Phylogenetic relationships of the New Zealand *Pittosporum* (Pittosporaceae) inferred from ITS sequences of rDNA

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Louise Angela Hathaway

The University of Waikato
Te Whare Wānanga o Waikato
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ABSTRACT

Sequence data from the internal transcribed spacer (ITS) region of nuclear ribosomal DNA was collected from almost all of the New Zealand *Pittosporum* species, as well as representatives from other areas and other genera within the Pittosporaceae and the outgroup *Pseudopanax discolor*. The variation in this region was used to examine the phylogenetic relationships of these species.

The New Zealand *Pittosporum* taxa did not form a monophyletic group, instead they were found in two distinct clades. One clade contained *P. cornifolium, P. pimeleoides* subsp. *pimeleoides* and *P. pimeleoides* subsp. *maius*. All other taxa formed a second monophyletic group.

The clade comprised of *P. cornifolium* and the two *P. pimeleoides* taxa appears to be a result of a dispersal event from New Caledonia. There is no clear indication of the ancestral source for the clade containing the rest of the New Zealand species. *Pittosporum bracteolatum* from Norfolk Island was found either within the main New Zealand clade, or as the sister group to it giving rise to the possibility that the main New Zealand clade and *P. bracteolatum* share a common Australian ancestor.

Both of the New Zealand radiations appear to be relatively recent events. The sequences of *P. cornifolium* and the two *P. pimeleoides* taxa are identical. The level of sequence differentiation within the main New Zealand clade is also relatively low, especially when compared to the New Caledonian and Australian *Pittosporum* taxa. A nucleotide substitution rate of approximately 1 bp per million years gives an age of around 22 million years for the main New Zealand radiation, which is consistent the fossil record.

The results of this study does not support previous hypothesises on relationships within the New Zealand *Pittosporum* based on morphology. The bivalved and trivalved species do not form monophyletic groups. Within the main New Zealand clade bivalve is ancestral and trivalve capsules have evolved twice. The species with a papery endocarp, the small leaved bivalved species and the species

thought to represent a closely related group showing a reduction in size do not form monophyletic groups, and are spread out throughout the New Zealand species.

The identical sequences found in *Pittosporum cornifolium* and *P. pimeleoides*, which was unexpected as these species have never been grouped based on morphology. Identical ITS sequences found in *P. pimeleoides* subsp. *pimeleoides* and *P. pimeleoides* subsp. *maius* and in *P. tenuifolium* subsp. *tenuifolium* and *P. tenuifolium* subsp. *colensoi*, and their similar morphology, supports their classification as subspecies. *Pittosporum rigidum 1, P. turneri* and *P. anomalum* had identical ITS sequences, but due to their distinct morphological difference, should retain their specific status. Two individuals of *P. rigidum* were sequenced and differed by one nucleotide. Based on this the South Island, smaller leaved individuals currently called *P. rigidum* may need to reinstated as *P. crassicaule.* The *Pittosporum* taxon found on Kermadec Islands identified as *P. crassifolium,* was most closely related to *P. fairchildii.*

Pittosporum bracteolatum from Norfolk Island is closely related to the New Zealand species, however its placement in relation to the New Zealand species varies. Four of the New Caledonian species form a distinct clade, with the fifth having no real indication of if its relationships to the other species. The Pacific species formed two clades, one appearing to be the result of a dispersal from Australia and the other from New Caledonia. *Citriobatus* was consistently found within the *Pittosporum.*

Character mapping indicates that both heteroblasty and the divaricating habit have each evolved at least three times independently in the New Zealand species. The darker flower colour is the more derived character in the New Zealand *Pittosporum.*

Because of the low level of sequence variation between most of the New Zealand taxa many relationships were unresolved. Hopefully future studies using a faster evolving marker and morphological characters will clarify these relationships.

ACKNOWLEDGEMENTS

Firstly thanks to my supervisor Chrissen Gemmill for starting me on this project at a time that seems so long ago. Thanks for your support and encouragement over this time.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

CHAPTER ONE
INTRODUCTION

1.1 NEW ZEALAND

New Zealand has a unique and distinctive flora. Various factors have contributed to this including its varied geological history, the wide range in latitude, from sub-tropical to sub-antarctic, and the numerous diverse environments, providing New Zealand with a wide range of habitats (Wardle 1991).

1.1.1 The Geographical Context

Until the end of the Triassic period, approximately 220 million years ago (mya), all the continents of today were joined in one large landmass, known as Pangea. The formation of the Tethys ocean at this time divided Pangea into two continents; Laurasia, comprising of almost all the current Northern Hemisphere landmasses, and Gondwana, which was comprised of the continents of the Southern Hemisphere and India (Stevens 1985). During the Jurassic period, which lasted from approximately 220 to 140 mya, Gondwana drifted south from its former mid-Northern Hemisphere position and began to fragment (Balance 1980).

The New Zealand region, which includes New Caledonia and the submerged continental crust of the Lord Howe Rise, Norfolk Ridge, Chatham Rise and Campbell Plateau (Figure 1.1), was located at the eastern edge of Gondwana, between New South Wales, Tasmania and Antarctica (Griffiths 1971). Until the early Cretaceous (approximately 140 mya) the land that was to become New Zealand was mostly submerged under water. New Zealand formed at this time from a combination of sediments being eroded from Australia, Tasmania and Antarctica and a period of mountain building known as the Rangitata Orogeny (Griffiths 1972). Over the Cretaceous period the mountains raised during the Rangitata Orogeny were progressively eroded and by the end of the period (approximately 65 mya) most of the land was flat and of low relief (Griffiths 1972).

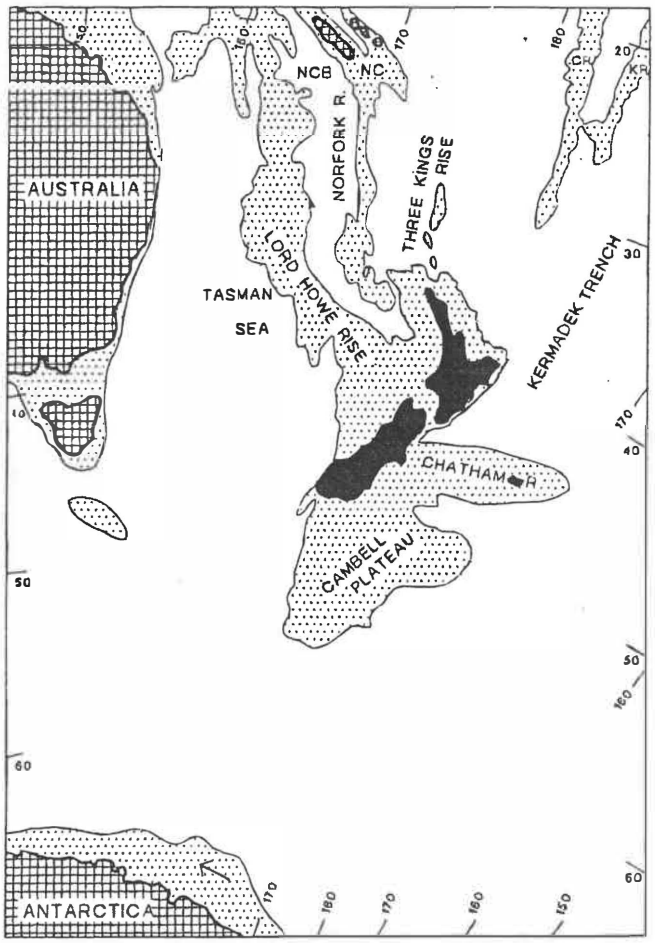


Figure 1.1. The New Zealand region. The stippled area is the 2000 m isobath, giving the approximate outline of the continental crust. Abbreviations as follows NC, New Caledonia; NCB, New Caledonia Basin; CR, Colville Ridge; KR, Kermadec Ridge. Modified from Cooper and Millener (1993).

The New Zealand region was separated from Gondwana about 80 mya by the formation of the Tasman sea and has remained isolated ever since (Cooper and Millener 1993). By the end of the Cretaceous New Zealand had moved about 1500 km away from west Antarctica and continued to move eastward throughout the Tertiary (from approximately 65 to 2 mya; Flemming 1979).

Over the Tertiary New Zealand underwent frequent changes in geography. From the Paleocene to the Oligocene (65 to 35 mya) the land was gradually reduced to a series of archipelagos. Then in the early Miocene (25 mya) the land began to rise again, with earth movements becoming more intense during the Kaikoura Orogeny, giving New Zealand its current geography (Flemming 1979). This was followed by a series of glaciations in the Pleistocene, the most recent of which (approximately 100 000-10 000 years ago) consisted of at least three interglacials, with the latest glacial period occurring between 25 000 to 15 000 years ago (McGlone 1985).

The current New Zealand archipelago consists of three main islands along with nearby smaller solitary islands or groups of islands (Kermadec, Chatham, Snares, Bounty, Antipodes, Auckland and Campbell Islands and the Australian administrated Macquarie Island). The three main islands and those close nearby have a combined area of 263 830 km^2 and extend in latitude from 24° 09' to 47°17' south. The outlying islands do not significantly increase the area but do considerably increase the range in latitude (Dawson 1988). Although these outlying islands are separated by relatively large distances from the main group, up to 1000 km, they all sit on the same extensive area of submarine plateaus and ridges – the Chatham rise and the Campbell Plateau - which are thought to have once been above sea level (Figure 1.1; Stevens 1989).

1.1.2 The New Zealand flora

Before human colonisation approximately 1000 years ago around 75% of the New Zealand mainland was covered in dense forests. The North Island and parts of the South Island were dominated by conifer broadleaf forest and beech forests (*Nothofagus*) dominated the South Island. This cover has been greatly reduced over the last 1000 years due to human impact. Despite this loss approximately 60% of New Zealand is still covered by native vegetation, although much of it is often greatly modified (Dawson 1988).

There are an estimated 1896 indigenous seed plants in New Zealand, 1876 species of angiosperms and 20 gymnosperms (Wilton and Breitwieser 2000). This is a relatively small flora (Godley 1979). For example Japan has 3561 indigenous angiosperm species and the island of Java, with an area half the size of New Zealand, 4303 (Fenner *et al.* 1997). 82.4% of the angiosperm species found in New Zealand are endemic (Wright *et al.* 2000), this number being much higher than other islands of comparable size. For example almost none of the vascular plant species in the British Isles are endemic. This level of endemism is second only to the Hawaiian Islands in which 92% of the angiosperm species are endemic (Wagner *et al.* 1990). The high proportion of endemism at the species level is a reflection of New Zealand's isolation (Dawson 1988).

Despite this high level of species endemism only 14.1% or 49 of the 341 indigenous genera in New Zealand are endemic and possibly only one of the 105 indigenous families is endemic (*Ixerba* may be a family; Kootz and Soltis 1999; Cameron 2000; Wilton and Breitwieser 2000).

Even though the main islands are almost 500 km from the tropics the flora has a distinct tropical element, with about a dozen tropical families having members in New Zealand (Good 1974; Dawson 1988). For example *Pittosporum,* which is well represented in New Zealand, is considered to be a tropical rain forest genus (van Balgooy 1966; Schodde 1972).

There is also an Antarctic element in the composition of the flora, which is mostly shared with temperate South America (Good 1974). One example of this is found in the distribution of the genus *Fuchsia.* This genus is represented in South America by sixty or more species, with the only Fuchsias species found naturally outside South America being the three species found in New Zealand (Dawson 1958). This Antarctic element is also represented in genera that are shared between South America, New Zealand, Tasmania and adjacent parts of Victoria and New South Wales. Forests of the genus *Nothofagus,* for example, are found in temperate South America, New Zealand, Tasmania and south east Australia, as well as New Caledonia and New Guinea (Stevens 1989).

New Zealand also shares about 300 species with Australia. There are, however, no genera which are widely distributed in or characteristic of both regions and many predominant Australian groups, for example *Eucalyptus*, are missing from New Zealand and conversely many characteristic New Zealand groups such as *Hebe* are not found in Australia (Good 1974).

1.1.2.1 Gondwana floral connections?

There is no doubt that long distance dispersal has played a large part in the development of New Zealand's flora with the ancestors of most species being dispersed to New Zealand from elsewhere (Winkworth *et al.* 1999). However there are a significant number of taxa considered unlikely to have reached New Zealand by long distance dispersal (Raven and Axelrod 1974; Burrows 1998).

For example, the generally short residence time of seeds in bird guts, usually no more than an hour or two (Howe 1986), means it is unlikely that the ancestors of species that have fleshy fruit with frugivorous bird dispersed seed, such as *Belschmiedia* and *Coprosma* or members of the Podocarpaceae, were carried by birds long distance to New Zealand (Burrows 1998). Its is also unlikely that the seeds of these fleshy fruited species or many dry fruited species with heavy fruit, such as *Nothofagus*, dispersed by flotation in ocean currents as the seeds do not float nor do they survive immersion in sea water (Preest 1963; Burrows 1998). It is often assumed that because of their low dispersal ability the presence of such genera indicates that these species must have 'rafted' with New Zealand when it broke off Gondwana (Pole 1994; Burows 1998). Although this is a possible scenario some authors cite evidence that New Zealand may have been completely submerged at some stage after the separation (Pole 1994). It has also been shown that *Nothofagus*, for example, can disperse long distances and conifers are found on islands which are known never to have had land connections such as *Araucaria heterophylla,* the Norfolk Island pine (Pole 1994; Macphail 1997).

1.1.2.2 Insular characteristics of theflora

New Zealand's flora exhibits many of the features characteristic of isolated islands including genera with a diverse range in morphology and ecology for such a small area, a high proportion of dioecy and small inconspicuous flowers and high levels of hybridisation (Dawson 1988).

Many genera in New Zealand exhibit wide morphological and ecological ranges. *Coprosma*, for example, includes small trees with large leaves found in lowland forests, small leaved shrubs of mostly open habitats and mat forming near herbs in mountainous areas. A wide of morphological and ecological range in also found in other genera, such as *Pittosporum* and *Hebe* (Dawson 1988). Due to New Zealand's isolation new arrivals were infrequent providing genera such as *Coprosma* with the opportunity to radiate out of their former forest habitats (Dawson 1988).

There is also a tendency towards small inconspicuous flowers on islands (Carlquist 1974). The flowers of New Zealand plants are generally small, simple and lacking in bright colours (Godley 1979; Lloyd 1985). In a study of flower conspicuousness 49% of the flowers of New Zealand angiosperms were classified in the third, or the least conspicuous, of the three classes (Thomson 1880). The small size of individual flowers is often offset by the formation of relatively large and dense infloresences (Lloyd 1985). In a survey of flower colour of 'attractive flowers' in New Zealand 60.6% where white compared to 25.1% in the British Isles (Godley 1979). There are some notable exceptions such as *Metrosideros* and *Sophora* but these are bird pollinated (Dawson 1988). A similar pattern of mostly small inconspicuous flowers, with a few exceptions, is also found in the Hawaiian Islands (Carlquist 1974). There are also notable examples of genera and families that often have white flowers in New Zealand but elsewhere have colourful flowers, one example being *Hebe* as compared to the closely related genus *Veronica* which is a predominantly northern hemisphere genus (Lloyd 1985). This lack of large and colourful flowers is thought to be correlated with a lack of specialised pollinators. For example, there are relatively few native bee species in New Zealand, all of which are short tongued and primitive (Webb and Kelly 1993).

A relatively high level of dioecy is often found on isolated islands (Carlquist 1974). It has been suggested that being dioecious is advantageous on isolated islands as it means that species must outcross, thereby increasing their variability. This then means that they are able to take advantage of unoccupied habitats and have the ability to adapt to change (Dawson 1988). The percentage of dioecy in the New Zealand flora is approximately 12% (Godley 1979; Lloyd 1985). Although not as high as within the Hawaiian Islands, where an estimated 27.7% of species are dioecious, the level of dioecy in New Zealand is much higher than that of continental areas and of other landmasses of comparable size. In Southwestern Australia for example 4.4% of the species are dioecious and in British Isles 3.1% (Carlquist 1974). The high level of dioecy is often particularly striking when comparing genera that are shared with other areas. For example, all species of *Clematis* and *Rubus* in New Zealand are dioecious while in other areas the dioecious condition is very rare (Dawson 1964).

Another characteristic of island floras is hybridisation, which is also thought to provide increased variability and ability to adapt to change. This is a major feature of the New Zealand flora and often occurs between species of widely different form and ecology (Dawson 1988). For example *Pittosporum obcordatum* - a small leaved divaricating shrub or small tree with scattered and restricted distribution - hybridises with *P. tenuifolium* subsp. *tenuifolium*, a non divaricating tree which is widely distributed throughout New Zealand (Clarkson and Clarkson 1994).

1.1.2.3 Tropical forest connections

As well as sharing taxa with the topic regions there is also a similarity in the structure of the New Zealand forest to that of the tropics(Dawson 1988). Tropical forest features such as several layers of stratification in the forest, a large number of epiphytes and laines on the trees, the production of infloresence on the trunk of large branches (caulifory), a particular method of bud protection and an evergreen habit are found in the New Zealand forests (Dawson and Sneddon 1969). There are some differences with New Zealand forests being lower in height, containing fewer species and having smaller leaves (Dawson and Sneddon 1969).

1.1.2.4 Divaricate growth form and heteroblasty

One of the most distinctive features of the New Zealand flora is the large number divaricating shrubs (Tomlinson 1978). Although definitions vary (McQueen 2000), they are best defined as small densely twiggy shrubs with wide branching angles, interlacing branches and very small leaves (5-20 mm long with an area <60 mm^2; Figure 1.2; (Kelly 1994). Approximately 54 species in New Zealand have the divaricate growth form and although they are almost identical in form they belong to 20 genera and 16 families, consisting of nearly 10% of the native woody flora (Went 1971; Greenwood and Atkinson 1977). The divaricating growth form is thought to be specialised and derived and to have evolved in New Zealand (Lloyd 1985). Almost all of the genera that contain divaricate species also have larger leaved normal growth form species in them and hybridisation of the divaricating species with these relatives is not uncommon (Greenwood and Atkinson 1977; Dawson 1988). The fact that hybridisation with larger leaved relatives occurs indicates that the evolution of this character is a relatively recent event (Dawson 1988).

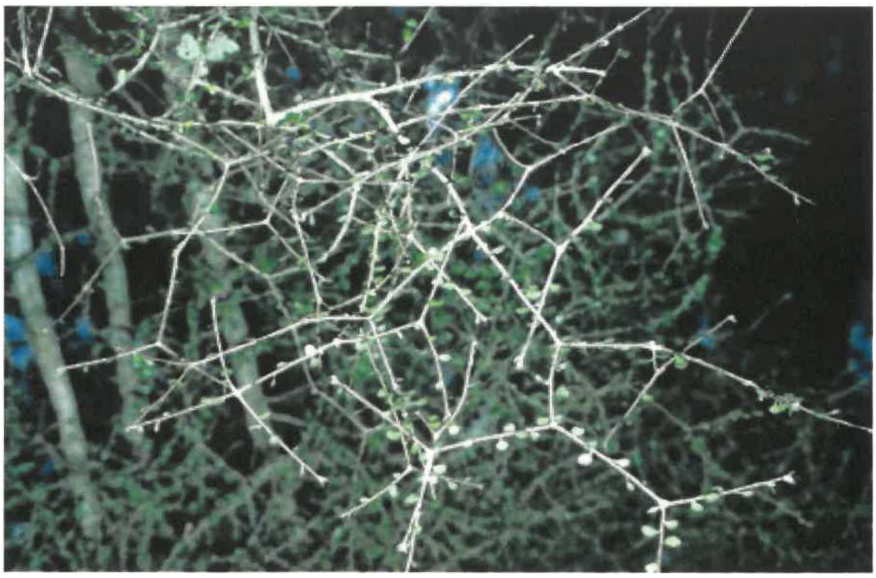


Figure 1.2. Close up of *Pittosporum obcordatum* showing the distinctive small leaves and wide branching angles of the divaricate growth form. Photo by C. Gemmill.

There are divaricating species elsewhere in the world but it is uncertain if they are equivalent to those found in New Zealand. In a list compiled of 53 so called divaricating species in California and Arizona, at least 44 have spiny branches or leaves and their average leaf size of 1.5cm is much larger than those in New Zealand (Tucker 1974). A study done on the divaricating species in Patagonia by McQueen (2000) found that most of the species with a divaricating form also have spines.

There are several theories on how the divaricating habit may have evolved with the two main ones being either an adaptation to climate or as a response to moa browsing. The climate hypothesis suggests that the divaricating habit is an adaptation to the variable extremes of wind, frost and drought found in New Zealand's mild, generally humid climate. It is thought that it is adaptation to this type of climate as the small-leaved divaricating growth form acts as a wind and frost screen and a heat trap to protect the internal leaves (McGlone and Webb 1981). Alternatively it has been suggested that the divaricating habit may have evolved as a defence mechanism in response to browsing by moa. The moa did not bite with a cutting action but clamped onto the branches and pulled to break off the foliage, and divaricate twigs are wiry and difficult to pull off and distangle from the bush (Greenwood and Atkinson 1977; Atkinson and Greenwood 1989). However, a study by Burrows (1980) showed evidence of clean cuts through the branches, which throws doubt on this hypothesis.

Also common in, although not unique to, the New Zealand flora are species with at least two distinct leaf forms found during ontogeny (Godley 1985). This is called heteroblasty, and is generally interpreted as a growth response by the apical meristem to changing physiological conditions within the plant and the subsequent interaction of endogenous factors such as hormones (Easu 1977). There are an estimated 200 heteroblastic species in the New Zealand flora and in most species there is a juvenile leaf form and an adult leaf form, often with an intermediate stage (Cockayne 1928). Figure 1.3 illustrates different leaf sizes found within one population of *Pittosporum virgatum*. In some species a different branching pattern is also found in the juvenile compared to that of the adult (Philipson 1964). *Pittosporum turneri*, for example, has a small leaved divaricating juvenile form and a large leaved normal growth form adult (Figure 1.4; Ecroyd 1994).

Several theories have been put forward for the evolution of heteroblasty. These include the hybridisation of two monoblastic species (Godley 1985), an adaptation to changing light conditions experienced at different heights (ages) in forest habitats (Day 1998) and for those species with divaricate juveniles, the fact that the divaricate form is resistant to moa browsing and the adult foliage occurs above moa browsing height (Greenwood and Atkinson 1977).

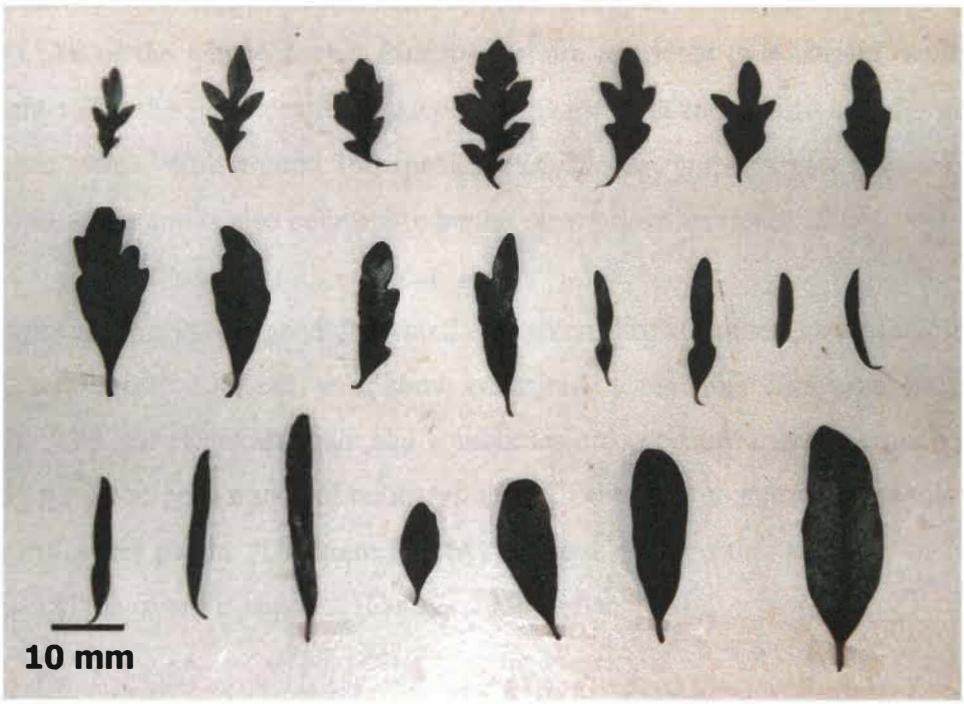


Figure 1.3. The different leaf sizes and shapes that were found in one population of *Pittosporum virgatum*, arranged from left to right, top to bottom in approximated order of increasing age

Figure 1.4. *Pittosporum turneri* foliage and branchlets, from left to right; small leaved divaricating juvenile, intermediate foliage and adult.

1.2 THE GENUS *PITTOSPORUM*

Pittosporum Banks ex Garten is a member of the Pittosporaceae, which contains nine genera and approximately 200 species (Cooper 1956; Allan 1961; Haas 1977). All of the genera except *Pittosporum* are restricted to Australia, which is thought to be the main centre of development for both the family and the genus (Cooper 1956). With around 160 species, *Pittosporum* is the largest genus in the Pittosporaceae and is also believed to be the most primitive (Haas 1977).

Pittosporum species are generally small evergreen shrubs, sometimes epiphytic, or small trees up to 10m tall, with some occasionally reaching 30m (van Balgooy 1966). The leaves are alternate and sometimes crowded towards the branch tips, giving them the appearance of being whorled. The calyx contains five sepals and the corolla five petals. There are five hypogynous stamens that alternate with the petals and the ovary is superior (Cooper 1956; Allan 1961).

The seeds of *Pittosporum* are held in woody capsules, which are usually either two or three valved (bivalved and trivalved respectively), but can occasionally number up to five valves (Figure 1.5; Allan 1961), and valve number is dependent upon carpel number (Haas 1977). Capsules are usually hard and woody or leathery and dehisence is loculicidal (splitting longitudinally along the midrib), with the valves opening widely exposing the seeds (Cooper, 1956). The seeds inside the capsule, often in large numbers (up to about 40) but can be as few as only one, are usually immersed in an aromatic, viscid, resinous fluid (Figure 1.6; Cooper 1956; Allan 1961). It is from this feature that the genus received its name, being derived from greek 'pitta', meaning resin, and 'spora', meaning seed (Gowda 1951; Haas 1977). The black or red seeds, embedded in the aromatic fluid, are thought to be attractive to birds and this has been suggested to be an adaptation to bird dispersal (Thomson 1906; Carlquist 1974; Townsend 1999). The wide distribution of the genus in the Pacific is considered to be attributable to long distance dispersal by birds (Haas 1977).

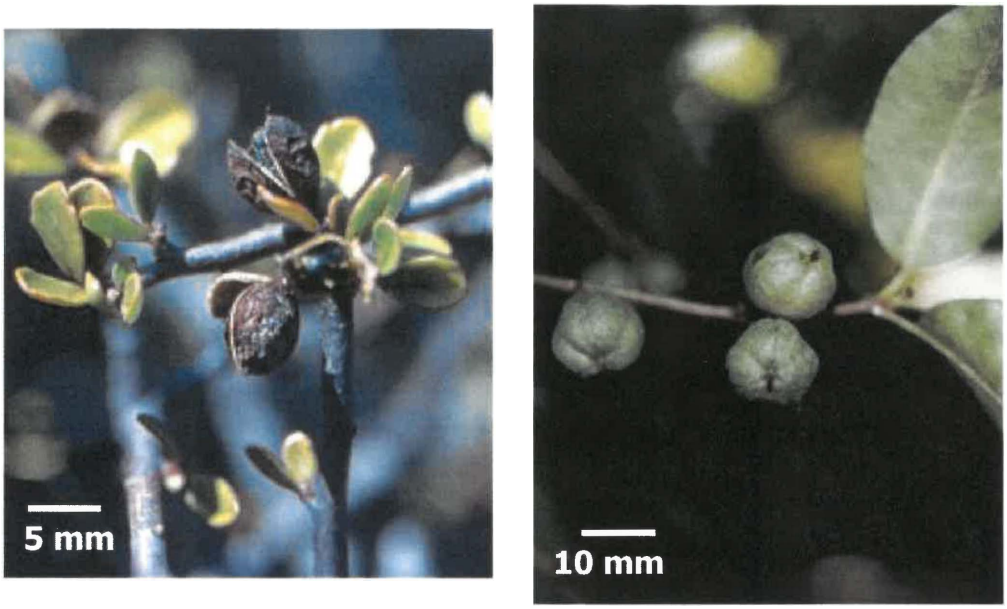


Figure 1.5. *Pittosporum* capsules. Bivaled capsules of *P. obcordatum* are shown on the left and on the right are trivaled capsules belonging to *P. tenuifolium* (*P. obcordatum* photo by C. Gemmill).

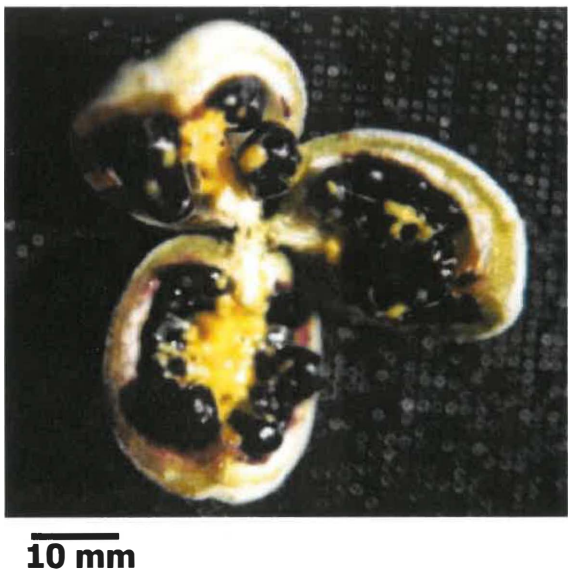


Figure 1.6. Almost mature capsules of *P. crassifolium*, cut open to show the black seeds immersed in a sticky, yellow resin.

The genus was split into two groups based on the valve number by Gowda (1951). He regarded this as an effective way of subdividing the entire genus, with the majority of the species being consistently bivalved, i.e. having two carpels. The rest of the species are termed trivalved, usually having three carpels. Gowda (1951) considered the trivalved form to be the more primitive form as they appear to have certain characters, such as large capsules, many seeds and funicles from the base to near the apex of the capsule valve, from which the characters of the bivalved species are derived. Haas (1977) considered this split to be artificial as it separates several pairs of closely related species. All of the Australian species are bivalved, with only a few species rarely having trivalve capsules as well (Bennett 19??). Since Australia is the centre of diversity for the Pittosporaceae family, and therefore the likely origin of the *Pittosporum* genus, this throws doubt on trivalve being the ancestral form.

A distinctive feature of the genus, and entire family, is the possession of schizogenous secretory canals found in the pericycle of the roots, stems and leaves, which contain ethereal oils, resins and mucilages (Jay 1969; Cronquist 1982). A wide range of secondary metabolites are found and common ones include flavines, caffeic and sinapic acids, saponins with phenolic triterpenes as well as free triterpenes (Wilkinson 1992).

A genus of the 'old world' (van Balgooy 1966), *Pittosporum* is found widely in the fragments of Gondwanaland and in the Pacific, with its absence from South America indicating an origin in east Gondwana (Crisp *et al.* 1989). *Pittosporum* is widespread in the tropic and temperate regions of both hemispheres, being widely distributed from New Zealand, Australia, the Pacific Islands up to the Hawaiian Islands, southeast Asia to Madagascar and Africa (Figure 1.7; Cooper 1956; Allan 1961; Haas 1977).

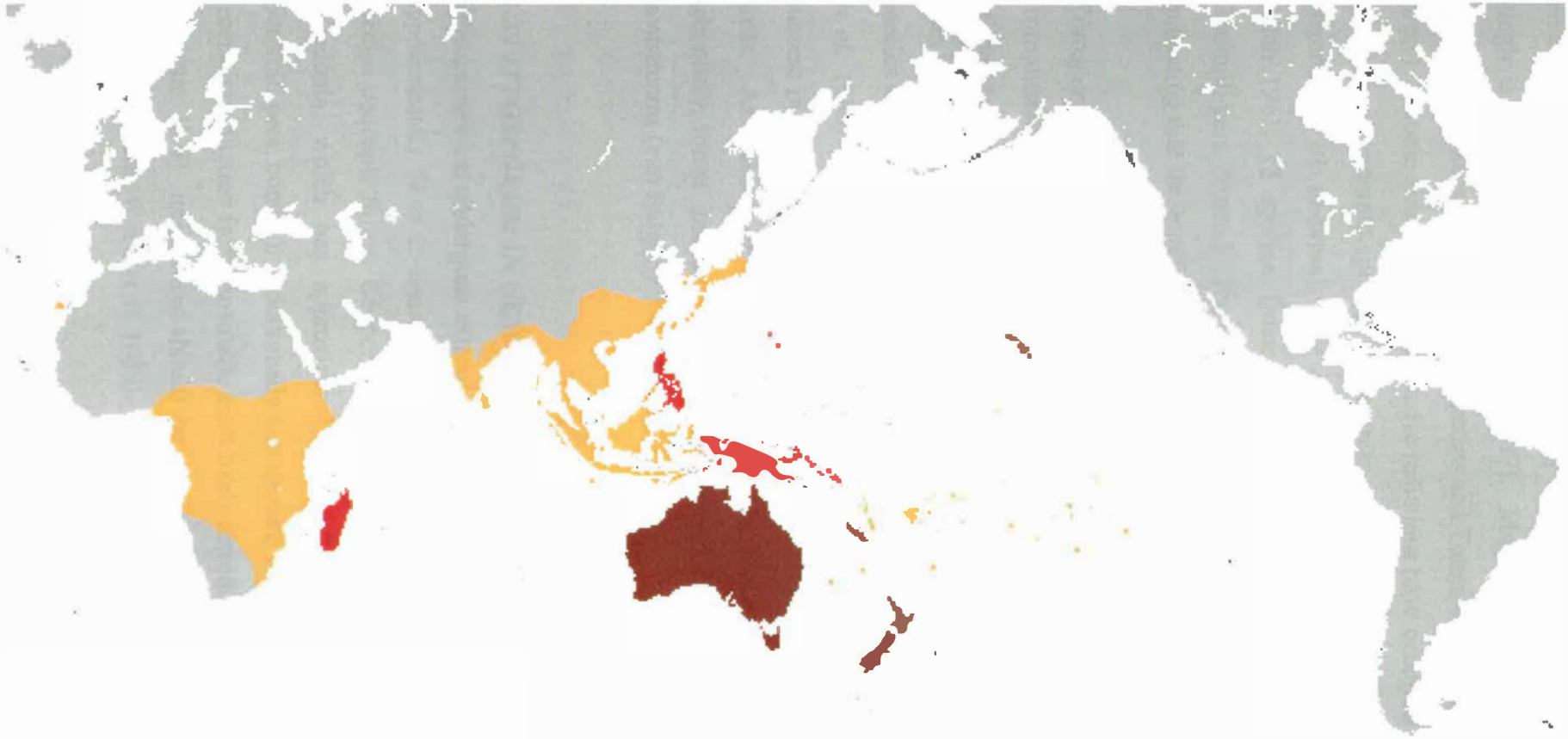


Figure 1.7. Map showing the world wide distribution of *Pittosporum*. Areas of highest species concentration are shown in brown, secondary concentrations in red, with yellow indicating presence in the area.

Throughout its geographical range *Pittosporum* shows, in general, high levels of endemism, especially in the Pacific region where most species are found only on a single island or island group (Haas 1977). Most areas have only one or a few species, for example Fiji has three species and Tonga only one (Gemmill *et al.* in press). In some areas however extensive radiations have occurred resulting in high numbers of endemic species. These areas are the Hawaiian Islands (11 species, Gemmill *et al.* in press), New Zealand (20 species, Cooper 1956; 26 species, Allan 1961; 18 species, Druce 1980) and New Caledonia (ca. 50 species, C. Gemmill pers. comm.). The radiation in Hawaii appears to be a very recent event, indicating that these radiations can be very rapid (Gemmill *et al.* in press).

Pittosporum species are found from sea-level to around 4000m in habitats ranging from monsoon forest to savannahs, rocky and sandy seashores and along the edge of mangroves and swamps with species also found in montane to subalpine forest (van Balgooy 1966). Most species are found in tropic to subtropic areas, although species are also found in temperate areas such as New Zealand (Allan 1961; Webb *et al.* 1988). *Pittosporum* is considered to be a tropical rain forest genus. Its success in rain forest is attributed to its ability to colonise marginal and disturbed areas. As a result of this ability *Pittosporum* species are also often found in secondary forest and as pioneers on disturbed sites such as land slips and lavastreams (van Balgooy 1966; Schodde 1972).

1.3 *PITTOSPORUM* IN NEW ZEALAND

Pittosporum has undergone extensive phyletic radiation within New Zealand with approximately 18 to 26 endemic species (Cooper 1956; Allan 1961; Druce 1980; species concepts follow Cooper (1956)). This radiation is second only to New Caledonia, which has approximately 50 endemic *Pittosporum* species (C. Gemmill pers. comm.). Additionally one species, *P. undulatum*, is thought to be a recent introduction from Australia, most likely introduced by birds. However it is uncertain if *P. undulatum* should be considered part of the naturalised or indigenous flora (Webb *et al.* 1988).

1.3.1 Habit

All New Zealand *Pittosporum* species are woody trees or shrubs. They range in form and size from epiphytic and dwarf (<0.8m tall) shrubs to medium sized trees around 15m tall (Cooper 1956; Allan 1961). The most common forms are low trees and shrubs between 2 to 8m in height (Cooper 1956). Some species can be highly variable in habit and size, for example *P. crassifolium* can be either a shrub or a small tree between 1 to 10m in height (Cooper 1956) (Table 1.1).

Five species of *Pittosporum* in New Zealand have the divaricating growth form, *P. anomalum, P. divaricatum, P. rigidum*, *P. obcordatum* and *P. crassicaule* (Table 1.1) (Wilson and Galloway 1993). This number is six if *P. lineare* is considered a distinct species (see Allan, 1961), however Wilson and Galloway (1993) did not consider *P. lineare* or *P. crassifolium* to be distinctive species, and included them both as slight morphological variations of *P. divaricatum.*

Heteroblasty is also common in the New Zealand *Pittosporum*, with eight species displaying strongly heteroblastic development (nine if *P. lineare* is included as a distinct species) (Table 1.1; Cooper 1956; Allan 1961). The juvenile leaves tend to be toothed or lobed and are often highly variable in outline, while the adult leaves have wavy or smooth edges and are generally larger. One species, *P. turneri*, has a distinctive divaricating, small-leaved juvenile form while the adult is larger leaved with a non-divaricating growth form (Cooper 1956; Wilson and Galloway 1993, Ecroyd 1994).

There are no divaricating *Pittosporum* species found outside New Zealand however some of the New Caledonian *Pittosporum* are heteroblastic (Cooper 1956; Hass 1977).

Table 1.1. Morphological and ecological characteristics of the New Zealand *Pittosporum*, species concepts follow Cooper (1956) (Kirk 1899; Cheeseman 1925; Lang and Gourlay 1935; Cooper 1956; Allan 1961; Eagle 1986; Webb *et al.* 1988; de Lang 1998; de Lange *et al.* 1999). Abbreviations are as follows: 2, bivalved capsules; 3, trivalved capsules; E, endangered; V, vulnerable; D, declining; R, recovering; CD, conservation dependant, require continuation of conservation measures; NU, naturally uncommon; S, sparse, small scattered populations; RR, range restricted; juv., juvenile.

Taxa	Distibution	Valve number	Conservation Status	Petal colour	Habit and maximum height	Development	Divaricate growth
P. anomalum	North Is., South Is.	2		yellow	shrub, 1 m	heteroblastic	yes
P. cornifolium	North Is., South Is.	2		yellow	epiphytic shrub, 2 m	monoblastic	no
P. crassicaule	South Is.	2		purple	shrub, 4 m	heteroblastic	yes
P. crassifolium	North Is., Kermadac Is. **a**	3		dark red to purple	shrub or small tree, 5 m	monoblastic	no
P. dallii	South Is.	2	V	white	small tree, 6 m	monoblastic	no
P. divaricatum	North Is., South Is.	2		purple	shrub, 2 m	heteroblastic	yes
P. ellipticum subsp. *ellipticum*	North Is.	2 & 3		red	small tree, 8 m	monoblastic	no
P. ellipticum subsp. *serpentinum*	North Is.	2	V	red	prostrate shrub	monoblastic	no
P. eugenioides	North Is., South Is.	2		yellow	small tree 12 m	monoblastic	no
P. fairchildii	Three Kings Is.	3	NU,RR	dark red	shrub 5m	monoblastic	no
P. huttonianum	North Is.	3		purple	shrub or small tree, 8 m	monoblastic	no
P. kirkii	North Is.	2		yellow	epiphytic shrub, 4 m	monoblastic	no
P. obcordatum	North Is., South Is.	2	R,CD	purple/yellow/white	shrub, 4m	heteroblastic	yes
P. patulum	South Is.	2	E	purple	shrub or small tree, 5 m	heteroblastic	no
P. pimeleoides subsp. *pimeleoides*	North Is.	2	NU,S	yellow	shrub, 2m	monoblastic	no
P. pimeleoides subsp. *maius*	North Is.	2	NU,RR	yellow	prostrate shrub	monoblastic	no
P. ralphii	North Is.	3		dark red to purple	shrub, 4 m	monoblastic	no
P. rigidum	North Is., South Is.	2		purple	shrub, 3 m	heteroblastic	yes
P. tenuifolium subsp. *tenuifolium*	North Is., South Is.	3		dark purple	small tree, 8 m	monoblastic	not
P. tenuifolium subsp. *colensoi*	North Is., South Is., Stewart Is.	3		dark purple	small tree, 10 m	monoblastic	not
P. turneri	North Is.	2 & 3	D	pink	juv. shrub, adult small tree, ? m	heteroblastic	juvenile
P. umbellatum	North Is.	3		pink	small tree, 7 m	monoblastic	not
P. virgatum	North Is.	2	NU,S	dark red to purple	small tree, 6 m	heteroblastic	not

a The *P. crassifolium* found on the Kermadec Islands has not yet been described and so its exact status, or in fact if it even is *P. crassifolium*, is unknown. The characters on this table are those of *P. crassifolium* found on the North Island, with which the taxa found on the Kermadec Islands is included.

1.3.2 Flower structure and breeding system

Within New Zealand inflorescences are terminal, lateral, or auxiliary, solitary to many flowered in fasicles, umbells or panicles (Cooper 1956; Allan 1961). Often a combination is found within one species, for example *P. huttonianum* has terminal or auxiliary flowers that are solitary or in fasicles of three (Cooper 1956).

Pittosporum flowers are small, about 10 to 2 mm long and 1 to 4mm broad. The five free sepals range between 5 to 11mm in length and 1.5 to 3mm broad and are linear to narrow ovate in shape. The tip is subacute to acuminate and some are imbriacte at the base, and in many species have brown or white tomentose (Cooper 1956; Allan 1961). The petals are longer than the sepals and range between 6 to 11mm in length and are between 2 to 4.5mm broad, and are linear lanceolate to oblong in shape, sometimes fused along some of their length, with the tips reflexed. Unlike most *Pittosporum* in other areas, which usually have white, greenish white or yellow corolla (Godwa 1951; Cooper 1956; Hass 1977), the corolla of New Zealand *Pittosporum* are mostly red to deep red or purple (Figure 1.8) in colour with only a few being yellow (Table 1.1; Cooper 1956; Allan 1961).

Figure 1.8. Flowers of *Pittosporum crassifolium* showing the dark corolla colour distinctive of the New Zealand species (photo by C. Gemmill).

The free five erect stamens are alternate to the petals. The introse anthers are two-celled (Cooper 1956) and dehiscence is via longitudinal slits (Hutchinson 1964; Watson and Dallwitz 1992 onwards). The sessile or stipulate ovary is superior and formed from 2 to 5 fused carpels, with axillary or partial placentation and several to numerous ovules. The stigma is acapitate to truncate and the style short (Cooper 1956; Allan 1961).

Although structurally *Pittosporum* flowers are perfect, in many species they are thought to be functionally unisexual. This has been observed in *Pittosporum* species in many areas, including New Zealand, and is thought to be both more derived than the bisexual condition and to have evolved independently in each area (Godwa 1951; Cooper 1956; Schodde 1972; Hass 1977). Those flowers that appear functionally unisexual often have either the male or female structures reduced (Cooper 1956). In male type flowers the style has an aborted stigma and the ovary is reduced in size Conversely in the female type flowers the anthers and filaments are reduced in size (Figure 1.9; Godley 1979).

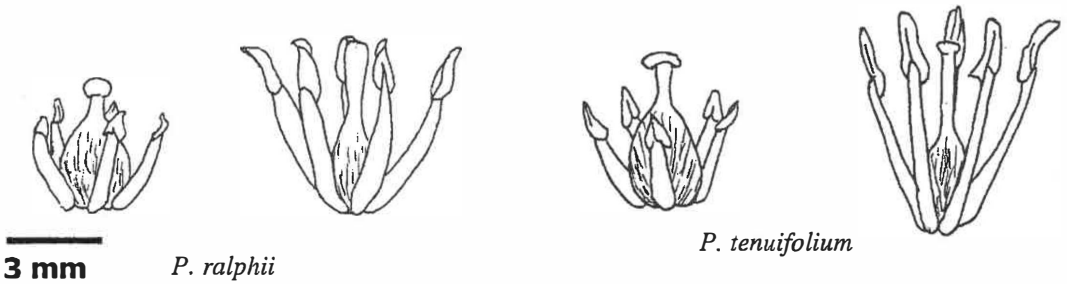


Figure 1.9. Diagrams showing the differences in size of the androecium and gynecium in the female (left) and male (right) flowers of two New Zealand *Pittosporum* species (redrawn from Godley 1979).

For example, male type *P. pimeleoides* flowers have ovules that are about half the size and number of those found in female flowers, while the female type flowers of *P. pimeleoides* are also smaller and bloom slightly later than the male type (Pickmere 1945). A study of a *P. cornifolium* population on Kawau Island found two distinct types of plants, which were identified as male and female by looking at the size of the floral structures. The male plants were also found to be less abundant and had less vigorous growth (Petrie 1920). Clarkson and Clarkson (1994) found a 1:1 ratio of female to male plants over a sample of eight populations of *P. obcordatum.*

It appears that despite the differentiation in floral parts, many species are not completely dioecious. Sometimes male plants can produce seed. Godley (1979) observed that male *P. crassifolium* plants (i.e. only produced male type flowers) produce fruit and Clarkson and Clarkson (1994) found that 6.66% of male plants of *P. obcordatum* produced seed capsules, although they were smaller than capsules of females growing nearby. These plants are termed inconstant males (Godley 1979). Inconstant females also occur, with female plants that were grown in the absence of male plants producing seed capsules (Cooper 1956).

There is much variation in types of flowers found on individual plants. For example in *P. eugenioides* some trees are practically dioecious while others have both male type and female type flowers along with perfect flowers. This has also been observed on other species of New Zealand *Pittosporum* (Kirk 1889).

Pickmere (1945) observed that *P. pimeleoides* seemed to require cross pollination for fertilisation. However, an isolated female *P. eugenioides* (i.e. only produced female type flowers) plant produced fruit (Petrie, 1920).

1.3.2.1 Pollination

The deep red or purple colouring found in most of the New Zealand *Pittosporum* is unusual as they are thought to be insect pollinated. Nocturnal moths have been observed visiting *P. fasciculatum, P. ralphii, P. pimeleoides* and *P. turneri* (Pickmere 1945; Godley 1979; Ecroyd 1994). Numerous flies have been seen visiting *P. eugenioides, P. tenuifolium* and *P. crassifolium* (Thomson 1880; Thomson 1925; Heine 1937) as well as two species of beetles on the flowers of *P. tenuifolium* and introduced honey bees have been observed visiting *P. obcordatum* and *P. crassifolium* (Heine 1937; Clarkson and Clarkson 1994). The flowers of *P. eugenioides, P. tenuifolium* and *P. obcordatum* are strongly fragrant, although the last two only in the dusk and evening (Thompson 1880; 1925; Heine 1937; Clarkson and Clarkson 1994). The flowers of *Pittosporum tenuifolium* have small beads of nectar between the bases of the ovary and filaments. The ovary is hairy and the hairs mean that only insects with a proboscis can reach the nectar. *Pittosporum eugenioides* also contain a large amount of nectar between the bases of the ovary and filaments (Thompson 1880; 1925).

1.3.3 Fruit

The capsules of the New Zealand species range in width from 40 to 300mm and are globose or subglobose and ovoid to obovoid in shape. The woody capsules are usually glabrous although some are pubescent, and are often granulate or furrowed. The larger capsules have thicker valves. The seeds vary from dark red to black in colour, and are round or irregular in shape and vary in number from one to 30 per capsule, with the larger capsules having more seeds (Cooper 1956; Allan 1961).

Both bivalve and trivalve capsules are found in the New Zealand *Pittosporum.* Two species, *P. ellipticum* and *P. turneri,* have inconsistent valve number, with both trivalve and bivalve capsules being found (Table 1.1). Allan (1961) described both species as having two valved capsules. Cooper (1956) included *P. ellipticum* in the trivalve, but stated that it was difficult to decide whether it belonged in the trivalve or bivalve group while he placed *P turneri* in the bivalve species group, describing it as two, rarely three valved.

Three of the New Zealand bivalved species, *P. dallii*, *P. eugenioides*. and *P. anomalum*, have distinctive capsules with a papery endocarp surrounding the seeds that remains intact when the valves separate (Moore and Adams 1949).

1.3.3.1 Seed dispersal

As with *Pittosporum* elsewhere, the seeds of the New Zealand species are probably dispersed by birds (Townsend 1999). For example, Ecroyd (1994) considered the most likely explanation for the wide distribution of *P. turneri* found under a stand of pines at Era to be dispersal of the seed by birds. The seeds of *P. obcordatum* are fully ripe while the capsule is still green, raising the possibility that that seed dispersal could also occur by birds eating the green capsules (Clarkson and Clarkson 1994).

1.3.4 Habitat

New Zealand *Pittosporum* are found in a wide variety of habitats ranging from early successional scrub to old growth forests.

Species of New Zealand *Pittosporum* species are frequently found on forest margins or in low growing bush and are especially common on the sides and spurs of gullies (Kirk 1871). In fact, in areas such as forest remnants, modified scrub, the edges of plantations and fields and on cliff faces *Pittosporum* species, such as *P. tenuifolium, P. eugenioides, P. crassifolium,* are among those woody plants most likely to be found (Webb *et al.* 1988).

There are also a number of small-leaved xeromorphic species. *Pittosporum pimeleoides* subsp. *pimeleoides* grows on ridges with *Agathis australis* where strongly leached podsolized and skeletal soils predominate (Cooper 1956). *Pittosporum divaricatum, P. rigidum* and *P. anomalum* are found on skeletal mountain and volcanic soils (Cooper 1956, Wilson and Galloway 1993).

Pittosporum ellipticum subsp. *serpentinum* and *P. pimeleoides* subsp. *maius* are both endemic to the ultramafic, serpentine rock and associated soils of the Surville Cliffs on the North Cape (Cooper 1956; de Lange 1998).

Pittosporum kirkii and *P. cornifolium* are usually epiphytic on the trunks and branches of *Metrosideros robusta* and various other large forest trees (Cheeseman 1914; Allan 1961).

1.3.5 Distribution

Pittosporum species are distributed over the whole of New Zealand (Table 1.1). Based on Cooper's (1956) classification there are nine taxa found only on the North Island, three restricted to the South Island and seven which are common to both. One species, *P. fairchildii* is endemic to the Three Kings Islands and *P. crassifolium* is found on the Kermadec Islands and the North Island (Cheeseman 1914; Allan 1961).

Only *P. eugenioides*, *P. tenuifolium* subsp. *tenuifolium* and *P. tenuifolium* subsp. *colensoi* are found throughout most of New Zealand. They are widely distributed and abundant over both main islands, with *P. tenuifolium* subsp. *colensoi* also found on Stewart Island (Cheeseman 1925; Cooper 1956; Allan 1961). Some species are distributed throughout the entire country, for example *P. tenuifolium* subsp. *tenuifolium* is found in costal to lower montane forest and *P. tenuifolium* subsp. *colensoi* and *P. eugenioides* in lowland to montane forest throughout almost the entire country (Allan 1961). In general though there seems to be a tendency for species to have restricted distributions, for example *P. turneri* is found at only nine sites on the Central North Island Plateau (Ecroyd 1994) and *Pittosporum crassifolium* is naturally confined to the coast north of 39° of latitude (Allan 1961).

The probable tropical origins of the genus mean that the warmer summers and milder winters of the North Island probably accounts for the fact that *Pittosporum* species tend to be concentrated in the north (Cooper 1956).

1.3.6 Fossils

The first leaf impressions of *Pittosporum* are first found in the late Oligocene (approximately 25 mya) and belong to a species named *P. elegans*, which resembles *P. tenuifolium* subsp. *colensoi* (Oliver 1950). The first pollen records attributed to *Pittosporum* in New Zealand are from the mid Miocene (approximately 15 mya; Flemming 1979).

1.3.7 Hybridisation and polymorphy

The confusion in New Zealand *Pittosporum* taxonomy and systematics is exaggerated by hybridisation and the large number of polymorphic characters.

Hybridisation does occur between species, some recorded hybrids include; *P. ralphii* X *P. tenuifolium* subsp. *tenuifolium, P. crassifolium* X *P. tenuifolium* subsp. *tenuifolium* (Druce 1980) *P. obcordatum* X *P. tenuifolium* subsp. *tenuifolium* (Clarkson and Clarkson 1994) and *P. turneri* X *P. divaricatum* (Ecroyd 1994), but the full extent of this is unknown (Allan 1961).

It may be possible that some described species are in fact hybrids. One example is *P. intermedium,* not only was this species based on a single tree form Kawau Island, which has since been destroyed, it shares morphological features with four different species; the foliage is similar to large forms of *P. tenuifolium,* it has the capsule characters of *P. crassifolium* and *P. ellipticum* and the petals and sepals resemble *P. umbellatum* in size and shape (Kirk 1871; Cooper 1956). Kirk (1871) gave this taxa specific status, although he did it with some hesitation, considering it to be a hybrid. Cooper (1956) considered it to be a doubtful species, and while Allan (1961) listed it in his flora he commented that it was possible the plant was a hybrid of *P. crassifolium* and *P. tenuifolium.* Both of these species are present on Kawau Island, *P. ellipticum* and *P. umbellatum* are not found on the island (Allan 1961), making them unlikely parents.

There is also a high proportion of intergrading forms such as the members of one species complex comprised of *P. tenuifolium* subsp. *tenuifolium, P. tenuifolium* subsp. *colensoi* and *P. fasciculatum* (Kirk 1871; note that he considered the first two taxa also to be separate species). Kirk (1871, page 262) commented "these forms vary considerably in all their parts, so that it would not be difficult to obtain a connected series of specimens, which should include the whole". Another example of variation in morphology causing confusion is shown in *P. obcordatum.* Once given varietal status, *P. obcordatum* var. *kaitaiaensis* is now seen as part of a morphological cline from north to south (Clarkson and Clarkson 1994).

1.3.8 Conservation

Pittosporum has a high proportion of species considered at risk (38% of species plus one subspecies). Three taxa, *P. patulum, P. dallii* and *P. ellipticum* subsp. *serpentinum,* are listed as threatened, and six, *P. turneri, P. obcordatum, P. pimeleoides* subsp. *pimeleoides, P. virgatum, P. fairchildii* and *P. pimeleoides* subsp. *maius* are listed as uncommon in the most recent list of the conservation status of New Zealand's vascular plants (de Lange *et al.* 1999).

1.4 RELATIONSHIPS AND TAXONOMY OF THE NEW ZEALAND *PITTOSPORUM*

Gowda (1951, page 263) stated that "most of the [*Pittosporum*] species are exceedingly variable and singularly lacking in the obvious characters which are useful for quickly identifying plants. In most regions where *Pittosporum* is well represented it is recognized by systematists as a confusing and difficult genus". This is certainly appropriate when considering the *Pittosporum* in New Zealand, as there is disagreement over the exact number of taxa and their status. Cooper (1956) in his revision of the New Zealand and Australian *Pittosporum* accepted 20 species while Allan (1961) listed 26 in his flora and a recent revision of the New Zealand flora by Druce (1980) contained 18 (Table 1.2). Allan (1961, page 318) stated that the whole genus "is in need of critical revision".

1.4.1 Relationships within New Zealand

Although no rigorous phylogenetic studies have been conducted on the relationships of the New Zealand *Pittosporum* various groupings of related taxa and complexes have been suggested based on morphology.

Cooper (1956) divided the New Zealand species into two groups on the basis of capsule morphology, i.e. trivalve and bivalve, which he considered to represent two distinct colonisations.

Allan (1961) divided the New Zealand *Pittosporum* into those with heteroblastic development and those without.

Cooper (1956) defined a group of species based on a comparison of leaf forms, infloresences, flowers and capsules. He suggested that *P. pimeleoides, P. patulum, P. turneri, P. virgatum* and *P. umbellatum* may have a common origin. Although other morphological characters and the geographical ranges of these taxa are quite distinct, they seem to represent a series of forms in which there has been an increase, or more likely a reduction, in size going from *P. pimeleoides* to *P. umbellatum*.

Table 1.2. New Zealand endemic *Pittosporum* taxa as proposed by Cooper (1956), Allan (1961) and Druce (1980).

Cooper, 1956	Allan, 1961	Druce, 1980
P. anomalum Laing et Gourlay	*P. anomalum* Laing et Gourlay	*P. anomalum* Laing et Gourlay
P. ~~cornifolium~~ A. Cunn	*P. cornifolium* A. Cunn	*P. cornifolium* A. Cunn
P. crassicaule Laing et Gourlay	*P. crassicaule* Laing et Gourlay	*P. rigidum* var. (*P. crassicaule*)
P. crassifolium Banks et Sol.	*P. crassifolium* Banks et Sol.	*P. crassifolium* var. *crassifolium* Banks et Sol.
		P. crassifolium var. (Surville Cliffs) **i.**
P. dallii Cheesem.	*P. dallii* Cheesem.	*P. dallii* Cheesem.
P. divaricatum Ckn.	*P. divaricatum* Ckn.	*P. divaricatum* Ckn.
	P. lineare Laing et Gourlay **ii.**	
P. ellipticum Kirk	*P. ellipticum* Kirk	*P. ellipticum* subsp. *ellipticum* Kirk
		P. ellipticum subsp. *serpentinum* de Lange **i.**
P. eugenioides A. Cunn.	*P. eugenioides* A. Cunn.	*P. eugenioides* A. Cunn.
P. fairchildii Cheesem.	*P. fairchildii* Cheesem.	*P. fairchildii* Cheesem.
P. huttonianum Kirk	*P. huttonianum* Kirk	*P. tenuifolium* subsp. (*P. huttonianum*) **iii.**
P. kirkii Hook. f. ex Kirk	*P. kirkii* Hook. f. ex Kirk	*P. kirkii* Hook. f. ex Kirk
P. obcordatum Raoul	*P. obcordatum* Raoul	*P. obcordatum* Raoul var. *obcordatum*
		P. obcordatum var. *kaitaiensis* Laing et Gourlay **iv.**
P. patulum Hook. f.	*P. patulum* Hook. f.	*P. patulum* Hook. f.
P. pimeleoides R. Cunn. subsp. *pimeleoides*	*P. pimeleoides* R. Cunn.	*P. pimeleoides* var. *pimeleoides* R.Cunn
P. pimeleoides subsp. *major* (Cheeseman) R.C. Cooper	*P. michiei* Allan	*P. pimeleoides* var. *maius* Cheeseman
P. ralphii Kirk	*P. ralphii* Kirk	*P. ralphii* Kirk
P. rigidum Hook. f.	*P. rigidum* Hook. f. var. *rigidum*	*P. rigidum* Hook. f. var. *rigidum*
	P. rigidum var. *majus* Allan	
P. tenuifolium Sol. ex. Gaerten subsp. t*enuifolium*	*P. tenuifolium* Sol. ex Gaerten	*P. tenuifolium* Sol. ex. Gaerten subsp. t*enuifolium*
P. tenuifolium subsp. *colensoi* (Hook.f.) Kirk	*P. colensoi* Hook. f.	*P. tenuifolium* subsp. *colensoi* (Hook.f.) Kirk
	P. buchananii Hook. f. **v.**	
	P. fasciculatum Hook. f. **v.**	
	P. intermedium Kirk **v.**	
P. turneri Petrie	*P. turneri* Petrie	*P. turneri* Petrie
P. umbellatum Banks et Sol. ex Gaertn.	*P. umbellatum* var. *umbellatum*	*P. umbellatum* var. *umbellatum*
	P. umbellatum var. *cordatum* Kirk	*P. umbellatum* var. *cordatum* Kirk
P. virgatum Kirk	*P. virgatum* var. *virgatum*	*P. virgatum* var. *virgatum*
	P. virgatum var. *matthewsii* (Petrie) Allan	*P. virgatum* var. *matthewsii* (Petrie) Allan
20 species, 2 with 2 subspecies	26 taxa, 3 with 2 varieties	18 taxa, 1 with 3 subspecies, 1 with 2 subspecies, 5 with 2 varieties **vi.**

i. These two taxa are the same, it was not known when Cooper (1956) and Allan (1961) published, Druce (1977) considered this taxon to be a variety of *P. crassifolium*, however de Lange (1998) after careful study described it as a subspecies of *P. ellipticum.*

ii. P. lineare is considered by Cooper (1956) and Druce (1980) to be synonym of *P. divaricatum* and *P. crassicaule.*

iii. Druce (1980) stated that *P. huttonianum*, which he considered a sub species of *P. tenuifolium,* was most similar to *P. tenuifolium* subsp. *colensoi*

iv. This taxa is no longer considered to be a variety, but to represent the extreme end of a morphological cline of P. obcordatum (Clarkson and Clarkson, 1994)

v. *P. buchanaii* and *P. intermedium* were considered doubtful, and *P. fasciculatum* a synonym of *P. tenuifolium* subsp. *tenuifolium* and *P. tenuifolium* subsp. *colensoi* by Cooper (1956) and Druce (1980)

vi. the two *P. crassifolium* varieties are not included in this count, see **i.**

Laing and Gourlay (1935) consider the small-leaved, bivalved species, *P. obcordatum, P. rigidum, P. divaricatum* and *P. anomalum,* as well as *P. crassiculae* and *P. lineare*, to be a natural group. They defined this group as the New Zealand taxa with juvenile leaves more or less pinnatifed, mature leaves no greater than 2.5 cm in length and flowers either solitary or in fascicles.

Pittosporum anomalum, P. dalliii and *P. eugeniodies* are thought to differ considerably from the other species and their relationships are uncertain (Allan, 1961). Allan (1961) went on to suggest that it may be necessary to create subgeneric classifications within the New Zealand *Pittosporum* to accommodate these species. Laing and Gourlay (1935) included *P. anomalum* in the small leaved, bivalved species group because of its small leaf size but then say that it "differs from all other species of *Pittosporum*" and that it "does not seem possible that it was developed in New Zealand from any known species, or from any species similar to those at present here." (Laing and Gourlay 1935, page 60). Its reduced capsule size, lack of 'mucilage' around the seed and reticulately marked testa are the primary characters that separate it from the other species in New Zealand (Laing and Gourlay 1935; Moore and Adams 1949). *Pittosporum anomalum* also does not fit within the usual limits for *Pittosporum,* and many of its characters are similar to those of the Australian genus *Bursaria*, also a member of the Pittosporaceae (Laing and Gourlay 1935). *Pittosporum eugenioides* is also very different from the other New Zealand species and may also represent a different genus (Allan 1961).

There are also several groups of taxa that are closely related but their status varies depending on the author. The two taxa that Cooper (1956) called *P. pimeleoides* subsp. *pimeleoides* and *P. pimeleoides* subsp. *maius* (which he called subsp. *major* but was changed to the more scientifically correct *maius* (Connor and Edgar 1987)), due to similarites in flowers and fruits despite different growth habit and leaf size, were considered by Allan (1961) to be distinct species. Druce (1980) gave them varietal status, as did Cheeseman (1925) in his manual of the New Zealand flora.

Another taxonomically troubling group is those taxa that are usually either allied to or included in *P. tenuifolium*. Kirk (1899) described *P. tenuifolium* var. *tenuifolium*, *P. tenuifolium* var. *colensoi* and *P. tenuifolium* var. *fasciculatum*. However, Cheeseman (1925), although stating they are very closely allied and connected many numerous intermediates, gave these three taxa species status. Cooper (1956) split *P. tenuifolium* into two subspecies, *P. tenuifolium* subsp. *tenuifolium* and *P. tenuifolium* subsp. *colensoi* and did not recognise *P. fasciculatum*. Allan (1961) gives all three taxa species status, while Druce (1980) gave them the same classification as Cooper (1956). *Pittosporum huttonianum* is generally considered to be a distinct species (see Kirk, 1899; Cheeseman, 1925; Cooper, 1956; Allan, 1961) although Druce (1980) considers it to be a subspecies of *P. tenuifolium*. *Pittosporum buchananii* and *P. intermedium* are recognised by Kirk (1899) Cheeseman (1925) and Allan (1961) with both Cooper (1956) and Druce (1980) rejecting them as doubtful taxa.

The status of members of the small leaved bivalved species group also varies depending on the author. When this group was originally described by Laing and Gourlay (1935) they considered it to contain six species. However, Cooper (1956) placed *P. lineare* as a synonym of *P. divaricatum* and *P. crassicaule*, although he did it with some hesitation. Allan (1961) gave all of these taxa species status. Druce (1980) also considered *P. lineare* as doubtful and *Pittosporum crassicaule* is now known as *P. rigidum*. This name was given to the plants south of Northwest Nelson that tend to be smaller leaved. Although worthy of recognition at the varietal level, the differences are not enough to consider it a distinct species (Druce 1980; Wilson and Galloway 1993).

1.4.2 Lineages of *Pittosporum* in New Zealand

It has been hypothesised that there could be as many as four lineages of *Pittosporum* in New Zealand, representing four unique colonisation events. These lineages are the bivalved species, the trivalved species, the species with a papery endocarp in the capsule and *P. undulatum* (B. Clarkson, University of Waikato, pers. comm.). However the possibility exists that the New Zealand *Pittosporum* are the result of a single long distance colonisation event, followed by extensive phyletic radiation within New Zealand.

Alternatively, the morphological diversity within New Zealand *Pittosporum* could be a result of vicariance, if the Pittosporaceae are an ancient lineage that evolved in Gondwanaland. Schodde (1972) suggested that the species diversity found in and between Africa, southeast Asia, Australia, Paupasia and New Zealand indicates that the presence of *Pittosporum* in these areas could be a result of ancient land connections. However, the relationships of the New Zealand species, with each other as well as species in other regions, have not yet been established (Schodde 1972).

1.5 INTERNAL TRANSCRIBED SPACERS OF NUCLEAR RIBOSOMAL DNA

Internal transcribed spacers (ITS) are located in the 18S – 26S nuclear ribosomal DNA (rDNA). The rDNA repeat unit consists of, from the 5' to the 3' end, an external transcribed spacer (ETS), the 18S gene, an internal transcribed spacer, known as ITS1, the 5.8S gene, a second internal transcribed spacer, ITS2, and the 26S gene separated by an intergenic spacer (IGS) (Figure 1.10; Jorgensen and Cluster 1988; Hamby and Zimmer 1992; Baldwin *et al.* 1995).

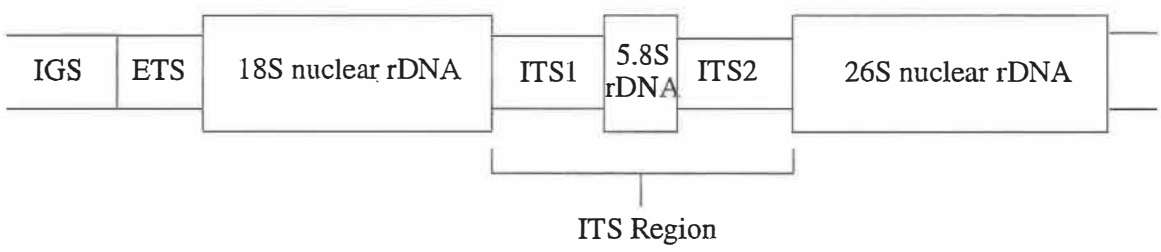


Figure 1.10. Diagrammatic representation of the 18S-26S nuclear ribosomal DNA repeat unit showing the location of the three ribosomal genes, the four spacers and the ITS region.

The spacers are transcribed along with the three genes but are not incorporated into mature ribosomes (Musters *et al.* 1990). Transcripts of the two internal spacers do however appear to have some function in the maturation of rDNA's, which indicates they are probably under some evolutionary constraint in structure and size. This is suggested by the similar size and G+C content found in comparisons among angiosperms (Baldwin *et al.* 1995).

There are several features of the ITS region, which consists of the two ITS spacers and the 5.8S rDNA gene (Figure 1.10), that make it a useful marker for phylogenetic studies. In the typical plant genome there are thousands of copies of the rDNA repeat unit arranged in tandem repeats at one or multiple chromosomal loci (Hamby and Zimmer 1992). This high copy number assists in the amplification of the ITS region (Baldwin *et al.* 1995). Due to unequal crossing over and gene conversion the rDNA repeat units undergo rapid concerted evolution (Jorgensen and Cluster 1988; Hillis and Dixon 1991; Hamby and Zimmer 1992), which is important for phylogeny reconstruction as it produces homogeneity of ITS sequences within an individual. As a result of this similarity PCR products can be sequenced directly (Baldwin *et al.* 1995).

In angiosperms the ITS region is usually less then 700 base pairs in size which makes it easy to amplify the entire region (Baldwin *et al.*1995). Because the three ribosomal RNA gene sequences which flank the spacers are very highly conserved the ITS region can be amplified using universal eukaryotic primers located in these regions developed by White *et al.* (1990) which means that it is not necessary to develop specific primers for the study group first (Baldwin *et al.* 1995).

The level of sequence variation found in the ITS region makes it a useful marker for investigating relationships within and among genera. Many studies have been conducted using ITS sequence data at the intra- and intergeneric level (eg Baldwin 1992; Baldwin 1993; Baldwin *et al.* 1995; Sang *et al.* 1995; Downie and Katz-Downie 1996; Choi and Kim 1997; Moller and Cronk 1997; Baldwin and Sanderson 1998; Gernandt and Liston 1999; Noyes and Rieseberg 1999; Ganders *et al.* 2000; Mitchell and Wagstaff 2000; Gemmill *et al.* in press), including studies on New Zealand taxa and often including related taxa in the pacific (eg Glenny and Wagstaff 1997; Mitchell and Wagstaff 1997; Wagstaff and Garnock-Jones 1998; Gatt and Hammett 2000; Mitchell and Heenan 2000; Wagstaff and Granock-Jones 2000; Wright *et al.* 2000).

Phylogenies constructed using ITS sequences are generally congruent with those using non-coding and coding regions of chloroplast DNA (eg the *trn*L intron (Gielly *et al.* 1996), the *rpl*16 intron (Baum *et al.* 1998), the *ndhF* gene (Smith 2000), the *matK* gene (Stanford *et al.* 2000) and the *rbcL* gene (Wagstaff and Dawson 2000) and chloroplast restriction site data (Baldwin 1992; Setoguchi and Watanabe 2000).

Gemmill *et al.* (in press) used the ITS region to investigate the origin and relationships of the Hawaiian and Pacific species of *Pittosporum.* They found that the morphologically diverse Hawaiian *Pittosporum* had identical ITS sequences. Three New Zealand species; *P. rigidum, P. turneri* and *P. cornifolium* were included in their analyses. They found that these three New Zealand species did not form a monophyletic group. *Pittosporum rigidum* and *P. turneri* tended to form a closely related clade, with a pairwise sequence divergence from each other of 0.25%, these two species both had a pairwise distance of about 10% from *P. cornifolium.* They hypothesized that there had been two colonisations into New Zealand, although there was no indication of the point of origin as they were unresolved within the clade of Pacific and New Caledonian species. They found that in most areas outside the Hawaiian Islands where they had sampled more than one taxon there appeared to have been more than one colonisation. This study does not support the hypothesis of a close relationship of the New Zealand bivalve species as hypothesized by Cooper (1956), or that of the bivalve species representing one colonization as all three of these species are bivalve. Both *P. turneri* and *P. rigidum* are heteroblastic, while *P. cornifolium* is monoblastic, which gives some support to grouping the New Zealand taxa into heteroblastic and monoblastic groups.

1.6 OBJECTIVES

The purpose of this research was to use DNA sequence variation of the ITS region to resolve the phylogenetic relationships within the New Zealand members of the genus *Pittosporum* and their relationships to *Pittosporum* in other areas.

The phylogenetic hypotheses generated from the ITS sequence data were used to address questions of taxonomy and phylogeny such as:

1. Do the New Zealand *Pittosporum* form a monophyletic group, and if not, how many colonisation events can be inferred?
2. Where did the colonising taxa originate from?
3. What are the phylogenetic relationships of the New Zealand species, to each other and to *Pittosporum* species elsewhere?
4. Are the current ideas on relationships and taxonomic status of *Pittosporum* based on morphology within New Zealand, and throughout its range and relationships within of the Pittosporaceae, supported by molecular data?
5. What patterns can be inferred about the distribution of *Pittosporum,* both within New Zealand and throughout its range?

The evolution of various characters of the *Pittosporum*, such as valve number and flower colour, were reconstructed, by mapping these characters onto the phylogenetic trees. These hypotheses were also used to investigate some distinctive features of the New Zealand flora, such as the prevalence of the divaricating habit and heteroblasty and all of which are found in this genus.

CHAPTER TWO
METHODS

2.1 SAMPLE COLLECTION

Leaf material was collected from one or several individuals from as many of the endemic New Zealand *Pittosporum* taxa as possible. *Pittosporum* species from Australia, New Caledonia, Norfolk Island, the Pacific Islands, Mauritius and Japan and representatives of several other genera in the Pittosporaceae were also included to test for the origins and relationships of the New Zealand species to *Pittosporum* elsewhere (Table 2.1; Figure 2.1).

Leaf material was collected from either wild populations or from botanical gardens. For those individuals acquired from botanical gardens provenance information was also obtained where possible. Sequences for *P. undulatum, P. balfouri,* the New Caledonian and Pacific Island species were obtained from Gemmill *et al.* (in press)/GenBank. The ITS sequence of *Pseudopanax discolor* was obtained from GenBank. The sequences of *P. tobira* and *Sollya heterophylla* were obtained from C. Gemmill (University of Waikato).

After collection the leaf material was either stored for up to a week in the fridge or for longer periods at –80°C or with silica gel until DNA extraction was performed.

2.2 DNA EXTRACTION

Total genomic DNA was extracted using a modified method of the CTAB procedure of Doyle and Doyle (1987; Appendix 1). Due to secondary compounds being co-extracted and co-preciptating with the DNA, observed as a change in colour from whitish to yellow or brown, a further PCI purification was performed on many of the extractions (Appendix 2).

Table 3.1 Taxa included in this study. See Table 1.2 for authorities of the New Zealand species. Abbreviations: NI, North Island; SI, South Island.

Taxon	Source	Collection Locality/Original Source	Collection/ Accesssion No.
New Zealand			
Pittosporum anomalum	wild	Castle Basin, SI	DG7505
P. cornifolium	Otari Native Botanical Garden	Waikanae, NI,	OBG9200494
P. crassifolium	wild	Kauaeranga, NI	CECG316
P. cf. *crassifolium*	Auckland Regional Botanical Garden	Raoul Island, Kermadec Islands	ABG942199
P. dalli	Landcare Lincoln	Cobb Dam, SI	LC16439
P. divaricatum	wild	Arthurs Pass, SI	CECG299
P. ellipticum	Auckland Regional Botanical Garden	Waitakere Ranges, NI	ABG941495
P. eugeniodies	cultivated	Chrustchurch, SI	CECG301
P. fairchildii	Otari Native Botanical Garden	Three Kings Islands	OBG8400050
P. huttonianum	wild	West Coast, NI	BCs.n.5
P. kirkii	Otari Native Botanical Garden	Great Barrier Island	OBG9100137
P. obcordatum	wild	Mr S Hain, Pehiri Rd, Waikura Valley, NI	CECG293
P. patulum	Landcare Lincoln	Lee Creek, Wairau River, SI	LC16564
P. pimeleoides subsp. *maius*	Landcare Lincoln	North Cape, NI	LC11564
P. pimeleoides subsp. *pimeleoides*	Landcare Lincoln	Timaru Botanic Gardens	LC15/90A
P. ralphii	wild	Raingatiki, NI	CECG291
P. rigidum 1	wild	West Coast, NI	BCs.n.4
P. rigidum 2	wild	Arthurs Pass, SI	CECG298
P. tenuifolium subsp. *colensoi*	wild	Pureora Forest Park, NI,	LAH16
P. tenuifolium subsp. *tenuifolium*	wild	Waipunga, NI	CECG290
P. turneri	wild	Ripia Valley, NI	CECG287
P. umbellatum	Auckland Regional Botanical Garden	Great Barrier Island	ABG940216
P. virgatum	Otari Native Botanical Garden	Puketi, NI	OBG8800096
Norfolk Island			
P. bracteolatum Endl.	Auckland Regional Botanical Garden	Norfolk Island National Park	ABG980883

Table 2.1 continued.

	Source	Collection Locality/Original Source	Collection/ Accesssion No.
New Caledoina			
P. coccineum Beauvis.	Gemmill *et al.* (in press)/GenBank	Poya, New Caledoina	AF302033
P. gatopenese Guillaumin	Gemmill *et al.* (in press)/GenBank	Poya, New Caledoina	AF302034
P. koghienese Guillaumin	Gemmill *et al.* (in press)/GenBank	Mt. Dmuzac, New Caledoina	AF302035
P. lanipetalum Tirel & Veillon sp. nov.	Gemmill *et al.* (in press)/GenBank	Roche Ouaieme, New Caledoina	AF302036
P. oreophilum Guillaumin	Gemmill *et al.* (in press)/GenBank	Mt. Koghi, New Caledoina	AF302037
Pacific Islands	Gemmill *et al.* (in press)/GenBank		
P. arborescens Rich ex A. Gray	Gemmill *et al.* (in press)/GenBank	Vava'u, Kingdom of Tonga	AF302026
P. arborescens	Gemmill *et al.* (in press)/GenBank	Rarotonga, Cook Islands	AF302025
P. hosmeri Rock	Gemmill *et al.* (in press)/GenBank	Hawai'i, Hawaiian Islands	AF302022
P. rhytidocarpum A. Gray	Gemmill *et al.* (in press)/GenBank	Lomilagi Mountain, Fiji	AF302029
P. tahitiense Putterl.	Gemmill *et al.* (in press)/GenBank	Mt. Oahia, Bora Bora, Society Islands	AF302027
P. yunkeri A.C. Smith	Gemmill *et al.* (in press)/GenBank	Eua, Kingdom of Tonga	AF302028
Australia			
P. moluccanum (Lam.) Miq.	Royal Botanical Gardens Sydney	Western Australia, Australia	RBGS940036
P. phylliraeoides DC.	Royal Botanical Gardens Sydney	Queensland, Australia	RBGS851162
P. undulatum Guill.	Gemmill *et al.* (in press)/GenBank	cultivlated, Australia	AF302014
Japan			
P. tobira Ait.	Missouri Botanical Gardens	unknown	MBG897147
Mauritius			
P. balfouri Cufod.	Gemmill *et al.* (in press)/GenBank	Mauritius	AF302015
Pittosporaceae			
Citriobatus spinescens (F.Muell.) Druce	Royal Botanical Gardens Sydney	Queensland, Australia	RBGS851161
Hymensporum flavum (Hook.) F. Muell.	Royal Botanical Gardens Sydney	New South Wales, Australia	RBGS861457
Sollya heterophylla Lind.	Missouri Botanical Gardens	unknown	MBG897138
Outgroup			
Pseudopanax discolor (Kirk) Harms	GenBank	cultivated	U63170

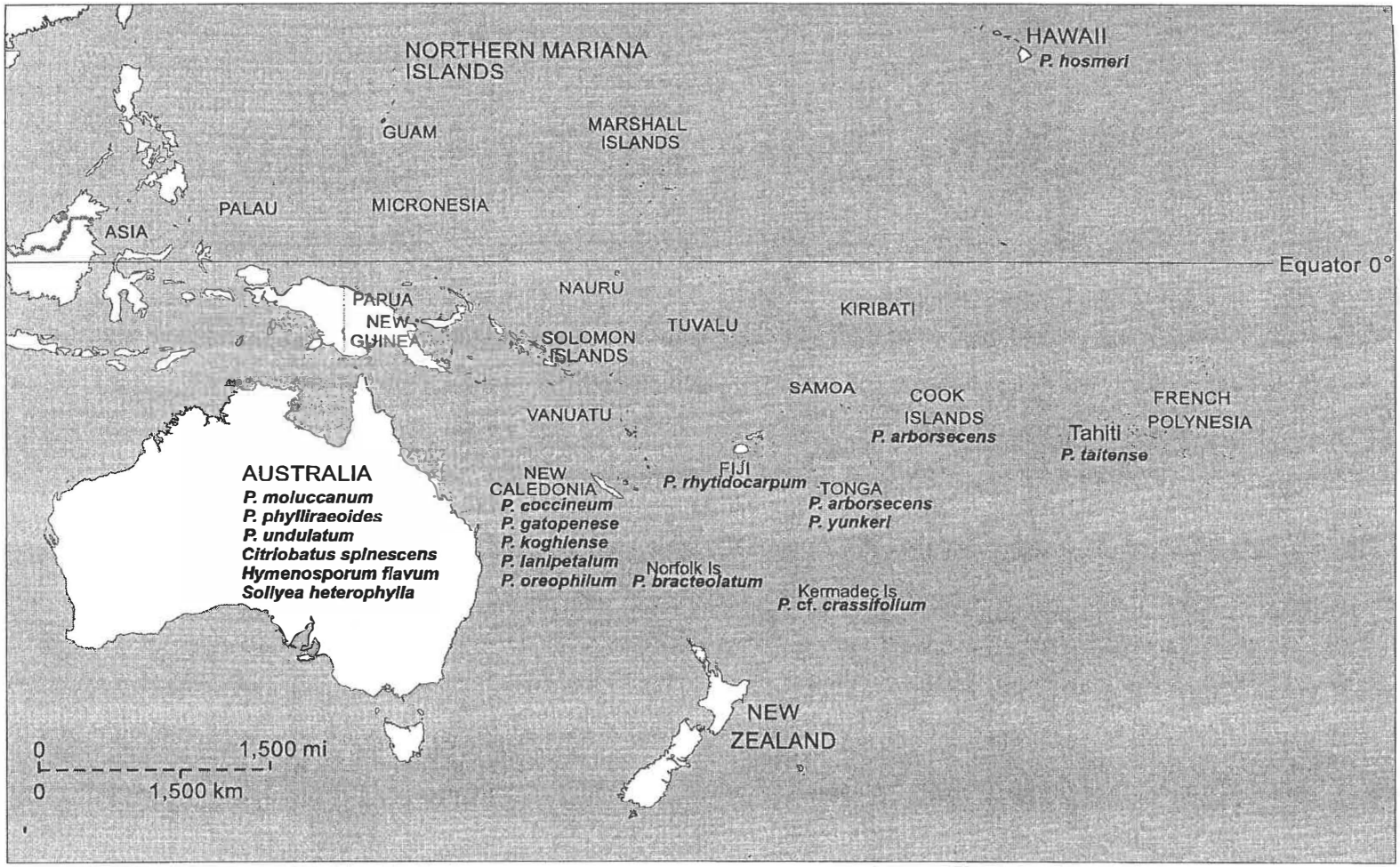


Figure 2.1. Map showing the localities of the non-New Zealand *Pittosporum* and Pittosporaceae taxa used in this study. Note that *P. balfouri* from the island of Mauritius of the eastern coast of Africa and *P. tobira* from Japan are not shown.

2.3 PCR AMPLIFICATION

The two ITS spacers, plus the intervening 5.8S gene (Figure 2.2), were amplified using the polymerase chain reaction (PCR) with primers ITS4 (White *et al.* 1990) and ITS5HP (Laboratory of Molecular Systematics, Smithisonian Institution (LMS)) (Table 2.2, Figure 2.2).

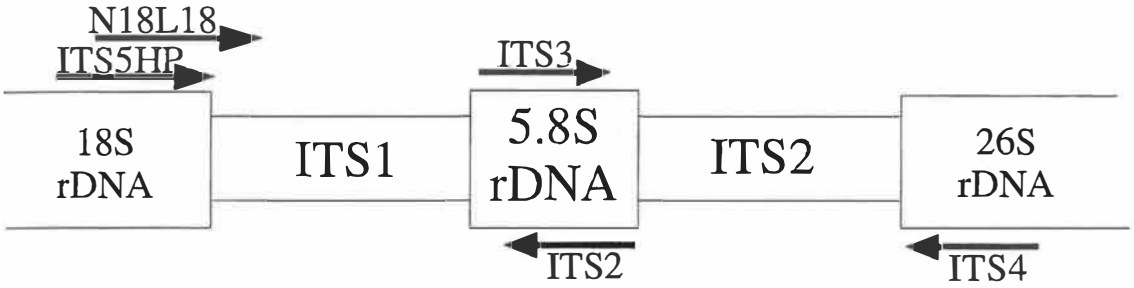


Figure 2.2. Diagrammatic representation showing the approximate positions of the primers used for PCR and sequencing.

Table 2.2. Primers used in this research. See Figure 2.2 for locations. ITS2, ITS3 and ITS4 are universal eukaryote primers from White *at al.* (1990), ITS5HP and N18L18 are higher plant specific primers from LMS.

ITS2 5' - GCT GCG TTC TTC ATC GAT GC - 3'
ITS3 5' - GCA TCG ATG AAG AAC GCA GC - 3'
ITS4 5' - TCC TCC GCT TAT TGA TAT GC - 3'
ITS5HP 5'- GGA AGG AGA AGT CGT AAC AAG G - 3'
N18L18 5'- AAG TCG TAA CAA GG - 3'

PCR was carried out in 100µL volumes and contained 43.5µL of sterile distilled water, 1X PCR buffer (50mM KCl, 10mM tris-HCl, pH 8.3), 250mM $MgCl_2$, 50µM ITS4, 50µM ITS5HP, 15mM dNTP's, 5% dimethylsulfoxide (DMSO), 2.5units of *Taq* DNA polymerase (Roche, Germany) and 4 µL of unquantified genomic DNA as template, with the stock DNA solution diluted if necessary. The dilution was done as the secondary compounds that were co-precipitating with the DNA prevented or inhibited its amplification. Diluting the stock DNA solution frequently overcame this problem and was done at either 1:10 or 1:100. The reactions were monitored by the inclusion of positive (sample that amplified well in a previous PCR) and negative (distilled water instead of DNA) controls. The PCR was performed in an Eppendorf Mastercycler Gradient thermal cycler (Eppendof, Germany) under the following conditions: an initial denaturation period of one cycle at 96^0C for 5 minutes, followed by 29 cycles of 95^0C for 30 seconds to denature the template DNA, 55^0C for 30 seconds to anneal primers to the single stranded template and 72^0C for 45 seconds for primer extension. This was followed by a final extension period of 72^0C for 10 minutes to allow for completion of polymerisation. The lid of the thermal cycler was heated to 105^0C during the PCR to prevent evaporation of the reactions.

To check the relative quantity and quality of the amplification products 5µl of each reaction, mixed with 2µl of gel loading buffer (0.0083% bromophenol blue, 2.5% ficol (MW 400 000), 5mM EDTA (disodium salt)), was electrophoresed on a 1.0% 1X TBE agarose (SeaKem LE) gel at 55 V for around 2 hours. The gels contained ethidium bromide (0.1mg/l) and the bands were visualised under UV light and photographed using an Eagle Eye II gel documentation system (Stratagene Inc., La Jolla). A 100 base pair ladder (3µl at a 1:10 dilution in TE buffer mixed with 2µl of 1X gel loading buffer) (New England Biolabs Inc., Massachusetts) was also run on the gel with the products. Successful PCR amplifications resulted in a single, bright band corresponding to about 750 base pairs. For those samples that did not amplify or amplified poorly, the reaction conditions were varied until an acceptable product was obtained.

Successful PCR amplifications where purified for sequencing using the QIAquick PCR purification kit (Qiagen Ltd., Australia) or the Concert PCR (Life Technologies) according to manufacturers directions or using a PEG precipitation protocol (Appendix 3).

2.4 DNA SEQUENCING

Proir sequencing the purified PCR products were quantified with a Hoefer DNA Fluorometer (Hoefer Scientific Instruments) to ensure the there was enough PCR product for the sequencing reaction to produce optimal results, at least 15ng/µl. Sequencing was performed by the University of Waikato DNA Sequencing Facility using the dideoxy termination method. Cycle sequencing reactions used BigDye Terminator chemistry® (ABI Prisim, Perkin Elmer Applied Biosystems, California), and included 1µl of 5% DMSO and initial denaturing period of 94°C for four minutes. Sequencing was performed twice for each individual, once with each of the ITS4 and ITS5HP primers to obtain sequence from both directions. This helped to ensure that unambiguous sequence information was obtained for the entire region by assisting in correct base calling. Some individuals were also sequenced using the more internal ITS2, ITS3 (White *et al.* 1990) and N18L18 (LMS) (Table 2.2; Figure 2.2) primers when ITS4 and ITS5HP did not give useable sequences. The sequencing reaction products were separated on a 4.5% polyacrylamide gel and analysed on an ABI 377 automated DNA sequencer (Perkin Elmer Applied Biosystems, California). Sequence data was collected in electropherograms both on hard copy printouts and electronically.

2.5 SEQUENCE ALIGENMENT

The sequences of the ITS region were edited and aligned manually using Sequencher 3.0 (Gene Codes Corporation). When more than one individual of a taxon was sequenced only a single sequence was used in further analysis if they were identical, however if they differed then both were used.

Two separate data matrices were produced, one containing only the New Zealand species, and the outgroup taxon *P. undulatum* (New Zealand only), and one containing the sequences of all the taxa, with *Pseudopanax discolor* designated as the outgroup (all taxa). The sequence matrices used in the analysis were truncated so all sequences started at the same position, and short sequences were made to the same length as the rest by inserting N's.

2.6 SEQUENCE CHARACTERISTICS

The number of nucleotides different between species and the uncorrected pairwise distance was calculated using PAUP* Version 4.0b3a (Swofford 1998). The g_1 statistic was calculated, using 10000 random trees, to investigate the level of phylogenetic signal in the data (Hillis and Huelsenbeck 1992) using PAUP*.

2.7 PHYLOGENETIC ANALYSES

Phylogenetic analyses were performed with PAUP* using maximum parsimony and neighbour joining.

2.7.1 Parsimony analysis

Maximum parsimony is one of the most widely used methods of inferring phylogenies, and selects for trees with minimal length, this being the lowest number of character state transitions required to explain the data (Quicke 1993; Swofford *et al.* 1996)

2.7.1.1 New Zealand only

Parsimony analysis of the New Zealand only matrix was performed using the heuristic search mode of PAUP*. The characters (i.e. nucleotides) were unordered and of equal weight, gaps were treated as missing data, with accelerated transformation (ACCTRAN) character state transformation, tree-bisection-reconnection (TBR) branch-swapping algorithm and simple addition.

2.7.1.2 All taxa

A second analysis was performed, using the all taxa matrix, to investigate the possible origins of the New Zealand species and to attempt to increase resolution within the New Zealand species. The same parameters were used as for the New Zealand only analysis.

Upon looking at the data matrix it appeared that many of the gaps inserted could be phylogenetically informative and tended to support groups found in the consensus trees. Subsequentially, an analysis was performed on the all taxa matrix, using the same parameters as the other analysis, except gaps were treated as a fifth base (gaps as fifth base). Although this did produce a significant reduction in the total number of trees obtained a relatively large number of trees was still found.

To investigate if the species with a large section of missing sequence were causing false patterns of relationships these species were removed from the all taxa matrix and an analysis performed using the same parameters as the initial analysis (minus poor sequences). Three New Zealand taxa were removed (*P. tenuifolium* subsp. *tenuifolium*, *P. crassifolium, P. patulum*) and one New Caledonian species (*P. oreophilum*).

It appeared that the New Zealand taxa may have been the cause of the large numbers of trees, especially since Gemmill *et al.* (in press) found only two most parsimonious trees. The only differences between this study and that of Gemmill *et al.* (in press) were the inclusion of a much larger number of the New Zealand species, two more Australian *Pittosporum,* a *Pittosporum* species from Japan and representatives of other genera in the Pittosporaceae, as well as the outgroup. To investigate the effect of the removal of the majority of the New Zealand taxa, almost all them were removed from the all taxa data set (removed New Zealand).

The selection of taxa to include was based on earlier analyses. Due to their obvious separation form the rest of the New Zealand species, *P. cornifolium, P. pimeleoides* subsp *pimeleoides, P. pimeleoides* subsp. *maius* were included. *Pittosporum dallii, P. kirkii* and *P. obcordatum* were included, as they appear to be the three most basal taxa. Also included were *P. tenuifolium* subsp. *colensoi, P. rigidum 2*, *P. umbellatum* and *P. eugenioides* as representatives of clades found within the main New Zealand clade in the other trees. This analysis was done using gaps as missing data with all other parameters the same as in other analyses.

100 bootstrap (BS) replicates where performed on the removed New Zealand data set to calculate relative support of the clades (Felsenstein 1985). This was done with this data set as due to both time and memory constraints this could only be done where a relatively low number of trees where found.

2.7.2 Neighbour joining

Neighbour joining is a distance based approach, conceptually relater to cluster analyses, where taxa are grouped according to overall similarity. It allows for unequal rates of change along branches (Avise 1994). The neighbour joining analysis was performed on the all taxa matrix.

2.8 CHARACTER MAPPING

Character mapping was done using MacClade Version 3.05 (Maddison and Maddison 1995), using either the entire tree obtained from analysis of the entire all taxa matrix with gaps as missing data, or the New Zealand species from this tree as this data set had more resolution than the one containing only the New Zealand species.

CHAPTER THREE
RESULTS

3.1 SPECIES REPRESENTATION

Of the endemic New Zealand taxa two of Cooper's (1956) species (*P. ellipticum* and *P. crassicuale*) were not included in the analysis, six of Allan's (1961; *P. ellipticum, P. crassicuale, P. lineare, P. buchananii, P. fasciculatum* and *P. intermedium*) and only one of Druce's (1980; *P. ellipticum*). Most of these were due to leaf material not being obtained. There was one species, *P. ellipticum,* which was collected however, repeated attempts at extraction and PCR failed.

Where more than one individual was sequenced for a species or subspecies the sequences were compared to see if they were identical. Only one New Zealand species, *P. rigidum,* was found to have differing sequences, with two individuals that differed from each other by one nucleotide. Both these individuals were therefore included in the analysis. *Pittosporum rigidum* from the North Island is called *P. rigidum 1*, while that from the South Island is called *P. rigidum 2.* Another species found to have different sequences was *P. arborescens*, with the individual from the Cook Islands *P. arborescens CI*, differing by seven bases from the one collected from Tonga, *P. arborescens T.*

There were several groups within the New Zealand species that had identical sequences: *P. tenuifolium* subsp. *tenuifolium, P. tenuifolium* subsp. *colensoi, P. crassifolium* and *P. huttonianum*; *P. rigidum 1*, *P. anomalum* and *P. turneri*; *P. cornifolium, P. pimeleoides* subsp. *pimeleoides* and *P. pimeleoides* subsp. *maius*.

3.2 SEQUENCE CHARACTERISTICS AND VARIATION

After truncating the sequences to the same length a matrix of 587 characters for the New Zealand only matrix and 604 characters for the all taxa matrix, including gaps to account for insertions or deletions (indels), was obtained for use in analysis (Appendix 4 and 5).

The two matrices contained most of ITS1 and ITS2 and the entire 5.8S gene. Based on the sequence of *Pseudopanax discolor*, which contained the full length of ITS1, ITS2 and the 5.8S gene, as well as the last 48 bases of the 18S gene and the first 58 of the 26S gene, the sequence of ITS1 used in this study starts 15 bases short and ITS2 is missing the last 27 bases (assuming no insertions or deletion events occurred in these regions).

3.2.1 INDELS

To align the New Zealand only sequence matrix a total of 27 gaps was required, 20 in ITS1 and 11 in ITS2. This involved the insertion of 17 indels, 6 in ITS1 and 11 in ITS2. Nine of these gaps, involving four indels, were needed only for the alignment of *P. undulatum*. Alignment of all the sequences required the insertion of a total of 95 gaps, 69 in ITS1 and 26 in ITS2, with a total of 37 indels, 18 in ITS1 and 19 in ITS2 (Table 3.1).

Most of the indels were one or two bases in length, 28 (69%) of these were single base indels, with nine of two bases. There were also two three-base indels, a 13 base pair deletion in *P. fairchildii* and a 35 base pair deletion shared by *P. rhytidocarpum* and *P. yunkeri*. Four of the single base indels and one three-base indels were within the 35 base deletion, and are not included in the total gap count.

Table 3.1. Indels needed for the aligenment of the taxa used in this study, for both the New Zealand only and all taxa matrices, informative sites highlighted in grey.

New Zealand only ITS1

size	position	characteristics
1	87	gap in *P. ralphii* , C in rest
	135	C in *P. tenuifolium, P. crassifolium, P. ralphii & P. huttonianum* gap in rest
	194	C in *P. kirkii* , gap in rest
	209	T in *P. tenuifolium, P. crassifolium, P. ralphii & P. huttonianum* gap in rest
3	207	CGG in *P. undulatum* , rest 3 gaps
13	92	*P. fairchildii* "deletion"

number of gaps: 20, 2 informative
number of indels: 6, 2 informative

New Zealand only ITS2

size	position	characteristics
1	392	G in *P.eugenioides* , A in *P. umbellatum* , gap in in rest
	396	gap *P. umbellatum,* C in rest
	404	C in *P. virgatum, P. fairchildii & P. dallii* , gap
	405	C in *P. dallii* , gap in rest
	412	gap in *P. umbellatum,* T in rest
	427	T in *P. kirkii* and *P. undulatum*
	452	gap in *P. undulatum, P. obcordatum, P. kirkii & P. dallii*
	461	T in *P.* cf. *crassifolium,* gap in rest
2	428	TG in *P. undulatum* , gap in rest
	422	CC in *P. undulatum* , gap in rest
	430	CA in *P. undulatum* , gap in rest
3	453	GGG in *P. eugenioides* , gap in rest

number of gaps:17, 2 informative
number of indels: 11, 2 informative

All taxa ITS1

size	position	characteristics
1	49	G in *Pseudopanax discolor* , gap in rest
	78	gap in *P. hosmeri, P. yunkeri & P. rhytidocarpum* *
	80	A in *Pseudopanax discolor,* gap in rest
	90	gap in *P. ralphii* , C in rest
	110	C in *C. spinescens* , gap in rest
	130	C in *Pseudopanx discolor* , gap in rest
	140	C in *P. tenuifolium, P. huttonianum P. crassifolium, P. ralphii & P. koghiense* , gap in rest
	162	gap in *P. tobira & P. balfouri* , A in rest
	180	gap in *Pseudopanax discolor* , T or G in rest#
	183	gap in *Pseudopanax discolor* , C in rest#
	190	gap in *P. coccineum* , C in rest#
	215	T in *P. tenuifolium, P. huttonianum P. crassifolium & P. ralphii* , gap in rest*#
	227	A in *P. moluccanum, C. spinescens & S. heterophylla* , gap in rest
2	36	gap in *Pseudopanax discolor* , A in rest
3	209	CGG in *P. gatopenese, P. oreophilum & P. undulatum* , AGG in *Pseudopanax discolor,* rest 3 gaps*#
	206	gap in *P. phylliraeodies* #
13	85	*P. fairchildii* "deletion"
35	182	*P. yunkeri & P. rhytidocarpum* "deletion"

number of gaps: 69, 43 informative
number of indels: 18, 7 informative

All taxa ITS2

size	position	characteristics
1	400	G in *P. eugenioides* , A in *P. umbellatum* gap in rest
	410	gap in *P. phylliraeoides* , C in rest
	411	gap in *P. bracteolatum, P. tobira, P. balfouri, P. phylliraeoides & H. flavum* , C in rest
	412	C in *P. dallii, P. fairchildii & P. virgatum* , gap in rest
	413	C in *P. dallii* , gap in rest
	419	gap in *P. undulatum*
	424	gap in *Pseudopanax discolo* r, A in rest
	443	gap in *Pseudopanax discolor* , T in rest
	431	gap in *H.flavum* & *S. heterophylla* , T in rest
	436	T in *P. undulatum P. ki* *seudopanax discolor* gap in rest
	450	gap in *Pseudopanax discolor* , G in rest
	460	*P. obcordatum, P. lanipetalum, P. oreophilum, P. balfouri, H. flavum & S. heterophylla* P. oreophilum & P. moluccanum G or A in rest*
	461	G in *P. eugenioides, P. bracteolatum, P. tobira,* gap in rest
2	428	TG in *P. undulatum* , gap in rest
	433	CC in *P. undulatum* , gap in rest
	431	CA in *P. undulatum* , gap in rest
	462	GG in *P. eugenioides* , gap in rest
	495	CG in *P. tobira* , gap in rest
3	509	AAA in *P. gatopenese* , gap in rest

number of gaps: 26, 6 informative
number of indels: 19, 6 informative

total number of gaps inserted in New Zealand only: 27, 4 informative
total number of indels inserted in New Zealand only: 17, 4 informative

total number of gaps in all taxa matrix: 95, 49 informative
total number of indels in all taxa matrix: 37, 13

*gaps/indels at a site which is informative when gaps treated
gaps/indels within 35 base deletion

3.2.2 Sequence length

Before truncation the length of the ITS1 spacer in the New Zealand taxa had an approximate range in length, based on the sequences of those in which the entire length of both spacers was obtained and *Pseudopanax discolor*, from 205 to 220 nucleotides, with an average length of 218. ITS2 was on average longer in length than ITS1, with an average of 231, which was found in the majority of species, and was also less variable in length than ITS1 with a range from 230 to 235. The 5.8S gene had a uniform length across all taxa of 163 nucleotides. The length of the two spacers combined with the 5.8S gene ranged from 600 to 616, with an average of 612 (Table 3.2).

The average length of ITS1 for all taxa used in this analysis was 217 nucleotides, with a range in length from 186 to 222. The average length of ITS2 for all taxa was also 231 nucleotides, which was still found in the majority of species, with a range in lengths from 228 to 237. The 5.8S gene had a length of 163 nucleotides in all taxa used. The length of the two spacers combined with the 5.8S gene ranged from 580 to 621, with an average of 611.

Pittosporum fairchildii which was 205 nucleotides in length had the shortest ITS1 sequence within the New Zealand species, while the shortest sequence in ITS1 was in both *P. rhytidocarpum* and *P. yunkeri* which were 186. All three of these species are characterised by large deletions. The longest ITS1 sequence in the New Zealand taxa at 220 was found in *P. tenuifolium* subsp. *tenuifolium, P. tenuifolium* subsp. *colensoi, P. huttonianum* and *P. crassifolium,* with the longest ITS1 sequence overall in *Sollya heterophylla* which had 222. The shortest sequence in ITS2 for the New Zealand taxa was *P. obcordatum* at 230, while 228 was the shortest overall, found in *Hymenosporum flavum* and *Pseudopanax discolor.* The longest ITS2 sequence in the New Zealand species was in *P. eugenioides* with 237 and *P. undulatum* (having the longest overall with 237.

Table 3.2. The length in nucleotides of the two spacers, the 5.8S gene and the entire ITS region for the taxa used in this study. Note that the first 15 bases of ITS1 and the last 27 ITS2 were not used in the analysis. Some of these are also estimated and are based on the those species which contained the entire length of both spacers and Pseudopanax discolor.

Taxon	ITS1	5.8S	ITS2	Entire Region
New Zealand				
P. anomalum	218	163	231	612
P. cf. *crassifolium*	220	163	231	614
P. cornifolium	218	163	231	612
P. crassifolium	218	163	231	612
P. dallii G42	218	163	232	613
P. divaricatum	218	163	231	612
P. eugeniodies	218	163	235	616
P. fairchildii	205	163	232	600
P. huttonianum	220	163	231	614
P. kirkii	218	163	231	612
P. obcordatum	218	163	230	611
P. patulum	218	163	231	612
P. pimeleoides subsp. *maius*	218	163	231	612
P. pimeleoidies subsp. *pimeleoides*	218	163	231	612
P. ralphii	219	163	231	613
P. rigidum 1	218	163	231	612
P. rigidum 2	218	163	231	612
P. tenuifolium subsp. *colensoi*	220	163	231	614
P. tenuifolium subsp. *tenuifolium*	220	163	231	614
P. turneri	218	163	231	612
P. umbellatum	218	163	231	612
P. virgatum	218	163	232	613
New Caledonia				
P. coccineum	220	163	233	616
P. gatopenese	217	163	231	611
P. koghienese	219	163	231	613
P. lanipetalum	218	163	230	611
P. oreophilum	221	163	232	616
Pacific Islands				
P. arborescens CI	218	163	231	612
P. arborescens T	218	163	231	612
P. hosmeri	217	163	231	611
P. rhytidocarpum	186	163	231	580
P. tahitiense	218	163	231	612
P. yunkeri	186	163	231	580
Norfolk Island				
P. bracteolatum	218	163	231	612
Japan				
P. tobira	216	163	233	612
Mauritius				
P. balfouri	217	163	229	609
Australia				
P. moluccanum	219	163	232	614
P. phylliraeoides	215	163	230	608
P. undulatum	221	163	237	621
Pittosporaceae				
Citriobatus spinescens	221	163	231	615
Hymenosporum falvum	218	163	228	609
Sollya heterophylla	222	163	229	614
Outgroup				
Pseudopanax discolor	216	163	228	607
average	217	163	231	611
minimun	186	163	228	580
maximum	222	163	237	621

3.2.3 GC content

There was a GC bias in the nucleotide content of the sequences, especially in the two spacers. The ITS1 GC content for the New Zealand species only had a mean of 64.3%, with a range from 60.1% to 69.5%. ITS2 had a slightly lower GC content, with a mean of 63.1% and a range from 57.8% to 65.5%. The GC content of the 5.8S gene had only a slight bias, with a mean of 54.3% and a range from 53.8 to 54.6% (Table 3.3).

When looking at the GC content across all taxa included ITS1 had a mean of 63.3%, with a range from 60.1% to 69.5%. ITS2 had a slightly lower GC content, with a mean of 61.9% and a range from 55.9% to 68.7%. The GC content of the 5.8S gene was only slightly biased, with a mean of 54.0% and a range of 52.1% to 55.2%. (Table 3.3).

Pittosporum cornifolium, P. pimeleoides subsp. *pimeleoides* and *P. pimeleoides* subsp. *maius* had the lowest GC content for ITS1 across all species, and the lowest GC content for ITS2 and the 5.8S gene for the New Zealand species. The lowest GC content in ITS2 was found in *P. hosmeri,* with *P. yunkeri* having the lowest in the 5.8S gene. *P. dallii* had the highest GC content in the two spacers within the New Zealand species. *Hymenosporum flavum* had the highest GC content in ITS1 and ITS2, while *Sollya heterophylla* had the highest in the 5.8S gene. *Sollya heterophylla* had the second highest GC content in the two spacers.

There was no correlation with GC content and spacer length, except in the three taxa that had the large deletions - *P. fairchildii, P. rhytidocarpum* and *P. yunkeri.* These taxa had GC contents in the ITS1 spacer and over the entire ITS region, that were lower than those found in the species they were found in the phylogenetic analysis to be most closely related to. The GC content of *P. fairchildii* was 63.2%, while the rest of the New Zealand species (not including *P. cornifolium* and the two *P. pimeleoides* taxa as they are not at all closely related to the rest of the New Zealand species) were between 64.2% and 66.5%. *Pittosporum rhytidocarpum* and *P. yunkeri* had GC contents of 56.7% and 56.5% respectively, while *P. hosmeri* was 60.8%.

Table 3.3. Percent of nucleotides which were either a G or a C in the two spacer regions, the 5.8S gene and across the entire region used in the analysis. for the taxa used in this study.

Taxon	ITS1	5.8S	ITS2	Entire Region
New Zealand				
P. anomalum	65.5	54.6	64.2	61.9
P. cf. *crassifolium*	64.5	54.6	64.2	61.6
P. cornifolium	60.1	52.8	57.8	57.2
P. crassifolium	64.4	54.6	63.7	60.1
P. dalli	66.5	54.6	64.9	62.9
P. divaricatum	64.9	54.6	64.5	61.8
P. eugeniodies	65.5	54.6	62.0	61.2
P. fairchildii	63.2	54.6	63.9	60.9
P. huttonianum	64.4	54.6	63.7	61.4
P. kirkii	65.5	54.6	61.7	61.2
P. obcordatum	65.5	54.6	65.5	61.7
P. patulum	65.5	54.6	64.2	60.8
P. pimeleoides subsp. *maius*	60.1	52.8	57.8	57.2
P. pimeleoidies subsp. *pimeleoides*	60.1	52.8	57.8	57.2
P. ralphii	64.2	54.6	63.7	61.3
P. rigidum 1	65.5	54.6	64.2	61.9
P. rigidum 2	65.5	54.6	63.7	61.8
P. tenuifolium subsp. *colensoi*	64.4	54.6	63.7	61.4
P. tenuifolium subsp. *tenuifolium*	64.4	54.6	63.7	61.2
P. turneri	65.5	54.6	64.2	61.9
P. umbellatum	65.0	54.0	64.2	61.4
P. virgatum	64.5	54.6	63.9	61.5
New Caledonia				
P. coccineum	62.4	53.4	61.3	59.4
P. gatopenese	62.9	54.0	59.2	59.1
P. koghienese	63.2	53.4	60.8	59.5
P. lanipetalum	65.5	54.0	61.1	60.6
P. oreophilum	58.8	54.0	57.4	57.4
Pacific Islands				
P. arborescens CI	59.9	54.0	60.1	58.2
P. arborescens T	60.6	54.0	59.3	58.2
P. hosmeri	60.8	52.8	55.9	56.7
P. rhytidocarpum	56.7	52.8	59.9	55.6
P. tahitiense	59.6	52.8	59.3	57.6
P. yunkeri	56.5	52.1	59.9	55.3
Norfolk Island				
P. bracteolatum	65.0	54.0	60.1	60.1
Japan				
P. tobira	63.2	53.4	59.7	59.1
Mauritius				
P. balfouri	63.1	54.6	59.4	59.1
Australia				
P. moluccanum	58.2	54.6	59.0	57.4
P. phylliraeoides	62.5	54.0	60.6	59.4
P. undulatum	65.0	54.6	61.4	60.8
Pittosporaceae				
Citriobatus spinescens	62.8	52.8	61.3	59.4
Hymenosporum falvum	69.5	54.6	68.7	64.9
Sollya heterophylla	67.8	55.2	68.3	64.4
Outgroup				
Pseudopanax discolor	62.4	54.0	62.6	60.1
mean	63.3	54.0	61.9	60.0
min	56.5	52.1	55.9	55.3
max	69.5	55.2	68.7	64.9

3.2.4 Sequence variation between species

Pairwise sequence divergence within the New Zealand species had an average of 3.1% (Table 3.4), with an average absolute distance, i.e. the number if bases that differ between two taxa, of 17.3 bases (Table 3.5). The range was between 0.0 and 9.3%, up to 53 bases different, which was the distance between *P. umbellatum* and *P. cornifolium*/the two *P. pimeleoides* taxa. The pairwise distance between these three taxa and the rest of the New Zealand species was between 6.8% and 9.3% or 34 to 53 bases, with an average distance of 8.3% or 47.3 bases. Excluding these three taxa lowered the average pairwise distance to 1.4%, a total distance of 7.8 bases and the maximum to 3.9% or 22 bases, the distance between *P. kirkii* and *P. umbellatum*.

The pairwise divergence of between all taxa used in the analysis had an average of 7.1%, (Table 3.4) with an average base difference of 39.6 (Table 3.5) and a maximum of 19.6% or 109 bases separating *Pseudopanax discolor* and *P. hosmeri*. Excluding *Pseudopanax discolor* lowered the average to 6.6% or 37 bases and the maximum pairwise divergence to 13.1%, the distance between *P. rhytidocarpum* and *P. cornifolium*/the two *P. pimeleoides* taxa and *P rhytidocarpum* and *P tobira*. The largest total distance was 72 bases, but was found between different taxa *P. hosmeri* and *P. tobira*. Excluding all non *Pittosporum* taxa lowered the average to 6.3%, an average of 35.2 bases. The maximum pairwise and total differences were still 13.1% and 72 bases, between the same groups of taxa as before the exclusion of the non *Pittosporum* taxa (Table 3.6).

Despite the relatively large pairwise and total base distance of *P. cornifolium* and the two *P. pimeleoides* taxa from the New Zealand species these distances were lower than those to all other taxa except for *P. lanipetalum* from New Caledonia. This species had a pairwise distance of 8.6% from these three taxa, which is within the range of that found for the New Zealand species. No species, or group of species, could be identified as having a lower sequence divergence than the rest relative to the New Zealand species.

Table 3.4. Pairwise sequence divergences for the ITS sequence region used in this analysis, see table 3.2 for distributions.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1 *P. anomalum*																						
2 *P. ~~cornifolium~~*	.082																					
3 *P. crassifolium*	.008	.068																				
4 *P. cf. crassifolium*	.004	.086	.010																			
5 *P. dallii*	.016	.084	.019	.019																		
6 *P. divaricatum*	.005	.077	.014	.009	.018																	
7 *P. eugenioides*	.014	.086	.024	.018	.026	.016				New Zealand												
8 *P. fairchildii*	.007	.088	.012	.005	.020	.013	.021															
9 *P. huttonianum*	.009	.084	.000	.012	.025	.014	.023	.016														
10 *P. kirkii*	.028	.086	.024	.032	.020	.030	.038	.029	.032													
11 *P. obcordatum*	.012	.084	.012	.016	.018	.018	.026	.016	.014	.025												
12 *P. patulum*	.004	.073	.012	.008	.014	.010	.020	.008	.012	.026	.012											
13 *P. ralphii*	.012	.088	.002	.016	.025	.018	.026	.016	.004	.032	.018	.014										
14 *P. rigidum 1*	.000	.082	.008	.004	.016	.005	.014	.007	.009	.028	.012	.004	.012									
15 *P. rigidum 2*	.002	.084	.010	.005	.018	.007	.016	.009	.011	.030	.014	.006	.014	.002								
16 *P. pimeleoides* subsp. *maius*	.082	.000	.068	.086	.084	.077	.086	.088	.084	.086	.084	.073	.088	.082	.084							
17 *P. pimeleoides* subsp. *pimeleoides*	.082	.000	.068	.086	.084	.077	.086	.088	.084	.086	.084	.073	.088	.082	.084	.000						
18 *P. tenuifolium* subsp. *colensoi*	.009	.084	.000	.012	.025	.014	.023	.016	.000	.032	.014	.012	.004	.009	.011	.084	.084					
19 *P. tenuifolium* subsp. *tenuifolium*	.008	.077	.000	.012	.023	.013	.023	.014	.000	.031	.012	.012	.004	.008	.010	.077	.077	.000				
20 *P. turneri*	.000	.082	.008	.004	.016	.005	.014	.007	.009	.028	.012	.004	.012	.000	.002	.082	.082	.009	.008			
21 *P. umbellatum*	.011	.093	.020	.011	.026	.016	.025	.014	.019	.039	.023	.016	.023	.011	.012	.093	.093	.019	.019	.011		
22 *P. virgatum*	.007	.088	.014	.007	.023	.012	.018	.011	.016	.035	.019	.010	.019	.007	.009	.088	.088	.016	.015	.007	.014	
23 *P. coccineum*	.062	.102	.053	.065	.056	.062	.072	.068	.065	.065	.062	.053	.069	.062	.063	.102	.102	.065	.056	.062	.072	.069
24 *P. gatopense*	.062	.100	.055	.065	.057	.062	.072	.066	.065	.063	.051	.054	.069	.062	.060	.100	.100	.065	.062	.062	.067	.069
25 *P. koghienese*	.053	.100	.052	.056	.047	.054	.063	.059	.061	.060	.054	.047	.065	.053	.054	.100	.100	.061	.054	.053	.063	.060
26 *P. lanipetalum*	.044	.086	.042	.047	.039	.044	.054	.052	.053	.055	.046	.033	.056	.044	.046	.086	.086	.053	.044	.044	.051	.051
27 *P. oreophilium*	.078	.108	.067	.082	.075	.080	.083	.087	.080	.085	.078	.066	.083	.078	.078	.108	.108	.080	.073	.078	.087	.083
28 *P. arborescens* Cl	.076	.109	.067	.078	.074	.072	.084	.079	.081	.083	.081	.062	.085	.076	.078	.109	.109	.081	.076	.076	.083	.081
29 *P. arborescens* T	.068	.100	.056	.070	.063	.065	.077	.071	.074	.072	.070	.051	.077	.068	.070	.100	.100	.074	.065	.068	.076	.074
30 *P. hosmeri*	.085	.122	.076	.088	.080	.080	.095	.090	.083	.085	.081	.075	.087	.085	.083	.122	.122	.083	.077	.085	.090	.092
31 *P. rhytidocarpum*	.087	.131	.073	.088	.084	.081	.098	.090	.088	.091	.085	.065	.092	.087	.085	.131	.131	.088	.076	.087	.091	.092
32 *P. tahitiense*	.072	.106	.059	.074	.067	.069	.081	.075	.074	.074	.071	.058	.078	.072	.074	.106	.106	.074	.066	.072	.080	.078
33 *P. yunckeri*	.085	.129	.077	.087	.082	.080	.096	.089	.087	.089	.083	.069	.091	.085	.083	.129	.129	.087	.081	.085	.089	.089
34 *P. tobira*	.077	.123	.071	.077	.065	.079	.077	.082	.083	.081	.076	.071	.086	.077	.079	.123	.123	.083	.077	.077	.083	.081
35 *P. balfouri*	.072	.116	.059	.072	.060	.074	.074	.077	.074	.074	.071	.064	.078	.072	.074	.116	.116	.074	.068	.072	.076	.076
36 *P. bracteolatum*	.053	.104	.048	.056	.048	.055	.042	.056	.062	.060	.055	.046	.065	.053	.055	.104	.104	.062	.052	.053	.063	.056
37 *P. moluccanum*	.077	.116	.053	.081	.079	.074	.075	.084	.076	.079	.079	.058	.079	.077	.079	.116	.116	.076	.062	.077	.086	.084
38 *P. phylliraeoides*	.078	.122	.066	.080	.071	.076	.081	.085	.082	.083	.078	.069	.085	.078	.080	.122	.122	.082	.074	.078	.087	.083
39 *P. undulatum*	.042	.100	.035	.046	.037	.044	.049	.050	.051	.049	.044	.030	.055	.042	.044	.100	.100	.051	.041	.042	.051	.049
40 *Citriobatus spinescens*	.075	.117	.064	.079	.067	.072	.082	.078	.077	.074	.072	.071	.081	.075	.077	.117	.117	.077	.071	.075	.084	.082
41 *Hymenosporum flavum*	.057	.109	.052	.060	.044	.058	.063	.060	.062	.057	.055	.049	.062	.057	.058	.109	.109	.062	.057	.057	.067	.064
42 *Sollya heterophylla*	.076	.123	.068	.079	.067	.074	.082	.079	.078	.081	.069	.068	.082	.076	.078	.123	.123	.078	.071	.076	.083	.083
43 *Pesudopanax discolor*	.158	.191	.126	.162	.153	.155	.158	.158	.160	.159	.155	.131	.161	.158	.160	.191	.191	.160	.145	.158	.165	.164

Table 3.4. continued

	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
23 *P. coccineum*																					
24 *P. gatopense*	.078																				
25 *P. koghienese*	.062	.077	New Caledonia																		
26 *P. lanipetalum*	.053	.067	.026																		
27 *P. oreophilium*	.089	.101	.062	.053																	
28 *P. arborescens CI*	.102	.097	.093	.081	.109																
29 *P. arborescens T*	.091	.086	.082	.070	.099	.012															
30 *P. hosmeri*	.099	.080	.087	.081	.119	.094	.087	Pacific Islands													
31 *P. rhytidocarpum*	.107	.083	.086	.081	.117	.088	.080	.009													
32 *P. tahitiense*	.099	.092	.086	.078	.100	.019	.007	.091	.084												
33 *P. yunckeri*	.101	.081	.085	.079	.121	.089	.081	.013	.008	.085											
34 *P. tobira*	.095	.092	.086	.081	.114	.106	.095	.128	.131	.097	.126										
35 *P. balfouri*	.094	.088	.081	.079	.109	.094	.083	.117	.117	.087	.115	.051									
36 *P. bracteolatum*	.086	.083	.074	.070	.099	.092	.086	.111	.117	.092	.115	.086	.085								
37 *P. moluccanum*	.100	.106	.093	.084	.103	.076	.065	.096	.097	.062	.100	.090	.081	.091							
38 *P. phylliraeoides*	.099	.101	.092	.083	.114	.094	.083	.121	.122	.087	.121	.096	.076	.092	.090	Australia					
39 *P. undulatum*	.067	.062	.054	.049	.083	.076	.065	.101	.105	.073	.104	.065	.058	.058	.078	.073					
40 *Citriobatus spinescens*	.095	.100	.091	.082	.111	.102	.091	.109	.101	.095	.096	.088	.088	.093	.097	.092	.075				
41 *Hymenosporum flavum*	.087	.081	.079	.071	.107	.108	.097	.114	.119	.099	.118	.094	.088	.081	.104	.094	.067	.093			
42 *Sollya heterophylla*	.106	.094	.093	.078	.119	.118	.109	.115	.123	.110	.122	.102	.099	.101	.114	.103	.086	.098	.051		
43 *Pesudopanax discolor*	.180	.186	.180	.169	.188	.168	.157	.196	.191	.158	.192	.175	.168	.175	.176	.171	.162	.176	.157	.157	

Table 3.5. Number of bases different between taxa over the region used for this study, see table 3.2 for distributions

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1 *P. anomalum*																						
2 *P. cornifolium*	47																					
3 *P. crassifolium*	4	34																				
4 *P. cf. crassifolium*	2	49	5																			
5 *P. dallii*	9	48	9	11																		
6 *P. divaricatum*	3	44	7	5	10																	
7 *P. eugenioides*	8	49	12	10	15	9																
8 *P. fairchildii*	4	49	6	3	11	7	12															
9 *P. huttonianum*	5	48	0	7	14	8	13	9														
10 *P. kirkii*	16	49	12	18	11	17	22	16	18													
11 *P. obcordatum*	7	48	6	9	10	10	15	9	8	14												
P. patulum	2	37	6	4	7	5	10	4	6	13	6											
13 *P. ralphii*	7	50	1	9	14	10	15	9	2	18	10	7										
14 *P. rigidum 1*	0	47	4	2	9	3	8	4	5	16	7	2	7									
15 *P. rigidum 2*	1	48	5	3	10	4	9	5	6	17	8	3	8	1								
16 *P. pimeleoides* subsp. *maius*	47	0	34	49	48	44	49	49	48	49	48	37	50	47	48							
17 *P. pimeleoides* subsp. *pimeleoides*	47	0	34	49	48	44	49	49	48	49	48	37	50	47	48	0						
18 *P. tenuifolium* subsp. *colensoi*	5	48	0	7	14	8	13	9	0	18	8	6	2	5	6	48	48					
19 *P. tenuifolium* subsp. *tenuifolium*	4	40	0	6	12	7	12	7	0	16	6	6	2	4	5	40	40	0				
20 *P. turneri*	0	47	4	2	9	3	8	4	5	16	7	2	7	0	1	47	47	5	4			
21 *P. umbellatum*	6	53	10	6	15	9	14	8	11	22	13	8	13	6	7	53	53	11	10	6		
22 *P. virgatum*	4	50	7	4	13	7	10	6	9	20	11	5	11	4	5	50	50	9	8	4	8	
23 *P. coccineum*	35	58	26	37	32	35	41	38	37	37	35	27	39	35	36	58	58	37	29	35	41	39
24 *P. gatopense*	35	57	27	37	32	35	41	37	37	36	29	27	39	35	34	57	57	37	32	35	38	39
25 *P. koghienese*	30	57	26	32	27	31	36	33	35	34	31	24	37	30	31	57	57	35	28	30	36	34
26 *P. lanipetalum*	25	49	21	27	22	25	31	29	30	31	26	17	32	25	26	49	49	30	23	25	29	29
27 *P. oreophilium*	44	61	33	46	42	45	47	48	45	48	44	33	47	44	44	61	61	45	38	44	49	47
28 *P. arborescens CI*	43	62	33	44	42	41	48	44	46	47	46	31	48	43	44	62	62	46	39	43	47	46
29 *P. arborescens T*	39	57	28	40	36	37	44	40	42	41	40	26	44	39	40	57	57	42	34	39	43	42
30 *P. hosmeri*	48	69	38	50	45	45	54	50	47	48	46	38	49	48	47	69	69	47	40	48	51	52
31 *P. rhytidocarpum*	46	70	34	47	44	43	52	47	47	48	45	31	49	46	45	70	70	47	37	46	48	49
32 *P. tahitiense*	41	60	29	42	38	39	46	42	42	42	40	29	44	41	42	60	60	42	34	41	45	44
33 *P. yunckeri*	45	69	36	46	43	42	51	46	46	47	44	33	48	45	44	69	69	46	39	45	47	47
34 *P. tobira*	44	70	35	44	37	45	44	46	47	46	43	36	49	44	45	70	70	47	40	44	47	46
35 *P. balfouri*	41	66	29	41	34	42	42	43	42	42	40	32	44	41	42	66	66	42	35	41	43	43
36 *P. bracteolatum*	30	59	24	32	27	31	24	31	35	34	31	23	37	30	31	59	59	35	27	30	36	32
37 *P. moluccanum*	44	66	26	46	45	42	43	47	43	45	45	29	45	44	45	66	66	43	32	44	49	48
38 *P. phylliraeoides*	44	69	32	45	40	43	46	47	46	47	44	34	48	44	45	69	69	46	38	44	49	47
39 *P. undulatum*	24	57	17	26	21	25	28	28	29	28	25	15	31	24	25	57	57	29	21	24	29	28
40 *Citriobatus spinescens*	43	67	32	45	38	41	47	44	44	42	41	36	46	43	44	67	67	44	37	43	48	47
41 *Hymenosporum flavum*	32	62	25	34	25	33	36	33	35	32	31	24	35	32	33	62	62	35	29	32	38	36
42 *Sollya heterophylla*	43	70	33	45	38	42	47	44	44	46	39	34	46	43	44	70	70	44	37	43	47	47
43 *Pesudopanax discolor*	89	107	61	91	86	87	89	87	90	89	87	65	90	89	90	107	107	90	74	89	93	92

New Zealand

Table 3.5 continued.

	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
23 *P. coccineum*																					
24 *P. gatopense*	44																				
25 *P. koghienese*	35	44																			
26 *P. lanipetalum*	30	38	15																		
27 *P. oreophilium*	50	57	35	30																	
28 *P. arborescens CI*	58	55	53	45	61																
29 *P. arborescens T*	52	49	47	40	56	7															
30 *P. hosmeri*	56	45	49	46	67	53	49														
31 *P. rhytidocarpum*	57	44	46	43	62	47	43	5													
32 *P. tahitiense*	56	52	49	44	56	11	4	51	45												
33 *P. yunckeri*	54	43	45	42	64	47	43	7	4	45											
34 *P. tobira*	54	52	49	46	64	60	54	72	70	55	67										
35 *P. balfouri*	53	50	46	45	61	53	47	66	62	49	61	29									
36 *P. bracteolatum*	49	47	42	40	56	52	49	63	62	52	61	49	48								
37 *P. moluccanum*	57	60	53	48	58	43	37	54	52	35	53	51	46	52							
38 *P. phylliraeoides*	56	57	52	47	64	53	47	68	65	49	64	54	43	52	51						
39 *P. undulatum*	38	35	31	28	47	43	37	57	56	41	55	37	33	33	44	41					
40 *Citriobatus spinescens*	54	57	52	47	63	58	52	62	54	54	51	50	50	53	55	52	43				
41 *Hymenosporum flavum*	49	46	45	40	60	61	55	64	63	56	62	53	50	46	59	53	38	53			
42 *Sollya heterophylla*	60	53	53	44	67	67	62	65	65	62	64	58	56	57	65	58	49	56	29		
43 *Pesudopanax discolor*	101	104	101	95	104	94	88	109	101	88	101	98	94	98	99	95	91	99	88	88	

New Caledonia

Pacific Islands

Australia

3.3 SEQUENCE VARIATION IN THE DATA MATRICES

3.3.1 New Zealand only

When only the New Zealand taxa were used (along with *P. undulatum* as the outgroup) 495 (84.3%) of the characters were constant and non-informative. Of the variable characters 37 (6.3%) were parsimony uninformative, and 55 (9.4%) parsimony informative. Table 3.6 shows the informative sites. Most of the variation was still in ITS1, which contained 52.7% of the informative characters, with ITS2 having 40 and the 5.8S gene only 7.3%.

3.3.2 All taxa

When gaps were treated as missing data when all taxa where included 355 (58.8%) of the characters were constant and non-informative. Of the variable characters 102 (16.9%) were autapomorphies, found in only one taxon, and therefore parsimony uninformative, and 147 (24.3%) synapomorphies, with at least two different nucleotide states in two or more sequences and were then parsimony informative. Most of the variation was in ITS1, which contained 51.7% of the informative characters, with ITS2 having 42.8% and the 5.8S gene only 5.5%.

Treating gaps as a fifth base for the all taxa matrix increased the amount of characters that were parsimony informative to 180 (29.8%), and 47 of the 91 (51.6%) gaps were parsimony informative (Table 3.3). Treating gaps as a 5th base in the New Zealand only matrix increased the number of being variable and parsimony informative sites to 61 (10.4%), with 5 of the 32 (15.6%) gaps being parsimony informative (Table 3.3).

3.3.2 g_1 Statistic

The g_1 value for the New Zealand only matrix was –2.461, while the value for the matrix including all taxa was –0.946.

Table 3.6. Variable and informative sites for the New Zealand only matrix. Numbers indicate the position of the informative site in the ITS region as aligned using the sequence of *Pseudopanax discolor*. Sites marked with an "*" are those which are informative when the sequences *P. cornifolium* and the two *P. pimeleoides* taxa are removed.

Taxon	Sequence
Region	ITS1 (sites 25–203) → 5.8S (224–400) ← ITS2 (412–599)
Site	25 27 44 48 50 55 57 63 67 68 73 78 81 84 89 106 107 112 114 128 141 146 164 170 172 181 190 197 203 224 321 360 387 400 412 439 446 447 451 475 480 502 507 513 549 554 564 565 566 575 579 584 592 594 599
Informative (*)	44 * 50 * 78 * 84 * 106 * 128 * 146 * 181 * 190 * 197 * 203 * 451 * 475 * 575 * 584 *
P. anomalum	TCCCGGCTAGCCCTCTGCGACCCCCTCGTCCCCCCTGGAGGGCGCAGCGCCCCTC
P. cornifolium	CACTAAGCCATCTGTTATACTCATTTTGTTTTTAAGTAGAGAAATCTTCTTTTCA
P. crassifolium	NNNNNNNNNNNNNNNTGCGACTCCCGTGTCCCCCCTGGAGGGCGCAGCGCCTCTC
P. cf. *crassifolium*	TCCCGGCTAGCCCTCTGCGACCCCCTCTTCCCCCCTGGAGGGCGCAGCGCCCCTC
P. dalli	CCCCGGCTAGCTCGCCGCGCCCCCCTCGCCCCCCCTGGGGGGCGCAGCGCCCCTC
P. divaricatum	TCCCGGCTAGCCCTCTGCGACCCTCTCGTCCCCCCTGGGGGGCGCAGCCCCCCTC
P. eugeniodies	TCCCGGCTAGCCCTCTGCGACCCCCTCGTCCCCCCTGAGTAGCGCAGCGCCCATC
P. fairchildii	TCCCGGCTAGCTCTCTGCGACCCCCTCTTCCCCCCTGGAGGGCGCAGCGTCCCTC
P. huttonianum	TCTCGGCTAGCCCTCTGCGACTCCCGTGTCCCCCCTGGAGGGCGCAGCGCCTCTC
P. kirkii	CCCCGGCTAGCTTGCCGCGCCACCCTCGCCCCCCCTGGGGGGCGCAGCGTCTCTC
P. obcordatum	CCTCGGCTAGCCCTCTGCGCCTCCCTCGCCCCCCCTGGAGGGCGCAGCGTCCCTC
P. patulum	NNNNNNNNNNNNNNCTGCGACCCCCTCGTCCCCCCTGGAGGGCGCAGCGCCCCTC
P. pimeleoides subsp. *maius*	CACTAAGCCATCTGTTATACTCATTTTGTTTTTAAGTAGAGAAATCTTCTTTTCA
P. pimeleoides subsp. *pimeleoides*	CACTAAGCCATCTGTTATACTCATTTTGTTTTTAAGTAGAGAAATCTTCTTTTCA
P. ralphii	TCTCGGCTAGCTCTCTGCGACTCCCGTGTCCCCCCTGGAGGGCGCAGCGCCTCTC
P. rigidum 1	TCCCGGCTAGCCCTCTGCGACCCCCTCGTCCCCCCTGGAGGGCGCAGCGCCCCTC
P. rigidum 2	TCCCGGCTAGCCCTCTGCGACCCCCTCGTCCCCCCTGGAGGGCGCAGCGCCCCTC
P. tenuifolium subsp. *colensoi*	NNNNNNTAGCCCTCTGCGACTCCCGTGTCCCCCCTGGAGGGCGCAGCGCCTCTC
P. tenuifolium subsp *tenuifolium*	TCTCGGCTAGCCCTCTGCGACTCCCGTGTCCCCCCTGGAGGGCGCAGCGCCTCTC
P. turneri	TCCCGGCTAGCCCTCTGCGACCCCCTCGTCCCCCCTGGAGGGCGCAGCGCCCCTC
P. umbellatum	TCCCGGCTAGCCCTCTGCGACCCCCTCTTCCCCCCTGGAGCGCGCAGCGCCCCTC
P. virgatum	TCCCGGCTAGCCCWCTGCGACCCCCTCTTCCCCCCTGGATGGCGCAGCGCCCCTC
P. undulatum	CACCGGTCAGCCCGCCGCGCCCCCCTCGCCCCCCCCGGGGAGCGCAGCGCCCCTC

3.4 PARSIMONY ANALYSIS

3.4.1 New Zealand only

Analysis of only the New Zealand only matrix found 23800 most parsimonious trees before the program ran out of memory. The length of these trees was 117 steps, with a consistency index (CI) of 0.838, a retention index (RI) of 0.877 and a rescaled consistency index (RC) of 0.735. One randomly chosen most parsimonious tree is shown in Figure 3.1. The 50% majority rule tree, showing relationships found in 50% or more of the most parsimonious trees is shown in Figure 3.2.

Pittosporum cornifolium, P. pimeleoides subsp. *pimeleoides* and *P. pimeleoides* subsp. *maius*, which all had identical sequences, form a clade with a branch of 43 bases leading to it. This difference is much longer than any changes within the rest of the New Zealand species, which in this tree from a clade, and the branch leading to *P. undulatum.* Most of the other branches are between zero to three bases in length, with a range from between zero to nine bases in the clade containing the rest of the New Zealand species (Figure 3.1).

The clade of *P. cornifolium, P. pimeleoides* subsp. *pimeleoides* and *P. pimeleoides* subsp. *maius* found in all of the equally parsimonious trees is unresolved in its placement in the 50% majority rule tree, while the rest of the New Zealand taxa form a clade in 99% of the trees (Figure 3.2). In 99% of the most parsimonious trees *P. dallii* and *P. kirkii* are sister taxa, a clade which in 99% of trees was a sister group to the rest of the New Zealand taxa, which formed a monophyletic group in all trees. *Pittosporum obcordatum* was the sister taxa to a clade of the rest of the New Zealand taxa in 99% of the trees. In this clade *Pittosporum anomalum, P. patulum, P. rigidum 2* and *P. turneri* where unresolved in their placement. *Pittosporum divaricatum* and *P. eugenioides* formed a clade in 96% of the most parsimonious trees. In 99% of the most parsimonious trees *P. fairchildii, P. virgatum, P. umbellatum* and *P.* cf. *crassifolium* formed a clade, within which 81% of the most parsimonious trees had *P fairchildii* as the sister to the other three taxa. *P. tenuifolium* subsp. *tenuifolium, Pittosporum tenuifolium* subsp. *colensoi, P. crassifolium* and *P. huttonianum* and *P. ralphii* formed a clade in all trees. The other groupings found in these trees were found in less than 80% of the equally parsimonious trees and therefore very weakly supported.

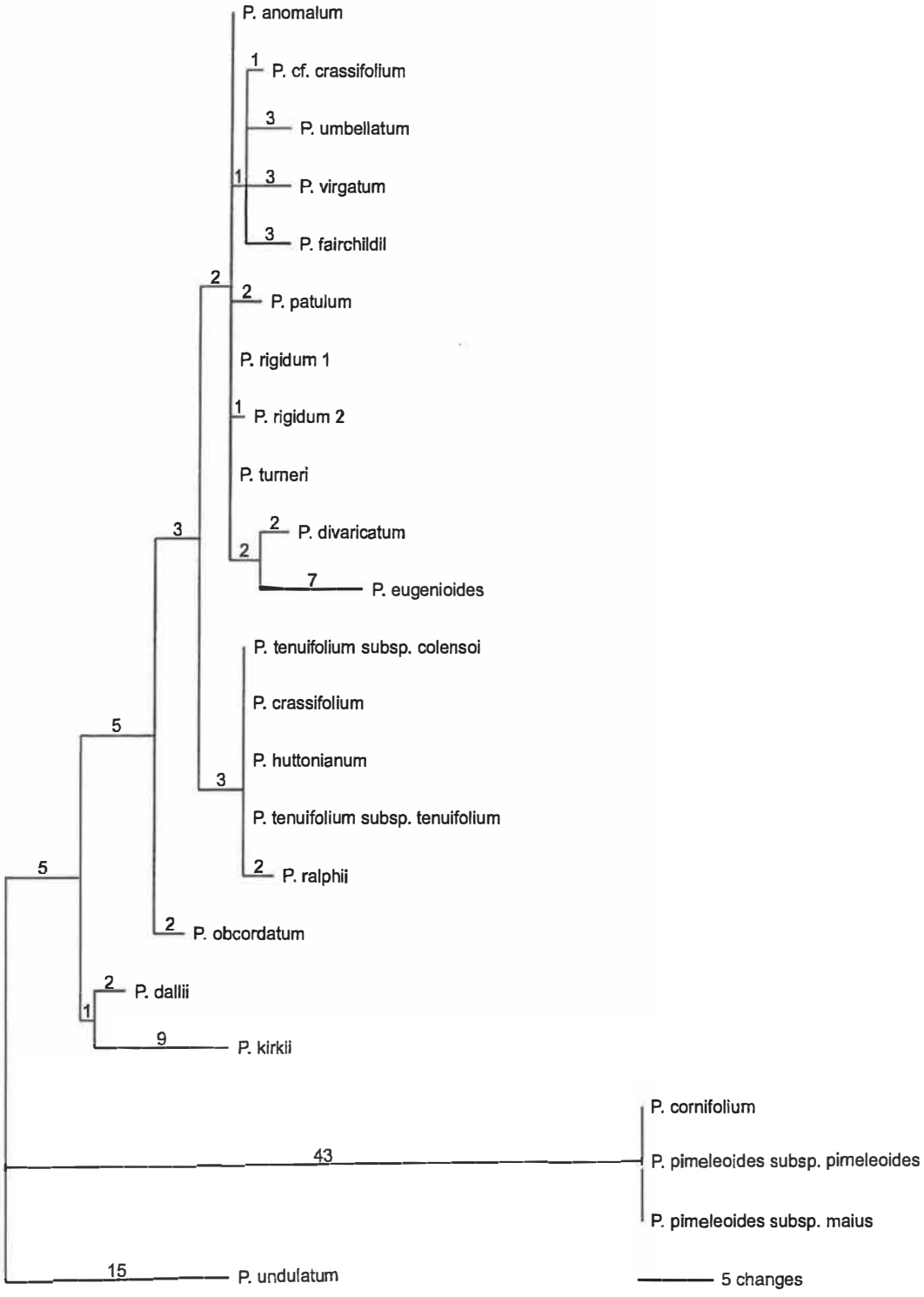


Figure 3.1. One if the 23800 most parsimonious trees obtained from analysis of the ITS region using the New Zealand only matrix. Tree length = 117 steps, CI = 0.838, RC = 0.735. Numbers above the branches represent branch lengths which correspond to the number of nucleotide changes along the branch.

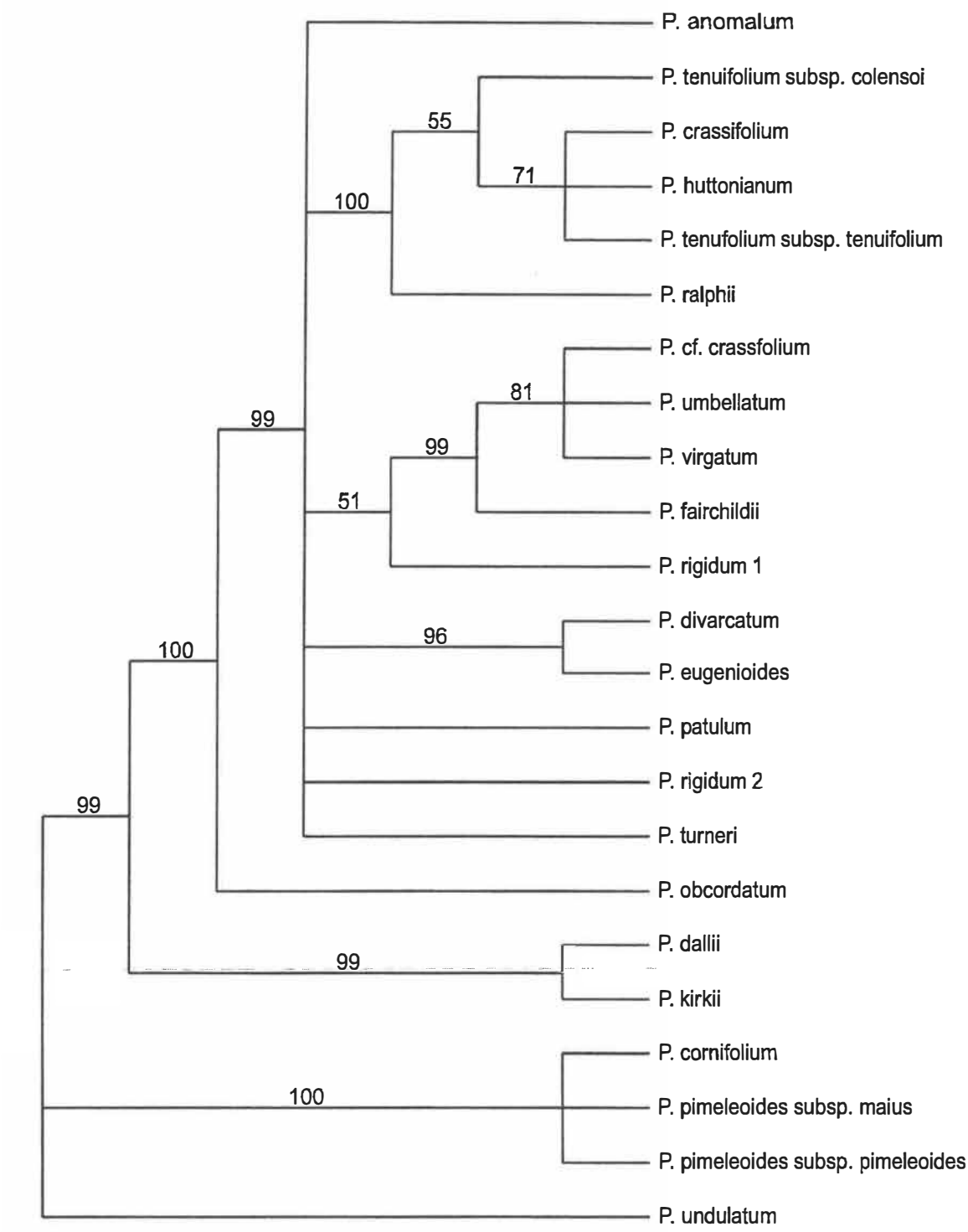


Figure 3.2. The 50% majority rule tree of the 23800 most parsimonious trees obtained from parsimony analysis of the New Zealand only matrix.

3.4.2 All taxa

Parsimony analysis of the entire region using gaps as missing data gave 15100 equally parsimonious trees before the program ran out of memory. The length of these trees was 557 steps, with a CI of 0.609, a RI of 0.694 and a RC of 0.422. One randomly chosen most parsimonious tree is shown in Figure 3.3. Figure 3.4 shows the 50% majority rule tree.

The branch lengths found in the most parsimonious tree shown in Figure 3.3 for the New Zealand species in the main clade are mostly between zero and three bases, with a range from zero to eight. One exception to the short branch lengths in this clade is the branch of 23 changes leading to *P. bracteolatum.* The lengths of the branches in the main New Zealand clade are relatively short compared to many found in the rest of the tree. Short branch lengths are found within the two groups of Pacific Island species, which are found on the end of relatively long branches. This is also found in the three New Zealand taxa with identical sequences which do not group with the rest of the New Zealand species - *P. cornifolium, P. pimeleoides* subsp. *pimeleoides* and *P. pimeleoides* subsp. *maius.*

In all of the most parsimonious trees the New Zealand species were not monophyletic and formed two distinct clades (Figure 3.4). These were a group of three taxa, *P. cornifolium, P. pimeleoides* subsp. *pimeleoides* and *P. pimeleoides* subsp. *maius*, and a clade containing the rest of the New Zealand species. Within this large New Zealand group *P. dallii* came out as being the sister group to the rest of the species in all most parsimonious trees, while *P. kirkii* was the second most basal species, followed by *P. obcordatum. Pittosporum tenuifolium* subsp. *tenuifolium, Pittosporum tenuifolium* subsp. *colensoi, P. crassifolium* and *P. huttonianum* and *P. ralphii* always formed a momophyletic group, which was the sister group to the rest of the species. *Pittosporum anomalum, P. patulum, P. rididum 1, P. rigidum 2* and *P. turneri* were unresolved. A clade of *P.* cf. *crassifolium, P. fairchildii, P umbellatum* and *P. virgatum* was found in all trees, within which *P.* cf. *crassifolium* and *P. fairchildii* formed a clade in 80% if the most parsimonious trees as did *P. umbellatum* and *P. virgatum. Pittosporum eugenioides* and *P. bracteolatum* from Norfolk Island, where sister taxa and had *P. divaricatum* as their sister taxon in all of the most parsimonious trees.

The main New Zealand clade appears to form a sister group to that containing all the other *Pittosporum* species included in the analysis in all of the most parsimonious trees (Figure 3.4). Within this group *P. cornifolium, P. pimeleoides* subsp. *pimeleoides* and *P. pimeleoides* subsp. *maius* had a clade of *P. gatopenese, P. hosmeri, P. rhytidocarpum* and *P. yunkeri* as its sister group, with a clade of four species from New Caledonia, *P. coccineum, P. koghienese, P. lanipeatalum* and *P. oreophilum* as the sister group to them. This clade is the sister group to a clade containing the rest of the *Pittosporum* species used in the analysis, which also contained *Citriobatus*. Within this clade *P. undulatum* was the sister taxon to a clade of *P. tobira* and *P. balfouri*, with a clade containing *Citriobatus spinescens* , *P. phylliraeoides* and *P. moluccanum, P. tahitiense* and the two *P. arborescens* individuals also found in all trees. *Hymenosporum falvum* is the sister taxa to the two clades within the *Pittosporum* in 99% of the most parsimonious trees, while *Soylla heterophylla* was unresolved.

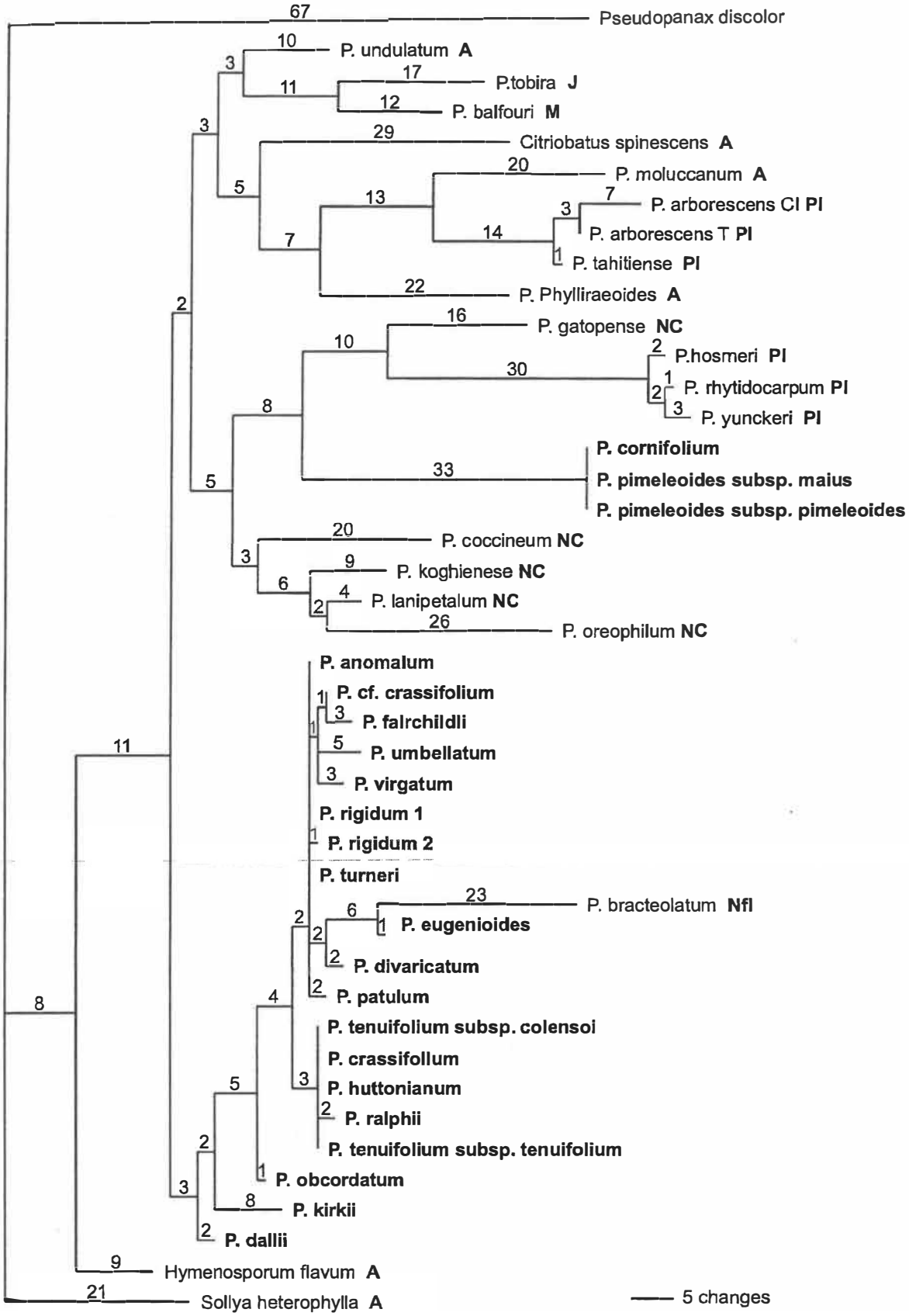


Figure 3.3. One of the 15100 most parsimonious trees obtained from analysis of the all taxa data matrix. Tree length = 577 steps, CI = 0.609, RC = 0.422. Numbers above the branches as in Figure 3.1. New Zealand species shown in bold. Distribution abbreviations as follows: A, Australia; NC, New Caledonia; NfI, Norfolk Island; PI, Pacific Islands; M, Mauritius; J, Japan.

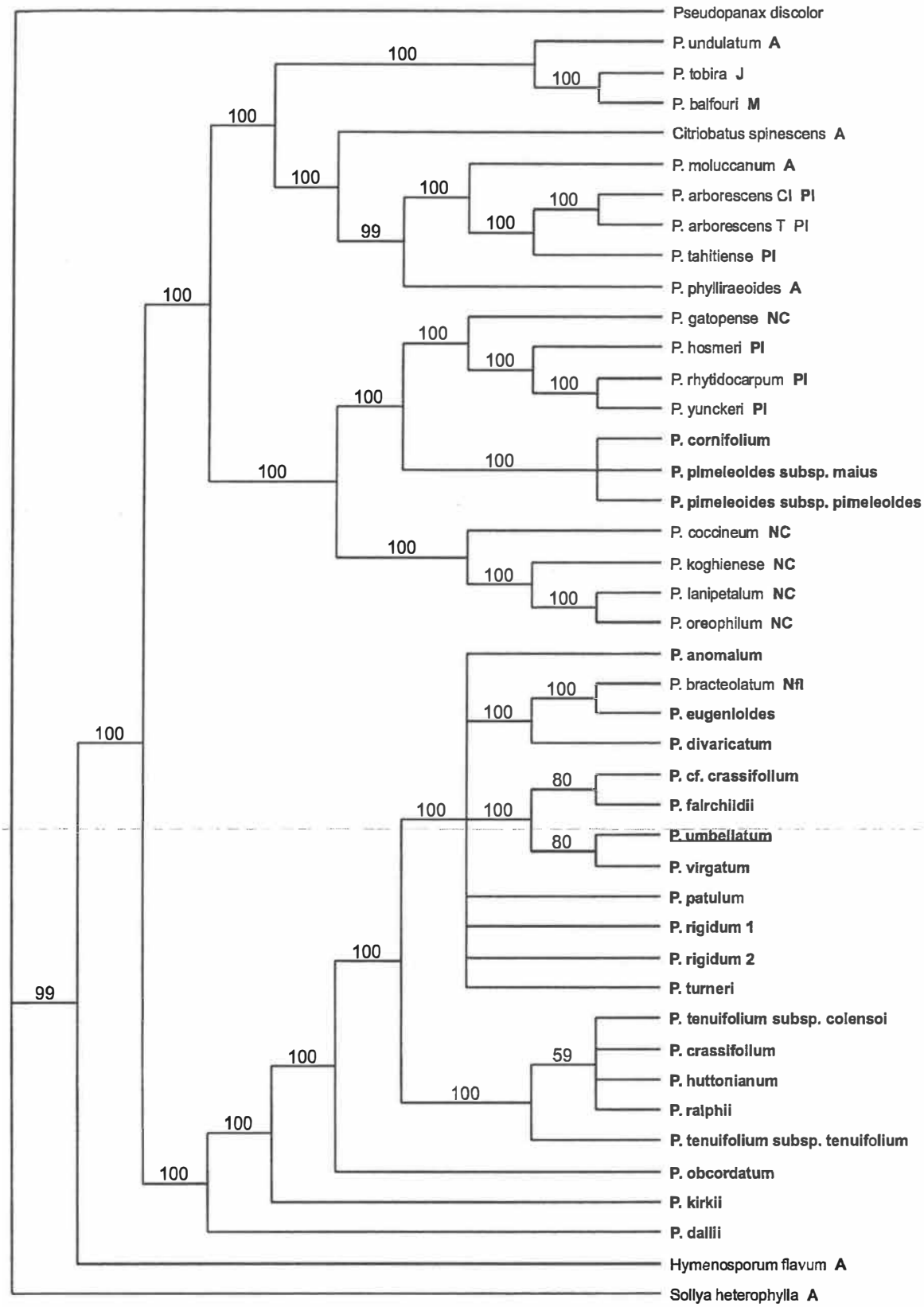


Figure 3.4. 50% majority rule tree of the 15100 most parsimonious trees obtained from analysis of the all taxa matrix. Numbers above the branches as in Figure 3.2, New Zealand taxa shown in bold, abbreviations as in Figure 3.3.

3.4.2.1 Gaps as a fifth base

When gaps were treated as a fifth base 896 equally parsimonious trees were obtained. The length of these trees was 689 steps, with a CI of 0.633, a RI of 0.682 and a RC of 0.431. Figure 3.5 shows the 50% majority rule tree.

Treating gaps as a fifth base consistently resulted in fewer equally parsimonious trees. It also resulted in a slight increase in the CI and RC. The same basic relationships and topologies were obtained in both the gaps as missing data and gaps as 5th base trees.

The New Zealand species formed two distinct clades, one of *P cornifolium* and the two *P pimeleoides* taxa, and one containing the rest of the species (main New Zealand clade). *Pittosporum dallii* and *P. kirkii* were both placed as sister taxa to the rest of the New Zealand taxa in the main New Zealand clade, however, they neither formed a clade as in the New Zealand only analysis (Figure 3.2), nor did *P. dallii* come out as more basal as it did in the all taxa gaps as missing data analysis (Figure 3.4). *Pittosporum obcordatum* was the sister group to the rest of the species. *Pittosporum anomalum, P. patulum, P. rididum 1, P. rigidum 2* and *P. turneri* were unresolved. *Pittosporum tenuifolium* subsp. *tenuifolium, Pittosporum tenuifolium* subsp. *colensoi, P. crassifolium*, *P. huttonianum* and *P. ralphii* formed a clade in all trees. *Pittosporum* cf. *crassifolium, P. fairchildii, P umbellatum* and *P. virgatum* also formed a monophyletic group, with *P. fairchildii* and *P. virgatum* forming a clade within this group in all trees. *Pittosporum eugenioides* and *P. bracteolatum* were sister taxa in all trees, and although relatively weakly supported, *P divaricatum* was the sister taxa to these to species in 76% if the equally parsimonious trees.

Pittosporum cornifolium, P. pimeleoides subsp. *pimeleoides* and *P. pimeleoides* subsp. *maius* had *P. gatopense, P. hosmeri, P. rhytidocarpum* and *P. yunkeri* as a sister group and a clade of four species from New Caledonia, *P. coccineum, P. koghienese, P. lanipeatalum* and *P. oreophilum* as the sister group to them. Although relatively weakly supported, as it was found in only 76% of the trees, the main New Zealand group and the clade containing the other three New Zealand taxa were sister groups.

Citriobatus spinescens was unresolved in its placement, with all the other taxa forming a clade in all trees. Within this, in all of the most parsimonious trees, *P. moluccanum, P. phylliraeoides, P. tahitiense* and the two *P. arborescens* taxa formed a clade, while the rest of the *Pittosporum* species, along with *Hymenosporum flavum* and *Sollya heterophylla*, also forming a monophyletic group. *Pittosporum undulatum, P. tobira* and *P. balfouri* formed the basal clade within this group in all trees, with the clade of *Hymenosporum flavum* and *Sollya heterophylla* forming a sister group to the clade containing the New Zealand species in 76% of the trees.

3.4.2.2 Removing taxa with bad sequences

Parsimony analysis with the four taxa which had bad sequences, using gaps as missing data gave 15100 trees before the program ran out of memory. The length of these trees was 529 steps, with a CI of 0.618, a RI of 0.697 and a RC of 0.422.

The relationships obtained in this analysis were exactly the same as those in the analysis including all taxa, with gaps as missing data.

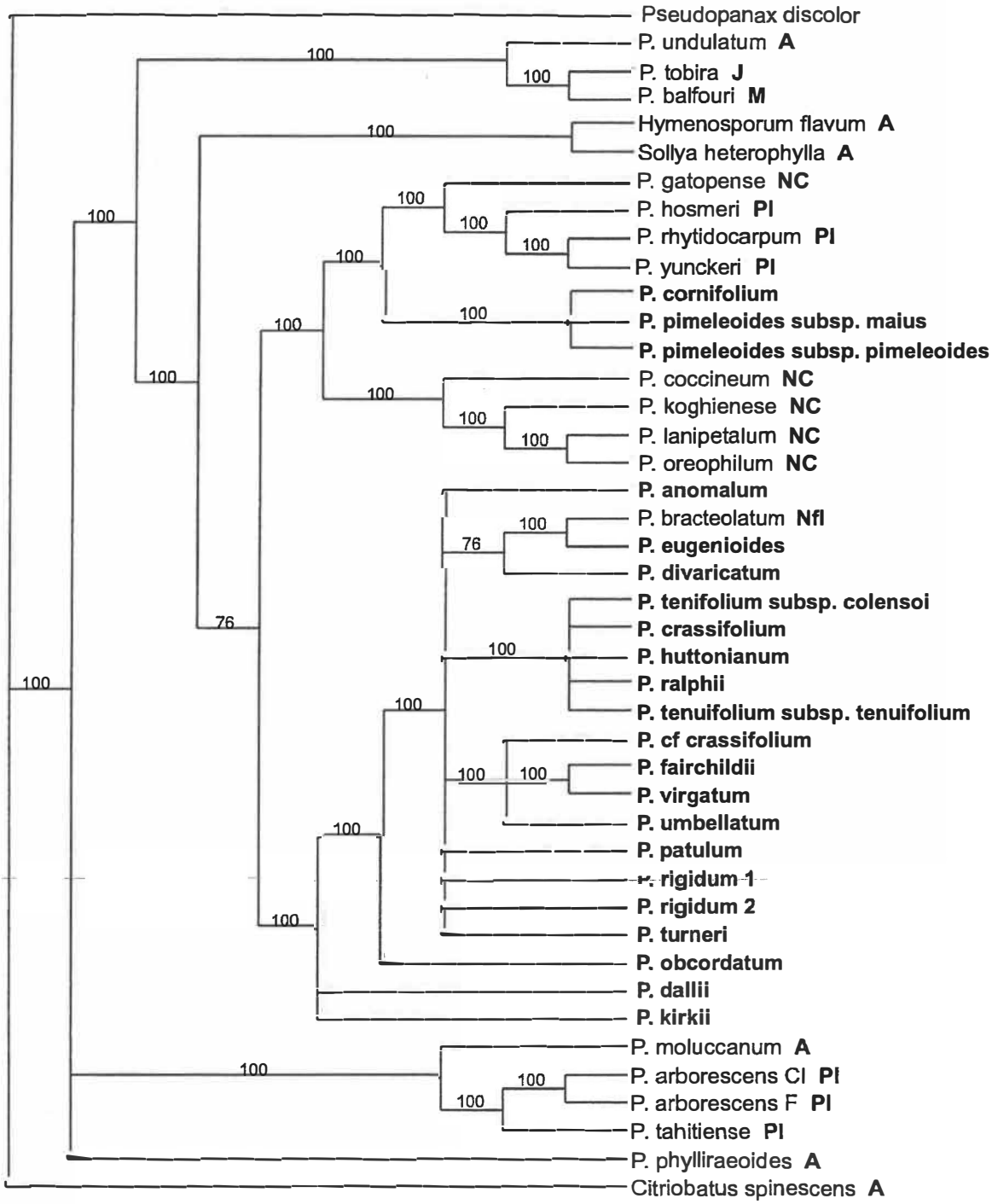


Figure 3.5. The 50% majority rule tree of the 896 most parsimonious trees obtained from analysis of the all taxa matrix using gaps as a fifth base. Tree length = 689, CI = 0.624, RI = 0.682, RC = 0.431. Numbers above the branches as in Figure 3.2, New Zealand taxa shown in bold, distribution abbreviations as in Figure 3.3.

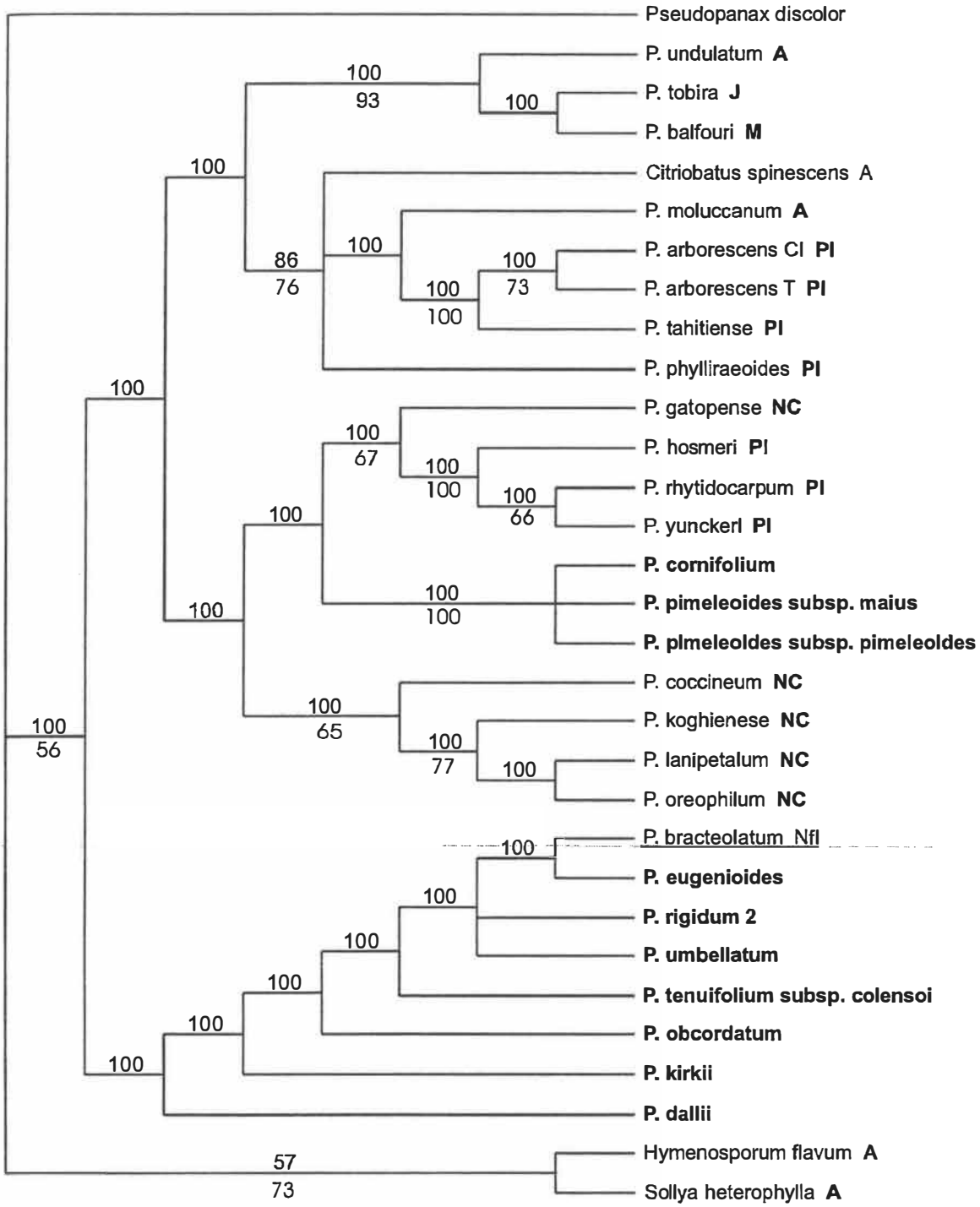
3.4.2.3 Removing New Zealand taxa

Removing many of the New Zealand species from the big data matrix resulted in a dramatic decrease in the number of most parsimonious trees obtained. Only 21 most parsimonious trees were found, with a length of 544 steps and a CI of 0.614 a RI of 0.660 and a RC of 0.405. The strict consensus and 50% majority rule trees are shown in Figure 3.6.

The relationships of the New Zealand species, in the main clade, have the same pattern in this analysis as in the all taxa analysis, with gaps as missing data. *Pittosporum dallii* is the most basal taxa, followed by *P. kirkii*, then *P. obcordatum*. *Pittosporum tenuifolium* subsp. *colensoi* is the sister group to a clade containg *P. rigidum 2* and *P. umbellatum* and a clade of *P. eugenioides* and *P. bracteolatum*. The relationships of the non New Zealand species are identical to those in the all taxa analysis, with gaps as missing data.

3.4.2.4 Bootstrap

The bootstrap numbers are mapped onto the trees in Figure 3.7. There was no bootstrap support for any grouping of species for the species in the main New Zealand clade used in this analysis. The clade of *P. cornifolium, P. pimeleoides* subsp. *pimeleoides* and *P. pimeleoides* subsp. *maius* had 100% bootstrap support, due to their sequences being identical. Other clades with 100% bootstrap support are the clade of *P. hosmeri, P. yunkeri* and *P. rhytidocarpum,* and one of *P. tahitiense*, and the two *P. arborescens* taxa. Other groups with relatively high bootstrap support are the clade of *P. koghienese, P. lanipetalum* and *P. oreophilum* (77%), *P. moluccanum* as the sister group to *P. tahitiense* and the two *P. arborescens* taxa (76%) and the clade of the two *P. arborescens* taxa (73%). Slightly lower bootstrap support was found for *P. gatopenese* as the sister group to the clade of *P. hosmeri, P. yunkeri* and *P. rhytidocarpum* (67%), the clade of *P. ynukeri* and *P. rhytidocarpum* (66%) and *P. coccineum* as the sister taxa to the clade of *P. koghienese, P. lanipetalum* and *P. oreophilum* (65%), and the clade of all the *Pittosporum* species, including *Citriobatus spinescens* (56%). Only one clade not found in all of the most parsimonious trees had bootstrap support. *Hymenosporum flavum* and *Sollya heterophylla* formed a clade in 57% of the most parsimonious trees, a grouping that had a bootstrap of 73% (Figure 3.7B).



igure 3.6. The 50% majority rule tree obtained from analysis of the all taxa matrix with less New Zealand taxa. Tree length = 544, CI = 0.502, RI = 0.660, RC = 0.405. Numbers above the branches as in Figure 3.2, numbers below the branches are bootstrap values, indicating the percentage occurrence of each clade in 100 replicates. New Zealand taxa shown in bold, distribution abbreviations as in Figure 3.3.

3.5 NEIGHBOUR JOINING

The relationships obtained from the neighbour joining analysis very similar to those obtained from the parsimony analysis. Ties were encountered during the, which resulted in more than one possible tree. Comparison of trees form several analyses indicates that the differences are not in topology but slight differences in branch lengths. Figure 3.8 shows one of the trees found.

The New Zealand species formed two distinct clades, with the main clade having the same relationships within it as found in the parsimony analyses. The main New Zealand group has a clade *P. bracteolatum* and *P. undulatum* as its sister group. *Pittosporum cornifolium* and the two *P. pimeleoides* taxa have the clade of the four New Caledonian species as their sister group, with *P. gatopenese, P. hosmeri, P. rhytidocarpum* and *P. yunkeri* as the sister group to these two clades. The main New Zealand clade and the clade containing *P. cornifolium* and *P. pimeleoides* are sister groups, which have the rest of the *Pittosporum* species as their sister group. The clade of the two *P. arborescens* taxa and *P. yunkeri* has *P. moluccanum* as their sister taxon. *Pittosporum balfouri* and *P. tobira* are sister taxa and have *P. phylliraeoides* as their sister taxon, with this group having *Citriobatus spinescens* as the sister taxon. *Hymenosporum flavum* and *Sollya heterophylla* form a clade that is unresolved in its placement.

Figure 3.7. A tree obtained from neighbour joining analysis of the all taxa data matrix. Branch lengths are a representation of the sequence distances between species. New Zealand taxa shown in bold, distribution abbreviations as in Figure 3.3.

CHAPTER FOUR
DISCUSSION

4.1 SAMPLE REPRESENTATION

Some of the New Zealand taxa could not included in this study. This was due to not being able to either acquire leaf material, extract DNA or to obtain a suitable PCR product. Most of taxa not included were varieties, i.e. *P. virgatum* var. *matthewsii*, *P. umbellatum* var. *cordatum*, and *P. rigidum* var. *majus*.

The only New Zealand species recognised by Cooper (1956), Allan (1961) and Druce (1980) not included was *P. ellipticum*. Repeated extractions on different individuals, using both the modified CTAB procedure of Doyle and Doyle (1987; Appendix 1) and the DNeasy Plant Mini Kit (Qiagen) on both fresh and herbarium material failed to extract DNA usable for PCR from this species. During the CTAB extraction large brown pellets were present, as a result of the secondary compounds co-precipitating with the DNA, this was also found in some of the other New Zealand taxa but not to the same extent. On electrophoresis after extraction no DNA was visible. This problem was found with both *P. ellipticum* subsp. *ellipticum* and *P. ellipticum* subsp. *serpentinum*.

Both *P. crassicaule* and *P. lineare* were not included in this study. Cooper (1956) and Allan (1961) both considered *P. crassicaule* to be a distinct species, although more recent authors (e.g. Druce 1980; Wilson and Galloway 1993) consider it to be just a smaller leaved form *P. rigidum*. Cooper (1956) includes *P. lineare* in *P. crassicaule* while later authors do not list *P. lineare* (Druce 1980; Wilson and Galloway 1993).

The other species not included are those considered closely related to *P. tenuifolium* but not recognised by any authors except Allan (1961). *Pittosporum fasciculatum* is similar to *P. tenuifolium* subsp. *tenuifolium* and *P. tenuifolium* subsp. *colensoi* in form but was established to include those individuals in which the flowers are terminal and axially in fascicles, since normally in *P. tenuifolium* subsp. *tenuifolium* and *P. tenuifolium* subsp. *colensoi* the flowers were axially and solitary (Cooper 1956; Allan 1961). However most authors do not consider these individuals to have separate species status as both *P. tenuifolium* subsp. *tenuifolium* and *P. tenuifolium* subsp. *colensoi* occasionally have fascicled flowers and other species of *Pittosporum* with simple inflorescences frequently have fascicled and solitary flowers (Cooper 1956).

Pittosporum buchananii is also similar in form to *P. tenuifolium* subsp. *tenuifolium* and *P. tenuifolium* subsp. *colensoi* and was established based on specimens cultivated in Wellington, which apparently came from near Tongariro. Specimens were later collected from Kaitaia and the Wellington district that were also identified as *P. buchananii* (Cooper 1956). Cooper (1956) considered its status as a species doubtful due to a lack of specimens and Allan (1961; page 318) stated, "Specimens attributed to this sp. show a considerable range of lf [sic] form and the status of the sp. requires further study". It is most likely that *P. buchananii,* and *P. fasciculatum* are at most varieties of *P. tenuifolium* subsp. *tenuifolium* and/or *P. tenuifolium* subsp. *colensoi.*

Pittosporum intermedium was known from only a single tree on Kawau Island, which was destroyed and no other individuals have been found. It is thought that it was most likely a hybrid between *P. tenuifolium* and *P. crassifolium* and possibly *P. ellipticum* or *P umbellatum* (Kirk 1871; Cooper 1956; Allan 1961).

If individuals of these taxa could have been collected and reliably identified then they would have been included to test their status. However, it is doubtful that by not including them a false picture of the relationships and evolution of the New Zealand *Pittosporum* that were included will be obtained. All of these taxa are considered by other authors to represent slight morphological variations of other taxa and are unlikely, based on the low sequence divergence found between most of the New Zealand taxa, to have any large or significant differences in their sequences.

In most New Zealand species where more than one individual was sequenced the two sequences were found to be identical. Considering the low level of sequence differentiation found between most of the New Zealand species this is not surprising. There were however, some species that were found to have different sequences. The two *P. rigidum* individuals sequenced, one from the North Island and one from the South Island, were found to differ by one base. The taxa on the Kermadec Islands thought to be *P. crassifolium* differed by five bases (1.0% sequence divergence) from the *P. crassifolium* collected from the North Island. This situation was also found in the Pacific Island species, where the two *P. arborescens* taxa sequenced, one from the Cook Islands and one from Tonga, differed by seven bases (1.2% sequence divergence). The specimen from the Cook Islands was once considered to be a separate species, *P. rarotongense* (Gemmill *et al.* in press), this situation is also found in *P. rigidum,* the smaller leaved South Island form was once given species status as *P. crassicaule* (Allan 1961; Cooper, 1956) with more recent authors considering it to a larger leaved from of *P. rigidum* (Druce 1980; Wilson and Galloway 1993). Therefore, although for most species sampling only one individual will provide a representative sample, where there are wide geographical, ecological or morphological ranges it is advisable to sample from the extremes of the range.

Hass (1977) stated that it is impossible to arrive at satisfactory classifications within the *Pittosporum* if the study was limited to fraction of their range. Cooper (1956) included information about the distribution of *Pittosporum* outside New Zealand and Australia, as well as the Pittosporaceae, as he felt the study must be done on a world wide basis, not a regional one. When commenting on the taxonomy of the New Zealand species, Allan (1961) stated that the genus is in need of critical revision, but that revision should be done along with a study of the entire genus. This research supports these authors and has clearly shown that species occurring in the same area may not necessarily be closely related, and that all species in a genus must be included in a phylogenetic analysis to obtain a correct picture of the relationships between species in an area, and to other species elsewhere. This holds not only for molecular studies but also morphological analyses. Hass (1977) recognised this when suggesting relationships for the Pacific species to those in other areas, stating that until studies of *Pittosporum* in other areas had been undertaken the Pacific species could not be associated with any certainty to any sectional alliances within the genus as a whole. A good example of the importance of including all species in a phylogenetic analysis is shown with the New Zealand species. *Pittosporum cornifolium* and *P. pimeleoides* are most closely related, on the basis of ITS sequences, to the *P. rhytidocarpum* group and the New Caledonian *Pittosporum* and are very distinct from the other New Zealand species. Not only were these two species never considered to be closely related, neither of them was ever identified as being distinct from the other New Zealand species in any way (see Cooper 1956; Allan 1961). In fact the species thought to be distinct to the other New Zealand species, *P. dallii, P. eugenioides* and *P. anomalum* came out as genetically closely related to the other New Zealand species, and interspersed within them.

4.2 SEQUENCE CHARACTERISTICS AND VARIATION

4.2.1 Sequence alignment

As with most other angiosperm ITS sequences, most of the variation between the species used in this study was due to point mutations, with a relatively low number of gaps indicating insertion or deletion events (see references in Baldwin *et al.* 1995). Approximately 50% of the informative sites were observed in ITS1, with ITS2 having approximately 40% of the informative sites (Table 3.6 for New Zealand only matrix; Appendix 4). Other studies have also found higher amounts of variation in ITS1 (e.g. Baldwin 1992; 1993; Baldwin *et al.* 1995 and references therein; Gielly *et al.* 1996). Indels were observed only in the two spacers. As found in most studies using this region, within genera or between closely related genera, most of these were single base indels (see references in Baldwin *et al.* 1995). Some of these single base indels did support clades found in the trees, and approximately 50% were phylogenetically informative in the all taxa matrix. This percentage was much lower in the New Zealand only matrix, at approximately 15% (Table 3.1).

4.2.2 Sequence length

Apart from the two taxa found to have a large indel of 35 bases in ITS1, the length of the ITS1 sequences, from 186 to 222 bp, falls within the range of those previously reported for angiosperms (187 to 298 bp). All of the ITS2 sequences lengths, of between 228 and 237 bp, fall within the range of those previously reported for angiosperms (187 to 252 bp; see references in Baldwin *et al.* 1995). However, due to truncation to obtain sequences of equal length for all species, neither all of ITS1 nor the ITS2 region was used in the analysis. Not including these sections is unlikely to have had any effect on the information content of the spacers, as the length removed is relatively short compared to region remaining. Due to constraints in secondary structure, the spacers are also more conserved near the genes, making it less likely that there will be significant levels informative characters (Baldwin *et al.* 1995). The 5.8S gene had a uniform length of 163 bp in all taxa used, similar to the 163 or 164 bp length usually found in angiosperms (see references in Baldwin *et al.* 1995).

4.2.3 GC content

The GC content of both ITS1, with an average of 69.5%, and ITS2, with an average of 68.7%, are very similar (Table 3.3). This similarity between spacers is consistent with what is found in angiosperm and most other eukaryote ITS sequences. At around 70% the GC content falls in the middle of the range of those found in other studies of angiosperms using ITS (Baldwin *et al.* 1995).

4.2.4 g_1 statistic

Most parsimonious trees, or the optimal tree obtained by any other criteria, are generally accepted to be a good estimate of phylogeny (Swofford *et al.* 1996). However, it is important to ensure that the variation among the taxa does contain phylogenetic signal and is not randomised in respect to the phylogenetic history. If there has been rapid evolution at the variable sites then there is no basis to assume that the most parsimonious tree is a good estimate of phylogeny (Swofford *et al.* 1996). The g_1 is a measure of the level of phylogenetic signal in a data set and is based on the skewness in length of a set of randomly generated trees. When g_1 is equal to 0 the tree length distribution is symmetrical, while a $g_1 < 0$ has a left skewed distribution and a $g_1 > 0$ is right skewed. The more left skewed the tree length distribution, i.e. the more negative the g_1 statistic, the more structured the data set, and the more likely that the most parsimonious tree/s represents the tree phylogeny (Hillis and Huelsenbeck 1992).

Simulations have shown that for a data set of 25 taxa, with a four character state data set of 500 characters, g_1 values of less than 0.09 are significantly more skewed than expected from random data ($p = 0.01$; Hillis and Huelsenbeck 1992).

The g_1 values for the New Zealand only (-2.461) and the all taxa (-0.946) matrices indicate that these data sets are significantly skewed from random and therefore contain a considerable level of phylogenetic signal. The New Zealand only data set appears to contain very high levels of phylogenetic signal. The g_1 of the New Zealand species is approximately 3.4 times higher than the of the entire date set and 2.7 times higher than the removed New Zealand tree. This is consistent with the higher CI and RC found for the New Zealand only data set.

4.3 CONSISTENCY INDICES

The CI is a measure of how well the data fits the tree topology and is used as an indicator of the level of homoplasy in the data (Kluge and Farris 1969; Forey *et al.* 1992). Homoplasy is the independent evolution of a character state in two or more lineages (Futuyma 1998). Although sometimes used to refer to any kind of convergence, parallelism or reversal, the term homoplasy in cladistic analysis has more specific connotations. It refers to features that are hypothesised at the beginning of an analysis to be homologous, i.e. derived from a common ancestor, but are then found to arise more than once over a cladogram or to have originated and then been lost (Sanderson and Donoghue 1989). The larger the CI the lower the level of homoplasy, with 1.0 being the highest possible value (Kluge and Farris 1969).

The CI values from this study are 0.838 for the New Zealand only matrix and 0.609 for the all taxa matrix. From these numbers it would appear that there is a lot less homoplasy in the New Zealand only matrix.

Although the CI is the most widely reported measure of fit between a data matrix and a tree (Sanderson and Donoghue 1989) it does have some drawbacks. The CI is influenced by the number of autapomorphies in a data matrix. Autapomorphies increase the CI without supporting any particular tree topology, resulting in a CI that gives more support than is actually present (Forey *et al.* 1992). One way to account for the autapomorphies is to remove them from the data matrix (Quicke 1993). Removing the uninformative sites resulted in a CI of 0.763 for the New Zealand only matrix and 0.493 for all taxa As expected these values have been lowered, but the New Zealand only matrix is still quite high and is not lowered as much, on a percentage basis, as that for the all taxa matrix.

Farris (1989) proposed the RI and the RC to try to overcome the problem of uninformative characters. The RI is a measure of the proportion of apparent synapomorphy, that represent true synapomorphies, so this measure excludes that part which can be attributed to homoplasy, i.e. characters shared due to convergence or parallel evolution.

The RC is the product of the CI and the RI, and has the advantage of excluding characters that do not contribute to the fit of the tree. It has several advantages to the method of removing autapomorphies as it not only removes these characters but also excludes totally homoplastic characters while allowing characters that are partly homoplastic yet provide partial support to the tree topology to contribute to the value (Farris 1989; Quicke 1993). The RC values were 0.735 for the New Zealand only matrix and 0.422 for all taxa. These values are lower again than the CI excluding uninformative characters but are likely to represent the best values for the fit of the data to the tree.

Since the CI is calculated by dividing the number of data columns by the length of the tree there is a negative relationship between the CI and the size of the data set (Forey 1992). This is because as more taxa or characters are included in an analysis more homoplasy will be encountered (Quicke 1993). Unfortunately this means that a direct comparison cannot be made between the CI values of the four data sets used in this analysis. They do show this pattern of decreasing CI as the size of the data set increases, although how much of this is attributable to this and how much, if any, to actual increases in homoplasy it not certain.

4.4 ORIGINS OF THE NEW ZEALAND *PITTOSPORUM*

The phylogenetic trees generated in this study gave results that both support and conflict with current ideas on the relationships of the New Zealand *Pittosporum* based on morphology.

4.4.1 Non-monophyly

All of the analyses support non-monophyly of the New Zealand species of *Pittosporum* and indicates that there have been two colonisation events onto New Zealand. One lineage has generated three taxa; *P. cornifolium, P. pimeleoides* subsp. *pimeleoides* and *P. pimeleoides* subsp. *maius*. The second event gave rise to all other New Zealand taxa (main New Zealand clade).

The clade of *P. cornifolium, P. pimeleoides* subsp. *pimeleoides* and *P. pimeleoides* subsp. *maius* is very distinct from the rest of the New Zealand species, with a very high average sequence divergence from the other New Zealand taxa of 8.3%, an average of 47.3 bases, compared to 1.4%, 7.8 bases, found between the all the other New Zealand species (Table 3.4; Table 3.5). The ITS sequences of these three taxa were identical so this relationship was found in all trees (Figure 3.2; Figure 3.4 to Figure 3.7) and had 100% bootstrap support (Figure 3.6).

The main New Zealand clade was found in 99% of the equally parsimony trees obtained from analysis of the New Zealand only matrix (Figure 3.2) and in all of the analyses using the all taxa matrix (Figure 3.4 to Figure 3.7) as well as the neighbour joining tree (Figure 3.7). However, this clade had no support in the bootstrap analysis (Figure 3.6).

4.4.2 Geographic origin

It appears that *P. cornifolium* and both *P. pimeleoides* subspecies are the result of a colonisation into New Zealand from New Caledonia. This is supported by the fact that in the strict consensus trees from the parsimony analysis *P. gatopenese* from New Caledonia is in their sister group and also that four New Caledonia taxa make up the sister group to the clade containing *P. cornifolium, P. pimeleoides* and their sister group (Figure 3.4 to Figure 3.6). Although neither of these groupings were found in the bootstrap tree (Figure 3.6), the hypothesis of colonisation from New Caledonia is given further support by the clade of the four New Caledonian taxa coming out as the sister group to *P. cornifolium* and the two *P. pimeleoides* taxa in the neighbour joining analysis (Figure 3.7).

There is no clear indication of the geographic origin of the main New Zealand clade in the parsimony analysis. It forms a sister group to a clade containing all of the other *Pittosporum* taxa used in the analysis (Figure 3.4 to Figure 3.6). The neighbour joining tree, which has a clade of *P. undulatum* and *P. bracteolatum* as sister group to the main New Zealand clade, does seem to provide some support for a dispersal into New Zealand from Australia, possibly via Norfolk Island. It is more likely however, that *P. bracteolatum* and the main New Zealand clade share a common Australian ancestor (Figure 3.7)

However, not all of the Australian *Pittosporum* were included, and also very few of the New Caledonian taxa. Both of these areas are highly likely to have been the origin for this New Zealand clade. Australia because, it has a large number of *Pittosporum* species, approximately 14 species, and it is the only place where other genera of the Pittosporaceae family are found (Cooper, 1956). This makes it the most likely place where the family, and the *Pittosporum* genus first arose and dispersed from. New Caledonia is also a possibility as it has a large number of species and it is almost certainly where the ancestor *P. cornifolium* and the two *P. pimeleoides* taxa dispersed from.

4.4.3 Age of the New Zealand radiations

The level of molecular divergence is considered to be approximately correlated with time, although it will not necessarily be constant over time (Swofford *et al.* 1996). From the very short branch lengths found separating the New Zealand taxa in the most parsimonious trees (Figures 3.1, Figure 3.3) and neighbour joining tree (Figure 3.7) it appears that the origin and radiation of this main New Zealand radiation is a relatively recent event. These branch lengths and the pairwise distances (Table 3.6) found within the main New Zealand clade are also all relatively very short compared to those in New Caledonia and Australia, also supporting a relatively recent radiation in New Zealand. *Pittosporum cornifolium* and the two *P. pimeleoides* taxa, due to their identical sequences, are the result of a very recent colonisation.

An asymmetric branching pattern characterised by short internal branch lengths and longer terminal branches was found in the main New Zealand clade. This pattern has also been found in other studies of New Zealand genera using ITS (*Anaphalis*, Glenny and Wagstaff 1997; *Pseudopanax*, Mitchell and Wagstaff 1997; *Hebe*, Wagstaff and Granock-Jones 1998; *Hebe* complex, Wagstaff and Granock-Jones 2000). This pattern is thought to reflect a relatively recent dispersal to New Zealand, followed by periods of rapid evolution and adaptation to the local ecological and climatic conditions and punctuated by extinction events in which all but one or two members of a lineage were eliminated (Wagstaff and Granock-Jones 1998; 2000).

A relatively recent arrival and radiation of *Pittosporum* in New Zealand is consistent with the fossil record. Most of the present New Zealand flora first makes its appearance in the fossil record during the late Miocene or Pliocene (approximately 10 to two mya; Pole 1994). The earliest fossils attributed to *Pittosporum* in New Zealand are leaf impressions, found in the late Oligocene, approximately 25 mya (Oliver 1950) and pollen in the mid Miocene, approximately 15 mya (Flemming 1975).

Some authors have attempted to estimate sequence divergence rates for the ITS region. For example Wright *et al.* (2000) calculated a minimum mutation rate for recently allopatric *Metrosideros* taxa of 1 bp per 1.5 million years and (Jobst *et al* 1997) calculated a nucleotide substitution rate range in ITS1-ITS2 for Cucurbitaceae of 0.8 –1.6 bp per million years. Because these dates are derived from the fossil record or geological events such as vicariance, which are very open to interpretation (Swofford *et al.* 1996), they are treated as estimates and not as the basis for a molecular clock.

The range in sequence divergence between the New Zealand species in the main clade is between zero and 22 bases. Using an estimated mutation rate of 1 bp per million years, gives an estimated age this radiation of 22 million years, which is consistent with the first appearance of *Pittosporum* in the fossil record in New Zealand.

Based on the relatively low level of sequence divergence found within the main New Zealand clade it is highly unlikely then that the morphological diversity within the New Zealand species is a result of vicariance, implying that *Pittosporum* were present in New Zealand when it broke off Gondwana 60 to 80 million years ago. Given the rate of sequence change calculated for the ITS region for other taxa, and the correlations of these rates with the fossil record the lineage within New Zealand does not appear to be old enough, unless, for some unknown reason, sequence change occurs very slowly in the New Zealand *Pittosporum*. However, there is no evidence of abnormally slow sequence change in any of the other studies of New Zealand genera (Glenny and Wagstaff 1997; Mitchell and

Wagstaff 1997; Wagstaff and Granock-Jones 1998; Gatt and Hammett 2000; Mitchell and Heenan 2000; Wagstaff and Granock-Jones 2000; Wright et al. 2000).

4.5 PREVIOUS HYPOTHESISES ON RELATIONSHIPS WITHIN NEW ZEALAND

4.5.1 Bivalved and tivalved goups

The hypothesis that the bivalved species and the trivalved species represent two separate monophyletic groups within New Zealand is not supported here, nor is the hypothesis that the trivalve taxa are ancestral.

Figure 4.1 shows the distribution of valve number within the New Zealand taxa. This clearly shows that the bivalve species within New Zealand are the result of two distinct colonisations. It also shows that within the big New Zealand clade bivalve is the ancestral form and that the trivalve habit has arisen twice within New Zealand, once leading to the *P. tenuifolium* clade, and once within the clade of containing *P. umbellatum, P. fairchildii, P. virgatum* and *P.* cf. *crassifolium*.

Figure 4.1. The distribution of valve number in the New Zealand *Pittosporum.*

Two members of the clade containing *P. umbellatum, P. fairchildii, P. virgatum* and *P.* cf. *crassifolium*; *P. fairchildii* and *P.* cf. *crassifolium* are trivalve. In 80% of the most parsimonious trees found in the analysis of the all taxa matrix, within this clade *P. umbellatum, P. virgatum* form a clade as do *P. fairchildii* and *P.* cf. *crassifolium* (Figure 3.4), with this second group also found in the neighbour joining tree (Figure 3.7). However in the all taxa using gaps a 5th base analysis *P. fairchildii* and *P. virgatum* form a clade in all of the most parsimonious trees (Figure 3.5) and in the New Zealand only analysis *P. fairchildii* is the sister group to the other three taxa in 81% of the most parsimonious trees (Figure 3.2). The grouping of *P. fairchildii* and *P. virgatum* in the gaps as a 5th base tree appears to be based on a deletion that these two taxa share, along with *P. dallii.* Because of this deletion being shared with *P. dallii,* which is, when compared to the difference between these four species, quite divergent from them, this deletion is not particularly informative. Therefore, based on the grouping found in the analysis excluding gaps and the neighbour joining analysis and valve number, splitting this clade into the two groups appears to be the correct representation of their relationships. Furthermore, as it appears that bivalve capsules are the ancestral form within New Zealand, and the trivalve form has arisen from it, it is hypothesised that the two trivalve species, *P. fairchildii* and *P.* cf. *crassifolium* have descended from either *P. umbellatum* or *P. virgatum* or an extinct bivalve species. Due to the low level of sequence divergence found within the New Zealand species it is not surprising that this pattern is not observed here. Perhaps analysis with a more variable marker would give a better indication of the relationships of these species.

Figure 4.2 shows the distribution of valve number throughout all the *Pittosporum* taxa used in this analysis. Apart from *P. tobira* from Japan, all non New Zealand species included in the analysis have bivalve capsules. This indicates that bivalve is the ancestral condition within the genus and trivalve capsules have risen independently in several areas. Cooper (1956) stated that trivalve taxa are found in Fiji and Hawaii, however Hass (1977) in a review of the Pacific species said only one species, *P. orohenese* from Tahiti has trivalved capsules, the rest being bivalve. Hass (1977) also noted that some taxa do occasionally produce anomalous trivalved capsules, giving rise to the possibility that carpel number is somewhat unstable. It seems most likely that specimens with anomalous capsule numbers lead Cooper (1956) to say that there were trivalve species in Hawaii and Fiji. This indicates that as Hass (1977) stated this is a poor character to split the genus into groups with.

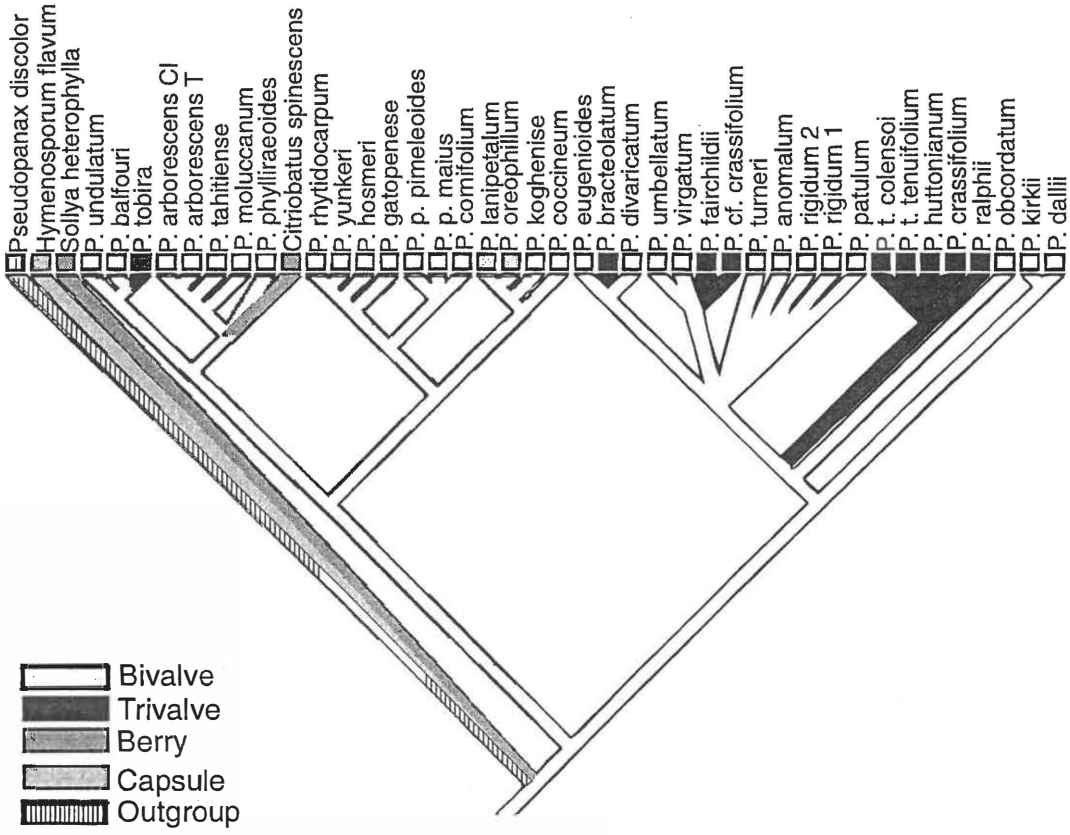


Figure 4.2. Distribution of valve number over all the taxa used in this analysis. Species which are bivalve and sometimes produce trivalve capsules are included in bivlave.

4.5.2 Papery endocarp

Three New Zealand species, *P. eugenioides, P. anomalum* and *P. dallii,* have distinctive capsules with a papery endocarp surrounding the seeds that remains intact when the valves separate (Moore and Adams 1941). These taxa did not form a monophyletic group in any of the analyses. Instead they where spread throughout the main New Zealand clade, with *P. dallii* and *P. anomalum* being two of the three most basal species, and *P. eugenioides* one of the most derived (Figure 3.2; Figure 3.4 to Figure 3.7) indicating that this papery endocarp may have evolved on three separate occasions.

4.5.3 Small leaved bivalved species

Only four of the six putative members of the small leaved bivalved species group, *P. obcordatum, P. rigidum, P. divaricatum* and *P. anomalum*, considered by Lanig and Gourlay (1935) to be closely related, were included in the analyses. These species did not form a monophyletic group in any of the analyses (Figure 3.2; Figure 3.4 to Figure 3.7). *Pittosporum rigidum 1* did have identical ITS sequence to *P. anomalum*, as well as *P. turneri,* with *P. rigidum 2* having a sequence divergence of 0.2% (Table 3.4), differing by one base (Table 3.4), from these two species. *Pittosporum divaricatum* and *P. obcordatum* had a sequence divergence of 0.5% and 1.4% respectively from *P. rigidum 1* and *P. anomalum*, which is quite high considering the low level found in the main New Zealand clade, with an average of 1.4%. *Pittosporum divaricatum* almost always formed a clade with *P. eugenioides*, while *P. obcordatum* was one of the most basal species. Based on these results it is unlikely that including the other two taxa would cause all these species to form a distinct clade.

4.5.4. Group showing decrease in size

The group of five species, *P. umbellatum, P. virgatum, P. turneri, P. patulum* and *P. pimeleoides,* which Cooper (1956) thought might be related based on an apparent decrease in size seen in various characters, did not form a clade in any of the analyses. *Pittosporum pimeleoides* does not even come out as closely related to the other New Zealand species. It is possible however, that *P. turneri* and *P. patulum* may be related to *P. virgatum* and *P. umbellatum*, possibly sharing a common ancestor, however the placement of the first two species is fairly unresolved. Possibly analysis with another marker, a more variable one, may also provide a better indication of the relationships of these four species.

4.6 TAXONOMY AND RELATIONSHIPS WITHIN NEW ZEALAND

4.6.1 *Pittosporum cornifolium* and *P. pimeleoides*

The identical ITS sequence found in *P. cornifolium* and *P. pimeleoides* was unexpected as no author has ever grouped *P. cornifolium* with *P. pimeleoides* based on morphology. Both Allan (1961) and Cooper (1956) considered *P. cornifolium* to be very similar to *P. kirkii* as both are epiphytic. Cooper (1956) considered *P. pimeleoides* to be related most closely to *P. patulum* while Allan (1961) grouped *P. pimeleoides* with the small leaved bivalved species, mainly because this species is heteroblastic.

Even if Pacific Island and New Caledonian species had not been included in the analysis it would still have been clear that these two species are genetically very different from the other New Zealand species. They differ from the other New Zealand species by between 34 and 53 bases, a sequence divergence of between 6.8% and 9.3%. The largest difference between the rest of the New Zealand species is 22 bases or 3.9% sequence divergence (Table 3.6).

Pittosporum cornifolium and *P. pimeleoides* appear to be the result of a very recent dispersal into New Zealand as indicated by their identical ITS sequences. A similar situation exists in the Hawaiian Islands. Gemmill *et al.* (in press) found that although the Hawaiian *Pittosporum* are morphologically quite diverse there was no variation in the ITS region. They hypothesised that this lack of resolution

indicated a relatively recent dispersal of *Pittosporum* to the Hawaiian Islands. This is given support by the fact that other molecular phylogenetic studies on insular Pacific taxa, including the Hawaiian Islands, find no or low levels of ITS sequence divergence (e.g. Baldwin and Sanderson 1998, Hawaiian silversword alliance; Ganders *et al.* 2000, *Bidens*; Wright *et al.* 2000, *Metrosideros*). The Hawaiian archipelago is also geologically very young and in other areas that are older, for example New Caledonia and Australia, there is much more sequence divergence between the *Pittosporum* taxa.

Pittosporum cornifolium and the two *P. pimeleoides* taxa are separated from their closest sister group by a very long branch. This pattern, of a long branch with a clade of species with little genetic divergence, is also seen with the clade of *P. hosmeri, P. rhytidocarpum* and *P. yunckeri*, and also, although to a lesser extent, in the clade of the two *P. arborescens* taxa and *P. tahitiense* (Figure 3.3, Figure 3.7). The inclusion of more taxa from the Pacific and also New Caledonia, in the case of *P. cornifolium* and *P. pimeleoides* and *P. hosmeri, P. rhytidocarpum* and *P. yunckeri;* and Australia, for the two *P. arborescens* taxa and *P. tahitiense,* may result in the placement of more taxa along these branches. This seems especially likely since so few of the New Caledonia and Pacific *Pittosporum* taxa included.

4.6.2 Status of the two *P. pimeleoides* taxa

Since *P. pimeleoides* subsp. *pimeleoides* and *P. pimeleoides* subsp. *maius* are very similar morphologically it is not surprising that they had identical sequences, especially considering the low genetic differentiation found between all the New Zealand taxa. Allan (1961) recognised these two taxa to as distinct species, while Cooper (1956) considered them to be conspecific due to their highly similar flower and fruit characteristics. These two taxa are different in growth form, *P. pimeleoides* subsp. *pimeleoides* is a shrub up to 2 m tall while *P.* pimeleoides subsp. *maius* has a prostrate habit. *Pittosporum pimeleoides* subsp. *maius* also has larger leaves. *Pittosporum pimeleoides* subsp. *maius* is found only on the Surville Cliffs at the North Cape, while *P. pimeleoides* subsp. *pimeleoides* is found throughout Northland. These two taxa hybridise freely to produce fertile offspring that are intermediate in form (Druce *et al.* 1979).

The prostrate growth habit of *P. pimeleoides* subsp. *maius* and *P. ellipticum* subsp. *serpentinum* is due to growing on the ultramafic substrate found at the Surville Cliffs. Soils derived from ultramafic rocks are highly infertile and contain high levels of toxic metals, such as nickel and chrome (Dawson 1988). This growth form is called semi-lianoid, where the 'elongated stems do not climb, but scramble or trail down the cliffs through other plants' (Druce *et al.* 1979). This habit is found in 12 of the 16 endemic shrubs found on the Surville Cliffs who have a lower stature than their nearest relative, a total of over 20% of the woody plants present, from 10 different families (Druce *et al.* 1979). Other taxa include *Metrosideros excelsa*, *Corokia cotoneaster* var. and *Pseudopanax lessonii* (Wheeler 1963; Druce *et al.* 1979). Such a degree of convergence on a common growth form indicates that there is intense selection pressure in this area. That there is such a high level of selection is supported by the fact that the two *P. pimeleoides* taxa have identical ITS sequences and hybridise freely, indicating that their divergence happened relatively recently. The prostrate habit of *P. pimeleoides* subsp. *maius* is maintained in cultivation. Most of the semi-lianoid taxa brought into cultivation maintain this habit, for example *Hebe brevifolia* and *Carex ophiolithica* (de Lange 1997; de Lange and Heenan 1997).

These results support the subspecies status of these two taxa, based on their similar morphology, ability to hybridise and produce viable offspring and identical ITS sequences.

Another subspecies of a more widely distributed species, *P. ellipticum* subsp. *serpentinum* is also found only on the Surville Cliffs. This taxon is also prostrate in habit, while the more widely distributed *P. ellipticum* subsp. *ellipticum* is a small tree up to 8 m tall. Multiple attempts at cultivation of *P. ellipticum* subsp. *serpentinum* have been unsuccessful however it will probably maintain its growth form in cultivation (de Lange 1998). No DNA suitable for PCR and sequencing could be extracted from either subspecies of *P. ellipticum*. However, due to similarities between the distribution and morphological differences between the two *P. ellipticum* taxa and the two *P. pimeleoides* taxa the two *P. ellipticum* subspecies are likely to have very similar sequences.

4.6.3 Status of the two *P. tenuifolium* taxa

The status given to *P. tenuifolium* subsp. *tenuifolium* and *P. tenuifolium* subsp. *colensoi* varies depending on the author. There is no doubt that these two species are closely related, as they are morphologically very similar. The ITS sequences of these two taxa are identical. Their identical sequences, in combination with their similar morphology, supports their classification as subspecies.

Two other New Zealand taxa had identical sequences to the two *P. tenuifolium* subspecies, *P huttonianum* and *P crassifolium*. *Pittosporum huttonianum* is very similar in morphology to the two *P. tenuifolium* subspecies, especially *P. tenuifolium* subsp. *colensoi* (Druce 1980). This species should therefore probably be given subspecies status within *P. tenuifolium*. *Pittosporum crassifolium* however is morphologically quite distinct from *P. tenuifolium* and because of this should retain it specific status.

4.6.4. Identical sequences in *P. rigidum 1, P. turneri* and *P. anomalum*

A third group of New Zealand species had identical sequences, *P. rigidum 1*, *P. anomalum* and *P. turneri*. As with *P. cornifolium* and *P. pimeleoides,* because of the distinct differences in morphology between these three species they should all be considered distinct, but closely related species. Once again a faster evolving marker may better resolve these relationships.

4.6.5 Different sequences in *P. rigidum*

Two individuals of *P. rigidum* were sequenced for this study, one from the North Island, *P. rigidum 1* and one from the South Island, *P. rigidum* 2, and were found to differ in their sequences by one base. Considering the low level of sequence divergence found within the New Zealand species this difference is probably quite significant. It is unlikely that this difference is a due to poor sequence as this site clearly had a different character state between the two species. *Pittosporum rigidum 1* also had identical sequence to *P. anomalum* and *P. turneri*. The smaller leaved form of *P. rigidum* found south of Northwest Nelson was considered by both Cooper (1956) and Allan (1961) to be members of a distinct species, *P. crassicaule,* however this species is no longer widely recognised (Druce 1980;

Wilson and Galloway 1983). The collection locality of the South Island *P. rigidum* (Arthurs Pass) indicates that it is probably a member of what was known as *P. crassicaule,* while the North Island individual is the more typical and more northerly distributed *P. rigidum* type. The one base pair difference supports the separation of these two morphological types into distinct species.

4.6.6 *Pittosporum* in the Kermadecs

The *Pittosporum* taxon found on Raoul Island was originally identified as *P. crassifolium*, or closely related taxon (Eagle 1982). However this study indicates that this is not the case, as it appears to be most closely related to *P. fairchildii* and not at all closely to *P crassifolium.* Further detailed morphological study is necessary and may reveal that this taxon is most morphologically similar to *P. fairchildii.* This situation is mirrored in the classification of *P. ellipticum* subsp. *serpentinum.* Until recently described by de Lange (1998) it had been identified as *P. umbellatum, P. crassifolium* and as a possible new species (see Wheeler 1963; Druce 1980; Druce *et al.* 1979; Eagle 1982; Cameron *et al.* 1993; Cameron *et al.* 1995). *Pittosporum fairchildii* differs from the taxon found on Raoul Island at three bases and lacks the 13 bp deletion found in *P. fairchildii.* Based on the level of sequence divergence found between the New Zealand taxa, and the fact that there are obvious morphological differences since the relationship between these two species has not earlier been made, I suggest that it is a distinct species, especially since it does not share the 13 bp deletion with *P. fairchildii.*

The colonisation of Raoul Island in the Kermadec Islands by *Pittosporum* appears to have been a relatively recent event. Due to the close genetic relationship between *P. fairchildii* and the taxon on Raoul Island, it is possible that the colonisation occurred from the population of *P. fairchildii*, found only on the Three Kings Islands. The fact that the Raoul Island *Pittosporum* does not have the 13 bp deletion does provide some conflict with this being a straight ancestor descendant relationship.

Another possibility is that *P. fairchildii* and the *Pittosporum* on Raoul Island share a common ancestor. Both *P. umbellatum* and *P. virgatum*, as well as a hypothetical, now extinct, bivalve species, are possibilities, especially since both these species are found in the North Auckland area. Although it was not included in the analysis, based on morphology, *P. ellipticum* would most likely group with these four species, probably closely with *P. virgatum,* to which it is most similar to in morphology (Cheeseman 1925). *Pittosporum ellipticum* usually has bivalve capsules, although it sometimes has trivalve capsules (Cooper 1956). It is also a possibility for the ancestral taxa to *P. fairchildii* and the Raoul Island *Pittosporum*. However, all of these would mean however that both species acquired trivalve capsules independently. Because of this a further possibility is that the ancestor of these two species is extinct and was trivalve.

4.7 RELATIONSHIPS OF NON NEW ZEALAND *PITTOSPORUM*

Some inferences can be made on the relationships of the other *Pittosporum* species included in this analysis, and from where they may have originated.

4.7.1 *Pittosporum bracteolatum*

The results of these analyses indicate that *P. bracteolatum* is closely related to the species in the main New Zealand clade. However, the placement of *P. bracteolatum* varied between the parsimony and neighbour joining analysis. In the parsimony analyses *P. bracteolatum* always formed a clade with *P. eugenioides* (Figure 3.4 to Figure 3.6), and was found within the New Zealand species. However in the neighbour joining analysis it was, with *P. undulatum*, a sister group to the main New Zealand clade (Figure 3.7).

Looking at the branch lengths found in the most parsimonious trees (Figure 3.3) shows an interesting and confounding branch length pattern. *Pittosporum bracteolatum* is in the middle of the main New Zealand clade, in which most branches are from one to three changes long, with many species having identical sequences, yet *P. bracteolatum* has a very long branch leading in the tree shown in with 23 changes (Figure 3.3). Therefore, despite *P. bracteolatum* coming out within the New Zealand species in the parsimony analyses, having it as the sister taxon to the main New Zealand clade, seems more consistent with the long branch

length found in the most parsimonious trees and the results from the neighbour joining analysis.

The most likely way to explain this relationship is that *P. bracteolatum* and the main New Zealand clade share the same ancestor and based on the neighbour joining tree, this ancestor was probably from Australia. It is also possible that dispersal to New Zealand from Australia may have occurred via Norfolk Island.

4.7.2 New Caledonia

Gemmill *et al.* (in press) hypothesised that there had been at least two dispersals onto New Caledonia. Unfortunately the results obtained in both this study and that by Gemmill *et al.* (in press), i.e. the apparent separation of *P. gatopenese* from the other New Caledonian species could not be properly compared to morphological differences. The New Caledonian *Pittosporum* species have not yet been fully studied, although a revision is currently being undertaken by Veillon and Tirel (C. Gemmill pers. comm.). However the pairwise distances between *P. gatopenese* and the other New Caledonian species do not appear to be much different than those separating the other species, in fact they fall within the range separating the other species. However, only four of the estimated 50 New Caledonian species were included here and inclusion of more species may find that the New Caledonian species are not monophyletic.

4.7.3 Pacific *Pittosporum*

Hass (1977) identified six possible evolutionary lines in the Pacific, indicating several introductions of the genus into the Pacific region. Members of only two of these putative lineages were included in this study, the *P. rhytidocarpum* group (*P. rhytidocarpum, P. yunckeri* and *P. hosmeri* as a representative of the Hawaiian species, which all had identical ITS sequences), and the *P. arborescens* group (*P. arborescens* from Fiji, *P. arborescens* from the Cook Islands (once considered a separate species *P. rarotongense* (Gemmill *et al.* in press)) and *P. tahitiense*). The study by Gemmill *et al.* (in press) also included these species and found that they formed separate clades, however there was no clear indication from where they may have dispersed into the Pacific.

As with the study by Gemmill *et al.* (in press) *P. gatopenese* from New Caledonia was found to be a possible sister taxon to the *P. rhytidocarpum* clade. Although this grouping was found in all analysis containing these species (Figure 3.3 to Figure 3.7) the bootstrap support is not high (67%; Figure 3.6). However, further support for New Caledonia being the origin of this radiation is provided by the other four New Caledonian species included in this study forming a sister group to the clade containing *P. gatopenese* and the *P. rhytidocarpum* clade (Figure 3.3 to Figure 3.7)

Hass (1977) stated that the *P. arborescens* group appeared to be most closely related to the Paupasian and Australian species placed in the section *Pseuditea* by Schodde (1972) and may be derived from or from an ancestor to this group. In this study the clade of the two *P. arborescens* taxa and *P. tahitiense* was found to have an Australian species *P. moluccanum* as its sister taxon. This grouping was found in all of the most parsimonious trees and the neighbour joining tree (Figures 3.3 to 3.7) and had bootstrap support of 78% (Figure 3.6). Another Australian species, *P. phylliraeoides*, was then the sister taxon to this group in 99% or 100% of the equally parsimonious trees (Figure 3.3 to Figure 3.6), although this grouping was not found in the bootstrap tree (Figure 3.6) or the neighbour joining tree (Figure 3.7). This indicates that this group of Pacific species is most likely the result of a colonisation into the Pacific from Australia.

4.8 NON-MONOPHYLY OF THE *PITTOSPORUM*

This study also does not support the monophyly of the *Pittosporum*. Analyses using the chloroplast genes *rbc*L, *mat*K and the *rpl*16 and *rpoC1* introns on relationships within the Apiales and Apiaceae included members of the Pittosporaceae. All three studies included a species of *Pittosporum, Hymenosporum* and *Sollya,* and found that *Pittosporum* and *Hymenosporum* formed a clade with *Sollya* as their sister taxon (Plunket *et al.* 1996; Plunket *et al.* 1997; Downie *et al.* 2000). In contrast this study placed *Hymenosporum* and *Sollya* as a clade. These studies do lend support to the all taxa parsimony analysis using gaps as missing data as the analysis with gaps as a fifth base tended to place these two genera within the *Pittosporum* (Figure 3.3; Figure 3.4).

The ITS data does not support groupings suggested based on morphology within the Pittosporaceae. The Pittosporaceae have been divided into two subfamilies. The Pittosporaceae, which included *Pittosporum, Hymenosporum, Marianthus, Cherianthera* and *Bursaria*, which have woody and leathery capsules, and the Billiardieae, containing *Citriobatus, Sollya, Billardiera* and *Pronaya*, which have succulent berries (Pax 1891; Hutchinson 1964). The results of this study are in conflict with this as it groups genera from the different subfamilies; *Citriobatus spinescens* comes out within the *Pittosporum* and *Hymenosporum* and *Sollya* from a clade. These morphological groupings are however, supported by the chloroplast studies. The study by Downie *et al.* (2000) included a representative of *Bursaria* found that it grouped with *Pittosporum* and *Hymenosporum* as expected based on morphology, and aslo a member of *Billardiea*, which formed a clade with *Soylla*.

Unfortunately none of the studies included a representative of *Citriobatus* so the placement of this species within the *Pittosporum* in this study could not be compared to other molecular analyses. However this study does support a cladistic analysis by Crisp *et al.* (1989) that used mostly morphological characters. Their study found that *Citriobatus* is most closely related to a subgroup of *Pittosporum*. Based on this they stated that *Citriobatus* should be included within the *Pittosporum* and this research supports their conclusion.

4.9 CHARACTER EVOLUTION IN NEW ZEALAND

4.9.1 Heteroblasty

Figure 4.4 shows the distribution of heteroblastic taxa in New Zealand. It appears that heteroblasty has evolved at least four times within the main New Zealand clade and also once in the *P. cornifolium* and *P. pimeleoides* clade

The independent development of heteroblastic development at least four times over the New Zealand *Pittosporum* and in what appears to be a very short time, indicates that there could strong selection pressure. All of the heteroblastic species are shrubs or small trees, with most having a maximum height of less than four metres (Cooper 1956; Allan 1961). They are found in both in forest under story and in scrub, so there is no apparent habitat correlation.

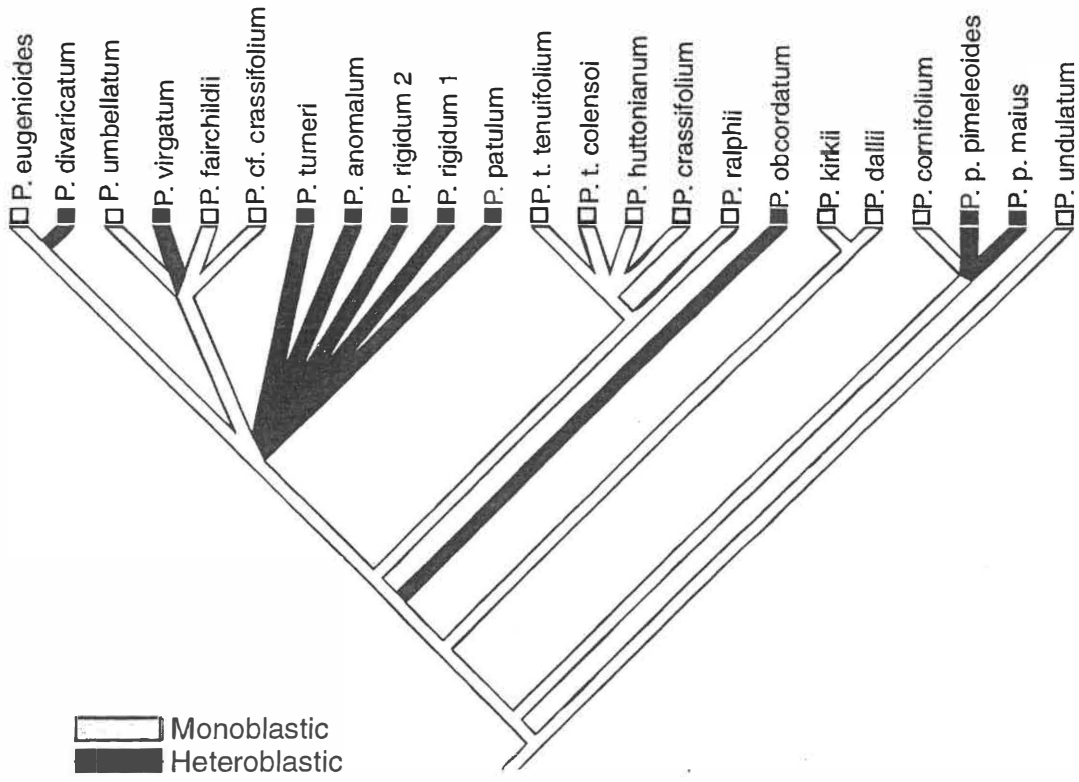


Figure 4.3. The distribution of heteroblasty throughout the New Zealand species

It has been hypothesised that heteroblastic species with a divaricating juvenile form, as found in *P. turneri,* are the result of hybridisation between a divaricating shrub and a tree without a divaricating juvenile form (Godley 1985). Based on morphology, putative small-leaved parent species for *P. turneri* are most likely to be *P. divaricatum* and *P. crassicaule*, as the juvenile of both these species have similar habit, leaf size, shape and outline to the juvenile of *P. turneri* (Allan 1961). *Pittosporum turneri* and *P. divaricatum* do hybridise and the hybrids flower (Ecroyd 1994), although it is not known if the hybrids produce viable gametes and/or if backcrossing can occur. *Pittosporum turneri* has identical ITS sequence to *P. rigidum 1* and *P. anomalum*, and differs by three bases from *P. divaricatum.* Based on molecular data it appears that the first two species are more closely related to *P. turneri* and therefore more likely to be potential parents. Individuals that are given the name *P. crassicaule* are found only in the South Island and this distribution makes them unlikely to be one of the parents, however all of the other three are found within the range of *P. turneri.* If the ITS sequence data does provide a true picture of the relationships then it would appear that either *P. rigidum* or *P. anomalum* are the most likely parents.

Pittosporum patulum has a similar leaf form to adult *P. turneri*, although the leaves of *P. turneri* are slightly smaller (Cheeseman 1925). Godley (1985) noted that in all the species that have divaricating juvenile forms the adult leaves are smaller than those of the hypothesised tree parents, and that this is what would be expected with hybridisation with a divaricating shrub due to the very small leaves. *Pittosporum patulum* differs by two bases from *P. turneri* and is also bivalve. Even though it is not found in the same localities as *P. turneri* it is possible that *P. patulum,* or possibly even a species ancestral or closely related to it, is most likely to be the non-divaricating tree parent.

4.9.2 Divaricating habit

It appears that the divaricating habit has arisen at least three times independently within the New Zealand *Pittosporum* (Figure 4.4).

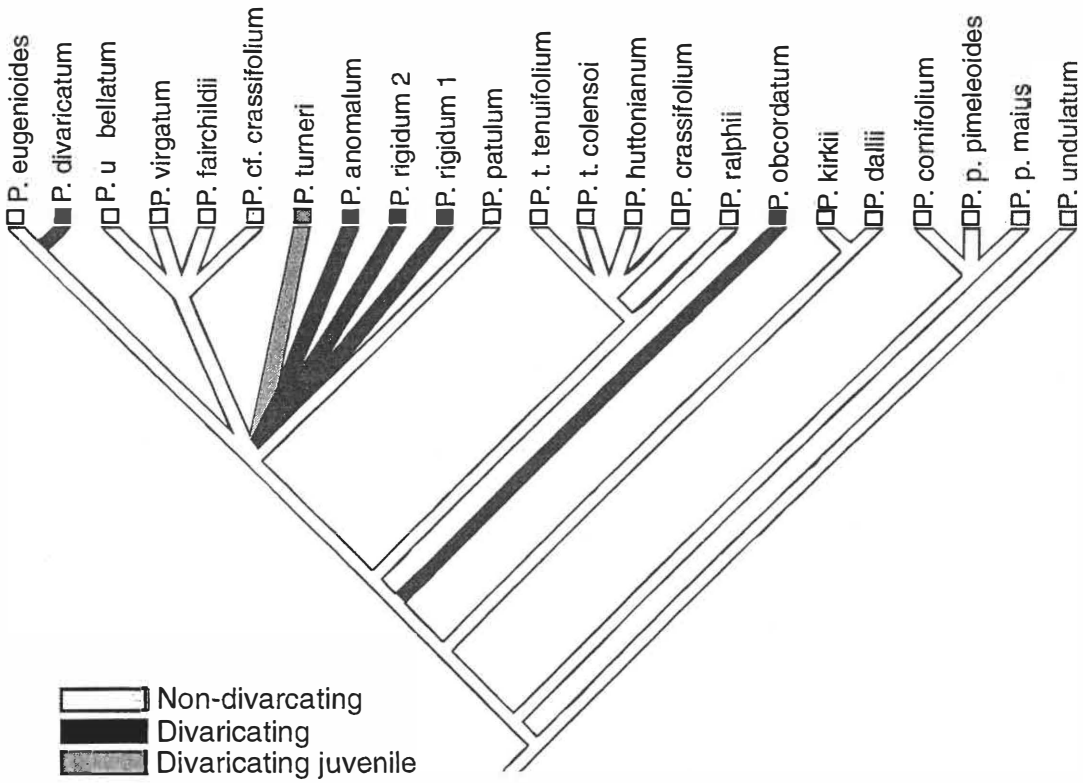


Figure 4.4. Distribution of the divaricating habit in the New Zealand *Pittosporum*

The divaricating habit is thought to be an evolutionary recent development. This theory is supported the fact that many divaricating shrubs can hybridise with their larger leaved relatives (Dawson 1988). This has been observed in the *Pittosporum*, for example, *P. obcordatum* hybridises with *P. tenuifolium* (Clarkson and Clarkson 1994).

The glaciations that periodically occurred over the Pleistocene (1.8 mya to 10 000 years ago) are thought to have provided the climate conditions responsible for the evolution of the divaricating habit (Wilson and Galloway 1993). Before this time New Zealand had a warm temperate climate (Flemming 1979). At the onset of the glaciations New Zealand had already moved to its current position (Stevens 1989). Because of this isolation plants already adapted the climatic conditions during the glaciations could not migrate here, and the divaricating habit is the response of a flora which consisting mainly of tropical forest species to these conditions (Wilson and Galloway 1993).

The *Pittosporum* species represent an example of a tropical group in New Zealand. Based on the total level of sequence divergence and the fossil record *Pitt* would have been present in New Zealand before the glaciations. Three of the four divaricating taxa, the two *P. rigidum* taxa and *P. anomalum*, definitely appear to have evolved very recently as they have almost identical sequences to each other and differ by very few bases to some of the non divaricating species (Table 3.3; Table 3.5). Although the age of the other two divaricating species do not give any clear indication of a very recent development, i.e. over the last 1.8 million years, there is no indication for them being a lot older than this.

This research provides support for the climate hypothesis for the evolution of the divaricating habit, and that there was intense selection pressure for this habit by the climatic conditions during the Pleistocene.

4.9.3 FLOWER COLOUR

In contrast to many other groups of New Zealand plants, which tend to have light coloured flowers (Godley 1979), many of the *Pittosporum* in New Zealand have red or dark red to purple flowers. Most of the *Pittosporum* found in other areas usually have yellow or white flowers(Gowda 1951; Cooper 1956; Hass 1977), which is also in contrast to many groups of New Zealand plants as usually the New Zealand members are lighter in colour than those elsewhere (Godley 1979, Dawson 1988).

Figure 4.5 shows the distribution of flower colour among the New Zealand species. *Pittosporum cornifolium* and the two *P. pimeleoides* taxa have yellow flowers, as do the three most basal species in the main New Zealand clade as well as *P. anomalum*, *P. eugenioides* and *P. bracteolatum.* Pink flowers are found in *P. turneri* and *P. umbellatum*, red or purple in *P. divaricatum,* the two *P. rigidum, P. patulum* and *P. fairchildii.* The species with dark red and purple flowers are the *P. tenuifolium* clade, and *P. virgatum* and *P.* cf. *crassifolium.*

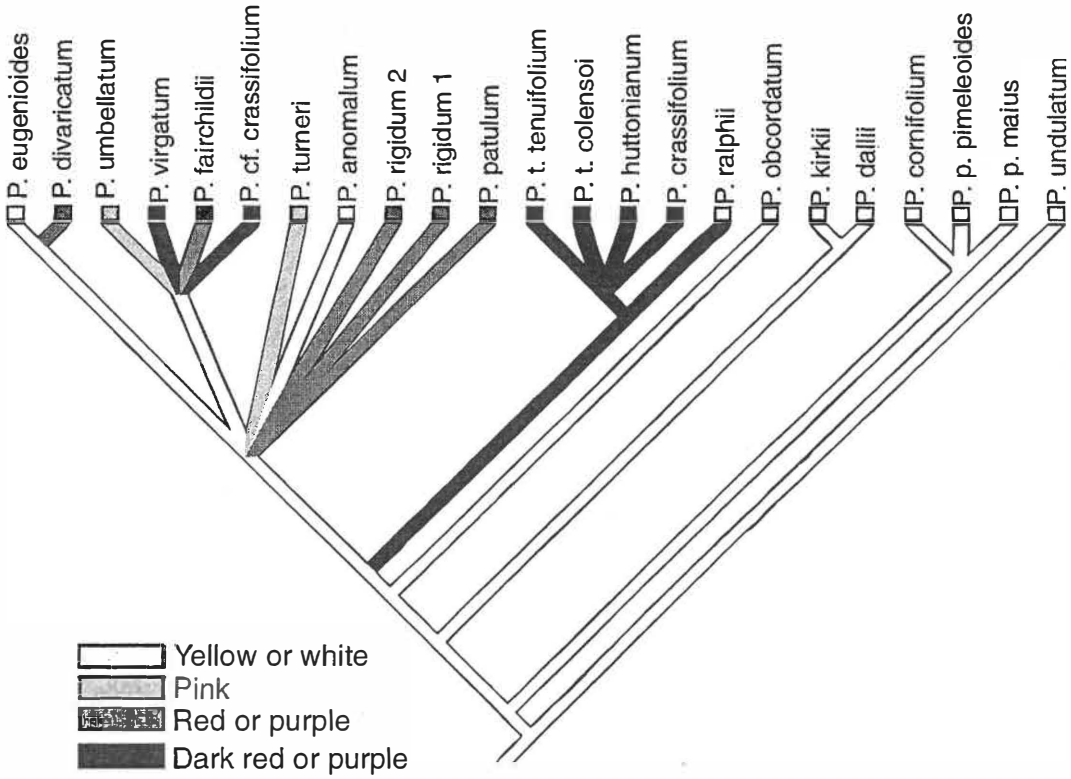


Figure 4.5. Distribution of corolla colour in the New Zealand *Pittosporum*

It appears that the darker flower colour found in some of the New Zealand species is the derived state within New Zealand, supported by the fact that the three most basal species have yellow flowers, as do most *Pittosporum* elsewhere. The dark red or purple flower colour appears to have arisen at least twice, once in the *P. tenuifolium* clade and maybe twice in *P. virgatum* and *P.* cf. *crassifolium.* Unfortunately, due to the lack of resolution in the tree, no further inferences can be made on the evolution of flower colour, making it impossible to determine wheter the flower colour gradually became darker or whether dark flowers developed directly from the yellow ones.

The flowers of *Pittosporum* in New Zealand are thought to insect pollinated, with a variety of insects, such as nocturnal moths, flies, beetles and honey bees observed visiting them (Thompson 1880; 1925; Heine 1937; Pickmere 1945; Godley 1979; Ecroyd 1994; Clarkson and Clarkson 1994). There are no other animals that could be potential pollinators for the New Zealand species, leading to the development of the red and purple flowers, especially considering the small size of the flowers.

4.10 FUTURE WORK

Although sufficient for resolving the relationships of some species within the New Zealand *Pittosporum*, the ITS region did not have enough variation to fully resolve relationships of all the New Zealand species. For example, there are three groups of species that have identical sequences. One marker that has been identified and may prove to have the resolution to provide a better indication of relationships within these three groups of species with identical sequences, and all the New Zealand species, is the external transcribed spacer (ETS) of nrDNA. The ETS regions has been shown to be more variable and phylogenetically informative than ITS in three genera, from three different tribes, in Asteraceae (Linder *et al.* 2000). Gemmill *et al.* (in press) are currently investigating the ETS region for use with the South Pacific and Hawaiian species. If this marker proves to have more phylogenetically informative variation than ITS for these species it should be sequenced for the New Zealand species. Not only would it hopefully provide more resolution of the relationships of the New Zealand *Pittosporum* it will provide a good comparison to relationships inferred from the ITS analysis.

A morphological cladistic analysis of the New Zealand *Pittosporum* also needs to be under taken to tie in with the molecular analyses and to provide support for the relationships. A molecular analysis using chloroplast DNA could be used to further investigate hybridisation, such as the hypothesised hybrid origins of *P. turneri.*

REFERENCES

Allan, H. H. 1961: *Flora of New Zealand, Volume I.* Government Printer, Wellington.

Atkinson, I. A. E. and Greenwood, R. M. 1989: Relationships between moas and plants. *New Zealand Journal of Ecology 12 (Supplement):* 67-98.

Avise, J.C. 1994: *Molecular markers, natural history and evolution.* Chapman and Hall, New York.

Baldwin, B. G. 1992: Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. *Molecular Phylogenetics and Evolution 1:* 3-16.

Baldwin, B. G. 1993: Molecular phylogenetics of *Calycadenia* (Compositae) based on ITS sequences of nuclear ribosomal DNA: chromosomal and morphological evolution reexamined. *American Journal of Botany 80:* 222-238.

Baldwin, B. G. and Sanderson, M. J. 1998: Age and rate of diversification of the Hawaiian silversword alliance (Compositae). *Proceedings of the National Academy of Sciences of the United States of America 95:* 9402-9406.

Baldwin, B. G.; Sanderson, M. J.; Porter, J. M.; Wojciechowski, M. F.; Cambell, C. S. and Donoghue, M. J. 1995: The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden 82:* 247-277.

Baum, D. A.; Small, R. L. and Wendel, J. F. 1998: Biogeography and floral evolution of Baobabs (*Adansonia,* Bombacaceae) as inferred from multiple data sets. *Systematic Biology 47(2):* 181-207.

Bennett, E. M. 19??: Pittosporaceae. *Australian Plants 15:* 20-37.

Burrows, C. J. 1980: Some empirical information concerning the diet of moas. *New Zealand Journal of Ecology 3:* 151-153.

Burrows, C.J. 1998: How did the New Zealand flora originate? *New Zealand Botanical Society Newsletter 52:* 14-16.

Cameron, E. K.; de Lange, P. J.; Given, D. R.; Johnson, P. N. and Ogle, C. C. 1993: Threatened and local plant lists (1993 revision). *New Zealand Botanical Society Newsletter 32:* 14-28.

Cameron, E. K.; de Lange, P. J.; Given, D. R.; Johnson, P. N. and Ogle, C. C. 1995: New Zealand Botanical Society threatened and local plant lists (1995 revision). *New Zealand Botanical Society Newsletter 39:* 15-28.

Cameron, E.K. 2000: New names, combinations, or comments from the journals (7). *New Zealand Botanical Society Newsletter 62:* 12-16.

Carlquist, S. 1974: *Island Biology.* Columbia University Press, New York.

Cheeseman, T. F. 1914: *Illustrations of the New Zealand flora.* Government Printer, Wellington.

Cheeseman, T.F. 1925: *Manual of the New Zealand Flora.* Government Printer, Wellington.

Choi, B. H. and Kim, J. H. 1997: ITS sequences and speciation on Far Eastern *Indigofera* (Leguminosae). *Journal of Plant Research 110:* 339-346.

Clarkson, B. D. and Clarkson, B. R. 1994: Ecology of an elusive endemic shrub, *Pittosporum obcordatum* Raoul. *New Zealand Journal of Botany 32:* 155-168.

Cockayne, L. 1928: *The vegetation of New Zealand.* Wilheim Engelmann, Leipzig.

Coleman, P. J. 1980: Plate tectonics background to biogeographic development in the southwest Pacific over the last 100 million years. *Palaeogeography, Palaeoclimatology, Palaeoecology 31:* 105-121.

Connor, H. E. and Edgar, E. 1987: Name changes in the indigenous New Zealand flora, 1969-1986, and Nomina Nova IV, 1983-1986. *New Zealand Journal of Botany 25:* 115-170.

Cooper, R. A. 1993: The New Zealand biota: historical background and new research. *Trends in Ecology and Evolution 8(12):* 429-433.

Cooper, R. C. 1956: The Australian and New Zealand species of *Pittosporum. Annals of the Missouri Botanical Garden 43:* 87-187.

Crisp, M. D.; Taylor, J. M. and Bennett, E. M. 1989: Pittosporaceae - an old east Gondwanan family? *Australian Systematic Botany Society Newsletter 60:* 20.

Cronquist, A. 1982: *An integrated system of classification of flowering plants.* Columbia University Press, New York.

Dawson, J. 1988: *Forest vines to snow tussocks.* Victoria University Press, Wellington.

Dawson, J. W. 1958: Interrelationships of the Australasian and South American floras. *Tuatara 7(1):* 1-6.

Dawson, J. W. 1964: Unisexuality in the New Zealand Umbelliferae. *Tuatara 12(2):* 67-68.

Dawson, J. W. and Sneddon, B. V. 1969: The New Zealand rain forest: a comparison with tropical rain forest. *Pacific Science 23:* 131-147.

Day, J. 1998: Light conditions and the evolution of heteroblasty (and the divaricate form) in New Zealand. *New Zealand Journal of Ecology 32(1):* 43-54.

de Lange, P. J. 1997: *Hebe brevifolia* (Scrophulariaceae) - an ultramafic endemic of the Surville Cliffs, North Cape, New Zealand. *New Zealand Journal of Botany 35:* 1-8.

de Lange, P. J. 1998: *Pittosporum ellipticum* subsp. *serpentinum* (Pittosporaceae) - a new ultramafic endemic from the Surville Cliffs, North Cape, New Zealand. *New Zealand Journal of Botany 36:* 389-397.

de Lange, P. J. and Heenan, P. B. 1997: *Carex ophiolithica* (Cyperaceae): a new ultramafic endemic form the Surville Cliffs, North Cape, New Zealand. *New Zealand Journal of Botany 35:* 429-436.

de Lange, P.J.; Heenan, P.B.; Given, D.R.; Norton, D.A.; Ogle, C.C.; Johnson, P.N. and Cameron, E.K. 1999: Threatened and uncommon plants of New Zealand. *New Zealand Journal of Botany 37:* 603-628.

Downie, S. R. and Katz-Downie, D. S. 1996: A molecular phylogeny of Apiaceae subfamily Apioideae: evidence form nuclear ribosomal DNA internal transcribed spacer sequences. *American Journal of Botany 83(2):* 234-251.

Downie, S. R.; Katz-Downie, D. S. and Watson, M. F. 2000: A phylogeny of the flowering plant family Apiaceae based on chloroplast DNA *rpl16* and *rpoC1* intron sequences: towards a suprageneric classification of subfamily Apioideae. *American Journal of Botany 87(2):* 273-292.

Doyle, J. J. and Doyle, J.L.. 1987: A rapid DNA isolation procedure for small quantities of fresh tissue. *Phytochemistry Bulletin 19:* 11-15.

Druce, A. P.; Bartlett, J. K. and Gardner, R. O. 1979: Indigenous vascular plants of the serpentine area of Surville Cliffs and adjacent Cliff tops, north-west of North Cape, New Zealand. *New Zealand Journal of Botany 35(429-436).*

Eagle, A. E. 1982: *Eagle's trees and shrubs of New Zealand, volume two.* William Collins Publishers Ltd., Auckland.

Easu, K. 1977: *Anatomy of seed plants.* John Wiley & Sons, Inc., USA.

Ecroyd, C. E. 1994: *Regeneration of Pittosporum turneri communities.* Department of Conservation, Wellington.

Farris, J. S. 1989: The retention index and the rescaled consistency index. *Cladistics 5:* 91-100.

Felsenstein, J. 1985: Confidence limits on phylogenies: an approach using the bootstrap. *Evolution 39(4):* 783-791.

Fenner, M.; Lee, W. G. and Barstow Wilson, J. 1997: A comparative study of the distribution of genus size in twenty angiosperm floras. *Biological Journal of the Linnean Society 62:* 225-237.

Flemming, C.A. 1975: The geological history of New Zealand and its biota. *In* G. Kuschel (ed) *Biogeography and ecology in New Zealand,* 1-86. Junk, The Hague.

Flemming, C.A. 1979: *The geological history of New Zealand and its life.* Auckland University Press, Auckland

Forey, P. L.; Humphries, C. J.; Kitching, I. L.; Scotland, R. W.; Siebert, D. J. and Williams, D. M. 1992: *Cladistics: a practical course in systematics.* Clarendon Press, Oxford.

Futuyma, D. J. 1998: *Evolutionary biology.* Sinauer Associates, Inc, Sunderland, Massachusetts.

Ganders, F. R.; Berbee, M. and Pirseyedi, M. 2000: ITS base sequence phylogeny in *Bidens* (Asteraceae): evidence for the continental relatives of Hawaiian and Marquesan *Bidens*. *Systematic Botany 25(1):* 122-133.

Gatt, M. K. and Hammett, K. R. W. 2000: Molecular phylogeny of the genus *Dahlia* Cav. (Asteraceae, Heliantheae-Coreopsidinae) using sequences derived from the internal transcribed spacers of nuclear ribosomal DNA. *Botanical Journal of the Linnean Society 133:* 229-239.

Gemmill, C. E. C.; Allan, G. J.; Wagner, W. L. and Zimmer, E. A. in press: Evolution of insular Pacific *Pittosporum* (Pittosporaceae): origin of the Hawaiian radiation. *Molecular Systematics and Evolution.*

Gernandt, D. S. and Liston, A. 1999: Internal transcribed spacer evolution in *Larix* and *Pseudotsuga*(Pinaceae). *American Journal of Botany 86(5):* 711-723.

Gielly, L.; Yuan, Y.-M.; Kupfer, P. and Taberlet, P. 1996: Phylogenetic use of noncoding regions in the genus *Gentiana* L.: chloroplast *trn*L (UAA) intron verses nuclear ribosomal internal transcribed spacer sequences. *Molecular Phylogenetics and Evolution 5:* 460-466.

Glenny, D. and Wagstaff, S. 1997: Evolution and biogeography of New Zealand *Anaphalis* (Asteraceae: Gnaphalieae) inferred form rDNA sequences. *New Zealand Journal of Botany 35:* 441-449.

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

Godley, E. J. 1985: Paths to maturity. *New Zealand Journal of Botany 23:* 687-706.

Good, R. 1974: *The geography of flowering plants.* Longman Group Limited, London.

Gowda, M. 1951: The genus *Pittosporum* in the Sino-Indian Region. *Journal of the Arnold Arboretum 32(263-343).*

Greenwood, R. M. and Atkinson, I. A. E. 1977: Evolution of divaricating plants in New Zealand in relation to moa browsing. *Proceedings of the New Zealand Ecological Society 24:* 21-33.

Griffiths, J.R. 1972: Plate tectonics and the evolution of New Zealand. *In* R. Fraser (ed.), *Oceanography of the Southern Pacific,* 263-267. New Zealand National Commission for UNESCO, Wellington.

Griffiths, J. R. 1971: Reconstruction of the South -West Pacific margin of Gondwanaland. *Nature 234:* 203-207.

Haas, J. E. 1977: The Pacific species of *Pittosporum* Banks ex Gaertn. (Pittosporaceae). *Allertonia 1(2):* 73-167.

Hamby, R. K. and Zimmer, E. A. 1992: Ribosomal DNA as a phylogentic tool in plant systematics. *In* P.S. Soltis; D.E. Soltis and J.J. Doyle (eds.), *Molecular systematics of plants*, 50-91. Chapman and Hall, New York.

Heine, E. M. 1937: Observations on the pollination of New Zealand flowering plants. *Transactions of the Royal Society of New Zealand 66:* 1-11.

Hillis, D. M. and Dixon, M. T. 1991: Ribosomal DNA: molecular evolution and phylogenetic inference. *Quarterly Review of Biology 66:* 411-453.

Hillis, D. M. and Huelsenbeck, J. P. 1992: Signal, noise and reliability in molecular phylogentic analyses. *Journal of Heredity 83(189-195).*

Howe, H.F. 1937: Seed dispersal by fruit eating birds and mammals. *In* D.R. Murrary (ed) *Seed dispersal,* 123-189. Academic Press, North Ryde, New South Wales.

Hutchinson, J. 1964: *The genera of flowering plants. II. Dicotyledons.* Claredon Press, Oxford.

Jay, M. 1969: Chemotaxonomic researches on vascular plants. XIX. Flavonoid distribution in the Pittosporaceae. *Botanical Journal of the Linnean Society 62:* 423-429.

Jobst, J.; King, K. and Hemleben, V. 1998: Molecular evolution of the internal transcribed spacers (ITS1 and ITS2) and phylogenetic relationships anoung species of the family Cucurbitaceae. *Molecular Phylogenetics and Evolution 9:* 204-219.

Jorgensen, R. A. and Cluster, P. D. 1988: Modes and tempos in the evolution of nuclear ribosomal DNA: new characters for evolutionary studies and new markers for genetic and population. *Annals of the Missouri Botanical Garden 75:* 1238-1247.

Kelly, D. 1994: Towards a numerical definition for divaricate (interlaced small-leaved) shrubs. *New Zealand Journal of Botany 32:* 509-518.

Kirk, T. 1871: On the New Zealand species of *Pittosporum*, with descriptions of new species. *Royal Society of New Zealand, Transactions and Proceedings 4:* 260-267.

Kirk, T. 1889: *The forest flora of New Zealand.* Government Printer, Wellington.

Kirk, T. 1899: *The student's flora of New Zealand and the outlying islands.* Government Printer, Wellington.

Kluge, A. G. and Farris, J. S. 1969: Quantative phyletics and the evolution of anurans. *Systematic Zoology 18:* 1-32.

Kootz, J.A. and Soltis, D.E. 1999: DNA sequence data reveal polyphyly of Brexioideae (Brexiaceae; Saxifragaceae sensu lato). *Plant Systematics and Evolution 219:* 219-208.

Laing, R. M. and Gourlay, H. W. 1935: The small-leaved species of the genus *Pittosporum* in New Zealand with descriptions of new forms. *Royal Society of New Zealand, Transactions and Proceedings 65:* 44-65.

Linder, C. R.; Goertzen, L. R.; Vandem Heuvel, B.; Francisco-Orega, J. and Jansen, R. K. 2000: The complete external transcribed spacer of 18S-26S rDNA: amplification and phylogenetic utility at low taxonomic levels in Asteraceae and closely allied families. *Molecular Phylogenetics and Evolution 14(2):* 285-303.

Lloyd, D. G. 1985: Progress in understanding the natural history of New Zealand Plants. *New Zealand Journal of Botany 23:* 707-722.

Macphail, M. K. 1997: Comment on M. Pole (1994): 'The New Zealand flora - entirely long distance dispersal?'. *Journal of Biogeography 24:* 113-117.

Maddisonm W.P. and Maddison, D.R. 1995: MacClade: analysis of phylogeny and character evolution. Version 3.05. Sinauer Associates, Sunderland, Massachusetts.

McGlone, M.S. 1985: Plant biogeography and the late Cenozoic history of New Zealand. *New Zealand Journal of Botany 23:* 723-749.

McGlone, M. S. and Webb, C. J. 1981: Selective forces influencing the evolution of divaricating plants. *New Zealand Journal of Ecology 4:* 20-28.

McQueen, D. R. 2000: Divaricating shrubs in Patagonia and New Zealand. *New Zealand Journal of Ecology 24(1):* 69-80.

Mitchell, A. D. and Heenan, P. B. 2000: Systematic relationships of New Zealand endemic Brassicaceae inferred from nrDNA ITS sequence data. *Systematic Botany 25(1):* 98-105.

Mitchell, A. D. and Wagstaff, S. J. 1997: Phylogenetic relationships of *Pseudopanax* species (Araliaceae) inferrred from parsimony analysis of rDNA sequence data and morphology. *Plant Systematics and Evolution 208(121-138).*

Mitchell, A. D. and Wagstaff, S. J. 2000: Phylogeny and biogeography of the Chilean *Pseudopanax laetevirens. New Zealand Journal of Botany 38:* 409-414.

Moller, M. and Cronk, Q. C. B. 1997: Origin and relationships of *Saintpaulia* (Gesneriaceae) based on ribosomal DNA internal transcribed spacer (ITS) sequences. *American Journal of Botany 84(8):* 956-965.

Moore, L. B. and Adams, N. M. 1949: Fruit characters of *Pittosporum dallii* Cheesem. *Transactions of the Royal Society of New Zealand 77(2):* 250-252.

Musters, W.; Boon, K.; van der Sande, C. A. F. M. and Planta, R. J. 1990: Functional analysis of transcribed spacers of yeast ribosomal DNA. *EMBO 9:* 3989-3996.

Noyes, R. D. and Rieseberg, L. H. 1999: ITS sequence data support a single origin for North American Astereae (Asteraceae) and reflect deep geographic divisions in *Aster* S.L. *American Journal of Botany 86(3):* 398-412.

Oliver, W. R. B. 1950: The fossil flora of New Zealand. *Tuatara 3(1):* 1-11.

Pax, F. 1891: Pittosporaceae. *In* A. Engler and K. Prantl (eds), *Die naturlichen pflanzenfamilien, 18a:* 265-286. Engelmann, Leipzig.

Petrie, D. 1920: Descriptions of new native flowering-plants, with a few notes. *Royal Society of New Zealand, Transactions and Proceedings 53(365-).*

Philipson, W. R. 1964: Habitat in relation to age in New Zealand shrubs. *Journal of the Indian Botanical Society 42A:* 167-179.

Pickmere, E. K. 1945: Note on *Pittosporum* pollination. *Wellington Botanical Society Bulletin 10:* 8.

Plunket, G. M.; Soltis, D. E. and Soltis, P. S. 1996: Higher level relationships of Apiales (Apiaceae and Araliaceae) based on phylogenetic analysis of *rbc*L sequences. *American Journal of Botany 83(4):* 499-515.

Plunket, G. M.; Soltis, D. E. and Soltis, P. S. 1997: Clarification of the relationship between Apiaceae and Araliaceae based on *mat*K and *rbc*L sequence data. *American Journal of Botany 84(4):* 565-580.

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

Preest, D.S. 1963: A note on the dispersal characteristics of the seed of the New Zealand podocarps and beeches and their biogeographical significance. *In* J.L. Gressitt (ed) *Pacific Basin biogeography,* 415-424. Bishop Museum Press, Honolulu.

Quicke, D. L. J. 1993: *Principles and techniques of contemporary taxonomy.* Blackie Academic and Professional, London.

Raven, P. H. and Axelrod, D.I. 1974: Angiosperm biogeography and past continental movements. *Annals of the Missouri Botanical Garden 61(3):* 539-673.

Sanderson, M. J. and Donoghue, M. J. 1989: Patterns of variation in levels of homoplasy. *Evolution 43(8):* 1781-1795.

Sang, T.; Crawford, D. J. and Stuessy, T. F. 1995: ITS sequences and the phylogeny of the genus *Robinsonia. Systematic Botany 20(1):* 55-64.

Schodde, R. 1972: A review of the family Pittosporaceae in Papuasia. *Australian Journal of Botany Supplement Number 3:* 1-60.

Setoguchi, H. and Watanabe, I. 2000: Intersectional gene flow between insular endemics of *Ilex* (Aquifoliaceae) on the Bonin Islands and the Ryukyu Islands. *American Journal of Botany 87(6):* 793-810.

Smith, J. F. 2000: Phylognetic resolution within the tribe Episcieae (Gesneriaceae): congruence of ITS and *ndhF* sequences from parsimony analysis. *Americam Journal of Botany 87(6):* 883-897.

Stanford, A. M.; Harden, R. and Parks, C. R. 2000: Phylogeny and biogeography of *Juglans*(Juglandaceae) based on *matK* and ITS sequence data. *American Journal of Botany 87(6):* 872-882.

Stevens, G. 1985: *Lands in collision: discovering New Zealand's past geography.* Science and Information Publishing Centre, DSIR, Wellington.

Stevens, G. R. 1989: The nature and timing of biotic links between New Zealand and Antactica in Mesozoic and early Cenzoic times. *In* J.A. Crane, *Origins and evolution of the Antarctic biota, Geological Society special publication No. 47,* 141-166. The Geological Society, London.

Swofford, D. L.; Olsen, G. J.; Waddell, P. J. and Hillis, D. M. 1996: Phylogenetic inference. *In* D.M. Hillis; C. Moritz and B.K. Mable (eds.), *Molecular Systematiccs*, 407-514. Sinauer Associates, Sunderland, Massachusetts.

Swofford, D.L. 1998: PAUP*. Phylogenetic analysis using parsimony (* and other methods) Version 4.0b3a. Sinaur Associates, Sunderland, Massachusetts.

Thomson, G. M. 1880: On the fertilization, etc., of New Zealand flowering plants. *Royal Society of New Zealand, Transactions and Proceedings 13:* 241-289.

Thomson, G. M. 1906: *Introductory class-book of botany.* Government Printer, Wellington.

Thomson, G. M. 1925: The pollination of New Zealand flowers by birds and insects. *Royal Society of New Zealand, Transactions and Proceedings 57:* 106-125.

Tomlinson, P. B. 1978: Some qualitative and quantitaive aspects of New Zealand divaricating shrubs. *New Zealand Journal of Botany 16:* 299-309.

Townsend, A. J. 1999: *Pittosporum patulum recovery plan, 1999-2009* Department of Conservation, Wellington.

Tucker, J. M. 1974: Patterns of parallel evolution of leaf form in new world oaks. *Taxon 23(1):* 129-154.

van Balgooy, M. M. J. 1966: 101. *Pittosporum* Banks ex Gaertn. Pacific plant areas II. *Blumea Supplement V:* 186-187 .

Wagner, W. L.; Herbst, D. R. and Sohmer, S. H. 1990: *Manual of the flowering plants of Hawaii.* University of Hawaii Press and bishop Museum (Special Publication 83), Honolulu, Hawaii.

Wagstaff, S. J. and Dawson, M. I. 2000: Classification, origin and patterns of diversification of *Corynocarpus* (Corynocarpaceae) inferred from DNA sequences. *Systematic Botany 25(1):* 134-149.

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

Wagstaff, S. J. and Granock-Jones, P. J. 2000: Patterns of diversification in *Chinohebe* and *Parahebe* (Scrophulariaceae) inferred from ITS sequences. *New Zealand Journal of Botany 38:* 389-407.

Wardle, P. 1991: *Vegetation of New Zealand.* Cambridge University Press, Cambridge.

Watson, L. and Dallwitz, M. J. (1992 onwards). The families of flowering plants: descriptions, illustrations, identification and information retrival. http://biodiversity.uno.edu/delta/.

Webb, C. J. and Kelly, D. 1993: The reproductive biology of the New Zealnd flora. *Trends in Ecology and Evolution 8(12):* 442-447.

Webb, C. J.; Sykes, W. R. and Granock-Jones, P. J. 1988: *Flora of New Zealand, Volume IV.* DSIR, Botany Division, Christchurch.

Went, F. W. 1971: Parallel evolution. *Taxon 20(2/3):* 197-226.

Wheeler, J. M. 1963: The vegetation of the North Cape area. *Tane 9(63-83).*

White, T. J.; Burns, T.; Lee, S. and Taylor, J. 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *In* M. Inns; C. Gelfand; J. Sninsky and T. J. White, *PCR protocols: a guide to methods and applications*, 315-322. Academic Press, San Deigo, pp. 315-322.

Wilkinson, H. P. 1992: Leaf anatomy of the Pittosporaceae R. Br. *Botanical Journal of Linnean Society 110:* 1-59.

Wilson, H. and Galloway, T. 1993: *Small-leaved shrubs of New Zealand.* Manuka Press, Christchurch.

Wilton, A. D. and Breitwieser, I. 2000: Composition of the New Zealand seed plant flora. *New Zealand Journal of Botany 38:* 537-549.

Winkworth, R. C.; Robertson, A. W.; Ehrendorfer, F. and Lockhart, P. J. 1999: The importance of dispersal and recent speciation in the flora of New Zealand. *Journal of Biogeography 26:* 1323-1325.

Wright, S. D.; Young, C. G.; Dawson, J. W.; Whittaker, D. J. and Gardner, R. C. 2000: Riding the ice age El Nino? Pacific biogeography and evolution of *Metrosideros* subg. *Metrosideros*(Myrtaceae) inferred from nuclear ribosomal DNA. *Proceedings of the National Acadamy of Sciences of the United States of America 97(8):* 4118-4132.

APPENDIX 1: DNA extraction with CTAB Buffer

Modified from Doyle and Doyle (1987)

1) For each sample add 5ml of 2X CTAB buffer, 0.3g of PVP-40 (polyvinylprrolidone, average molecular weight 40 000) and 50µl of β-mercaptoethanol to a bottle. Mix on a magnetic stir plate until all the PVP-40 is dissolved. Incubate in a 60°C water bath for 45 minutes to warm solution, ensuring that water line is above liquid level in the bottle and shaking every now and then

2) Label four 15mL polypropylene conical tubes for each sample. Then add 5ml of hot CTAB buffer and 50µL of 10mg/mL Proteinase K to one tube for each sample and put in –60°C water bath

3) Weigh out 0.5g of leaf material and grind it into a fine powder in a clean, room temperature mortar with liquid Nitrogen

4) Scrape ground leaf material into the conical tube containing the CTAB buffer and proteinase K, using a pre-chilled, clean spatula and place in a rack in a 60°C water bath for 60 minutes ensuring that water line is above liquid level in the tube. Shake the tubes every 10 minutes.

5) (optional step) Spin tubes in Eppendorf 5810R centrifuge at 2400 rpm for 10 minutes at room temperature. Transfer supernatant to a new polypropylene tube using a pipette with plugged tips.

6) Add 2/3 to 1 volume (3.33-5ml) chloroform:isoamyl alcohol (24:1 v/v). Invert till a good immersion is obtained and then gas off the tube

7) Spin in Eppendorf 5810R centrifuge at 2400 rpm for 10 minutes at room temperature.

8) Transfer only the upper aqueous phase to a new polypropylene tube with a pipette using wide bore tips (cut end of pipette tip).

9) Repeat steps 6-8

10) Fill tubes with cold (-20°C) ethanol, mix gently by inverting several times and precipitate at –20°C for at least 30 minutes (usually left overnight)

11) Spin in Eppendorf 5810R centrifuge at 4000rpm for 5 minutes at -0°C. Tip of supernatant and leave tubes to air dry

12) Resuspend pellet completely in 200µl of sterile water (Use the 37°C water bath to help resuspend if necessary). Move sample to new 1.5ml tube

13) Add 700µl of –20°C 7.5M NH_4AcO, 95% ethanol solution (1:6). Invert a few times and precipitate at –20°C for at least 20 minutes

14) Spin in Eppendorf 5810R centrifuge at 4000rpm for 5 minutes at 0°C

15) Pour of supernatant and dry in Savant DNA120 Speed Vac, with medium heat (43°C) for 5 minutes and resuspend in 200µl sterile water

16) Add 1/10th volume (20µL) 2.5 M NaAcO then add 440µl –20°C 95% ethanol. Precipitate at –20°C for at least 20 minutes

17) Spin in Eppendorf 5810R centrifuge at 4000rpm for 5 minutes at 0°C. Pour off supernatant

18) Fill tubes with –20°C 70% ethanol, invert

19) Repeat step 17

20) Dry in Savant DNA120 Speed Vac, with medium heat (43°C) until pellet is dry and no traces of ethanol remain

21) Resuspend pellet in 100-200µl of sterile water depending on the size of the pellet. (If necessary put in 37°C water bath)

33) RNase samples by adding 1/100th volume of 10mg/ml RNase A to the DNA. Incubate at 37°C for one hour

34) Electrophorese on a 1% (1XTBE) agarose (SeaKem LE) gel at 55V using 5µl of DNA and 2µl of loading dye

APPENDIX 2: PCI EXTRACTION

From Laboratory of Molecular Systematics, Smithsonian Institution.

1) Add an equal volume of phenol:chlorofom:isomayl alcohol (25:24:1) to the sample
2) Vortex briefly and spin in Eppendorf 5414D centrifuge at maximum speed for 5 minutes
3) Remove supernatant to a new tube
4) Add an equal volume of chloroform:isomayl alcohol (24:1)
5) Vortex briefly and spin Eppendorf 5414D centrifuge at maximum speed for 5 minutes
6) Remove supernatant to a new tube
7) Repeat steps 4-6
8) Precipitate by adding 1ml of –20°C 95% ethanol. Incubate at –20°C overnight
9) Spin in Eppendorf 5414D centrifuge at maximum speed for 5 minutes
10) Pour off supernatant and leave tubes upside down on kimwipes for 15-30 min
11) Dry in Savant DNA120 Speed Vac on medium heat (43°C) until no traces of ethanol are left (10-15 minutes)
12) Resuspend in 25µl sterile water (E-pure)
13) Electrophorese on a 1% (1XTBE) agarose (SeaKem LE) gel at 55V using 5µl of DNA and 2µl of loading dye

APPENDIX 3: PEG PRECIPITATION

From Laboratory of Molecular Systematics, Smithsonian Institution.

1) Add an equal volume of PEG/NaCl (20% PEG 8000 (Polyethylene Glycol/Sodium Chloride), 2.5M Sodium Chloride) to the PCR
2) Vortex briefly and put in a 37°C water bath for 15 minutes
3) Spin in Eppendorf 5414D centrifuge at maximum speed for 15 minutes
4) Pipet of supernatant
5) Wash pellet with 200µl of –20°C 70% ethanol by slowly pipetting up and down
6) Spin in Eppendorf in Eppendorf 5810R centrifuge at 4000rpm for 5 minutes at 4°C
7) Remove ethanol
8) Repeat steps 5-7
9) Dry in Savant DNA120 Speed Vac on medium heat (43°C) until no traces of ethanol are left (10-15 minutes)
10) Resuspend in 25µl sterile water (E-pure)
11) Electrophorese on a 1% (1XTBE) agarose (SeaKem LE) gel at 55V using 5µl of DNA and 2µl of loading dye

APPENDIX 4: ALIGNED DATA MATRICES

The two data matrices of aligned sequences from the ITS regions and 5.8S gene used in this study. For ambiguous nucleotides the IUPAC (IUB) code was followed: N = A/G/C/T, K = G/T, S = C/G, R = A/G, Y = C/T, M = A/C, W = A/T, : = gap (insertion or deletion). Abbreviations are; p. = *pimeleoides* subsp., t. = *tenuifolium* subsp., *Citriobatus* = *Citriobatus spinescens, Hymenosporum* = *Hymenosporum flavum*, *Sollya* = *Sollya heterophylla*, *Pseudopanax* = *Pseudopanax discolor*. Numbers indicate the relative position in the ITS region, based on sequence of *Pseudopanax discolor* and those taxa which contained the entire region. Sequence data obtained in this research will be deposited in GenBank on publication.

New Zealand only **ITS1** →

	85
P. anomalum	AGC GAC CAG TGC ACT CGT AAA ACA CAT CCG GCC GGC GG: GAC GGA GGT GAC AGC TTC CCC AGC CGC CGT
P. cornifolium	AGC GAC CAG CGA ACT CGT AAA ACA CAT CCG GCT GAC GGC AAG GGA GGC GAC CAC TTC TCC AGC CGT CGG
P. crassifolium	NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN
P. cf. crassifolium	AGC GAC CAG TGC ACT CGT AAA ACA CAT CCG GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC AGC CGC CGT
P. dalli	AGC GAC CAG CGC ACT CGT AAA ACA CAT CCG GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC AGT CGC TGG
P. divaricatum	AGC GAC CAG TGC ACT CGT AAA ACA CAT CCG GCC GGY GGC GAC GGA GGT GAC AGC TTC CCC AGC CGC CGT
P. eugeniodies	AGC GAC CAG TGC ACT CGT AAA ACA CAT CCG GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC AGC CGC CGT
P. fairchildii	AGC GAC CAG TGC ACT TGT AAA ACA CAT CCG GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC AGT CGC CGT
P. huttonianum	AGC GAC CAG TGC ACT CGT AAA ACA CAT CTG GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC AGC CGC CGT
P. kirkii	AGC GAC CAG CGC ACT CGT AAA ACA CAT CCG GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC AGT TGT CGG
P. obcordatum	AGC GAC CAG CGC ACT CGT AAA ACA CAT CTG GCC GGA GGC GAC GGA GGT GAC AGC TTC CCC AGC CGC CGT
P. patulum	NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN
P. p. maius	AGC GAC CAG CGA ACT CGT AAA ACA CAT CCG GCT GAC GGC AAG GGA GGC GAC CAC TTC TCC AGC CGT CGG
P. p. pimeleoides	AGC GAC CAG CGA ACT CGT AAA ACA CAT CCG GCT GAC GGC AAG GGA GGC GAC CAC TTC TCC AGC CGT CGG
P. ralphii	AGC GAC CAG TGC ACT CGT AAA ACA CAT CTG GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC AGT CGC CGT
P. rigidum 1	AGC GAC CAG TGC ACT CGT AAA ACA CAT CCG GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC AGC CGC CGT
P. rigidum 2	AGC GAC CAG TGC ACT CGT AAA ACA CAT CCG GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC AGC CGC CGT
P. t. colensoi	NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNN NNA GGT GAC AGC TTC CCC AGC CGC CGT
P. t. tenuifolium	AGC GAC CAG TGC ACT CGT AAA ACA CAT CTG GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC AGC CGC CGT
P. turneri	AGC GAC CAG TGC ACT CGT AAA ACA CAT CCG GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC AGC CGC CGT
P. umbellatum	AGC GAC CAG TGC ACT CGT AAA ACA CAT CCG GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC AGC CGC CGT
P. undulatum	AGC AAC CAG CGA ACT CGT AAT ACA CAT CCG GTC GGC GGT GAT GGA GGC AAC AGC TTC CCC ATC CGC CGG
P. virgatum	AGC GAC CAG TGC ACT CGT AAA ACA CAT CCG GCC GGC GGC GAC GGA GGT GAC AGC TTT CCC AGC CGC CGW

P. anomalum	CCC ACG GAC GGG GAG TGC CCT TGG GCG CTG CTC GAC CGA AAA CAA AAC CCC :GG CGC GGA ACG CGC CAA
P. cornifolium	CCC ATG GAC GGG GAG TGC CCT TAG GCG TTA CTC GAC CGA AAA CCA AAC CCC :GG CGT GGA ACG CGC CAA
P. crassifolium	NNN NNN NNN NNN NAG TGC CCT TGG GCG CTG CTC GAC CGA AAA CAA AAC CCC CGG CGC GGA ATG CGC CAA
P.cf. crassifolium	CCC ACG GAC GGG AAG TGC CCT TGG GCG CTG CTC GAC CGA AAA CAA AAC CCC :GG CGC GGA ACG CGC CAA
P. dalli	CCC ACG GAC GGG GAG TGC CCC CGG GCG CTG CTC GAC CGA AAA CCA AAC CCC :GG CGC GGA ACG CGC CAA
P. divaricatum	CCC ACG GAC GGG GAG TGC CCT TGG GCG CTG CTC GAC CGA AAA CAA AAC CCC :GG CGC GGA ACG CGC CAA
P. eugeniodies	CCC ACG GAC GGG GAG TGC CCT TGG GCG CTG CTC GAC CGA AAA CAA AAC CCC :GG CGC GGA ACG CGC CAA
P. fairchildii	CCC ACG G:: ::: ::: ::: ::T TGG GCG CTG CTC GAC CGA AAA CAA AAC CCC :GG CGC GGA ACG CGC CAA
P. huttonianum	CCC ACG GAC GGG GAG TGC CCT TGG GCG CTG CTC GAC CGA AAA CAA AAC CCC CGG CGC GGA ATG CGC CAA
P. kirkii	CCC ACG GAC GGG GAG TGC CCT CGG GCG CTG CTC GAT CGA AAA CCA AAC CCC :GG CGC GGA AAG CGC CAA
P. obcordatum	CCC ACG GAC GGG GAG TGC CCT TGG GCG CTG CTC GAC CGA AAA CCA AAC CCC :GG CGC GGA ATG CGC CAA
P. patulum	CCC ACG GAC GGG GAG TGC CCT TGG GCG CTG CTC GAC CGA AAA CAA AAC CCC :GG CGC GGA ACG CGC CAA
P. p. maius	CCC ATG GAC GGG GAG TGC CCT TAG GCG TTA CTC GAC CGA AAA CCA AAC CCC :GG CGT GGA ACG CGC CAA
P. p. pimeleoides	CCC ATG GAC GGG GAG TGC CCT TAG GCG TTA CTC GAC CGA AAA CCA AAC CCC :GG CGT GGA ACG CGC CAA
P. ralphii	CC: ACG GAC GGG GAG TGC CCT TGG GCG CTG CTC GAC CGA AAA CAA AAC CCC CGG CGC GGA ATG CGC CAA
P. rigidum 1	CCC ACG GAC GGG GAG TGC CCT TGG GCG CTG CTC GAC CGA AAA CAA AAC CCC :GG CGC GGA ACG CGC CAA
P. rigidum 2	CCC ACG GAC GGG GAG TGC CCT TGG GCG CTG CTC GAC CGA AAA CAA AAC CCC :GG CGC GGA ACG CGC CAA
P. t. colensoi	CCC ACG GAC GGG GAG TGC CCT TGG GCG CTG CTC GAC CGA AAA CAA AAC CCC CGG CGC GGA ATG CGC CAA
P. t. tenuifolium	CCC ACG GAC GGG GAG TGC CCT TGG GCG CTG CTC GAC CGA AAA CAA AAC CCC CGG CGC GGA ATG CGC CAA
P. turneri	CCC ACG GAC GGG GAG TGC CCT TGG GCG CTG CTC GAC CGA AAA CAA AAC CCC :GG CGC GGA ACG CGC CAA
P. umbellatum	CCC ACG GAC GGG GAG TGC CCT TGG GCG CTG CTC GAC CGA AAA CAA AAC CCC :GG CGC GGA ACG CGC CAA
P. undulatum	CCC ACG GAC GGG GAG TGC CCT CGG GCG CTG CTC GAC CAA AAA CCA AAC CCC :GG CGC GGA ACG CGC CAA
P. virgatum	CCC ACG GAC GGG GAG TGC CCT TGG GCG CTG CTC GAC CGA AAA CAA AAG CCC :GG CGC GGA ACG CGC CAA

P. anomalum	GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC CCC GTT CGC GGG CGG C:G GTG GCG ::: TCA TTC CAT AAC
P. cornifolium	GGA ACT CAA AAT GAA TTG TAC GTC TCC TCC CCC GTT TGC GGG CGG C:G GTG GCG ::: TCA TTC CAT AAC
P. crassifolium	GGA ACT CAA ACT GAA TCG CAC GTC TCC GCC CCC GTT TGC GGG CGG C:G GTG GCG ::T TCA TTC CAT AAC
P.cf. crassifolium	GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC CCC GTT CGC GGG CTG C:G GTG GCG ::: TCA TTC CAT AAC
P. dalli	GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC CCC GTT CGC GGG CGG C:G GCG GCG ::: TCA TTC CAT AAC
P. divaricatum	GGA ACT CAA ACT GAA TTG CAC GTC TCC TCC CCC GTT CGC GGG CGG C:G GTG GCG ::: TCA TTC CAT AAC
P. eugeniodies	GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC CCC GTT CGC GGG CGG C:G GTG GCG ::: TCA TTC CAT AAC
P. fairchildii	GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC CCC GTT CGC GGG CTG C:G GTG GCG ::: TCA TTC CAT AAC
P huttonianum	GGA ACT CAA ACT GAA TCG CAC GTC TCC GCC CCC GTT TGC GGG CGG C:G GTG GCG ::T TCA TTC CAT AAC
P. kirkii	GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC CCC GTT CGC GGG GGG CCG GCG GCG ::: TCA TTC CAT AAC
P. obcordatum	GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC CCC GTT CGC GGG CGG C:G GCG GCG ::: TCA TTC CAT AAC
P. patulum	GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC CCT GTT CGC GGG CGG C:G GTG GCG ::: TCA TTC CAT AAC
P. p. maius	GGA ACT CAA AAT GAA TTG TAC GTC TCC TCC CCC GTT TGC GGG CGG C:G GTG GCG ::: TCA TTC CAT AAC
P. p. pimeleoides	GGA ACT CAA AAT GAA TTG TAC GTC TCC TCC CCC GTT TGC GGG CGG C:G GTG GCG ::: TCA TTC CAT AAC
P. ralphii	GGA ACT CAA GCT GAA TCG CAC GTC TCC GCC CCC GTT TGC GGG CGG C:G GTG GCG ::T TCA TTC CAT AAC
P. rigidum 1	GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC CCC GTT CGC GGG CGG C:G GTG GCG ::: TCA TTC CAT AAC
P. rigidum 2	GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC CCC GTT CGC GGG CGG C:G GTG GCG ::: TCA TTC CAT AAC
P. t. colensoi	GGA ACT CAA ACT GAA TCG CAC GTC TCC GCC CCC GTT TGC GGG CGG C:G GTG GCG ::T TCA TTC CAT AAC
P. t. tenuifolium	GGA ACT CAA ACT GAA TCG CAC GTC TCC GCC CCC GTT TGC GGG CGG C:G GTG GCG ::T TCA TTC CAT AAC
P. turneri	GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC CCC GTT CGC GGG CGG C:G GTG GCG ::: TCA TTC CAT AAC
P. umbellatum	GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC CCC GTT CGC GGG CTG C:G GTG GCG ::: TCA TTC CAT AAC
P. undulatum	GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC CCC GTT CGC GGG CGG C:G GCG GCG GCG TCA TTC CAT AAC
P. virgatum	GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC CCC GTT CGC GGG CTG C:G GTG GCG ::: TCA TTS CAT AAC

5.8S →

292

P. anomalum	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. cornifolium	ATA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. crassifolium	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P.cf. crassifolium	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. dalli	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. divaricatum	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. eugeniodies	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. fairchildii	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. huttonianum	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. kirkii	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. obcordatum	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. patulum	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. p. maius	ATA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. p. pimeleoides	ATA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. ralphii	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. rigidum 1	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. rigidum 2	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. t. colensoi	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. t. tenuifolium	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. turneri	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. umbellatum	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT TGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. undulatum	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT
P. virgatum	ACA AAC GAC TCT CGG CAA CGG ATA TCT CGG CTC TCG CAT CGA TGA AGA ACG TAG CGA AAT GCG ATA CTT

	361
P. anomalum	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC
P. cornifolium	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATT GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCT
P. crassifolium	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC
P.cf. crassifolium	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC
P. dalli	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC
P. divaricatum	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC
P. eugeniodies	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC
P. fairchildii	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC
P. huttonianum	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC
P. kirkii	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC
P. obcordatum	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC
P. patulum	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC
P. p. maius	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATT GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCT
P. p. *pimeleoides*	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATT GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCT
P. ralphii	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC
P. rigidum 1	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC
P. rigidum 2	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC
P. t. colensoi	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC
P. t. tenuifolium	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC
P. turneri	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC
P. umbellatum	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC
P. undulatum	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC
P. virgatum	GGT GTG AAT TGC AGA ATC CCG TGA ACC ATC GAG TCT TTG AAC GCA AGT TGC GCC CGA AGC CAT TAG GCC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430
P. anomalum GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC G:C GTC GCC CCC C:: AAC CCT CCC CAT CCC ::A TC: :A:
P. cornifolium GAG GGC ACG TCT GCC TGG GCG TCA CGT ATC G:C GTC GCC ACC C:: AAC CCT ACC CAT CCC ::A TC: :A:
P. crassifolium GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC G:C GTC GCC CCC C:: AAC CCT CCC CAT CCC ::A TC: :A:
P.cf. crassifolium GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC G:C GTC GCC CCC C:: AAC CCT CCC CAT CCC ::A TC: :A:
P. dalli GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC G:C GTC GCC CCC CCC AAC CCT CCC CAT CCC ::A TC: :A:
P. divaricatum GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC G:C GTC GCC CCC C:: AAC CCT CCC CAT CCC ::A TM: :A:
P. eugeniodies GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC GGC GTC GCC CCC C:: AAC CCT CCC CAT CCC ::A TT: :A:
P. fairchildii GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC G:C GTC GCC CCC CC: AAC CCT CCC CAT CCC ::A TC: :A:
P. huttonianum GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC G:C GTC GCC CCC C:: AAC CCT CCC CAT CCC ::A TC: :A:
P. kirkii GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC G:C GTC GCC CCC C:: AAC CCT CCC CAT CCC ::A TC: :AT
P. obcordatum GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC G:C GTC GCC CCC C:: AAC CCT CCC CAT CCC ::A TC: :A:
P. patulum GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC G:C GTC GCC CCC C:: AAC CCT CCC CAT CCC ::A TC: :A:
P. p. maius GAG GGC ACG TCT GCC TGG GCG TCA CGT ATC G:C GTC GCC ACC C:: AAC CCT ACC CAT CCC ::A TC: :A:
P. p. pimeleoides GAG GGC ACG TCT GCC TGG GCG TCA CGT ATC G:C GTC GCC ACC C:: AAC CCT ACC CAT CCC ::A TC: :A:
P. ralphii GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC G:C GTC GCC CCC C:: AAC CCT CCC CAT CCC ::A TC: :A:
P. rigidum 1 GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC G:C GTC GCC CCC C:: AAC CCT CCC CAT CCC ::A TC: :A:
P. rigidum 2 GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC G:C GTC GCC CCC C:: AAC CCT CCC CAT CCC ::A TC: :A:
P. t. colensoi GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC G:C GTC GCC CCC C:: AAC CCT CCC CAT CCC ::A TC: :A:
P. t. tenuifolium GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC G:C GTC GCC CCC C:: AAC CCT CCC CAT CCC ::A TC: :A:
P. turneri GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC G:C GTC GCC CCC C:: AAC CCT CCC CAT CCC ::A TC: :A:
P. umbellatum GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC GAC GT: GCC CCC CC: AAC CC: CCC CAT CCC ::A TC: :A:
P. undulatum GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC G:C GTC GCC CCC C:: AAC CCT CCC TAT CCC TGA TCC CAT
P. virgatum GAG GGC ACG TCT GCC TGG GCG TCA CGC ATC G:C GTC GCC CCC CC: AAC CCT CCC CAT CCC ::A TC: :A:

499

P. anomalum	::G GGG TGC TGA GGG CGG GGG AG: ::C GGA T:A CTG GCC TCC CGT GCC TCG ATG TGC GGT TGG CCC AAA
P. cornifolium	::G GGG TGC GGA GGG CTA GGG GG: ::C GGA T:A CTG GCC TCC CGT ACC TCG ATG TGC GGT TGG CCC AAA
P. crassifolium	::G GGG TGC TGA GGG CGG GGG AG: ::C GGA T:A CTG GCC TCC CGT GCC TCG ATG TGC GGT TGG CCC AAA
P.cf. crassifolium	::G GGG TGC TGA GGG CGG GGG AG: ::C GGA TTA CTG GCC TCC CGT GCC TCG ATG TGC GGT TGG CCC AAA
P. dalli	::G GGG TGC TGA GGG CGG GGG G:: ::C GGA T:A CTG GCC TCC CGT GCC TCG ATG TGC GGT TGG CCC AAA
P. divaricatum	::G GGG TGC TGA GGG CGG GGG GG: ::C GGA T:A CTG GCC TCC CGT GCC TCG ATG TGC GGT TGG CCC AAA
P. eugeniodies	::G GGG TGT TGA GGG CGA GGG GGG GGC GGA T:A CTG GCC TCC CGT TCC TCA ATG TGC GGT TGG CCC AAA
P. fairchildii	::G GGG TGC TGA GGG CGG GGG AG: ::C GGA T:A CTG GCC TCC CGT GCC TCG ATG TGC GGT TGG CCC AAA
P. huttonianum	::G GGG TGC TGA GGG CGG GGG AG: ::C GGA T:A CTG GCC TCC CGT GCC TCG ATG TGC GGT TGG CCC AAA
P. kirkii	::G GGG TGC TGA GGG CGG GGG G:: ::C GGA T:A CTG GCC TCC CGT GCC TCG ATG TGC GGT TGG CCC AAA
P. obcordatum	::G GGG TGC TGA GGG CGG GGG A:: ::C GGA T:A CTG GCC TCC CGT GCC TCG ATG TGC GGT TGG CCC AAA
P. patulum	::G GGG TGC TGA GGG CGG GGG AG: ::C GGA T:A CTG GCC TCC CGT GCC TCG ATG TGC GGT TGG CCC AAA
P. p. maius	::G GGG TGC GGA GGG CTA GGG GG: ::C GGA T:A CTG GCC TCC CGT ACC TCG ATG TGC GGT TGG CCC AAA
P. p. pimeleoides	::G GGG TGC GGA GGG CTA GGG GG: ::C GGA T:A CTG GCC TCC CGT ACC TCG ATG TGC GGT TGG CCC AAA
P. ralphii	::G GGG TGC TGA GGG CGG GGG AG: ::C GGA T:A CTG GCC TCC CGT GCC TCG ATG TGC GGT TGG CCC AAA
P. rigidum 1	::G GGG TGC TGA GGG CGG GGG AG: ::C GGA T:A CTG GCC TCC CGT GCC TCG ATG TGC GGT TGG CCC AAA
P. rigidum 2	::G GGG TGC TGA GGG CGG GGG AG: ::C GGA T:A CTG GCC TCC CGT GCC TCG ATG TGC GGT TGG CCC AAA
P. t. colensoi	::G GGG TGC TGA GGG CGG GGG AG: ::C GGA T:A CTG GCC TCC CGT GCC TCG ATG TGC GGT TGG CCC AAA
P. t. tenuifolium	::G GGG TGC TGA GGG CGG GGG AG: ::C GGA T:A CTG GCC TCC CGT GCC TCG ATG TGC GGT TGG CCC AAA
P. turneri	::G GGG TGC TGA GGG CGG GGG AG: ::C GGA T:A CTG GCC TCC CGT GCC TCG ATG TGC GGT TGG CCC AAA
P. umbellatum	::G GGG TGC TGA GGG CGG GGG AG: ::C GGA T:A CTG GCC TCC CGT GCC TTC ATG TGC GGT TGG CCC AAA
P. undulatum	CAG GGG TAC CGA GTG CGG GGG G:: ::C GGA T:A TTG GCC TCC CGT GCC TCA ATG TGC GGT TGT CCC AAA
P. virgatum	::G GGG TGC TGA GGG CGG GGG AG: ::C GGA T:A CTG GCC TCC CGT TCC TCG ATG TGC GGT TGG CCC AAA

568

P. anomalum	TGC GAG TCC TCG GCG ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CGG
P. cornifolium	TGC AAG TCA TCG GCA ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCT TCT TCT CAT GTC GTT TCG
P. crassifolium	TGC GAG TCC TCG GCG ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CGG
*P.*cf. *crassifolium*	TGC GAG TCC TCG GCG ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CGG
P. dalli	TGC GAG TCC TCG GCG ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CGG
P. divaricatum	TGC GAG TCC TCG GCG ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CCG
P. eugeniodies	TGC GAG TCC TCG GCG ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CGG
P. fairchildii	TGC GAG TCC TCG GCG ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CGG
P. huttonianum	TGC GAG TCC TCG GCG ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CGG
P. kirkii	TGC GAG TCC TCG GCG ATG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CGG
P. obcordatum	TGC GAG TCC TCG GCG ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CGG
P. patulum	TGC GAG TCC TCG GCG ACG TAT GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CGG
P. p. maius	TGC AAG TCA TCG GCA ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCT TCT TCT CAT GTC GTT TCG
P. p. *pimeleoides*	TGC AAG TCA TCG GCA ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCT TCT TCT CAT GTC GTT TCG
P. ralphii	TGC GAG TCC TCG GCG ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CGG
P. rigidum 1	TGC GAG TCC TCG GCG ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CGG
P. rigidum 2	TGC GAG TCC TCG GTG ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CGG
P. t. colensoi	TGC GAG TCC TCG GCG ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CGG
P. t. tenuifolium	TGC GAG TCC TCG GCG ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CGG
P. turneri	TGC GAG TCC TCG GCG ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CGG
P. umbellatum	TGC GAG TCC TCG GCG ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CGG
P. undulatum	TGC GAG TCC TCG GCG ACA TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CGG
P. virgatum	TGC GAG TCC TCG GCG ACG TAC GTC ACG ACA AGT GGT GGT TGT CAA AGG CCC TCT TAT CAT GTC GTG CGG

603

P. anomalum	TCG AAT GCC GCC AGA GCG ATC TCA CGT GAC CCT GT
P. cornifolium	TCG AAT GTC GCT AGA GTG ATC TCA TGC GAC CAT GT
P. crassifolium	TCG AAT GCC GCC AGA GTG ATC TCA CGT GAC CCT GT
P. cf. *crassifolium*	TCG AAT GCC GCC AGA GCG ATC TCA CGT GAC CCT GT
P. dalli	TCG AAT GCC GCC AGA GCG ATC TCA CGT GAC CCT GT
P. divaricatum	TCG AAT GCC GCC AGA GCG ATC TCA CGT GAC CCT GT
P. eugeniodies	TCG AAT GCC GCC AGA GCG ATC TCA ATT GAC CCT GT
P. fairchildii	TCG AAT GTC GCC AGA GCG ATC TCA CGT GAC CCT GT
P. huttonianum	TCG AAT GCC GCC AGA GTG ATC TCA CGT GAC CCT GT
P. kirkii	TTG AAT GTC GCC AGA GTG ATC TCA CGT GAC CCT GT
P. obcordatum	TCG AAT GTC GCC AGA GCG ATC TCA CGT GAC CCT GT
P. patulum	TCG AAT GCC GCC AGA GCG ATC TCA CGT GAC CCT GT
P. p. maius	TCG AAT GTC GCT AGA GTG ATC TCA TGC GAC CAT GT
P. p. *pimeleoides*	TCG AAT GTC GCT AGA GTG ATC TCA TGC GAC CAT GT
P. ralphii	TCG AAT GCC GCC AGA GTG ATC TCA CGT GAC CCT GT
P. rigidum 1	TCG AAT GCC GCC AGA GCG ATC TCA CGT GAC CCT GT
P. rigidum 2	TCG AAT GCC GCC AGA GCG ATC TCA CGT GAC CCT GT
P. t. colensoi	TCG AAT GCC GCC AGA GTG ATC TCA CGT GAC CCT GT
P. t. tenuifolium	TCG AAT GCC GCC AGA GTG ATC TCA CGT GAC CCT GT
P. turneri	TCG AAT GCC GCC AGA GCG ATC TCA CGT GAC CCT GT
P. umbellatum	TCG AAT GCC GCC AGA GCG ATC TCA CGT GAC CCT GT
P. undulatum	TCG AAT GCC GCC AGA GCG ATC TCA CGT GAC CCT GT
P. virgatum	TCG AAT GCC GCC AGA GCG ATC TCA CGT GAC CCT GT

All taxa	ITS1 ▶ 103
P. anomalum	AGA GCG ACC AGT GCA CTC GTA AAA CAC ATC C:G GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC A:G CCG CCG TCC CAC GGA CGG GGA
P. cornifolium	AGA GCG ACC AGC GAA CTC GTA AAA CAC ATC C:G GCT GAC GGC AAG GGA GGC GAC CAC TTC TCC A:G CCG TCG GCC CAT GGA CGG GGA
P. crassifolium	ANN NNN NNN NNN NNN NNN NNN NNN NNN NNN N:N NNN NNN NNN NNN NNN NNN NNN NNN NNN N:N NNN NNN NNN NNN NNN NNN NNA
P. cf. crassifolium	AGA GCG ACC AGT GCA CTC GTA AAA CAC ATC C:G GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC A:G CCG CCG TCC CAC GGA CGG GAA
P. dalli	AGA GCG ACC AGC GCA CTC GTA AAA CAC ATC C:G GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC A:G TCG CTG GCC CAC GGA CGG GGA
P. divaricatum	AGA GCG ACC AGT GCA CTC GTA AAA CAC ATC C:G GCC GGY GGC GAC GGA GGT GAC AGC TTC CCC A:G CCG CCG TCC CAC GGA CGG GGA
P. eugeniodies	AGA GCG ACC AGT GCA CTC GTA AAA CAC ATC C:G GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC A:G CCG CCG TCC CAC GGA CGG GGA
P. fairchildii	AGA GCG ACC AGT GCA CTT GTA AAA CAC ATC C:G GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC A:G TCG CCG TCC CAC GG: ::: :::
P. huttonianum	AGA GCG ACC AGT GCA CTC GTA AAA CAC ATC T:G GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC A:G CCG CCG TCC CAC GGA CGG GGA
P. kirkii	AGA GCG ACC AGC GCA CTC GTA AAA CAC ATC C:G GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC A:G TTG TCG GCC CAC GGA CGG GGA
P. obcordatum	AGA GCG ACC AGC GCA CTC GTA AAA CAC ATC T:G GCC GGA GGC GAC GGA GGT GAC AGC TTC CCC A:G CCG CCG TCC CAC GGA CGG GGA
P. patulum	ANN NNN NNN NNN NNN NNN NNN NNN NNN NNN N:N NNN NNN NNN NNN NNN NNN NNN NNN NNN N:N NNN NNN NCC CAC GGA CGG GGA
P. p. maius	AGA GCG ACC AGC GAA CTC GTA AAA CAC ATC C:G GCT GAC GGC AAG GGA GGC GAC CAC TTC TCC A:G CCG TCG GCC CAT GGA CGG GGA
P. p. pimeleoides	AGA GCG ACC AGC GAA CTC GTA AAA CAC ATC C:G GCT GAC GGC AAG GGA GGC GAC CAC TTC TCC A:G CCG TCG GCC CAT GGA CGG GGA
P. ralphii	AGA GCG ACC AGT GCA CTC GTA AAA CAC ATC T:G GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC A:G TCG CCG TCC :AC GGA CGG GGA
P. rigidum 1	AGA GCG ACC AGT GCA CTC GTA AAA CAC ATC C:G GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC A:G CCG CCG TCC CAC GGA CGG GGA
P. rigidum 2	AGA GCG ACC AGT GCA CTC GTA AAA CAC ATC C:G GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC A:G CCG CCG TCC CAC GGA CGG GGA
P. t. colensoi	AGA GCG ACC AGT GCA CTC GTA AAA CAC ATC T:G GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC A:G CCG CCG TCC CAC GGA CGG GGA
P. t. tenuifolium	ANN NNN NNN NNN NNN NNN NNN NNN NNN NNN N:N NNN NNN NNN NNN NNA GGT GAC AGC TTC CCC A:G CCG CCG TCC CAC GGA CGG GGA
P. turneri	AGA GCG ACC AGT GCA CTC GTA AAA CAC ATC C:G GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC A:G CCG CCG TCC CAC GGA CGG GGA
P. umbellatum	AGA GCG ACC AGT GCA CTC GTA AAA CAC ATC C:G GCC GGC GGC GAC GGA GGT GAC AGC TTC CCC A:G CCG CCG TCC CAC GGA CGG GGA
P. virgatum	AGA GCG ACC AGT GCA CTC GTA AAA CAC ATC C:G GCC GGC GGC GAC GGA GGT GAC AGC TTT CCC A:G CCG CCG WCC CAC GGA CGG GGA
P. coccineum	AGA GCG ACC AGC GAA CTC GTA AAA CAC ATC A:G CTC GGC GGC GCT GGA GGC AAC AGC TTC CCC A:G CCG CCG GCC CAC GGA TGG GGA
P. koghienese	AGA GTG ACC AGC GAA CTC GTA AAA CAC ATT C:G GTC GGC GGC GAT GGA GGC GAC AGC TTC CCC A:G CCG CCG GCC CAC GGA CGG GGA
P. lanipetalum	AGA GCG ACC AGC GAA CTC GTA AAA CAC ACT C:G GTC GGC GGC GAT GGA GGC GAC AGC TTC CCC A:G CCG CCG GCC CAC GGA CGG GGA
P. gatopenese	AGA GCG ACC AGC GAA CTC GTA AAA CAC ATC T:G GCC GGT GGI AAC GGA GGC AAC AAC TTC CCC A:G CCG CCG GCT CAC GGA CGG GGA
P. oreophilum	ANN NNN NNN AGA GAA CTC GTA AAA CAC ATT C:G GTC GGC GAC GAT GGA GGC GAC AAC TTC CCT A:G CCG CTG GCC CAC GGA CGG GGA
P. arborescens C	AGA CCA ACC AGT GAA CTC GTA ATA CAC ATC C:G GTC GGC AGC GAS GGA AGC GAC AGC TTC CCT A:G CCA CTG GCC CAC GGA CGG GGA
P. arborescens F	AGA CCA ACC AGC GAA CTC GTA ATA CAC ATC C:G GTC GGC AGC GAC GGA AGC GAC AGC TTC CCT A:G CCA CTG GCC CAC GGA CGG GGA
P. hosmeri	ANN NAG ACC AGC GAA CTG GTA AAA CAC ATC T:G GCC AGT GGC AAC GGA GGT GAC AGC TTC CC: A:G CCA CCG GCC CAC GGA CGG GGA
P. rhytidocarpum	AAG CAG ACC AGC GAA CTG GTA AAA CAC ATC T:G GCC AGT GGC AAC GGA AGT GAC AGC TTC CC: A:G CCA CCG GCC CAC GGA CGG GGA
P. yunkeri	ANN NAG ACC AGC GAA CTG GTA AAA CAC ATC T:G GCC AGT GGC AAC GGA AGT GAC AGC TTC CC: A:G CCA CCG GCC CAC GGA CGG GGA
P. tahitiense	AGA CCA ACC AGC GAA CTC GTA ATA CAC ATC C:G GTC GGC AGC GAC GGA AGT GAC AGC TTC CCT A:G CCA CTG GCC CAC GGA CGG GGA
P. balfouri	AGA GCG ACC AGC GAA CTC GTA ATA CAC ATC C:G GTC GGC GGI GAC AGA GGC AAT AGC TTC CCC A:G CCG CTG GCC CAT GGA CGG GGA
P. tobira	AGA GCG ACC AGC GAA CTC GTA ATA CAC ATC C:G GTC GGC GGI GAC AGA GGC AAC AGC TTC CCC A:G CCG CTG GCC CAT GGA CGG GGA
P. bracteolatum	AGA GCA ACC AGT GCA CTC GTA AAA CAC ATC C:G GTC AGC GGI CGA GGA GGC GAC AGC TTC CCC A:G CCG CCG GCC CAC GGA CGA GGA
P. moluccanum	GAA CCA ACC AGC GAA CTC GTA ATA CAC ATT C:G GTC GGY GGI GAC GGA AGT GAC AGC TTC CCT A:G CCA TCG GCC CAC GGA TGG GGA
P. phylliraeoides	AGA GCG ACC AGC GAA CTC GTA ATA CAC ATA C:G GTC GGC GGI GAT GGA GGC GAA AGC TTA CCC A:G CCA CCG GCC CAC GGA CGG GCA
P. undulatum	AGA GCA ACC AGC GAA CTC GTA ATA CAC ATC C:G GTC GGC GGI GAT GGA GGC AAC AGC TTC CCC A:T CCG CCG GCC CAC GGA CGG GGA
Citriobatus	AGA GCG ACC AGC GAA CTT GTA ATA CAC ATC C:G GTC GGT GGC GAC GGA GGC GAC AGC TTC CCC A:G CCA CCG GCC CAT GGA AGG GGA
Hymenosporum	AGA GCG ACC AGC GAA CCC GTA AAA CAC ATC G:G GCC GAC GGC GAC GGA GGC GAG AGC CTC CCC A:G TCG CCG GCC CAC GGA CGG GGA
Sollyea	AGA GCG ACC AGC GAA CCT GTA AAA CAC ACA TCG GCC GGG GGC GAC GGA GGC GAC AGC CTC CCC A:G CCG CCG GCC CAT GGA CGG GGA
Pseudopanax	AGA ACG ACC CGC GAA CAC GT: :TA CAA CAC C:G GGT GAG GGA CGA AGG GTG CGC AAG CTC CCC AAG TCG CGA ACC CAT GGT CGG GGA

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P. anomalum          GTG CCC TT: GGG CGC TGC TCG ACC GAA :AA CAA AAC CCC :GG CGC GGA ACG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC
P. cornifolium       GTG CCC TT: AGG CGT TAC TCG ACC GAA :AA CCA AAC CCC :GG CGT GGA ACG CGC CAA GGA ACT CAA AAT GAA TTG TAC GTC TCC TCC
P. crassifolium      GTG CCC TT: GGG CGC TGC TCG ACC GAA :AA CAA AAC CCC CGG CGC GGA ATG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC GCC
P. cf. crassifolium  GTG CCC TT: GGG CGC TGC TCG ACC GAA :AA CAA AAC CCC :GG CGC GGA ACG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC
P. dalli             GTG CCC CC: GGG CGC TGC TCG ACC GAA :AA CCA AAC CCC :GG CGC GGA ACG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC
P. divaricatum       GTG CCC TT: GGG CGC TGC TCG ACC GAA :AA CAA AAC CCC :GG CGC GGA ACG CGC CAA GGA ACT CAA ACT GAA TTG CAC GTC TCC TCC
P. eugeniodies       GTG CCC TT: GGG CGC TGC TCG ACC GAA :AA CAA AAC CCC :GG CGC GGA ACG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC
P. fairchildii       ::: ::: TT: GGG CGC TGC TCG ACC GAA :AA CAA AAC CCC :GG CGC GGA ACG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC
P. huttonianum       GTG CCC TT: GGG CGC TGC TCG ACC GAA :AA CAA AAC CCC CGG CGC GGA ATG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC GCC
P. kirkii            GTG CCC TC: GGG CGC TGC TCG ATC GAA :AA CCA AAC CCC :GG CGC GGA AAG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC
P. obcordatum        GTG CCC TT: GGG CGC TGC TCG ACC GAA :AA CCA AAC CCC :GG CGC GGA ATG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC
P. patulum           GTG CCC TT: GGG CGC TGC TCG ACC GAA :AA CAA AAC CCC :GG CGC GGA ACG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC
P. p. maius          GTG CCC TT: AGG CGT TAC TCG ACC GAA :AA CCA AAC CCC :GG CGT GGA ACG CGC CAA GGA ACT CAA AAT GAA TTG TAC GTC TCC TCC
P. p. pimeleoides    GTG CCC TT: AGG CGT TAC TCG ACC GAA :AA CCA AAC CCC :GG CGT GGA ACG CGC CAA GGA ACT CAA AAT GAA TTG TAC GTC TCC TCC
P. ralphii           GTG CCC TT: GGG CGC TGC TCG ACC GAA :AA CAA AAC CCC CGG CGC GGA ATG CGC CAA GGA ACT CAA GCT GAA TCG CAC GTC TCC GCC
P. rigidum 1         GTG CCC TT: GGG CGC TGC TCG ACC GAA :AA CAA AAC CCC :GG CGC GGA ACG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC
P. rigidum 2         GTG CCC TT: GGG CGC TGC TCG ACC GAA :AA CAA AAC CCC :GG CGC GGA ACG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC
P. t. colensoi       GTG CCC TT: GGG CGC TGC TCG ACC GAA :AA CAA AAC CCC CGG CGC GGA ATG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC GCC
P. t. tenuifolium    GTG CCC TT: GGG CGC TGC TCG ACC GAA :AA CAA AAC CCC CGG CGC GGA ATG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC GCC
P. turneri           GTG CCC TT: GGG CGC TGC TCG ACC GAA :AA CAA AAC CCC :GG CGC GGA ACG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC
P. umbellatum        GTG CCC TT: GGG CGC TGC TCG ACC GAA :AA CAA AAC CCC :GG CGC GGA ACG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC
P. virgatum          GTG CCC TT: GGG CGC TGC TCG ACC GAA :AA CAA AAG CCC :GG CGC GGA ACG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC
P. coccineum         GTG CCC CT: GGG CAC TGC TCG ACT GAA :AA CCA AAC CCC :GG CGT AGA ACA CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC
P. koghienese        GTG TCC CT: GGG CGC TGC TCG ACC AAA :AA CCA AAC CCC CGG CGC GGA ACG CGC CAA GGA ACT CAA ATT GAA TCG CAC GTC TCC TCC
P. lanipetalum       GTG CCC CT: GGG CGC TGC TCG ACC AAA :AA CCA AAC CCC :GG CGC GGA ACG CGC CAA GGA ACT CAA ATT GAA TCG CAC GTC TCC TCC
P. gatopenese        GTG CCC AT: GGG GAC TTC TCG ACC GAA :A: CCA AAC CCC :GG CGC GGA AAG CGC CAA GGA ACT CAA ACT GAA TCG TAC GTC TCC TCC
P. oreophilum        GTG CCC TT: GGG CGC TGC TCG ACC AAA :AA CCA AAC CCC :GG CGC GAA ATG TGC CAA GGA ACT CAA ATT GAA TCG CAC GTC TCC TCC
P. arborescens C     GTG CCC TC: GGG TGT TGY TCG ACC GAA :AA CCA AAC CCC :GG CGC GGA ACG CGT CAA GGA ACT CAA ACT GAA TTG AAC GTC ACC TCC
P. arborescens F     GTG CCC TC: GGG TGT TGC TCG ACC GAA :AA CCA AAC CCC :GG CGC GGA ACG CGT CAA GGA ACT CAA ACT GAA TTG AAC GTC ACC TCC
P. hosmeri           ATG CCC CT: GGG GGT TGC TCG ACC GAA :AA CTA AAC CCC :GG CGC GGA ACG CGC CAA GGA ACT CAA ACT GAA TTG CAC GTC TCC TCT
P. rhytidocarpum     ATG CCC CT: GGG GGT TGC TCG ACC GAA :AA CTA AAC CCC :GG CGC GGA ACG CGC CAA GGA AAT CAA ACT GAA TTG CAC GTC ::: :::
P. yunkeri           ATG CCC CT: GGG GGT TGC TCG ACC GAA :AA CTA AAA CCC :GG CGC GGA ACG CGC CAA GGA ACT CAA ACT GAA TTG CAC GTC ::: :::
P. tahitiense        GTG CCC TC: GGG TGT TGC TCG ACC GAA :AA CCA AAC CCC :GG CGC GGA ATG CGT CAA GGA ACT CAA ACT GAA TTG AAC GTC ACC TCC
P. balfouri          GTG CCC CC: GGG CGT TGC TCG ACC GAA :AA CCA AAC CCC :GG CGC GGA ATG CGC CAA GGA :CC CAG ATT GAA TCG CAC GTT TGC TCC
P. tobira            GTG CCC CC: GGG CGC TTC TCG ACC GAA :AA CCA AAC CCC :GG CGC GGA ATG CGC CAA GGA :CT CAG ATT GAA TCG CAC GTC TCC TCC
P. bracteolatum      GTG CCC CC: GGG CGC TGT TTG ACC GAA :AA CCA AAC CCC :GG CGC GGA ACG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC
P. moluccanum        GTG CCC TT: GGG CGC TGC TCC ACC GAA :AA CCA AAC CCC :GG CGC GGA ATG CGC CAA GGA ACT CAA ATT GAA TTG CAC GTC TCC TCC
P. phylliraeoides    GTG CCC CC: GGG TGT TGC TCG ACC GAA :AA CCA AAC CCC :GG CGC GGA ATG CGC CAA GGA ACC CAA ACT GAA TTG CAC GTC TCC TCC
P. undulatum         GTG CCC TC: GGG CGC TGC TCG ACC AAA :AA CCA AAC CCC :GG CGC GGA ACG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC
Citriobatus          GTG CCC CCC GGG CGC TGC TCG ACC GAA :AA CCA AAC CCC :GG CGC CAA ATG CGC CAA GGA ACT CAA ACT GAA TTG CAC GTC TTC GCC
Hymenosporum         GCG CCC CC: GGG CGC CGC CCG ACC GAA :AA CCA AAC CCC :GG CGC GGA AAG CGC CAA GGA ACT CAA ACT GAA TCG CAC GTC TCC TCC
Sollyea              GCG CCC CC: GGG TGC TGC CCG GCC GAA :AA CCA AAC CCC :GG CGC GGA ATG CGC CAA GGA ACT CAA ATT GAA CTG CAC GTC TCC TCC
Pseudopanax          CTG CCC TC: GGG TGT TCC TCG TCC GAA CAA CGA CCC CCC :GG CGC GGA ATG CGC CAA GGA AAT CAA ACT GAA CTG CAC GCA TCC :CC
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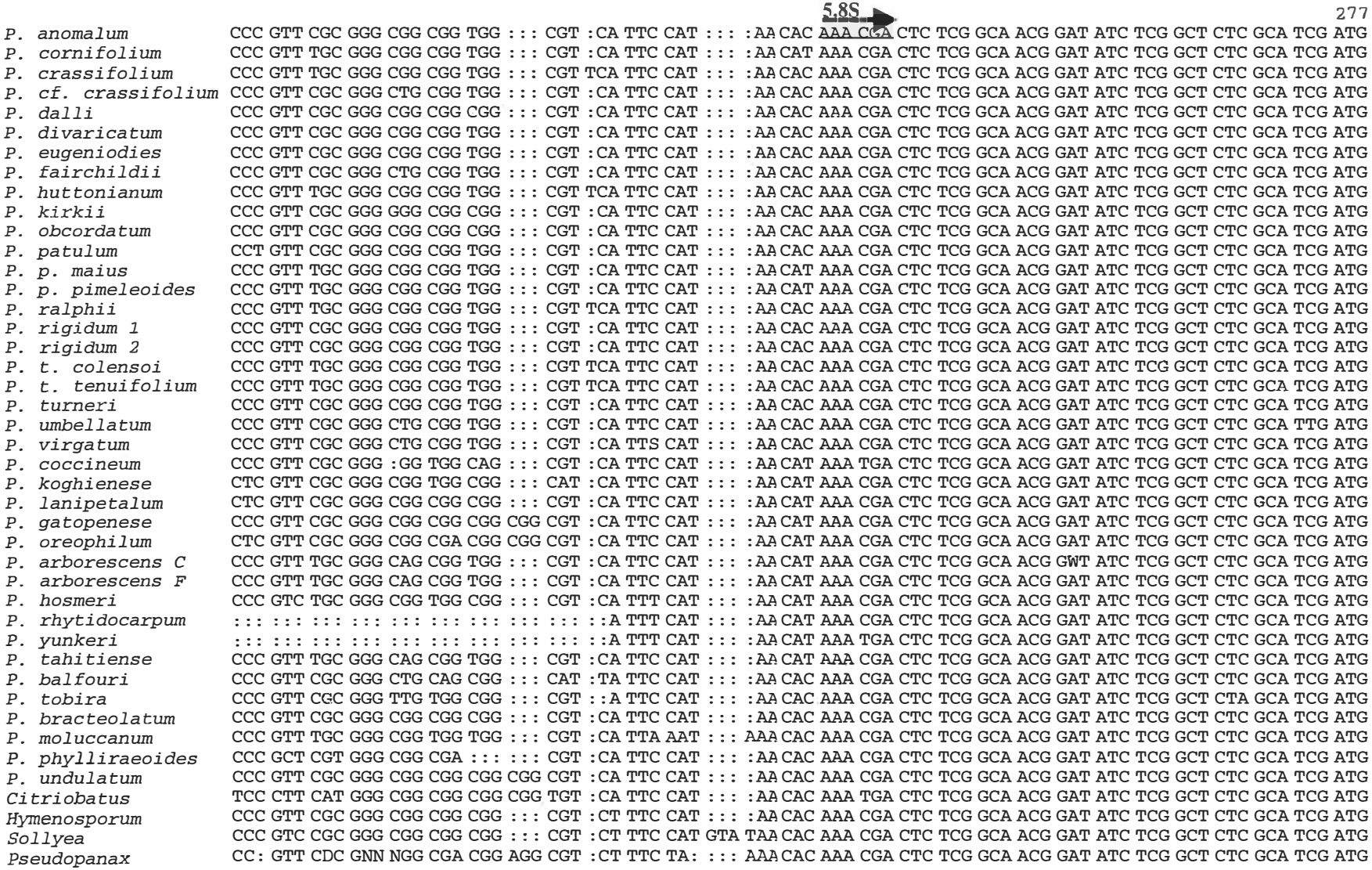
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P. cornifolium CCC GTT TGC GGG CGG CGG TGG ::: CGT :CA TTC CAT ::: :AA CAT AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. crassifolium CCC GTT TGC GGG CGG CGG TGG ::: CGT TCA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. cf. crassifolium CCC GTT CGC GGG CTG CGG TGG ::: CGT :CA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. dalli CCC GTT CGC GGG CGG CGG CGG ::: CGT :CA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. divaricatum CCC GTT CGC GGG CGG CGG TGG ::: CGT :CA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. eugeniodies CCC GTT CGC GGG CGG CGG TGG ::: CGT :CA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. fairchildii CCC GTT CGC GGG CTG CGG TGG ::: CGT :CA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. huttonianum CCC GTT TGC GGG CGG CGG TGG ::: CGT TCA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. kirkii CCC GTT CGC GGG GGG CGG CGG ::: CGT :CA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. obcordatum CCC GTT CGC GGG CGG CGG CGG ::: CGT :CA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. patulum CCT GTT CGC GGG CGG CGG TGG ::: CGT :CA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. p. maius CCC GTT TGC GGG CGG CGG TGG ::: CGT :CA TTC CAT ::: :AA CAT AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. p. pimeleoides CCC GTT TGC GGG CGG CGG TGG ::: CGT :CA TTC CAT ::: :AA CAT AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. ralphii CCC GTT TGC GGG CGG CGG TGG ::: CGT TCA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. rigidum 1 CCC GTT CGC GGG CGG CGG TGG ::: CGT :CA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. rigidum 2 CCC GTT CGC GGG CGG CGG TGG ::: CGT :CA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. t. colensoi CCC GTT TGC GGG CGG CGG TGG ::: CGT TCA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. t. tenuifolium CCC GTT TGC GGG CGG CGG TGG ::: CGT TCA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. turneri CCC GTT CGC GGG CGG CGG TGG ::: CGT :CA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. umbellatum CCC GTT CGC GGG CTG CGG TGG ::: CGT :CA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TTG ATG
P. virgatum CCC GTT CGC GGG CTG CGG TGG ::: CGT :CA TTS CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. coccineum CCC GTT CGC GGG :GG TGG CAG ::: CGT :CA TTC CAT ::: :AA CAT AAA TGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. koghienese CTC GTT CGC GGG CGG TGG CGG ::: CAT :CA TTC CAT ::: :AA CAT AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. lanipetalum CTC GTT CGC GGG CGG CGG CGG ::: CGT :CA TTC CAT ::: :AA CAT AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. gatopenese CCC GTT CGC GGG CGG CGG CGG CGG CGT :CA TTC CAT ::: :AA CAT AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
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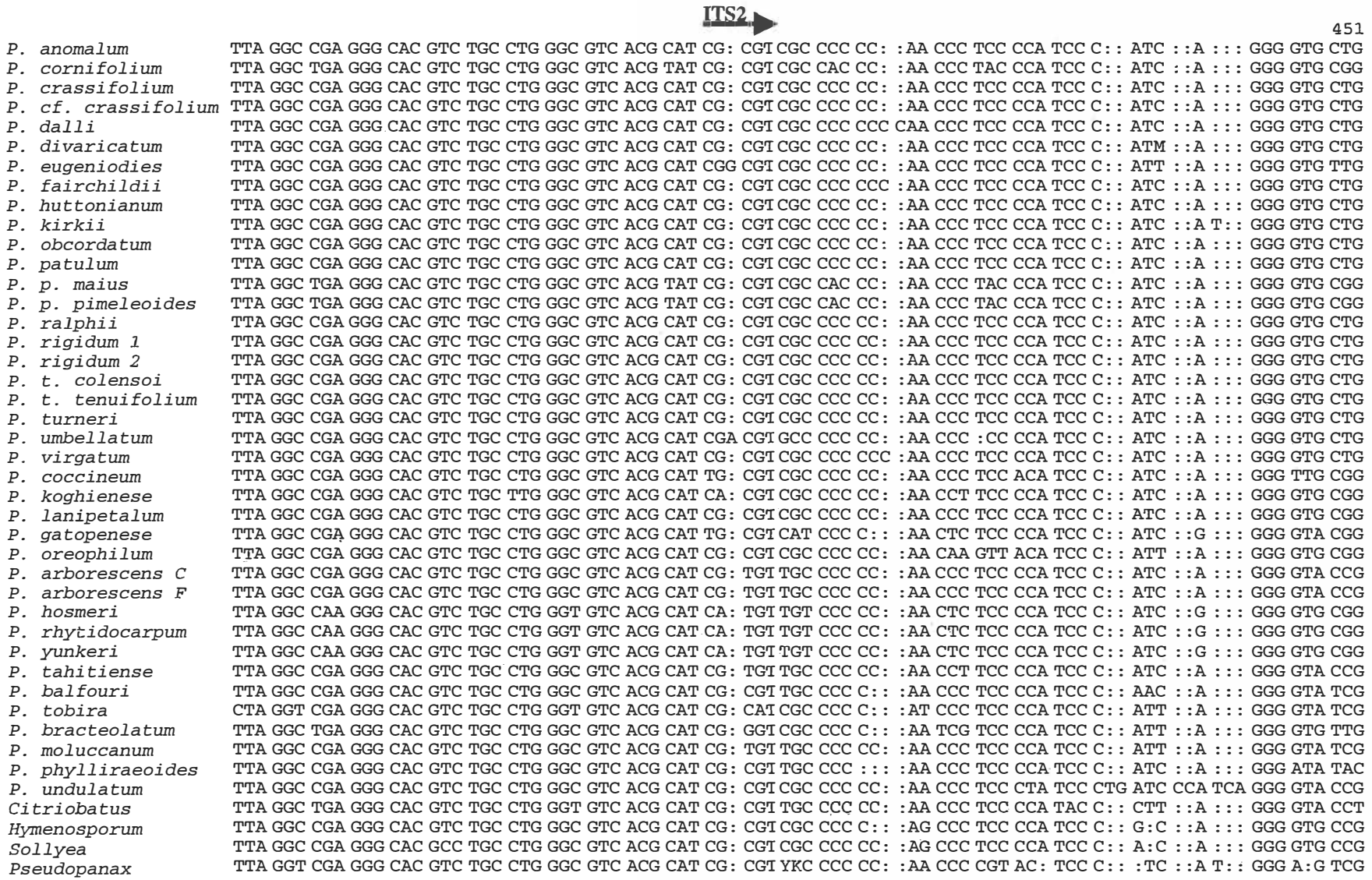
P. arborescens C CCC GTT TGC GGG CAG CGG TGG ::: CGT :CA TTC CAT ::: :AA CAT AAA CGA CTC TCG GCA ACG GWT ATC TCG GCT CTC GCA TCG ATG
P. arborescens F CCC GTT TGC GGG CAG CGG TGG ::: CGT :CA TTC CAT ::: :AA CAT AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. hosmeri CCC GTC TGC GGG CGG TGG CGG ::: CGT :CA TTT CAT ::: :AA CAT AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. rhytidocarpum ::: ::: ::: ::: ::: ::: ::: ::: ::: ::A TTT CAT ::: :AA CAT AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. yunkeri ::: ::: ::: ::: ::: ::: ::: ::: ::: ::A TTT CAT ::: :AA CAT AAA TGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. tahitiense CCC GTT TGC GGG CAG CGG TGG ::: CGT :CA TTC CAT ::: :AA CAT AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. balfouri CCC GTT CGC GGG CTG CAG CGG ::: CAT :TA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. tobira CCC GTT CGC GGG TTG TGG CGG ::: CGT ::A TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTA GCA TCG ATG
P. bracteolatum CCC GTT CGC GGG CGG CGG CGG ::: CGT :CA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. moluccanum CCC GTT TGC GGG CGG TGG TGG ::: CGT :CA TTA AAT ::: AAA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. phylliraeoides CCC GCT CGT GGG CGG CGA ::: ::: CGT :CA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
P. undulatum CCC GTT CGC GGG CGG CGG CGG CGG CGT :CA TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
Citriobatus TCC CTT CAT GGG CGG CGG CGG CGG TGT :CA TTC CAT ::: :AA CAC AAA TGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
Hymenosporum CCC GTT CGC GGG CGG CGG CGG ::: CGT :CT TTC CAT ::: :AA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
Sollyea CCC GTC CGC GGG CGG CGG CGG ::: CGT :CT TTC CAT GTA TAA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG
Pseudopanax CC: GTT CDC GNN NGG CGA CGG AGG CGT :CT TTC TA: ::: AAA CAC AAA CGA CTC TCG GCA ACG GAT ATC TCG GCT CTC GCA TCG ATG

P. anomalum	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. cornifolium	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT TGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. crassifolium	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. cf. crassifolium	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. dalli	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. divaricatum	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. eugeniodies	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. fairchildii	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. huttonianum	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. kirkii	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. obcordatum	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. patulum	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. p. maius	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT TGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. p. pimeleoides	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT TGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. ralphii	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. rigidum 1	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. rigidum 2	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. t. colensoi	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. t. tenuifolium	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. turneri	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. umbellatum	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. virgatum	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. coccineum	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. koghienese	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. lanipetalum	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. gatopenese	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. oreophilum	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT TGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. arborescens C	AAR AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. arborescens F	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. hosmeri	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. rhytidocarpum	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. yunkeri	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. tahitiense	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT YGA GTY TTT GAA MGC AAK TTG CGC CCG AAG CCA
P. balfouri	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. tobira	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. bracteolatum	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. moluccanum	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. phylliraeoides	AAG AAC GTA GCA AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
P. undulatum	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
Citriobatus	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
Hymenosporum	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
Sollyea	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA
Pseudopanax	AAG AAC GTA GCG AAA TGC GAT ACT TGG TGT GAA TTG CAG AAT CCC GTG AAC CAT CGA GTC TTT GAA CGC AAG TTG CGC CCG AAG CCA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P. cornifolium TTA GGC TGA GGG CAC GTC TGC CTG GGC GTC ACG TAT CG: CGT CGC CAC CC: :AA CCC TAC CCA TCC C:: ATC ::A ::: GGG GTG CGG
P. crassifolium TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CC: :AA CCC TCC CCA TCC C:: ATC ::A ::: GGG GTG CTG
P. cf. crassifolium TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CC: :AA CCC TCC CCA TCC C:: ATC ::A ::: GGG GTG CTG
P. dalli TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CCC CAA CCC TCC CCA TCC C:: ATC ::A ::: GGG GTG CTG
P. divaricatum TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CC: :AA CCC TCC CCA TCC C:: ATM ::A ::: GGG GTG CTG
P. eugeniodies TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CGG CGT CGC CCC CC: :AA CCC TCC CCA TCC C:: ATT ::A ::: GGG GTG TTG
P. fairchildii TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CCC :AA CCC TCC CCA TCC C:: ATC ::A ::: GGG GTG CTG
P. huttonianum TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CC: :AA CCC TCC CCA TCC C:: ATC ::A ::: GGG GTG CTG
P. kirkii TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CC: :AA CCC TCC CCA TCC C:: ATC ::A T:: GGG GTG CTG
P. obcordatum TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CC: :AA CCC TCC CCA TCC C:: ATC ::A ::: GGG GTG CTG
P. patulum TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CC: :AA CCC TCC CCA TCC C:: ATC ::A ::: GGG GTG CTG
P. p. maius TTA GGC TGA GGG CAC GTC TGC CTG GGC GTC ACG TAT CG: CGT CGC CAC CC: :AA CCC TAC CCA TCC C:: ATC ::A ::: GGG GTG CGG
P. p. pimeleoides TTA GGC TGA GGG CAC GTC TGC CTG GGC GTC ACG TAT CG: CGT CGC CAC CC: :AA CCC TAC CCA TCC C:: ATC ::A ::: GGG GTG CGG
P. ralphii TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CC: :AA CCC TCC CCA TCC C:: ATC ::A ::: GGG GTG CTG
P. rigidum 1 TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CC: :AA CCC TCC CCA TCC C:: ATC ::A ::: GGG GTG CTG
P. rigidum 2 TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CC: :AA CCC TCC CCA TCC C:: ATC ::A ::: GGG GTG CTG
P. t. colensoi TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CC: :AA CCC TCC CCA TCC C:: ATC ::A ::: GGG GTG CTG
P. t. tenuifolium TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CC: :AA CCC TCC CCA TCC C:: ATC ::A ::: GGG GTG CTG
P. turneri TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CC: :AA CCC TCC CCA TCC C:: ATC ::A ::: GGG GTG CTG
P. umbellatum TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CGA CGT GCC CCC CC: :AA CCC :CC CCA TCC C:: ATC ::A ::: GGG GTG CTG
P. virgatum TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CCC :AA CCC TCC CCA TCC C:: ATC ::A ::: GGG GTG CTG
P. coccineum TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT TG: CGT CGC CCC CC: :AA CCC TCC ACA TCC C:: ATC ::A ::: GGG TTG CGG
P. koghienese TTA GGC CGA GGG CAC GTC TGC TTG GGC GTC ACG CAT CA: CGT CGC CCC CC: :AA CCT TCC CCA TCC C:: ATC ::A ::: GGG GTG CGG
P. lanipetalum TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CC: :AA CCC TCC CCA TCC C:: ATC ::A ::: GGG GTG CGG
P. gatopenese TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT TG: CGT CAT CCC C:: :AA CTC TCC CCA TCC C:: ATC ::G ::: GGG GTA CGG
P. oreophilum TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CC: :AA CAA GTT ACA TCC C:: ATT ::A ::: GGG GTG CGG
P. arborescens C TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: TGT TGC CCC CC: :AA CCC TCC CCA TCC C:: ATC ::A ::: GGG GTA CCG
P. arborescens F TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: TGT TGC CCC CC: :AA CCC TCC CCA TCC C:: ATC ::A ::: GGG GTA CCG
P. hosmeri TTA GGC CAA GGG CAC GTC TGC CTG GGT GTC ACG CAT CA: TGT TGT CCC CC: :AA CTC TCC CCA TCC C:: ATC ::G ::: GGG GTG CGG
P. rhytidocarpum TTA GGC CAA GGG CAC GTC TGC CTG GGT GTC ACG CAT CA: TGT TGT CCC CC: :AA CTC TCC CCA TCC C:: ATC ::G ::: GGG GTG CGG
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P. tahitiense TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: TGT TGC CCC CC: :AA CCT TCC CCA TCC C:: ATC ::A ::: GGG GTA CCG
P. balfouri TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT TGC CCC C:: :AA CCC TCC CCA TCC C:: AAC ::A ::: GGG GTA TCG
P. tobira CTA GGT CGA GGG CAC GTC TGC CTG GGT GTC ACG CAT CG: CAT CGC CCC C:: :AT CCC TCC CCA TCC C:: ATT ::A ::: GGG GTA TCG
P. bracteolatum TTA GGC TGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: GGT CGC CCC C:: :AA TCG TCC CCA TCC C:: ATT ::A ::: GGG GTG TTG
P. moluccanum TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: TGT TGC CCC CC: :AA CCC TCC CCA TCC C:: ATT ::A ::: GGG GTA TCG
P. phylliraeoides TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT TGC CCC ::: :AA CCC TCC CCA TCC C:: ATC ::A ::: GGG ATA TAC
P. undulatum TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CC: :AA CCC TCC CTA TCC CTG ATC CCA TCA GGG GTA CCG
Citriobatus TTA GGC TGA GGG CAC GTC TGC CTG GGT GTC ACG CAT CG: CGT TGC CCC CC: :AA CCC TCC CCA TAC C:: CTT ::A ::: GGG GTA CCT
Hymenosporum TTA GGC CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC C:: :AG CCC TCC CCA TCC C:: G:C ::A ::: GGG GTG CCG
Sollyea TTA GGC CGA GGG CAC GCC TGC CTG GGC GTC ACG CAT CG: CGT CGC CCC CC: :AG CCC TCC CCA TCC C:: A:C ::A ::: GGG GTG CCG
Pseudopanax TTA GGT CGA GGG CAC GTC TGC CTG GGC GTC ACG CAT CG: CGT YKC CCC CC: :AA CCC CGT AC: TCC C:: :TC ::A T:: GGG A:G TCG

538

P. anomalum	AGG GCG GGG GAG ::: CGG ATA CTG GCC TCC CGT GCC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ACG TCA
P. cornifolium	AGG GCT AGG GGG ::: CGG ATA CTG GCC TCC CGT ACC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCA AGT CAT CGG CAA CGT ACG TCA
P. crassifolium	AGG GCG GGG GAG ::: CGG ATA CTG GCC TCC CGT GCC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ACG TCA
P. cf. crassifolium	AGG GCG GGG GAG ::: CGG ATA CTG GCC TCC CGT GCC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ACG TCA
P. dalli	AGG GCG GGG GG: ::: CGG ATA CTG GCC TCC CGT GCC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ACG TCA
P. divaricatum	AGG GCG GGG GGG ::: CGG ATA CTG GCC TCC CGT GCC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ACG TCA
P. eugeniodies	AGG GCG AGG GGG GGG CGG ATA CTG GCC TCC CGT TCC TCA ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ACG TCA
P. fairchildii	AGG GCG GGG GAG ::: CGG ATA CTG GCC TCC CGT GCC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ACG TCA
P. huttonianum	AGG GCG GGG GAG ::: CGG ATA CTG GCC TCC CGT GCC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ACG TCA
P. kirkii	AGG GCG GGG GG: ::: CGG ATA CTG GCC TCC CGT GCC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA TGT ACG TCA
P. obcordatum	AGG GCG GGG GA: ::: CGG ATA CTG GCC TCC CGT GCC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ACG TCA
P. patulum	AGG GCG GGG GAG ::: CGG ATA CTG GCC TCC CGT GCC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ATG TCA
P. p. maius	AGG GCT AGG GGG ::: CGG ATA CTG GCC TCC CGT ACC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCA AGT CAT CGG CAA CGT ACG TCA
P. p. pimeleoides	AGG GCT AGG GGG ::: CGG ATA CTG GCC TCC CGT ACC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCA AGT CAT CGG CAA CGT ACG TCA
P. ralphii	AGG GCG GGG GAG ::: CGG ATA CTG GCC TCC CGT GCC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ACG TCA
P. rigidum 1	AGG GCG GGG GAG ::: CGG ATA CTG GCC TCC CGT GCC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ACG TCA
P. rigidum 2	AGG GCG GGG GAG ::: CGG ATA CTG GCC TCC CGT GCC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG TGA CGT ACG TCA
P. t. colensoi	AGG GCG GGG GAG ::: CGG ATA CTG GCC TCC CGT GCC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ACG TCA
P. t. tenuifolium	AGG GCG GGG GAG ::: CGG ATA CTG GCC TCC CGT GCC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ACG TCA
P. turneri	AGG GCG GGG GAG ::: CGG ATA CTG GCC TCC CGT GCC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ACG TCA
P umbellatum	AGG GCG GGG GAG ::: CGG ATA CTG GCC TCC CGT GCC TTC ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ACG TCA
P. virgatum	AGG GCG GGG GAG ::: CGG ATA CTG GCC TCC CGT TCC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ACG TCA
P. coccineum	AGG GCT GGG GGG ::: CGG ATA CTG GCC TCC CGT GCC TCG ATG TGC A:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ACG TCA
P. koghienese	AGG GTT GGG GGG ::: CGG ATA TTG GCC TCC TGT GCC TCG ATG TGC A:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA TGT ACG TCA
P. lanipetalum	AGG GTT GGG GG: ::: CGG ATA CTG GCC TCC TGT GCC TTG ATG TGC A:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ATG TCA
P. gatopenese	AGG GCG GGG GGA ::: CGG ATA TTG GCC TCC CGT GCC TTG ATG TGC G:: GTT GGC CAA AAA AAT GCG AGT CCT CGG TGA CGT ACG TCA
P. oreophilum	AGG GTT GGG GGG G:: CGG ATA CTG GCC TCC TTT ACC TCG ATG TGC A:: GTT GGC CCA AA: ::T GCG AGT CCT CGG AAT CGT ATG TCA
P. arborescens C	AGG GGT GGG GGG ::: CGG ATA TTG GCC TCC CGT GCC TSG ATG TGC G:: GTT GGG GAA AA: ::T GGG AGT CCG GGG CGA CGT ATG TCA
P. arborescens F	AGG GGT GGG GGG ::: CGG ATA TTG GCC TCC CGT GCC TCG ATG TGC G:: GTT GGC CAA AA: ::T GCG AGT CCT TGG CGA CGT ATG TCA
P. hosmeri	AGG GAT GGG GGA ::: CGG ATA TTG GCC TCC CGT GCC TTG ATG TGC G:: GTT GGC CCA AA: ::T GCG AAT CCT CGG TGA TGT ATG TCA
P. rhytidocarpum	AGG GTT GGG GGA ::: CGG ATA TTG GCC TCC CGT GCC TTG ATG TGC G:: GTT GGC CCA AA: ::T GCG AAT CCT CGG TGA TGT ATG TCA
P. yunkeri	AGG GTT GGG GGA ::: CGG ATA TTG GCC TCC CGT GCC TTG TTG TGC G:: GTT GGC CCA AA: ::T GCG AAT CCT CGG TGA TGT ATG TCA
P. tahitiense	AGG GGT GGG GGG ::: CGG ATA TTG GCC TCC CGT GCC TCG ACG TGC G:: GTT GGC CAA AA: ::T GCG AGT CCT TGG CGA CGT ATG TCA
P. balfouri	AGG GCG GGG GG: ::: CGG ATA TTG GCC TCC CGT GCC TCA ATG TGT G:: GTT GGC CCA AA: ::T ACG AGT CCT CGG CGA CGT ACG TCA
P. tobira	AGG GCG GGG GGG G:: CGG ATA TTG GCC TCC CGT GCC TCT TGA TGT GCG GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT ACG TCA
P. bracteolatum	AGG GCG AGG GGG G:: CGG ATA TTG GCC TCC CGT TCC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA CGT RCG TCA
P. moluccanum	AGG GCG AGG GGG G:: CGG ATA TTG GCC TCC CGT GCC TCG ACG TGT G:: GTT GGC CCA AA: ::T ACG AGT CCT CGG CGA CGC ATG TCA
P. phylliraeoides	TGA GGG CGG GGG ::: CGG ATA CTG GCC TCC CGT GCC TCG ATG TGT G:: GTT GGC CGA AA: ::T GCG AGA CCT CGG CGA CGA ACG TCA
P. undulatum	AGT GCG GGG GG: ::: CGG ATA TTG GCC TCC CGT GCC TCA ATG TGC G:: GTT GTC CCA AA: ::T GCG AGT CCT CGG CGA CAT ACG TCA
Citriobatus	AGG GCG GGG GGG ::: CGG ATA CTG GCC TCC CGT GCC TCG ATG TGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGA TGT ACG TCA
Hymenosporum	AGG GCG AGG GG: ::: CGG ATA CTG GCC TCC CGT GCC TCG ACG CGC G:: GTT GGC CCA AA: ::T GCG AGT CCT CGG CGG CGT ACG TCA
Sollyea	AGG GCG AGG GG: ::: CGG ATA CTG GCC TCC CGT GCC CTG ACG CGC G:: GTT GGC CCA AA: ::T GCG AGT CCC CGG CGA CGT ACG TCA
Pseudopanax	AG: GCG GAG GGG ::: CGG ATA CTG GCC TCC CGT GTC TCA CCG CGC G:: GTT GGC CCA AA: ::T GTG AGT CCT TGK CGA CGG ACG TCA

620

P. anomalum	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG CCG CCA GAG C:: GAT CTC ACG TGA CCC :TG T
P. cornifolium	CGA CAA GTG GTG GTT GTC AAA GGC CTT CTT CTC ATG TCG TTT CGT CGA ATG TCG CTA GAG T:: GAT CTC ATG CGA CCA :TG T
P. crassifolium	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG CCG CCA GAG T:: GAT CTC ACG TGA CCC :TG T
P. cf. crassifolium	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG CCG CCA GAG C:: GAT CTC ACG TGA CCC :TG T
P. dalli	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG CCG CCA GAG C:: GAT CTC ACG TGA CCC :TG T
P. divaricatum	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC CGT CGA ATG CCG CCA GAG C:: GAT CTC ACG TGA CCC :TG T
P. eugeniodies	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG CCG CCA GAG C:: GAT CTC AAT TGA CCC :TG T
P. fairchildii	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG TCG CCA GAG C:: GAT CTC ACG TGA CCC :TG T
P. huttonianum	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG CCG CCA GAG T:: GAT CTC ACG TGA CCC :TG T
P. kirkii	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT TGA ATG TCG CCA GAG T:: GAT CTC ACG TGA CCC :TG T
P. obcordatum	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG TCG CCA GAG C:: GAT CTC ACG TGA CCC :TG T
P. patulum	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG CCG CCA GAG C:: GAT CTC ACG TGA CCC :TG T
P. p. maius	CGA CAA GTG GTG GTT GTC AAA GGC CTT CTT CTC ATG TCG TTT CGT CGA ATG TCG CTA GAG T:: GAT CTC ATG CGA CCA :TG T
P. p. pimeleoides	CGA CAA GTG GTG GTT GTC AAA GGC CTT CTT CTC ATG TCG TTT CGT CGA ATG TCG CTA GAG T:: GAT CTC ATG CGA CCA :TG T
P. ralphii	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG CCG CCA GAG T:: GAT CTC ACG TGA CCC :TG T
P. rigidum 1	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG CCG CCA GAG C:: GAT CTC ACG TGA CCC :TG T
P. rigidum 2	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG CCG CCA GAG C:: GAT CTC ACG TGA CCC :TG T
P. t. colensoi	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG CCG CCA GAG T:: GAT CTC ACG TGA CCC :TG T
P. t. tenuifolium	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG CCG CCA GAG T:: GAT CTC ACG TGN NNN :NN N
P. turneri	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG CCG CCA GAG C:: GAT CTC ACG TGA CCC :TG T
P. umbellatum	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG CCG CCA GAG C:: GAT CTC ACG TGA CCC :TG T
P. virgatum	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG CCG CCA GAG C:: GAT CTC ACG TGA CCC :TG T
P. coccineum	CGA CAA GTG GTG GTT GTG AAA GGC CCT CTT ATC ATG TCG TGC AGT GGA ATG CCG CCA GAG T:: GAT CTC ACG TGA TCC :TG T
P. koghienese	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG CCG CCA GAG C:: AAT CTC ACG TGA CCC :TG T
P. lanipetalum	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGT AGT CGA ATG CCG CCA GAG C:: GAT CTC ACG TGA CCC :TG T
P. gatopenese	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GCT CGA ATG TCG CCA GAG C:: GAT CTC ACG TGA CCC :TG T
P. oreophilum	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTC ATC ATG TCG TGC GGT CGA ATG CCG CCA TAG T:: GAT CTC ACG TGA CCC :TG T
P. arborescens C	CGA CAA GTG GTG GTT GTC ARA GGC CCT CTT ATC ATG TCG TGC AGT TGA ATG TTG CCA GAG C:: GAT CTC ATG TGA CCC :TG T
P. arborescens F	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC AGT TGA ATG TCG CCA GAG C:: GAT CTC ATG TGA CCC :TG T
P. hosmeri	CGA CAA GTG GTG GTT GTC AAA GGA CCT CTT ATC ATG TCG TGC AGT TGA ATG CTG ACA GAG T:: GAT CTC ACG TGA CCC :TG T
P. rhytidocarpum	CGA CAA GTG GTG GTT GTC AAA GGA CCT CTT ATC ATG TCG TGC AGT TGA ATG CTG CCA GAG C:: GAT CTC ACG TGA CCC :TG T
P. yunkeri	CGA CAA GTG GTG GTT GTC AAA GGA CCT CTT ATC ATG TCG TGC AGT TGA ATG CTG CCA GAG C:: GAT CTC ACG TGA CCC :TG T
P. tahitiense	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC AGT TGA ATG TCG CCA GAG C:: GAT CTC ATG TGA CCC :TG T
P. balfouri	CGA CAA GTG GTG GTT GTC AAA GGC CCT ATT ATC ATG TCG TGC GGT CAA ATG CCG CCA GAG T:: GAT CTC ATG TGA CCC :TG T
P. tobira	CGA CAA GTG GTG GTT GTT AAA GGC CCT CTT ATC ATG TCA TGT GGT CAA ATG CCG CCA GAG C:: GAT CTC ATG TGA CCC :TG T
P. bracteolatum	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG TCA CCA GAG C:: GAT CTC AAT TGA CCC :TG T
P. moluccanum	CGA CAA GTG GTG GTT GTC AAA GGC CCT TTT ATC ATG TCG TGC AGT TGA ATG CCG CCA GAG T:: GAT CTC ATG TGA CCC :TG T
P. phylliraeoides	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATT CCG CCG GAG A:: GAT CTC ATG TGA CCC CTG T
P. undulatum	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GGT CGA ATG CCG CCA GAG C:: GAT CTC ACG TGA CCC :TG T
Citriobatus	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTC ATC ATG TCG TGT GGT TTA ATG CCG CTA GAG C:: GAT CTC ACG TGA CCC :TG T
Hymenosporum	CGA CAA GCG GTG GTT GTC AAA GGC CCT CTT ATC ATG TCG TGC GAT CGA ACG CCG CCG GAG C:: GAT CTC ACG TGA CCC C:G T
Sollyea	CGA CAA GTG GTG GTT GTC AAA GGC CCT CTT ATC ACG TCG TGC GAT CCA ACG CCG CCG CGG C:: GAT CTC ACG TGA CCC :TG T
Pseudopanax	CGA CAA GTG GTG GTT GTA AAA AGC CCT CTT CTC CTG TCG TGC AAT GAC CCG TCG CCA GCG AAA GAC CTC ::G TGA CCC :TG T