Systematics, Biology and Ecology of New Zealand's Pygmy Mistletoes (*Korthalsella:* Viscaceae)

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Abstract

New Zealand's pygmy mistletoes belong to the genus *Korthalsella* Tieghem, which comprises about 30 species ranging from Malesia to Hawaii, the Marquesas and Henderson Islands in the east, Japan in the north, Australia, New Zealand in the south, and Ethiopia and Madagascar to the west. Mainland Australia, Hawaii, Malesia and Madagascar all have high levels of species richness. This thesis shows that *Korthalsella* has high levels of regional endemism and has widespread parallelism and supports the biogeographic model of speciation, whereas, the traditional sections based on morphology are not supported.

Korthalsella is represented in New Zealand by a monophyletic clade of three species *K. clavata* (Kirk) Cheeseman, *K. lindsayi* (Oliver ex J. D. Hooker) Engl., and *K. salicornioides* (A. Cunningham) Tiegh. *Korthalsella clavata* and *K. lindsayi* are both generalists with relatively broad host ranges whereas *K. salicornioides* is a specialist species with most host records from two myrtaceous genera *Kunzea* Rchb. (kanuka) and *Leptospermum* J. R. Forst & G. Forst (manuka). Cross-infection experiments in *Korthalsella salicornioides* indicate the presence of putative *Kunzea*- and *Leptospermum*-specific races with better success rates of seedling survival when maternal and recipient hosts were the same. However, genetic data based on nuclear internal transcribed spacer (ITS) and chloroplast *trnQ-rps16* spacer sequences does not support the presence of host related variability and the within-species genetic structure appears to be geographic rather than host-based. My study shows that both cpDNA haplotypes and ITS sequence types are shared between *K. clavata* and *K. lindsayi,* which possiby is an outcome of hybridisation and introgression between the two species. *Korthalsella salicornioides* is an ambophilous species relying both on insects and on wind for effective pollination, whereas *K. lindsayi* is an anemophilous species. This study confirms the presence of explosive seed discharge in both species with dispersal distances ranging up to 4 and 7 m in *K. lindsayi* and *K. salicornioides,* respectively. However, dispersal distances of around 1 m are more typical. This study also describes the first account of clonal propagation by means of proliferation of endophyte and formation of adventitious sprouts in these species.

Several new natural enemies specialising on New Zealand *Korthalsella* were discovered - two specialist armoured scales (*Leucaspis albotecta* Henderson and *L. trilobata* Henderson), a felt scale (*Eriococcus korthalsellae* Henderson), and two pathogenic ascomycetes *Guignardia korthalsellae* Sultan, Johnston, Park & Robertson and *Rosenscheldiella korthalsellae* Sultan, Johnston, Park & Robertson. Other natural enemies include a specialist gall mite *Aceria korelli* Manson, whereas generalists include native and adventive scale insects and generalist Lepidoptera.

Demographic study of several populations revealed that all species have high growth rates and high ramet turnover. *Korthalsella salicornioides* and *K. clavata* had relatively stable recruitment rates in all size/stage classes whereas *K. lindsayi* populations were apparently declining at the two study sites.

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Contents

Chapter 1: Introduction

The parasitic habit has evolved independently in twelve different angiosperm lineages (Nickrent 2012). Parasitic plants constitute about 1% of the flowering plants (Heide-Jorgensen 2008) and belong to 276 genera in 30 families (Nickrent 2012). Holoparasites lack chlorophyll and are entirely dependent on their host plant, whereas, hemiparasites are chlorophyllous and photosynthetic yet draw nutrients and water from the host. Mistletoes are obligate stem-hemiparasites and are represented by c. 1600 species in 88 genera (Nickrent 2011) belonging to families Misodendraceae, Santalaceae, Amphorogynaceae, Loranthaceae and Viscaceae in the order Santalales (Nickrent 2010). The mistletoe habit first evolved about 80 million years ago (Mya) in the South American genus *Misodendrum* and subsequently in Viscaceae (72 Mya), Santalaceae ("Eremolepidaceae") (53 Mya), Amphorogynaceae (46 Mya), and Loranthaceae (28 Mya) (Vidal-Russell and Nickrent 2008). Among the mistletoe families, Loranthaceae and Viscaceae are the most speciose with c. 973 and 573 species, respectively (Nickrent 2012). The family Viscaceae comprises seven genera: *Arceuthobium, Dendrophthora, Ginalloa*, *Korthalsella, Notothixos, Phoradendron* and *Viscum*.

The Viscaceae had long been treated as sub-family Viscoideae within the Loranthaceae until it was formally resurrected as a family in its own right by Barlow (1964). Subsequently Viscaceae was placed in synonymy under Santalaceae by the APGIII (2003) but Mathiasen *et al.* (2008) suggest that sinking the well-characterised Viscaceae into a more heterogeneous Santalaceae is undesirable. More recently, Nickrent *et al.* (2010) in a revised classification of Santalales recognised the family status of Viscaceae. Barlow's (1964) classification included Eremolepidaceous genera in Viscaceae, however, these were included in the Santalaceae in Nickrent *et al.* (2010).

New Zealand's pygmy mistletoes belong to the genus *Korthalsella*. Aiken (1957) used the name pygmy mistletoes to refer to New Zealand's *Korthalsella* species. Pygmy mistletoes are leafless, aerial hemiparasites, having terete or flattened internodes with flowers borne on the tip of internodes in the axils of rudimentary leaves or on specialized inflorescence branches. *Korthalsella* has an unusual, scattered and discontinuous distribution and the genus is thought to have originated in Malesia (Molvray *et al.* 1999) (Malesian region comprises Malaysia, Brunei Darussalam, Philippines, Singapore, Indonesia and New Guinea). The distribution extends from Malesia to Hawaii, the Marquesas and Henderson Islands in the east, Japan in the north, Australia, New Zealand in the south, and Ethiopia and Madagascar to the west (Burrows 1996, Molvray 1999).

New Zealand is home to three pygmy mistletoe species; *K. salicornioides*, *K. clavata* and *K. lindsayi*, though there is some debate about the status of the latter two as separate species. *Korthalsella clavata* was regarded as a distinct species by Danser (1940), in a supplement to monograph of the genus, while Allan (1961) treated K. *clavata* as *K. lindsayi* var. *clavata*. Molvray (1997) and Molvray *et al.* (1999) also circumscribed *K. clavata* as a variety of *K. lindsayi.* Moreover, the circumscription of *Korthalsella amentacea* of New Caledonia in *K. lindsayi,* of Malagasy *K. madagascarica* and New Caledonian *K. striata* in *K. salicornioides* by Molvray (1997) raises questions regarding endemism of the New Zealand taxa and the extremely discontinuous distribution of *K. salicornioides* (New Zealand - New Caledonia – Madagascar). Barlow (1996) also reported *K. salicornioides* from New Caledonia whereby he placed *K. striata* in synonymy with *K. salicornioides*.

Korthalsella populations of all three New Zealand species are declining because of habitat transformation caused by fire and vegetation clearance (Department of Conservation 2001). *Korthalsella salicornioides* is classified as “naturally uncommon-sparse” in the threatened and uncommon plants list for New Zealand (de Lange *et al.* 2009). *Korthalsella salicornioides* is subject to extreme population fluctuations (unnatural/natural fluctuations overlaying human induced declines) and is listed in threatened plants of Northland conservancy (Department of Conservation 2004). Vegetation succession caused by domination of broadleaved forest, and thus the replacement of *Kunzea* hosts by overtopping, may possibly lead to local extinction of *K. salicornioides* on Kapiti Island (Department of Conservation 2001). Death of hosts caused by natural senescence coupled with lack of natural regeneration is leading to decline in overall mistletoe populations in Wairarapa Conservancy, while cutting of *Kunzea* and *Leptospermum* scrub for firewood is a threat to already declining *K. salicornioides* populations (Department of Conservation 2001). *Korthalsella clavata* is quite rare in the entire North Island (Rebergen and Sawyer 2005) and is regarded as a regionally threatened plant in Wellington Conservancy (Department of Conservation 2001). Host range studies and distribution surveys are important towards devising conservation management strategies, while demographic monitoring gives insight into recruitment and mortality rates, as well as age-group structure of mistletoe populations and is an important tool in determining the efficacy of management strategies for threatened populations.

SYSTEMATICS OF KORTHALSELLA AND OTHER GENERA IN VISCACEAE

In a molecular phylogenetic study by Nickrent and Soltis (1995) based on 18S nrDNA and plastid *rbcL* data, *Ginalloa* and *Korthalsella* formed a well supported sister group, while *Phoradendron* and *Dendrophthora* also formed a well supported relationship within the family Viscaceae. *Viscum* and *Arceuthobium* formed a clade in this study (Nickrent and Soltis 1995), while *Notothixos* was sister to this clade comprising *Viscum* and *Arceuthobium,* however these relationships were not well supported. A later molecular phylogenetic study (Nickrent and Duff 1996), including New Zealand species *K. lindsayi* and Hawaiian *K. complanata,* showed that the genus was monophyletic. In a more recent study by Mathiasen *et al*. (2008), based on nuclear 5.8S and small-subunit rDNA and the chloroplast gene *rbcL*, the viscaceous genera were resolved into *Phoradendron-Dendrophthora*, *Korthalsella-Ginalloa*, *Viscum-Notothixos* and *Arceuthobium* clades. Based on molecular evidence tribal classification proposed by Barlow (1964) (Table 1) can be revised as follows:

Phoradendreae: *Arceuthobium, Phoradendron, Dendrophthora*
Visceae: *Viscum, Notothixos*
Korthalselleae: *Ginalloa, Korthalsella*

The only species level molecular phylogenetic study of the genus *Korthalsella* is that of Molvray *et al.* (1999) based on the ITS region and the plastid encoded *trnL-F*. Molvray *et al.,* (1999) rooted the tree with *K. papuana,* with differentiated inflorescence branches and eight or more vascular bundles. A clade comprising the New Zealand taxa (*K. salicornioides*, *K. lindsayi* and *K. lindsayi* var. *clavata*) with four or fewer vascular bundles was resolved. Molvray *et al.* (1999) viewed all New Zealand taxa as possessing differentiated inflorescence branches. The remaining species examined with undifferentiated inflorescence branches and four or more vascular bundles formed a clade. This study suggested that the morphological characters used up to that time probably were unreliable indicators of phylogenetic relationships and that the previous classification based on morphology (Danser 1937, 1940) was not supported.

Table 1. Viscaceae: Tribal classification (after Barlow 1964)

	Tribe Phoradendreae	Tribe Arceuthobieae	Tribe Visceae
Subtribes and Genera	Ginalloinae: *Ginalloa*, Korthalsellinae: *Korthalsella*, Phoradendrinae: *Phoradendron*, *Dendrophthora*	*Arceuthobium*	*Viscum, Notothixos*

Other species level molecular phylogenetic investigations in viscaceous genera include a study involving all *Arceuthobium* species, based on nuclear ITS and chloroplast *trnL* region (Nickrent *et al*. 2004), while *Phoradendron* and related *Dendrophthora* were investigated (Ashworth 2000 a, b) using nuclear ITS and 26S rDNA sequences.

ECONOMIC IMPORTANCE

Viscaceous mistletoes as forest and plantation pests

Dwarf mistletoes *(Arceuthobium* spp). are damaging pathogens of coniferous forest trees (Hawksworth and Wiens 1996) often predisposing the trees to insect attack and fungal pathogens. Dwarf mistletoes may have a substantial influence on forest structure as infection is correlated with stunted growth, reduced seed production and mortality of their host trees (Bennetts *et al*. 1996).

Viscum album infests a wide range of cultivated and forest trees. *Viscum album* subsp. *album* parasitises a wide range of deciduous and evergreen trees, whereas *V. album* subsp. *austriacum* and *V. album* subsp. *abietis* parasitise pines and firs, respectively (Zuber 2004). *Viscum cruciatum* parasitises olives in the Mediterranean (Aparicio 1995) and wild olives and poplar in temperate Asia (Khan 1980).

Some *Korthalsella* species have been recorded as pests of a number of introduced and economically important trees (Table 2). *Korthalsella disticha* (= *K. articulatum*) infection was correlated with mortality in lemons, oranges and peaches (Laing 1914). *Korthalsella complanata* infection on *Acacia koa* is correlated with reduced wood strength (Nelson and Friday 2011). *Korthalsella japonica* has an overall wide host range, but Himalayan populations of *K. japonica* predominantly occur on *Quercus* spp., which are a valuable source of fodder during winter months (Khan 1980, Zakaullah 1988). *Phoradendron* spp. are damaging to coffee, avocado, teak, citrus and various forest trees in North America, Central America and the Caribbean (Knutson 1983).

Table 2. *Korthalsella* species as pests of introduced and economically important plants

Korthalsella spp.	Region	Hosts	References
K. breviarticulata	Australia	*Citrus aurantium**	Downey 1998
K. disticha	Norfolk Island	*Citrus* spp*. (lemons, oranges), *Prunus persica** *Nestegis ligustrina**	Laing 1914 Downey 1998
K. japonica	India Taiwan Himalaya	Apricot*, Tea-oil camillea, *Quercus* spp.	Gupta 1974; Sun 2006; Khan 1980, Zakaullah 1988
K. rubra	Australia	*Prunus persica**	Downey 1998
K. complanata	Hawaii	*Acacia koa*	Wagner *et al.* 1999
K. complanata	Hawaii	*Ficus**	Wagner *et al.* 1999
K. complanata	Hawaii	*Solanum sodomeum**	Wagner *et al.* 1999, Herbst 1980
K. salicornioides	New Zealand	*Erica lusitanica**, *Erica vagans**	Bannister 1989
K. platycaula	Fiji	*Inocarpus fagifera*	Smith 1985

* instances where *Korthalsella* species were recorded on non-native plants

Viscaceous mistletoes as medicinal plants

Viscum album has a long history of usage in the folklore medicine for therapeutic properties in a variety of ailments (Büssing 2000). Somewhat similar properties have been associated with *Phoradendron* species in native American ethnobotany (Moerman 1998), including usage of dried berries as starvation food. More recently, *Viscum album* has yielded promising compounds for the treatment of cancer (Kirsch 2007, Kienle and Kiele 2010). Similarly the possibility of utilising extracts and compounds isolated from *Korthalsella japonica* (Park 2000) and *Phoradendron* spp. (Endo *et al.* 1989, Varela *et al.* 2004) has also been explored.

Viscaceous mistletoes as keystone resources for wildlife

Many mistletoe species are important nutritional resources for frugivorous birds and larval forms of insects. *Viscum, Notothixos* and *Korthalsella* are larval food plants for *Delias* butterflies (Braby 2006). At least twelve insect species including the purple hair streak (*Atlides halesus*) exclusively depend on *Phoradendron tomentosum* for larval food (Whittaker 1984). Witches' brooms caused by dwarf mistletoes and mistletoe clumps provide nesting sites for birds and refuge in inclement weather (Watson 2004).

LITERATURE ON NEW ZEALAND KORTHALSELLA

Stevenson's (1934) work on New Zealand *Korthalsella* is the most detailed account on the biology of these plants. Her study focussed mostly on morphological and anatomical examination of vegetative and reproductive features and anatomical aspects of host-parasite interface. Her study lacks aspects relating to overall host range, dependent fauna/natural enemies of these mistletoes and had limited discussion on pollination and dispersal biology. Most of the more recent work on *Korthalsella* in New Zealand has focused on various anatomical aspects of the host-parasite interface. Coetzee and Fineran (1989) found that phloem was generally absent in the host/parasite interface of *K. lindsayi* and there were few xylem contacts, thus transport of host materials is implicated apoplastically possibly through plasmatubules. This apoplastic continuum is provided by walls of the haustorial parenchyma cells, which have convoluted appearance of the plasmalemma often differentiated into plasmatubules (Coetzee and Fineran 1987). Fineran (1995) reported the presence of chloroplasts in the haustorium of New Zealand *Korthalsella*. He assumed that these chloroplasts perform a low level of photosynthesis and thus might provide a local source of osmotically active material assisting transport between host and parasite.

AIMS OF THE THESIS

Several key taxa from Malesia, Australia, New Caledonia, Norfolk, Lord Howe, South Pacific and Indian Ocean Basin were missing from the only phylogenetic study on *Korthalsella* (Molvray *et al.* 1999). In the current study, an attempt has been made to include those missing taxa to infer the phylogenetic affinities of representatives from those regions. Knowledge on host range, host specificity and local host preferences for New Zealand's pygmy mistletoes was also lacking. This study has attempted to address these important aspects of the biology of these parasites. Similarly, there have not been any in-depth studies on the reproductive biology, demographic population structure and biodiversity associated with New Zealand's pygmy mistletoes. An attempt has been made to investigate these poorly-known aspects of the biology of New Zealand's pygmy mistletoes.

This study focuses on the hitherto unexplored aspects of the systematics, biology and ecology of New Zealand's pygmy mistletoes and comprises the following sections:

- Molecular phylogeny of *Korthalsella*
- Host range, host specificity, regional host preferences and distribution segregation in New Zealand's pygmy mistletoes
- Experimental evidence for the presence of potential host races in *Korthalsella salicornioides*
- Reproductive biology of *Korthalsella salicornioides* and *K. lindsayi*
- Insect/mite fauna and fungal pathogens of New Zealand's pygmy mistletoes
- Demographic population structure of New Zealand's pygmy mistletoes

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Chapter 2: Molecular phylogeny of *Korthalsella* (Viscaceae) based on nuclear Internal Transcribed Spacer (ITS) and chloroplast *trn*L-*trn*F regions

INTRODUCTION

Korthalsella Tieghem (Viscaceae) is a genus of about 30 species (Danser 1937, 1940, Barlow 1983a) of scale-leaved mistletoes with cylindrical or flattened stems and diminutive unisexual flowers, distributed in Africa, Madagascar, Australasia and islands of the Pacific (Molvray *et al.* 1999, Burrows 1996, Fig. 1). These mistletoes differ from leafless *Viscum* L. species by the absence of inflorescence bracts and from dwarf mistletoes (*Arceuthobium* M. Bieb.) in parasitising predominantly angiosperm hosts and being monoecious, compared to parasitising gymnosperm hosts and being dioecious in *Arceuthobium*. Mainland Australia, Hawaiian Islands, Malesia and Madagascar have the highest levels of species richness in *Korthalsella* with seven (Barlow 1983a, Cranfield 2002), six (Wagner *et al.* 1999), five (Barlow 1997) and at least four (Callmander *et al.* 2010) species, respectively.

Flowers in this genus are trimerous, comprising three petals that completely enclose the carpel in the female flowers except the sessile umbonate stigma. Anthers are bi-sporangiate and the three connate anthers form a disc-like structure called a synandrium, which is not found in other angiosperms (Molvray *et al.* 1999). Pollen exudes from a central pore in the synandrium in small nectar droplets. Female flowers in the smaller cylindrical species are only c. 0.3-0.6 mm across in size and male flowers are c. 0.6-0.9 mm wide.

Parallelism refers to the independent evolution of the same derived trait through the same developmental changes, whereas convergence refers to superficially similar traits that have a distinct developmental basis (Yoon 2004). Whipcord (scale-leaved) hebes (*Veronica* L., Plantaginaceae) and *Cassiope fastigiata* (Wall) D. Don (Ericaceae) represent an example of convergence as both have opposite, decussate scale-like leaves. Cushion-forming plants *Thylacospermum caespitosum* Fenzl., *Scleranthus* L. species (Caryophyllaceae), *Androsace* L. species (Primulaceae), *Saxifraga* L. species (Saxifragaceae), *Raoulia* Hook. f. ex Raoul species (Asteraceae) and *Haastia* Hook. f. species (Asteraceae) also have similar growth forms. A study in the alpine genus *Androsace* (Roquet *et al.* 2013) has demonstrated that cushions, which appeared independently in Asia and Europe, enhanced species diversification in alpine regions.

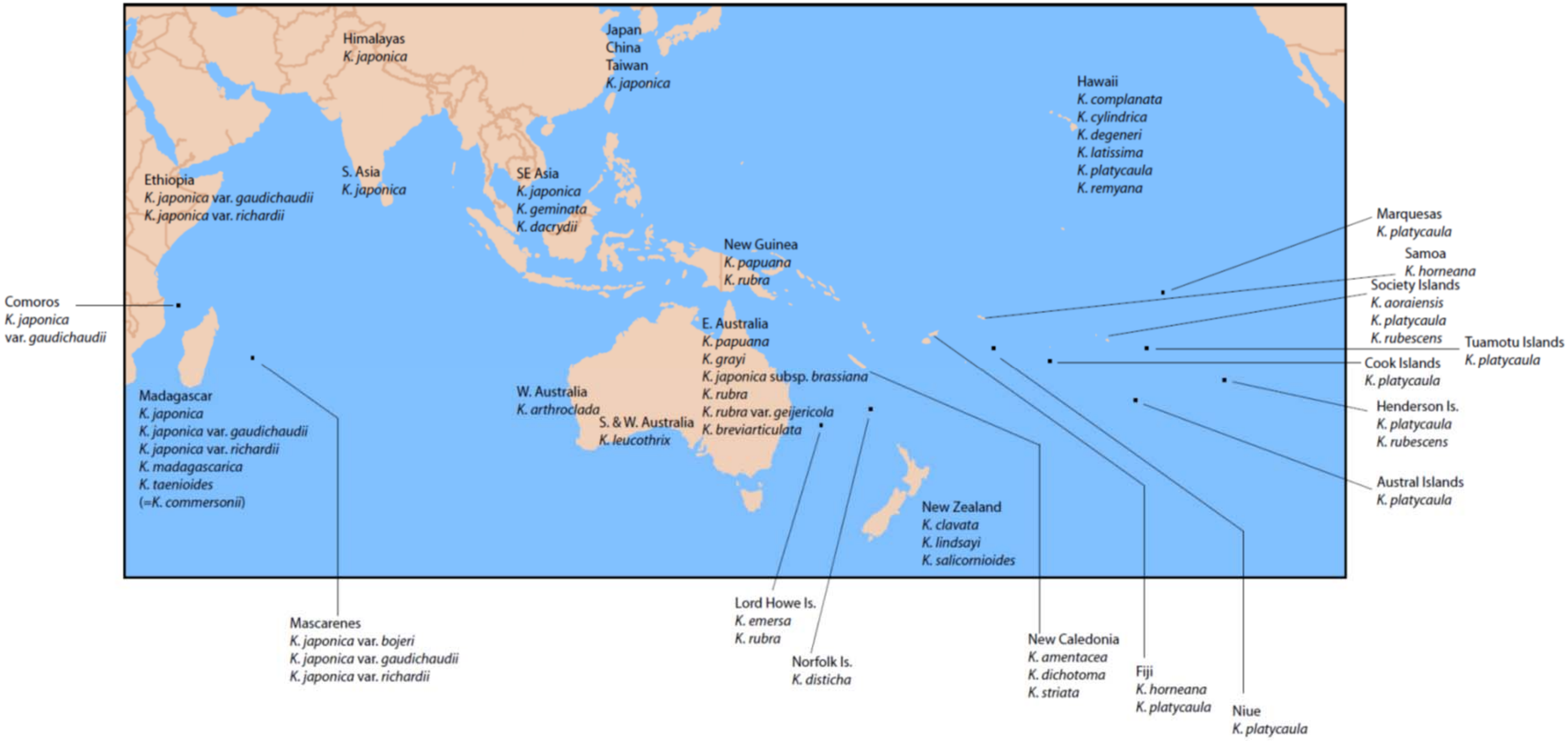

Fig. 1 Global distribution of *Korthalsella* species (taxon names correspond to those in Danser (1937, 1940) and Barlow (1983a)).

Their study (Roquet *et al.* 2013) shows that repeated emergence of the cushion life form in *Androsace* was a convergent key innovation that fostered radiation into alpine habitats. Amongst the parasitic plants *Cuscuta* L. (Convolvulaceae) and *Cassytha* Mill. (Lauraceae) represent a striking example of morphological convergence (Nickrent 2002), as both are characterised by wiry stems and stem parasitism. A study in the parasitic genus *Pedicularis* (Scrophulariaceae) showed that almost all main clades recovered in the ITS tree were not consistent with the sub-generic classification, reflecting extensive parallelism in floral morphology of the genus (Yang 2003). Similar morphologies within *Korthalsella* are known from different geographical regions, e.g., *K. latissima* (from Hawaii) and *K. breviarticulata* (from Australia) (Danser 1937), which both have stems modified into phyllocladia. The presence of similar morphologies in *Korthalsella* from different geographical regions represents an example of parallelism likely resulting from the limited architecture given the leafless nature and simple stem morphology. Studies in the *Viscum album* L. complex show that forms with very similar morphologies can only be differentiated genetically, but these apparently specialise on different host lineages (Zuber and Widmer 2000, Böhling *et al.* 2002, Mejnartowicz 2006).

Species delimitation within *Korthalsella* has been very challenging given the diminutive floral features (Fig. 2), simple morphology and the occurrence of similar morphological forms in different regions/island groups. Because of the highly reduced flowers, a greater reliance on vegetative characters has been used in species classification. However, the presence or absence of specialised inflorescence branches (spike-like inflorescences), the number of flowers in each floral cluster, the colour of trichomes in the floral clusters (Fig. 3) and the emergence of floral cushions from axils have been used in various classification schemes (Danser 1937, Barlow 1983a). Vegetative features used to delimit taxa include decussate vs. distichous cladotaxy, flattened vs. cylindrical stems, integration of internodes into phyllocladia, the plane of flattening of branches compared to the parent axis (transverse vs. flattening in the same plane), the number of prominent veins on the internodes, the presence of an acute or obtuse leaf apex, and the internode shapes and dimensions (Danser 1937, Barlow 1983a).

The taxa currently circumscribed in *Korthalsella* include Van Tieghem's (1896) genera *Korthalsella* Tieghem, *Heterixia* Tieghem and *Bifaria* Tieghem. *Heterixia* comprised species bearing flowers on distinct spicate inflorescences and having strongly flattened distichous stems (Fig. 4). *Korthalsella* and *Bifaria* included species lacking sharply distinct inflorescences and either decussate (*Korthalsella*, Fig. 5) or distichous cladotaxy (*Bifaria*, Figs. 6-8). Engler (1897) included *Heterixia* and *Bifaria* as sections within *Korthalsella*. In a monograph of the genus, Danser (1937, 1940) used the same characters to delimit sections *Heterixia* Engler, *Bifaria* Engler and *Eukorthalsella* Engler. However, he suggested separating New Zealand's *K. lindsayi* (Oliver ex Hooker) Engl. and *K. clavata* from *Heterixia* into an independent section, because both of these species possess superposed flower-bearing axils, as opposed to the decussate flower-bearing axils found in *K. geminata* and *K. papuana*. He also used a biogeographic species concept, delimiting species on the basis of geographic distribution, as well as morphology (see key to species in Danser 1937). For example, *K. disticha* (Endl.) Engl. and *K. dichotoma* (Tiegh.) Engl. are considered to be conspecific by Barlow (1996) but were considered to be distinct species in Danser (1937), endemic to Norfolk Island and New Caledonia, respectively. Danser (1940) recognised twenty-three species in the genus (Table 1). Barlow (1983a) added two new species and one new subspecies from mainland Australia and one new species from Lord Howe Island (Table 1). More recently, Cranfield (2002) described a new species from Western Australia, *Korthalsella arthroclada* Cranfield, which is characterised by cylindrical stems and acute leaf tips.

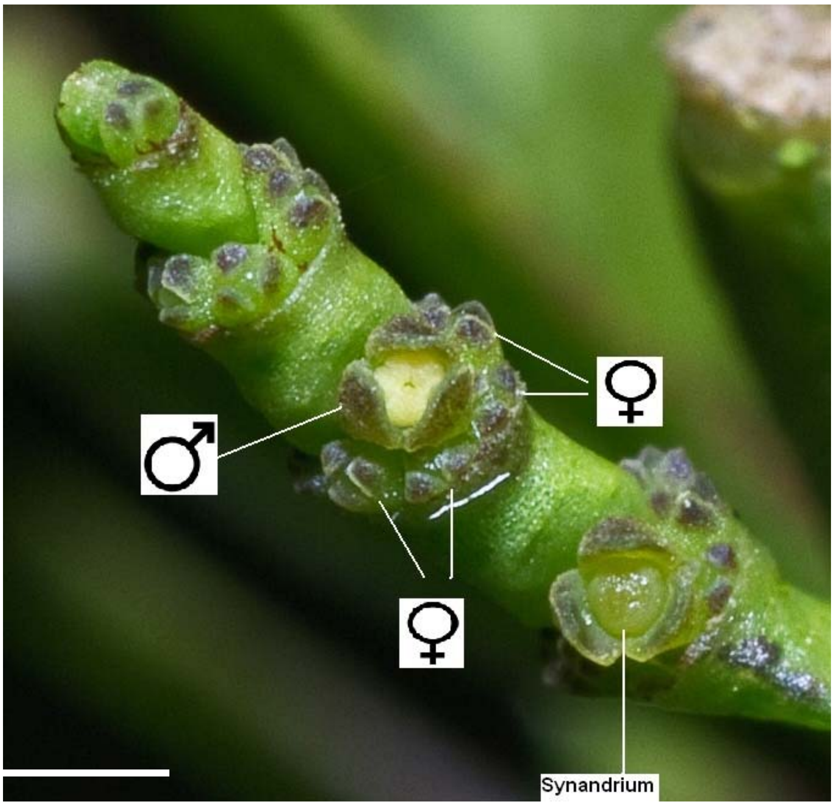

Fig. 2. Inflorescence of *Korthalsella clavata* (Kirk) Cheeseman, each floral cluster comprises one male and four female flowers, scale bar= c. 1mm.

Fig. 3. Floral clusters of *Korthalsella salicornioides* (Cunningham) Tiegh. interspersed with reddish brown trichomes (according to Barlow (1997) these structures are probably very densely fimbriate floral bracts), on each side of trichomes in the figure there are two female flowers, scale bar=c. 1mm.

Fig. 4. *Korthalsella geminata,* on *Garcinia* sp., Cardamon Mountains near Phnom Samkos in SW Cambodia, with distinct cylindrical inflorescences and strongly flattened internodes (photo by Philip Thomas (E)), scale bar=c. 1 cm.

Fig. 5. *Korthalsella salicornioides* from New Zealand, an example of decussate cladotaxy, scale bar=c. 5 mm.

Fig. 6. *Korthalsella cylindrica* (Tiegh.) Engl. from Hawaii, an example of distichous cladotaxy (photo by Jean-Yves Meyer, Department of Research, Government of French Polynesia), scale bar=c. 1 cm.

Fig. 7 *Korthalsella japonica* from Madagascar, corresponding to collection Callmander *& al.* 640, an example of distichous cladotaxy and flattened stems, (photo by Martin Callmander (MO)), scale bar= c. 5 mm.

Fig. 8. *Korthalsella taenioides* (=*K. commersonii*) from Madagascar corresponding to collection Antilahimena & al. 7526, (an example of distichous cladotaxy and modification of stems into phyllocladia), photo by Patrice Antilahimena scale bar= c. 1cm.

Molvray (1997) took quite a different view of the circumscription of *Korthalsella* and published a synopsis of the genus recognising only eight species (Tables 1, 2). Her classification was based on an anatomical study (Touw 1984), taking into account the number of main vascular bundles in the stem, and the results of a molecular study that was published later (Molvray *et al.* 1999). She placed several species from the Pacific archipelagos, Oceania, mainland Africa and the Indian Ocean basin as forms into a very broadly circumscribed and highly polymorphic *K. taenioides*. Most of these species were placed in synonymy under the main form *K. taenioides* (Comm. ex DC.) f. *taenioides* (Molvray 1997). She placed *K. madagascarica* Danser (Madagascar) and *K. striata* Danser (New Caledonia) in synonymy under *K. salicornioides* (New Zealand) and *K. amentacea* (Tiegh.) Engl., a poorly known species from Art Island, New Caledonia under *K. lindsayi* (New Zealand). *Korthalsella clavata* was also placed under *K. lindsayi* as a variety. Australian *K. rubra* subsp. *rubra* and *K. grayi* Barlow were reduced to forms of *K. japonica,* whereas, *K. leucothrix* Barlow, *K. japonica* (Thunb.) Engl. subsp. *brassiana* (Blakely) Barlow and *K.* "*opuntia*" var. *fasciculata* from China were placed under *K. japonica* f. *japonica*.

The biogeographic distribution of *Korthalsella* is interesting in that it has been suggested mainly to be the result of long distance dispersal (LDD) to southern land-masses and to remote Pacific and Indian Ocean islands from the core Malesian region.

Table 1. *Korthalsella* species recognised by Danser (1937, 1940) and Barlow (1983) compared to Molvray (1997).

Danser (1937, 1940) & Barlow (1983)	Molvray (1997)
Section *Heterixia* (specialised inflorescences, flattened stems)	
K. amentacea (Tiegh.) Engl.	*K. lindsayi* (Oliver ex J. D. Hooker) Engl. var. *lindsayi*
K. clavata (Kirk) Cheeseman	*K. lindsayi* (Oliver ex J. D. Hooker) Engl. var. *clavata* (Kirk) Danser*
K. geminata (Korthals) Engl.	*K. geminata* (Korthals) Engl.
K. lindsayi (Oliver ex J. D. Hooker) Engl.	*K. lindsayi* (Oliver ex J. D. Hooker) Engl. var. *lindsayi*
K. papuana Danser	*K. papuana* Danser
Section *Korthalsella* (decussate cladotaxy)	
K. dacrydii (Ridley) Danser	*K. dacrydii* (Ridley) Danser
K. horneana Tiegh.	*K. taenioides* (Comm. ex DC.) Engl. f. *horneana* (Tiegh.) Molvray
K. madagascarica Danser	*K. salicornioides* (A. Cunningham) Tiegh.
K. salicornioides (A. Cunningham) Tiegh.	*K. salicornioides* (A. Cunningham) Tiegh.
K. striata Danser	*K. salicornioides* (A. Cunningham) Tiegh.
K. remyana Tiegh.	*K. taenioides* (Comm. ex DC.) Engl. f. *remyana* (Tieghem) Molvray
K. remyana Tiegh. var. *wawrae* (Tiegh.) Danser	*K. taenioides* (Comm. ex DC.) Engl. f. *taenioides*
Section *Bifaria* (distichous cladotaxy)	
Stems cylindrical	
K. aoraiensis (Nadeaud) Engl.	--
K. cylindrica (Tiegh.) Engl.	*K. cylindrica* (Tiegh.) Engl.
K. cylindrica (Tiegh.) Engl. var. *planiuscula* Danser	*K. taenioides* (Comm. ex DC.) Engl. f. *taenioides*
K. degeneri Danser‡	*K. taenioides* (Comm. ex DC.) Engl. f. *horneana* (Tiegh.) Molvray
K. grayi Barlow	*K. japonica* f. *grayi* (Barlow) Molvray
K. leucothrix Barlow	*K. japonica* (Thunb.) Engl. f. *japonica*
Stems flattened modified into phyllocladia	
K. breviarticulata (Tiegh.) Danser	*K. taenioides* (Comm. ex DC.) Engl. f. *pendula* (Wawra) Molvray
K. complanata (Tiegh.) Engl.	*K. taenioides* (Comm. ex DC.) Engl. f. *taenioides*
K. latissima (Tiegh.) Danser	*K. taenioides* (Comm. ex DC.) Engl. f. *pendula* (Wawra) Molvray

Danser (1937, 1940) & Barlow (1983)	Molvray (1997)
K. latissima (Tiegh.) Danser var. *crassa* (Tiegh.) Danser	*K. taenioides* (Comm. ex DC.) Engl. f. *taenioides*
K. taenioides (Juss.) Engl. (=*K. commersonii* (Tiegh.) Danser)†	*K. taenioides* (Comm. ex DC.) Engl. f. *taenioides*
Stems flattened not modified into phyllocladia	
K. dichotoma (Tiegh.) Engl.	*K. taenioides* (Comm. ex DC.) Engl. f. *taenioides*
K. dichotoma (Tiegh.) Engl. var. *balansae* (Tiegh.) Danser	*K. taenioides* (Comm. ex DC.) Engl. f. *taenioides*
K. disticha (Endl.) Engl.	*K. taenioides* (Comm. ex DC.) Engl. f. *disticha* (Endl.) Molvray
K. emersa Barlow	*K. taenioides* (Comm. ex DC.) Engl. f. *emersa* (Barlow) Molvray
K. japonica (Thunb.) Engl. (=*K. opuntia* (Thunb.) Merrill)	*K. japonica* (Thunb.) Engl. f. *japonica*
K. japonica (Thunb) Engl. subsp. *brassiana* (Blakeley) Barlow	*K. japonica* (Thunb.) Engl. f. *japonica*
K. "*opuntia*" var. *bojeri* (Tiegh.) Danser	*K. taenioides* (Comm. ex DC.) Engl. f. *taenioides*
K. "*opuntia*" var. *fasciculata* (Tiegh.) Danser	*K. japonica* (Thunb.) Engl. f. *japonica*
K. "*opuntia*" var. *gaudichaudii* (Tiegh.) Danser	*K. taenioides* (Comm. ex DC.) Engl. f. *taenioides*
K. "*opuntia*" var. *richardii* (Tiegh.) Danser	*K. taenioides* (Comm. ex DC.) Engl. f. *taenioides*
K. platycaula (Tiegh.) Engl.var platycaula	*K. taenioides* (Comm. ex DC.) Engl. f. *taenioides*
K. platycaula (Tiegh.) Engl. var. *rapensis* (F. Brown) Danser	*K. taenioides* (Comm. ex DC.) Engl. f. *taenioides*
K. platycaula (Tiegh.) Engl. var. *vitiensis* (Tiegh.) Danser	*K. taenioides* (Comm. ex DC.) Engl. f. *taenioides*
K. rubescens (Tiegh.) Lecomte	
K. rubra (Tiegh.) Engl. subsp. *rubra*	*K. japonica* (Thunb.) Engl. f. *rubra* (Tiegh.) Molvray
K. rubra subsp. *geijericola* Barlow	*K. taenioides* (Comm. ex DC.) Engl. f. *taenioides*

* Danser (1937) considered *K. clavata* to be a variety of *K. lindsayi*, however, later (Danser, 1940) resurrected the species rank of *K. clavata* when he actually cited a specimen of *K. clavata*.

†*K. taenioides* is the legitimate name for the Malagasy entity *K. commersonii* in Danser (1937, 1940), see Callmander *et al*. (2010) for details.

‡Molvray (1997) placed different forms of *K. degeneri* under *K. taenioides* f. *horneana,* and *K. taenioides* f. *taenioides*.

According to Molvray *et al*. (1999), the Malesian region appeared to be the centre of origin of *Korthalsella,* because *K. papuana* Danser, which was sister to all other *Korthalsella* in their phylogenetic study, and the most closely related genus, *Ginalloa* Korth, are both found in this region. *Korthalsella* is the most widely dispersed mistletoe genus and often occupies remote oceanic islands (Barlow 2012a, Fig. 1). In the Pacific, it has a distribution extending to Hawaii, Marquesas and Henderson island through New Caledonia, Fiji, Samoa, Niue, Cook Islands, Society Islands, Austral Islands and Tuamotu Islands and southwards it has a distribution through eastern, western and southern Australia, Lord Howe Island and Norfolk Island to New Zealand. Northwards, the distribution extends to Japan through Taiwan and China and westwards to eastern Africa, Comoros and Madagascar through Southeast Asia, Himalaya, southern India and Mascarenes (Fig. 1). If *Korthalsella* is regarded as a non-Gondwanan group as suggested by Barlow (1983b), then the current disjunct distribution of the genus cannot be explained as relictual occurrences from Gondwana super-continent following vicariance events. Although, there is some evidence for Australia-New Guinea distributions by vicariance (Sanmartin *et al.* 2006), Molvray *et al*. (1999) hypothesised that dispersal of the genus proceeded outward from Malesia; the agents of long distance dispersal (LDD) to remote oceanic islands being birds and possibly butterflies.

Table 2. Sectional arrangement of *Korthalsella* according to Molvray (1997)

Section *Heterixia* (branch vascular bundles 8 or more, rarely 7 or 6; specialised inflorescences)
K. geminata (Korthals) Engler
K. papuana Danser

Section *Korthalsella* (branch vascular bundles 8 or more, rarely 7 or 6; lacking specialised inflorescences)
K. cylindrica (Tiegh.) Engler
K. taenioides (Comm. ex DC.) Engl. f. *taenioides*
K. taenioides (Comm. ex DC.) Engl. f. *disticha* (Endl.) Molvray
K. taenioides (Comm. ex DC.) Engl. f. *emersa* (Barlow) Molvray
K. taenioides (Comm. ex DC.) Engl. f. *horneana* (Tiegh.) Molvray
K. taenioides (Comm. ex DC.) Engl. f. *pendula* (Wawra) Molvray
K. taenioides (Comm. ex DC.) Engl. f. *remyana* (Tieghem) Molvray

Section *Bifaria* (branch vascular bundles 4 or fewer)
Specialised inflorescences:
K. lindsayi (Oliver ex J. D. Hooker) Engler
Lacking specialised inflorescences:
K. dacrydii (Ridley) Danser
K. salicornioides (A. Cunningham) Tiegh.
K. japonica (Thunb.) Engl. f. *japonica*
K. japonica (Thunb.) Engl. f. *grayi* (Barlow) Molvray
K. japonica (Thunb.) Engl. f. *rubra* (Tiegh.) Molvray

A general pattern of dispersal of Southeast Asian elements, possibly of Laurasian origin, into Australian areas can be expected, and a reverse pattern can be expected for Australian elements, possibly of Gondwanan origin, apart from any patterns of vicariance (Turner *et al.* 2001). While the occurrence of Australian and Southeast Asian elements in the Pacific is result of westward movement of the Pacific plate which brought the different islands within reach of Southeast Asia and Australia (Turner *et al.* 2001). Thus, the occurrence of *Korthalsella* in Australia and western Pacific islands is suggestive of dispersal of Laurasian origin taxa to these regions. The development of *Korthalsella* in the Hawaiian Islands is considered a relatively recent secondary radiation (Barlow 1983b). According to Carlquist (1967), the current distribution of *Korthalsella* on the Hawaiian Islands is the result of two independent bird-mediated LDD events, though which species are thought to be in each group is not clear. The similarity of Pacific islands flora and fauna to those of Mascarene Islands is suggestive of the fact that islands in both the Pacific and Indian oceans have been colonised by groups from Indo-Malaya capable of LDD (Carlquist 1967).

Barlow (2012a) suggested that the distribution of *Korthalsella* on remote Pacific and Indian Ocean archipelagos conformed to the movement of wide ranging, island-dwelling seabirds that are potential LDD agents of these mistletoes, whereas the continental distribution patterns of other species conform to movement of dispersing land birds which move through tropical and temperate forests in these regions (Barlow 2012a). Some of the wide-ranging species also inhabit remote oceanic islands and geographical regions. For example, amongst the Pacific species, *K. platycaula* (Tiegh.) Engl. is the most widely distributed species and is found on many Pacific archipelagos (Fig. 1) including Fiji, Niue, Cook islands, Hawaii, the Society Islands, the Austral islands, the Tuamotu islands, Marquesas and Henderson island (Danser 1937, 1940, Sykes 1970). However, across its distribution, distinct morphological forms inhabiting different islands/archipelagos were recognised by Danser (1940). *Korthalsella platycaula* (Tiegh.) Engl. var. *vitiensis* (Tiegh.) Danser, in which the largest internodes are obovate-oblong or oblongate-lanceolate, has a distribution in Fiji, Austral Islands, Tuamotu Islands, Tahiti and in Henderson Island (Danser 1940). *Korthalsella platycaula* (Tiegh.) Engl. var. *rapensis* (F. Brown) Danser, with stiffer stems and shorter internodes, is only found on the Austral Islands, whereas the main form with long and narrow articulations forming long ribbons occurs in Tahiti, Marquesas, Hawaiian Islands, Tuamotu Archipelago and the Cook Islands (Danser 1940). Among the other Pacific species, *K. rubescens* (Tiegh.) Lecomte also has a disjunct distribution and is known from Tahiti and from Henderson Island (Danser 1937).

Korthalsella japonica (Thunb.) Engl. (=*K. opuntia*) is another apparently widespread species occurring from Japan, Taiwan, China, through SE and South Asia westward to Madagascar. *Korthalsella brassiana* Blakely from Queensland, Australia is also considered a subspecies of *K. japonica* (*K. japonica* (Thunb) Engl. subsp. *brassiana* (Blakeley) Barlow, see Barlow 1983). Some distinct forms from the Mascarenes, Madagascar and the Comoros have also been treated as varieties of *K. japonica* (*K.* "*opuntia*" var. *bojeri* (Tiegh.) Danser, *K.* "*opuntia*" var. *gaudichaudii* (Tiegh.) Danser, and *K.* "*opuntia*" var. *richardii* (Tiegh.) Danser) (Danser 1937, 1940).

Mainland African populations resemble the descriptions of *K.* "*opuntia*" var. *gaudichaudii* (specimen: de Wilde 4526 in Kew (K) from Ethiopia) and *K.* "*opuntia*" var. *richardii* (specimen: Pichi-Sermolli 2488 in K from Ethiopia), whereas a recent collection from Madagascar (Callmander *& al.* 640) is similar to the main Asian form of this species (Callmander *et al.* 2010). The populations of *K. japonica* from China and the Himalayas were also considered a distinct variety (*K.* "*opuntia*" var. *fasciculata* (Tiegh.) Danser) by Danser (1937). Other disjunct distributions of *Korthalsella* species include the occurrences of *K. papuana* in New Guinea and Queensland, Australia; *K. geminata* (Korthals) Engl. in Borneo, Peninsular Malaysia, Flores, Sumatra, plus a recent collection from Cambodia; *K. dacrydii* (Ridley) Danser which occurs in Java, Sumatra, Peninsular Malaysia, Borneo and Timor (Wasscher 1941, Barlow 1997); and *K. rubra* (Tiegh.) Engl., which occurs in New Guinea, Eastern Australia and in Lord Howe Island (Barlow 1997).

A previous species level molecular phylogenetic study (Molvray *et al.* 1999) supported monophyly of *Korthalsella*, but relationships within the genus remain obscure. Molvray *et al.* (1999) did not include any species from Polynesia, New Caledonia, Norfolk Island, Lord Howe Island, Madagascar or mainland Africa. Moreover, two key species from the Malesian region, *K. geminata* and *K. dacrydii,* were also missing from their study. The species that were not included in that study create a large knowledge gap with regard to infra-generic relationships and hinder the ability to evaluate species delimitations, relationships, biogeography, and morphological evolution. Moreover, testing monophyly of the classification scheme as proposed by Molvray (1997) requires much broader sampling than was included in the 1999 study.

The goal of this study was to sample more broadly within *Korthalsella* and reconstruct molecular phylogenetic hypotheses from nuclear and chloroplast markers. These phylogenies would then be used to 1) test the monophyly of the sectional classification scheme of Molvray (1997, Table 2) and 2) assess intra-generic relationships to determine if morphology or geographic distribution was a better indicator of relatedness amongst species. With regard to the latter, the phylogenies would be used to determine support for the species concepts of either Molvray (1997) (i.e., broadly circumscribed entities and similar morphological forms inhabiting different geographical regions (e.g., *K. madagascarica*, *K. salicornioides* and *K. striata/ K. breviarticulata* and *K. latissima*) are conspecific) or Danser (1937) and Barlow (1983a) (i.e., most *Korthalsella* species inhabiting different geographical regions are specialised regional endemics and therefore show morphological parallelism across the distribution).

MATERIALS AND METHODS

The taxa sampled are listed in Table 3. New sequences were generated for 33 *Korthalsella* individuals (Table 3) and the remaining sequences originated from previous studies and were acquired from GenBank (Molvray *et al.* 1999, Papadopulos *et al.* 2011, Table 4). Sixteen taxa not included in the phylogenetic study of Molvray *et al.* (1999) were included here. The names of the taxa correspond to those in Danser (1937, 1940) and Barlow (1983a). However, *K. taenioides* is adopted as a legitimate name for the Malagasy endemic named *K. commersonii* in Danser (1937, 1940) (see Callmander *et al.* 2010) and *K. opuntia* in Danser (1937 1940) is considered an illegitimate name (see Barlow 1983a). *Phoradendron rhipsalinum* and *P. robinsonii* sequences from GenBank were used as outgroup taxa in the ITS analysis; these taxa were also used as outgroups for *Korthalsella* in Papadopulos *et al.* (2011). *Viscum album* subsp. *album* and *Arceuthobium douglasii* sequences were used as outgroup taxa in the *trnL-F* analysis. *Viscum album* was also used as an outgroup for *trnL-F* analyses in Molvray *et al.* (1999). In the combined ITS and chloroplast analysis only *V. album* subsp. *album* was used as an outgroup, because both ITS and *trnL-F* sequences were available for this taxon in GenBank. DNA was extracted from silica gel-dried stem tissue or recent herbarium specimens by a modified CTAB method (Doyle and Doyle 1987) of Loockerman and Jansen (1996). For older herbarium specimens, DNA was extracted using a Qiagen DNeasy Plant Mini Kit (Qiagen Inc., Valencia CA) followed by multiple displacement amplification (MDA) using a Qiagen REPLI-g kit (Brockington *et al.* 2008).

The nuclear ribosomal internal transcribed spacer region (ITS1, 5.8S, and ITS2) was amplified using primers 7A (5′-GAGTCATCAGCTCGCGTTGACTA-3′, A. Plovanovich and J. Panero, unpub.) and 4 (5′-TCCTCCGCTTATTGATATGC-3′, White *et al.* 1990). The chloroplast *trnL-F* intergenic spacer was amplified using primers ‘e’ (5′-GGTTCAAGTCCCTCTATCCC-3′, Taberlet *et al.* 1991) and ‘f’ (5′-ATTTGAACTGGTGACACGAG-3′, Taberlet *et al.* 1991). Both ITS and *trnL-F* were amplified in a 25 µl total volume comprising 10x ThermoPol reaction buffer (New England BioLabs), 10 mM dNTPs, 4 µM forward and reverse primers, 5M Betaine, 0.5 unit NEB TAQ polymerase and c. 50 ng template. Cycling parameters for ITS were 95^{o}C for 1 min, 53^{o}C for 1 min. and 72^{o}C for 1 min for five cycles, followed by a decrease in the annealing temperature to 48^{o}C for another 44 cycles, and with a 7 min final extension at 72^{o}C. The cpDNA region was amplified using 95^{o}C for 1 min, 50^{o}C for 1 min followed by 65^{o}C for 4 min for 34 cycles, and a 5 min final extension at 72^{o}C. Amplification products were separated on a 1% agarose gel, stained with ethidium bromide and visualised with UV on a transilluminator. For amplifying the ITS region from older herbarium specimens, internal primers were used to amplify ITS in two pieces: ITS1: ITS 7A (A. Plovanovich and J. Panero, unpub.) and ITS 2B (CTCGATGGAACACGGGATTCTGC, based on Kim and Jansen 1994); ITS2: ITS 3 (GCATCGATGAAGAACGCAGC, Kim and Jansen 1994) and ITS 4 (White *et al.* 1990). Bovine serum albumin (BSA) was used as an adjuvant at a concentration of 1% to avoid misamplification from fungal contamination in the ITS reactions (de Miranda *et al.* 2010) which yielded a product in the form of a smear.

Table 3. Voucher information for new *Korthalsella* species sequenced for molecular phylogenetic analysis. Taxon names followed by an asterisk (*) were collected by the author in the field, samples with a dagger (†) were collected in the field by collaborators, and taxa with a yen sign (¥) following the name were sampled from existing herbarium specimens.

Taxon name	Herbarium/ Herbarium ID	Collector/s, year	Number	DNA accession	Locality	Host
K. aoraiensis (Nadeaud) Engl. †	MPN	Meyer, J. Y., 2011	3175	K227	Society Islands, Tahiti, trail to Mt. Aorai	*Metrosideros collina* A. Gray
K. arthroclada Cranfield¥	PERTH 7399081	Marchant, N. G., 1976	76/127	K223	Western Australia, Lake Logue	*Melaleuca lanceolata* Otto
K. breviarticulata (Tiegh.) Danser¥	BRI: AQ0771489	Cause, T.		K232	Australia, Queensland, Boonah	*Citrus* L. sp.
K. clavata (Kirk) Cheeseman*	MPN	Sultan, A. & Robertson, A., 2008		K16	New Zealand, South Island, Castle Hill Basin	*Coprosma propinqua* A. Cunn.
K. clavata (Kirk) Cheeseman¥	AK 304816	de Lange, P. J., 2009	7839	K181	New Zealand, North Island, Whakamaru, Mangakowhiriwhiri Gorge	*Coprosma propinqua*
K. dacrydii (Ridley) Danser*	MPN	Iskandar E., Hambali, G. & Sultan, A., 2011	EA 315	K166	Java, Gunung Gede Pangrango National Park, trail above Cibodas Mountain Gardens	*Podocarpus imbricatus* Blume
K. degeneri Danser¥	AK 29113	Degener, O., Park, K. & Nitta, Y., 1932		K197	Hawaii, Oahu	*Sapindus oahuensis* Hillebr.
K. degeneri Danser†	MPN	Lau, J. Q. C., 2012		K234	Hawaii, Oahu	*Sapindus oahuensis, Pouteria* Aubl. sp.
K. dichotoma (Tiegh.) Engl. †	MO	Callmander, M. W. & al., 2010	911	K49	New Caledonia, South Province, Port boisé	*Halfordia* F. Muell.
K. dichotoma (Tiegh.) Engl. †	MPN	Callmander, M. W., 2010		K50	New Caledonia	

K. disticha (Endl.) Engl. ¥	MPN 24043	Petterson, J. A., & McCoy, H.		K28	Norfolk Island, Mt Pitt	
K. disticha (Endl.) Engl. ¥	AK 237512	de Lange, P, Crowcroft, G., 1998	NF44	K222	Norfolk Island, Saddle between Mount Bates & Mount Pitt	*Melicytus ramiflorus* J. R. Forst & G. Forst.
K. disticha (Endl.) Engl. ¥	AK 297254	Platt, G. C., 1998		K176	Norfolk Island, Mt Pitt summit	*Coprosma pilosa* Endl.
K. geminata (Korthals) Engl. ¥	KEP	Awa, D., & Lee, B., 1985	S 50969	K175	Borneo, Sarawak, Bario, Gunung Batu Buli	
K. geminata (Korthals) Engl. †	E	Thomas, P., 2011		K226	Cambodia, Cardamon Mountains near Phnom Samkos	*Garcinia* L. sp.
K. grayi Barlow¥	CNS 130825	Zich, F. A. & Harrington, M., 2009	652	K173	Australia, Queensland, Mt Bellenden Ker	*Symplocos ampulliformis* C.T. White
K. horneana Tiegh.*	MPN	Sultan, A. & Asotasi, I., 2011		K225	Samoa, Upolu, Mt Sina'ele	*Dysoxylum huntii* Merr. ex Setch.
K. leucothrix Barlow¥	PERTH 05853818	Edinger, D.J., Backhouse, B. & B. & Marsh, G., 2001	DJE 2479 A	K224	Western Australia, Track to Baker Lake	*Acacia aneura* F. Muell. ex Benth.
K. lindsayi (Oliver ex J. D. Hooker) Engl.*	MPN	Sultan, A., Robertson, A. & Tate, J., 2009		K74	New Zealand, North Island, Coles Bush, near Rongotea	*Coprosma rigida* Cheeseman
K. lindsayi (Oliver ex J. D. Hooker) Engl.*	MPN	Sultan, A., & Mahmood, F., 2010		K108	New Zealand, South Island, Aramoana	*Myrsine australis* (A. Rich.) Allan
K. japonica (Thunb.) Engl.*	MPN	Iskandar, E., Hambali, G. & Sultan, A., 2011	EA 314	K165	Java, Cibodas Mountain Gardens	*Altingia excelsa* Noronha
K. japonica (Thunb.) Engl. ¥	MO	Callmander, M. W., Jo Vasaha & Malaza, 2006	640	K26	Madagascar, Antsiranana, DIANA region	*Vaccinium* L. sp.
K. "*opuntia*" var. *bojeri* (Tiegh.) Danser ¥	MAU 24845	Pynee, K. & Bone, R., 2007		K230	Mauritius, Mt Le Pouce	*Eugenia* L. sp.
K. "*opuntia*" var. *gaudichaudii* (Tiegh.) Danser¥	MAU 24307	Pynee, K. & Chitbauhaal, A., 2005		K231	Mauritius, Plaine Champagne,	*Nuxia verticillata* Lam.
K. "*opuntia*" var. *richardii* (Tiegh.) Danser¥	MAU 24938	Pynee, K. & Beetun, D. R., 2010		K167	Mauritius, Mt. Cocotte	*Nuxia verticillata*
K. madagascarica Danser¥	MO	Jongkind, C. C. H, Andriantiana, J., &	3529	K235	Madagascar, Mahajanga, Tsingy de Bemaraha, South of	*Cynometra* L. sp.

		Razanatsoa, H. E., 1996			Manambolo river	
K. platycaula (Tiegh.) Engl. (Tiegh.) Danser†	MPN	Meyer, J. Y., 2011	3177	K229	Society Islands, Tahiti, Anaorii Plateau, Papenoo Valley	*Crossostylis biflora* Forst.
K. rubescens (Tiegh.) Lecomte†	MPN	Meyer, J. Y., 2011	3178	K228	Society Islands, Moorea, Paoroa valley, trail to Mt Tohiea	*Metrosideros collina* A. Gray
K. rubra (Tiegh.) Engl. ¥	BRI: AQ 771312	Jensen, R., 2002	1325	K233	Australia, Queensland, Gadgarra	*Elaeocarpus grandis* F. Muell.
K. rubra subsp. *geijericola* Barlow¥	AK 297162	Brown, E. A., Cuddy, W. S., & McPherson, H., 2002	2002/42	K177	Australia, NSW, 52km SE of Coonamble	*Acacia* Mill. sp.
K. salicornioides (A. Cunningham) Tiegh.*	MPN	Sultan, A., 2010		K30	New Zealand, North Island, Bay of Islands, Kerikeri Inlet Road	*Leptospermum scoparium* s.l.
K. salicornioides (A. Cunningham) Tiegh.*	MPN	Robertson, A., 2011		K168	New Zealand, South Island, Kaiteriteri	*Kunzea ericoides* s.l.
K. taenioides (Juss.) Engl. (=*K. commersonii* (Tiegh.) Danser) ¥	MO	Antilahimena, P. & al., 2010	7526	K25	Madagascar, Ambotavy near Moramanga	*Rhodolaena bakeriana* Baill.

Table 4. GenBank accession numbers for *Korthalsella* and outgroup species included in the study.

Taxon name	Collector No./ID	*trnL-trnF*	ITS
K. complanata (Tiegh.) Engl.	308	AF055688	AF051966
K. complanata (Tiegh.) Engl.	313	AF055689	AF051967
K. cylindrica (Tiegh.) Engl.	303	AF055682	AF051960
K. cylindrica (Tiegh.) Engl.	310	AF055681	AF051959
K. emersa Barlow	Papadopulos AP710	JF950942	JF950771
K. japonica (Thunb.) Engl.	K & L 228	AF055696	AF051974
K. japonica (Thunb.) Engl.	INO	AF055697	AF051975
K. japonica (Thunb) Engl. subsp. *brassiana* (Blakeley) Barlow	358a	AF055690	AF051968
K. "*opuntia*" var. *bojeri* (Tiegh.) Danser	LR 19	AF055694	AF051972
K. "*opuntia*" var. *richardii* (Tiegh.) Danser	STR	AF055695	AF051973
K. "*opuntia*" var. *richardii* (Tiegh.) Danser	L1967	AF055693	AF051971
K. latissima (Tiegh.) Danser	304	AF055687	AF051965
K. latissima (Tiegh.) Danser	309	AF055684	AF051962
K. papuana Danser	359	AF055673	AF051951
K. platycaula (Tiegh.) Engl.	302	AF055685	AF051963
K. platycaula (Tiegh.) Engl.	307	AF055686	AF051964
K. remyana Tiegh.	312	AF055683	AF051961
K. rubra (Tiegh.) Engl.	786344	JF950891	JF950772
K. rubra subsp. *geijericola* Barlow	361	AF055692	AF051970
K. rubra subsp. *geijericola* Barlow	362	AF055691	AF051969
Arceuthobium douglasii	DOU1949	AY288216	
Phoradendron rhipsalinum			AF178719
P. robinsonii			AF178718
Viscum album subsp. *album*	Hapl E	AF180535	
Viscum album subsp. *album*			AF180530

Unincorporated dNTPs and excess primers were removed by adding 5 units of exonuclease I and 0.5 unit shrimp alkaline phosphatase to 8 µL of PCR product and incubating at 37°C for 30 min followed by 80°C for 15 min. PCR products were sequenced using forward and reverse primers. Cleaned products were sequenced on an ABI 3770 sequencer at the Massey Genome Service (Massey University, Palmerston North, New Zealand) following the manufacturer's recommendation (Applied Biosystems, Foster City, California, U.S.A.). Sequences were edited by aligning forward and reverse sequences in Sequencher v.4.8 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.). Edited sequences were aligned in BioEdit v.7.0.5.3 (Hall 1999) using the ClustalW option, with manual adjustments as needed. Both the ITS and cp *trnL-F* alignments are included as electronic appendices 2.1 and 2.2.Parsimony analyses were conducted on separate ITS and chloroplast data sets in PAUP* v. 4.10 (Swofford 2002) using heuristic searches with tree-bisection-reconnection (TBR) branch-swapping, gaps coded as missing and with maximum trees setting of 50000. Bootstrap support for clades was determined on the basis of 1000 replicates (Felsenstein 1985). To determine if the nuclear and chloroplast data sets could be concatenated, the incongruence length difference (ILD) test (Farris *et al.* 1994) was conducted in PAUP* using a heuristic search with stepwise addition, 1000 random addition sequence replicates, tree-bisection-reconnection branch swapping and gaps coded as missing. Combined ITS and cpDNA data sets were analysed using the same search criteria as for the separate regions.

Bayesian analyses were conducted on separate and concatenated ITS and cpDNA data sets using MrBayes v.3.1.2 (Huelsenbeck & Ronquist 2001). The best fit model for each region (ITS1, 5.8S, ITS2, *trnL-F*) was determined using MrModeltest v.2.3 (Nylander 2004) prior to Bayesian phylogeny estimation. ITS1, 5.8S and ITS2 were analysed together, specifying the best fit model for each partition (GTR + G for ITS1 & 2, K80 + I + G for 5.8S). For the chloroplast data set, the best fit model selected was GTR + G. MrBayes was run for at least 10^6 generations or until the average standard deviation of the split frequencies approached 0.001. Trees corresponding to the burn-in period (the first 25%) were discarded and a majority rule consensus constructed from the remaining trees.

RESULTS

The ITS matrix had a total aligned length of 759 bp (excluding outgroups), with 323 parsimony-informative characters (including outgroups). *Korthalsella arthroclada* had the longest sequence comprising 685 bp (with ITS1 region extending for 256 bp and ITS2 for 262 bp). *Korthalsella leucothrix* had the shortest sequence length at 626 bp (with ITS 1 including 219 bp and ITS 2 with 240 bp). All New Zealand taxa have an 11 bp deletion at positions 84-94 and another 6 bp deletion at 208-213. A 6 bp deletion was common to *K. grayi* and *K. japonica* subsp. *brassiana* at 86-91. Two 2 bp insertions at 104-105, 260-261 and a 28 bp deletion at position 227 were common to all Mauritius and Reunion taxa. A *Korthalsella platycaula* collection from Tahiti had a 15 bp deletion beginning at position 107 relative to others. All Hawaiian species except *K. remyana* Tiegh. have a 6 bp deletion at 122-127. Two *K. disticha* collections (K176 and K222) are missing from ITS data as reliable ITS sequences for these collections could not be obtained.

The *trnL-F* matrix had a total aligned length of 1112 bp (excluding outgroups) with 189 parsimony-informative characters (including the outgroups). *Korthalsella leucothrix* had the longest sequence comprising 894 bp. *Korthalsella arthroclada* had the shortest sequence length of 638 bp. *Korthalsella madagascarica, K. japonica* subsp. *brassiana*, *K. dacrydii*, *K. grayi, K. rubra* subsp. *geijericola* (#362 & 361), and *K. rubra* also have relatively shorter sequences with lengths of 749, 756, 761, 765, 785, 789 and 803 bp respectively. All Mauritius and Reunion taxa share an 8 bp deletion beginning at position 292 and a 4 bp insertion at 616-619 relative to other *Korthalsella* species. *Korthalsella japonica, K. dichotoma, K. disticha* and *K. taenioides* share a 4 bp deletion at 312-315, whereas, all *Korthalsella japonica* accessions have a 6 bp insertion at 601-606. Similarly, all Hawaiian species have a 2 bp deletion at 178-179 and another 6 bp deletion at 353-358. A 2 bp insertion at 559-560 is common to *K. papuana* and all New Zealand species and a 10 bp insertion at 743-752 is common to *K. geminata*, *K. papuana, K. leucothrix* and all New Zealand species. A 5 bp deletion at 805-809 is shared by *K. rubra* subsp. *geijericola* (361 & 362) and *K. rubra* subsp. *rubra*.

The parsimony analysis of ITS resulted in six equally parsimonious trees. The parsimony tree has a length of 1021 steps and a consistency index (CI) of 0.66 and a rescaled consistency index (RC) of 0.52. Parsimony analysis of ITS reconstructed the ingroup taxa into four clades (Figs. 9). *Korthalsella arthroclada* from Western Australia was sister to all *Korthalsella* species (bootstrap support [BS]=100). *Korthalsella arthroclada* has decussate axils and cylindrical stems with acute leaves. *Korthalsella geminata* and *K. papuana* formed a clade (BS=92) sister to a New Zealand clade. Both *K. geminata* and *K. papuana* have specialised inflorescences, decussate inflorescence axils and strongly flattened vegetative branches. All New Zealand species formed a strongly supported clade (BS=91), with *K. lindsayi* and *K. clavata* forming a strongly supported sub-clade (BS=100). Both *K. lindsayi* and *K. clavata* are characterised by specialised inflorescences, superposed inflorescence axils, and strongly flattened vegetative branches. Collections from the Indian Ocean Basin plus *K. leucothrix* from southern and western Australia formed an unsupported clade. Taxa from the Mascarenes (*K. "opuntia"* var. *bojeri,* var. *gaudichaudii* and var. *richardii*) formed a strongly supported sub-clade within the Indian Ocean Basin clade (BS =100). Asian populations of *K. japonica* formed a well-supported clade (BS=100). *Korthalsella dacrydii*, along with collections from Norfolk Island, New Caledonia, Lord Howe Island, Queensland and New South Wales (NSW) formed a weakly supported clade (BS=58). Within this clade, *K. grayi* and *K. japonica* subsp. *brassiana* from Queensland formed a strongly supported sub-clade (BS=100). Similarly, *K. emersa* Barlow from Lord Howe Island and *K. breviarticulata* from Queensland/NSW also formed a strongly supported clade (BS=100). *Korthalsella disticha* from Norfolk Island and a collection of *K. dichotoma* (K50) from New Caledonia also formed a strongly supported sub-clade (BS=100), whereas, *K. rubra* subsp. *rubra* and *K. rubra* subsp. *geijericola* were both polyphyletic. All collections from the Society Islands and *K. horneana* from Samoa formed a strongly supported clade (BS=96). Within this South Pacific clade, *K. platycaula* and *K. aoraiensis* from Tahiti formed a weakly supported sub-clade (BS=69). *Korthalsella horneana* from Samoa was sister to this Tahitian sub-clade. *Korthalsella rubescens* from Moorea was sister to the Tahiti + *K. horneana* sub-clade. All Hawaiian species formed a strongly supported clade (BS=98). Within this Hawaiian clade, two *K. cylindrica* (Tiegh.) Engl. samples grouped together (BS=77) while *Korthalsella complanata* (Tiegh.) Engl., *K. latissima* (Tiegh.) Danser and *K. platycaula* were polyphyletic.

The parsimony and Bayesian phylogenies were largely congruent, with the latter less resolved than the former (compare Figs. 9 and 10). The two differed in their placement of *Korthalsella arthroclada* as either sister to all other *Korthalsella* (parsimony) or as sister to a clade composed of New Zealand, Australian, and southeast Asian species (Bayesian). The position of *K. leucothrix* also differed in the two analyses: included within the clade of Malagasy and Mauritius taxa (parsimony) or as sister to that clade (Bayesian).

The parsimony analysis of the plastid *trnL-F* data resulted in 4555 equally parsimonious trees of 695 steps and a CI of 0.74 and a RC of 0.57. Because the resulting consensus tree was highly unresolved, only the results from the Bayesian analyses are considered further.

Bayesian analyses (Fig. 11) of the plastid *trnL-F* data set reconstructed three major clades comparable to clades A, B and C in Molvray *et al.* (1999) and similar to the four major clades in the ITS phylogeny (Figs. 9, 10). *Korthalsella papuana* and *K. geminata* form a strongly supported clade (posterior probability [PP] = 0.93) sister to the rest of the genus. The New Zealand species are monophyletic (PP=1, BS=97). Taxa from Madagascar, Mauritius and the Reunion islands form a weakly supported clade. A New Caledonia-Norfolk Island sub-clade was strongly supported (PP=1, BS=91) compared to the ITS phylogeny where this sub-clade was not supported. *Korthalsella dacrydii* and collections from Lord Howe Island, Queensland and New South Wales formed a sub-clade (PP=0.98). *Korthalsella leucothrix* and *K. arthroclada* had divergent positions compared to other collections from Australia/Lord Howe island, whereas, *Korthalsella grayi* and *K. rubra* subsp. *rubra* from Lord Howe Island formed a weakly supported sub-clade (PP=0.57) compared to the ITS phylogeny in which *K. grayi* was sister to *K. japonica* subsp. *brassiana*. A collection of *Korthalsella degeneri* (K197) and *K. japonica* from Madagascar (K26) are missing from the plastid data matrix as reliable sequences for these two collections could not be obtained from available herbarium specimens.

ITS and cpDNA data sets were concatenated as there was not significant conflict between them as determined by the incongruence length difference test (P=0.436). Because *trnL-F* sequences were not obtained from *Korthalsella degeneri* (K197) or *K. japonica* from Madagascar (K26), these were not included in the combined analysis. The combined ITS and cpDNA data matrix had 407 parsimony-informative characters. The parsimony analysis resulted in three equally parsimonious trees, with a length of 1613 steps and a CI of 0.69 and a RC of 0.51 (not shown).

Overall, the concatenated ITS+cpDNA Bayesian phylogeny (Fig. 12) is similar to the ITS Bayesian phylogeny, however, *K. arthroclada* had a divergent position compared to the ITS phylogeny where it was sister to *K. papuana* + *K. geminata* and the New Zealand clades.

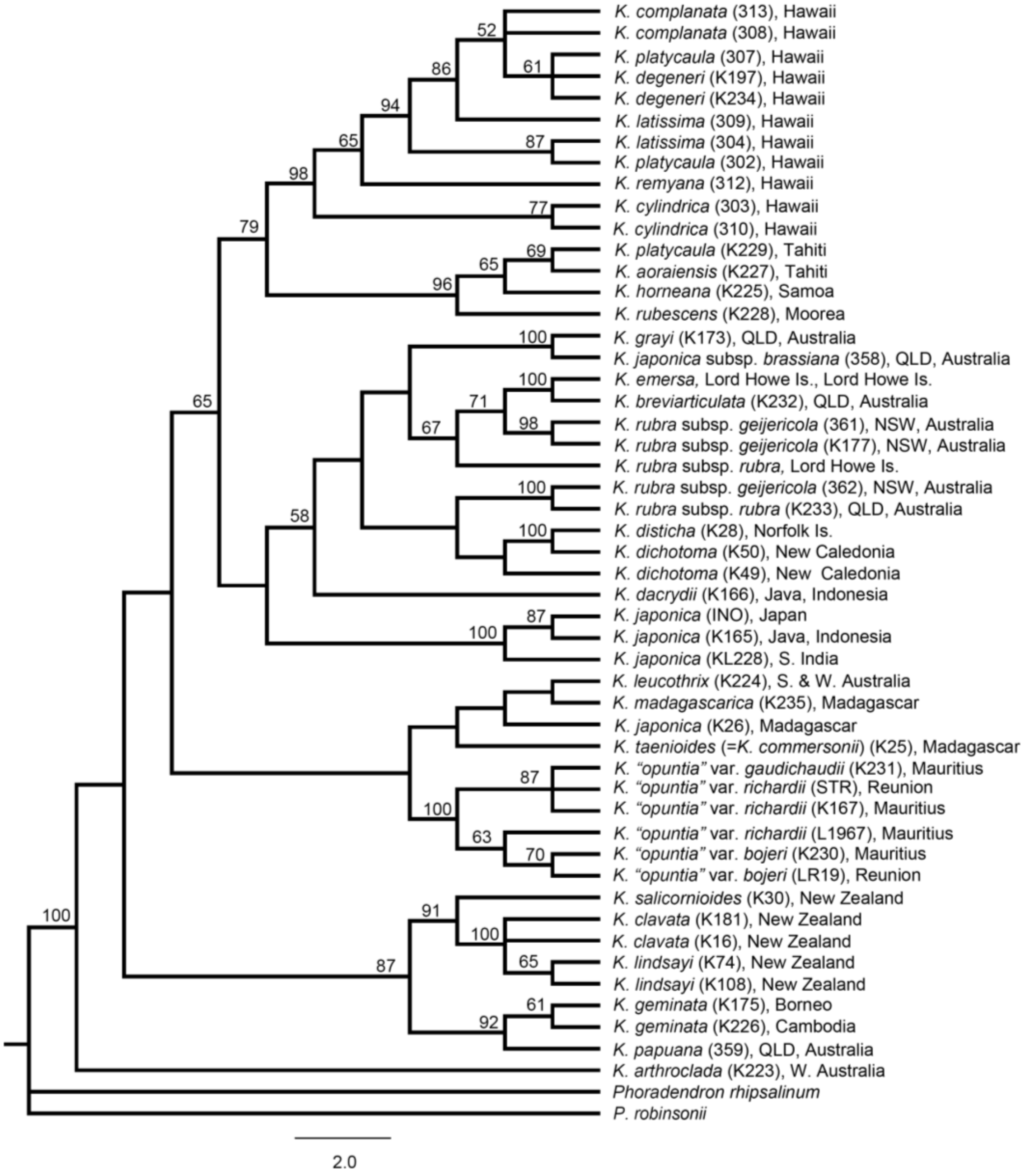

Fig. 9. ITS phylogeny of *Korthalsella* species (one of six equally parsimonious trees), CI=0.66, number of steps=1021. Numbers above the branches represent parsimony bootstrap support values.

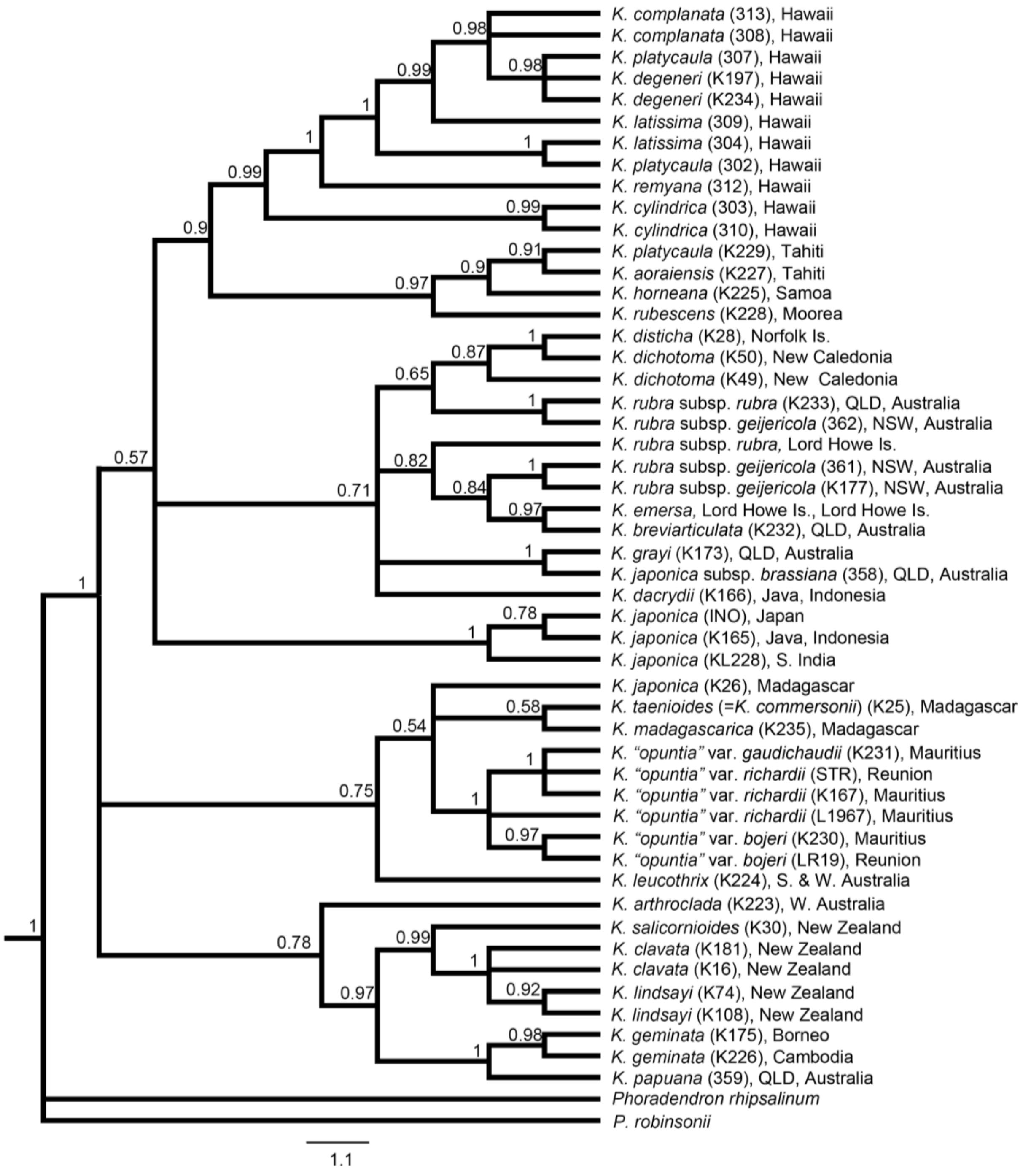

Fig. 10. ITS phylogeny of *Korthalsella* species (Bayesian consensus tree). Numbers above the branches represent Bayesian posterior probabilities.

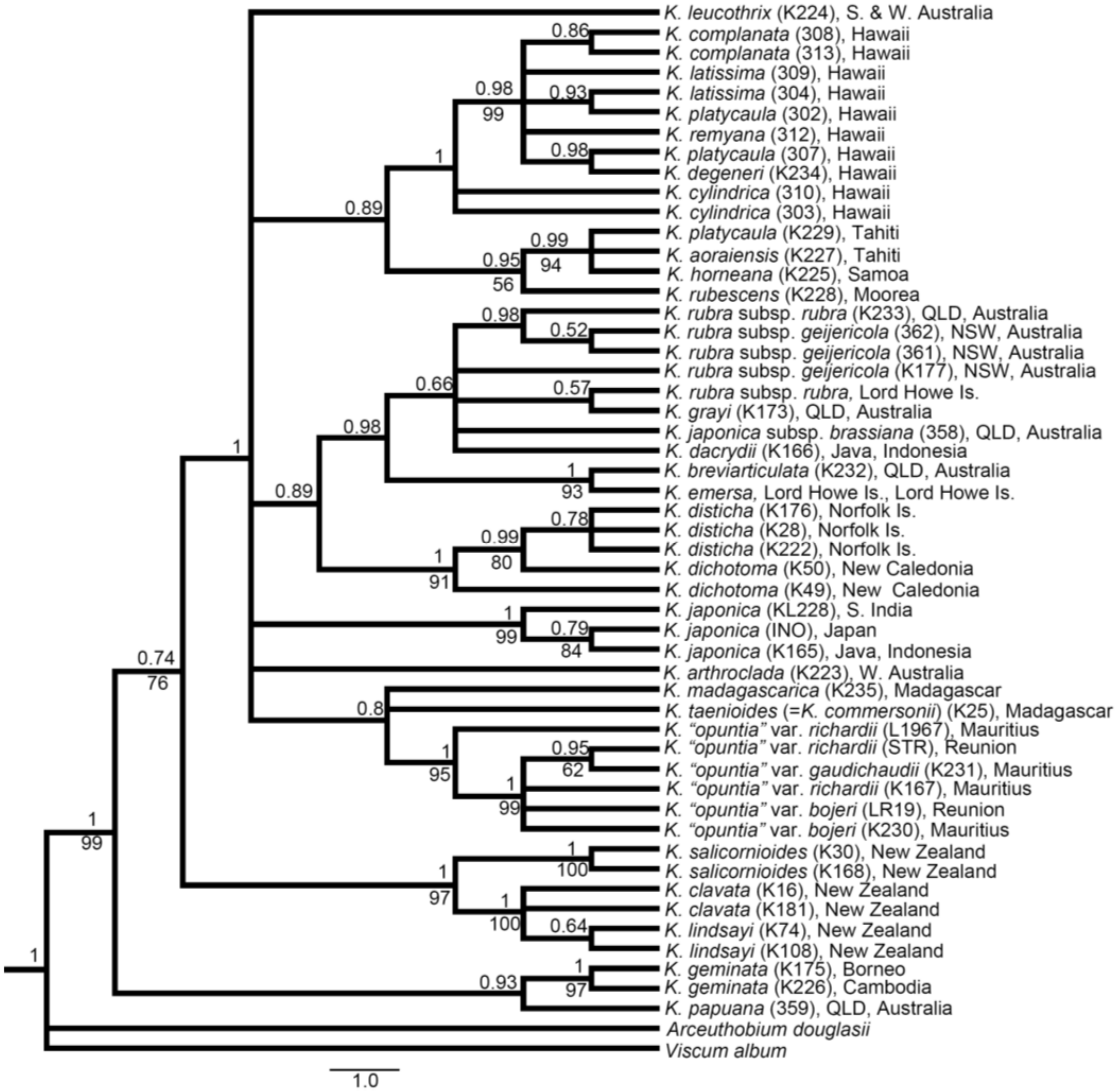

Fig. 11. Plastid phylogeny of *Korthalsella* species (Bayesian consensus tree). The numbers above the branches represent Bayesian posterior probabilities and the numbers below the branches represent parsimony bootstrap support values, respectively.

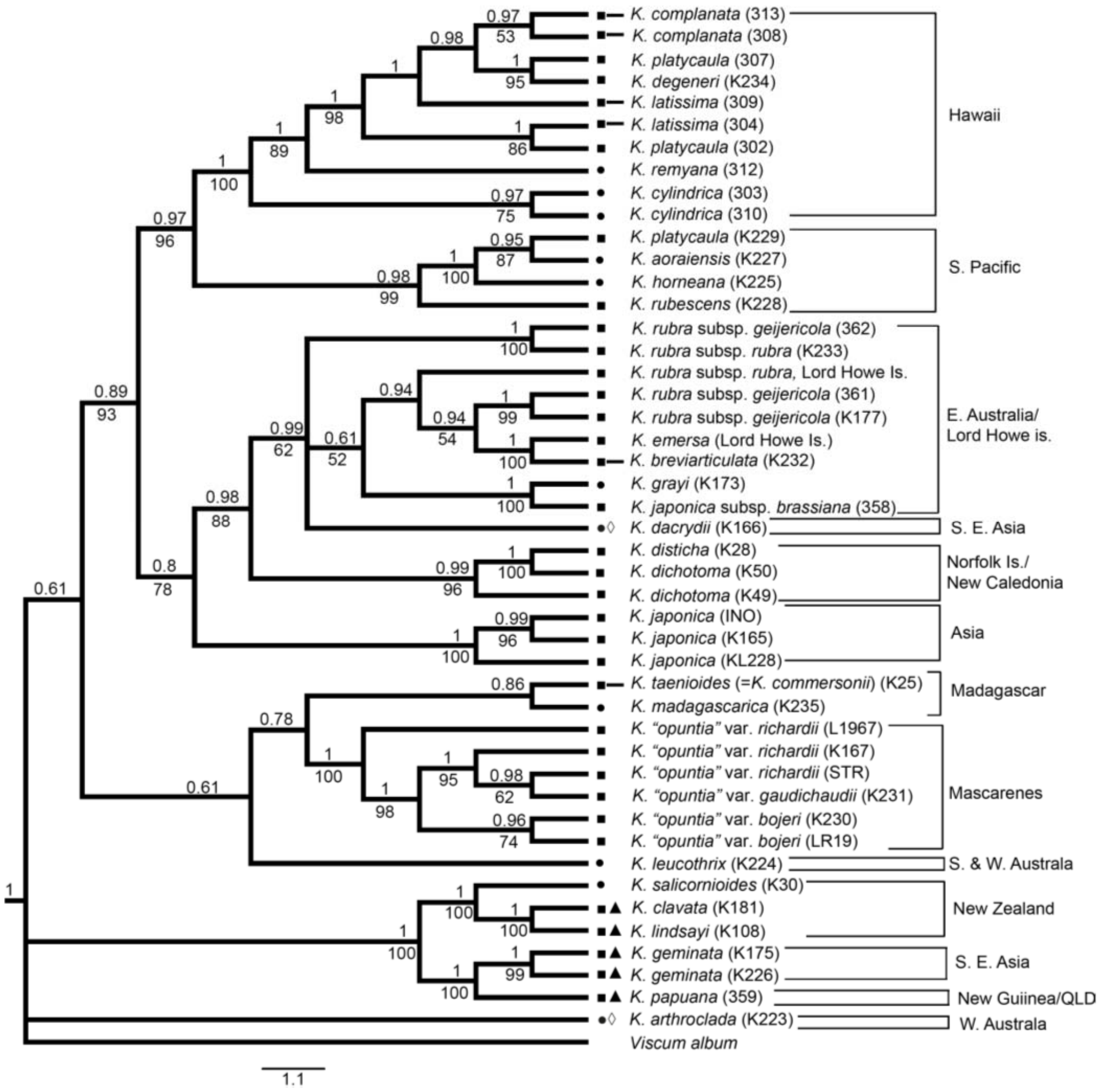

Fig. 12. Combined ITS and plastid phylogeny of *Korthalsella* species (Bayesian consensus tree). The numbers above the branches represent Bayesian posterior probabilities and the numbers below the branches represent parsimony bootstrap support values, respectively. A collection of *Korthalsella japonica* (K26) & a collection of *K. degeneri* (K197) included in ITS analysis were excluded from the combined dataset as plastid sequences for these collections were missing. (▲=Specialised inflorescences, ◊=Leaves acute, •=Stems terete, ■=Stems flattened, ■▬ =Stems flattened, modified into phyllocladia)

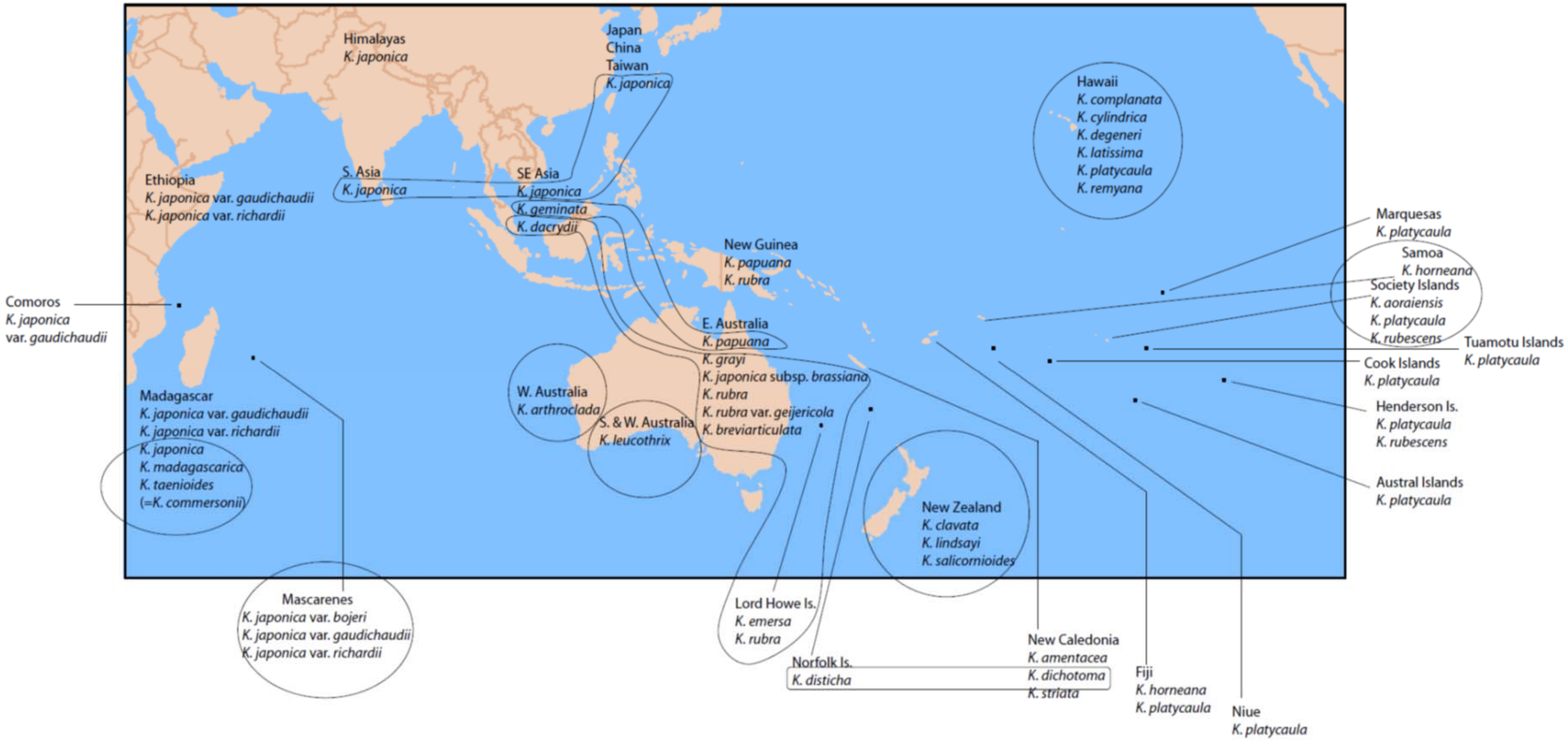

Fig. 13. Grouping of sampled taxa corresponding to clades in the phylogenetic analyses. Uncircled taxa/geographical locations were not sampled.

DISCUSSION

This study supports the biogeographic species concept (i.e., similar morphologies inhabiting different regions are distinct species as recognised by Danser 1937, 1940), whereas, the traditional sections based on morphology are not supported and thus resemblance in morphology is a consequence of parallelism in this genus. This study confirms the sister relationship of *K. papuana* and *K. geminata* as indicated by Molvray *et al.* (1999). *Korthalsella dacrydii* (the only species parasitic on gymnosperms) had a divergent position in a clade comprising collections from Queensland, New South Wales, Lord Howe Island, Norfolk Island and New Caledonia in both the ITS and combined ITS and plastid phylogenies (Figs. 9, 10, 12). In the plastid phylogeny, this species grouped with collections from Queensland, New South Wales and Lord Howe Island (Fig. 11).

For most of the collections, ITS, plastid, and combined ITS and plastid reconstructions strongly support biogeographical affinities within the genus (Fig. 13). The cause of the patchy distribution of these mistletoes on remote Indian Ocean and Pacific Islands remains elusive. Viscaceae are considered to be a non-Gondwanan group as they do not have relictual occurrences on the southern landmasses (Barlow 1983b). Phytogeographic evidence does not support the idea that Viscaceae may have had an earlier history in western Gondwana and reached Laurasia by rafting with India (Barlow 1983b), at least as inferred from the phylogeny of *Korthalsella*. Rather progenitor Viscaceae probably evolved in Laurasia and are likely to have reached the limits of distribution in the southern lands (Barlow 1983b). A more recent study (Vidal-Russell and Nickrent 2008) suggests that Viscaceae diverged 72 Mya and had a northern hemisphere origin. Molvray *et al.* (1999) also considered the Malesian region to be centre of origin of *Korthalsella.*

Carlquist (1967), Burrows (1996), and Barlow (2012a), all propose that migratory birds are potential vectors of long distance dispersal (LDD) in *Korthalsella,* carrying seeds by adherence to plumage after weakly explosive discharge of seeds. Speciation in this genus then most likely followed independent introductions and specialisation on different hosts. For example, *Korthalsella breviarticulata* from eastern Australia, *K. latissima* from Hawaii and *K. taenioides* (=*K. commersonii*) from Madagascar represent examples of parallelism in different geographical regions, with stems modified into phyllocladia and an overall close resemblance in gross morphology. These species group with their biogeographic counterparts in different clades/subclades. Thus, phylloclades evolved independently in at least three different lineages. Based on these apparent similarities, Molvray (1997) reduced *K. breviarticulata* and *K. latissima* to synonyms of *K. taenioides* (Comm. ex DC.) Engl. f. *pendula* (Wawra) Molvray and the similar Malagasy endemic *K. taenioides* (= *K. commersonii*) was placed under a broadly circumscribed *K. taenioides* f. *taenioides*. However, *Korthalsella taenioides* is a legitimate name for a Malagasy endemic, which was named *K. commersonii* in Danser (1937, 1940) (see Callmander *et al.* 2010). Similarly, *Korthalsella aoraiensis* from Tahiti and Moorea and *K. cylindrica* from the Hawaiian Islands both have distichous cylindrical stems and are usually parasitic on *Metrosideros* species, yet they group with other neighbouring species from the south Pacific and Hawaiian Islands, respectively.

Korthalsella madagascarica (Fig. 14), *K. striata* and *K. salicornioides* with decussate cladotaxy, cylindrical stems and a relatively small plant size, represent another example of parallelism in the genus. Danser (1937, 1940) recognized *K. madagascarica, K. striata* and *K. salicornioides* as distinct species endemic to Madagascar, New Caledonia and New Zealand respectively. However, Barlow (1996) considered *K. striata* and *K. salicornioides* to be conspecific and placed New Caledonian *K. striata* in synonymy under the New Zealand *K. salicornioides*. Molvray (1997) also treated *K. striata* and *K. madagascarica* as synonyms of *K. salicornioides*, rendering the latter species with a highly disjunct distribution. *Korthalsella striata* is missing from our data set, as it is known only from a few isolated records. Despite considering *K. striata* to be conspecific with *K. salicornioides,* Barlow (1996) noted differences like thinner basal internodes and more pronounced ribs on New Caledonian material compared to specimens from New Zealand. *Korthalsella madagascarica* groups with the other Malagasy taxa in the analysis of both genes (Figs. 9-12). Moreover, examination of a herbarium specimen of *K. madagascarica* (isosyntype, Humbert 14060 in B) that was cited in the original description of the species has revealed a significant number of morphological characters that differ from *K. salicornioides*. Flowering in *K. madagascarica* is continuous, whereas in *K. salicornioides* flowering time is restricted to summer. Female flowers in *K. madagascarica* are pedicellate while these are sessile in *K. salicornioides*. The inflorescence trichomes in *K. salicornioides* are fewer and reddish-brown in colour, while in *K. madagascarica* these are colourless and more numerous. In *K. madagascarica* flowers originate as a cluster of three (two female, one male) or five (four female, one male), and more female flowers continue to develop below these (Fig.

15), thus the number of flowers in each axil is variable, while the number of flowers in each floral cluster of *K. salicornioides* is always five (four female, one male). *Korthalsella salicornioides* shares this characteristic (number of flowers) with the other New Zealand species, which means that each node bears a maximum of eight fruits all of the same age (Fig. 16).

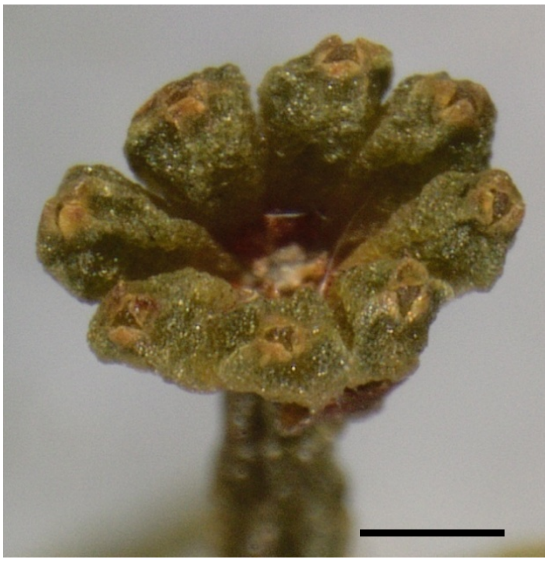

Fig. 14 (left). *K. madagascarica* parasitic on *Cynometra*, (corresponding to collection Jongkind *et al.* 3529), photo courtesy Folkert Aleva, scale bar= c. 5 mm, **Fig. 15 (right above).** Developing fruits and newly emerging flowers of *K. madagascarica* (photographed by Sarah Bollendorf (B) from isosyntype: Humbert 14060), scale bar= c. 1 mm, **Fig. 16. (right below)** Developing fruits of *K. salicornioides* (apical part of stem removed), scale bar= c. 1mm.

Thus, closer examination into inflorescence/floral micro-morphology could potentially reveal more taxonomically important characters. The occurrence of *K. madagascarica* on non-Myrtaceous hosts *Philippia* Klotzsch (Ericaceae), *Leptolaena* Thou. (Sarcolaenaceae), *Diospyros* L. (Ebenaceae) (Balle 1964) and *Cynometra* (Fabaceae) (Jongkind *et al*. 3529 in MO, WAG) as opposed to predominantly Myrtaceous hosts for *K. salicornioides* (*Kunzea ericoides* s.l. and *Leptospermum scoparium* s.l.) also suggests divergence in terms of host preferences. Thus, *K. madagascarica* is not conspecific with *K. salicornioides* given the genetic affinity with Malagasy taxa, distinct ecology, and differences in floral characters. Instead, both of these species are specialised regional endemics.

Korthalsella arthroclada from Western Australia, which is parasitic on *Melaleuca lanceolata* (Cranfield 2002), was considered to be possibly conspecific with *K. dacrydii* (Barlow 2012b, Watson 2011), as both species have acute leaves. However, the ITS and plastid phylogenies show that both of these species are not closely related. *Korthalsella arthroclada* is a much larger species with distinctive yellowish-green branches (Fig. 17) compared to the smaller *K. dacrydii* (Fig. 18 a, b). *Korthalsella arthroclada* has a single row of female flowers in each axil (Cranfield 2002) compared to several female flowers developing in more than one row in *K. dacrydii* (Fig. 19a). Morphological examination of *K. dacrydii* flowers shows that male flowers in this species have a distinctive synandrium with a raised central pore (Fig 19b), whereas, the central pore in *K. arthroclada* is not raised.

Korthalsella japonica subsp. *brassiana*, *K. grayi* from Queensland and *K. leucothrix* from Southern and Western Australia are not conspecific with Asian collections of *K. japonica* as considered by Molvray (1997). The Malagasy collection of *K. japonica* (Callmander & *al*. 640 in MO), although it resembles the main Asiatic form, is not closely related to it.

In some cases, distinct morphologies from the same geographical region were genetically very similar. For example, *Korthalsella lindsayi,* having obovate internodes and producing single axillary and apical inflorescences mostly in two's and three's, and *K. clavata,* having much narrower spathulate internodes and solitary apical inflorescence, are polyphyletic (see chapter 3). This may be the result of occasional gene flow between populations particularly when the two species occur in sympatry. Both of these species also significantly differ in their host preferences (see chapter 3).

Fig 17. *K. arthroclada*, corresponding to collection Marchant, 76/127, photo courtesy David Watson & Raymond Cranfield, scale bar= c. 5 mm

Fig 18a. *Korthalsella dacrydii* parasitic on *Podocarpus imbricata*, Gunung Gede Pangragno National Park (photo by John Dransfield), scale bar= c. 5 mm

Fig. 18b. *Korthalsella dacrydii* parasitic on *Dacrydium*, Mt Tahan (photo by Yao Tze Leong (KEP)), scale bar= c. 5 mm

Fig. 19a. Floral cluster of *K. dacrydii,* scale bar= c. 0.5 mm

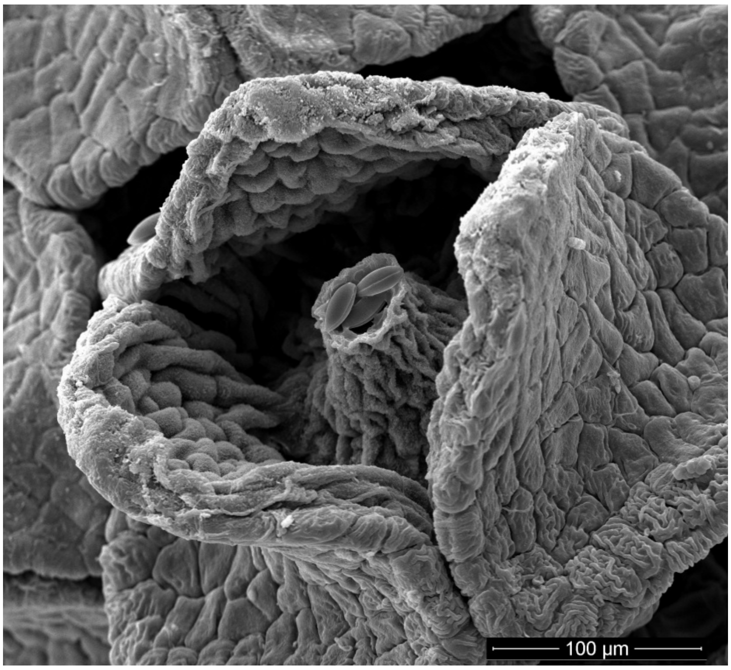

Fig. 19b. SEM of male *K. dacrydii* flower

Korthalsella "*opuntia*" var. *gaudichaudi* from the Mascarenes and *K.* "*opuntia*" var. *richardii* also from the Mascarenes differ in having much broader internodes in the former, with usually five prominent ribs as opposed to much narrower internodes and fewer (1-3) ribs in the latter. Both of these taxa have similar ITS sequences. Perhaps these morphologies have evolved recently. Danser (1937) noted that amongst the specimens of var. *gaudichaudii* and of var. *richardii* that he studied there were several that showed transitions towards other varieties.

Collections of New Caledonian *K. dichotoma* were paraphyletic within the Norfolk Island-New Caledonia clade. *Korthalsella disticha* from Norfolk Island has overall larger dimensions with larger dark olive-green internodes compared to smaller yellow/green internodes in *K. dichotoma*. Danser (1937) recognised two forms of *K. dichotoma.* The typical form has obovate to lanceolate-obovate internodes, whereas *K. dichotoma* (Tiegh.) Engl. var. *balansae* (Tiegh.) Danser has oblong-lanceolate internodes (Danser, 1937). A collection from New Caledonia included in the current study (Callmander & *al*. 911) matches the description of *K. dichotoma* var. *balansae* whereas another collection (Callmander, K50) matches the typical form of this species. *Korthalsella disticha* and *K. dichotoma* were considered conspecific by Barlow (1996), however, he noted that New Caledonian collections were polymorphic. Similarly, the Hawaiian species are also polyphyletic within the Hawaiian clade. Danser (1937, 1940) recognised distinct morphological varieties within Hawaiian *K. latissima*, *K. cylindrica* and considered *K. complanata* to be a highly polymorphic species.

This study confirms that most *Korthalsella* species are specialised regional endemics and geographically proximal species are more closely related to one another than morphology suggests given the frequent instances of parallelism in this group. In a phylogenetic study of *Arceuthobium* (Viscaceae), Nickrent *et al*. (2004) also found that Old and New World species were phylogenetically distinct (using ITS data) thus making subgenus *Arceuthobium* paraphyletic.

Based on the current evidence, the species concepts in Danser (1937, 1940) and Barlow (1983a) are supported rather than those of Molvray (1997). However, Danser's (1937, 1940) sectional arrangement is not supported since these do not form monophyletic clades within any of these trees, consistent with the findings of Molvray *et al.* (1999). For example, within the New Zealand clade, *K. salicornioides* has decussate cladotaxy and inflorescences that are not sharply distinct from the vegetative branches (section *Korthalsella* in Danser 1937, 1940), whereas *K. clavata* and *K.*

lindsayi (section *Heterixia* in Danser 1937, 1940) have distichous cladotaxy and specialized inflorescences (see Fig. 12). Molvray *et al.* (1999) considered *K. salicornioides* to be a specialised form of *Heterixia* in which vegetative branches have secondarily become terete. Similarly within the E. Australian/Lord Howe Island, South Pacific and Hawaiian clades, *K. dacrydii, K. horneana* and *K. remyana* have decussate cladotaxy (section *Korthalsella* in Danser 1937, 1940) and the remaining taxa have distichous cladotaxy (section *Bifaria* in Danser 1937, 1940) (see Fig. 12). The New Zealand, Indian Ocean Basin, E. Australia/Lord Howe Island, South Pacific, and the Hawaiian clades have both flattened-stemmed and cylindrical-stemmed forms. Thus flattened- and terete-stemmed forms have evolved independently multiple times in several clades. In a molecular phylogenetic study of Phoradendreae (Viscaceae), Ashworth (2000a) also found that the three major clades identified in the molecular study could not be readily characterised morphologically. In another molecular phylogenetic study of the North American species of *Phoradendron,* Ashworth (2000b) found that species lacking cataphylls were polyphyletic given the divergent position of *P. californicum* Nutt. relative to other species lacking cataphylls. This study also showed a sister relationship between *P. rhipsalinum* Rzed. and *P. brachystachyum* (DC) Nutt. that was not evident from morphology or host associations.

My study shows that there is need for further sampling and a complete taxonomic revision of the genus as was also suggested by Molvray *et al.* (1999). While cladotaxy and internode characteristics are not reliable characters in terms of determining phylogenetic affinities within the genus, these can still be useful in regional identification. Additional characters, such as flower number in each floral cluster and inflorescence characters, may prove to be useful in conjunction with the host range of each species.

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Chapter 3: Host Range, Host Specificity, Regional Host Preferences and Distribution Segregation in Pygmy Mistletoes of New Zealand (*Korthalsella*: Viscaceae)

INTRODUCTION

Parasitic organsims can be generalists parasitising a wide range of unrelated hosts or can be specialists exhibiting high levels of host specificity sometimes utilising a single host species. Even in generalists, sometimes only a component of the available host range is preferentially utilised in a particular region. Intrinsic biological properties of host-parasite associations and emergent properties of their ecological and evolutionary relationships determine patterns of host specificity (Dick and Patterson 2007).

Host specificity is encountered in many phytophagous insects, plant-parasitic fungi and parasitic flowering plants. For example, many leaf beetles (family Chrysomelidae) are known to specialise on a single host plant species (Rane *et al.* 2000, Borowiec *et al.* 2008). Although the distribution of such specialists is dependent on the availability and abundance of the host species, which can also have a restricted range host specialisation can be advantageous as it minimises the competition from other species particularly when successful utilisation involves overcoming the defence mechanisms of a particular host species. *Danaus* Kluk butterflies for example are capable of utilising milkweeds (Asclepiadoideae: Apocynaceae) as larval food plants and their digestive proteases are able to break down the defensive proteins found in the latex of these plants (Pereira *et al.* 2010). Among the phytopathogenic fungi, "special forms" or *formae speciales* of the rust fungus *Puccinia graminis* Pers. are known to specialise on different grass hosts. *Puccinia graminis* f. sp. *tritici* Erikss. & Henning is parasitic on wheat, *P. graminis* f. sp. *avenae* Erikss. & Henning on oats and *P. graminis* f. sp. *secalis* Erikss. & Henning on rye (Alexopoulos *et al.* 1996). Each *forma specialis* has different physiological races; *P. graminis* f. sp. *tritici* for example, has about 350 races and different wheat cultivars show varying levels of susceptibility to these (Alexopoulos *et al.* 1996).

Parasitic plants constitute about 1% of the flowering plants (Heide-Jorgensen 2008) belonging to 276 genera in 27 families (Nickrent 2012), and these display varying degrees of host specificity. Stem parasites like *Cassytha* L. species (Lauraceae) have broad host ranges, while there are generalist as well as specialist species in *Cuscuta* L. (Convolvulaceae). Studies in *Cuscuta costaricensis* Yuncker (Kelly *et al.* 1988) show that it prefers to use just a subset of the potential hosts. *Rafflesia* R. Br. species (Rafflesiaceae) are restricted to members of the vitaceous genus *Tetrastigma* Planch. (Vitaceae) and each species is restricted to one or two of the 95 *Tetrastigma* species

(Nais 2001, Barcelona and Fernando 2002, Barcelona *et al.* 2006, Barcelona *et al.* 2008). Root parasites generally are capable of parasitising a wide range of unrelated hosts. *Dactylanthus taylorii* Hook. f. (Balanophoraceae), for example, parasitises a wide range of trees and shrubs (Ecroyd 1996). However, host specificity is known in some taxa, e.g., in *Orobanche amethystea* Thuill. (Orobanchaceae) (Heide-Jorgensen 2008) and *Cytinus hypocistis* L. (Cytinaceae) (Tutin *et al.* 1993).

Amongst the stem parasites (the mistletoes), *Viscum album* subsp. *album* L. (Viscaceae) has been recorded on numerous hosts (Barney 1998, Zuber and Widmer 2000), whereas, *V. minimum* Harv. parasitises only succulent *Euphorbia* L. species (Heide-Joregensen 2008). The Australian Christmas tree (*Nuytsia floribunda* (Labill.) R.Br. ex G.Don), an unusual member of Loranthaceae, has a remarkably diverse host range (Hopper 2010), while *Tristerix aphyllus* (Miers ex D.C.) van Tiegh. ex Barlow et Wiens, another loranthaceous mistletoe, is parasitic on the cacti, *Echinopsis* Zucc. spp. and *Eulychinia acida* Phil. (Kraus *et al.* 1995). Co-evolution of mistletoes and host species may over time lead to a tendency of having host specificity. Norton and de Lange (1999) examined the extent of host specificity in New Zealand's loranthaceous mistletoes and suggested that stability of host availability through space and time is the key factor in host specificity patterns. Host specificity is particularly advantageous when mistletoes parasitise homogeneous forests (Norton and de Lange 1999), thus enabling the mistletoes to utilise the most abundant host species. In mixed forest communities with high tree species diversity, many Viscaceae tend to have low host specificity and thus parasitise a wide range of hosts. Conversely in open forests or woodlands with low species diversity high host specificity is encountered (Barlow 1997).

Among the viscaceous mistletoes, host specificity has developed sufficiently within some species to produce host-specific races, while in other cases the specificity is at the species-level and the species are restricted to almost a single host species or group of related host taxa. *Arceuthobium* M. Bieb. species are parasitic only on conifers (Pinaceae and Cupressaceae) (Hawksworth and Wiens 1996). Mexican *Phoradendron brachystachyum* Nutt. is parasitic on the cactus *Pachycereus pringlei* Britton & Rose (Mauseth and Rebman 2008). European populations of *Viscum album* comprise three subspecies: *V. album* subsp. *album* is parasitic on deciduous trees, *V. album* subsp. *austriacum* on pines (*Pinus* spp.) and *V. album* subsp. *abietis* on firs (*Abies* Mill. spp.) (Zuber and Widmer 2000, Mejnartowicz 2006). Some *Viscum* L., *Notothixos* Oliver, *Phoradendron* Nutt. and *Dendrophthora* Eichler species are epiparasitic on other mistletoes.

Hosts parasitised by different species of the genus *Korthalsella* Tieghem throughout its global distribution belong mostly to families within the Magnoliids, Asterids, Asterids I and II, Rosid I/Fabidae and Rosid II/Malvidae clades in the APG III classification (Angiosperm Phylogeny Group III 2009) (see Table 1). The Malesian species, *Korthalsella dacrydii* (Ridley) Danser is the only *Korthalsella* species parasitic on conifers (genera *Dacrydium* Sol. ex G. Forst and *Podocarpus* L'Her. ex Pers.) (Barlow 1997).

Herbst (1980) collated the host records of Hawaiian *Korthalsella* spp. and Downey (1998) collated those for Australian viscaceaous and loranthaceous mistletoes, while the host range of New Zealand's loranthaceous mistletoes was compiled by de Lange *et al.* (1997). Only sporadic and scattered accounts of hosts of New Zealand's pygmy mistletoes have been made. This chapter represents the first attempt to document 1) the host range of each species of New Zealand's pygmy mistletoes, 2) the degree of host specificity in each species based on occurrence, 3) regional host preferences and 4) the potential for host specificity using molecular markers.

Table 1. Frequently parasitised host families of *Korthalsella* (based on literature) in APG III classification (Angiosperm Phylogeny Group III 2009).

Clade	Order	Family
Magnoliids	Laurales	Lauraceae
Rosid I/Fabidae	Celastrales	Celastraceae
	Oxalidales	Elaeocarpaceae
	Malpighiales	Euphorbiaceae
	Fabales	Fabaceae
	Fagales	Fagaceae
Rosid II/Malvidae	Myrtales	Myrtaceae
	Sapindales	Rutaceae
		Sapindaceae
Asterids	Ericales	Ebenaceae
		Ericaceae
		Myrsinaceae
		Sapotaceae
Asterid I	Gentianales	Apocynaceae
		Rubiaceae
	Lamiales	Oleaceae
Asterid II	Aquifoliales	Aquifoliaceae

MATERIALS AND METHODS

Host range and host specificity

Host records were collated from c. 800 herbarium sheets (Table 2) in New Zealand herbaria by examining every specimen for mistletoe species name and host species latin/common name. Records were also obtained from the literature and from the Department of Conservation database (Bioweb). In some instances data were acquired electronically from curators (64 records representing collections at NZFRI, UNITEC, WAIK, WELTU).

A host identification was made at the time if the host was visualised on the herbarium sheet and had not already been identified. *Kunzea* Rchb. and *Leptospermum* J. R. Forst & G. Forst hosts (Myrtaceae) were identified by Peter de Lange. As the taxa in the *Kunzea ericoides* (A. Rich.) Joy Thomps. complex remain unresolved, all *Kunzea–K. salicornioides* (Cunningham) Tiegh. combinations at CHR and AK were identified as provisional segregates - *Kunzea* aff. *ericoides* (a), *K.* aff. *ericoides* (b), *K.* aff. *ericoides* (c), *K.* aff. *ericoides* (d), *K. ericoides* var. *linearis* (Kirk) W. Harris, *K. ericoides* (A. Rich.) Joy Thomps. s.s. and *K. ericoides* var. *microflora* (G. Simpson) W. Harris (de Lange and Murray 2004, de Lange *et al.* 2005). *Coprosma* J. R. & G. Forst (Rubiaceae) hosts were identified by David Glenny (CHR). Peter Heenan (CHR) identified and/or confirmed the identity of *Carmichaelia* R. Br. and *Sophora L.* (Fabaceae) hosts. Synonymies and spelling mistakes of host names were checked from the New Zealand Plants website at Landcare Research (http://nzflora.landcareresearch.co.nz/). In some instances, host common names were transcribed into Latin names. Host records were categorised following de Lange *et al.* (1997) viz., records supported by a herbarium specimen showing the host branch with mistletoe (category 1), records based on communication from a reliable source (category 2), record on the herbarium sheet without host being collected (category 3) and literature records (category 4). Literature records are regarded as the least reliable sources of host information, unless these could be verified by voucher specimens showing the host-mistletoe association (de Lange *et al.* 1997).

A Shannon-Wiener index was used to infer the level of host specificity following Norton and de Lange (1999) who used this index to determine the level of host specificity in loranthaceous mistletoes of New Zealand. All *Kunzea ericoides* s.l. hosts that could not be ascribed to provisional segregate species in the genus *Kunzea* were not included when determining the Shannon-Wiener index value (i.e., observations of occurrences on *Kunzea* hosts for example not supported by herbarium specimens).

Table 2. Numbers of herbarium specimens of each *Korthalsella* species examined from different New Zealand herbaria. The numbers in braces represent the number of sheets out of the total where the host was not present on the sheet/ or was present but could not be identified yet was stated on the sheet. The numbers in parentheses represent the number of sheets out of the total where the host was not present/ or was present but could not be identified nor was stated on sheet.

	AK	CANU	CHBG	CHR	LINC	MPN	NZFRI	OTA	WAIK	WELT	WELTU	Unitec	Total
K. clavata	28 [1]	12	1	118 [2]		2	3	2	1	7 (1)	5		179
K. lindsayi	67 (2)	15	2	171 [8](3)	3	10	5	14 [1]	3	43	8	1	342
K. salicornioides	108	4	3	112 (2)	1	14	14	6	9	53 [1]	15		339
Total	203	31	6	401	4	26	22	22	13	103	28	1	860

AK	Auckland War Memorial Museum
CANU	University of Canterbury Herbarium
CHBG	Christchurch Botanic Gardens, Christchurch
CHR	Allan Herbarium, Landcare Research, Lincoln
LINC	Lincoln University Herbarium, Lincoln
MPN	Dame Ella Campbell Herbarium, Massey University, Palmerston North
NZFRI	National Forestry Herbarium, Rotorua
OTA	University of Otago Herbarium, Dunedin
UNITEC	Unitec Herbarium, Auckland
WAIK	University of Waikato Herbarium, Hamilton
WELT	Museum of New Zealand - Te Papa Tongarewa, Wellington
WELTU	HD Gordon Herbarium, Victoria University of Wellington

To compile regional host preferences, all records were mapped; records without grid references were assigned approximate place marks and the respective ecological regions (ERs) were determined using DOC-GIS (http://gis.doc.govt.nz/docgis/). On the basis of relative host record numbers, the most commonly encountered hosts were designated primary hosts, frequently encountered hosts (second to primary hosts in the number of records) were categorised as secondary hosts, hosts recorded from relatively few locations were considered tertiary hosts, whereas, hosts not usually parasitised and only parasitised in locations where they co-occur with mistletoe populations were categorised as occasional hosts. Hosts known from unique or rare co-occurrences were considered to be rare hosts. Distribution maps for each host were generated in ARC-GIS software. Host distributions were determined based on herbarium specimens of hosts in CHR.

Molecular genetic analysis of regional variability

Silica gel-dried samples of all three species were collected from New Zealand-wide surveys. Permits were obtained from the Department of Conservation to collect from reserves. Since these permits were intended for collection of silica-gel samples, entire mistletoes were not collected for the purpose of herbarium vouchers, so as not to unnecessarily deplete mistletoe biomass, though many of these sites have herbarium sheets made by other collectors. Recent herbarium specimens were also sampled to represent some populations (Appendix 1). These samples represent twenty-five populations of *K. clavata* (Kirk) Cheeseman, twenty-nine populations of *K. lindsayi* (Oliver ex J. D. Hooker) Engl. and fifty populations of *K. salicornioides*. Wherever possible an attempt was made to sample all host-mistletoe combinations from each population. Collection details for these samples are given in Appendix 1. A different chloroplast spacer region - *trnQ-rps16* was used in this study as a potentially more rapidly evolving region than *trnL-F* (alignments are provided as electronic appendices 3.1 and 3.2). Nuclear ITS and chloroplast *trnQ-rps16* regions were amplified in a 25 µl total volume comprising 10X ThermoPol reaction buffer (New England BioLabs), 10 mM dNTPs, 5 µM forward (nr ITS 7A: GAGTCATCAGCTCGCGTTGACTA, A. Plovanovich and J. Panero, unpub.; cp *trnQ*$^{(UUG)}$: GCGTGGCCAAGYGGTAAGGC, Shaw *et al.* 2007) and reverse primer (nr ITS 4: TCCTCCGCTTATTGATATGC, White *et al.* 1990; cp *rpS16x1*: GTTGCTTTYTACCACATCGTTT, Shaw *et al.* 2007), 5M Betaine, 0.5 unit NEB TAQ polymerase and c. 50 ng DNA template. For amplifying the ITS region, cycling parameters were 95°C for 1 min, 53°C for 1 min. followed by 72°C for 1 min for five cycles, followed by a decrease in the annealing temperature to 48°C for another 44 cycles. Cycling was followed by a 7 min final extension at 72°C. The cpDNA region was amplified using 95°C for 1 min, 50°C for 1 min. followed by 65°C for 4 min for 34 cycles. Cycling was followed by a 5 min final extension at 72°C. PCR products were verified by agarose gel electrophoresis. PCR products were then prepared for Sanger sequencing by treating with 5 units of exonuclease I and 0.5 unit shrimp alkaline phosphatase and incubating at 37°C for 30 min followed by 80°C for 15 min. These products were then sequenced on an ABI3730 using Big Dye (v.3.1) Terminator chemistry at Massey Genome Service (Palmerston North). Plastid and nuclear sequences were sequenced with both forward and verse primers and contigs were assembled in Sequencher v. 4.8 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.). The edited sequences were aligned in BioEdit (Hall 1999) using ClustalW and additional adjustments made manually. Parsimony analyses were conducted on separate ITS and chloroplast data sets in PAUP* v. 4.10 (Swofford 2002) using heuristic searches with tree-bisection-reconnection (TBR) branch-swapping, gaps coded as missing and with maximum trees setting of 50000. Bootstrap support for clades was determined on the basis of 1000 replicates (Felsenstein 1985).

Bayesian analyses were conducted on separate ITS and cpDNA data sets using MrBayes v.3.1.2 (Huelsenbeck & Ronquist 2001). The best fit model for each region (ITS1, 5.8S, ITS2, *trnQ-rps16* spacer) was determined using MrModeltest v.2.3 (Nylander 2004) prior to Bayesian phylogeny estimation. ITS1, 5.8S and ITS2 were analysed together, specifying the best fit model for each partition. MrBayes was run for at least 10^6 generations or until the average standard deviation of the split frequencies approached 0.001. Trees corresponding to the burn-in period (the first 25%) were discarded and a majority rule consensus constructed from the remaining trees. Haplotype data files were generated in Dna SP v. 5 (Librado and Rozas 2009) and median-joining networks were created in Network v. 4.6.1.1. (Bandelt *et al*. 1999).

RESULTS

Host range and regional host preferences

The hosts recorded for *Korthalsella clavata* include 42 taxa from 13 genera in 11 families (Table 3). *Coprosma propinqua* A. Cunn. (Rubiaceae) is the primary host, *C. dumosa* (Cheeseman) J. T. Jane is the secondary host, whereas, *Aristotelia fruticosa* Hook. f. (Elaeoacarpaceae), *Discaria toumatou* Raoul. (Rhamnaceae), *C. rigida* Cheeseman, *C. wallii* Petrie, and *C. virescens* Petrie are tertiary hosts for *K. clavata.* The only instance where *K. clavata* was recorded on a non-native host was from Fabaceae: *Ulex europaeus* L. (Fabaceae) (Fig. 1, Appendix 2). The primary, secondary and tertiary hosts (Table 6) parasitised by *K. clavata* belong to either the asterid I or rosid I clades following the APG III classification (Fig. 1).

The hosts recorded for *Korthalsella lindsayi* include 45 taxa from 20 genera in 15 families (Table 4). *Melicope simplex* A. Cunn. (Rutaceae) is the primary host, *Coprosma crassifolia* Colenso (Rubiaceae), *Lophomyrtus obcordata* (Sol. ex A. Cunn.) Burret (Myrtaceae), *Myrsine australis* (A. Rich.) Allan and *M. divaricata* A. Cunn. (Myrsinaceae) are the secondary hosts. *Coprosma areolata* Cheeseman, *C. linariifolia*

Hook. f., *C. rigida, C. rotundifolia* A. Cunn., *C. virescens, Muehlenbeckia complexa* (A. Cunn.) Meisn. (Polygonaceae) and *Sophora microphylla* Aiton (Fabaceae) are tertiary hosts for *K. lindsayi*. The primary, secondary and tertiary hosts (Table 7) parasitised by *K. lindsayi* belong to the rosid II, asterids, asterid I and rosid I clades in the APG III classification (Fig. 1). Non-native hosts recorded for *K. lindsayi* include *Cytisus scoparius* (L.) Link (Fabaceae) (Appendix 2: Fig. 2) (Fabaceae), *Olearia phlogopappa* (Labill.) DC. (= *Eurybia gunniana*) (Asteraceae) and *Syringa vulgaris* L. (Oleaceae) (Appendix 2: Fig. 3). Amongst the older literature records, the association of *K. lindsayi* on *Melicytus ramiflorus* J. R. Forst & G. Forst (Violaceae) (Chilton 1924) and *Sophora tetraptera* J. F. Mill. (Fabaceae) (Danser, 1937) could not be verified by a herbarium specimen. *Korthalsella lindsayi* has been occasionally recorded as a hyperparasite on the loranthaceous mistletoe *Ileostylus micranthus* (Hook. f.) Tiegh. (Loranthaceae) (CHR 22408, Appendix 2: Fig. 4). *Korthalsella lindsayi* has also been observed as a hyperparasite on *K. clavata* (CHR 286231, Appendix 2: Fig. 5). Auto-parasitism was also noted in *K. lindsayi* (CHR 608049, Appendix 2: Fig. 6).

The hosts recorded for *Korthalsella salicornioides* include 26 taxa from six genera in five families (Table 5). *Leptospermum scoparium* J. R. Forst & G. Forst s.l. (Myrtaceae) is the primary host, *Kunzea* aff. *ericoides* (b) (Myrtaceae) is the secondary host, whereas, *Kunzea* aff. *ericoides* (a) is the tertiary host for *K. salicornioides*. The primary, secondary and tertiary hosts (Table 6) parasitised by *K. salicornioides* all belong to the rosid II clade in the APG III classification (Fig 1). *Leptospermum scoparium* s.l. is the most favoured host followed by *Kunzea* aff. *ericoides* (b) and *Kunzea* aff. *ericoides* (a) (Table 8). Exotic hosts recorded for *Korthalsella salicornioides* include *Erica lusitanica* Rudolphi (Ericaceae), *E. arborea* L. (Appendix 2: Fig. 7) and *E. vagans* L. *Gaultheria* Kalm ex L. (Ericaceae) is mentioned as a host for *K. salicornioides* in Hooker's Flora Novae-Zelandiae (1853), while *Dracophyllum* Labill. (Ericaceae) was mentioned as a host for *K. salicornioides* in Hooker's Handbook of the New Zealand Flora (1864). However, no herbarium sheet in New Zealand herbaria, the British Museum (BM) nor Kew Botanic Gardens (K) was found showing any association either with *Gaultheria* or *Dracophyllum*. The mention of *Gaultheria* and *Dracophyllum* as hosts for *K. salicornioides* in Cheeseman (1906) and Allan (1961) probably also stems from these unverified historical records. The occurrence of *K. salicornioides* on *Myrsine australis* (Myrsinaceae) (Eagle 2006) also could not be verified by a herbarium specimen.

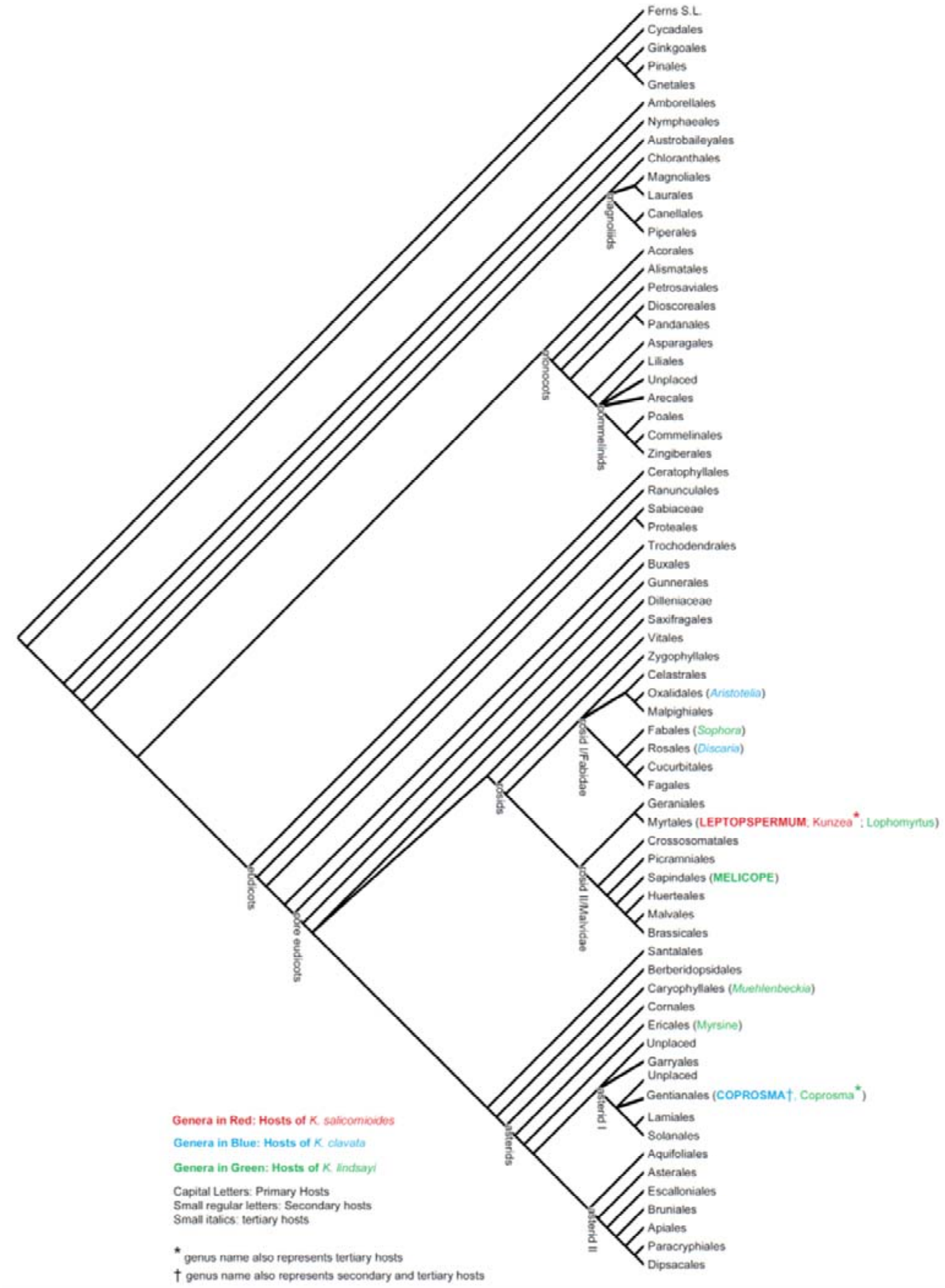


Fig. 1. Orders representing primary, secondary and tertiary hosts of New Zealand species of *Korthalsella* (tree redrawn from Stevens 2001 - onwards).

Double parasitism was noted in a few instances, whereby *K. salicornioides* and *K. lindsayi* were seen parasitising a single *Melicope simplex* (Rutaceae) (Appendix 2: Fig. 8), similarly *K. clavata* and *K. lindsayi* were seen on the same branch of *M. simplex* (Appendix 2: Fig. 9), *Muehlenbeckia* Meisn. sp. (Polygonaceae) (Appendix 2: Fig. 10) and *Sophora microphylla* Aiton x *prostrata* Buchanan (Fabaceae) (Appendix 2: Fig. 11). These examples of dual parasitism are very rare and are known from only a few localities having sympatric populations of *K. salicornioides* and *K. lindsayi* /*K. lindsayi* and *K. clavata*.

The host families Apiaceae, Polygonaceae, Ranunculaceae and Rhamnaceae have not been documented as hosts for any species of *Korthalsella* outside New Zealand, thus the vines like *Scandia geniculata* (G. Forst.) J. W. Dawson (Apiaceae) (Appendix 2: Fig. 12), *Muehlenbeckia complexa*, *M. australis* (G. Forst.) Meisn. and *Clematis marata* J. B. Armstr. (Ranunculaceae) are unique host examples amongst the Korthal mistletoes.

Korthalsella salicornioides is the most host-specific among the three species with a Shannon-Wiener index value of 1.17. *Korthalsella clavata* and *K. lindsayi* are generalist species with Shannon-Wiener index values of 2.88 and 2.84, respectively.

Table 3. Host list for *Korthalsella clavata.* (in the current and following two tables, 1=records supported by a herbarium specimen showing the host branch with mistletoe, 2=records based on communication from a reliable source, 3=record on the herbarium sheet without host being collected and 4=literature record).

Host Family	Host genus	Host Species	Reliability level		
			1	2	4
Asteraceae	*Olearia* Moench	*O. avicenniifolia* (Raoul) Hook. f.	CHR 286181		
		O. fimbriata Heads	CHR 574669		
		*O. fragrantissima*Petrie	AK 238639		
		O. nummulariifolia Hook. f.	CHR 416703		
		O. paniculata (J. R. et G. Forst.) Druce	CHR 188760		
		O. solandri Hook. f.	CHR 529058		
Elaeocarpaceae	*Aristotelia* L'Hér.	*A. fruticosa* Hook. f.	CHR 107889		
Fabaceae	*Carmichaelia* R. Br.	*C. australis* R. Br.		A. Sultan pers.obs	
		C. petriei Kirk	CHR 286199		
	Sophora L.	*S. microphylla* Aiton	CHR 286182		
		S. microphylla x *prostrata*	CHR 286224		
		S. prostrata Buchanan			New Zealand Botanical Society 2007
	Ulex L.	*U. europaeus* L.*	CHR 535286		
Myrsinaceae	*Myrsine* L.	*M. australis* (A. Rich.) Allan	CHR 278928		
		M. divaricata A. Cunn.	CHR 116318		
Pittosporaceae	*Pittosporum* Banks & Sol. ex Gaertn.	*P. tenuifolium* Sol. ex Gaertn.	CHR 286113		
Polygonaceae	*Muehlenbeckia* Meisn.	*M. australis* (G. Forst.) Meisn.	CHR 286115		
		M. complexa (A. Cunn.) Meisn.	CHR 286211		

Host Family	Host genus	Host Species	Reliability level		
			1	2	4
Ranunculaceae	*Clematis* L.	*C. marata* J. B. Armstr.	CHR 184313		
Rhamnaceae	*Discaria* Hook.	*D. toumatou* Raoul.	OTA 004226		
Rubiaceae	*Coprosma* J. R. & G. Forst	*C. areolata* Cheeseman	CHR 568810		
		C. ciliata Hook. f.	CHR 536534		
		C. colensoi Hook. f.	CHR 178951		
		C. crassifolia Colenso	CHR 41272		
		C. decurva Heads	CHR 366233		
		C. dumosa (Cheeseman) J. T. Jane†	CHR 212998		
		C. elaterioides de Lange & A, S, Markey	CHR 366221		
		C. foetidissima J. R. Forst & G. Forst.	CHR 363576		
		C. linariifolia Hook. f.	NZFRI 20322		
		C. microcarpa Hook. f.	NZFRI 9203		
		C. obconica Kirk	AK 235472		
		C. pedicellata Molloy, de Lange & B. D. Clarkson	AK 228392		
		C. propinqua A. Cunn.var. *propinqua*	CHR 291036		
		C. propinqua var. *latiuscula* Allan	CHR 200115		
		C. rigida Cheeseman	AK 261237		
		C. rotundifolia A. Cunn.	CHR 537555		
		C. rugosa Cheeseman	CHR 252870		
		C. tenuicaulis Hook. f.	CANU 25711		
		C. virescens Petrie	CHR 537565		
		C. wallii Petrie	CHR 269216		
Rutaceae	*Melicope* J. R. Forst. & G. Forst.	*M. simplex* A. Cunn.	CHR 573250		
Thymelaeaceae	*Pimelea* Sol. ex	*P. oreophila* C. J. Burrows x *P.*	CHR 608048		

Host Family	Host genus	Host Species	Reliability level		
			1	2	4
	Gaertn.	*declivis* C. J. Burrows			
11 families	13 genera	42 taxa	40	1	1
Indigenous	12 genera	41 taxa	39	1	1
Exotic	1 genus	1 species	1		

† *Coprosma tayloriae* has been synoymised with *C. dumosa* (Glenny and Cruickshank 2011)

Table 4. Host list for *Korthalsella lindsayi*.

Host Family	Host genus	Host Species	1	2	3	4
Apiaceae	*Scandia* J. W. Dawson	*S. geniculata* (G. Forst.) J. W. Dawson (=*Angelica geniculata* Forst. f. (Hook. f.)	CHR 22409			
Araliaceae	*Pseudopanax* K. Koch	*P. ferox* Kirk	WELT SP 087688			
Asteraceae	*Olearia*	*Olearia phlogopappa* (Labill.) DC. (=*Eurybia gunniana; Olearia gunniana*)*	WELT SP42541			
		Olearia solandri	MPN 45522			
	Helichrysum Mill.	*H. lanceolatum* (Buchanan) Kirk (= *H. glomeratum*)				Stevenson 1934, Barkla 2009
Fabaceae	*Carmichaelia*	*C. australis*	CHR 174109			
		C. petriei (=*C. ramosa*)	CHR 342367			
	Cytisus Desf.*	*C. scoparius* (L.) Link*	CHR 215913			
	Sophora	*S. godleyi* Heenan & de Lange	CHR 33917			
		S. microphylla (=*Edwardsia microphylla*)	CHR 129466			
		S. microphylla x prostrata	CHR 286223			
		S. prostrata	CHR 462574			
Loranthaceae	*Ileostylus* Tiegh.	*I. micranthus* (Hook. f.) Tiegh. (=*Loranthus micranthus*)	CHR 22408			
Myrsinaceae	*Myrsine*	*M. australis* (=*Myrsine urvillei*)	CHR 232461			
		M. divaricata	CHR 286114			
Myrtaceae	*Lophomyrtus* Burret	*L. bullata* (Sol. ex A. Cunn.) Burret (=*Myrtus bullata*)	CHR 33925			
		L. bullata x *obcordata*	CHR 473598			
		L. obcordata (Raoul) Burret (=*Myrtus obcordata*)	CHR 33920			
	Metrosideros Banks ex Gaertn.	*M. colensoi* Hook. f.			CHR 33928^{+}	
		M. diffusa (G, Forst.) Sm. (=*M. hypericifolia*)	CHR 33922			
	Neomyrtus Burret	*Neomyrtus pedunculata* (Hook. f.) Allan	WELT SP053941			

Host Family	Host genus	Host Species	1	2	3	4
Oleaceae	*Syringa* L.*	*S. vulgaris* L.*	CHR 286145			
Pittosporaceae	*Pittosporum*	*P. cornifolium* A. Cunn.	CHR 416850			
		P. obcordatum Roul	CHR 484537			
Polygonaceae	*Muehlenbeckia*	*M. astonii* Petrie	CHR 165214			
		M. australis	AK 230829			
		M. complexa	CHR 22413			
Rhamnaceae	*Discaria*	*D. toumatou*	CHR 268921			
Rubiaceae	*Coprosma*	*C. areolata*	CHR 552681			
		C. crassifolia	CANU 3499b			
		C. decurva		JW Barkla, pers. comm.		
		C. linariifolia	OTA 017953			
		C. obconica	CHR 286225			
		C. propinqua	AK 281594			
		C. rhamnoides A. Cunn.	CHR 33928			
		C. rigida	CHR 33923			
		C. rotundifolia	CHR 107893			
		C. rubra Petrie	AK 231902			
		C. dumosa	CHR 171988			
		C. virescens	CHR 269215			
		C. wallii	AK 283292			
Rutaceae	*Melicope*	*M. simplex*	AK 232463			
		M. simplex A. Cunn. x *ternata* J. R. Forst. & G. Forst.				Druce 1966
Viscaceae	*Korthalsella*	*K. clavata*(Kirk) Cheeseman	CHR 286231			
14 families	19 genera	44 taxa	40	1	1	2
Indigenous	17 genera	41 taxa	37	1	1	2
Exotic	2 genera	3 species	3			

[+] The host recorded for CHR 33928 is *Coprosma rhamnoides*, *Metrosideros colensoi* was listed as a host on the sheet by AG Healy

Table 5. Host list for *Korthalsella salicornioides.*

Host Family	Host genus	Host Species	1	2	4
Ericaceae	*Erica* L.*	*E. arborea* L.*	CHR 499558		
		E. lusitanica Rudolphi*			Nickrent 2012, Bannister 1989
		E. vagans L.*			Bannister 1989
Fabaceae	*Sophora*	*S. chathamica* Cockayne		Peter de Lange, pers. comm.	
		S. microphylla	CHR 35289		
Myrtaceae	*Kunzea*	*K.* aff. *ericoides* (a)	AK 281702		
		K. aff. *ericoides* (a) x *K. ericoides* var. *linearis* ?	AK 24435		
		K. ericoides (A. Rich.) Joy Thomps. s.str.	AK 282237		
		K. ericoides s.str. x *K.* aff. *ericoides* (b)	CHR 33188		
		K. ericoides var. *linearis* (Kirk) W. Harris	AK 248067		
		K. aff. *ericoides* (b)	AK 232919		
		K. aff. *ericoides* (b) x *K.* aff. *ericoides* (a) ?	AK 232145		
		K. aff. *ericoides* (c)	AK 247672		
		K. aff. *ericoides* (b) x *K.* aff. *ericoides* (c)	CHR 479494		
		K. sinclairii (Kirk) W. Harris	AK 237879		
		K. sinclairii x *K.* aff. *ericoides* (b)		Peter de Lange, pers. comm.	
		K. ericoides var. *microflora* x *K.* aff. *ericoides* (b)	AK 256158		
		K. ericoides var. *microflora* (G. Simpson) W. Harris	AK 303695		
		K. aff. *ericoides* (d)	AK 300906		

Host Family	Host genus	Host Species	1	2	4
	Leptospermum J. R. Forst & G. Forst	*L. scoparium* J. R. Forst & G. Forst s.str.	CHR 87369		
		L. scoparium var. *incanum* Cockayne	AK 301701		
		L. aff. *scoparium* (a)	AK 211611		
Rubiaceae	*Coprosma*	*C. propinqua*		Amir Sultan, pers. obs.	
		C. rhamnoides		Townsend A, pers. comm.	
		C. tenuicaulis	CHR 572247		
Rutaceae	*Melicope*	*M. simplex*	AK 230860		
5 families	6 genera	26 taxa	20	4	2
Indigenous	5 genera	23 taxa	19	4	2
Exotic	1 genus	3 species	1		

Regional host patterns of K. clavata:

The dominant host genus for *K. clavata* is *Coprosma* (Rubiaceae) (c. 66% of records) followed by the genus *Aristotelia* (Elaeocarpaceae) (8.5%), *Discaria* (Rhamnaceae) (6%), *Olearia* (Asteraceae) (4.5%), *Myrsine* (Myrsinaceae) (3.5%), *Muehlenbeckia* (Polygonaceae) (3%) and *Melicope* (Rutaceae) (2%).

2A. *Coprosma propinqua*

2B. *C. dumosa*

2C. *Aristotelia fruticosa*

2D. *C. rigida*

2E. *C. wallii* 2F. *C. virescens*

2G. *Discaria toumatou*

Fig. 2 Distribution of *K. clavata* on primary, secondary & tertiary hosts. For each *K. clavata*-host combination ecological regions (ERs) in dark grey represent the overall known distribution of the host based on herbarium records, ERs in light grey are those where the host is absent, in Fig 2A ● represents a herbarium record of mistletoe-host combination, ✚ represents an observation of mistletoe-host combination not based on a herbarium record, in Figs. 2B-2G ● represents a herbarium record of mistletoe-host combination where mistletoe is not known to occur on the primary host (*Coprosma propinqua*), ◉ represents a herbarium record of mistletoe-host combination where the mistletoe is known to occur on the primary host also, ✚ represents an observation of mistletoe-host combination not based on a herbarium record where the mistletoe is not known to occur on the primary host while ⊕ represents an observation of mistletoe-host combination not based on a herbarium record where the mistletoe is known to occur on the primary host.

Coprosma propinqua is the primary *Coprosma* host (c. 40% of all *Coprosma* hosts), followed by *C. dumosa* (16%). *C. wallii* (11%), *C. rigida* (8%), *C. virescens* (4.5%) (Table 6).

Regional patterns are discussed below with regard to the botanical provinces (see also Table 6) of New Zealand (see Wardle 1991 for details on botanical provinces).

On the Volcanic Plateau where *K. clavata* is rare (Central Volcanic Plateau, Tongariro and Moawhango ERs) *Coprosma* hosts are predominant (3 of the 4 records), with a single record is from *Myrsine divaricata*. *Coprosma* hosts recorded are *Coprosma colensoi, C. propinqua* and *C. dumosa*.

Taranaki: All records from Rangitikei ER are from *Coprosma* hosts. Half of the records are on *Coprosma wallii* (4/8 records), other species parasitised include *C. propinqua*, *C. linariifolia*, *C. obconica* and *C. dumosa*.

Southern North Island (records from Wairarapa Plains and Tararua ERs): *Coprosma propinqua* (4/6 records) is the predominant host in Wairarapa Plains ER, *Myrsine australis* and *Muehlenbeckia complexa* are also parasitised. There is a single record each from *Coprosma propinqua* and *Olearia solandri* in Tararua ER.

Sounds Nelson: Records from Nydia Bay area in Sounds-Wellington ER are from *Coprosma propinqua* (2/5 records), *Olearia solandri* (2/5 records) and a single record from *Ulex europaeus*.

Marlborough (records from Inland Marlborough ER): Recorded on *Coprosma propinqua, C. foetidissima* and *Sophora prostrata* in Marlborough.

Canterbury (records from Lowry, Puketeraki, Canterbury Foothills, Canterbury Plains, Banks, D'Archiac, Heron, Pareora, Mackenzie and Kakanui ERs): *Coprosma* hosts predominate (61/109 records). *Coprosma propinqua* is the most common *Coprosma* host (28/61 of *Coprosma* hosts). *Coprosma dumosa* (11/61) and *C. rigida* (6/61) are also often parasitised. Sometimes *C. linariifolia, C. obconica, C. crassifolia, C. virescens, C. pedicellata, C. rugosa, C. ciliata* and *C. areolata* are parasitised. Genera utilised occasionally or rarely include *Muehlenbeckia* (*M. complexa* and *M. australis*), *Myrsine* (*M. divaricata*), *Carmichaelia* (*C. australis* and *C. petriei*), *Sophora* (*S. microphylla*, *S. microphylla* x *prostrata*), *Olearia* (*O. paniculata, O. nummulariifolia, O. avicenniaefolia*), *Melicope* (*M. simplex*), *Pittosporum* (*P. tenuifolium*) and *Pimelea* (*P. oreophila* x *declivis*).

Otago (records from Lakes, Central Otago and Otago Coast ERs): *Coprosma* hosts comprise 3 of 7 records (*C. propinqua,* 2/3 and *C. virescens,* 1/3 of *Coprosma* hosts). Other records are from *Aristotelia fruticosa, Discaria toumatou, Olearia fragrantissima* and *O. fimbriata*.

Westland (records from North Westland, Spenser, Whataroa and Aspiring ERs): *Coprosma* hosts are predominant (33/35 records). *Coprosma propinqua* (10/33 of *Coprosma* hosts), *C. dumosa* (8/33), *C. wallii* (7/33) are the main *Coprosma* hosts. Other *Coprosma* spp. parasitised include *C. rigida, C. elatirioides, C. decurva, C. tenuicaulis, C. microcarpa* and *C. ciliata.* Sometimes *Myrsine australis* is also parasitised.

Fiordland: *Coprosma* hosts comprise three of five records (*C. rigida* 2/3, *C. wallii* 1/3). Non-*Coprosma* hosts include *Aristotelia fruticosa* and *Myrsine divaricata*.

Southland (records from Catlins, Mavora, Southland Hills, Te Wae Wae and Makarewa ERs): *Coprosma* hosts predominate (14/17 records). *Coprosma propinqua, C. virescens, C. wallii, C. obconica, C. rotundifolia* and *C. rigida* are the *Coprosma* hosts parasitised. Other hosts include *Melicope simplex* and *Myrsine divaricata*.

Korthalsella clavata is rare in the North Island and therefore, not surprisingly, some mistletoe-host combinations for *K. clavata* are restricted to the South Island even though the mistletoe and host both have a wider range than this (*Aristotelia fruticosa* Fig. 2C, *Coprosma rigida* Fig 2D, *C. virescens* Fig. 2F, and *Discaria toumatou* Fig. 2G). On the other hand, although in many cases the use of the secondary, tertiary and occasional or rare hosts occurs in sites where the primary host is also being utilised, some records on secondary and tertiary hosts may be the result of range extension since they occur at sites where the primary host is apparently not being utilised (symbols ● & ✚ in Fig 2D, E, F, G). With regard to occurrences in different ecological regions, records on *Coprosma dumosa* in Moawhango ER; on *C. virescens* and *Discaria toumatou* in Lowry ER; on *C. rigida* and *C. dumosa* in Canterbury Plains ER; on *C. virescens* in Banks and Otago Coast ERs; on *Aristotelia fruticosa, C. rigida* and *C. wallii* in Fiord ER and on *C. rigida, C. wallii* and *C. virescens* in Te Wae Wae ER represent range extensions through secondary/tertiary hosts only.

Regional host patterns of K. lindsayi:

The dominant host genus for *K. lindsayi* is also *Coprosma* (Rubiaceae) (c. 36% of total records, see Fig. 16M for distribution on *Coprosma* hosts) followed by *Melicope*

(Rutaceae) (22%), *Lophomyrtus* (Myrtaceae) (13%), *Myrsine* (Myrsinaceae) (13.5%), *Muehlenbeckia* (Polygonaceae) (4%) and *Sophora* (Fabaceae) (3%).

Melicope simplex is the dominant host in terms of records on a single host species and is therefore the primary host (see Fig 3A for distribution on *M. simplex*). *Coprosma crassifolia* is the dominant *Coprosma* host (c. 33% of all *Coprosma* hosts), followed by *Coprosma areolata* (14%). *Coprosma rigida, C. virescens*, *C. rotundifola* and *C. linariifolia,* comprising 10, 9, 7 and 6% respectively of all *Coprosma* hosts. Regional patterns in different botanical provinces of New Zealand are discussed below (see also Table 7).

Gisborne (records from Urewera and Wairoa ERs): *Melicope simplex* comprises 2 of 8 records. Other hosts include *Coprosma virescens*, *Lophomyrtus obcordata*, *Myrsine australis*, *M. divaricata*, and *Pittosporum obcordatum*.

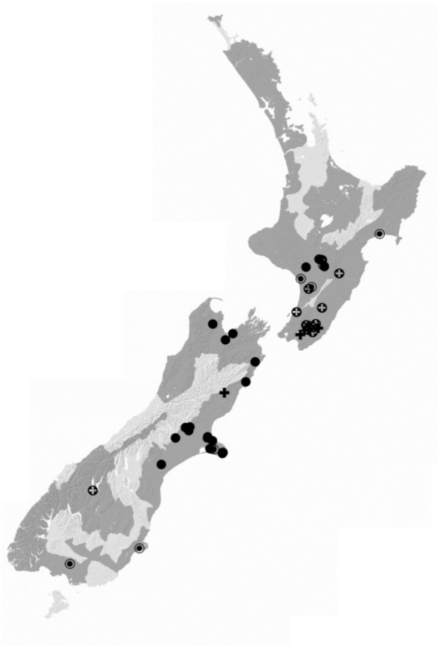
Volcanic Plateau: The only record is from Mount Pureora in Western Volcanic Plateau ER on *Myrsine divaricata*.

Taranaki (records from Rangitikei ER): Melicope simplex comprises 21 of 66 records. *Coprosma* hosts comprise 28 records. *Coprosma* species parasitised include *C. crassifolia*, *C. areolata*, *C. linariifolia*, *C. rhamnoides*, *C. rigida*, *C. rotundifolia*, *C. virescens*, *C. obconica*, *C. rubra* and *C. wallii*. The genus *Myrsine* comprises 10 records (*M. divaricata* 8, *M. australis* 2) and *Lophomyrtus obcordata* comprises 6 records.

Southern North Island (records from Ruahine, Hawkes Bay, Manawatu, Pahiatua, Eastern Hawkes Bay, Eastern Wairarapa, Wairarapa Plains, Tararua ERs and Wellington ED): *Coprosma* hosts comprise 62 of 168 records. Main *Coprosma* hosts include *C. crassifolia* (20/62 of *Coprosma* records)*, C. areolata* (10/62 of *Coprosma* records) and *C. rigida* (9/62 of *Coprosma* records). *Coprosma virescens, C. rotundifolia, C. propinqua, C. rhamnoides, C. obconica, C. rubra* and *C. linariifolia* are sometimes also parasitised. *Melicope* comprises 30 records (*M. simplex* 29, *M. simplex* x *ternata* 1), *Myrsine* 29 (*M. divaricata* 21, *M. australis* 8), *Lophomyrtus* 22 (*L. obcordata* 19*, L. bullata,* 2*, L. bullata* x *obcordata,* 1), *Muehlenbeckia* 14 (*M. complexa* 8, *M. australis* 3, *M. astonii* 3) and *Sophora* comprises two records (*S. microphylla*, 1, *S. godleyi*, 1). *Olearia solandri, Pittosporum obcordatum, Carmichaelia australis, Metrosideros diffusa, Ileostylus micranthus* and *Scandia geniculata* are also rarely used as hosts.

3A. *Melicope simplex*

3B. *Coprosma crassifolia*



3C. *Lophomyrtus obcordata*

3D. *Myrsine australis*

3E. *M. divaricata*

3F. *Coprosma areolata*

3G. *C. linariifolia*

3H. *C. rigida*

3I. *C. rotundifolia*

3J. *C. virescens*

3K. *Muehlenbeckia complexa*

3L. *Sophora microphylla*

3M. All *Coprosma* hosts

Fig. 3 Distribution of *K. lindsayi* on primary, secondary and tertiary hosts. For each *K. lindsayi*-host combination, ecological regions (ERs) in dark grey represent the overall known host distribution based on herbarium records, ERs in light grey are those where the host is absent, in Fig 3A ● represents a herbarium record of mistletoe-host combination, ✚ represents an observation of mistletoe-host combination not based on a herbarium record, in Figs 3B-3M ● represents a herbarium record of mistletoe-host combination where mistletoe is not known to occur on the primary host (*Melicope simplex*), ◉ represents a herbarium record of mistletoe-host combination where mistletoe is known to occur on the primary host also, ✚ represents an observation of mistletoe-host combination not based on a herbarium record where the mistletoe is not known to occur on the primary host while ⊕ represents an observation of mistletoe-host combination not based on a herbarium record where the mistletoe is known to occur on the primary host

Sounds Nelson (records from Cook Strait, Sounds EDs, Richmond and Nelson ERs): *Melicope simplex* comprises 4/18 records. *Coprosma* hosts comprise 5 of 18 records (*C. crassifolia*, 2, *C. linariifolia*, 1, *C. virescens*, 1, *C. rubra*, 1). *Lophomyrtus* comprises 7 of 18 records (*L. obcordata* 2, *L. bullata* 4, *L. bullata* x *obcordata,* 1). *Muehlenbeckia complexa* is sometimes also parasitised and a record from Nukuwaiata Island is from *Pittosporum cornifolium*.

Marlborough (records from Wairau, Inland Marlborough, Molesworth and Kaikoura ERs): *Sophora* hosts comprise 4 of 11 records (*S. microphylla*, 2, *S. prostrata*, 2). *Lophomyrtus obcordata* comprises 3 records, *Coprosma crassifolia* comprises 2 records and *Myrsine australis* comprises 1 record.

Western Nelson (records from North-West Nelson ER): All five records from Takaka and Cobb valleys are from *Lophomyrtus obcordata*.

Canterbury (records from Lowry, Canterbury Foothills, Canterbury Plains, Banks, Pareora, Wainono and Mackenzie ERs): *Coprosma* hosts comprise 40 of 103 records. Main *Coprosma* hosts include *C. crassifolia, C. rotundifolia, C. areolata, C. rigida* and *C. virescens*. *Coprosma linariifolia, C. propinqua, C. rhamnoides, C. rubra, C. obconica* and *C. dumosa* are sometimes also utilised. *Melicope simplex* comprises 17 of 103 records, *Lophomyrtus obcordata* 20, *Myrsine* 8, (*M. australis* 4, *M. divaricata* 4) and *Sophora* comprises 8 of 103 records (*S. microphylla* 4, *S. microphylla* x *prostrata* 2, *S. prostrata* 3). *Muehlenbeckia complexa*, *Carmichaelia australis, Cytisus scoparius, Syringa vulgaris, Ileostylus micranthus* and *Discaria toumatou* are occasional or rare hosts.

Otago (records from Lakes, Central Otago and Otago Coast ERs): *Melicope simplex* is the predominant host (23 of 60 records). *Coprosma* hosts comprise 17 records (*C. crassifolia* 9, *C. areolata* 4, *C. propinqua* 1, *C. linariifolia* 1, *C. decurva* 1, *C. virescens* 1). The genus *Myrsine* comprises 7 records (*M. divaricata* 4, *M. australis* 3). *Lophomyrtus obcordata* comprises 2 records. *Sophora microphylla, Muehlenbeckia australis, Carmichaelia petriei, Discaria toumatou, Helichrysum glomeratum, Pseudopanax ferox* and *Neomyrtus pedunculata* are sometimes also parasitised.

Westland: The only record is from Landsborough Station in Aspiring ER on *Coprosma crassifolia*.

Southland (records from Catlins, Gore, Southland Hills, Te Wae Wae and Makarewa ERs): *Melicope simplex* comprises 6 of 14 records. *Coprosma* hosts comprise 6 records (*C. crassifolia* 1, *C. linariifolia* 2, *C. areolata* 1, *C. obconica* 1, *C. virescens* 1). *Lophomyrtus obcordata, Myrsine divaricata* and *Sophora microphylla* constitute 1 record each.

Fiordland: The only record from Fiordland is from *Myrsine divaricata*.

Some records on secondary and tertiary hosts account for range extension through occurrence on non-primary hosts only (symbols ● & ✚ in Fig. 3C, E, G, H, K, L; Table 7). With regard to occurrences in different ecological regions, records of *K. lindsayi* on *Myrsine divaricata* in Western Volcanic Plateau and Fiord ERs, on *Coprosma crassifolia, C. areolata, C. virescens* and *Muehlenbeckia complexa* in Tararua ER represent range extensions through secondary/tertiary hosts on the North Island. Records on *C. crassifolia* in Wairau and Aspiring ERs, on *Lophomyrtus obcordata* in Inland Marlborough and North-West Nelson ERs, on *Sophora microphylla* in Molesworth ER, on *C. crassifolia, L. obcordata, Myrsine australis* and *S. microphylla* in Kaikoura ER, on *C. crassifolia* and *L. obcordata* in Lowry ER, on *C. crassifolia, C. rotundifolia* and *L. obcordata* in Canterbury Foothills ER and on *C. linariifolia, C. rigida, M. divaricata, M. complexa* and *S. microphylla* in MacKenzie ER represent range extensions through secondary/tertiary hosts on the South Island.

Regional host patterns of K. salicornioides:

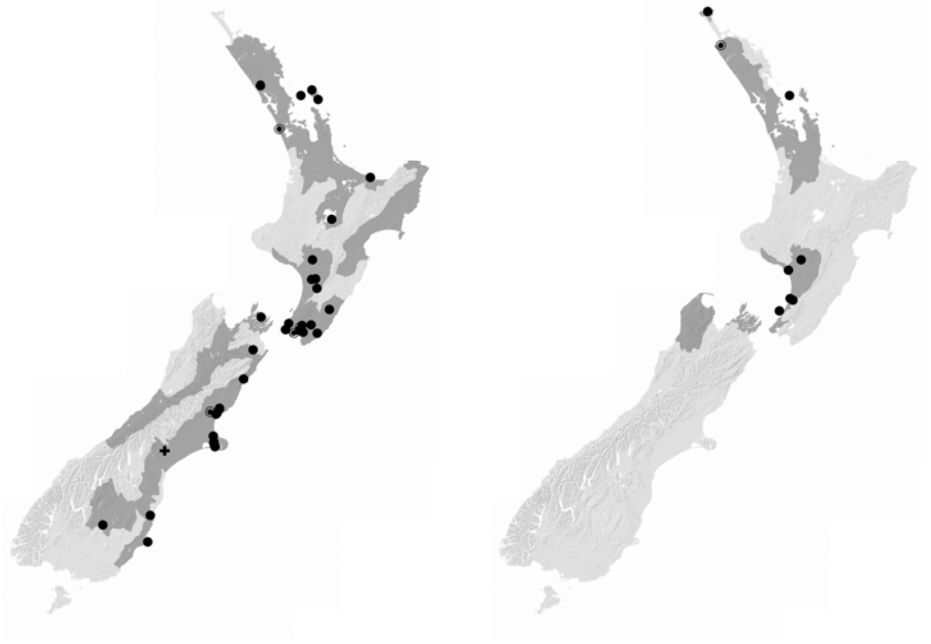
The primary host for *K. salicornioides* is *Leptospermum* (Myrtaceae) (c. 57% of total records) followed by members of the genus *Kunzea* (Myrtaceae) (c. 39% of total records). *Kunzea* aff. *ericoides* (b) is the dominant *Kunzea* host within the *Kunzea ericoides* complex (c. 51% of total *Kunzea* hosts examined at CHR and AK), followed by *Kunzea* aff. *ericoides* (a) (c. 16%).

Regional patterns are discussed below with regard to the botanical provinces (see also Table 8) of New Zealand.

In Northland (records from Te Paki, Western Northland and Eastern Northland Ecological Regions): *Leptospermum* comprised the dominant host genus (45/57). The dominant *Leptospermum* taxa in Northland are *L. scoparium* var. *incanum* and *L.* aff. *scoparium* (a) and of the unidentified *K. salicornioides* on *Leptospermum* records from Northland, most are expected to belong to either of these two taxa. The dominant *Kunzea* taxon in Northland is *K. ericoides* var. *linearis,* followed by *K.* aff. *ericoides* (a), and of all *Kunzea* records from Northland most are expected to be from *K. ericoides* var. *linearis*. *Coprosma rhamnoides* is parasitised along with *L. scoparium* var. *incanum* at Mahinepua Peninsula in Eastern Northland ER.

In Auckland (records from Kaipara, Auckland, Coromandel, Waikato and Tainui ERs): *Leptospermum* is the dominant host genus in the Auckland province (46/64) followed by *Kunzea* hosts (17/64). The main *Kunzea* host in Auckland Province is *Kunzea* aff. *ericoides* (b) followed by *Kunzea* aff. *ericoides* (a) while a few historic gatherings (1866) are from *K. ericoides* var. *linearis*. *Coprosma rhamnoides* is rarely parasitized in Auckland ER. *K. sinclairii* and its hybrid with *K.* aff. *ericoides* (b) are sometimes parasitised on Mount Young on Great Barrier Island. Peninsular populations of *K. salicornioides* in Coromandel ER are known from *Leptospermum* hosts only; Mayor Island, Little Barrier Island populations are parasitic on *Kunzea* hosts, while Great Barrier Island populations exist both on *Kunzea* and *Leptospermum* hosts. The only record from Tainui ER is from a *Kunzea* host.

4A. *Leptospermum scoparium* s.l. 4B. *Kunzea ericoides* s.l.



4C. *Kunzea* aff. *ericoides* (b) 4D. *Kunzea* aff. *ericoides* (a)

Fig. 4 Distribution of *K. salicornioides* on primary, secondary & tertiary hosts. For each *K. salicornioides*-host combination ecological regions (ERs) in dark grey represent the overall known distribution of host, ERs in light grey are those where the host is absent, in Fig 4A ● represents a herbarium record of mistletoe-host combination, ✚ represents an observation of mistletoe-host combination not based on a herbarium record, in Figs 4B-4D ● represents a herbarium record of mistletoe-host combination where mistletoe is not known to occur on the primary host (*Leptospermum scoparium* s.l.), ◉ represents a herbarium record of mistletoe-host combination where mistletoe is known to occur on the primary host also, ✚ represents an observation of mistletoe-host combination not based on a herbarium record where the mistletoe is not known to occur on the primary host while ⊕ represents an observation of mistletoe-host combination not based on a herbarium record where the mistletoe is known to occur on the primary host

On the Volcanic Plateau and the Bay of Plenty, *Leptospermum* is the main host (32/51). *Kunzea* hosts comprise of 19 out of 51 records; *K. ericoides* var. *microflora* and *K.* aff. *ericoides* (b) being the main *Kunzea* hosts. *K. ericoides* var. *microflora* hybrid with *K.* aff. *ericoides* (b) and *K.* aff. *ericoides* (d) are sometimes also parasitized.

Gisborne: The only historic record from Wairoa ER in the Gisborne province is from a *Kunzea* host.

Taranaki: Records from Rangitikei ER in Taranaki province are from *Kunzea* hosts [*Kunzea* aff. *ericoides* (a) and *K.* aff. *ericoides* (b)] and from *Melicope simplex.*

In southern North Island (Manawatu, Eastern Wairarapa, Wairarapa Plains, Aorangi, Tararua ERs and Wellington ED) *Kunzea* hosts comprise 56 of 110 records closely followed by *Leptospermum* records (48 of 110). The main *Kunzea* host is *K.* aff. *ericoides* (b) followed by *K.* aff. *ericoides* (a). Records from the Manawatu Plains ED and Eastern Wairarapa ER are from *Kunzea* hosts only.

In the Sounds/Nelson region (Cook Strait, Sounds, D'Urville EDs, Richmond and Nelson ERs), *Leptospermum* is the predominant host (18 of 30 records) followed by *Kunzea* (12 of 30). *Kunzea ericoides* s.s. is the main *Kunzea* host in Nelson and Richmond ERs followed by *K.* aff. *ericoides* (b). *Kunzea ericoides* s.s. hybrid with *K.* aff. *ericoides* (b) and *K.* aff. *ericoides* (a) are sometimes also parasitized. Kapiti Island and Adele Island records are from *Kunzea* hosts while D'Urville Island records are from *Leptospermum* hosts.

In Marlborough (Wairau, Inland Marlborough, Clarence and Kaikoura ERs) *Kunzea* hosts comprise 8 of the 11 records and *Leptospermum* hosts comprise 3 records. The *Kunzea* hosts parasitised are *K.* aff. *ericoides* (b) and *K.* aff. *ericoides* (c).

In Canterbury (Lowry, Puketeraki, Canterbury Foothills, Banks, Pareora, Wainono and Kakanui ERs): *Kunzea* hosts again predominate, comprising 27 of 31 records, with only 4 records from *Leptospermum*. Among the *Kunzea* hosts, *K.* aff. *ericoides* (b) is the main host, and there are some records from *K.* aff. *ericoides* (c) (in Lowry and Puketeraki ERs). Records from Banks ER and Peel Forest (Pareora ER) are all from *Kunzea* aff. *ericoides* (b).

In Otago (records from Lakes, Central Otago and Otago Coast ERs): *Kunzea* hosts comprise 19 of 29 records in the Otago province, while *Leptospermum* hosts comprise 6 records.

In Western Nelson (records from North-West Nelson ER, Foulwind and Punakaiki EDs): *Leptospermum* and *Kunzea* hosts each comprise 5 records while in Westland (records from Spenser, Whataroa, Aspiring and Olivine ERs) *Leptospermum* hosts predominate (8 of 9 records and only one *Kunzea* record). All west coast populations have been recorded on *Leptospermum* hosts only (Fig. 4A) even though *Kunzea* is present all along the northern half of the west coast (Fig. 4B).

In Fiordland, Southland and Rakiura (Fiord ER, Makarewa ER, Stewart Island, Codfish Island and Big South Cape Island) all populations are from *Leptospermum* because *Kunzea* is absent from these regions (Fig. 4A and B).

With regard to occurrences in different ecological regions, records of *K. salicornioides* on *Kunzea ericoides* s.l. in Tainui, Wairoa, Clarence and Wainono ERs, on *K.* aff. *ericoides* (b) in Kaikoura, Banks, Pareora, Kakanui and Central Otago ERs and on *Kunzea* aff. *ericoides* (a) and *Kunzea* aff. *ericoides* (b) in Rangitikei ER represent examples of range extension through secondary/tertiary hosts.

Table 6. The distribution and host range of *Korthalsella clavata* in New Zealand. The numbers and records in braces are the mistletoe-host associations not supported by a herbarium specimen.

	Primary Host	Secondary Host	Tertiary Hosts					Occasional Hosts	Rare Hosts
Ecological region	*Coprosma propinqua*	*C. dumosa*	*Aristotelia fruticosa*	*Coprosma rigida*	*C. wallii*	*C. virescens*	*Discaria toumatou*		
Central Volcanic Plateau	1								
Tongariro									*Coprosma colensoi*
Moawhango		1						*Myrsine divaricata*	
Rangitikei	[1]	1			4			*Coprosma linariifolia*, *C. obconica*	
Wairarapa plains	4							*Muehlenbeckia complexa*, [*Myrsine australis*]	
Tararua	1								*Olearia solandri*
Sounds-Wellington	2								*O. solandri*, *Ulex europaeus*
Inland Marlborough	1								*C. foetidissima*, [*Sophora prostrata*]
North Westland	2	3			[1]				*C. elatirioides*, *C. tenuicaulis*
Spenser	4	2		1	3			*Myrsine australis*	*C. microcarpa*, *C. decurva*
Whataroa	3								
Aspiring	1	3		1	3				*C. ciliata*
Lowry						[1]	[1]	[*Muehlenbeckia complexa*]	[*Carmichaelia australis*], *Coprosma*

	Primary Host	Secondary Host	Tertiary Hosts					Occasional Hosts	Rare Hosts
Ecological region	*Coprosma propinqua*	*C. dumosa*	*Aristotelia fruticosa*	*Coprosma rigida*	*C. wallii*	*C. virescens*	*Discaria toumatou*		
									areolata, [*C. crassifolia*]
Puketeraki	15[1]		8				2[2]	*Coprosma linariifolia*, *Muehlenbeckia complexa*, [*Myrsine divaricata*]	*Clematis marata*, *Coprosma propinqua* var. *latiuscula*, [*Olearia avicenniaefolia*], *O. paniculata*, *Pimelea oreophila* x *declivis*
Canterbury Foothills	1	3		1				*Coprosma obconica*	*Coprosma pedicellata*
Canterbury Plains		4		1				*Melicope simplex*	
Banks						1			
D’Archiac	1	2	2[1]	[1]			1	*Coprosma rugosa*	[*Coprosma ciliata*], *Olearia nummulariifolia*, *Pittosporum tenuifolium*
Heron	3[2]						[1]		
Pareora	1	1							
MacKenzie	3[1]	1	3	3			3	*Coprosma linariifolia*, *Muehlenbeckia complexa*	*Carmichaelia petriei*, [*Coprosma crassifolia*], *Muehlenbeckia australis*, *Olearia avicenniaefolia*, *O. nummulariifolia*, *Pittosporum tenuifolium*, *Sophora microphylla*, *S.*

Ecological region	Primary Host	Secondary Host	Tertiary Hosts					Occasional Hosts	Rare Hosts
	Coprosma propinqua	*C. dumosa*	*Aristotelia fruticosa*	*Coprosma rigida*	*C. wallii*	*C. virescens*	*Discaria toumatou*		
									microphylla x *prostrata*
Kakanui								*Melicope simplex*	
Lakes	1								*Olearia fimbriata*
Central Otago	1		1				1		
Otago Coast						[1]			*Olearia fragrantissima*
Catlins									[*Coprosma* sp.]
Fiord			[1]	2	1			[*Myrsine divaricata*]	
Mavora	[1]								
Southland Hills	[1]								
Te Wae Wae				1	2	2		*Coprosma obconica, Melicope simplex*	*Coprosma rotundifolia*
Makarewa	[1]					1		[*Coprosma obconica, Melicope simplex, Myrsine divaricata*]	*Coprosma rotundifolia*
Total	45[8]	21	14[3]	10[1]	13[1]	4[2]	8[4]		

Table 7. The distribution and host range of *Korthalsella lindsayi* in New Zealand. The numbers and records in braces are the mistletoe-host associations not supported by a herbarium specimen.

	Primary Host	Secondary Hosts				Tertiary Hosts							Occasional Hosts	Rare Hosts	Host not recorded
Ecological Region	*Melicope simplex*	*Coprosma crassifolia*	*Lophomyrtus obcordata*	*Myrsine australis*	*M. divaricata*	*Coprosma areolata*	*C. linariifolia*	*C. rigida*	*C. rotundifolia*	*C. virescens*	*Muehlenbeckia complexa*	*Sophora microphylla*			
W. Volcanic Plateau					1										
Urewera	1			1											
Wairoa	1		1		1					2				*Pittosporum obcordatum*	
Ruahine	1												*Coprosma rhamnoides*		
Hawkes Bay	3		[1]			[1]							[*Coprosma rhamnoides*]		
Rangitikei	18[3]	5[1]	4[2]	1[1]	6[2]	3	3[1]	3[1]	2	1[1]			*Coprosma obconica*, [*Coprosma rhamnoides*], *C. rubra*	*Coprosma wallii*, *Pittosporum obcordatum*	
Manawatu	5[3]	1[2]	3[2]	2[1]		4[1]		1[1]	2		[1]		*Coprosma rhamnoides*, *Lophomyrtus bullata*	[*Lophomyrtus bullata* x *obcordata*], [*Melicope simplex* x	

Ecological Region	Primary Host	Secondary Hosts				Tertiary Hosts							Occasional Hosts	Rare Hosts	Host not recorded
	Melicope simplex	*Coprosma crassifolia*	*Lophomyrtus obcordata*	*Myrsine australis*	*M. divaricata*	*Coprosma areolata*	*C. linariifolia*	*C. rigida*	*C. rotundifolia*	*C. virescens*	*Muehlenbeckia complexa*	*Sophora microphylla*			
														ternata], *Metrosideros diffusa*, *Sophora godleyi*	
Pahiatua	1[1]				1										
Eastern Hawkes Bay	2	1			3										
Eastern Wairarapa	4[2]	1[3]	[4]	[2]	3[4]	[2]	1	[2]	[1]	1	[1]	[1]	*Carmichaelia australis*, *Coprosma obconica*, [*C. propinqua*, *C. rhamnoides*], *C. rubra*	[*Pittosporum obcordatum*]	
Wairarapa Plains	[6]	1[7]	[9]	2[1]	[10]			[5]		[2]			[*Lophomyrtus bullata*], *Muehlenbeckia australis*	*Pittosporum obcordatum*	
Tararua		2[2]				2				[1]	2[4]		*Coprosma propinqua*, [*C. rhamnoides*]	*Ileostylus micranthus*, *Muehlenbeckia astonii*, *Olearia*	

	Primary Host	Secondary Hosts				Tertiary Hosts							Occasional Hosts	Rare Hosts	
Ecological Region	*Melicope simplex*	*Coprosma crassifolia*	*Lophomyrtus obcordata*	*Myrsine australis*	*M. divaricata*	*Coprosma areolata*	*C. linariifolia*	*C. rigida*	*C. rotundifolia*	*C. virescens*	*Muehlenbeckia complexa*	*Sophora microphylla*			Host not recorded
														solandri, Scandia geniculata	
Sounds-Wellington	1										1		*Lophomyrtus bullata*	*Pittosporum cornifolium*	
Richmond	2[1]													*Lophomyrtus bullata* x *obcordata*	
Wairau		[1]													
Inland Marlborough			1												
Molesworth												1	*Sophora prostrata*		
Kaikoura		[1]	2	1								1	*Sophora prostrata*	*Coprosma* sp.	
North-west Nelson			5												
Nelson	1	2	2				1			1			*Coprosma rubra*		
Aspiring		1													
Lowry		1	[1]										*Carmichaelia australis, Sophora*		

	Primary Host	Secondary Hosts				Tertiary Hosts							Occasional Hosts	Rare Hosts	
Ecological Region	*Melicope simplex*	*Coprosma crassifolia*	*Lophomyrtus obcordata*	*Myrsine australis*	*M. divaricata*	*Coprosma areolata*	*C. linariifolia*	*C. rigida*	*C. rotundifolia*	*C. virescens*	*Muehlenbeckia complexa*	*Sophora microphylla*			Host not recorded
													prostrata		
Canterbury Foothills		1[1]	3						1				*Sophora prostrata*		
Canterbury Plains	1	7	4	1									*Coprosma propinqua*	*C. dumosa*, *Cytisus scoparius*, *Syringa vulgaris*	
Banks	10[1]	3[1]	9[1]	2	1	4			3	3	[1]	2	*Coprosma rhamnoides*	*Coprosma* sp., [*Ileostylus micranthus*]	
Pareora	5	[1]	1[1]	1	[1]	1		2	2			[1]	[*Coprosma rubra*]	*Coprosma dumosa*, *Discaria toumatou*	
Wainono															1
MacKenzie					2		1	1			1	1	*Coprosma obconica*	*Discaria toumatou*, *Sophora microphylla* x *prostrata*	

Ecological Region	Primary Host	Secondary Hosts				Tertiary Hosts							Occasional Hosts	Rare Hosts	Host not recorded
	Melicope simplex	*Coprosma crassifolia*	*Lophomyrtus obcordata*	*Myrsine australis*	*M. divaricata*	*Coprosma areolata*	*C. linariifolia*	*C. rigida*	*C. rotundifolia*	*C. virescens*	*Muehlenbeckia complexa*	*Sophora microphylla*			
Lakes	[1]	[1]	[1]		[1]		[1]						[*Coprosma propinqua*, *Muehlenbeckia australis*]	*Carmichaelia petrei*, *Discaria toumatou*, [*Helichrysum glomeratum*], *Pseudopanax ferox*	
Central Otago	1														
Otago Coast	19[2]	6[2]	1	2[1]	1[2]	3[1]				1		1		[*Coprosma decurva*, *Helichrysum glomeratum*], *Neomyrtus pedunculata*, *Olearia phlogopappa*	
Catlins	3	1								1					
Fiord					1										
Gore	1														
Southland Hills	1														

	Primary Host	Secondary Hosts				Tertiary Hosts							Occasional Hosts	Rare Hosts	Host not recorded
Ecological Region	*Melicope simplex*	*Coprosma crassifolia*	*Lophomyrtus obcordata*	*Myrsine australis*	*M. divaricata*	*Coprosma areolata*	*C. linariifolia*	*C. rigida*	*C. rotundifolia*	*C. virescens*	*Muehlenbeckia complexa*	*Sophora microphylla*			
Te Wae Wae							1						*Coprosma obconica*		
Makarewa	1		1		1	1	1					1			
	82 [20]	33 [23]	31 [22]	13 [6]	22 [20]	18 [5]	8 [2]	7 [9]	10 [1]	10 [4]	4 [7]	7 [2]			

Table 8. The distribution and host range of *Korthalsella salicornioides* in New Zealand, ● =at least one of the *Leptospermum* host was identified as *L. scoparium* var. *incanum,*▲ =at least one of the *Leptospermum* host was identified as *L*. aff. *scoparium* (a), ■ =at least one of the *Leptospermum* host was identified as *L. scoparium* s.s, numbers in braces are the mistletoe-host associations not supported by a herbarium specimen, dotted lines for *Kunzea ericoides* s.l. hosts column indicates that these records could belong to any of the other provisional segregates from this taxon, known from a particular region.

	Primary Host	Secondary Host	Tertiary Host		Occasional hosts	Rare hosts	
Ecological Region	*Leptospermum scoparium* s.l.	*Kunzea* aff. *ericoides* (b)	*K.* aff. *ericoides* (a)	*K. ericoides* s.l.			Host not recorded
Te Paki	4[2] ●		1		*Kunzea ericoides* var. *linearis*	*K*. aff. *ericoides* (a) x *K. ericoides* var. *linearis?*	
Western Northland	13[3] ●		1	1			
Eastern Northland	20[3] ●▲			1 [2]	*K. ericoides* var. *linearis*	[*Coprosma rhamnoides*]	
Kaipara	2	1					
Auckland	19[5]▲	2			*K. ericoides* var. *linearis*	[*Coprosma rhamnoides*]	
Coromandel	13 [3]▲	3	3	3 [1]		*Kunzea sinclairii,* [*K. sinclairii* x *K. aff. ericoides* (b)]	

	Primary Host	Secondary Host	Tertiary Host		Occasional hosts	Rare hosts	Host not recorded
Ecological Region	*Leptospermum scoparium* s.l.	*Kunzea* aff. *ericoides* (b)	*K.* aff. *ericoides* (a)	*K. ericoides* s.l.			
Waikato	4						
Tainui				1			
Northern Volcanic Plateau	18[3] ■			2[4]	*K. ericoides* var. *microflora*		
Whakatane	[4]	1		[4]		*K.* aff. *ericoides* (d), *K. ericoides* var. *microflora* x *K.* aff. *ericoides* (b)	
Central Volcanic Plateau	4[2]	1		[1]	*K. ericoides* var. *microflora*		
Tongariro	1			[1]			
Wairoa				1			
Rangitikei		1	1	[1]	*Melicope simplex*		
Manawatu	3 [3]	4	5	1[2]		[*Coprosma sp., C. propinqua, Sophora* sp.], *S. microphylla*	
Eastern Wairarapa	4[8]	3		[5]			
Wairarapa Plains	8[18]	5		2[25]		*Coprosma tenuicaulis, K.* aff. *ericoides* (b) x (a)?	

Ecological Region	Primary Host *Leptospermum scoparium* s.l.	Secondary Host *Kunzea* aff. *ericoides* (b)	Tertiary Host *K.* aff. *ericoides* (a)	*K. ericoides* s.l.	Occasional hosts	Rare hosts	Host not recorded
Aorangi							1
Tararua	2	1					
Sounds-Wellington	10[3]	2	1	1[1]		[*Sophora chathamica*]	
Richmond	4 ■			2	*K. ericoides* s.s.	*K. ericoides* s.s. x *K.* aff. *ericoides* (b)	
Wairau	[1]				*K.* aff. *ericoides* (c)		
Inland Marlborough	2	1		2			
Clarence				1			
Kaikoura		1		1			
North-west Nelson	3			2[2]	*K. ericoides* s.s.		
Nelson	2[1]			[1]	*K. ericoides* s.s.		
North Westland	2						
Spenser	2			[1]			
Whataroa	2						
Aspiring	[1]						

Ecological Region	Primary Host *Leptospermum scoparium* s.l.	Secondary Host *Kunzea* aff. *ericoides* (b)	Tertiary Host *K.* aff. *ericoides* (a)	*K. ericoides* s.l.	Occasional hosts	Rare hosts	Host not recorded
Lowry	2	4		1	*K.* aff. *ericoides* (c)		
Puketeraki				[1]	*K.* aff. *ericoides* (c)		
Canterbury Foothills	2 ■	1		[1]			
Banks		5		3[2]		*K.* aff. *aricoides* (b) x *K.* aff. *ericoides* (c)	
Pareora		[1]		1			
Wainono				[1]			
Kakanui		1		[1]			
Lakes	[1]			[1]	*K.* aff. *ericoides* (c)		
Central Otago		1		[3]			
Otago Coast	5	1		7[5]	*Melicope simplex*	*Erica arborea*, [*E. lusitanica*, *E. vagans*]	
Olivine	1[2]						
Fiord	18[1]						
Makarewa	2						
Rakiura	7[8]						

Ecological Region	Primary Host	Secondary Host	Tertiary Host		Occasional hosts	Rare hosts	Host not recorded
	Leptospermum scoparium s.l.	*Kunzea* aff. *ericoides* (b)	*K.* aff. *ericoides* (a)	*K. ericoides* s.l.			
	179[72]	39[1]	12	33[66]			

Phylogenetic analyses of New Zealand Korthalsella

Plastid *trnQ-rps16* sequences were generated for 45 collections of *K. clavata*, 63 samples of *K. lindsayi*, and 55 samples of *K. salicornioides*, whereas, nuclear ITS sequences were generated for 45 collections of *K. clavata*, 64 samples of *K. lindsayi*, and 56 samples of *K. salicornioides*, representing the diversity of host-mistletoe combinations across their geographic ranges in New Zealand. The *trnQ-rps16* data set included 741 characters (including outgroups), with 39 parsimony-informative characters (including the outgroups). The ITS data set included 783 characters (including outgroups), with ITS1 region extending for 358 bp and ITS2 for 258 bp and had 49 parsimony-informative characters (including the outgroups).

The parsimony analysis of *trnQ-rps16* data set resulted in two equally parsimonious trees. The parsimony tree has a length of 90 steps and a consistency index (CI) of 1 and a rescaled consistency index (RC) of 1. Parsimony analysis of *trnQ-rps16* data set reconstructed the ingroup taxa into two main clades - a *K. clavata/K. lindsayi* clade (boostrap support [BS]=100) and a *K. salicornioides* clade (BS=100). Within the *K. clavata/K. lindsayi* clade *K. clavata* and *K. lindsayi* collections were polyphyletic. *Korthalsella clavata* and *K. lindsayi* collections from South Island formed a clade (BS=87), the only exception was a collection of *K. lindsayi* from Waimakariri Gorge in the South Island (K119) that did not group within this South Island clade. Within the *K. salicornioides* clade collections from the North Island grouped as a clade (BS=86) and were thus distinct from the main South Island haplotype and some Wairarapa Plains, Eastern Wairarapa collections (K53, K54 and K192), which were closer to the South Island haplotype.

The topologies of the parsimony and Bayesian plastid phylogenies were similar, therefore only the Bayesian phylogeny is presented (Fig. 5).

The parsimony analysis of the ITS data set resulted in 3615 equally parsimonious trees. The parsimony tree has a length of 117 steps and a consistency index (CI) of 0.96 and a rescaled consistency index (RC) of 0.94. Because the resulting consensus tree was highly unresolved, only the results from the Bayesian analyses are considered further.

Bayesian analysis of the ITS data set reconstructed the ingroup taxa into two main clades - a *K. clavata/K. lindsayi* clade (PP=1, BS=100) and a *K. salicornioides* clade (PP=1, BS=100). Within the *K. clavata/K. lindsayi* clade *K. clavata* and *K.*

lindsayi collections were again polyphyletic (Fig. 6). Within the *K. salicornioides* clade a collection of *K. salicornioides* from North Cape (K179) and a sequence type representing *K. salicornioides* collections from Whakatane, a collection from Eastern Northland (K33) and a collection from Northern Volcanic Plateau ER formed a clade (PP=0.97, BS=62). A collection of *K. salicornioides* from Kaipara ER (K187) and a sequence type representing *K. salicornioides* collections from Coromandel Peninsula and a collection from Rangitikei ER also formed a clade (PP=0.99, BS=64).

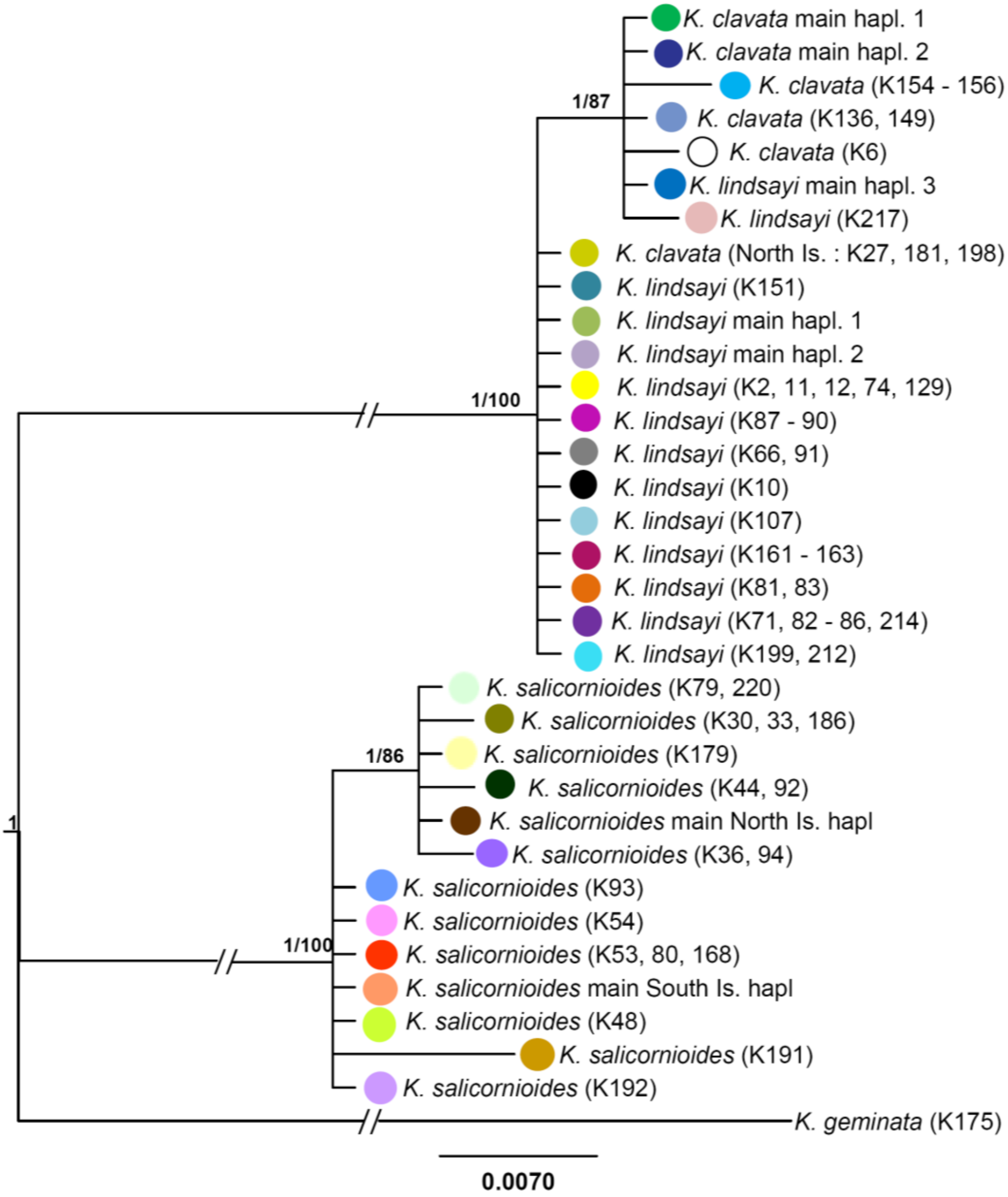


Fig. 5. cpDNA Bayesian phylogeny of New Zealand species, numbers above the branches represent Bayesian posterior probabilities and parsimony bootstrap support values, respectively

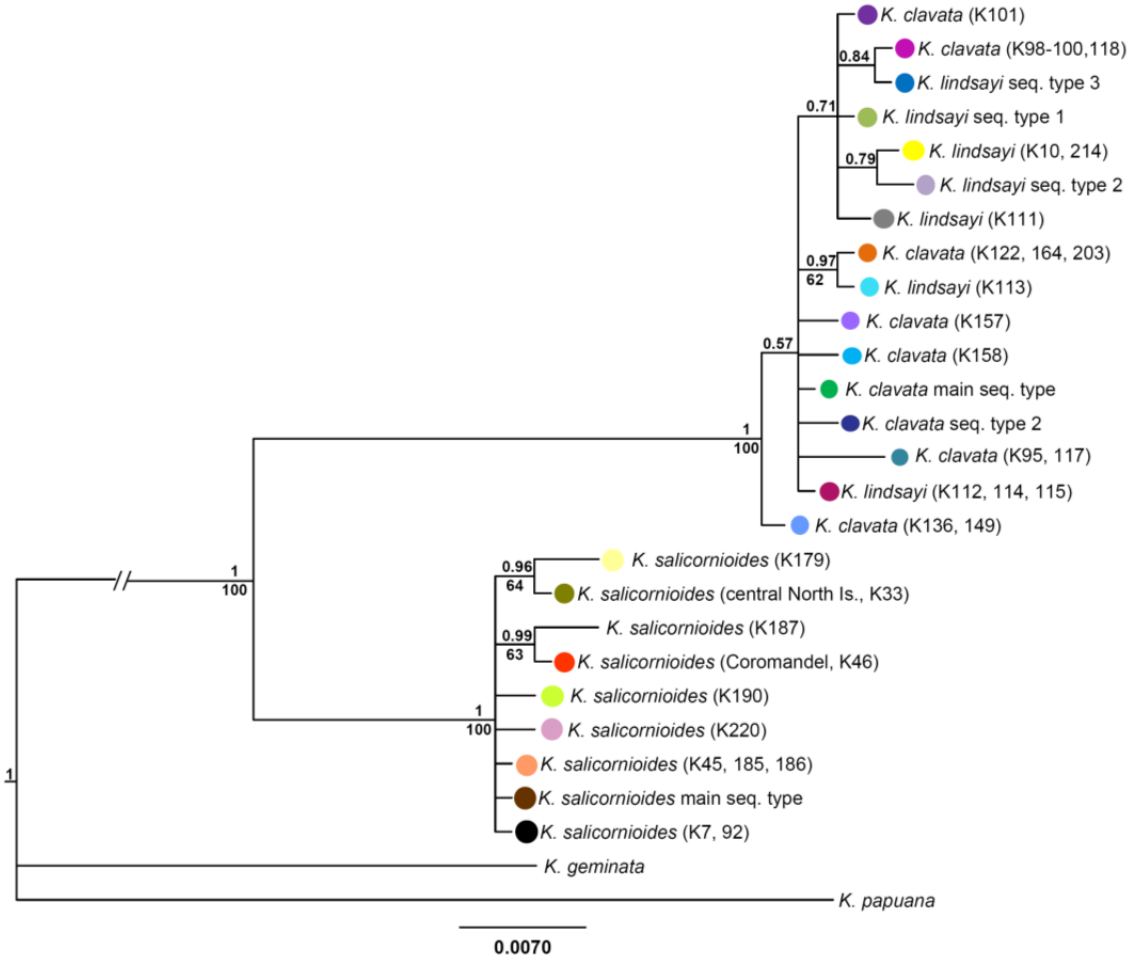


Fig. 6. ITS Bayesian phylogeny of New Zealand species, numbers above the branches represent Bayesian posterior probabilities and parsimony bootstrap support values, respectively

Network analyses of regional molecular variability

***Korthalsella clavata* - Chloroplast sequences:** All North Island collections had identical sequences (K27-181-198, Figs. 7, 9) and most of the South Island populations had very similar sequences. The relationship of different *K. clavata* haplotypes with each other and to *K. lindsayi* haplotypes is illustrated in Fig. 7. Mistletoes with the main haplotype 1 had *Coprosma* as well as *Discaria*, *Aristotelia* and rarely *Pimelea* and *Carmichaelia* hosts. Mistletoes with the main haplotype 2 also had *Coprosma*, *Discaria* and *Aristotelia* and rarely *Olearia* as hosts. Some collections from the Spenser, Puketeraki and Te Wae Wae ERs showed some slight variations compared to the main haplotypes 1 and 2 (Figs. 7, 9).

***Korthalsella clavata* - ITS sequences:** Collections from twelve different populations throughout the North and South Islands have identical ITS sequences (main sequence type 1 in Figs. 8, 10). These collections were predominantly from *Coprosma* hosts and rarely from *Pimelea* and *Melicope*. Some collections from the Canterbury Foothills, Puketeraki, Heron and MacKenzie ERs had identical sequences (sequence type 2 in Figs. 8, 10). These collections had *Coprosma*, *Myrsine*, *Discaria*, *Aristotelia*, *Muehlenbeckia* and *Olearia* as hosts. Collections from Wairarapa Plains, Spenser, Lowry, Canterbury Foothills, Puketeraki, MacKenzie, Otago Coast and Makarewa ERs had some mutations differentiating them from the other two main sequence types (Figs. 8, 10). The relationship of different *K. clavata* sequence types with each other and to *K. lindsayi* sequence types is given in Fig. 8.

Korthalsella lindsayi **- Chloroplast sequences:** Collections from six populations in the Hawkes Bay, Eastern Hawkes Bay, Wairarapa Plains and Banks ERs had identical chloroplast sequences (main haplotype 1 in Figs. 7, 11). These had *Myrsine*, *Melicope*, *Coprosma*, *Lophomyrtus*, *Muehlenbeckia* and *Ileostylus micranthus* as hosts. Collections from four populations in Pareora, Lakes and Otago Coast ERs had identical sequences (main haplotype 2 in Figs. 7, 11). These had *Myrsine*, *Melicope*, *Coprosma*, *Sophora*, *Lophomyrtus* and *Carmichaelia* hosts. Collections from four populations in MacKenzie, Otago Coast and Te Wae Wae ERs had identical sequences (main haplotype 3 in Figs. 7, 11). These had *Myrsine*, *Sophora*, *Coprosma* and *Melicope* hosts. Collections from two populations in Rangitikei and Manawatu ERs had identical sequences. These had *Melicope*, *Lophomyrtus* and *Coprosma* hosts. Collections from three populations on *Melicope*, *Myrsine*, *Lophomyrtus*, *Coprosma* and *Olearia* hosts in Eastern Wairarapa and Tararua had similar sequences. A few collections from Rangitikei, Otago Coast, Eastern Hawkes Bay, Banks and Catlins ERs showed some variability compared to the other sequence types. The relationship of different *K. lindsayi* haplotypes with each other and to *K. clavata* haplotypes is given in Fig. 7.

Korthalsella lindsayi **- ITS sequences:** Collections from sixteen populations in Rangitikei, Hawkes Bay, Eastern Hawkes Bay, Manawatu, Eastern Wairarapa, Wairarapa Plains, Tararua, Lowry, Banks, Lakes and Otago Coast ERs had identical ITS sequences (sequence type 1 in Figs. 8, 12). These populations had *Coprosma*, *Myrsine*, *Melicope*, *Lophomyrtus*, *Sophora*, *Muehlenbeckia*, *Olearia*, *Ileostylus* and *Carmichaelia* as hosts. Collections from ten populations in Western Volcanic Plateau, Wairoa, Rangitikei, Hawkes Bay, Manawatu, Eastern Wairarapa, Wairarapa Plains had identical sequences (sequence type 2 in Figs. 8, 12). These had *Myrsine*, *Melicope*, *Coprosma* and *Lophomyrtus* as hosts. Collections from nine populations in Banks, Pareora, MacKenzie, Otago Coast, Te Wae Wae and Catlins ERs also had an identical sequence type (sequence type 3 in Figs. 8, 12). These populations were parasitic on *Coprosma*, *Myrsine*, *Sophora*, *Lophomyrtus*, and *Melicope* hosts. Few collections from Rangitikei, Tararua, Pareora and Otago Coast ERs had some variability compared to the three main sequence types. The relationship of different *K. lindsayi* sequence types with each other and to *K. clavata* sequence types is given in Fig. 8.

Korthalsella salicornioides **- Chloroplast sequences:** Collections from seventeen populations in Eastern Northland and Islands, Western Northland, Auckland, Whakatane, Northern Volcanic Plateau, Central Volcanic Plateau, Rangitikei, Manawatu, Eastern Wairarapa and Wairarapa Plains Ecological regions (ERs) had identical sequences (main North Island haplotype in Figs. 13, 15) These had *Leptospermum scoparium* s.l., *Leptospermum scoparium* var. *incanum*, *Coprosma rhamnoides, Leptospermum scoparium* s.s., *Kunzea ericoides* s.l., *Kunzea* aff. *ericoides* (b), *Kunzea ericoides* var. *microflora*, *Kunzea* aff. *ericoides* (a) hosts and a single population from *Melicope simplex*. Collections from Nelson, Wairau, Spenser, Puketeraki, Whataroa, Wainono, Central Otago, Fiord, Otago Coast and Makarewa ERs representing twelve populations had identical sequences (main South Island haplotype in Figs. 13, 15). Hosts parasitised by the main South Island haplotype include *Kunzea ericoides* s.l., *K. ericoides*. s.s., *Kunzea* aff. *ericoides* (b), *Kunzea* aff. *ericoides* (c), *Leptospermum scoparium* s.l. and a single occurrence on *Erica arborea*. Populations from Te Paki, Coromandel, Rangitikei, North West Nelson and Banks Ecological regions and some collections from Wairau, Eastern Wairarapa, Wairarapa Plains ERs, showed some variations of the two main haplotypes (Figs. Figs. 13, 15). The relationship of different *K. salicornioides* haplotypes to each other is given in Fig. 13.

Korthalsella salicornioides **- ITS sequences:** Most of the collections from the North and South Islands had identical ITS sequences (main sequence type in Figs. 14, 16). Collections from Coromandel, one collection each from Te Paki, Western Northland, Kaipara, Auckland, Central Volcanic Plateau, Rangitikei and collections from Manawatu ERs had some variability compared to the main sequence type. A collection from Eastern Northland, collections from Whakatane and a collection from Northern Volcanic Plateau ER had identical sequences (central North Island-K 33 sequence type in Figs. 14, 16). The relationship of different *K. salicornioides* sequence types to each other is given in Fig. 14.

Korthalsella clavata and K. lindsayi combined network analysis

Because *Korthalsella clavata* and *K. lindsayi* were not reciprocally monophyletic in the resulting phylogenies, combined network analyses were conducted for these two species. The combined network of the plastid data of *K. clavata* and *K. lindsayi* accessions (Fig. 7), shows that three *K. clavata* accessions from the North Island (K27, K181 and K210) are identical to two *K. lindsayi* accessions (K66, K91), also from the North Island and are thus closer to *K. lindsayi* collections rather than the conspecific collections. However, corresponding ITS sequences for these North Island collections of *K. clavata* were identical to main sequence type 1 of *K. clavata* (Fig. 8). Similarly three *K. lindsayi* accessions from Mackenzie, Otago Coast and Te Wae Wae Ecological Regions in South Island had CP sequences identical to main haplotype 1 of *K. clavata* (Fig. 7). However, their ITS sequences comprised main sequence type 3 of *K. lindsayi* and were thus closer to conspecific ITS sequences (Fig. 8).

The combined network of ITS data (Fig. 8) also shows a similar pattern with four *K. lindsayi* collections (K112, K113, K114, K115) from Peel Forest in South Island being identical to *K. clavata* sequences, while another four *K. lindsayi* collections from the same site (K132, K133, K134, K145) had ITS sequences that were identical to sequence type 3 of *K. lindsayi*. However, the corresponding CP sequences of all accessions from Peel Forest comprised main haplotype 2 of *K. lindsayi* (Fig. 7). Similarly one *K. clavata* collection from Broken River (K100) and three collections from Mt Alexander (K98, K99, K118) in the South Island had ITS sequences identical to the main sequence type 3 of *K. lindsayi* which is confined to South Island (Fig. 8). Another *K. clavata* collection from Broken River (K101) had ITS sequence identical to the main sequence type 2 of *K. lindsayi*, while still another *K. clavata* collection from the same site (K164) had an ITS sequence identical to two *K. clavata* collections from Balclutha (K122, K203) (Fig. 8). A *K. clavata* collection from Mt Alexander (K117) had an ITS sequence identical to another *K. clavata* collection from Lake Tekapo (K95) (Fig. 8). However, corresponding plastid sequences of all collections from Broken River and Mt Alexander were identical to main haplotype 1 of *K. clavata* (Fig. 7). Thus, a mosaic of ITS sequences identical to conspecific collections or to ITS sequences from other species existed at these sites.

Overall, most of the ITS sequence variability in *K. clavata* is concentrated in the South Island (Fig. 10) and in *K. salicornioides,* sequence variability is concentrated in the North Island (Fig. 16). In *K. lindsayi,* sequence type 1 is represented by collections from both the North and South Island, while sequence types 2 and 3 are restricted to the North and South Islands, respectively (Fig. 12).

It is noteworthy that in all of these instances where collections from a particular site had CP sequences identical to those of the other species, their ITS sequences matched or were closer to conspecific specimens. A similar situation was seen for collections having ITS sequences identical to those of other species, their respective CP sequences matched or were closer to conspecific collections.

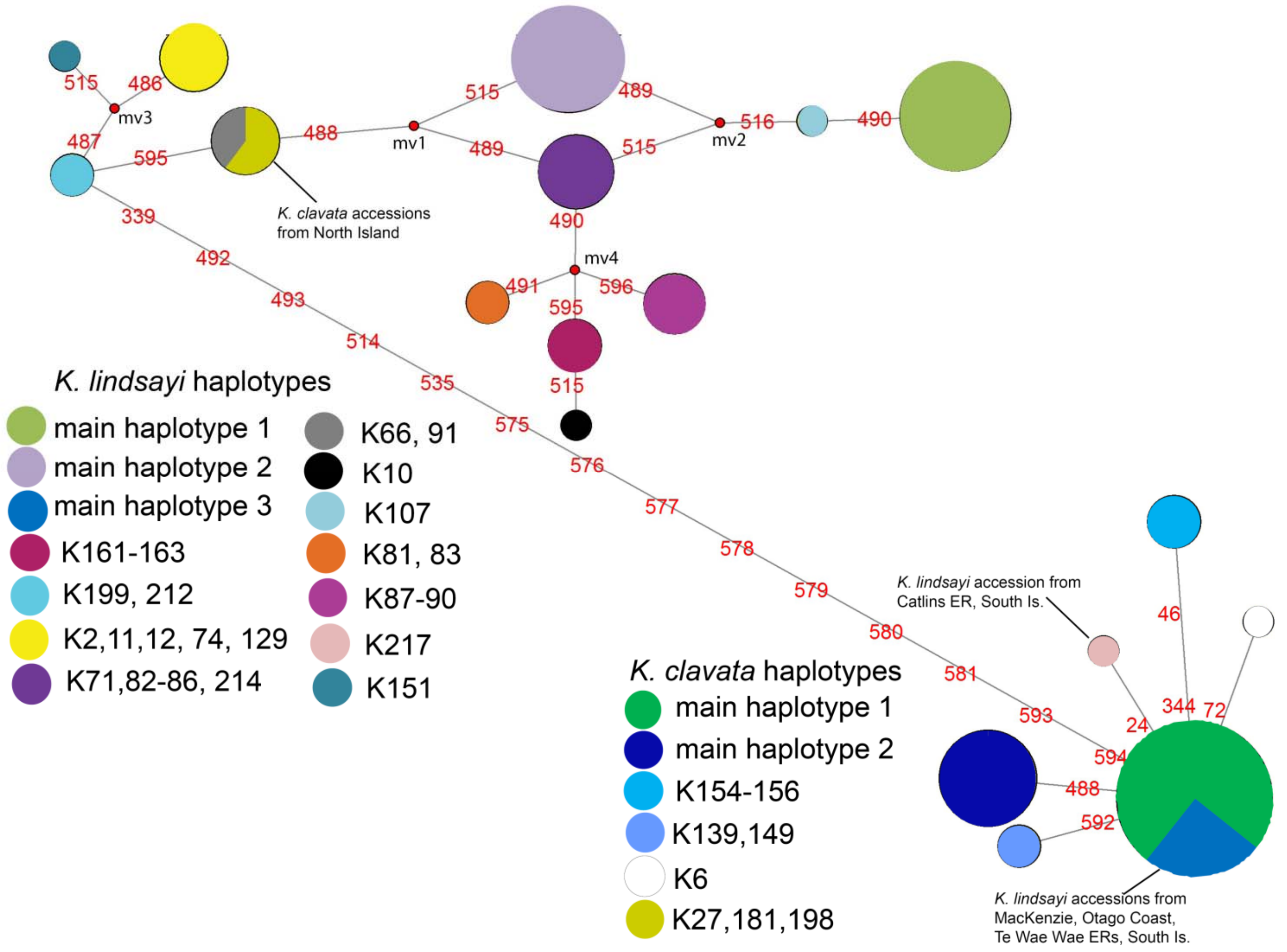


Fig. 7. Network showing relationship of cpDNA haplotypes of *K. clavata* and *K. lindsayi* (node size proportional to haplotype frequency, nodes with two colours represent haplotypes shared by the two species).

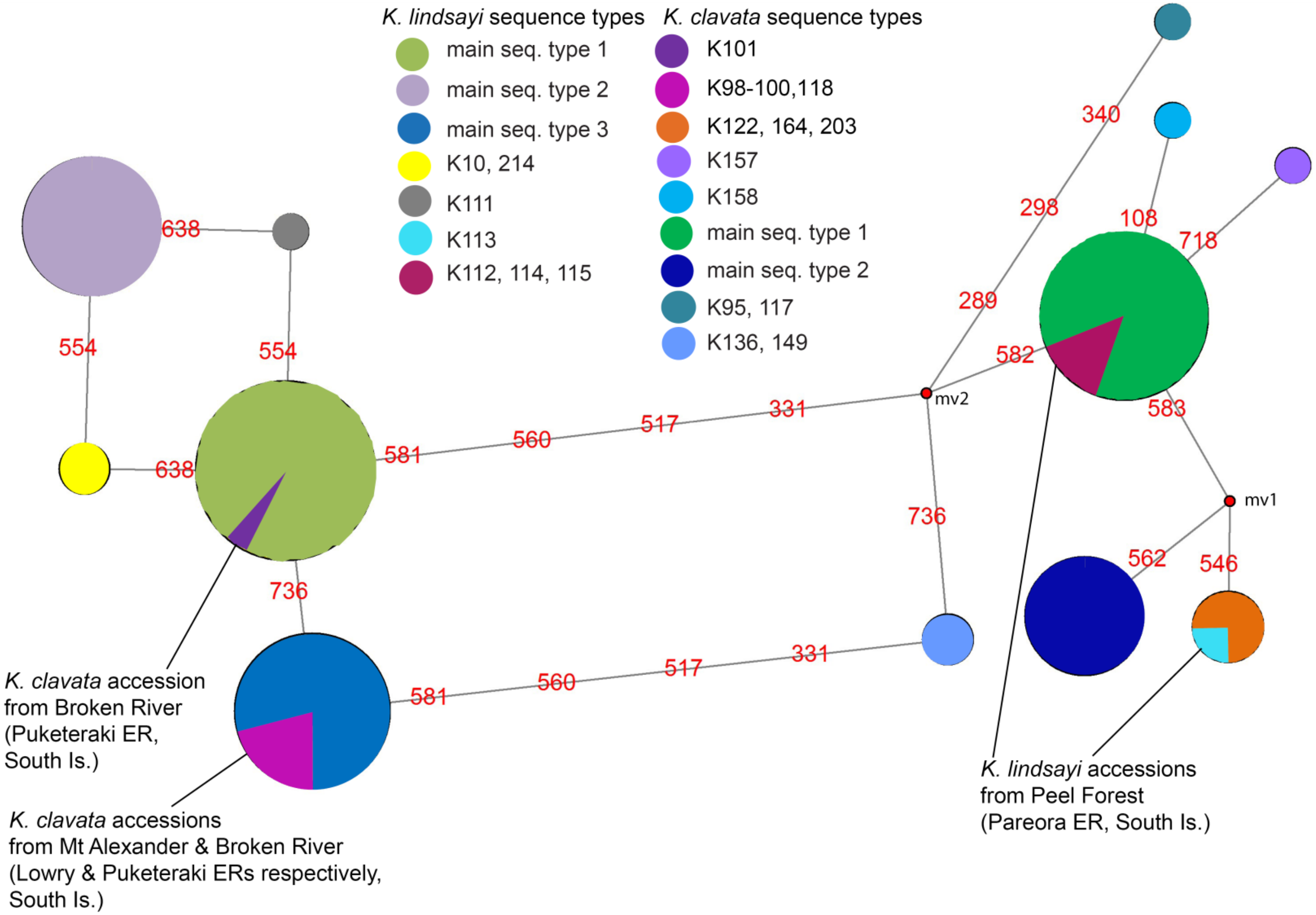


Fig. 8. Network showing the relationship of ITS sequence types of *K. clavata* and *K. lindsayi* (node size proportional to sequence type frequency, nodes with two colours represent sequence types shared by the two species).

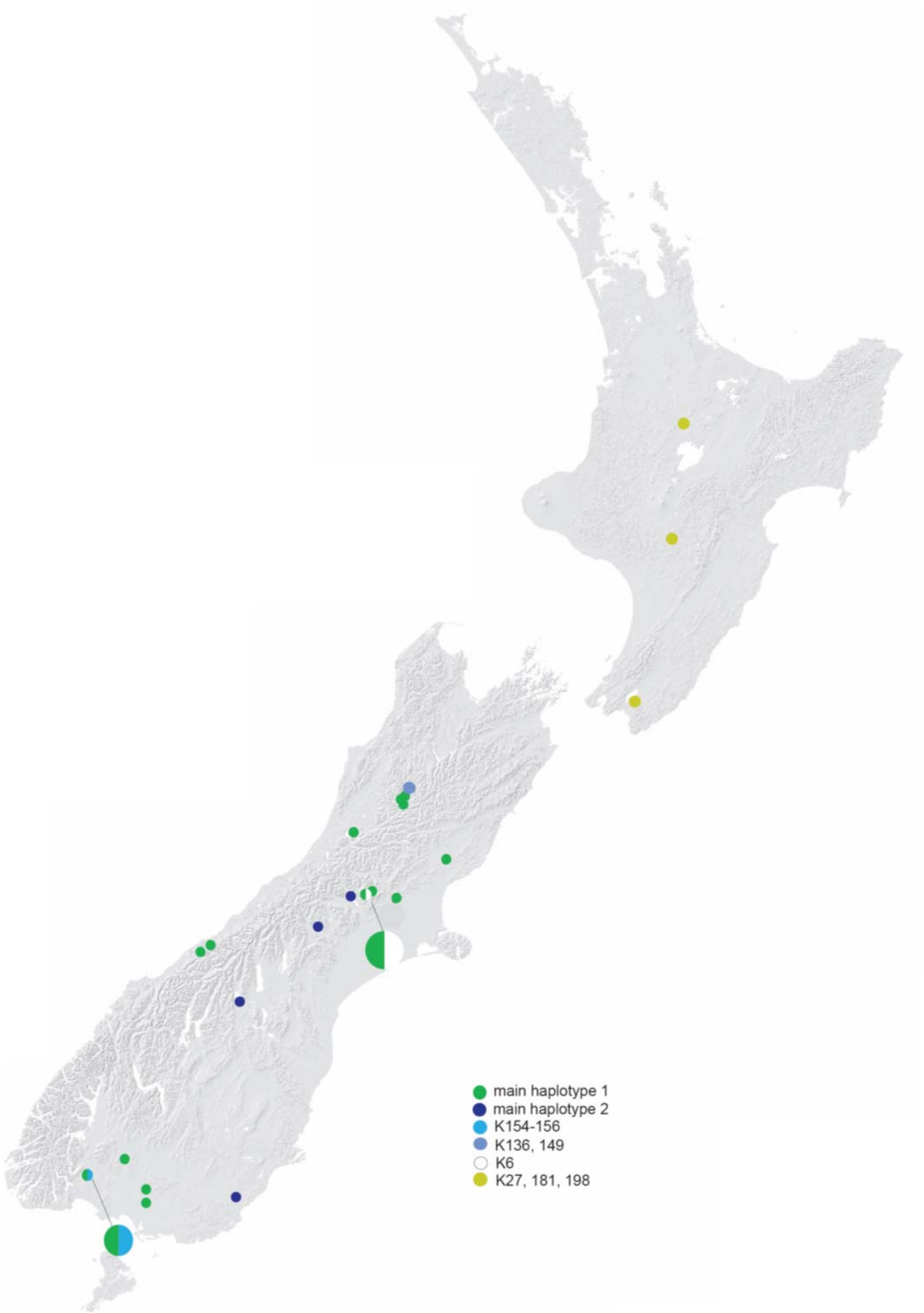


Fig. 9 cpDNA haplotype diversity in *K. clavata*; symbols with two colours show the presence of more than one haplotype at a particular location.

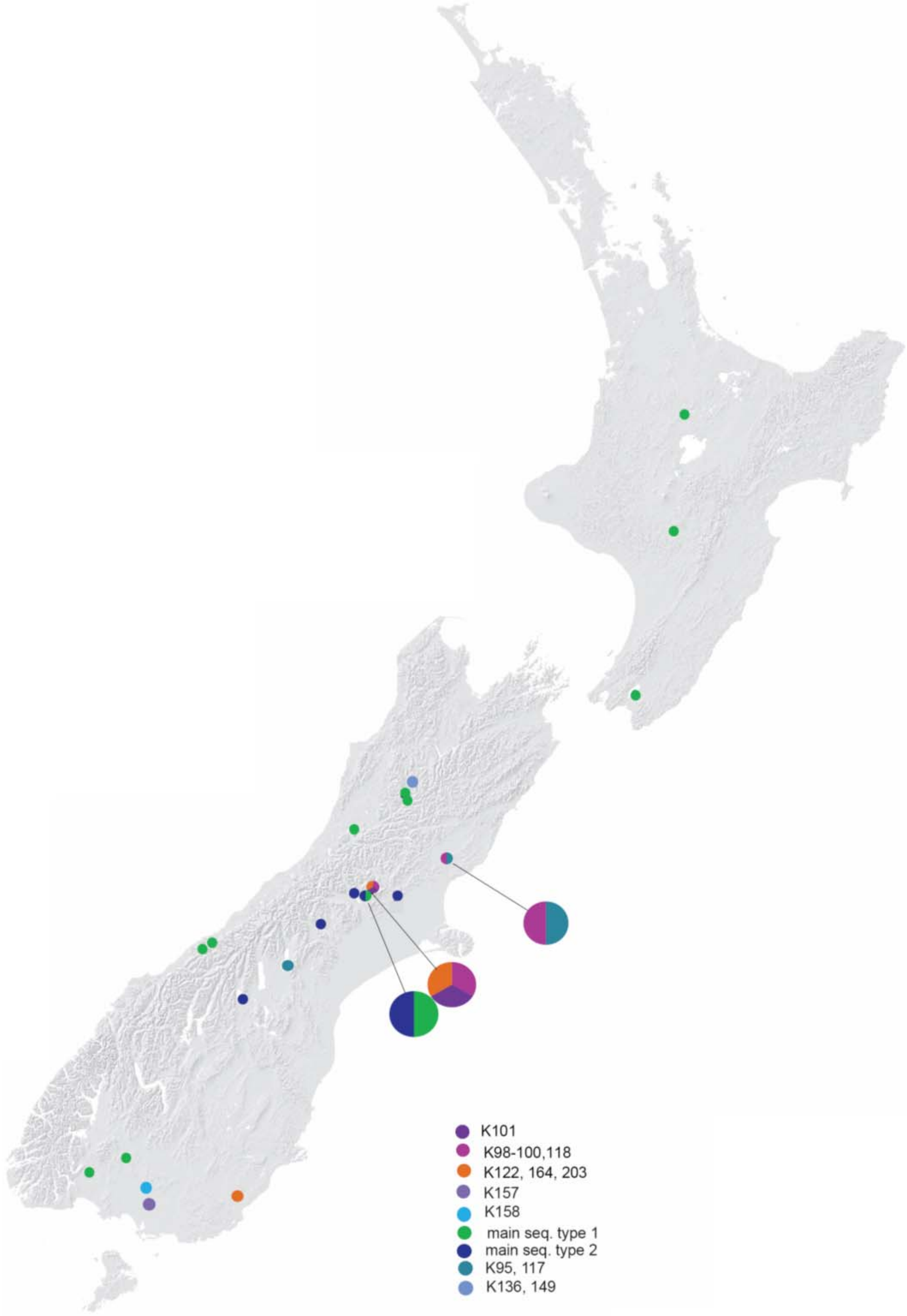


Fig. 10. ITS sequence type diversity in *K. clavata,* symbols with two or more colours show the presence of more than one sequence type at a particular location

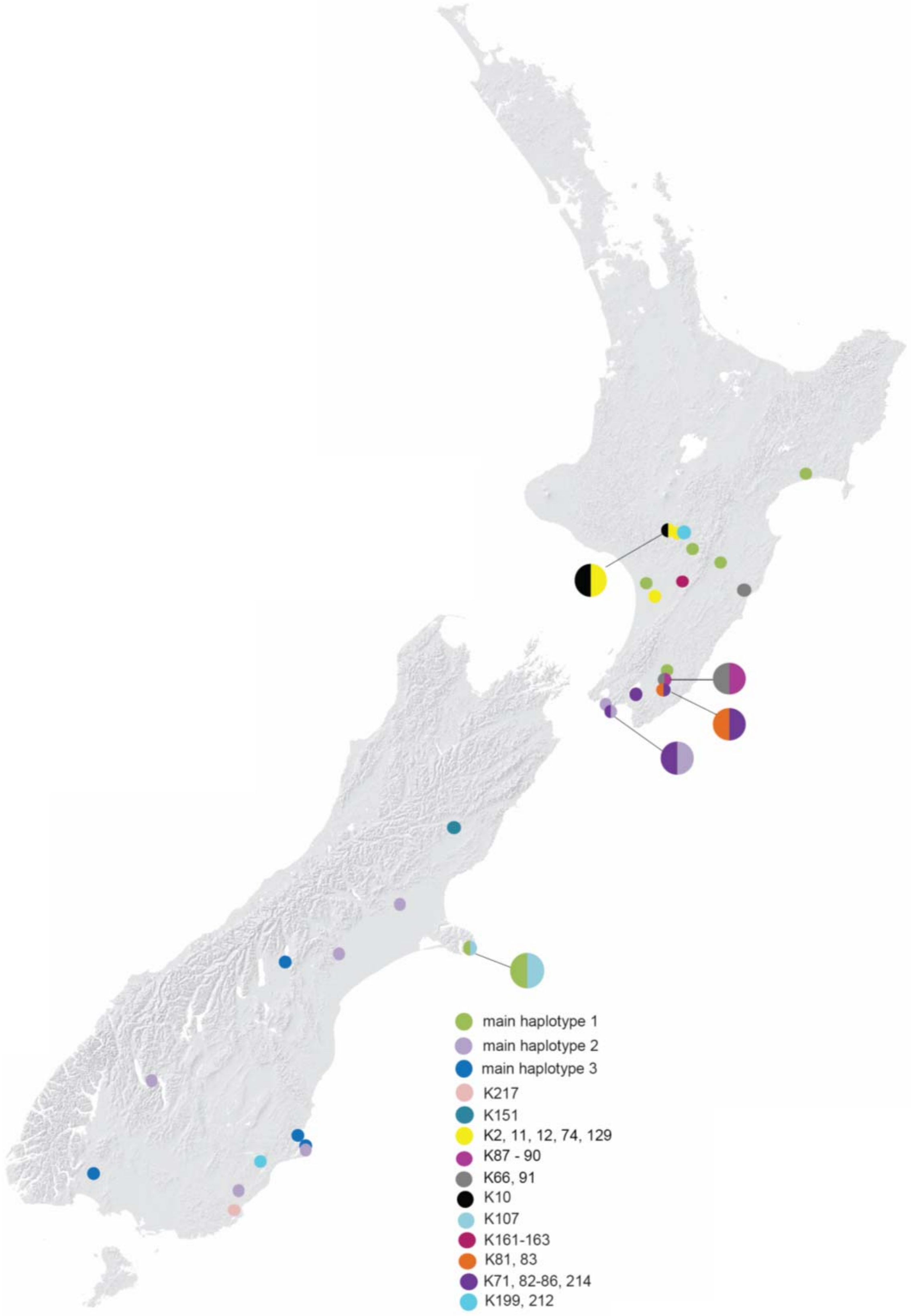


Fig. 11. cpDNA haplotype diversity in *K. lindsayi*, symbols with two colours show the presence of more than one haplotype at a particular location

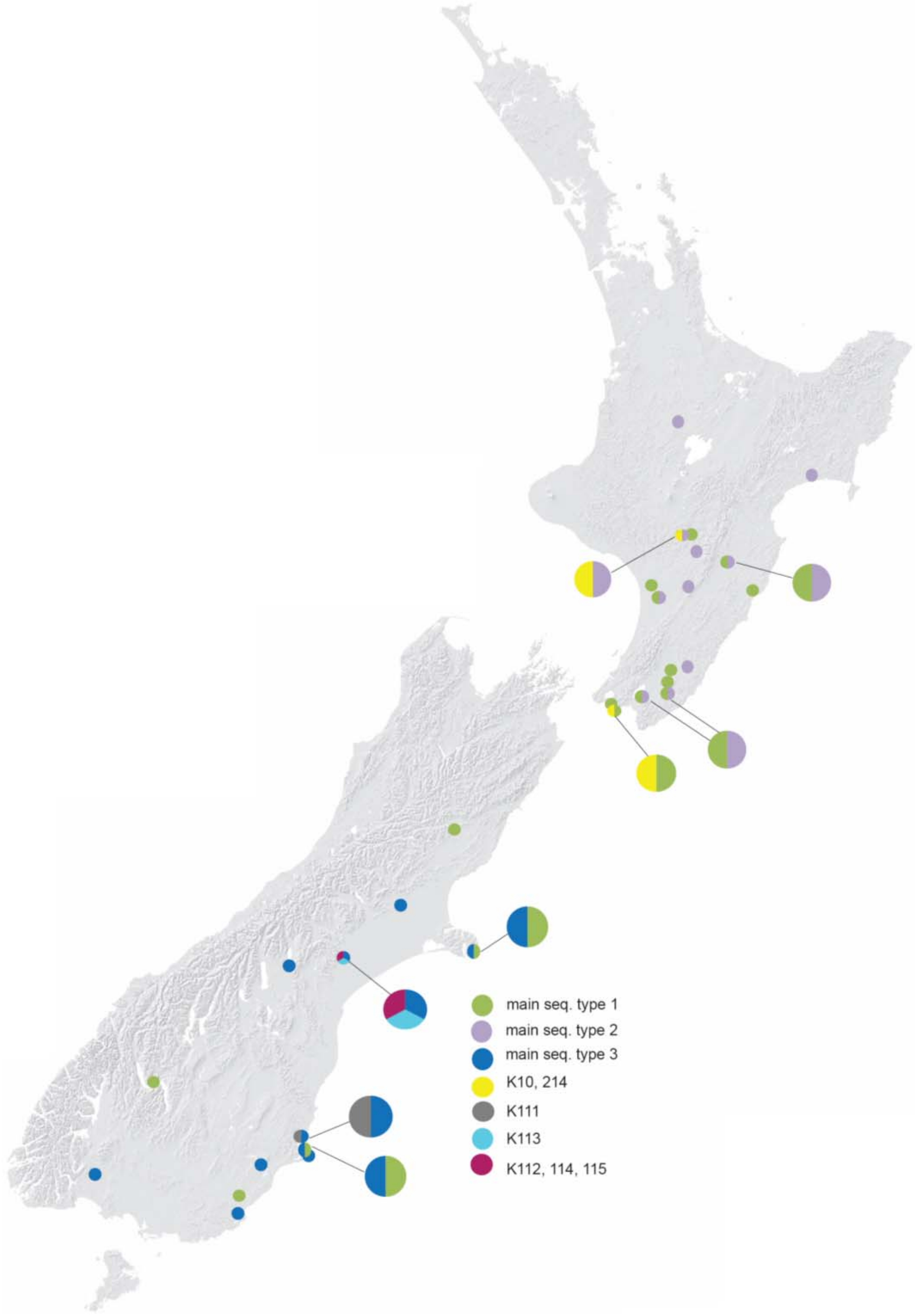


Fig. 12. ITS sequence type diversity in *K. lindsayi,* symbols with two or three colours show the presence of more than one sequence type at a particular location

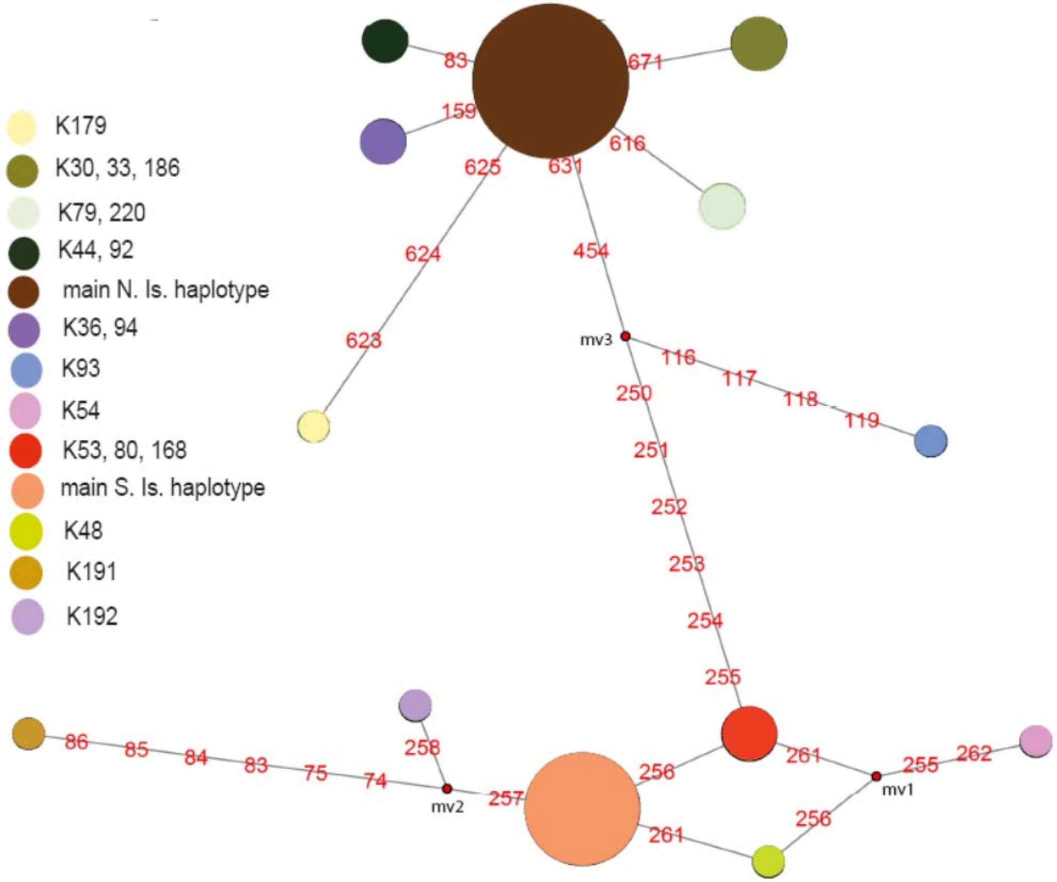


Fig. 13. Network showing relationship of cpDNA haplotypes of *K. salicornioides* (node size proportional to haplotype frequency)

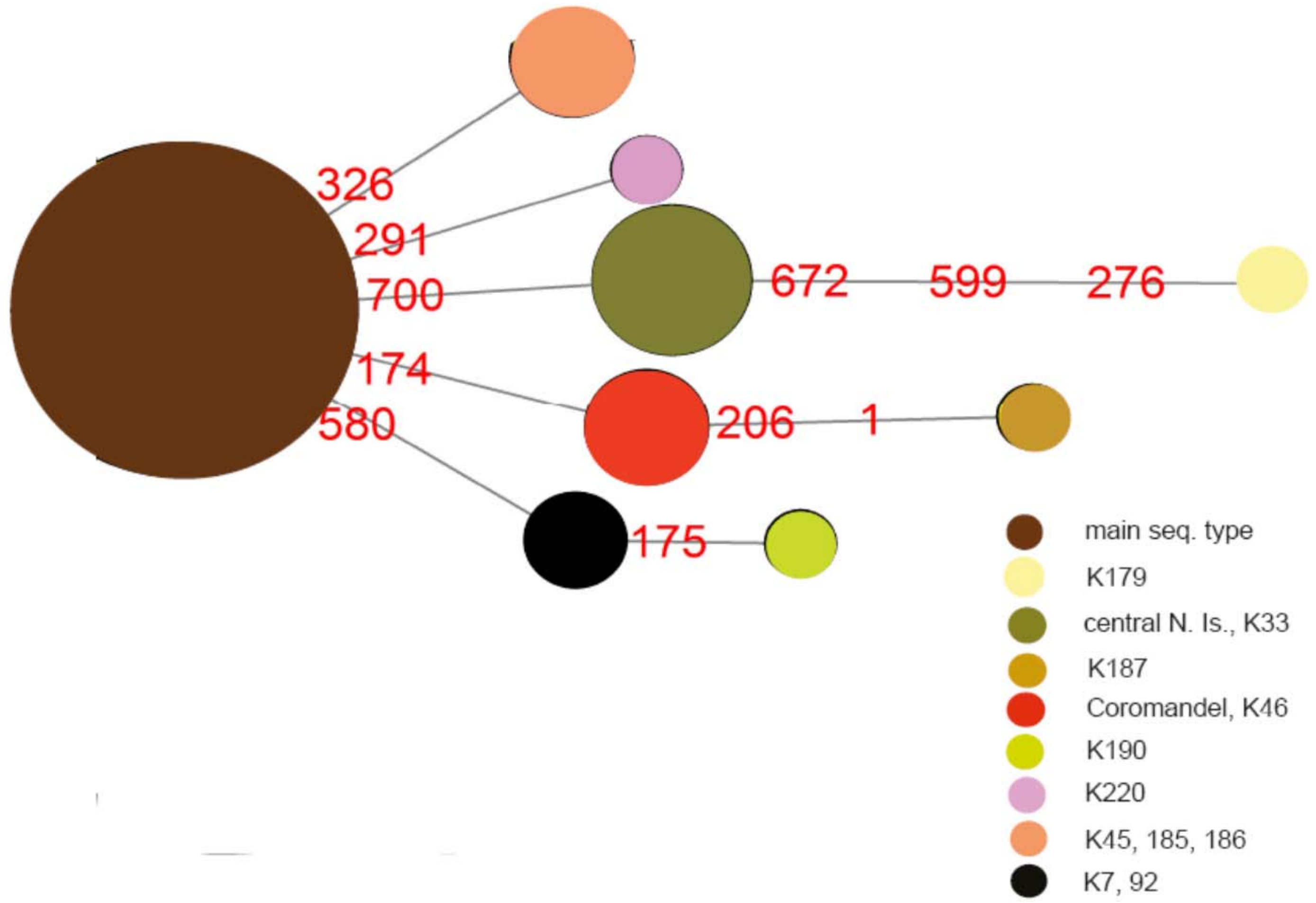


Fig. 14. Network showing relationship of ITS sequence types of *K. salicornioides* (node size proportional to haplotype frequency)

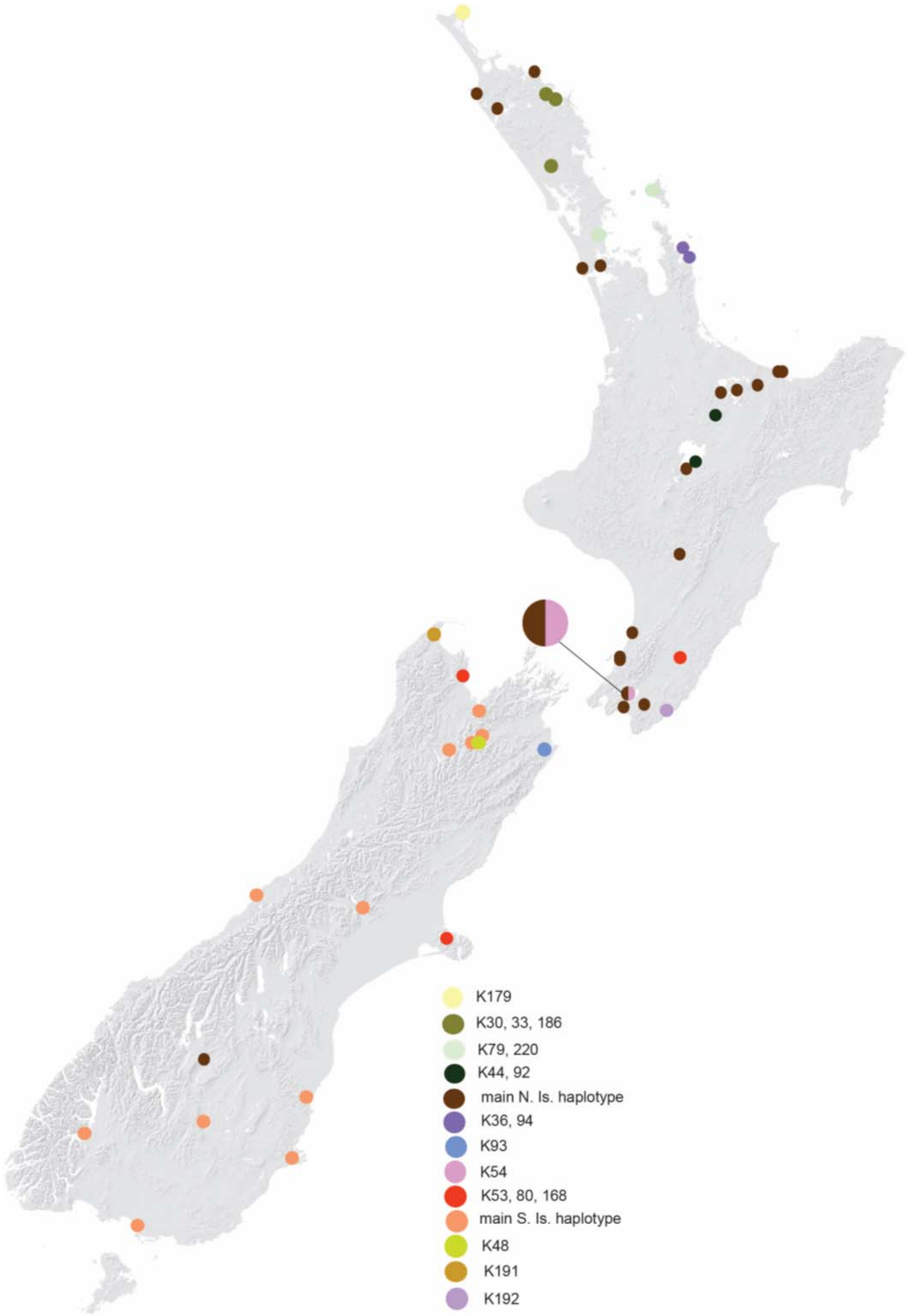


Fig. 15. cpDNA haplotype diversity in *K. salicornioides*, symbol with two colours shows the presence of more than one haplotype at a particular location

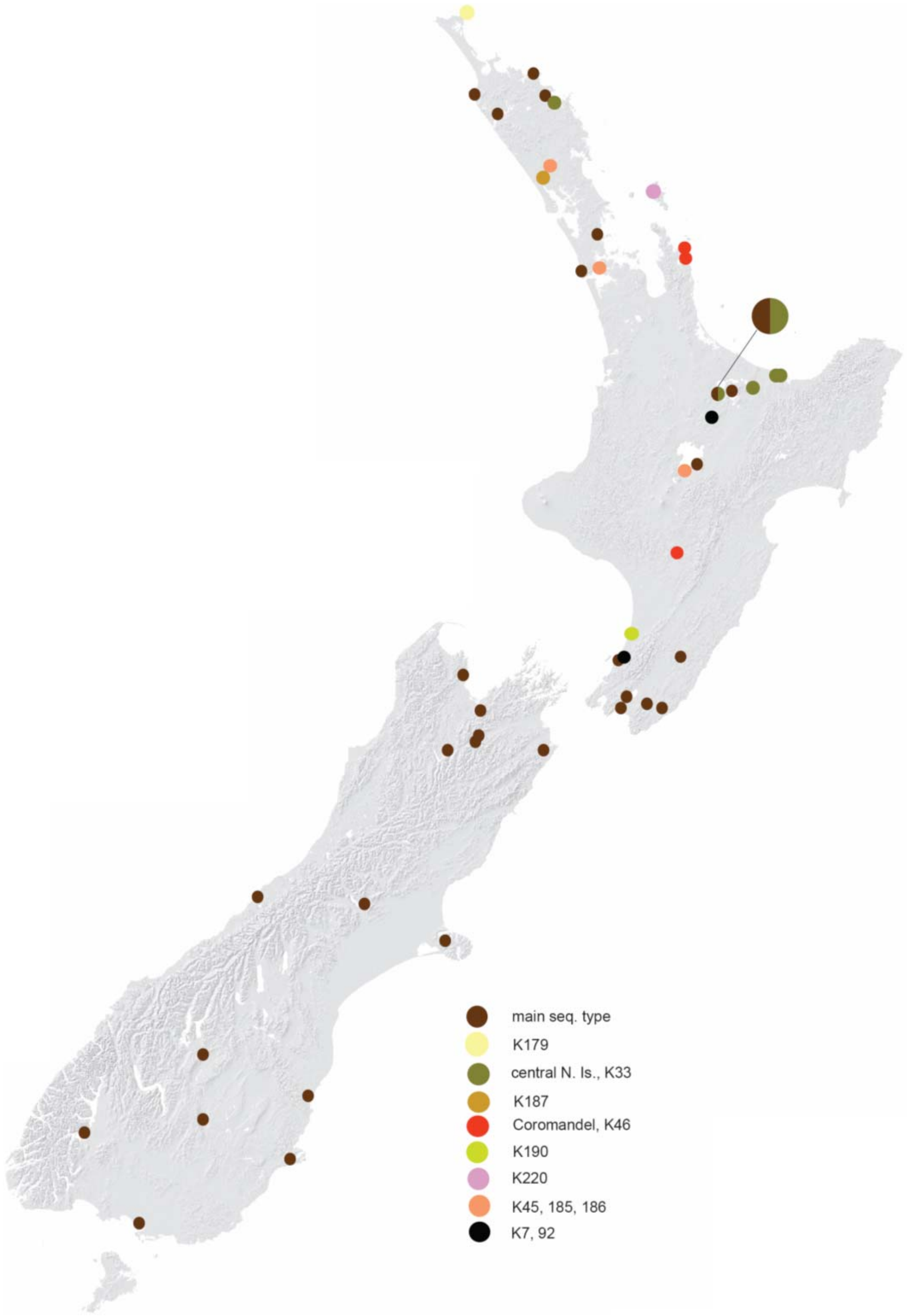


Fig. 16. ITS sequence type diversity in *K. salicornioides;* symbols with two colours show the presence of more than one sequence type at a particular location

DISCUSSION

Host specificity in New Zealand Korthalsella

This study shows that most of the primary and secondary hosts of the three New Zealand species are common species and are quite widespread, suggesting that pygmy mistletoe distributions are probably not usually limited by the absence of hosts, although suitable hosts may be comparatively rarer in some of the regions (Figs. 2A-G, 3A-L, 4A-D). Nevertheless, there are some noticeable gaps in the distributions of these mistletoes. For example, all three species are absent from the East Cape, Raukumara, King Country, Taranaki and Egmont ERs (Fig. 17) despite most of the main hosts being present in all of these regions. Similarly, the northern limit recorded for *K. clavata* is near Lake Whakamaru in the Central Volcanic Plateau ER, while *K. lindsayi* has not been recorded north of Pureora State Forest in the Western Volcanic Plateau ER and Waikura Valley in the Wairoa ER, even though the main hosts of both of these species extend further north and as far as North Cape in some cases. *Korthalsella lindsayi* is also absent from the west coast of the South Island despite the fact that its primary host *Melicope simplex* is found down most of the West Coast. *Korthalsella salicornioides* is the most widespread species, occurring from North Cape to Invercargill and on many off-shore islands (Little Barrier, Great Barrier, Mayor, Kapiti, D' Urville, Adele, Stewart, Big South Cape and Codfish Islands) but is also not as widespread as its main hosts.

Korthalsella clavata was once thought to be a strictly calciphile species (Wall 1920), as it was known only from shrubs growing amongst limestone rocks. From the current known distribution, it appears that although it has a preference for this habitat type, it is not always restricted to shrubs in this situation. It can sometimes be found up to elevations of 900 m or more; the highest elevation recorded is 1066 m in the Kaimanawa Ranges. In contrast, *K. lindsayi* is mostly a lowland species, the only high altitude record for this species is from Pureora State Forest at 1000 m. *Korthalsella salicornioides* is also mostly a lowland species, found up to 640 m and 675 m on Banks Peninsula and Stewart Island, respectively. The disjunct distribution of *K. clavata* in the North Island suggests that it might have had a broader range in earlier times and may have been eliminated from the eastern North Island by clearance of lowland forests and could potentially still be found in some suitable locations in eastern North Island with further searching (Ogle and Barkla 1995) but there is also evidence that these North

Island provenances may have a hybrid origin and may represent recent range expansion through hybridisation with *K. lindsayi* (see discussion below).

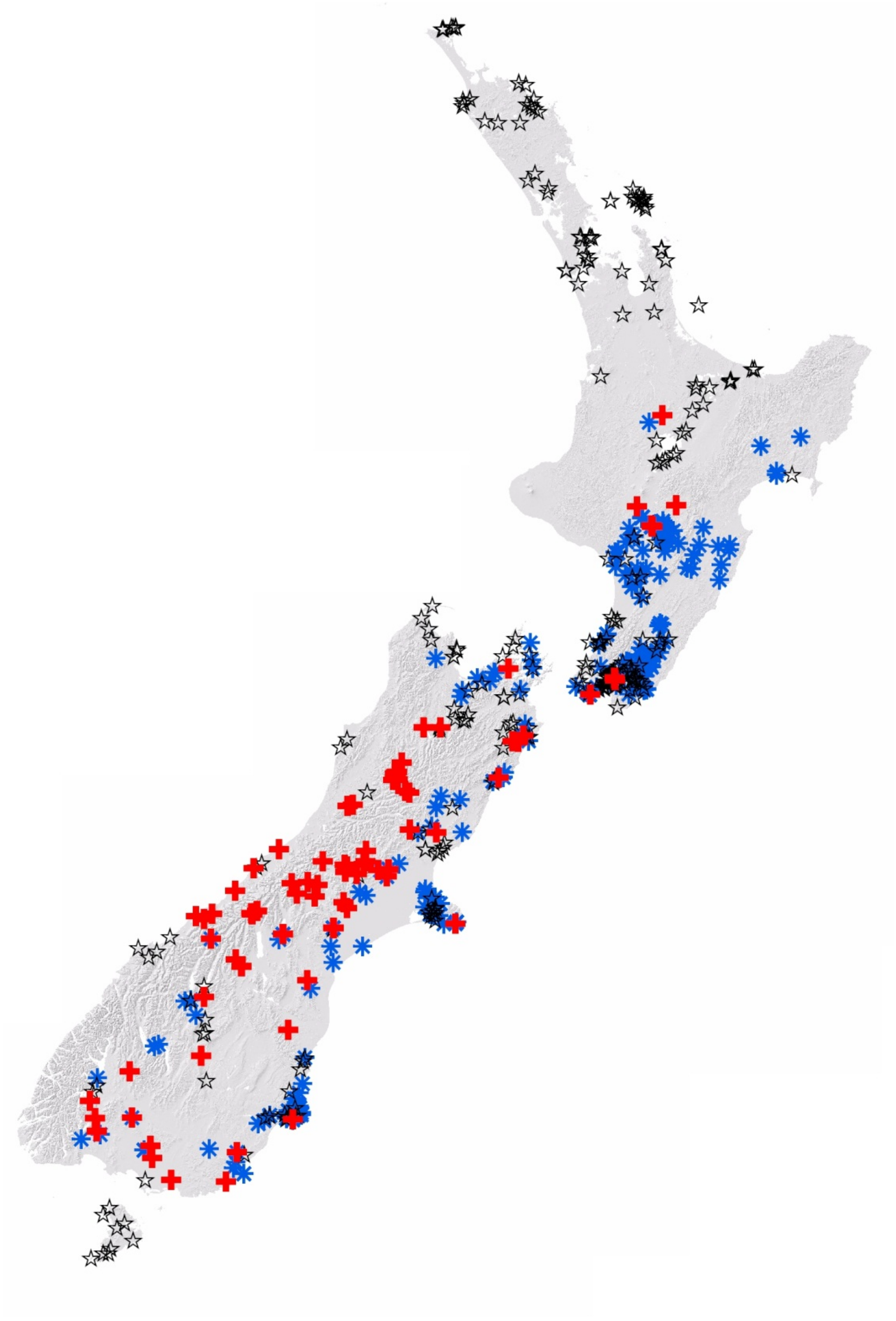


Fig. 17. Distribution of *K. clavata* (red crosses), *K. lindsayi* (blue asterisks) and *K. salicornioides* (black stars) in New Zealand

Korthalsella salicornioides is the most host-specific of the three New Zealand species specialising on *Leptospermum-Kunzea* hosts (and has a corresponding relatively low Shannon-Wiener index of 1.17). Ninety-six percent of all records for *K. salicornioides* are from these two myrtaceous genera. *Korthalsella clavata* and *K. lindsayi* are more generalised (with Shannon-Wiener indices of 2.88 and 2.84 respectively). However, despite the wide host range, *Korthalsella clavata* preferentially parasitises *Coprosma* species, with about 66% of total records from different species in this genus and non-*Coprosma* hosts constituting about one third of the total records. *Korthalsella lindsayi* preferentially occurs on *Melicope simplex* and *Coprosma* hosts, followed by *Lophomyrtus* and *Myrsine* hosts. Similar trends were also discernible in loranthaceous mistletoes of New Zealand (Norton and de Lange 1999). *Peraxilla colensoi* (Hook. f.) Tiegh., *P. tetrapetala* (L. f.) Tiegh. and *Alepis flavida* (Hook. f.) Tiegh. were specialists on *Nothofagus* Blume species (with diversity values of 0.71, 1.47 and 0.80 respectively), while *Tupeia antarctica* (G. Forst.) Cham. & Schltdl. and *Ileostylus micranthus* are generalists (with diversity values of 2.27 and 3.37 respectively) and utilise a wide range of un-related hosts. Host specificity is advantageous in terms of being capable of utilising the most common available host, while generalists can be more successful in habitats with a more diverse floristic composition (Norton and de Lange 1995). Kanuka-manuka (*Kunzea-Leptospermum*) heaths are very common in seral vegetation, are capable of responding vigorously to disturbance and owing to more tolerance to wetland conditions, *Leptospermum* is also very common in swamps and wetlands (Wardle 1991). Hence the wider distribution of *K. salicornioides* may be explained by its abundant, wide-ranging hosts.

Though *K. lindsayi* is recorded on 47 host species (Table 4) and *K. salicornioides* is the most host-specific species, the conviction that *K. lindsayi* can parasitise anything except a concrete post (Elder 1984) does not hold. Hosts for New Zealand taxa belong to APG clades Rosid I, Rosid II, Asterids, Asterid I, Asterid II with a single occurrence on the Eudicot *Clematis*. No gymnosperms or monocots are parasitised by New Zealand's pygmy mistletoes. In contrast, some of New Zealand's loranthaceous mistletoes use gymnosperms or monocots as hosts. For example, several species of native and introduced gymnosperms are used by *Ileostylus micranthus,* while Cabbage tree, *Cordyline* Comm. ex. R. Br. sp. was noted hosting *I. micranthus* at Dunedin Botanic Gardens (Tom Myers of Dunedin Botanic Gardens pers. comm.). *Prumnopitys taxifolia* (D. Don) de Laub. (de Lange *et al.* 1997) and *Ripogonum* *scandens* J. R. Forst & G. Forst (Ripogonaceae) (Smart 1952) were recorded as hosts for *Tupeia antarctica.*

Despite some host overlap, the three species amply demonstrate taxonomic host partitioning in terms of utilisation of the available flora since *K. clavata* and *K. lindsayi* share hosts only at the tertiary level and beyond, and *K. salicornioides* only occasionally uses the main hosts of the other two species (Fig. 18). This virtually eliminates inter-specific competition between species.

There is no clear pattern on the taxonomic affinity of the hosts for *K. clavata* and *K. lindsayi* other than they are all dicots (there are host combinations which are unique to each of these two species, e.g., occurrence of *K. clavata* on *Aristotelia fruticosa* and of *K. lindsayi* on *Lophomyrtus* species). The most conspicuous feature is that they are all shrubs or small trees and are frequently found on forest edges. This presumably helps keep mistletoes in high light environments with actively growing but moderately long-lived hosts as a result. The occasional or rare occurrence of *K. salicornioides* on *Melicope simplex*, *Coprosma* spp. and *Sophora* spp. is probably indicative of the occurrence of these hosts in the proximity of parasitised *Leptospermum* or *Kunzea* hosts. Seeds dispersed by the weak explosive mechanism (see chapter 5 for an account of the seed dispersal mechanism) from mistletoes growing on tall *Leptospermum* or *Kunzea* hosts are inevitably occasionally lodged onto other shrubs growing nearby and thus occasional success on these hosts is encountered (Appendix II: Fig. 13).

Host-mistletoe combinations are dynamic and expansion of the host range may also occur through pressure from land use change (e.g., resulting from population fragmentation and cultivation of new exotic and indigenous species around fragmented populations) and the activity of avian dispersers. In the absence of host inventories and information on regional host preferences, some of the new hosts may be overlooked (Downey 2004). The occurrence of Korthal mistletoes on rare and exotic hosts suggests that the potential host range is large, but these mistletoes are constrained by the fact that the seeds are rarely disseminated to acceptable hosts. Records of *Solanum sodomeum* L. and *Ficus* L. as hosts for the Hawaiian species *Korthalsella complanata* (Tiegh.) Engl. (Wagner *et al.* 1999) are examples of acceptability of hosts from families not usually utilised by the genus. The only other example where a *Korthalsella* species was recorded occurring naturally on a solanaceous plant is of *K. rubra* (Tiegh.) Engl. on *Lycium* L. (Downey 1998), while there is no other record of any of the *Korthalsella* species on *Ficus* or other Moraceae. Similarly, the occurrence of *K. disticha* (Endl.)

Engl. (Laing 1914) and *K. rubra* on peach (Downey 1998) and of *K. japonica* on apricot (Gupta 1974) are other examples of hosts from a family (Rosaceae) that is not otherwise parasitised by other species. Thus the restriction of mistletoes to certain native hosts does not necessarily mean that other species might not be acceptable as alternative hosts, as has also been shown by artificial inoculation and survival of *Viscum verrucosum* Harv. on succulent non-natural hosts (Heide-Jorgensen 2008); the usual hosts for *V. verrucosum* are *Acacia* Mill. species (Polhill and Wiens 1998). Similarly, the South African mistletoe *Viscum minimum* occurs on *Euphorbia polygona* Haw. and *E. horrida* Boiss. Host range experiments involving the germination and establishment of *V. minimum* on other *Euphorbia* hosts, showed that a number of succulent *Euphorbia* species were acceptable as hosts while non-succulent herbaceous *Euphorbia* species were not (Heide-Jorgensen 2008).

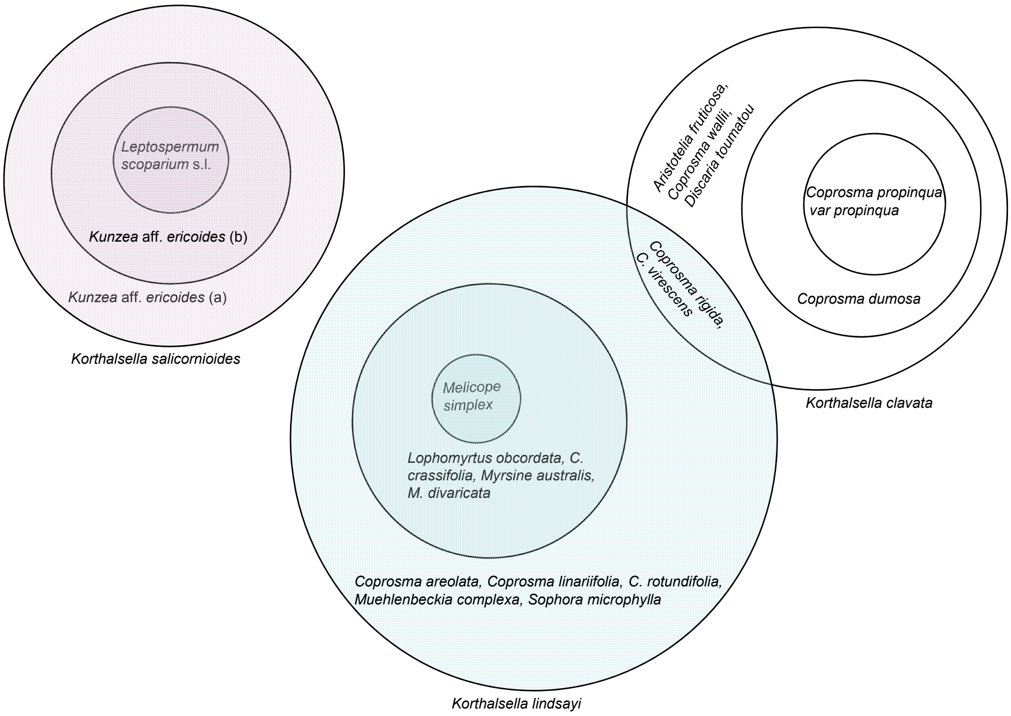


Fig. 18. Primary (inner most circle), secondary (middle circle) and tertiary hosts (outer most circle) of *Korthalsella salicornioides* (left), *K. clavata* (right) and *K. lindsayi* (middle) showing very little overlap in main hosts.

In more extensively collected regions, the number of host species encountered is obviously greater than in poorly collected areas (Downey 2004). That fewer host taxa were identified in some of the regions (Tables 6-8) might also reflect the lack of adequate collecting; more thorough surveys could potentially reveal additional hosts. *Korthalsella salicornioides* was classified as sparse under the 'naturally uncommon' category of threatened and uncommon plants of New Zealand by de Lange *et al.* (2009). *K. clavata* is a regionally threatened plant in Wellington Conservancy (Department of Conservation 2001) and is apparently rare in the whole of the North Island. It is hoped that this work will stimulate further studies on the regional host range and host preferences of New Zealand's pygmy mistletoes and will help in devising conservation management strategies for vulnerable populations of mistletoes.

Molecular genetic variability in New Zealand Korthalsella

Korthalsella clavata and *K. lindsayi* are polyphyletic both in plastid and ITS phylogenies. Results of the phylogenetic study (chapter 2) of the entire genus show that Hawaiian species *K. latissima* (Tiegh.) Danser and *K. platycaula* (Tiegh.) Engl. are also polyphyletic. Stevenson (1934) considered *K. clavata* to be a distinct species intermediate in morphological characters between *K. salicornioides* and *K. lindsayi.* Wilson (1996) postulated that *K. clavata* originated as a rare hybrid resulting from introgression by *K. salicornioides* genes into *K. lindsayi* populations, this is not supported by genetic data presented here. Temporal separation in flowering periods of *K. lindsayi* and *K. salicornioides* (see chapter 5) also precludes the chances of hybridisation between the two species. Most of the *K. salicornioides* collections from North Island formed a distinct clade in the plastid phylogeny, similarly most of the *K. clavata/K. lindsayi* collections from South Island also formed a distinct clade. Chloroplast *trnQ-rps16* data show geographically-based genetic structure rather than host-based structure. There are distinct North and South Island haplotypes in *K. salicornioides* and *K. clavata.* Moreover there is a distinct haplotype in the southern South Island in *K. lindsayi.* The genetic data based on nuclear ITS sequences show that most of the variability in *K. salicornioides* is concentrated on the North Island, whereas in *K. clavata* sequence type variability is concentrated on the South Island. Thus perhaps, *K. salicornioides* had a longer presence in the North Island compared to the South Island and *K. clavata* had a longer presence in the South Island compared to the North Island.

Incongruence between cpDNA and ITS data within *K. clavata* and *K. lindsayi* is possiby an outcome of hybridisation and introgression between *K. clavata* and *K. lindsayi*. Main haplotype 3 of *K. lindsayi* representing four South Island populations was similar to main haplotype 1 of *K. clavata* and a collection from Catlins in the South

Island (K217) was also closer to main haplotype 1 of *K. clavata* than conspecific collections. All North Island collections of *K. clavata* (representing three populations) had haplotypes similar to haplotype K66, 91 of *K. lindsayi.* A similar situation was apparent in South American species of *Nothofagus* where CP haplotypes were associated with geographic locations rather than to taxonomic relationships (Acosta and Premoli 2009). Acosta and Premoli (2009) considered this species-independent cpDNA phylogeographic structure to be a consequence of repeated chloroplast capture caused by replacement of cytoplasm of one species by that of another through hybridisation/introgression events over geological time. Chloroplast capture could occur frequently in species with sympatric distribution and reproductive compatibility (Acosta and Premoli 2009). In a later study Premoli *et al*. (2011) have shown that cpDNA haplotypes of five sympatric species in the subgenus *Nothofagus* were shared by all present species within a given area. Chloroplast capture has also been observed in *Alocasia* (Schott) G. Don species (Nauheimer *et al.,* 2012). Genetic signal left by hybridisation event may persist for considerable span of time even in occasionally hybridising plants with localised seed dispersal and obligate out-breeding (Nauheimer *et al.,* 2012). A variety of processes may cause cytoplasmic gene flow in the absence of significant nuclear gene flow and it can occur fairly rapidly leading to complete replacement of cytoplasm of one species by that of another, as a result unexpected chloroplast capture can occur among closely related species at different taxonomic ranks (Rieseberg and Soltis 1991). Since *K. clavata* and *K. lindsayi* populations are often sympatric (in fact, rarely are both species present on the same host or same host branch right next to each other, see Appendix II: Figs. 9-11) and since both are early spring flowering species, hybridisation/introgression are not unexpected between the two. My study shows that both cpDNA haplotypes and ITS sequence types are shared between *K. clavata* and *K. lindsayi* (Figs. 7, 8). The presence of sequences of the other species was more prevalent in cpDNA compared to ITS. However, given their varied ecologies and morphological differences both *K. clavata* and *K. lindsayi* are considered distinct species with occasional hybrids in a few locations.

The level of intraspecific variation encountered in this study corroborates a recent study by Bromham *et al.* (2013) which shows that parasitic plants have increased rates of molecular evolution compared to non-parasitic lineages. Their study suggests that consistent differences in rate of molecular evolution in parasitic lineages should be considered when interpreting molecular phylogenies and date estimates.

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APPENDIX I: Collection details for DNA accessions of New Zealand species of *Korthalsella* included in the molecular study. All samples were collected by the author, unless indicated by an herbarium accession number.

Collection details for *Korthalsella clavata* DNA accessions included in the molecular study

Ecological region	Locality	Host	Accesion No. when sampled from a herbarium specimen	DNA Accession	ITS sequence type	CP haplotype
North Island						
Central Volcanic Plateau	Mangakowhiriwhiri Gorge, Whakamaru	*Coprosma propinqua*	AK 304816	K181	main seq type	K27, 181, 198
Rangitikei	Mataroa, Hautapu River	*Coprosma obconica*	AK 283293	K198	main seq type	K27, 181, 198
Wairarapa Plains	Lake Wairarapa	*Muehlenbeckia complexa*	AK 232723	K210	main seq type	
	Oporua	*Coprosma propinqua*		K27	main seq type	K27, 181, 198
South Island						
Spenser	Rappahannock River, Maruia	*Coprosma propinqua*		K149	K136, 149	K136, 149
	Rappahannock River, Maruia	*Coprosma dumosa*		K136	K136, 149	K136, 149
	Maruia Valley	*Coprosma wallii*		K150	main seq type	main hapl 1
North Westland	Maruia	*Coprosma dumosa*		K145	main seq type	main hapl 1
	Buller, West Bank Maruia	*Coprosma tayloriae*		K146	main seq type	main hapl 1
	Buller, West Bank Maruia	*Coprosma propinqua*		K147	main seq type	main hapl 1
	Lake Brunner	*Coprosma propinqua*		K139	main seq type	main hapl 1
	Lake Brunner	*Coprosma dumosa*		K144	main seq type	main hapl 1
	Lake Brunner	*Coprosma tenuicaulis*		K17	main seq type	main hapl 1
Lowry	Mt Alexander	*Discaria toumatou*		K98	K98, 99, 100, 118,	main hapl 1

Ecological region	Locality	Host	Accesion No. when sampled from a herbarium specimen	DNA Accession	ITS sequence type	CP haplotype
					119	
	Mt Alexander	*Coprosma virescens*		K117	K95, 117	main hapl 1
	Mt Alexander	*Muehlenbeckia complexa*		K99	K98, 99, 100, 118, 119	main hapl 1
	Mt Alexander	*Carmichaelia australis*		K116		main hapl 1
	Mt Alexander	*Coprosma crassifolia*		K118	K98, 99, 100, 118, 119	main hapl 1
Canterbury Foothills	Cooper's Creek, Karetiana Maori Reserve	*Coprosma pedicellata*	AK 228392	K207	seq type 2	main hapl 1
Puketeraki	Broken River	*Aristotelia fruticosa*		K101	K101	main hapl 1
	Broken River	*Coprosma propinqua*		K100	K98, 99, 100, 118, 119	main hapl 1
	Broken River	*Discaria toumatou*		K164	K122, 164, 203	main hapl 1
	Castle Hill	*Myrsine divaricata*		K6	seq type 2	K6
	Castle Hill	*Coprosma propinqua*		K16	seq type 2	main hapl 1
	Castle Hill	*Pimelea oreophila* x *P. declivis*		K97	main seq type	main hapl 1
	Red Lakes near Lake Coleridge	*Discaria toumatou*		K4	seq type 2	main hapl 2
Heron	Potts River Valley, Rangitata Catchment	*Discaria toumatou*		K1	seq type 2	main hapl 2
	Potts River Valley, Rangitata Catchment	*Coprosma propinqua*		K5	seq type 2	main hapl 2

Ecological region	Locality	Host	Accesion No. when sampled from a herbarium specimen	DNA Accession	ITS sequence type	CP haplotype
Aspiring	Paringa Bridge SR, South Westland	*Coprosma dumosa*		K141	main seq type	main hapl 1
	Windbag Creek, Paringa, South Westland	*Coprosma dumosa*		K138	main seq type	main hapl 1
MacKenzie	Motuariki Island, Lake Tekapo	*Coprosma linariifolia*		K95	K95, 117	
	Glen Lyon Road, Lake Ohau	*Discaria toumatou*		K126	seq type 2	main hapl 2
	Glen Lyon Road, Lake Ohau	*Aristotelia fruticosa*		K128	seq type 2	main hapl 2
	Glen Lyon Road, Lake Ohau	*Olearia nummulariifolia*		K125	seq type 2	main hapl 2
	Glen Lyon Road, Lake Ohau	*Muehlenbeckia complexa*		K127	seq type 2	main hapl 2
	Glen Lyon Road, Lake Ohau	*Coprosma propinqua*		K123		main hapl 2
	Glen Lyon Road, Lake Ohau	*Coprosma crassifolia*		K124	seq type 2	main hapl 2
Otago Coast	Balclutha	*Olearia fragrantissima*	AK 238639	K203	K122, 164, 203	main hapl 2
	Balclutha	*Coprosma virescens*		K122	K122, 164, 203	main hapl 2
Te Wae Wae	Dean Burn Forest	*Coprosma obconica*	AK 281582	K208	main seq type	main hapl 1
	Dean Burn Forest	*Melicope simplex*		K156	main seq type	K154, 155, 156
	Dean Burn Forest	*Coprosma rotundifolia*		K21	main seq type	main hapl 1

Ecological region	Locality	Host	Accesion No. when sampled from a herbarium specimen	DNA Accession	ITS sequence type	CP haplotype
	Dean Burn Forest	*Coprosma wallii*		K155	main seq type	K154, 155, 156
	Dean Burn Forest	*Coprosma virescens*		K154	main seq type	K154, 155, 156
Makarewa	Otapiri Stream	*Coprosma virescens*		K158	K158	main hapl 1
	Tussock Creek	*Coprosma propinqua*		K157	K157	main hapl 1
Southland Foothills	Castle Rock, Dipton	*Coprosma propinqua*		K120	main seq type	main hapl 1

Collection details for *Korthalsella lindsayi* DNA accessions included in the molecular study

Ecological region	Locality	Host	Accesion No. when sampled from a herbarium specimen	DNA accession	ITS sequence type	CP haplotype
North Island						
Western Volcanic Plateau	Mt Pureora	*Myrsine divaricata*	AK 252125	K180	seq type 2	
Wairoa	Wairoa	*Myrsine divaricata*		K65	seq type 2	main hapl 1
Rangitikei	Te One Bush	*Melicope simplex*		K159	seq type 2	main hapl 1
	Te One Bush	*Coprosma rubra*		K160	seq type 2	main hapl 1
	Paengaroa Scenic Reserve	*Melicope simplex*		K11	seq type 2	K2, 11, 12, 74, 129
	Paengaroa Scenic Reserve	*Myrsine divaricata*		K10	K10, 214	K10
	Paengaroa Scenic Reserve	*Coprosma virescens*		K129	seq type 2	K2, 11, 12, 74, 129
	Rowland's Bush	*Coprosma linariifolia*	AK 314941	K199	seq type 1	K199 212
	Totara Reserve	*Melicope simplex*		K161	seq type 2	K161, 162, 163
	Totara Reserve	*Coprosma rhamnoides*		K163	seq type 2	K161, 162, 163
	Totara Reserve	*Myrsine australis*		K162	seq type 2	K161, 162, 163
Hawkes Bay	Avoca River	*Melicope simplex*		K67	seq type 2	main hapl 1
	Avoca River	*Lophomyrtus obcordata*		K68	seq type 2	main hapl 1
	Avoca River	*Coprosma areolata*		K69	seq type 1	main hapl 1
	Avoca River	*Coprosma rhamnoides*		K70	seq type 2	main hapl 1
Eastern Hawkes Bay	Huatokitoki Stream	*Myrsine divaricata*		K66	seq type 1	K66, 91
Manawatu	Tutaenui Stream Bulls	*Melicope simplex*		K64	seq type 1	main hapl 1

Ecological region	Locality	Host	Accesion No. when sampled from a herbarium specimen	DNA accession	ITS sequence type	CP haplotype
	Coles Bush near Rongotea	*Melicope simplex*		K2	seq type 1	K2, 11, 12, 74, 129
	Coles Bush near Rongotea	*Lophomyrtus obcordata x bullata*		K12	seq type 1	K2, 11, 12, 74, 129
	Coles Bush near Rongotea	*Coprosma rigida*		K74	seq type 2	K2, 11, 12, 74, 129
Eastern Wairararapa	Strong Creek a tributary of Kaumingi Stream Te Kanuka Stream	*Coprosma linariifolia*	AK 256621	K205	seq type 2	
	Glenside Station Mangahuia Stream Bush	*Lophomyrtus obcordata*		K90	seq type 1	K87, 88, 89, 90
	Glenside Station Mangahuia Stream Bush	*Melicope simplex*		K91	seq type 1	K66, 91
	Glenside Station Mangahuia Stream Bush	*Sophora microphylla*		K88	seq type 1	K87, 88, 89, 90
	Glenside Station Mangahuia Stream Bush	*Coprosma rhamnoides*		K89	seq type 1	K87, 88, 89, 90
	Glenside Station Mangahuia Stream Bush	*Muehlenbeckia complexa*		K87	seq type 1	K87, 88, 89, 90
	Ahi Paku	*Melicope simplex*		K82	seq type 1	K71, 82, 84, 85, 86, 214
	Ahi Paku	*Lophomyrtus obcordata*		K81	seq type 1	K81 83
	Ahi Paku	*Myrsine divaricata*		K83	seq type 2	K81 83
Wairarapa Plains	Martinborough - Masterton Road Ruamahanga River	*Muehlenbeckia australis*	AK 230829	K200	seq type 1	main hapl 1

Ecological region	Locality	Host	Accesion No. when sampled from a herbarium specimen	DNA accession	ITS sequence type	CP haplotype
	Oporua	*Myrsine australis*		K84	seq type 1	K71, 82, 84, 85, 86, 214
	Oporua	*Lophomyrtus bullata*		K85	seq type 2	K71, 82, 84, 85, 86, 214
	Oporua	*Coprosma rigida*		K86	seq type 2	K71, 82, 84, 85, 86, 214
Tararua	Wainuiomata	*Coprosma propinqua*		K72	seq type 1	main hapl 2
	Cape Turakirae	*Olearia solandri*		K71	seq type 1	K71, 82, 84, 85, 86, 214
	Cape Turakirae	*Olearia solandri*	AK 306384	K214	K10, 214	K71, 82, 84, 85, 86, 214
South Island						
Lowry	Marble Point	*Coprosma prostrata*		K151	seq type 1	K151
Canterbury Foothills	Waimakariri Gorge Bridge	*Sophora prostrata*		K119	seq type 3	main hapl 2
Banks	Otanerito Bay	*Lophomyrtus obcordata*		K105	seq type 1	main hapl 1
	Otanerito Bay	*Coprosma crassifolia*		K103	seq type 3	main hapl 1
	Otanerito Bay	*Melicope simplex*		K107	seq type 1	K107
	Otanerito Bay	*Muehlenbeckia complexa*		K104	seq type 1	main hapl 1
	Otanerito Bay	*Ileostylus micranthus*		K106	seq type 1	main hapl 1
Pareora	Peel Forest	*Myrsine australis*		K135	seq type 3	main hapl 2
	Peel Forest	*Melicope simplex*		K113	K113	main hapl 2
	Peel Forest	*Melicope simplex*		K114	K112, 114, 115	main hapl 2
	Peel Forest	*Myrsine divaricata*		K115	K112, 114, 115	main hapl 2
	Peel Forest	*Coprosma rubra*		K112	K112, 114, 115	main hapl 2
	Peel Forest	*Sophora microphylla*		K133	seq type 3	main hapl 2

Ecological region	Locality	Host	Accesion No. when sampled from a herbarium specimen	DNA accession	ITS sequence type	CP haplotype
	Peel Forest	*Lophomyrtus obcordata*		K132	seq type 3	main hapl 2
	Peel Forest	*Coprosma crassifolia*		K134	seq type 3	main hapl 2
MacKenzie	Motuariki Island Lake Tekapo	*Myrsine divaricata*		K109		main hapl 3
	Motuariki Island Lake Tekapo	*Sophora microphylla x prostrata*		K110	seq type 3	main hapl 3
Lakes	Lake Wakatipu	*Carmichaelia petrei*		K78	seq type 1	main hapl 2
Otago Coast	Okia Reserve	*Coprosma crassifolia*	AK 281594	K202	seq type 3	main hapl 2
	Guilds Hill	*Coprosma crassifolia*		K73	seq type 3	main hapl 3
	Guilds Hill	*Melicope simplex*		K111	K111	main hapl 3
	Aramoana	*Coprosma crassifolia*		K130	seq type 3	main hapl 3
	Aramoana	*Coprosma areolata*		K131	seq type 3	main hapl 3
	Aramoana	*Myrsine australis*		K108	seq type 1	main hapl 3
	Waipori Valley Kowhai Spur	*Coprosma virescens*	AK 288681	K212	seq type 3	K199 212
	Balclutha	*Myrsine divaricata*		K121	seq type 1	main hapl 2
Te Wae Wae	Waiau Broadlands Bush	*Coprosma obconica*	AK 252527	K209	seq type 3	main hapl 3
	Waiau Broadlands Bush	*Melicope simplex*		K137	seq type 3	main hapl 3
Catlins	Glenomaru Valley 8 km north of Owaka	*Melicope simplex*	AK 281597	K217	seq type 3	K217

Collection details for *Korthalsella salicornioides* DNA accessions included in the molecular study

Ecological region	Locality	Host	Accesion No. when sampled from a herbarium specimen	DNA accession	ITS sequence type	CP haplotype
North Island						
Te Paki	Surville Cliffs Plateau	*Leptospermum scoparium* s.l.	AK 319669	K179	K179	K179
Western Northland	Gumfields Road, Ahipara	*Leptospermum scoparium* s.l.		K29	main seq type	main North Is hapl
Eastern Northland Islands	Mahinepua Peninsula	*Leptospermum scoparium* var. *incanum*		K31	main seq type	main North Is hapl
	Mahinepua Peninsula	*Coprosma rhamnoides*		K32	main seq type	main North Is hapl
	Kerikeri Inlet Road	*Leptospermum* aff. *scoparium* (a)		K30	main seq type	K30, 33, 186
	Te Haumi, trackside Opua-Paihia walkway	*Kunzea ericoides* var. *linearis*		K33	central N Is, K33	K30, 33, 186
Western Northland	N Hokianga harbour, edge of peninsula near Panguru	*Leptospermum scoparium* s.l.		K59	main seq type	main North Is hapl
	Curnow Road	*Leptospermum scoparium* s.l.	AK 300261	K186	K45, 185, 186	K30, 33, 186
Kaipara	Maungaraho Rock	*Kunzea* aff. *ericoides* (b)	AK 232713	K187	K187	Not available
Cormondel	Motu Kaikoura Island, near Great Barrier Island	*Kunzea ericoides* s.l.	AK 328166	K220	K220	K79, 220
Auckland	Leigh Road, Silverdale	*Leptospermum scoparium* s.l.		K79	main seq type	K79, 220
	Lynfield, Manukau Domain	*Leptospermum scoparium* s.l.		K185	K45, 185, 186	main North Is hapl
	Waitakere Ranges, Winstone Track	*Kunzea* aff. *ericoides* (b)		K34	main seq type	main North Is hapl
	Waitakere Ranges, Winstone Track	*Leptospermum scoparium* s.l.		K35	main seq type	main North Is hapl

Ecological region	Locality	Host	Accesion No. when sampled from a herbarium specimen	DNA accession	ITS sequence type	CP haplotype
Cormondel	Black Jack Road	*Leptospermum scoparium* s.l.		K94	Coromandel K46	K36, 94
	Gemstone Bay	*Leptospermum scoparium* s.l.		K36	Coromandel K46	K36, 94
Whakatane	beside Whakatane Estuary, Piripai Spit	*Kunzea* aff. *ericoides* (e)	AK 300906	K189	central N Is, K33	
	beside Whakatane Estuary, Piripai Spit	*Kunzea* aff. *ericoides* (b)		K37, K38	central N Is, K33	main North Is hapl
	Kohi Point Scenic Reserve, between Whakatane and Ohope	*Leptospermum scoparium* s.l.		K39	central N Is, K33	main North Is hapl
	Monica Lanham Reserve, Kawerau township	*Kunzea ericoides* s.l.		K40	central N Is, K33	main North Is hapl
Northern Volcanic Plateau	Lake Okataina Scenic Reserve	*Kunzea ericoides* s.l.		K43	main seq type	main North Is hapl
	Te Puia, Geothermal Field, Whakarewarewa	*Kunzea ericoides* var. *microflora*		K41	central N Is, K33	main North Is hapl
	Te Puia, Geothermal Field, Whakarewarewa	*Leptospermum scoparium* s.s.		K42	main seq type	main North Is hapl
Cental Volcanic Plateau	Te Kopia Scenic Reserve	*Kunzea ericoides* s.l.		K92	K7, 92	K44, 92
	Lake Taupo, near Jellicoe Point, Motutere Bay	*Kunzea* aff. *ericoides* (b)		K44	main seq type	K44, 92
	Motuoapa	*Leptospermum scoparium* s.l.		K45	K45, 185, 186	main North Is hapl
Rangitikei	Mangaweka Scenic Reserve	*Melicope simplex*		K46	Coromandel K46	main North Is hapl
Manawatu	Horowhenua, Hokio Rd, Hokio	*Kunzea* aff. *ericoides* (a)	AK 281702	K190	K190	main North Is hapl
	Waikanae	*Leptospermum scoparium* s.l.		K7	K7, 92	main North Is hapl

Ecological region	Locality	Host	Accesion No. when sampled from a herbarium specimen	DNA accession	ITS sequence type	CP haplotype
	Waikanae	*Leptospermum scoparium* s.l.		K76	main seq type	main North Is hapl
	Waikanae	*Kunzea ericoides* s.l.		K75	main seq type	main North Is hapl
Eastern Wairarapa	Rata Hills	*Kunzea ericoides* s.l.		K53	main seq type	K53, 80, 168
	Pahaoa Gorge, Pahaoa River	*Kunzea* aff. *ericoides* (b)	AK 232915	K192	main seq type	K192
	Dry River Road	*Kunzea ericoides* s.l.		K58	main seq type	main North Is hapl
Wairarapa Plains	Lake Wairarapa, Western Lake Road	*Kunzea ericoides* s.l.		K54	main seq type	K54
	Lake Wairarapa, Western Lake Road	*Leptospermum scoparium* s.l.		K55	main seq type	main North Is hapl
	South Wairarapa, Western Lake Road	*Coprosma tenuicaulis*		K57	main seq type	main North Is hapl
	South Wairarapa, Pounui Lagoon	*Leptospermum scoparium* s.l.		K56	main seq type	main North Is hapl
South Island						
Northwest Nelson	Kaituna Track, Knuckle Hill	*Kunzea ericoides* s.l.	AK 281675	K191		K191
	Kaiteriteri	*Kunzea ericoides* s.l.		K168	main seq type	K53, 80, 168
Nelson	Hackett Creek, Bryant Range	*Kunzea ericoides* s.s.		K152	main seq type	main South Is hapl
Wairau	Upper Flaxbourne River	*Leptospermum scoparium* s.l.		K93	main seq type	K93
	Wairau River, Transverse Swamp	*Leptospermum scoparium* s.l.		K142	main seq type	main South Is hapl
	Branch River between Goat Stm Fuchsia Stm	*Kunzea* aff. *ericoides* (c)		K48	main seq type	K48
	Junction b/w Branch and	*Kunzea* aff. *ericoides* (c)		K153	main seq type	main South Is

Ecological region	Locality	Host	Accesion No. when sampled from a herbarium specimen	DNA accession	ITS sequence type	CP haplotype
	Leatham Rivers					hapl
Spenser	Teetotal Flats,Upper Buller River	*Kunzea ericoides* s.l.		K19	main seq type	main South Is hapl
	Lake Rotoiti	*Leptospermum scoparium* s.l.		K148	main seq type	main South Is hapl
Puketeraki	Lake Coleridge	*Kunzea* aff. *ericoides* (c)		K3	main seq type	main South Is hapl
Whataroa	Pakahi near Okarito	*Leptospermum scoparium* s.l.		K140	main seq type	main South Is hapl
Banks	Mt Herbert	*Kunzea ericoides* s.l.		K80	main seq type	K53, 80, 168
Wainono	Herbert, Mill House	*Kunzea ericoides* s.l.		K63	main seq type	main South Is hapl
Central Otago	Elbow Creek, Lake Roxburgh	*Kunzea* aff. *ericoides* (b)		K62	main seq type	main South Is hapl
	Poison Creek Conservation Area, Upper Clutha Valley	*Kunzea ericoides* s.l.		K61	main seq type	main North Is hapl
Fiord	Shallow Bay, Lake Manapouri	*Leptospermum scoparium* s.l.		K23	main seq type	main South Is hapl
Otago Coast	Northern Cemetry Dundein	*Erica arborea**		K47	main seq type	main South Is hapl
	Northern Cemetry Dundein	*Kunzea ericoides* s.l.		K77	main seq type	main South Is hapl
Makarewa	Grant Road, Bushy Point, Otatara, Invercargill	*Leptospermum scoparium* s.l.		K143	main seq type	main South Is hapl

* introduced exotic

Appendix II: Images of rare host combinations

Appendix II: Fig. 1. *Korthalsella clavata* on *Ulex europaeus* (CHR 535286)

Appendix II: Fig. 2. *Korthalsella lindsayi* on *Cytisus scoparius* (CHR 215913)

CHR 286145 BOTANY DIVISION, D.S.I.R., NEW ZEALAND

Korthalsella lindsayi (D. Oliv.) Engl.

Loc. Riccarton Bush Reserve, Christchurch c. 60 ft. S84/972567

Hab. Shrub in House grounds. Host – Syringa vulgaris

Coll. B.P.J. Molloy Date: 5-10-75

Det. B.P.J. Molloy Date: 8-2-77

Remarks:

Appendix II: Fig. 3. *Korthalsella lindsayi* on *Syringa vulgaris* (CHR 286145)

Appendix II: Fig. 4. *Korthalsella lindsayi* on *Ileostylus micranthus* (CHR 22408)

Appendix II: Fig. 5. *Korthalsella lindsayi* on *K. clavata* (CHR 286231)

Appendix II: Fig. 6. *Korthalsella lindsayi* on *K. lindsayi*

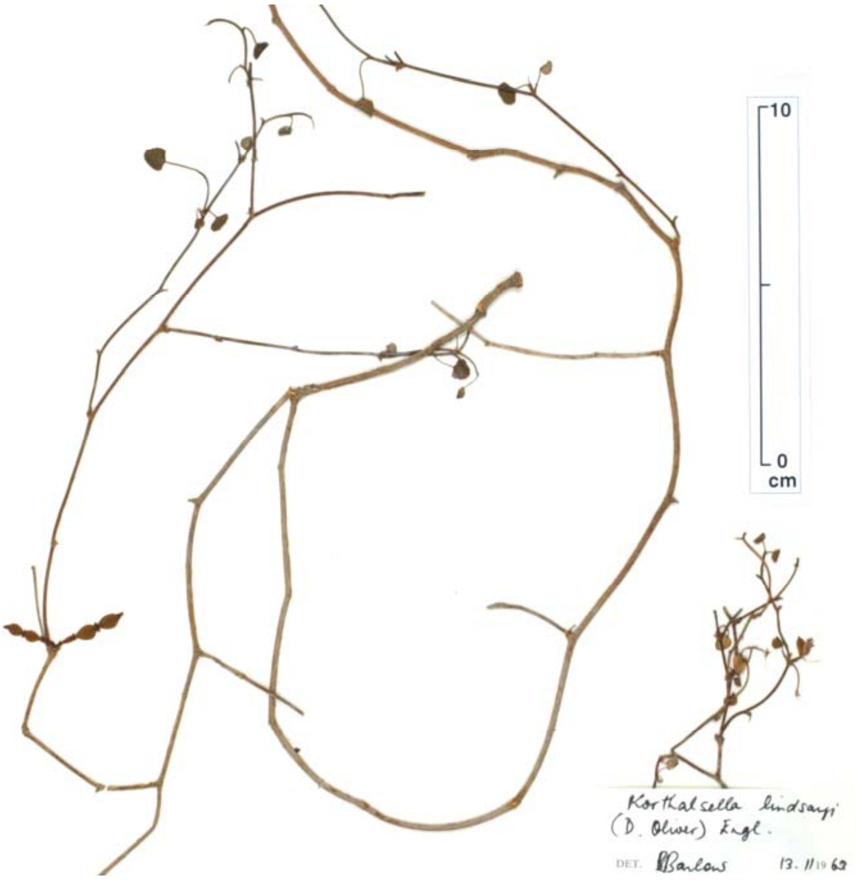
Appendix II: Fig. 7. *K. salicornioides* on *Erica arborea*

Appendix II: Fig. 8. *K. lindsayi* (left) and *K. salicornioides* (right) on *Melicope simplex* (AK 3876)

Appendix II: Fig. 9. *K. lindsayi* (left) and *K. clavata* (right) on *Melicope simplex* (CHR 286139)

Appendix II: Fig. 10. *K. lindsayi* (left) and *K. clavata* (right) on *Muehlenbeckia* sp. (CHR 286231)

Appendix II: Fig. 11. *K. lindsayi* (upper) and *K. clavata* (lower) on *Sophora microphylla* x *prostrata* (CHR 286224)



Appendix II: Fig. 12. *Korthalsella lindsayi* on *Scandia geniculata* (CHR 22409)

Appendix II: Fig. 13. *Korthalsella salicornioides* on *Coprosma propinqua*

Chapter 4: Experimental evidence for presence of potential host races in *Korthalsella salicornioides*

INTRODUCTION

Parasites can be more infective on hosts from local populations than on hosts from different locations (Lively *et al.* 1989). Reciprocal cross-infection experiments of parasites on hosts from different locations are a means of determining relative host specificity; better infectiveness on local host populations is suggestive of a genetic basis for host specialisation (Lively *et al*. 1989).

Host populations may contain different genotypes and correlated population variation of parasites might be suggestive of adaptation to local host populations (Hawksworth and Wiens 1996). Moreover, localised gene flow and spatial segregation of host species can also promote differentiation of parasite populations occurring on different host species (Clay *et al.* 1985). Genetic differentiation of plant parasites on different host species may be reinforced by localised dispersal of seeds onto the same host species and by temporal separation of anther dehiscence, stigma receptivity and fruit ripening of parasites growing on different host species, thus limiting the inter-host gene flow (Clay *et al.* 1985). A study on *Phoradendron villosum* in California (Thomson and Mahall 1983) showed that of the geographic range sampled, coast live oak (*Quercus agrifolia*) was infested only in the southern sites and remained uninfested in the northern sites, despite its presence in the northern parts. Conversely, blue oak (*Q. douglasii*) and valley oak (*Q. lobata*) were infested both at northern and southern sites. Similarly studies on *Phoradendron flavescens* (=*P. leucocarpum*) showed that elms (*Ulmus americana* and *U. serotina*) (Rucker and Hemmerly 1976), hickory (*Carya ovata*) (Brown and Hemmerly 1979) and black gum (*Nyssa sylvatica*) were each the most frequent host in different regions of Tennessee (Hemmerly *et al.* 1979). Thus the hosts used by a given parasite population may represent just a portion of the entire host range for a given species. The absence on a usually frequently utilised host suggests that the host population in that region is genetically different and resistant to infection or that the parasite has become locally adapted to an alternate host or the parasite is perhaps locally absent.

Extensions in the distribution of New Zealand’s Pygmy mistletoe populations may occur when non-primary hosts are parasitised in the absence of the main hosts. Once established within the new range, localised selection can work on variations in infective abilities on the new host increasing the ability to efficiently colonise it and local adaptations to the new host can gradually accumulate. An examination of host provenances of *Korthalsella salicornioides* in different ecological regions suggests selective preference for *Leptospermum* hosts over *Kunzea* hosts in some of the regions and vice versa in other regions, while in some regions both *Kunzea* and *Leptospermum* parasitic populations coexist (see Chapter 3). These host combinations may be genetically determined and may represent *Kunzea*- or *Leptospermum*-specific races. The presence of provisional segregate species within *Leptospermum scoparium* s.l. (Peter de Lange pers. comm.) and *Kunzea ericoides* s.l. (de Lange and Murray 2004, de Lange *et al.* 2005) in different ecological regions further supports the idea that differentially adapted populations might be present in *K. salicornioides*. However, none of the genetic markers screened so far support the presence of host races and any within-species genetic structure is geographic rather than host-based (see Chapter 3).

Several studies suggest the presence of host races in different viscaceous mistletoes. Based on cross-infection and establishment experiments in *Phoradendron californicum,* Overton (1997) reported the presence of *Prosopis*- and *Acacia*-types, *Prosopis*-type infecting *Prosopis* and *Acacia*-type infecting *Acacia* and *Cercidium*. Reproductive isolation was also recorded between these host specific types as the *Prosopis*-type flowered later than the mistletoes on other hosts (Overton 1997). This study corroborated the study of Glazner *et al.* (1988) which indicated genetic and morphological differentiation in *Phoradendron californicum* occurring on *Acacia* and *Prosopis* hosts. Clay *et al.* (1985) suggested that populations of the mistletoe *Phoradendron tomentosum* infesting different hosts were genetically differentiated as seedling development was greatest when maternal host and seedling host were the same. Infraspecific entities specialising on different hosts are also known in European mistletoe *Viscum album* (Zuber and Widmer 2000, Böhling *et al*. 2002, Mejnartowicz 2006), dwarf mistletoe *Arceuthobium americanum* (Jerome and Ford 2002), *Phoradendron bolleanum* and *P. juniperinum* (Wiens 1964).

The aim of this study was to determine the presence of potential *Leptospermum*- or *Kunzea*-adapted races in *K. salicornioides* by comparing the rate of successful establishment of *K. salicornioides* seedlings on *Leptospermum* and *Kunzea* hosts, in reciprocal and in corresponding maternal and seedling hosts.

MATERIALS AND METHODS

Reciprocal and corresponding seed planting was carried out for *K. salicornioides*, to test for the presence of *Kunzea*- or *Leptospermum*-adapted races. Seeds of mistletoes were collected from *Leptospermum scoparium* s.l. and *Kunzea* aff. *ericoides* (b) host populations at Waikanae and Lake Wairarapa, respectively, by bagging the mistletoes with fruits that were approaching maturity during the second half of February 2010. The bags were left on the plants for at least a week and then individual seeds were removed from inside the bags by soaking them in water for 10-15 minutes, which caused swelling of the viscin on the seeds and allowed easy removal. The sticky seeds were transferred to suitable branches on pot-grown hosts using a small wire loop. The eco-sourced potted *Leptospermum scoparium* s.l. and *Kunzea* aff. *ericoides* (b) hosts used in this study were acquired from native plant nurseries Newbury Gardens and Manawatu Native Plant Nursery, respectively. Ten *Kunzea* aff. *ericoides* (b) and six *Leptospermum scoparium* s.l. hosts were used in this study and 1560 seeds were planted.

The seeds were monitored for two years after planting and then scored for the rate of establishment on different potted hosts. The success rate was analysed using a binomial generalised linear model (GLM), treating each host plant as a replicate and comparing the success on each of the four combinations of mistletoe provenance and host type, using least square differences post-hoc tests to identify significantly different means.

RESULTS

Overall, only a small proportion of the transplanted *Korthalsella salicornioides* survived and established in the two years following transplant. However, the seeds had a significantly better success rate when the source provenance and host type were the same. Seeds collected from *Leptospermum* mistletoes and planted onto *Leptospermum* hosts (*Leptospermum* x *Leptospermum*) had a success rate of 4.74% compared to only a 1% success rate in *Kunzea* x *Leptospermum* seed plantings (Fig. 1). Similarly, the *Kunzea* x *Kunzea* seed plantings had a success rate of 8.65% compared to the *Leptospermum* x *Kunzea* combination with a success rate of 0.58 % (Fig. 1, Table 1).

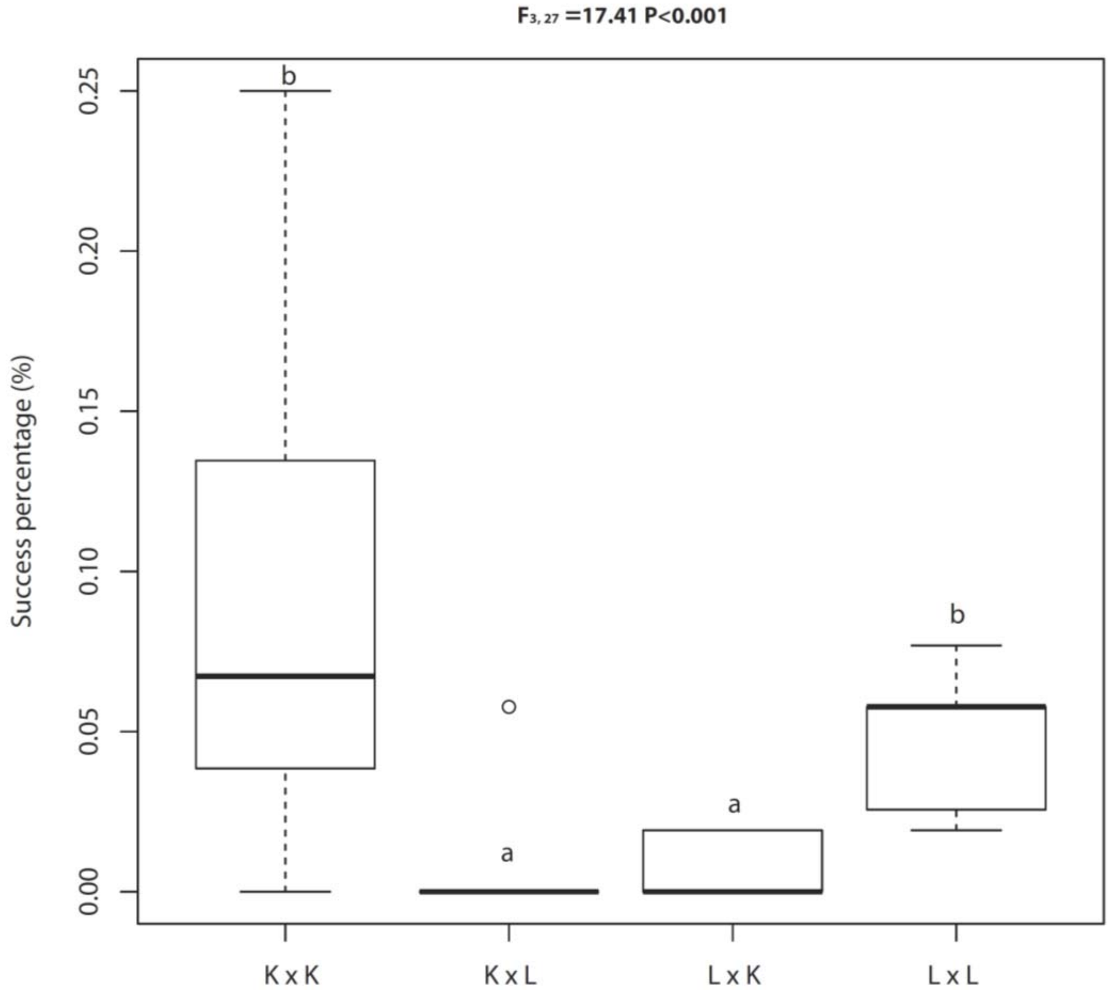


Fig. 1. Success rates in *Kunzea* x *Kunzea* (K x K), *Leptospermum* x *Leptospermum* (L x L) and reciprocal seed plantings (*Kunzea* x *Leptospermum*= K x L; *Leptospermum* x *Kunzea*= L x K). The boxes that share letters are not significantly different from each other.

Table. 1. Numbers of seeds planted and successes in each combination

	No. of seeds planted	No. of successes
Kunzea on *Kunzea*	520	45
Kunzea on Leptospermum	520	3
Leptospermum on *Leptospermum*	234	11
Leptospermum on *Kunzea*	286	3

DISCUSSION

The differential success rates in the two *K. salicornioides* provenances tested in this study support the hypothesis that ecotypes exist within these species that are adapted to different host types. The difference in success rates were large and strongly significant and despite heavy losses in all cases, 6-7% of transplants succeeded in the matched pairings. A similar study in *Phoradendron tomentosum* (Clay *et al.* 1985) showed that development of haustorial disks was significantly greater when source and experimental host species were the same compared to when experimental and source host species were different. However, their study was not conclusive in terms of successful establishment of seedlings as it terminated due to inclement weather conditions causing high mortalities in the developing seedlings. In my study of *K. salicornioides,* the first shoots were produced in about 12-15 months and about 11% of the successful mistletoes attained reproductive status within two years from initial planting, thus allowing for observations on successful establishment of mistletoes onto different hosts. In the unmatched pairs, the success rate was much lower (less than 1% survived to 2 years and none of these had flowered in the *Kunzea* x *Leptospermum* combination). Thus, there appears to be significant barriers preventing these mistletoes from switching to a different host under these experimental conditions.

However, two caveats to this conclusion must be added. First, molecular data - including both a nuclear and a chloroplast region (nrITS and cp *trnQ-rps16,* respectively) do not support the presence of *Leptospermum*- or *Kunzea*- adapted forms in *K. salicornioides* (chapter 3). The molecular data, especially the chloroplast data, support the presence of distinct geographically-based haplotypes in the North and South Islands, but both haplotypes appear to be equally able to colonise *Leptospermum* and *Kunzea* hosts. Similarly, the North and South Island populations of *K. clavata* are also distinct haplotypes, but there are no apparent host races within these geographic regions (host range, distribution chapter). The southern South Island populations of *K. lindsayi* contain a distinct haplotype and there are no apparent host races here either.

Second, since only one population each of *Kunzea* and *Leptospermum* mistletoes and only one provenance of each host was tested, the conclusion of significant differences in infectability in the mistletoe provenances must be regarded as preliminary. An experiment including a wider range of *K. salicornioides* populations in different ecological regions and parasitic on different *Kunzea* and *Leptospermum* hosts should give more conclusive answers.

More genetic work is also needed. The regions used in the molecular study are variable among species but there is little within-species diversity and so are probably too conservative to detect population-level differences and thus may have missed host-associated genetic diversity. More rapidly evolving regions, such as microsatellites, may help to elucidate the presence of host races in different ecological regions.

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Chapter 5: Reproductive Biology of *Korthalsella salicornioides* and *K. lindsayi*

INTRODUCTION

Reproductive biology studies in parasitic plants help elucidate important aspects of the life history of these elusive plants. These studies are not only important in developing strategies for management of parasitic plants that take a toll on economically important plants, but they are also are helpful in devising conservation management for threatened parasitic plants. The reproductive biology of some viscaceous mistletoes is well studied. *Arceuthobium* contains ambophilous (Penfield *et al.* 1976, Gilbert and Punter 1984, 1990, Gilbert 1988), predominantly anemophilous (Player 1979), as well as predominantly entomophilous species (Gregor *et al.* 1974). Studies in the genus *Viscum* suggest that *V. cruciatum* is predominantly entomophilous (Aparicio *et al.* 1995), whereas some studies in *V. album* suggest that it is an anemophilous species (Hatton 1964) and several studies support an entomophilous pollination syndrome in this species (Lawalree 1952, Kay 1989). Members of *Viscum, Notothixos* and *Phoradendron* depend on avian frugivores for seed dispersal, while *Arceuthobium* has ballistic fruits that disperse seeds by an explosive mechanism where seeds are typically dispersed to 10 m or less this way (Hawksworth and Wiens 1996).

Colonising new host individuals periodically is vital for parasitic plant populations to survive, as heavy parasite load can eventually lead to host/host branch mortality and thus parasitic plants have to be well dispersed and reproductively efficient. Effective pollination is a prerequisite to seed production and effective mechanisms of seed dispersal to suitable hosts are essential for perpetuation of parasitic plant populations. Enticing insects to visit tiny, inconspicuous flowers is not easy however, and birds are unlikely to disperse small unattractive fruits. Alternate methods such as wind pollination and explosive seed dispersal have been employed in parasitic plants (*Arceuthobium*) facing this kind of dilemma.

The only detailed study on the biology of *Korthalsella* spp. in New Zealand is that of Stevenson (1934), but this study lacked discussion of most aspects relating to the reproductive biology of these species, particularly pollination biology, and it had a limited discussion on dispersal in these species.

The aim of the current study is to investigate the reproductive biology of New Zealand *Korthalsella*. These studies were carried out on *K. lindsayi* and *K. salicornioides* in the lower North Island.

MATERIALS AND METHODS

The pollination biology of *K. salicornioides* was studied at Waikanae where it is parasitic on *Leptospermum scoparium* s.l. The dispersal studies in *K. salicornioides* were carried out at western shore of Lake Wairarapa.The study site selected for *K. lindsayi* was Coles Bush, near Palmerston North, where it is parasitic on *Melicope simplex, Lophomyrtus obcordata, L. obcordata* x *bullata*, *Coprosma areolata, C. crassifolia, C. rigida*, *Myrsine australis* and *Muehlenbeckia complexa.* Pollination and dispersal studies were both conducted at the site.

Experimental manipulations to determine pollination requirements in both species were carried out using mesh bags to exclude insects and breathable plastic bags to exclude conspecific pollen. The latter were acquired from Proseed New Zealand who extensively use these bags in pine breeding. These experiments were carried out over a four year period from spring 2008-spring 2011. The treatments comprised mesh bagging, pollen proof bagging, open hand selfing, open hand crossing, and combinations of these treatments namely hand self with mesh bagging, hand cross with mesh bagging and hand self with pollen-proof bagging. Emasculation and hand crossing were also attempted but were discontinued as female flowers adjacent to male flowers aborted in the process of emasculation presumably as a result of physical injury. Indices of autonomous selfing (pollen proof bag/cross ratio), self-compatibility (hand selfed/hand crossed ratio) and pollen limitation (natural/cross ratio) were determined from fruit set values in pollination experiments (Newstrom and Robertson 2008). A wind pollination index (mesh bagged/natural ratio) was also calculated.

Measurements of floral features, seed and fruit size were made using Motic Plus software and a stereo dissecting microscope. Nectar samples were collected by absorbance and subsequent drying of nectar contents from male and female flowers onto small chromatography paper wicks as outlined in McKenna and Thompson (1988). These wicks were mounted onto pins and stored in plastic containers lined with polystyrene block that was divided into a square grid, each labelled square cell receiving one nectar sample to air dry. Each wick was later re-hydrated in a known volume of water separately in small glass vials to dissolve the nectar absorbed. The sugar content of the resulting wash was quantified by a sulphuric acid anthrone colourimetric assay (McKenna and Thomson 1988) in comparison with sucrose standard solutions.

Pollinating insects collected by sweep netting during each flowering season were kept in 70% ethanol in small glass vials or were pinned and stretched until dry for final storage. Each specimen was initially sorted to family or sometimes to genus level. Jo Berry (Landcare Research, Auckland), Anthony Harris (Otago Museum, Dunedin) and Barry Donovon (Donovan Scientific Insect Research, Canterbury Agriculture and Science Centre, Lincoln) confirmed the identity of hymenopterous pollinators to species level and dipterous pollinators were identified by Ian Andrew (Institute of Natural Resources, Massey University, Palmerston North).

Ultrastructural studies on pollen morphology, mode of pollen exudation and stigmatic surfaces in *K. salicornioides* were done on a FEI Quanta 200 Environmental Scanning Electron Microscope at Manawatu Microscopy and Imaging Centre, Massey University. Samples were cut to appropriate size and fixed in a primary fixative comprising 3% glutaraldehyde, 2% formaldehyde, in 0.1M phosphate buffer (pH 7.2) for 24-48 hours at room temperature. The fixed samples were given three buffer washes for 10-15 minutes each wash. Samples were then dehydrated through an ethanol series: 25%, 50%, 75%, 95%, 2 x 100% and critical-pointdried using liquid CO_2. Dried samples were mounted on aluminium support stubs using double-sided tape or conductive silver paint and sputter coated with gold.

Field experiments to determine the explosive nature of *Korthalsella* fruits were done using vinyl acetate sheets that were fixed at varying distances (7 cm to 35 cm) from the mistletoes when the fruits were approaching maturity. The sheets were observed after eight days for the presence of seeds adhering to the surface facing the mistletoes.

A laboratory experiment was also carried out to determine the explosive nature of *Korthalsella* fruits. This laboratory experiment was intended to measure dispersal distance without any influence of wind to propel the seed. Several branches with mature *K. salicornioides* fruits were kept in a small plastic vial lined with moist tissue paper, the vial was attached horizontally using double-sided tape to a wall in the laboratory and the seeds ejected were observed the following day on the surface of the desk below. Dispersal distances in the field were determined by recording seed rain shadow on cloth sheets 3, 5 and 8 meters long depending on the height of the host canopy. For taller hosts, longer sheets were used. Mistletoe height within the host canopy ranged from 1.5 to ~ 4 m in *K. lindsayi* and 2.5 to ~ 6 m in *K. salicornioides*. Host shrubs or trees on the margins of the forest or isolated trees were used in this study to avoid seed rain from adjacent mistletoe infected hosts. The orientation of the sheets was dependent on the spatial distribution of mistletoes within the hosts and on the direction of the prevailing wind.

For life cycle studies, seeds were collected by bagging the mistletoes for at least a week with fruits approaching maturity. Individual seeds were removed from inside the bags by soaking the bags in water for 10-15 minutes, which allowed for swelling of the viscin on seeds and easy removal of the seeds. The sticky seeds were then manually transferred onto pot-grown host branches using a small wire loop. Eco-sourced potted hosts used in this study were acquired from Manawatu Native Plant Nursery and from Newbury Gardens. *Korthalsella lindsayi* seeds were planted onto *Melicope simplex* (Rutaceae), *Lophomyrtus obcordata, L. bullata*, *Metrosideros diffusa* (Myrtaceae), *Myrsine australis* (Myrsinaceae), *Muehlenbeckia astonii* (Polygonaceae) and *Olearia solandri* (Asteraceae). *Korthalsella salicornioides* seeds were planted onto *Kunzea ericoides* s.l. and *Leptospermum scoparium* s.l. The seeds were periodically observed for germination and establishment on the different hosts for three years following transfer.

The fruit set in different experimental manipulations to determine pollination requirements was analysed using a binomial generalised linear model (GLM), treating each mistleote as a replicate and comparing the fruit set in each of different treatments, using least square differences post-hoc tests to identify significantly different means. The impact of gall mite *Aceria korelli* (Eriophyidae) on reproductive output was also studied.

RESULTS

Floral Biology and Pollination

Korthalsella salicornioides is an Austral summer flowering species, which starts flowering around mid-November and continues until mid-December. The plants are monoecious and flowers develop as a cluster of five, with one axillary male flower and two female flowers on each side (Fig. 1). There are two clusters of five flowers on each node and these clusters are oriented in a decussate manner along the flowering branches. The flowers are interspersed with reddish brown trichomes. Flowers are produced in the upper axils only; lower branching axils do not produce flowers. The flowers of both sexes have three minute petals - the female flowers are about 0.7-0.8 x 0.5-0.6 mm and are sessile while male flowers are about 1 x 0.8-0.9 mm and are shortly pedicellate. The pollen is tricolporate and subprolate and has a smooth exine (Fig. 2). The anthers are bisporangiate and all six sporangia of the three anthers fuse forming a disc-like structure called a synandrium, which is about 0.29-0.45 mm in diameter. The pollen exudes from a central pore (Fig. 3). In the female flowers the corolla is adnate to the gynoecium and only the sessile stigma surface is evident in the form of a convex warty protuberance (Fig. 4). The fruit (a berry) is pale green, c. 1.5 x 1 mm and each contain a single seed which is c. 0.9 x 0.7 mm (Fig. 5).

Both male and female flowers exude nectar (Fig. 6a) and attract hymenoptera and diptera (Fig. 6b-d; Table 1). *Lasioglossum sordidum, Spilogona* sp. and *Dilophus* sp. were found to be important insect pollinators for *K. salicornioides* at the Waikanae study site. *Podagritus albipes* was also a regular visitor on flowering *K. salicornioides* plants at the study site, preferentially probing male flowers for nectar (Fig. 6b).

Nectar sucrose equivalent averages in female flowers were 4.76 µg (std error =0.89, n=10), 8.24 µg (std error=1.48, n=10) and ranged from 7.75 µg (std error =0.75, n=10) to 9.03 µg (std error =1.84, n=8) in male flowers.

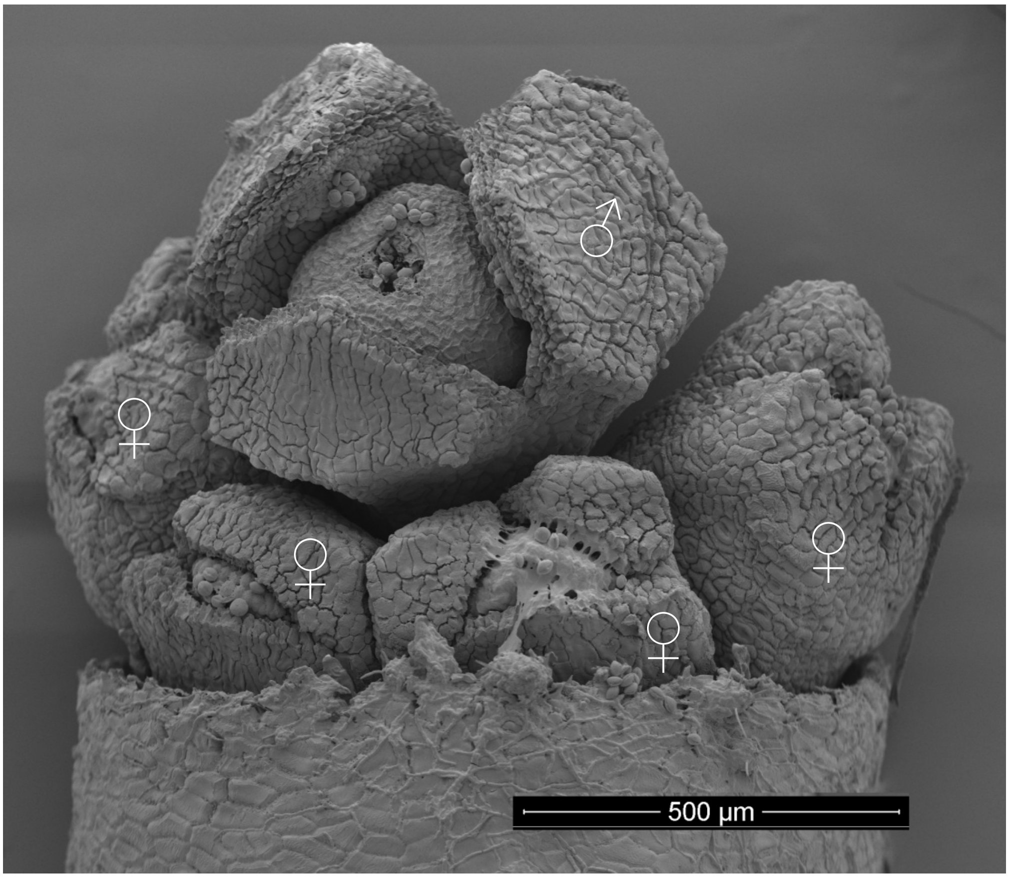
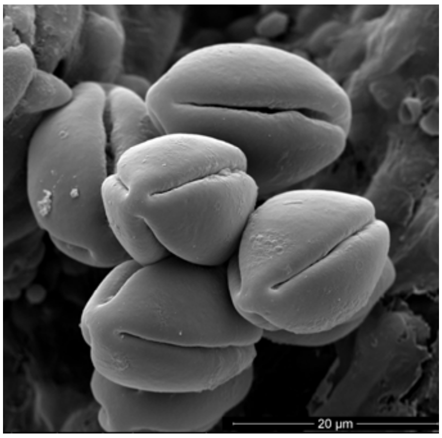


Fig 1. Scanning electron micrograph of a *Korthalsella salicornioides* floral cluster



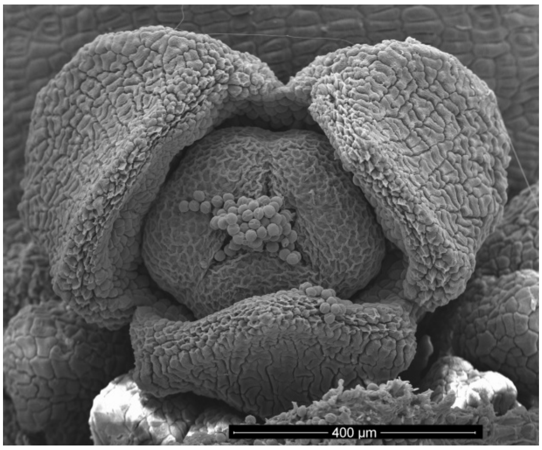


Fig 2. Scanning electron micrograph of *Korthalsella salicornioides* pollen grains (scale bar = 20 µm)

Fig 3. Scanning electron micrograph of a *Korthalsella salicornioides* male flower (scale bar = 400 µm)

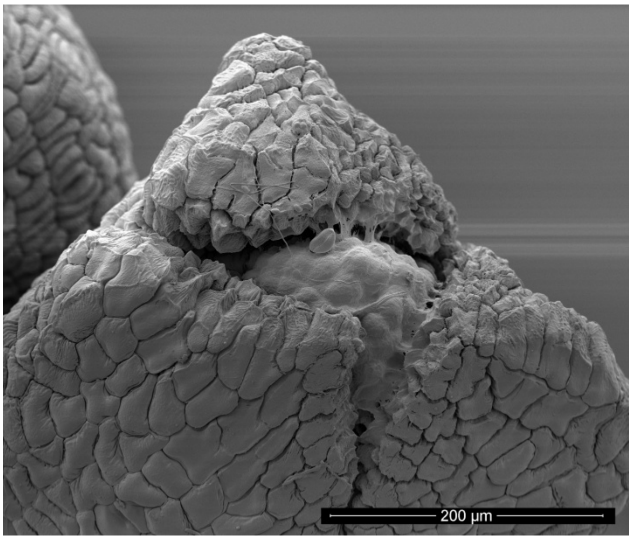


Fig 4. Scanning electron micrograph of a *Korthalsella salicornioides* female flower (scale bar=200 µm)

Fig 5. Close-up of a *Korthalsella salicornioides* seed (v=viscin layer)

Fig 6a (above left). *Korthalsella salicornioides* with a nectar droplet (arrow) in one of the male flowers, Fig 6b (above right). *K. salicornioides* flowers visited by *Podagritus albipes,* Fig 6c (bottom left). *K. salicornioides* flowers visited by *Pales* sp., Fig 6d (bottom right). *K. salicornioides* flowers visited by *Spilogona* sp.

Table 1. Insect pollinators recorded on *K. salicornioides* at the Waikanae site

	Family	Pollinator species
Diptera	Bibionidae	*Dilophus* sp.
	Muscidae	*Spilogona* sp. ♀
	Syrphidae	*Allograpta* sp.
	Tachinidae	*Mallochomacquartia vexata*
		Pales sp. ♂
	Tipulidae	*Leptotarus* (*Macromastix*) sp.
Hymenoptera	Sphecidae	*Podagritus albipes* ♂
	Apidae	*Apis mellifera*
	Colletidae	*Hylaeus relegatus*
	Halictidae	*Lasioglossum sordidum*

When plants were covered with mesh bags, fruit set was significantly reduced compared to natural fruit set (Fig. 7, Table 2). However, during 2010, in mesh bags, and in treatment where plants were hand selfed in mesh bags, the fruit set was anomalously high compared to natural fruit set. Hand crossed plants without mesh bags had reduced fruit set compared to natural fruit set during the same year (2009), however, the difference was not significant. When plants were covered with pollen-proof bags fruit set was significantly reduced compared to natural control. However in 2009, in treatment where plants were hand selfed and covered with pollen proof bags, fruit set significantly increased compared to when plants were covered with pollen proof bags without selfing.

Table 2. Percent fruit set in *K. salicornioides* and *K. lindsayi* in different pollination treatments, ASI = autonomous selfing index, SCI = self-compatibility index, PLI = pollen limitation index, WPI = wind pollination index

Treatment	*K. salicornioides*			*K. lindsayi*			
	2008	2009	2010	2008	2009	2010	2011
Pollen excluded	27.66	6.54		4.4	4.11	16.87	
Insects excluded	22.58	20.1	46.45	11.8	36.94	32.26	
Hand Crossed		48.7			47.67	60.68	
Hand Selfed & insect excluded			54.24				45.47
Hand Crossed & insect excluded			39.1				60.51
Hand Selfed & pollen excluded		28.82			22.95	49.16	
Natural Control	62.56	54.62	42.8	52.2	57.19	66.45	63.99
ASI		0.13			0.09	0.28	
SCI			1.39				0.75
PLI		-0.12			-0.2	-0.1	
WPI	0.36	0.37	1.1	0.23	0.6	0.5	

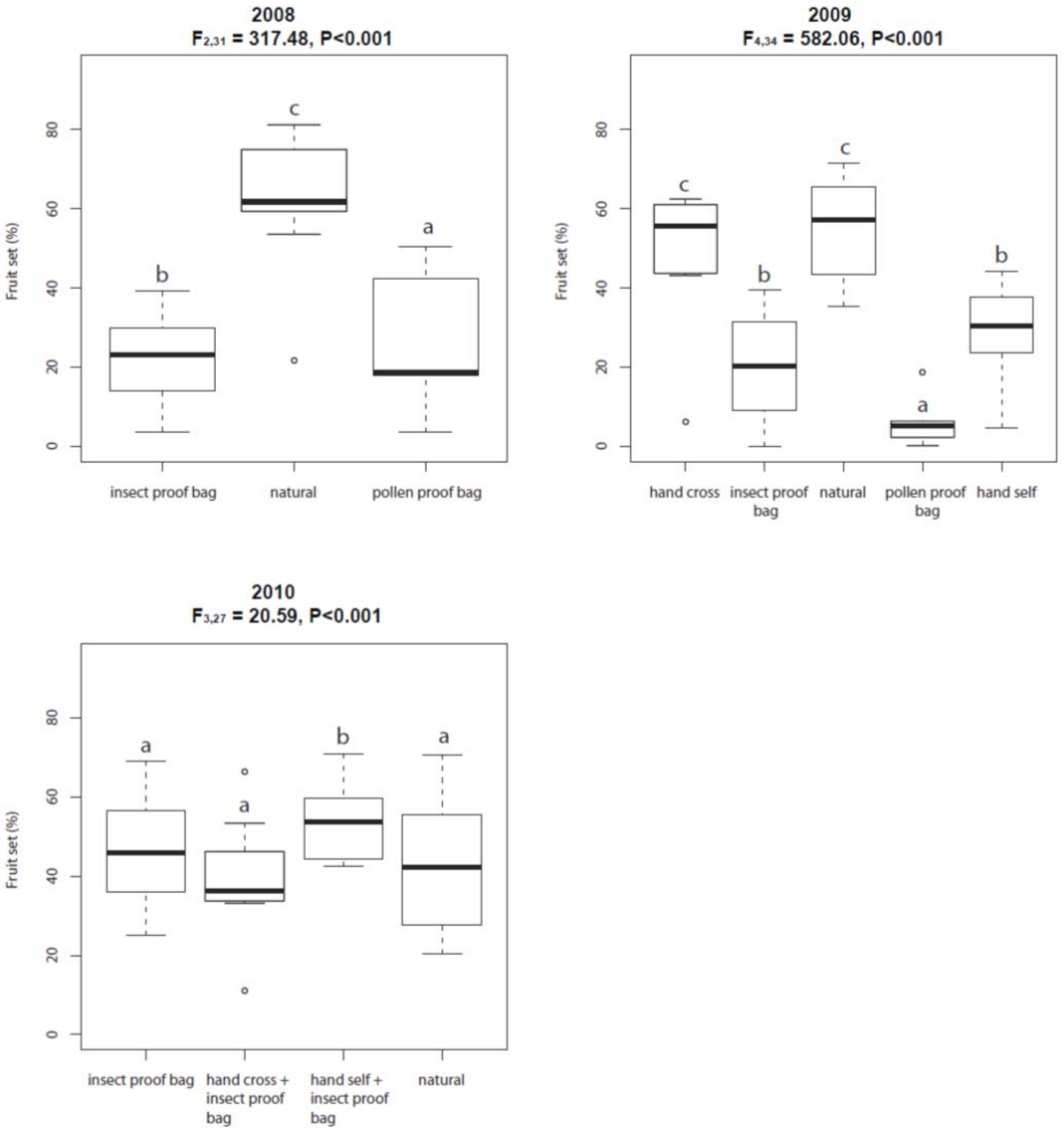


Fig. 7. Percent fruit set in *K. salicornioides* in different pollination treatments. Treatments that share a letter are not significantly different.

Korthalsella lindsayi is an Austral spring flowering species, which starts flowering in late August and continues to flower into September. Flowers also develop as a cluster of five, with one axillary male flower and two female flowers on each side. There are two clusters of five flowers on each node and are borne on definite inflorescence branches that are distinct from vegetative branches and the floral clusters are superposed (Fig 8). The flowers are again interspersed with reddish brown trichomes. Female flowers are 0.6 x 0.4-0.5 mm and are shortly pedicellate. Male flowers are 0.7-0.8 x 0.5-0.6 mm and are shortly pedicellate. The synandrium is about 0.4 mm in diameter. The fruit (a berry) is pale green, c. 1.5 x 1 mm, and the single seed is c. 0.9 x 0.7 mm. Nectar sucrose equivalent in female flowers averaged 13 µg (std error=0.84, n=16) and in male flowers 9 µg (std error=0.7, n=14). No insects were seen visiting *K. lindsayi* inflorescences.

Fig. 8. *K. lindsayi* inflorescence

When plants were covered with mesh bags the fruit set was significantly reduced compared to the natural fruit set (Fig. 9, Table 2). When plants were hand selfed and covered with mesh bags fruit set was significantly reduced compared to natural fruit set during the same year (2011). Whereas, when plants were hand crossed and covered with mesh bags fruit set was slightly reduced compared to natural fruit set during the same year (2011), however, the difference was not significant. Hand crossed plants without mesh bags had reduced fruit set compared to natural fruit set during the same years however the difference was not significant. When plants were covered with pollen proof bags fruit set was significantly reduced compared to natural control and compared to all other treatments except during 2008 when it was not significantly lower than mesh bag covered plants. However, when plants were hand selfed and covered with a pollen-proof bag the fruit set was significantly increased compared to when plants were covered with pollen proof bags without selfing. This fruit set was significantly lower than natural fruit set during the same years.

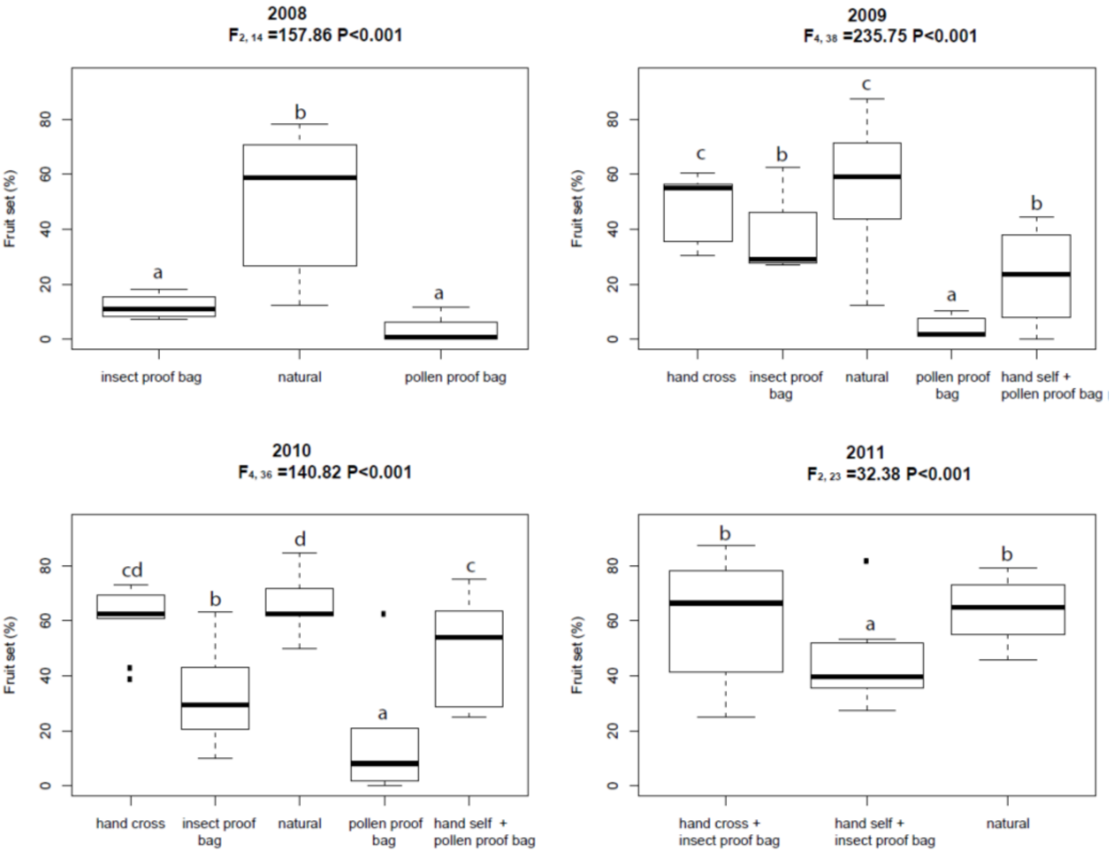


Fig. 9. Percent fruit set in *K. lindsayi* in different pollination treatments. Treatments that share a letter are not significantly different.

Dispersal Biology

This study confirms explosive seed discharge in both species studied. All vinyl acetate sheets were covered with numerous seeds adhering on the side facing the mistletoes, thus confirming explosive seed dispersal in *Korthalsella*. Some seeds drifted for some distance along the surface of the vinyl acetate sheet after hitting the sheet (Fig. 10). In the laboratory experiment, the lateral distance measured from the point of ejection (c. 22 cm high) for two different *K. salicornioides* seeds was found to be 17 and 38 cm respectively (Fig. 11a). The seeds exit from the stigmatic end and seeds are ejected with the radical end upwards. Sometime the remaining fruit wall is also dislodged when the seed exits. The dispersal distance studies show that seeds are dispersed to at least about 4 and 7 meters in *K. lindsayi* and *K. salicornioides* respectively, depending on the height of mistletoes in the host canopy and the direction of prevailing wind (Fig. 11b, c). The median dispersal distance in *K. lindsayi* ranged from 1 to 1.9 m and in *K. salicornioides* from 1.3 to 2.3 m. In *K. lindsayi,* seed discharge occurs during December-January and in *K. salicornioides* seed discharge occurs during February-March. Like other mistletoes, the viscin layer on the seeds helps them adhere to the host branches.

Fig. 10. *K. salicornioides* seeds on vinyl acetate sheets

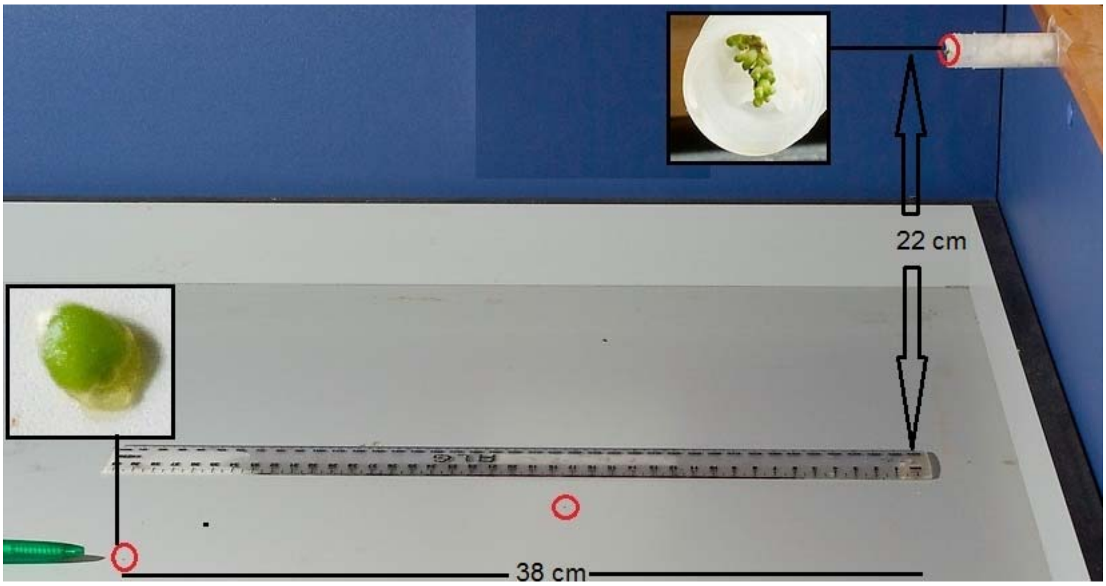


Fig. 11a. Set up of laboratory experiment on seed dispersal in *K. salicornioides*

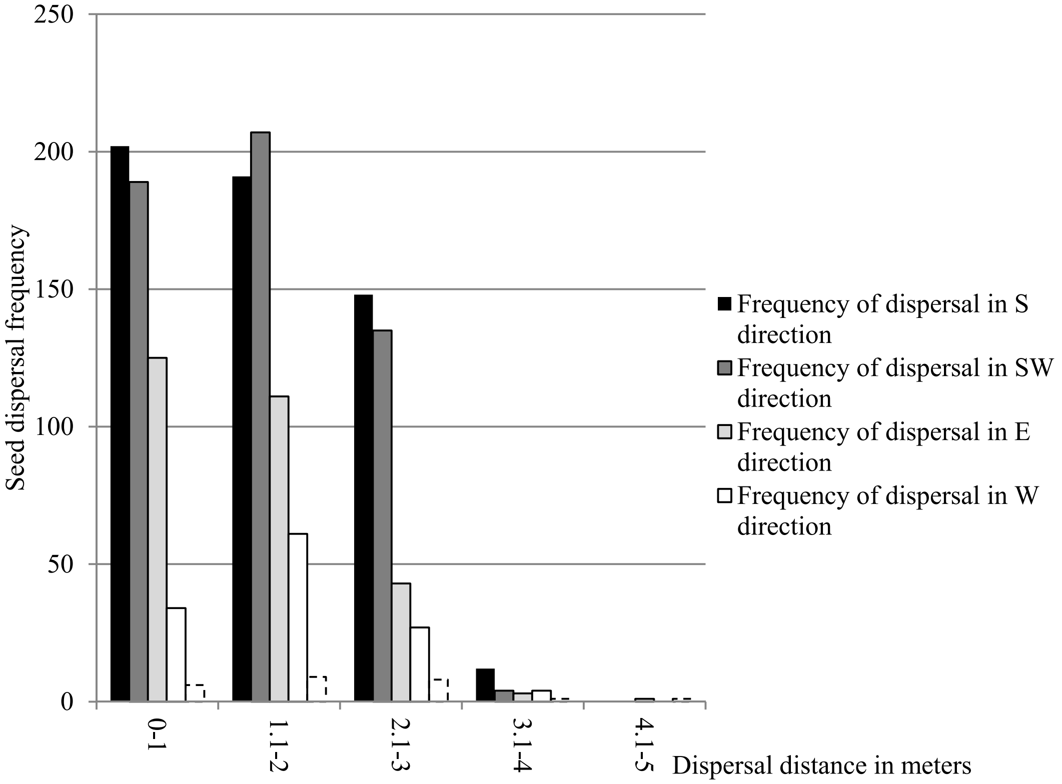


Fig. 11b. Frequencies of seed dispersal in *K. lindsayi* in different directions

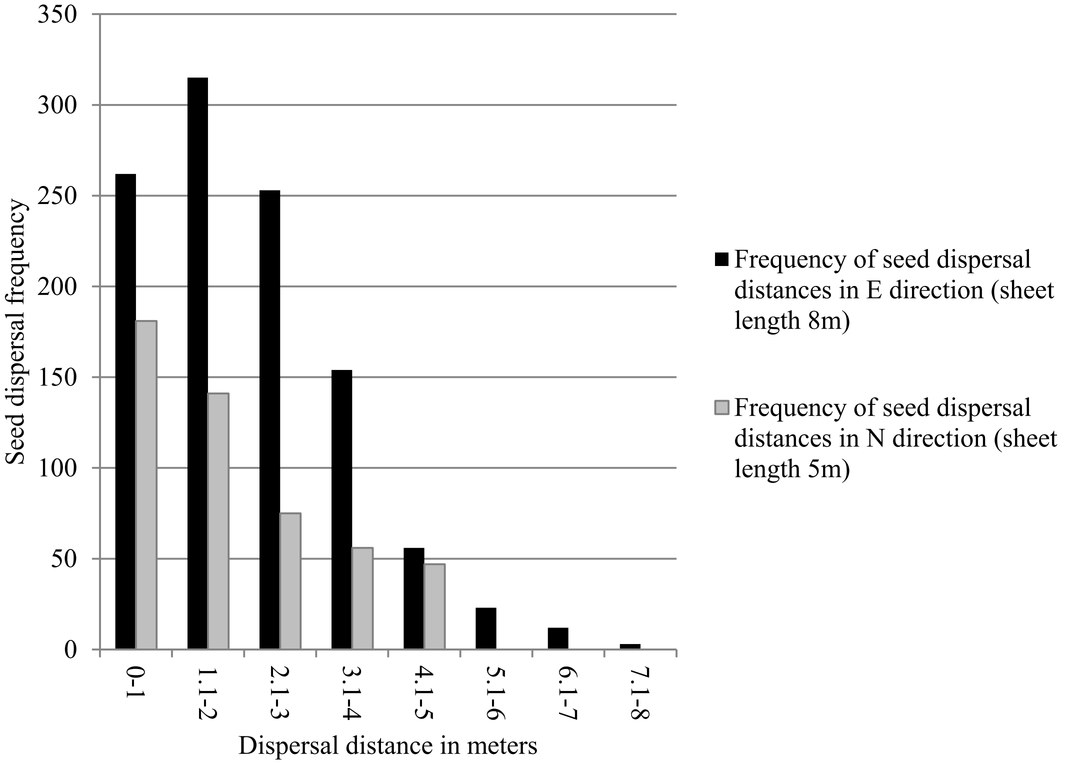


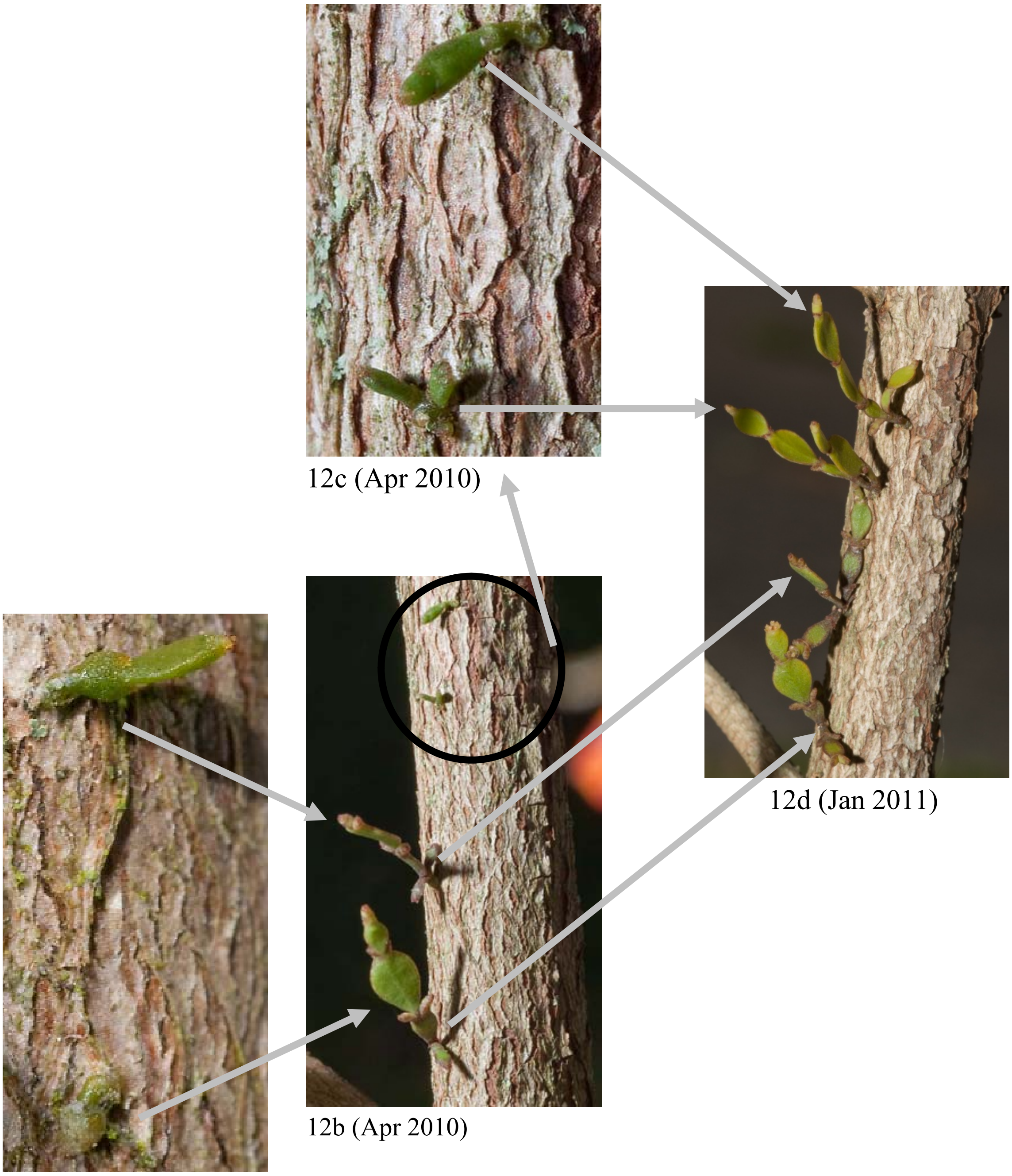
Fig. 11c. Frequencies of seed dispersal in *K. salicornioides* in east and north directions

Establishment and Vegetative Reproduction

Korthalsella seeds lack a true seed coat since, like other Viscaceae, the embryo sacs lack recognizable nucellus and integuments; and instead develops in a dome-like structure termed a “mamelon” (Bhandari and Vohra, 1983), “ovarian papilla” (Gordon and Jones, 1965) or placental-nucellar complex (Ross and Sumner, 2004). This organ is like a vestigial placenta (Kuijt, 1969). The seeds are supplied with a copious amount of viscin (Fig. 5) that helps adherence to host branches. The endosperm tissue is chlorophyllous and seeds can survive for several months without establishment of contact with host tissue. Once contact with the host is established, the endophyte proliferates within the host and over the next five to six months, the establishing plants begin to develop internodes and, in *K. lindsayi,* the first inflorescence branch primordia are produced.

Vegetative reproduction by proliferation of endophytic tissue within the host plant and the production of sprouts from adventitious buds was also recorded (Fig 12). Sprouts can be distinguished from seedlings by the absence of a cotyledonary collar and often by the multiple shoots that appear soon after emergence from the host tissue (Fig 12c, see basal sprout). The growth of *K. lindsayi* seed transplants on potted hosts was recorded over a period of three years and the first vegetative sprouts were produced in the second year following seed transplant. Thus, seeds transplanted in December 2008 produced the first internodes during September-October 2009 after establishing contact with the host. Primordia of the first inflorescence branches and sprouts could be seen in April 2010, i.e., sixteen months after seed transplant. During spring 2010 (September i.e., 18 months from seed transplant) these mistletoes produced the first flowers and by summer 2010 (December i.e., 2 years from seed transplant) the first seeds were dispersed. By January 2011, the first vegetative sprouts had almost attained the size of the parent plants and developed primordial inflorescence branches, which flowered during spring 2011 (33 months from seed transplant).

A relatively slower growth was recorded in *K. salicornioides,* where the first internodes were produced in about 12-15 months from planting, and whereas in *K. lindsayi* almost all established mistletoes produced inflorescences in the second year from planting, only about 11% mistletoes produced inflorescences in *K. salicornioides* during the second year of establishment.



12c (Apr 2010)

12d (Jan 2011)

12b (Apr 2010)

12a (Oct 2009)

Fig 12. a.10 month old seedlings of *K. lindsayi* on a *Lophomyrtus bullata* host, b. 14 month old *K. lindsayi* plants with the first vegetative sprouts, c. close up of sprouts, d. 2 year old parent plants (bottom two plants) and one year old sprouts (upper two plants)

The impact of the gall mite Aceria korelli on reproductive output

The gall mite *Aceria korelli* (Eriophyidae), which infects all three New Zealand *Korthalsella* species, transforms both floral and vegetative branches into galls. Sometimes entire floral whorls are transformed (Fig 13a and b). In Fig 13b, some of the

K. lindsayi inflorescences are completely transformed into galls whereas some are partially transformed. Its occurrence on mistletoes seems to be patchy as often mistletoes on a host or on hosts growing in close proximity are infected while other hosts a few meters away are free of galls.

Fig 13a (above) A gall mite (*Aceria korelli*) infected *K. salicornioides* plant (photo courtesy Jeremy Rolfe), Fig 13b (below) Gall mite (*A. korelli*) infected *K. lindsayi* plant (photos courtesy Don Pittham)

DISCUSSION

Insect visitation in *K. salicorniodies* despite diminutive inconspicuous flowers and small nectar amounts with low sugar content; explosive seed dispersal to a distance of several feet and vegetative reproduction by sprouts in both species are important findings of this study.

Experimental manipulations to determine pollination requirements show that *K. salicornioides* has a low autonomous selfing index (<0.13), but is fully self-compatible (self-compatibility index>0.8) and is not pollen limited (pollen limitation index = -0.12). The wind pollination index values show that mesh-bagged plants generally had lower fruit set than open natural fruit set suggesting that either wind alone does not produce the full complement of seed, or that mesh bag somehow impedes the passage of pollen. Indeed, the frequent insect visitation in *K. salicornioides* suggests that it is an ambophilous species relying both on wind and insects for pollination (Fig 6b-d, 7). However, Player (1979) noted a similar situation in wind-pollinated dwarf mistletoe species and considered the filtration effect from mesh forcing a large component of wind with its pollen load around the cage and altered wind velocity around the caged plant to be possible explanations for low fruit set.

Korthalsella lindsayi also has low autonomous selfing index (<0.1), and is only partially self-compatible (self-compatibility index<0.8) but again is not pollen limited (pollen limitation index = -0.1, -0.2). The wind pollination indices show that mesh bagged plants had lower fruit set than open natural fruit set. However, the lack of insect visitation suggests a predominantly anemophilous pollination system in *K. lindsayi* (Fig 9).

Although bagged *K. lindsayi* and *K. salicornioides* plants will have pollen available from the same plant, unlike the dioecious dwarf mistletoes, reduction of the wind velocity coupled with dichogamy (temporal separation of male and female phase) in floral whorls possibly reduces the dispersal of pollen to pistillate flowers on the same plant. The male flowers are initiated before the female flowers in *Korthalsella* (Kuijt 1969) but we have not observed a consistent pattern of either protandry or protogyny in floral clusters, and whorls appear to be variably protogynous or protandrous. Rutishauser (1935) noted protogyny in *K. dacrydii*. In either case, dichogamy and the spatial separation of male and female parts appear to be effective mechanisms in preventing autogamy.

Kuijt (1969) considered viscaceous flowers to be insect-pollinated mainly by Hymenoptera. However, anemophily seems to be far more important in the Viscaceae, especially in the dioecious species (Molau 1995). Player (1979) stated that the floral morphology in *Korthalsella* is suggestive of wind as being important in shaking the pollen out of the central pore of the fused anthers. Considering the inconspicuous nature of the flowers and the occurrence of *Korthalsella* spp. in comparatively open parts, or on the outside edges of bush, Thomson (1880) also suggested that New Zealand species of *Korthalsella* are wind-pollinated. However, Molvray (1999) regarded thrips, mites and similar arthropods with limited mobility to be pollinating agents for the genus. Stevenson (1934 p. 183) stated that "seeds are produced in large numbers in proportion to the size of the plants" which is indicative of the effectiveness of pollination as well as the reliance on the photosynthates from the host. As *K. lindsayi* is an early spring-flowering species, lack of insect visitation on its flowers can be correlated with lack of insect activity during the flowering period. The presence of sugar-rich exudates in male and female *K. lindsayi* flowers and the comparatively lower fruit set in bagging experiments apparently suggest an ambophilous pollination syndrome in this species also, but lack of insect visitation in the current study indicates a possible shift to anemophily. Sugar-rich nectar secreted by wind-pollinated plants could possibly be indicative of an entomophilous past (Culley *et al*. 2002). Alternatively, Player (1979) suggests that the presence of a secretary central cushion in staminate flowers of wind pollinated dwarf mistletoe species may be important in preventing pollen lysis under conditions of high rainfall, while the presence of stigmatic exudate is important in pollen retention. The entomophilous and anemophilous characteristics of *Korthalsella* flowers are summarised in Table 3 and are compared with the dwarf mistletoe genus *Arceuthobium*.

Ambophily is an advantage in adapting to different environments with spatial variation in conditions that favour either anemophily or entomophily and in situations where there is temporal variation in pollinator abundance for example low and sporadic pollinator numbers during spring in alpine habitats (Culley *et al*. 2002). It is also advantageous in conditions where there is competition with other entomophilous species flowering at the same time and offering more reward to pollinators (Culley *et al*. 2002).

Studies in other viscaceous mistletoes suggest that different genera may have a suite of anemophilous, entomophilous and ambophilous species. Several studies suggest that *Arceuthobium* species are ambophilous (Penfield *et al.* 1976, Gilbert and Punter, 1984, 1990, Gilbert 1988). Player (1979) studied pollination in *A. douglasii* and *A. strictum* and found these two species to be primarily anemophilous. However, Gregor *et al.* (1974) studying pollination in *A. americanum* found that it is primarily pollinated by insects that effectively pollinated every pistillate flower and that the wind dispersal of pollen was limited. They also noted nectar production, and a pungent, citrus-like odour emitted by both pistillate and staminate flowers. Various Diptera and ants were found to be the most common visitors of *A. americanum* flowers. Coppola (1989) studied the wind dispersal of *A. americanum* pollen and found that pollen was effectively dispersed up to a distance of 512 m. Baker *et al.* (1985) studied pollination in *A. pusillum* and considered entomophily to be the most important pollination mechanism for that species.

Hatton (1964) reported a greater efficiency of wind than insect pollination for *Viscum album*. However, Kay (1989) considered *V. album* flowers to be ill-adapted for wind pollination and found several fly species visiting both male and female flowers of which *Dasyphora cyanella* (Muscidae) was found to be an effective pollinator in this species. According to Lawalree (1952), *V. album* is pollinated by Diptera and wind. Aparicio *et al.* (1995) regarded insects to be absolutely necessary for pollination success in the related *V. cruciatum*. Male flowers in *V. cruciatum* produce a perceptible lemon-scent and female flowers secrete nectar in small amounts (Aparicio *et al.* 1995). Horwood (1983) reported that large nectar drops in both male and female flowers of *V. minimum* that attracted small pollinating insects. Cupedo (1985), considering the small foraging range of pollinating flies, regarded close clustering of *Viscum album* plants as a prerequisite for effective pollination. Similarly, the distribution of *Korthalsella lindsayi* and *K. salicornioides* tends to be in dense localised patches, which presumably helps ensure effective pollination, particularly for the latter species when the pollinators such as diptera have a relatively small foraging range.

Compared to the average nectar sugar production in insect pollinated species visited by bees (0.76 mg), butterflies (0.43 mg) and hawkmoths (5.41 mg) (Cruden 1976), *K. salicornioides* nectar has very low sugar levels (sugar equivalent averages in female flowers were 4.76 μg, 8.24 μg and in male flowers averages ranged from 7.75-9.03 μg). These sugar contents are again very low when compared to nectar sugar content in dwarf mistletoe *Arceuthobium abietinum* (=*A. campylopodum*) female flowers, in which 0.24 μl of nectar was found to have 58-92% sugar content (c. 250 μg).

However, despite these low sugar levels, the diminutive floral dimensions, and the inconspicuous corolla, the ability of *K. salicornioides* flowers to attract pollinating insects might also be attributable to floral volatiles. Although tiny (male flowers are only ca 1mm across), *Korthalsella salicornioides* flowers are not the smallest flowers able to attract insect pollinators. Duckweed (*Wolffia* sp.) have possibly the smallest angiosperm flowers (c. 0.3mm), also produce nectar on the stigma mouth and have brightly coloured pollen; pollination is thought to be entomophilous initially brought about by mites and aphis (Ivanova 1970, Cross 2002). As the pollen becomes less brightly coloured later, pollination becomes wind-dependent.

Vegetative Reproduction

This study represents the first record of vegetative reproduction from endophytic tissue in *Korthalsella* as no vegetative reproduction was seen in a study on *Korthalsella dacrydii* (Rutishauser 1935). While colonisation of different branches within a host or adjacent hosts is probably usually accomplished by explosive seed dispersal, vegetative reproduction by ramification of the endophyte and sprouting maximises the colonisation of individual branches and enhances the reproductive output of individual mistletoes. The daughter ramets are presumably subsidised by the parent plants, thus allowing rapid growth and relatively earlier production of inflorescences in the sprouts compared to seedlings established from seeds. During the initial establishment (2-3 years), the number of sprouts apparently does not surpass the number of founder seedling plants, however, continued proliferation of the endophyte within the host may continue to add more sprouts, thus maximising the overall reproductive output of the genet. This phenomenon also helps in the persistence of the genet, as loss of parent plants by herbivory or mortality caused by natural senescence is compensated by the presence of ramets. In more rapidly growing hosts, rapid stem elongation may result in the loss of contact between the sprouts and parent plants and splitting of endophyte may encourage more sprouts at various points along the stem. *Viscum album* also produces adventitious shoots from cortical strands, particularly in response to cutting of the main mistletoe or when it is broken away or gets frozen (Zuber 2004). Isophasic parasitism in some dwarf mistletoe (*Arceuthobium*) species is a highly specialised manifestation of endophytic growth, where the endophyte grows in synchrony with the host apical meristem (Lye 2006, Vidal-Russel and Nickrent 2008).

Dispersal Biology

Stevenson (1934) indicated that the small *Korthalsella* seeds are not adapted to bird attraction and are dispersed by a weakly explosive mechanism. She stated that the swelling of mucilaginous tissue develops weak internal pressure, thus the seed is forced out through the ruptured tip of the fruit, falls nearby, often infecting the mother plant.

There are some reports of explosive seed dispersal in other *Korthalsella* spp. (Sahni 1933, Danser 1940, Ohwi 1965, Herbst 1980, Whistler 2011) but Nickrent (1996, 2012) doubts the explosive nature of *Korthalsella* fruits. Herbst (1980) indicated that as the fruit approaches maturity, internal water pressure increases causing the fruit to burst, ejecting the seed several feet, while Sahni (1933) reported that seeds are ejected to a distance of two feet or more in *Viscum japonicum* (=*Korthalsella japonica*). This mechanism, however, is only useful for short distances. In the current study the median dispersal distance in *K. lindsayi* in the field ranged from 1 to 1.9 m and in *K. salicornioides* from 1.3 to 2.3 m depending on direction and height of host canopy. Dispersal distances of 17 and 38 cm were recorded from a height of about 22 cm in the laboratory study with *K. salicornioides*. Thus they may only be good at dispersing within a shrub or to adjacent hosts as Zakaullah (1988) observed that the mode of spread of *Korthalsella opuntia* (=*K. japonica*) on oaks was from tree to tree. For longer distances, some other kind of dispersal mechanism is required. Perhaps birds move seed either internally after swallowing the fruits, or externally on their feathers or feet, or perhaps wind can blow seeds far enough. Nelson and Friday (2009) regard birds, as well as water, strong winds and explosive seed discharge, as agents of seed dispersal in Hawaiian *Korthalsella* species. Hambali (1977) assumed that *K. dacrydii* seeds are dispersed by small birds, such as the pygmy Bush tit *Psaltria exilis* (Paridae), which frequently visit the foliage of many trees in their search for small insects, while Degener (1973) indicated that the seeds of Hawaiian spp. of *Korthalsella* are dispersed by certain birds which feed upon the fruits. These events are presumably rare and account for some of the patchiness. Under this scenario, some suitable hosts will not be colonised by chance and must wait for these rare events to start a new population. Such events also account for occasional records on non-native hosts. Long distance dispersal of *Korthalsella* to remote oceanic islands is also postulated to be a consequence of secondary dispersal by migratory birds (Carlquist 1967, Burrows 1996, Barlow 2012) or birds/butterflies (Molvray *et al*. 1999).

Explosive seed discharge has evolved independently in two different lineages within Viscaceae, as explosive dispersal is also the usual mode of dispersal in *Arceuthobium* spp. Dispersal biology in *Arceuthobium* is discussed for a comparison of explosive dispersal mechanisms, otherwise, the two genera are not closely related (see Nickrent 1996, Mathiasen *et al.* 2008). The exceptional seed dispersal mechanism in *Arceuthobium* is regarded as one of the most efficient in the whole plant kingdom (Hinds *et al.* 1963). High hydrostatic pressure develop in the viscin cells surrounding the seed cause the seed to explosively exit from the recurved fruit at the pedicel end (Hinds *et al.* 1963). Seed discharge velocities ranged from about 22m/s to 27 m/s in four Colorado species (Hinds and Hawksworth 1965). Dispersal distances of 10 m or less are more typical (Hawksworth and Wiens 1996). Gilbert (1988) reports a dispersal up to 18 m for *A. americanum* while Weir (1916 p. 34) reports a dispersal distance of 66 feet (= 20 m) in an unnamed species from a height of 8 feet. He also describes strong wind as an important factor in the dispersal quoting an instance of dispersal of larch mistletoe (*Arceuthobium laricis*) to a distance of one quarter of a mile (400 m) from the nearest infected tree, thus the combined effect of strong winds and explosive seed discharge may carry seeds for unusually long distances. Local dispersal and intensification of the dwarf mistletoes is almost exclusively due to mechanical seed expulsion (Hinds and Hawksworth, 1965) which accounts for the occurrence of slowly expanding foci resulting from a founder long-distance dispersalist followed by gradual expansion through short distance dispersal. Conversely, mistletoes depending on birds do not show advancing fronts of this sort and occur erratically or in patterns determined by the habits of birds (Kuijt 1969). Birds are potential vectors of long and medium-range seed dispersal carrying the seeds on the plumage beyond the range of explosive seed dispersal of dwarf mistletoes (Hudler *et al.* 1979, Nicholls *et al.* 1984, Johnson *et al.* 1989, Punter and Gilbert 1989). This was indicated by the occurrence of many satellite pockets of infection too far from large infection centres to have arisen from explosively discharged mistletoe seed alone (Nicholls *et al.* 1984). Apart from birds, mammals can also vector dwarf mistletoe seeds and establish new infection centres well beyond the range of explosive seed dispersal (Nicholls *et al.* 1984). As seed dispersal often coincides with a period of peak animal activity, some of the discharged seeds directly stick to the fur or feathers of animals. Moreover, previously discharged seeds adhering to the branches can also be inadvertently picked up under conditions of high humidity on the bodies of animals using infected trees for nesting and foraging and subsequent deposition onto healthy trees being affected by preening and bill wiping/grooming habits of animals (Ostry *et al*. 1983). Migratory birds feeding upon insects associated with mistletoe-killed branches were extremely active in mistletoe- infected trees and were found carrying mistletoe seeds (Ostry and Nicholls 1979).

In *Arceuthobium verticilliflorum,* which has the largest seeds in the genus, the weakly explosive mechanism is sufficient to remove the pericarp exposing the seeds to birds which are the primary seed dispersers in this species (Hawksworth and Wiens 1996). Nickrent (2012) observed a similar situation in *Korthalsella latissima* (or *K. complanata*) whereby seeds just oozed from the stigmatic end of fruits. The seeds then probably disperse by adherence to plumage of birds perched near mistletoes in this species. Western bluebirds feed upon *A. vaginatum* fruits (Pinkowski 1981). According to Hudler *et al*. (1979), *A. vaginatum* seeds are occasionally ingested by birds but are not viable when voided.

In the current study, small insectivorous/omnivorous birds like the silvereye (*Zosterops lateralis*) and grey warbler (*Gerygone igata*) were noticed visiting *K. salicornioides*-parasitised manuka hosts which possibly visit mistletoe-infected branches in search of insects. Kakapo (*Strigops habroptila*) reportedly feeds on *K. salicornioides* (Wilson *et al.* 2006) and New Zealand pigeon (*Hemiphaga novaeseelandiae*) on *K. lindsayi* and *K. salicornioides* (Baker 2013). These birds can potentially carry *Korthalsella* seeds on their plumage.

The fruits of New Zealand *Korthalsella* species may be the smallest of the known explosive fruits, which rely on hydrostatic pressure caused by swelling of viscin layer. A viscin layer might also act as a slip zone for discharge of seeds from the apical part of the fruit thus minimizing the friction during ejection process. Thus *Korthalsella* species studied here exhibit elaborate reproductive features for effective pollination, seed dispersal and perpetuation of the endophyte.

Table 3. Entomophilous and anemophilous features of *Korthalsella* flowers

Floral morphology	Entomophilous Features of flowers as given in Culley *et al.*, 2002	Entomophilous features in dwarf mistletoe flowers (Hawksworth and Wiens 1996)	Presence or absence of same features in *Korthalsella*	Anemophilous Features of flowers as given in Culley *et al.*, 2002	Anemophilous features in dwarf mistletoe flowers (Hawksworth and Wiens, 1996)	Presence or absence of same features in *Korthalsella*
Stigmas	Simple	Non-plumose stigma	+	Feathery	-	-
Pollen:ovule ratio	Low	Low pollen production (> 11,000 grains per flower)	+	High	-	-
					Single-"ovule" ovaries	+
Pollen diameter	Highly variable (often >60 µm)	-	-	10–50 µm	Pollen size in the general range of wind-pollinated species (10 to 60 µm)	+
Pollen ornamentation	Often elaborate with pollenkitt	Spined pollen	-	Smooth with reduced/absent pollenkitt	-	+
Pollen aperture, number and type	Numerous, elongate		+, apertures are elongate yet few	Few, circular		+, apertures are only three, yet are elongate
		Clustering of pollen by mucous strands	+?		Long-distance transport of pollen (several kilometres) and its occurrence in the fossil palynological record	?

Floral morphology	Entomophilous Features of flowers as given in Culley *et al*., 2002	Entomophilous features in dwarf mistletoe flowers (Hawksworth and Wiens 1996)	Presence or absence of same features in *Korthalsella*	Anemophilous Features of flowers as given in Culley *et al*., 2002	Anemophilous features in dwarf mistletoe flowers (Hawksworth and Wiens, 1996)	Presence or absence of same features in *Korthalsella*
Stamen filaments	Variable	Sessile anthers	+	Long	-	-
					Exposed anthers	-
Nectaries	Present	Nectar production in staminate flowers and stigmatic exudate in pistillate flowers	+	Absent or reduced		
Fragrance	Present	Faint odour production by both pistillate and staminate flowers	?	Absent or reduced		
Perianth	Showy			Absent or reduced		+, perianth reduced
Flower type	Usually bisexual			Usually unisexual	Unisexual flowers	+, unisexual
Inflorescence structure	Variable, sometimes simple and diffuse		+	Pendulous, catkin-like, often condensed		
Inflorescence position	Variable		+	Held away from vegetation		
Habitat						
Optimum wind speed	Zero to low			Low to moderate		+
Humidity	Medium to high		+	Low		
Precipitation	Infrequent to common		+	Infrequent		
Surrounding	Open to closed			Open		+

Floral morphology	Entomophilous Features of flowers as given in Culley *et al*., 2002	Entomophilous features in dwarf mistletoe flowers (Hawksworth and Wiens 1996)	Presence or absence of same features in *Korthalsella*	Anemophilous Features of flowers as given in Culley *et al*., 2002	Anemophilous features in dwarf mistletoe flowers (Hawksworth and Wiens, 1996)	Presence or absence of same features in *Korthalsella*
vegetation						
Plant density	Low to high			Moderate to high	Localized, dense population structure	+
					Temporal separation of flowering periods from those of their wind–pollinated hosts	+
					Temporal partitioning of flowering periods when two dwarf mistletoe species are sympatric	+, b/w *K. lindsayi* & *K. salicornioides* and b/w *K. clavata* & *K. salicornioides*
					Sexual dimorphism with open and spreading staminate plants (favouring the release of pollen with minimum filtration effect from branches of the staminate plant) and compact, densely branched pistillate plants	-, *Korthalsella* spp. are monoecious, however occurrence on comparatively open parts, or on the outside edges of host bushes suggestive of anemophily

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Chapter 6: Insect/Mite Fauna and Fungal Pathogens of New Zealand’s Pygmy Mistletoes

INTRODUCTION

Across their range, mistletoes are key resources for wildlife (Watson 2004). Many mistletoe species are important nutritional resources for vertebrates as well as invertebrates, including larval forms of insects. The pygmy mistletoes are generally more nutritious than their hosts (Bannister 1989) and evolution of crypsis and host mimicry are postulated as possible mechanisms for avoidance of herbivory from extinct birds like moa (Bannister 1989, Burns 2010). This is remarkably exhibited by the resemblance of linear internodes of *K. salicornioides* to the linear leaves of *Kunzea ericoides* (Bannister 1989) and *K. lindsayi* internode shape to *Lophomyrtus obcordatus* leaves which is a frequent host (Fineran 1995).

There have been a few sporadic records of herbivores and fungal pathogens associated with *Korthalsella* spp. globally. Larvae of the Pierid butterfly *Delias nysa* feed on *Korthalsella breviarticulata*, *K. rubra* (Herbison-Evans and Crossley 2012) and *K. japonica* (=*K. opuntia*) (Common and Waterhouse 1972) in Australia. A polyphagous scale insect *Aulacaspis* (=*Phenacaspis*) *vitis* (Diaspididae) was recorded on *K. opuntia* (=*K. japonica*) in Pakistan (Baloch et al. 1975). The Hawiian endemic hemiptera, *Sarona flavidorsum* and *S. gagnei* (Miridae) have been recorded on *Korthalsella* spp. (Asquith 1994). An unidentified micro-lepidoptera was also associated with *K. complanata* in Hawaii (Kliejunas et al. 1979). Fungal hyperparasites reported on Hawaiian *Korthalsella* spp. include *Curcurbitaria obducens* (=*Teichospora obducens*), *Pleospora* sp., (Kliejunas *et al*. 1979), *Meliola visci* (Stevens 1925) and *Echidnodes* (Petrak 1953). Apparently there is insufficient data on the occurrence of specialists exclusively dependent on *Korthalsella* spp. globally. Herbarium specimens of *Korthalsella disticha* from Norfolk Island, *K. japonica* from Japan and *K. platycaula* from Cook Islands in CHR have armoured scale insects belonging to genus *Lepidosaphes* that are potentially undescribed specialists of these species.

In New Zealand, caterpillars of two species of polyphagous moths, *Ctenopseustis obliquana* and *Harmologa oblongana* (Tortricidae), have been recorded on *K. lindsayi* (Patrick and Dugdale 1997). The Eriophyid gall mite *Aceria korelli* has been reported on *Korthalsella salicornioides, K. lindsayi* and *K. clavata* (Manson 1984); this species is known from New Zealand's pygmy mistletoes only and is the first specialist recorded on *Korthalsella*. On New Zealand's outlying Stewart and Codfish Islands, *K. salicornioides* is a component of the diet of the critically endangered bird, *Strigops habroptila*, commonly known as kakapo (Wilson *et al*. 2006). *Korthalsella* has also been found to be a component of the diet of rats on Stewart Island (Sturmer 1988). Lately, New Zealand pigeons (*Hemiphaga novaeseelandiae*) have been reported feeding on *K. lindsayi* and *K. salicornioides* (Baker 2013).

Colonising hosts and maintaining populations can be challenging for specialist natural enemies of widely dispersed and often-sparse host plants. The occurrence of New Zealand's pygmy mistletoes in declining, fragmented populations highlights that the dependent native invertebrate fauna and fungal pathogens may also be range-restricted and threatened. Conversely, exotic natural enemies may also pose serious threats to hosts of specialist feeders as mistletoes have not evolved to defend against them, and they may also displace native natural enemies by competition. Adventive natural enemies have the advantage of being generalised and therefore not having to rely on finding a single target host but can opportunistically adopt mistletoes by host-shifting from other host species. The aim of the current study is to record and characterise the herbivores and fungal pathogens associated with New Zealand's pygmy mistletoes throughout their distribution.

MATERIALS AND METHODS

Pygmy mistletoe populations throughout New Zealand were explored for the presence of invertebrate herbivores and fungal pathogens. Scale insects were collected either directly in 70 % ethanol or mistletoe fragments with scale insects were collected, dried and subsequently isolated under stereomicroscope and transferred to 70 % ethanol. In some cases, scale insects were also obtained from herbarium specimens of *Korthalsella* spp. Scale insects were initially identified by Rosa Henderson (Landcare Research, Auckland). This study resulted in the discovery of one new species of felt scale (Eriococcidae) and two new species of armoured scales (Diaspididae), all specialising on New Zealand *Korthalsella*. These species have now been named and described in:

> Henderson, R. C., Sultan, A. and Robertson, A. W. 2010. Scale insect fauna (Hemiptera: Sternorrhyncha: Coccoidea) of New Zealand's pygmy mistletoes (*Korthalsella*: Viscaceae) with description of three new species: *Leucaspis albotecta, L. trilobata* (Diaspididae) and *Eriococcus korthalsellae* (Eriococcidae). Zootaxa 2644: 1–24.

The descriptions of three new scale insect species in the above mentioned paper were made by Rosa Henderson but I made the observations of where they had been found and also identified the exotic scale insects. The present chapter reports scale insects reported in the above mentioned paper as well as additional scale insect observations made afterwards. The type specimens of new scale insect species as well other scale insects recorded were deposited in New Zealand Arthropod Collection (NZAC) at Landcare Research, Auckland.

Leptidopterous larvae found feeding on *Korthalsella* spp. were reared in captivity until pupation and emergence of imago. Tortricid moths were identified by John Dugdale (ex-associate of Landcare Research), whereas, a noctuid species recorded on *K. lindsayi* was identified by Robert Hoare (Landcare Research, Auckland).

Fungal pathogens were also recorded and collected during these surveys, and this study resulted in the discovery of two new ascomycetes specialising on New Zealand *Korthalsella*. Both species have now been named and described in collaboration with Peter Johnston and Duckchul Park (Landcare Research, Auckland) in:

> Sultan, A., Johnston, P. R., Park, D. and Robertson, A. W. 2011. Two new pathogenic ascomycetes on New Zealand's Pygmy Mistletoes (*Korthalsella*: Viscaceae) in the genera *Guignardia* and *Rosenscheldiella*. Studies in Mycology, 68: 237-247.

The type specimens of these new species were deposited in New Zealand Fungal Herbarium (PDD) at Landcare Research, Auckland.

This chapter adds further observational records to the occurrence of each specialist and the occurrence of Lepidopterous larvae on these mistletoes and also adds distribution maps for gall mite *Aceria korelli* and fungal pathogens. Since we relied on herbarium specimens for the presence or absence of specialist natural enemies in certain regions, records cited in this chapter do not necessarily represent an exhaustive compilation on the occurrence of each of specialist natural enemy. Thus, range extensions from current known distribution might be expected with more targeted systematic searches within each mistletoe population.

RESULTS

Scale Insects (Hemiptera: Sternorrhyncha: Coccoidea) specialising on New Zealand Korthalsella

Family Diaspididae

Leucaspis albotecta (Fig. 1, 8)

This newly described armoured scale is currently known only from *K. salicornioides* and has a distribution in both North and South Islands.

Specimens deposited in NZAC: NEW ZEALAND, CENTRAL OTAGO, Lake Roxburgh, 11 Jan 2010, A. Sultan, on *K. salicornioides* parasitic on *Kunzea ericoides* s.l., MARLBOROUGH, Wairau Valley, Branch River, Feb 2010, A.W. Robertson, on *K. salicornioides*; WAIRARAPA, Lake Wairarapa, Western Lake Road, Lake Shore Scenic Reserve, March 2010, A. Sultan; BAY OF PLENTY, Rotorua Whakarewarewa Geothermal Reserve, 10 Apr 2010, A. Sultan, on *K. salicornioides*; as previous except Jan 1920, D. Petrie, from herbarium sheet WELT SP031442; [no collection data] *K. salicornioides*, from herbarium sheet MPN 31332.

To the above can be added following observation:

NEW ZEALAND: WAIRARAPA: Dry River Road, 19 Mar 2010, A. Sultan on *K. salicornioides* parasitic on *Kunzea ericoides* s.l.

Leucaspis trilobata (Fig. 2, 9)

This newly described armoured scale is currently known from a single collection on *K. clavata* and a few collections from *K. lindsayi,* all from the southern South Island.

Specimens deposited in NZAC: NEW ZEALAND: DUNEDIN, Aramoana, 12 Jan 2010, A. Sultan, on *K. lindsayi*, parasitic on *Coprosma areolata*; SOUTH CANTERBURY, Peel Forest, Te Wanahu Flat, 5 Jan 2010, A. Sultan, on *K. lindsayi*, parasitic on *Lophomyrtus obcordata*; MACKENZIE, Motuariki Island in Lake Tekapo, 9 Oct 1973, B.P.J. Molloy, on *K. lindsayi* and *K. clavata* parasitic on *Muehlenbeckia*, from herbarium sheet CHR 286231; as previous except small island near Motuariki Island, 9 Jan 2010, A. Sultan, on *K. clavata*; as previous except on *K. lindsayi*; SOUTHLAND, Winton, 8 Dec 1882, T. Kirk, on *K. lindsayi*, from herbarium sheet WELT SP031420.

Family Eriococcidae

Eriococcus korthalsellae (Fig. 3, 10)

This newly described felt scale species has been recorded on all three *Korthalsella* species. Nymphs and young scales feed within the nodes of vegetative and flowering mistletoe shoots, whereas older gravid females can often be found on the basal internodes.

Specimens deposited in NZAC: NEW ZEALAND, TAUPO, Turangi, Motuoapa, 19 March 2009, A. Sultan, on *K. salicornioides* parasitic on *Leptospermum scoparium,* BAY OF PLENTY, Rotorua, Whakarewarewa Geothermal Reserve, 10 Apr 2010, A. Sultan, on *K. salicornioides*; WAIRARAPA, Gladstone, Longbush Road, Mangahuia Stream Bush, 26 Feb 2010, A. Sultan, on *K. lindsayi*; MID CANERBURY, Banks Peninsula, Otanerito Bay, 4 Jan 2010, A. Sultan, on *K. lindsayi*; MACKENZIE, Lake Tekapo, small island near Motuariki Island, 9 Jan 2010, A, Sultan, on *K. lindsayi*.

To the above can be added following observations:

NEW ZEALAND: WAIRARAPA: Rata Hills, 27 Nov 2010, A. Sultan, on *K. salicornioides;* Pounui Lagoon, 26 Feb 2010, A. Sultan on *K. salicornioides*; MID CANTERBURY: Lake Coleridge, Feb 2008, A. Robertson on *K. salicornioides.*

Other scale insect species

Family Coccidae

Ceroplastes sinensis (Chinese wax scale) is an adventive species recorded on 21 native plant species from 18 genera in 16 families (including one fern sp.) (Martin 1999, Hodgson and Henderson 2000, Morrison 2007, Anon. 2009, Anon. 2010, Landcare Research 2012). It has also been recorded on 11 cultivated/exotic species in 11 genera from seven families (Hodgson and Henderson 2000, Bain 2008, Philip 2008, Anon. 2009, Hedley-Stevens 2009, Anon. 2010, Landcare Research 2012). Young scales were recorded on *K. salicornioides,* while adults were seen on the mistletoe host *Leptospermum scoparium* in the present study. These are new host records for this scale.

Specimens deposited in NZAC: NEW ZEALAND, COROMANDEL, Gemstone Bay, on *Korthalsella salicornioides*, 11 Apr 2010, A. Sultan.

Fig. 1. Female (upper) and male (lower) *Leucaspis albotecta* (photo courtesy Rosa Henderson)

Fig. 2. Female (darker) and male (paler) *Leucaspis trilobata* (photo courtesy Rosa Henderson)

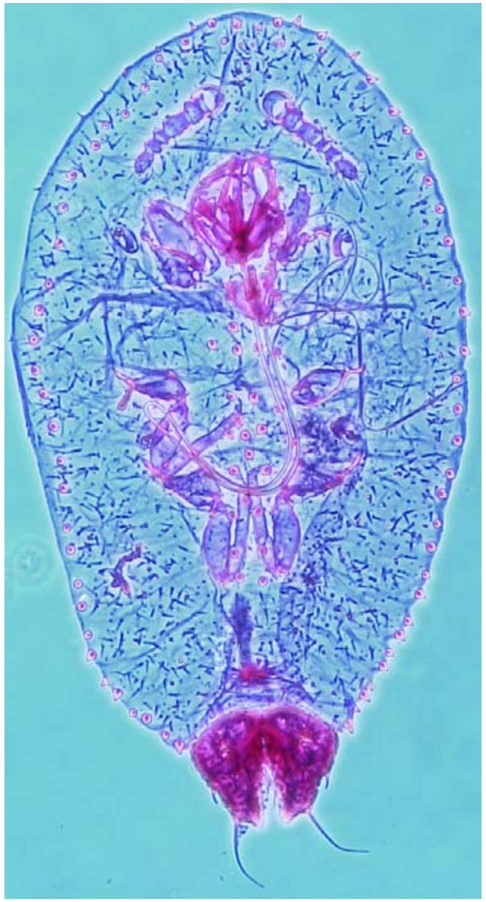

Fig. 3. Slide mounted young female of *Eriococcus korthalsellae* (photo courtesy Rosa Henderson)

Coccus hesperidum (soft brown scale) is an adventive species known to infest a wide range of native and exotic plants. Host recorded for soft brown scale comprise 27 native species from 17 families and 19 genera (including two fern species) (de Lange 1997, Martin 1999, Hodgson and Henderson 2000, Walker 2008, Anon. 2009, Hedley-Stevens 2009, Landcare Research 2012). Cultivated/introduced hosts recorded comprise 34 spp. from 30 genera in 20 families (Hodgson and Henderson 2000, Lester *et al.* 2003, Anon. 2009, Landcare Research 2012). *Korthalsella clavata, K. lindsayi,* and *K. salicornioides* are new host records for this species.

Specimens deposited in NZAC: NEW ZEALAND, WANGANUI, Rongotea, Coles Bush, on *K. lindsayi*, 18 Jul, 2009, A. Sultan; Rangitikei, Mangaweka Scenic Reserve, on *K. salicornioides*, 8 May, 2010, A. Sultan.

To the above can be added following observation:

NEW ZEALAND, MID CANTERBURY, Castle Hill, Jan 2011, A. Sultan & A.W. Robertson, on *K. clavata*.

Kalasiris perforata (fringed scale) is the only native coccid recorded on a *Korthalsella* sp. so far. Hosts recorded for this scale comprise 17 native spp. from 10 genera in 10 families (Hodgson and Henderson 2000, Anon. 2011, Landcare Research 2012). In the present study, it was recorded on *K. lindsayi*, which is a new host record for this coccid.

Specimens deposited in NZAC: NEW ZEALAND, WANGANUI, Rongotea, Coles Bush, on *K. lindsayi*, Oct 2009, A. Sultan.

Saissetia coffeae (hemispherical scale). Hosts recorded for this adventive species in New Zealand include 21 native species from 17 genera in 16 families (including six fern species) (Hodgson and Henderson 2000, Landcare Research 2012). Cultivated/exotic species infested comprise 21 species in 20 genera from 16 families (Hodgson and Henderson 2000, Lester *et al.* 2003, Winks *et al.* 2004a, Bain 2008, Philip 2008, Anon 2009, Landcare Research 2012). In the present study, this scale was recorded on *K. salicornioides*. This is a new host record for this scale.

Specimens deposited in NZAC: NEW ZEALAND, COROMANDEL, Gemstone Bay, on *K. salicornioides*, 11 Apr 2010, A. Sultan.

Saissetia oleae (black scale, olive scale, 'H' scale) is an adventive species. Previous host records for this species include 22 native plants from 17 families and 21 genera (Hodgson and Henderson 2000, Anon. 2009, Landcare Research 2012). Cultivated/exotic hosts recorded for black scale comprise 25 exotic spp. in 25 genera from 18 families (Hodgson and Henderson 2000, Winks *et al*. 2004a, b, Hedley-Stevens 2009, Anon. 2011). *Korthalsella lindsayi* is a new host record for this species.

Specimens deposited in NZAC: NEW ZEALAND, WANGANUI, Rongotea, Coles Bush, on *K. lindsayi*, 30 Aug 2009, A. Sultan.

Family Diaspididae

Hemiberlesia lataniae (latania scale) is an adventive species, recorded on 24 native spp. from 23 genera in 21 families (including one conifer species: *Dacrycarpus dacrydioides*) (Charles and Henderson 2002, Walker 2008, Anon. 2009, Landcare Research 2012). Also recorded on 28 exotic/cultivated species from 24 genera in 22 families (Hill *et al.* 2007, McKenna *et al.* 2009, Henderson 2011, Landcare Research 2012). In the present study, it was recorded on *K. salicornioides* which is a new host record for this species.

Specimens deposited in NZAC: NEW ZEALAND, COROMANDEL, Black Jack Road, on *K. salicornioides*, 11 Apr 2010, A. Sultan.

Hemiberlesia rapax (greedy scale) is an adventive armoured scale species. Hosts recorded for this scale include 73 native species from 39 families and 55 genera (Charles and Henderson 2002, Hedley-Stevens 2009, Henderson 2011, Landcare Research 2012). Thirty-five exotic spp., in 33 genera from 25 families are also infested (Bain 2008, Hedley-Stevens 2008, McKenna *et al*. 2009, Henderson 2011, Landcare Research 2012). *Korthalsella lindsayi* and *K. salicornioides* are new host records for this species.

Specimens deposited in NZAC: NEW ZEALAND, BAY OF PLENTY, Kohi Point walkway near Whakatane, on *K. salicornioides*, 10 Apr 2010, A. Sultan; TAUPO, Turangi, Motuoapa, on *K. salicornioides*, 19 Mar 2009, A. Sultan; WANGANUI, Rongotea, Coles Bush, on *K. lindsayi*, 7 Aug 2008, A. Sultan; WAIRARAPA, Glenside Station, Mangahuia Stream Bush near Gladstone, on *K. lindsayi*, 26 Feb 2010, A.

Sultan; Oporua (east of Lake Wairarapa), on *K. clavata*, 27 Feb 2010, A. Sultan; KAIKOURA, upper Flaxbourne River, on *K. salicornioides*, 1 Jan 2010, A. Sultan.

Aspidiotus nerii (oleander scale) is an adventive species recorded on a wide range of hosts including 49 native plant species from 37 genera in 32 families (Charles and Henderson 2009, Hedley-Stevens 2009, Henderson 2011, Landcare Research 2012), while 36 cultivated/exotic species in 35 genera from 24 families were also recorded as hosts (Kay and Smale 1990, Bain 2008, Henderson 2011).

In the present study it was recorded on *K. clavata*, *K. lindsayi* and *K. salicornioides* which are new host records for this species.

Specimens deposited in NZAC: NEW ZEALAND, RANGITIKEI, Mangaweka Scenic Reserve, on *Korthalsella salicornioides*, 8 May, 2010, A. Sultan; WAIRARAPA, Oporua (east of Lake Wairarapa), on *K. clavata*, 27 Feb 2010, A. Sultan; KAIKOURA, Marble Point, on *K. lindsayi*, Feb 2010, A.W. Robertson; SOUTH CANTERBURY, Te Wanahu Flat, Peel Forest, on *K. lindsayi*, 5 Jan 2010, A. Sultan.

Family Eriococcidae

Eriococcus campbelli. The presence of *E. campbelli* females on *K. salicornioides* is a result of individuals becoming vagrant from *Leptospermum scoparium* which is the main host for this species.

Specimens deposited in NZAC: NEW ZEALAND, TAUPO, Turangi, Motuoapa, on *Korthalsella salicornioides* parasitic on *Leptospermum scoparium,* 19 Mar 2009, A. Sultan; FIORDLAND, Supply Bay, Lake Manapouri, on *K. salicornioides*, Feb 2010, A. Robertson.

Eriococcus leptospermi. The presence of *E. leptospermi* females on *K. salicornioides* is a result of individuals becoming vagrant from *Leptospermum scoparium* which is the main host for this species.

Specimens deposited in NZAC: NEW ZEALAND, NORTHLAND, Gumfields Rd, Ahipara, on *K. salicornioides*, 14 Apr 2010, A. Sultan; MARLBOROUGH, Wairau River, on *K. salicornioides*, Feb 2010, A. Robertson.

Eriococcus pallidus. This native felt scale has been recorded on 18 native plant species from 15 genera in 12 families (Hoy 1962, Landcare Research 2012). Cultivated/exotic species infested by this scale include *Cytisus* sp., *Elaeagnus* x *reflexa*, and *Escallonia* sp. (Hoy 1962). In the present study, it was recorded on *K. lindsayi*, which is a new host record for this species.

Specimens deposited in NZAC: NEW ZEALAND, WAIRARAPA, Oporua (east of Lake Wairarapa), on *K. lindsayi*, 27 Feb 2010, A. Sultan.

Family Pseudococcidae

Paracoccus glaucus. This native mealybug has been recorded on 25 native plant species in 22 genera from 20 families (including a fern and a club moss species) (Cox 1987, Landcare Research 2012). Cultivated species infested include grapefruit (*Citrus* x *paradisi*) (Cox 1987). In the present study, it was recorded on *K. lindsayi* which is a new host record for this species.

Specimens deposited in NZAC: NEW ZEALAND, WAIRARAPA, Oporua (east of Lake Wairarapa), on *K. lindsayi*, 27 Feb 2010, A. Sultan.

Lepidoptera associated with New Zealand Korthalsella spp.

Family Noctuidae

Meterana ochthistis is the only noctuid recorded so far on a *Korthalsella* sp. Previous host records for this species include 13 native hosts in 12 genera belonging to 12 families (Gaskin 1966, White 2002); also recorded on *Mida* sp. (J. Dugdale, pers. comm.) and *Parsonsia* sp. (R. Hoare, pers. comm.). It was recorded on *K. lindsayi* in the current study (Fig. 4) which is a new host record for this species.

Specimen reared from host: NEW ZEALAND, WANGANUI, Rongotea, Coles Bush, on *K. lindsayi*, Mar 2009, A. Sultan.

Family Torticidae

Harmologa oblongana is a native polyphagous species known to use 14 endemic species, in 9 genera from 9 families as larval food plants (Patrick and Dugdale 1997, Patrick 2000, White 2002, Landcare Research 2012). European larch (*Larix decidua*), tree lupin (*Lupinus arboreus*), apple (*Malus* x *domestica*), apricot (*Prunus armeniaca*) and lawn daisy (*Bellis perennis*) are also used as larval food plants (White 2002, Landcare Research 2012). This species was also recorded by Patrick and Dugdale (1997) on *K. lindsayi.*

Specimen reared from host: NEW ZEALAND, WANGANUI, Rongotea, Coles Bush, on *K. lindsayi*, Mar 2009, A. Sultan.

Ctenopseustis obliquana is a native polyphagous leaf-roller species with a very broad host range. Forty-six native species, in 37 genera from 31 families (including four fern species and two conifers) are known to be used as larval food plants (Green and Dugdale 1982, Hutchinson 1992, Patrick and Dugdale 1997, Berndt *et al*. 2006, Landcare Research 2012). Seventy-one species in 54 exotic genera from 37 families (including four conifer species) are also used as larval food plants (Green and Dugdale 1982, Lo *et al*. 1995, Suckling *et al*. 1998, Winks and Fowler 2000, Winks *et al*. 2001, Winks *et al*. 2003, Smith *et al*. 2004, Winks *et al*. 2004a, b, McKenna and Stevens 2007, Anon. 2009, McKenna *et al*. 2009, Anon. 2011, Landcare Research 2012). This species was also recorded by Patrick and Dugdale (1997) on *K. lindsayi.*

Specimen reared from host: NEW ZEALAND, WANGANUI, Rongotea, Coles Bush, on *K. lindsayi*, Mar 2009, A. Sultan.

Gall mite Aceria korelli (Arachnida: Acari: Eriophyidae) (Fig. 11)

Aceria korelli is a specialist mite colonising all three *Korthalsella* species (Manson 1984). It transforms vegetative and flowering shoots into galls (Fig. 5). The impact on reproductive output is given in the reproductive biology chapter. It was recorded from the following locations:

NEW ZEALAND, MID CANTERBURY, Castle Hill Basin, on *K. clavata,* Jan 2008, A. Sultan and A. Robertson; RANGITIKEI, Te One Bush, on *K. lindsayi,* Mar 2011, A. Sultan; Paengaroa Scenic Reserve, on *K. lindsayi,* Nov 2008, A. Sultan; WANGANUI, Rongotea, Coles Bush, on *K. lindsayi,* 6 Dec 2008; WAIRARAPA, Glenside Station, Mangahuia Stream Bush near Gladstone, on *K. lindsayi*, 26 February 2010, A. Sultan; SOUTH CANTERBURY, Peel Forest, on *K. lindsayi,* 5 Jan 2010, A. Sultan; BAY OF PLENTY, Lake Okataina Scenic Reserve, on *K. salicornioides,* 13 Apr 2010, P. Cashmore; WELLINGTON, Ngarara Road, Waikanae, on *K. salicornioides*, 16 Dec 2007, A. Sultan; CENTRAL OTAGO, Poison Creek Conservation area, Upper Clutha Valley, on *K. salicornioides,* 11 Jan 2011, A. Sultan, DUNEDIN, Northern Cemetery, Dunedin, on *K. salicornioides,* 29 Jan 2009, A. Robertson.

Fungal Pathogens

Guignardia korthalsellae (Fig. 6, 12)

This newly described ascomycete is a specialist on New Zealand *Korthalsella* spp. The fungus produces black, erumpent fruiting bodies that are solitary or coalescing (Fig. 6a). Fruiting bodies develop within the epidermal layer. Hyphae within the plant develop between 2-3 layers of hypodermal cells, thus the host tissue is disrupted to a minimal extent. *Guignardia korthalsellae* has *Phyllosticta* (macroconidial) and *Leptodothiorella* (microconidial) imperfect states.

Specimens in PDD.

NEW ZEALAND, WANGANUI, vic. Palmerston North, Coles Bush, 22 Nov 2008, A. Sultan, PDD 94922 (holotype), on *K. lindsayi*; vic. Palmerston North, Coles Bush, Oct 2008, A. Sultan, PDD 94884, on *K. lindsayi*; vic. Palmerston North, Coles Bush, 23 Dec 2008, A. Sultan, PDD 95152, on *K. lindsayi*; BAY OF PLENTY, Paengaroa Scenic Reserve, Oct/Nov 2008, A. Sultan, PDD 94900, on *K. lindsayi*; MID CANTERBURY, Christchurch, Riccarton Bush, 11 Apr. 1996, R.C. Close, PDD 65953, on *K. lindsayi*.

To the above can be added following observations:

NEW ZEALAND, NORTHLAND, Gumfields Road, Ahipara, 14 Apr 2010, A. Sultan, on *K. salicornioides*, COROMANDEL, Black Jack Road, 11 Apr 2010, A. Sultan, on *K. salicornioides*, TAUPO, Lake Taupo, near Jellicoe Point, Motutere Bay, Apr 2010, A. Sultan, on *K. salicornioides*; HAWKES BAY, Huatokitoki Stream, Jan 2011, A. Sultan, on *K. lindsayi*, RANGITIKEI, Te One Bush, Mar 2011, A. Sultan, on *K. lindsayi*; Avoca River, Jan 2011, A. Sultan, on *K. lindsayi*; Ngamatea Farm, Whangaehu River Valley, Wanganui, 28 Jan 1995, C. Ogle, on herbarium specimen of *K. lindsayi* (CHR 505986); Totara Reserve, March 2011, A. Sultan, on *K. lindsayi*; WELLINGTON, Waikanae, Feb 2010, A. Sultan; WAIRARAPA, Oporua, 27 Feb 2010, A. Sultan, on *K. clavata*,; Glenside Station, Mangahuia Stream Bush near Gladstone, 26 Feb 2010, A. Sultan, on *K. lindsayi*; Ahi Paku, 19 Mar 2010, A. Sultan, on K. lindsayi; Lake Wairarapa, Western Lake Road, 17 Mar 2010, A. Sultan; MARLBOROUGH, Transverse Swamp, Wairau River, Feb. 2010, A. Robertson, on *K. salicornioides*; NELSON, Kaiteriteri, April 2011, A. Robertson, on *K. salicornioides*;

Teetotal Flats, Upper Buller River, Feb 2010, A. Robertson, on *K. salicornioides*; BULLER, Lake Rotoiti, Feb 2010, A. Robertson, on *K. salicornioides*; Maruia Valley, Feb 2010, A. Robertson, on *K. clavata*; Lake Brunner, Feb 2010, A. Robertson, on K. *clavata*; MID CANTERBURY, Banks Peninsula, Otanerito Bay, 4 Jan 2010, A. Sultan, on *K. lindsayi*; NORTH CANTERBURY, Mt Alexander, Jan 2010, A. Sultan, on *K. clavata*; SOUTH CANTERBURY, Peel Forest, 5 Jan 2010, A. Sultan, on *K. lindsayi*; DUNEDIN, Mill House, Herbert, Jan 2010, A. Sultan, on *K. salicornioides*; Guilds Hill, 13 Jan 2010, A. Sultan, on *K. lindsayi*; Aramoana, 12 Jan 2010, A. Sultan, on *K. lindsayi*; Dunedin Botanical Gardens, on herbarium specimen of *K. lindsayi* (CANU 3507); WESTLAND, Paringa Bridge Scenic Reserve, Feb 2010, A. Robertson, on *K. clavata*; Windbag Creek, Paringa, Feb 2010, A. Robertson, on *K. clavata*; Pakahi near Okarito, Feb 2010, A. Robertson, on *K. salicornioides*; FIORDLAND, Dean Burn Forest, Feb 2010, A. Robertson, on *K. clavata*; Broadlands Bush, Waiau, 16 Jun 2000, B. D. Rance, on herbarium specimen of *K. lindsayi* (AK 252527); SOUTHLAND, Tussock Creek, Feb 2010, A. Robertson, on *K. clavata*; Grant Road, Bushy Point, Otatara, Invercargill, Feb 2010, A. Robertson, on *K. salicornioides*.

***Rosenscheldiella korthalsellae* (Fig. 7, 13)**

This newly described ascomycete is a specialist on New Zealand *Korthalsella* spp. Ascomata are globose, initially reddish later brown coloured (Fig. 7a). Hyphae within the plant tissue are confined to the substomatal cavity and to the immediate area around the ascomata and are intercellular. The other *Rosenscheldiella* species occurring in New Zealand is *R. brachyglottidis* which is associated with *Brachyglottis repanda* (Asteraceae) (Laundon 1971).

Specimens in PDD.

NEW ZEALAND, RANGITIKEI, vic. Taihape, Paengaroa Scenic Reserve, 3 Nov 2008, A. Sultan, PDD 94885 (holotype), on *Korthalsella lindsayi*; WANGANUI, vic. Palmerston North, Coles Bush, 22 Nov 2008, A. Sultan, PDD 94923, on *K. lindsayi*; MACKENZIE, Lake Ohau, Jan 2009, A.W. Robertson, PDD 95153, on *K. clavata*; MID CANTERBURY, Banks Peninsula, Price's Valley, 2 Sep 1995, J.E. Braggins, PDD 65042, on *K. lindsayi*; Castle Hill, 17 Jan 2008, A. Sultan & A.W. Robertson, PDD 95150, on *K. clavata*; SOUTH CANTERBURY, Peel Forest, 22 Jun

1995, B. P. J. Molloy, PDD 35039, on *K. lindsayi*; Taupo, vic. Motuoapa, 3 Apr 2008, A. Sultan, PDD 95151, on *K. salicornioides*.

To the above can be added the following observations:

AUCKLAND, Winstone Track, Waitakere Ranges, 15 Apr 2010, A. Sultan, on *K. salicornioides*; RANGITIKEI, Avoca River, Jan 2011, A. Sultan, on *K. lindsayi*; WAIRARAPA, Glenside Station, Mangahuia Stream Bush near Gladstone, 26 Feb 2010, A. Sultan, on *K. lindsayi*; Ahi Paku, 19 Mar 2010, A. Sultan, on *K. lindsayi*; MARLBOROUGH, Transverse Swamp, Wairau River, Feb 2010, A. Robertson, on *K. salicornioides*; Wairau Valley, Branch River, between Goat Stream and Fuchsia Stream, Feb 2010, A. Robertson, on *K. salicornioides*; NELSON, Teetotal Flats, Upper Buller River, Feb 2010, A. Robertson, on *K. salicornioides*; BULLER, Maruia Valley, Feb 2010, A. Robertson, on *K. clavata*, Lake Brunner, Feb 2010, A. Robertson, on *K. clavata*; NORTH CANTERBURY, Mt Alexander, Jan 2010, A. Sultan, on *K. clavata*; MID CANERBURY, Banks Peninsula, Otanerito Bay, 4 Jan 2010, A. Sultan, on *K. lindsayi*; DUNEDIN, Mill House, Herbert, Jan 2010, A. Sultan, on *K. salicornioides*; Guilds Hill, 13 Jan 2010, A. Sultan, on *K. lindsayi*; WESTLAND, Pakahi near Okarito, Feb 2010, A. Robertson, on *K. salicornioides*; FIORDLAND, Dean Burn Forest, Feb 2010, A. Robertson, on *K. clavata*; Broadlands Bush, Waiau, 16 Jun 2000, B. D. Rance, on herbarium specimen of *K. lindsayi* (AK 252527); SOUTHLAND, Tussock Creek, Feb 2010, A. Robertson, on *K. clavata*; Castle Rock, Dipton, Mar 2009, A. Robertson, on *K. clavata*.

Fig. 4. *Meterana ochthistis* larva recorded on *Korthalsella lindsayi* at Coles bush

Fig. 5. Galls induced by *Aceria korelli* on *Korthalsella clavata* at Castle Hill

Fig. 6a. *Guignardia korthalsellae* on *K. lindsayi* at Coles bush

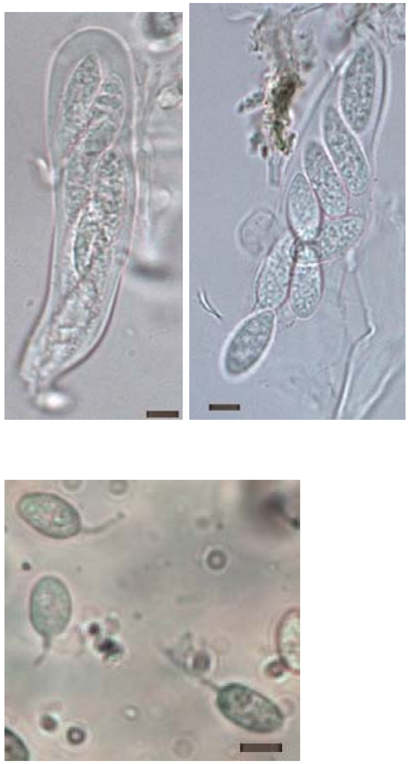

Fig. 6b (upper left) *G. korthalsellae* ascus, Fig. 6c (upper right) ascospores and Fig. 6d (bottom) conidia, scale bar= approx. 10 µm

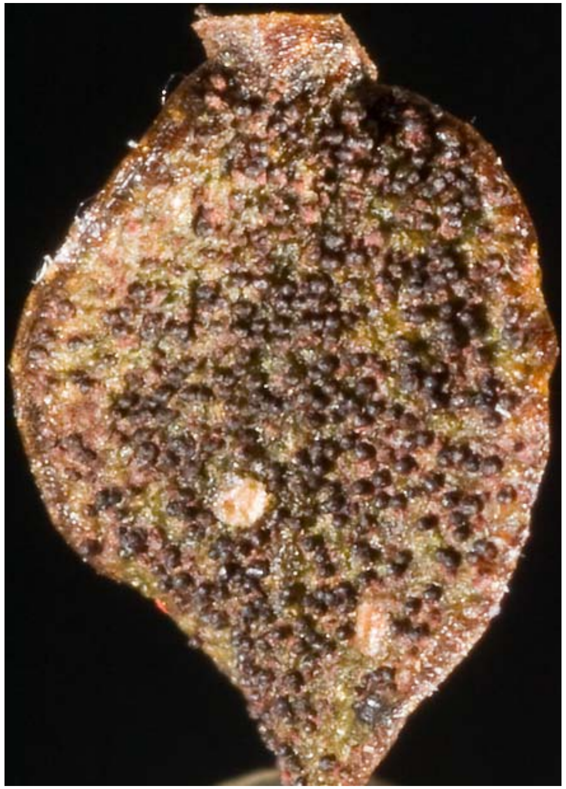

Fig. 7a. *Rosenscheldiella korthalsellae* on *K. lindsayi* at Paengaroa

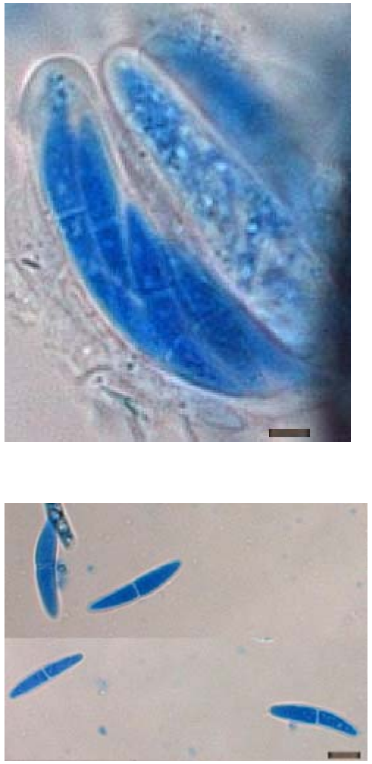

Fig. 7b (upper) *R. korthalsellae* ascus and Fig. 7c (bottom) ascospores, scale bar=approx. 10 µm

DISCUSSION

The natural enemies recorded on New Zealand *Korthalsella* in this study comprise native specialists, native generalists and exotic generalists (Table 1).

Table 1. Checklist of natural enemies of New Zealand's pygmy mistletoes

	K. clavata	*K. lindsayi*	*K. salicornioides*
Fungi: Ascomycota			
Family Botryosphaeriaceae			
Guignardia korthalsellae Sultan, Johnston, Park & Robertson†	+	+	+
Family Mycosphaerellaceae			
Rosenscheldiella korthalsellae Sultan, Johnston, Park & Robertson†	+	+	+
Arachnida: Acari: Eriophyoidea			
Family Eriophyidae			
Aceria korelli Manson†	+	+	+
Insecta: Hemiptera: Sternorrhyncha: Coccoidea			
Family Eriococcidae			
Eriococcus korthalsellae Henderson†		+	+
E. pallidus Maskell ◊		+	
Family Coccidae			
Kalasiris perforata (Maskell) ◊		+	
Ceroplastes sinensis Del Guercio*			+
Coccus hesperidum Linnaeus*	+	+	+
Saissetia coffeae (Walker)*			+
S. oleae (Olivier)*		+	
Family Diaspididae			
Leucaspis albotecta Henderson†			+
L. trilobata Henderson †	+	+	
Aspidiotus nerii Bouché*		+	+
Hemiberlesia lataniae (Signoret)*			+
H. rapax (Comstock)*		+	+
Family Pseudococcidae			
Paracoccus glaucus (Maskell) ◊		+	
Insecta: Lepidoptera			
Family Noctuidae			
Meterana ochthistis (Meyrick) ◊		+	
Family Tortricidae			
Ctenopseustis obliquana (Walker) ◊		+	
Hormologa oblongana (Walker) ◊		+	

* adventives species – all generalists
† endemic specialists on New Zealand *Korthalsella*
◊ native generalists

Native Specialists

Leucaspis albotecta is found only on *K. salicornioides* and in both North and South Islands (Fig. 8) whereas *L. trilobata* is, so far, restricted to the southern South Island on *K. clavata* and *K. lindsayi* (Fig. 9). *Leucaspis albotecta* may possibly be found in some locations in the Wairarapa region on *K. clavata* and *K. lindsayi,* however, *K. salicornioides* is the preferred host species for this scale throughout its range in New Zealand. The distribution of *Eriococcus korthalsellae* is also stretched between the North and South Islands (Fig. 10) and its host preferences included both *K. lindsayi* and *K. salicornioides*. The two new *Leucaspis* species and *E. korthalsellae* are specific to the genus, which possibly implies a long association between these scale insects and the New Zealand pygmy mistletoes.

The gall mite *Aceria korelli* is found on all three *Korthalsella* species and occurs both in the North and South Islands (Fig. 11).

Both the fungal pathogens *Guignardia korthalsellae* and *Rosenscheldiella korthalsellae* develop within living host tissue and cause minimal damage. These highly developed biological relationships support host specialisation in both of these newly described species. Both fungal pathogens are found on all three *Korthalsella* spp. and their distributions stretch between both the North and South Islands (Fig. 12-13).

This study highlights the significance of conserving the declining mistletoe populations as most of specialist invertebrate and fungal associates of New Zealand's pygmy mistletoes particularly the two *Leucaspis* species are quite rare. *Leucaspis trilobata* is the most threatened species, currently known from only three sites in southern South Island. It is recommended that *L. trilobata* be added to high priority conservation concerns in the threatened arthropods of New Zealand and active measures be taken to protect its populations. A systematic survey of current *Korthalsella* populations is a pre-requisite to determine the true range, which may be larger but since the species is easily overlooked.

The fragmentation evident in many mistletoe populations and the limited dispersal potential of the specialist invertebrates results in populations of these specialist invertebrates being constrained to colonising new mistletoe populations. According to the rarity classification of Rabinowitz (1981) based on the geographic range, habitat specificity and local population size, natural enemies of New Zealand's pygmy mistletoes can be categorised as:

- locally abundant over a large range in a specific habitat (least at risk): *Aceria korelli*
- constantly sparse in a specific habitat but over a large range: *Leucaspis albotecta, Guignardia korthalsellae* and *Rosenscheldiella korthalsellae*
- constantly sparse and geographically restricted in a specific habitat (most at risk): *L. trilobata*

Thus, the specialists that have adopted pygmy mistletoes must have a difficult time persisting and warrant classification as vulnerable if not higher since they can never be very common.

Native Generalists

The coccid *Kalasiris perforata*, the felt scale *Eriococcus pallidus*, the mealy bug *Paracoccus glaucus*, and the larvae of the tortricid moths *Ctenopseustis obliquana*, *Hormologa oblongana* and of noctuid moth *Meterana ochthistis* are the native generalists recorded on *K. lindsayi* in this study. They are probably opportunistic feeders and never seem particularly common or dense on pygmy mistletoes.

Exotic Generalists

The records of adventive scale insect species on New Zealand's pygmy mistletoes, illustrate their vulnerability to outbreaks of these generalists, which may potentially be threatening to existence of localised populations. Although levels of infestation for these exotic scale insects were not very high, they represent a significant threat to already declining mistletoe populations. These scale insects might have shifted from mistletoe hosts to the mistletoes and thus represent a threat to the mistletoe hosts also. Inadvertent introduction of exotic scale insects can be extremely deleterious to plants lacking defence mechanisms against them as is exemplified by infestation and rapid decline of native *Cycas micronesiaca* in Guam by introduction of armoured scale *Aulacaspis yasumatsui* (Marler and Lawrence 2012). de Lange (1997) reported an infestation of *Coccus hesperidum*, soft brown scale, *Saissetia oleae*, black scale, and an *Eriococcus* sp. on New Zealand's loranthaceous mistletoe *Ileostylus micranthus* and the presence of a *Ctenochiton* sp. and black scale on herbarium specimens of New Zealand's extinct loranth *Trilepidea adamsii* (*Eriococcus* spp. can be native or adventive and *Ctenochiton* spp. are endemic). The role of such invertebrates, particularly introduced species, in the decline of these mistletoes is largely unknown (de

Lange 1997) but de Lange suggested that scale insects are possibly one of the several causes that have led to dramatic decline in the populations of *Ileostylus micranthus* in the Wellington region and presumed extinction of *Trilepidea adamsii* (de Lange 1997). Exotic scale insects may also potentially displace the specialist scale insects of New Zealand's pygmy mistletoes by competition. The occurrence of greedy scale, soft brown scale, oleander scale and black scale on *K. lindsayi* and of Chinese wax scale, latania scale, oleander scale and greedy scale on *K. salicornioides* are the first records of these exotic scale insects feeding on *Korthalsella* spp. in New Zealand. This may be the result of habitat fragmentation and the cultivation of infected exotics in the vicinity of the remnant mistletoe populations.

This study represents the first account of natural enemies of New Zealand's pygmy mistletoes and will hopefully be a precursor to further studies on the occurrence of these species.

Fig. 8. Distribution of *L. albotecta* (square represents records on *K. salicornioides*)

Fig. 9. Distribution of *Leucaspis trilobata,* (circle represents records on *K. clavata*, triangle represents records on *K. lindsayi*)

Fig. 10. Distribution of *Eriococcus korthalsellae* (triangle represents records on *K. lindsayi*, square represents records on *K. salicornioides*)

Fig. 11. Distribution of *Aceria korelli* (circle represents record on *K. clavata*, triangle represents records on *K. lindsayi* and square represents records on *K. salicornioides*)

Fig. 12. Distribution of *Guignardia korthalsellae,* (circle represents record on *K. clavata*, triangle represents records on *K. lindsayi* and square represents records on *K. salicornioides*)

Fig. 13. Distribution of *Rosenscheldiella korthalsellae* (circle represents record on *K. clavata*, triangle represents records on *K. lindsayi* and square represents records on *K. salicornioides*)

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Chapter 7: The Demographic Population Structure of New Zealand’s Pygmy Mistletoes

INTRODUCTION

Reproductive capacity to at least replace adults as they die is a key factor for the local success of a species (Sarukhan 1974). Demographic studies of plant species give insights into population dynamics based on mortality and recruitment rates and incidence of diseases/pests. These studies provide the foundation for conservation and management of a given species by addressing the questions relating to its population status, as well as stages/demographic processes having the greatest impact on the population growth rate and probabilities of local extinction (Mondragón 2011). The structure of a plant population can be described in terms of the ages, sizes and forms of the constituting individuals (Harper and White 1974). Size-based age estimation in non-arborescent perennials is subject to errors, which accounts for the scarcity of information on age-structure in non-woody perennials and on the correlation of age with physiological and/or ecological traits like fecundity (Dawson *et al.* 1990). Moreover, reproductive activity in plants cannot always be reliably correlated with age of individuals as it can instead be a function of plant density (Harper and White 1974). Population models based on stage (size) distribution may give more satisfactory results compared to models based on chronological age (Werner and Caswell 1977) and the long=term observation of marked individuals may represent the only means of assembling age distributions particularly for plants that do not exhibit annual growth rings (Harper and White 1974).

Studies on population structure in parasitic plants are challenging because parasite demography is also dependent on the demographic aspects of their hosts. Mistletoes can be classified into stage classes of development including attached seeds, seedlings, juveniles, immature, reproductive, and “subsenile” [subsenescent] and “senile” [senescent] states (Pinto 2005). Demographic studies in mistletoes based on size are preferable to those based on age, as age determination by counting the number of annual growth rings of host traversed by the mistletoe haustorium is destructive and is often unsuitable since many host species do not exhibit annual growth rings (Pinto 2005). Dawson *et al.* (1990) used a non-destructive aging index based on the number of bifurcating, branching events on the longest mistletoe stem, which was shown to be highly correlated with the age determined by the number of annual growth rings of the host traversed by mistletoe haustoria. Norton *et al.* (1997a) found that host stem diameter immediately below the mistletoe haustorium to be the best indicator of age in New Zealand’s loranthaceous mistletoes in *Nothofagus* forests.

Mistletoe volume was also found to be a good age predictor (Norton *et al.* 1997a) and thus size-class distributions can be used as surrogates for mistletoe age distributions (Norton *et al.* 1997b).

Many plant species rely both on seed and vegetative propagation while others may be exclusively vegetatively reproducing (Sarukhan 1974). For example, reproduction in *Viola blanda* is predominantly by vegetative production of new ramets, whereas, *V. sororia* reproduces exclusively by sexual means (Newell *et al.* 1981). Clonal growth further complicates demographic monitoring and interpretation since the fate of individuals depends on the combined success of ramets and it is usually very difficult to identify the extent and age of genets particularly when ramet turnover is high. The physiological organisation of a vegetatively reproducing plant thus has important repercussions on plant demography (Sarukhan 1974). Population dynamics can be followed at the genet level (or genotype - a genetically distinct individual), considering the generation of new ramets to be growth process of the genet, and/or at the ramet level (the shoots that together make up the genet), considering the generation of new ramets as the generation of new individuals (Mondragón 2011). The presence of clonal reproduction by sprouting in New Zealand's pygmy mistletoes (Chapter 5) probably allows relatively rapid colonisation of host branches and reduces the demographic dependence on seed for population persistence. In other plants, the rapid decay of connecting stolons, for example, in *Ranunculus repens,* confers independence to the established clonal daughter plants and thus means minimal physiological inter-dependence of the daughter plants (Sarukhan 1974). Conversely, physiological integration of intraclonal ramets means that daughter ramets can be subsidised at least initially by the parent ramets. For example, a study in *Cornus dummondii* indicated that that daughter ramet growth is reliant on the connective vasculature in the rhizomes (Killian *et al.* 2012). Genets may be very extensive and long-lived in grasses as was indicated in a study on *Sesleria albicans* where distances of over 1 m were recorded for ramets belonging to the same genet (Janišová and Gömöry 2007). In mistletoes, connectivity from older ramets to daughter sprouts along the cortical strands within the host tissue may allow substantial integration and cross-subsidy between ramets . Adventitious shoots may develop near the mother plant in *Viscum album* and it often takes place in response to breakage or freezing of the main shrub (Zuber 2004). The reproductive output of the genet may be maximised by an early onset of flowering in the sprouts compared to mistletoes originating from seeds (Chapter 5).

Natural enemies can regulate the population density or reproductive productivity of a species by affecting the vigour and survival of its individuals. For example, caterpillars of the nocutid specialist *Cyathissa percara* often completely defoliate *Phoradendron tomentosum* plants sometimes causing mortality of mistletoes (Whittaker 1984). However, specialist invertebrate feeders and fungal pathogens of New Zealand's pygmy mistletoes (Chapter 6) appear to cause minimal damage to their hosts. One way of detecting negative effects would be to monitor growth rates and reproductive output of infected versus non-infected plants. In addition, the occurrence of New Zealand's pygmy mistletoe hosts in seral vegetation means that hosts are prone to domination by other vegetation types and elimination by over-shadowing and natural age-related senescence of both the host and the mistletoes.

This chapter represents the first study of the demographic population structure of New Zealand's pygmy mistletoes. The aim of this study is to record the recruitment/mortality rates and relative growth rates of different size/stage classes of New Zealand species of *Korthalsella* and to study the impact of natural enemies on the population dynamics of each of the three species.

MATERIALS AND METHODS

Five sites were selected for demographic population structure monitoring of New Zealand *Korthalsella* (Table 1). A haphazardly chosen but typical subset of parasitised hosts was included in each population to represent the fate of a set of hosts and associated mistletoes. In most cases, only a section of each host was followed by marking branches and annually monitoring all the mistletoes present on each branch and adding any recruits noted on these branches in subsequent years. From 2008-2011, individual mistletoes were monitored by recording host node levels, compass aspects of host branches and mapping the location of individual mistletoes along the host branches. *Korthalsella clavata* was monitored in January during fruiting season so plants could be classified as being young (seedlings or sprouts), juveniles - larger plants, but not yet fruiting, and adults with fruits. In some cases, mistletoes were not noted until they were of juvenile size or even adults. In these cases, the ramet was assumed to have been initiated the year before but missed in the census due to its small size. As a result, these plants were added as inferred young to the previous year's count of young plants. In addition, the size of each mistletoe was recorded to the nearest mm in three dimensions – the longest axis and the two orthogonal dimensions and converted to cubic centimetres assuming that the mistletoe volume was approximated by a spheroid shape.

For *K. lindsayi* and *K. salicornioides*, which were monitored in winter in all but the last season, plants were categorised into young, small and large size classes based on volume, because at the time of year, both species were not flowering/fruiting and distinction into juveniles/adults could not be made. During the last year of study reproductive status of all species at all sites was recorded, to determine the relative numbers of reproductive mistletoes in different size classes. Categorisation into arbitrary size classes was made subsequently for these two species. In *K. lindsayi*, plants up to 0.02 cm^3 were considered seedlings/sprouts, plants >0.02 cm^3 and up to 1 cm^3 were considered small mistletoes, whereas plants larger than 1 cm^3 were considered large mistletoes. For *K. salicornioides,* plants up to 0.04 cm^3 were considered seedlings/sprouts, plants >0.04 cm^3 and up to 2 cm^3 were considered small mistletoes, whereas plants larger than 2 cm^3 were considered large mistletoes. The presence of any natural enemies was noted on each mistletoe.

Since there was relatively high mortality rate in both *K. lindsayi* sites in the first year of the study, additional host branches and associated mistletoes were added to the set of monitored plants to maintain a reasonable sample size. These additional plants were monitored in the same way as the other plants from 2009 onwards.

Following classification into three stage classes, the population growth trends were modelled in STELLA® (Isee Systems 2005) by recording the transition frequencies between classes ("births" and deaths, stasis within a stage, progression to a larger class, or regression to a smaller class) in each of the three years monitored. The annual relative growth rate of each mistletoe was also measured as:

$$\log_e \text{re-measured volume} - \log_e \text{initial volume}$$

When the study was designed, I was unaware of the clonality of these mistletoes so each ramet was treated as an individual. Thus the rate of growth and transition frequencies of true seedlings and of sprouts, which are likely to be quite different (see chapter 5), could not be distinguished. However, in order to estimate the initial frequency of the two types of young (seedlings and sprouts) once they had been recognised was attempted in the final season of monitoring. The earliest stages of sprouts and seedlings can be distinguished by the presence of a cotyledonary collar at the base of a seedling whereas sprouts lack a cotyledonary collar and tend to have multiple shoots. Even so, not all young plants could be unambiguously assigned, so the relative frequencies of the two types of shoots is not precisely known, nor is their relative growth rates or survival rates.

Table 1. Sites for demographic studies in each species.

Korthalsella species	Study site	Hosts
K. clavata	Castle Hill (South Island)	*Coprosma propinqua, Myrsine divaricata*
K. lindsayi	Coles Bush near Rongotea (North Island)	*Coprosma areolata, Melicope simplex, Lophomyrtus obcordata, L. bullata* x *obcordata, Muehlenbeckia complexa* and *Myrsine australis*
	Paengaroa Scenic Reserve (North Island)	*Melicope simplex, Coprosma rotundifolia, C. virescens* and *Myrsine divaricata*
K. salicornioides	Waikanae (North Island)	*Leptospermum scoparium* s.l.
	Motuoapa near Turangi (North Island)	*Leptospermum scoparium* s.l.

RESULTS

Korthalsella clavata

The likelihood of progression, regression, stasis, mortality and growth rates were fairly constant and positive over the three years of study at the Castle Hill study site (Fig 1). There was a steady rise in the number of ramets in each size class over the course of the study. The 2009-2010 year was particularly productive in terms of growth rates in this population with relatively low mortality rates and a high rate of class progression.

A total of 71 seedlings/sprouts were recorded on the hosts included in the study at the Castle Hill site during the first census in 2008. Of these 42.5% progressed to juveniles and 20.3% progressed to adults, whereas 9.9% stayed in the seedling/sprout phase during 2008-2009 study period. Seedling/sprout mortality was 22.5%. The average growth rate of the seedlings/sprouts during this first year was 1.6 cm^3 (std error=0.52). The total number of juveniles recorded was 63 and of these, 57% progressed to adults and 12.7% stayed in the juvenile phase. A juvenile mortality of 30.2% was recorded during this period. The average growth rate of juveniles was 1.8 cm^3 (std error=0.29). Of the 100 adults recorded during the first census in 2008, 12% regressed to juvenile size, 55% were recorded as adults in stasis, whereas adult mortality was 33% at the end of first census year (Fig. 1a). An average growth rate of -0.3 cm^3 (std error=0.19) was recorded for the adults.

During the second census (2009) a total of 136 seedlings/sprouts were recorded, of these 44% progressed to juveniles and 47% grew into adults at the end of 2009-2010 study period and seedling/sprout mortality was 6%. Average seedling/sprout relative growth rate was 3.7 cm^3 (std error=0.43). The number of juveniles recorded was 50, of these 70% grew into adults, 12% stayed as juveniles and juvenile mortality was 18%. Average juvenile relative growth rate was 1.8 cm^3 (std error=0.24). Of the 109 adults recorded, the majority (71.6%) stayed adults, 7.3% regressed to juveniles and adult mortality was 21% (Fig. 1b). Average adult relative growth rate was 0.05 cm^3 (std error=0.14).

During the third census (2010) a total of 168 seedling/sprouts were recorded, out of these 31% progressed to juveniles, 26.2% progressed to adults and 23.2% stayed in seedling/sprout phase at the end of the third census year (2010-2011). Seedling/sprout mortality was 19.6%. Average seedling/sprout relative growth rate was 2.1 cm^3 (std error=0.3). Of the 74 juveniles recorded during this year, 62.2% progressed to adults, 6.8% stayed in stasis and juvenile mortality was 31.1%. The average juvenile relative growth rate was 1.8 cm^3 (std error=0.2). Of the 177 adults recorded, 58.2% stayed in stasis as adults, 5.6% regressed to juveniles and adult mortality was 36.2% (Fig. 1c). The average adult relative growth rate was -0.2 cm^3 (std error=0.1). This three year study suggests a positive population growth trend at the Castle Hill site (Fig. 1e).

Most of the breeding mistletoes were within the size range of c. 0.02-20 cm^3 (largest dimension c. 1-4.5 cm) at this site (Fig. 1f-i).

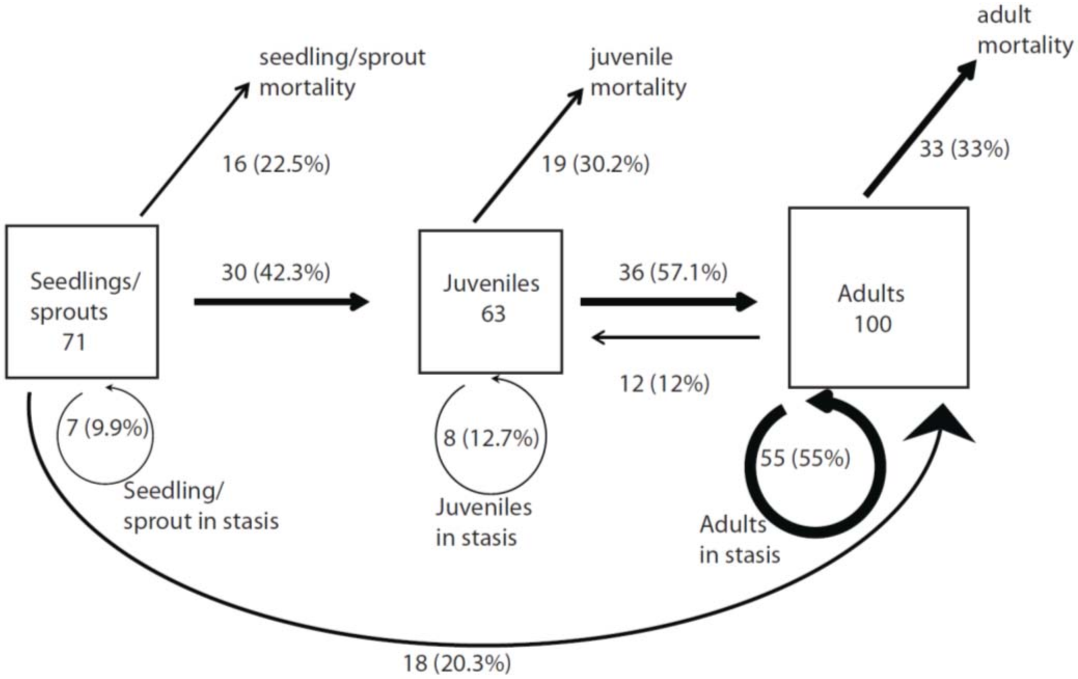

Fig. 1a. Demographics of *K. clavata* during 2008-2009 at the Castle Hill site. Size of arrows indicate the relative numbers of mistletoes in transition and similarly in size of squares indicates the relative number of mistletoes in each class.

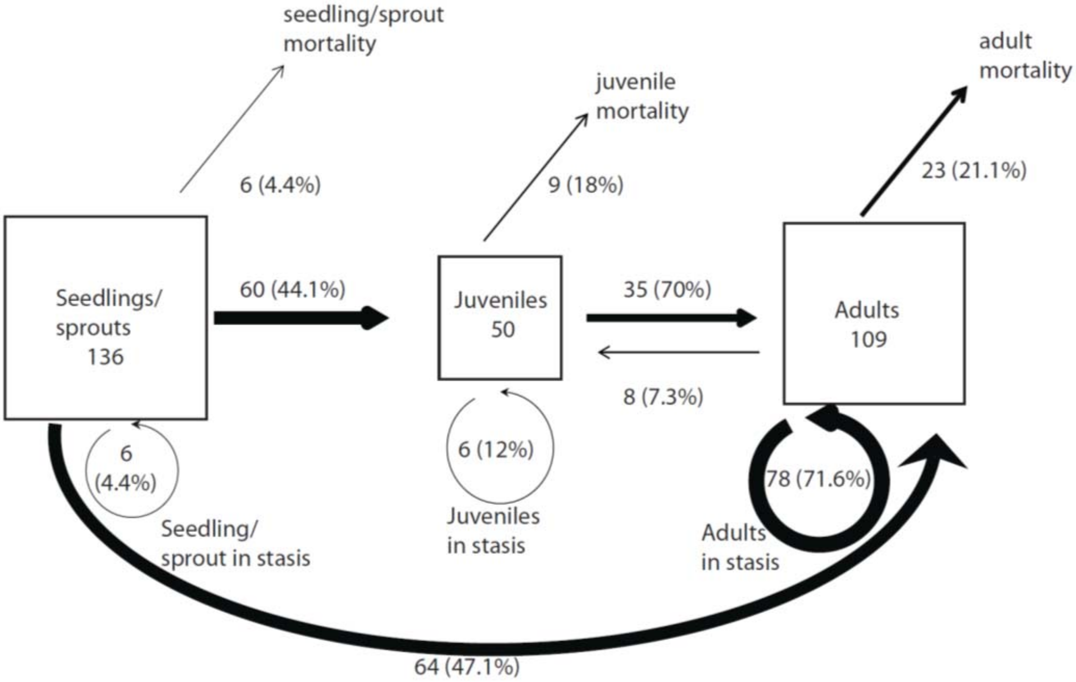

Fig. 1b. Demographics of *K. clavata* during 2009-2010 at the Castle Hill site

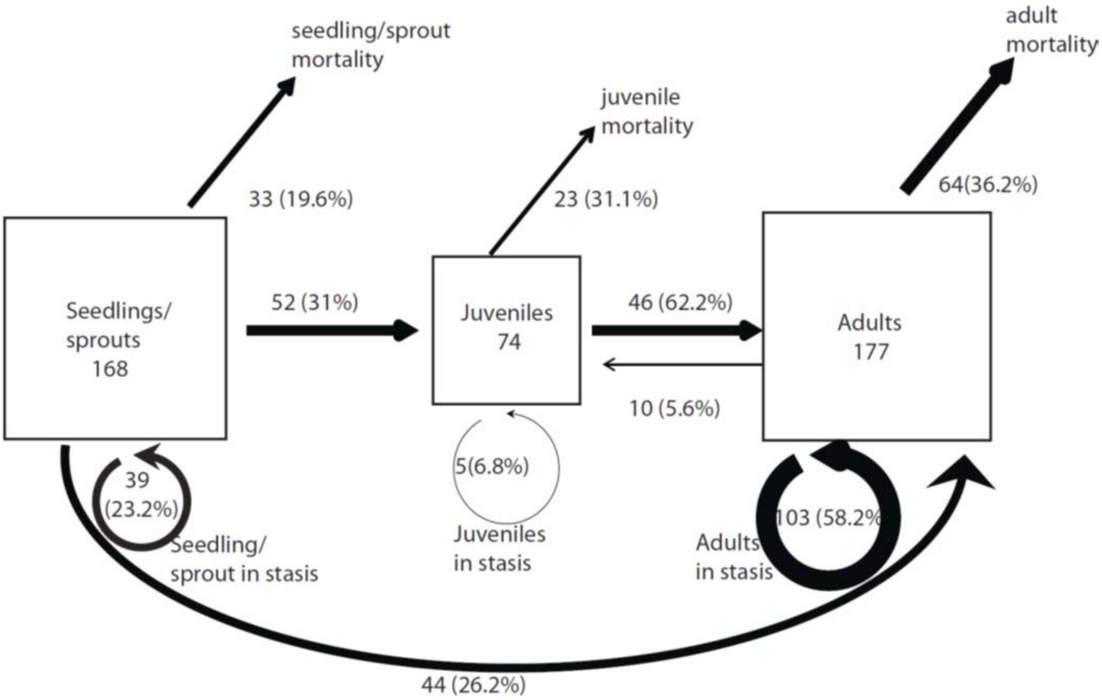

Fig. 1c. Demographics of *K. clavata* during 2010-2011 at the Castle Hill site

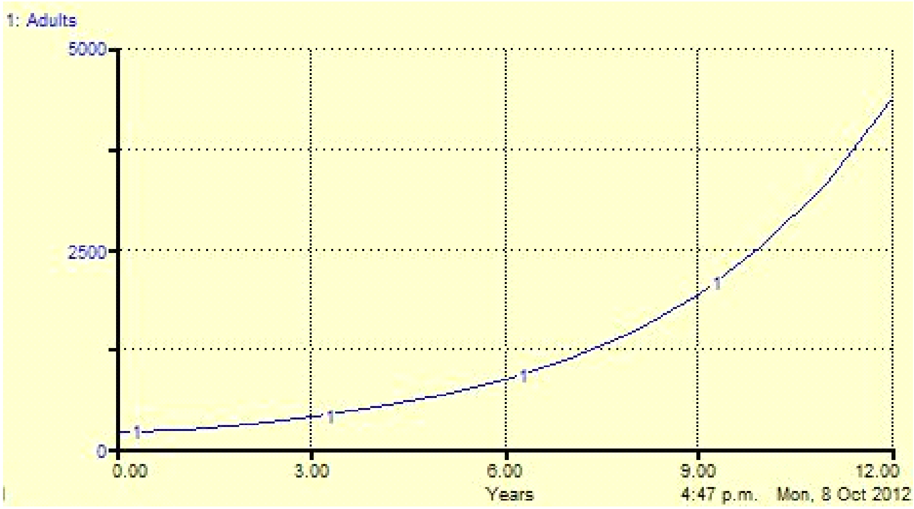

Fig. 1e. Projected population growth curve for *K. clavata* at the Castle Hill site assuming a starting population from the 2010 census plants and the average transition probabilities noted over the 3 seasons of monitoring.

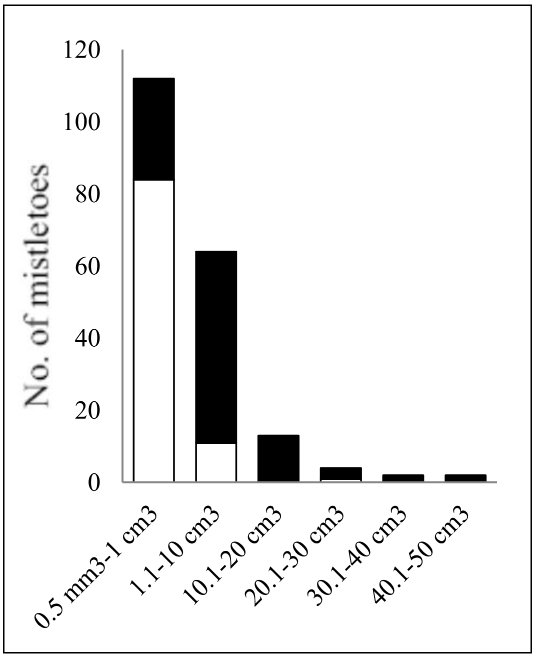

Fig. 1f. Size class distribution of breeding and non-breeding mistletoes in *K. clavata* at Castle Hill during 2008, □: number of non-breeding mistletoes, ■: number of breeding mistletoes

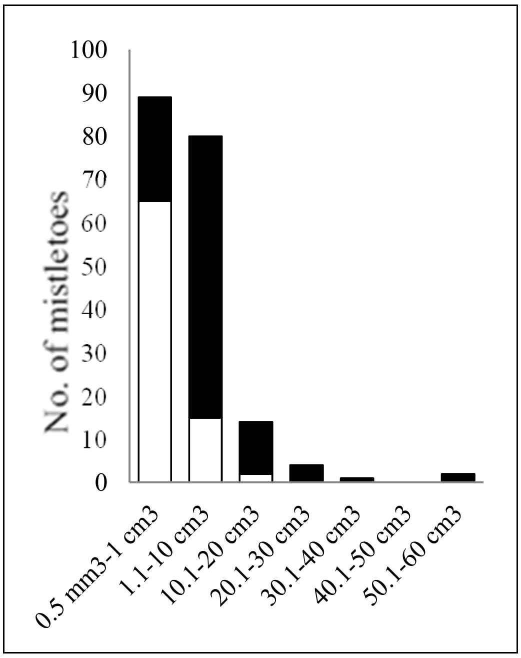

Fig. 1g. same as 1f. except during 2009

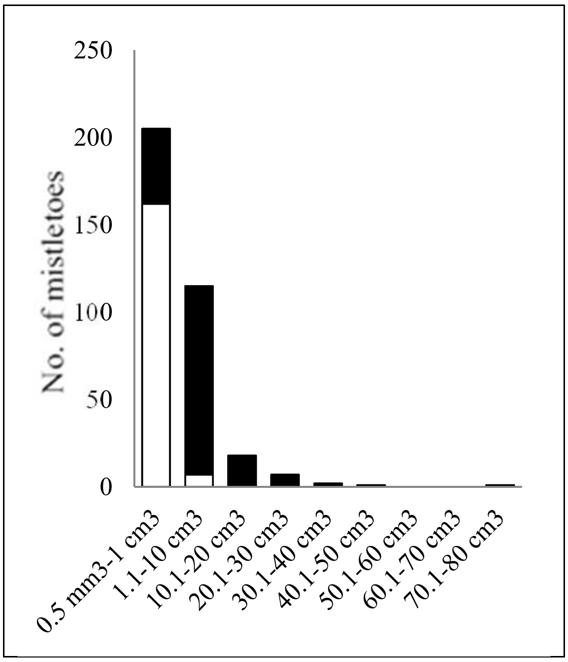

Fig. 1h. same as 1f. except during 2010

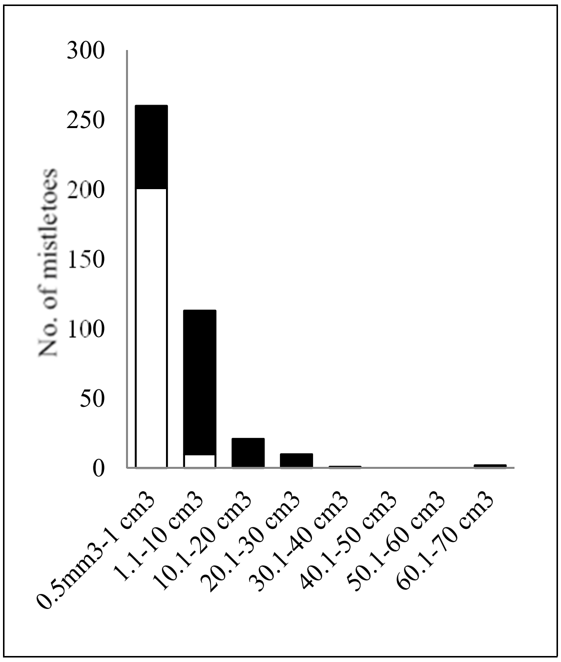

Fig. 1i. same as 1f. except during 2011

Korthalsella lindsayi

Coles Bush site

Compared to the *K. clavata* population, higher death rates (average adult mortality of 60.1% compared to 30.1% in *K. clavata*), low rates of progression of small plants to large and more frequent regression to smaller classes were observed in this *K. lindsayi* population.

During the first census year (2008) a total of 37 seedlings/sprouts were recorded on the hosts included in the study at the Coles Bush site, of which 43.2% progressed to small mistletoes (size to 1 cm^3), 2.7% progressed to large mistletoes (size > $1cm^3$), 8.1% stayed in stasis and seedling/sprout mortality was 45.9% by the end of first census year. An average relative growth rate of 2 cm^3 (std error=0.5) was recorded for seedling/sprouts. Of the 53 small mistletoes 17% progressed to large mistletoes, 30.2% stayed in stasis, 9.4% regressed and small mistletoe mortality was 43.4%. Average small mistletoe relative growth rate was 0.8 cm^3 (std error=0.4). Of the 22 large mistletoes, 22.7% stayed in stasis, 9.1% regressed to small mistletoe size and a further 2.7% regressed to seedling/sprout size. Large mistletoe mortality was 68.2% (Fig. 2a) and the average large mistletoe relative growth rate was -0.1 cm^3 (std error=1).

Of the 37 seedlings/sprouts recorded during the second census, 45.9% progressed to small mistletoes, a further 18.9% grew into large mistletoes, 2.7% stayed in stasis and seedling/sprout mortality was 32.4% by the end of the second census year. An average seedling/sprout relative growth rate of 3.02 cm^3 (std error=0.93) was recorded during this year. Of the 46 small mistletoes 23.9% stayed in stasis, 2.2% progressed to large mistletoes, 13% regressed to seedling/sprout size and small mistletoe mortality was 60.9%. An average small mistletoe relative growth rate of -1.28 cm^3 (std error=0.72) was recorded during this year. Of the 32 large mistletoes recorded, 34.4% stayed in stasis, 6.3% regressed to small mistletoe size and large mistletoe mortality was 59.4% (Fig. 2b). An average large mistletoe relative growth rate of -1.81 cm^3 (std error=0.55) was recorded during the second year of the study.

During the third census year, of the 49 seedlings/sprouts recorded 49% progressed to small mistletoes, a further 20.4% grew into large mistletoes and 4.1% stayed in stasis as seedlings and sprouts. Seedling/sprout mortality was 26.5%. Average seedling/sprout relative growth rate was 1.75 cm^3 (std error=0.6). Of the 30 small mistletoes 26.7% stayed in stasis, 10% progressed to large mistletoes and small mistletoe mortality was 56.7%. Average small mistletoe relative growth rate was 0.67 cm^3 (std error=0.76). Of the 19 large mistletoes, 31.6% stayed in stasis, 15.8% regressed to small mistletoes and large mistletoe mortality was 52.6% (Fig. 2c). An average large mistletoe relative growth rate of -1.15 cm^3 (std error=0.96) was recorded during this year. This study suggests a declining *K. lindsayi* population at the Coles Bush site (Fig. 2d). Most of the breeding mistletoes were within the size range of 0.03-5 cm^3 (largest dimension c. 0.8-3 cm) and also in size range of 41-42 cm^3 (largest dimension 5-6.5 cm) at Coles Bush site (Fig. 3).

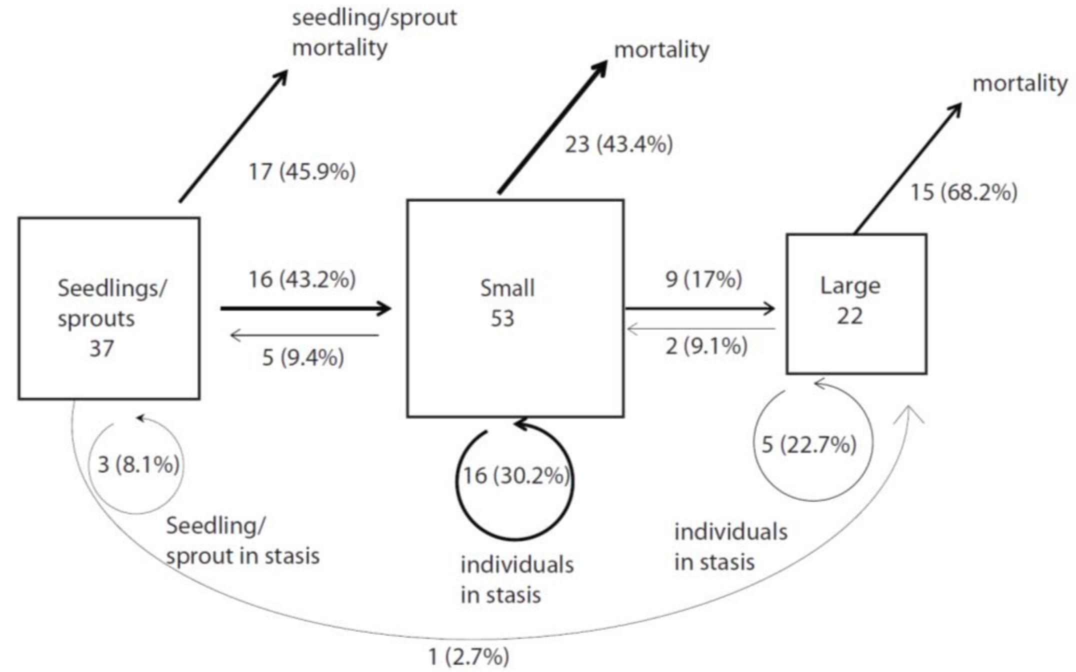

Fig. 2a. Demographics of *K. lindsayi* during 2008-2009 at the Coles Bush site

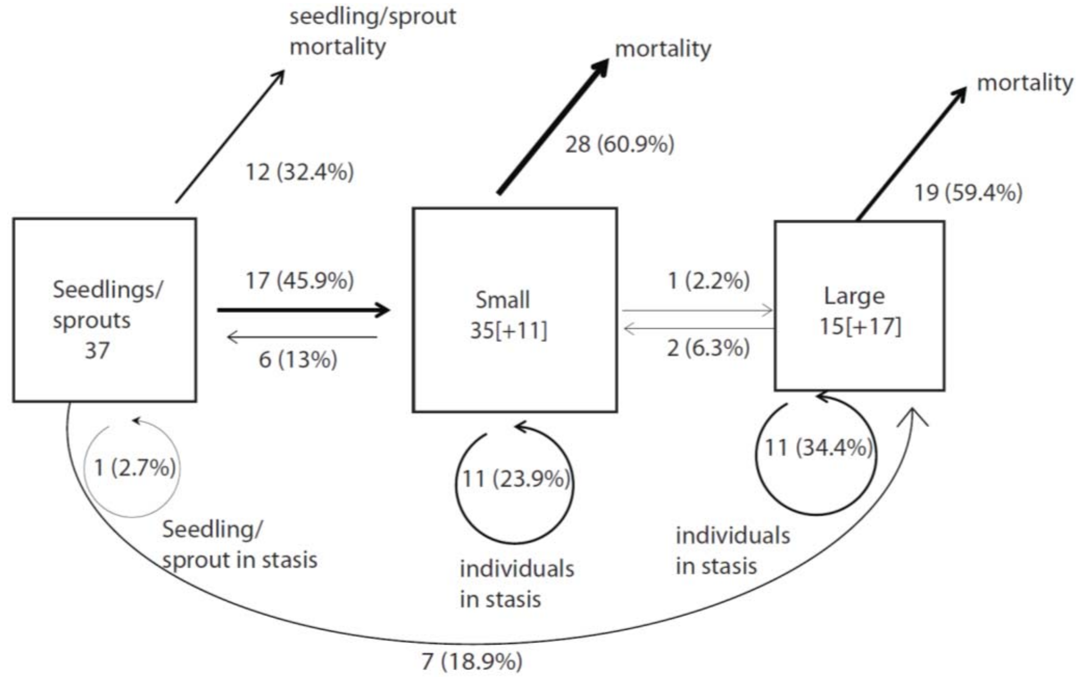

Fig. 2b. Demographics of *K. lindsayi* during 2009-2010 at the Coles Bush site, numbers in square brackets represent plants newly recruited into study

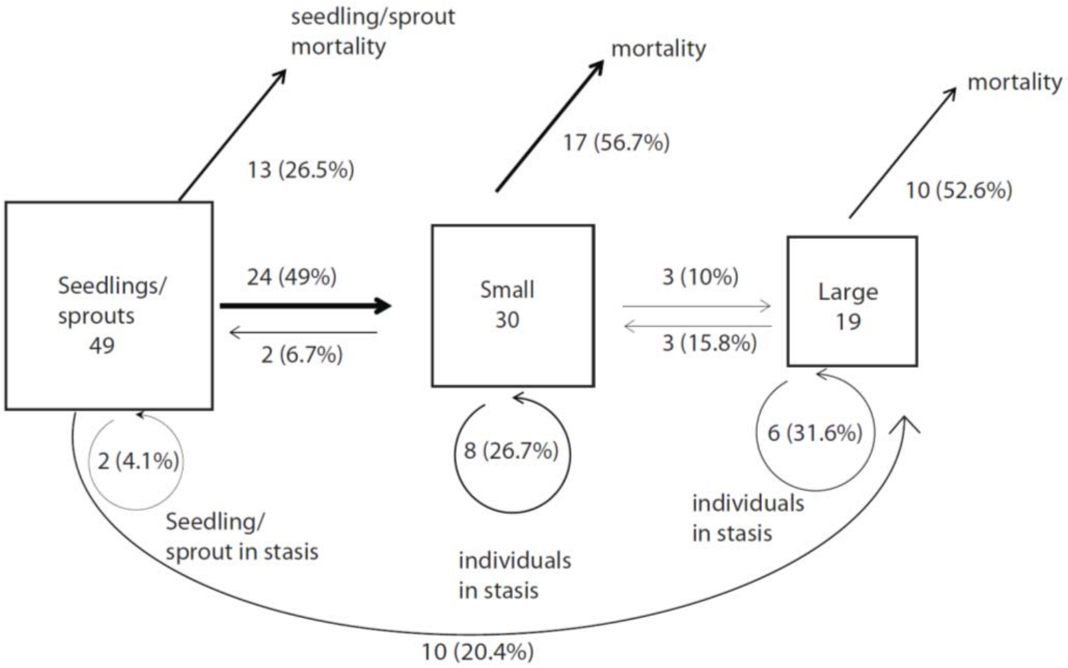

Fig. 2c. Demographics of *K. lindsayi* during 2009-2010 at the Coles Bush site

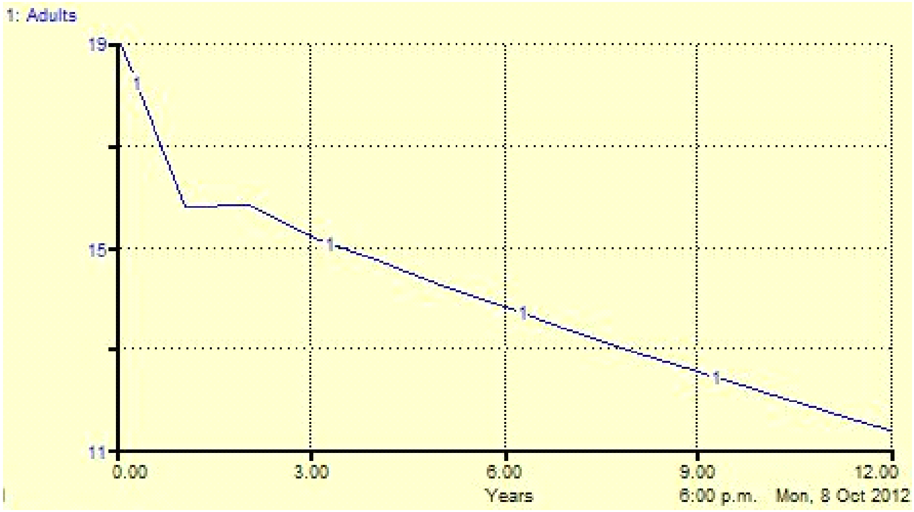

Fig. 2d. Projected population growth curve for *K. lindsayi* at the Coles Bush site

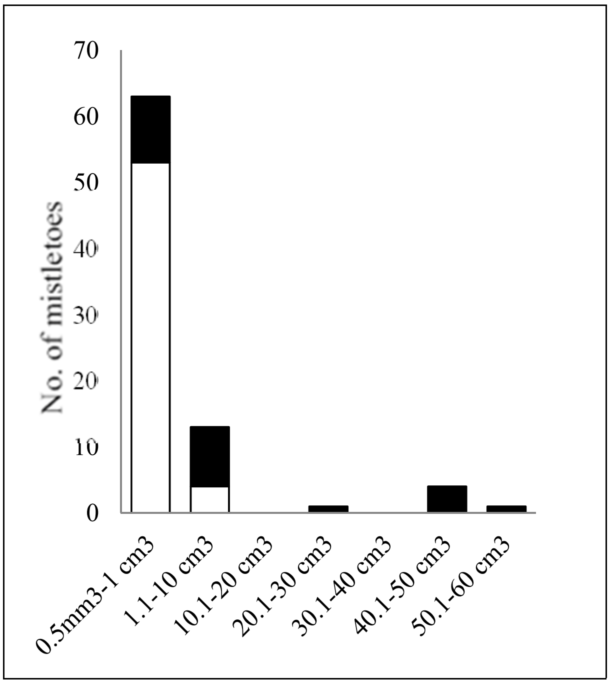

Fig. 3. Size class distribution of breeding and non-breeding mistletoes in *K. lindsayi* at Coles Bush site during 2011, □: number of non-breeding mistletoes, ■: number of breeding mistletoes

Paengaroa site

Compared to the Coles Bush site there was low seedling/sprout recruitment at this site leading to population decline rather than high rates of mortality noted in Coles Bush site. During the first census in 2008 at this site, out of 15 *K. lindsayi* seedlings/sprouts recorded on the hosts included in the study, 46.7% progressed to small mistletoes and 6.7% stayed in stasis, while seedlings/sprout mortality was 46.7% by the end of first census year. Average seedling/sprout relative growth rate was 0.85 cm^3 (std error=0.37). Out of 40 small mistletoes recorded 30% progressed to large mistletoes, 45% stayed in stasis and small mistletoe mortality was 17.5%. Average small mistletoe relative growth rate was 1.12 cm^3 (std error=0.34). Out of 48 large mistletoes recorded, most (62.5%) stayed in stasis, 30% regressed to small mistletoes and large mistletoe mortality was 35.4% (Fig. 4a). The average large mistletoe growth rate was 0.13 cm^3 (std error=0.12).

A total of 30 seedlings/sprouts were recorded during the second census (2009), of which 43.5% progressed to small mistletoes, a further 16.7% grew into large mistletoes, 36.7% stayed in stasis and a seedling/sprout mortality of 3.3% was recorded by the end of the second census year. Average seedling/sprout relative growth rate was 0.81 cm^3 (std error=0.43). Out of 31 small mistletoes recorded, about one third (32.3%) progressed to large mistletoes, 35.5% stayed in stasis, 3.2% regressed to seedling/sprout size and small mistletoe mortality was 29%. Average small mistletoe relative growth rate was 1.39 cm^3 (std error=0.32). Out of 56 large mistletoes recorded, the majority (66.1%) stayed in stasis, 8.9% regressed to small mistletoes and large mistletoe mortality was 25% (Fig. 4b). The average large mistletoe relative growth rate was -0.13 cm^3 (std error=0.20).

A total of 22 seedlings/sprouts were recorded during the third census (2010), of which 54.5% progressed to small mistletoes, 4.5% grew into large mistletoes, 9.1% stayed in stasis and a seedling/sprout mortality of 31.8% was recorded by the end of third census year. Average seedling/sprout relative growth rate was 0.42 cm^3 (std error=0.39). Out of 29 small mistletoes recorded, 31% progressed to large mistletoes, 31% stayed in stasis, 17.2% regressed to seedling/sprout size and small mistletoe mortality was 20.7%. The average small mistletoe relative growth rate was 0.57 cm^3 (std error=0.51). Out of 52 large mistletoes recorded, 26.9% stayed in stasis, 27.6% regressed to small mistletoes and large mistletoe mortality was 57.7% (Fig. 4c). Average large mistletoe relative growth rate was -0.47 cm^3 (std error=0.18). This study suggests a declining *K. lindsayi* population at the Paengaroa site (Fig. 4d).

At Paengaroa site most of the breeding mistletoes were within the size range of 0.1-19 cm^3 (largest dimension c. 2-4.5 cm) and some in the size range of 42-57 cm^3 (largest dimension > 6 cm) (Fig. 5).

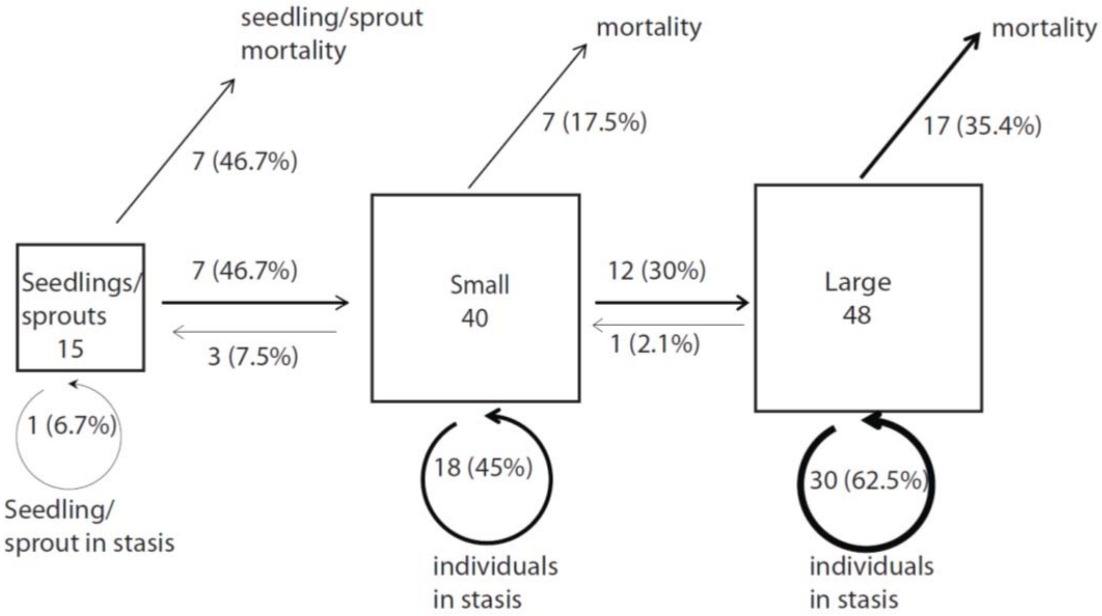

Fig. 4a. Demographics of *K. lindsayi* during 2008-2009 at the Paengaroa site

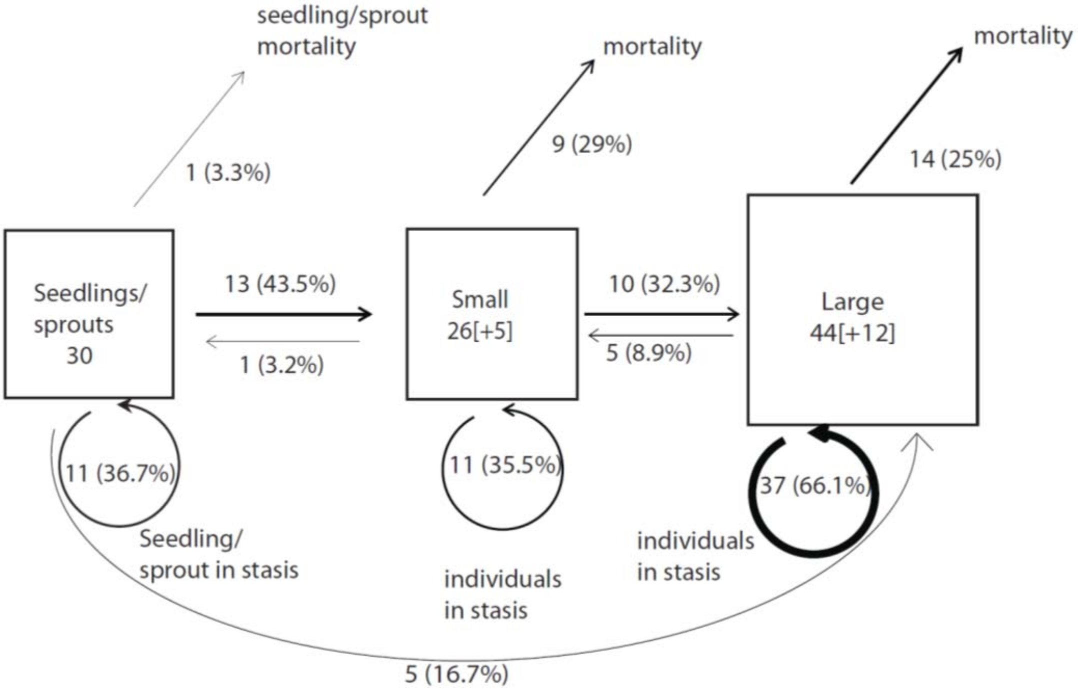

Fig. 4b. Demographics of *K. lindsayi* during 2009-2010 at the Paengaroa site, numbers in square brackets represent plants newly recruited into study

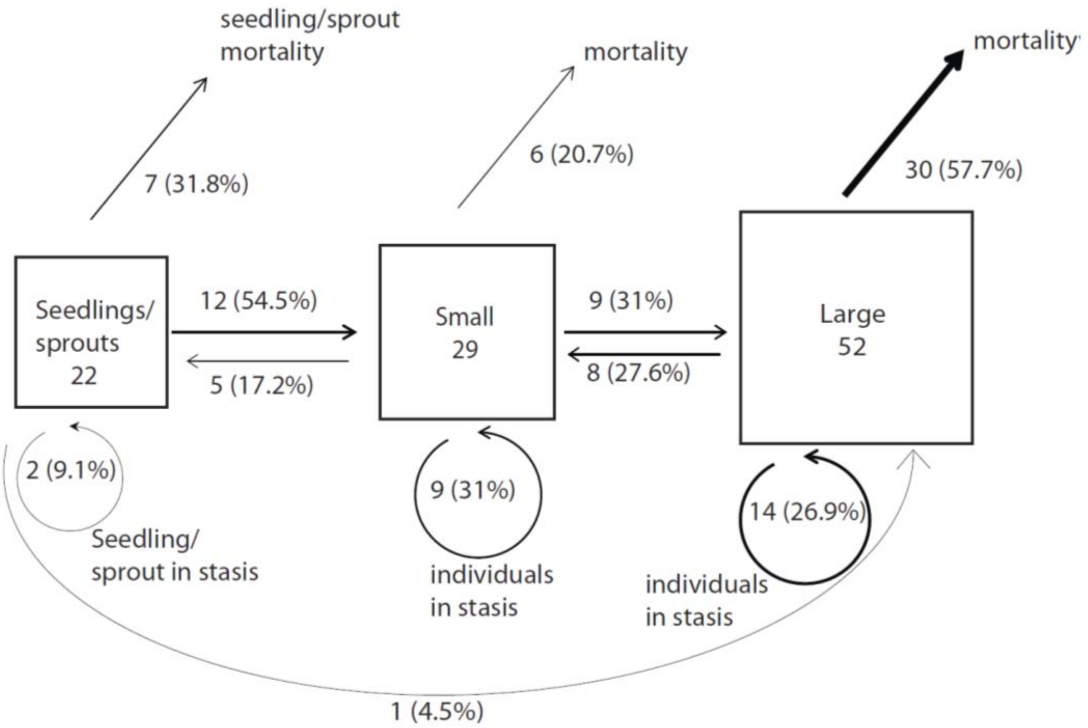

Fig. 4c. Demographics of *K. lindsayi* during 2010-2011 at the Paengaroa site

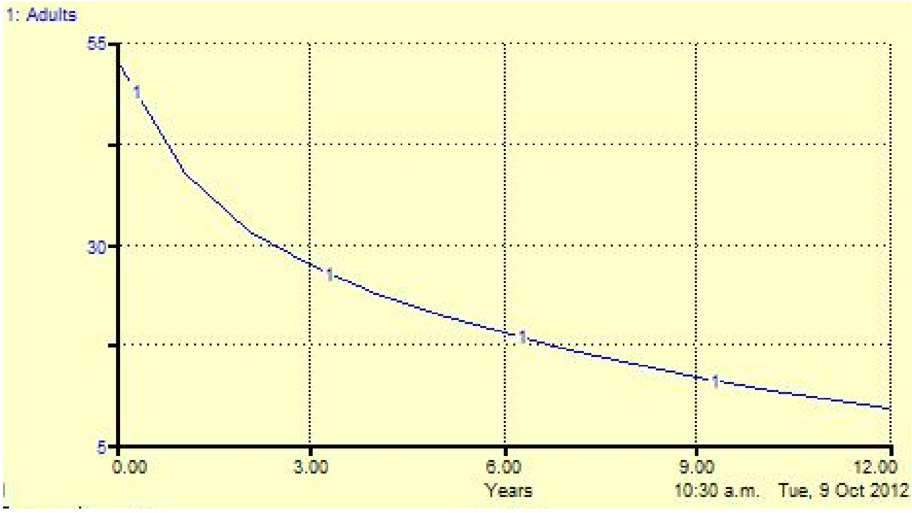

Fig. 4d. Projected population growth curve for *K. lindsayi* at the Paengaroa site

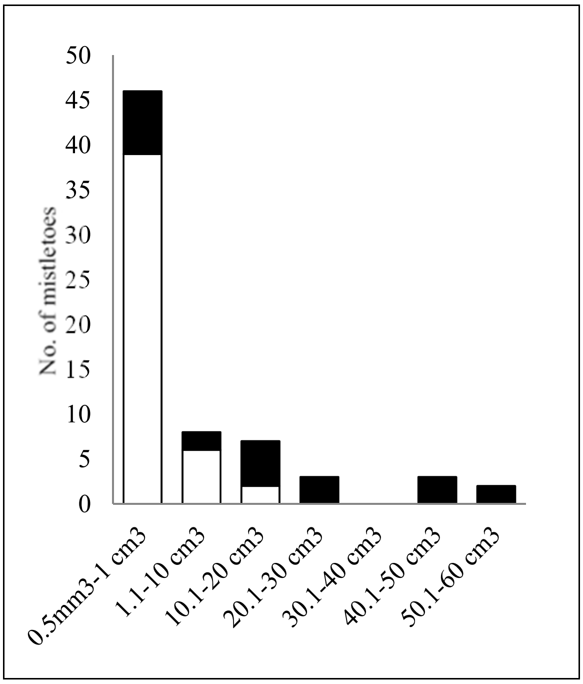

Fig. 5. Size class distribution of breeding and non-breeding mistletoes in *K. lindsayi* at Paengaroa site during 2011, □: number of non-breeding mistletoes, ■: number of breeding mistletoes

Korthalsella salicornioides

The *Korthalsella salicornioides* population at Motuoapa had relatively high recruitment rates, rapid seedling/sprout growth rates compared to the other sites and other species, rapid turnover and short-life span of plants. At this site, during the first census (2008) a total of 75 *K. salicornioides* seedlings/sprouts were recorded on the hosts included in the study. Of these 25.3% progressed to small mistletoes, 32% grew into large mistletoes, 16% stayed in stasis and seedling/sprout mortality was 26.7% by the end of first census year. Average seedling/sprout relative growth rate was 5.07 cm^3 (std error=0.57). Of the 27 small mistletoes recorded, 11.1% progressed to large mistletoes, 29.6% stayed in stasis, 6% regressed to seedling/sprout size and small mistletoe mortality was 48.1%. The average small mistletoe relative growth rate was -0.01 cm^3 (std error=0.7). Of the 39 large mistletoes recorded, 35.9% stayed in stasis, 12.8% regressed to small mistletoes and large mistletoe mortality was 51.3% (Fig. 6a). Average large mistletoe relative growth rate was -0.23 cm^3 (std error=0.22).

During the second census (2009) a total of 62 seedlings/sprouts were recorded. Of these 24.2% progressed to small mistletoes, 25.8% grew into large mistletoes and seedling/sprout mortality was 32.3% by the end of second census year. Average seedling/sprout relative growth rate was 6.06 cm^3 (std error=0.37). Of the 33 small mistletoes recorded, 18.2% progressed to large mistletoes, 12.1% stayed in stasis, 3% regressed to seedling/sprout size and small mistletoe mortality was 66.7%. Average small mistletoe relative growth rate was 0.61 cm^3 (std error=0.72). Of the 52 large mistletoes recorded, 34.6% stayed in stasis, 9.6% regressed to small mistletoes and large mistletoe mortality was 55.8% (Fig. 6b). The average large mistletoe relative growth rate was -0.14 cm^3 (std error=0.28).

During the third census (2010) a total of 79 seedlings/sprouts were recorded. Of these 69.6% progressed to small mistletoes, 6.3% grew into large mistletoes and seedling/sprout mortality was 21.5% by the end of third census year. Average seedling/sprout relative growth rate was 5.52 cm^3 (std error=0. 5). Of the 25 small mistletoes recorded, 24% progressed to large mistletoes, 24% stayed in stasis, 16% regressed to seedling/sprout size and small mistletoe mortality was 36%. The average small mistletoe relative growth rate was 0.28 cm^3 (std error=0.51). Of the 40 large mistletoes recorded, 55% stayed in stasis, 17.5% regressed to small mistletoes and large mistletoe mortality was 27.5% (Fig. 6c). Average large mistletoe relative growth rate was -0.23 cm^3 (std error=0.2). This three year study suggests a slight positive population growth trend at the Motuoapa site (Fig. 6d).

At the Motuoapa site, most of the breeding mistletoes were within the size range of 0.01-20 cm^3 (largest dimension c. 1-5 cm) (Fig. 7).

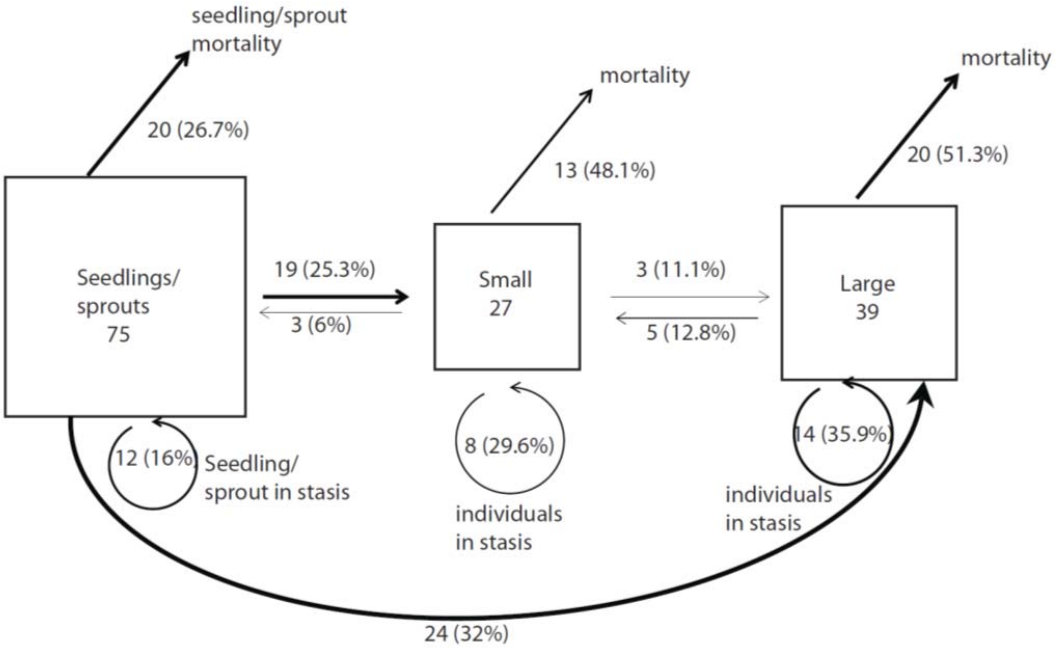

Fig. 6a. Demographics of *K. salicornioides* during 2008-2009 at the Motuoapa site

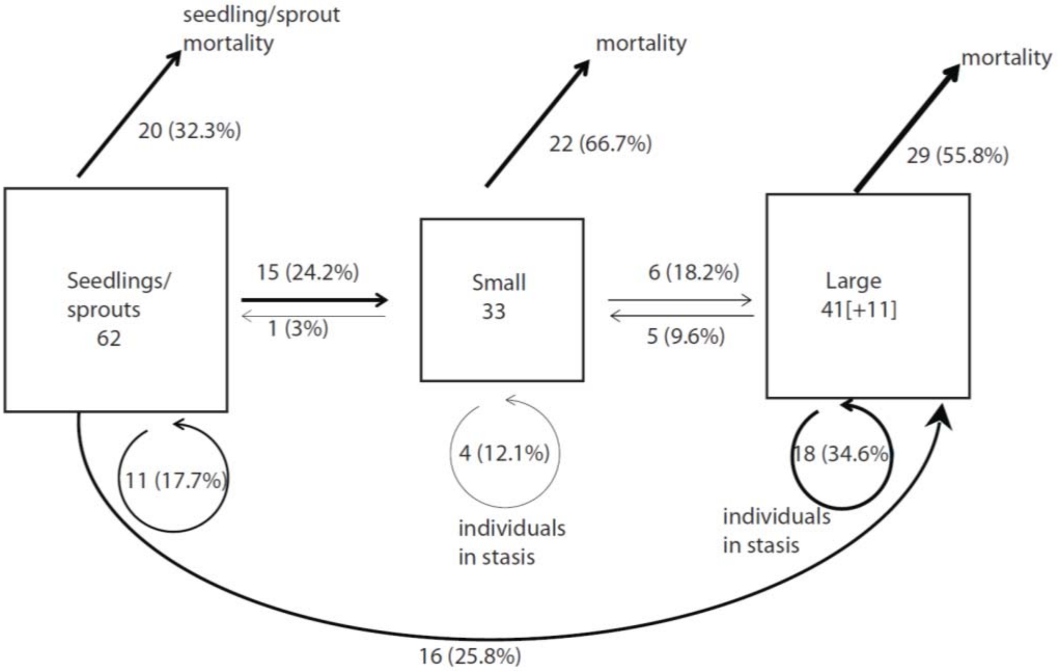

Fig. 6b. Demographics of *K. salicornioides* during 2009-2010 at the Motuoapa site, numbers in square brackets represent plants newly recruited into study

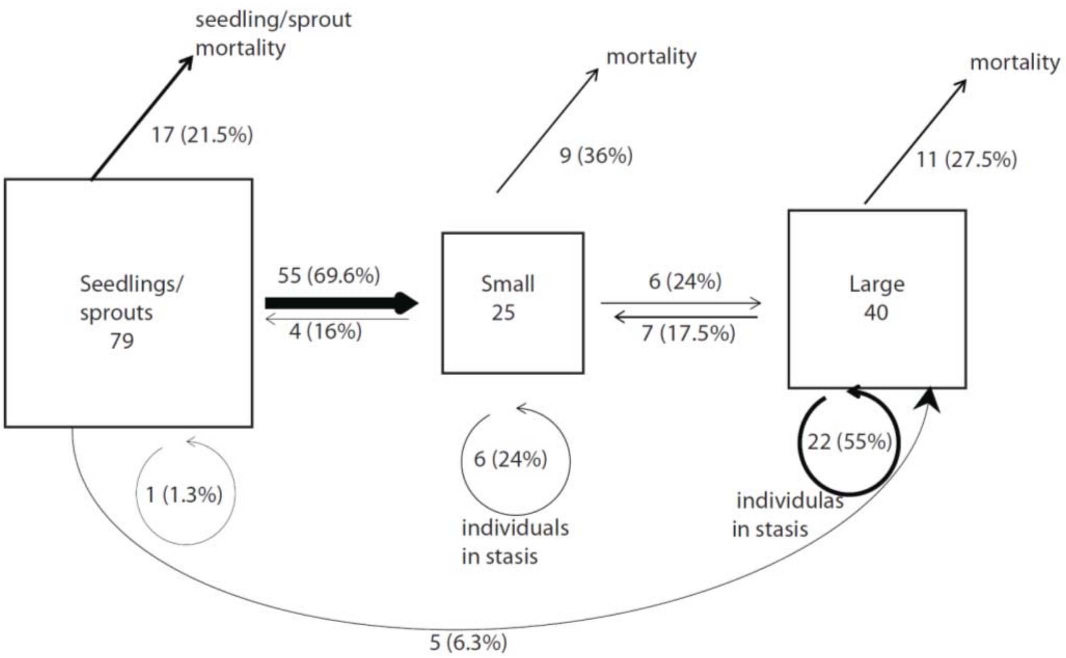

Fig. 6c. Demographics of *K. salicornioides* during 2010-2011 at the Motuoapa site

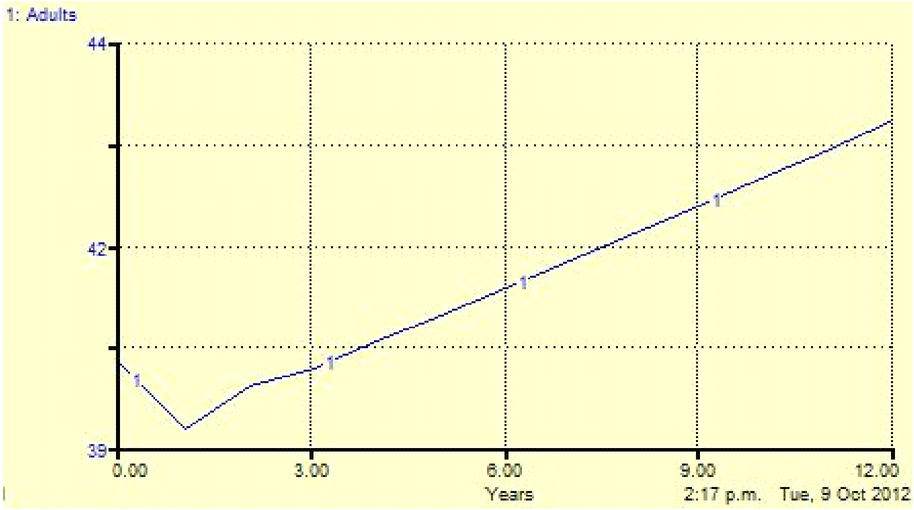

Fig. 6d. Projected population growth curve for *K. salicornioides* at the Motuoapa site

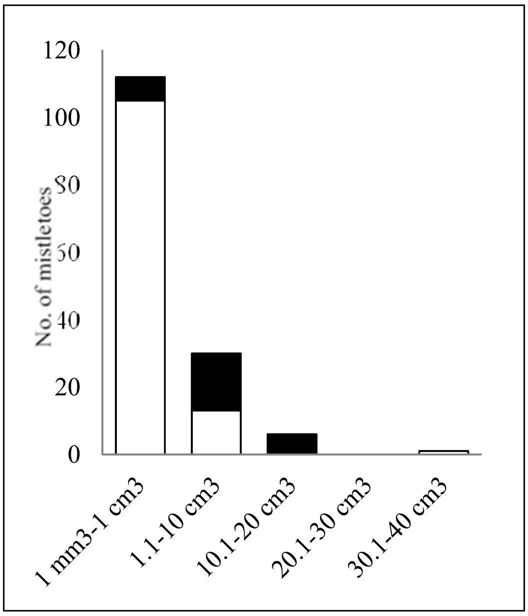

Fig. 7. Size class distribution of breeding and non-breeding mistletoes in *K. salicornioides* at Motuoapa site during 2011, □: number of non-breeding mistletoes, ■: number of breeding mistletoes

At the Waikanae site, the fate of individual mistletoes could not be followed as the rapid host growth did not permit tracking of individual mistletoes since the host branches elongated so rapidly in successive years that it was very hard to consistently locate individual mistletoes. However, the relative numbers of seedlings/sprouts, small and large mistletoes over three years (Table 2) suggest positive population growth at this site. Most of the breeding mistletoes were within the size range of 0.3-40 cm^3 (largest dimension 1.2-5 cm) at the Waikanae site (Fig. 8).

Table 2. Relative numbers of seedlings/sprouts, small and large mistletoes for *K. salicornioides* at the Waikanae site during different years of the demographic study

	Seedlings/sprouts	Small	Large
2008	28	19	61
2009	41	49	91
2010	47	46	90
2011	44	50	103

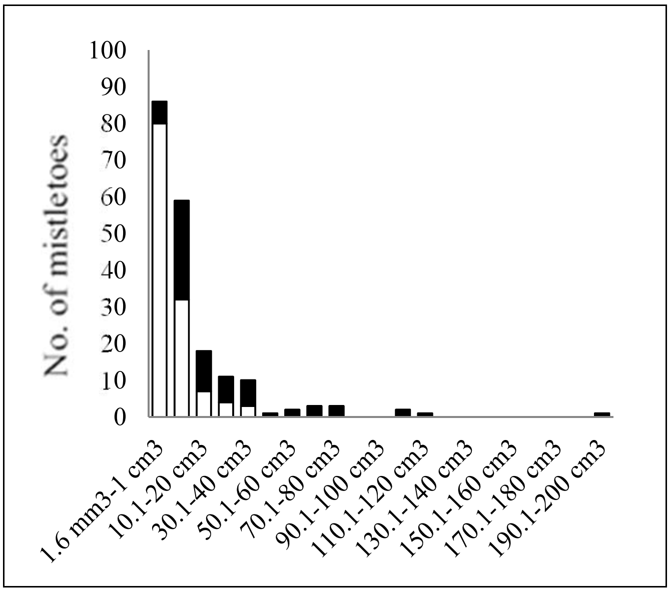

Fig. 8. Size class distribution of breeding and non-breeding mistletoes in *K. salicornioides* at Waikanae site during 2011, □: number of non-breeding mistletoes, ■: number of breeding mistletoes

Seedling-Sprout Ratios

At Castle Hill and Waikanae, relatively high numbers of sprouts were recorded (Table 3). At the Castle Hill site, high levels of sprouting were observed on gall mite *Aceria* infected hosts. This is possibly a consequence of suppression of growth of infected mistletoes as apical and lateral buds modified into galls cease to grow, and thus sprouting is promoted. Whereas at the Waikanae site, rapid host growth caused rapid host branch elongation, which apparently resulted in fragmentation of cortical strands within elongating branches and thus resulted in high levels of mistletoe sprouting.

Table 3. Seedling sprout ratios at different study sites in the 2011 census

	Site	Seedlings	Sprouts	Ambiguous
K. clavata	Castle Hill	42	45	60
K. lindsayi	Coles Bush	1	1	26
K. lindsayi	Paengaroa	1	-	15
K. salicornioides	Motuoapa	49	7	26
K. salicornioides	Waikanae	13	11	20

Relative Growth Rates

High relative seedling/sprout growth rates were observed at Castle Hill, Motuoapa and Coles Bush sites compared to Paengaroa site, where seedling/sprout showed little progression (Fig. 9). Adult/large mistletoes generally show a regression in size, which apparently is a result of loss of reproductive branches after fruiting is over. Overall, the mistletoes exhibited very slow growth at the Paengaroa site and at Coles Bush site many plants became smaller, especially in 2009-2010 where on average only the young plants showed positive growth.

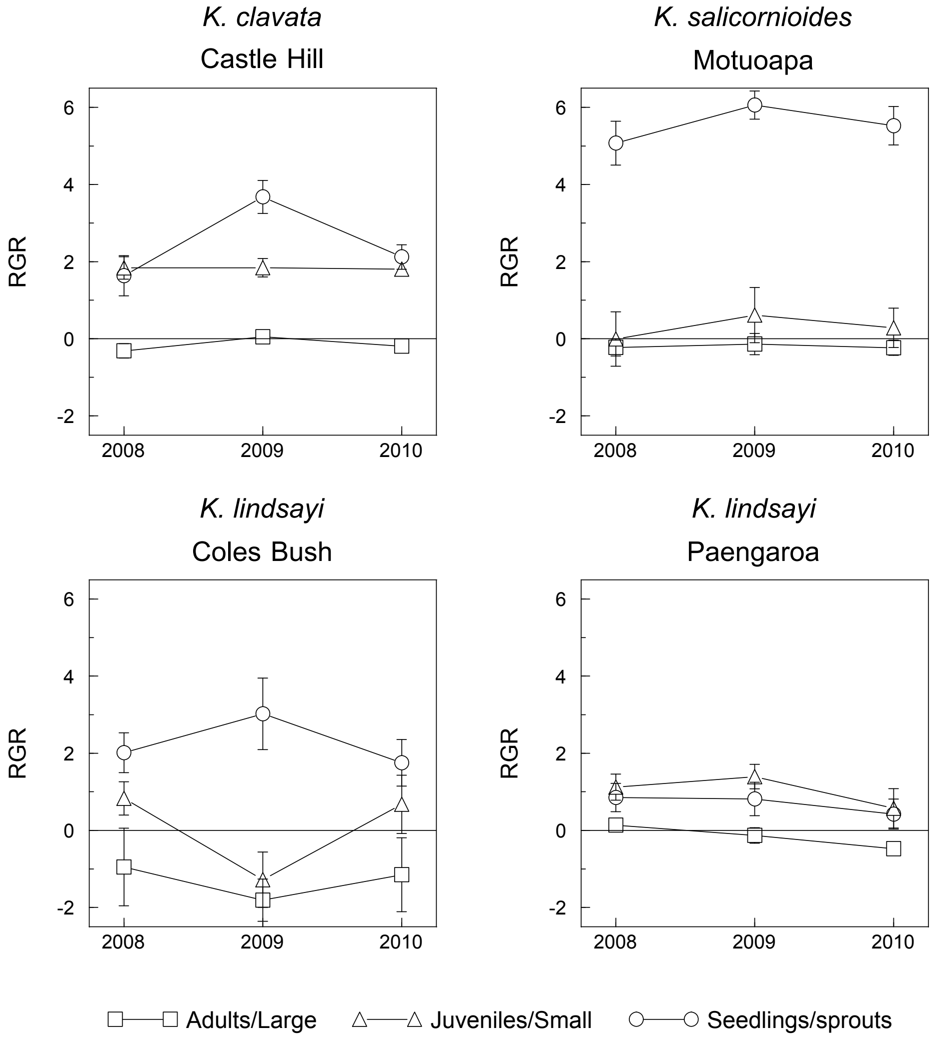

Fig. 9. Relative growth rates at different sites for each size class or stage. The errors bars are standard errors.

Occurrence of Invertebrates and Fungal Pathogens at the Study Sites

The gall mite *Aceria korelli* and the ascomycete fungus *Rosenscheldiella korthalsellae* were associated with *K. clavata* at the Castle Hill site. However, *R. korthalsellae* was sparse at this site. *Aceria korelli* was abundant but patchy at this site being restricted to mistletoes on certain hosts. Its occurrence did not appear to impact the reproductive output of *K. clavata* at the study site judging by the similar size versus fecundity plot of infected and uninfected plants (Fig. 10). Instead, if anything, it increased the overall reproductive output perhaps by modifying the apical buds of mistletoes into galls and thus by promoting sprouting (sprout: seedling ratios were 39:12 and 6:30 in hosts with *Aceria* infected and non-infected mistletoes respectively). *Aceria korelli* and *Guignardia korthalsellae* were associated with *K. lindsayi* at the Coles Bush site, whereas at Paengaroa site *Rosenscheldiella korthalsellae* was also prevalent in addition to *A. korelli* and *G. korthalsellae*. At Waikanae, *A. korelli* was associated with *K. salicornioides* and at Motuoapa site *Eriococcus korthalsellae* and quite rarely *R. korthalsellae* was associated with *K. salicornioides.* Larvae of native tortricids and a native nocutid (Chapter 6) were also recorded at Coles Bush on *K. lindsayi* and different adventive scale insects were also recorded at all study sites (Chapter 6), however, infestation levels were not very high to cause any serious threats to mistletoe populations.

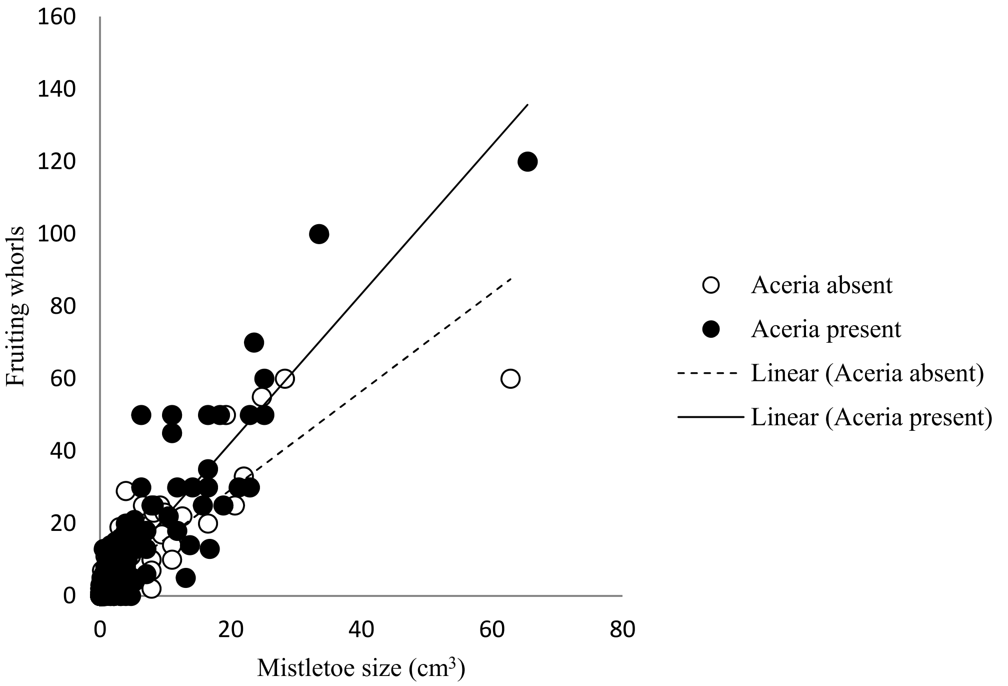

Fig. 10. A comparison of relative size and fruiting whorl numbers in *Aceria* infected and non-infected *K. clavata* plants at Castle Hill site during 2011

DISCUSSION

The turnover of ramets was surprisingly high in all three species and with annual mortality rates typically around 25-55% - it is unlikely many ramets would last more than about 5 years. This is in strong contrast with the age estimates of New Zealand's loranthaceous mistletoes. Genets may be much longer lived; however, as the true extent of clonality is not known beyond the shoot initiation stage, it is not possible to infer the longevity of genets.

Most of the mortality at all sites resulted from host branch or entire host death caused by natural branch or host senescence. New Zealand's pygmy mistletoes are benign parasites and usually do not kill the host branch or host. Similarly, the natural enemies of New Zealand's pygmy mistletoes also do not appear to cause frequent mistletoe mortality. However, monitoring of infestation levels of adventive scale insects might be important as their outbreaks may have deleterious impacts on the survival of the mistletoes. In a study on the population ecology of *Phoradendron anceps* and its host *Pisonia albida,* Pinto (2005) also found that *P. anceps* was a benign parasite.

Korthalsella salicornioides on rapidly growing younger *Leptospermum scoparium* s.l. hosts had more sprouts and relatively higher recruitment rates were recorded compared to older hosts, which may be attributable to rapid host branch elongation and splitting of the endophyte within the host, thus promoting sprouting. Relatively low seedling recruitment was recorded on older *Leptospermum scoparium* s.l. hosts compared to younger hosts. This may possibly be attributable to the high incidence of *Leptospermum scoparium* s.l. blight (manuka blight) caused by felt scales (*Eriococcus* spp.) and resultant deposition of sooty moulds (*Cladosporium* spp.) on the older host stems. The sparcity (see de Lange *et al.* 2009) of *K. salicornioides* is attributable to elimination of *Leptospermum-Kunzea* scrubs by succession, vegetation clearance and mortality coupled with lack of regeneration (Department of Conservation 2001). Vegetation clearance and habitat destruction by fire means that elimination of mistletoe populations also eliminates the possibility of new mistletoe recruitments as all seeds will be present on the host branches. Given the fact that bird mediated dispersal is by chance only (birds are not known to feed on fruits, and perhaps can only disperse seeds by adherence to plumage, see Chapter 5), thus the regenerated host patches have very little likelihood of reinfection.

In *K. lindsayi,* mortality caused by host/branch death were more common on *Coprosma* and *Muehlenbeckia complexa* hosts, compared to *Melicope simplex* in which lower branch mortality was seen. Host branch death related mortalities were also seen in *Lophomyrtus* hosts. Although mistletoe growth is relatively slow on *Melicope simplex,* this host species provides a more reliable habitat in terms of host longevity. Mistletoes growing on *Myrsine australis* exhibited the most luxuriant growth compared to all other hosts studied. However, the projected population growth rates indicate that both the populations of *K. lindsayi* are declining, which is attributable to low host recruitment at these sites and high rates of mortality at Coles Bush. This is possibly because of the fact that most of the host species of *K. lindsayi* occur in seral vegetation which are prone to elimination by over-topping from larger trees. Thus succession related elimination coupled with little host regeneration in fragmented populations means that declining populations may need to be augmented with host reintroductions and reinfection (by methods outlined in Chapter 5) if the population is to persist at both sites. In *K. clavata,* again more mistletoe mortalities occurred on *Coprosma propinqua* hosts, which comprised the principal host species at the study site compared to *Myrsine divaricata.*

Despite high adult /large mortalities, (averages ranging from 30.1% in *K. clavata* at Castle Hill site, 39.4% in *K. lindsayi* at Paengaroa site, 44.9% in *K. salicornioides* at Motuoapa site to 60.1 % in *K. lindsayi* at Coles Bush site) relatively stable adult/large numbers are indicative of high turnover on live host branches. In a similar study, Pinto (2005) also found high turnover in *Phoradendron anceps.* That study also showed that bird mediated dispersal of seeds was localised in this species. This study indicated that small mistletoe populations might be susceptible to elimination.

The relatively rapid onset of the reproductive phase in some of the mistletoes classified as seedlings/sprouts is most likely because of sprouts exhibiting relatively rapid growth and thus early transition from vegetative to reproductive status. Sprouts may have a comparative advantage of availability of metabolites from established parent plants compared to seedlings, which rely on a long phase of establishment of haustorial contact with the host for active growth.

Clonally reproducing plants were found to have more abundant adult populations compared to plants lacking vegetative reproduction, which had more abundant seedling populations (Forbis 2003). In this study, the aggregate numbers of seedling/sprouts and juveniles/small mistletoes were always higher compared to adults/large mistletoes for all species at all sites. However, the exact ratio of seedlings and sprouts could not be determined reliably as there were young mistletoes that could not be clearly identified as seedlings or sprouts.

Effective pollination, high fecundity levels and vegetative reproduction by sprouting are the key factors in the perpetuation of each of these species; however, these mistletoes are constrained by relatively low dispersal potential to more distant host populations as explosive seed dispersal is effective for shorter distances carrying seeds to adjacent hosts only.

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Chapter 8: Synthesis

New Zealand’s pygmy mistletoes exhibit general flexibility as far as host range is concerned. They are not completely host specific either at the species level or subspecific level. This presumably helps in maximising the chances of dispersing to a suitable host. In mixed communities viscaceous mistletoes tend to have relatively low host specificity (Barlow 1997), as is exhibited by *K. clavata* and *K. lindsayi* in the current study, both species having relatively broad host ranges. Whereas in homogeneous communities viscaeous mistletoes tend to be more host specific (Barlow 1997), and *K. salicorniodes* represents an example of the latter. Even though it is predominantly confined to members of the *Kunzea ericoides* complex and to *Leptospermum scoparium* s.l., its occurrence on non-myrtaceous genera suggests that an occasional or rare host switch may occur through long distance dispersalist birds by adherence to plumage. But these examples are exceedingly rare in this case. Somewhat similar host use patterns were also discernible in New Zealand’s loranthaceous mistletoes. *Ileostylus micranthus* and *Tupeia antarctica* both have relatively broad host ranges whereas the beech (*Nothofagus*) specialising mistletoes *Peraxilla colensoi, P. tetrapetala* and *Alepis flavida* have high host specificity (Norton and de Lange 1999). So, specialists appear to have a comparative advantage in terms of having more reliable host availability both temporally and spatially (Norton and de Lange 1999). Flexibility in host use is advantageous in communities that are prone to disturbance, and also when hosts are predominantly seral species, as such communities tend to be more diverse. The divaricate shrubs parasitised by New Zealand’s pygmy mistletoes mostly occur in seral vegetation. This flexibility is also advantageous in situations where a varied set of host species is available to mistletoe populations in different regions or when local populations of a given host may be genetically different and have resistance to infection from mistletoes. For example, *Phoradendron anceps* predominatly occurs on *Pisonia albida* (Nyctaginaceae) in Guánica forest in southwestern Puerto Rico whereas *Bursera simaruba* (Burseraceae), which was also sympatric at this site, was not parasitised though it was a reported host in other locations (Pinto 2005).

The three species have little niche overlap and so are not in direct competition. *Kunzea* and *Leptospermum* the main host genera for *K. salicornioides* are not parasitised by the other two species. At locations where *K. clavata* and *K. lindsayi* occur sympatrically they tend to parasitise different sets of hosts. For example at the Paengaroa site where both species are found within a short distance from each other, *K. lindsayi* occurs on *Melicope* *simplex*, *Myrsine divaricata*, *Coprosma virescens* and *C. rotundifolia* whereas *K. clavata* occurs on *C. wallii* and *C. obconica*. Similarly at a site in Wairarapa, *K. clavata* occurs mainly on *Coprosma propinqua* whereas *K. lindsayi* was found growing on *Lophomyrtus bullata* and *Myrsine australis*. A remnant population of *K. clavata* at Motuariki Island (within Lake Tekapo) was parasitic on *Coprosma linariifolia*, whereas that of *K. lindsayi* was parasitic on *Myrsine divaricata* and *Sophora prostrata* x *microphylla*. Both of these species have host genera unique to each of these species. *Aristotelia fruticosa* is often used by *K. clavata* and is never known to be parasitised by *K. lindsayi.* Similarly *K. lindsayi* is frequently parasitic on *Lophomyrtus obcordata* which is never parasitised by *K. clavata*. Thus, these two species clearly exemplify taxonomic resource partitioning. In fact both species also exhibit varied ecologies in terms of habitat preferences. *Korthalsella clavata* tends to be a more open shrub land species whereas *K. lindsayi* can be found in more shaded locations and appears to be more shade tolerant than *K. clavata*. Niche partitioning at a different level was reported in New Zealand’s loranthaceous mistletoes, which tend to partition the canopy of the same host species. *Alepis flavida* was found to be more common on the periphery of the host trees whereas *Peraxilla colensoi* was more prevalent on inner branches and tree trunks (Norton *et al*. 1997).

Genetic data based on nrITS and cpDNA *trnQ-rps*16 shows that any variability in mistletoe populations is geographic and not host-associated. In a similar study in *Viscum album* Zuber and Widmer (2009) found that independent of host race, mistletoe haplotypes from Turkey were distinct and distant from those found in Europe, which was suggestive of the presence of highly differentiated populations, at the range limit of the species. Cross-infection experiments in *Korthalsella salicornioides* provide some insight into the presence of putative host races, as better success rates were evident when seed source and recipient host were the same. Perhaps more rapidly evolving markers, such as microsatellites, might help elucidate the host-associated divergence. In a study in the root parasite *Dactylanthus taylorii,* McLay *et al.* (2012) found ten polymorphic microsatellite sites which might prove to be useful in genetic studies at population level for this parasite.

My study has added molecular sequence data from several important *Korthalsella* taxa across its entire distribution and helped resolve the major clades within the genus. *Korthalsella dacrydii* and *K. geminata* are two important Malesian species that were missing from the previous phylogenetic study by Molvray *et al*. (1999). My study shows that *K. dacrydii,* which is the only species parasitic on gymnosperms in the genus, has affinities with taxa from Eastern Australia, Norfolk and from Lord Howe. *Korthalsella geminata* with specialised inflorescences is a sister species to *K. papuana* – a species which also has specialised inflorescences and has a distribution in Papua New Guinea and Queensland. Collections from the South Pacific, including representatives from Western Samoa, Tahiti and Moorea, constituted a South Pacific clade. *Korthalsella arthroclada* and *K. leucothrix* from western and southern Australia, respectively, had divergent positions compared to other collections from Australia/Lord Howe Island. *Korthalsella grayi* from Queensland was related to other collections from Eastern Australia and to collections from Norfolk/ Lord Howe compared to Asian collections of *K. japonica* and is thus not related to the latter species as was considered by Molvray (1997). Similarly, *K. rubra* collections were related to their bio-geographic counterparts and were not related to Asian populations of *K. japonica* as was considered by Molvray (1997). Likewise, *K. madagascarica* considered to be conspecific with *K. salicornioides* by Molvray (1997) is not related to *K. salicornioides*. This study thus shows that there is widespread regional endemism and parallelism in this genus. Thus, similar forms have evolved independently in different regions in a process that possibly corresponds to co-evolution with their hosts. *Korthalsella breviarticulata* from E. Australia, *K. latissima* from Hawaii and *K. taenioides* (=*K. commersonii*) from Madagascar represent an interesting example of parallelism within different lineages. Similarly, *K. madagascarica* and *K. salicornioides* are another striking example of parallelism. Likewise, *K. japonica* from Madagascar, despite having similar morphology to Asiatic collections of *K. japonica,* is in fact in a phylogenetically quite different position.

Regarding the un-sampled regions/taxa, my work suggests that *K. striata* (considered to be conspecific with *K. salicornioides*) will most likely group with other New Caledonian species. Likewise, *K. platycaula* from Cook Islands, Niue, Henderson Island, Australs etc. are also most likely to be parts of the South Pacific clade. There may be more species embedded in *Korthalsella platycaula* in these Pacific islands. Mainland African populations of *Korthalsella* and populations from the Comores might also represent independent lineages.

Although *K. clavata* and *K. lindsayi* are polyphyletic and introgressed, they have a myriad of morphological differences and have varied ecologies in terms of host preferences. For these reasons these ought to be considered distinct species. The non-monophyly of these two species might be the result of occasional gene flow between populations particularly when the two species occur in sympatry.

Thus the speciation pattern in this genus is one of colonisation of geographic areas by one or two clades only, followed by local differentiation and taxonomy has been misled by frequent instances of parallelism.

Studies on the reproductive biology of *Korthalsella salicornioides* show it is an ambophilous species (relying both on wind and insects for pollination). It is fully self-compatible but shows a low rate of autonomous selfing and so is dependent on pollen vectors. In the field site in the current study, there was little or no pollen limitation, suggesting that pollen supply is seldom limiting. Insects and wind appear to be both implicated in supplying this pollen. Insect visitors of *K. salicornioides* flowers comprise dipterous and hymenopterous insects. *Korthalsella lindsayi* appears to be a more anemophilous species with little autonomous selfing, high self-compatibility and little evidence of pollen limitation.

This study confirmed the presence of explosive seed discharge in both species. The median dispersal distance in *K. lindsayi* in the field ranged from 1 to 1.9 m and in *K. salicornioides* from 1.3 to 2.3 m, whereas maximum dispersal distances were at least about 4 and 7 m in *K. lindsayi* and *K. salicornioides* respectively depending on direction and height of host canopy.

This study represents the first account of the occurrence of vegetative reproduction by proliferation of the endophyte in the genus. Thus, New Zealand's pygmy mistletoes have a flexible reproductive ecology having a suite of ambophily/anemophily, self-compatibility and some selfing, and clonal growth. Dispersal is mostly abiotic allowing spread through a population, but the small sticky seeds probably allow occasional long distance dispersal on birds. Flexibility in pollination ecology is particularly advantageous when there are temporal and spatial variations affecting the relative abundance of pollinators (Culley *et al.* 2009).

Studies on the biodiversity associated with New Zealand *Korthalsella* has interestingly resulted in the discovery of three specialist scale insect species depending exclusively on New Zealand's pygmy mistletoes. These comprise one felt scale *Eriococcus* *korthalsellae* and two armoured scales viz., *Leucaspis albotecta* and *L. trilobata.* Additonally, two pathogenic fungi *Guignardia korthalsellae* and *Rosenscheldiella korthalsellae* depending exclusively on New Zealand's pygmy mistletoes were also discovered and described. The two *Leucaspis* species and particularly *K. trilobata* is quite rare. Other natural enemies recorded included a specialist gall mite *Aceria korelli* and several generalist exotic and native scale insects. Generalist Lepidoptera were also recorded on *K. lindsayi.* It appears that most of the specialist natural enemies are constrained by limited dispersal capacity and occurrence of pygmy mistletoes in fragmented and widely dispersed populations is an added reason for limited dispersal potential. The specialist natural enemies recorded on the pygmy mistletoes incite minimal damage to their hosts. Occurrence of exotic scale insects on pygmy mistletoes is a point of concern as potential outbreaks can be very deleterious to already declining populations given the fact that most of these are generalists and can affect both the hosts and the mistletoes. Moreover, these adventive species can eliminate the native specialists by competition.

The demographic work shows very high growth rates and turnover of mistletoe ramets. *Korthalsella clavata* and *K. salicornioides* had positive population growth at the sites included in the study whereas *K. lindsayi* populations at both the study sites are declining and, if typical for this species, suggest it may be vulnerable to population extinction. In a similar study Pinto (2005) found that *Phoradendron anceps* subpopulations exhibited different size structures; some were stable, others increased and still other declined thus produce an overall net dynamic metapopulation structure with a high turnover rate. The methods developed in the reproductive biology studies to grow these mistletoes on captive hosts could be applied to the translocation of mistletoe populations to areas where they have disappeared or are declining.

FUTURE WORK

Inclusion of missing taxa from my phylogenetic study, particularly from South Pacific, New Caledonia, Indian Ocean Basin and mainland Africa, will further help address the questions regarding regional endemism in this widespread genus.

More work is needed to elucidate the possible introgression between *K. clavata* and *K. lindsayi* and to determine the genetic basis of host specificity in *K. salicornioides.* As suggested, more rapidly evolving regions like microsatellites should be useful in addressing these questions.

In poorly collected areas within New Zealand, more targeted surveys will be useful to better understand the regional host use patterns.

The study of the presence of floral volatiles in nectar, particularly in the ambophilous *K. salicornioides,* will also constitute an interesting study. Similarly, the regional patterns of insect visitation on *K. salicornioides* flowers is another interesting area to be studied.

More targeted surveys are needed to determine the true range of native scale insects specialising on New Zealand *Korthalsella* and to determine the levels of incidence and threat posed by adventive scale insects.

More work is needed to determine the ratio of seedlings and sprouts. The demographic study suggests that population growth dynamics of different populations need to be studied and the fate of sprouts and seedlings demonstrated. For instance, if seedlings have a worse prognosis than sprouts, the colonisation process of new hosts may be more difficult than the rapid spread of mostly vegetative ramets within a host suggests. More population monitoring will help determine the typical population growth trends of each species and will help those who devise appropriate conservation management plans.

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