Population genetics and autecology of the endemic shrub epiphyte *Pittosporum cornifolium*

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Fiona Marie Clarkson

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Abstract

A comprehensive investigation of the population genetics and autecology of the endemic New Zealand shrub epiphyte *Pittosporum cornifolium* (Pittosporaceae) is presented. *Pittosporum cornifolium* has a wide geographic range and is well adapted to a variety of lifestyles namely terrestrial, rupestral, and more commonly, epiphytic. The primary habitats of *P. cornifolium* are lowland and coastal ecosystems which, in recent times (<200 years) have been subjected to widespread clearance and fragmentation resulting in major reductions to the species potential population range.

The study focused on five populations in the North Island from Coromandel to Taranaki where habitat loss of lowland and coastal ecosystems has been significant. Population-level genetic analysis using Inter-Simple Sequence Repeat (ISSR) markers revealed that while *P. cornifolium* exhibited high genetic diversity at the species-level, genetic diversity was relatively low at the population-level. The outcrossing dioecious breeding system and unique evolutionary history of *P. cornifolium* are likely to be key factors influencing the observed high intra-specific diversity whereas reduced genetic diversity at population-level is probably due to geographic isolation caused by recent habitat fragmentation.

Ecological parameters were investigated to determine the current ecological status of the five populations and results did not reveal any substantial ecological impediments to regeneration and dispersal modes. Ecological data were incorporated with information from national data sets to provide a more comprehensive overview of *P. cornifolium* autecology and to develop a predicted environmental distribution map. Key findings indicate *P. cornifolium* is typically affiliated with old growth forest systems and well drained low nutrient substrates, while low mean daily temperatures (<0.6°C) restrict environmental distribution.

Both genetic and autecological research was applied to determine levels of intra-specific divergence in cultivated *P. cornifolium* individuals from the Poor Knights Islands (outer Hauraki Gulf), which are morphologically distinct from mainland forms. The Poor Knights Islands individuals were the most genetically distinct as revealed by ISSR analysis, having higher pairwise levels of genetic distance than

mainland populations as well as more unique loci. A single mutation in the sequence of the Internal Transcribed Spacer (ITS) region was revealed in the Poor Knights Islands individuals, distinguishing them from mainland *P. cornifolium* and additional members of a monophyletic clade which have shared ITS sequences. Furthermore, *P. cornifolium* from the Poor Knights Islands have significant morphological and anatomical differences such as larger leaves and leaf tissue depths. Long term isolation on the offshore islands is likely to have had the most significant effect on this population divergence.

The differences in the Poor Knights Islands individuals may warrant the delineation of a new subspecies or even species. However, a more comprehensive examination of the taxon across its mainland range, the Poor Knights Island group, and other northern offshore islands where the species is present is recommended to clarify current inferences.

The results of this research have provided a framework for the development of species specific conservation and restoration strategies for *P. cornifolium* and reveal the importance of provenance and microhabitat (lifestyle) when sourcing seed for reintroduction projects.

Keywords: autecology, *Pittosporum cornifolium*, population genetics, conservation, restoration.

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1 Chapter One: Introduction

1.1 Overview

This thesis investigates the population genetics and autecology of *Pittosporum cornifolium* A. Cunn. (Pittosporaceae) based mainly on the results of field surveys of five populations located in the North Island, New Zealand. Also included were cultivated samples of the larger-leaved morphological variant from the Poor Knights Island group, Hauraki Gulf, New Zealand. Additional ecological information was derived from five New Zealand herbaria, National Vegetation Survey Databank (NVS), Land Use and Carbon Analysis System (LUCAS), publications and unpublished records. These data were compiled and analysed to extend autecological findings to a national scale and develop a quantitative environmental profile for *P. cornifolium*.

Pittosporum cornifolium is an endemic shrub epiphyte, typically perched on forest canopy trees but it also grows on the forest floor (terrestrial lifestyle) or on rocks (rupestral). Its primary habitats are lowland and coastal forest ecosystems, which have been subjected to widespread clearance and fragmentation. As a result, the potential population range of *P. cornifolium* has been severely reduced. To date little quantitative research has been undertaken regarding the biology and ecology, and genetic diversity of this widely distributed species. The goal of this research is to characterise the population genetics and autecology of *P. cornifolium* in order to inform restoration and conservation management.

1.2 Conservation biology and restoration ecology

Conservation biology and restoration ecology serve primarily to aid in the understanding of the consequences of anthropogenic impacts that have impacted upon the planet's natural ecosystems (Falk *et al.* 1996). There are many parallels and shared goals between conservation biology and restoration ecology, but there are also important differences (Young 2000). Conservation biology can be described as the science of preservation of original habitats in the face of biodiversity and habitat degradation. It is focused on the present day threats of permanent loss to biodiversity and the dominant conceptual theme is centred on population viability and dynamics. Restoration ecology on the other hand can be

described as the science of habitat and biodiversity recovery. The focus of restoration ecology is long term and on a much broader scale, encompassing community and ecosystem level dynamics, with an overarching focus on community succession and assembly (Young 2000).

1.3 Vascular epiphytes

Vascular epiphytes are plants that use botanical hosts (supporting plants) solely for mechanical support (Benzing 2004). Constituting approximately 10% of all vascular plant species (Benzing 1990), epiphytes play an integral role in the maintenance and functioning of forest ecosystems though contributions to species diversity, primary productivity, biomass, litter fall, water retention, and providing substrate for nitrogen fixing bacteria (Benzing 1998, Munoz *et al.* 2003). Epiphytes also provide habitat to canopy fauna (Cruz-Angon & Greenberg 2005; Freeman *et al.* 2005; Ellwood *et al.* 2002). Given the functional benefits that epiphytes provide to ecosystem processes and services, their consideration is imperative for the successful conservation and restoration of complex forest communities (Cummings *et al.* 2006).

In New Zealand, temperate rainforest canopies host a rich diversity of vascular epiphytes, which is comparable to that of tropical rainforests (Zotz 2005). Oliver (1930) described this wealth of vascular epiphytes, of which many are endemic, as a conspicuous feature of the New Zealand rainforest. The present study investigates one such endemic element, the shrub epiphyte *P. cornifolium*.

1.4 *Pittosporum cornifolium*

Pittosporum cornifolium is one of four shrub epiphytes endemic to New Zealand (Oliver 1930). This species has a distinctive whorled leaf arrangement, reddish brown flowers (Hooker 1832), and produces capsules with sticky ‘pitch’ seeds characteristic of the genus *Pittosporum* (Poole & Adams 1994) (Figure 1.1). It is frequently recorded growing in epiphytic congregations with nest epiphyte species *Astelia solandri* and *Collospermum hastatum* (Oliver 1930; Clarkson 1985; Burns & Dawson 2005).

Pittosporum cornifolium has been described by Oliver (1930) as a typical epiphyte in that it is habitually epiphytic. However, it can also be found growing in terrestrial (Salmon 1986) and rupestral lifestyles (Petrie 1921). It occupies a wide geographic range throughout the North Island to the northern reaches of the South Island and on various northern offshore islands (Poole & Adams 1994). Within this range it occupies habitats within diverse lowland forest ecosystems and coastal zones (Cooper 1956; Poole & Adams 1994). *Pittosporum cornifolium* is also known to be palatable to the introduced pest mammal the possum (Ravine 1995; Mitcalfe & Horne 2005).

Figure 1. 1 *Pittosporum cornifolium* images clockwise from left: mainland individual with immature capsules, mainland individual with dehisced capsule revealing seeds embedded in sticky resin and young leaves in whorled leaf arrangement (photo: T. Foster), Poor Knights Islands individual in flower, mainland individual in flower (photo: C. E. C. Gemmill).

Pittosporum cornifolium from the Poor Knights Islands have larger leaves compared to mainland plants, and bright yellow flowers, as opposed to the reddish brown colour of the mainland form (Smith 2004) (Figure 1.1).

1.5 Research objectives

The present study employs genetic and autecological population-level assessments to provide a framework for the development of species specific conservation and restoration strategies. The focus of this research was initially intended to be concentrated in the Waikato region where *P. cornifolium* is becoming locally rare (B. D. Clarkson, University of Waikato, pers. comm. 2008) and only 6% of the former lowland and coastal forests remain unmodified (Leathwick *et al*. 1995). However, it soon became evident that the number of individuals was insufficient to adequately assess population genetic diversity and species biology and ecology. The survey was therefore extended to include sites in Coromandel and Taranaki because of their close proximity to the Waikato and reconnaissance surveys there revealed the presence of more substantial populations. Cultivated individuals sourced from the Poor Knights Islands were also included in this research to quantify levels of morphological and genetic variability and to investigate whether this taxon is conspecific with the mainland form of *P. cornifolium*.

The specific aim of the genetic research was to estimate genetic variation of selected mainland and offshore populations of *P. cornifolium* in order to 1) reveal patterns of genetic variation within and among populations of *P. cornifolium* to inform conservation status and restoration management of the species and 2) assess the genetic distinctness, and potential taxonomic recognition of the off-shore populations.

The specific aim of the autecological research was to quantify a range of ecological parameters in order to 1) estimate the current ecological health of these populations, 2) relate population attributes to wider data sets to provide a more comprehensive overview of *P. cornifolium* autecology, 3) characterise the microclimate of the plant's habitat, and 4) determine the biological distinctness of mainland individuals compared with the Poor Knights Islands plants in order to determine the potential for separate taxonomic recognition.

1.6 Study area

Potential population study sites within the Waikato region and wider North Island were chosen using information from herbarium records (Allan herbarium (CHR), Auckland herbarium (AK), New Zealand Forest Research Institute herbarium (NZFRI), Te Papa herbarium (WELT) and The University of Waikato herbarium (WAIK)), site species lists (e.g. Gudex 1955, 1959, 1962, & 1963), and field observations. Access to individuals was via either tree climbing techniques, by kayak (Figure 1.2) or collection from ground depending on the lifestyle of located individuals.

Figure 1. 2 Collection methods of *Pittosporum cornifolium* individuals. Left: tree climbing to access an epiphytic individual, right: kayaking to access rupestral individuals.

Sites investigated within the Waikato region included: Maungatautari Scenic Reserve (hence forth Maungatautari), Mt Pirongia, Mt Karioi, Hakarimata Scenic Reserve walkway, Te Miro Scenic Reserve, Raglan Harbour, Kawhia Harbour, and Aotea Harbour. Raglan Harbour had the largest collection size of 25 individuals (Table 1.1), which accounted for the majority of the population present. In the southern enclosure of Maungatautari nine individuals were located for survey purposes and, of these eight were collected for genetic analysis (Table 1.1). Mt Pirongia had very few (<4) individuals that we were able to locate, and all of these were inaccessible. At all other sites no individuals were located.

Because of low collection numbers in the Waikato region numerous sites from the Coromandel region were investigated. These sites were located throughout the Coromandel Forest Park and included: Twin Kauri walkway, Square Kauri, The 309 Road, Waiomu Kauri Grove, and Taumatawahine. Large populations were located at both The 309 Road and Square Kauri sites with 24 and 18 individuals collected respectively (Table 1.1).

The Taranaki region was selected to compare inter-regional genetic diversity and to assess gene flow across the central North Island as well as provide a broader range for ecological assessments. In total, 24 individuals were collected from six small forest patches located in and around New Plymouth city (Table 1.1). These patches were all within close proximity to one another and samples were combined to represent a fifth population.

Finally, individuals from the Poor Knights Island group (outer Hauraki Gulf) were selected for comparison for their distinct morphological variation. Because access to the island group is strictly controlled by the Department of Conservation *P. cornifolium* plants were sourced from three different plant nurseries, with a total of 8 collected (Table 1.1).

Table 1. 1 *Pittosporum cornifolium* population sites on mainland New Zealand and original seed source for cultivated individuals from the Poor Knights Island group. Showing New Zealand Map Grid (NZMG) coordinates, and number of samples collected with sample IDs.

Region	Population / source	Sample I.D.	NZMG east	NZMG north	No. of samples
Hauraki Gulf	Poor Knights Islands	PK	2668100	6636700	8
Coromandel	The 309 Road	C	2738149	6481567	24
Coromandel	Square Kauri	S	2739270	6464682	17
Waikato	Raglan Harbour	R	2674437	6377361	25
Waikato	Maungatautari	M	2735418	6346749	8
Taranaki	Huatoki		2603383	6234672	5
Taranaki	Te Henui		2604860	6236899	3
Taranaki	Vogeltown		2803360	6236349	3
Taranaki	Sheppard’s Bush		2602688	6235396	1
Taranaki	Ratapihipihi		2600137	6233017	8
Taranaki	Everett Park		2620913	6230574	4
Taranaki	All Taranaki sites	T			24

1.6.1.1 Raglan Harbour

Raglan Harbour is the most northerly of three adjacent harbours on the west coast of the central North Island and covers approximately 33 km² (Sherwood & Nelson 1979). The harbour's rupestral population of *Pittosporum cornifolium* is located along the northern mouth, growing from calcareous rock outcrops for a stretch of approximately 1.5km (Figure 1.3). Individuals appeared to grow exclusively on outcrops that are separated from the mainland, or were found clinging to the sides of vertical cliffs. Their abundance on these sites may be the result of reduced accessibility of browsers such as possum. The Raglan Harbour population of *P. cornifolium* was accessed by kayak and collections were made using pole pruners and/or secateurs.

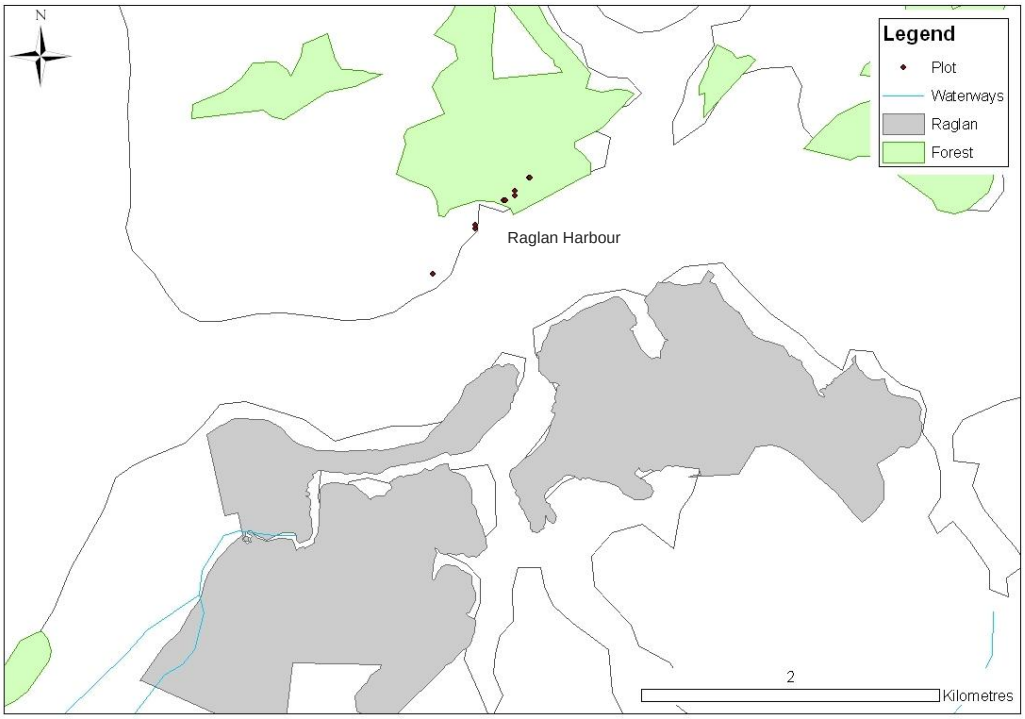

Figure 1. 3 Map showing collection (plot) sites at Raglan Harbour, Waikato.

1.6.1.2 Maungatautari

Maungatautari, a highly eroded, andesitic volcanic cone located in central Waikato supports approximately 3,400 ha of native forest or regenerating native bush (Speedy *et al.* 2007). The forest changes with altitude from lowland rimu (*Dacrydium cupressinum*) / tawa (*Beilschmiedia tawa*) forest to upland forest dominated by tawari (*Ixerba brexioides*) – kamahi (*Weinmannia racemosa*) and

tawheowheo (*Quintinia serrata*) (Clarkson 2002). In an attempt to remove all introduced mammalian pests from the mountain, the Maungatautari Ecological Island Trust (MEIT) constructed a 47 km Xcluder™ pest-proof fence enclosing the native forest which was completed in August 2006 (MEIT 2006; Speedy *et al.* 2007). Smaller 'pilot' enclosures were constructed in 2004 enclosing 35 ha (Northern Enclosure) and 65 ha (Southern Enclosure) of lowland native forest. Within these enclosures eradication procedures were tested and successful strategies were then applied to the larger scale pest control attempts on the main mountain (Speedy *et al.* 2007). Because *P. cornifolium* is susceptible to possum browse, searching and collection efforts were focused within these two smaller enclosures that have been pest free since early 2005 (MEIT 2005; Speedy *et al.* 2007). Unfortunately, no *P. cornifolium* individuals were located within the northern enclosure, which is perhaps due to the younger age of forest at this site. Finding individuals within the southern enclosure (Figure 1.4) proved challenging as many were located in the crowns of large emergents such as rimu and rewarewa (*Knightia excels*a).

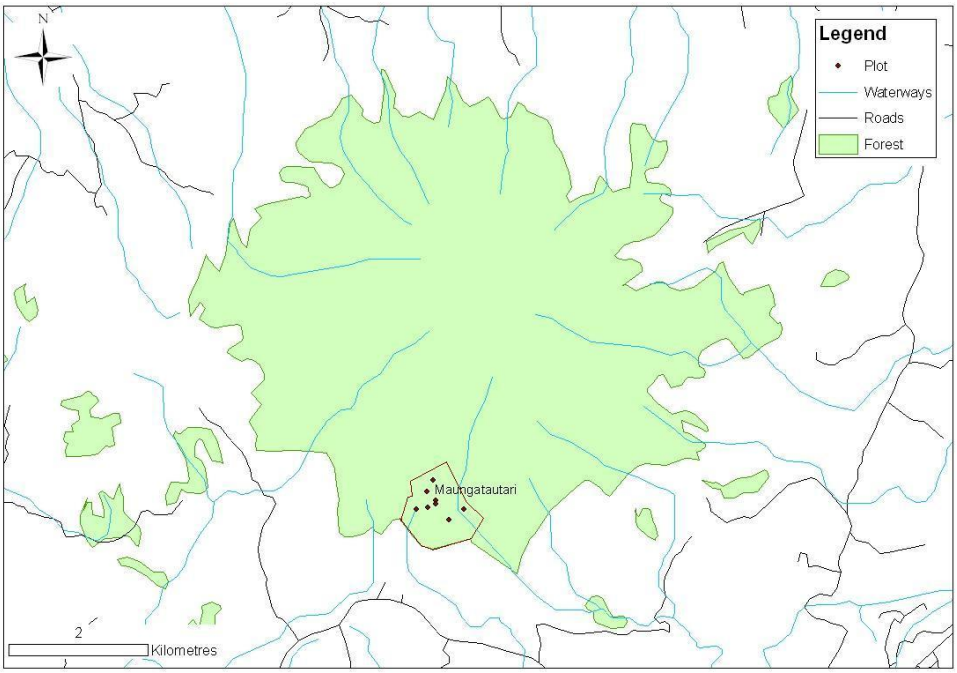

Figure 1. 4 Map showing collection (plot) sites at Maungatautari, Southern enclosure, Waikato.

1.6.1.3 The 309 Road

The 309 Road kauri grove is located 7.5 km along The 309 Road, at the northern end of the Coromandel Forest Park, and is one of the few mature stands of kauri (*Agathis australis*) on the Coromandel peninsula (Department of Conservation 2009). Numerous *P. cornifolium* individuals are found throughout the kauri grove, predominantly within, or in close proximity to, the older emergent kauri trees. Long term possum control in the area (C. Friis, Department of Conservation, pers. comm. 2010), and favourable habitat (i.e. sparse understory, thick detritus, and an open canopy), allow *P. cornifolium* individuals to thrive in the terrestrial lifestyle. Leaves were collected from 19 terrestrial individuals (Figure 1.5). Collections were also made from epiphytic individuals growing from the trunks of tree ferns; *Cyathea cunninghamii* and *Cyathea dealbata* (4 and 2 respectively). No collections were permitted from the numerous epiphytic individuals in emergent kauri trees due to the protected status of these trees.

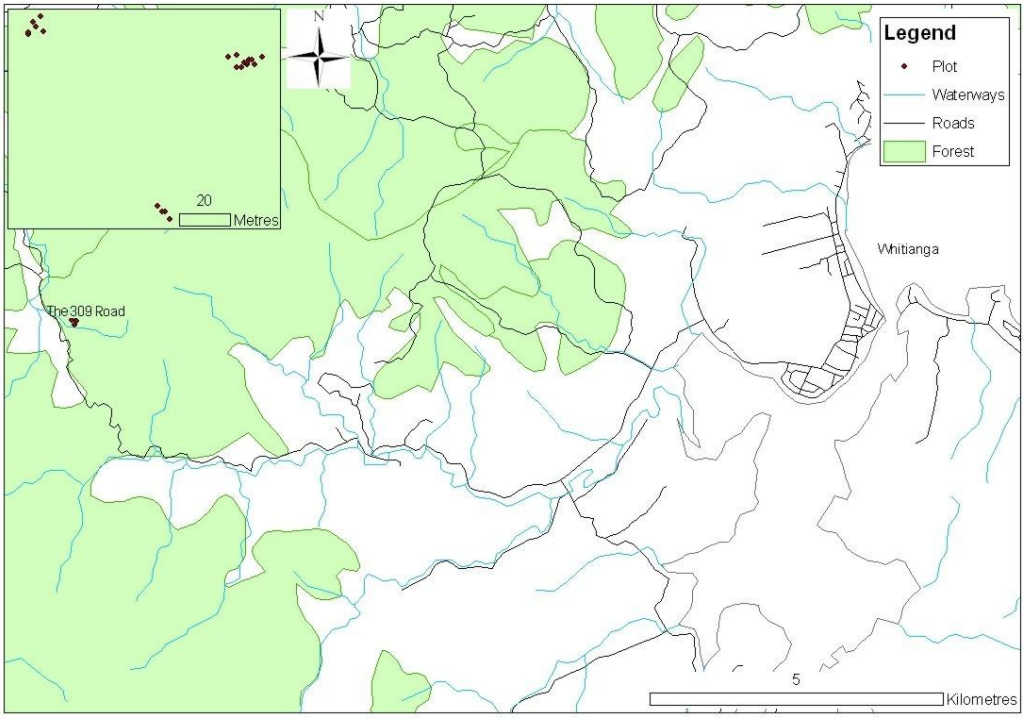

Figure 1. 5 Map showing collection (plot) sites at The 309 Road, Coromandel.

1.6.1.4 Square Kauri

The Square Kauri track is a short walk situated to the west of the Tapu-Coroglen Road summit, in the central range of Coromandel Forest Park. The 15th largest kauri (*Agathis australis*) on the peninsula, which is estimated to be 1200 years old,

is located on a steep ridge at the end of the Square Kauri track (Department of Conservation 2009). This single ancient kauri is host to several *P. cornifolium* individuals which reside among the various levels of its branches, from approximately 14 metres high to upwards of 40 metres. Directly beneath the canopy, which spans approximately 30 metres in diameter, live a terrestrial community of around 20 individuals of *P. cornifolium*. Only terrestrial individuals were collected from this site due to the protected status of the Square Kauri tree (Figure 1.6).

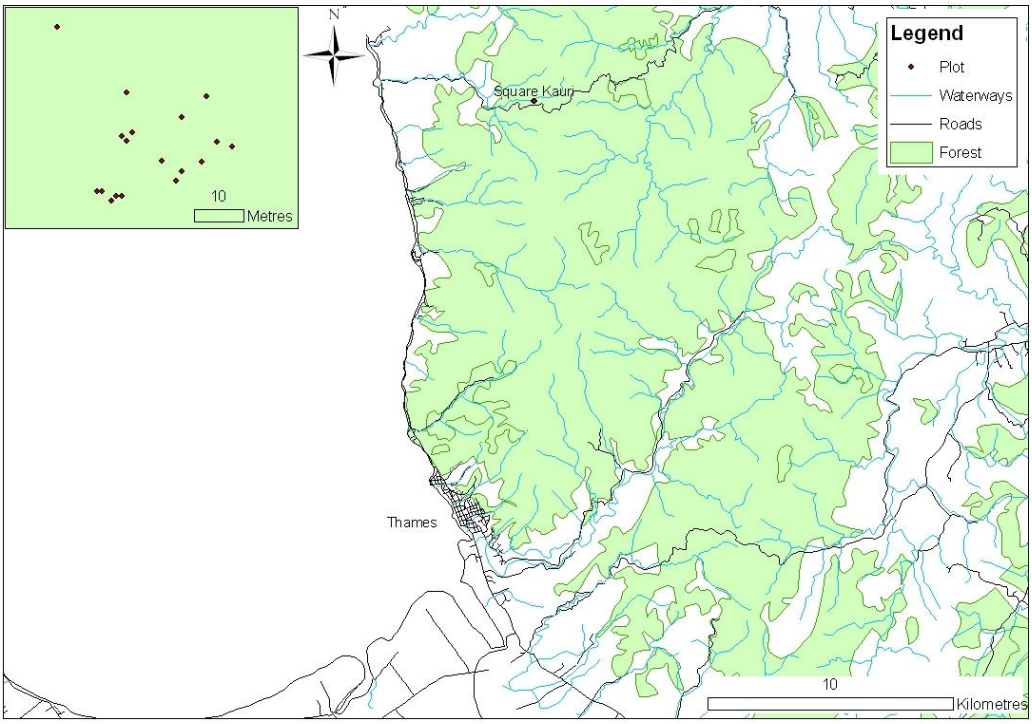

Figure 1. 6 Map showing collection (plot) sites at Square Kauri, Coromandel.

1.6.1.5 Taranaki

Of the six sites located within the Taranaki region (Figure 1.7), four are urban forest patches located within New Plymouth city; these include the Te Henui forest, Sheppard's Bush, Vogeltown Park, and Huatoki Scenic Reserve. Two considerably larger patches, Ratapihipihi Scenic Reserve and Everett Park Scenic Reserve, are located 5 km and 18 km respectively from New Plymouth city centre.

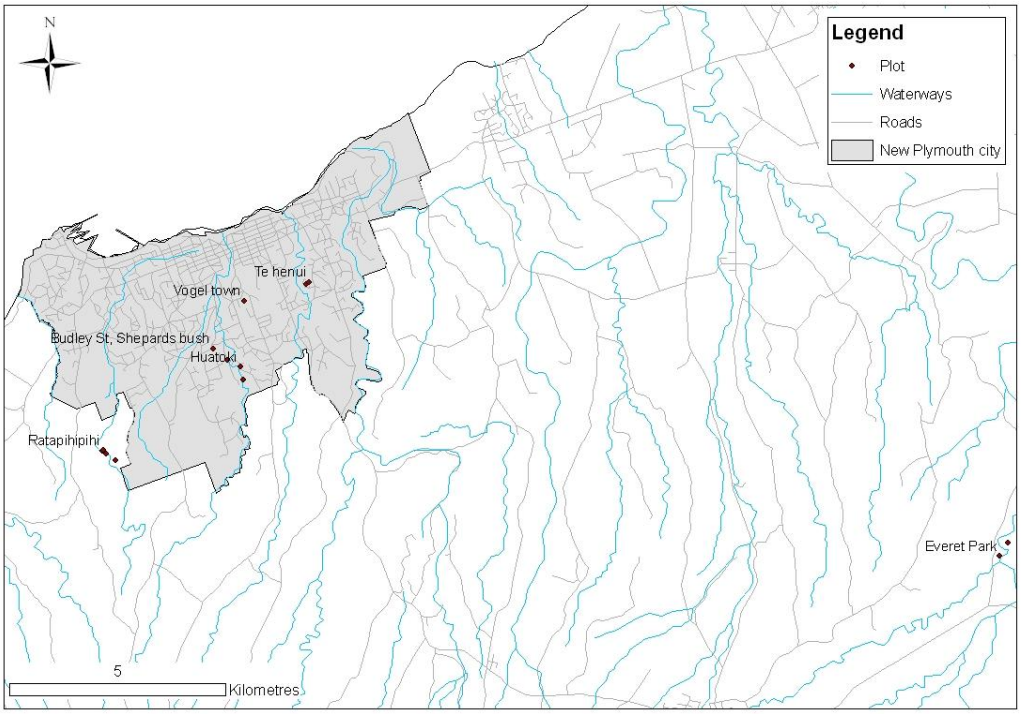

Figure 1. 7 Map showing collection (plot) sites in and around New Plymouth city, Taranaki.

The Te Henui forest is a 14 hectare urban remnant adjacent to the Te Henui Stream and walkway which runs through New Plymouth city. The vegetation consists of a broadleaved dominant canopy including tawa and pukatea (*Laurelia novae-zelandiae*), reaching up to 20m in height. Collections were made from two *P. cornifolium* individuals growing from large pukatea. A third individual was located growing from a fallen nest epiphyte on the edge of the walkway.

Sheppard's bush consists of an urban wetland and native forest remnant, with a combined area of approximately 3 hectares (New Plymouth District Council 2009). Swamp maire (*Syzygium maire*) dominates the wetland while the forest remnant is mixed broadleaved with large titoki (*Alectryon excelsus*) and tawa. A single *P. cornifolium* individual was located growing on a swamp maire amongst nest epiphyte *C. hastatum*.

The Huatoki Scenic Reserve is a 16 hectare semi coastal forest remnant located on the outskirts of New Plymouth city. The broadleaf dominant canopy reaches up to 20 metres high and comprises mainly tawa and pukatea (Clarkson *et al.* 1982). Four epiphytic individuals were collected from this site.

The Ratapihipihi Scenic Reserve is a 22 ha semi-coastal tawa broadleaved forest with a canopy up to 20 metres high. Logging in the late 1850s has left residual emergent rimu and canopy puriri (*Vitex lucens*) scattered throughout the reserve (Clarkson *et al.* 1982). Six of the eight individuals collected were found in emergent rimu, while the remaining two were collected from kohekohe (*Dysoxylum spectabile*) and pukatea. All individuals were associated with nest epiphytes.

Everett Park Scenic Reserve, located in Inglewood County, is a 77 ha remnant of kahikatea (*Dacrycarpus dacrydioides*) / tawa forest. The canopy ranges from 12 – 20 metres in height with emergents exceeding 25 metres, particularly kahikatea (Clarkson *et al.* 1982). Collections were made from epiphytic individuals located on emergent rimu, as well as canopy totara (*Podocarpus totara*).

1.6.1.6 The Poor Knights Island group

The Poor Knights Islands are located in the outer (northern) Hauraki Gulf, 20 km east of Northland. The island group comprises two main islands, Tawhiti Rahi (163 ha) and Aorangi (110 ha), as well as several smaller islands and islets (de Lange & Cameron 1999). Vegetation is dominated by dense pohutukawa (*Metrosideros excelsa*) forest giving way to subtropical broadleaved forest further inland (de Lange & Cameron 1999; Bowden 2010).

Figure 1. 8 Map showing position of the Poor Knights Island group from mainland New Zealand. Insert: the Poor Knights Island group, outer Hauraki Gulf.

1.7 Thesis outline

Chapter One provides background information on conservation and restoration, and epiphytes before introducing the study species *P. cornifolium.* The major research objectives of the thesis are then outlined for the two chosen lines of research; population genetics and autecology. Chapter One concludes by describing the North Island study sites that are relevant to both Chapters Two and Three. Research on the population genetics and autecology of *P. cornifolium* is presented in Chapters Two and Three respectively. Chapter Two outlines quantitative research measuring the genetic diversity and structure of five mainland populations as well as eight Poor Knights Islands samples. In Chapter Three ecological parameters are investigated to determine the ecological status of mainland populations. Ecological data were then incorporated with information from national data sets to determine species specific autecology and to develop observed and predicted distribution maps. Collation of existing information and collection of new information presented in these chapters was used to develop a contribution for the New Zealand Biological Flora Series. This has been prepared in the format for submission to the New Zealand Journal of Botany and is presented as Chapter Four. A synthesis of the key findings and future directions for research are outlined in Chapter Five.

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2 Chapter Two: Population genetics of *Pittosporum cornifolium*

This chapter investigates the population genetics of *Pittosporum cornifolium* with regard to five mainland North Island populations, and cultivated samples from the Poor Knights Islands variant. *Pittosporum cornifolium* is one of four New Zealand indigenous shrubs well adapted to the epiphytic mode (Oliver 1930; Wardle 1991; Dawson & Lucas 1993), which has been subjected to major reductions in potential population range due to the widespread clearance and fragmentation of lowland forest ecosystems (McGlone 1989) that host this elusive species. However, to date no quantitative research has been undertaken regarding the genetic diversity and structure of this widely distributed species with distinct morphological variability. The goal of this chapter is to characterise the genetic diversity within and among different populations of this species in order to inform conservation management.

2.1 Introduction

Globally the widespread degradation and destruction of biodiversity, which is a direct or indirect consequence of human actions, has highlighted the importance of conservation and restoration biology (Frankham 1995a; Falk *et al.* 1996; Harris *et al.* 2006). In New Zealand the impact of human settlement over the last 1000 years has surpassed anything brought about by natural processes over the past three millennia (McGlone 1989). Currently over a third (38%) of New Zealand's vascular flora, which is rich in endemism and diversity, is classified as threatened or uncommon (de Lange *et al.* 2009) and the risk of extinction means that many species now require the development of management strategies and conservation intervention to ensure their long term viability (Falk & Holsinger 1991; Wallis 1994; Frankham 1995a). In light of this it is imperative that conservation and restoration projects are underpinned with sound scientific research into the evolutionary significance of genetic variability, ecology, and reproductive biology (Falk & Holsinger 1991).

Epiphytic plants are important ecosystem components that are often overlooked in conservation and restoration biology (Zotz 2005). Epiphytes play a fundamental role in the maintenance and functioning of forest ecosystems though contributions to biodiversity, habitat for fauna, and ecosystem processes and services (Benzing 1998, Munoz *et al.* 2003). New Zealand temperate rainforests are host to a rich diversity of vascular epiphytes, which is comparable to that of tropical rainforests (Zotz 2005). Unfortunately, information on canopy species such as epiphytes and their associated ecosystems are scarce and/or outdated, which is due at least in part to the extreme difficulty in accessing them (Zotz 2005). Without adequate research into the extant distribution and state of extant epiphyte species we risk losing the inconspicuous elements of New Zealand's unique flora.

The New Zealand endemic shrub epiphyte *Pittosporum cornifolium* A. Cunn. (Pittosporaceae) is one such inconspicuous element for which limited information is available. *Pittosporum cornifolium* is a dioecious, evergreen shrub with reddish pink to light pink and yellow flowers and a distinctive architecture of sub-whorled branching and whorled leaf arrangement (Cooper 1956). Individuals of this species growing on the Poor Knights Island group (outer Hauraki Gulf) express morphologically distinct character states including larger more coriaceous leaves and bright yellow flowers (Smith 2004). The known range of *Pittosporum cornifolium* extends throughout the North Island to the Marlborough Sounds in the South Island, New Zealand (Poole & Adams 1994). Despite having a wide geographic range, *P. cornifolium* is localised in distribution and thought to be declining in numbers in the Waikato region, North Island, New Zealand (B. D. Clarkson, University of Waikato, pers. comm. 2008). Within the Waikato region (based on herbarium records and earlier publications, e.g., Gudex 1955, 1959, 1962, & 1963) it can be found growing in rupestral communities and lowland forests. Significant clearance and fragmentation of lowland habitat in the Waikato region (Leathwick *et al.* 1995) has potentially impacted on many of the historic populations. Furthermore, lowland forest ecosystems, the primary habitat of this species, have been severely reduced and fragmented at a national scale (McGlone1989). Habitat loss, fragmentation, and subsequent geographic isolation may cause severe ecological and genetic consequences leading to genetic impoverishment and rapid population decline (Barrett & Kohn 1991; Ellstrand & Elam 1993; Willi *et al.* 2006). However, no research has been undertaken

regarding the viability or genetic diversity of the Waikato or other populations where there has been significant habitat loss or fragmentation of forest. Nor has any research been conducted into the potential genetic divergence of the Poor Knights Islands variant.

The present research aims to bridge this gap through the application of comprehensive population-level genetic analyses employing Inter-simple Sequence Repeat (ISSR) markers. The discipline of population genetics allows for the assessment of genetic variation at the most intimate levels of divergence: within and among populations of single species (Wallis 1994; Gillespie 2004; Beebee & Rowe 2008). Population genetics attempts to describe the genetic structure of populations, and from these structures theorize on the evolutionary forces acting on populations (Gillespie 2004). This has proven to be highly valuable in assessing the conservation needs of individual species, especially in the case of fragmented and/ or isolated species distributions (Falk & Holsinger 1991; Frankham *et al.* 2004; Ci *et al.* 2008; Ramp Neale *et al.* 2008; Reunova *et al.* 2009; Hu *et al.* 2010; Zhou *et al.* 2010). The ISSR technique uses the Polymerase Chain Reaction (PCR) to amplify the genomic DNA between inversely oriented microsatellite regions (Zietkiewicz *et al.* 1994; Pradeep Reddy *et al.* 2002; Nybolm 2004; Rakoczy-Trojanowska 2004; Gronzalez *et al.* 2005). ISSR fragments segregate as dominant markers following simple mendelian inheritance to produce a DNA fingerprint. The technique has been shown to exhibit substantially higher levels of polymorphism than other markers such as Restriction Fragment Length Polymorphism (RFLP's) (Kantety *et al.* 1995; Salimath *et al.* 1995), Random Amplification of Polymorphic DNA (RAPD's) (Salimath *et al.* 1995; Yang *et al.* 1996), and Simple Sequence Repeats (SSR's) (Zhou *et al.* 2005). Other positive attributes of this technique include its high reproducibility, and broad applicability (Zietkiewicz *et al.* 1994; Pradeep Reddy *et al.* 2002; Nybolm 2004; Rakoczy-Trojanowska 2004; Gronzalez *et al.* 2005).

The present study also utilizes the Internal Transcribed Spacer (ITS) region to resolve broader scale relationships between the mainland form and the Poor Knights Islands individuals. The nuclear Internal Transcribed Spacers are located in the 18S – 26S nuclear ribosomal DNA (rDNA) region which consists of three components: the 5.8S subunit and two spacers' ITS–1 and ITS–2 (Baldwin *et al.*

1995). The ITS region has proven useful in resolving species-level phylogenetic relationships in angiosperm families (Baldwin *et al.* 1995), and has been shown to depict relationships more accurately within New Zealand *Pittosporum* than the plastid *trn*T–*trn*L region, another common region used in resolving angiosperm phylogenies (Carrodus 2009). Results of this research can be applied to developing conservation strategies tailored to the specific needs of endangered populations to effectively ensure the long term viability of this unique species.

The specific aims of the research were to estimate genetic variation of selected mainland and off-shore populations of *P. cornifolium* to 1) reveal patterns of genetic variation within and among populations of *P. cornifolium* to inform conservation status and restoration management of the species and 2) to assess the genetic distinctness, and potential taxonomic recognition of the off–shore populations. It is hypothesised that: 1) smaller populations have less genetic diversity and 2) that isolated populations will be genetically distinct from contiguous populations due to reduced gene flow.

2.2 Methods

Samples were Collected at five mainland populations: The 309 Road, Coromandel; Square Kauri, Coromandel; Maungatautari, Waikato; Raglan Harbour, Waikato; and sites located in and around New Plymouth city, Taranaki (see Chapter 1 for further detail).

The main techniques used to locate and access *P. cornifolium* individuals were binocular inspection, tree climbing methods and by kayak. Collections of 1–2 leaves per individual were made using either secateurs or pole pruners. Collections were also made from eight nursery propagated individuals of the Poor Knights Islands variant. A sample from cultivated specimens of both *P. pimeleoides* subspecies were also collected for ITS analysis to confirm recent findings of Hathaway (2001) who discovered identical DNA sequences for both *P. pimeleoides* subspecies and *P. cornifolium*.

2.2.1 DNA extraction

Total genomic DNA was extracted from approximately 5 g of fresh and/ or frozen leaf tissue using a modified CATB-based protocol similar to that described in Doyle & Doyle (1987; Appendix 1). Samples were then treated with RNase, and run out on a 1% agarose/1×TBE gel via gel electrophoresis to assess the quality and quantity of DNA.

2.2.2 ISSR amplification and scoring

Twenty ISSR primers (Invirtogen) were initially screened, and of these, ten yielded bright, distinct, reproducible bands with high levels of polymorphism (Table 2.1).

Table 2. 1 ISSR primers used for *Pittosporum cornifolium* population genetic research, showing primer sequence, annealing temperature, total number of bands per primer and number of polymorphic bands per primer.

Primer	Primer sequence	Annealing temp (°C)	No. of bands	No. polymorphic bands
ISSR 1	$(CA)_6GG$	50.0	16	15
ISSR 4	$(CA)_6AC$	53.4	15	14
ISSR 7	$(CA)_6GT$	50.0	17	16
ISSR 8	$(GA)_6GG$	53.4	12	8
ISSR 9	$(GT)_6GG$	58.7	14	13
ISSR 10	$(GA)_7CC$	50.0	17	15
ISSR 13	$(GAG)_3GC$	50.0	19	17
ISSR A	$(CA)_7TC$	40.1	14	14
ISSR E	$(CA)_6GC$	41.0	15	14
ISSR F	$(GAG)_4GC$	43.3	22	20
Total			161	146

PCR was performed in 25 µl reaction volumes containing final concentrations of 1×PCR buffer (Invitrogen), 4 mM $MgCl_2$, 200 µM of each dNTP, 0.2µl of 1% bovine serum albumin (BSA), 10 pM of each primer, 0.5 units of *Taq* DNA polymerase (Invitrogen), and 1 µl of unquantified DNA (diluted 1:10). PCR Amplification was performed on all collected individuals, from all populations, for all 10 ISSR primers (Table 2.2). A positive control using the isolated DNA of a cultivated *P. cornifolium* sample was included in every PCR run and visualised

alongside tested individuals to check reproducibility. Likewise, products that were amplified twice (replicates) were subsequently run side by side with original products to check reproducibility. A negative control (containing no DNA) was also added to each run to test for contamination in reagents.

Reactions were run on an Eppendorf mastercycler program of: 4 min at 94°C (initial denaturation); 35 cycles of denaturation period of 40 s at 94°C, 45 s at appropriate annealing temperature (Table 2.1), and 1 min 30s at 72°C, followed by a final extension of 5 min at 72°C. PCR amplification products were then mixed with 8 µl loading dye, and 16 µl was loaded on a 2% agarose/1×TBE gel containing ethidium bromide. A 100 base pair ladder (Invitrogen) was also loaded on the first and last lanes of a gel to be used as a reference for sizing of bands.

Table 2. 2 Number of PCR amplifications (PCRa) per primer per population: PK (Poor Knights Islands), C (The 309 Road), S (Square Kauri), R (Raglan), M (Maungatautari), T (Taranaki). Note: where PCRa is double the population number (n) replicates have been performed.

Primer	PK (n=8) PCRa	C (n=24) PCRa	S (n=17) PCRa	R (n=25) PCRa	M (n=8) PCRa	T (n=24) PCRa	Total PCRa
ISSR 1	8	24	17	50	8	24	131
ISSR 4	8	24	17	25	8	24	106
ISSR 7	8	24	17	25	8	24	106
ISSR 8	8	24	17	25	8	24	106
ISSR 9	8	24	17	50	8	24	131
ISSR 10	8	24	17	25	8	24	106
ISSR 13	8	24	17	50	8	24	131
ISSR A	8	24	17	50	8	24	131
ISSR E	8	24	17	25	8	24	106
ISSR F	8	24	17	50	8	24	131
Total							1185

Each gel was run for between 2 to 4 hours at 2.5 V/cm, then visualized under ultraviolet light and photographed using an Alpha imager (Alpha Innotech) (Figure 2.1). Resulting bands ranged from 200 – 2,000 base pairs.
Each gel was scored manually for presence and absence of alleles. A data matrix of allele frequencies within each population was then produced. Replicates of primers ISSR 1, ISSR 9, ISSR 13, ISSR A, and ISSR F within the Raglan population (see Table 2.2) showed there were no discrepancies among the scored

bands. Fragment profiles of both positive controls and replicate samples were highly reproducible.

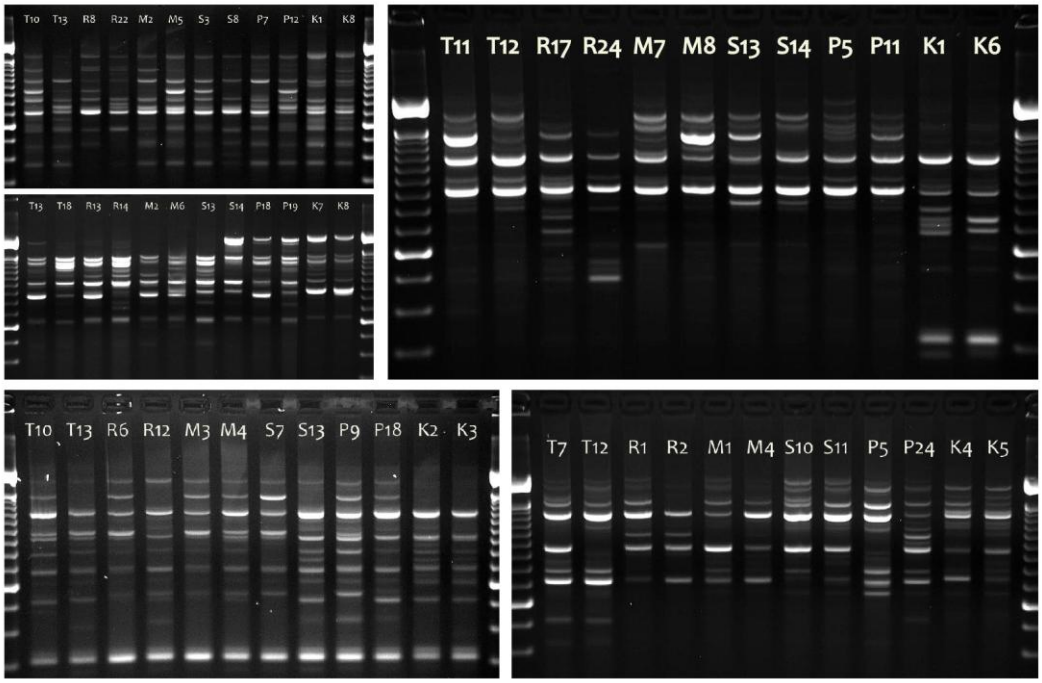

Figure 2. 1 Agarose gels showing ISSR products, clockwise from left: ISSR 8, ISSR F, ISSR 4, ISSR 13, & ISSR 10. In all images: Lane 1: ladder, Lane 2–3: Taranaki individuals, lane 4–5: Raglan individuals, Lane 6–7: Maungatautari individuals, Lane 8–9: Square Kauri individuals, Lane 10–11: The 309 Road individuals, Lane 12–13: Poor Knights Islands individuals, Lane 14: Ladder.

2.2.3 ITS region amplification

A total of four samples were selected for DNA sequencing of the nr ITS region, two of these were from mainland populations (Raglan (R1) and The 309 Road (C18)), and two were nursery propagated individuals from the Poor Knights Islands (PK1 and PK8). Both *P. pimeleoides* subsp. *pimeleoides* & *P. pimeleoides* subsp. *majus* were also re–sequenced to confirm results of Hathaway (2001) who found identical ITS sequences between these two subspecies and *P. cornifolium*. The ITS region was amplified by PCR with the 3′ universal eukaryote primer ITS–4 (5′ –TCCTCCGCTTATTGATATGC–3′) (White *et al.* 1990), and the higher–plant primer ITS–5 HP, (5′ –GGAAGGAGAAGTCGTAACAAGG–3′) (Laboratory of Molecular Systematics (LMS), Smithsonian Institution). PCR was performed in 25 µL volumes with final concentrations of 1×PCR buffer (Invitrogen), 7.5 mM MgCl2, 200 µM each dNTP, 0.2 µL 1% BSA, 10 pM of

each primer, 1 unit of *Taq* (Invitrogen) and 1 μL of unquantified DNA (diluted 1:100). Reactions were run on an Eppendorf thermocycler program of: 94°C for 5 min (initial denaturation), 30 cycles of denaturation period of 94°C for 1 min, a primer annealing period of 53.1°C for 1 min, an extension of 72°C for 1 min), followed by a final extension of 72°C for 10 min. To check quality and quantity of amplifications 3 μl of product was mixed with 2μl of loading buffer was loaded onto a 1.8% agarose/ 1×TBE gel stained with ethidium bromide. Gels were run out via electrophoresis for 1 to 2 hours at 2.5 V/cm. A 100 base pair ladder (Invitrogen) was used to determine the size of the PCR product. Gels were visualized and photographed under UV light (Alpha Innotech Corporation). Products that produced a single clear band of around 700 base pairs were purified using EXO–SAP (Invitrogen). Sequencing was performed at the University of Waikato DNA sequencing facility using both ITSHP5 and ITS4 primers with Big Dye chemistry v 3.1 on an Applied Biosystems 3130×1 Genetic Analyzer.

2.2.4 Genetic Analyses

From the ISSR data matrix, standard diversity indices were calculated including the percent of polymorphic loci (P), and Nei's (1978) gene diversity (*h*) in Arlequin ver. 3.5 (Excoffier & Lischer 2010). To determine whether levels of polymorphism were attributed to larger sample size populations were also assessed using eight randomly selected individuals per population (or total collection sizes of eight as with Maungatautari and the Poor Knights Islands). The hierarchical Analysis of Molecular Variance (AMOVA), which is based on Euclidian distance, was used to statistically assess how genetic variation is partitioned within and among populations. Associated fixation indices (F_{st}) (Wright 1951, 1965) were based on pairwise distance between individuals (number of shared ISSR bands). A distance matrix using Nei's (1978) unbiased genetic distance was constructed from ISSR data to produce a UPGMA (unweighted pair group method algorithm) dendrogram in TFPGA 1.3 (Miller 1997). A Mantel test was conducted using Arlequin ver. 3.5 to determine correlations between genetic and geographic distance matrices. ITS sequences were edited and aligned using Geneious 5.2.1 (Biomatters Ltd).

2.3 Results

2.3.1 ISSR Population–level Genetic Diversity and Structure

A total of 161 ISSR loci which were highly reproducible were included in the analyses (Table 2.1). Of those 161 loci, 146 were polymorphic across all populations (90%). Percentages of polymorphic loci (PL) among *P. cornifolium* populations ranged from 60.9% in Raglan (n = 25) to 16.8% in the Poor Knights Islands population (n = 8) (Table 2.3). The populations with larger sample sizes (n=17–25) including Taranaki, The 309 Road, Square Kauri, and Raglan, shared relatively higher levels of polymorphism (52.2–60.9%). While the two populations with small sample size (Maungatautari and the Poor Knights Islands (n=8 for each)) expressed relatively lower levels of polymorphism (43% and 16.8% respectively). Percent polymorphisms decreased an average of 11.8% among populations with larger (>8) sample size (Taranaki, The 309 Road, Square Kauri, and Raglan) when sample sizes were reduced to a randomly selected subset of eight individuals. All mainland populations share a relatively similar polymorphism level (42.2–46.6%), while the Poor Knights Islands population has considerably lower levels of observed polymorphism (16.8%). These data are further supported by higher gene diversity indices (Nei 1978) for mainland populations (0.17–0.20) compared to a relatively lower gene diversity indices for off shore island population the Poor Knights Islands (0.07) (Table 2.3). The Poor Knights Islands population also had the highest number of unique population specific bands (n = 18). Additional unique bands were observed only in two other populations, The 309 Road (n = 2) and Raglan (n = 1) (Table 2.3).

Genetic distance among populations were examined by UPGMA cluster analysis using a pairwise genetic distance matrix (Nei 1978) (Table 2.4; Figure 2.2). Nei's (1978) pairwise genetic distance varied between 0.0497. (Raglan vs. Maungatautari) and 0.4121 (Maungatautari vs. Poor Knights Islands). The Poor Knights Islands population was the most distinct population with both an average pairwise genetic distance and UPGMA node distance of 0.3663 from mainland populations. Mainland populations clustered together with a UPGMA node distance of 0.1059. The lowest pairwise genetic distance was observed between Raglan and Maungatautari (0.0497), with the next lowest pairwise genetic distance between The 309 Road and Square Kauri population (0.0595). Taranaki

was most similar to the Raglan and Maungatautari populations, with a maximum pairwise genetic distance of 0.0946, and UPGMA node distance of 0.0891. Furthermore, a Mantel test revealed a significant correlation between genetic and geographic distance matrices (r = 0.646683, p = 0.004, 1000 permutations) (see Appendix 2 for geographic distance matrix).

Table 2. 3 Population genetic statistics for six populations of *Pittosporum cornifolium.* Abbreviations as follows: # PL (%) = the number of polymorphic loci (percentage polymorphic loci), # U (%) = the number of unique loci per population (percentage unique loci), GD = Nei's (1978) gene diversity shown ± one standard deviation.

Region	Hauraki Gulf	Coromandel		Waikato		Taranaki
Population site	Poor Knights Islands	The 309 Road	Square Kauri	Raglan Harbour	Maungatautari	Taranaki
Sample size	8	24	17	25	8	24
# PL (%)	27 (16.8)	92 (57.1)	92 (57.1)	98 (60.9)	70 (43.5)	84 (52.2)
# U (%)	18 (11.2)	2 (1.0)	0 (0)	1 (0.6)	0 (0)	0 (0)
GD	0.07 ± 0.04	0.20 ± 0.01	0.19 ± 0.10	0.17 ± 0.09	0.18 ± 0.10	0.17 ± 0.09
Sample size	8	8	8	8	8	8
# PL (%)	27 (16.8)	72 (44.7)	75 (46.6)	75 (46.6)	70 (43.5)	68 (42.2)
GD	0.07 ± 0.04	0.20 ± 0.11	0.19 ± 0.11	0.17 ± 0.10	0.18 ± 0.10	0.17 ± 0.09

Table 2. 4 Nei's (1978) pairwise genetic distance of *Pittosporum cornifolium* populations

	The 309 Road	Square Kauri	Maungatautari	Raglan	Taranaki	Poor Knights Islands
The 309 Road	–					
Square Kauri	0.0595	–				
Maungatautari	0.1229	0.0878	–			
Raglan	0.1239	0.1039	0.0497	–		
Taranaki	0.0771	0.1199	0.0837	0.0946	–	
Poor Knights Islands	0.3335	0.3656	0.3546	0.3659	0.4121	–

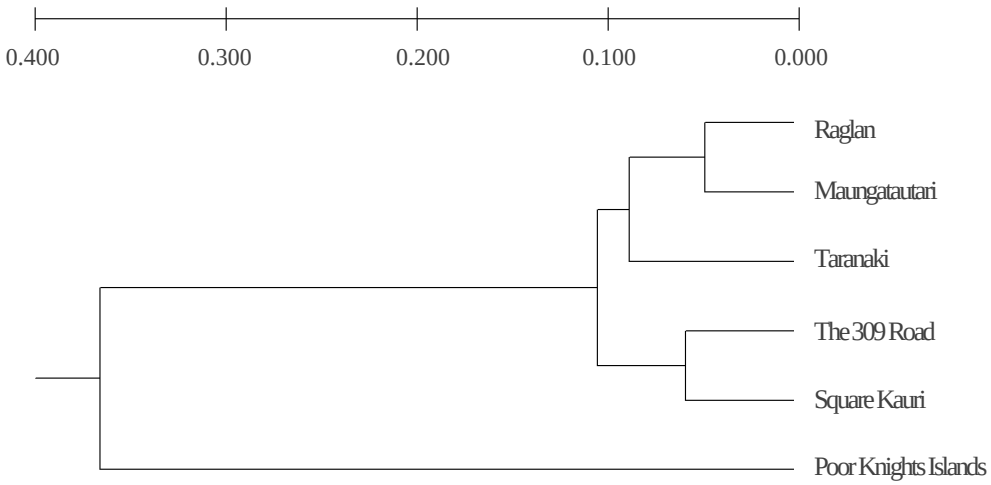

Figure 2. 2 UPGMA dendrogram using Nei's (1978) unbiased genetic distance between populations of *Pittosporum cornifolium* based on ten ISSR primers.

The AMOVA revealed that 40.19% of variation was partitioned among populations, while 59.81% was partitioned within populations (Table 2.5). To examine population structure further a series of AMOVA analyses were undertaken to determine any significant population groupings. The most statistically significant structure was comprised of three separate groups: Group 1, The 309 Road and Square Kauri (Coromandel – East Coast); Group 2, Maungatautari, Raglan and Taranaki (Waikato and Taranaki – West Coast); and Group 3, the Poor Knights Islands (off shore/ Hauraki Gulf) (Table 2.6). AMOVA analysis showed that 26.92% of the variation was partitioned among these groups ($p = 0.01$), 18.25% was partitioned among populations within these groups ($p = 0.00$), and 54.83% could be explained within populations ($p=0.00$) (Table 2.6). The associated population-level F_{st} value of 0.45172 was statistically significant ($p = 0.00$).

Table 2. 5 AMOVA analysis of genetic variation within and among all populations of *Pittosporum cornifolium.*

Source of variation	d.f.	Sum of squares	Variance components	Variation (%)
Among populations	5	863.222	9.30755 V	40.19
Within populations	100	1385.41	13.85410 V	59.81

Table 2. 6 AMOVA analysis of three groups of *Pittosporum cornifolium*: Group 1, The 309 Road and Square Kauri; Group 2, Taranaki, Maungatautari, and Raglan; Group 3, the Poor Knights Islands.

Source of variation	d.f.	Sum of squares	Variance components	Variation (%)
Among groups	2	569.382	6.80298 V	26.92
Among populations within groups	3	293.84	4.61122 V	18.25
Within populations	100	1385.41	13.85410 V	54.83

2.3.2 ITS sequence results

The matrix of aligned sequences was 685 base pairs in length. Sequences of mainland *P. cornifolium* samples (R1 and C18) and each of the *P. pimeleoides* subspecies were identical. Sequence alignment of the Poor Knights Islands individuals (PK1 and PK8) revealed a single point mutation (T>C) at 583 base pairs for the Poor Knights Islands samples.

2.4 Discussion

2.4.1 Genetic diversity of Pittosporum cornifolium

From a theoretical standpoint, it is assumed that species with smaller, more fragmented population structures will possess low levels of genetic diversity due to factors such as founder effects, genetic drift, and inbreeding (Barrett & Kohn 1991; Ellstrand & Elam 1993). While much research supports this theory (e.g. Moran & Hopper 1983; Frankham 1995b; Xiao *et al.* 2004), several other studies have revealed that rare species can share equally high levels, or higher levels of genetic diversity than their widespread congeners (e.g., Vogelmann & Gastony 1987; Ranker 1994; Young and Brown 1996). Other factors which influence genetic diversity of extant populations include modes of reproduction, as outcrossing species commonly have considerably higher levels of genetic diversity (Hamrick & Godt 1989), and long term evolutionary history, such as shifts in distribution (Schaal *et al.* 1998).

In the case of *P. cornifolium* genetic diversity was extremely high at species-level with 90% PL. This result is comparable to other plant species with the same breeding systems (dioecious outcrossing), e.g. *Litsea szemaois*, Lauraceae (87% PL) (Ci *et al.* 2008) (Table 2.7).

Table 2. 7 Population and species–level comparisons of percent polymorphic loci values (%PL) and Nei's (1978) unbiased genetic distances (GD) using ISSR's observed in natural populations of flowering plants which have experienced severe habitat loss and fragmentation. Interspecific comparisons were chosen to compare genetic distances between closely related congeners of *Pittosporum cornifolium*. Primary breeding system (PBS) is also recorded, abbreviations are as follows: D, dioecious, O, outcrossing, S, self pollination, V, vegetative reproduction. Some values were not reported for all taxa. *combined ISSR and AFLP data.

Taxon	P.B.S	Level	% PL	GD
Pittosporaceae (Apiales)				
Pittosporum cornifolium	D–O	Species	90.7	
		Population (range)	16.8–60.9	0.05–0.41
		Population (mean)	47.9	0.18
Pittosporum cornifolium (Mainland)		Population (range)	43.5-60.9	0.05-0.12
		Population (mean)	54.2	0.09
Pittosporum cornifolium (Poor Knights Islands)		Population (range)	16.8	0.33-0.41
		Population (mean)	16.8	0.37
P. colensoi – P. divaricatum –P. turneri[a]	D–O	Interspecific (range)		0.19–0.24
		Interspecific (mean)		0.22
Araliaceae (Apiales)				
Oplopanax elatus[b]	V	Species	43.2	
		Population (range)	21.6–32.4	
		Population (mean)	27.3	
Aristolochiaceae (Piperales)				
Saruma henryi[c]	S	Species	73.7	
		Population (range)	10.3–36.6	
		Population (mean)	22.8	
Asteraceae (Asterales)				
Lasthenia conjugens[d]	O	Species	100	
		Population (range)	68.2–86.9	
		Population (mean)	76.2	
Lauraceae (Laurales)				
Litsea szemaois[e]	D–O	Species	87	
		Population (range)	18.2–72.7	0.06–0.42*
		Population (mean)	38	0.25*
Polygonaceae (Caryophyllales)				
Rheum tanguticum[f]	O	Species	92.9	
		Population (range)	25.8–58.9	0.06–0.18
		Population (mean)	46.5	0.13

[a], Carrodus 2009; [b], Reunova *et al.* 2010; [c], Zhou *et al.* 2010; [d], Ramp Neale *et al.* 2008; [e], Ci *et al.* 2008; [f], Hu *et al.* 2010.

The outcrossing dioecious breeding system of *P. cornifolium* is likely to be one of the most important factors influencing the observed high intra-specific diversity. Other factors may include its unique evolutionary history. The family is likely to have east Gondwanan, or solely Australian origin (post Gondwana) from which it arrived in New Zealand via long distance dispersal (Chandler *et al.* 2007). Proposed dispersal events into an insular system led to adaptive radiation and speciation resulting in high endemism among New Zealand *Pittosporum* (Gemmill *et al.* 2002). *Pittosporum cornifolium* was historically found growing in abundance across its known range of diverse lowland forest ecosystems to coastal zones from the top of the North Island to the northern half of the South Island (Cooper 1956; Poole & Adams 1994). Such extreme environmental heterogeneity may lead to accumulation of genetic variability through environment specific genetic adaptations (Givnish 1998). Maintenance of high genetic variability at species-level would have been aided by dioecious breeding systems and high gene flow through once-continuous ranges of lowland and coastal forests.

2.4.2 Genetic differentiation among six populations of *Pittosporum cornifolium*

In contrast to high intra-specific genetic diversity, population-level genetic diversity was relatively low (16.8–60.9% PL (total sample size)). The populations with relatively large sample sizes (Taranaki, Raglan, The 309 Road and Square Kauri) appear to have higher genetic variation than those with small sample size (Maungatautari, and Poor Knights Islands) (52.2–60.9% PL vs. 16.8–43% respectively). However, when all population sizes were reduced to eight samples and reassessed, mainland populations shared comparable levels of genetic diversity (42.2–46.6% PL), while the off shore island population of the Poor Knights Islands remained lower by a considerable amount (>25% less PL). Lower population-level genetic diversity (relative to intra-specific genetic diversity) is observed in many species with geographically isolated populations which have resulted from habitat fragmentation (Table 2.7). For example, Ci *et al.* (2008) used the ISSR technique to investigate the genetic diversity of highly fragmented populations of the Chinese endemic tree *Litsea szemaois.* Results

revealed a mean population-level PL of 31% vs. 87% PL at species-level (Ci *et al.* 2008). Similarly, recent habitat fragmentation and loss has severely reduced and isolated remaining populations of *Saruma henryi* Aristolochiaceae, an endangered Chinese endemic herb (Zhou *et al.* 2010). ISSR analysis of *S. henryi* populations revealed that on average 22.82% PL were expressed at population-level vs. 73.71% PL at species-level. The clearance and fragmentation of lowland forest systems which has lead to geographic isolation on the mainland is likely to have had the most significant influence on the partitioning of genetic diversity among *P. cornifolium* populations. This is because geographic isolation limits the amount of gene flow via both pollen and seeds (Pfeifer & Jetschke 2006). Moreover, given geographic isolation is a major factor in limiting gene flow, it is not surprising that the off shore island population of the Poor Knights Islands expresses the lowest levels of genetic diversity compared with mainland populations. Island populations are usually more differentiated and contain less genetic diversity than comparable mainland samples, these genetic differences may result from stochastic processes operating during founder events and/ or periods of small population size (Barrett *et al.* 1996; Frankham 1998). However, low genetic diversity in the Poor Knights Islands individuals may also be an artefact of sampling a few cultivated individuals, which likely are a cohort of closely related individuals (i.e. full and half siblings).

Partitioning and structures of genetic diversity were obtained by conducting an AMOVA, which revealed that 59.81% of variation was partitioned within populations, and 40.19% of variation was expressed among populations. Higher population differentiation may be attributed to geographic isolation impinging gene flow as discussed above. Population differentiation is reinforced by a high F*st* value of 0.45, as when F*st* estimates exceed 0.15 it is an indication of significant differentiation (Wright 1978).

Statistically significant population groupings were attained by combining the two Coromandel populations (east coast grouping), the Waikato and Taranaki populations (west coast grouping), with the Poor Knights Islands population remaining in its own grouping. AMOVA analysis revealed that 26.92% of the variation could be described among these groups, and a lesser 18.25% observed among populations within these groups. The significance of this population

structuring is reinforced by the topography of the UPGMA dendrogram. Both AMOVA groupings and UPGMA topologies within mainland populations appear to cluster with respect to geographic location, in that the most closely aligned sites are generally also close to one another with respect to geographic distance. The Coromandel/ east coast sites are approximately 20km in distance from one another (the closest of all sites) and share the second lowest genetic distance value of 0.0595. Similarly, the two Waikato sites are approximately 70km apart (second closest sites) and share the lowest genetic distance of 0.0497. Taranaki is geographically closest to the Waikato populations (approximately 150km to Raglan Harbour and 170km to Maungatautari) and has a mean genetic distance of 0.08915 from these sites. Finally, the Poor Knights Islands population was the most genetically distant population from all others with an average distance of 0.36634 and is approximately 180km from the closest mainland population and is isolated by ocean. Moreover, Mantel test confirmed significant correlation between genetic and geographic distances ($r=0.685287$, $P=0.008$). The relatively higher pairwise genetic distance values for Coromandel populations (with respect to extremely close geographic location) may be attributed to the relatively insular nature of these populations. The 309 Road and Square Kauri populations were established when small portions of old growth forest became isolated, thus restricting gene flow between populations and enhancing the potential for breeding between closely related individuals.

Population structures and the correlation between geographic isolation and genetic diversity as revealed by AMOVA, UPGMA and Mantel tests, support the main hypothesis that geographic isolation is the main contributing factor to population-level differentiation in *P. cornifolium* populations. Geographic isolation among mainland populations is due to considerable habitat loss and fragmentation which been a direct or indirect result of human settlement over the last 1000 years. The impact on the once vast lowland ecosystems has surpassed anything brought about by natural processes over the past 3 millennia (McGlone 1989). While the life span of *P. cornifolium* is unknown, it is probable that it has a similar lifespan to that of congener *P. turneri,* of approximately 90 years (Ecroyd 1994). Habitat fragmentation and loss over the course of 1000 years (and upwards of 10 generations), has possibly initiated some levels of inbreeding and genetic drift causing observed population differentiation. This is because insular populations

are more susceptible to genetic drift and genetic decay over subsequent generations (Loveless & Hamrick 1984).

With respect to the Poor Knights Islands individuals, higher population differentiation could possibly have arisen from stochastic processes operating during founder events and/or periods of small population size (Barrett *et al.* 1996; Frankham 1998). A small number of founders can lead to rapid differentiation between source and founder populations due to changes in allele frequencies caused the differential representation of alleles in the founding pool (Barrett *et al.* 1996). Differentiation may be further enhanced by random genetic drift leading to loss and fixation of alleles and increased rate of inbreeding (Suzuki *et al.* 1981), and/ or ecological divergence driven by natural selection (Givnish 1998). The Poor Knights Islands were formed in the late Miocene (approximately 5–12 m.y.a.) and have been isolated from the mainland for less than one million years (Hayward 1986). The islands remained isolated when sea levels rose at the peak of the last glaciation (18,000–20,000 m.y.a.). The Poor Knights Islands variant exhibits high susceptibility to frost mortality (Smith 2004) which may indicate recent colonisation (post glaciations) and subsequent divergence from the mainland forms. Furthermore, the observed level of Nei's (1978) unbiased genetic distance is comparable to species-level genetic distance between other members of New Zealand *Pittosporum*. When comparing ISSR profiles of putative hybrid *P. turneri*, to potential parents *P. colensoi* and *P. divaricatum* as well as outgroup taxon *P. cornifolium*, Carrodus (2009) found an average species genetic distance (Nei's 1978 unbiased genetic distance) of 0.4376. However, only one to three samples per taxon (each from a single population) were included in this portion of Carrodus's (2009) research, which is therefore limiting when drawing comparisons with this current research. High genetic differentiation is emphasized by the presence of 18 unique loci in the Poor Knights Islands individuals, 11% of total bands scored across *P. cornifolium* are unique to this single population.

While geographic isolation is perhaps the most significant factor influencing population-level genetic variation, ecological factors may also play an important role in the partitioning of genetic diversity, in particular the modes of dispersal and pollination. Field observations at sites in the Coromandel revealed entire

terrestrial communities of *P. cornifolium* confined to the area below canopy spans of single large kauri trees in which numerous epiphytic individuals were located above. This suggests that seed dispersal in these communities was initially by bird dispersal then largely by gravity dispersal. Such limited dispersal could significantly influence the genetic variation within and between populations by means of increasing the likelihood of pollination between closely related individuals (inbreeding), and limiting emigration to surrounding populations (reduced gene flow between populations). Similarly, individuals in the Raglan Harbour population were confined to a few small rock outcrops, dispersal by gravity is also a plausible explanation for such limited distribution in this system. Although no records of specific insect pollinators exist, *P. cornifolium* is thought to be entomophilous due to small flower size and absence of features that are adapted to pollination by birds (Webb *et al.* 1999). Pollen dispersal is limited in insect pollinated plants when compared to the distances travelled by wind dispersed pollen; the increase in dispersal distance is directly correlated with an increase in population differentiation due to higher levels of gene flow between unrelated individuals (Hamrick & Godt 1989; Loveless & Hamrick 1984).

2.4.3 ITS sequence divergence of the Poor Knights Islands variant

Hathaway's (2001) research into the phylogeny of the New Zealand *Pittosporum* found that *P. cornifolium* shared identical ITS sequences with both *P. pimeleoides* subspecies, this was also confirmed by the sequence alignment of these species in this research. Identical DNA sequences lead Hathaway (2001) to conclude that *Pittosporum cornifolium* and both *P. pimeleoides* subspecies are likely to be the result of a very recent colonization event to New Zealand. Hathaway (2001) suggests that this lineage is the result of colonization from New Caledonia. The concept of recent colonization in this grouping is especially compelling when compared with the main radiation of *Pittosporum* taxa in New Zealand which are estimated to be approximately 22 million years in age (Hathaway 2001). This estimate is consistent with the first appearance of *Pittosporum* in the fossil record (late Oligocene approximately 25 mya) (Oliver 1950).

Interestingly, additional alignment of two *P. cornifolium* individuals from the Poor Knights Islands population revealed a single point mutation (T>C) at 583

base pairs. This was an unexpected result, especially considering the identical ITS sequences of mainland *P. cornifolium* with both *P. pimeleoides* subspecies. Calculations by Wright *et al.* (2000) estimated that ITS sequence divergence rates in *Metrosideros* constituted 1bp mutation every 1.5 million years. Using this as a rough guide (not as a basis for a molecular clock) it is possible to estimate that colonization and subsequent sequence divergence was extremely recent i.e. in the last 1.5 million years. However, it is also important to note that while the level of molecular divergence is considered to be approximately correlated with time, it will not necessarily be consistent over time (Swoffort *et al.* 1996). In consideration of this, and the fact that the Poor Knights Island group have been isolated from mainland New Zealand for less than one million years, it is therefore hypothesised that sequence divergence in this offshore island variant would have a maximum age consistent with isolation – less than 1 million years. Inconsistent mutation rates are also perhaps an explanation as to why two morphologically distinct species do not express sequence level differentiation. This result is similar to findings by Gemmill *et al.* (2002) who reported 0% ITS sequence divergence between Hawaiian members of *Pittosporum*. The putative single colonisation onto the Hawaiian archipelago lead to rapid radiation and morphological diversification but no genetic divergence in the rapidly evolving ITS region.

2.4.4 Conservation, restoration and future study implications for *Pittosporum cornifolium*

In general, the first, most relevant action when endeavouring to conserve rare species and prevent extinction is habitat preservation (Templeton 1991). The importance of habitat preservation is emphasised by relatively high levels of genetic variation expressed across the different populations of *P. cornifolium*, thus each site is of extremely high conservation value. Fortunately, the majority of remaining *P. cornifolium* population sites surveyed in this research are protected and well managed. For example, Maungatautari is a 32 ha area of protected native forest which is being managed intensively to eliminate mammalian pest species. Similarly, sites such as the Poor Knights Islands (Hauraki Gulf), Coromandel, and most sites in the Taranaki region are protected and undergoing intensive pest management regimes. However, one of the most unique and rare

ecosystems, the calcareous rock outcrops of Raglan Harbour, does not have an official protected status, however, the isolated and rugged nature of this site may mean populations remain relatively undisturbed. Ex-situ conservation could be considered for this vulnerable Raglan population to preserve genetic legacy. To date, cuttings from the five largest individuals (to ensure minimal damage to individual plants) have been propagated at the University of Waikato glasshouse. While this is only a small subset of the genotypes in the current population, further collections can be made if required.

As we endeavour to preserve the scarce indigenous ecosystems that remain, the fact remains that the loss of biodiversity in New Zealand and at a global scale is severe and in dire need of restoration intervention. The sheer loss of lowland habitat in the Waikato region (Leathwick *et al.* 1995) and in the majority of ecological regions around New Zealand (McGlone 1989), has meant the historical range of *P. cornifolium* and its populations are largely depleted. In light of widespread ecosystem degradation, various independent and government funded restoration initiatives are emerging nationwide. An example of a high profile restoration project in the Waikato region is that of Waiwhakareke Natural Heritage Park in Hamilton city. The project aims to completely reconstruct a variety of native ecosystems once common in the Hamilton Basin (Clarkson & McQueen 2004). To ensure the long term health and viability of native populations, it is imperative that practitioners of restoration projects source native plants with respect to provenance and associated locally adapted ecotypes, ecological microhabitats, and the levels and structure of genetic variation in wild populations (Wilkinson 2001; Bischoff *et al.* 2010).

The significant correlation between geographic distance and population differentiation among populations of *P. cornifolium* highlights the importance of plant provenance and associated ecotypes when sourcing seed for restoration. The concept of ecological microhabitats is also important considering the many exhibited lifestyles of *P. cornifolium* in a range of lowland and coastal ecosystems (epiphytic, rupestral and terrestrial). Ideally, source sites would be both the closest to the restored site (to maintain provenance) and be a similar ecosystem to the one needed restoring. Using the Waiwhakareke project as an example, a key ecosystem to be restored is that of lowland mixed tawa–rimu forest, much like the

forest in Maungatautari (where *P. cornifolium* is commonly epiphytic), hence, seeds would be sourced from one of two close populations to account for local provenance, which has the equivalent lifestyle or ecological microhabitat (epiphytic). Furthermore, seeds should be collected from as many individuals as possible to maintain levels of genetic diversity similar to that of source populations (Ávila-Díaz & Oyama 2007).

Finally, the genetically distinct population structure and ITS sequence divergence of the Poor Knights Islands population raise some interesting questions with regard to species delineations. Such distinct genetic profiles found among island individuals and the fact that island populations are more vulnerable to extinction (as discussed above) merit a high conservation value. The observed genetic differentiation may warrant the delineation of new subspecies or even species, but a more comprehensive assessment (including extensive morphological and anatomical assessment of a larger subset individuals, and breeding experiments) will be required to determine the true status of these relationships with respect to other species and subspecies throughout the New Zealand *Pittosporum.*

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3 Chapter Three: Autecology of *Pittosporum cornifolium*

This chapter outlines aspects of autecology of *P. cornifolium* based on the results of a field survey of five mainland North Island populations, collection of microclimate data, and a compilation of herbarium, National Vegetation Survey databank (NVS), Land Use and Carbon Analysis System (LUCAS), and both published and unpublished records. *Pittosporum cornifolium* is considered distinctive as one of four indigenous shrubs well adapted to the epiphytic mode (Wardle 1991; Dawson 1993). However, to date little quantitative autecological information has been published on the species. The goal of this chapter is to characterise the autecology and habitat preferences of the species in order to inform conservation management.

3.1 Introduction

Autecology (species biology and ecology) is the study of the interactions of a single species with the living and non-living factors of its environment (Losos 2009). Good autecological information is a prerequisite for effectively managing species and their populations. Comprehensive autecological studies are lacking in New Zealand apart from a limited number on physionomically important trees e.g. rimu (Norton & Herbert 1988), beeches (Wardle 1984), kauri (Bergin & Steward 2004) and kahikatea (Stephens *et al.* 1999), and on threatened species such as *Pittosporum obcordatum* (Clarkson & Clarkson 1994), *Olearia hectorii* (Rogers 1996), *Clianthus puniceus* (Shaw & Burns 1997), *Hebe cupressoides* (Widyatmoko & Norton 1997), *Coprosma pedicellata* (Clarkson *et al.* 2003), and *Pittosporum patulum* (Rogers & Walker 2005). To date, no publication on the autecology of any New Zealand epiphyte species has been produced.

Comparative autecological studies are also important as they enable the ecologist to identify characteristics of life history and physiology which determine fitness (or lack of fitness) in particular habitats (Grime 1979). Similarly, with rare or uncommon species, comparative studies that contrast the biologies of rare taxa with those of related common ones are particularly valuable (Krunkeberg & Rabinowitz 1985). Many factors have been identified as contributing to the decline of threatened and local species. The most commonly mentioned are habitat loss and fragmentation, competition from invasive species, browsing by

introduced animals, and loss of pollinators or dispersers (Burgman 2002; Heywood & Iriondo 2003). These multiple factors interact over time at a range of scales, making causal relationships complex and difficult to determine (Didham *et al.* 2007).

The aim of the present research is to understand the aspects of the biology and ecology of *P. cornifolium* that determine population survival and success. The increasing threat of isolation, population reduction, and mammalian browse may result in severe ecological consequences for *P. cornifolium.* These ecological consequences may include restricted recolonisation, limited regeneration (resulting in skewed population structures), and uneven sex ratios, all of which increase the likelihood of local extinction (Gilpin and Soulé 1986; Soulé 1987; He *et al.* 2003). In recent years several observers in New Zealand have commented on the decline of rare and local species that are palatable to the introduced possum and specifically referred to *P. cornifolium* (Ravine 1995; Mitcalfe & Horne 2005).

Five North Island populations in Waikato (2), Coromandel (2), and Taranaki, encompassing the range of habitat from coastal to inland were sampled. The populations included all three known lifestyles of *P. cornifolium* i.e. epiphytic, rupestral, and terrestrial. The specific aims of the research were to quantify the ecological parameters to 1) estimate the current ecological health of these populations, 2) relate population attributes to wider data sets to provide a more comprehensive overview of *P. cornifolium* autecology, 3) characterise the microclimate of the plant's habitat, and 4) determine the biological distinctness of mainland individuals compared with Poor Knights Islands plants in order to determine the potential for separate taxonomic recognition. It is hypothesised that 1) the specialised nature of the lifestyles of *P. cornifolium* constrain population abundance and regeneration, and 2) isolated populations have developed distinct morphological and anatomical characteristics.

Determining the relative importance of limiting factors such as habitat range and specificity, browsing pressure and reproductive capacity was expected to inform the development of species recovery plans if required (for example see Townsend 1999: *Pittosporum patulum* recovery plan, 1999–2009). Collation of existing information and collection of new information was also expected to contribute

towards a publication for the New Zealand Biological Flora Series. Only eleven contributions have been completed in New Zealand to date, whereas in the British Isles more than 300 accounts of individual species have been published in the Journal of Ecology (British Ecological Society 2011).

3.2 Methods

3.2.1 Ecological survey

A field survey was conducted between November 2009 and September 2010 of the five mainland populations already described in Chapter 1: The 309 Road, Coromandel; Square Kauri, Coromandel; Maungatautari, Waikato; Raglan Harbour, Waikato; and Taranaki sites located in and around New Plymouth city.

The main techniques used to locate and measure *P. cornifolium* individuals were binocular inspection, tree climbing methods and by kayak. Once all possible individuals had been located, habitat and vegetation type were categorised, and host trees as well as direct species associations were listed. To inform population structure assessments, the height and width of all *P. cornifolium* individuals were estimated, and reproductive status noted. Plants were classified as male or female depending on the presence or absence of capsules. Later in the year during the flowering season (August and September*)* sex ratios were reassessed at the Raglan Harbour and Square Kauri sites by inspecting flower structures.

Detailed information was obtained for 99 individuals (309 Road: 24; Square Kauri: 18; Maungatautari: 9; Raglan Harbour: 25; Taranaki: 23) and host species information collected for an additional 11 individuals (309 Road: 4; Square Kauri: 7).

3.2.2 Herbarium records and supplementary data

Herbarium records of *P. cornifolium* were requested from the Allan Herbarium (NZCHR), Auckland Museum Herbarium (AK), Waikato University Herbarium (WAIK), Forest Research Institute Herbarium (NZFRI) and Te Papa Herbarium (WELT). Some 186 database records of variable quality were obtained. Limitations included lack of information on the specific host, vegetation type, and

whether the plant was of terrestrial, rupestral or epiphytic lifestyle. As noted later, other data sources including published and unpublished records were used to supplement this data where required e.g. distribution, phenology etc. Supplementary data sets were obtained from the National Vegetation Survey databank (NVS), Land Use and Carbon Analysis Systems (LUCAS), and both published and unpublished records from reliable field botanists to aid in the production of maps of observed distribution and predicted distribution.

3.2.3 Microclimate

Hygrochron Temperature and Humidity iButtons (hence forth data loggers) were first calibrated for relative humidity (RH) and temperature and then deployed at 3 of the 5 mainland sites; Square Kauri, Raglan Harbour, and Maungatautari. These sites were chosen to cover a wide range of lowland systems but also to compare the microclimates of the three distinct lifestyles of *P. cornifolium*: rupestral (Raglan Harbour), terrestrial (Square Kauri), and epiphytic (Maungatautari). Two data loggers per site were emplaced either on the ground or up on the host branches to best characterise the actual habitat in which *P. cornifolium* plants grew (internal data loggers). At each site another data logger was placed just outside the forest or shrubland perimeter in an open/ exposed site as a comparative control (exposed data loggers). To provide more accurate temperature data, data loggers were placed in solar radiation shields. Internal data loggers were moved once every two months to new positions where different *P. cornifolium* individuals were growing to ensure the data set entailed a range of site specific microclimates. Calibration for RH entailed logger rotation through four desiccation chambers of known RH and constant temperature using NaCl, KCl, MgCl and $Mg(NO_3)_2$. For temperature calibration, the loggers were moved between two consistent temperatures of 36°C and 4°C. From these results one logger was selected as a standard for both temperature and RH and all others were plotted against the results from this standard and fitted with a trend line. The slope and intercept of each trend line were used to correct all subsequent results.

3.2.4 Leaf morphology

In total, 39 individuals were sampled from field collections, glass house and shade house cultivated specimens, and herbarium vouchers. On each individual 10 fully

grown leaves were randomly selected and leaf length and width measured and recorded. Observations on leaf shape were also recorded.

3.2.5 Anatomy

Initially, anatomical comparisons were conducted on two *P. cornifolium* individuals; one mainland form and one Poor Knights Islands form (from the University of Waikato glasshouse collection). Comparisons of tissue layers of leaves, stems and roots were made by transverse hand section using toluidine blue stain. Initial results prompted further investigation into comparative leaf anatomies of mainland and Poor Knights Islands forms. Twelve individuals from three localities (Poor Knights Islands, Raglan, and Waitakere Ranges – 4 individuals per locality) were then propagated from cutting and grown in a controlled glasshouse environment to counteract potential range in environmental plasticity. After approximately 8 months of growth, the first fully expanded leaf from each individual was selected for sectioning. Transverse sections were cut at the centre of the leaf and the differing tissue layers of the leaf midrib were recorded for comparison.

3.3 Data analysis

All data collected were entered into a Microsoft Excel spreadsheet for subsequent analysis. Descriptive statistics, graphs and histograms were used to explore and interpret patterns in the data.

3.3.1 Population (life stage) structure

Height and width estimates of *P. cornifolium* individuals obtained from the ecological survey were multiplied to give a single cover value in square metres. These values in conjunction with reproductive status were explored to determine the best way of interpreting population (life stage) structure. Seedlings were defined as individuals less than 0.02m², this size is indicative of recent germination (<12 months old) and individuals were reproductively immature. Juveniles consisted of individuals ranging from 0.02m² to 0.25m², two out of 18 individuals were confirmed as having reached reproductive maturity so this size

class can only be considered a general representation of juveniles in populations. The final category ‘adult’ included all individuals of more than 0.25m² cover.

3.3.2 Herbarium records and supplementary data

Herbarium records were reviewed to determine which data elements were of use for further analysis, and data that was sufficient was amalgamated with the field survey data. A conservative approach was taken to any information that was ambiguous or incomplete and it was not used. As a result, the sample sizes reported below vary for different data elements.

Pittosporum cornifolium locations were overlain on Land Environments of New Zealand (LENZ) environmental surfaces (Leathwick *et al.* 2003) to obtain minimum and maximum values of selected environmental variables in ARCVIEW ver. 3.2. Environmental variables included total annual rainfall (r), mean October vapour pressure deficits at 0900 hours (vpd), mean annual temperature (mat), mean minimum daily temperature of the coldest month (tmin), elevation, mean annual solar radiation (mas), and mean minimum daily solar radiation in June (junes). Using these variables the spatial expression of minimum and maximum values were mapped in ARCVIEW ver. 3.2, and summary statistics from these values were tabulated in Microsoft Excel 2007.

3.3.3 Microclimate

Mean daily averages of temperature and vapour pressure deficits were calculated for external readings, and combined averages for internal readings, at each population. From these results, seasonal means were calculated. The averaged total data sets of internal loggers at each site as well as external data sets were used to compare minimum and maximum ranges for temperature and vapour pressure deficits between internal and external microclimates.

3.3.4 Leaf morphology and anatomy

Leaf length and width measurements were averaged for each individual and used to construct a scattergram. The scattergram was inspected to determine whether the variation in leaf size was continuous or clumped. Tables of leaf tissue depth

per individual and mean leaf tissue depth per locality were constructed and reviewed.

3.4 Results

3.4.1 Distribution

The 186 herbarium records spanned the range of *P. cornifolium* distribution from Northland to the West Coast of the South Island but grid references were available only for 65 specimens. These records were supplemented with 37 records (GPS readings) from my field survey, 76 records from the National Vegetation Survey databank (NVS), 4 records from Land Use and Carbon Analysis System (LUCAS), and a further 47 published and unpublished records from reliable field botanists to produce a distribution map showing 229 unique locations (Figure 3.1).

Pittosporum cornifolium is distributed widely in the coastal and lowland zones of the North Island. It also occurs on numerous northern offshore islands, including the Poor Knights Islands, Hen Island, Little Barrier Island, Great Barrier Island, Kawau Island, and Waiheke Island. In the South Island, *P. cornifolium* is restricted to Marlborough and the West Coast and its southern limit is near Barrytown (Figure 3.1).

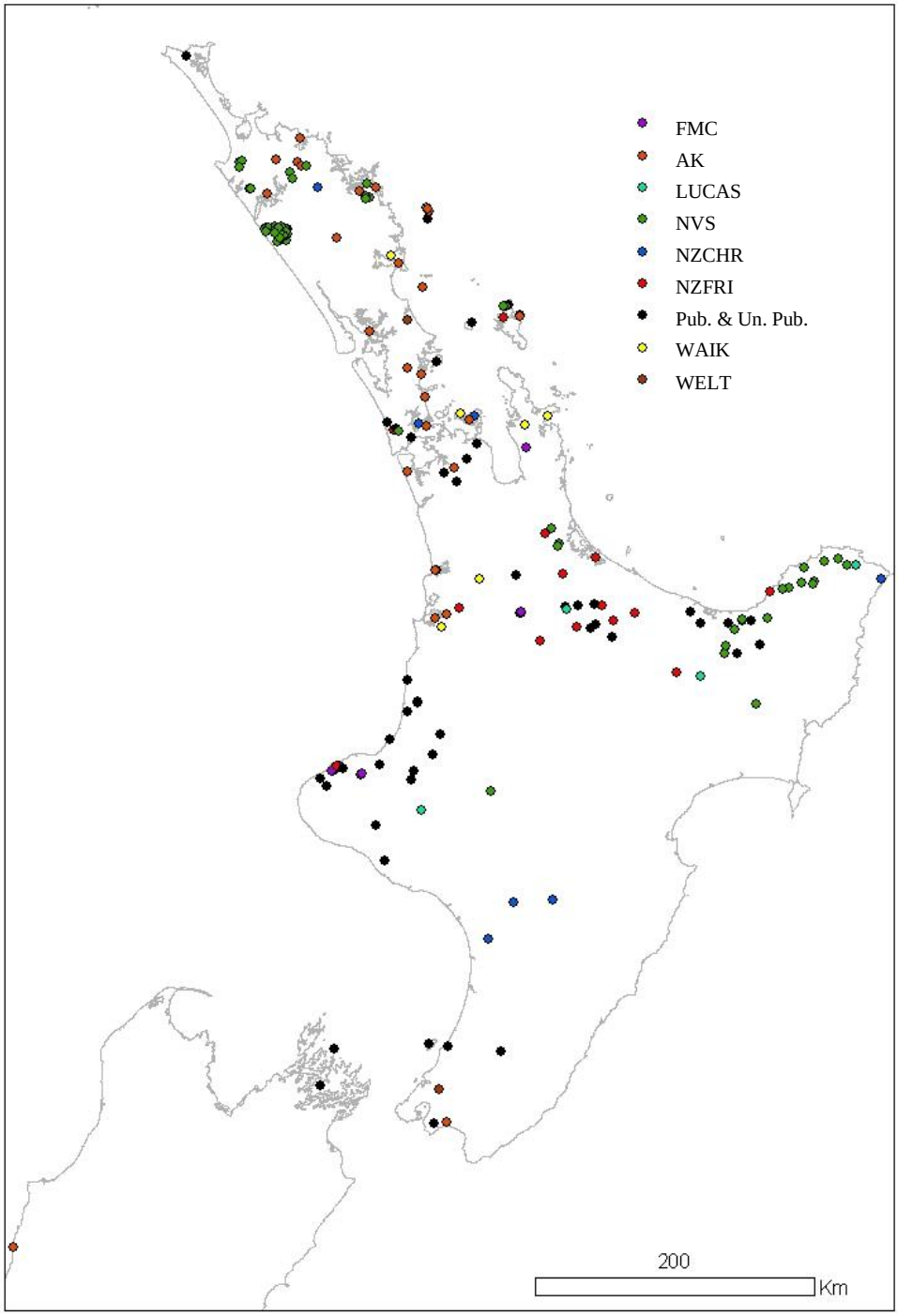

Figure 3. 1 Observed distribution of *Pittosporum cornifolium* based on plot locations obtained from field survey results (FMC), Auckland herbarium (AK), Land Use and Carbon Analysis Systems (LUCAS), National Vegetation Survey data bank (NVS), Christchurch herbarium (NZCHR), New Zealand Forest Research Institute (NZFRI), Published and unpublished records from field botanists (Pub. & Un. Pub.), Waikato herbarium (WAIK), and Wellington herbarium (WELT).

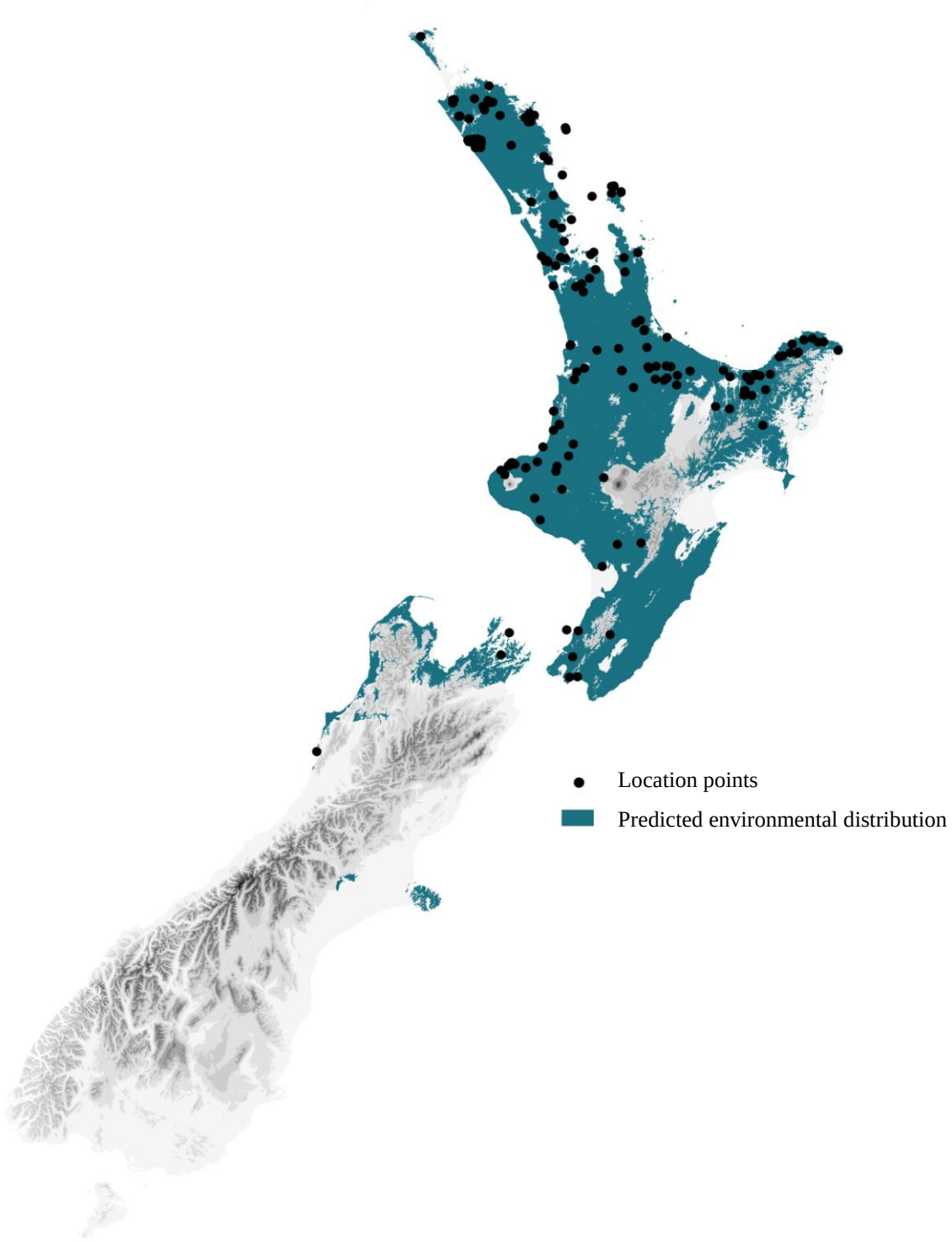

Figure 3. 2 Predicted environmental distribution of *Pittosporum cornifolium* based on environmental variables including total annual rainfall, mean October vapour pressure deficits at 0900 hours, mean annual temperature, mean minimum daily temperature of the coldest month, elevation, mean annual solar radiation, and mean minimum daily solar radiation in June.

3.4.2 Predicted environmental distribution

Thirty-three unique locations obtained from field survey results were added to a supplementary data set sourced from herbarium records (n=62), published and unpublished records from reliable field botanists (n=46), the National Vegetation Survey data bank (NVS) (n=76) and Land Use and Carbon Analysis systems (LUCAS) (n=4). Eight data points positioned close to the coast line in the distribution map (Figure 3.1) were excluded from the predicted environmental distribution map (Figure 3.2) as these points did not produce any environmental variables when overlain on environmental surfaces. From 221 data points the predicted environmental distribution of *P. cornifolium* was mapped and summary statistics of environmental variables were tabulated (Figure 3.2; Table 3.1). Mean annual rainfall (rain) across all sites was 1841.7 mm (1SD=380.9 mm) and ranged from 937–3321 mm. Mean October vapour pressure deficits at 0900 hours (vpd) across all sites were 0.3 kPa (1SD = 0.04 kPa) with a range of 0.2–0.5 kPa. The mean annual temperature of combined plots was 13.2 °C (1SD = 1.2 °C) with a range from 9.6–15.7 °C. Mean minimum daily temperature of the coldest month (tmin) across all sites was 5°C (1SD = 1.7 °C) with a range of 0.6–8.7. The mean altitude (elevation) was 247.8 m (1SD = 178.1 m) and ranges from 1–786 m a.s.l. Mean annual solar radiation was 14.8 kJ (1SD = 0.3 kJ) with a range of 13.1–15.5 kJ. Mean minimum daily solar radiation in June was 5.9 kJ (1SD = 0.6 kJ) 4.2–7.1 kJ. The predicted environmental distribution of *P. cornifolium* excludes several sites in the North Island including a large portion of the East Coast, the Central North Island volcanoes, the Kaimanawa and Ikawhenua Ranges, the Wairoa lowlands, Mt Taranaki, and the majority of Northland north of Kaitaia (Figure 3.2). The predicted environmental distribution of *P. cornifolium* is extremely limited in the South Island but includes Marlborough, the West Coast just south of Barrytown and Banks Peninsula on the East coast (Figure 3.2). The environmental variable that most determined predicted absence of environmental distribution was tmin, which excluded the majority of the South Island and was the sole variable in excluding the central North Island uplands. Vpd was also important and solely responsible for excluding the Wairoa, Gisborne and Waiapu lowlands on the East Coast of the North Island. Lower temperatures (both mat and tmin) in combination with higher altitude restricted predicted distributions for North Island mountains. Large areas in the South Island were restricted by not

only minimum temperatures but by a combination of mean annual temperature, mean annual rainfall, solar radiation, and elevation.

Table 3. 1 Summary statistics of environmental variables from known plot locations of *Pittosporum cornifolium*. Environmental variable include: total annual rainfall (r), mean October vapour pressure deficits at 0900 hours (vpd), mean annual temperature (mat), mean minimum daily temperature of the coldest month (tmin), elevation, mean annual solar radiation (mas), and mean minimum daily solar radiation in June (junes).

	rain (mm)	vpd (kPa)	mat (°C)	tmin (°C)	elevation (m)	mas (kJ/m²/day)	junes (MJ/m²/day)
Mean	1841.7	0.32	13.2	5.0	247.8	14.8	5.9
Standard Error	25.6	0.00	0.1	0.1	12.0	0.0	0.0
Median	1822.0	0.33	13.4	5.6	229.0	14.8	6.0
Mode	1362.0	0.35	13.6	5.8	220.0	14.7	6.3
Standard Deviation	380.9	0.04	1.2	1.7	178.1	0.3	0.6
Range	2384.0	0.24	6.1	8.1	785.0	2.4	2.9
Minimum	937.0	0.23	9.6	0.6	1.0	13.1	4.2
Maximum	3321.0	0.47	15.7	8.7	786.0	15.5	7.1
Count	221.0	221.0	221.0	221.0	221.0	221.0	221.0

3.4.3 Life style

Field survey *P. cornifolium* (*n* = 110) exhibited three distinct lifestyles (Figure 3.3): epiphytic (49), terrestrial (37) and rupestral (24).

The population in the rimu/tawa lowland forest of the Southern Enclosure at Maungatautari comprised entirely epiphytic plants, growing between 5 and 20 m above the ground (Table 3.2) and was found in the branches of large emergents (*Dacrydium cupressinum* and *Knightia excelsa*) and canopy trees (*Beilschmiedia tawa*, *Laurelia novae-zelandiae* and *Litsea calicaris*). Despite extensive searching, no individuals were located in similar but younger forest in the Northern Enclosure of Maungatautari.

The lowland forest patches located in and around New Plymouth city, Taranaki, comprised mixed broadleaved, tawa–broadleaved, kahikatea–tawa dominant, and swamp maire forest. All except one plant recorded in Taranaki were growing epiphytically (between 1 and 20 m above the ground; Table 3.2). The single terrestrial individual found was the result of a recent (in the last few years based

on plant condition) fall. The most common host trees across all Taranaki sites were emergent rimu, which accounted for 45% (10/22) of total host trees surveyed. Individual emergent rimu were recorded hosting up to four *P. cornifolium* plants. Other host trees consisted of a mix of podocarp and broadleaved species, which were all large canopy or emergent individuals.

At the northern mouth of Raglan Harbour *P. cornifolium* was found as a rupestral (*n* = 24) in shrubland communities restricted to a 1.5km stretch of limestone rock stacks, disconnected from the mainland. These communities are co-dominated in parts by *P. cornifolium*, with other dominant species including *Astelia banksii* and *Griselinia lucida*. The harbour fringe supports occasional wind-shorn *Vitex lucens*, which sometimes host epiphytic *P. cornifolium*. The single epiphytic *P. cornifolium* individual measured was at a height of 10 m (Table 3.2) on the host tree, *V. lucens.*

In the lowland old growth kauri forests of the Coromandel (The 309 Road and Square Kauri), two lifestyles, terrestrial (*n* = 36) and epiphytic (*n* = 17) were both well represented (Table 3.2). The terrestrial plants were found mainly on steep ridges in association with *Astelia trinervia* and *Brachyglottis kirkii*. Epiphytic individuals were not confined to kauri, but were also found growing directly on the trunks of the tree ferns; *Cyathea dealbata* and *Cyathea cunninghamii*. Searches in the adjoining regenerating second growth forest failed to detect any additional populations.

Herbarium data with adequate information on *P. cornifolium* lifestyle (n = 92) revealed 58 were epiphytic, 15 were terrestrial and 19 were rupestral. Terrestrial plants were recorded in a wider range of settings notably pohutukawa forest, hard beech forest and kamahi–tawari forest, while rupestral forms were found on Waiheke Island, Rakitu Island, and the Poor Knights Islands on greywacke and volcanic rock.

The overall lifestyle statistics were 107 (53%) epiphytic, 52 (26%) terrestrial, and 43 (21%) rupestral.

Figure 3. 3 Images displaying the range of lifestyles and growing substrates of *Pittosporum cornifolium*. Clockwise from top left: epiphytic individual growing from the base of nest epiphyte *Astelia solandri* lodged on the trunk of a host tree, epiphytic individual growing from the trunk of a tree fern, rupestral individuals growing in clumps of *Astelia banksii* on limestone rock out-crops, terrestrial individual growing on detritus soils of a kauri forest.

Table 3. 2 *Pittosporum cornifolium* habitat location from ground (height class). individuals recorded <1m were rupestral in Raglan Harbour populations and terrestrial in all other populations with the exception of 1 low lying epiphytic individual in Taranaki. All individuals located above 1m from ground were epiphytic.

Height class (m)	Maungatautari	Square Kauri	309 Road	Raglan Harbour	Taranaki	Total
< 1	0	18	21	24	2	65
1–5	0	0	3	0	2	5
5–10	2	0	0	1	9	12
10–15	5	1	0	0	7	13
15–20	2	3	4	0	3	12
20–25	0	2	0	0	0	2

3.4.4 Vegetation type

Field survey *Pittosporum cornifolium* were recorded in 11 vegetation types (Table 3.3), all of which were forest except for rupestral shrubland. The three most common types were kauri forest, confined to the Coromandel sites, rupestral shrubland, confined to Raglan Harbour, and rimu/tawa forest found at Maungatautari and Taranaki. Herbarium data with adequate vegetation type information (n = 142) revealed 20 different vegetation types including 9 not encountered in the field survey. Overall, this did not alter the rank order of preferred vegetation type but did reveal a wider range of types including pohutukawa forest, hard beech forest, kanuka-manuka scrub, and matai dominated forest.

Table 3. 3 Vegetation types containing *Pittosporum cornifolium*

Vegetation types	Count
kauri forest	58
rupestral shrubland	30
rimu/tawa forest	16
Tawa-broadleaved forest	8
pohutukawa forest	4
Titoki-pukatea forest	3
Tawa-mixed podocarp forest	3
puriri forest	3
Pukatea-tawa forest	3
hard beech forest	2
Matai-titoki forest	2
kanuka-manuka scrub	2
swamp maire forest	1
Alder-willow forest	1
mangeao-tawa forest	1
hinau/kanuka forest	1
northern rata/tawa forest	1
Kamahi-ixerba forest	1
Tawa-taraire forest	1
Broadleaved-conifer forest	1

3.4.5 Host trees

The tall often emergent tree species, kauri, rimu, and pukatea were the most common hosts for the epiphytic individuals (n = 49) of *P. cornifolium* in the field survey (Figure 3.4). The herbarium data that recorded the host plant (*n* = 35)

included 20 tree species and one sedge *Carex secta*, which has a fibrous short trunk.

The total data (*n* = 84) confirmed the importance of pukatea (15), rimu (12), and kauri (11) as the main hosts, but also revealed northern rata (10), tawa (7), puriri (5) and titoki (3) as important hosts (Figure 3.5).

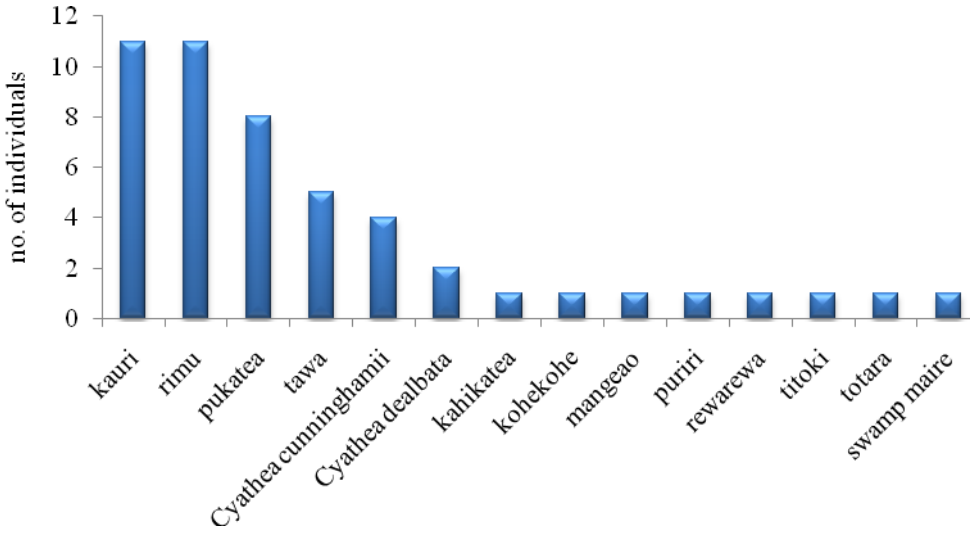

Figure 3. 4 Host tree species of *Pittosporum cornifolium* field survey records

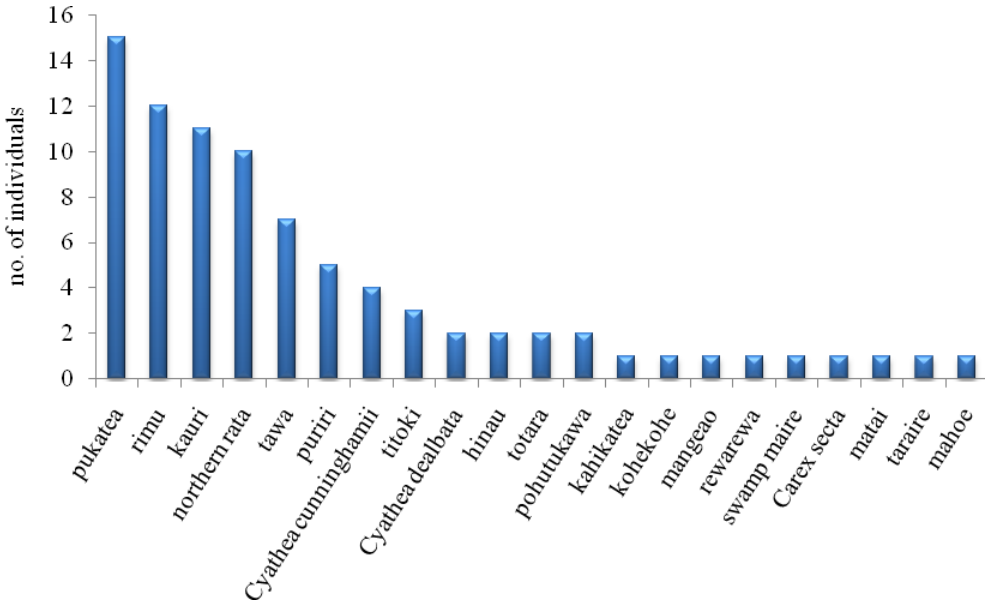

Figure 3. 5 Host tree species of *Pittosporum cornifolium* field survey and herbarium records

Table 3. 4 *Pittosporum cornifolium* host tree height means, minimums and maximums across five North Island populations

	The 309 Road	Square Kauri	Raglan Harbour	Maungatautari	Taranaki	Combined
Mean	18	40	16	21	21	23
Min.	2	40	16	14	14	2
Max.	40	40	16	25	25	40

Table 3. 5 *Pittosporum cornifolium* host tree diameter at breast height means, minimums and maximums across five North Island populations

	The 309 Road	Square Kauri	Raglan Harbour	Maungatautari	Taranaki	Combined
Mean	98	290	60	128	146	152
Min.	7	290	60	50	42	7
Max.	250	290	60	205	202	290

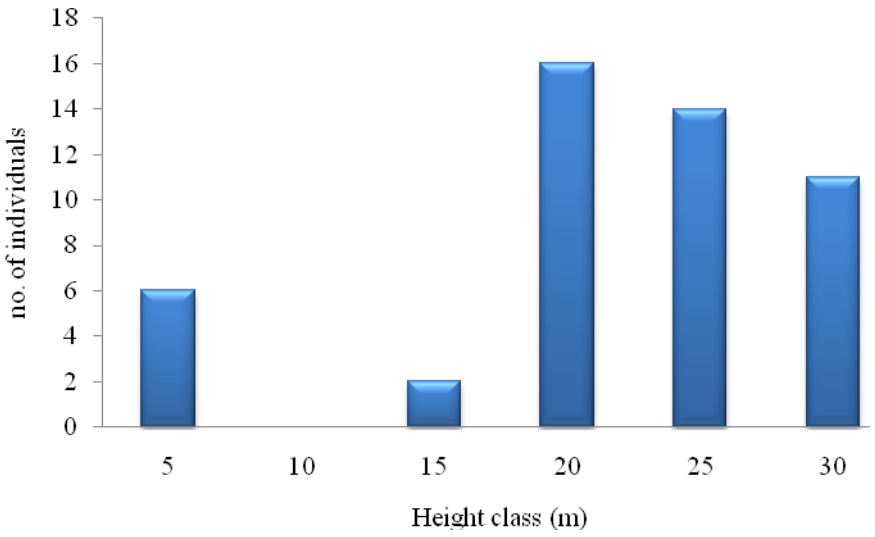

Figure 3. 6 Number of *Pittosporum cornifolium* host trees in differing height classes

Host heights and diameters (at breast height) were also recorded in the field survey in order to determine whether there was any relationship with host tree size. The mean host height and diameter for trees recorded within each mainland population is presented in Tables 3.4 & 3.5, and host heights across all populations are presented in Figure 3.6. Mean host tree height and diameter ranged from 16–40 m and 60–290 cm respectively, and the majority (83%) of host trees were found within the higher height ranges >15 m (Figure 3.6). Within the kauri forest of Coromandel, the majority of epiphytic individuals were found growing in large emergent kauri trees. Kauri trees at The 309 Road and Square

Kauri sites yielded the maximum height and diameter values recorded (40 & 40 m; 250 & 290 cm, respectively). Mean height and diameter in The 309 Road population were considerably lower than these maximums (18 m; 98 cm) due to 6 epiphytic individuals being recorded on tree ferns. The tree fern species *Cyathea dealbata* and *Cyathea cunninghamii* are of smaller stature and reside in sub canopy tiers of lowland forest systems. These represented the minimum height value of 2 m and the minimum diameter of 7 cm (Tables 3.4 & 3.5). Relatively high mean height and diameter measurements were recorded for hosts within the Southern enclosure of Maungatautari (21 m; 128 cm), the majority of these host species were either large canopy or emergent individuals. Emergent rimu were by far the most common host species in Taranaki sites accounting for 45% of all host trees surveyed. Like Maungatautari, the majority of individuals at Taranaki sites were found in large canopy or emergent trees. In Raglan Harbour only one epiphytic *P. cornifolium* was recorded, growing from a puriri (16 m height; 60 cm diameter) adjacent to rock outcrops (Tables 3.4 & 3.5).

3.4.6 Substrate

While *P. cornifolium* was associated with different host trees it was never recorded directly attached to host tree trunk or bark. Instead, *P. cornifolium* individuals (n = 110) were directly rooted in three types of substrate; nest host, ground detritus and tree fern trunk. Nest hosts included nest epiphytes *Collospermum hastatum* (40) and *Astelia solandri* (4), and the rupestral species *Astelia banksii* (21). Nest hosts were the most common substrate and accounted for 52% of the total individuals surveyed (Figure 3.7), these included the majority of epiphytic, and all rupestral individuals surveyed. Ground detritus accounted for all the terrestrial individuals growing beneath kauri forest in the Coromandel populations. The final, least common category was 'tree fern trunk' and accounted for individuals located in the Coromandel populations, which were found growing directly from the trunks of tree fern species *Cyathea cunninghamii* (7) and *Cyathea dealbata* (3).

As well as the nest hosts and tree fern trunks, to which *P. cornifolium* was directly attached, some 21 additional species were recorded as being in close contact. The

most frequent were *Metrosideros fulgens* (9), *Astelia trinervia* (8), *Asplenium polyodon* (7), *Brachyglottis kirkii* (6), and *Microsorum pustulatum* (5).

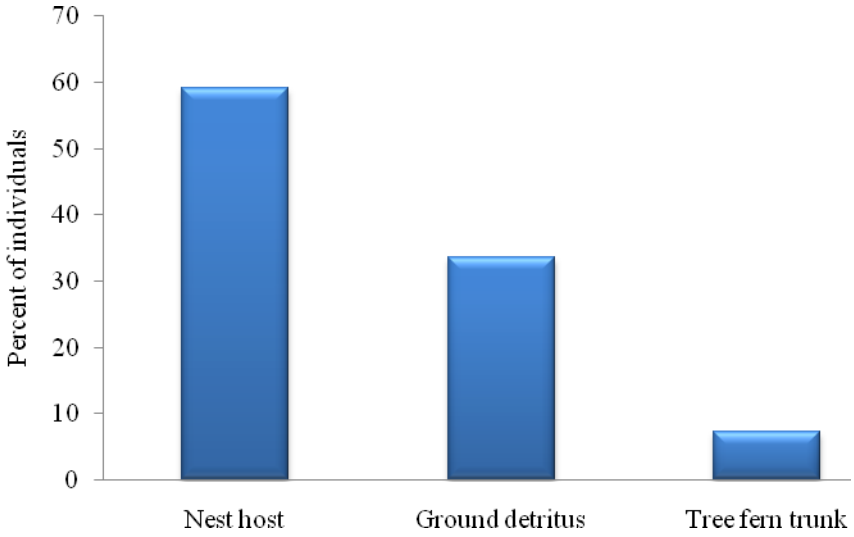

Figure 3. 7 Growing substrate of *Pittosporum cornifolium* from five North Island populations

3.4.7 Population (life stage) structure

The five populations displayed a range of life stage structures (Figure 3.8). Only two of the populations exhibited recent regeneration (Square Kauri and The 309 Road), with a total of 5 seedlings (20% of population) present in the Square Kauri population, and one seedling (4% of population) present in The 309 Road population (Table 3.6, Figure 3.8). Of the remaining three populations, only Raglan Harbour had individuals in the juvenile size class, while the remaining two populations, Maungatautari and Taranaki, were entirely composed of adult individuals (Table 3.6, Figure 3.8).

Table 3. 6 Life stage structure of five North Island *Pittosporum cornifolium* populations

Population	Seedling	Juvenile	Adult	Total
The 309 Road	1	8	15	24
Square Kauri	5	3	13	21
Raglan Harbour	0	4	21	25
Maungatautari	0	0	9	9
Taranaki	0	0	23	23

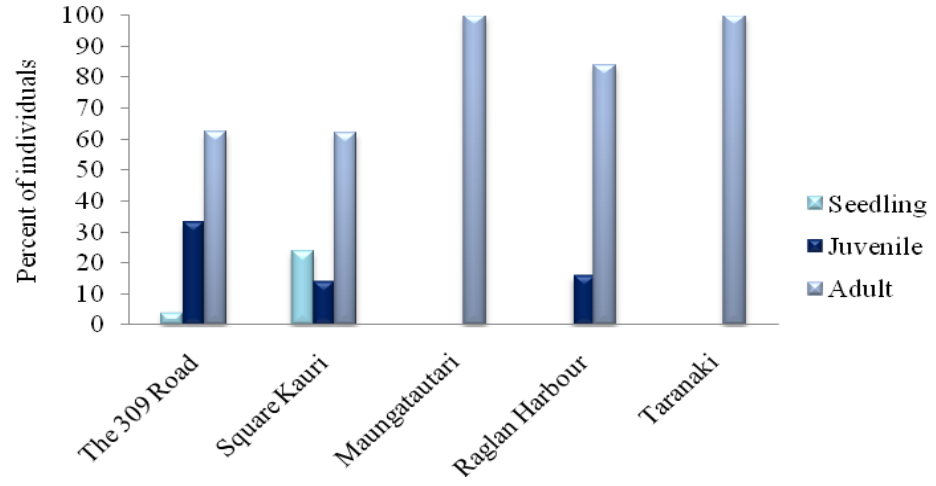

Figure 3. 8 Life stage structure of five North Island *Pittosporum cornifolium* populations

3.4.8 Phenology

A combination of my own field data, herbarium records, and observations of other field botanists was compiled (n=105) to produce a phenology calendar (Figure 3.9).

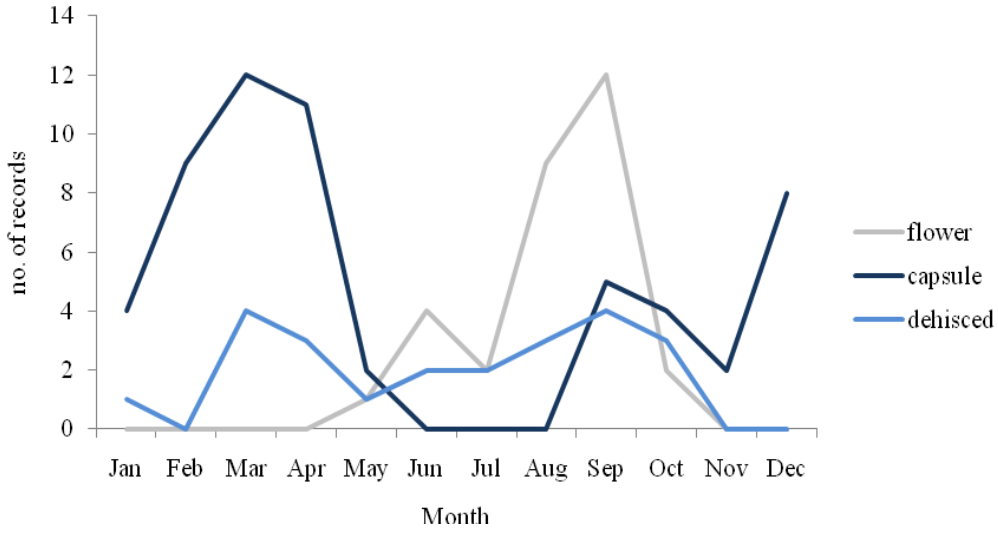

Figure 3. 9 Phenology calendar of *Pittosporum cornifolium*

The number of records for most months appears to be adequate, but is poor for November. *Pittosporum cornifolium* flowers from May to October with peak flowering in September and an abrupt cessation of flowering. Observations (2009–2010) from my glasshouse collection showed that the mainland plants flowered earlier (early July to late September) while the Poor Knights Islands plants flowered later (mid August to late October). Capsules can be found on

female plants all year round and often green capsules are present from the current flowering year together with dehisced capsules from the previous year.

3.4.9 Sex ratios

Four out of the five mainland populations had a higher number of male individuals, while only one population, Square Kauri, had a higher proportion of female individuals (Figure 3.10). The combined sex ratio of all mainland populations was 39 female: 61 male (Figure 3.10). It should be noted that while individual plants are typically dioecious, cuttings from an apparently dioecious male plant have produced infrequent capsules (University of Waikato Glasshouse collections). Godley (1979) has described this 'inconstant male' trait among other dioecious genera in New Zealand.

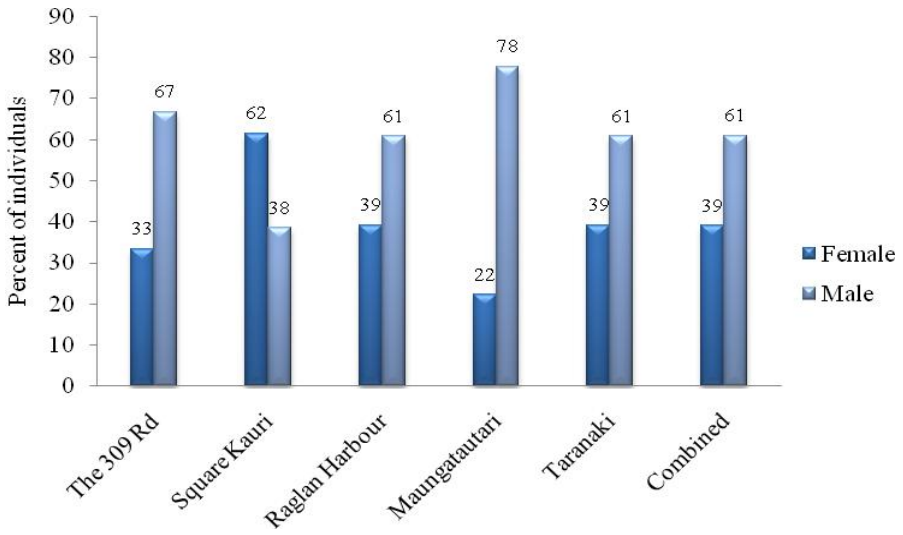

Figure 3. 10 Sex ratios of five North Island *Pittosporum cornifolium* populations

1.3.2 Microclimate

The data logger results indicate that, of the three mainland populations investigated, Raglan had both the highest mean temperatures (Figure 3.11) and highest mean vapour pressure deficits (Figure 3.12) across all three seasons. Canopy loggers at Maungatautari consistently recorded the lowest mean temperatures across all three seasons, and while mean vapour pressure deficits across autumn and winter were also the lowest recorded means, spring vapour pressure deficit were relatively high with Coromandel populations expressing the lowest.

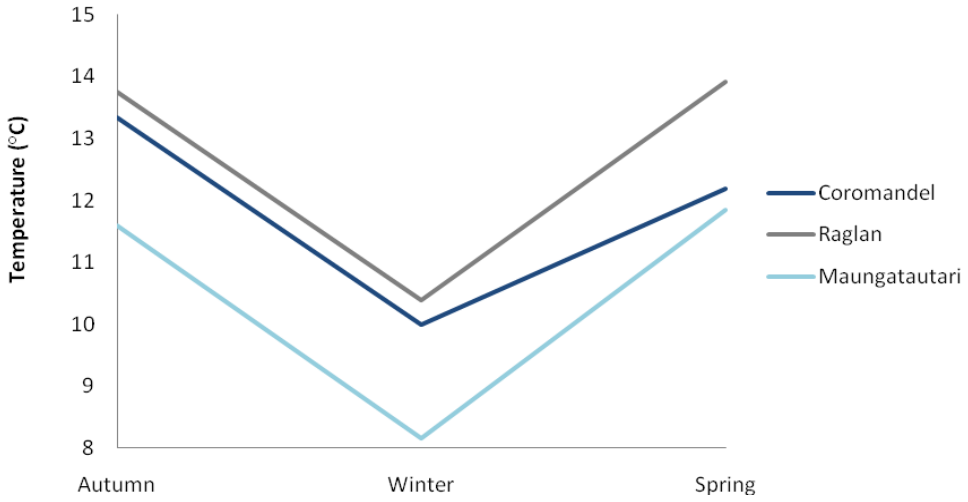

Figure 3. 11 Mean temperature of three *Pittosporum cornifolium* population sites over three seasons, North Island.

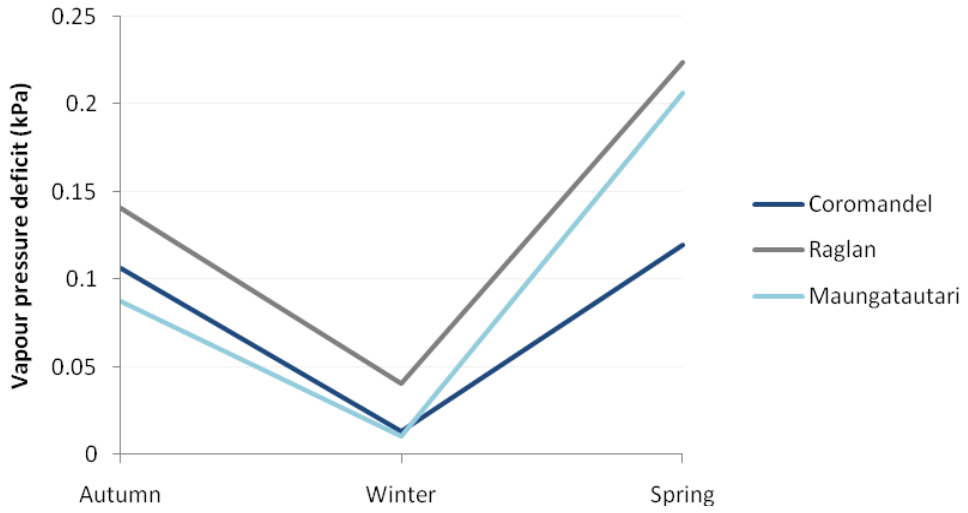

Figure 3. 12 Mean vapour pressure deficits of three *Pittosporum cornifolium* population sites over three seasons, North Island.

When comparing interior sites to exterior/ exposed sites both overall temperature and vapour pressure deficit means were higher than the means of their associated interior sites (Tables 3.7 & 3.8). Figure 3.13 shows exterior sites have consistently higher maximums and lower minimums for total temperature data sets when compared with associated interior sites. Similarly, exterior sites demonstrate higher maximum vapour pressure deficit from total data sets when compared with relevant interior sites (Figure 3.14). The total range for the majority of the data (25–75%) is wider in exterior sites than associated interior sites (Figures 3.13 & 3.14).

Table 3. 7 Comparison of exterior and interior temperatures (°C) for three *Pittosporum cornifolium* population sites in the North Island over three seasons.

	Coromandel		Raglan		Maungatautari	
	Int.	*Ext.*	*Int.*	*Ext.*	*Int.*	*Ext.*
Mean	11.51	11.96	12.45	12.46	10.30	10.55
Minimum	2.75	2.00	0.36	0.05	0.69	0.28
Maximum	23.41	28.46	23.59	24.45	24.37	25.91

Table 3. 8 Comparison of exterior and interior vapour pressure deficits (kPa) for three *Pittosporum cornifolium* population sites in the North Island over three seasons.

	Coromandel		Raglan		Maungatautari	
	Int.	*Ext.*	*Int.*	*Ext.*	*Int.*	*Ext.*
Mean	0.07	0.13	0.13	0.18	0.10	0.12
Minimum	-0.08	-0.08	-0.07	-0.07	-0.06	-0.08
Maximum	1.30	1.86	1.19	1.41	1.60	1.97

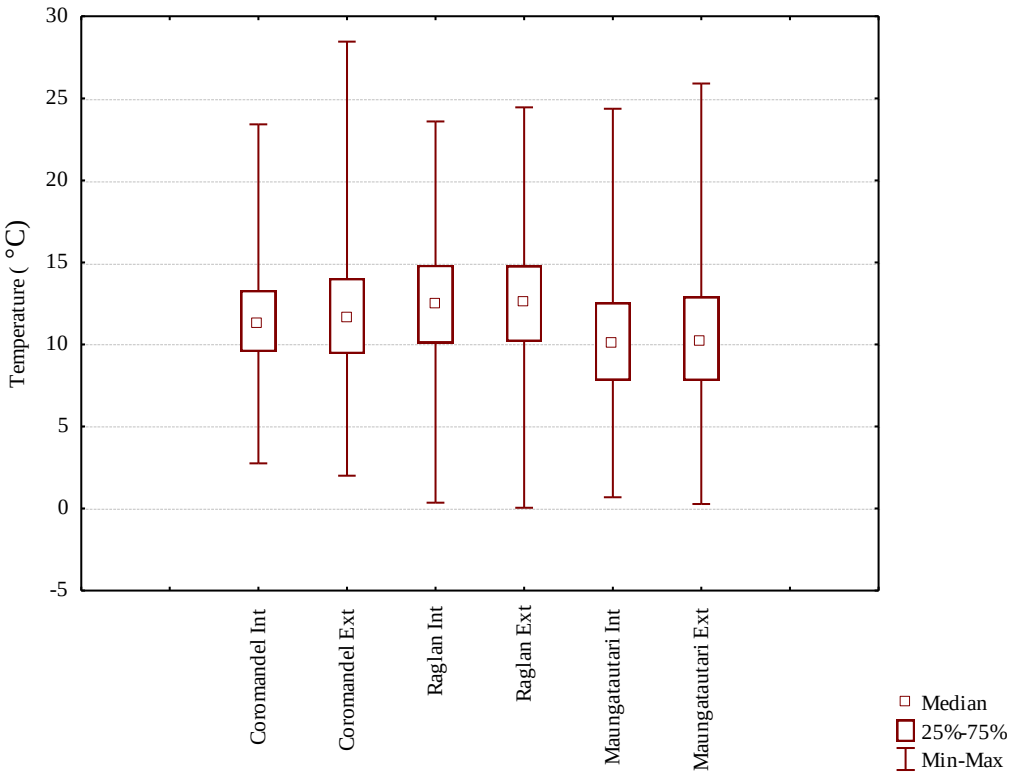

Figure 3. 13 Comparison of exterior and interior temperatures for three *Pittosporum cornifolium* population sites, North Island.

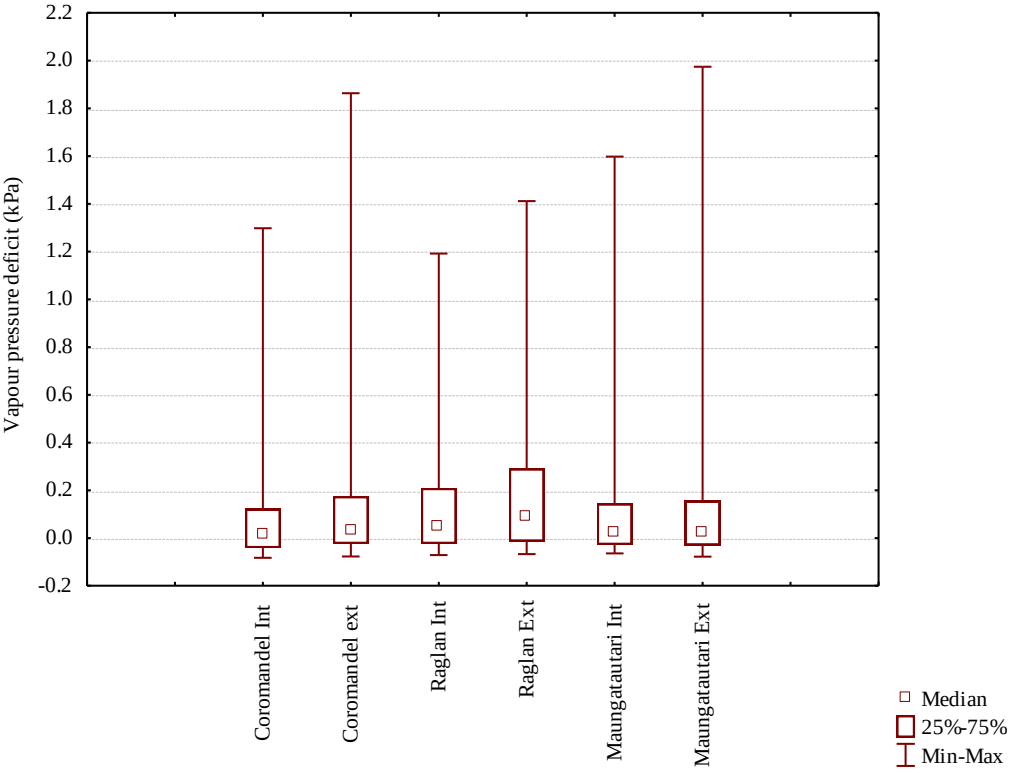

Figure 3. 14 Comparison of exterior and interior vapour pressure deficits for three *Pittosporum cornifolium* population sites, North Island.

3.4.10 Leaf morphology

Pittosporum cornifolium leaf length and width data shows continuous variation from smaller mainland individuals at a wide range of sites to larger off shore island individuals entirely from the Poor Knights Islands (Figure 3.15). The Poor Knights Islands individuals are distinguished mainly by their mean width which ranges from 27.1–36.7 mm, but also by their generally greater length ranging from 53.7– 0.8mm (Figure 3.15). Mainland individuals leaves were mostly shaped elliptic-lanceolate to obovate (acute to subacuminate at apex, and acute to obtuse at base) as described previously by Cooper (1956). While Poor Knights Islands individuals leaves appear more obovate to rhomboid (subacuminate to obtuse at apex, and acute to obtuse at base).

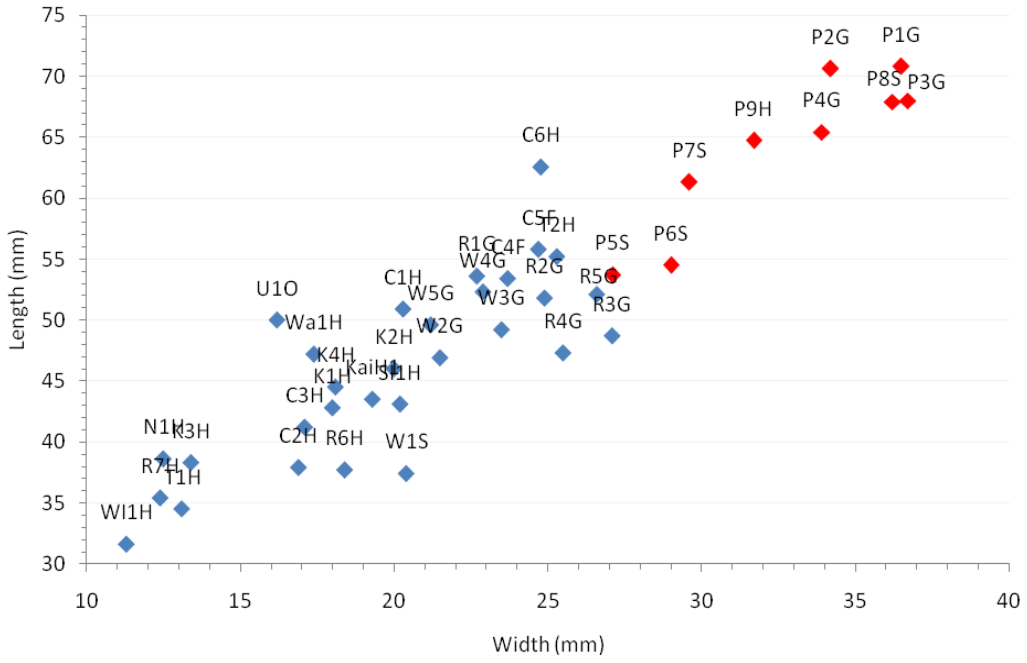

Figure 3. 15 Length and width comparisons of *Pittosporum cornifolium* leaves (blue = mainland individuals; red = Poor Knights Islands individuals). First letter/ letters represent original source of individual; C; Coromandel, K; Kawhia, Kai; Kaipara, N; Whangarei, P; Poor Knights Islands, R; Raglan, SI, Barrytown (South Island), T; Tairua, U; University of Waikato, W; Waitakere filter station, WI; Waiheke Island. Number represents number of samples per source. Last letter represents current collection source; G; University of Waikato glasshouse, H; Herbarium record, S; University of Waikato shade house, O; University of Waikato grounds.

3.4.11 Anatomy

Initial anatomical comparisons between a single Poor Knights Islands individual and mainland individual revealed a key distinction between the leaves of the different forms; hypodermal layers adjacent to the midrib revealed two cell layers in the Poor Knights Islands form as opposed to a single cell layer in the mainland form (Figure 3.16). Hypodermal cell layers in mainland leaf samples were on average approximately 40 µm thick, compared with Poor Knights Islands leaf hypodermis which were on average approximately double that (80 µm). Both individuals had secretory ducts located in vascular bundles throughout the leaf; these appeared to contain an epithelium which indicates schizogenous formation (Evert *et al.* 2006).

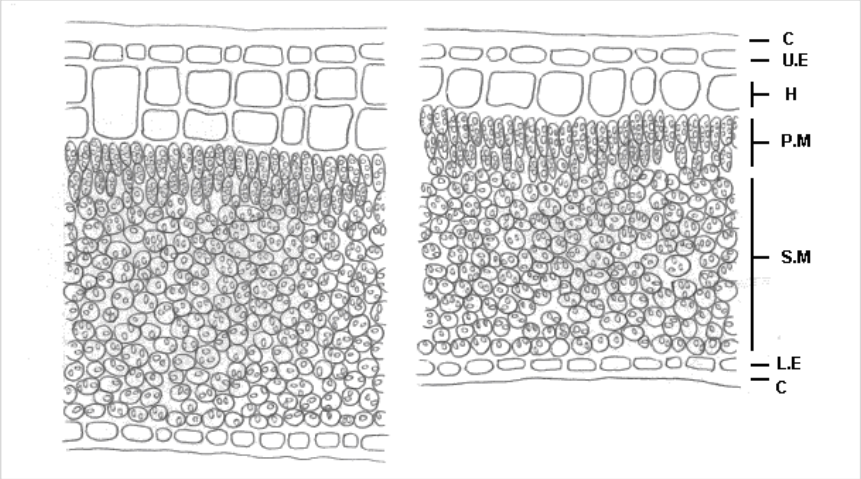

Figure 3. 16 Drawing depicting transverse sections of *Pittosporum cornifolium* leaves – adjacent to midrib, showing difference in hypodermal layer of Poor Knights Islands form (left) and mainland form (right) (not to scale). C; cuticle, U.E; upper epidermis, H; Hypodermis, P.M; palisade mesophyll, S.M; spongy mesophyll, L.E; lower epidermis.

No key distinctions were apparent in stem and root sections between the Poor Knights Islands individual and mainland individual (Figure 3.17). Root and stem sections for both individuals' revealed thick peridermal layers (comprising of phellogen (cork cambium), phelloderm (secondary cortex) and phellem (cork)) (Figure 3.17). Stem sections also displayed a ring of secretory ducts between the primary phloem and cortex tissue layers; these appear to lack an epithelium which indicates lysigenous formation (Figure 3.17) (Evert *et al.* 2006).

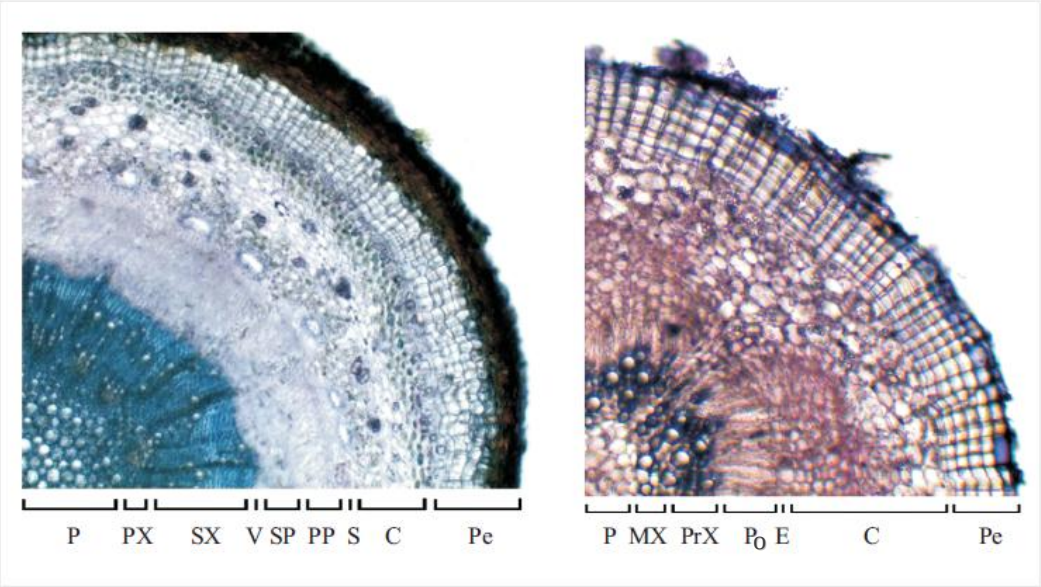

Figure 3. 17 *Pittosporum cornifolium* stem cross section (left) and root cross section (right). P; pith, PX; primary xylem, SX; secondary xylem, V; vascular cambium, SP; secondary phloem, PP; primary phloem, S; ring of secretory ducts, C; cortex, Pe; periderm, MX; meta xylem, PrX; proto xylem, Po; Phloem, E; endodermis.

More comprehensive comparisons of *P. cornifolium* leaf tissue thickness revealed continuous variation from smaller mainland individuals to larger Poor Knights Islands individuals across all tissue layers (Table 3.9). Despite this continuous variation, the Poor Knights Islands individuals consistently express higher thickness values across the majority of tissue types (Table 3.9), and have higher mean tissue thickness values across all layers when compared with the combined mean of both North Island localities (Table 3.9). Depths of hypodermal layers at the midrib and adjacent to midrib were variable across all localities, but Poor Knights Islands individuals generally expressed higher values and had on average a much thicker hypodermal layer when compared with other mainland localities (Table 3.10). The Poor Knights Islands individuals also exhibited an additional upper hypodermal cell layer in the leaf midrib and two additional lower hypodermal cell layers when compared with mainland individuals (with one exception in upper hypodermal cells from mainland sample Raglan 3) (Table 3.10). However, measurements adjacent to the leaf midrib were consistently one layer thick from all individuals at all localities (Table 3.10) unlike initial screening results (Figure 3.16).

Table 3. 9 *Pittosporum cornifolium* lamina tissue depths at midrib (µm), mean lamina tissue depths at midrib (µm), and mean for combined North Island sites; R+W (Raglan + Waitakere). Upp. Cut.; upper cuticle, Upp. Epi.; upper epidermis, Upp. Hyp; upper hypodermis, Pal. Mes.; palisade mesophyll, Upp. B.S.C.; upper bundle sheath cells, Xyl.; xylem, Phl.; phloem, Low. B.S.C.; lower bundle sheath cells, Low. Hyp.; lower hypodermis, Low. Epi.; lower epidermis, Low. Cut.; lower cuticle.

	Total	Upp. Cut.	Upp. Epi	Upp. Hyp.	Pal. Mes.	Upp. B.S.C.	Xyl.	Phl.	Low. B.S.C.	Low. Hyp.	Low. Epi.	Low. Cut.
Poor Knights 1	875	8	38	69	100	100	138	188	144	63	25	4
Poor Knights 2	557	8	25	50	38	44	113	88	121	55	13	4
Poor Knights 3	629	10	31	38	88	38	125	100	125	56	13	6
Poor Knights 4	821	8	31	75	88	125	125	125	150	63	25	6
Raglan 1	463	6	31	25	63	25	125	25	113	25	19	6
Raglan 2	411	6	25	19	50	38	75	88	88	13	8	3
Raglan 3	660	6	31	25	88	50	125	175	125	13	19	4
Raglan 4	587	8	25	19	88	63	63	138	138	25	19	4
Waitakere 1	544	6	19	31	38	50	75	150	125	25	19	6
Waitakere 2	455	4	31	13	38	38	81	125	88	15	19	4
Waitakere 3	408	4	25	19	63	25	63	88	75	19	25	4
Waitakere 4	477	4	25	25	56	38	69	113	100	25	19	4
Poor Knights mean	720	9	31	58	78	77	125	125	135	59	19	5
Raglan mean	530	7	28	22	72	44	97	106	116	19	16	4
Waitakere mean	471	5	25	22	48	38	72	119	97	21	20	5
R+W mean	501	6	27	22	60	41	84	113	106	20	18	4

Table 3. 10 *Pittosporum cornifolium* adaxial (upper) and abaxial (lower) hypodermal cell layers at midrib, and adaxial (upper) hypodermal cells adjacent to leaf midrib per individual, and location averages (AV).

	layers of cells at midrib (n)		depth of cells at midrib (μm)		layers of cells adjacent (n)	depth of cells adjacent (μm)
	upper	*lower*	*upper*	*lower*	*upper*	*upper*
Poor Knights 1	3	3	69	63	1	50
Poor Knights 2	3	3	50	55	1	63
Poor Knights 3	3	3	38	56	1	75
Poor Knights 4	3	3	75	63	1	75
Poor Knights AV	3	3	58	59	1	66
Raglan 1	2	1	25	25	1	56
Raglan 2	2	1	19	13	1	50
Raglan 3	2	1	25	13	1	38
Raglan 4	2	1	19	25	1	44
Raglan AV	2	1	22	19	1	47
Waitakere 1	2	1	31	25	1	50
Waitakere 2	2	1	13	15	1	44
Waitakere 3	2	1	19	19	1	50
Waitakere 4	2	1	25	25	1	44
Waitakere AV	2	1	22	21	1	47

3.5 Discussion

3.5.1 Distribution

Pittosporum cornifolium is distributed widely in the coastal and lowland zones of the North Island. However, there are some obvious gaps in both its observed distribution and its predicted distribution. Gaps in its observed distribution that are inconsistent with potential environmental distribution include the Waikato region where environmental variables are favourable but the observed absence is due to the area being formerly dominated by extensive wetland systems (Clarkson 2002). The environment is suitable south of the Wairoa lowlands in the Wairarapa but there are no current records probably because nearly all lowland forest in the area has been cleared (Nicholls 1980). Similarly, much of the Taupo Volcanic Zone is favourable in terms of the predicted environmental distribution of *P. cornifolium*, but observed absences are likely to be due to the ecological impact caused by the 186 AD Taupo eruption (Lowe & de Lange 2000). Both Mahia in the North Island and Banks Peninsula in the South Island are also environmentally favourable locations where no records of *P. cornifolium* have been found. The often companion obligate epiphyte *Griselinia lucida* is present at both of these

localities (Laing 1919 (Banks Peninsula) & Whaley *et al.* 2001 (Mahia)), and many predominantly North Island subtropical species such as nikau (*Rhopalostylis sapida*) are found growing in the Banks Peninsula area (Department of Conservation 2010). Searches should be undertaken at these locations, especially Mahia, to determine if *P. cornifolium* is present. *Pittosporum cornifolium* is largely unrecorded from the drier eastern side of the North Island where native forests have been almost completely removed, and the low rainfalls are not conducive to rich epiphyte floras (Clarkson & Clarkson 1991). Absence from the Wairoa, Gisborne and Waiapu lowlands was predicted due to the high vapour pressure deficits in the area. *Pittosporum cornifolium* was absent from the Kaimanawa and Ikawhenua ranges in the central North Island in both observed and predicted distribution maps; this is because of the low temperature minimums in the area. *Pittosporum cornifolium* has restricted observed distributions and restricted predicted distributions in the South Island; these restrictions are set primarily by low temperature minimums.

3.5.2 Habitat

Pittosporum cornifolium occupies a range of habitats from coastal rock systems, to a variety of lowland forest ecosystems (1–786 m a.s.l.) where it exhibits three distinct lifestyles; epiphytic, terrestrial and rupestral. *Pittosporum cornifolium* has been described by Oliver (1930) as a typical epiphyte in that the species is habitually epiphytic. However, while it is more commonly epiphytic it can also be found growing in abundance in terrestrial and rupestral lifestyle. In consideration of this diverse lifestyle preference, *P. cornifolium* may be considered a facultative epiphyte as defined by Benzing (1990). Benzing (1990) states that epiphytes become increasingly facultative as environmental conditions in tree canopies converge on terrestrial environmental conditions. Within this extensive range, *P. cornifolium* appears to be tolerant of both high light and relatively shaded habitat, as it is found growing on exposed rock stacks in Raglan Harbour, as well as in semi-shaded understory of kauri forest. Within the lowland forest systems, where it can be found both in epiphytic and terrestrial lifestyle, the species appears to have an affinity with older growth forest and/ or remnant old growth trees. Both the Southern and Northern enclosures at Maungatautari are of similar vegetative composition, but the southern enclosure where *Pittosporum*

cornifolium was located is much older growth than that of the northern enclosure. The lowland forest systems located in the Coromandel were composed of mainly second growth lowland forest with interspersed patches of remnant old growth kauri stands. *Pittosporum cornifolium* individuals were concentrated within and beneath the old growth kauri trees, but were absent from surrounding second growth forest. Taranaki sites supported a range of secondary and older growth forest, with the majority of trees hosting *P. cornifolium* being large canopy or emergent individuals, particularly emergent rimu. A key contributing factor in noticeable preference and abundance in large old growth trees is that there has been greater time available for colonization and a larger potential colonization surface (Benzing 1990).

In distinct contrast to these lowland forest systems were the coastal rock systems of Raglan Harbour, where *P. cornifolium* grows as a co-dominant species amongst scrub communities of *Astelia banksii* and *Griselinia lucida*. These communities were also the most exposed, with the individuals growing on open exposed rock stacks, which could potentially receive direct light throughout much of the day.

The strong habitat specificity of *P. cornifolium* restricts it to habitats of very limited extent which are widely and discontinuously distributed. In terms of the interpretation of Drury (1974), the species has evolved to use the resources of an extreme habitat at the cost of being able to compete in biologically favourable habitats. According to Rabinowitz's (1991) typology of rarity, the species is locally sparse, of large range, with a specific habitat.

3.5.3 Growing substrate

The noted affinities with old growth systems and older trees are also likely to result from the successional pattern of epiphytic establishment. This is evident due to the common association of *P. cornifolium* with nest hosts *Astelia solandri* and *Collospermum hastatum* when an epiphyte, a relationship frequently mentioned in the literature (see Oliver 1930; Clarkson 1985; Burns & Dawson 2005). Establishment of these nest epiphytes may facilitate the establishment of larger shrub epiphytes like *P. cornifolium* by providing an increase in organic matter and moisture thereby enhancing microclimate (see Dickinson *et al.* 1993).

The growth substrate provided by these nest hosts is therefore composed entirely of organic matter (dead leaves and roots) which, by decomposition processes, result in a fine, low nutrient gritless soil that collects in large quantities (Oliver 1930) and has low nutrient status (Dickinson *et al.* 1993). Not surprisingly, the accumulation of epiphyte associations like this are more common on larger older trees and therefore even more so in old growth systems (Benzing 1990). The rupestral individuals at Raglan Harbour were also found growing in association with nest host *Astelia banksii*, and facilitation by the nest host is also a plausible explanation of such associations, as *P. cornifolium* individuals were notably absent from raw rock surfaces. Similar organic soil types would also result from decomposition of organic matter provided by this nest host.

The detritus substrate of terrestrial individuals growing beneath kauri stands in the Coromandel are classified as mainly brown loam soils, which are typically low nutrient and acidic (Molloy 1998). The large kauri trees contribute to the low fertility of these soils by producing a deep layer of litter composed of mostly leaves, as well as woody components (bark, branchlets, and cones), and kauri gum, that is slow to decay (Orwin 2009). This litter eventually develops into highly acidic humus that is slow to release nutrients (Orwin 2009). Detritus substrates are a result of long term soil development by gradual erosion and build up of organic matter provided by old growth kauri forest systems. This emphasises the affiliation with old growth systems, and subsequent facilitative processes of suitable substrate formation. The prevalence of these terrestrial individuals within kauri forests is likely to be a result of two key interactions allowing higher light penetration at ground level. First; the relatively open canopy of this forest type which is due to a kauri's ability to dominate and enhance the high acidity and low nutrient status of soil types, inhibits other large broadleaved and podocarp trees. Second, the low nutrient, highly acidic soils minimise intra specific competition within the shrub layer, as only a limited number of species tolerate these soil conditions. It is not surprising then that *Pittosporum cornifolium* and other typically epiphytic species such as *Brachyglottis kirkii* are present as these soil conditions are comparable to both epiphytic and rupestral substrates discussed above.

The final, least common growing substrate was that of tree fern trunk. Because tree ferns do not undergo secondary growth they do not possess wood or bark. Instead, structural support is provided by a dense mass of intertwined adventitious roots (often referred to as a root mantle) that surround the stem (Roberts *et al*. 2005). These adventitious roots provide a moisture retaining organic stratum in which epiphytes can take hold and embed their roots (Oliver 1930). Tree fern trunks are therefore a readymade growth substrate for epiphytic species without need for the either the breakdown of mineral substrate or the build up of organic matter as initiated by facilitation on primary substrates like rock or bark.

Despite this range of growing substrates, *P. cornifolium* was consistently found growing on well drained substrates. In the canopy, wind exposure and direct sun induce high evaporation, the limited arboreal soil restricts moisture absorption and water retention, and gravity ensures rain water rapidly drains to the ground (Dawson & Lucas 2005). The vertical trunks of tree ferns would also be exposed to rapid draining by gravity and the exposed rupestral substrates of limestone rock in the Raglan Harbour system would experience similar evaporation due to coastal winds and solar radiation. These surfaces are also sloped and contain numerous crevices, which is indicative of a well drained substrate. The terrestrial communities of *P. cornifolium* in the Coromandel were located on well drained steep ridges, characteristic of kauri forest habitat, and the prominent relief type throughout the Coromandel Peninsula (McLintock 1966).

3.5.4 Host species

The range of host tree species was diverse, and host preference appears to be strongly correlated with larger tree size (and therefore older trees) as there was an abundance of *P. cornifolium* individuals on the most common emergent individuals in the lowland forest systems surveyed; rimu, kauri, and pukatea. An affiliation with older remnant trees accords with epiphytic lichen distribution, in that old trees which have survived natural disaster or forest clearance can act as refugia and propagule sources (Neitlech & McCune 1997; Peck & McCune 1997; Peterson & McCune 2001). This is likely to be the case in Coromandel populations where remnant old growth kauri trees are surrounded by recovering second growth forests. These few remnant kauri are host to abundant epiphytic

populations, and complete terrestrial communities reside directly beneath the canopy span of these large trees, which is likely to be a result of seed dispersal by gravity. In this case, entire populations appear to have resulted from old growth refugia of emergent kauri. It is possible that this is also the case for many large emergent trees which survived logging efforts throughout lowland forest systems. In Ratapihipihi (a key Taranaki site) for example, large emergent rimu that remained unlogged during the 1850s (Clarkson *et al.* 1982) were the most common host trees, and in a single tree up to four *P. cornifolium* individuals were recorded. Burns & Zotz (2010) hypothesise that clumped epiphyte distributions like these are caused by higher proportions of seed dispersal within the host tree than among other potential host trees.

3.5.5 Population structure

While juveniles and seedlings were not strongly represented across all of the populations measured there is no strong evidence of a significant regeneration gap. Epiphytic seedlings and juveniles would be very difficult to detect from the ground and even with the use of tree climbing there is no guarantee they would be detected. In contrast to this, both seedlings and juveniles on the forest floor of Coromandel populations were relatively easy to locate, confirming recent regeneration. At the Raglan Harbour population, no seedlings were recorded, but restricted access to these rocks stacks means it is possible that small seedlings were hidden within dense clusters of *Astelia banksii* and remained unrecorded. All of the populations contained reproductively mature individuals (females) which flower regularly producing capsules and seed (see section 3.5.6).

The epiphytic populations in Maungatautari and Taranaki are widely dispersed on large tree hosts. The spatial distribution of plants is significantly different in the kauri forest populations of Coromandel where clusters of terrestrial individuals are found beneath the canopy span of remnant trees harbouring epiphytic individuals. Similar to Coromandel distributions, the Raglan Harbour system exhibits clumped distribution structure, but does exhibit some more widely dispersed individuals.

The concentration of individuals within and around remnant kauri trees is likely to be a result of host tree refugia and subsequent dispersal by gravity, as well as more favourable growing conditions at ground level (open canopies and organic soils) as discussed earlier. Zotz *et al.* (1999) also noted epiphyte species exhibiting highly clumped distributions, suggesting a predominance of very local dispersal within a tree crown, infrequently interrupted by successful long-distance dispersal between crowns.

The much denser forest canopies of Maungatautari and Taranaki, in combination with high interspecific competition at shrub layer, are likely to restrict the potential growth of terrestrial individuals dispersed by gravity. Terrestrial growth restrictions means already established individuals must rely exclusively on either dispersal by gravity to lower tiers of an individual host tree or by dispersal by bird to different host trees. Suggested infrequency of successful long distance dispersal among epiphytic species (Zotz *et al.* 1999) may account for the lower density and widely dispersed population structures of Maungatautari and Taranaki.

Raglan Harbour limestone outcrops present similar clumped modes of distribution to Coromandel populations. Large proportions of the population (10/25 individuals) were represented on a single outcrop, the majority of the population (20/25) was interspersed on both the heavily populated outcrop and other smaller outcrops or cliff faces within a 500 m stretch of the harbour system, and the final five individuals were distributed across a 1.5 km stretch of the harbour. The clumped distributions exhibited on single outcrops are likely to be the result of dispersal by gravity, whereas dispersal to different rock outcrops up to 1.5 km apart would be by bird.

3.5.6 Sex ratios

Sex ratios for combined populations are slightly skewed with a higher proportion of male to female individuals (61:39). Lower proportions of female individuals is potentially the result of greater reproductive investment by females which can lead to a higher mortality rates, and thus to populations with male-biased sex ratios (Meagher 1981; Waser 1984; Bierzychudek & Eckhart 1988; Allen & Antos 1993). Despite these slightly lower proportions of female individuals, all

identified females were recorded producing multiple fruits almost immediately after an extensive flowering season. Moreover, relatively even proportions promote extensive potential for crossing between a range of different males and females within a single population, reducing the risk of inbreeding. Furthermore, the demonstrated ability within this species of male individuals to inconstantly produce seed may also reduce risks of inbreeding (provided they produce a viable seed, which is still unproven) should ratios become more skewed. Maungatautari had the most uneven sex ratio of all populations 78:22 male: female individuals, but this ratio is more likely an artefact of limited access and identification. Not all individuals were accessed by tree climbing, and some had to be viewed using binoculars from the ground. It was difficult to determine whether these individuals had capsules or not and female numbers may have been underestimated. Furthermore, the total number of plants identified in the population at Maungatautari was only 9, this will be only a subset of a much larger and more widely dispersed population.

A range of sex ratios has been recorded in other New Zealand *Pittosporum* species including close to 50 male: 50 female for *P. turneri* (Ecroyd 1994) and 45:55 for *P. patulum* (Rogers & Walker 2005). Although the overall sex ratio in *P. obcordatum* was almost 50:50, severely unbalanced outlier populations of 100:0, and 16:84 were recorded and considered a potential limiting factor contributing to the species vulnerability (Clarkson & Clarkson 1991). For *P. cornifolium*, current sex ratios do not appear to limit regeneration.

3.5.7 Phenology

Pittosporum cornifolium has an autumn–spring flowering season extending from May–October with peak flowering in September and an abrupt cessation of flowering. The flowering time is somewhat longer than the June-September period reported by Cooper (1956) and Allan (1961). Within the genus at least eight out of approximately twenty New Zealand species, including shrubs and small trees, have a similar flowering time, peaking in September, but most of these persist in summer (Cooper 1956; Allan 1961). Most of the species with a similar flowering season are characteristic of coastal and lowland bioclimatic zones (Cooper 1956; Allan 1961). On Little Barrier Island, which has a mild

coastal climate, the majority of 18 tree and shrub species flowered in August and September (Gravatt 1970). Five unrelated species out of 33 trees, shrubs, and lianes documented by Leathwick (1984) in four Central North Island forests show peak flowering in September. These included; *Aristotelia serrata*, *Fuchsia extorticata*, *Melicope simplex*, *Macropiper excelsum*, and *Rubus cissoides*.

Capsules can be found on female plants all year round and often green capsules are present from the current flowering year and dehisced capsules from the previous year so there a ready and continuous seed supply.

Observations from my glasshouse collection showed that the mainland plants flowered earlier (early July to late September) while the Poor Knights Islands plants flowered later (mid August to late October) providing further support for formal recognition of the Poor Knights Islands form.

3.5.8 Microclimate

The microclimate data confirms that *P. cornifolium* tolerates a wide range of temperatures and vapour pressure deficits within the coastal and lowland zones. The coastal Raglan Harbour site recorded the highest means for temperature and vapour pressure deficit across all three recorded seasons. Higher mean temperatures and vapour pressure deficits are likely to be attributed to higher radiation and wind exposure in these more open sites than in sheltered canopies of lowland forest systems. Dickinson *et al.* (1993) compared total radiation at an exposed coastal site to both canopy and ground level sites in lowland forest. Consistently higher radiation readings were observed at the coastal site across all measured months, with the highest values during peak sun hours in the summer month of December (Dickinson *et al.* 1993). Furthermore, consistently higher average temperatures were recorded at the coastal site than in internal forest data loggers (Dickinson *et al.* 1993).

Comparisons of internally and externally positioned data loggers at the three sites suggest that internal sites are buffered, as temperature and vapour pressure deficits for external sites revealed more extreme maximum values and a wider range of values.

3.5.9 Leaf morphology

Continuous variation from smaller mainland leaf forms to the larger Poor Knights Islands leaf forms in *P. cornifolium* is a common phenomenon observed across multiple species and genera in relation to the northern offshore islands of New Zealand (Beever 1986). For some species, the large-leaved forms have been accorded formal subspecific status, e.g., *Macropiper excelsum* var. *psittacorum*, and *Solanum aviculare* var. *latifolium* (Beever 1986). Some of the large-leaved forms may be a response to the favourable environmental conditions of a phenotypically plastic species (Beever 1986). In the present case however, genetic divergence is a more plausible explanation considering the majority of Poor Knights Islands individuals sampled and many of the mainland individuals were propagated in controlled glasshouse environments, yet still retained distinctly different leaf sizes. The differences then are more likely to warrant formal recognition at least at the subspecies level.

3.5.10 Anatomy

The stem and root anatomies of *P. cornifolium* appear typical of xerophytic dicotyledons due to the thick peridermal layers. Peridermal layers are protective tissues which prevent water loss (Peterson *et al.* 2008).

The leaf anatomy of *P. cornifolium* was previously described by Oliver (1930) and Wilkinson (1992) from transverse sections. The leaf tissue layers recorded in this research are consistent with the descriptions of both Oliver (1930) and Wilkinson (1992). However, Wilkinson (1992) recorded a leaf thickness range at the midrib of 329–427µm which is relatively low compared to the range of this research (408–875µm), but the noted higher values were recorded in the Poor Knights Islands variant. *Pittosporum cornifolium* leaf anatomy is consistent with typical xeromorphic form, particularly due to the presence of hypodermal leaf tissue layers, as this layer may function as both a water storing tissue and as a supporting tissue when cell walls become considerably thickened (Fahn 1982; Cutler *et al.* 2007).

The comparative leaf anatomy of the Poor Knights Islands form and the mainland form revealed a trend towards thicker leaf blades and leaf tissue layers in the Poor Knights Islands form. Thicker leaf blades have been related with greater degrees of xeromorphy and associated drought tolerance (Petrova 1988 as cited in Hameed *et al.* 2002, Venora & Calcagno 1991; Hameed *et al.* 1995). Two tissue layers that are also related to greater degrees of xeromorphy include hypodermal/ aquias layers (Fahn 1982; Cutler *et al.* 2007) and bundle sheath cells composed of sclerenchyma (Ridley & Todd 1966), both of which were significantly thicker in Poor Knights Islands individuals. The higher hypodermal depths and cell counts observed in Poor Knights Islands individuals suggest an adaptation to drought tolerance, as a well-developed hypodermis is believed to play an important role in drought resistance (Ridley & Todd 1966; Rojas *et al.* 1983; Petrova 1988 as cited in Hameed *et al.* 2002). Genetic divergence is plausible explanation with respect to the comparative leaf anatomy considering all individuals sampled were propagated in controlled glasshouse environments, yet still retained distinct differences in leaf and tissue layer thickness. The differences then are more likely to warrant formal recognition at least at the subspecies level. Initial screening found secondary cell layers in the Poor Knights Islands variants hypodermal layers (of transverse sections adjacent to midrib) but this was not confirmed in the more comprehensive survey. This is possibly a result of phenotypic plasticity, as all individuals were propagated in controlled conditions, whereas initial screening individuals were not.

3.5.11 Conservation and restoration implications

Pittosporum cornifolium can be locally uncommon but does not currently meet the specific criteria to be considered regionally or nationally threatened. However, there is no doubt that its range has been reduced and that it has been lost from a number of sites where it was previously collected. Anecdotal observations have suggested that it is palatable to possums, and possum browsing may have contributed to the reduction of some populations (Ravine 1995; Mitcalfe & Horne 2005). No evidence of possum browse was detected during the present survey, however, all but one of the study sites had adequate to good pest control. The site which did not have pest management was Raglan Harbour; these individuals were

located on limestone outcrops which were detached from the mainland and therefore have natural protection in that they are inaccessible to possums.

Sites where *P. cornifolium* have been lost are a priority for reintroduction. If a reintroduction were proposed, key considerations would be local sourcing of propagules, and determination of both lifestyle and growth substrate most suitable for the receiving site. Evidence from the previous research (Chapter 2) showed a significant correlation between geographic distance and genetic differentiation among populations of *P. cornifolium.* This highlights the importance of plant provenance and associated ecotypes when sourcing seed for restoration. Evidence from the ecological survey (present chapter) suggests habitat type and associated canopy components such as canopy species and density will predict which lifestyle is most suitable for reintroduction. For example, in dense lowland forest with old growth canopy species such as rimu or tawa, the epiphytic lifestyle would be the most suitable and possibly the only viable option. This is because terrestrial individuals would not survive high interspecific competition and limited light environments characteristic of dense lowland forest floors. Once site specific lifestyles have been identified, direct growing substrate for reintroductions should be considered. Reintroductions should be established in preferable growing substrates such as nest hosts, detritus substrates, or tree fern trunks. Where suitable growing substrates are not available, artificial growing substrates could be developed with respect to natural substrate composition (e.g. well drained, low nutrient).

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4 Chapter Four: Biological Flora of New Zealand. *Pittosporum cornifolium*, Tawhiri karo, Cornel-leaved pittosporum.

4.1 Abstract

A comprehensive review of the morphology, anatomy, taxonomy, chemistry, and ecology of the endemic New Zealand shrub epiphyte *Pittosporum cornifolium* (Pittosporaceae) is presented. *Pittosporum cornifolium* has a wide geographic range in which it is well adapted to a variety of lifestyles including terrestrial, rupestral, and more commonly, epiphytic. Strong habitat specificity restricts this species to lowland forest and coastal habitats of very limited extent, which are widely yet discontinuously distributed. Environmental factors that may further restrict distribution include high vapour pressure deficits, cooler temperatures, limited rainfall, limited solar radiation and higher elevations. Edaphic range is characterised by low nutrient well-drained substrate. Significant morphological variability is evident in leaves and flowers especially with respect to plants from the Poor Knights Islands. Population genetics of mainland and offshore island populations revealed relatively low genetic diversity at population-level which is likely to be the result of geographic isolation. Significant genetic distinctiveness and reduced genetic variability were observed in the Poor Knights Islands individuals. Molecular phylogenetic studies of *P. cornifolium* suggest a New Caledonian origin with close affinities to both *P. pimeleoides* subspecies. Several lines of evidence suggest recognition of the Poor Knights Islands entity as a species or subspecies. However, additional morphological, reproductive and molecular analyses across the total geographic range will be required to confirm current inferences.

Key Words: anatomy, distribution, morphology, Pittosporaceae, *Pittosporum cornifolium,* population genetics, reproductive biology, taxonomy and relationships.

4.2 Morphology

Pittosporum cornifolium A. Cunn. (Pittosporaceae) is a perennial, evergreen shrub reaching up to 2.5 m in height, with a distinctive whorled architecture (Cooper 1956). Both polygamous and dioecious individuals are present, however, it appears that the dioecious form occurs more frequently (Petrie 1921).

Branches are dark brown, glabrous and grow in forked or whorled arrangements (Cooper 1956), and leaf scars are often present (Kirk 1871). Leaves are entire with slightly revolute margins and grow in whorled arrangement (Cooper 1956). They are ciliate when young, but soon become glabrate, coriaceous, with a glossy cuticle (Cooper 1956; Poole & Adams 1994), and have a well raised midrib above (immersed below), and distinct secondary veins below (Cooper 1956). Leaves are 3.5–7.5 cm long, and 1.5–3.5 cm broad (Allan 1961), and are shaped elliptic-lanceolate to obovate (acute to subacuminate at apex, and acute to obtuse at base) (Cooper 1956). Secondary nerves are obscure above, distinct beneath. Petioles are broad and glabrous, approximately 0.5 - 3.0 mm. long and 0.5 - 2.0 mm wide (Cooper 1956).

Figure 4. 1 *Pittosporum cornifolium* in flower. From left: reddish pink colouring (photo: Kerry Jones, Little Barrier Island), reddish brown colouring (photo: Rob L. Suisted, Wilton's Bush Reserve, Wellington), and yellow colouring (individuals from the Poor Knights Islands).

Inflorescences are terminal, 1–10-flowered, usually umbelliform (Cooper 1956; Petrie 1921). Flowers (Figure 4.1) are a reddish brown (type form described by Hooker 1932) to reddish pink colour (Cooper 1956; Petrie 1921). Flower pedicels

are 2–15 mm long and are subtended by a whorl of leaves (Cooper 1956). Flower sepals are not imbricate, of narrow-lanceolate shape, acute at apex and are 4–7 mm long (Cooper 1956). Petals are broad, coherent in a tube formation with reflexed tips, shaped linear-lanceolate, acute to acuminate at the apex, and 8–12 mm in length (Cooper 1956). Flowers are perfect but are generally unisexual in function; male flowers have a reduced ovary and stigmatic surface, while female flowers have reduced filaments and anthers (Figure 4.2; Clarkson 2011). Stamens are 4–6 mm long, anthers sagittiform to elliptic-oblong, 1–2 mm long, and 0.5–1.0 mm broad (Cooper 1956). Gynoecia are a similar length to stamens, the ovaries are broad and covered with villous hairs, 1.5–3 mm long and 0.5–2 mm broad, styles are 2.5–4 mm long, and stigmas range from capitate (2-lobed) to truncate (Cooper 1956).

Figure 4. 2 *Pittosporum cornifolium* flowers showing reproductive structure. Left, young female flower showing large ovary and reduced stamen; Right: young male flower showing large stamen and reduced ovary.

Capsules are 2-valved (occasionally 3), approximately 1 cm in diameter and ovoid to ellipsoid in shape (Cooper 1956; Poole & Adams 1994). Capsules have valves which are less than 1 mm thick, and a basal placenta bearing thick strap-like funicles up to 5 mm in length (Cooper 1956). Capsules have vermilion stained interiors with characteristic sticky ‘pitch’ seeds of the genus *Pittosporum* (Poole & Adams 1994), which are black and irregular (4–8 per capsule) (Cooper 1956).

Morphological variants of *P. cornifolium* have been described on Kawau Island (Hauraki Gulf) with yellow flowers (Petrie 1921), and the Poor Knights Island group (Hauraki Gulf) with yellow flowers and larger more coriaceous leaves (Smith 2004). Poor Knights Islands individuals leaves are shaped obovate to rhomboid (subacuminate to obtuse at apex and acute to obtuse at base) (Clarkson 2011).

4.3 Anatomy

The stem and root anatomies of *P. cornifolium* appear typical of xerophytic dicotyledons. Both have thick peridermal layers, comprising phellogen (cork cambium), phelloderm (secondary cortex) and phellem (cork) (Clarkson 2011). The periderm is a protective tissue that prevents water loss (Peterson *et al.* 2008). A ring of secretory ducts is present in the stem between the primary phloem and cortex tissue layers (Clarkson 2011). Because these ducts appear to lack an epithelium, this indicates lysigenous formation (Evert *et al.* 2006).

The leaf anatomy of *P. cornifolium* was previously described by Oliver (1930) and Wilkinson (1992) from transverse sections and more recently by Clarkson (2011). Leaf tissue layers adjacent to the leaf midrib comprise an upper cuticle, upper epidermis, a thick 'aqueous layer' / hypodermis, a closely packed mesophyll layer composed of palisade and spongy tissue, lower epidermis, and lower cuticle. *Pittosporum cornifolium* leaf anatomy is consistent with xenomorphic form, particularly due to the presence of hypodermal leaf tissue layers, as this layer may function as both a water storing tissue and as a supporting tissue when cell walls become considerably thickened (Fahn 1982; Cutler *et al.* 2007). Furthermore, secretory ducts were noted in the midrib and scattered vascular bundles throughout the leaf (Wilkinson 1992; Clarkson 2011), these appear to contain an epithelium which indicate schizogenous formation (Evert *et al.* 2006).

Comparative leaf anatomy of the Poor Knights Islands form and the mainland form revealed a trend towards thicker leaf blades and leaf tissue layers in the Poor Knights Islands form (Clarkson 2011).

4.4 Cytology

The chromosome number of *P. cornifolium* is 2n = 24 (de Lange *et al.* 2004). Chromosome counts of 2n = 24 have also been reported in all members of New Zealand *Pittosporum* (de Lange *et al.* 2004).

4.5 Taxonomy and relationships

4.5.1 Apiales

The angiosperm order Apiales comprise five major lineages: core Apiaceae, core Araliaceae, Pittosporaceae, and the *Mackinlaya* and *Myodocarpus* groups (Chandler & Plunkett 2004). Taxonomic research investigating molecular, morphological, and phytochemical similarities has validated this placement of the Pittosporaceae within the Apiales (Plunkett *et al.* 2004).

Early evidence supporting this relationship came from morphological, cytological and phytochemical data including; stem structure (presence of schizogenous secretory canals (Van Tieghem 1884 as cited in Plunkett *et al.* 2004)), wood anatomy (vessel element and ray morphology (Carlquist 1981)), ovular structure and development (Jurica 1922), chromosome number (Jay 1969), combinations of secondary compounds (Hegnauer 1969 as cited in Carlquist 1981; Hegnauer 1972) and the presence of flavonoids; quercetin and kaempferol (Jay 1969).

This evidence is reinforced by more recent molecular data using both nuclear RNA and plastid regions (e.g. Plunkett *et al.* 1996; Plunkett 2001; Plunkett & Lowry 2001; Chandler & Plunkett 2004), and ever increasing morphological data including homology in ovary positioning (Erbar & Leins 1995; 1996) and fruit anatomy (Lui *et al.* 2006). Four core families represented within order Apiales (including Pittosporaceae) are now recognized under new suborder Apiineae based on cohesion of both molecular and morphological data (Plunkett *et al.* 2004).

4.5.2 Pittosporaceae

The family Pittosporaceae is relatively small, consisting of nine genera (Cayzer 1997) and around 200–240 species (Carlquist 1981). Early morphological discrimination between genera proved daunting due to a large overlap in morphological traits between genera, and the inability to distinguish whether observed variation was genetically or ecophenetically generated (Wilkinson 1992). However, recent taxonomic revisions and phylogenetic studies based on more comprehensive morphological data sets have served to redefine the limits of the genera within Pittosporaceae (Chandler *et al.* 2007). Such revisions include the establishment of a new genus *Auranticarpa* (segregated from *Pittosporum*) (Cayzer *et al.* 2000a), and the inclusion of genus *Citriobatus* within *Pittosporum* (Cayzer *et al.* 2000b). More recent molecular phylogenies (constructed with nuclear Internal Transcribed Spacer (ITS) regions and plastid *trn*L–*trn*F sequence data) are closely correlated with morphological data sets and support most of the taxonomic revisions (including that of Cayzer 2000a; 2000b listed above) (Chandler *et al.* 2007).

Two key theories as to the origins of Pittosporaceae are suggested; first, a purely Australian origin post Gondwana followed by dispersal (Raven & Axelrod 1974; Webb *et al.* 1986), and second, an east Gondwanan origin, with dispersal and subsequent vicariance (Crisp *et al.* 1989). Chandler *et al.* (2007) conducted a preliminary molecular assessment of biogeographic origins. Their findings were consistent with a single origin of the family in Australia, and with the more recent view that Pittosporaceae are an old east Gondwanan family. However, Chandler *et al.* (2007) indicates that formal biogeographic analysis will require a greater representation of taxa from the Indian Ocean, South-east Asia, New Caledonia and New Zealand. Currently, genera within Pittosporaceae are almost entirely endemic to Australia apart from *Auranticarpa* which may extend into Malesia (Cayzer 2000a); *Hymenosporum* which is also represented in New Guinea, and the type genus *Pittosporum* which is represented not only in Australia but is widespread throughout the Pacific (as far east as the Hawaiian Islands), eastern Africa, and Asia (Haas 1977; Cayzer 1997).

4.5.3 *Pittosporum*

Pittosporum Banks ex Garthen is derived from the Greek words "pitta" meaning resin and "spora" meaning seed (Gowda 1951; Bennett 1988), referring to the characteristic resinous seeds of most species within the genus (Cooper 1956). *Pittosporum* comprises over 100 species (including *Citriobatus sensu* Chandler *et al.* 2007) of evergreen shrubs or trees (up to 30m high) (Cooper 1956). Leaves are alternate or whorled, and flowers are perfect but typically of unisexual function (Cooper 1956). The fruits are generally woody or coriaceous, two to five valved capsules, which remain fused at the base (Bennett 1988). While *Pittosporum* is abundant in Australia (Gowda 1951), the highest levels of endemism occur in New Caledonia (50 species) (Morat 1993), New Zealand (21 species) (de Lange *et al.* 2006), and the Hawaiian Islands (11 species) (Wagner *et al.* 1999). High endemism in such insular Pacific systems is attributed to species radiations (Gemmill *et al.* 2002) after establishment via suspected long distance dispersal of resinous seeds by birds (Carlquist 1974).

Early classification systems within *Pittosporum* were based on morphological characteristics such as leaf size and shape, capsule number, inflorescence type, placenta size (Cooper 1956), and various anatomical features such as leaf tissue layers (Wilkinson 1992). Furthermore, relationships between species were postulated based on valve number – and grouped within either bivalved or trivalved groups (Gowda 1951). However, morphological studies within the genus have been complicated by the abundance of phenotypic plasticity (ecophenetic or heteroblastic) (Chandler *et al.* 2007), and both hybridism and introgression, which skew well defined discriminating characteristics (Allan 1961). Recent molecular phylogenies are not consistent with initially proposed bivalve and trivalve clades (Hathaway 2001), but do support the hypothesis of a single colonization event onto the Hawaiian Islands (Gemmill *et al.* 2002). However, many of the relationships between species of *Pittosporum* remain unclear or unresolved.

4.5.4 New Zealand *Pittosporum*

Of the 21 New Zealand species of *Pittosporum*, all are endemic (de Lange *et al.* 2006). Nine of these are endemic to the North Island, and two to the South Island (Eagle 1982; Eagle 2006). There are two further entities that require resolution: *P.*

aff. *crassifolium* from Raoul Island (see Eagle 2006) and the *Pittosporum* found on Stephens Island which is intermediate in appearance between *P. crassifolium* and *P. tenuifolium* (B. D. Clarkson, pers. comm., University of Waikato 2010). Historic relationships among New Zealand *Pittosporum* were inferred using bi-valved and tri-valve group segregation (Gowda 1951). However, recent molecular data does not support this grouping (Hathaway 2001; Chandler *et al.* 2007). Phylogenetic relationships among New Zealand *Pittosporum* have been characterised using the Internal Transcribed Spacer (ITS) region of nuclear ribosomal DNA (nrDNA), supporting an Australian origin, with all other colonisation events from Australia or from island hopping (Chandler *et al.* 2007). New Zealand taxa form two distinct clades; one clade contains *P. cornifolium*, *P. pimeleoides* subsp. *pimeleoides* and *P. pimeleoides* subsp. *majus*, while all other taxa form a second monophyletic group (main clade). Within this main clade, the bivalve capsule appears as an ancestral form from which trivalve capsules have evolved twice (Hathaway 2001). Other morphological characteristics previously used for inferring relationships among New Zealand taxa (such as presence or absence of papery endocarps, small leaved bivalved species, heteroblastic vs. monoblastic development, and divaricating growth form) are also not consistent with molecular data in that they do not form monophyletic groups (Hathaway 2001).

Both of the proposed New Zealand radiations appear to be relatively recent events due to the limited level of ITS sequence divergence. An average of 1.4% sequence divergence between species in the main radiation make it an estimated 22 million years old. These estimates are consistent with the arrival of *Pittosporum* in the fossil record (Hathaway 2001). The clade containing *P. cornifolium* and *P. pimeleoides* was unexpected as these species have not previously been grouped based on morphology. However, this clades grouping was consistent due to identical sequences and an average of 8.3% (47.3 bases) sequence divergence from all other New Zealand taxa (Hathaway 2001). Hathaway (2001) proposed that *P. cornifolium* and both *P. pimeleoides* subspecies are the result of a more recent colonisation into New Zealand from New Caledonia, due to their close affinities with *P. gatopenese* and three other New Caledonian taxa.

Some four interspecific wild hybrids are known within the New Zealand *Pittosporum* (Druce 1977; Ecroyd 1994; Clarkson & Clarkson 1994), but no hybrid involving *P. cornifolium* has been recorded and, in particular, no hybrid with *P. pimeleoides*.

4.5.5 *Pittosporum cornifolium* nomenclature

Pittosporum cornifolium A. Cunningham was described and illustrated (plate 3161) by W. J. Hooker in 1832 in Curtis's Botanical Magazine, volume 59 (Figure 4.3). According to Cooper (1956, page 163),

> "the species was described by W. J. Hooker from material grown at the Royal Botanical Gardens, Kew, and from Allan Cunningham's specimens and notes made by him in New Zealand in 1826. Two "type" sheets in the herbarium of the Royal Botanic Gardens, Kew, bear five labels, two sterile specimens, a fruiting specimen and fragments of flowers. One label is dated 1826, one 1833, two 1838, and one is undated. As the species was described in 1832 only part of the material can have been available to W. J. Hooker".

Allan (1961, page 316) gives the type locality as "in humid woods on the banks of the Kanakana [Kawakawa] and other rivers, Bay of Islands, &c." and the type specimen as "British Museum, A. Cunningham, 1826". The specific epithet *cornifolium* refers to leaves resembling the cornel or dogwood tree belonging to the genus *Cornus* (Laing & Blackwell 1957).

Common English names used are cornel-leaved pittosporum (Hooker 1832; Cooper 1956; Laing & Blackwell 1957), perching pittosporum (Beever 1991) and straggling pittosporum (Anderson 1926).

Common Maori names used are tawhiri karo, karo, and wharewhareatua (Beever 1991).

Both perching kohuhu and perching kohukohu are common combined English – Maori names that have been applied (Cockayne 1967; Landcare Research 2010).

Figure 4. 3 *Pittosporum cornifolium* type speciman illustrated (plate 3161) by W. J. Hooker in 1832 in Curtis's Botanical Magazine, volume 59.

4.5.6 *Pittosporum cornifolium* intraspecific variation

Pittosporum cornifolium shows significant morphological variability in leaves, inflorescences, flowers and capsules, anatomical variability in leaves, and genetic variability across widely distributed populations, but especially with respect to the Poor Knights Islands individuals.

Capsule valve numbers within this species are inconsistent, with capsules typically being two-valved but occasionally producing three (Petrie 1921). Inflorescence type throughout the species ranges from terminal umbels with 2–10 flowers, to single terminal flowers (Cooper 1956; Petrie 1921). Flower colour is variable, with mainland forms bearing reddish pink (Salmon 1986) to a mix of light red and reddish brown (Hooker 1932), and the Poor Knights Islands forms bearing bright yellow flowers slightly later in the year (Smith 2004). Observations (2009–2010) from glasshouse collections showed that the mainland plants sourced from the Central North Island flowered earlier (early July to late September) while the Poor Knights Islands plants flowered later (mid August to late October) (Clarkson 2011).

Continuous variation of mean leaf length and width measures are observed from smaller mainland leaf forms (34.5–62.6 mm and 12.4–27.1 mm respectively) to the larger Poor Knights Islands leaf forms (53.7–70.8 mm 27.1–36.7 mm respectively) (Clarkson 2011). The Poor Knights Islands individuals are mainly distinguished by their greater mean width. Furthermore, maximum leaf length and width measures were significantly lower for mainland individuals when compared with Poor Knights Islands individuals (76 mm, 35 mm and 104 mm, 50 mm respectively) (Clarkson 2011).

Mean leaf blade thickness measures were significantly higher in the Poor Knights Islands form when compared with the mainland form (720 µm and 501 µm respectively). Thicker leaf blades have been related with greater degrees of xeromorphy and associated drought tolerance (Petrova 1988 as cited in Hameed *et al.* 2002, Venora & Calcagno 1991; and Hameed *et al.* 1995). Two tissue layers that are also related to greater degrees of xeromorphy include hypodermal/ aquias layers (Fahn 1982; Cutler *et al.* 2007) and bundle sheath cells composed of sclerenchyma (Ridley & Todd 1966), both of which were significantly thicker in

Poor Knights Islands individuals when compared with mainland individuals (mean values of 58.5 µm vs. 21 µm and 106 µm vs. 73.5 µm respectively) (Clarkson 2011). The greater hypodermal thickness observed in Poor Knights Islands individuals may indicate an adaptation to drought tolerance, as a well-developed hypodermis is suggestive of drought resistance (Ridley & Todd 1966; Rojas *et al.* 1983; Petrova 1988 as cited in Hameed *et al.* 2002). The Poor Knights Islands form is also more susceptible to frost, and unlike the mainland form it does not cease growth in winter months (Smith 2004).

Population genetic analyses were conducted on five North Island populations and eight propagated individuals sourced from the Poor Knights Islands using a genetic fingerprinting technique known as Inter-Simple Sequence Repeats (ISSR) (Clarkson 2011). Results indicated that genetic diversity was extremely high at species-level (90% polymorphic loci) but much lower at population-level (16.8–60.9% polymorphic loci). The outcrossing dioecious breeding system of *P. cornifolium* is likely to be one of the most important factors influencing the observed high species-level diversity (Clarkson 2011). Lower population-level genetic diversity (relative to intra-specific genetic diversity) is likely to be attributed to geographic isolation, this is because geographic isolation limits the amount of gene flow via both pollen and seeds (Pfeifer & Jetschke 2006). On the mainland, population isolation is due to the clearance and fragmentation of lowland ecosystems that host *P. cornifolium*, whereas the Poor Knights Island group is isolated from the mainland by ocean. Overall, the Poor Knights Islands plants exhibited the lowest levels of genetic diversity compared with mainland populations and were by far the most genetically distant population. Island populations are usually more differentiated and contain less genetic diversity than comparable mainland samples. These genetic differences may result from stochastic processes operating during founder events and/ or periods of small population size (Barrett *et al.* 1996; Frankham 1998). Results also revealed that populations appeared to cluster with respect to geographic location; in that in general the most closely aligned sites genetically were also close to one another with respect to geographic distance. This further supports the main hypothesis that geographic isolation is the main contributing factor to population-level differentiation in *P. cornifolium* populations (Clarkson 2011).

The ITS region comparisons of both mainland and Poor Knights Islands *P. cornifolium* individuals revealed a single point mutation (T>C) at 583 base pairs (Clarkson 2011). This was an unexpected result, especially considering the identical ITS sequences of mainland *P. cornifolium* with both *P. pimeleoides* subspecies. It is hypothesised that sequence divergence in this offshore island variant would have a maximum age that is consistent with the isolation of the offshore island group from the mainland – less than 1 million years (Clarkson 2011).

The distinct population genetic structure, leaf anatomy and morphology, and ITS sequence divergence of the Poor Knights Islands individuals may warrant the delineation of a new subspecies, or even species.

4.6 Chemistry

Phytochemical research has been undertaken within the wider family of Pittosporaceae (e.g. Hegnauer 1969 as cited in Carlquist 1981; Jay 1969), and even within *Pittosporum* (e.g. Nemethy & Calvin 1982; Jay 1969; Rao *et al.* 1990), but very little research has been specific to *Pittosporum cornifolium*.

Combinations of secondary compounds have been identified in Pittosporaceae including; flavines, caffeic and sinapic acids, cumarin, furano-cumarin, saponins, phenolic triterpenes (Hegnauer 1969 as cited in Carlquist 1981), flavinols, quercertin, and kampferol (Jay 1969).

Within *Pittosporum* monoterpenes, myrcene and α-pinene have been extracted from the fruit oil of *P. resiniferum*, and limonene from *P. undulatum* (Nemethy & Calvin 1982). Flavonoids such as quercertin, and kampferol are found consistently throughout members of *Pittosporum* and have been recorded in some of the New Zealand taxa (*P. crassifolium, P. eugenioides, P. dallii,* and *P. tenuifolium*) (Jay 1969). Flavonoid isorhamnetin has been identified in *P. eugenioides*, and flavone apigenin has been found in *P. tenuifolium* (Jay 1969). Phytosterols have been isolated from the bark of *P. colensoi* and *P. eugenioides* (Cambrie & Parnell 1969), and polyacetylenes from the root of *P. crassifolium* (Bohlmann & Zdero 1975 as cited in Nemethy & Calvin 1982). Greshoff (1909)

conducted a photochemical investigation of over 90 genera (including *Pittosporum*) in which he found saponin and tannin in the leaves of *P. cornifolium* (as well as *P. crassifolium, P. erioloma, P. eugenioides, P. huttonianum, P. rhombiflorum, P. tobira,* and *P. undulatum*).

4.7 Reproductive biology

4.7.1 Flowering

Pittosporum cornifolium flowers annually and has an autumn–spring flowering season extending from May–October with peak flowering in September (Clarkson 2011). This flowering duration is somewhat longer than reported by Cooper (1956) and Allan (1961). Specimens of the Poor Knights Islands form grown in the University of Waikato glasshouse complex have been recorded flowering from mid August to late October (the male individual was first to flower in mid August, while the female individual started late September). Mainland specimens growing alongside these island forms flowered much earlier, starting early July (the male individual flowered first in early July, finishing late September, and the inconstant male individual started late July) (Clarkson 2011). Petrie (1921) noted the tendency for male plants to produce more flowers than female plants, with male plants producing terminal umbels with up to 10 flowers (usually 6–8), and female plant producing single terminal flowers.

4.7.2 Pollination and seeding

Individual plants are typically dioecious (Petrie 1921). However, cuttings from an apparently dioecious male plant have produced infrequent capsules (Clarkson 2011). Godley (1979) has described this ‘inconstant male’ trait among other dioecious genera in New Zealand. Thus, *P. cornifolium* is sub-dioecious, with out-crossing being the preferential mode of fertilization but self fertilization may also be possible (controlled studies have yet to be undertaken to confirm this) (Clarkson 2011).

While no records of specific insect pollinators exist, *P. cornifolium* is thought to be entomophilous due to its small flower size and absence of features that are adapted to pollination by birds (Webb *et al.* 1999). These characteristics are

consistent throughout New Zealand *Pittosporum* taxa (Webb *et al.* 1999). Insect visitations have been recorded for *P. tenuifolium* by beetle species, *Erirhinus limbatus* and *Tigones caudata* (Thomson 1926), and numerous *Diptera* (fly) species (Heine 1937); for *P. crassifolium* by fly species, *Calliphora stygia* and *Syrphus novae–zelandiae*, members of families Tachinidae and Opomyzidae, the introduced bee *Apis mellifera* (Heine 1937) and insect orders Coleoptera, Hymenoptera, and Hemiptera (Anderson 2003); and for *P. eugenioides* by introduced flies (Thomson 1926). However, Anderson (2003) has also recorded bird visitations to *P. crassifolium* by endemic honey eaters, tui (*Prosthemadera novaeseelandiae*) and bellbird (*Anthornis melanura*); this association was previously underestimated and suggests birds may be active pollinators among other members of the New Zealand *Pittosporum*. Castro & Robertson (1997) document visitation of *P. cornifolium* flowers by endemic honeyeaters hihi (*Notiomystis cincta*) and bellbird; this further suggests birds may have a larger influence in the active pollination of *Pittosporum* than first suspected.

After fertilization, the ovules develop into black seeds which are irregular in shape (4–8 per capsule), capsules open to reveal vermilion stained interiors with seeds immersed in a sticky resin (Cooper 1956; Poole & Adams 1994). *Pittosporum cornifolium* is thought to be bird dispersed due to its resinous seeds (Oliver 1930; Burrows 1994), the vermilion red inner-capsule coating may also act as an attractant. Oliver (1930) records *P. cornifolium* seeds being accidentally attached to feathers which would serve as a dispersal mode, and Powlesland (1987) has recorded consumption of the seed by native kokako (*Callaeas cinerea*), however, tests to determine whether seeds are viable once passed through the gut have yet to be conducted.

No information is available on seed viability and seed germination rates but results for *P. obcordatum* (Clarkson & Clarkson 1994) show that seed from female plants geminates readily but seed collected from inconstant males failed to germinate.

4.8 Distribution

4.8.1 Geographic range

The known range of *Pittosporum cornifolium* extends from the North Cape (North Island) to the Marlborough Sounds and Paparoa Range (South Island) (Poole & Adams 1994) with a southern limit near Barrytown on the West Coast (Figure 4.4) (Clarkson 2011). It also occurs on numerous northern offshore islands such as Poor Knights Islands, Hen Island, Little Barrier Island, Great Barrier Island, Kawau Island, and Waiheke Island (Figure 4.4) (Clarkson 2011).

4.8.2 Environmental range

Throughout its geographic range, *P. cornifolium* can be found in a variety of rupestral and forest ecosystems within an elevation range of 0–786 m above sea level and a mean elevation of 248 m above sea level (Clarkson 2011). Environmental factors that may restrict distribution include high vapour pressure deficits, cooler temperatures, limited rainfall, limited solar radiation and higher elevations (Clarkson 2011). *Pittosporum cornifolium* is distributed widely in the coastal and lowland zones of the North Island. However, there are some obvious gaps in both its observed distribution and its predicted distribution (Figure 4.4). Gaps in its observed distribution that are inconsistent with potential environmental distribution include the Waikato basin where environmental variables are favourable but the observed absence is due to the area being formerly dominated by extensive wetland systems (Clarkson 2002). The environment is suitable south of the Wairoa lowlands in the Wairarapa but there are no current records probably because nearly all lowland forest in the area has been cleared (Nicholls 1980). Similarly, much of the Taupo Volcanic Zone is favourable in terms of the predicted environmental distribution of *P. cornifolium*, but observed absences are likely to be due to the ecological impact caused by the 186 AD Taupo eruption (Lowe & de Lange 2000). Both Mahia in the North Island and Banks Peninsula in the South Island are also environmentally favourable locations where *P. cornifolium* has not been recorded. The often companion obligate epiphyte *Griselinia lucida* is present at both of these localities: Banks Peninsula (Laing 1919) and Mahia (Whaley *et al.* 2001). As well, many predominantly North Island subtropical species such as nikau (*Rhopalostylis sapida*) are found growing in the Banks Peninsula area (Department of Conservation 2010). *Pittosporum*

cornifolium is largely unrecorded from the drier eastern side of the North Island where native forests have been almost completely removed, and the low rainfalls are not conducive to rich epiphyte floras (Clarkson & Clarkson 1991). Absence from the Wairoa, Gisborne and Waiapu lowlands was predicted due to the high mean October vapour pressure deficits exceeding 0.47 kPa in the area (Clarkson 2011). *Pittosporum cornifolium* was absent from the Kaimanawa and Ikawhenua ranges in the Central North Island in both observed and predicted distribution maps. This is because of mean daily temperature minimums for the coldest month that were lower than 0.6°C in the area. *Pittosporum cornifolium* has both restricted observed distributions and predicted distributions in the South Island; these limits are set primarily by low mean daily temperature minimums of the coldest month (<0.6°C), but also from a combination of low mean annual temperature (9.6°C), low mean annual rainfall (<937 mm), low mean annual solar radiation (<13.1 kJ/m²/day), low mean minimum solar radiation in June (<4.2 kJ/m²/day) and high elevation (>786 m) (Clarkson 2011).

Pittosporum cornifolium is classified as a sun epiphyte due to its pronounced xerophytic features such as its thick cuticle and water storage tissue (Oliver 1930). *Pittosporum cornifolium* is commonly recorded in high light habitats such as exposed rock outcrops and the crowns of emergent or canopy trees as well as semi-shaded habitats such as in the understory of relatively open canopy kauri (*Agathis australis*) forests and the lower tiers of large canopy trees in a range of lowland forest types (Clarkson 2011).

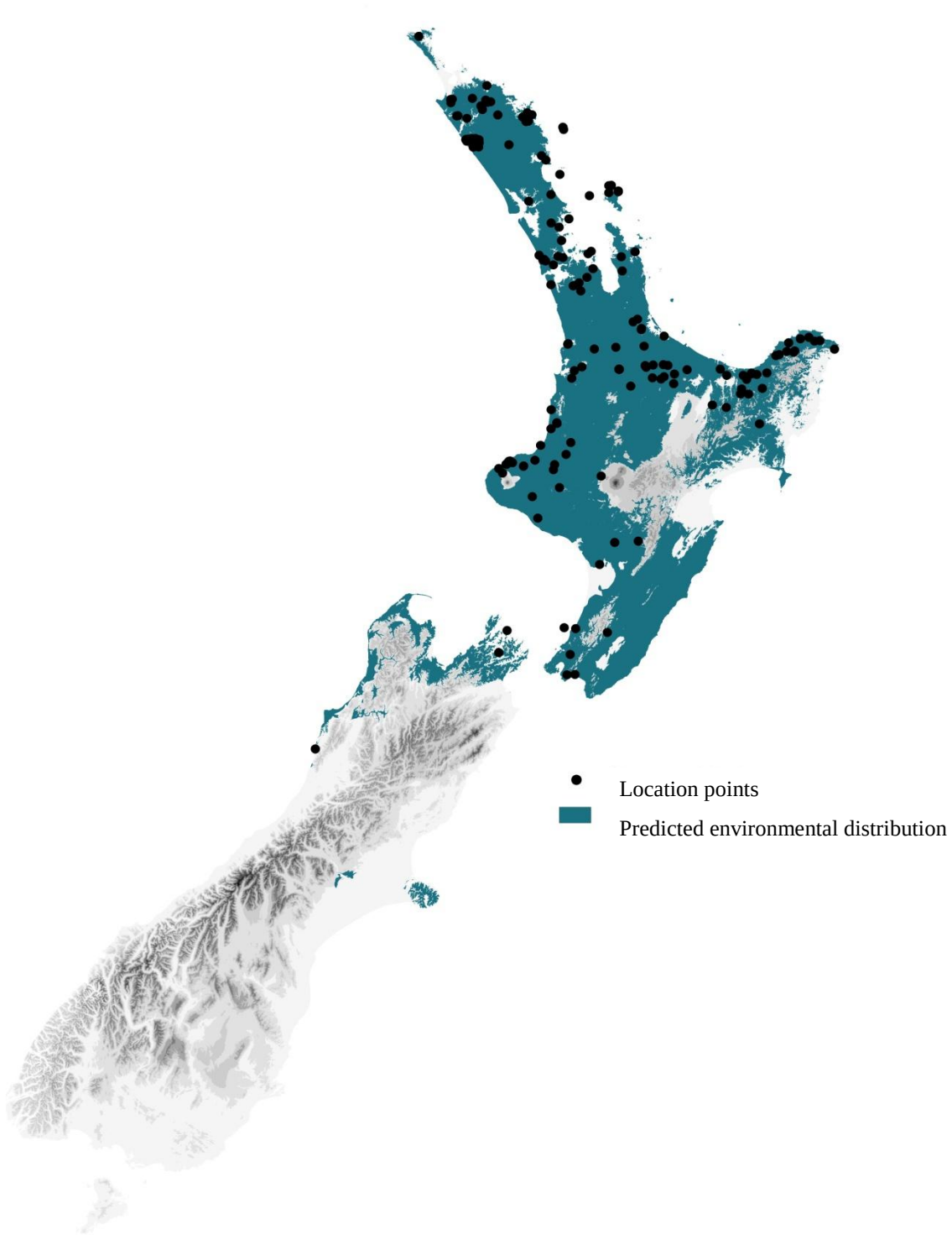

Figure 4. 4 Observed distribution (location points) and predicted environmental distribution of *Pittosporum cornifolium* based on environmental variables including total annual rainfall, mean October vapour pressure deficits at 0900 hours, mean annual temperature, mean minimum daily temperature of the coldest month, elevation, mean annual solar radiation, and mean minimum daily solar radiation in June.

4.8.3 Edaphic range

Although soil composition varies with respect to habitat type, *P. cornifolium* is consistently found growing on well drained low nutrient substrates. In the canopy, wind exposure and direct sun induce high evaporation, the limited arboreal 'soil' restricts moisture absorption and water retention, and gravity ensures rain water drains rapidly to the ground (Dawson & Lucas 2005). In these canopy systems *P. cornifolium* is found growing almost exclusively within large 'nest' epiphytes including *Astelia solandri* and *Collospermum hastatum* (Oliver 1930; Clarkson 1985; Burns & Dawson 2005; Clarkson 2011). Establishment of these 'nest' growth forms facilitate the establishment of larger shrub epiphytes like *P. cornifolium* by providing an increase in organic matter and moisture thereby enhancing microclimate (Dickinson *et al.* 1993). The growth substrate provided by these nest hosts is therefore composed entirely of organic matter (dead leaves and roots) by which decomposes into a fine, low nutrient gritless soil that collects in large quantities (Oliver 1930) with low nutrient status (Dickinson *et al.* 1993).

Pittosporum cornifolium also grows epiphytically directly on the root mantle (trunk) of tree ferns including *Cyathea dealbata* and *Cyathea cunninghamii*. The vertical trunks of tree ferns would also be exposed to rapid draining by gravity and are composed of purely organic (and therefore low nutrient) adventitious roots (Clarkson 2011).

Similarly, *P. cornifolium* grows on exposed coastal rock substrates which experience high evaporation rates due to strong coastal winds and high solar radiation levels. *Pittosporum cornifolium* has been recorded growing on silicic (rhyolite) rock, greywacke rock, and calcareous (limestone) rock outcrops (Clarkson 2011). The individuals found on calcareous rock outcrops of Raglan Harbour were growing within 'nest' host communities of *Astelia banksii.* Decomposition of organic matter provided by *A. banksii* would produce low nutrient soil types similar to those resulting from canopy 'nest' associations.

Records of terrestrial *P. cornifolium* indicate similar preference for sloping well drained soil types, i.e. hard beech (*Nothofagus truncata*) dominant ridges and kauri forest ridges (Clarkson 2011). Both kauri and beech forests are associated with low fertility, well drained soils (Orwin 2009a,b).

4.9 Plant communities

Pittosporum cornifolium is represented in a range of lowland and coastal forest, and rupestral ecosystems. Within these ecosystems, *P. cornifolium* exhibits three distinct lifestyles; epiphytic, terrestrial and rupestral, the most common being the epiphytic lifestyle (Clarkson 2011). While it is never a dominant component of these ecosystems, it occasionally attains co-dominant status in the rupestral communities of Raglan Harbour (Clarkson 2011).

4.9.1 Lowland and coastal forest communities

Pittosporum cornifolium occurs in several lowland forest types including kauri, mixed podocarp, coastal *Metrosideros* and broadleaved forest types. Within these lowland forest systems, where it exhibits both epiphytic and terrestrial lifestyles, the species appears to have an affinity to older growth forest and/ or remnant old growth trees (Clarkson 2011).

The tall kauri forests of the North Island, which usually occur on well drained hill sides (Oliver 1930), host *P. cornifolium* of both terrestrial and epiphytic lifestyles. Within this forest type *P. cornifolium* has been found growing abundantly in the crowns and directly beneath the crowns of large old growth kauri trees, on the trunks of tree fern species *C. dealbata* and *C. cunninghamii*, and in association with *C. hastatum, A. solandri, Astelia trinerva* and *Brachyglottis kirkii* (Clarkson 2011).

Podocarp dominant lowland forests, with their dense canopy of foliage, contain the greatest diversity of epiphytes (Oliver 1930). Within these forests, *P. cornifolium* can be found on a variety of host trees including: rimu (*Dacrydium cupressinum*), totara (*Podocarpus totara*), matai (*Prumnopitys taxifolia*), and kahikatea (*Dacrycarpus dacrydioides*), and grow primarily in association with *C. hastatum* and *A. solandri* (Clarkson 2011).

Broadleaved dominant lowland forests also provide a range of canopy hosts. These include tawa (*Beilschmiedia tawa*), taraire (*Beilschmiedia taraire*), pukatea (*Laurelia novae–zelandiae*), puriri (*Vitex lucens*), and titoki (*Alectryon excelsus*), and again *P. cornifolium* grows primarily in association with *C. hastatum* and *A. solandri* (Clarkson 2011).

The main coastal forest hosts are pohutukawa (*Metrosideros excelsa)* and puriri (Clarkson 2011). Kirk (1872) noted abundant epiphytic *G. lucida*, *P. cornifolium* and *A. solandri* on Tarawera lake shore pohutukawa prior to the 1886 eruption. Large puriri growing in the Raglan Harbour also host epiphytic *P. cornifolium* (Clarkson 2011).

Other associated species in lowland and coastal forests include *Metrosideros fulgens, Asplenium polyodon* and *Microsorum pustulatum* (Clarkson 2011).

4.9.2 Rupestral communities

Pittosporum cornifolium can be found growing as a rupestral in coastal rock communities including Maunganui Bluff (G. Bowden, Tawapou Nursery, pers. comm. 2010), Waiheke Island, Poor Knights Islands, and Raglan Harbour where *P. cornifolium* grows as a co-dominant species amongst scrub communities of *A. banksii* and *G. lucida*. (Clarkson 2011).

4.10 Succession

Oliver (1930) describes the general pattern of epiphyte succession in New Zealand forests as follows: primary colonisation of bark by small lichens and mosses act to facilitate (by substrate alteration) the establishment of ferns or orchid species (which can colonise almost bare bark surfaces). The increase in community composition encourages further establishment (i.e. lichens and mosses adhere to fern rhizomes or aerial roots of orchids), while exfoliated bark and fallen leaves are caught and decay to produce a rich soil humus (vertical branches may support soil layers as thick as a third of the branch diameter). Moisture is retrieved directly via rainfall or indirectly via trunk runoff, while evaporation is retarded by the covering of foliose lichens (Oliver 1930). The epiphytic habit of

Pittosporum cornifolium is termed ‘proto-epiphyte’ in that it acquires nourishment from the surface of its supporting structure and the atmosphere (Oliver 1930), therefore establishment generally occurs late in the successional sequence as it needs a reasonable formation of humus to gain this required nourishment.

Establishment in late successional communities by *P. cornifolium* is evidenced by its common association with the nest hosts *A. solandri* and *C. hastatum* when an epiphyte (Clarkson 2011). Establishment of these nest epiphytes may facilitate the establishment of larger shrub epiphytes like *P. cornifolium* by providing an increase in organic matter and moisture thereby enhancing microclimate (see Dickinson *et al.* 1993).

In contrast to nest epiphytes, the mantle of adventitious roots of tree ferns provides moisture retaining organic stratum in which epiphytes can directly take hold and embed their roots (Oliver 1930). Tree fern trunks are a ready-made growth substrate for epiphytic species without need for the either the breakdown of mineral substrate or the build up of organic matter as initiated by facilitation on primary substrates like rock or bark (Clarkson 2011).

Rupestral individuals at Raglan Harbour were also found growing in association with nest host species (*A. banksii*), and facilitation by the nest host is likely, as *P. cornifolium* individuals were notably absent from raw rock surfaces (Clarkson 2011).

The detritus substrate of terrestrial individuals growing beneath kauri stands in the Coromandel is composed of mostly brown loam soils (Molloy 1998). Detritus substrates are a result of long term soil development by gradual erosion and build up of organic matter provided by old growth kauri forest systems. Therefore the build up of detritus soils in old growth systems may facilitate the establishment of terrestrial *P. cornifolium* as does substrate development in canopy and rupestral systems (Clarkson 2011).

4.11 Conservation and restoration

4.11.1 Conservation status

The loss of lowland and coastal habitat in the Waikato region (Leathwick *et al.* 1995), and in the majority of ecological regions around New Zealand (McGlone 1989), has meant the historical range of *P. cornifolium* is significantly reduced and its populations depleted. Additionally, the strong habitat specificity of *P. cornifolium* restricts it to habitats of very limited extent (Clarkson 2011).

Pittosporum cornifolium can be locally uncommon but does not currently meet the specific criteria (see de Lange *et al*. 2009) to be considered regionally or nationally threatened (Clarkson 2011). However, there is no doubt that its range has been reduced and that it has been lost from a number of sites where it was previously collected (Clarkson 2011). Anecdotal observations have suggested that it is palatable to possums, and possum browsing may have contributed to the reduction of some populations (Ravine 1995; Mitcalfe & Horne 2005).

4.11.2 Conservation and restoration recommendations

Conservation efforts should be focused on retaining substantial populations in large reserves while restoration is required where numbers are reduced or the species has been recently lost.

The significant correlation between geographic distance and genetic differentiation among populations of *P. cornifolium* highlights the importance of plant provenance and associated ecotypes when sourcing seed for restoration (Clarkson 2011). The concept of ecological microhabitats is also important considering the different lifestyles of *P. cornifolium* in a range of lowland and coastal ecosystems (epiphytic, rupestral and terrestrial). Ideally, source sites would be both the closest to the restored site (to maintain provenance) and be a similar ecosystem to the one in need of restoring (Clarkson 2011). Furthermore, seeds should be collected from as many individuals as possible to maintain levels of genetic diversity similar to that of source populations (Clarkson 2011).

The distinct population genetic structure, leaf anatomy and morphology, and ITS sequence divergence of the Poor Knights Islands individuals raise some

interesting questions with regard to species delineations. Such distinctions among island individuals and the fact that island populations are more vulnerable to extinction (due to stochastic processes operating during founder events and/ or periods of small population size (Barrett *et al.* 1996)) merit a high conservation value.

4.11.3 Conservation and restoration to date

To date only a few restoration projects around New Zealand have considered reintroduction of *P. cornifolium.* These include Karori Sanctuary, Matakohe Island and Motuihe Island. Karori Sanctuary, Wellington, comprises 225 ha of regenerating lowland forest and wetlands protected by an 8.6 km predator-proof fence, specially designed to exclude non-native mammals ranging from hedgehogs to possums (Karori Sanctuary Trust 2008). Currently one mature *P. cornifolium* specimen is present within the sanctuary so propagation is underway for further reintroductions (Karori Sanctuary Trust 2008). The restoration plan for Matakohe/ Limestone Island (a 37 ha island located in Whangarei Harbour, Northland), has incorporated introduction of *P. cornifolium.* Although not currently present on the island, there is suitable habitat available and it is a locally native species (Ritchie 2000). The plan recognises the importance of local provenance by advocating collection of seeds and cuttings from the Eastern Northland and Islands Ecological District. It has also incorporated the concept of maintaining the genetic variability of collections by collecting seeds from as many genotypes as possible (Ritchie 2000). Finally, Motuihe Island, a 179ha island situated 4.5km off Musick Point in the Inner Gulf Islands Ecological District, where the majority of forest has been converted to pasture is currently being restored and *P. cornifolium* is proposed for reintroduction (Hawley 2005).

4.12 Conclusion

Pittosporum cornifolium is an endemic shrub that is well adapted to a variety of lifestyles including terrestrial, rupestral, and, more commonly, epiphytic. The strong habitat specificity of *P. cornifolium* restricts it to lowland and coastal habitats of very limited extent, which are widely yet discontinuously distributed. Environmental factors that may restrict distribution include high vapour pressure deficits, cooler temperatures, limited rainfall, limited solar radiation and higher

elevations, and edaphic range is characterised by low nutrient well drained substrate types. The distinctive leaf morphology and anatomy as well as the differentiated population genetic structure and ITS sequence divergence in the Poor Knights Islands individuals suggest recognition at subspecies or even species-level may be warranted. However, more detailed examination of the taxon across its mainland range, the Poor Knights Island group, and other northern offshore islands where the species is present is needed. While *P. cornifolium* can be locally uncommon across its known range, it does not currently meet the specific criteria to be considered regionally or nationally threatened. However, there is no doubt that its range has been reduced and that it has been lost from a number of sites where it was previously collected. Key considerations for the conservation and restoration of this species in areas in which populations have been depleted or completely displaced include sourcing for local provenance and specific microhabitats and associated lifestyles.

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5 Chapter five: Synthesis

5.1 Main findings

This research has contributed to our understanding of the population genetics and autecology of the endemic shrub epiphyte *Pittosporum cornifolium.* Genetic analysis has revealed patterns of genetic variation within and among populations of *P. cornifolium* with regard to mainland New Zealand and individuals from the Poor Knights Island group (Chapter 2). Ecological parameters were used to determine the current ecological status of five mainland populations (Chapter 3). Ecological data were then incorporated with information from national data sets to provide a more comprehensive overview of *P. cornifolium* autecology (Chapter 3). Environmental mapping has identified the major environmental determinants of species distribution throughout New Zealand (Chapter 3). Both genetic and autecological research have contributed to identifying major biological distinctiveness of mainland individuals compared with the Poor Knights Islands individuals (Chapters 2 & 3). The results of this research have provided a framework for the development of species specific conservation and restoration strategies for *P. cornifolium*.

Pittosporum cornifolium exhibits high genetic diversity at species-level. Both the outcrossing dioecious breeding system and unique evolutionary history of *P. cornifolium* are likely to be key factors influencing the observed high intra-specific diversity. In contrast, population-level genetic diversity was relatively low across all populations. The clearance and fragmentation of lowland forest systems, which has lead to geographic isolation, is likely to have had the most significant influence on the partitioning of genetic diversity among mainland *P. cornifolium* populations. This is because geographic isolation limits the amount of gene flow via both pollen and seeds (Pfeifer & Jetschke 2006). Similarly, the long history of isolation by ocean is likely to be a key influence in reduced population genetic diversity of samples from the Poor Knights Islands individuals. However, the observed low genetic diversity of the Poor Knights Islands individuals may also be due to the small sample size, and the fact that all samples were derived from propagated individuals.

Genetic results also revealed that populations appear to cluster with respect to geographic location in that the most closely aligned sites genetically were generally also close to one another with respect to geographic distance. This further supports the main hypothesis that geographic isolation is the main contributing factor to population-level differentiation in *P. cornifolium* populations. This significant correlation between geographic distance and genetic differentiation among populations of *P. cornifolium* highlights the importance of plant provenance and associated ecotypes when sourcing seed for restoration projects.

The ecological assessment of five mainland populations of *P. cornifolium* in the Central North Island did not reveal any substantial ecological impediments to regeneration and dispersal modes. While juveniles and seedlings were not strongly represented across all of the populations measured, there is no solid evidence of a significant regeneration gap. The surveyed individuals were generally concentrated in close proximity to one another and are likely to be part of more widely dispersed populations within the broader collection areas. Total proportions of female individuals across populations were lower but all identified females were recorded producing multiple fruits almost immediately after an extensive flowering season, thus indicating high probability for ongoing regeneration. Noted high concentrations of individuals in the Coromandel populations beneath emergent kauri trees indicates prevalence of dispersal by gravity, but does not discount bird dispersal over greater distances.

Pittosporum cornifolium was described as a typical epiphyte by Oliver (1930), but it can also be found growing in abundance in terrestrial and rupestral lifestyles and may be considered a facultative epiphyte as defined by Benzing (1990). *Pittosporum cornifolium* is present in a range of lowland and coastal ecosystems, being typically affiliated with old growth forest systems and well drained low nutrient substrates. Environmental factors that may restrict distribution include cool temperatures, high vapour pressure deficits, limited rainfall, limited solar radiation and high elevations. Evidence from the ecological survey suggests habitat type and associated species and density can predict which lifestyle is most suitable when considering reintroduction for restoration projects.

Pittosporum cornifolium shows significant morphological, anatomical, and genetic variability with respect to the Poor Knights Islands individuals. These had greater mean leaf widths, and higher maximum leaf length and width measures compared with mainland forms. Similarly, mean leaf blade thickness measures were significantly higher in the Poor Knights Islands form than in the mainland form. As well, leaf tissue layers including the hypodermis and bundle sheath cells were significantly thicker in Poor Knights Islands individuals. Greater hypodermal thickness may indicate an adaptation to drought tolerance (Ridley & Todd 1966; Rojas *et al.* 1983; Petrova 1988 as cited in Hameed *et al.* 2002). The Poor Knights Islands individuals were the most genetically distinct from all other populations, having higher levels of genetic distance than mainland populations as well as more unique loci. Comparisons of the Internal Transcribed Spacer (ITS) region of both Poor Knights Islands and mainland individuals revealed a single point mutation (T>C) at 583 base pairs for the former. This is an important difference when considering the identical ITS sequences of mainland *P. cornifolium* with and both *P. pimeleoides* subspecies. It is hypothesised that sequence divergence in this offshore island variant would have a maximum age consistent with the isolation of the offshore island group from the mainland, i.e. less than 1 million years. Such distinctions among island individuals merit a high conservation value for the Poor Knights Islands form.

5.2 Directions for further research

Perhaps the most interesting findings of this research are the significant differences demonstrated across multiple lines of evidence (genetics, morphology and anatomy) with respect to the Poor Knights Islands individuals. Such distinctions suggest recognition at subspecies or even species level may be warranted. However, a more detailed examination of the taxon across its mainland range, the Poor Knights Island group, and other northern offshore islands where the species is present is needed. Breeding experiments would also be necessary within *P. cornifolium* and closely affiliated members of *Pittosporum* to determine functional levels of divergence. Finally, continued ecological research on this species that would be useful for conservation and restoration initiatives include germination and reintroduction trials.

The current findings on potential taxonomic separation are hinged on a very small subset of propagated individuals from the Poor Knights Islands. These individuals may not be representative of the total range in genotypes and phenotypes present in the natural populations. Further research is required to rule out the possibility of continuous variation from the mainland form to the currently defined variant on the island group. Furthermore, *P. cornifolium* has been recorded on a number of other northern offshore islands and research would be needed to determine whether these islands host similar or even intermediate morphological and genetic variations of the species. Of particular interest is Kawau Island where *P. cornifolium* have been identified with yellow flowers (Petrie 1921) and Hen Island where individuals that have been propagated have larger, thicker leaves (S. Bartlam, Landcare Research, pers. comm. 2011). It is also possible that the island forms and even the plants at coastal mainland sites such as Raglan Harbour have physiological ecotypic adaptation to tolerate high salinity given their close proximity to the sea; salt tolerance experiments could be undertaken.

Breeding experiments would also be necessary in determining appropriate levels of taxonomic recognition within the current species (de Queiroz 2007), as intrinsic reproductive isolation is paramount in determining biological species delineation (Mayr 1942; Dobzhansky 1950). Breeding experiments between mainland and offshore variants of *P. cornifolium*, as well as between *P. cornifolium* and *P. pimeleoides* subspecies, would be necessary to clarify species and subspecies functional relationships within the relevant New Zealand *Pittosporum* clade.

Future ecological research is proposed to aid reintroduction efforts for conservation and restoration projects. Preliminary germination trials were undertaken as part of this research but no seeds have germinated thus far. More extensive germination trials would be useful in determining the best conditions for propagation of this species for underpinning reintroduction efforts. Additionally, trials on species establishment would also be required to develop best practice techniques to maximise restoration success.

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Appendix 1: Isolation of DNA procedure

Adjusted from a method obtained from Ray Cursons and Richard Wilkins, The University of Waikato 2008

HOMOGENISATION BUFFER (H. Buffer):

- EDTA Ethylenediaminetetraacetic acid 20 mM
- CTAB Hexadecyltrimethylammonium bromide 2 %
- PVP–40 Polyinvylpyrrolidone MW 40,000 w/v
- DIECA Diethyldithiocarbamic acid 4 mM
- Tris– HCl (pH 8) 100 mM
- NaCl 1.42 M

100ml solution of H. Buffer:

- 10 ml 1M Tris–HCl
- 4 ml 0.5M EDTA
- 2 g CTAB
- 28.4 ml 5M NaCl
- 2 g PVP–40
- 0.069 g DIECA

Other Chemicals needed for procedure:

- 2–mercaptoethanol (3.49 µl per reaction)
- Proteinase K (0.5 µl per reaction)
- RNase A (10 mg/ml)
- Chloroform: Isoamyl alcohol (24:1)
- 7.5M NH_4Aco: 95% ethanol solution (1:6) – stored at -20°C
- (stored at -20°C)
- Isopropanol
- 100% ethanol
- 70% ethanol
- TE buffer

<u>PREPARATION:</u>

- Label and leave under ultra violet light for 5 mins 2×1.5ml Microcentrifuge tubes per sample
- Collect ice and Liquid Nitrogen
- Collect Mortar and Pestles (one per sample)
- Add 500 µl (× no. of samples) of H. Buffer to a15 ml Conical tube, Then add 3.49 µl of 2–mercaptoethanol (× no. of samples) & 0.5 µl of Proteinase K (× no. of samples) to the conical tube and Invert to mix **NB:** add ingredients for 1 extra sample for pipetting error
- Aliquot 500µl of H. Buffer + 2–mercaptoethanol mix into the first set of U.Ved microcentrifuge tubes and sit on ice.

STEPS:

1: Weigh out about 0.7g (this varies with species) of frozen leaf material per sample then grind to a fine powder using Liquid Nitrogen. Scrape powder into microcentrifuge tube on ice Mix thoroughly by flicking tube (the buffer and leaf material shoµld make a thick solution), then return to ice until all samples have been ground.

2: Vortex samples at high speed then incubate in the Thermomixer at 60°C for 10–15 minutes (more for fibrous leaves).

3: Add 500µl of Chloroform: Isoamyl alcohol (24:1), cap tube and mix vigorously using Vortex at high speed.

4: Centrifuge at Max speed for 10 minutes – you should now have two distinct layers in the tube with an interface between them that may look like skin. The DNA is in the top (Aqueous) layer, Debris type plant material in the interface and proteins etc in the lower (Chloroform) layer.

5: Recover DNA by gently sucking off the top layer and transferring it to a fresh 1.5ml microcentrifuge tube – Try to recover as much of the top layer without sucking up any of the interface or lower layer (NOTE: if supernatant appears cloudy or had debris left in it REPEAT steps **3 – 5**).

6: Add equal volume of Isopropanol (400 µl?) and invert to mix, then sit samples for 10 minutes.

7: Centrifuge at max for 10 minutes.

8: Locate the fine whitish pellet near the bottom of the tube, then use a fine tip pipette to remove all supernatant without disturbing pellet (which contains the DNA!).

9: Resuspend DNA in 20µl MQ H_2O (use thermo–mixer at 37°C) then add 70µl NH_4Aco: 95% EtOH (1:6) and invert afew times. Precipitate at –20°C for at least 20 mins (overnight if necessary).

10: Spin for 5 mins at maximum, pipette supernatant (leaving pellet) and dry in speed vac.

11: Add 500 µl of 100% ethanol, centrifuge at max for 1 min and again locate the pellet and remove the supernatant as above in **8.**

12: Add 500 µl of 70% ethanol to samples, centrifuge at max for 1 min – again locate pellet and remove supernatant as in **8** – Re-spin at max briefly to bring liquid from sides down – remove excess liquid without disturbing pellet.

13: Sit on bench for 5 mins to dry off any ethanol. Then add 100µl of TE buffer and 1µl of RNase A (10mg/ml) – Shake tube in thermomixer at 37 °C for 1hour+ to resuspend DNA.

Appendix 2: Geographic distance matrix

Appendix Table 1 Geographic distance matrix (km) of six *Pittosporum cornifolium* populations used in Mantel test.

	Taranaki	The 309 Road	Square Kauri	Raglan Harbour	Maungatautari	Poor Knights
Taranaki	0					
The 309 Road	300	0				
Square Kauri	280	20	0			
Raglan Harbour	160	120	110	0		
Maungatautari	175	140	120	70	0	
Poor Knights	400	180	200	270	300	0