZ_31, NZ_64), *L. potentillina, L. serrulata, L. tenella*). The ploidy level of the populations NZ_25 *(L. dendyi)*, NZ_57 *(L. filiformis)*, A567, and A755 *(L. maniototo)* could not be estimated, but tetraploid ploidy level could be

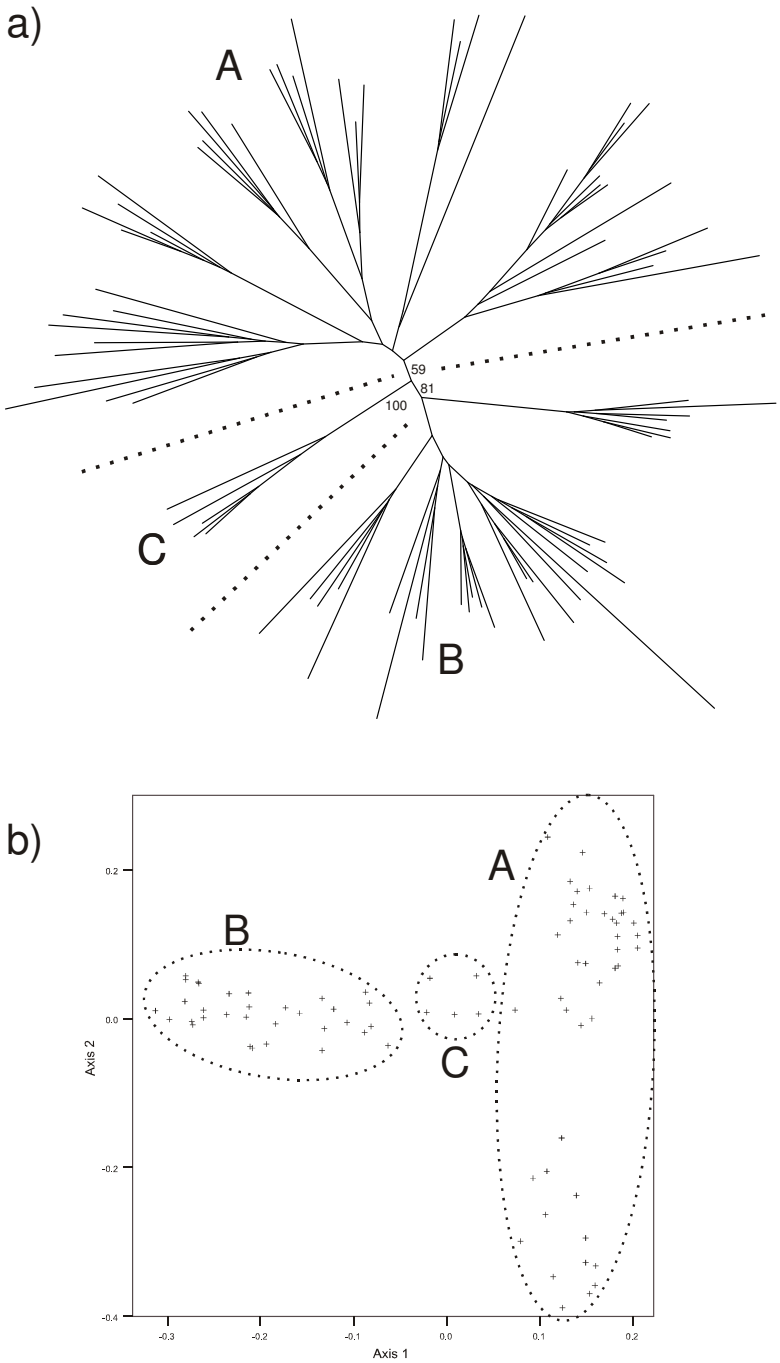

Fig. 4-2: AFLP analysis of tetraploid individuals (81, individuals, 15 taxa) of *Leptinella* main clade. Letters indicate groups which discussed in the text.
a) Unrooted neighbour-joining tree using Nei-Li distances. Bootstrap values for basal branches are indicated.
b) Axes 1 and 2 of the principal coordinate analysis.

confirmed for other populations of these taxa by FCM, and by chromosome counts in literature (Lloyd 1972c, Beuzenberg and Hair 1984). We also did not include *L. featherstonii*, because our investigated sample is octoploid in contrast to four chromosome counts published by Dawson (1995).

Using three primer pairs 1054 fragments were scored for the dataset of tetraploid taxa (81 individuals of 15 *Leptinella* taxa). The unrooted NJ is shown in Fig. 4-2. It shows three main clusters (taxon group A-C) corresponding to the analysis of the complete dataset. These clades have moderate to high bootstrap support (59-100 %). Group A consists of 12 taxa *(L. albida, L. atrata* subsp. *atrata, L. conjuncta, L. dendyi, L. featherstonii, L. filiformis, L. goyenii, L. longipes, L. maniototo, L. minor, L. nana, L. pectinata* subsp. *villosa, L. pyrethrifolia)*. Three species belong to group B *(L. dispersa, L. potentillina, L. tenella)*, and *L. serrulata* is the only tetraploid member of group C. The tree resulting from the MP analysis shows similar results (not shown).

The first three axes of the PCoA explain 32.5 % of the variation (15.3 %, 9.8 %, 7.2 %, respectively). The first two components are plotted in Fig. 4-2. The three taxon groups (A-C), which were found in the analysis of the complete dataset, are also found in the dataset with tetraploid taxa, but the borders between these groups are more blurred. Group A is divided into two subgroups: a) *L. filiformis, L. minor,* and *L. nana,* and b) *L. albida, L. atrata* subsp. *atrata, L. dendyi, L. goyenii, L. longipes, L. maniototo*, and *L. pectinata* subsp. *villosa* (corresponding to both subgroups of group A of the complete dataset, which are separated by the third component). Group B comprises the same taxa as in the analysis of the complete dataset. Group C is only represented by *L. serrulata* in the tetraploid dataset.

Relationships within taxon group A. Using three primer pairs 935 fragments were scored for the dataset of taxon group A (66 individuals of 16 *Leptinella* taxa). The midpoint rooted NJ tree of group A is shown in Fig. 4-3. Within the tree, there are some subgroups recognizable *(L. albida, L. pectinata* subsp. *villosa; L. conjuncta, L. pectinata* subsp. *pectinata; L. goyenii; L. pyrethrifolia* (both subspecies); *L. atrata* subsp. *atrata, L. dendyi; L. maniototo; L. filiformis, L. minor, L. nana).* Most taxa with more than one individual/population turned out to be 'monophyletic', with a wide range of bootstrap support (>50 - 100 %). One exception is *L. pectinata* subsp. *villosa* that groups with *L. albida*; the others are the intermixed taxa *L. atrata* subsp. *atrata and L. dendyi*. The individuals of 8 of the 11 populations cluster together (>50 - 100 % BS). The tree resulting from the MP analysis shows similar results (not shown).

The first three axes of the PCoA explain only 28.0 % of the variation (13.0 %, 8.2 %, 6.8%, respectively). The first two components are plotted in Fig. 4-6, and three different groups may be distinguished: Group A1 comprises *L. filiformis, L. minor* and *L. nana*.

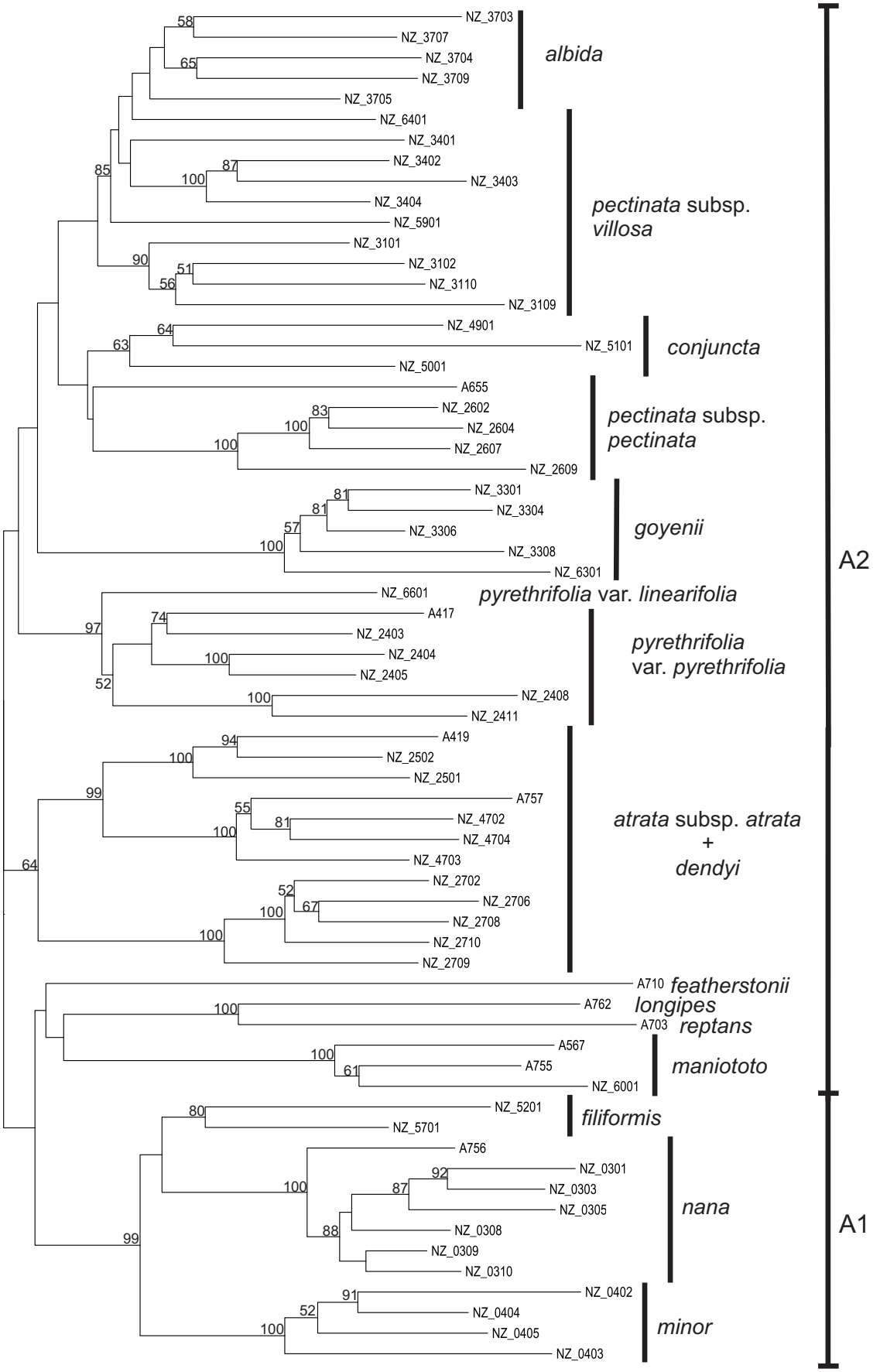

Fig. 4-3. Midpoint rooted neighbour-joining tree using Nei-Li distances of taxon group A. Bootstrap values > 50 are indicated. The left bars indicate taxa or taxon groups. The right bars correspond to groups founded in the PCoA.

Group A2 is represented by *L. albida, L. atrata, L. conjuncta, L. dendyi, L. goyenii, L.maniototo, L. pectinata*, and *L. pyrethrifolia*. Within the groups, the taxa are not separated from each other on the first tree components. The single investigated individuals of *L. featherstonii, L. longipes*, and *L. reptans* belong to group A2. However, these three species showed obviously different AFLP profiles in comparison to the other members of group A.

Relationships within taxon group B. Using three primer pairs 631 fragments were scored for the dataset of taxon group B (32 individuals of 4 *Leptinella* taxa). The NJ tree based of group B is illustrated in Fig. 4-4. The tree resulting from the MP analysis shows similar results (not shown). In the NJ tree three different groups may be distinguished: Group B1 is represented by *L. dispersa* (both subspecies) and *L. tenella* from the southern part of the North Island. Group B2 comprises a population of *L. dispersa* subsp. *dispersa* from Stewart Island. *L. potentillina* is placed in group B3. The five populations *of L. dispersa* and *L. tenella* are 'monophyletic'. The three investigated populations of *L. potentillina* are intermixed.

The first three axes of the PCoA explain 52.7 % of the variation (28.0 %, 14.3 %, 10.3 %, respectively). The first two components are plotted in Fig. 4-6, in which the same groups as in the NJ tree are distinguished.

Relationships within taxon group C. Using three primer pairs 989 fragments were scored for the dataset of taxon group C (138 individuals of 14 *Leptinella* taxa). The NJ tree based of group C is shown in Fig. 4-5. The tree resulting from the MP analysis shows similar results (not shown). The basal branches have mostly low bootstrap support, and there are only few taxon groups recognizable. One group comprises *L. calcarea, L. pusilla, L. serrulata* and one individual of *L. dioica* subsp. *dioica* (NZ_4501). Some taxa are 'monophyletic' (e.g. *L. dioica* subsp. *manoica, L. rodundata)*, whereas other taxa split into different groups (e.g. *L. dioica* subsp. *dioica,* both subspecies of *L. squalida*). In the analysis, we have included 30 populations having more than one individual, 20 of them form a cluster. The other are intermixed with individuals of other populations, either from the same taxa (e.g. NZ_05/NZ_06; *L. dioica* subsp. *manoica)* or from a other taxa (e.g. NZ_02/NZ_22; *L. dioica* subsp. *dioica* and *L. squalida* subsp. *mediana*). The first three axes of the PCoA explain only 17.8 % of the variation (7.4 %, 5.7 %, 4.7 %, respectively).

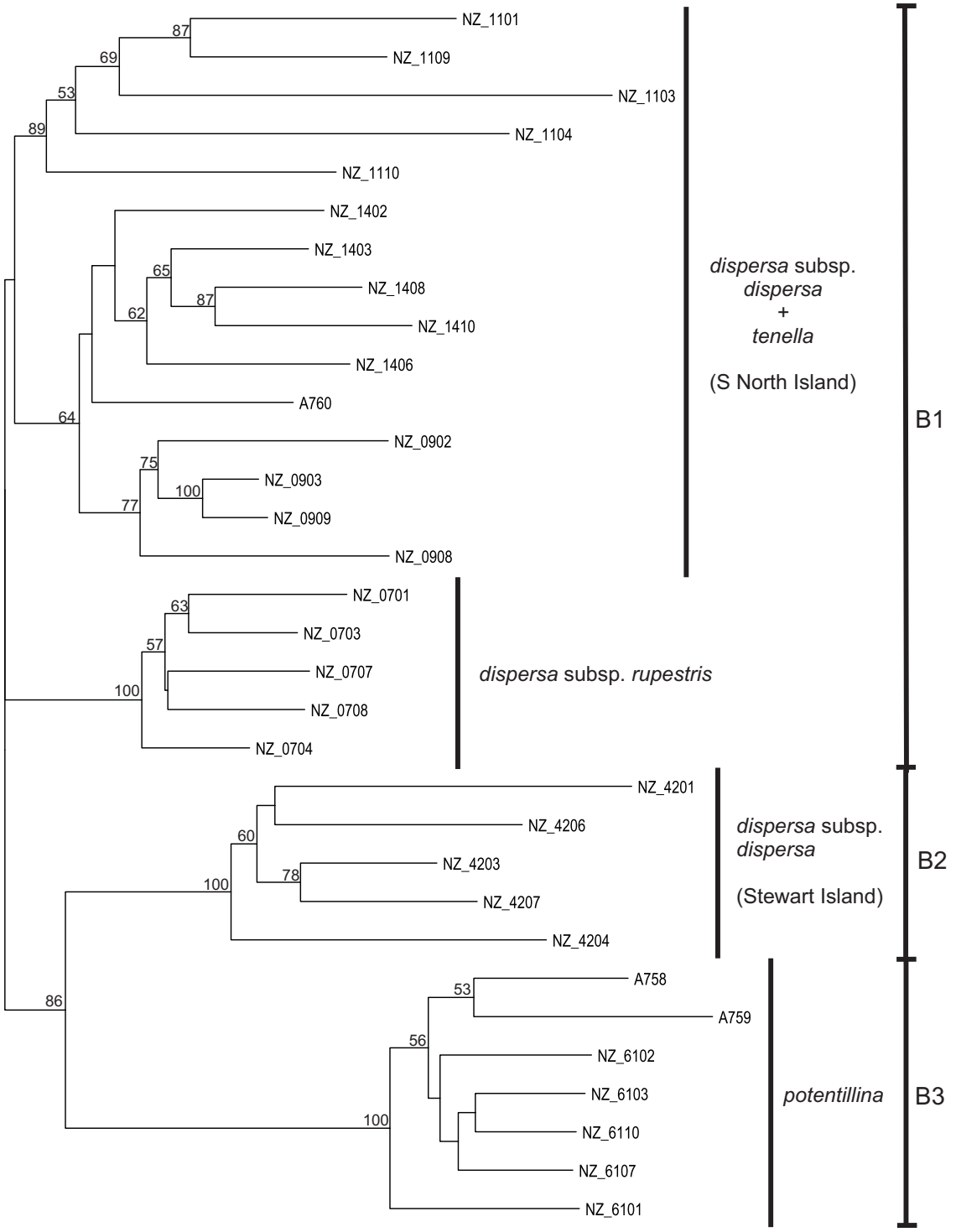

Fig. 4-4: Midpoint rooted neighbour-joining tree using Nei-Li distances of taxon group B. Bootstrap values > 50 are indicated. The left bars indicate taxa or taxon groups. The right bars correspond to groups founded in the PCoA.

The first two components are plotted in Fig. 4-6, where more or less two groups are distinguished: Three taxa *(L. calcarea, L. pusilla, L. serrulata;* C1) are separated by the second component from the other taxa of group C (group C2); however, the two groups are not clearly separated from each other. Additionally, *L. dioica* subsp. *manoica* is separated by the third axis (not shown). It is not possible to separate further taxa within group C. Some species (e.g. *L. dioica* subsp. *dioica, L. pusilla*) are scattered widely within their group.

Discussion

Flow cytometry. Use of flow cytometry (FCM) in plant biology research has expanded in the last years (Suda and Trávnícek 2006, Kron et al. 2007). Information gained about ploidy level has been usefully utilized in plant systematic, and several authors have recently used FCM to determined ploidy levels of taxa in their study group (e.g. Torrell and Valles 2001, Šmarda 2006, Dixon et al. 2009). Generally, only fresh leaf samples were used for ploidy level estimation, but a comprehensive study by Suda and Trávnícek (2006) showed that dehydrated plant tissues (herbarium vouchers or silica dried material) could be also used for FCM. The authors also showed that dried samples have a lifetime of 3 years. However, a shift of the peak position and a higher coefficient of variation were observed (Suda and Trávnícek 2006, Šmarda 2006, R. Hößl (Germany), personal communication). Several processes could be considered to explain the pattern, e.g. the loss of DNA during the death of cells, presence of secondary metabolites originating during cell degradation, different concentrations of metabolites in natural conditions and in plants from cultivation (Šmarda 2006). In low polyploids the variation between fresh and dried samples is negligible. More care should be given to the discrimination of high polyploids because the relative between cytotyp difference in DNA amount decrease with increasing ploidy level (DNA downsizing; Suda and Trávnícek 2006, Leitch and Bennett 2004). In our study, the determination of 4*x*, 8*x*, 12*x* is unambiguously possible, but the determination of the higher polyploids is more difficult and not in all case unambiguous. Beside the problem with differences in fresh and dried material, there are also other possible reasons for difficulties in determining ploidy levels by FCM in taxa with high ploidy level AT- amount of different taxa (especially a problem with DAPI staining, Caronel et al. 2007), the possibility of unexpected and so far unreported ploidy levels in *Leptinella* (e.g. 18*x*, 22*x*),

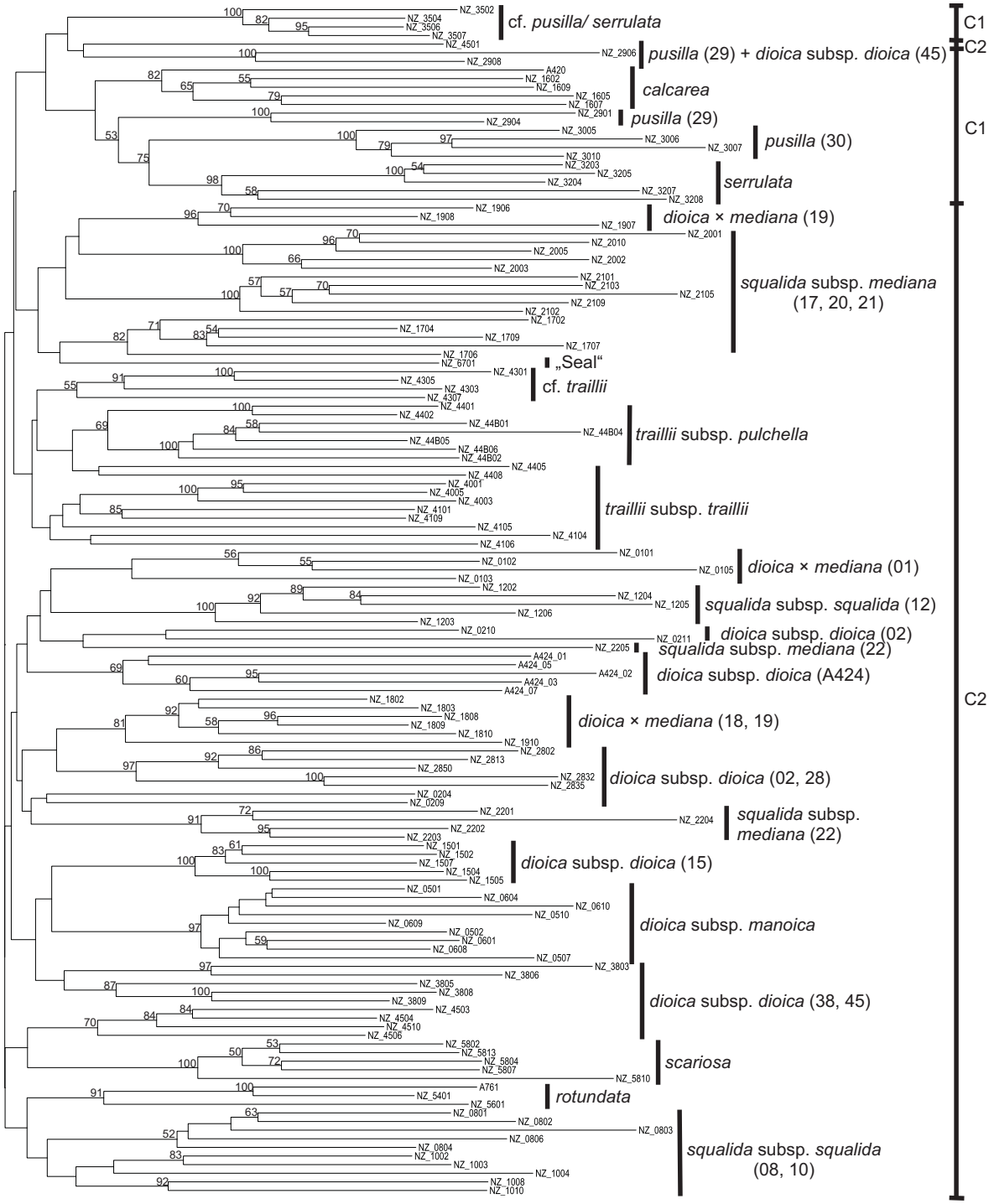

Fig. 4-5. Midpoint rooted neighbour-joining tree using Nei-Li distances of taxon group C. Bootstrap values > 50 are indicated. The left bars indicate taxa or taxon groups. The right bars correspond to groups founded in the PCoA.

and genome modifications like gain or loss of chromosomes as reported for *L. featherstonii* and *L. scariosa* by Dawson (1995) and Moore (1981). Nevertheless, there are several interesting and new results. Different ploidy levels were found for both subspecies of *L. squalida*. Lloyd (1972c) reported three different chromosome numbers for *L. squalida* subsp. *mediana* (12*x*, 16*x*, 20*x*), the latter is missing in our study. Druce (1987) suggested, that the different cytotypes may be correspondence to three so far unrecognised entities (*L. squalida* subsp. *mediana* s.s., *L.* "high altitude", *L.* "seep"). We have found also two different cytotypes in *L. squalida* subsp. *squalida*. Two populations from the coast of Wellington and Taranaki (NZ_08, NZ_12) have 20*x* as reported by Lloyd (1972c). A population from Mount Taranaki (NZ_10) has >20*x*. The later population is morphologically similar to the undescribed entity *L.* "Volcanic Plateau" mentioned by Druce (1993) of the Volcanic Plateau of the North Island (C. C. Ogle (New Zealand), personal communication). Lloyd (1972c) also mentioned these morphologically deviating populations with barely apparent differentiation of the terminal pinnae and reduced hairier leaves (that converge remarkably on *L. pusilla*). Presumably, the population with the different cytotype belongs also to the undescribed entity *L.* "Volcanic plateau", for which we have not included a population. Different ploidy levels were also found in *L. pectinata* subsp. *villosa*, which was also reported by Lloyd (1972c). On the other side, all 9 investigated population of the morphologically diverse taxon *L. dioica* subsp. *dioica* were found to have a uniform ploidy level (20*x*; see Tab. 4-1 and unpublished data).

The FCM analysis suggested that our investigated sample of *L. featherstonii* is octoploid. This result is in contrast to the chromosome counts of four different plants by Dawson (1995), who found they to be tetraploid with $2n = 54$ instead of $2n = 52$ chromosomes as in other taxa of *Leptinella*. *L.* "Seal" (Druce 1993) from Seal Island on the West Coast of South Island has 20*x* like populations of *L. dioica* and *L. squalida*. The investigated plant has another ploidy level as *L. potentillina* (4*x*) from the Auckland and Chatham islands, to which *L.* "Seal" is superficially similar (New Zealand Plant Conservation Network 2009).

The population NZ_35 is morphological similar to *L. pusilla* and *L. serrulata* but has another ploidy level compared to these taxa, and the population NZ_43 (*L.* cf. *traillii*) has another ploidy level as both subspecies of *L. traillii*. Maybe these populations represented new cytotyps for these taxa or they are so far unrecognized entities.

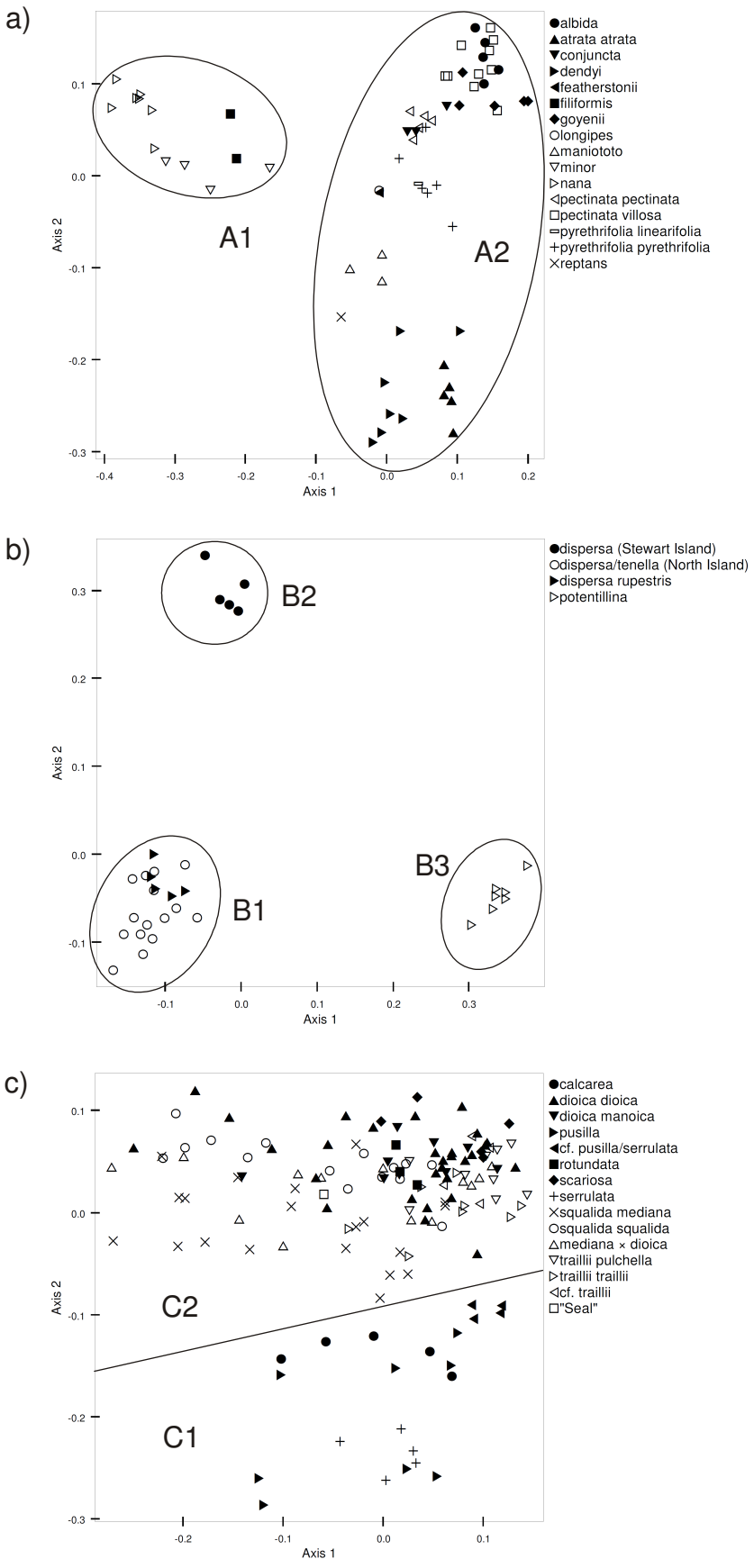

Fig. 4-6: Axes 1 and 2 of the principal coordinate analysis for a) taxon group A, b) taxon group B, and c) taxon group C. Letters indicate groups which discussed in the text.

AFLP-phylogeny. The AFLP techniques is widely used in phylogenetic studies of more or less closely related taxa (e.g. Koopman et al. 2001, Mitchell and Heenan 2002, Pelser et al. 2003, Guo et al. 2005, Koopman et al. 2008, Meudt and Bayly 2008, Pleines and Blattner 2008, Schenk et al. 2008, Perrie and Shepherd 2009). Compared to DNA sequences, AFLPs have the advantage that they are more variable and that they are sampled across the entire genome (Koopman 2005, Meudt and Clarke 2007). Beside these advantages, some authors discussed the drawbacks of AFLP in phylogenetic studies, especially a possible lack of homology of fragments across different taxa (reviewed in Koopman 2005, Meudt and Clarke 2007). Several studies have shown that homology assignment between fragments decrease with increasing evolutionary distance among taxa (Pelser et al. 2003, Koopman 2005, Althoff et al. 2007). Koopman (2005) compared AFLP variation with ITS sequence divergence from a large number of studies and concluded that AFLPs are reliable phylogenetic markers for plant taxa with ITS sequences differing up to 30 - 35 nucleotides. A survey of the dataset from chapter 4 revealed that ITS sequence differences among taxa of the *Leptinella* main clade ranged from 0 to 47 nucleotides (substitution and indels), which is higher than the maximum of Koopman (2005). In taxon group A it range from 0 to 47. When we exclude *L. featherstonii*, *L. longipes* and *L. reptans* the range is only between 0 and 36 differences. These three species showed very distinctly AFLP profiles. On the other side, the ITS sequence differences among taxa of groups B and C are much lower (0 - 6, 0 - 18, respectively). However, Koopman et al. (2008) have used the AFLP technique for a phylogenetic analysis in the genus *Rosa* [a genus with higher ITS sequence variation as suggested by Koopman (2005)]. The authors found that AFLPs were useful to resolve the relationships within the genus and the results are comparable to sequence and morphological analyses.

Although the ITS sequence differences among all taxa of the *Leptinella* main clade is higher as Koopman (2005) suggested for phylogenetic AFLP analyses, we think that AFLPs are useful to determine the main taxon groups within the complete dataset. But to have a more detailed look on the relationships of taxa within these main groups, we have split the dataset to avoid non-homology of fragments. In these groups the ITS sequence variation is more or less within the range defined by Koopman (2005). Therefore, it is expected that AFLP marker variation in our data set of group A to C is a suitable indicator of relationship within these groups. In the divided dataset, the bootstrap supports were found to be higher for most basal and derived branches. Furthermore, it was possible to

determine further subgroups in the PCoA which were not observable in the analysis of the complete dataset.

Our AFLP data distinguish three main groups and different subgroups. However, we could not unambiguously resolve relationships among these groups and subgroups. Due the postulated and observed extensive hybridization, introgression and polyploidyzation within the *Leptinella* main group (Lloyd 1972c, 1975a, chapter 3); good support for the relationships is not always to be expected. The phylogeny obtained from AFLP data is partially congruent with the molecular phylogeny based on sequence information (chapter 3). However, there are some differences (see discussion below).

Additionally, a problem could be that we have included only a few populations per taxon in our analysis, these populations do not cover the complete distribution area and the morphological variation of some taxa (e.g. *L. pusilla, L. pectinata*). To keep this in mind, a more comprehensive sampling of the taxon groups A-C would be necessary.

To summarize this section: The AFLP data are useful to investigate the relationships and the phylogeny within the genus *Leptinella*, but it is not possible to untangle the complex relationships within *Leptinella* completely with this method. The sequencing of single copy genes and/or a microsatellite analysis of related taxa may be further methods to investigate the relationships within *Leptinella*. Both methods were used successfully by several authors (e.g. Joly et al. 2006, Brysting et al. 2007, Edwards et al. 2008).

Polyploidy and hybridization. Taxon groups A and C contain tetraploid and higher polyploid taxa (see Fig. 5-1). While, tetraploid taxa predominantly are in group A, higher polyploids are mainly found in group C. Taxon group B exclusively contains tetraploid species. However, *L. intermedia* (12*x;* not included in the present study) may be also a member of the latter group according to sequence information (chapter 3).

The results of the complete and the tetraploid dataset are comparable, both showing a split of the *Leptinella* main clade into three distinct groups. There is no evidence for intermediate individuals between the three groups, indicating no hybridization or allopolyploidization events between members of the different groups. Extensive hybridization in combination with polyploidization may be occurring mainly between the taxa of one group. However, occasional hybridization between representatives of the three main groups were reported (e.g. *L. dioica* × *L. dispersa, L. dispersa* × *L. plumosa;* Lloyd 1972c), and artificial crosses are possible (Lloyd 1975a).

Group A contains only few higher polyploid taxa [*L. albida* (4*x*, 8*x*), *L. conjuncta* (8*x*), all three subspecies of *L. pectinata* (4*x*, 8*x*), both varieties of *L. pyrethrifolia* (12*x,* 16*x*)] in addition to its 10 tetraploid taxa. Natural hybrids are rare in this group and most taxa are well circumscribed and easily determined (Lloyd 1972c). Most taxa of group A are 'monophyletic' in the present AFLP analysis, suggesting low level of hybridization and polyploidization between the taxa and a single origin of most taxa.

On the other side, hybrids are common between members of group C, even between taxa with different ploidy level (Lloyd 1972c). Additionally, most taxa are morphologically very similar to each other and difficult to discriminate. This group shows also a higher variation in ploidy levels ranging from tetraploid (only *L. serrulata*) to a chromosome set of $2n = 24x$ (11 higher polyploid taxa).

Several taxa of group C are widespread throughout New Zealand or on one of the both islands (e.g. *L. dioica* subsp. *dioica, L. pusilla,* both subspecies of *L. squalida)*. Some of these taxa are morphologically and cytogenetically variable (Lloyd 1972c). For example, *L. dioica* subsp. *dioica* shows extreme variation in morphology (see the different leaf shapes illustrated in Fig. 1-4), but no variation in cytology. On the other hand, *L. squalida* (both subspecies) shows cytologically and morphologically variation (Lloyd 1972c, Druce 1987, 1992, own observation). These taxa are not 'monophyletic': In the PCoA, individuals of these taxa are scattered widely within taxon group C and are also found in several clades in the NJ tree. Results based on sequence information (chapter 3) also showed a complex and reticulate evolutionary history of this group. We think that our results indicate a multiple origin of these taxa by hybridization and polyploidization events.

Winkworth et al. (2005) suggested a potential model for the rapid diversification of New Zealand alpine plant lineages during the Pliocene and Pleistocene. Repeated cycles of range expansion and contraction associated with climate fluctuations during the glaciation cycles may promote diversification, especially when interactions (e.g. hybridization or introgression) between previously isolated forms are possible. Although, group C contains mostly non alpine taxa, we think such processes as descried by Winkworth et al. (2005) could lead to the observed patterns in this group.

On the other hand, some taxa of group C are restricted to very small areas where only few morphologically and cytologically uniform populations occur (e.g. *L. calcarea, L. dioica* subsp. *manoica, L. rotundata*; Lloyd 1972c). These taxa are 'monophyletic' in the AFLP analysis, suggesting a single origin of these taxa. *L. scariosa* is widespread in the

southern parts of South America, but this taxon shows no geographic variation throughout its range as was found in widespread *Leptinella* taxa of New Zealand (Lloyd 1972c). A single origin and a subsequently long distance dispersal event from New Zealand to South America is assumed for *L. scariosa.*

Our results indicate different evolutionary histories of group A and C. Taxon group C is characterised by extensive hybridization and polyploidization, whereas both processes are less frequent in group A. Both groups vary in several features (see below), but it is not clear which of them explain the difference.

Taxonomy. Taxon group A contains all members of subgenus *Radiata* and two members of subgenus *Oligoleima* sensu Lloyd (1972c). The AFLP profiles of the two taxa of subgenus *Oligoleima* from Australia *(L. longipes, L. reptans)* are obviously different from the other profiles of this group. These taxa show many sequence differences compared to the other taxa of the *Leptinella* main clade and they differ also in morphology (Lloyd 1972c, van Royen and Lloyd 1975, see chapter 3).

In an analysis using nuclear and chloroplast sequence information, the subgenus *Radiata* was found not to be monophyletic and subsequently was split into four more or less supported monophyletic groups (chapter 3). In our present study, we included three of these groups (not included are the two taxa of the *plumosa*-group from the sub-Antarctic islands). In contrast to the results of sequencing, all investigated taxa of subgenus *Radiata* cluster together in the AFLP analysis with modest bootstrap support. Subgenus *Radiata* is characterised by clustered branches with short rhizome internodes, usually showy capitula, rhizomes with eight vascular bundles, and a more or less patchy growth habit (Edgar 1958, Lloyd 1972c; see Fig. 1-2, d-f). The taxa of group A predominantly occur in mountain and alpine habitats and are monoecious or rarely paradioecious (Lloyd 1972c).

Clearly separated from the other taxa of group A are *L. filiformis, L. minor* and *L. nana*. This was also found in the molecular phylogeny based on sequence information (*minor*-group; chapter 4). On the other hand, the *pectinata*- and *pyrethrifolia*-group are not confirmed. However, both groups are not well supported in the phylogenetic study based on sequence information (chapter 4).

Within taxon group A, the AFLP analysis shows some taxon relationships: A close relationship of *L. albida* and *L. pectinata* subsp. *villosa* is observed in the NJ tree. Both taxa can not always be reliably distinguished, because morphological intermediate forms

occur and both taxa have populations with tetraploid and octoploid chromosome numbers (Lloyd 1972c, New Zealand Plant Conservation Network 2009).

L. goyenii with its palmate leaves is morphologically unique within *Leptinella*. This taxon was clearly separated from all other taxa of the *Leptinella* main clade in the ITS dataset, but a more closely relationship to other taxa of the *pectinata*- and *pyrethrifolia*-group was indicated by the chloroplast dataset (chapter 3). The closer relationship to other taxa of subgenus *Radiata* has been confirmed by the AFLP analysis.

The results of chapter 3 suggest a hybrid origin of the range restricted *L. pyrethrifolia* var. *linearifolia*. ITS clones of this taxon were found in two different groups (*pectinata*-, *pyrethrifolia*-group), while it is characterised by a chloroplast haplotyp of the *pectinata* group. However, the AFLP data do not provide evidence for this hypothesis. The only investigated individual of *L. pyrethrifolia* var. *linearifolia* was found clustering with individuals of the typical variety.

In contrast to taxon group C (see below) most taxa of this group are 'monophyletic' with low to high statistical support, which means that all individuals of one taxon cluster together. However, there are two exceptions (*L. atrata* subsp. *atrata*, *L. dendyi*; *L. albida*, *L. pectinata* subsp. *villosa*). Hybridization and polyploidization was found to be less common in this group (see above), and this may explain that the relationships in this group is better resolved and the taxa are mostly 'monophyletic'.

Taxon group B and C of the present AFLP analysis form a well supported monophyletic group in the phylogenetic analysis based on sequence information (*dioica*-group; chapter 3). In the phylogenetic tree of the maximum parsimony and Bayesian analyses and especially in the ITS distance network, a subdivision into two subgroups was observed. These groups are identical with taxon group B and C of the AFLP analysis. Both groups share a similar morphology and rhizome anatomy (Edgar 1958, Lloyd 1972c). All taxa have single branches and long rhizome internodes, inconspicuous capitula, rhizomes with four vascular bundles, and a grass-like growing form (for illustration see Fig 1-2, a-c). The taxa are distributed in coastal to montane habitats. Group B contains monoecious and dioecious taxa, and group C is characterised by dioecy (with three exceptions).

Taxon group B: This group comprises the tetraploid species *L. dispersa* (with two subspecies), *L. potentillina* and *L. tenella*. The PCoA and the NJ tree show a further division into three supported subgroups. All three investigated populations of *L. potentillina* form a well supported group. The relationship between *L. dispersa* and *L. tenella* is more complex: In the northern part of the North Island, both species are very

distinct in habit and morphology, but in the southern part of the North Island around Wellington, it is difficult to discriminate among the two taxa (Lloyd 1972c, own observation). Additionally, both taxa hybridize in this area (Lloyd 1972c). On the South Island, both taxa are geographically separated. Our analyses show that the population of *L. dispersa* subsp. *dispersa* from Stewart Island (where *L. tenella* does not occur) is separated from the investigated populations of the southern North Island. All four populations (including one population of *L. dispersa* subsp. *rupestris*) of the southern part of the Northern Island group together. Unfortunately, we did not include *L. tenella* from northern North Island, because these plants presumably could be the "true" *L. tenella*, and the plants from Stewart Island could be the "true" *L. dispersa* subsp. *dispersa*. While the plants of the southern North Island are be intermediate. However, there is also another possible explanation: *L. dispersa* subsp. *dispersa* and *L. tenella* are widespread but sparsely distributed and both taxa show extreme geographical variation (Lloyd 1972c) maybe every population group/geographic area representing an own "taxa". To clarify the relationship of *L. dispersa* and *L. tenella*, a more comprehensive sampling of populations from the whole distribution area would be necessary.

L. dispersa subsp. *rupestris*, which is not clearly separated in the PCoA, is morphological distinct from *L. dispersa* subsp. *dispersa* and *L. tenella*. The plants are smaller in all parts (Lloyd 1972c).

Taxon group C: The phylogenetic analysis based on sequence information could not identify subgroups within group C. Little sequence variation was found between the species and most taxa were not monophyletic (chapter 3). The observed non-monophyly could be explained by the lack of sufficient synapomorphies (as it is expected in recenly evolved lineages), and/or by hybridization and polyploidization (chapter 3).

In the PCoA of the AFLP patterns, a slight split into two subgroups could be observed. One subgroup contains *L. calcarea, L. pusilla* and *L. serrulata*, these three taxa clustering also together in the NJ tree with low bootstrap support. There are also some morphological evidence for separation of these three taxa from the other taxa: the pale and wiry rhizomes are well buried and have no or only few leaves (Lloyd 1972c). The remaining taxa of group C have green to brown rhizomes near the soil surface (Lloyd 1972c). Within the latter subgroup, some interesting details are observed: All five investigated populations of *L. traillii* from southern Southland and Stewart Island clustered together, and all entities (subsp. *traillii*, subsp. *pulchella*, cf. *traillii*) form their own subcluster. All three groups are distinguished by morphology and ploidy level (Lloyd

1972c, own results and observations). However, Wilson (1994) had found plants which are morphologically intermediate between the two subspecies of *L. traillii*.

L. dioica subsp. *manoica* was not recognized as being different from *L. dioica* subsp. *dioica* by de Lange et al. (2009) and the New Zealand Plant Conservation Network (2009). According to Lloyd (1972c), both subspecies differ in leaf shape (blade 0-5 pairs of lobes, lateral lobes entire and smaller than terminal lobes vs. blade 4-15 pairs of lobes, lateral lobes often toothed and larger than or equal to terminal lobes) and breeding system (monoecious vs. dioecious). However, there were found also dioecious populations with leaf shapes similar to *L. dioica* subsp. *manoica* (P. de Lange, (New Zealand) personal communication). In our analysis, *L. dioica* subsp. *manoica* forms a supported clade in the NJ tree and is separated from all other taxa of group C by the third component in the PCoA. However, since we only included few populations of *L. dioica*, the inclusion of more populations would presumably blur the differences in the AFLP analysis. Especially, *L. dioica* subsp. *dioica* shows geographic variation with a clinal variation pattern (Lloyd 1972c). The extreme forms are distinctly different, especially in leaf size, division and shape (e.g. pinnatifid leaves in Southland vs. nearly entire leaves on the coast of banks Peninsula; see Fig. 1-4, leaves 2 - 4). However, the extreme forms are linked by a series of intermediate populations (Lloyd 1972c). In the PCoA, individuals of *L. dioica* subsp. *dioica* are scattered widely within taxon group C and are also found in several clades in the NJ tree. Such geographical variation is also observed in other taxa (e.g. *L. squalida, L. pusilla)*. These widespread, morphologically and partly cytologically variable taxa form no 'monophyletic' clusters in the present AFLP analysis.

L. "Seal" (20*x*) is a member of group C and is not closely related to the tetraploid *L. potentillina* (group B) as suggested by the New Zealand Plant Conservation Network (2009). This confirms the result of the sequencing analysis (chapter 3).

The morphological discrimination of taxa of group C is not easy (Lloyd 1972c, own observation). Especially, the paucity of universally applicable differential characters, the extreme morphological plasticity, the geographical variation, and the occurrence of hybridization obscure the typical differences among species. For example, Lloyd (1972c) wrote that four different species *(L. dioica, L. pusilla, L. scariosa, L. traillii)* could be easily confused with *L. squalida*. Additionally, hybridization is very common among species of group C (e.g. between *L. squalida* and *L. dioica* or *L. pusilla*; Lloyd 1972c). Lloyd (1972c) reported hybrid swarms between these taxa, as well as more uniform hybrid segregates in some localities, but on other localities each taxon coexisted with little or no

hybridization. Hybrids could also occur between taxa with different chromosome numbers (Lloyd 1972c, 1975a).

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Chapter 5

Evolution of dimorphic sex expression and polyploidy in *Leptinella*

Introduction

While the majority of flowering plants are hermaphrodite, gender dimorphism (e.g. dioecy, gynodioecy, androdioecy, paradioecy) is found only in approximately 7 % of flowering plant species (Renner and Ricklefs 1995). Dioecy is the most common mode of dimorphic sex expression, Renner and Ricklefs (1995) suggested that about 6 % of species are dioecious. Such gender dimorphism occurs in nearly half of all angiosperm families, suggesting numerous independent origins (Renner and Ricklefs 1995).

The classical explanation for the evolution of dimorphic sex expression is that it is a mechanism to promote outcrossing (Lloyd 1976, Charlesworth and Charlesworth 1978, Thomson and Brunet 1990, Miller and Venable 2000). Alternatively, female plants may be favoured in a population because of changes in resource allocation (Charnov 1982, Webb 1999). These females may be favoured if they can produce over twice as many seeds as hermaphrodite plants. This additional seed production of females has to be compensated by hermaphrodites, favouring male function in these plants, and this may result in complete male plants. Various authors have made suggestions about the evolutionary pathways that may have led to dioecious sex expression. Five pathways to dioecy are well documented: from hermaphroditism or monoecy via gynodioecy or androdioecy, from monoecy via paradioecy, from heterostyly, and from duodichogamy or heterodichogamy to dioecy (reviewed in Webb 1999).

Remarkable is the high level of dimorphic plants on islands, especially on the Hawaiian Islands (20.7 % of genera, 14.7 % of species; Sakai et al. 1995a,b) and on New Zealand (23 % of genera, 12 - 13 % of species; Godley 1979, Webb and Kelly 1993, Webb et al. 1999). On the other hand, there are several islands with a lower than the average number of dioecious plants, e.g. the Galapagos Islands, Iceland, and the Azores (2 - 3 % of species; Baker and Cox 1984). The high frequency of dioecy on some islands has fascinated numerous botanist, and many hypotheses have been put forward to explain this correlation (see Baker 1967, Baker and Cox 1984, Sakai et al. 1995a,b, Webb 1999).

The genus *Leptinella* is one of the New Zealand genera with dimorphic sex expression. The genus has its centre of diversity in New Zealand with 29 taxa being endemic, but occurs also in Australia, New Guinea, South America, on the Chatham Islands, and on the sub-Antarctic islands. The sex expression of *Leptinella* was studied intensively in the field and in the glasshouse by Lloyd (1972a,b,c, 1975a,b, 1980). He found that several different modes of sex expression are realised in the genus: monoecy,

paradioecy, dioecy and a number of different intermediate conditions (Lloyd 1972a, 1975b). Dimorphic populations are observed in 16 of the 42 taxa (12 dioecious, 4 paradioecious). Lloyd (1975a, 1980) and Webb (1999) suggested that monoecy is the ancestral condition in the genus and that dioecy may have evolved from monoecy via the paradioecy pathway. Webb (1999) pointed out that *Leptinella* is one of the best documented examples for this pathway. The paradioecious pathway starts with monoecious populations in which the individuals are already pollen or seed sterile. Divergence in the ratio of female and male florets may then lead to sex specialization of the plants (Webb 1999). Sex inconstancy in both genders, which was found in *Leptinella*, is characteristic for this pathway (Lloyd 1980, Webb 1999). Sex inconstancy means that few individuals bear a very small number of florets of the opposite gender. Additionally, Lloyd (1975b) proposed also transitions from dioecy to monoecy in some taxa. Monoecy may evolve from dioecy via unisexual male populations, which have a few inconstant male plants. Monoecious plants may evolve from the inconstant male plants by changing of gender ratio.

Beside the variation in sex expression, *Leptinella* forms also an impressive polyploid complex with ploidy levels ranging from tetraploid to a chromosome set of $2n = 24x$ (Hair 1962, Lloyd 1972c, Lloyd 1975b, Moore 1981, Beuzenberg and Hair 1984, Dawson 1995, chapter 4). The lowest chromosome number found in *Leptinella* is $2n = 52$, which would indicate that the basic number for the genus is $x = 26$. However, this is a relatively high number and suggests that this is a secondary basic number, following a polyploidization event. The basic number of the genus is therefore $x = 13$ (Hair 1962, Lloyd and Webb 1987) and tetraploids could possibly be interpreted as functional diploids.

The occurrence of dimorphic sex expression and polyploidy within the genus, makes *Leptinella* a fascinating study object. The assumed connection of sex expression and polyploidy was discussed by several authors. However, until now there are only few molecular studies that deal with this subject (e.g. *Lycium* (Solanaceae), Miller and Venable 2000, Yeung et al. 2005; *Mercurialis* (Euphorbiaceae), Pannell et al. 2004, Obbard et al. 2006; *Bryonia* (Curbitacaea), Volz and Renner 2008; *Melicytus* (Violaceae), Mitchell et al. 2009a). Some authors suggested that polyploidization may break down dimorphic breeding systems (Westergaard 1958, Richards 1997). For example, *Empetrum nigrum* (Ericaceae) is diploid and dioecious and *E. hermaphroditum* is tetraploid and hermaphrodite (Richards 1997). Mitchell et al. (2009a) found that in the Australasian genus *Melicytus* the change in sex expression is from dioecism and mostly tetraploid (functionally diploid) to

hermaphroditism and predominately octoploid, which suggests also a break down of dimorphic sex expression after polyploidization. However, there are also three exceptions in the genus: hermaphrodite tetraploids and one dioecious dodecaploid taxon, demonstrating that the change in chromosome number is not necessary a causal factor in the switch from one sex expression to another (Mitchell et al. 2009a).

Miller and Venable (2000) suggested that polyploidy is a trigger of unrecognized importance for the evolution of gender dimorphism, by disrupting self-incompatibility and leading to inbreeding depression. Subsequently, dioecy may evolve to recover outcrossing. The authors could show that gender dimorphism in North American *Lycium* evolved in polyploid, self-compatible taxa while the closest relatives are hermaphrodite, self-incompatible diploids. Population studies in *Lycium californicum* confirm this hypothesis, because there were found either diploid and hermaphrodite or tetraploid and gender dimorphic populations (Yeung et al. 2005). Additionally, Miller and Venable (2000) presented evidence for this pathway for further 12 genera. Volz and Renner (2008) and Pannell et al. (2008) found no direct correlation between sexual system and ploidy level in *Bryonia* or *Mercurialis*. However, the complexities involved in sex expression and in polyploidy make general conclusions difficult (Pannell et al. 2004). Thus, polyploidization may be invoked as a cause of transition from monomorphic sex expression to dioecy and vice versa.

The present chapter will focus on the phylogenetic history of dimorphic sex expression in the genus *Leptinella*. We will use the phylogenetic tree presented in chapter 3 to test several hypotheses with regard to chromosome number and sex expression. The first question is how many times dimorphic conditions have evolved. Secondly we will discuss the origin of these breeding systems and the evolutionary pathways leading to them. Additionally, we will have a look at the connection between breeding system and ploidy level.

Methods

Character state. We have used the description of the mode of sex expression for every taxon of *Leptinella* by Lloyd (1972a,b,c, 1975a,b, 1980), van Royen and Lloyd (1975), Thompson (2007), Heenan (2009), and New Zealand Plant Conservation Network (2009). The used terminology follows Lloyd (1975b) and Sakai and Weller (1999). Short descriptions of the different conditions in *Leptinella* are present in Tab. 1-2.

The ploidy levels of *Leptinella* taxa were either determined by flow cytometry (chapter 4) or were taken from chromosome counts of several authors (Hair 1962, Lloyd 1972c, 1975b, Moore 1981, Beuzenberg and Hair 1984, Dawson 1995, Heenan 2009, New Zealand Plant Conservation Network 2009). The ploidy level estimation by flow cytometry for *L. filicula* and *L. filicula* are new to science (for a description of the method see chapter 4).

Character state evolution. A molecular study based on nrDNA and cpDNA sequence information including most taxa of *Leptinella* are show in chapter 3. However, the phylogenetic analyses in chapters 3 have shown that most taxa of *Leptinella* are not monophyletic. Different accessions and/or clones of these taxa form no monophyletic cluster and individuals or clones of these taxa are scattered widely within the tree. Due the lack of a species tree, it is impossible to perform a character state reconstruction with maximum parsimony or maximum likelihood (as implemented in Mesquite, Maddison and Maddison 2006). Therefore, sex expression and ploidy levels could be mapped only on the phylogenetic tree. For this purpose, we used the majority consensus tree from the Bayesian analysis of the combined dataset (ITS, *psb*A-*trn*H, *trn*C-*pet*N; chapter 3).

Tab. 5-1: Sex expression and ploidy level of *Leptinella* taxa according to Hair (1962), Lloyd (1972a,b,c, 1975a,b), Moore (1981), Beuzenberg and Hair (1984), Dawson (1995), Thomson (2007), Heenan 2009, New Zealand Plant Conservation Network (2009), and chapter 4. The result for *L. filicula* and *L. longipes* are new is new to science. For the different groups within *Leptinella* see chapter 3.

Taxon	Breeding system	Ploidy level
***Leptinella* main group**		
***dioica*-group**		
L. calcarea (D. G. Lloyd) D. G. Lloyd & C. J. Webb	D	8
L. dioica Hook. f. subsp. *dioica*	D	20
L. dioica subsp. *manoica* (D. G. Lloyd) D. G. Lloyd & C. J. Webb	M	20
L. dispersa (D. G. Lloyd) D. G. Lloyd & C. J. Webb subsp. *dispersa*	M/D	4
L. dispersa subsp. *rupestris* (D. G. Lloyd) D. G. Lloyd & C. J. Webb	D	4
L. intermedia (D. G. Lloyd) D. G. Lloyd & C. J. Webb	M	12
L. potentillina F. Muell.	M	4
L. pusilla Hook. f.	D	8
L. rotundata (Cheeseman) D. G. Lloyd & C. J. Webb	M	24
L. scariosa Cass.	D	16/20
L. serrulata (D. G. Lloyd) D. G. Lloyd & C. J. Webb	D	4
L. squalida subsp. *mediana* (D. G. Lloyd) D. G. Lloyd & C. J. Webb	D	12/16/20
L. squalida Hook. f. subsp. *squalida*	D	20/>20
L. tenella (A. Cunn.) D. G. Lloyd & C. J. Webb	M	4
L. traillii subsp. *pulchella* (Kirk) D. G. Lloyd & C. J. Webb	D	>20/24
L. traillii (Kirk) D. G. Lloyd & C. J. Webb subsp. *traillii*	D	>20
L. "Seal"	D	20
***minor*-group**		
L. filiformis (Hook. f.) D. G. Lloyd & C. J. Webb	M	4
L. minor Hook. f.	M	4
L. nana (D. G. Lloyd) D. G. Lloyd & C. J. Webb	M	4
***plumosa*-group**		
L. lanata Hook. f.	M	4
L. plumosa Hook. f.	M	4
***pectinata*-group**		
L. albida (D. G. Lloyd) D. G. Lloyd & C. J. Webb	M	4/8
L. atrata (Hook. f.) D. G. Lloyd & C. J. Webb subsp. *atrata*	M	4
L. conjuncta Heenan	M	8
L. dendyi (Cockayne) D. G. Lloyd & C. J. Webb	PD	4
L. pectinata (Hook. f.) D. G. Lloyd & C. J. Webb subsp. *pectinata*	M	8
L. pectinata subsp. *villosa* (D. G. Lloyd) D. G. Lloyd & C. J. Webb	M	4/8
L. pectinata subsp. *willcoxii* (Cheeseman) D. G. Lloyd & C. J. Webb	M	8
***longipes*-group**		
L. drummondii (Benth.) D. G. Lloyd & C. J. Webb	M	n/a
L. longipes Hook. f.	M	4
L. reptans (Benth.) D. G. Lloyd & C. J. Webb	M	n/a
***pyrethrifolia*-group**		
L. atrata subsp. *luteola* (D. G. Lloyd) D. G. Lloyd & C. J. Webb	M	4
L. featherstonii F. Muell.	M	4/8
L. pyrethrifolia var. *linearifolia* (Cheeseman) D. G. Lloyd & C. J. Webb	M/PD	12
L. pyrethrifolia (Hook. f.) D. G. Lloyd & C. J. Webb var. *pyrethrifolia*	M/PD	12/16
ungrouped		
L. goyenii (Petrie) D. G. Lloyd & C. J. Webb	PD	4
L. maniototo (Petrie) D. G. Lloyd & C. J. Webb	M	4
***filicula*-group**		
L. filicula (Hook. f.) Hook. f.	M	~12
L. sarawaketensis (P. Royen & D. G. Lloyd) D. G. Lloyd & C. J. Webb	M	n/a
L. wilhelminensis (P. Royen) D. G. Lloyd & C. J. Webb	M	n/a

D - dioecious, M - monoecious, PD - paradioecious

Results and Discussion

The mode of sex expression of all included taxa of *Leptinella* is shown in Tab. 5-1. A summary of sex expressions in the genus is given in Tab. 5-2. Most of the taxa are monoecious, but there are also 16 taxa with dimorphic populations. Species, subspecies and other entities of *Leptinella* are mostly strict dioecious, paradioecious or monoecious. There are three noteworthy exceptions: monoecy and dioecy occurs in *L. dispersa* subsp. *dispersa* and monoecy and paradioecy occurs in both varieties of *L. pyrethrifolia*. Fig. 5-1 shows the phylogenetic tree of the Bayesian analysis of the combined dataset from chapter 3. The sex expression and the ploidy level are mapped to the tree.

Tab. 5-2: Summary of sex expressions in *Leptinella.*

Sex expression		Number of taxa
monomorphic	monoecious	27
dimorphic	dioecious	11
	paradioecious	2
mixed	dioecious + monoecious	1
	paradioecious + monoecious	2

Sex expression. The phylogeny presented here for *Leptinella* infers that the ancestral sex expression is monoecy and that dioecy and paradioecy are derived conditions (Fig. 5-1). The basal *filicula*-group (including *Cotula alpine*) is monoecious, and the earlier diverging lineages are also mostly (*pectinata*-, *pyrethrifolia*-group) or entirely monoecious (*longipes-plumosa*-, *minor*-group). However, there are four taxa in these lineages with paradioecious populations (*L. dendyi, L. goyenii,* both varieties of *L. pyrethrifolia*). Dioecy is only found in the terminal *dioica*-group. These results support the hypotheses that dioecy and paradioecy evolved from monoecy (Lloyd 1972a, 1980, Webb 1999).

The morphologically similar dioecious taxa cluster together and there is low sequence variation among these taxa (chapter 3). Two species in the *dioica*-group have the lowest reported chromosome number $2n = 4x$, while the other taxa represent five higher ploidy levels (up to $2n = 24x$). The sex expression, the secondary sexual characters, and the genetic basis of sex determination are similar in all species (Lloyd 1975a). These suggest that dioecy evolved from monoecy at the tetraploid level and was retained during the evolution of the higher ploidy levels.

Within the *dioica*-group, there are also six taxa with non-dioecious populations. *L. dioica* subsp. *manoica* is monoecious or complex-monoecious and *L. rotundata* is complex-monoecious. *L. dispersa* has the greatest diversity in sex expression: There are

dioecious, 'pseudo-monomorphic dioecious', unisexual male, unisexual female and monoecious populations (Lloyd 1975b). Geographical, morphological evidence and data from crossing experiments suggested that the direction of changes have been from dioecy to monoecy in these three taxa, and not vice-versa. On the other side, Lloyd (1975a) suggested that the other three monoecious taxa of the *dioica*-group (*L. intermedia, L. potentillina, L. tenella*) show the ancestral sex expression and have not evolved from dioecy. However, our molecular data can not help to verify the evolutionary direction in the *dioica*-group, because sequence divergence is too low in this group and most taxa are not monophyletic.

In contrast to the dioecious taxa, the four at least partly paradioecious taxa were found in three different groups in the phylogenetic tree, suggesting several independent origins. The paradioecious taxa could be seen either as intermediate conditions along the pathway from monoecy to dioecy or as the final state of sex expression in these taxa.

Our data suggest that dimorphic sex expression has evolved independently at least four times (*dioica*-, *pectinata*-, *pyrethrifolia*-group, *L. goyenii)* and that these conditions have evolved autochthonously in New Zealand, as in other 16 of the 83 New Zealand genera with gender dimorphic species (Webb et al. 1999). The dioecious taxa *L. scariosa* from South America is nested within the other dioecious taxa from New Zealand in the phylogentic analyses based on DNA sequencing and on AFLP fingerprinting (chapters 3 and 4), suggesting a long distance dispersal event from New Zealand to South America. *L. scariosa* is very similar in morphology and sex dimorphism to the other dioecious taxa, suggesting that dioecy has evolved in New Zealand and that the already dioecious *L. scariosa* or its ancestor dispersed to South America.

Polyploidy. Taxa of *Leptinella* show a wide range of different ploidy levels (see Tab. 5-1). Unfortunately, chromosome counts for most members of the basal *filicula*-group and the *longipes*-group are yet missing. However, flow cytometry analysis suggests that the Australian taxa *L. filicula* is polyploid (~12*x*) and *L. longipes* is tetraploid. When chromosome numbers are mapped onto the phylogenetic tree (see Fig. 5-1), the tetraploid and octoploid number are predominant in the earlier-branching lineages (*minor*-, *pectinata*-, *plumosa*-, *pyrethrifolia*-group). In contrast, the higher ploidy levels occur predominantly in the most terminal clade (*dioica*-group). Though, some taxa with higher ploidy levels occur also in other groups, suggesting several independent polyploidization events.

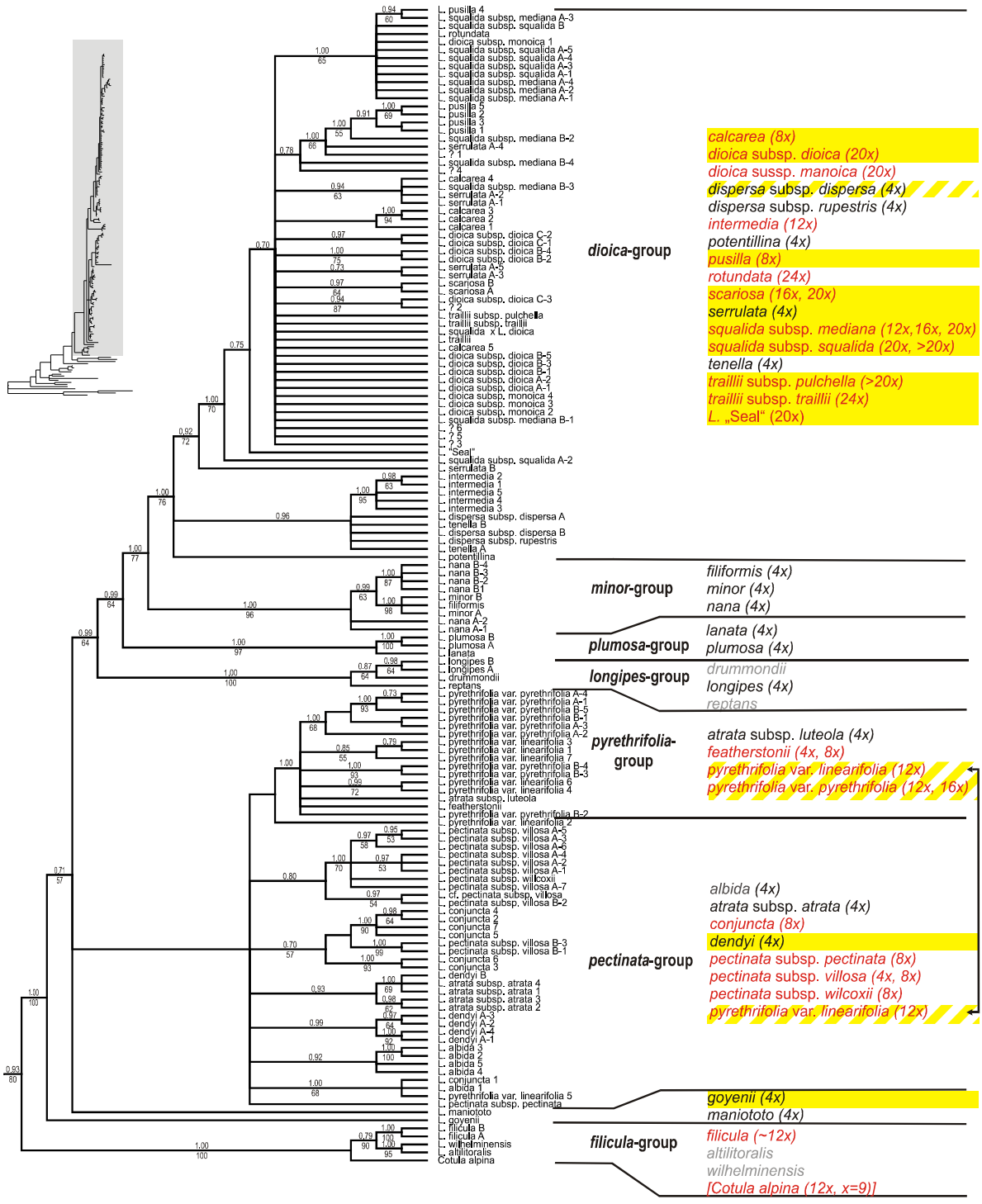


Fig. 5-1: Phylogenetic tree from the Bayesian analysis of the combined dataset from chapter 3 (only the clade of *Leptinella* + *Cotula alpina* are shown). The member of each group (see chapter 3) are shown alphabetically on the right side with their ploidy level and sex expression. Taxa in black are tetraploid, taxa in red have a higher ploidy level, and taxa in grey are not counted yet. Yellow boxes indicate dimorphic (dioecy, paradioecy), white boxes monomorphic (monoecy), and dashed yellow boxes mixed sex expression. The arrow shows the position of the clones of *L. pyrethrifolia* var. *linearifolia* (see discussion in chapter 3).

Association between polyploidy and sexual system. There is a correlation between dimorphic sex expression and high ploidy level, as 11 of the 16 taxa with dimorphic sex expression are octoploid or have even a higher ploidy level. However, four dimorphic taxa are tetraploid, and eight monoecious taxa are at least octoploid, revealing no firm relationship between polyploidy and sex expression.

Polyploidization may lead to a break down of dioecy (Westergaard 1958, Smith 1958, 1969, Richards 1997). In *Leptinella,* this does not seem to be the case, since most dioecious members of the *dioica*-group are octoploid or have even higher chromosome numbers. Additionally, Lloyd (1975a) has done several inter-specific crosses in which the species of the pollen donator had a chromosome number that was either higher, lower, or equal to the number of the ovule parent. In all cases, the resulting offspring were either female or male and no break down of the dimorphic sex expression was found.

The hypothesis that polyploidy may favour dioecy as an outcrossing mechanism because polyploidization led to a break down of the self-incompatible system (Miller and Venable 2000, Yeung et al. 2005) is not likely in *Leptinella,* which seems to be self-compatible. The so far tested monoecious and tetraploid taxa (*L. atrata, L. minor, L. pectinata*) are self-compatible, although the seed set and subsequent germination percentages are slightly lower after self-pollination in comparison with cross-pollination (Lloyd 1972b).

However, the origin of dioecy in *Leptinella* could be interpreted as a promotion of outcrossing to avoid inbreeding depression, which Lloyd (1972b) had observed in some taxa. But polyploidy dos not seem to be a trigger in the evolution of dioecy in *Leptinella* as suggested by Miller and Venable (2000). Another mechanism beside dimorphic sex expression to favour outcrossing in *Leptinella* is the absence of overlap in the anthesis of the female and male florets within one capitulum (Lloyd 1972a, 1980a).

Chapter 6

Conclusion

Conclusion and perspectives

The aim of the present thesis was to generate molecular data to 1) investigate the intergeneric and infrageneric relationships of the southern hemisphere genus *Leptinella,* 2) elucidate the origin, the biogeography, and divergence time of the genus, and 3) reconstruct the evolution of sex expression and polyploidy in *Leptinella*. For this purpose, two different molecular methods (DNA sequencing, AFLP fingerprinting) were used on different taxonomical levels.

Intergeneric relationships. One region each from nuclear (ITS) and chloroplast genome (*ndh*F) were chosen for amplification and sequencing of DNA to reconstruct the molecular phylogeny of the southern hemisphere members of the tribe Anthemideae (chapter 2). The markers used are suitable to analyze the relationships within the study group and have given more or less well resolved and supported trees. The analyses show that the subtribes sensu Bremer and Humphries (1993) are polyphyletic. As a consequence of the non-monophyletic nature of these subtribes, an alternative generic grouping of the southern hemisphere Anthemideae is discussed (*Osmitopsis*, *Cotula*-group, *Athanasia*-grade, *Pentzia*-clade; see chapter 2).

The genus *Leptinella* is a member of the basal *Cotula*-group which also contains seven southern African genera, the South American genus *Soliva*, and the widespread southern hemisphere genus *Cotula*. The monophyly of this clade suggested by the molecular results is corroborated by the apomorphies of epaleate receptacles and the formation of 4-lobed corollas in tubular florets (with exceptions to this in *Adenanthellum* and *Hippia*). In all analyses, *Leptinella* cluster together with *Cotula* and *Soliva*. The close morphological relationship of these three genera was observed by different authors (Lloyd 1972c, Bremer and Humphries 1993, Oberprieler et al. 2006). The results obtained in chapter 2 in combination with an expanded ITS phylogeny, which included more Mediterranean and Eurasian genera, was used to propose a new subtribal classification of the tribe Anthemideae (Oberprieler et al. 2007). Following the cited study, *Leptinella* is a member of the subtribe Cotulinae Kitt., which corresponds to the *Cotula*-group in chapter 2.

The results of chapter 3 based on nuclear and chloroplast sequence information show that *Leptinella* is nested within *Cotula* and that *Leptinella* in the circumscription of Lloyd and Webb (1987) is not monophyletic. *Leptinella* + *Cotula alpina* are monophyletic in the

combined dataset with modest support, but not monophyletic in the cpDNA and ITS datasets. However, there is morphological and cytological evidence that *Leptinella* is monophyletic (Lloyd 1972c, Lloyd and Webb 1987). For example the inflated corolla of the female disc florets and the basic chromosome number $x = 13$. Both characteristics are unique within the tribe Anthemideae.

Infrageneric relationships. Two different molecular methods (DNA sequencing, AFLP fingerprinting) were used to reconstruct the phylogeny of *Leptinella*. In chapter 3, three non-coding DNA markers (nrDNA ITS, cpDNA *psb*A-*trn*H, *trn*C-*pet*N) were sequenced for *Leptinella* and members of the *Cotula*-group. Additionally, AFLP fingerprinting was used within the monophyletic *Leptinella* main group (chapter 4).

The results of the two analyses based on sequence information and on AFLP fingerprinting are summarized in Fig. 6-1. The analyses show that the subgenera according to Lloyd (1972c) are only partly monophyletic. Sequence data (chapter 3) suggests that subgenus *Leptinella* is monophyletic, and the other two subgenera are not. However, the subgenus *Leptinella* is split in two distinct groups in the AFLP analysis. Several taxon groups are found within *Leptinella*. The analyses based on sequence information show that *Leptinella* is split into two well supported groups: the basal *filicula*-group and the *Leptinella* main group. The *filicula*-group comprises taxa from Australia and New Guinea. All taxa from New Zealand, the sub-Antarctic islands, South America, and three taxa from Australia belong to the *Leptinella* main group. Within the latter group, six subgroups were found: The *dioica*-, *longipes*-, *minor*-, *plumosa*-, and the *pyrethrifolia*-group are well supported in the analyses. The *pectinata*-group is only monophyletic in the distance network of the ITS dataset.

The AFLP analysis of the *Leptinella* main clade resulted in three main groups (taxon groups A-C). This AFLP phylogeny is partly congruent with the results from the sequencing analysis, but there are some differences (see Fig. 6-1). The *dioica*-group is split into two well distinguishable groups in the AFLP analysis (taxon group B and C). This split is also indicated in the ITS distance network in chapter 3, but it is not well supported there. The close relationship among the taxa of the *minor*-group is supported in both analyses. The *pectinata*- and the *pyrethrifolia*-group are not supported by the AFLP analysis. However, there are also differences between the nuclear and chloroplast dataset: whereas both groups are separated in the ITS network, the groups are intermixed in the chloroplast network.

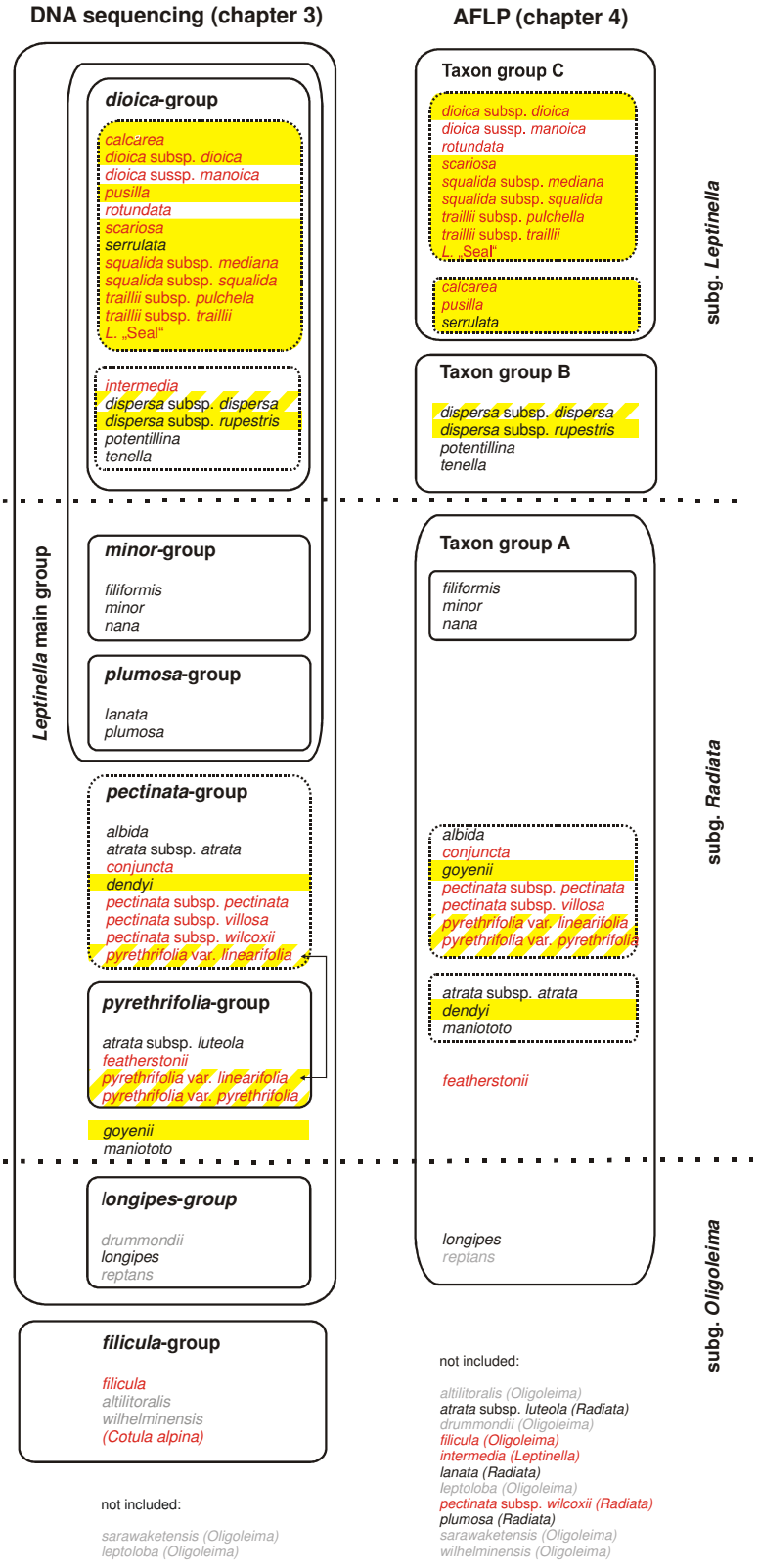


Fig. 6-1: Results of the DNA sequencing and AFLP fingerprinting analyses of *Leptinella* (chapters 3 and 4). Boxes indicate groups that were found in the analyses (solid lines: well supported groups, dashed line: low supported groups). The right pattern shows the subgenera classification according to Lloyd (1972c) based on morphological data. Taxa in black are tetraploid, taxa in red have a higher ploidy level, and taxa in grey are not counted until now. Yellow boxes indicate dimorphic (dioecy, paradioecy), white boxes monomorphic (monoecy), and dashed yellow boxes mixed sex expression. The arrow shows the position of the clones of *L. pyrethrifolia* var. *linearifolia*.

Different phylogenetic positions are also found for the morphological aberrant *L. goyenii*: This taxon has a basal and distinct position in the ITS and combined dataset, but shows more closely relationships to other taxa in the AFLP analysis and in the chloroplast dataset.

Both used methods are suitable to determine groups within *Leptinella*, but failed to resolve the relationships of single taxa. The extensive hybridization and polyploidization in combination with low genetic variation due the young age of *Leptinella* may be the main factors to explain the observed patterns (for details see discussion in chapters 3 and 4).

Clear evidence for hybridization is rare in the molecular dataset. A hybrid origin may be assumed for *L. pyrethrifolia* var. *linearifolia*, because ITS clones of this taxon are found in two different groups in the sequencing analysis (chapter 3). However, it can be assumed that hybridization with polyploidization is common in *Leptinella*. Because, several taxa are not monophyletic in both analyses and the individuals and clones of these taxa are scattered widely in the phylogenetic trees or in the PCoA. Even clones of a single individual do not form a monophyletic clade. Additionally, some taxa are found in different positions in the networks based on either nrDNA or cpDNA dataset (e.g. *L. goyenii*, *L. atrata* subsp. *luteola*; chapter 3). However, the results suggested hybridization to be more common within groups instead than among them. Especially, the AFLP analysis shows a strict separation of the taxon groups, indicating only little hybridization between these groups.

The mentioned patterns were found also in several other studies dealing with New Zealand groups. Especially, low sequence divergence among species is a common phenomenon (e.g. Breitwieser et al. 1999, Mitchell and Heenan 2000, Lockhardt et al. 2001, Meudt and Simpson 2006, Ford et al. 2007). Molecular dating analyses show that most of these lineages evolved recently in the Pliocene and Pleistocene by adaptive radiation (e.g. Wagstaff et al. 2006, Barker et al. 2007, Mitchell et al. 2009a).

The important role of hybridization in the evolution of New Zealand endemic plants and animals has been highlighted by recent genetic studies. Morgan-Richards et al. (2009) stated in their review of genetic analyses of hybridization in New Zealand, that hybridization is a common and important evolutionary process in New Zealand and elsewhere. Hybridization of at least 19 pairs of endemic species (plants, insects, fishes, birds), has been confirmed using genetic markers (Morgan-Richards et al. 2009). Hybridization could occur in combination with allopolyploidization. The ferns from

Australia and New Zealand are the only well investigated group in which polyploidy is a common phenomenon. All species of *Asplenium* are at least tetraploid, and of the 17 species in the Australian group, nine are octoploid. Chloroplast and nuclear sequences indicate that most of the octoploids are autopolyploid (Perrie and Brownsey 2005, Shepherd et al. 2008a,b). In some cases the analyses also indicate repeated polyploidization events (Perrie and Brownsey 2005). Allopolyploidy has also been documented in the fern genus *Polystichum* (Perrie et al. 2003). Hybridization following by a chromosome doubling has been presumed in *Anaphalioides* and *Ranunculus* (Breitwieser et al. 1999, Carter 2006).

Origin, biogeography and divergence time of *Leptinella*. The estimated crown age for the Anthemideae is 26.0 Ma. The clade which contains *Cotula and Leptinella* has originated in the Miocene (13.9 Ma). The several groups of *Leptinella* mentioned above are even younger (10.3 - 3.6 Ma). The estimated divergence times of the Anthemideae, *Cotula*, and *Leptinella* in chapter 3 are much younger than the supposed break-up of the Gondwana continent (c. 85 Ma Gondwana and New Zealand, 35-28 Ma South America and Antarctica; McLoughlin 2001, Winkworth et al. 2002a). Therefore, the current distribution of the *Cotula*-group in Australia, New Guinea, New Zealand, South America, southern Africa, and on several southern hemisphere islands could be explained only by long distance dispersal events between the different continents and islands. The suggested biogeographic history of *Leptinella* and related genera is illustrated in Fig. 6-1.

The *Cotula*-group originated in southern Africa and dispersed to South America (*Soliva*, *Cotula mexicana*) or Australasia/New Guinea (several taxa of *Cotula*, *Leptinella*). The basal *filicula*-group of *Leptinella* is distributed in Australia and New Guinea, from there a long distance dispersal event to New Zealand may have occurred. Additionally, our data suggest several long distance dispersals from New Zealand to Australia, Chatham Islands, South America, and the sub-Antarctic islands.

The outcomes of the present thesis (chapters 2 and 3) confirm previous results found in other groups: Most New Zealand plant lineages are very young and originated in the Miocene to Pleistocene. Many of these groups arrived by long distance dispersal from Africa, Asia, Australia, and South America (see Winkworth et al. 2002a, Sanmartin and Ronquist 2004, Sanmartin et al. 2007, Trewick et al. 2007, Goldberg et al. 2008, Bergh and Linder 2009). These studies also suggest several long distance dispersal events from New Zealand to other southern hemisphere continents and islands.

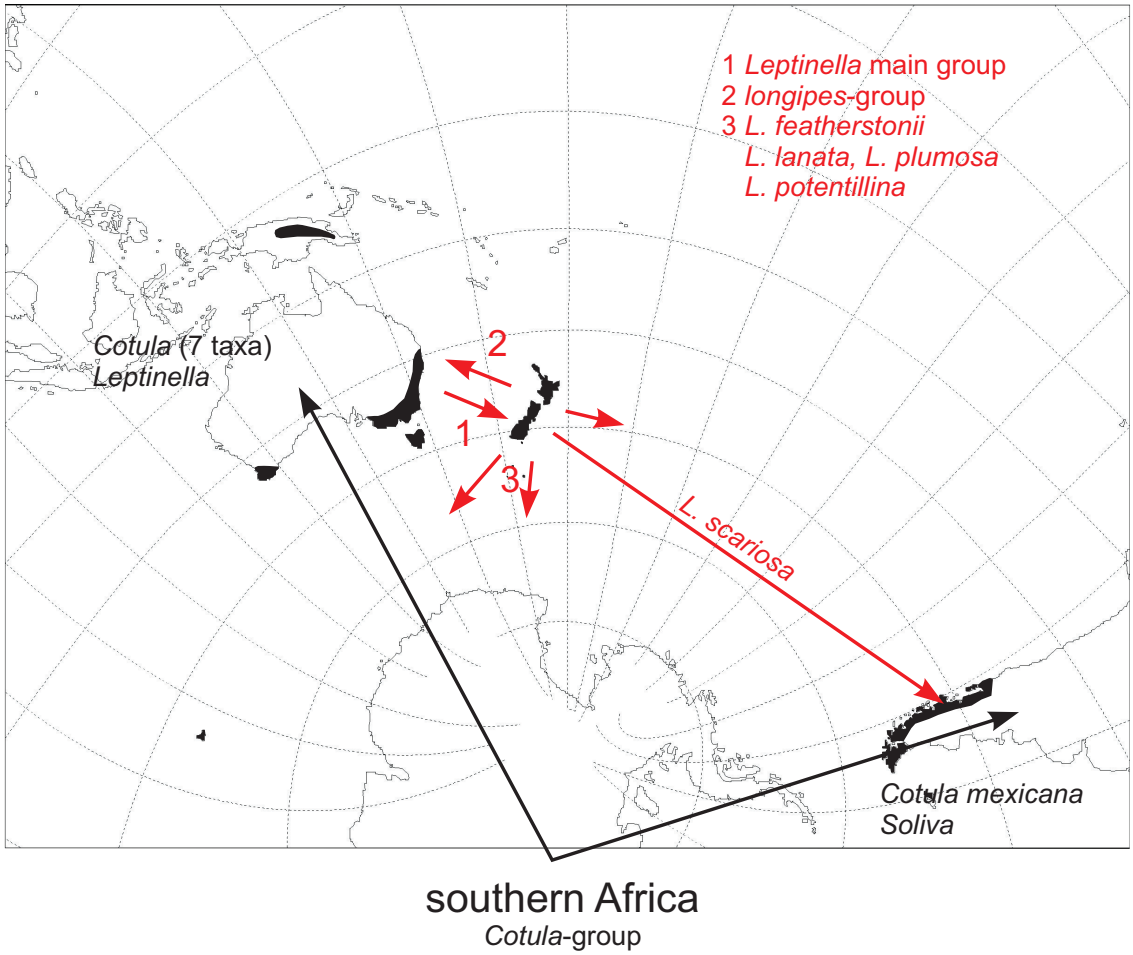


Fig. 6-2: Long distance dispersal events within the *Cotula*-group (black arrows) and *Leptinella* (red arrows) as suggested by molecular phylogenies (chapters 2 and 3).

Evolution of dimorphic sex expression

"To study the evolution of dioecy in plants, detailed comparative analyses are need of taxa whose biology is well understood, and in which objective data - such as DNA sequences - are used to estimate phylogenies." - Charlesworth (2001).

The sex expression of *Leptinella* was studied intensively in the field and in the glasshouse by David Lloyd. He published his results in a series of papers (Lloyd 1972a,b, 1975a,b, 1980). Lloyd studied the distribution and the sexual dimorphism of female and male florets in hundreds of capitula to determine the sex expression of plants, populations, and species. Additionally, he carried out several crossing experiments to determine the genetic background of dimorphic sex expression in the genus. The results of these studies together with cytological, morphological, and taxonomical data (Lloyd 1972c) were used

by Lloyd (1972a, 1975b) to suggest evolutionary pathways that may have led to the complex and diverse sex expression system in *Leptinella*.

These extensive studies by Lloyd (1972a,b,c, 1975a,b, 1980) in combination with the molecular phylogeny of the genus presented in chapter 3 allow us to test several hypotheses regarding to the evolution of dimorphic sex expression. Unfortunately, it was not possible to obtain a well resolved species tree due the low sequence variation and extensive hybridization and polyploidization in *Leptinella* (chapter 3). As a consequence, it was not possible to verify all pathways proposed by Lloyd (1975b). However, chapter 5 shows that dimorphic sex expression (dioecy, paradioecy) has evolved several times from the ancestral monoecious sex expression. Additionally, a weak correlation was found between dimorphic sex expressions and polyploidy. However, the two diametrical hypotheses about the correlation of dioecy and polyploidy by Westergaard (1958; break down of dimorphic sex expression through polyploidization) and Miller and Venable (2000; polyploidy as a trigger of the evolution of dioecy) could not explain the observed patterns in *Leptinella*. Nethertheless, the evolution to the dimorphic sex expression could be interpreted as promotion of outcrossing to avoid in breeding depression.

Summary

The genus *Leptinella* Cass. (Compositae, Anthemideae) is widely distributed in the southern hemisphere (Australia, Chatham Islands, New Guinea, New Zealand, South America, sub-Antarctic Islands). *Leptinella* comprises 42 taxa and consists of small perennial and predominantly procumbent herbs. The genus is characterised by a remarkable variety in sex expression: there are populations with monoecious, paradioecious, and dioecious plants. Additionally, *Leptinella* forms an impressive polyploid complex with chromosome numbers ranging from tetraploid to a chromosome set of $2n = 24x$.

In the present thesis, different molecular methods are used to reconstruct the phylogenies of the genus *Leptinella* and related genera. The obtained molecular phylogenies are then used to a) investigate the intergeneric and infrageneric relationships of *Leptinella*, b) elucidate the origin, the biogeography and the divergence time of the genus, and c) reconstruct the evolution of polyploidy and sex expression in *Leptinella*.

Chapter 2 deals with the intergeneric relationships of *Leptinella*. One region from the nuclear (ITS) and the chloroplast genome (*ndh*F) were chosen for amplification and sequencing to reconstruct the molecular phylogeny of the southern hemisphere members of the tribe Anthemideae. The analyses show that the subtribes sensu Bremer and Humphries (1993) are polyphyletic. As a consequence of the non-monophyletic nature of these subtribes, an alternative generic grouping of the southern hemisphere Anthemideae is discussed (*Osmitopsis*, *Cotula*-group, *Athanasia*-grade, *Pentzia*-clade). The study shows that the genus *Leptinella* is a member of the basal *Cotula*-group which also contains seven southern African genera, the South American genus *Soliva*, and the widespread southern hemisphere genus *Cotula.*

Chapter 3 and 4 deal with the infrageneric phylogeny of the genus *Leptinella*. For this purpose two different methods were used: DNA sequencing and AFLP fingerprinting. Both methods are suitable for the determination of taxon groups within *Leptinella*, but failed to resolve the relationships of single taxa. The extensive hybridization and polyploidization in combination with low genetic variation due the young age of *Leptinella* may be the main points to explain the observed patterns.

In chapter 3, three different markers from the nuclear (ITS) and from the chloroplast genome (*psbA*-*trn*H, *trn*C-*pet*N) were sequenced for *Leptinella* and other members of the *Cotula*-group. The analyses show that *Leptinella* is not monophyletic and the genus is nested within *Cotula*. However, *Leptinella* and *Cotula alpina* form a moderate support monophyletic clade in the combined dataset. In the cpDNA and nrDNA dataset this group is not monophyletic. The division of *Leptinella* into three subgenera according to Lloyd (1972c) is only partly supported by the molecular data. The genus *Leptinella* is split in two well supported groups: The *filicula*-group contains taxa from New Guinea and one taxon from Australia. The remaining taxa of *Leptinella* from New Zealand, South America, the Chatham Islands, the sub-Antarctic islands, and three further taxa from Australia belong to the *Leptinella* main clade. The latter group is divided into six further groups *(dioica-, longipes-, minor-, plumosa-, pectinata-, pyrethrifolia-group).* Within these groups most taxa are not monophyletic.
The study shows that *Leptinella* has radiated in the Miocene to Pleistocene. The estimated divergence time for *Leptinella* is much younger as the supposed break-up of the Gondwana continent. Therefore, the current distribution of *Leptinella* could be explained only by long distance dispersal events.

In chapter 4, AFLP fingerprinting was used to study the phylogeny of the *Leptinella* main group. The AFLP analysis resulted in three main groups (taxon group A-C) and several subgroups. These groups are partly congruent with the results of the sequencing analysis presented in chapter 3. The results indicate different evolutionary histories of group A and C. Taxon group C is characterised by extensive hybridization and polyploidization, whereas both processes are less frequent in taxon group A. Furthermore, new ploidy level estimations for *Leptinella* are provided this chapter.

Chapter 5 focuses on the evolution of dimorphic sex expression and polyploidy in *Leptinella*. For this purpose, sex expression and ploidy levels are mapped on the phylogenetic tree of chapter 3. The study shows that monoecy is the ancestral sex expression in *Leptinella* and dimorphic sex expression (dioecy, paradioecy) has evolved independently at least four times from monoecy.
Additionally, a weak correlation was found between dimorphic sex expression and polyploidy. However, neither of two diametrical hypotheses about the correlation of dioecy and polyploidy by Westergaard (1958; break down of dimorphic sex expression through

polyploidization) and Miller and Venable (2000; polyploidy as a trigger of the evolution of dioecy) was able to explain the observed patterns in *Leptinella*.

Zusammenfassung

Die südhemisphärische Gattung *Leptinella* Cass. (Compositae, Anthemideae) umfasst 42 Taxa und ist in Australien, Neuguinea, Neuseeland, Südamerika, auf den Chatham Islands und den subantarktische Inseln verbreitet. *Leptinella* besteht aus kleinen mehrjährigen und meist niederliegenden Kräutern. Die Gattung zeigt eine erstaunliche Vielfalt in den Geschlechterverhältnissen: es gibt Populationen mit monözischen, paradiözischen und diözischen Pflanzen. Außerdem bildet *Leptinella* einen umfangreichen Polyploid-Komplex ($4x$ - $24x$).

In der vorliegenden Arbeit wurden verschiedene molekulare Methoden verwendet um Phylogenien der Gattung *Leptinella* und verwandeter Gruppen zu rekonstruieren. Basierend auf diesen molekularen Phylogenien sollen a) die Verwandtschaftsverhältnisse einerseits innerhalb der Gattung *Leptinella* und anderseits zwischen den Gattungen der südhemisphärischen Anthemideae rekonstruiert werden, b) die Abstammungsgeschichte, die Biogeographie und die divergence time der Gattung *Leptinella* untersucht werden und c) die Evolution von Polyploidie und Geschlechterverhältnissen in *Leptinella* rekonstruiert werden.

Kapitel 2 beschäftigt sich mit der molekularen Phylogenie der südhemisphärischen Anthemideae. Es wurde jeweils ein Marker aus dem Kern- (ITS) und dem Chloroplast-Genome (*ndh*f) für alle südhemisphärischen Gattungen sequenziert. Die Analysen zeigen, dass die Subtriben sensu Bremer und Humphries (1993) nicht monophyletisch sind. In der vorliegenden Arbeit wird deshalb eine alternative Gliederung diskutiert (*Osmitopsis*, *Cotula*-group, *Anthanasia*-grade, *Pentzia*-group). Die Gattung *Leptinella* gehört zur basalen *Cotula*-group. Diese Gruppe besteht aus sieben südafrikanischen Gattungen, der südamerikanischen Gattung *Soliva* und der in der Südhemisphäre weitverbreiteten Gattung *Cotula*.

Kapitel 3 und 4 beschäftigen sich mit der Phylogenie von *Leptinella*. Hierfür wurden zwei verschiedene molekulare Methoden angewandt: DNA-Sequenzierung und AFLP-Fingerprinting. Beide Methoden sind geeignet zur Identifizierung von Artengruppen innerhalb der Gattung *Leptinella*, es ist jedoch mit Hilfe beider Methoden nicht gelungen, die Beziehung der einzelnen Arten untereinander aufzuklären. Die wahrscheinlichen

Gründe sind die extensive Hybridisierung und Polyploidisierung sowie die geringe genetische Variation zwischen den Arten.

In Kapitel 3 werden die Ergebnisse der Sequenzierung von drei verschiedenen Markern (ITS, *psb*A-*trn*H, *trn*C-*pet*N) für *Leptinella* und andere Gattungen der *Cotula*-group dargestellt. Die Analysen zeigen, das *Leptinella* nicht monophyletisch ist. Jedoch ist *Leptinella* + *Cotula alpina* monophyletisch im kombinierten Datensatz, aber nicht in den einzelnen Datensätzen. Die Untergliederung von *Leptinella* in drei Untergattungen (Lloyd 1972c) wird durch die molekularen Daten nur teilweise bestätigt. Die Gattung *Leptinella* ist in zwei gut gestützte Gruppen geteilt: Die basale *filicula*-group beinhaltet Arten aus Neuguinea und eine australische Art. Die restlichen Arten aus Australien, Neuseeland, Südamerika, den Chatham Islands und den subantarktischen Inseln gehören zur *Leptinella* main group. Die zuletzt genannte Gruppe kann noch in sechs weitere Untergruppen gliedert werden *(dioica-, longipes-, minor-, plumosa-, pectinata,* und *pyrethrifolia*-group). Die verschieden *Leptinella*-Gruppen entstanden im Miozän, Pliozän und Pleistozän. Dieser Zeitraum ist deutlich jünger als die Aufspaltung des Gondwana-Kontinents; deshalb kann das Verbreitungsmuster von *Leptinella* nur durch verschiedene long distance dispersal Ereignisse erklärt werden.

In Kapitel 4 werden die Ergebnisse der AFLP-Analyse der *Leptinella* main group dargestellt. Mit Hilfe der AFLP-Analyse wurde eine Untergliederung dieser Gruppe in drei Hauptgruppen (Taxon-Gruppen A, B, C) und verschieden Untergruppen gefunden. Die Ergebnisse der AFLP-Analyse sind allerdings nur teilweise kongruent zu den Resultaten der Sequenzierung in Kapitel 3. Taxon-Gruppe C ist durch intensive Hybridisierung und Polyploidizierung gekennzeichnet, diese beiden Prozesse sind weniger häufig in Gruppe A. Desweiteren beinhaltet Kapitel 4 zahlreiche neue Ploidiestufen Bestimmungen mittels flow cytometry.

Die Evolution der Geschlechterverhältnisse und von Polyploidie in *Leptinella* werden in Kapitel 5 behandelt. Zu diesem Zweck wurden Geschlechterverhältnisse und Chromosomennummern auf den phylogenetischen Stammbaum geplottet. Die Studie zeigt, dass Monözie der ursprüngliche Zustand in *Leptinella* ist, und dass sich Diözie und Paradiözie mindestens viermal unabhängig von Monözie entwickelt haben.

Es wurde eine schwache Korrelation zwischen diözischen Geschlechterverhältnis und Polyploidie gefunden. Allerdings kann keine der beiden gegensätzlichen Hypothesen zu Diözie und Polyploidie von Westergaard (1958; Zusammenbruch von Diözie durch Polyploidisierung) beziehungsweise von Miller und Venable (2000; Polyploidie fördert Entstehung von Diözie) das beobachtete Muster in *Leptinella* erklären.

References

Ainsworth, C. (2000) Boys and girls come out to play: The molecular biology of dioecious plants. Annals of Botany 86: 211-221

Albach, D. C., Utteridge, T., Wagstaff, S. J. (2005) Origin of Veroniceae (Plantaginaceae, formerly Scrophulariaceae) on New Guinea. Systematic Botany 30: 412–423

Althoff, D. M., Gitzendanner, M. A., Segraves, K. A. (2007) The utility of amplified fragment length polymorphisms in phylogenetics: a comparison of homology within and between genomes. Systematic Biology 56: 477–484

Anderberg, A. A., Eldenäs, P., Bayer, R. J., Englund, M. (2005) Evolutionary relationships in the Asteraceae tribe Inuleae (incl. Plucheeae) evidenced by DNA sequences of *ndh*F; with notes on the systematic positions of some aberrant genera. Organisms Diversity & Evolution 5: 135-146

Anderberg, A. A., Swenson, U. (2003) Evolutionary lineages in Sapotaceae (Ericales): a cladistic analysis based on *ndh*F sequence data. International Journal of Plant Sciences 164: 763-773

Baagøe, J. (1977) Taxonomical application of ligule microcharacters in Compositae. I Anthemideae, Heliantheae, Tageteae. Botanisk Tidsskrift 71: 393-223

Baker, H. G. (1967) Support for Baker's law - as a rule. Evolution 21: 853-856

Baker, H. G., Cox, P. A. (1984) Further thoughts on dioecism and islands. Annals of the Missouri Botanical Garden 71: 244-253

Barker, N. P., Weston, P. H., Rutschmann, F., Sauquet, H. (2007) Molecular dating of the 'Gondwanan' plant family Proteaceae is only partially congruent with the timing of the break-up of Gondwana. Journal of Biogeography 34: 2012-2027

Bayer, R. J., Breitwieser, I., Ward, J. M., Puttock, C. (2006) Tribe Gnaphalieae (Cass.) Lecoq & Juillet. In: Kadereit, J., Jeffrey, C. (eds.) The families and genera of vascular plants 8. Springer, Berlin, 246-284

Bayer, R. J., Soltis, D. E., Soltis, P. S. (1996) Phylogenetic inferences in *Antennaria* (Asteraceae: Gnaphalieae: Cassiniinae) based on sequences from nuclear ribosomal DNA internal transcribed spacers (ITS). American Journal of Botany 83: 516-527

Beauverd, G. (1915) Contribution á letude des Composées. Bulletin de la Société botanique Genève (ser 2) 7: 21-56

Beier, B.-A., Chase, M. W., Thulin, M. (2003) Phylogenetic relationships and taxonomy of subfamily Zygophylloideae (Zygophyllaceae) based on molecular and morphological data. Plant Systematics and Evolution 240: 11-39

Bentham, G. (1867) Flora Australiensis 3. Reeve, London

Bentham, G. (1873) Compositae. In: Bentham, G., Hooker, J. D. (eds.) Genera Plantarum Vol 2, no. 2. Reeve & Co., London, 163-533

Bergh, N. G., Linder, H. P. (2009) Cape diversication and repeated out-of-southern-Africa dispersal in paper daisies (Asteraceae-Gnaphalieae). Molecular Phylogenetics and Evolution 51: 5-18

Beuzenberg, E. J. (1961) Observations on sex differentiation and cytotaxonomy of the New Zealand species of Hymenantherinae (Violaceae). New Zealand Journal of Science 4: 337-349

Beuzenberg, E. J., Hair, J. B. (1984) Contributions to a chromosome atlas of the New Zealand flora - 27. Compositae. New Zealand Journal of Botany 22: 353-356

Bohlmann, F., Burkhardt, T., Zdero, C. (1973) Naturally occurring acetylenes. Academic press, London

Bohlmann, F., Grenz, M. (1975) Neue Sesquiterpenlactone aus *Athanasia*-Arten. Chemische Berichte 108: 357-361

Bohlmann, F., Rao, N. (1972) Neue Furansesquiterpene aus *Athanasia*-Arten. Tetrahedron Letters 1972: 1295–1296

Bohlmann, F., Zdero, C. (1972a) Zwei neue Sesquiterpen-Lactone aus *Lidbeckia pectinata* Berg. und *Pentzia elegans* DC. Tetrahedron Letters 1972: 621–624

Bohlmann, F., Zdero, C. (1972b) Ein neues Furansesquiterpen aus *Phymaspermum* parvifolium. Tetrahedron Letters 1972: 851-852

Bohlmann, F., Zdero, C. (1974) Neue Acetylverbindungen aus südafrikanischen Vertretern der Tribus Anthemideae. Chemische Berichte 107: 1044-1048

Bohlmann, F., Zdero, C. (1977) Ein neues Guajanolid aus *Matricaria zuubergensis*. Phytochemistry 16: 136-137

Bohlmann, F., Zdero, C. (1978a) New Furansesquiterpenes from *Eumorphia* species. Phytochemistry 17: 1155-1159

Bohlmann, F., Zdero, C. (1978b) New sesquiterpenes and acetylenes from *Athanasia* and *Pentzia* species. Phytochemistry 17: 1595-1599

Bohlmann, F., Zdero, C. (1982) Sesquiterpene lactones from *Inezia integrifolia*. Phytochemistry 21: 2743-2745

Boissier, E. (1849) Diagnoses plantarum orientalium novarum, vol 11. Ducloux, Paris,

Breitwieser, I., Glenny, D., Thorne, A., Wagstaff, S. J. (1999) Phylogenetic relationships in Australian Gnaphalieae (Asteraceae) inferred from ITS sequences. New Zealand Journal of Botany 37: 399-412

Bremer, K. (1972) The genus *Osmitopsis* (Compositae). Botaniska Notiser B125: 9-48

Bremer, K. (1976) New taxa of *Osmitopsis* (Compositae). Botaniska Notiser 129: 21-24

Bremer, K., Humphries, C. J. (1993) Generic monograph of the Asteraceae-Anthemideae. Bulletin of of the Natural History Museum: Botany series 23: 71-177

Bremer, K., Källersjo, M. (1985) Taxonomic notes on *Hymenolepis* (Asteraceae-Anthemideae). Nordic Journal of Botany 5: 27-30

Brouillet, L., Lowrey, T. K., Urbatsch, L. E., Wagstaff, S. J., Karaman, V. (2006) The tribe Astereae. The International Compositae Alliance Barcelona, 25

Bruhl, J. J., Quinn, C. J. (1990) Cypsela anatomy in the ´Cotuleae´(Asteraceae-Anthemideae). Botanical Journal of the Linnean Society 102: 37-59

Bruhl, J. J., Quinn, C. J. (1991) Floral morphology and a reassessment of affinities in the 'Cotuleae' (Asteraceae). Australian Journal of Botany 4: 637-654

Brysting, A. K., Oxelman, B., Huber, K. T., Moulton, V., Brochmann, C. (2007) Untangling complex histories of genome mergings in high polyploids. Systematic Biology 56: 467 - 476

Cabrera, A. L. (1949) Sinopsis del Género *Soliva* (Compositae). Notes del Museo de la Plata 14: 123-139

Campbell, H. J. (1998) Fauna and flora of the Chatham Islands: less than 4 My old? Geological Society of New Zealand Miscellaneous publication 97: 15-16

Campbell, H. J., Begg, J. G., Beu, A. G., Carter, R. M., Davies, G., Holt, K., Landis, C., Wallace, C. (2006) On the turn of a scallop. Geological Society of New Zealand Miscellaneous publication 121: 9

Caronel, J. D., Greilhuber, J., Suda, J. (2007) Estimation of nuclear DNA content in plants using flow cytometry. Nature Protocols 2: 2233-2244

Carter, R. J. (2006) An investigation into the evolutionary relationships of the North Island alpine Ranunculus. Unpublished MSc thesis, Massey University, New Zealand

Cassini, H. (1816) Anthémidées. In: Cuvier, G. L. C. F. D. (eds.) Dictionaire des sciences naturelles, 2nd edn, 2 suppl. Paris, 73–75

Cassini, H. (1817) Apercu des Genres nouveaux formés par M. Henri Cassini dans la famille des Synanthérés. Bull Sci Soc Philom Paris 1817:

Cassini, H. (1822) Proposition d'un nouveau genre de plantes (*Leptinella*). Bulletin de la Societe Philomathique de Paris 3: 127-129

Chamberlain, C. P., Poage, M. A. (2000) Reconstructing the paleotopography of mountain belts from the isotopic composition of authigenic minerals. Geology 28: 115-118

Charlesworth, D. (1999) Theories of the evolution of dioecy. In: Geber, M. A., Dawson, T. E., Delph, L. F. (eds.) Gender and sexual dimorphism in flowering plants. Springer, Berlin, 33-60

Charlesworth, D. (2001) Evolution: An exception that proves the rule. Current Biology 11: R13-R15

Charlesworth, D., Charlesworth, B. (1978) Population genetic of partial male-sterility and the evolution of monoecy and dioecy. Heredity 41: 137-153

Charnov, E. (1982) Monographs in population biology 18: The theory of sex allocation. Princeton University Press, New Jersey

Cooper, R. A., Millener, P. R. (1993) The New Zealand biota - historical background and new research. Trends in Ecology & Evolution 8: 429-433

Darwin, C. (1877) The different forms of flowers on plants of the same species. John Murray, London

Dawson, M. I. (1995) Contributions to a chromosome atlas of the New Zealand flora - 33. Miscellaneous species. New Zealand Journal of Botany 33: 477-487

Dawson, M. I. (2000) Index of chromosome numbers of indegenous New Zealand spermatophytes. New Zealand Journal of Botany 38: 47-150

de Lange, P. J., Heenan, P. B., Keeling, D. J., Murray, B. G., Smissen, R. D., Sykes, W. R. (2008) Biosystematics and conservation; A case study with two enigmatic and uncommon species of *Crassula* from New Zealand. Annals of Botany 101: 881-899

de Lange, P. J., Nort on, D. A., Courtney, S. P., Heenan, P. B., Barkla, J. W., Cameron, E. K., Liston, A., Hitchmough, R., Temsch, E. M., Townsend, A. J. (2009) Threatened and uncommon plants of New Zealand (2008 revision). New Zealand Journal of Botany 47: 61-96

Delph, L. F. (1990a) The evolution of gender dimorphism in New Zealand *Hebe* (Scrophulariaceae) species. Evolutionary Trends in Plants 4: 85-97

Delph, L. F. (1990b) Sex-ratio variation in the gynodioecious shrub *Hebe strictissima* (Srophulariaceae). Evolution 44: 134-142

Delph, L. F., Lloyd, D. G. (1991) Enviromental and genetic control of gender in the dimorphic shrub *Hebe subalpina*. Evolution 45: 1957-1964

Demesure, B., Comps, B., Petit, R. J. (1995) A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. Molecular Ecology 4: 129-131

Doyle, J. J., Doyle, J. S. (1987) A rapid DNA isolation procedure for small quantatities of fresh leaf tissue. Phytochemical Bulletin 19: 11-15

Druce, A. P. (1987) Vegetation and flora of tertiary calcareous rocks in the mountains of western Nelson, New Zealand. New Zealand Journal of Botany 25: 41-78

Druce, A. P. (1992) Indigenous psilopsids, lycopods, quillworts, ferns, gymnosperms, & flowering plants of the mountains of inland Otago and N Southland. Unpublished checklist 292. Copy held at Landcare Research, Lincoln, New Zealand

Druce, A. P. (1993) Indigenous vascular plants of New Zealand, 9th revision september 1993. Unpublished checklist. Copy held at Landcare Research, Lincoln, New Zealand

Drummond, A. J., Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7: 214

Eastwood, A., Gibby, M., Cronk, Q. C. B. (2004) Evolution of St Helena arborescent Astereae (Asteraceae): relationships of the genera Commidendrum and Melanodendron. Botanical Journal of the Linnean Society 144: 69-83

Edgar, E. (1958) Studies in New Zealand *Cotulas*. Transactions of the Royal Society of New Zealand 85: 357-377

Edwards, C. E., Soltis, D. E., Soltis, P. S. (2008) Using patterns of genetic structure based on microsatellite loci to test hypotheses of current hybridization, ancient hybridization and incomplete lineage sorting in *Conradina* (Lamiaceae). Molecular Ecology 17: 5157–5174

Eldenäs, P., Källersjö, M., Anderberg, A. A. (1999) Phylogenetic Placement and Circumscription of Tribes Inuleae s. str. and Plucheeae (Asteraceae): Evidence from Sequences of Chloroplast Gene *ndh*F. Molecular Phylogenetics and Evolution 13: 50-58

Farris, J. S., Källersjö, M., Kluge, A. G., Bult, C. (1994) Testing significance of incongruence. Cladistics 10: 315-319

Felsenstein, J. (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. Journal Molecular Ecology 17: 368-376

Felsenstein, J. (1985) Confidence limits of phylogenies: an approach using the bootstrap. Evolution 39: 783-791

Ford, K., Ward, J. M., Smissen, R. D., Wagstaff, S. J., Breitwieser, I. (2007) Phylogeny and biogeography of *Craspedia* (Asteraceae: Gnaphalieae) based on ITS, ETS and psbA-trnH sequence data. Taxon 56: 783-794

Francisco-Ortega, J., Barber, J. C., Santos-Guerra, A., Febles-Hernandez, R., Jansen, R. K. (2001) Origin and evolution of the endemic genera of Gonosperminae (Asteraceae: Anthemideae) from the Canary Islands: evidence from nucleotide sequences of the internal transcribed spacers of the nuclear ribosomal DNA. American Journal of Botany 88: 161-169

Francisco-Ortega, J., Santos-Guerra, A., Hines, A., Jansen, R. K. (1997) Molecular evidence for a Mediterranean origin of the Macaronesian endemic genus *Argyranthemum* (Asteraceae). American Journal of Botany 84: 1595-1613

Funk, D. J., Omland, K. E. (2003) Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. Annual Review of Ecology, Evolution, and Systematics 34: 397–423

Funk, V. A. et al. (in press) Compositae Meta-supertree: The Next Generation. In: Funk, V. A., Susanna, A., Stuessy, T. (eds.) Systematics and Evolution of the Compositae. IAPT, Vienna

Funk, V. A., Bayer, R. J., Keeley, S., Chan, R., Watson, L., Gemeinholzer, B., Schilling, E., Panero, J. L., Baldwin, B. G., Garcia-Jacas, N., Susanna, A., Jansen, R. K. (2005) Everywhere but Antarctica: Using a supertree to understand the diversity and distribution of the Compositae. Biologiske skrifter 55: 343-374

Funk, V. A., Chan, R., Keeley, S. C. (2004) Insights into the evolution of the tribe Arctoteae (Compositae: subfamily Cichorioideae s.s.) using trnL-F, ndhF and ITS. Taxon 53: 637-655

Gadek, P. A., Bruhl, J. J., Quinn, C. J. (1989) Exine structure in the 'Cotuleae' (Anthemideae, Asteraceae). Grana 28: 163-178

Geber, M. A. (1999) Theories of the evolution of sexual dimorphism. In: Geber, M. A., Dawson, T. E., Delph, L. F. (eds.) Gender and sexual dimorphism in flowering plants. Springer, Berlin, 97-122

Gemeinholzer, B., Oberprieler, C., Bachmann, K. (2006) Using GenBank data for plant identification: possibilities and limitations using the ITS 1 of Asteraceae species belonging to the tribes Lactuceae and Anthemideae. Taxon 55: 173-187

Godley, E. J. (1979) Flower biology in New Zealand. New Zealand Journal of Botany 17: 441-466

Goertzen, L. R., Cannone, J. J., Gutell, R. R., Jansen, R. K. (2003) ITS secondary structure derived from comparative analysis: implications for sequence alignment and phylogeny of the Asteraceae. Molecular Phylogenetics and Evolution 29: 216-234

Goldberg, J., Trewick, S. A., Paterson, A. M. (2008) Evolution of New Zealand's terrestrial fauna: a review of molecular evidence. Philosophical Transactions of the Royal Society of London B 363: 3319–3334

Graham, A. (1996) A contribution to the geologic history of the Compositae. In: Hind, D. J. N., Beentje, H. J. (eds.) Proceedings of the International Compositae Conference Kew, 1994, vol. 1. Royal Botanic Gardens Kew, London, 123–140

Grant, S. R. (1999) Genetics of gender dimorphism in higher plants. In: Geber, M. A., Dawson, T. E., Delph, L. F. (eds.) Gender and sexual dimorphism in flowering plants. Springer, Berlin, 247-274

Guibert, C., Civeyrel, L., Linder, P. (2009) Male and female separation event trapped in a species tree. Taxon 58: 172-180

Guo, Y.-P., Ehrendorfer, F., Samuel, R. (2004) Phylogeny and systematics of *Achillea* (Asteraceae-Anthemideae) inferred from nrITS and plastid trnL-F sequences. Taxon 53: 657-672

Guo, Y.-P., Saukel, J., Ehrendorfer, F. (2008) AFLP trees versus scatterplots: evolution and phylogeography of the polyploid complex *Achillea millefolium* agg. (Asteraceae). Taxon 57: 153-169

Guo, Y.-P., Saukel, J., Mittermayr, R., Ehrendorfer, F. (2005) AFLP analyses demonstrate genetic divergence, hybridization, and multiple polyploidization in the evolution of *Achillea* (Asteraceae-Anthemideae). New Phytologist 166: 273-290

Hair, J. B. (1962) Basic chromosome number in *Cotula*. Chromosome information service 3: 41-42

Hall, T. A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symposium series 41: 95-98

Hamilton, M. B. (1998) Four primer pairs for the amplification of chloroplast intergenic regions with intraspecific variation. Molecular Ecology 8: 513-525

Hedren, M., Fay, M. F., Chase, M. W. (2001) Amplified fragment lenght polymorphisms (AFLP) reveal details of polyploid evolution in *Dactylorhiza* (Orchidaceae). American Journal of Botany 88: 1868-1880

Heenan, P. B. (2009) *Leptinella conjuncta* (Asteraceae), a diminutive new species from arid habitats in the South Island, New Zealand. New Zealand Journal of Botany 47: 127-132

Heenan, P. B., Lockhart, P. J., Kirkham, N., McBreen, K., Havell, D. (2006) Relationships in the alpine *Ranunculus haastii* (Ranunculaceae) complex and recognition of R. piliferus and *R. acraeus* from southern New Zealand. New Zealand Journal of Botany 44: 424-441

Hershkovitz, M. A., Arroyo, M. T. K., Bell, C., Hinojosa, L. F. (2006) Phylogeny of *Chaetanthera* (Asteraceae; Mutisieae) reveals both ancient and recent origins of the high elevation lineages. Molecular Phylogenetics and Evolution 41: 594-605

Heywood, V. H., Humphries, C. J. (1977) Anthemideae - systematic review. In: Heywood, V. H., Harborne, J. B., Turner, B. L. (eds.) The Biology and Chemistry of the Compositae 2. Academic Press, London,

Himmelreich, S., Källersjö, M., Eldenäs, P., Oberprieler, C. (2008) Phylogeny of southern hemisphere Compositae-Anthemideae based on nrDNA ITS and cpDNA *ndh*F Sequence information. Plant Systematics and Evolution 272: 131-153

Hoffmann, O. (1894) Compositae. In: Engler, A., Prantl, K. (eds.) Die natürlichen Pflanzenfamilien nebst ihren Gattungen und wichtigeren Arten insbesondere den Nutzpflanzen 4/5. Wilhelm Engelmann, Leipzig, 87-394

Holland, B. R., Clarke, A. C., Meudt, H. M. (2008) Optimizing automated AFLP scoring parameters to Improve phylogenetic resolution. Systematic Biology 57: 347-366

Hooker, J. D. (1864) Handbook of the New Zealand flora. Reeve, London

Huelsenbeck, J. P., Ronquist, F. (2001) MrBayes: Bayesian Inference of Phylogenetic Trees. Computer software available at http://brahms.biology.rochester.edu/software.html,

Huson, D. H., Bryant, D. (2006) Application of Phylogenetic Networks in Evolutionary Studies. Molecular Biology and Evolution 23: 254-267

Jobb, G. (2004) TREEFINDER version of November 2004, www.treefinder.de

Joly, S., Starr J, Lewis W, A., B. (2006) Polyploid and hybrid evolution in roses east of the Rocky Mountains. American Journal of Botany 93: 412-425

Kadereit, G., Mucina, L., Freitag, H., 2006. (2006) Phylogeny of Salicornioideae (Chenopodiaceae): diversification, biogeography, and evolutionary trends in leaf and flower morphology. Taxonomy 55: 617–642

Källersjö, M. (1985) Fruit structure and generic delimitation of *Athanasia* (Asteraceae - Anthemideae) and related South African genera. Nordic Journal of Botany 5: 11-26

Källersjö, M. (1988) A generic re-classification of *Pentzia* Thunb. (Compositae-Anthemideae) from southern Africa. Botanical Journal of the Linnean Society 96: 299-322

Källersjö, M. (1991) The genus *Athanasia* (Compositae-Anthemideae). Opera Botanica 106: 5-75

Kim, K.-J., Choi, K.-S., Jansen, R. K. (2005) Two chloroplast DNA inverstions originated simultaneously during the early evolution of the sunflower family (Asteraceae). Molecular Biology and Evolution 22: 1783–1792

Kim, K.-J., Jansen, R. K. (1995) *ndh*F sequence evolution and the major clades in the sunflower family. Proceedings of the National Academy of Sciences of the United States of America 92: 10379-10383

Kinney, M. S., Columbus, J. T., Friar, E. A. (2007) Dicliny in *Bouteloua* (Poaceae: Chloridoideae): Implications for the evolution of dioecy. Aliso 23: 605-614

Kishino, H., Hasegawa, M. (1989) Evaluation of the maximum likelihood estimate of the evolutionary tree topology from DNA sequence data, and the branching order of Hominoideae. Journal Molecular Ecology 29: 170-179

Knapp, M., Mudaliar, R., Havell, D., Wagstaff, S. J., Lockhart, P. J. (2007) The drowning of New Zealand and the problem of *Agathis*. Systematic Biology 56: 862-870

Knapp, M., Stöckler, K., Havell, D., Delsuc, F., Sebastiani, F., Lockhart, P. J. (2005) Relaxed molecular clock provides evidence for long-distance dispersal of *Nothofagus* (Southern Beech). PLoS Biology 3: e14

Koopman, W. J. M., Wissemann, V., De Cock, K., Van Huylenbroeck, J., De Riek, J., Sabatino, G. J. H.-., Visser, D., Vosman, B., Ritz, C. M., Maes, B., Werlemark, G., Nybom, H., Debener, T., Linde, M., Smulders, M. J. M. (2008) AFLP markers as

tool to reconstruct complex relationships: A case study in *Rosa* (Rosaceae). American Journal of Botany 95: 353-366

Koopman, W. J. M., Zevenbergen, M. J., Van den Berg, R. G. (2001) Species relationships in *Lactuca* s.l. (Lactuceae, Asteraceae) inferred from AFLP fingerprints. American Journal of Botany 88:

Koopmann, W. J. M. (2005) Phylogenetic Signal in AFLP Data Sets. Systematic Biology 54: 197-217

Kornkven, A. B., Watson, L. E., Estes, J. R. (1998) Phylogenetic analysis of *Artemisia* section *Tridentatae* (Asteraceae) based on sequences from the internal transcribed spacers (ITS) of nuclear ribosomal DNA. American Journal of Botany 85: 1787-1795

Kovach, M. L. (1999) MVSP - A multi statistical package for Windows 3.1., Kovach Computing Service,

Kress, W. J., Wurdack, K. J., Zimmer, E. A., Weigt, L. A., Janzen, D. H. (2005) Use of DNA barcodes to identify flowering plants. PNAS 102: 8369-8374

Kron, P., Suda, J., Husband, B. C. (2007) Applications of flow cytometry to evolutionary and population biology. Annual Review of Ecology, Evolution, and Systematics 38: 847-876

Landis, C. A., Campbell, H. J., Begg, J. G., Mildenhall, D. C., Paterson, A. M., Trewick, S. A. (2008) The Waipounamu Erosion Surface: questioning the antiquity of the New Zealand land surface and terrestrial fauna and flora. Geological Magazine 145: 173-197

Lee, C., Wen, J. (2004) Phylogeny of *Panax* using chloroplast *trn*C-*trn*D intergenic region and the utility of *trn*C-*trn*D in interspecific studies of plants. Molecular Phylogenetics and Evolution 31: 894-903

Leitch, I. J., Bennett, M. D. (2004) Genome downsizing in polyploid plants. Biological Journal of the Linnean Society 82: 651-663

Lewis, P. O. (2001) Phylogenetic systematics turns over a new leaf. Trends in Ecology & Evolution 16: 30-37

Linder, H. P. (2008) Plant species radiations: where, when, why? Philosophical Transactions of the Royal Society of London B 363: 3097-3105

Liu, J. Q., Gao, T. G., Chen, Z. D., Lu, A. M. (2002) Molecular phylogeny and biogeography of the Qinghai-Tibet Plateau endemic *Nannoglottis* (Asteraceae). Molecolar Phylogenetics and Evolution 23: 307-325

Lloyd, D. G. (1972a) Breeding systems in *Cotula* L. (Compositae, Anthemideae). I. The array of monoclinous and diclinous systems. New Phytologist 71: 1181-1194

Lloyd, D. G. (1972b) Breeding systems in *Cotula* L. (Compositae, Anthemideae). II. Monoecious populations. New Phytologist 71: 1195-1202

Lloyd, D. G. (1972c) A revision of the New Zealand, Subantarctic, and South American species of *Cotula*, section *Leptinella*. New Zealand Journal of Botany 10: 277-372

Lloyd, D. G. (1975a) Breeding systems in *Cotula* III. Dioecious populations. New Phytologist 74: 109-123

Lloyd, D. G. (1975b) Breeding systems in *Cotula* IV. Reversion from dioecy to monoecy. New Phytologist 74: 125-145

Lloyd, D. G. (1976) The transmission of genes via pollen and ovules in gynodioecious angiosperms. Theoretical Population Biology: 299-316

Lloyd, D. G. (1980) The distributions of gender in four angiosperm species illustrating two evolutionary pathways to dioecy. Evolution 34: 123-134

Lloyd, D. G. (1981) Evolution of prostrate and erect habitus in *Cotula* section *Leptinella* and other New Zealand plant groups. New Zealand Journal of Botany 19: 247-253

Lloyd, D. G. (1982) Variation and evolution of plant species on the outlying islands of New Zealand, with particular reference to *Cotula featherstonii*. Taxon 31: 478-487

Lloyd, D. G., Webb, C. J. (1977) Secondary sex characters in seed plants. Botanical Review 43: 177-216

Lloyd, D. G., Webb, C. J. (1987) The reinstatement of *Leptinella* at generic rank, and the status of the ´Cotuleae´ (Asteraceae, Anthemideae). New Zealand Journal of Botany 25: 99-105

Lo Presti, R. M., Oberprieler, C. (2009) Evolutionary history, biogeography and eco-climatological differentiation of the genus *Anthemis* L. (Compositae, Anthemideae) in the circum-Mediterranean basin. Journal of Biogeography 36: 1313-1332

Lockhart, P. J., McLenachan, P. A., Havell, D., Glenny, D., Huson, D., Jensen, U. (2001) Phylogenety, radiation, and transoceanic dispersal of New Zealand alpine buttercups: Molecular evidence under split composition. Annals of the Missouri Botanical Garden 88: 458-477

Maddison, W. P., Maddison, D. R. (2006) Mesquite: a modular system for evolutionary analysis version 1.1, http://mesquiteproject.org

Markos, S., Baldwin, B. G. (2001) Higher-Level Relationships and Major Lineages of *Lessingia* (Compositae, Astereae) Based on Nuclear rDNA Internal and External Transcribed Spacer (ITS and ETS) Sequences. Systematic Botany 26: 168-183

McGlone, M. S. (2005) Goodbey Gondwana. Journal of Biogeography 32: 739-740

McLoughlin, S. (2001) The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. Australian Journal of Botany 49: 271-300

Meister, J., Hubaishan, M., Kilian, N., Oberprieler, C. (2006) Temporal and spatial diversication of the shrub *Justicia areysiana* Deflers (Acanthaceae) endemic to the monsoon affected coastal mountains of southern Arabian Peninsula. Plant Systematics and Evolution 262: 153-171

Meudt, H. M., Bayly, M. J. (2008) Phylogeographic patterns in the Australasian genus *Chionohebe* (*Veronica* s.l., Plantaginaceae) based on AFLP and chloroplast DNA sequences. Molecular Phylogenetics and Evolution 47: 319-338

Meudt, H. M., Clarke, A. C. (2007) Almost Forgotten or Latest Practice? AFLP applications, analyses and advances. Trends in Plant Science 12: 106-117

Meudt, H. M., Simpson, B. B. (2006) The biogeography of the austral, subalpine genus *Ourisia* (Plantaginaceae) based on molecular phylogenetic evidence: South American origin and dispersal to New Zealand and Tasmania. Biological Journal of the Linnean Society 87: 479-513

Miller, J. S., Venable, D. L. (2000) Polyploidy and the evolution of gender dimorphism in plants. Science 289: 2335-2338

Ming, R., Wang, J., Moore, P. H., Paterson, A. H. (2007) Sex chromosomes in flowering plants. American Journal of Botany 94: 141-150

Mitchell, A. D., Heenan, P. B. (2000) Systematic relationships of New Zealand endemic Brassicaceae inferred from nrDNA ITS sequence data. Systematic Botany 25: 98-105

Mitchell, A. D., Heenan, P. B. (2002a) Genetic variation within the *Pachycladon* (Brassicaceae) complex based on fluorescent AFLP data. Journal of the Royal Society of New Zealand 32: 427-443

Mitchell, A. D., Heenan, P. B. (2002b) *Sophora* sect. *Edwardsia* (Fabaceae): further evidence from nrDNA sequence data of a recent and rapid radiation around the Southern Oceans. Botanical Journal of the Linnean Society 140: 435-441

Mitchell, A. D., Heenan, P. B., Murray, B. G., Molloy, B., De Lange, P. J. (2009a) Evolution of the south-western Pacific genus *Melicytus* (Violaceae): evidence from DNA sequence data, cytology and sex expression. Australian Systematic Botany 22:

Mitchell, A. D., Heenan, P. B., Paterson, A. H. (2009b) Phylogenetic relationships of *Geranium* species indigenous to New Zealand. New Zealand Journal of Botany 47: 21-31

Moore, D. M. (1981) Chromosome numbers of Fuegian angiosperms. Boletim da Sociedade Broteriana 53: 995-1012

Morgan-Richards, M., Smissen, R. D., Shepherd, R. D., Wallis, G. P., Hayward, J. J., Chan, C.-H., Chambers, G. K., Chapman, H. M. (2009) A review of genetic analyses of hybridisation in New Zealand. Journal of the Royal Society of New Zealand 39: 15-34

Morgan, D. R. (1997) Reticulate Evolution in *Machaeranthera* (Asteraceae). Systematic Botany 22: 599-615

Mummenhoff, K., Franzke, A. (2007) Gone with the birds: late Tertiary and Quaternary intercontinal long-distance dispersal and allopolyploidization in plants. Systematics and Biodiversity 5: 255-260

Munoz, J., Felicisimo, A. M., Cabezas, F., Burgaz, A. R., Martinez, I. (2004) Wind as a long-distance dispersal vehicle in the Southern Hemisphere. Science 304: 1144-1147

Navajas-Perez, R., de la Herran, R., Lopez Gonzalez, G., Jamilena, M., Lozano, R., Ruiz Rejon, C., Ruiz Rejon, M., Garrido-Ramos, M. A. (2005) The evolution of reproductive systems and sex-determining mechanisms within *Rumex* (Polygonaceae) Inferred from nuclear and chloroplastidial sequence data. Molecular Biology and Evolution 22: 1929-1939

Neall, V. E., Trewick, S. A. (2008) The age and origin of the Pacific islands: a geological overview. Phil. Trans. R. Soc. B 363: 3293–3330

Nei, M., Li, W. H. (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences of the United States of America 76: 5269-5273

Nesom, G., Robinson, H. (2006) Tribe Astereae Cass. In: Kadereit, J., Jeffrey, C. (eds.) The families and genera of vascular plants 8. Springer, Berlin, 284-342

New Zealand Plant Conservation Network (2009) Plant fact sheets. http://www.nzpcn.org.nz [accessed May 2009]

Nordenstam, B. (1976) Re-classification of *Chrysanthemum* L. in South Africa. Botaniska Notiser 129: 137-164

Noyes, R. D., Rieseberg, L. H. (1999) ITS sequence data support a single origin for North American Astereae (Asteraceae) and reflect deep geographic divisions in Aster s.L. American Journal of Botany 86: 398-412

Obbard, D. J., Harris, S. A., Buggs, R. J. A., Pannell, J. R. (2006) Hybridization, polyploidy, and the evolution of sexual systems in *Mercurialis* (Euphorbiaceae). Evolution 60: 1801-1815

Oberprieler, C. (2001) Phylogenetic relationships in *Anthemis* L. (Compositae, Anthemideae) based on nrDNA ITS sequence variation. Taxon 50: 745-762

Oberprieler, C. (2004a) On the taxonomic status and the phylogenetic relationships of some unispecific Mediterranean genera of Compositae-Anthemideae I. *Brocchia*, *Endopappus* and *Heliocauta*. Willdenowia 34: 39-57

Oberprieler, C. (2004b) On the taxonomic status and the phylogenetic relationships of some unispecific Mediterranean genera of Compositae-Anthemideae II. *Deveaua*, *Leucocyclus* and *Nananthea*. Willdenowia 34: 341-350

Oberprieler, C. (2005) Temporal and spatial diversification of Circum-Mediterranean Compositae-Anthemideae. Taxon 54: 951-966

Oberprieler, C., Himmelreich, S., Vogt, R. (2007) A new subtribal classification of the tribe Anthemideae (Compositae). Willdenowia 37: 89-114

Oberprieler, C., Vogt, R. (2000) The position of *Castrilanthemum* Vogt & Oberprieler and the phylogeny of Mediterranean Anthemideae (Compositae) as inferred from nrDNA ITS and cpDNA *trn*L/*trn*F IGS sequence variation. Plant Systematics and Evolution 225: 145-170

Oberprieler, C., Vogt, R. (2002) A phylogenetic analysis of *Chamaemelum* Mill. (Compositae: Anthemideae) and related genera based upon nrDNA ITS and cpDNA *trnL*/*trn*F IGS sequence variation. Botanical Journal of the Linnean Society 138: 255-273

Oberprieler, C., Vogt, R. (2006) The taxonomic position of *Matricaria macrotis* (Compositae-Anthemideae). Willdenowia 36: 329-338

Oberprieler, C., Vogt, R., Watson, L. E. (2006) Tribe Anthemideae Cass. (1819). In: Kadereit, J. W., Jeffrey, C. (eds.) The Families and Genera of Vascular Plants 8. Springer, Berlin, 342-374

Panero, J. L., Funk, V. A. (2002) Toward a phylogenetic subfamilial classification for the Compositae (Asteraceae). Proceedings of the Biological Society of Washington 115: 909-922

Pannell, J., Dorken, M., Pujol, B., Berjano, R. (2008) Gender variation and transitions between sexual systems in *Mercurialis annua* (Euphorbiaceae). International Journal of Plant Sciences 169: 129-139

Pannell, J. R., Obbard, D. J., Buggs, R. J. A. (2004) Polyploidy and the sexual system: what can we learn from *Mercurialis annua*? Biological Journal of the Linnean Society 82: 547-560

Pelser, P. B., Gravendeel, B., von der Meijden, R. (2003) Phylogeny reconstruction in the gap between too little and too much divergence: the closest relatives of *Senecio jacobaea* (Asteraceae) according to DNA sequences and AFLPs. Molecular Phylogenetics and Evolution 29: 613-628

Perrie, L., Brownsey, P. (2007) Molecular evidence for long-distance dispersal in the New Zealand pteridophyte flora. Journal of Biogeography 34: 2028-2038

Perrie, L., Brownsey, P. J. (2005) Insights into the biogeography and polyploid evolution of New Zealand *Asplenium* from chloroplast DNA sequence data. American Fern Journal 95: 1-21

Perrie, L. R., Brownsey, P. J., Lockhart, P. J., Large, M. F. (2003) Evidence for an allopolyploid complex in New Zealand *Polystichum* (Dryopteridaceae). New Zealand Journal of Botany 41: 189-215

Perrie, L. R., Shepherd, L. D. (2009) Reconstructing the species phylogeny of *Pseudopanax* (Araliaceae), a genus of hybridising trees. Molecular Phylogenetics and Evolution 52: 774-783

Peterson, A., Harpke, D., Peruzzi, L., Levichev, I. G., Tison, J.-M., Peterson, J. (2009) Hybridization drives speciation in *Gagea* (Liliaceae). Plant Systematics and Evolution 278: 133-148

Pleines, T., Blattner, F. R. (2008) Phylogeographic implications of an AFLP phylogeny of the American diploid *Hordeum* species (Poaceae: Triticeae). Taxon 57: 875-881

Pole, M. (1994) The New Zealand flora - entirely long-distance dispersal? Journal of Biogeography 21: 625-635

Posada, D., Crandall, K. A. (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14: 817-818

Renner, S. S., Ricklefs, R. E. (1995) Dioecy and its correlates in the flowering plants. American Journal of Botany 82: 596-606

Richards, A. J. (1997) Plant breeding systems. Chapman & Hall, London

Robinson, H., Brettell, R. D. (1973) Tribal revisions in the Asteraceae VIII. A new tribe, Ursinieae. Phytologia 26: 76-85

Rogstad, S. (2003) Plant DNA extraction using silica. Plant Molecular Biology Reporter 12: 463a-463g

Ronquist, F., Huelsenbeck, J. P. van der Mark, P., (2005) MrBayes 3.1.2. http://mrbayes.csit.fsu.edu/manual.php

Rydin, C., Pedersen, K. R., Friis, E. M. (2004) On the evolutionary history of *Ephedra*: Cretaceous fossils and extant molecules. PNAS 101: 16571-16576

Sakai, A. K., Wagner, W. L., Ferguson, D. M., Herbst, D. R. (1995a) Origins of the dioecy in the Hawaiian Flora. Ecology 76: 2517-2529

Sakai, A. K., Wagner, W. L., Ferguson, D. M., Herbst, D. R. (1995b) Biogeographical and ecological correlates of dioecy in the Hawaiian flora. Ecology 76: 2530-2543

Sakai, A. K., Weller, S. G. (1999) Gender and sexual dimorphism in flowering plants: review of terminology, biogeographic patterns, ecological correlates, and phylogenetic approaches. In: Geber, M. A., Dawson, T. E., Delph, L. F. (eds.) Gender and sexual dimorphism in flowering plants. Springer, Berlin, 1-31

Sanmartin, I., Ronquist, F. (2004) Southern hemisphere biogeography inferred by event-based models: plants versus animal patterns. Systematic Biology 53: 216-243

Sanmartin, I., Wanntorp, L., Winkworth, R. C. (2007) West wind drift revisited: testing fr directional dispersal in the Southern Hemisphere using event-based tree fitting. Journal of Biogeography 34: 398-416

Schenk, M. F., Thienpont, C.-N., Koopman, W. J. M., Gilissen, L. J. W. J., Smulders, M. J. M. (2008) Phylogenetic relationships in *Betula* (Betulaceae) based on AFLP markers. Tree Genetics & Genomes 4: 911-924

Seehausen, O. (2004) Hybridization and adaptive radiation. Trends Ecol. Evol. 19: 198-207

Shaw, J., Lickey, E. B., Beck, J. T., Farmer, S. B., Liu, W., Miller, J., Siripun, K. C., Winder, C. T., Schilling, E. E., Small, R. L. (2005) The tortoise and the hare II: relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analysis. American Journal of Botany 92: 142-166

Shaw, J., Lickey, E. B., Schilling, E. E., Small, R. L. (2007) Comparison of whole chloroplast genome sequences to choose noncoding regions for phylogenetic studies in angiosperms: the tortoise and the hare III. American Journal of Botany 94: 275-288

Shepherd, L. D., Perrie, L. R., Brownsey, P. (2008a) Low-copy nuclear DNA sequences reveal a predominance of allopolyploids in a New Zealand Asplenium fern complex. Molecolar Phylogenetics and Evolution 49: 20-248

Shepherd, L. D., Perrie, L. R., Brownsey, P. J. (2008b) Conflict amongst chloroplast DNA sequences obscures the phylogeny of a group of Asplenium ferns. Molecolar Phylogenetics and Evolution 48: 176-187

Simmons, M., Ochoterena, H. (2000) Gaps as characters in sequence-based phylogenetic analyses. Systematic Biology 49: 369-381

Šmarda, P. (2006) DNA ploidy levels and intraspecific DNA content variability in Romanian fescues (Festuca, Poaceae) measured in fresh and herbarium material. Folia Geobotanica 41: 417-432

Smissen, R. D., Breitwieser, I., Ward, J. M. (2004) Phylogenetic implications of trans-specific chloroplast DNA sequence polymorphism in New Zealand Gnaphalieae (Asteraceae). Plant Systematics and Evolution 249: 37-53

Smissen, R. D., Breitwieser, I., Ward, J. M. (2006) Genetic diversity in the New Zealand endemic species *Helichrysum lanceolatum* (Asteraceae: Gnaphalieae). New Zealand Journal of Botany 44: 237-247

Smith , B. W. (1958) Sex chromosomes and natural polyploidy in dioecious *Rumex*. Journal of Heredity 46: 226 – 232

Smith , B. W. (1969) Evolution of sex-determining mechanisms in *Rumex*. Chromosomes Today 2: 172 – 182

Soltis, D. E., Soltis, P. S., Pires, J. C., Kovarik, A., Tate, J. A., Mavrodiev, E. (2004) Recent and recurrent polyploidy in *Tragopogon* (Asteraceae): cytogenetic, genomic and genetic comparisons. Biological Journal of the Linnean Society 82: 485-501

Stix, E. (1960) Pollenmorphologische Untersuchungen an Compositen. Grana Palynol 2: 39-114

Stuessey, T. F. (2009) Plant Taxonomy. Columbia University Press, New York

Suda, J., Trávnícek, P. (2006) Reliable DNA ploidy determination in dehydrated tissues of vascular plants by DAPI flow cytometry-new prospects for plant research. Cytometry A 69A: 273 - 280

Swenson, U., Backlund, S., McLoughlin, S., Hill, R. S. (2001) *Nothofagus* biogeography revisited with spatial emphasis on the enigmatic distribution of subgenus Brassosperma in New Caledonia. Cladistics 17: 2-47

Swofford, D. L. (2002) PAUP* Phylogenetic Analysis Using Parsimony (*and other Methods) 4.0b10, Sinauer Associates,

Syring J, Farrell K, Businsky R, Cronn R, A, L. (2007) Widespread genealogical nonmonophyly in species of *Pinus* subgenus *Strobus*. Systematic Biology 56: 163-181

Taberlet, P., Gielly, L., Pautou, G., Bouvet, J. (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Plant Molecular Biology 17: 1105-1109

Thompson, I. R. (2007) A taxonomic treatment of the tribe Anthemideae (Asteraceae) in Australia. Muelleria 25: 21-58

Thomson, J. D., Brunet, J. (1990) Hypotheses for the evolution of dioecy in seed plants. Trends in Ecology & Evolution 5: 11-16

Tkach, N. V., Hoffmann, M. H., Röser, M., Korobkov, A. A., von Hagen, B. K. (2007) Parallel evolutionary patterns in multiple lineages of arctic *Artemisia* L. (Asteraceae). Evolution 62: 184–198

Torices, R., Anderberg, A. A. (2009) Phylogenetic analysis of sexual systems in Inuleae (Asteraceae). American Journal of Botany 96: 1011-1019

Torrell, M., Valles, J. (2001) Genome size in 21 *Artemisia* L. species (Asteraceae, Anthemideae): systematic, evolutionary, and ecological implications. Genome 44: 231-238

Trewick, S. A., Morgan-Richards, M. (2005) After the deluge: mitochondrial DNA indicates Miocene radiation and Pliocene adaptation of tree and giant weta (*Orthoptera*: Anostostomatidae). Journal of Biogeography 32: 295-309

Trewick, S. A., Paterson, A. M., Campbell, H. J. (2007) Hello New Zealand. Journal of Biogeography 34: 1-6

Turner, B. L. (1970) Chromosome numbers in the Compositae. XII. Australian species. American Journal of Botany 57: 382-389

Turner, P. A. M., Scott, J. J., Rozefelds, A. C. (2006) Probable long distance dispersal of *Leptinella plumosa* Hook.f. to Heard Island: habit, status and discussion of its arrival. Polar Biology 29: 160-168

Unwin, M., Bayer, R. J., Ward, J. M., Breitwieser, I., Koekemoer, M., Galbany, M. (2006) Phylogenetic relationships in the Gnaphalieae (Asteraceae). The International Compositae Alliance Barcelona, 24

Vallès, J., Torrell, M., Garnatje, T., Garcia-Jacas, N., Vilatersana, R., Susanna, A. (2003) The genus *Artemisia* and its allies: phylogeny of the subtribe Artemisiinae (Asteraceae, Anthemideae) based on nucleotide sequences of nuclear ribosomal DNA Internal transcribed spacers (ITS). Plant Biology 5: 274-284

van Royen, P., Lloyd, D. (1975) The genus *Cotula* (Asteraceae) in New Guinea. Sertulum Papuanum 21. Blumea 22: 197-206

Vogt, R., Oberprieler, C. (2006) The genus *Plagius* (Compositae, Anthemideae). Willdenowia 36: 47-67

Volz, S. M., Renner, S. S. (2008) Hybridization, polyploidy, and evolutionary transitions between monoecy and dioecy in *Bryonia* (Cucurbitaceae). American Journal of Botany 95: 1297-1306

von Hagen, K. B., Kadereit, J. W. (2001) The phylogeny of *Gentianella* (Gentianaceae) and its colonization of the southern hemisphere as revealed by nuclear and chloroplast DNA sequence variation. Organisms Diversity & Evolution 1: 61-79

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M. (1995) AFLP: A new technique for DNA fingerprinting. Nucleic Acids Research 23: 4407-4414

Wagstaff, S. J. (2004) Evolution and biogeography of the austral genus *Phyllocladus* (Podocarpaceae). Journal of Biogeography 31: 1569-1577

Wagstaff, S. J., Breitwieser, I. (2002) Phylogenetic relationships of New Zealand Asteraceae inferred from ITS sequences. Plant Systematics and Evolution 231: 203-224

Wagstaff, S. J., Breitwieser, I. (2004) Phylogeny and classification of *Brachyglottis* (Senecioneae, Asteraceae): An example of a rapid species radiation in New Zealand. Systematic Botany 29: 1003-1010

Wagstaff, S. J., Breitwieser, I., Swenson, U. (2006) Origin and relationships of the austral genus *Abrotanella* (Asteraceae) inferred from DNA sequences. Taxon 55: 95-106

Wagstaff, S. J., Garnock-Jones, P. J. (1998) Evolution and biogeography of the *Hebe* complex (Scrophulariaceae) inferred from ITS sequences. New Zealand Journal of Botany 36: 425-437

Wagstaff, S. J., Wege, J. (2002) Patterns of diversification in New Zealand Stylidiaceae. American Journal of Botany 89: 865-874

Watson, L., Bates, P., Evans, T., Unwin, M., Estes, J. (2002) Molecular phylogeny of Subtribe Artemisiinae (Asteraceae), including *Artemisia* and its allied and segregate genera. BMC Evolutionary Biology 2: 17

Watson, L. E., Evans, T. M., Boluarte, T. (2000) Molecular phylogeny and biogeography of tribe Anthemideae (Asteraceae), based on chloroplast gene *ndh*F. Molecular Phylogenetics and Evolution 15: 59-69

Webb, C. J. (1979) Breeding systems and evolution of dioecy in New Zealand apioid Umbelliferae. Evolution 33: 662-672

Webb, C. J. (1992) Sex ratio from seed in six families of *Scandia geniculata*. New Zealand Journal of Botany 30: 401-404

Webb, C. J. (1999) Empirical studies: evolution and maintenance of dimorphic breeding systems. In: Geber, M. A., Dawson, T. E., Delph, L. F. (eds.) Gender and sexual dimorphism in flowering plants. Springer, Berlin, 61-95

Webb, C. J., Kelly, D. (1993) The reproductive biology of the New Zealand flora. Trends in Ecology & Evolution 8: 442-447

Webb, C. J., Lloyd, D., Delph, L. F. (1999) Gender dimorphism in indigenous New Zealand seed plants. New Zealand Journal of Botany 37: 119-130

Webb, C. J., Simpson, M. J. A. (2001) Seeds of New Zealand gymnosperms and dicotyledons. Manuka Press and Caxton Press, Christchurch

Webb, C. J., Sykes, W. R., Garnock-Jones, P. J. (1988) Flora of New Zealand 4, Naturalised Pteridophytes, Gymnosperms, Dicotyledons. Botany Division, Department of Scientific and Industrial Research, Wellington

Weiss-Schneeweiss, H., Tremetsberger, K. (2008) Karyotype diversication and evolution in diploid and polyploid South American *Hypochaeris* (Asteraceae) inferred from rDNA localization and genetic fingerprint data. Annals of Botany 101: 909-918

Westergaard, M. (1958) The mechanism of sex determination in dioecious flowering plants. Advances in Genetics 9: 217-281

White, T. J., Bruns, T., Lee, S., Taylor, J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M., Gelfund, D., Sninsky, J., White, T. (eds.) PCR Protocols: A Guide to Methodes and Applications. Academic Press, San Diego, 315-322

Wilson, H. D. (1994) Field guide: Stewart Island plants. Manuka Press, Christchurch

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

Winkworth, R. C., Wagstaff, S. J., Glenny, D., Lockhart, P. J. (2002a) Plant dispersal N.E.W.S. from New Zealand. Trends in Ecology & Evolution 17: 514-520

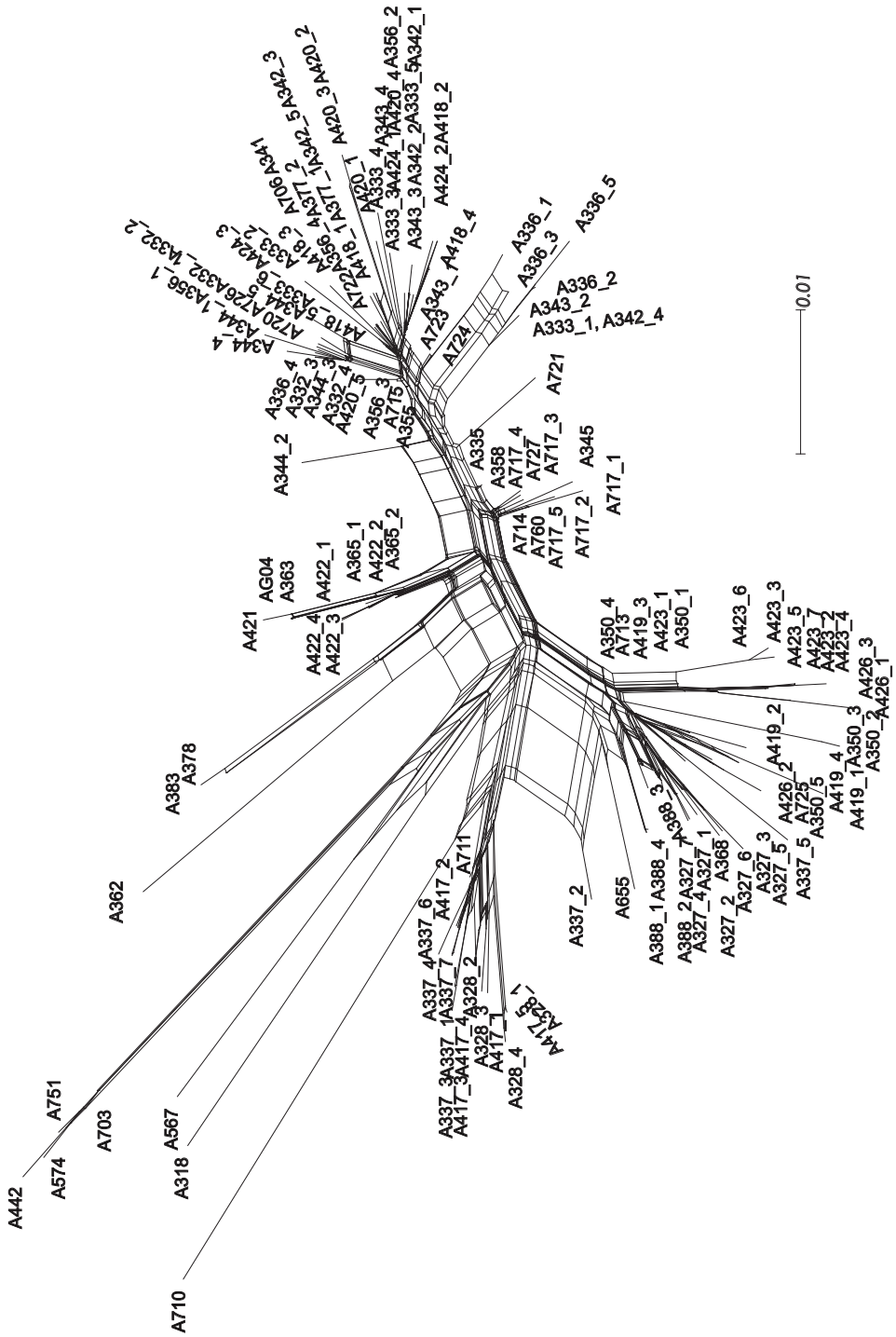
Winkworth, R. C., Wagstaff, S. J., Glenny, D., Lockhart, P. J. (2005) Evolution of the New Zealand mountain flora: Origins, diversification and dispersal. Organisms Diversity & Evolution 5: 237-247

Yampolsky, C., Yampolsky, H. (1922) Distribution of sex forms in the phanerogamic flora. Bibliotheca Genetica 3: 1-62

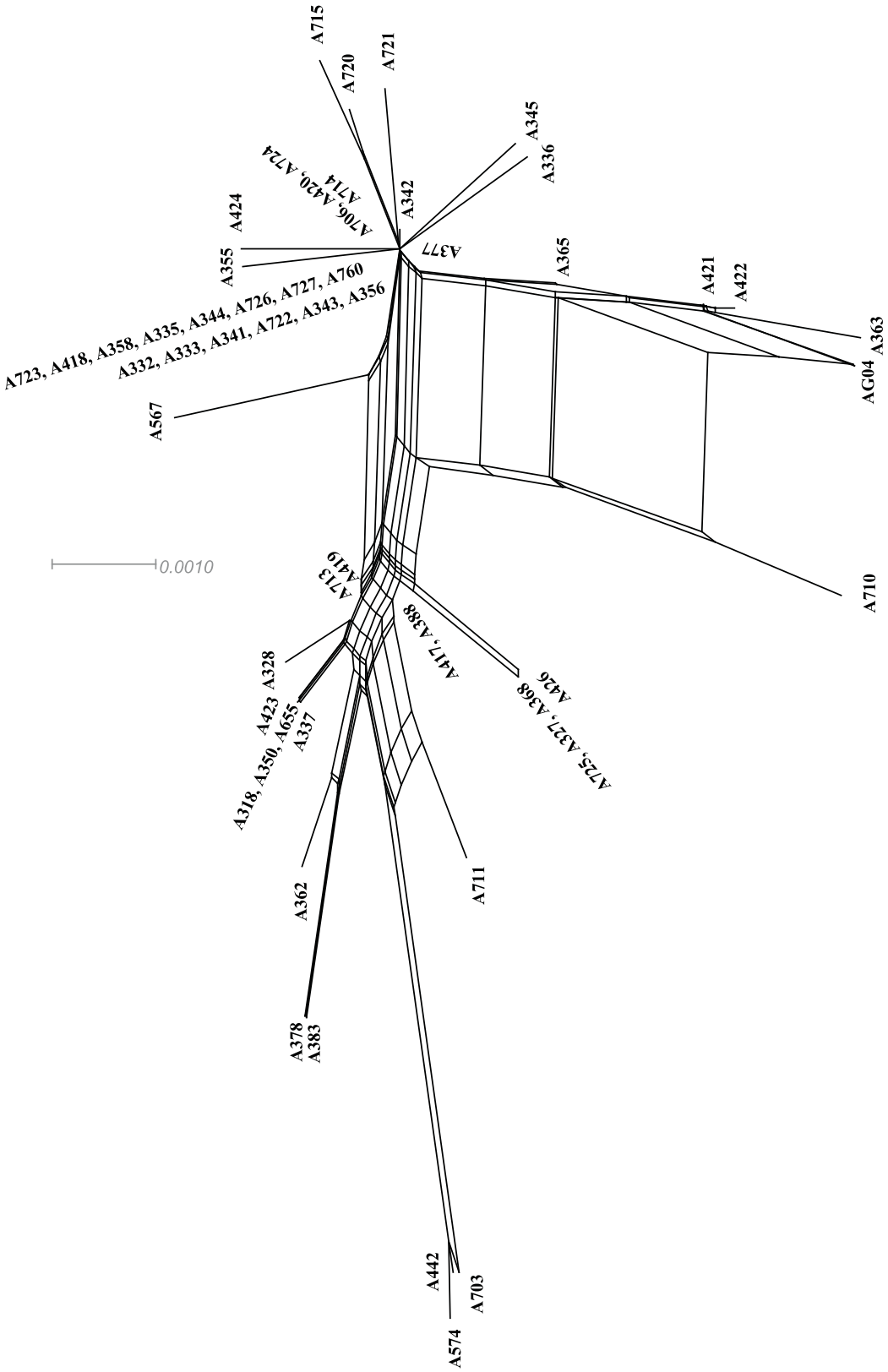
Yeung, K., Miller, J. S., Savage, A. E., Husband, B. C., Igic, B., Kohn, J. R. (2005) Association of ploidy and sexual system in *Lycium californicum* (Solanaceae). Evolution 59: 2048-2055

Young, N., Healy, J. (2003) GapCoder automates the use of indel characters in phylogenetic analysis. BMC Bioinformatics 4: 6

Zhang, L.-B., Simmons, M. P., Kocyan, A., Renner, S. S. (2006) Phylogeny of the Cucurbitales based on DNA sequences of nine loci from three genomes: Implications for morphological and sexual system evolution. Molecular Phylogenetics and Evolution 39: 305-322



Appendix 3-1: Phylogenetic network based on ITS data of the *Leptinella* main group with codes of the individuals (For numbers see appendix 3-3).



Appendix 3-2: Phylogenetic network based on cpDNA data of the *Leptinella* main group with codes of the individuals (For numbers see appendix 3-3).

Appendix 3-3: Individuals of the *Leptinella* main clade used in the phylogenetic networks.

Nr.	Taxon
A350	L. albida
A419	L. atrata subsp. atrata
A711	L. atrata subsp. luteola
A420	L. calcarea
A423	L. conjuncta
A388	L. dendyi A
A713	L. dendyi B
A377	L. dioica subsp. dioica A
A418	L. dioica subsp. dioica B
A424	L. dioica subsp. dioica C
A356	L. dioica subsp. monoica
A760	L. dispersa subsp. dispersa A
A714	L. dispersa subsp. dispersa B
A358	L. dispersa subsp. rupestris
A751	L. drummondii
A710	L. featherstonii
A421	L. filiformis
A318	L. goyenii
A717	L. intermedia
A362	L. lanata
A442	L. longipes A
A574	L. longipes B
A567	L. maniototo
A363	L. minor A
AG04	L. minor B
A365	L. nana A
A422	L. nana B
A655	L. pectinata subsp. pectinata
A327	L. pectinata subsp. villosa A
A426	L. pectinata subsp. villosa B
A725	L. cf. pectinata subsp. villosa
A368	L. pectinata subsp. willcoxii
A378	L. plumosa A
A383	L. plumosa B
A335	L. potentillina
A336	L. pusilla
A333	L. cf. pusilla/serrulata
A337	L. pyrethrifolia var. linearifolia
A328	L. pyrethrifolia var. pyrethrifolia A
A417	L. pyrethrifolia var. pyrethrifolia B
A703	L. reptans
A720	L. rotundata
A341	L. scariosa A
A706	L. scariosa B
A342	L. serrulata A
A721	L. serrulata B
A722	L. squalida subsp. mediana × L. dioica subsp. dioica
A332	L. squalida subsp. mediana A
A343	L. squalida subsp. mediana B
A344	L. squalida subsp. squalida A
A726	L. squalida subsp. squalida B
A345	L. tenella A
A727	L. tenella B
A715	L. traillii
A724	L. traillii subsp. pulchella
A723	L. traillii subsp. traillii
A355	L. "Seal"