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Identifying Host Species of *Dactylanthus taylorii* using DNA Barcoding

A thesis submitted in partial fulfilment

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A note on formatting:

This thesis is a thesis by publication, with each of the research chapters forming the basis of late publications. Chapters Two and Three are intended to be sufficiently detailed to be considered as stand-alone pieces.

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Chapter One: Introduction

1.1 Parasitic Plants

Approximately one percent of angiosperms have evolved to exploit other species of angiosperms through parasitism. Parasitic plants gain some or all of their nutrients and/or water through a physical connection with a host plant (Aly 2012). Parasitic plants interact with host species in a manner that can be considered equivalent to that of an herbivore, as it removes similar levels of carbon and induces similar defense responses (Pennings 2002). Parasitism is a heterotrophic lifestyle, as opposed to the autotrophic lifestyle of most plants (Graves 1995). In order to be classified as parasitic, the parasitic plant must possess an organ known as a haustorium. This distinction separates parasitic plants from mycotrophic plants, which gain their nutrients indirectly from another plant, through the mycorrhizal fungi of that plant. The haustorium (or its precursor root) breaks into host tissues and forms connections with the vascular system of the host. The haustorium is the interface between host and parasitic plant, allowing the exchange of water, sugars, even genetic material (Hibberd 2001).

Parasitic plants occur in 19 angiosperm families, and are a diverse and polyphyletic group, with 12 orders known to contain parasitic members (Kujit 1969). Approximately one percent of the world's angiosperms are parasitic, a total of over 4000 species (Heide-Jørgensen 2008). Parasitism has evolved in at least twelve independent lineages at the level of order, with parasitic plants showing great diversity genetically, physically and ecologically (Figure One). Barkman et al (2007) suggest eight origins for holoparasites, with endoparasitism occurring in four clades. Parasitic plants can be found in nearly every habitat across the globe, with immense diversity allowing them to thrive wherever suitable hosts can be found. Of the 12 orders, five show a global distribution of parasitic members. The orders which contain parasitic

plants, and the number of genera within orders are shown, along with a crude distribution.

Table 1: Angiosperm orders that contain parasitic plants, and their locations and number of known parasitic containing genera. Information taken from Heide-Jorgenson 2008.

Order	**Distribution of parasitic genera**	**Number of genera containing parasitic plants**
Boraginales	America (North & South)	2
Cucurbitales	America (North & South)	3
Ericales	Global	1
Lamiales	Global	90
Laurales	Tropics	1
Malpighiales	Malaysia, Asia	3
Malvales	Global	2
Piperales	Tropics, Africa	2
Santalales	Global	>100
Saxifragales	Europe, Mediterranean, Central Asia	1
Solanales	Global	1
Zygophyllales	America (North & South)	1

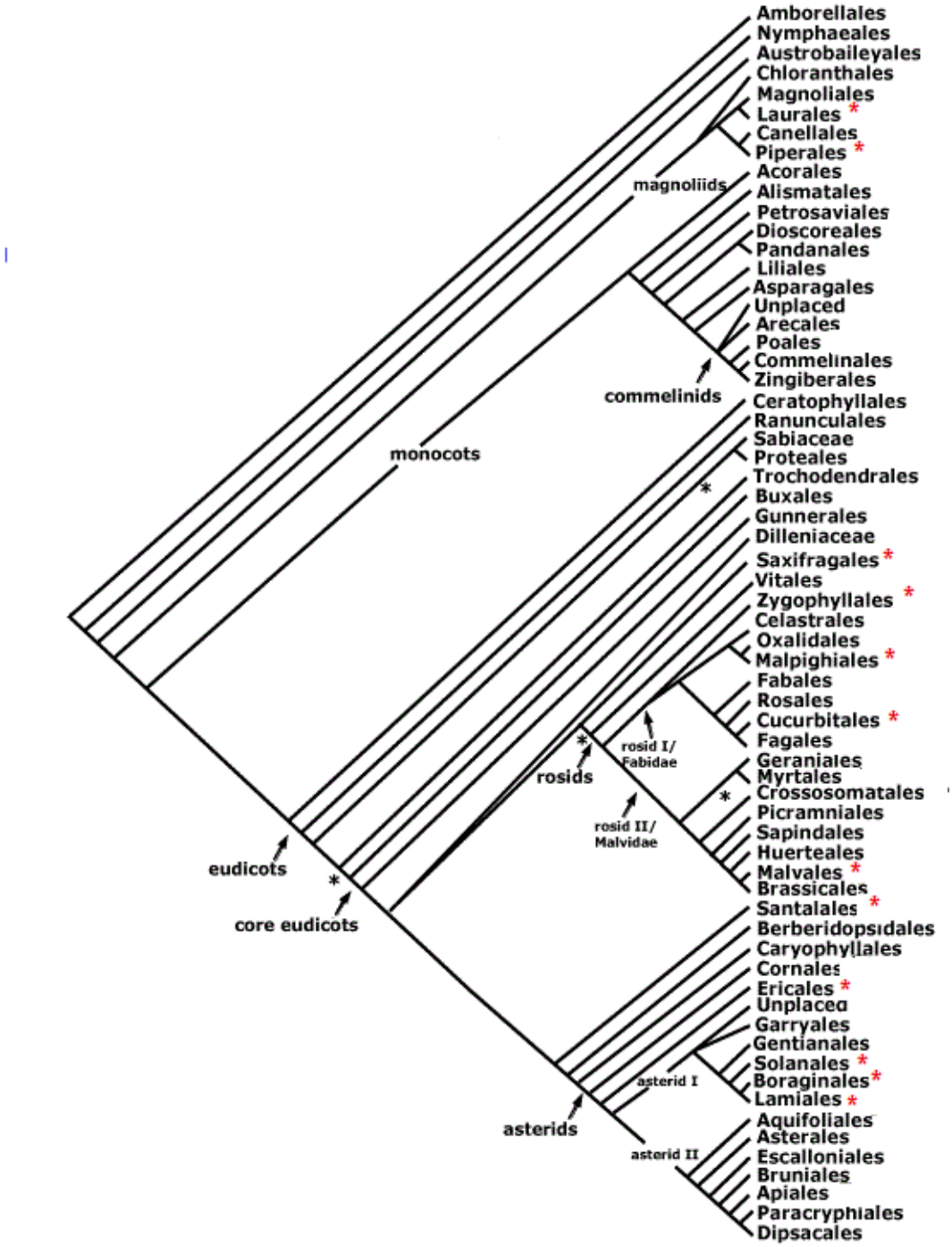

Figure 1: A phylogeny of the angiosperm orders, showing the orders which contain parasitic species, highlighting the diverse nature of parasitic plants as a group. Image adapted from Stevens, P. F. (2001 onwards). Angiosperm Phylogeny Website. Version 12, July 2012.

The Santalales contains seven families, containing the highest number of parasitic genera. Over 100 genera within the Santalales contain parasitic species. A phylogenetic tree showing parasitic families (Figure 1) highlights the multiple occurrences of parasitism throughout the angiosperms (Heide-Jorensen 2008). Research into the evolution of parasitism has only begun in recent years; the current literature is only able to suggest that the transition from autotroph to parasitic plant must involve a set of genes that are able to adapt a root into a haustorium (Westwood 2003). Much of the research has focused on the parasitic plants targeting crop species, but many species have been described, and some important economic plants are parasitic. *Santalum album* L. (Santalaeae), commonly known as sandalwood, is one such taxon, with Australian sandalwood comprising a multi-million dollar market (Carpenter 1998). Though parasitic plants are still a vastly understudied area, the research that has been conducted has revealed many fascinating and exciting phenomenon – including the exchange of genetic material between multiple parasitic plant and host species (Mower 2010).

Generalizing amongst such immense diversity is difficult, but several basic artificial classifications exist. Each of these classifications is somewhat artificial, as they do not capture the range of intermediates that exist, and classify parasitic plants into broad groups. These classifications remain useful however, as an initial means of understanding the physiology and ecology of a parasitic species. Parasitic plants can be classified into stem or root parasites, based on which part of the host the parasitic plant infects (Graves 1995). This classification is not always robust, as some species parasitize any host tissue that they come into contact with. Mistletoes such as *Viscum album* are examples of stem parasites, while *Rafflesia arnoldii*, famous for bearing the world's largest fowers, is an example of a root parasite. *Cuscuta europaea* is an example of a parasitic plant which can be found penetrating virtually all parts of the host – the genus is also noted for its hyperparasitism,

and will readily parasitize itself and other parasitic species (Kujit 1969). Parasitic plants can also be divided into holo and hemi parasites (Kuijt 1969). Holoparasites do not photosynthesize, and rely on their host for all nutrients and water. In some cases, holoparasitic species have lost their chloroplasts completely, and genes associated with photosynthesis are no longer functional. Hemi parasites retain some photosynthetic ability and are not completely reliant on their host. The mistletoe species *Viscum album* is an example of a hemiparasite, while *Raflessia arnoldii* is a holoparasite. Hemiparasites are further divided into facultative or obligate parasites. Facultative parasites are able to complete their life cycle in the absence of a host, while obligate parasites cannot do so. Parasitic plants can exist as epiparasites, with their vegetative form largely outside of host tissues, or as endoparasites, with a majority of vegetative tissue contained within the host. The species *Pilostyles thurberi* A. Gray (Apodanthaceae) is an endoparasite, only visible during flowering and fruiting.

1.2 Ecological Significance of Parasitic Plants

Despite the broad and diverse range of parasitic plants, there is only a small body of research regarding the role of parasitic plants in their communities. Literature tends to focus on a few agricultural parasitic plants, such as *Striga* species, dodders (*Cuscuta* spp.) and mistletoes (predominantly members of the Viscaceae) (Pennings 2002, Press 2005). These species are usually root hemi-parasites which parasitize agricultural species in Africa.

Parasitic plants have the ability to alter soil water, leaf temperatures and nutrient cycling (Bardgett 2012, Callaghan 2005). Because of this, the presence of a parasitic plant species within a system may result in an alteration of functions in that community (Spasojevic 2011). The structuring role that parasitic plants play in their communities is of great importance (Reed 2012). Research conducted on non-agricultural parasitic species has found evidence of a keystone role for parasitic plants within their communities. Reed (2012) found that parasitic plants promote diversity and evenness within a community. The species studied were members of the genus Castilleja, present in montane meadow ecosystems in North America. This is usually accomplished indirectly through the suppressing effect of parasitic plants on a dominant species, by reducing their competitive advantage (Phoenix 2005). Additionally, some species were found to play a role in zonation, with *Cuscuta salina* extending the range and cover of *Arthocnemum subterminale* by suppressing *Salicornia virginica* (Callaway 1998). It can safely be said that most parasitic plants are beneficial to their communities, despite being harmful to the host plants (Watson 2009).

It is interesting to note that parasitic plants trigger the same chemical responses within a host plant as an herbivore does (Pennings 2002). Upon invasion of host tissues, defense compounds such as cis jasmonic acid are released (Runyon 2010). These compounds are also released during insect attack (Runyon 2010). Other similarities include alteration of host resource

allocation, community level effects, and host preference (Pennings 2002). Decreased flowering and fruiting are observed, though there is no detectable change in solute concentration of the leaves to indicate why flowering and fruiting are reduced (Phoenix 2005). Parasitic plants have also been shown to reduce mychorrhizal fungi through competition for host carbon (Davies 1998; Pennings 2002).

Bell (2010) found that the presence of parasitic plants reduces the productivity and biomass of a community (Bell 2010). This is due in part to the inefficient resource use of parasitic plants. Many hemi-parasitic species will keep their stomata open in conditions that would cause an autotrophic plant to close its stomata (Bell 2010). Bells work also showed that parasitic plants often have inefficient metabolisms, resulting in less biomass produced in relation to an autotrophic plant (Bell 2010). Parasitic plants can acquire up to 20% of the host plants water, and reduced host photosynthesis is common (Ehleringer 1986, Cameron 2007, Watling 2001). Movement of sugars through the haustorium to the parasitic plant has been measured, using isotope analysis to determine carbon influx from the host. Uptake of host sugars accounts for anywhere from 5% to 100% of the parasitic plants energy needs (Aly 2012, Bell 2010). The presence of metabolites not naturally produced by the host, such as manitol, indicates the photosynthetic activity occurring in the parasitic plant (Press 1991).

The availability of a viable population of preferred hosts in good health is essential to the continuation of any species of parasitic plant. Parasitic plants require a large selection of healthy host individuals of multiple host species, in order to be able to select the best host for the current environment (Huang 2005). Some species of parasitic plants will use chemical cues to select a healthy host of a preferred species (Bickford 2005, Mescher 2006). After the parasitic plant undergoes germination, the root which will become the haustorium grows towards chemical signals that indicate the presence of a preferred host. This allows parasitic plant species to select the best available

host given the limitations posed by a seeds inability to move. Through these cues it may be possible to determine if a host is suffering from a heavy insect burden, is stricken by disease, or if the individual has hosted parasitic plants previously (Shen 2006). The alterations in plant physiology caused by parasitism make it easier for future parasitic plants to invade the host, with chemical defenses proving less effective after the first infection (Garcia-Franco 2007).

Additional host properties may also be of importance, given the ability of secondary metabolites to pass through the connection and the impact of host properties on host success, which impacts the success of the parasitic plant (Ture 2010). Some parasitic species utilize host metabolites for defence, attraction of pollinators and increasing their own growth rate (Schadler 2005; Alder 2002; Adler 2003). In a study by Press et al. (1993) the performance of two hemiparasitic species (*Bartsia trixago* (Scrophulariaceae) and *Parentucellia viscosa* (Orobanchaceae)) was examined. The results show that both species were advantaged if connected to a nitrogen fixing host (Press et al. 1993). Research on the hemiparasite *Olax phyllanthi* (Olacaceae) has shown a significant difference in the ability of the host to provide adequate water across a year (Cernusak 2004). Deep-rooted hosts were best for long term survival, as they had access to water deep within the soil (Bolin 2010). Where parasites are able to infect multiple hosts consecutively or concurrently, a range of hosts may allow them to reap the unique benefits of each species. Healthy and diverse host populations increase the chances of a parasitic plant persisting at the site (Carpenter 1998).

1.3 The Balanophoraceae

The Balanophoraceae (Richard) is a family of root holoparasites, found predominantly in dark tropical forests, often at high elevations (Kujit 1969). The family contains 17 genera, with 11 of those genera being monotypic, and the remainder containing four species or less (Goto 2011, Heide-Jorgenson 2008). Balanophora is the exception to this, with 15 species distributed across multiple continents (Goto 2011).

Members of Balanophoraceae are often described as looking like fungi. The family is known for its atypical appearance, and highly reduced and very small flowers. The name Balanophoraceae is a reference to the inflorescences, which have been described as appearing to be covered in barnacles (Heide-Jorgenson 2008). Most species take a tuberous achlorophyllus form, growing up to 60cm in diameter (Heide-Jorgenson 2008). Tubers can be divided into simple tubers, made up of parasitic tissues, or complex tubers, made up of both host and parasitic tissues (Heide-Jorgenson 2008). It is also notable that many members of Balanophoraceae have endogenous inflorescences, which emerge from within the tuber after a period of growth (Goto 2011). Some species are thought to be capable of growing through the host as an endophyte within host tissue, with the parasitic plants flowers emerging directly from the host plant (Heide-Jorgenson 2008). There has been speculation that this could lead to inflorescences in two separate locations belonging to one individual - perhaps even separate male and female inflorescences originating from the same individual (Heide-Jorgenson 2008). This has implications for sex distributions and population censuses.

Balanophora is present throughout the Old World tropics, South China and Japan. There are six genera of Balanophoraceae present in the Americas. Scybalium and Ombrophytum are the most specious genera, with four members each (Heide-Jorgenson 2008). Lophophytum contains three species, while Helosis, Corynea and Lathrophytum are monotypic (Heide-Jorgenson 2008). Lathrophytum may be extinct, as no plants have been

observed since collection of specimens in 1886 (Heide-Jorgenson 2008). Africa is home to seven genera of Balanophoraceae, with Mystropetalon containing two species. Balanophora, Scarophyte, Chlamydophytum, Thonningia and Langsdorffia are all represented by a single species (Heide-Jorgenson 2008). Seven genera are present in Southeast Asia and the Pacific, with all genera being monotypic. As well as Dactylanthus, Balanophora, Ombrophytum, Langsdorffia, Rhopalocnemis, Exorhopala and Hachettea can be found in the region.

Dactylanthus taylorii is the southernmost genus, and occurs in a more temperate environment than its relatives (Kujit 1969). The species has relatively large tubers, growing to a known maximum of 50cm in diameter (Kujit 1969). Characteristic of Balanophoraceae, *D. taylorii* has numerous reduced flowers in large inflorescences. The male flowers of *D. taylorii* are among the most reduced male flowers of any angiosperm species (Heide-Jorgenson 2008). Sarcophyte and Balanophora contain the most reduced female flowers of any angiosperm species, some consisting of an ovary and a stigma only (Heide-Jorgenson 2008). *Sarcophyte sanguinea* has flowers which occur in inflorescences of up to 150 flowers, with an appearance similar to a raspberry. These inflorescences are then clustered in groups of up to 200, to form a large mass which is essentially all stigma (Heide-Jorgenson 2008). This species is fly pollinated, and relies on scent to lure its pollinators (Heide-Jorgenson 2008). Another member of Balanophoraceae may occur at the highest altitude of any parasitic plant. The species Ombrophytum subterraneum is found at elevations of 3800m, in the Andes (Heide-Jorgenson 2008). Unusually, this species carries out its entire life cycle underground, and may be pollinated by burrowing insects (Heide-Jorgenson 2008).

1.4 Dactylanthus taylorii

1.4.1 – Basic Biology & History

First described by Sir Joseph Hooker in 1859, *Dactylanthus taylorii* Hook F.(Balanophoraceae) is known as pua o te reinga (flower of the underworld) or wae-wae-atua (fingers or toes of the god) by Māori (Holzapfel 2001). The species is colloquially referred to as 'dactylanthus' or 'wood rose' in English. The plant takes the form of a subterranean rhizome, buried just below the soil. (Figure 2, Figure 3). The tuber has a warty appearance caused by papillae and old flowering and fruiting scars. Shoots appear yearly during flowering, and emerge from the base of tubers. These shoots are covered in membranous, scale leaves between 5 and 20mm long, and 5-9mm wide. Scale leaves and bracts are usually a range of brown, cream, red and yellow, and do not change in colour throughout their life. Lacking photosynthetic capacity, the scale leaves act as floral bracts (Figure 3), and lack stomata (Ecroyd 1996). Being a holoparasite, the tuber has no need to gather water from the soil, and lacks a well-developed root system, sending out small infectious roots once established. Tubers grow at a rate of 1.2cm in diameter per year, up to 50cm in diameter for an individual tuber (Ecroyd 1996). Because of their somewhat cryptic appearance, the species is most easily visible from January to August when the inflorescences emerge from the soil (Holzapfel 2001). The species is considered dioecious, though hermaphroditic inflorescences have been found (Ecroyd 1996). There are 15-28 spikes per inflorescence, with each spadice bearing numerous flowers. Individual flowers and pollen are white, bright at first but becoming more cream or yellow as they age. A clump of inflorescences is shown below, these are older inflorescences as shown by the yellowish cream colour of the visible pollen (Figure 4). *Dactylanthus taylorii* has small fruit (<2mm) which are purple-black to brown-black in colour. These fruits are rapidly lost, exposing a seed which is <2mm, elliptic, glaborous and red-brown to black-brown (Hooker 1859, Holzapfel 2001).

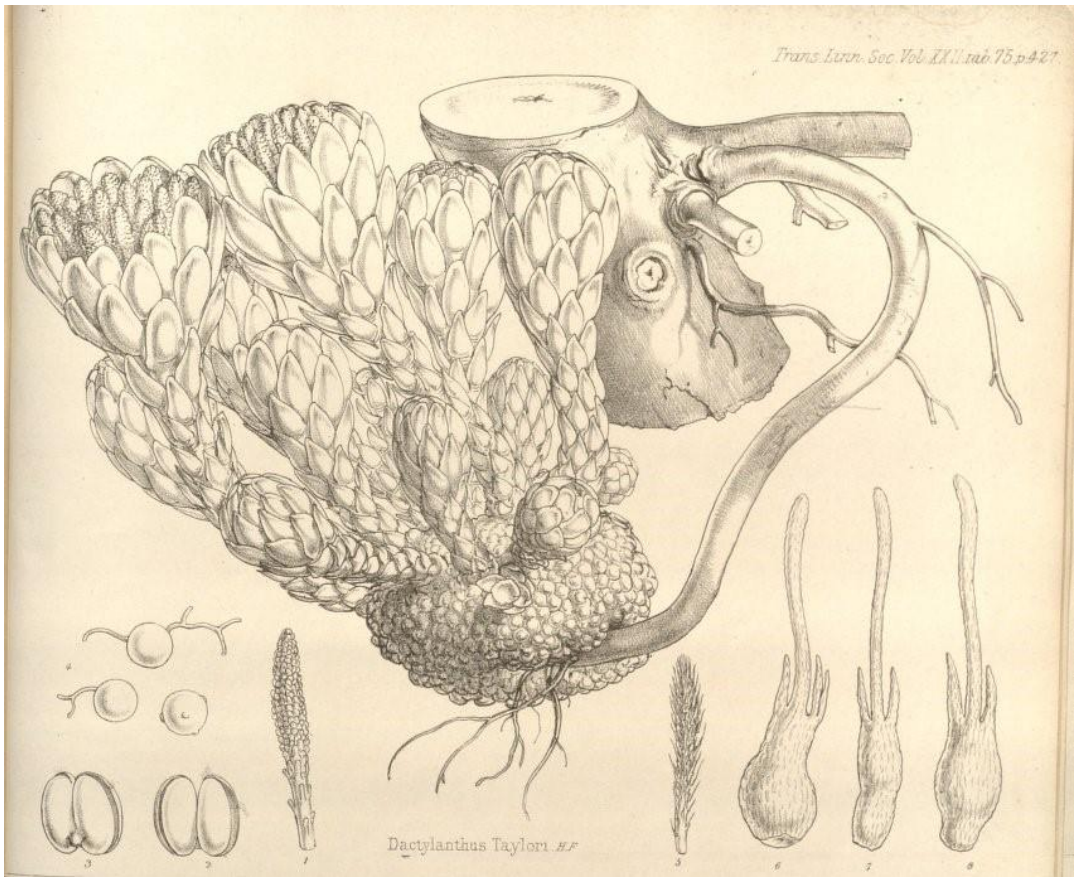

Figure 2: Original drawing of Dactylanthus taylorii, taken from Hooker (1859), showing the rhizome attached to the terminal end of a host root, with buds and inflorescences shown.

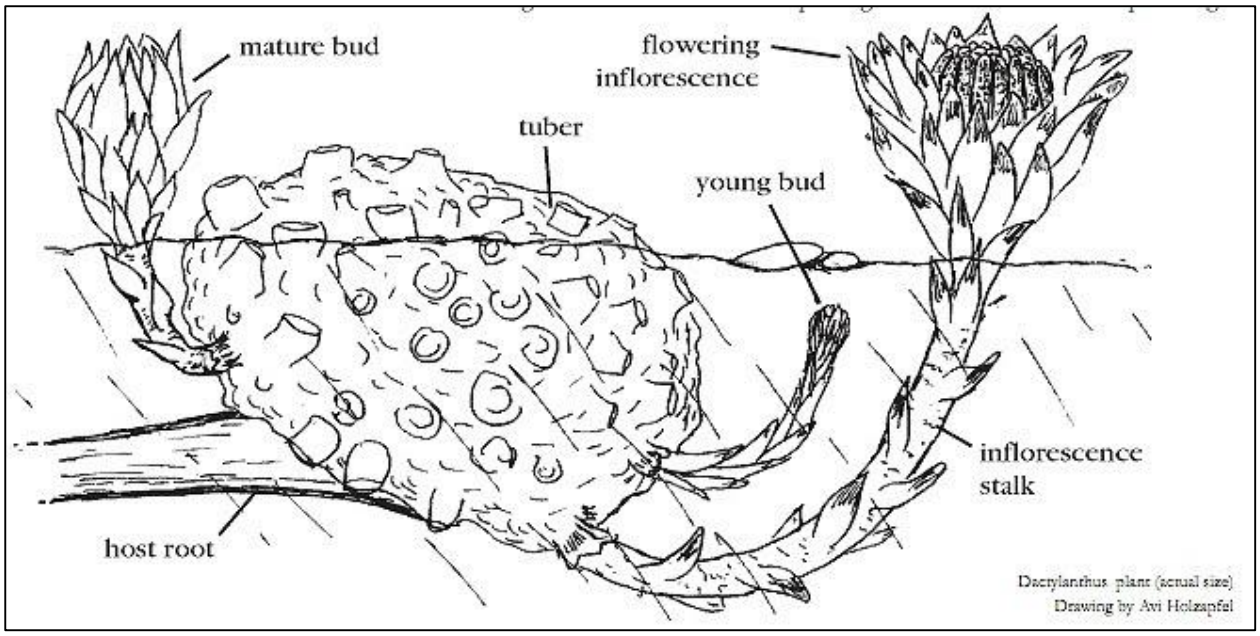

Figure 4: Dactylanthus taylorii showing inflorescences and scars from previous flowerings. Image by Avi Holzapfel.

Figure 3: A group of inflorescences of Dactylanthus taylorii showing the yellow/cream colouration of old pollen. Photo credit: Abe Coffin, 2013

Initially, the parasitic root will penetrate a young host root at its terminal end, and form a haustorium. After a connection is established, *D. taylorii* tubers alter root morphology, with changes including a broadening and flaring of the host root. The resulting host root structure is referred to as a wood rose – this is the origin of the common name 'wood rose' for *D. taylorii*. The wood rose is a flattened disk with ridges and valleys (Figure 5).

Figure 5: A 'wood rose' structure of host root, created during infection of the host by D. taylorii. Photo credit: Phil Bendle.

1.4.2 – Ecology & Conservation

The species favours forest margins and gaps, and has a current host list of ~35 species (Figure 7). Little is known about which host species are favoured, the host ecology of the species is under researched. The species is the subject of conservation efforts. It now occupies just 4% of its former range, though the species was once widespread, with populations found across the entire North Island, as well as the Northern tip of the South Island (Figure 6). Despite how broad its range was, it had a somewhat discontinuous distribution, both spatially and temporally, as a result of its favoured habitat (Holzapfel 2001). Because *D. taylorii* produces copious, heavily scented nectar, it is attractive to its endangered natural pollinator, *Mystacina tuberculata* (Mystacinidae), the short tailed bat. Unfortunately, this scented nectar contains components similar to those found in a mammalian scent. This makes the plant a target for exotic mammalian species (Ecroyd 1996). Possums (*Trichosurus Vulpecula)*, rats (*Rattus rattus, Rattus norvegicus, Rattus exulans)* and mice (*Mus musculus*) are all known to browse on flowers, as well as seeds. It was previously estimated that less than 1% of unprotected inflorescences survive to produce viable seed (Ecroyd 1996). The species is capable of vegetative reproduction, allowing localized recruitment that may explain the persistence of heavily browsed populations (Ecroyd 1996). The decline of the short tailed bat is also cause for concern as *D. taylorii* suffers from recruitment failure; poor bat pollination may be part of the reason for the failure of populations to maintain over time (Holzapfel 2001). Seed dispersal could potentially be a part of the problem, as it is unknown how seed was dispersed in the past. Currently gravity, rats and water appear to be the means of dispersal, with earthworms potentially dispersing seed (Holzapfel 2001, Meys 2003).

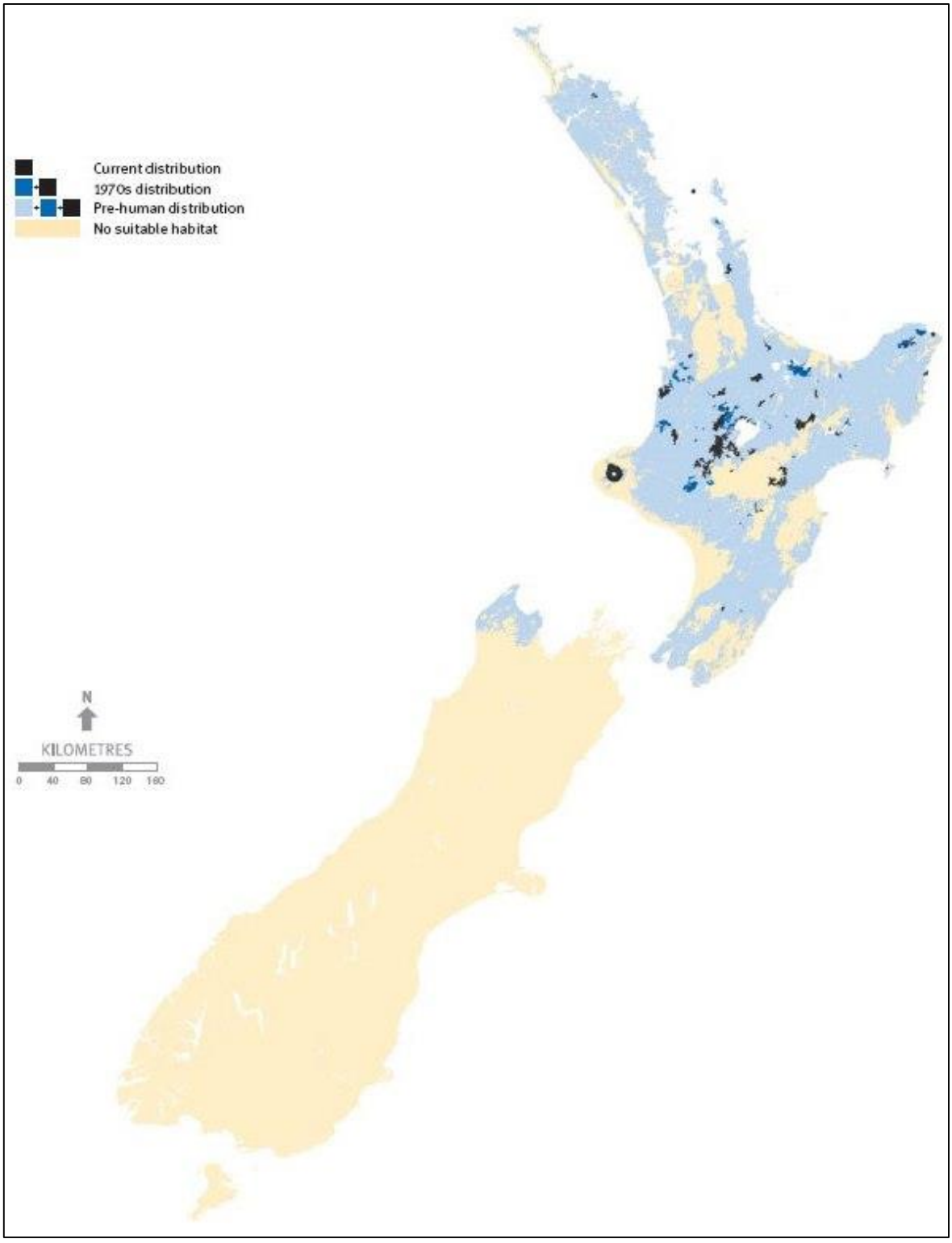

Figure 6: Historic distribution of D. taylorii, with current distribution overlaid. Image taken from https://www.mfe.govvt.nz/environmental-reporting/land/distribution-seven-native-species-indicator/woodrose/

Exact tuber counts are difficult, owing to the near invisibility of tubers, as well as the inability to accurately determine the number of individual tubers in a clump. Tubers will often form dense groups in which individuals are impossible to delineate, requiring the whole group to be counted as one clump. Outside of flowering it can be difficult to determine if tubers are still living. Populations appear to senesce as the forest front moved forward, or as succession took place in a gap. This senescence can be identified by the uneven sex ratios of a population - younger populations have more females than males, while older populations may be almost entirely male. Loss of suitable habitat, collection for wood rose structures by humans, and browsing by pest animal species have all impacted *D. taylorii* (Ecroyd 1995).

Dactylanthus taylorii is among the highest priority species for conservation efforts. The current recovery plan focuses on preserving current diversity, improving recruitment, and restoring the species within its former range. Classified in 2012 as being 'Nationally Vulnerable', the species still needs protection if it is to survive (de Lange et al 2012). The recovery plan calls for replanting to preserve genetic diversity and expand the current range of the species to match its historic range (Ecroyd 1995). As part of the research towards achieving this goal, seeding trials were conducted. These trials served to identify what seeding strategies work best with the most well-known host species. The trials were successful, and new tubers have been grown in the trial plots. Four habitat types were selected, testing for exposure and dominant host species. As well as this, different methods of seed sowing were tested.

Dactylanthus taylorii is often present in young communities, as forest margins and gaps are normally early successional communities. This may be a consequence of the preferred habitat of the hosts of *D. taylorii*. There is evidence that some parasitic species can influence community development. In the grassland systems of North America, some species require their natural parasitic species to be present during restoration (Joshi 2000, Bardgett 2006,

Bardgett 2012). Beyond that, evidence suggests that diverse communities are more resilient to invasion by exotic species. It is worth investigating the long term interactions between *D. taylorii* and its host species and community members. If *D. taylorii* is responsible for promoting diversity within New Zealand forests, its absence may result in forest margins which are more vulnerable to invasion by pest species. Without accurate host identification, it would be virtually impossible to investigate community level effects.

For a population of parasitic plants to establish and persist, seed dispersal mechanisms, viable seed, appropriate pollinators and suitable hosts must all occur in the same area (Watson 2009). Without the presence of all four factors, the population of parasitic plants may experience recruitment failure. Our lack of knowledge regarding seed dispersal for *D. taylorii* prevents us from monitoring seed dispersal mechanisms easily. Given the intention of restoring populations, viable seed must be transferred to an area in which a selection of preferred host species in good health are present. Ideally the location chosen would also be home to pollinators of *D. taylorii*, such as the short tailed bat.

BOTANICAL NAME	COMMON NAME
Aristotelia serrata	wineberry
Brachyglottis repanda	rangiora
Carpodetus serratus	putaputaweta
Coprosma arborea	mamangi
Coprosma grandifolia	kanono
Coprosma sp. (aff. *C. parviflora*)	small-leaved coprosma
Coprosma tenuifolia	
Coriaria arborea (1)	tutu
Geniostoma rupestre var. *ligustrifolium*	hangehange
Griselinia littoralis	broadleaf
Hebe stricta (2)	koromiko
Hedycarya arborea	pigeonwood
Melicytus ramiflorus	mahoe
Myrsine australis	mapou
Myrsine salicina	toro
Nothofagus sp. (3)	beech
Phyllocladus trichomanoides	tanekaha
Pittosporum ellipticum	
Pittosporum eugenioides	lemonwood
Pittosporum ralphii	
Pittosporum tenuifolium	kohuhu
Pseudopanax anomalus	
Pseudopanax arboreus	fivefinger
Pseudopanax colensoi	mountain fivefinger
Pseudopanax crassifolius	lancewood
Pseudopanax edgerleyi	raukawa
Pseudopanax simplex	haumakaroa
Pseudowintera sp. (4)	horopito
Quintinia serrata (5)	tawheowheo
Schefflera digitata	pate
Streblus heterophyllus	turepo
Weinmannia racemosa	kamahi

Figure 7: List of potential hosts, used in the New Zealand Department of Conservation recovery plan for D. taylorii. Sourced from Ecroyd 1995.

1.4.3 – Putative Host List

The literature reveals little about how the host list used in the previous recovery plans (see Figure Seven) was compiled. Some species have recently been confirmed through seeding trials, or through tree fall exposing roots with tubers attached (Dodgson 2004). Knowledge of host species of a parasitic plant is important for many reasons, including guiding conservation efforts. In order to successfully replant across the entire historical range of *D. taylorii*, a complete host list will be needed. This host list should ideally include information about host preference and environmental factors. Optimal hosts promote growth and successful reproduction, leading to self-sustaining populations (Hautier 2010).

Given the evidence that host preference changes between sites, often as a result of environmental conditions, it is vital to determine if there is a link between host preference and environmental conditions in *D. taylorii* (Krasnov 2011, Dean 1994). The main environmental factor implicated by these studies was water availability – in drier environments, parasitic plants favoured hosts which had traits related to continued access to water. Some species of parasitic plants are able to select between multiple hosts, using chemical cues to select for an individual of a favoured species which is in good health (Mescher 2006). Because of the lack of literature it is unknown whether similar preferences (and the drivers for these preferences) are operating in *D. taylorii.* Hence further research is warranted.

The unknown degree of host specificity has consequences for management, at present it is impossible to determine which host species are a priority for conservation efforts and which potential planting areas are most suitable. A higher preference for a particular host, that is, a more specialist nature, would increase the importance of thorough site selection. If no strong host preference is shown, literature highlights the importance of ensuring a range of functionally diverse hosts (Norton 1997). As well as this, there is evidence that host selection can drive divergence in parasitic plants, as different hosts

may inhabit different niches (Thorogood 2008). If host use differs between populations, it may be wise to use locally sourced *D. taylorii* seeds. Guiding replanting efforts is the most important reason for producing an updated host list. A host list with information on relative preference for each host species in a given environment would be a vital resource which could aid conservation efforts into the future, and is one of the key drivers of the research presented here.

1.5 Molecular Identification of Hosts

DNA barcoding is being utilized globally as a method to genetically identify organisms to species level (Hebert et al. 2003, Valentini 2009). Barcoding involves using a short DNA sequence to identify taxa (Hebert et al 2003). The process of DNA barcoding allows the sequence of a small region of an organism's DNA to be matched against a database to confirm species identity (Joly 2014). This technique is now one of the most widely used approaches to identification as it is accurate, fast, and relatively inexpensive. From a small sample of tissue, DNA can be extracted and a target region amplified and sequenced to generate a "barcode" (Hebert 2003, Mitchell 2008).

A barcode is a small region of DNA whose sequence is conserved within species, but varies between species, allowing identification to species level (Taberlet 2007). Mitochondrial or chloroplast genes are often used as these genes have an appropriate level of variation (Fazekas 2009, Valentini 2009). The ideal marker would be short (under 150bp), contain enough information to distinguish between species, and be robust and reliable (Valentini 2009). The ideal marker for plants has not been found yet, and may not even exist (Valentini 2009). A standard marker used in animal studies, CO1, evolves too slowly in plants to be informative (Fazekas 2009). Alternative regions suitable for use include *mat*K and *rbc*L (Fazekas 2009).

One of the ultimate goals for DNA barcoding is to create a library of standardized barcodes for every species, reducing the need for expert physical identification of specimens (Savolainen 2005). Morphological identification, particularly of species of insects and cryptic species, is time consuming and requires expert knowledge (Casiraghi 2010). Phenotypic plasticity can make accurate identification difficult; barcoding is a way to counter this uncertainty (Joly et al 2014). DNA barcoding is a way to counter the need for specialized morphological identification, increasing accuracy and reducing workloads (Valentini 2009, Joly et al 2014). Because it uses short fragments, DNA barcoding is suitable for use with degraded or environmental

DNA. This offers a range of possibilities, including the analysis of samples to detect invasive species, microbial community, and species diversity (Hajibabaei et al 2007, Valentini 2009). DNA barcoding can also be used to identify the diet of a species by analysis of its fecal material (Joly 2014).

The main disadvantages of DNA barcoding stem from the fact that it is a single locus identification system (Valentini 2009). As a result, problems arising from introgression, incomplete lineage sorting, nuclear contamination and heteroplasmy can occur (Valentini 2009). Introgression is the introduction of a gene from another species as a result of mating between an interspecific hybrid and one of its parent species. This can result in an individual which would align with its own species at all loci except the swapped gene – if this is the area in which the barcode is located, the individual will be incorrectly identified. Heteroplasmy refers to the potential for multiple variants of an organelles genome to exist (Valentini 2009). This results in one individual having multiple mitochondrial/chloroplast genomes, which may confound results. Incomplete lineage sorting occurs when the evolutionary history of a gene does not match the phylogenetic relationships of the species (Rogers 2014). These problems must be considered when selecting a barcode or group of barcodes, given the lack of a universal plant barcode.

The marker psbA-trnH has been rejected by CBoL as a candidate for a universal plant marker. While studies found that the marker is able to correctly differentiate between many species, it lacks the resolution to accurately identify all species (Fazekas et al 2009). As well as this, it is longer than the ideal plant marker, contains numerous indels, is variable in length, contains mono repeats, and is prone to palindromes (Savolainen et al 2005, Fazekas et al 2009). Mono repeats and palindromes can cause errors in sequencing, making it difficult to get an accurate read. The variation in length, and the presence of indels makes it hard to align sequences from different species (Joly et al 2014). This limits the accuracy of the marker, and makes it more difficult to use universally.

The marker *trnH-psbA* has good success at discriminating between species. This marker is relatively common, but a lack of prior barcoding work in New Zealand means a reference library will need to be built. As well as being fast, accurate and reliable, this method minimizes contamination of the sample DNA by foreign DNA. Working in the soil exposes the sample to foreign DNA sources, including soil microorganisms, but these sources lack chloroplast DNA. Any potential contamination from the *Dactylanthus taylorii* tuber itself is unlikely, as D. taylorii is not known to merge with its host (Heide-Jørgensen 2008). The minimization of contamination was the major factor in choosing *trnH-psbA* over ITS.

1.6 Thesis Overview

Chapter One: An overview of the literature reviewed for this thesis.

Chapter Two: The main objective of this study is to identify the host species of *Dactylanthus taylorii* using a DNA barcoding approach. It aims to produce an accurate, genetically confirmed host list which will aid conservation efforts as well as increasing our knowledge of the species. DNA barcoding will be used to identify host species at two sites within the study location, Pureora Forest Park, North Island, New Zealand. Environmental conditions will be recorded at these sites, to examine any correlation between host preference and environmental conditions and to capture the number of host species and individuals present.

Chapter Two: Flowering and seed set for a population located in Pureora Forest Park will be examined using statistical analysis to process eight years of monitoring data. The data set was collected over several years by the New Zealand Department of Conservation, using a specialized data collection sheet. The monitoring protocol was also examined to highlight areas for improvement. All results will be passed on to the Department of Conservation, for use in future management.

Chapter Four: This research is intended to contribute valuable information to the conservation and restoration of *Dactylanthus taylorii*. This chapter summarizes the findings of both studies, and details potential applications and future research.

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Chapter Two: Using DNA barcoding techniques to identify the host species of *Dactylanthus taylorii* in Pureora Forest Park

2.0 – Acknowledgements

Beginning with a pilot study in 2012, this project will reach completion with the publication of a paper in early 2016. A joint project between the New Zealand Department of Conservation and the University of Waikato, this study aims to establish a protocol for the accurate identification of the host species of *Dactylanthus taylorii* tubers. The ultimate goal is to produce an accurate host list for use by management staff. This chapter is the foundation for the final paper, which will be authored by Dr Chrissen Gemmill, Dr Avi Holzapfel, Cass Parker, and Kevin Maurin.

This work was carried out under a New Zealand Department of Conservation research permit (National Authorisation Number: 38860-RES).

2.1 – Abstract

The population of the endangered endemic holoparasitic plant, *Dactylanthus taylorii* Hook F. (Balanophoraceae), in Pureora Forest Park was selected as the trial population for developing a method to accurately identify host species using DNA barcoding methods. The marker used was *psb*A-*trn*H, and ten samples were able to be successfully identified using the protocol trialed. Of these samples, nine were identified as *Pseudopanax arboreus*, with one result of *Podocarpus totara*.

The marker used (*psb*A-*trn*H) provided adequate levels of variation to discriminate between the host species sequenced in the pilot study. Further work will focus on refining the protocol and trialing a second region for greater resolution of the *Pseudopanax* species cluster. The method used is relatively simple to employ, and resulted in no mortality of host or tuber.

The ability to accurately identify hosts paves the way for the creation of an accurate host list which reflects the true host range and preferences of *Dactylanthus taylorii*. Globally, few host lists are accurate, and this poses significant challenges for managers.

Future work will expand upon this study, collecting host root samples from populations across New Zealand to determine host preferences.

2.2 - Introduction

Parasitic plants live a heterotrophic lifestyle, gaining some or all of their nutrients and water from a connection with a host plant (Kujit 1969). *Dactylanthus taylorii* (Balanophoraceae) Hook F. is a unique and endangered holoparasitic plant, endemic to New Zealand. It is the southernmost member of Balanophoraceae, and the only holoparasite in New Zealand (Kujit 1969). Described as having a warty, tuber like appearance, the plant lacks any photosynthetic tissue (Hooker 1859). Tubers are found in forest margins and gaps, usually submerged beneath the soil, and the species is somewhat cryptic (Kujit 1969). The species has been the focus of a New Zealand Department of Conservation recovery plan for over two decades, and was ranked as Nationally Vulnerable by the Department in 2012 (La Cock 2004, de Lange et al 2012). Unable to photosynthesize, *D. taylorii* is completely dependent on its hosts. Because of the obligate nature of the relationship between *D. taylorii* and its hosts, it is vital to protect suitable host species alongside protection of the species itself (Marvier & Smith 1997). Work to identify host species has been hindered by the sheer number of roots present in these communities, and the inability to trace a host root to its origin without causing significant disturbance. The current list of suggested hosts is largely based on older botanical reports, and needs to be updated using more accurate and reliable techniques (Ecryod 1995).

As the focus of conservation efforts, there has been research on *Dactylanthus taylorii* which has furthered our understanding – usually undertaken with the goal of informing management. The New Zealand Department of Conservation recovery plan for *D. taylorii* sets goals to guide conservation efforts. One of the goals listed in the recovery plan is to seed appropriate habitat with *D. taylorii*, to facilitate the establishment of populations (La Cock 2004). Work conducted on this aspect of the recovery plan has been successful. It is likely that the goal of re-establishing populations is achievable in the long term. As well as confirming it is possible

to seed an area successfully, and optimizing seeding methods, a knowledge of host preferences is needed. Selecting appropriate areas to seed requires knowledge of the preferred habitat of *D. taylorii*, as well as a more accurate host list which reflects any preference in certain habitat types. Host lists are a key resource for managers of parasitic plant populations, yet all too often are outdated and misleading (Marvier & Smith 1997).

There are several problems with the current list, some of which are common to virtually all known host lists for species across the world. As understanding of host-parasitic plant interactions has increased, it has revealed trends in host use linked to environmental factors, evolutionary history and has highlighted problems with many of the current host lists for parasitic plant species across the globe (Marvier & Smith 1997, Bell & Adams 2011). The current host list was assembled on the basis of early studies, though it has been acknowledged to be potentially incorrect. Methods used to confirm hosts include examination of uprooted trees, proximity of tubers to a potential host, and seeding trials (Ecryod 1995, Holzapfel & Dodgson 2004). These methods vary in their effectiveness, with the majority of hosts being tentative at best. It is difficult to trace a root from the tuber to its parent tree, and visual identification based on the appearance of the root is unlikely to be accurate. A method which allows an accurate identification of the root at the point of connection will vastly improve our ability to confirm host species. A new host list, based on DNA collected from the actual host root, will increase the usefulness of the host list and improve its accuracy. Furthermore, there is little evidence regarding host preference. Many parasitic species have host lists that encompass a range of species that are rarely used, while a small number of host species account for the majority of individual hosts (Cuevas-Reyes et al, 2011). It is even possible for some parasitic plant species to infect a host that does not provide the parasitic plant sufficient energy to reproduce (Kujit, 1969). To avoid the pitfalls of earlier lists, the host list needs to be accurate, listing species which have been scientifically confirmed to be

hosts, as well as giving some indication of how commonly used each host species appears to be (Marvier & Smith, 1997).

An updated, accurate host list could be produced by sampling host roots, extracting and sequencing DNA. The use of DNA barcoding will allow researchers to accurately and easily identify the host species of individual tubers, building a host list for the species. DNA barcoding involves using a small region of DNA to identify a specimen as belonging to a taxonomic group (Hajibabaei et al 2007). DNA barcoding is being used to create a catalogue of life, in which each species has a unique code based on a universal barcode, but there remains no single ideal plant barcode (Savolanen et al 2005). For plants, chloroplast markers such as *rbc*L and *trn*H-*psb*A are used, often in conjunction, as the chloroplast has an appropriate level of variation over time and these regions come close to meeting the requirements for a universal plant barcode (Savolanen et al 2005, Taberlet et al 2007). The method needs to allow for accurate identification from a small sample, while minimizing contamination. The sample taken must be small enough that it won't damage the host root and risk killing the tuber, and can be taken from the host root within 10cm of the tuber. Because it is difficult to isolate a clean piece of root, steps must be taken to minimize the potential for contamination. Though not strictly a barcoding project, this project aims to lay the foundations for a host barcode library. This library will be used in conjunction with the methods detailed here to identify the host species of individual tubers within populations across New Zealand.

The aim of this study is to develop a protocol for the accurate identification of host species, which can be implemented across New Zealand to create an accurate host list for *Dactylanthus taylorii*.

2.3 – Methods

2.3.1 2.3.1 – Study Sites

Host root samples were collected from Pikiariki Ecological Area, Pureora Forest Park, Pureora Ecological District, Western Volcanic Plateau (Region), North Island, New Zealand. Sites were selected based on the ease of access, presence of healthy *Dactylanthus taylorii* populations and the extent to which they represented a variety of different environmental conditions and community compositions. The area selected for this study was Pureora Forest Park, with multiple sites sampled within the park. *Dactylanthus taylorii* is known to grow across a variety of altitudes, climates and regions. It prefers areas with good drainage which are not drought prone, with little groundcover underneath a moderately dense canopy (Ecroyd 1996). A number of clumps are monitored, and these guided the selection of microsites, as well as the preferences indicated by Ecroyd.

2.3.2 2.2.2 – Tuber Selection

Tubers or clumps were selected based on the ease with which a host root connection could be verified and sampled from. For this to occur, the tuber needed to be somewhat independent of other tubers, and either close to the surface or embedded in a soil soft enough to gently brush away to reveal the host root. Prior to collection, appropriate tubers were tagged with two colours of marking tape and GPS coordinates recorded, courtesy of David Mudge, Thomas Emmitt and a group of volunteers from Auckland Zoo. Each tuber had its connection to the host root verified. Verification was achieved by visual observation of the connection between the tuber and host root, with characteristic flaring observed, or by palpation of the connection, feeling for the flared host root. Care was taken to avoid damaging the tuber or its connection to the host root. A small pilot study resulted in no mortality of tubers, confirming that this method is safe to use when carried out correctly.

2.3.3 2.2.3 – Sample Collection

Once a connection between the host root and the tuber had been confirmed, the host root was traced back to find a secondary root. If a secondary root was found within 15cm, it was cut off and placed in a labelled ziplock bag. If no secondary root was present, a small slither of the main root was removed with a sterile blade. The sample size is small enough to ensure that damage to the host is minimal and unlikely to result in any long term damage. After sampling, the tuber and host root are returned to their pre-sampling state - any displaced litter and soil is replaced and compacted, and any other disturbance is minimized. Host roots were marked with yellow tape with the sample number written on it, to allow for long term monitoring. GPS co-ordinates of sampled roots were taken, but are unable to be published here due to the endangered status of *Dactylanthus taylorii*.

A 10x10m plot was used to assess community at each different habitat type, with altitude, trees, canopy and ground cover measured during sampling. The sampled host root(s) were the approximate center of the plot. Data loggers recording temperature and humidity were also placed at each site, to measure differences between sites. This data will be used in a future phase of this project. As well as this, specimens of community members were collected to enter the WAIK herbarium, and form the basis for the host species reference library.

2.3.4 2.2.4 – DNA extraction, amplification & sequencing

Samples were prepared for extraction by homogenization with liquid nitrogen. This resulted in a find powder of root material. Prior to homogenization, roots were cleaned with Millique water to remove soil and other contaminants. Where the surface of the root was too rough to clean, a sterile scalpel was used to strip away the outer layer. Once homogenized, root samples were

subjected to the extraction protocol outlined by the Bioline plant DNA minikit. Lysis time was extended from 10 minutes to three hours, as previous trials had found this to be optimal. Extraction resulted in 100uL of stock DNA.

After extraction, the host root DNA was amplified for *trn*H-*psb*A. Primers *trn*H and *psb*A were used. The thermal cycling protocol consisted of a 5 min at 94°, 35 cycles of 30 sec at 94°, 30 sec at 49°, 1 min at 74°, with a final extension of 10 minutes at 72°.

Success of amplification was assessed by running 5uL of the sample DNA with 3uL of loading buffer on a 1% agarose gel containing 4.5uL of ethidium bromide. The gel was then photographed. Bright single bands indicated good amplification. Absence of a band showed PCR had failed, but did not mean DNA had not been extracted.

The successful PCR products were prepared for sequencing using an ExoSAP protocol. ExoSAP uses two enzymes (Exonuclease I & Shrimp Alkaline Phosphatase) to strip the DNA of unwanted dNTPs and primers. 0.2uL of Exo, 0.1uL of SAP, 2.7uL of MQ H_2O and 10uL of product are mixed. The mixture is then incubated at 37° for 20min, followed by 15min at 80°.

Once they had been prepared for sequencing, the samples were sent to the University of Waikato sequencing facility to be sequenced using the same primers as the PCR. Dideoxy termination using ABI PRISM BigDye V.3 terminator chemistry was used, the products were analysed using an ABI377 automated DNA sequencer. PCR products were sequenced bidirectionally.

2.3.5 2.2.5 – Analysis

Sequences were edited and aligned using Sequencher. The edited sequences were then compared to those in the Gen Bank database using a BLASTn optimized using the megablast search criterion (Altschul 1990). Sequences were also compared against the reference sequences we obtained from known samples of potential hosts.

2.4 – Results

Of the 48 samples collected and processed, 10 samples were successfully sequenced. It is likely that some samples failed due to the condition of the material collected, as some samples were noted as being decayed. These samples were collected to test the limits of the protocol, and no dead material was able to be successfully identified. Samples D0001 – D0033 may have failed due to an unexpected delay between collection and processing.

Table 2: Samples codes and results, ordered by processing date. Note that co-ordinates cannot be included due to the endangered/rare status of D. taylorii.

Sample	*Results*
D005/D027	Failed
D006	*Psuedopanax arboreus*
D007/D028	Failed
D008/D029	Failed
D011	Failed
D012/D031	Failed
D013	*Psuedopanax arboreus*
D014/D032	Failed
D015	*Psuedopanax arboreus*
D016/D033	Failed
D017	*Psuedopanax arboreus*
D018	*Psuedopanax arboreus*
D020	Failed
D021	*Podocarpus totara*
D022	*Psuedopanax arboreus*
D023	*Psuedopanax arboreus*
D024	*Psuedopanax arboreus*
D025	Failed
D026	*Psuedopanax arboreus*
D0001	Awaiting sequence

D0002	Awaiting sequence
D0003	Awaiting sequence
D0004	Awaiting sequence
D0005	Awaiting sequence
D0006	Awaiting sequence
D0008	Awaiting sequence
D0013	Awaiting sequence
D0014	Awaiting sequence
D0033	Failed
D0007	Failed
D0009	Failed
D0010	Failed
D0011	Failed
D0012	Failed
D0015	Failed
D0016	Failed
D0017	Failed
D0018	Failed
D0019	Failed
D0020	Failed
D0021	Failed
D0022	Failed
D0023	Failed
D0024	Failed
D0025	Failed
D0026	Failed
D0032	Failed
D0034	Failed

Table 3: Identified host samples.

Sample	**Family**	**Genus**	**Species**
D021	Podocarpaceae	*Podocarpus*	*totara*
D006	Araliaceae	*Psuedopanax*	*arboreus*
D017	Araliaceae	*Psuedopanax*	*arboreus*
D013	Araliaceae	*Psuedopanax*	*arboreus*
D022	Araliaceae	*Psuedopanax*	*arboreus*
D018	Araliaceae	*Psuedopanax*	*arboreus*
D015	Araliaceae	*Psuedopanax*	*arboreus*
D024	Araliaceae	*Psuedopanax*	*arboreus*
D023	Araliaceae	*Psuedopanax*	*arboreus*
D026	Araliaceae	*Psuedopanax*	*arboreus*

Nine of the identified host samples were *Pseudopanax arboreus* (L. f) Allan (Araliaceae). The result of a single host sample as *Podocarpus totara* G.Benn. ex D.Don (Podocarpaceae) is unusual. The protocol was not observed to result in mortality of tuber or host, the in-situ collection technique is simple enough to be carried out at an estimated rate of 100 samples a day by previously untrained staff, providing host roots to sample have been collected first. Identification of species had good confidence, there was an appropriate level of variation between the sampled host species. Further resolution may be necessary for the *Pseudopanax* species complex and trials are underway to test an alternate barcoding region.

2.5 – Discussion

It has been noted that conservation of parasitic plants is particularly difficult, because the usual problems associated with small populations are encountered, but additional problems must also be managed (Marvier & Smith, 1997). Part of the challenge of managing endangered parasitic plants is ensuring that they have access to a range of suitable hosts in good health (Bickford et al 2005, Watson 2009). As studies conducted on other parasitic species has revealed preferences for particular hosts, despite potentially broad host lists, it is important to have information on the relative preference for each host species (Gibson & Watkinson 1989, Huang et al 2012). Certain species may prove to promote healthier populations, with some species experiencing greater reproductive success when the parasitic plant was hosted by a preferred species (Dean et al 1994, Kavanagh & Burns 2012). It is even possible for differences in host use to occur in geographical separate locations, create host specific races, driving speciation (Thorogood et al 2008, Thorogood et al 2009). The host species most commonly used by *Dactylanthus taylorii* at Pureora Forest Park should be abundant in any areas considered for seeding. For the population at Pureora, *Pseudopanax arboreus* appears to be a common host. Areas with *P. arboreus* present should be able to support a population of *D. taylorii* tubers, provided the general area matches the preferences noted by Ecroyd (1996). This may present problems for managers of populations without high levels of pest control, as *Pseudopanax arboreus* is known to be a well utilized food source for possums in some areas (Payton et al 1997). Poor host health is one reason why parasitic plant species may do poorly, and ensuring adequate numbers of healthy host plants are available is one of the challenges of conservation management for parasitic plant species (Bickford et al 2005). Some host species are more resistant to infection by parasitic plants, and parasitic plants hosted by these species tend to show poor health (Cameron et al 2006, Pennings & Simpson 2008). The ability to identify host species for individual tubers creates an opportunity to investigate which hosts promote

healthier populations. Further work needs to be done to establish if a true preference for *P. arboreus* exists, or if it is simply the most abundant host species at the study site. This should include vegetation surveys to determine the relative abundance of *P. arboreus* and other host species. If a host species is used more frequently than expected based on the presence of that species, it is evidence that the parasitic plant species favours it as a host.

A single sample returned a sequence identifying it as a totara (*Podocarpus totara*). Though *Nothofagus* Spp (Nothofagaceae) are recorded in the host list, it was on the basis of anecdotal testimony and there has been no confirmation of *Dactylanthus taylorii* being hosted by any gymnosperm species. This sample may be the first such confirmation, but further study is needed. It is unlikely that this sample was contaminated, as the protocol used was designed to minimize contamination. There was no contamination of other samples processed in the facility at the time of this work. Root specimens were collected in situ and placed directly into a sterile storage bag labelled with the sample number - there was little chance of non-sample root material being collected in parallel. Further, it is unlikely that this is a result of mis-sampling, as the characteristic flaring found at the join between host and tuber was identified before samples were taken. Care was taken to minimize contamination, but until further samples are found to be podocarps, this is a single instance and cannot be used as proof that podocarps are hosts. There have been anecdotal reports of tubers on *Podocarpus totara*, but these individuals appear to die far earlier than expected and stay small in size (David Mudge, Levin, pers. comm.). The location at which this has been observed would be a prime candidate for further testing. It may also be suitable to establish a sowing/seeding trial around *P. totara*.

A literature search contained no examples of a similar protocol to the one trialed here being used for host identification. The databases JSTOR, Wiley Online Library and Science Direct were searched using combinations of the following keywords: ‘genetic’, ‘molecular’ ‘host’ ‘host species’ ‘host list’ ‘barcoding’ ‘parasitic plants’ and ‘Balanophoraceae’ in varying combinations. No relevant results were returned, this study represents the first of its kind involving a member of the Balanophoraceae. This methodology could be adapted for host identification for other root parasitic species, and can be used across New Zealand to update the host list. The method laid out here was able to successfully ID ten samples from the samples collected. The sample collection method is simple, and as many as 100 samples could be collected in a day from a population which had been screened for accessible host roots. Lab work for 100 samples could be completed in 2 months, dependent on the facility used to process the samples. Samples which were extracted within 24 hours of collection returned the highest success rates, and the failure of samples D0001 – D0033 may be due to an unexpected delay between collection and processing. Protocol optimization is ongoing, with particular emphasis on minimizing handling times. The high number of *Pseudopanax arboreus* results has led to the development of a trial that will use ITS to distinguish between closely related species and/or hybrids, which are known to be common in New Zealands *Pseudopanax* species complex (Payton et al 1997). Potential difficulties of this method may include a bias in which samples are able to be successfully identified, as some species of plants may contain inhibitory agents which prevent amplification. Further, certain host species may have more accessible roots leading to a bias in the host species sampled. It has not yet been tested on tuber/host roots which are more submerged, which account for a significant proportion of most *Dactylanthus taylorii* populations.

2.6 - Conclusions

The method described here will allow managers to work with molecular biology facilities to identify the host species for individual tubers, scaling up to generalize about host use/preference in a population. The field techniques for collection are simple, and require minimal training of staff. There is little risk to host and tuber, provided the method is carried out as described here, with zero tuber or host mortality as a result of sampling.

Of the 48 samples taken, ten samples were able to be identified. The identified samples show nine samples are *Pseudopanax arboreus,* with one sample identified as *Podocarpus totara*. The number of failures indicates that the protocol needs adjusting and refining to ensure a high identification rate, but is partially explained by poor sample quality and unexpected delays in processing time.

The high number *Pseudopanax arboreus* identified in the successful samples may indicate a preference for *P. arboreus* as a host, but further testing is needed to rule out a bias in the samples which were able to be successfully sequenced. The proportion of *P. arboreus* in the community in comparison to other hosts must also be established. Despite the large list of potential hosts, it may be possible that in the areas sampled, *P. arboreus* was the only host species present in high density.

The result of *Podocarpus totara* warrants further investigation, though only a single sample, this result is unlikely to be the result of contamination or error. A site suggested by anecdotal report is a good candidate for further sampling, as it may include more examples of *Podocarpus totara* as a host.

This study began in 2012 with an initial pilot study, and is going to continue to completion, with the aim of publishing results in early 2016, and eventually establishing a national host species list.

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Chapter Three – Assessing the results of routine monitoring of flower and seed set.

3.1 – Abstract

A long term data set of flowering and seed set of a population of *Dactylanthus taylorii* at Pureora Forest Park, collected through routine monitoring, was analyzed to investigate correlations between inflorescence damage and seed set. The data was modelled using a generalized linear mixed model (GLMM). To cope with probable overdispersion due to an abundance of zero seed set counts, an analysis of the factors that influence the probability of producing seed sets was performed separately from an analysis of the factors that influence the number of seed sets produced when seed production is successful. For the population at Pureora, rotten unidentified inflorescences are correlated with a decrease in the likelihood of any seed set. The number of infructescences produced (assuming at least one instance of seed set) is negatively affected by rat damage to male and female inflorescences.
Seed set could be further improved by reducing the number of inflorescences browsed by rats. Rot has been observed to occur as quickly as 12 hours after rainfall. Managers should be aware that heavy rain in flowering season is likely to result in a poorer seed set.
The monitoring program collected useable and relevant data which could be used for more in depth analysis of the factors affecting seed set, if supplemented by climate and pest data.

3.2 - Introduction

Dactylanthus taylorii Hook F (Balanophoraceae) is New Zealands only endemic root holoparasite, and the southernmost member of the Balaphoraceae (Hansen 1980). The species is classified as endangered, in part due to recruitment failure (La Cock 2005). Conservation efforts targeting the species are guided by a New Zealand Department of Conservation recovery plan, which outlines goals for management (La Cock 2005). An ongoing objective of management is to restore populations where appropriate, and ensure populations are maintained (La Cock 2005). *Dactylanthus taylorii* is dioecious, with male and female inflorescences on separate plants, and flowering occurs between January and April, with fruiting occurring February to May (Ecroyd 1996). When *D. taylorii* is able to bear fruit successfully, a single inflorescence may produce approximately 3600 viable seeds (Ecroyd 1996). The species is pollinated by the native short tailed bat *Mystacina tuberculate* (Mystacinidae) also endangered (Ecroyd 1996). Other species may be pollinators, but in areas where short tailed bats are absent, seed set is still occurring, but it I unclear which species are pollinating. Seed production can vary, and anecdotal observations suggest that spontaneous abortion of all developing seed on a plant can occur (David Mudge, Levin, pers. comm.). It is not known how seeds are dispersed over long distances, but seeds are dispersed over short distances by water or gravity (Ecroyd 1996). It is known that possums, rats and insects can browse the inflorescences, potentially reducing seed set. To counter this, some populations were caged to prevent access by possums (Ecroyd 1995, Ferreira 2005). In areas with limited pest numbers it appears that *D. taylorii* is able to regenerate and maintain healthy populations, but seed set still varies (Ecroyd 1996). Seed set may also be affected by the rot or molding of inflorescences following rain. Inflorescences will begin to rot or mold in as little as 12 hours after rain, if heavy or prolonged rain is followed by warmer, drier weather (David Mudge, Levin, pers. comm.).

One of the healthiest remaining populations of *Dactylanthus taylorii* is located in Pureora Forest Park, and is managed by Department of Conservation staff (La Cock 2005). Year to year variation in the numbers of inflorescences and infructescences of individual clumps has been observed, but it is not clear what is driving these trends (David Mudge, Pers. Comm). The monitoring program collects data on 60 tagged clumps each flowering and fruiting period, dividing seed set into categories based on the visual guide (FIG 1). Inflorescences are categorized as male, female, unknown or bud. Damage to inflorescences is also recorded, broken down into rat damaged, possum damaged, rotted or intact. Managing a population for recruitment failure would be virtually impossible if no data were collected regarding reproductive attempts. It may also play a part in re-establishing *Dactylanthus taylorii* across its previous range, which will be accomplished by spreading seed in suitable areas, following a protocol established in a trial conducted in 2004 (Holzapfel & Dodgson 2004, La Cock 2005). As the population at Pureora is one of the largest and healthiest, it is a prime candidate for seed collection, and monitoring seed set may inform seed collection.

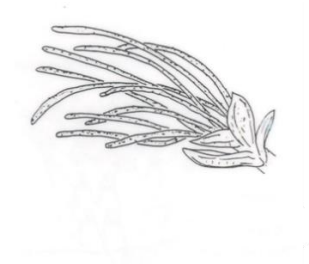
Condition	Visual guide
No seed set	
Light seed set	
Medium seed set	
Heavy seed set	
Post-dispersal/seeds shed	

Figure 8: Visual guide to seed set categories of Dactylanthus taylorii infructescences. Adapted from Meys 2004.

Monitoring is a vital part of good conservation practice (Clewell & Winterhalder 2004). The New Zealand Department of Conservation practices monitoring across a broad range of species and habitats for a diverse variety of goals. Above all, the usefulness of the data collected is deemed to be the priority when designing a monitoring program (Conroy et al 2012). Data must be easy to collect despite unpredictable conditions and staff turnover, while still being relevant to the question(s) that prompted monitoring in the first place (Kull et al 2008, Greene 2012). In order to be useful to managers, data must be statistically sound, and regular analysis of collected data must take place (Legg & Nagy, 2006, Greene 2012). It is important to remain consistent and collect data according to the methodology laid out during planning, but this does not mean that a faulty or difficult protocol cannot be updated (Greene 2012). Monitoring programs must undergo scrutiny to ensure data is fit for the purpose intended (Greene 2012).

Well-designed monitoring programs provide a wealth of data that can be utilized by researchers seeking to answer such questions (Nichols & Williams 2006). The benefits of well implemented monitoring has been documented repeatedly, but monitoring programs are often severely limited for a variety of reasons (Haughland 2010, Lindenmayer & Likens 2010). For larger, ecosystem based restoration projects it is often difficult to allocate staff time because of the wide range of species and potential data to be collected (Haughland 2010). Deciding which species or factors to monitor can be made easier by weighing up the costs of the monitoring program compared to the potential benefits. While real world limitations must be taken into account, it is possible to use regular monitoring as a way to move towards research active restoration projects (Kull et al 2008, Lindenmayer et al 2011). Long term monitoring data can be used to investigate long term trends, and ideally, can be collected in a way which allows retrospective use of the data to investigate related questions (Haughland 2010). Data sets collected through monitoring may not always be suitable for statistical analysis, and part of the challenge of

moving towards a research centered conservation system is to build in naturally easy to achieve and highly useable data collection via monitoring (Lindenmayer & Likens 2009, Blanco et al 2012).

The aims of this study are to use monitoring data to identify trends in flowering and seed set, and to examine the monitoring protocol to ensure it is meeting the criteria for good monitoring. The findings will be used to inform and improve monitoring at Pureora Forest Park.

3.3 - Methods

To investigate the relationship between flowering and seed set of *Dactylanthus taylorii*, a data set collected via routine monitoring was analyzed. Over a period of eight years, a dataset of flowering and seed set for tagged clumps from populations within Pureora Forest Park was compiled. This data contains information on the number of inflorescences, possum and rat browse, rotted inflorescences and the number of infructescences. It was unclear from available literature what criteria were used to identify each type of damage (Meys 2004). Seed set was assessed using a visual guide, with each infructescence being classified as light, medium or heavy seedset. Five locations within Pureora Forest Park were represented: Cabins, Firestation, Kotukunui Road, Over the Stream and Plains Road. The number of monitored clumps at each site varied from 2 clumps to 32 clumps. Each location was monitored for an 8 year period, spanning from 2005-2012. Clumps were tagged with a four digit code on a metal tag affixed to a stake next to the clump. A clump was defined as one or more individuals of either sex (some of which may be submerged) within a 30cm radius (Meys 2004). Monitoring involves two visits to each clump, with flowering and seed set recorded on a specialized spreadsheet.

This data set was used to investigate the factors contributing to variation in seed set. The assumptions made when working with this data were that females could contribute to seed set, even if browsed, as could intact buds, while unknowns were removed from the pool of candidates. The statistical program R version 3.1 was used to handle the data.

The design structure suggested by this arrangement was tagged clumps (tags) nested within locations, all crossed with years. The natural variation between years, between locations, and within clumps over time needs to be accounted for, but is not of primary interest. This variation was modelled as random effects for this reason. To examine the effects of rat damage, possum damage and rot damage on seed set, a Generalised Linear Mixed Model

(GLMM) was used. This was selected because of the high likelihood that the number of infructescences produced would not follow a normal distribution. infructescences were recorded as count data, but due to the large number of zero counts, a Poisson distribution was not suitable due to probable over-dispersion.

	0 instances	1 or more instances
Low	446	65
Medium	433	78
High	372	139

Table 4: Presence or absence of infructescences in each category

Modelling was performed in two parts to account for the large number of zero counts. Whether any infructescences were observed was modelled, with seed presence or absence coded by 1 and 0, assuming a binomial distribution for the response variable in the GLMM. Conditional on infructescences being present, the number of infructescences was modelled with a GLMM, assuming a Poisson distribution for the response. This attempted to account for over-dispersion by disregarding cases where no seed sets were observed. The response variable was investigated using the total number of infructescences, and the number of low, medium and high infructescences individually. Fixed effects included the number of rat damaged inflorescences (including female, male and unknown), the number of possum damaged inflorescences, and the number of rotten inflorescences, intact inflorescences and intact buds.

3.4 - Results & Discussion

For all results, effects are estimated assuming all other variables are held constant; significance is taken at the 5% level; "95% CI" refers to the 95% confidence interval for the average multiplicative effect of increasing the predictor variable by one unit on the odds of producing seeds. The odds of producing seeds is defined to be the probability of producing seeds divided by the probability of not producing seeds.

Probability of producing any seeds

There were 185 instances when seeds were observed, 36% of the total sample of 511. The likelihood of producing any seeds was significantly positively impacted by the number of intact female and intact male inflorescences. There was a significant negative effect from the number of rotten unidentified inflorescences. For each intact female inflorescence, the odds of producing seeds increases by 18.1% - 47.5%, p value <0.001 (95% CI, 1.18-1.47) if all else remains constant. The odds of producing seeds increases by 1.2% - 17.7%, p value 0.012 (95% CI, 1.01-1.18) for each additional intact male inflorescence if all else remains constant. For each rotten unidentified inflorescence the odds of producing seeds decreases by 6.0% - 40.9%, p value 0.008 (95% CI, 0.591-0.94) if all else remains constant. The negative correlation between intact female inflorescences and rotten unidentified inflorescences is relatively large (-0.379), and may indicate that rotten unidentified inflorescences are more likely to be female than male. This would mean that a higher number of rotten unidentified inflorescences represents a loss of female inflorescences, hence a loss in seed production. It is notable that the number of rotten female inflorescences does not have a significant effect on seed set, given the variables previously mentioned are in the model.

The variance component attributable to year-to-year variation was 2.604, almost twice that attributable to tag-to-tag variation (1.442). Because there is

more variation between tagged clumps than between years, more information is obtained by favouring a greater number of tagged clumps as opposed to more frequent sampling. Very little variation was attributable to locations, suggesting little benefit to taking observations over more locations.

3.4.1 Probability of producing low seeds

Of 511 instances of infructescence production, 65 were classified as low seed set (13% of infructescences produced). The number of intact buds had a significant positive effect on the likelihood of producing low seed set, increasing the odds of low seed set by 1.7% – 16.1%, p value = 0.014 (95% CI, 1.017-1.161). The number of intact female and intact male inflorescences had no significant effect. Low seed set may be a consequence of mistimed flowering, clumps which flower early or late may go on to produce infructescences with low seed set.

Variance attributable to tag-to-tag variation is greater than that attributable to yearly variation or location. This further supports the idea that the number of tagged clumps should be prioritized over other factors.

3.4.2 Probability of producing medium seeds

Of 511 instances 78 were observations of medium seeds (15%). The only significant effect identified was a positive effect of intact female inflorescences. For each additional intact female inflorescence, the odds of medium seed set increase by 0.8 – 12.9%, p value 0.026 (95% CI, 1.008-1.129) all else remaining constant. The variance component attributable to tag-to-tag variation (1.117) is slightly larger than that attributable to year-to-year variation (0.7998). This suggests that there is more benefit to prioritizing a greater number of tagged clumps over a more frequent monitoring schedule. Very little variation was attributable to location.

3.4.3 Probability of producing high seeds

There were 139 of 511 instances (27%) when high seed set was observed. The number of intact female inflorescences had a significant positive effect, with each intact female inflorescence increasing the odds of producing high seed set increasing by 0.1 – 14.0%, p value = <0.001 (95% CI, 1.117-1.140). Though not significant, the positive effect of intact male inflorescences and the negative impact of rotten unidentified inflorescences border significance at the 5% level. For each additional intact female inflorescence, the odds of high seed set was increased by 11.7-31.9%, all else remaining constant. For each additional rotten unidentified inflorescence, the odds of producing high seed set alters by -34.7% - 0.4% (95% CI) if all else remains constant. Each intact male inflorescence increases the odds of producing high seed set by -0.1 – 14.0%, 95% CI, all else remaining constant.

The variance component attributable to year-to-year variation (1.951) is almost twice as large as that attributable to tag-to-tag variation (0.9926). This matches the analysis for other levels of seed set, and suggests that the number of tagged clumps should be a priority when monitoring.

3.4.4 Number of any seeds produced

Conditional on there being at least one instance of seed production for a tagged clump at a particular site in a particular year. Given seeds are produced, the factors the affect the number of infructescences produced are the number of intact female inflorescences and intact buds, and the number of rat damaged inflorescences had a negative effect. For each additional intact female inflorescence, the number of seeds produced increases by 2.8-4.4%, p value = <0.001 (95% CI, 1.028-1.044). For each additional intact bud, the number of seeds produced increases by 1.8-4.5%, p value = <0.001 (95% CI, 1.018-1.045). In contrast, each additional rat damaged female inflorescence decreases the chance of seeds by 15.5-35.5%, and each additional rat damaged male inflorescence decreases the chance of seeds by 5.8-17.9% on average. Most random variation is explained by between tag variation, as with previous analyses.

3.4.5 Number of low seeds produced

In 65 cases at least one low seed set infructescences was produced. The number of intact female inflorescences has a significant positive effect on infructescences production, with the average number of low seed set infructescences increasing by 0.1-4.2%, p value = 0.023 (95% CI, 1.001-1.042) for each additional female inflorescence. Most variation is tag-to-tag.

3.4.6 Number of medium seeds produced

The only positive effect observed for medium seed set infructescences was the number of intact female inflorescences – for each additional intact female inflorescence the number of medium seed set infructescences increases by 1.7-5.2%., p value = <0.001 (95% CI, 1.017-1.052). Most variation is between tagged clumps.

3.4.7 Number of high seeds

High seed set infructescences were observed more frequently than medium or low seed set. The results for high seed set mirror those of “any seeds”. For each intact female inflorescence the number of high seed set infructescences increases by 3.0-5.0%, p value = >0.001 (95% CI, 1.030-1.040) on average, and for each additional intact bud the number of high seed set infructescences increases by 0.9-4.0%. For each additional rat damaged female inflorescence, the number of high seed set infructescences decreases by 7.6-31.1%, p value = 0.003, (95% CI, 0.689-0.924). For each additional rat damaged male inflorescence, the number of high seed set infructescences decrease by 7.6-22.3%, p value = 0.002 (95% CI, 0.777-0.924) on average. Tag to tag variation was the major source of variation.

The findings of this analysis fit with the current understanding and management of *Dactylanthus taylorii*. A greater number of female inflorescences increases the probability of infructescences developing, and increases the number of infructescences produced. Rotten unidentified inflorescences/buds decrease the probability of producing infructescences when all data is pooled. This may reflect a higher number of females in this category, but further work is needed to clarify the matter. Rat damage significantly reduced the number of infructescences, by as much as 35.5%, which is good support for continued monitoring and pest control in the area. The idea of rodents as pollinators has been put forward (INSERT PAPER HERE), however it appears that rats are best excluded from *Dactylanthus taylorii* inflorescences at Pureora. The population of short tailed bats (*Mystacina tuberculata*) within the Park appears to be sufficient for pollination of the local *D. taylorii*, removal of rats is unlikely to reduce pollination and may improve the number of infructescences produced. Elsewhere, this may not be the case, and managers need to balance outcomes for their populations on a case by case basis. Interestingly, rotten inflorescences did not have a

significant effect on the number of infructescences produced. This may be because one male inflorescence is able to provide pollen to multiple female inflorescences, so high male mortality due to rot doesn't prevent pollination, and female inflorescences are thought to lose only external floral parts to rot, which aren't needed after pollination.

In this study, rotten inflorescences were used as a proxy for rainfall, but future studies should collect or obtain climate data to investigate the link between rainfall and success. It is known that pollen deteriorates rapidly after rain, which may be impacting reproductive success. This study was not able to address this issue, but has confirmed the suitability of the flowering/seed set data set for analysis. Despite the presumed overdispersion, the data collected was suitable for an initial investigation, and could be used for a more detailed study utilizing climate and pest population data. No changes to the current procedure are advised, and on the basis of this study, the data collected is adequate to monitor yearly success and investigate the factors influencing seed set. The monitoring required to collect data on flowering and seed set is minimal, and as the Department of Conservation and community groups look towards introducing *Dactylanthus taylorii* to new areas, it is useful to keep records on available seed.

The available literature detailing the methods used for monitoring lacked criteria for categorizing inflorescences as rat, possum or rot damaged. This information may be available elsewhere, but ideally needs to be included in an updated version of the monitoring guidelines established by Meys (2004). It has been noted that distinguishing between some types of browse or damage can be difficult –for example, rat browse is

3.5 - Conclusions

The data used for this project was collected as part of a monitoring program in place at Pureora Forest Park. Though it contained many zero values, the design of the data collection was good, and allowed the data to be used for purposes other than those intended by those monitoring. Monitoring the same tags each year is advised. Location showed little influence on results, monitoring more sites for the purpose of data collection is not advised. If resources devoted to monitoring decrease, it would be acceptable to monitor fewer locations to obtain flowering and seed set data. It is preferable to add more tagged clumps at existing locations, rather than expanding the local range of monitoring. Differences between tagged clumps explained most of the variation, increasing the number of clumps monitored would add strength to this analysis. Year to year variation was somewhat significant, it is advisable to monitor as frequently as possible (i.e. yearly), while prioritising the number of clumps monitored. It is better to monitor more clumps less frequently than to monitor fewer clumps more regularly.

This monitoring program is relatively simple to carry out, and has collected sound data for eight years. It answers basic questions about yearly success, as well as providing a data set useful for investigation of the factors influencing seed set. Despite the success of the population as a result of the high level of pest management carried out at Pureora Forest Park, it would be worthwhile to continue with this monitoring program. The data collected may be used to inform seeding projects, and could be used to investigate more thoroughly the factors which affect seed set. Further studies could use the same data set, but include estimates of rat and possum densities and climate data to further explain the variation in seed set. In particular, rainfall data would allow a more detailed investigation of the relationship between rainfall and seed set. Time constraints prevented expansion of this study to include such data.

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Chapter 4 - Summary and Recommendations

4.1 - Summary of Findings

The management of endangered parasitic plants poses many of the same challenges that face non-parasitic endangered plant management, with additional issues arising from the requirement to protect host plants. *Dactylanthus taylorii* is under Department of Conservation management, and is subject to routine monitoring. This research project was comprised of two parts, with both studies aiming to improve monitoring and knowledge of *D. taylorii*. The first study used DNA barcoding methods to accurately identify the hosts of *D. taylorii*, developing a methodology which can be used across New Zealand to create an accurate host list which reflects any trends in host use. The second study analyzed a data set collected via monitoring of flowering and seed set, and investigated how the monitoring program could be improved.

The host barcoding project was successful in developing a method for the genetic identification of hosts, but the method needs refining to reduce the rate of failure. Of the ten samples that were successfully identified, nine were *Pseudopanax arboreus*. The tenth sample was identified as *Podocarpus totara*, an exciting result that warrants further study. Collection of low quality material and delays in processing may have increased the failure rate. Low quality material was collected intentionally to test the limits of the process, and it is apparent that only live or very recently dead material is suitable using this method.., but further optimization trials will be conducted to minimize failures and improve resolution of the *Pseudopanax* species cluster. This technique enables managers to identify the host species being utilized by their populations, sampling of the hosts of individual tubers as a subpopulation. The current has list contains approximately 30 species, but this study found a majority of hosts were *P. arboreus*, suggesting that not all hosts are used equally. An accurate host list will allow managers to select

optimal sites for seeding, and may allow managers to better understand the ongoing status of their population, as parasitic plant health and reproductive success is influenced by host health (Bickford et al 2005). Based on a multiple database search, this study appears to be the first attempt to identify host species of a member of the Balanophoaraceae using DNA barcoding methodology. The databases searched were JSTOR, Wiley Online Library, and Science Direct using the key words ‘genetic’, ‘molecular’ ‘host’ ‘host species’ ‘host list’ ‘barcoding’ ‘parasitic plants’ and ‘Balanophoraceae’ in varying combinations repeated across each database. No relevant results were obtained. The method used here could be employed to generate accurate host lists for a range of root holoparasites.

Flowering and fruiting records from eight years of monitoring a Pureora population was analyzed to determine the success of the monitoring program, what data could be collected to enhance monitoring, and to identify trends captured in the data. Analysis of the data showed that higher numbers of intact inflorescences increased the probability of achieving good seed set, and rot and rat browse decrease the probability of any seed set occurring. The lack of information on how inflorescences were categorized complicates interpretation of the analysis, as it is unclear if it is possible to accurately differentiate between types of browse (Dr Avi Holzapfel, Hamilton, pers. Comm.). This information should be added to the methodology, to avoid misidentification of browse/damage by future surveyors. The program was found to have been successful at collecting data suitable for statistical analysis, but key areas for improvement were identified. The most crucial area for improvement was the definition of basic terms and the expansion of the methodology to ensure that the monitoring surveys are conducted identically across years.

4.2 - Discussion

Both studies were concerned with management of *D. taylorii* (Balanophoraceae) at Pureora Forest Park, North Island, New Zealand. This site represents one of the strongholds of *D. taylorii*, with possum numbers kept so low that caging isn't a requirement as it is at most sites. With over 1000 clumps present, the population of Pureora is a likely location for sourcing of seed for reintroduction projects and seed sowing trials have been conducted (Holzapfel & Dodgson 2004). Pureora Forest Park has been a key conservation site for over two decades, and as a result, there is a relative wealth of information on the site and its management (Beveridge et al. 2000). This information provides a background for future studies. The management methodologies practiced at Pureora can be used for other populations with similar requirements.

The identification of host species at each population across New Zealand could be achieved within five years, providing information to managers and increasing understanding of the ecology of *D. taylorii,* particularly host ecology. Vegetation surveys and environmental data should be collected for Tubers are relatively long lived, meaning that any tagged individuals/clumps that have an identified host will likely be able to be monitored over long periods of time without having to repeat sampling of hosts. Trends in the success of *D. taylorii* linked to host species may be able to be investigated using monitored plants with known hosts.

Management is reliant on good monitoring. In order for management to be responsive and effective, managers must be able to identify changes in their populations, and respond accordingly. By ensuring the basic components of a wider management plan are up to standard, it is possible to build good quality, long term datasets which can be used to better inform long term management and expand upon current knowledge. The addition of host information to a long term dataset collected by monitoring could allow the investigation of trends in success related to host species. Parasitic plant

species often have a broad range of hosts, many of which are rarely used (Gibson & Watkinson 1989). Studies of other parasitic plants have revealed differences in success between individual parasitic plants which are hosted by different species (Pennings & Simpson 2008, Huang et al 2012). Host species differ in their ability to avoid parasitic infection, and defend their resources from attached parasitic plants, which can result in poor performance of the parasitic plant (Cameron et al 2006 Secondary metabolites are passed from host to parasitic plant, with different host species producing different secondary metabolites (Birschwilks 2006). This has been shown to alter attractiveness of hemi-parasitic species to herbivores and pollinators (Marvier 1996). It is becoming more evident that the host species of an individual parasitic plant can play a large role in its success through means other than simply providing sugars and water. With the addition of host species data to our current monitoring data, it could be possible to determine if reproductive success is linked to host identity.

4.3 - Future Directions

Host identification should be expanded to include sites across the current range of *D. taylorii*. Differences in host species used across a parasitic plants range have been documented for some species (Huang et al 2012). Building a database of hosts used at each location, alongside community and environmental data, will allow further investigation into the host ecology of *D. taylorii*. Current understanding of host ecology is poor - the host list is unlikely to be accurate, beyond that, little literature exists regarding host selection, the interchange between host and parasitic plant, host preference, and host use in response to environment. The relationship between parasitic plant species and their host species influences their ecosystem, and to leave host ecology unstudied is to risk missing a vital piece of the puzzle (Marvier 1997, Bell & Adams 2011). Once a population has had its hosts identified, routine monitoring programs can be tailored to collect information with the goal of better understanding the ecology of the area. For the population at Pureora Forest Park, the flowering/fruiting data set could be used in conjunction with a list of host species for the monitored clumps to investigate the potential link between host species and reproductive success.

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